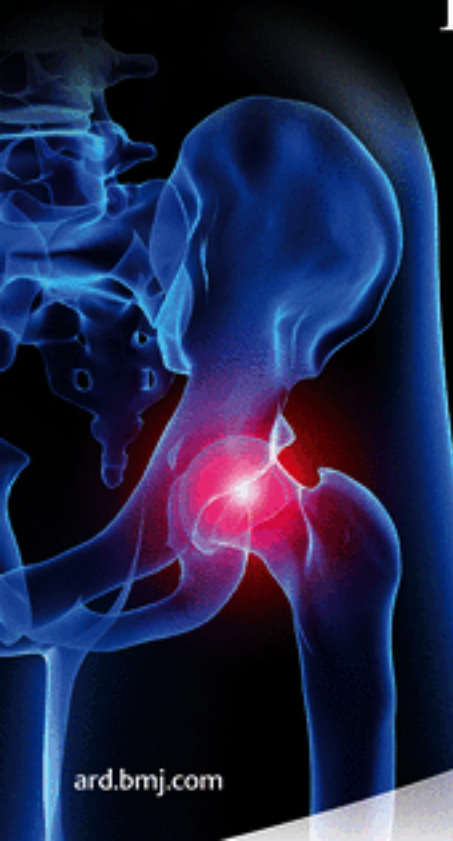
May 2018 Volume 77 Issue 5

Impact Factor 12.811

Annals of the Rheumatic Diseases

The EULAR Journal



ard.bmj.com

eular BMJ

ARD
The EULAR Journal

Guidelines for Authors and Reviewers

Full instructions are available online at http://ard.bmj.com/pages/authors. Articles must be submitted electronically at http://mc.manuscriptcentral.com/ard. Authors retain copyright but are required to grant ARD an exclusive licence to publish. (http://authors.bmj.com/submitting-your-paper/copyright-and-authors-rights).

Annals of the Rheumatic Diseases publishes original work on all aspects of rheumatology and disorders of connective tissue. Laboratory and clinical studies are equally welcome

Subscription Information

ARD is published monthly; subscribers receive all supplements
ISSN 0003-4967 (print); 1468-2060 (online)

Institutional Rates 2018

Print
£986

Online
Site licences are priced on FTE basis and allow access by the whole institution. Details available online at http://journals.bmj.com/content/subscribers or contact the Subscription Manager in the UK (see above right)

Personal Rates 2018

Print (includes online access at no additional cost)
£407

Online only
£173

Eular congress delegates
Delegates receive a Continuous Professional Development package that includes a 12 month complimentary subscription to *ARD* in print and/or online

Personal print or online only and institutional print subscriptions may be purchased online at http://journals.bmj.com/content/subscribers (payment by Visa/Mastercard only)

Residents of some EC countries must pay VAT; for details, call us or visit http://journals.bmj.com/content/subscribers

Contact Details

Editorial Office
Annals of the Rheumatic Diseases
BMJ Publishing Group Ltd
BMA House
Tavistock Square
London WCIH 9JR,UK
T: **+44 (0)20 7383 6250**
E: **ard@bmj.com**

Permissions
http://www.bmj.com/company/products-services/rights-and-licensing/permissions

Supplement Enquiries
T: **+44 (0)20 7383 6057**
E: **journals@bmj.com**

Subscriptions
For all subscription enquiries and orders
T: **+44 (0)20 7111 1105**
E: **http://ard.bmj.com/pages/subscribe**

Display Advertising Sales
Sophie Fitzsimmons
T: **+44 (0)20 7383 6783**
E: **sfitzsimmons@bmj.com**
http://www.bmj.com/company/raise-visibility-and-reach

Online Advertising Sales
Marc Clifford
T: **+44 (0) 20 7383 6161**
E: **mclifford@bmj.com**
http://www.bmj.com/company/raise-visibility-and-reach

Display & Online Advertising Sales (USA)
American Medical Communications (AMC)
T: **+1 973 214 4374**
E: **rgordon@americanmedicalcomm.com**

Author Reprints
Reprints Administrator
E: **admin.reprints@bmj.com**

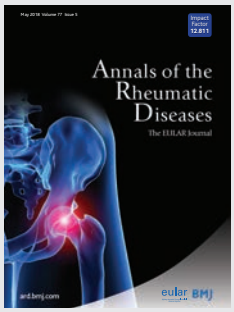
Commercial Reprints (except USA & Canada)
Nadia Gurney-Randall
T: **+44 (0)20 8445 5825**
M: **+44 07866 262344**
E: **ngurneyrandall@bmj.com**

Commercial Reprints (USA & Canada)
Ray Thibodeau
T: **+1 267 895 1758**
M: **+1 215 933 8484**
E: **ray.thibodeau@contentednet.com**

EULAR
EULAR Executive Secretariat
Seestrasse 240, 8802 Kilchberg, Switzerland
E: **eular@eular.org**
www.eular.org

For all other ARD contacts
http://ard.bmj.com/contact-us

ARD
The Eular Journal



Editorial office
Annals of the Rheumatic Diseases
BMJ Publishing Group Ltd
BMA House
Tavistock Square
London WCIH 9JR,UK
T: **+44 (0)20 7383 6250**
F: **+44 (0)20 7383 6668**
E: **ard@bmj.com**
Twitter: **@ARD_BMJ**
ISSN: 0003-4967 (print)
ISSN: 1468-2060 (online)
Impact Factor: 12.811

ARD is published by BMJ Publishing Group Ltd typeset by Exeter Premedia Services Private Ltd, Chennai, India and printed in the UK on acid-free paper.

Annals of the Rheumatic Diseases (ISSN No: 0003-4967) is published monthly by BMJ Publishing Group and distributed in the USA by Air Business Ltd. Periodicals postage paid at Jamaica NY 11431 POSTMASTER: send address changes to *Annals of the Rheumatic Diseases*, Air Business Ltd, c/o Worldnet Shipping Inc., 156-15, 146th Avenue, 2nd Floor, Jamaica, NY 11434, USA.

Editorial

Views on news

Recommendation

Clinical and epidemiological research

MORE CONTENTS ▶

Basic and translational research

Letters

Corrections

Electronic pages

Pneumocystis and glucocorticoid use: to prophylax or not to prophylax (and when?); that is the question

Kevin L Winthrop,[1] John W Baddley[2]

Pneumocystis jiroveci is an opportunistic fungus with the ability to cause lethal pneumonia in those with advanced immunosuppression.[1] Fortunately, this outcome is preventable with prophylaxis. Unfortunately, however, deciding who is immunosuppressed enough to justify prophylaxis can be a confusing subject, particularly among rheumatology patients where immunosuppression waxes and wanes based on the use of immunosuppressive therapies and the contribution of the underlying inflammatory disease. Foggy notions persist regarding who is at risk, the level of absolute risk where the risk-benefit of using trimethoprim/sulfamethoxazole (TMP/SMX) or other prophylaxis is worthwhile, and when can prophylaxis be safely stopped.[2] The article by Park *et al* published in the *Annals of Rheumatic Diseases*[3] sheds light on these important questions, such that a picture of how to approach this issue clinically might finally be more clear for the practicing rheumatologist.

WHAT IS THE BENEFIT OF TMP/SMX PROPHYLAXIS?

Given the difficulty studying these questions in a randomised controlled trial fashion, comparative effectiveness studies such as this one might provide the next best thing. In the article, the authors address the following questions: in patients starting high-dose glucocorticoids and taking them greater than 4 weeks, what is the risk of pneumocystic jiroveci pneumonia (PJP) (and the risk factors for it) and how does risk relate to dose? Further, they evaluate the efficacy of TMP/SMX prophylaxis. To answer these questions, they retrospectively identified an institutional cohort of rheumatology patients in Korea treated with 'high-dose' glucocorticoids (≥30 mg/day) for 4 or more weeks. Within this cohort, they selected patients offered TMP/SMX prophylaxis and compared their incidence of PJP with the remainder of the group that did not receive prophylaxis. There were important underlying differences between the groups, as one might expect, and it was clear that the treating physicians had generally chosen to give TMP/SMX to those they had perceived at higher risk for PJP. These risk factors included lymphopaenia, greater glucocorticoid use in the past, concomitant use of cyclophosphamide and the presence of dermatomyositis, microscopic polyangiitis (MPA) or granulomatosis polyangiitis. These factors being more prevalent within the prophylaxis group create 'confounding by indication' or 'channelling bias' such that one might expect a higher incidence of PJP in the group receiving prophylaxis, making it difficult to ascertain any protective effect of prophylaxis. This bias is the bane of observational and pharmacoepidemiological studies, as researchers struggle to compare 'apples with apples' and overcome this bias. In this case, the researchers used propensity scores to adjust for differences in groups and created two groups similar in their likelihood to receive prophylaxis based on disease characteristics and underlying risk factors for PJP (those risk factors beyond the use of high-dose glucocorticoids, a risk that was present in all participants). Crude incidence among the unprophylaxed was significantly higher, and when controlling for bias through propensity scores, use of TMP/SMX was associated with a 93% decrease in incidence of PJP. Only one case occurred in the prophylaxis group, and this after initial TMP/SMX was stopped due to an adverse drug reaction. The protection therefore appeared nearly complete and those at highest risk were protected. The benefits of TMP/SMX cannot be understated, and this study adds to others showing similarly high levels of protective effects within different settings of immunosuppression.[4] So, we know that it works, but what other conclusions can we draw from this experience that are relevant to the practice of rheumatology?

WHEN TO START PROPHYLAXIS?

The observations from these researchers confirm the answer is glucocorticoid dose-dependent. This is intuitive, although to our knowledge this has not been shown previously with regard to time to event, in that patients starting on 30 mg dosing took several months longer on average to develop PJP than those who started 60 mg/day. This in part could be related to the fact that patients starting higher doses take much longer to taper below a threshold level of risk. They spend longer times at risk. While Park *et al*'s data suggest you have longer to make a decision regarding

Table 1 Proposed PJP* prophylaxis with glucocorticoid use

	Prophylaxis at glucocorticoid dose (Y/N)†		Discontinuation of prophylaxis at glucocorticoid dose (Y/N)
Underlying disease	**15–30 mg**	**>30 mg**	**<15 mg**
Granulomatosis with polyangiitis	Y	Y	Y‡
Microscopic polyangiitis	Y	Y	Y‡
Systemic sclerosis	Y§	Y	Y
Dermatomyositis/ polymyositis	Y§	Y	Y
Systemic lupus erythematosus	N	Y	Y
Rheumatoid arthritis	N	Y§	Y

*Based on limited data and expert opinion, the authors advocate additional studies to further refine recommendations in this area.
†Requires prolonged glucocorticoids (≥4 weeks).
‡Conditional on <2 additional risk factors at time of discontinuation: baseline lymphopaenia, low CD4 count, cyclophosphamide use, anti-TNF or rituximab use, initial glucocorticoid dose of >60 mg.
§Conditional on at least one additional risk factor: baseline lymphopaenia, low CD4 count, cyclophosphamide use, anti-TNF or rituximab use, initial glucocorticoid dose of >60 mg.

[1]Division of Infectious Diseases, Oregon Health and Sciences University, Portland, Oregon, USA
[2]Division of Infectious Diseases, University of Alabama, Birmingham, Alabama, USA

Correspondence to Dr Kevin L Winthrop, Division of Infectious Diseases, Oregon Health and Sciences University, Portland, Oregon 97239-3098, USA; winthrop@ohsu.edu

prophylaxis start in those using 30 mg (versus 60 mg for example), it is clear that in any patient where such a dose is envisioned for 4 weeks or longer, that prophylaxis should be initiated.

WHEN TO STOP PROPHYLAXIS?

The concept of a risk 'threshold' has forever been a 'holy grail' type of question, with initial thresholds drawn at 15 mg or 20 mg per day for greater than 3 weeks. These were based on initial case series data that suggested most cases occur at these dose levels or higher after a prolonged time period.[5] The current study is consistent with these prior case series and, importantly, highlights that this threshold with regard to dose and time is not uniform. Three (10%) of the cases within this series were diagnosed with PJP after tapering to doses below 15 mg/day; however, each case had at least one other risk factor for PJP. We agree with the authors that at this level of glucocorticoid use, the overall risk is much lower (90% of the cases occurred at dose levels above 15 mg/day), but these cases illustrate that the risk of a certain dosage of glucocorticoids is likely modified by other PJP risk factors. The benefit of prophylaxis is almost certainly different in an 80 year old with lymphopaenia and vasculitis using 10 mg/day of glucocorticoids as compared with a 50 year old with rheumatoid arthritis (RA) using the same dose who lacks other risk factors. We suggest that for patients receiving prophylaxis that the treating physician consider stopping TMP/SMX once doses have been tapered to 15 mg/day, but that strong consideration be given to continuing it until lower doses are achieved if other PJP risk factors are present.

WHAT IS THE RISK-BENEFIT OF TMP/SMX?

The risk-benefit of TMP/SMX prophylaxis has been debated, given the high incidence of side effects reported with this compound.[6] Prior analyses suggest that the benefit outweighs the risk only in certain inflammatory disease conditions such as vasculitis or dermatomyositis, largely because the frequency of PJP is higher in these conditions.[4 7] Notably, prophylaxis may not be favourable in rheumatoid arthritis where the risk is low and number needed to treat (NNT) is much higher.[4] While this analysis is limited to those using high-dose glucocorticoids for greater than 1 month, it supports these ideas and provides both NNT and numbers needed to harm (NNH) information within some disease subgroups. Overall, this cohort tolerated TMP/SMX fairly well, with approximately 15% of patients developing AEs attributable to TMP/SMX. This was similar to findings from other analyses of rheumatic disease patients using TMP/SMX prophylaxis. The authors did not report what percentage of those using TMP/SMX withdrew drug due to adverse events; however, the incidence of serious adverse events attributable to TMP/SMX was low (n=2 events), such that the NNTs in order to prevent one PJP case were lower than the NNH with regard to serious adverse events. Not surprisingly, this benefit-risk scenario varied by disease state where the NNT was markedly lower for the higher risk diseases such as MPA or systemic lupus erhythematosus (SLE).

There were some important limitations to this analysis. Most PJP cases were diagnosed by use of PCR testing on induced sputum or bronchoalveolar lavage fluid, a situation in which it is sometimes difficult to distinguish between colonisation and definitive PJP. While there should be no differential bias between the two exposure groups that would affect one's ability to judge TMP/SMX effectiveness, the study might overestimate the risk of PJP and hence NNT calculations if some of these cases were only colonisation. It is also unclear if these data can generalise outside of Korea where the prevalence of PJP colonisation (and therefore the risk of developing disease) might differ. In addition, the analysis did not evaluate the risk of biological therapies. It is possible that their use might modify the risk of glucocorticoids, and that this could vary by their mechanism of action. Further, the analysis only addressed risk among a high-dose glucocorticoid using population, and it did not report PJP incidence in rheumatology patients who were not using high-dose glucocorticoids. The regimen studied was single-strength daily TMP/Sulfa. The risk/benefit of intermittent (three times a week) double strength TMP/SMX may be different, although experience from other settings of immunosuppression would suggest similar efficacy as daily single strength therapy.[8] Last, it is unclear if prophylaxis with other regimens (eg, dapsone, atovaquone) is of similar utility or should be employed if patients cannot tolerate TMP/SMX.

While these and other clinical questions remain, our opinion is that the current analysis provides strong guidance in terms of who to select for prophylaxis. It supports the efficacy of TMP/SMX use among those starting regimens of Prednisone>30 mg/day who continue for greater than 1 month and particularly those starting higher doses such 60 mg/day where the short-term risk of PJP is much greater. This is no matter their underlying disease state, although the benefits of prophylaxis are greater in those with higher risk diseases (eg, vasculitis) and other risk factors. We recommend such prophylaxis should continue until doses are below 15 mg/day, and even at this level if other PJP risk factors such as cyclophosphamide use, lymphopaenia or underlying vasculitis are present (table 1). While the absolute risk of PJP is considered to be low within rheumatology, this analysis clearly shows the risk is substantial within certain subgroups of diseases. This is a potentially lethal and preventable infection, and the Park *et al* analysis suggests there is little reason to take a risk with a month or more of high-dose glucocorticoids.

Handling editor Tore K Kvien

Contributors KLW and JWB shared all writing responsibilities for the editorial.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests KLW has been a consultant and/or investigator for Pfizer, AbbVie, Lilly, BMS, Galapagos and UCB. JWB has been a consultant for Pfizer, Merck and R-Pharm.

Provenance and peer review Commissioned; externally peer reviewed.

To cite Winthrop KL, Baddley JW. *Ann Rheum Dis* 2018;**77**:631–633

Linked

► http://dx.doi.org/10.1136/annrheumdis-2017-211796

Ann Rheum Dis 2018;**77**:631–633. doi:10.1136/annrheumdis-2017-212588

REFERENCES

1 Catherinot E, Lanternier F, Bougnoux ME, *et al*. Pneumocystis jirovecii Pneumonia. *Infect Dis Clin North Am* 2010;24:107–38.
2 Cettomai D, Gelber AC, Christopher-Stine L. A survey of rheumatologists' practice for prescribing pneumocystis prophylaxis. *J Rheumatol* 2010;37:792–9.
3 Park JW, Curtis JR, Moon J, *et al*. Prophylactic effect of trimethoprim-sulfamethoxazole for pneumocystis pneumonia in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoids. *Ann Rheum Dis* 2018;77:664–9.
4 Green H, Paul M, Vidal L, *et al*. Prophylaxis of Pneumocystis pneumonia in immunocompromised non-HIV-infected patients: systematic review and meta-analysis of randomized controlled trials. *Mayo Clin Proc* 2007;82:1052–9.

5 Yale SH, Limper AH. Pneumocystis carinii pneumonia in patients without acquired immunodeficiency syndrome: associated illnesses and prior corticosteroid therapy. *Mayo Clin Proc* 1996;71:5–13.
6 Wolfe RM, Peacock JE. Pneumocystis Pneumonia and the Rheumatologist: Which Patients Are At Risk and How Can PCP Be Prevented? *Curr Rheumatol Rep* 2017;19:35.
7 Rodriguez M, Fishman JA. Prevention of infection due to Pneumocystis spp. in human immunodeficiency virus-negative immunocompromised patients. *Clin Microbiol Rev* 2004;17:770–82.
8 Stern A, Green H, Paul M, *et al*. Prophylaxis for Pneumocystis pneumonia (PCP) in non-HIV immunocompromised patients. *Cochrane Database Syst Rev* 2014:Cd005590.

How the gut inflames the joints

A study by Pianta *et al* published in the *Journal of Clinical Investigation* provides new evidence that the pathogenesis of rheumatoid arthritis (RA) may involve molecular mimicry, one of the most venerable models for the aetiology of rheumatic disease.[1] Molecular mimicry represents the development of cross-reactive B or T cell responses to a component of an infecting agent. As shown in this study, the agent in question may be a commensal in the gut microbiome rather than a bacterium or virus inducing clinical disease.

While a violation of tolerance, molecular mimicry can occur due to the sharing of amino sequences by proteins from pathogen and host. If an immune response during infection goes awry, autoreactivity to the shared sequence can ensue. The most dramatic example of molecular mimicry is acute rheumatic fever (ARF), still a major problem worldwide. The scenario is now classic: within weeks of an infection with group A *Streptococcus*, usually pharyngitis, an inflammatory syndrome encompassing arthritis strikes a susceptible person. In ARF, the infection is obvious; the culprit bacterial antigen is the M protein.[2]

In a novel approach to finding molecular mimics, Pianta *et al* used mass spectrometry to characterise peptides bound to HLA-DR molecules of cells from synovial tissue, synovial fluid or peripheral blood of patients with RA.[1 3] This analysis demonstrated the presence of DR-presented peptides derived from previously unidentified autoantigens called N-acetylglucosamine-6-sulfatase (GNS) and filamin A (FLNA). Importantly, peptides from GNS and FLNA have sequence homology to proteins of *Prevotella* and other gut species. The homology is notable since the microbiomes of patients with new-onset RA have expansion of *Prevotella copri*.[4]

Other studies in this paper showed that sera from patients with RA contain IgG and IgA antibodies to GNS and FLNA, with levels correlated with those of *P. copri*. Among patients lacking anti-citrullinated protein antibodies (ACPA) or rheumatoid factor, over half had IgG and/or IgA antibodies to GNS or FLNA. Furthermore, GNS and FLNA peptides could stimulate in vitro responses of T cells. Overall, B and T cell responses to GNS and FLNA were present in 52% and 56% of patients, respectively, providing impressive evidence for a role of gut antigens in driving events in RA.

While RA, like ARF, may involve molecular mimicry, the clinical situations of RA and ARF are vastly different. With ARF, the infection is readily apparent and the onset of disease is sudden, making it easy to postulate a link. In the case of RA, the 'infection' is dysbiosis, a shift in the composition in the microbiome, in this case, increased *Prevotella* sp. The onset of RA is gradual and years may pass before an autoantibody response to proteins, especially citrullinated versions, develops and culminates in arthralgia and arthritis. Since the microbiome is populated early in life, the basis of this long evolution is unclear.

Molecular mimicry is only one mechanism by which dysbiosis can impact the pathogenesis of autoimmunity. Studies, especially in animal models, have suggested other intriguing possibilities. Thus, genetic factors may affect the composition of the microbiome; dysbiosis may perturb the poise of the immune system; and inflammation may lead to dysbiosis. The interplay between host and organism is thus likely to be complicated and dynamic.[5 6]

While the gut microbiome attracts great attention, it is only one of several. The mouth and the upper airways are others. A role of the gut microbiome in RA pathogenesis must therefore be incorporated into models of RA based on a role of periodontal and pulmonary infection in inducing protein citrullination and subsequent ACPA production.[7] If, as some data suggest, the gut and the oral cavity may have common organisms in their microbiomes, there can be two-way traffic of organisms in the body as well as traffic of pathogenic T cells emerging in either locale to journey into the joint.[8]

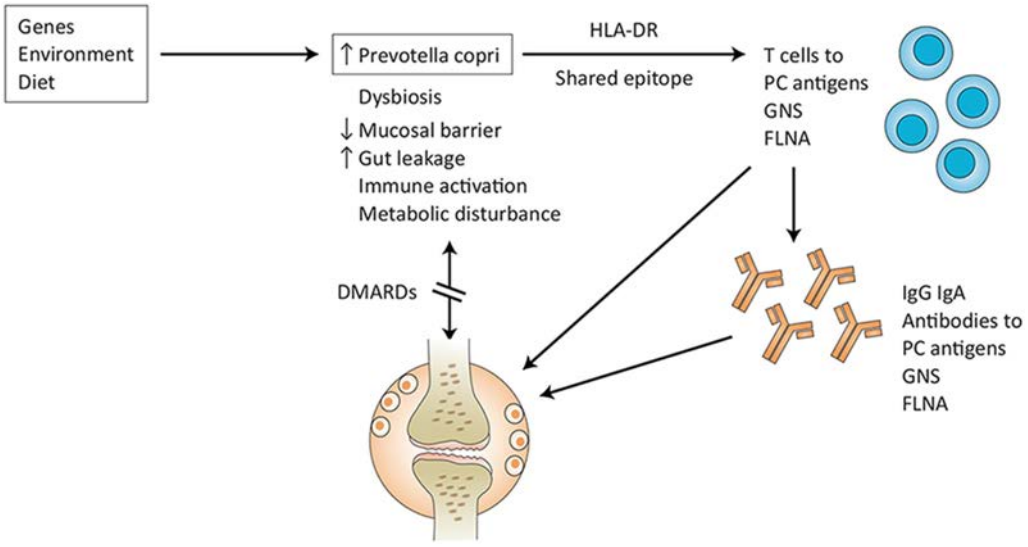


Figure 1 The role of the microbiome in the pathogenesis of rheumatoid arthritis (RA). In the pathogenesis of RA and other autoimmune and inflammatory conditions, dysbiosis can result from the interplay of genetic and environmental factors including diet. Dysbiosis can lead to changes in the mucosal barrier; leakage of bacteria and bacterial products into the blood; immune activation; induction of cytokines; and metabolic disturbance. In RA, dysbiosis can encompass an increase in *Prevotella copri* (PC) as well as a decrease in *Bacteroides* sp. Depending on genetic factors, most prominently the HLA-DR shared epitope, exposure to bacterial antigens can induce the activation of T cells that react to proteins from *Prevotella* as well as cross-reactive proteins N-acetylglucosamine-6-sulfatase (GNS) and filamin A (FLNA). These T cells can provide help to B cells which produce IgG and IgA antibodies to these antigens by molecular mimicry. Both T cell and B cell autoreactivity to GNS and FLNA in the joint can drive synovitis. Disease-modifying antirheumatic drugs (DMARD) can ameliorate inflammation as well as impact on dysbiosis.

Practically, these studies are important in identifying new target antigens for serological assessment in RA, especially for those patients who are ACPA negative. In addition, the phenomenon of dysbiosis raises the possibility of therapy to improve this state although the changes with disease-modifying antirheumatic drugs suggest a return to more normal gut composition can occur even with current approaches.[8] Figure 1 highlights these mechanisms. Whatever the direction of future therapy, this important study suggests that the boundary between foreign and self is not complete and that various encounters with bacterial organisms can lead to mimicry of the pathological kind.

David S Pisetsky

Correspondence to David S Pisetsky, Department of Medicine, Medical Research Service, Durham VA Medical Center, Duke University Medical Center, Durham, NC 27705, USA; david.pisetsky@duke.edu

Contributors DSP is sole author of this article.

Funding This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Pisetsky DS. *Ann Rheum Dis* 2018;**77**:634–635..

Received 16 January 2018
Accepted 17 January 2018
Published Online First 2 March 2018

REFERENCES

1 Pianta A, Arvikar SL, Strle K, *et al*. Two rheumatoid arthritis-specific autoantigens correlate microbial immunity with autoimmune responses in joints. *J Clin Invest* 2017;127:2946–56.
2 Carapetis JR, Beaton A, Cunningham MW, *et al*. Acute rheumatic fever and rheumatic heart disease. *Nat Rev Dis Primers* 2016;2:15084.
3 Pianta A, Arvikar S, Strle K, *et al*. Evidence of the Immune Relevance of Prevotella copri, a Gut Microbe, in Patients With Rheumatoid Arthritis. *Arthritis Rheumatol* 2017;69:964–75.
4 Scher JU, Sczesnak A, Longman RS, *et al*. Expansion of intestinal Prevotella copri correlates with enhanced susceptibility to arthritis. *Elife* 2013;2:e01202.
5 Longman RS, Littman DR. The functional impact of the intestinal microbiome on mucosal immunity and systemic autoimmunity. *Curr Opin Rheumatol* 2015;27:381–7.
6 Maeda Y, Kurakawa T, Umemoto E, *et al*. Dysbiosis contributes to arthritis development via activation of autoreactive t cells in the intestine. *Arthritis Rheumatol* 2016;68:2646–61.
7 Catrina AI, Deane KD, Scher JU. Gene, environment, microbiome and mucosal immune tolerance in rheumatoid arthritis. *Rheumatology* 2016;55:391–402.
8 Zhang X, Zhang D, Jia H, *et al*. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. *Nat Med* 2015;21:895–905.

EULAR recommendations for the use of imaging in large vessel vasculitis in clinical practice

Christian Dejaco,[1,2] Sofia Ramiro,[3] Christina Duftner,[4] Florent L Besson,[5,6] Thorsten A Bley,[7] Daniel Blockmans,[8] Elisabeth Brouwer,[9] Marco A Cimmino,[10] Eric Clark,[11] Bhaskar Dasgupta,[12,13] Andreas P Diamantopoulos,[14] Haner Direskeneli,[15] Annamaria Iagnocco,[16] Thorsten Klink,[7] Lorna Neill,[17] Cristina Ponte,[18,19] Carlo Salvarani,[20,21] Riemer H J A Slart,[22,23] Madeline Whitlock,[12] Wolfgang A Schmidt[24]

Handling editor Josef S Smolen

For numbered affiliations see end of article.

Correspondence to
Dr Christian Dejaco, Department of Rheumatology and Immunology, Medical University Graz, Graz 8036, Austria; christian.dejaco@gmx.net

Received 4 November 2017
Revised 13 December 2017
Accepted 2 January 2018
Published Online First 22 January 2018

ABSTRACT

To develop evidence-based recommendations for the use of imaging modalities in primary large vessel vasculitis (LVV) including giant cell arteritis (GCA) and Takayasu arteritis (TAK). European League Against Rheumatism (EULAR) standardised operating procedures were followed. A systematic literature review was conducted to retrieve data on the role of imaging modalities including ultrasound, MRI, CT and [^{18}F]-fluorodeoxyglucose positron emission tomography (PET) in LVV. Based on evidence and expert opinion, the task force consisting of 20 physicians, healthcare professionals and patients from 10 EULAR countries developed recommendations, with consensus obtained through voting. The final level of agreement was voted anonymously. A total of 12 recommendations have been formulated. The task force recommends an early imaging test in patients with suspected LVV, with ultrasound and MRI being the first choices in GCA and TAK, respectively. CT or PET may be used alternatively. In case the diagnosis is still in question after clinical examination and imaging, additional investigations including temporal artery biopsy and/or additional imaging are required. In patients with a suspected flare, imaging might help to better assess disease activity. The frequency and choice of imaging modalities for long-term monitoring of structural damage remains an individual decision; close monitoring for aortic aneurysms should be conducted in patients at risk for this complication. All imaging should be performed by a trained specialist using appropriate operational procedures and settings. These are the first EULAR recommendations providing up-to-date guidance for the role of imaging in the diagnosis and monitoring of patients with (suspected) LVV.

INTRODUCTION

Large vessel vasculitis (LVV) is the most common form of primary vasculitis comprising giant cell arteritis (GCA) and Takayasu arteritis (TAK).[1 2] The field of GCA and LVV has undergone rapid expansion. Ultrasound-guided fast-track strategies have led to a reduction of irreversible vision loss, and the concept of imaging confirmed large vessel (LV-)GCA with or without cranial disease, has been added to the disease definition.[3–5] Based on these considerations, the importance of imaging modalities including ultrasound, MRI, CT and [^{18}F]-fluorodeoxyglucose positron emission tomography (PET) has steadily increased.[6 7] These techniques enable the assessment of cranial and extracranial arteries and the aorta and are less invasive, more sensitive and more quickly available than temporal artery biopsy (TAB) and conventional angiography, which have been the sole diagnostic standards in GCA and TAK, respectively, for decades.

In TAK, temporal arteries are usually spared, and extracranial artery biopsies are rarely feasible. Angiography visualises luminal changes caused by vasculitis such as stenosis or occlusion but cannot delineate vessel wall pathology. Besides, angiography bears the risk of allergic reactions, haematoma, iatrogenic embolisation and arterial dissection. Modern imaging methods have therefore almost replaced catheterised angiography unless it is performed for therapeutic vascular interventions.[8]

These advances have brought along significant controversy and uncertainty about when to use which imaging technique, whether imaging might be helpful during follow-up to assess disease activity and damage and whether imaging results might predict future outcomes.

The objective of this project was to provide user-friendly, evidence-based recommendations for the use of imaging modalities for diagnosis, monitoring and outcome prediction of primary LVV.

METHODS

After approval by the EULAR Executive Committee, the convenors (ChristiaD and WAS) and methodologist (SR) led a task force guided by the 2014 updated EULAR standardised operating procedures.[9] The 20 task force members consisted of rheumatologists, radiologists, nuclear medicine specialists, patient representatives, an internist, a methodologist, a healthcare professional and EMerging EUlar NETwork representatives from 10 countries. All members disclosed their potential conflicts of interest before the start of the process. Two task force meetings took place.

At the first task force meeting, the panel agreed on four key questions covering the following aspects: the role of imaging techniques (including ultrasound, MRI, CT and PET) in (1) diagnosis and (2) monitoring of inflammation and damage, (3) prediction of outcome and (4) required technical standards for imaging.

To cite: Dejaco C, Ramiro S, Duftner C, *et al*. *Ann Rheum Dis* 2018;**77**:636–643.

Table 1 Oxford Centre for Evidence-Based Medicine 2011 levels of evidence for diagnostic studies (modified according to ref 13)

Level	Definition
1	Evidence from a systematic review of cross-sectional studies with consistently applied reference standard and blinding.
2	Individual cross-sectional studies with consistently applied reference standard and blinding.
3	Non-consecutive studies or studies without consistently applied reference standards.
4	Case–control studies or poor or non-independent reference standard.
5	Mechanism-based reasoning.

Level of evidence may be downgraded based on study quality, imprecision, indirectness, because of inconsistency between studies or because the absolute effect size is very small. Level may be upgraded if there is a large or very large effect size.

The systematic literature review (SLR) was conducted by two task force members (ChristinD and ChristiaD) under the guidance of the methodologist. Only prospective studies conducted in >20 patients with suspected and/or established primary LVV were included. The evidence summarised in the SLR was presented to the task force in the form of tables summarising the findings, including an assessment of the risk of bias of the studies.[10 11] The SLR is published separately[12]; however, the SLR and the present recommendations manuscript form an integral and inseparable part and should be read as such.

At the second meeting, the task force formulated the recommendations based on the evidence and expert opinion in a process of discussion and consensus, followed by final voting on the recommendations. Consensus was accepted if >75% of the members voted in favour of the recommendation at the first round, >67% at the second round and at a third round >50% was accepted. The Oxford Centre for Evidence-Based Medicine levels of evidence (LoE) derived from the SLR were added to each recommendation (table 1).[13]

Finally, each task force member anonymously indicated the level of agreement via e-mail (LoA, 0–10 numeric rating scale with 0=do not agree and 10=fully agree). The mean and SD of the LoA, as well as the percentage of task force members with an agreement ≥8 are presented.

Based on the gaps in the evidence and the issues of controversy, a research agenda was formulated. The final manuscript was reviewed and approved by all task force members and approved by the EULAR Executive Committee.

RESULTS

General aspects

These recommendations are intended to advise physicians on the use of imaging modalities (including ultrasound, MRI, CT and PET) when making a clinical diagnosis of LVV and when to apply imaging for monitoring of disease activity and damage. CT and MRI also refer to specific angiography techniques such as CT angiography (CTA) and MR angiography (MRA), and PET is commonly used in conjunction with CT or CTA.

These recommendations are not intended to cover all aspects of diagnosis and management of LVV and particularly do not discuss in full the role of TAB for GCA diagnosis.

The targeted users of these recommendations are secondary and tertiary care physicians including rheumatologists, ophthalmologists, neurologists, radiologists, nuclear medicine specialists, vascular surgeons, angiologists, geriatricians and other specialists in general (internal) medicine. The target population is patients with suspected or established primary LVV, specifically GCA or TAK. These recommendations may also inform patients participating in shared decision making, primary care physicians and healthcare providers organising care of patients with LVV.

Recommendations

A total of 12 recommendations have been formulated that are summarised in table 2 (including the LoE and LoA) and are discussed in detail below.

Recommendation 1: in patients with suspected GCA, an early imaging test is recommended to complement the clinical criteria for diagnosing GCA, assuming high expertise and prompt availability of the imaging technique. Imaging should not delay initiation of treatment.

This recommendation is general in its nature and intended to provide a framework for the subsequent specific recommendations on different imaging modalities. The choice of the individual imaging method depends on the predominant clinical symptoms and local settings as specified below. Early confirmation or exclusion of GCA by a diagnostic test is essential in order to prevent disease complications such as blindness or toxicity from unnecessary treatment.[14] The task force members recognised that many physicians still consider TAB as the gold standard test for the diagnosis of GCA. The present (and subsequent) proposition(s) should not be understood as a recommendation against performing TAB. In settings where imaging modalities are not readily available or expertise with imaging in GCA is questionable, a biopsy should still be favoured in first place. Besides, if positive histology is already available, additional imaging may not be needed for the diagnosis. In centres, however, where imaging (and TAB) is readily available and performed with high quality, the task force recommends that imaging should be preferred as the first test because of low invasiveness, ready availability of imaging results and assessment of a larger extent of potentially inflamed arteries at the same examination, thus contributing to a lower number of false negative results.

Imaging should be performed before or as early as possible after initiation of therapy, best within 1 week, because treatment with glucocorticoids rapidly reduces the sensitivity of imaging.[15–18] Treatment, however, should never be delayed in patients with a strong suspicion of GCA due to outstanding imaging or other diagnostic tests, because ischaemic complications such as blindness occur almost exclusively before initiation of therapy.[14]

The procedural risk of TAB is low; however, there is burden to patients and resource use.[19] Ultrasound in all patients with suspected GCA has been reported as cost-effective compared with biopsy plus clinical judgement alone with a net monetary benefit of £485 (€~550/US$~600) per patient.[15] Modelling of cost-effectiveness analysis considered the costs of the tests as well as the consequences of correct and incorrect diagnosis resulting in drug toxicity or vision loss that might have been prevented by one or the other test. Marking arterial segments with ultrasound to guide subsequent biopsy failed to increase the sensitivity of TAB in one randomised study[20]; however, additional research is necessary to better investigate this issue.

In patients with predominately LV-GCA, a lower sensitivity of TAB has been reported as compared with cranial GCA.[21–23] As TAB has not been conducted systematically in these studies, future studies should be conducted to investigate the diagnostic value of TAB for LV-GCA.

Recommendation 2: in patients in whom there is a high clinical suspicion of GCA and a positive imaging test, the diagnosis

Table 2 EULAR recommendations for the use of imaging in LVV in clinical practice

Statement	LoE	LoA
1. In patients with suspected GCA, an early imaging test is recommended to complement the clinical criteria for diagnosing GCA, assuming high expertise and prompt availability of the imaging technique. Imaging should not delay initiation of treatment.	1	9.2 (2.1) 90% ≥8
2. In patients in whom there is a high clinical suspicion of GCA and a positive imaging test, the diagnosis of GCA may be made without an additional test (biopsy or further imaging). In patients with a low clinical probability and a negative imaging result, the diagnosis of GCA can be considered unlikely. In all other situations, additional efforts towards a diagnosis are necessary.	2	9.4 (1.0) 90% ≥8
3. Ultrasound of temporal±axillary arteries is recommended as the first imaging modality in patients with suspected predominantly cranial GCA*. A non-compressible 'halo' sign is the ultrasound finding most suggestive of GCA.	1	9.7 (0.6) 100% ≥8
4. High resolution MRI† of cranial arteries‡ to investigate mural inflammation may be used as an alternative for GCA diagnosis if ultrasound is not available or inconclusive.	2	9.2 (1.1) 90% >8
5. CT† and PET† are not recommended for the assessment of inflammation of cranial arteries.	5	9.5 (1.2) 95% >8
6. Ultrasound, PET, MRI and/or CT may be used for detection of mural inflammation and/or luminal changes in extracranial arteries to support the diagnosis of LV-GCA. Ultrasound is of limited value for assessment of aortitis.	3 (PET and CT) and 5 (MRI and ultrasound)	9.8 (0.6) 100% ≥8
7. In patients with suspected TAK, MRI to investigate mural inflammation and/or luminal changes should be used as the first imaging test to make a diagnosis of TAK, assuming high expertise and prompt availability of the technique.	3	9.1 (1.4) 90% >8
8. PET, CT and/or ultrasound may be used as alternative imaging modalities in patients with suspected TAK. Ultrasound is of limited value for assessment of the thoracic aorta.	3 (CT) and 5 (PET and ultrasound)	9.4 (0.8) 100% ≥8
9. Conventional angiography is not recommended for the diagnosis of GCA or TAK as it has been superseded by the previously mentioned imaging modalities.	5	9.8 (0.6) 100% ≥8
10. In patients with LVV (GCA or TAK) in whom a flare is suspected, imaging might be helpful to confirm or exclude it. Imaging is not routinely recommended for patients in clinical and biochemical remission.	5	9.4 (0.8) 100% ≥8
11. In patients with LVV (GCA or TAK), MRA, CTA and/or ultrasound may be used for long-term monitoring of structural damage, particularly to detect stenosis, occlusion, dilatation and/or aneurysms. The frequency of screening as well as the imaging method applied should be decided on an individual basis.	5	9.3 (1.2) 95% ≥8
12. Imaging examination should be done by a trained specialist using appropriate equipment, operational procedures and settings. The reliability of imaging, which has often been a concern, can be improved by specific training. Suggestions for technical and operational parameters are depicted in box 1.	5	9.8 (0.6) 100% ≥8

Numbers in column 'LoA' indicate the mean and SD (in parentheses) of the LoA, as well as the percentage of task force members with an agreement ≥8.
*Cranial symptoms of GCA include headache, visual symptoms, jaw claudication, swelling and/or tenderness of temporal arteries.
†CT and MRI also refers to specific angiography techniques such as CT angiography (CTA) and MR angiography (MRA), and PET is commonly combined with CT or CTA.
‡Cranial arteries: superficial temporal, occipital and facial, usually all visible in one examination in MRI.
EULAR, European League Against Rheumatism; GCA, giant cell arteritis; LoA, level of agreement; LoE, level of evidence; LV-GCA, large vessel GCA; LVV, large vessel vasculitis; PET, positron emission tomography; TAK, Takayasu arteritis.

of GCA may be made without an additional test (biopsy or further imaging). In patients with a low clinical probability and a negative imaging result, the diagnosis of GCA can be considered unlikely. In all other situations, additional efforts towards a diagnosis are necessary.

The performance of a diagnostic test depends on its sensitivity and specificity and on the clinical situation where it is applied, that is, on the particular pretest probability.[24] For example, a patient with 50 years of age, with chronic unspecific headache and normal inflammatory markers has a very low pretest clinical probability for the presence of GCA. Assuming a pretest probability of 5% and a positive ultrasound result (which has a 77% sensitivity and a 96% specificity),[12] the post-test probability would increase to 50% only.[24] In case of a negative test, however, the diagnosis of GCA is very unlikely with a post-test probability of 1.3%. In patients with a high clinical suspicion of GCA (>50%), for example, in case of new-onset headache, visual symptoms, jaw claudication and elevated erythrocyte sedimentation rate (ESR) and C reactive protein, a positive ultrasound would result in a post-test probability of >95%. A negative examination decreases the probability to 20%, hence, GCA is still a possible option and further investigation is necessary. In clinical practice, the pretest probability needs to be determined case by case since a clinical probability score, as it has been published for other diseases,[25] is not yet available for GCA. Estimating the pretest probability for predominately LV-GCA might be particularly challenging because symptoms of LV-GCA are often vague.

The task force clearly emphasised that in all cases, where GCA cannot be confirmed or excluded based on clinical, laboratory and imaging results, every effort towards a diagnosis should be made including additional tests such as TAB and/or additional imaging.

Recommendation 3: ultrasound of temporal±axillary arteries is recommended as the first imaging modality in patients with suspected predominantly cranial GCA.[i] A non-compressible 'halo' sign is the ultrasound finding most suggestive of GCA.

Ultrasound should be the primary imaging test in patients with suspected GCA presenting predominantly with cranial symptoms because of a high LoE of good test performance, easy access, absence of radiation or other procedural risks and the relative low costs as compared with other modalities.

The 'halo' sign of temporal arteries is the most relevant ultrasound finding in GCA. Recently, it has been defined by an Outcome Measures in Rheumatology (OMERACT) working group as a 'homogenous, hypoechoic wall thickening that is well delineated towards the luminal side that is visible both in longitudinal and transverse planes, most commonly concentric in

[i]Cranial symptoms of GCA include headache, visual symptoms, jaw claudication, swelling and/or tenderness of temporal arteries.

transverse scans'.[26] The 'halo' sign at temporal arteries revealed a pooled sensitivity of 77% and a pooled specificity of 96% as compared with the clinical diagnosis of GCA.[12] These values remained consistent in a series of sensitivity analyses.[12] The persistence of a hypoechoic swelling despite the compression of the artery lumen with the ultrasound probe (termed 'compression' sign) is a variant of the 'halo' sign revealing sensitivities of 77%–79% and a specificity of 100%.[27 28] The detection of temporal artery stenosis or occlusion did not increase the diagnostic yield over the halo sign alone.

False-positive halos might occasionally be detected in other forms of vasculitis (eg, in ANCA-associated vasculitis), in infectious diseases or in patients with (severe) arteriosclerosis.[29–31] Ultrasound results should therefore always be interpreted together with clinical and laboratory findings as stated above.

According to expert opinion, examination of axillary arteries is particularly helpful in patients with suspected GCA but negative or inconclusive temporal artery ultrasound. The SLR revealed only a slight increment in the sensitivity (2%) in one study that considered the axillary arteries as compared with the assessment of temporal arteries alone.[31] The recommendation of the task force is therefore to primarily investigate the temporal arteries. Where this examination is non-diagnostic and a clinical suspicion of GCA remains, additional vessels such as axillary or other cranial and/or extracranial arteries should be scanned.

Recommendation 4: high resolution MRI of cranial arteries[ii] to investigate mural inflammation may be used as an alternative for GCA diagnosis if ultrasound is not available or inconclusive.

High resolution MRI of superficial cranial arteries should be considered as an alternative to ultrasound. The diagnostic value of both modalities is comparable (pooled sensitivity of MRI: 73%; specificity: 88%).[12] Similarly, a retrospective direct comparison of MRI and ultrasound revealed a similar sensitivity (69% and 67%, respectively) and specificity (both 91% and 91%) of both techniques.[32]

The main limitations of MRI are restricted availability, costs and possible adverse effects of contrast agents. MRI might only be feasible if emergency referrals for GCA can be implemented. It is strongly advised not to delay GC therapy due to outstanding imaging, and MRI of cranial arteries needs to be performed immediately within the first days of GC therapy in order to avoid false-negative results.

The advantages of MRI over ultrasound are a higher standardisation of data acquisition and the possibility to investigate multiple cranial and extracranial arteries including the aorta at the same time, which might reduce the probability of missing inflammation in case of skip lesions. This requires specific technical settings with multiple coils and a long time on the MRI scanner, which is not always feasible. MRI can also assess intracranial arteries, which may be affected in GCA. Other intracranial vasculopathies such as primary cerebral angiitis, atherosclerosis or reversible cerebral vasoconstriction syndrome must be differentiated from an intracranial manifestation of GCA. In a small prospective study, the examination of intracranial arteries did not increase the diagnostic yield for GCA[33]; however, further studies are needed to investigate this issue.

[ii]Cranial arteries: superficial temporal, occipital and facial, usually all visible in one examination in MRI.

Recommendation 5: CT and PET are not recommended for the assessment of inflammation of cranial arteries.

The task force does not recommend CT or PET for the assessment of cranial arteries because of lack of evidence, radiation exposure and high resource use. No studies have been conducted on these imaging modalities for the assessment of cranial arteries in GCA.[12] The use of PET is limited by the proximity of the brain; hence, superficial cranial vessels cannot be distinguished from the brain.

Recommendation 6: ultrasound, PET, MRI and/or CT may be used for detection of mural inflammation and/or luminal changes in extracranial arteries to support the diagnosis of LV-GCA. Ultrasound is of limited value for assessment of aortitis.

This recommendation is mainly based on expert opinion. The best imaging technique for patients with suspected LV-GCA and predominantly systemic symptoms is unclear and depends on local settings and expertise. While ultrasound has advantages as outlined above, it has limited access to the thoracic aorta. Besides, the exact sensitivity and specificity of ultrasound for LV-GCA is unknown because this subgroup of patients has not been analysed separately in ultrasound studies.[31 34]

The major advantage of PET in patients with systemic symptoms is the ability to identify GCA along with other serious pathology such as infections or tumours. This may be particularly relevant in elderly patients with constitutional symptoms without specific clinical features of GCA and/or PMR. Two clinical studies reported divergent sensitivities (67%–77%) and specificities (66%–100%) for PET, which may be explained by the small sample size, the lack of independence between index test and the reference standard, use of GC as well as the circular application of different criteria for GCA.[35 36] Disadvantages of PET are high costs, lower availability and radiation exposure. Inexperienced readers may misinterpret atherosclerosis as LVV.[37] Missing information on wall-thickness and luminal changes can be overcome by combining PET with CT.

The advantages of MRI are the absence of radiation and the contemporaneous detection of structural lesions (such as vessel wall thickening and luminal stenosis/occlusion) and contrast enhancement of the arterial wall, which is presumed (but not proven) to reflect active inflammation. Specific sequences are required to image both the arterial wall and the arterial lumen as outlined in box 1. The SLR did not retrieve any study investigating the use of MRI in LV-GCA.[12]

CT may also be useful to detect structural lesions and wall inflammation and enables a higher resolution and shorter procedural time than MRI; however, this is at the cost of radiation exposure. Evidence from literature is scarce with only a single small study indicating a sensitivity of 73% and a specificity of 78% of CTA for the diagnosis of LV-GCA.[35]

Recommendation 7: in patients with suspected TAK, MRI to investigate mural inflammation and/or luminal changes should be used as the first imaging test to make a diagnosis of TAK, assuming high expertise and prompt availability of the technique.

This recommendation is almost entirely based on expert opinion and current clinical practice. A technique without radiation exposure is preferable over other modalities because of the young age of patients with TAK. Besides, MRI enables assessment of the vessel wall and luminal changes, which are both relevant for TAK. In one study, MRA yielded a sensitivity and specificity of 100% for TAK using conventional angiography as the reference standard.[38] The most important limitation of MRI is the restricted availability as compared with ultrasound or CT.

Box 1 Suggestions for technical and operational parameters on imaging modalities in large vessel vasculitis

Ultrasound

- High-quality, modern equipment is essential. Linear probes are recommended for supra-aortic arteries, sector or convex probes for ascending aorta and aortic arch and convex probes for abdominal aorta. Settings may slightly vary according to different equipment.
- The B-mode frequency should be ≥15 MHz for temporal arteries and 7–15 MHz for extracranial supra-aortic arteries. Image depth should be 10–20 mm for temporal arteries and 30–40 mm for extracranial supra-aortic arteries.
- The focus should be at the level of the artery. The B-mode gain should be adjusted to avoid anechoic appearance of the artery wall. The colour Doppler gain should be adjusted to avoid underfilling or overfilling of the vessel lumen.
- Colour Doppler mode is preferred over power Doppler mode. Tissue harmonic imaging may improve delineation of the intima-media complex.
- Doppler frequencies of 7–12 MHz and 4–8 MHz should be applied for the temporal and for the extracranial supra-aortic arteries, respectively. PRF should be 2–3.5 kHz and 3–4 kHz, respectively. The angle between sound waves and artery should be ≤60°.

CT

- Multislice CT scanner should be used.
- Collimation 0.6 mm, tube voltage 120 kV, tube current time product (mAs) determined by automatic dose modulation.
- Reconstruction slice thickness should be between 0.5 mm and 1.0 mm.
- Body-weight adapted injection of 60–120 mL of non-ionic iodinated contrast agent (≥ 350 mg/mL) using a power injector (≥ 4 mL/s).
- Arterial phase: bolus-tracking method (threshold of 100 HU); ECG triggering.
- Venous phase: 50 s after finishing the arterial phase acquisition.

MRI

Cranial MRI technique:

- 1.5 T, preferentially 3.0 T MRI scanner, minimum 8-channel head-coil.
- T1-weighted spin echo, gadolinium contrast-enhanced, fat-suppressed, high-resolution (inplane << 1 mm^2, for example, 195×260 µm, slice thickness 3 mm, repetition time (TR)/echo time (TE) 500/22 ms).
- T2-weighted turbo spin echo (TSE), non-contrast-enhanced imaging (TR/TE 9000/143 ms) is significantly less sensitive.
- Transversal slices angulated parallel to skull base.

Body MRI technique:

- 1.5 T, preferentially 3.0 T MRI scanner, minimum 8-channel head and neck coil and 16-channel body coil.
- MR angiography of aorta and major branches from carotid bifurcation to iliac arteries in coronal acquisition to include axillary and brachial arteries → detection of vessel lumen (stenosis, occlusion and aneurysm).
- T1-weighted, fat-suppressed, contrast-enhanced, black blood imaging (eg, navigated three-dimensional TSE, spatial resolution 1.2×1.3×2 mm^3, TR/TE 1000/35 ms) → assessment of mural inflammation.
- T2-weighted TSE imaging for oedema detection in mural inflammation is less sensitive and more prone to artefacts.

[^{18}F]-Fluorodeoxyglucose (FDG) positron emission tomography (PET)

- Hybrid PET with low-dose CT.
- Blood glucose levels: preferred <7 mmol/L (126 mg/dL), <10 mmol/L (180 mg/dL) acceptable.
- Interval between FDG infusion and image acquisition should be at least 60 min, preferably 90 min.
- Position of patient is supine, position of the arms should be arms down.
- Body parts to include: from top of head to at least midthigh, preferably to below the knees.
- Scoring FDG uptake: qualitative visual grading; if result is unclear, compare it with the liver background (grading 0–3).

Recommendation 8: PET, CT and/or ultrasound may be used as alternative imaging modalities in patients with suspected TAK. Ultrasound is of limited value for assessment of the thoracic aorta.

This recommendation is also based on expert opinion. The task force felt that PET might be particularly valuable in patients with unspecific symptoms to detect alternative causes of illness. CT (also in conjunction with PET) enables visualisation of vessel wall and luminal changes and is widely available. However, it is associated with significant radiation. Only a single small study was available for CTA yielding a sensitivity and specificity of 100% for the diagnosis of TAK using conventional angiography as the reference standard.[39] No studies were available for PET and for ultrasound. Ultrasound might be particularly valuable in patients with claudication of upper and/or lower limbs.

Recommendation 9: conventional angiography is not recommended for the diagnosis of GCA or TAK as it has been superseded by the previously mentioned imaging modalities.

Although conventional angiography has not been included formally as one of the key questions to guide the SLR, the task force felt it was necessary to make a statement. Conventional angiography has been the gold standard for several decades in the diagnosis of LVV, but it is very invasive and involves high resource use and a higher procedural risk as compared with other imaging modalities. Besides, it provides no information about wall morphology, although luminal changes are depicted with detail. The main indication for conventional angiography in LVV is currently as part of vascular interventions such as percutaneous transluminal balloon angioplasty or stenting.[40]

Recommendation 10: in patients with LVV (GCA or TAK) in whom a flare is suspected, imaging might be helpful to confirm

Box 2 Future research agenda

- To define a gold standard for the diagnosis, particularly of large vessel giant cell arteritis (LV-GCA).
- To directly and prospectively compare the diagnostic value of ultrasound and MRI of cranial arteries for diagnosis of GCA.
- To investigate the diagnostic value of CT of cranial arteries for diagnosis of GCA.
- To compare the value of imaging for the diagnosis of GCA when performed by examiners with low versus high expertise in large vessel vasculitis (LVV) imaging.
- To investigate the additional value of imaging of axillary arteries in all patients with suspected GCA versus performing it in those without a positive imaging of temporal arteries.
- To investigate the value of standardised assessment of different vascular beds by imaging for the diagnosis of GCA.
- To investigate the role of MRI in the diagnosis of GCA in patients with a negative ultrasound.
- To investigate the possible relevance of the assessment of intracranial arteries for the diagnosis and prognosis of GCA.
- To investigate the role of ultrasound, MRI, positron emission tomography (PET) and CT in the diagnosis of LVV with predominantly systemic symptoms.
- To investigate the role of ultrasound, MRI, PET and CT in the diagnosis of Takayasu arteritis.
- To develop tools for the assessment of disease activity in LVV and to agree on definitions of remission and relapse (to better investigate the role of imaging for monitoring of LVV).
- To investigate the additional value of the different imaging modalities in the assessment of disease activity during follow-up over clinical and laboratory assessment alone.
- To investigate the value of imaging (eg, assessment of the extent of vascular involvement) as well as individual vasculitis signs (eg, 'halo' sign, contrast enhancement as compared with wall thickening) as a prognostic factor for LVV outcomes.
- To further study the possibility of differentiating persistent mural inflammation from vascular remodelling (eg, persistent fluorodeoxyglucose uptake in patients in clinical remission).
- To investigate the number of patients needed to screen with imaging methods for identifying cases with aortic complications.
- To define standardised, well-validated scoring methodologies for all imaging modalities and to develop composite scores for imaging-based monitoring of patients with LVV. To compare different imaging modalities for monitoring of aortic complications.
- To study whether therapy should be modified based on imaging results alone.
- To compare therapy modification based on traditional clinical evaluation versus evaluation that includes results of additional imaging.
- To study the value of novel technical developments for diagnosis and monitoring of LVV such as contrast-enhanced ultrasound or PET with ligands specifically targeting immune cells.

or exclude it. Imaging is not routinely recommended for patients in clinical and biochemical remission.

This recommendation is based on expert opinion. In certain situations, for example when clinical and laboratory parameters are inconclusive, imaging may determine the decision whether to change treatment. The primary choice between different imaging modalities depends again on the clinical situation, local availability and expertise. In individual patients, imaging methods might also be complementary, given that the information provided is different (such as local glucose consumption/metabolism by PET or perfusion by contrast-enhanced imaging).

In patients with a clear-cut clinical flare, as well as in patients in clinical and biochemical remission, the role of additional imaging to determine disease activity is currently unknown.

In one PET study, scans were performed in all newly diagnosed patients with GCA at baseline and during follow-up.[41] While PET scores significantly dropped from baseline to 3 months, there was no further reduction at 6 months. Up to two-thirds of patients in full clinical remission still revealed a positive PET at both follow-up visits, and PET scores did not significantly differ at times of remission and relapse. Whether ongoing tracer uptake in patients in full clinical remission is caused by low-grade inflammation or remodelling and whether these findings have any impact on future vascular outcomes are issues that have to be clarified by future studies.

Ultrasound studies in GCA reported the disappearance of the ' halo' sign in temporal arteries in the majority of patients after 2–4 weeks of GC therapy.[16 17 34 42–48] In extracranial arteries, residual changes often remained visible for several months. The examination of these vessels might be of potential value for monitoring purposes; however, none of these studies addressed whether ultrasound was helpful for the assessment of relapse.

In summary, the limited literature is mainly descriptive and does not add further insights into the additional value of imaging compared with only clinical definition of flare. Further research is urgently needed to address this issue.

Recommendation 11: in patients with LVV (GCA or TAK), MRA, CTA and/or ultrasound may be used for long-term monitoring of structural damage, particularly to detect stenosis, occlusion, dilatation and/or aneurysms. The frequency of screening as well as the imaging method applied should be decided on an individual basis.

The task force suggests, based on expert opinion, that regular screening for structural damage might be performed in GCA and TAK patients with signs or symptoms of stenosis/occlusion or aneurysms, as well as in those with recurrent or persistent inflammation of large arteries and/or the aorta. The choice of the imaging method depends on the vessel(s) affected, local settings and expertise. Monitoring of a patient with inflammation and/or dilatation of the aorta, for example, requires MRI or CT, whereas a stenosis of the axillary/subclavian arteries could be followed up by ultrasound.

The frequency of imaging assessments for vasculitic stenoses should also be decided on an individual basis, as there is currently insufficient data to frame a recommendation.[12]

The development of aortic aneurysms has been reported in patients with GCA despite the absence of ongoing clinical activity. Aortic dilatation might occur even years after disease outset.[49] Aortic inflammation at baseline as well as male sex, hypertension and smoking history have been described as risk

factors for aortic dilatation.[50 51] However, whether and how often imaging of the aorta should be repeated remains an uncertain decision. A chest X-ray and abdominal ultrasound every other year in patients at low risk for aortic aneurysms is current clinical practice in some countries.[52] However, there are no data demonstrating that such a strategy would have a sufficient sensitivity, specificity and cost-effectiveness.[53] This is an area that requires further robust research.

Recommendation 12: imaging examination should be done by a trained specialist using appropriate equipment, operational procedures and settings. The reliability of imaging, which has often been a concern, can be improved by specific training. Suggestions for technical and operational parameters are depicted in box 1.

The task force unanimously agreed that the standardisation of investigational procedures as well as the definition of minimal technical and training requirements is essential to produce sensitive, specific and reliable imaging results.[15 54] The development of specific training programmes as well as national and international courses for imaging in LVV (particularly for ultrasound) should have a high priority in order to facilitate implementation of these recommendations in clinical practice.

The items listed in box 1 are almost entirely based on expert consensus.

No recommendation was made on the prognostic value of imaging modalities in patients with established GCA and TAK because of the absence of evidence and experience. Based on the discussions and the areas of uncertainty, a research agenda has been proposed, which is depicted in box 2.

DISCUSSION

These are the first EULAR recommendations providing up-to-date guidance for the role of imaging in the diagnosis and monitoring of patients with (suspected) LVV, recognising recent progress in the field. Imaging enables rapid diagnosis of LVV with low burden to patients and should therefore be used as the first diagnostic test provided it is readily available and performed with high quality. To implement the recommendations in clinical practice, training programmes for imaging are required. These principles are reflected in both the recommendations and the research agenda, acknowledging also the gaps in evidence that include direct comparisons of different imaging modalities, the diagnostic value of imaging for predominantly LV-GCA and TAK, as well as the specific value of imaging for monitoring and outcome prediction. Some of the recommendations were mainly based on clinical experience and consensus. Good quality studies are now required to answer the numerous questions raised in the research agenda, so that future recommendations can be upgraded and based on more solid evidence. The present recommendations nevertheless represent a step forward in the approach to patients with (suspected) LVV, and we believe that their implementation will improve patient care.

Previous EULAR recommendations for the management of LVV, already from 2009, recognised the possible value of MRI and PET for the diagnosis and assessment of TAK similar to the present prepositions, whereas for GCA, TAB was previously considered as the only reliable diagnostic test.[55] The present article is not intended to discredit the role of biopsy as clearly explained in the recommendations; nevertheless, the task force felt that TAB may be dispensable in cases where GCA is confirmed or excluded based on clinical, laboratory and imaging results.

In summary, we developed 12 recommendations on the use of imaging for the diagnosis and monitoring of LVV. These recommendations are supported by evidence along with expert consensus. Unresolved issues and areas of further study have been depicted in the research agenda. We expect that much progress continue to take place in the area of imaging in LVV, and we will carefully follow developments in the field, assuming that an amendment of these recommendations may be needed within a few years.

Author affiliations
[1]Department of Rheumatology and Immunology, Medical University Graz, Graz, Austria
[2]Department of Rheumatology, South Tyrol Health Trust, Hospital of Bruneck, Bruneck, Italy
[3]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[4]Department of Internal Medicine VI, Medical University Innsbruck, Innsbruck, Austria
[5]Department of Biophysics and Nuclear Medicine, Hôpitaux Universitaires Paris-Sud, Assistance Publique-Hôpitaux de Paris, Le Kremlin-Bicetre, Île-de-France, France
[6]IR4M-UMR8081, Université Paris-Saclay, Orsay, France
[7]Department of Diagnostic and Interventional Radiology, University Medical Center Wuerzburg, Wuerzburg, Germany
[8]General Internal Medicine Department, University Hospital Gasthuisberg, Leuven, Belgium
[9]Department of Rheumatology and Clinical Immunology, University Medical Center Groningen, Groiningen, The Netherlands
[10]Research Laboratory and Academic Division of Clinical Rheumatology, Department of Internal Medicine, University of Genova, Genova, Italy
[11]Patient Charity Polymyalgia Rheumatica and Giant Cell Arteritis UK, London, UK
[12]Department of Rheumatology, Southend Hospital NHS Trust, Westcliff-on-Sea, UK
[13]Anglia Ruskin University, Chelmsford, UK
[14]Department of Rheumatology, Martina Hansens Hospital, Baerum, Norway
[15]Division of Rheumatology, Department of Internal Medicine, Marmara University, Istanbul, Turkey
[16]Rheumatology Unit, Universita degli Studi di Torino Dipartimento di Scienze Mediche, Torino, Italy
[17]Polymyalgia Rheumatica and Giant Cell Arteritis Scotland (PMR-GCA Scotland), Perth, UK
[18]Rheumatology Research Unit, Instituto de Medicina Molecular, Faculty of Medicine, University of Lisbon, Lisbon, Portugal
[19]Rheumatology Department, Hospital de Santa Maria – Centro Hospitalar Lisboa Norte, Lisbon, Portugal
[20]Department of Rheumatology, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy
[21]Dipartimento di Scienze della Vita, Universita degli Studi di Modena e Reggio Emilia, Reggio Emilia, Italy
[22]Department of Nuclear Medicine and Molecular Imaging, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands
[23]Department of Biomedical Photonic Imaging, University of Twente, Enschede, The Netherlands
[24]Medical Centre for Rheumatology Berlin-Buch, Immanuel Krankenhaus Berlin, Berlin, Germany

Correction notice This article has been corrected since it published Online First. The text in box 1 has been corrected.

Acknowledgements The authors would like to thank Louise Falzon for her work in the development of the literature search strategy, and Alexandre Sepriano for his help with the statistical analysis of the systematic literature review.

Contributors All authors were involved in the discussion and formulation of the recommendations. ChristiaD wrote the first version of the manuscript. All authors reviewed it and made extensive comments and appropriate changes to it. All authors approved the final version of the manuscript.

Funding Funding was provided by the European League Against Rheumatism.

Competing interests TAB received research grants from Deutsche Forschungsgemeinschaft (DFG) and Siemens Healthineers and received consultancies and speaker fees from HeartFlow, GSK, MSD, Roche, Bayer, Bracco, Guerbet and Siemens. EB received consultancies and speaker fees from Roche, which were paid to the University Medical Center Groningen. BD has received consultancies from Roche, GSK and Munidipharma. APD received speaker fees from Roche. WS received consultancies and speakers fee from Roche and GSK. All other authors have no competing interest.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No additional data are available.

REFERENCES

1 Smeeth L, Cook C, Hall AJ. Incidence of diagnosed polymyalgia rheumatica and temporal arteritis in the United Kingdom, 1990-2001. *Ann Rheum Dis* 2006;65:1093–8.
2 Lawrence RC, Felson DT, Helmick CG, *et al*. Estimates of the prevalence of arthritis and other rheumatic conditions in the United States. Part II. *Arthritis Rheum* 2008;58:26–35.
3 Patil P, Williams M, Maw WW, *et al*. Fast track pathway reduces sight loss in giant cell arteritis: results of a longitudinal observational cohort study. *Clin Exp Rheumatol* 2015;33.
4 Diamantopoulos AP, Haugeberg G, Lindland A, *et al*. The fast-track ultrasound clinic for early diagnosis of giant cell arteritis significantly reduces permanent visual impairment: towards a more effective strategy to improve clinical outcome in giant cell arteritis? *Rheumatology* 2016;55:66–70.
5 Dejaco C, Duftner C, Buttgereit F, *et al*. The spectrum of giant cell arteritis and polymyalgia rheumatica: revisiting the concept of the disease. *Rheumatology* 2017;56:506–15.
6 Prieto-González S, Arguis P, Cid MC. Imaging in systemic vasculitis. *Curr Opin Rheumatol* 2015;27:53–62.
7 Monti S, Floris A, Ponte C, *et al*. The use of ultrasound to assess giant cell arteritis: review of the current evidence and practical guide for the rheumatologist. *Rheumatology* 2017 [Epub ahead of print].
8 Direskeneli H. Clinical assessment in Takayasu's arteritis: major challenges and controversies. *Clin Exp Rheumatol* 2017;35:189–93.
9 van der Heijde D, Aletaha D, Carmona L, *et al*. 2014 Update of the EULAR standardised operating procedures for EULAR-endorsed recommendations. *Ann Rheum Dis* 2015;74:8–13.
10 Whiting PF, Rutjes AW, Westwood ME, *et al*. QUADAS-2: a revised tool for the quality assessment of diagnostic accuracy studies. *Ann Intern Med* 2011;155:529–36.
11 Hayden JA, Côté P, Bombardier C. Evaluation of the quality of prognosis studies in systematic reviews. *Ann Intern Med* 2006;144:427–37.
12 Duftner C, Dejaco C, Sepriano A, *et al*. Imaging in diagnosis, monitoring and outcome prediction of large vessel vasculitis: a systematic literature review and meta-analysis informing the EULAR recommendations. *RMD open*. submitted.
13 OCEBM Levels of Evidence Working Group. The Oxford 2011 levels of evidence, 2011. http://www.cebm.net/index.aspx?o=5653
14 Salvarani C, Cimino L, Macchioni P, *et al*. Risk factors for visual loss in an Italian population-based cohort of patients with giant cell arteritis. *Arthritis Rheum* 2005;53:293–7.
15 Luqmani R, Lee E, Singh S, *et al*. The role of ultrasound compared to biopsy of temporal arteries in the diagnosis and treatment of giant cell arteritis (TABUL): a diagnostic accuracy and cost-effectiveness study. *Health Technol Assess* 2016;20:1–238.
16 Schmidt WA, Kraft HE, Vorpahl K, *et al*. Color duplex ultrasonography in the diagnosis of temporal arteritis. *N Engl J Med* 1997;337:1336–42.
17 Karahaliou M, Vaiopoulos G, Papaspyrou S, *et al*. Colour duplex sonography of temporal arteries before decision for biopsy: a prospective study in 55 patients with suspected giant cell arteritis. *Arthritis Res Ther* 2006;8:R116.
18 Hauenstein C, Reinhard M, Geiger J, *et al*. Effects of early corticosteroid treatment on magnetic resonance imaging and ultrasonography findings in giant cell arteritis. *Rheumatology* 2012;51:1999–2003.
19 Gunawardene AR, Chant H. Facial nerve injury during temporal artery biopsy. *Ann R Coll Surg Engl* 2014;96:257–60.
20 Germanò G, Muratore F, Cimino L, *et al*. Is colour duplex sonography-guided temporal artery biopsy useful in the diagnosis of giant cell arteritis? A randomized study. *Rheumatology* 2015;54:400–4.
21 Schmidt WA, Seifert A, Gromnica-Ihle E, *et al*. Ultrasound of proximal upper extremity arteries to increase the diagnostic yield in large-vessel giant cell arteritis. *Rheumatology* 2008;47:96–101.
22 Muratore F, Kermani TA, Crowson CS, *et al*. Large-vessel giant cell arteritis: a cohort study. *Rheumatology* 2015;54:463–70.
23 Brack A, Martinez-Taboada V, Stanson A, *et al*. Disease pattern in cranial and large-vessel giant cell arteritis. *Arthritis Rheum* 1999;42:311–7.
24 Griner PF, Mayewski RJ, Mushlin AI, *et al*. Selection and interpretation of diagnostic tests and procedures. Principles and applications. *Ann Intern Med* 1981;94:557–92.
25 Rudwaleit M, van der Heijde D, Khan MA, *et al*. How to diagnose axial spondyloarthritis early. *Ann Rheum Dis* 2004;63:535–43.
26 Chrysidis S, Duftner C, Dejaco C, *et al*. Definitions and reliability assessment of elementary ultrasound lesions in giant cell arteritis: a study from the OMERACT Large Vessel Vasculitis Ultrasound Working Group. *RMD open*;2017. submitted.
27 Aschwanden M, Daikeler T, Kesten F, *et al*. Temporal artery compression sign–a novel ultrasound finding for the diagnosis of giant cell arteritis. *Ultraschall Med* 2013;34:47–50.
28 Aschwanden M, Imfeld S, Staub D, *et al*. The ultrasound compression sign to diagnose temporal giant cell arteritis shows an excellent interobserver agreement. *Clin Exp Rheumatol* 2015;33.
29 Nesher G, Shemesh D, Mates M, *et al*. The predictive value of the halo sign in color Doppler ultrasonography of the temporal arteries for diagnosing giant cell arteritis. *J Rheumatol* 2002;29:1224–6.
30 Pfadenhauer K, Weber H. Ultrasonography of the temporal, periorbital and carotid arteries in the diagnosis of giant cell arteritis and its neuroophthalmological complications. *Ultraschall Med* 2006;27:329–35.
31 Diamantopoulos AP, Haugeberg G, Hetland H, *et al*. Diagnostic value of color Doppler ultrasonography of temporal arteries and large vessels in giant cell arteritis: a consecutive case series. *Arthritis Care Res* 2014;66:113–9.
32 Bley TA, Reinhard M, Hauenstein C, *et al*. Comparison of duplex sonography and high-resolution magnetic resonance imaging in the diagnosis of giant cell (temporal) arteritis. *Arthritis Rheum* 2008;58:2574–8.
33 Siemonsen S, Brekenfeld C, Holst B, *et al*. 3T MRI reveals extra- and intracranial involvement in giant cell arteritis. *AJNR Am J Neuroradiol* 2015;36:91–7.
34 Aschwanden M, Kesten F, Stern M, *et al*. Vascular involvement in patients with giant cell arteritis determined by duplex sonography of 2x11 arterial regions. *Ann Rheum Dis* 2010;69:1356–9.
35 Lariviere D, Benali K, Coustet B, *et al*. Positron emission tomography and computed tomography angiography for the diagnosis of giant cell arteritis: A real-life prospective study. *Medicine* 2016;95:e4146.
36 Blockmans D, Stroobants S, Maes A, *et al*. Positron emission tomography in giant cell arteritis and polymyalgia rheumatica: evidence for inflammation of the aortic arch. *Am J Med* 2000;108:246–9.
37 Puppo C, Massollo M, Paparo F, *et al*. Giant cell arteritis: a systematic review of the qualitative and semiquantitative methods to assess vasculitis with 18F-fluorodeoxyglucose positron emission tomography. *Biomed Res Int* 2014;2014:1–11.
38 Yamada I, Nakagawa T, Himeno Y, *et al*. Takayasu arteritis: diagnosis with breath-hold contrast-enhanced three-dimensional MR angiography. *J Magn Reson Imaging* 2000;11:481–7.
39 Yamada I, Nakagawa T, Himeno Y, *et al*. Takayasu arteritis: evaluation of the thoracic aorta with CT angiography. *Radiology* 1998;209:103–9.
40 Gülcü A, Gezer NS, Akar S, *et al*. Long-term follow-up of endovascular repair in the management of arterial stenosis caused by takayasu's arteritis. *Ann Vasc Surg* 2017;42:93–100.
41 Blockmans D, de Ceuninck L, Vanderschueren S, *et al*. Repetitive 18F-fluorodeoxyglucose positron emission tomography in giant cell arteritis: a prospective study of 35 patients. *Arthritis Rheum* 2006;55:131–7.
42 De Miguel E, Roxo A, Castillo C, *et al*. The utility and sensitivity of colour Doppler ultrasound in monitoring changes in giant cell arteritis. *Clin Exp Rheumatol* 2012;30:S34–8.
43 Habib HM, Essa AA, Hassan AA. Color duplex ultrasonography of temporal arteries: role in diagnosis and follow-up of suspected cases of temporal arteritis. *Clin Rheumatol* 2012;31:231–7.
44 Pérez López J, Solans Laqué R, Bosch Gil JA, *et al*. Colour-duplex ultrasonography of the temporal and ophthalmic arteries in the diagnosis and follow-up of giant cell arteritis. *Clin Exp Rheumatol* 2009;27:S77–82.
45 Pfadenhauer K, Weber H. Duplex sonography of the temporal and occipital artery in the diagnosis of temporal arteritis. A prospective study. *J Rheumatol* 2003;30:2177–81.
46 Salvarani C, Silingardi M, Ghirarduzzi A, *et al*. Is duplex ultrasonography useful for the diagnosis of giant-cell arteritis? *Ann Intern Med* 2002;137:232–8.
47 Schmidt WA, Moll A, Seifert A, *et al*. Prognosis of large-vessel giant cell arteritis. *Rheumatology* 2008;47:1406–8.
48 Schmidt WA, Natusch A, Möller DE, *et al*. Involvement of peripheral arteries in giant cell arteritis: a color Doppler sonography study. *Clin Exp Rheumatol* 2002;20:309–18.
49 García-Martínez A, Arguis P, Prieto-González S, *et al*. Prospective long term follow-up of a cohort of patients with giant cell arteritis screened for aortic structural damage (aneurysm or dilatation). *Ann Rheum Dis* 2014;73:1826–32.
50 Blockmans D, Coudyzer W, Vanderschueren S, *et al*. Relationship between fluorodeoxyglucose uptake in the large vessels and late aortic diameter in giant cell arteritis. *Rheumatology* 2008;47:1179–84.
51 Robson JC, Kiran A, Maskell J, *et al*. The relative risk of aortic aneurysm in patients with giant cell arteritis compared with the general population of the UK. *Ann Rheum Dis* 2015;74:129–35.
52 Dasgupta B, Borg FA, Hassan N, *et al*. BSR and BHPR guidelines for the management of giant cell arteritis. *Rheumatology* 2010;49:1594–7.
53 Mackie SL, Hensor EM, Morgan AW, *et al*. Should I send my patient with previous giant cell arteritis for imaging of the thoracic aorta? A systematic literature review and meta-analysis. *Ann Rheum Dis* 2014;73:143–8.
54 De Miguel E, Castillo C, Rodríguez A, *et al*. Learning and reliability of colour Doppler ultrasound in giant cell arteritis. *Clin Exp Rheumatol* 2009;27:S53–8.
55 Mukhtyar C, Guillevin L, Cid MC, *et al*. EULAR recommendations for the management of large vessel vasculitis. *Ann Rheum Dis* 2009;68:318–23.

EXTENDED REPORT

Prophylactic effect of trimethoprim-sulfamethoxazole for pneumocystis pneumonia in patients with rheumatic diseases exposed to prolonged high-dose glucocorticoids

Jun Won Park,[1] Jeffrey R Curtis,[2] Jinyoung Moon,[1] Yeong Wook Song,[1] Suhnggwon Kim,[3,4] Eun Bong Lee[1]

Handling editor Tore K Kvien

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-211796).

[1]Division of Rheumatology, Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea
[2]Division of Clinical Immunology and Rheumatology, University of Alabama at Birmingham, Birmingham, Alabama, USA
[3]Division of Nephrology, Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea
[4]Seoul Kidney Clinic, Seoul, Republic of Korea

Correspondence to
Dr Eun Bong Lee, Division of Rheumatology, Department of Internal Medicine, Seoul National University College of Medicine, Seoul 110-744, Republic of Korea; leb7616@snu.ac.kr

Received 15 May 2017
Revised 1 October 2017
Accepted 22 October 2017
Published Online First 1 November 2017

ABSTRACT

Objectives To investigate the efficacy and safety of trimethoprim/sulfamethoxazole (TMP-SMX) as primary prophylaxis for pneumocystis pneumonia (PCP) in patients with rheumatic diseases receiving high-dose steroids.

Methods The study included 1522 treatment episodes with prolonged (≥4 weeks) high-dose (≥30 mg/day prednisone) steroids in 1092 patients over a 12-year period. Of these, 262 treatment episodes involved TMP-SMX (prophylaxis group) while other episodes involved no prophylaxis (control group). Differences in 1-year PCP incidence and its mortality between the two groups were estimated using Cox regression. To minimise baseline imbalance, propensity score matching was performed and efficacy outcome was mainly assessed in the postmatched population (n=235 in both groups).

Results During a total of 1474.4 person-years, 30 PCP cases occurred with a mortality rate of 36.7%. One non-fatal case occurred in the prophylaxis group. TMP-SMX significantly reduced the 1-year PCP incidence (adjusted HR=0.07(95% CI 0.01 to 0.53)) and related mortality (adjusted HR=0.08 (95% CI 0.0006 to 0.71)) in the postmatched population. The result of the same analysis performed in the whole population was consistent with that of the primary analysis. Incidence rate of adverse drug reactions (ADR) related to TMP-SMX was 21.2 (14.8–29.3)/100 person-years. Only two serious ADRs (including one Stevens-Johnson syndrome case) occurred. The number needed to treat for preventing one PCP (52 (33–124)) was lower than the number needed to harm for serious ADR (131 (55–∞)).

Conclusion TMP-SMX prophylaxis significantly reduces the PCP incidence with a favourable safety profile in patients with rheumatic disease receiving prolonged, high-dose steroids.

INTRODUCTION

Pneumocystis pneumonia (PCP) caused by *Pneumocystis jirovecii* is a common but potentially life-threatening infection in immunocompromised patients.[1] Although it had been the most common cause of death in patients infected by HIV, the advent of effective HIV treatment and prophylactic strategy led to marked fall of its incidence.[2] However, it remains a significant cause of pneumonia in non-HIV immunocompromised patients. In addition, PCP in non-HIV patients usually shows more severe manifestations and carries a higher mortality rate than that in HIV-infected patients.[3–5]

The most important risk factor for PCP in non-HIV patients is the use of immunosuppressive drugs, especially corticosteroids. Prolonged treatment with high-dose steroids is a significant risk factor for PCP in patients with haematologic malignancies, solid organ transplants and rheumatic diseases.[4 6 7] Thus, current guidelines recommend PCP prophylaxis for patients receiving immunosuppressive drugs, including steroids.[8] However, there is no consensus on PCP prophylaxis for patients with rheumatic diseases because the absolute incidence of PCP in this group is unclear[9] and no risk-benefit assessment for prophylactic regimen has been performed. Thus, this has led to different opinions among rheumatologists regarding PCP prophylaxis.[10]

To find the answers to these problems, we examined the incidence of PCP in patients diagnosed with a rheumatic disease and receiving prolonged high-dose steroid treatment. Patients were recruited from a large tertiary referral centre over a 12-year period. In addition, we evaluated the efficacy and safety of PCP prophylaxis to enable a useful risk-benefit assessment.

METHODS

Patients and clinical data

The electronic medical database at Seoul National University Hospital was examined, and patients with a rheumatic disease treated with high-dose steroid for more than 4 consecutive weeks (defined as a treatment episode) between January 2004 and December 2015 were identified. High-dose steroid was defined as ≥30 mg/day prednisone or equivalent, as suggested by Buttgereit *et al*.[11] The ICD-10 (International Classification of Diseases, 10th Revision) codes used for case identification are presented in online supplementary text. Patients with a history of PCP, HIV infection, current cancer, or a solid organ transplant, or those less than 18 years of age were excluded. Next, all treatment episodes were classified into two groups (control group vs prophylaxis group) according to whether a patient receiving high-dose steroid had started primary PCP prophylaxis.

The baseline date was defined as the first day of PCP prophylaxis (prophylaxis group) or high-dose

► http://dx.doi.org/10.1136/annrheumdis-2017-212588

Check for updates

To cite: Park JW, Curtis JR, Moon J, *et al*. *Ann Rheum Dis* 2018;**77**:644–649.

steroid (control group). Each patient should maintain high-dose steroid for at least 4 weeks from the baseline date. The observation period for each treatment episode was 1 year from the baseline date because previous studies suggest that most PCP cases occur within this period.[4 12 13] Therefore, prolonged high-dose steroid treatment which started within the last 1 year from the baseline date in the prophylaxis group could not be entered into the observation period of the control group. But if a patient restarted prolonged high-dose steroid treatment after more than 1 year from the baseline date, it was counted as a separate treatment episode. The primary outcome was the incidence of PCP in each group during the observation. Secondary outcomes included PCP-related mortality and incidence of adverse drug reactions (ADR) related to PCP prophylaxis. All suspected ADRs were reviewed and assigned a probability of causation based on the timing and known patterns of adverse effects. Probable/likely or certain causality was regarded as an ADR.[14]

Patient consent was waived by the IRB due to the retrospective nature of the study.

Detection of PCP during treatment episodes

A complex algorithm (see online supplementary figure S1) was used to capture all PCP cases during the observation. Briefly, data from confirmatory microbiologic tests such as PCR and direct fluorescent antibody staining of induced-sputum or bronchoalveolar lavage fluid were collected. The medical records of patients with positive results and fulfilling the criteria for analysis were then reviewed to ascertain whether they showed features consistent with PCP, such as fever or acute dyspnea, along with characteristic radiographic findings. A positive PCR result in the absence of clinical manifestations was not considered as PCP.

PCP prophylaxis

Trimethoprim/sulfamethoxazole (TMP-SMX) was the only agent used for PCP prophylaxis in this study and was given as one double-strength tablet three times a week or as one single-strength tablet per day. Selection of patients for PCP prophylaxis and its duration were mainly determined by the treating physician. TMP-SMX was started on the first day of high-dose steroid treatment in most cases (unless contraindicated) and was stopped when the daily steroid dose (based on prednisone) was tapered: to 30 mg in 35 (13.6%) treatment episodes, 25 mg in 6 (2.3%), 20 mg in 26 (10.1%), 15 mg in 53 (20.6%) and <15 mg/day in 113 (44.0%). For patients with renal insufficiency, the TMP-SMX dose was adjusted accordingly (determined by creatinine clearance, n=23). Second-line antibiotics against PCP such as dapsone, atovaquone or aerosolised pentamidine were not used for primary prophylaxis against PCP during the observation period.

Statistical analysis

Continuous or dichotomous baseline data were compared using Student's t-test or the χ^2 test as appropriate. Cox proportional hazards regression models were used to estimate the effect of TMP-SMX on outcome. The HR was adjusted for baseline clinical factors that showed a significant association (P<0.1) with outcome. In addition, the final model was adjusted for intracluster correlation as some patients may have undergone multiple treatment episodes. With respect to PCP-related mortality, which showed the complete separation of outcome, Firth's penalised maximum likelihood was used to reduce statistical bias.[15]

Since there were differences between the groups in terms of baseline characteristics, the same survival analyses were undertaken after applying 1:1 propensity score (PS) matching. This was carried out using the patients' age, cumulative steroid dose during the 6 months prior to baseline, concomitant use of immunosuppressants (cyclophosphamide and steroid pulse), lymphopenia (<800/μL) and the presence of certain underlying diseases as predictors of a requirement for prophylaxis; the selected calliper was 0.2. After matching, 235 treatment episodes from each group were selected for use as the postmatched populations (see online supplementary figure S2). Although a comparison of PCP incidence and related mortality was performed before and after matching, primary outcome was mainly assessed in the postmatched population because it was expected to have less statistical bias regarding the number of covariates per case. All statistical analyses were performed using R V.3.3.1 software, and a P value <0.05 was considered statistically significant.

RESULTS

Patient characteristics

A total of 1522 treatment episodes from 1092 patients were fulfilled the criteria for analysis. TMP-SMX prophylaxis was performed in 262 treatment episodes, with a mean (SD) duration of 237.0 (272.2) days. Patients received daily single-strength TMP-SMX regimen in most treatment episodes (251/262, 95.8%). Prophylaxis began on the first day of high-dose steroid treatment (except in nine cases in which TMP-SMX prophylaxis was delayed by more than 1 month from the initiation of high-dose steroid due to acute kidney injury (n=4), leucopenia (n=3) or pregnancy (n=2)).

The baseline characteristics of the control and prophylaxis groups are shown in table 1. Patients in the prophylaxis group were older, more likely to have lymphopenia and to be treated with secondary immunosuppressive agents. In addition, the proportion of patients with diseases associated with a high risk of PCP, such as granulomatosis with polyangiitis (GPA), microscopic polyangiitis (MPA) and dermatomyositis, was significantly higher in the prophylaxis group. The cumulative steroid dose administered during the entire observation period was also higher in the prophylaxis group (based on prednisone, 7158±4552 mg vs 8202±5145 mg, P=0.001). There were no significant differences in the above-mentioned clinical factors in the postmatched population (table 2).

Incidence of PCP

During the observation period of 1474.4 person-years, there were 30 PCP cases in 30 patients: the incidence rate (95% CI) in the control group was 2.37 (1.59–3.41)/100 person-years. When the whole population was stratified according to underlying disease, the incidence of PCP was highest in those with GPA and MPA (12.14 (95% CI 3.94 to 28.33) per 100 person-years), followed by those with systemic sclerosis (10.88 (95% CI 2.24 to 31.80) per 100 person-years), dermatomyositis (3.11 (95% CI 0.64 to 9.07) per 100 person-years) and systemic lupus erythematosus (SLE) (2.42 (95% CI 1.36 to 4.00) per 100 person-years). The mean time interval between baseline and PCP was 3.4 (SD=2.5, min=0.9, max=10.8) months and 27 (90.0%) cases occurred within the first 6 months. The mean (SD) dose of steroid (based on prednisone) at the time of PCP diagnosis was 31.3 (SD=20.1, min=5, max=80) mg; 15 (50%) cases occurred when the dose was ≥30 mg/day, 12 cases when 15–30 mg/day and 3 cases when <15 mg/day. Twenty-nine cases of PCP developed in the control group, whereas only one case occurred in the prophylaxis group. However, in this case, TMP-SMX was discontinued prematurely due to ADR. Among all PCP cases,

Table 1 Baseline* characteristics of the whole population

(n=number of treatment episodes)	Control group (n=1260)	Prophylaxis group (n=262)	P value
Male gender, n (%)	374 (29.7)	89 (34.0)	0.170
Age, year, mean (SD)	41.2 (15.2)	46.2 (16.0)	<0.001
Disease duration, year, mean (SD)	3.0 (3.8)	2.5 (4.0)	0.053
Underlying disease			
Systemic lupus erythematosus, n (%)	636 (50.5)	122 (46.8)	0.249
Systemic sclerosis, n (%)†	30 (2.4)	5 (1.9)	0.642
Dermatomyositis, n (%)	100 (7.9)	38 (14.5)	0.001
Polymyositis, n (%)	54 (4.3)	12 (4.6)	0.831
GPA, n (%)	38 (3.0)	18 (6.9)	0.003
MPA, n (%)	9 (0.7)	11 (4.2)	<0.001
EGPA, n (%)	43 (3.4)	7 (2.7)	0.541
Polyarteritis nodosa, n (%)	17 (1.3)	7 (2.7)	0.118
Rheumatoid arthritis, n (%)†	58 (4.6)	10 (3.8)	0.575
Adult-onset Still's disease, n (%)	31 (2.5)	9 (3.4)	0.369
Behcet's disease, n (%)	182 (14.4)	12 (4.6)	<0.001
Cryoglobulinaemic vasculitis, n (%)	1 (0.1)	2 (0.8)	0.023
Ankylosing spondylitis, n (%)	12 (1.0)	0 (0.0)	0.113
Primary Sjogren's syndrome, n (%)	3 (0.2)	0 (0.0)	0.429
Others, n (%)‡	47 (3.7)	9 (3.4)	0.817
Initial steroid dose of 30–45 mg PD, n (%)	426 (33.8)	88 (33.6)	0.945
Initial steroid dose of 45–60 mg PD, n (%)	141 (11.2)	42 (16.0)	0.028
Initial steroid dose of ≥60 mg PD, n (%)	696 (55.0)	132 (50.4)	0.172
Concomitant immunosuppressive treatment			
Steroid pulse treatment, n (%)	164 (13.0)	99 (37.8)	<0.001
Oral cyclophosphamide, n (%)	49 (3.9)	34 (13.0)	<0.001
Cyclophosphamide pulse treatment, n (%)	99 (7.9)	67 (25.6)	<0.001
Cumulative steroid dose, mean (SD)§	1597.1 (1568.7)	3119.7 (1821.5)	<0.001
Lymphopenia, n (%)¶	283 (22.5)	87 (33.2)	<0.001

*The baseline date was defined as the day on which PCP prophylaxis (prophylaxis group) or high-dose steroid (control group) was started.
†The main reason for the use of high-dose steroids in these diseases was associated interstitial lung disease.
‡Including Takayasu's arteritis, temporal arteritis and relapsing polychondritis.
§Cumulative steroid (prednisone) dose during the previous 6 months.
¶Defined as <800 lymphocytes/mL.
EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; PCP, pneumocystis pneumonia; PD, prednisone.

16 (53.3%) received mechanical ventilation and 11 (36.7%) expired. All PCP-related deaths occurred in the control group. Clinical features of PCP cases at baseline and PCP occurrence are summarised in online supplementary tables S1 and S2, respectively.

The incidence of PCP tended to increase according to the increase in the initial steroid dose. Patients receiving ≥60 mg/day prednisone showed a significantly higher PCP incidence than those in other subgroups (figure 1).

Efficacy of TMP-SMX prophylaxis in the PS-matched population

Univariable analysis in the PS-matched population revealed that the 1-year incidence of PCP significantly decreased with prophylaxis (HR=0.07; 95% CI 0.01 to 0.54). This result was also consistent with the result of multivariable analysis including age and MPA as covariates (adjusted HR=0.07; 95% CI 0.01 to 0.53). PCP-related mortality in the prophylaxis group fell significantly in both univariable analysis (HR=0.07, 95% profile likelihood CI 0.0005 to 0.55) and multivariable analysis (adjusted HR=0.08; 95% profile likelihood CI 0.0006 to 0.71) (table 3). The HR and its significance level for other covariates are presented in online supplementary table S3.

Table 2 Baseline* characteristics of the PS-matched population

(n=number of treatment episodes)	Control group (n=235)	Prophylaxis group (n=235)	P value
Male gender, n (%)	173 (73.6)	161 (68.5)	0.222
Age, year, mean (SD)	45.8 (16.3)	45.5 (15.7)	0.843
Disease duration, year, mean (SD)	3.1 (4.0)	2.6 (3.9)	0.200
Underlying disease			
Systemic lupus erythematosus, n (%)	109 (46.4)	112 (47.7)	0.782
Systemic sclerosis, n (%)†	6 (2.6)	5 (2.1)	0.760
Dermatomyositis, n (%)	34 (14.5)	34 (14.5)	1.000
Polymyositis, n (%)	17 (7.2)	10 (4.3)	0.165
GPA, n (%)	16 (6.8)	13 (5.5)	0.565
MPA, n (%)	8 (3.4)	8 (3.4)	1.000
EGPA, n (%)	6 (2.6)	7 (3.0)	0.779
Polyarteritis nodosa, n (%)	7 (3.0)	6 (2.6)	0.779
Rheumatoid arthritis, n (%)†	9 (3.8)	9 (3.8)	1.000
Adult-onset Still's disease, n (%)	2 (0.9)	8 (3.4)	0.106
Behcet's disease, n (%)	11 (4.7)	12 (5.1)	0.831
Cryoglobulinaemic vasculitis, n (%)	1 (0.4)	2 (0.9)	0.562
Ankylosing spondylitis, n (%)	3 (1.3)	0 (0.0)	0.248
Primary Sjogren's syndrome, n (%)	1 (0.4)	0 (0.0)	0.317
Others, n (%)‡	5 (2.1)	9 (3.8)	0.278
Initial steroid dose of 30–45 mg PD, n (%)	70 (29.5)	72 (30.9)	0.747
Initial steroid dose of 45–60 mg PD, n (%)	29 (12.2)	39 (16.7)	0.165
Initial steroid dose of ≥60 mg PD, n (%)	138 (58.2)	122 (52.4)	0.201
Concomitant immunosuppressive treatment			
Steroid pulse treatment, n (%)	84 (35.7)	80 (34.0)	0.699
Oral cyclophosphamide, n (%)	20 (8.5)	25 (10.6)	0.433
Cyclophosphamide pulse treatment, n (%)	54 (23.0)	54 (23.0)	1.000
Cumulative steroid dose, mean (SD)§	2696.6 (2123.1)	2898.6 (1558.8)	0.240
Lymphopenia, n (%)¶	73 (31.1)	76 (32.3)	0.766

*The baseline date was defined as the day on which PCP prophylaxis (prophylaxis group) or high-dose steroid (control group) was started.
†The main reason for the use of high-dose steroids in these diseases was associated interstitial lung disease.
‡Including Takayasu's arteritis, temporal arteritis and relapsing polychondritis.
§Cumulative steroid (prednisone) dose during the previous 6 months.
¶Defined as <800 lymphocytes/mL.
EGPA, eosinophilic granulomatosis with polyangiitis; GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; PCP, pneumocystis pneumonia; PD, prednisone; PS, propensity score.

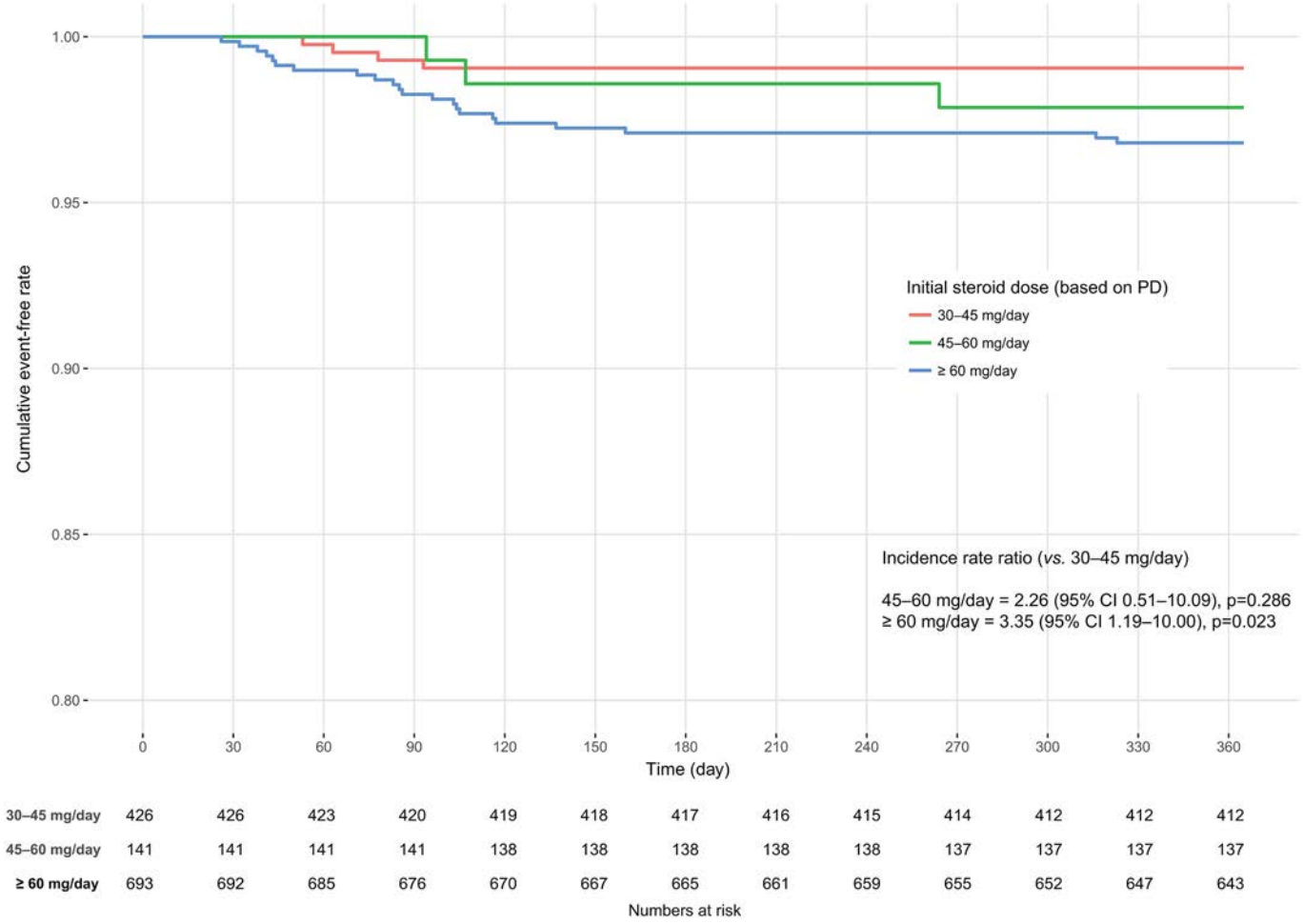


Figure 1 Kaplan-Meier curve showing pneumocystis pneumonia (PCP)-free survival according to the initial dose of steroids (30–45 mg/day prednisone, 45–60 mg/day and ≥60 mg/day) in the whole population. PD, prednisone.

Since the incidence of PCP increased according to the increase in the initial steroid dose, we next examined the efficacy of TMP-SMX prophylaxis after stratifying all treatment episodes by this factor. In the subgroup with a higher initial steroid dose (≥60 mg/day prednisone) (n=261), TMP-SMX led to a significant reduction in PCP incidence after adjusting for GPA (adjusted HR=0.05; 95% profile likelihood CI 0.0004 to 0.40). However, the effectiveness was not apparent in the subgroup receiving a lower initial steroid dose (HR=0.36; 95% profile likelihood CI 0.04 to 2.21).

Table 3 Effect of TMP-SMX prophylaxis on 1-year PCP incidence and related mortality in the propensity score-matched population (n=470)

	1-year PCP incidence		1-year PCP-related mortality*	
	HR (95% CI)		HR (95% profile likelihood CI)	
	Univariable analysis	Multivariable analysis†	Univariable analysis	Multivariable analysis‡
TMP-SMX prophylaxis	0.07 (0.01 to 0.54)	0.07 (0.01 to 0.53)	0.07 (0.0005 to 0.55)	0.08 (0.0006 to 0.71)
P value for HR	0.010	0.010	0.007	0.019

*Firth's penalised maximum likelihood was used due to complete separation of outcome.
†Included age and MPA as covariates, and was also adjusted for clustering.
‡Included age, GPA and MPA as covariates, and was also adjusted for clustering.
GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; PCP, pneumocystis pneumonia; TMP-SMX, trimethoprim-sulfamethoxazole.

Efficacy of TMP-SMX prophylaxis in the whole population

In the whole population, the 1-year incidence of PCP tended to decrease with prophylaxis (HR=0.17; 95% CI 0.02 to 1.22). MPA, higher steroid dose, concomitant cyclophosphamide pulse and baseline lymphopenia were associated with an increased incidence of PCP (see online supplementary table S4). After adjusting for these factors, the prophylaxis group showed a significantly lower incidence of PCP than control group (HR=0.06; 95% CI 0.004 to 0.66) (table 4). As in the PS-matched population, TMP-SMX significantly reduced PCP incidence only in the subgroup with a higher initial steroid dose (n=825) (adjusted HR=0.02; 95% profile likelihood CI 0.0001 to 0.24).

TMP-SMX was also associated with a reduction in PCP-related mortality after adjusting for age, GPA, MPA and concomitant steroid pulse treatment (adjusted HR=0.09; 95% profile likelihood CI 0.0007 to 0.76) (table 4).

ADRs associated with prophylactic TMP-SMX

During the 170.1 person-year duration of TMP-SMX prophylaxis, 36 ADRs (of any type) occurred in 32 patients (21.2/100 person-years; 95% CI 14.8 to 29.3). The most common ADRs were elevated (>1.5 the upper normal range) serum alanine transaminase levels and a skin rash (3.5/100 person-years for both), followed by thrombocytopenia (1.8/100 person-years) and hyperkalaemia

Table 4 Effect of TMP-SMX prophylaxis on 1-year PCP incidence and related mortality in the whole population (n=1522)

	1-year PCP incidence		1-year PCP-related mortality*	
	HR (95% CI)		HR (95% profile likelihood CI)	
	Univariable analysis	Multivariable analysis†	Univariable analysis	Multivariable analysis‡
TMP-SMX prophylaxis	0.17 (0.02 to 1.22)	0.06 (0.004 to 0.66)	0.21 (0.002 to 1.61)	0.09 (0.0007 to 0.76)
P value for HR	0.078	0.022	0.165	0.023

*Firth's panelised maximum likelihood was used due to complete separation of outcome.
†Included age, MPA, initial steroid dose (≥60 mg/day prednisone vs not), concomitant cyclophosphamide pulse and baseline lymphopenia as covariates, and was also adjusted for clustering.
‡Included age, GPA, MPA and concomitant steroid pulse as covariates, and was also adjusted for clustering.
GPA, granulomatosis with polyangiitis; MPA, microscopic polyangiitis; PCP, pneumocystis pneumonia; TMP-SMX, trimethoprim-sulfamethoxazole.

(1.8/100 person-years) (table 5). There were no lupus flares during prophylaxis. In most cases, ADR severity was mild to moderate (34/36, 94.4%). There were only two cases of serious ADRs that led to prolonged hospitalisation (one case of pancytopenia and one case of Stevens-Johnson syndrome) (1.2/100 person-years, 95% CI 0.1 to 4.2). However, they resolved shortly after discontinuation of TMP-SMX.

Risk-benefit analysis of TMP-SMX prophylaxis

Based on the two cases of serious ADR, the number needed to harm (NNH) was 131 (55–∞). By contrast, the number needed to treat (NNT) to prevent one case of PCP in the whole population was 52 (33–124). After stratification according to each underlying disease, the NNT in patients with SLE (43 (28–85)) or MPA (3 (1.6–39.4)) was lower than the NNH. The same was true for other diseases; however, the 95% CI for absolute risk reduction extended from a negative number to a positive number, making it irrelevant. Interestingly, when we stratified treatment episodes according to initial steroid dose (≥60 mg/day prednisone vs other), the NNT for the subgroup receiving a higher steroid dose was 32 (22–54), whereas that for the subgroup receiving a lower steroid dose was 215 (45–∞), which is higher than the NNH for serious ADRs.

Table 5 Incidence of adverse drug reactions caused by trimethoprim/sulfamethoxazole prophylaxis

	Number of cases*	Incidence rate (95% CI)†
Adverse drug reactions	34	20.6 (14.3 to 28.6)
Anaemia	2	1.2 (0.1 to 4.2)
Leucopenia	1	0.6 (0.0 to 3.3)
Thrombocytopenia	3	1.8 (0.4 to 5.2)
GI problems	2	1.2 (0.1 to 4.2)
LFT abnormality	6	3.5 (1.3 to 7.7)
Skin rash	6	3.5 (1.3 to 7.7)
Azotaemia	5	3.0 (1.0 to 7.1)
Hyperkalaemia	3	1.8 (0.4 to 5.2)
Others‡	6	3.5 (1.3 to 7.7)
Serious adverse drug reactions	2	1.2 (0.1 to 4.2)
Pancytopenia	1	0.6 (0.0 to 3.3)
Stevens-Johnson syndrome	1	0.6 (0.0 to 3.3)

*Total observation period was 170.1 person-years for 262 cases.
†Rate per 100 person-years.
‡Including headache (1), anorexia (1), eosinophilia (1), tingling sensation (1) and pruritus (2).
GI, gastrointestinal; LFT, liver function test.

Sensitivity analysis

Because differences in the dosing regimens of TMP-SMX could have influenced its efficacy, we performed the same Cox regressions after excluding subgroups with atypical TMP-SMX dosing, including (1) twenty-three treatment episodes with a renal dose adjustment, (2) ten with a thrice weekly TMP-SMX regimen and (3) nine with more than a month's delay in prophylaxis. The result from each of these analyses was consistent with the original one (data not shown).

Since patients in the prophylaxis group discontinued TMP-SMX at various times, we analysed the data using a censoring scheme based on tapering of steroids (eg, 30 mg/day and 15 mg/day prednisone). The prophylactic effect of TMP-SMX was unchanged (see online supplementary figure S3). In addition, to minimise the effect of heterogeneity in the duration of prophylaxis, we also performed the same analysis using 6 and 3-month observation periods, respectively. Using these censoring schemes, the mean (SD) proportion of time that TMP-SMX was administered was significantly increased (0.50 (0.33) in the original analysis vs 0.70 (0.32) and 0.86 (0.25) in 6-month and 3-month time frames, respectively, P<0.001). However, the efficacy of prophylaxis was unaffected by the change in observation period (see online supplementary figure S4).

DISCUSSION

Systemic high-dose steroid treatment is one of the most important weapons against rheumatic diseases; however, it is a risk factor for PCP. Many studies describe an association between PCP and steroid use in patients with rheumatic disease, but few have investigated the prophylactic effects in such populations.[16–18] To the best of our knowledge, this is the largest study conducted to investigate the efficacy and safety of TMP-SMX prophylaxis in patients with rheumatic diseases who received prolonged high-dose steroids. The incidence of PCP in the control group was 2.37/100 person-years, which is consistent with previous reports.[19]

TMP-SMX was highly effective at preventing PCP and related mortality. In contrast, compared with that reported in other studies of HIV-positive patients, TMP-SMX showed a lower incidence of ADRs.[20] Recent meta-analyses on the efficacy of PCP prophylaxis in patients with haematologic malignancy or post-transplantation suggest that TMP-SMX should be considered when the NNT is balanced against the NNH for severe ADRs.[21 22] Overall, the NNT herein was 52, whereas that for severe ADRs was 131, illustrating that the benefit of TMP-SMX prophylaxis was greater than the risk of potential harm to the patient. Interestingly, in the subgroup that received a higher initial steroid dose, the NNT was even lower. This demonstrates that, in patients receiving ≥60 mg/day prednisone, the benefits of TMP-SMX prophylaxis outweigh the risks. This result suggests that initial steroid dose may identify patients who would derive maximum benefit from TMP-SMX prophylaxis.

The optimal time to stop PCP prophylaxis in non-HIV patients receiving high-dose steroids remains unclear. Expert opinion suggests that prophylaxis should be continued until the CD4 T cell count rises above 200/mm^3 for 6 consecutive months.[23] However, the correlation between this factor and the risk for PCP is less clear in patients without HIV.[24] In that context, it is noteworthy that most PCP cases (90.0%) in the present study occurred when

a patient received ≥15 mg/day prednisone or equivalent, which is in line with the findings of previous studies.[13 18 24 25] This suggests that tapering the dose of steroid down to <15 mg/day might be a relevant point at which to consider stopping prophylaxis. In agreement with previous reports, we found that PCP showed a significant association with concomitant cyclophosphamide, lymphopenia and old age, and at least one of these risk factors was present in all instances of PCP in patients receiving <15 mg/day prednisone.[13 23 26] However, because of the small number of PCP cases, it should be precautious to define relevant time point of stopping prophylaxis with this result alone.

This study has some limitations. First, the baseline characteristics of the prophylaxis and non-prophylaxis groups were not fully balanced, a limitation inherent to observational studies. To overcome this limitation, primary analysis was performed based on PS-matching population; however, unmeasured confounders such as physician's preference cannot be completely balanced without randomisation. Second, the number of PCP cases in this study was rather small so we could not perform a precise risk-benefit assessment for some rheumatic diseases. In addition, because this was not a randomised controlled study, we could not compare the prevalence of adverse events between the two groups; therefore, the NNH was based on the ADR from the prophylaxis group alone.

In conclusion, we show here the benefit of TMP-SMX as primary prophylaxis for PCP in patients with rheumatic diseases who were treated with prolonged high-dose steroids; this was particularly true for patients receiving an initial steroid dose ≥60 mg/day prednisone or equivalent. Although the results should be confirmed in a future randomised study, the data may impact the use of PCP prophylaxis for patients with rheumatic diseases.

Contributors EBL had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: EBL, JRC, SK and JWP. Acquisition, analysis or interpretation of data: JWP, JRC, JM, YWS and EBL. Drafting of the manuscript: EBL, JRC and JWP. Critical revision of the manuscript for important intellectual content: JWP, JRC, JM, YWS, SK and EBL. Statistical analysis: EBL, JRC and JWP.

Funding This research was partly supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2016R1D1A1A02937044).

Competing interests EBL has acted as a consultant to Pfizer, and received research grants from Green Cross Corp and Hanmi Pharm Company. The other authors declare no conflicts of interest.

Patient consent Informed consents were waived based on the retrospective nature of the study.

Ethics approval The study was approved by the Institutional Review Board of the Seoul National University Hospital (IRB 1508-050-694) and was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Thomas CF, Limper AH. Pneumocystis pneumonia. *N Engl J Med* 2004;350:2487–98.
2 Morris A, Lundgren JD, Masur H, *et al*. Current epidemiology of Pneumocystis pneumonia. *Emerg Infect Dis* 2004;10:1713–20.
3 Mansharamani NG, Garland R, Delaney D, *et al*. Management and outcome patterns for adult Pneumocystis carinii pneumonia, 1985 to 1995: comparison of HIV-associated cases to other immunocompromised states. *Chest* 2000;118:704–11.
4 Yale SH, Limper AH. Pneumocystis carinii pneumonia in patients without acquired immunodeficiency syndrome: associated illness and prior corticosteroid therapy. *Mayo Clin Proc* 1996;71:5–13.
5 Sepkowitz KA. Opportunistic infections in patients with and patients without Acquired Immunodeficiency Syndrome. *Clin Infect Dis* 2002;34:1098–107.
6 Maeda T, Babazono A, Nishi T, *et al*. Quantification of the effect of chemotherapy and steroids on risk of Pneumocystis jiroveci among hospitalized patients with adult T-cell leukaemia. *Br J Haematol* 2015;168:501–6.
7 Martin SI, Fishman JA. Pneumocystis pneumonia in solid organ transplantation. *Am J Transplant* 2013;13(Suppl 4):272–9.
8 Limper AH, Knox KS, Sarosi GA, *et al*. An official american thoracic society statement: Treatment of fungal infections in adult pulmonary and critical care patients. *Am J Respir Crit Care Med* 2011;183:96–128.
9 Godeau B, Coutant-Perronne V, Le Thi Huong D, *et al*. Pneumocystis carinii pneumonia in the course of connective tissue disease: report of 34 cases. *J Rheumatol* 1994;21:246–51.
10 Cettomai D, Gelber AC, Christopher-Stine L. A survey of rheumatologists' practice for prescribing pneumocystis prophylaxis. *J Rheumatol* 2010;37:792–9.
11 Buttgereit F, da Silva JA, Boers M, *et al*. Standardised nomenclature for glucocorticoid dosages and glucocorticoid treatment regimens: current questions and tentative answers in rheumatology. *Ann Rheum Dis* 2002;61:718–22.
12 Eitner F, Hauser IA, Rettkowski O, *et al*. Risk factors for Pneumocystis jiroveci pneumonia (PcP) in renal transplant recipients. *Nephrol Dial Transplant* 2011;26:2013–7.
13 Lertnawapan R, Totemchokchyakarn K, Nantiruj K, *et al*. Risk factors of Pneumocystis jeroveci pneumonia in patients with systemic lupus erythematosus. *Rheumatol Int* 2009;29:491–6.
14 Edwards IR, Aronson JK. Adverse drug reactions: definitions, diagnosis, and management. *Lancet* 2000;356:1255–9.
15 Heinze G, Schemper M. A solution to the problem of separation in logistic regression. *Stat Med* 2002;21:2409–19.
16 Vananuvat P, Suwannalai P, Sungkanuparph S, *et al*. Primary prophylaxis for Pneumocystis jirovecii pneumonia in patients with connective tissue diseases. *Semin Arthritis Rheum* 2011;41:497–502.
17 Ogawa J, Harigai M, Nagasaka K, *et al*. Prediction of and prophylaxis against Pneumocystis pneumonia in patients with connective tissue diseases undergoing medium- or high-dose corticosteroid therapy. *Mod Rheumatol* 2005;15:91–6.
18 Chew LC, Maceda-Galang LM, Tan YK, *et al*. Pneumocystis jirovecii pneumonia in patients with autoimmune disease on high-dose glucocorticoid. *J Clin Rheumatol* 2015;21:72–5.
19 Roux A, Gonzalez F, Roux M, *et al*. Update on pulmonary Pneumocystis jirovecii infection in non-HIV patients. *Med Mal Infect* 2014;44:185–98.
20 Rodriguez M, Fishman JA. Prevention of infection due to Pneumocystis spp. in human immunodeficiency virus-negative immunocompromised patients. *Clin Microbiol Rev* 2004;17:770–82.
21 Green H, Paul M, Vidal L, *et al*. Prophylaxis of Pneumocystis pneumonia in immunocompromised non-HIV-infected patients: systematic review and meta-analysis of randomized controlled trials. *Mayo Clin Proc* 2007;82:1052–9.
22 Stern A, Green H, Paul M, *et al*. Prophylaxis for Pneumocystis pneumonia (PCP) in non-HIV immunocompromised patients. *The Cochrane database of systematic reviews* 2014;01:CD005590.
23 Suryaprasad A, Stone JH. When is it safe to stop Pneumocystis jiroveci pneumonia prophylaxis? Insights from three cases complicating autoimmune diseases. *Arthritis Rheum* 2008;59:1034–9.
24 Overgaard UM, Helweg-Larsen J. Pneumocystis jiroveci pneumonia (PCP) in HIV-1-negative patients: a retrospective study 2002-2004. *Scand J Infect Dis* 2007;39(6-7):589–95.
25 Roblot F, Le Moal G, Kauffmann-Lacroix C, *et al*. Pneumocystis jirovecii pneumonia in HIV-negative patients: a prospective study with focus on immunosuppressive drugs and markers of immune impairment. *Scand J Infect Dis* 2014;46:210–4.
26 Teichtahl AJ, Morrisroe K, Ciciriello S, *et al*. Pneumocystis jirovecci pneumonia in connective tissue diseases: Comparison with other immunocompromised patients. *Semin Arthritis Rheum* 2015;45:86–90.

EXTENDED REPORT

Patient characteristics influence the choice of biological drug in RA, and will make non-TNFi biologics appear more harmful than TNFi biologics

Thomas Frisell,[1] Eva Baecklund,[2] Karin Bengtsson,[3] Daniela Di Giuseppe,[1] Helena Forsblad-d'Elia,[4] Johan Askling,[1,5] on behalf of the ARTIS Study group

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212395).

[1]Clinical Epidemiology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm, Sweden
[2]Rheumatology Unit, Department of Medical Sciences, Uppsala University, Uppsala, Sweden
[3]Department of Rheumatology and Inflammation Research, Institute of Medicine, Sahlgrenska Academy at University of Gothenburg, Göteborg, Sweden
[4]Department of Public Health and Clinical Medicine, Rheumatology, Umeå University, Umeå, Sweden
[5]Department of Rheumatology, Karolinska University Hospital, Stockholm, Sweden

Correspondence to
Dr Thomas Frisell, Clinical Epidemiology Unit, Department of Medicine Solna, Karolinska Institutet, Stockholm 17176, Sweden; thomas.frisell@ki.se

Received 15 September 2017
Revised 23 November 2017
Accepted 27 November 2017
Published Online First 13 December 2017

To cite: Frisell T, Baecklund E, Bengtsson K, *et al*. *Ann Rheum Dis* 2018;**77**:650–657.

ABSTRACT

Objectives With the wide range of biological disease-modifying anti-rheumatic drugs (bDMARDs) available for treating rheumatoid arthritis (RA), and limited evidence to guide the choice for individual patients, we wished to evaluate whether patient characteristics influence the choice of bDMARD in clinical practice, and to quantify the extent to which this would bias direct comparisons of treatment outcome.

Methods Register-based study of all Swedish patients with RA initiating necrosis factor inhibitor (TNFi), rituximab, abatacept or tocilizumab in 2011–2015 as their first bDMARD (n=6481), or after switch from TNFi as first bDMARD (n=2829). Group differences in demographics, clinical characteristics and medical history were assessed in multivariable regression models. Predicted differences in safety and treatment outcomes were calculated as a function of patient characteristics, through regression modelling based on observed outcomes among patients with RA starting bDMARDs 2006–2010.

Results Patients starting non-TNFi were older than those starting TNFi, had lower socioeconomic status, higher disease activity and higher burden of diseases including malignancy, serious infections and diabetes. Differences were most pronounced at first bDMARD initiation. These factors were linked to treatment outcome independent of therapy, yielding worse apparent safety and effectiveness for non-TNFi biologics, most extreme for rituximab. Standardising to the age/sex distribution of the TNFi group reduced differences considerably.

Conclusions There was significant channelling of older and less healthy patients with RA to non-TNFi bDMARDs, in particular as first bDMARD. Whether this channelling represents a maximised benefit/risk ratio is unclear. Unless differences in age, medical history and disease activity are accounted for, they will substantially confound non-randomised comparative studies of available bDMARDs' safety and effectiveness.

BACKGROUND

Following a rapid development over the past two decades, a wide range of biological disease-modifying anti-rheumatic drugs (bDMARDs) are currently available for the treatment of rheumatoid arthritis (RA). In many countries, Sweden included, RA treatment guidelines have expanded the recommended options for first bDMARD in recent years, from necrosis factor inhibitor (TNFi) drugs exclusively to include abatacept, tocilizumab and rituximab,[1–4] ranking the drugs as comparable in overall safety and efficacy. For historical reasons, TNFi drugs remain the most common choice as first bDMARD, but many patients will switch from their initial bDMARD,[5] and similar to the first, the choice of the next bDMARD (eg, switching to another TNFi or to another mode of action) is seldom strictly regulated. In clinical practice, perceived or established differences between bDMARD options lead to a non-random allocation of treatment. Although many clinicians may be aware of the *existence* of such channelling, its *magnitude* (ie, how different treatment outcomes it will give rise to) is seldom quantified, yet essential for a correct evaluation of the drugs' relative effectiveness and safety.[6 7]

In all situations with an element of preference-guided choice of therapy, it is important to monitor which patient gets which therapy for at least two reasons. First, if physicians' show a preference for a specific drug for certain patients, it should be a research priority to tell whether this is motivated by an increased tolerability or efficacy in this group, or merely a misconception about the drug's (side) effects, potentially leading to inequities in care.[8] Second, non-random choice of therapy will hamper studies of RA therapies by introducing *confounding by indication*, which occurs when factors associated with the choice of therapy are also predictors of the studied outcome, and is generally considered the major limitation of non-randomised comparisons of therapies.[9] The case of bDMARDs in RA illustrates both of these needs.

Thus, the objective of this paper is twofold. First, to describe baseline patient characteristics at initiation of different bDMARDs at two clinically distinct and common time points: (1) at first bDMARD initiation, (2) at switch to a second bDMARD after having used a TNFi as first bDMARD. Second, to estimate the potential of the observed channelling to confound comparative safety and effectiveness studies in RA.

METHODS

Data on clinical characteristics, demographics and medical history among all patients with RA in Sweden who initiated a first or second bDMARD therapy during 2011–2015 were identified by linking the Swedish Rheumatology Quality register (SRQ) to nationwide Swedish healthcare registers.

Data sources

The database used for this study has been described previously.[10 11] Briefly, the SRQ is a clinical register with longitudinal data on disease activity and treatment at each rheumatology visit,[12] with a national coverage for bDMARD treatment in RA of 95%.[13] The National Patient Register provided all diagnoses set in inpatient and non-primary outpatient visits; validation against medical files has found a high positive predictive value (85%–95%) for diagnoses in inpatient care.[14 15] The Prescribed Drug Register provided all dispensed prescriptions in Sweden since July 2005; the register has virtually complete coverage.[16] The Swedish Cancer Register contains clinical data on all cancers since 1958; estimated coverage is greater than 95%.[17] Registers on communicable diseases provided dates for verified tuberculosis, hepatitis B and C. Socioeconomic data were available through census registers.

Covariates

We considered an inclusive list of baseline characteristics to capture factors that we a priori considered may influence choice of therapy, safety or treatment outcome. Variables included sociodemographic background (highest education, country of birth), RA-specific clinical characteristics (rheumatoid factor (RF), disease duration, Health Assessment Questionnaire - Disability Index (HAQ-DI), Disease Activity Score - 28 joints (DAS28) with components, visual analogue scale (VAS) pain), concomitant treatment with non-steroidal anti-inflammatory drugs (NSAIDs,) glucocorticosteroids, conventional synthetic DMARDs and medical history. Disease activity and current therapy was extracted from the visit in the SRQ with valid data on each variable closest to treatment start (within −90 to +14 days, chosen to increase data availability, while avoiding values influenced by the treatment effect). Medical history was measured as having been diagnosed with either of a range of specific conditions (definitions in online supplementary table s1), within 5 years before treatment start, except for serious infections (defined as 'recent' within 1 year, and 'non-recent' within 1 to 5 years) and malignancy ('recent' within 5 years, and 'non-recent' more than 5 years earlier). Analysis of individual conditions was preferred over a combined comorbidity score since the latter would mask disease-specific associations and increase risk for residual confounding.[18] We used three continuous measures intended to capture patients' general health: (1) number of days hospitalised, (2) days of lost work due to sick leave or disability pension (only for those aged 25–65 years) and (3) total healthcare costs. Healthcare costs were calculated by summing costs for dispensed drugs and visits in inpatient and non-primary outpatient care, weighted by disease-related group with annual national tariffs, inflation corrected to 2012.

Statistics

To assess differences in patient characteristics across biologics, we tabulated means and proportions of baseline covariates, with adjusted differences for each non-TNFi bDMARD compared with TNFi, modelled in multivariable linear regression models with bootstrapped CIs.[19 20] The main model was adjusted for sex, age and geographic region, and a supplemental model further adjusted for country of birth, education level, RF, disease duration, ertythrocyte sedimentation rate (ESR), DAS28calculated with C-reactive protein (DAS28-CRP), recent infections, recent malignancy, joint surgery, chronic lung disease and acute coronary syndrome. The choice of covariates in model 2 was based on observed differences and availability of data.

Therapy after switch from TNFi was defined as the first bDMARD therapy started within 1 year of discontinuing an initial TNFi as the first ever bDMARD. The main analysis focused on the difference between abatacept, rituximab, tocilizumab and the class of TNFi (adalimumab, certolizumab pegol, etanercept, infliximab and golimumab). Supplementary analyses were performed comparing individual TNFi drugs.

The expected impact of confounding was assessed through a series of prediction models. Logistic regressions were used to estimate associations of patient characteristics and treatment outcomes among all individuals with RA starting a bDMARD (to maximise cohort size and precision, we included up to third bDMARD) in the years 2006–2010 (immediately prior to our study period). We defined safety outcomes as the proportions experiencing the following events within 5 years of starting therapy: (1) death, (2) serious infection, (3) major acute cardiovascular event (MACE), (4) non-benign malignancy (definitions in supplementary table s2). Similarly, we defined treatment effectiveness outcomes as the proportion: (1) discontinuing therapy before 1 year and (2) remaining on therapy and having reached good European League Against Rheumatism (EULAR) response after 1 year. Separate models were created for each outcome using the full list of covariates. To allow some deviation from linearity, continuous variables were entered as second-degree polynomials; the only included interaction was between age and sex. Line of therapy was included as a binary variable (biologics naive vs not). Work loss was excluded from model building, since it is restricted to those of working age. The coefficients from the final models were used to calculate the predicted probability of each outcome, by treatment, in our main cohort. Since specific bDMARDs were not included in the prediction models, these predicted probabilities will reflect the proportions expected only from baseline characteristics, averaged across all bDMARDs. To further assess how much of the predicted difference between treatments would be removed by adjustment for age and sex rather than by other patient characteristics (eg, medical history), we standardised each treatment group to the age (in 10-year categories) and sex distribution in the largest group (TNFi as first bDMARD).

Linear regression with bootstrapped CIs was made using the boot package in R V.3.3.1. SAS V.9.4 was used for all other analyses.

RESULTS

Patient characteristics at start of first bDMARD

We identified 6481 patients with RA starting a first bDMARD between 1 January 2011 and 31 December 2015. Most started a TNFi (n=5307, 82%), with all available TNFi in common use, ranging from etanercept (n=1502, 28% of all TNFi) to golimumab (n=745, 14%). The most common non-TNFi was rituximab (n=655, 10% of all first bDMARD). Demographical and clinical characteristics are shown in table 1. Initiators of non-TNFi therapy were older and less well educated than those starting a TNFi, with largest difference for rituximab. Compared with those starting TNFi, rituximab initiators were also more often seropositive, had longer disease duration and slightly higher ESR. Abatacept initiators were similar to the TNFi group, but had higher ESR. Tocilizumab initiators were most extreme in terms of disease activity, with significantly higher ESR and CRP, and borderline higher tender joint counts and HAQ. Initiators of non-TNFis had lower use of concomitant methotrexate.

There were substantial differences in baseline medical history, with those starting rituximab or abatacept more often having

Table 1 Patient characteristics at start of bDMARD therapy, among all bionaïve Swedish patients with RA, 2011–2015

	TNFi	Rituximab	Mean difference versus TNFi (95% CI)	Abatacept	Mean difference versus TNFi (95% CI)	Tocilizumab	Mean difference versus TNFi (95% CI)
Patients, n	5307	655		274		245	
Demographics							
Age, mean (SD)	55.2 (14.1)	65.1 (12.5)	10.0 (9.0 to 11.1)	61.4 (13.3)	6.2 (4.7 to 7.8)	58.5 (13.7)	3.3 (1.7 to 5.1)
Female, n (%)	3929 (74.0)	471 (71.9)	1.3 (–2.4 to 5.3)	202 (73.7)	1.7 (–3.4 to 7.2)	190 (77.6)	4.5 (–0.7 to 10.1)
Highest education, n (%)							
9 years or less	908 (21.2)	165 (33.0)	5.7 (1.6 to 10.1)	60 (29.0)	4.2 (–1.5 to 10.3)	44 (25.1)	2.0 (–4.8 to 8.5)
10 to 12 years	2091 (48.9)	219 (43.8)	–2.6 (–7.4 to 1.9)	102 (49.3)	1.2 (–5.5 to 8.2)	87 (49.7)	2.0 (–5.5 to 9.9)
>12 years	1279 (29.9)	116 (23.2)	–3.1 (–7.1 to 0.9)	45 (21.7)	–5.4 (–11.1 to 0.3)	44 (25.1)	–4.0 (–10.6 to 2.7)
Country of birth, n (%)							
Sweden	485 (9.1)	82 (12.5)	–6.9 (–10.1 to –3.3)	21 (7.7)	–1.6 (–5.8 to 2.7)	27 (11.0)	–1.6 (–6.3 to 3.1)
Scandinavia	267 (5.0)	54 (8.2)	1.6 (–0.6 to 3.9)	19 (6.9)	1.1 (–2.2 to 4.1)	13 (5.3)	–0.3 (–3.3 to 2.4)
Other	4555 (85.8)	519 (79.2)	5.3 (2.5 to 7.8)	234 (85.4)	0.5 (–2.9 to 3.5)	205 (83.7)	1.9 (–2.3 to 5.7)
Medical history							
Days hospitalised last 5 years, mean (SD)	5.5 (16.5)	15.2 (30.5)	8.0 (5.4 to 10.2)	14.9 (29.0)	8.4 (4.7 to 11.5)	7.5 (16.6)	1.1 (–1.2 to 3.1)
Days of work loss last 5 years, mean (SD)	363.1 (588.2)	587.2 (703.2)	183.0 (103.1 to 264.7)	560.2 (715.8)	151.1 (45.6 to 261.9)	407.9 (651.3)	20.4 (–77.7 to 116.2)
Healthcare costs last 5 years, TSEK, mean (SD)	123.3 (133.4)	228.0 (234.2)	85.4 (66.6 to 102.6)	213.0 (216.0)	77.6 (52.5 to 103.3)	136.0 (143.1)	2.5 (–16.7 to 20.7)
RA clinical characteristics							
Rheumatoid factor positive (%)	3886 (75.2)	566 (88.2)	10.4 (7.6 to 13.3)	206 (76.6)	–0.5 (–6.0 to 4.9)	182 (75.2)	–0.7 (–6.4 to 4.9)
Disease duration, years, mean (SD)	9.1 (9.8)	12.9 (12.7)	2.1 (1.0 to 3.1)	9.6 (11.6)	–0.7 (–2.0 to 0.6)	7.3 (10.2)	–2.3 (–3.6 to –1.1)
HAQ, mean (SD)	1.02 (0.61)	1.16 (0.66)	0.06 (–0.00 to 0.13)	1.12 (0.61)	0.07 (–0.02 to 0.16)	1.14 (0.55)	0.10 (0.02 to 0.17)
DAS28, mean (SD)	4.70 (1.28)	4.83 (1.28)	–0.04 (–0.17 to 0.09)	4.95 (1.23)	0.16 (–0.01 to 0.34)	5.16 (1.13)	0.39 (0.23 to 0.55)
Tender joints, 0–28, mean (SD)	6.9 (5.6)	6.7 (5.6)	–0.1 (–0.6 to 0.5)	7.0 (5.5)	0.2 (–0.6 to 0.9)	7.9 (6.0)	0.9 (–0.0 to 1.7)
Swollen joints, 0–28, mean (SD)	6.3 (4.8)	6.1 (4.6)	–0.4 (–0.9 to 0.0)	6.7 (5.0)	0.3 (–0.4 to 1.0)	6.8 (5.1)	0.5 (–0.2 to 1.2)
Global health, mean (SD)	54.4 (24.8)	53.6 (24.8)	–1.3 (–3.6 to 1.3)	56.4 (23.5)	1.5 (–1.5 to 4.5)	57.3 (21.8)	2.7 (–0.4 to 5.7)
ESR, mean (SD)	24.2 (20.4)	30.6 (22.5)	2.2 (0.1 to 4.4)	30.4 (22.2)	4.0 (1.1 to 6.9)	33.4 (26.3)	7.7 (4.1 to 11.2)
CRP, mean (SD)	15.9 (21.9)	18.8 (22.4)	0.7 (–1.4 to 2.8)	20.6 (25.9)	3.4 (–0.1 to 6.7)	23.3 (28.0)	7.4 (3.6 to 10.9)
VAS pain, mean (SD)	54.8 (24.9)	53.5 (24.5)	–1.7 (–4.1 to 0.8)	56.9 (24.1)	1.8 (–1.5 to 5.3)	57.3 (23.8)	2.2 (–1.1 to 5.5)
Conc. use of MTX, n (%)	3812 (71.8)	352 (53.7)	–17.8 (–21.8 to –13.6)	161 (58.8)	–12.6 (–18.7 to –6.7)	135 (55.1)	–15.5 (–21.7 to –9.4)
Conc. use of non-MTX csDMARD, n (%)	1035 (19.5)	166 (25.3)	6.9 (3.2 to 10.6)	54 (19.7)	0.4 (–5.1 to 5.2)	28 (11.4)	–6.9 (–11.2 to –2.9)
Conc. use of oral steroids, n (%)	2692 (50.7)	375 (57.3)	4.0 (–0.0 to 8.0)	139 (50.7)	–1.7 (–7.8 to 4.2)	116 (47.3)	–4.3 (–10.8 to 2.3)
Conc. use of NSAIDs, n (%)	1573 (29.6)	128 (19.5)	–8.4 (–11.7 to –5.0)	60 (21.9)	–6.1 (–11.2 to –1.2)	56 (22.9)	–4.3 (–9.9 to 0.9)

Mean differences are compared with TNFi in multivariable linear regression adjusted for age, sex and geographical region with bootstrapped CIs.
bDMARD, biological disease-modifying anti-rheumatic drug; csDMARD, conventional synthetic disease modifying anti-rheumatic drug; RA, rheumatoid arthritis; TNFi, tumour necrosis factor inhibitor; TSEK, Thousand SEK; MTX, methotrexate.

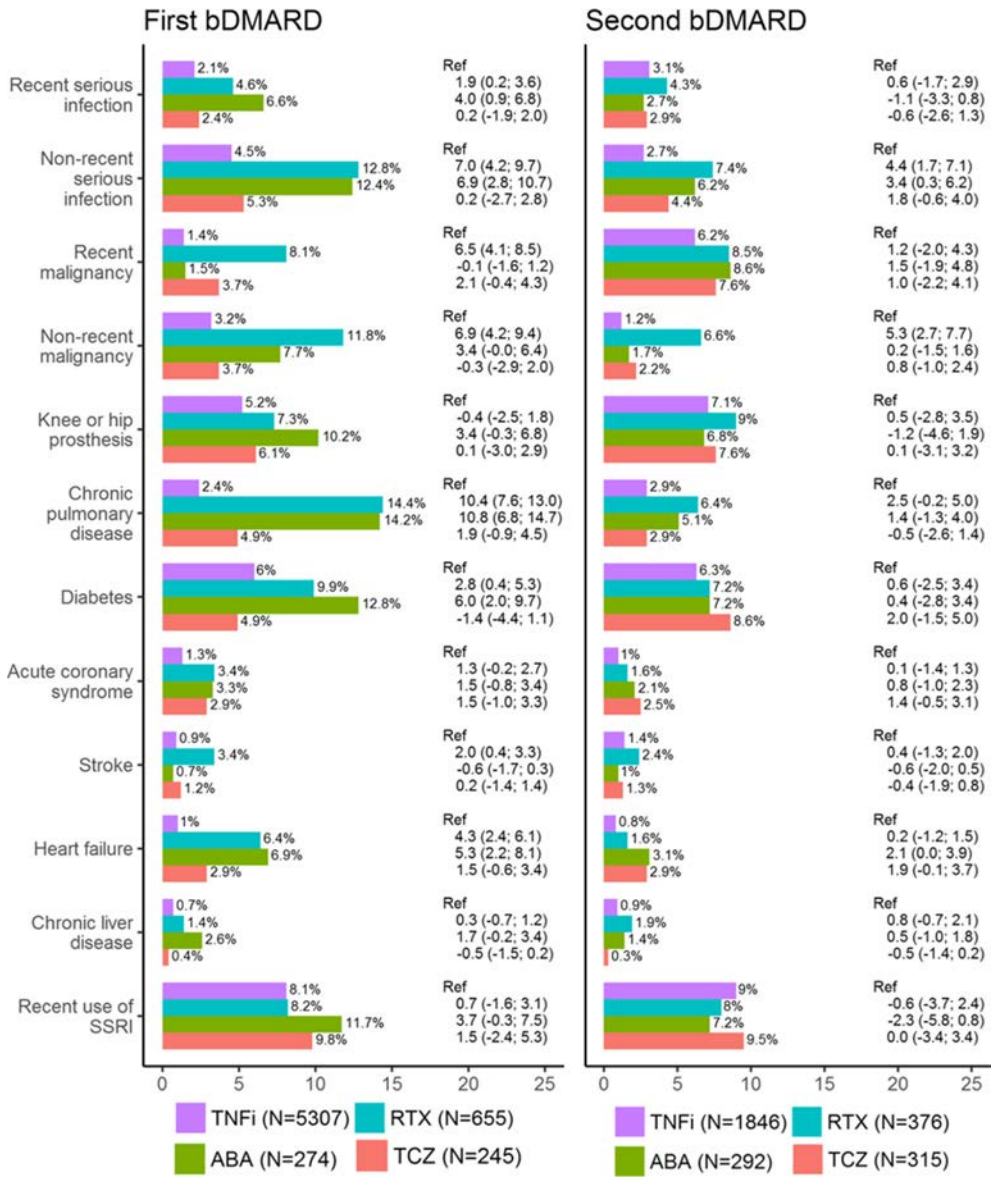


Figure 1 History of disease at treatment start of bDMARD therapy among all patients with rheumatoid arthritis in the SRQ, 2011–2015. Differences in proportion (with 95% CIs) are with reference to TNFi, and adjusted for age, sex and geographical region. bDMARD, biological disease-modifying anti-rheumatic drug; SRQ, Swedish Rheumatology Quality register; SSRI, selective serotonin reuptake inhibitor; TNFi, tumour necrosis factor inhibitor.

a history of the assessed diseases, and having consumed more healthcare resources before treatment start (table 1 and figure 1). Adjusting for age, sex and geographical region decreased these differences, but most of the associations remained. The same was not seen for tocilizumab, where baseline medical history was more similar to the TNFi group. Of particular note, rituximab had a higher proportion with recent (within 5 years) or non-recent (more than 5 years before) malignancy at treatment start (8.1% with recent malignancy, compared with 1.4% on TNFi) (figure 1). Due to low numbers, it was not possible to assess a difference in history of tuberculosis (n=9 patients had tuberculosis before starting first bDMARD), hepatitis B (n<5), hepatitis C (n=6) or multiple sclerosis/demyelinating events (n=14). For brevity, these conditions and other inflammatory conditions are presented in online supplementary table s3-s5.

In sensitivity analyses, further adjustment for demographics, disease activity and medical history did not materially alter the observed pattern of differences in baseline characteristics (online supplementary table s3). Comparisons of individual TNFis revealed few noteworthy differences (online supplementary table s4), but those starting infliximab were slightly older and had slightly higher disease activity compared with the others, while those starting etanercept more often were female, and had accrued higher healthcare costs.

Patient characteristics at switch from first TNFi

We identified 2829 patients with RA who initiated a second ever bDMARD within 1 year of discontinuing a first TNFi. (For reference, during the same period, 1144 patients discontinued a first TNFi without starting a bDMARD within 1 year). It was common to start a second TNFi (n=1846, 65%), regardless of recorded reason for discontinuing the first TNFi. The switch cohort was more homogenous than the first bDMARD cohort, with overall smaller differences across therapies (table 2 and figure 1). Patients starting rituximab and abatacept were older than those starting a TNFi, and had a higher proportion with recent serious infections. Those starting rituximab had a

Table 2 Demographics and clinical characteristics at start of second bDMARD therapy, among Swedish patients with RA switching from TNFi 2011–2015

	TNFi	Rituximab	Mean difference versus TNFi (95% CI)	Abatacept	Mean difference versus TNFi (95% CI)	Tocilizumab	Mean difference versus TNFi (95% CI)
Patients, n	1846	376		292		315	
Demographics							
Age, mean (SD)	55.1 (14.4)	60.3 (12.3)	5.1 (3.6 to 6.5)	58.7 (13.3)	3.7 (2.0 to 5.4)	56.6 (13.8)	1.6 (–0.0 to 3.2)
Female, n(%)	1408 (76.3)	270 (71.8)	–2.6 (–7.6 to 2.5)	233 (79.8)	5.0 (–0.0 to 9.9)	241 (76.5)	1.0 (–3.8 to 5.8)
Highest education, n (%)							
9 years or less	318 (21.7)	82 (26.5)	1.2 (–4.0 to 6.3)	57 (26.1)	2.3 (–4.1 to 8.1)	70 (27.5)	4.1 (–1.8 to 10.0)
10 to 12 years	705 (48.1)	156 (50.3)	2.9 (–3.5 to 8.9)	111 (50.9)	4.1 (–3.1 to 11.3)	110 (43.1)	–4.1 (–10.6 to 2.3)
>12 years	443 (30.2)	72 (23.2)	–4.1 (–9.2 to 1.2)	50 (22.9)	–6.4 (–12.0 to –0.4)	75 (29.4)	0.1 (–6.0 to 6.2)
Country of birth, n (%)							
Sweden	145 (7.9)	28 (7.4)	–1.8 (–5.6 to 2.0)	31 (10.6)	–4.0 (–8.7 to 0.6)	35 (11.1)	–2.3 (–6.5 to 2.1)
Scandinavia	90 (4.9)	24 (6.4)	0.5 (–2.2 to 3.2)	15 (5.1)	0.2 (–2.5 to 2.8)	11 (3.5)	–1.2 (–3.6 to 1.1)
Other	1611 (87.3)	324 (86.2)	1.3 (–1.8 to 4.1)	246 (84.2)	3.8 (0.1 to 7.4)	269 (85.4)	3.5 (–0.5 to 7.1)
Medical history							
Days hospitalised last 5 years, mean (SD)	6.9 (14.7)	11.0 (20.7)	3.1 (0.7 to 5.3)	8.8 (18.3)	1.5 (–0.8 to 3.5)	9.9 (19.4)	2.3 (0.0 to 4.4)
Days of work loss last 5 years, mean (SD)	478.6 (623.9)	615.1 (723.0)	183.0 (103.1 to 264.7)	564.2 (661.3)	20.4 (–76.8 to 118.1)	486.0 (667.6)	–46.3 (–144.5 to 43.7)
Healthcare costs last 5 years, TSEK, mean (SD)	319.2 (256.8)	370.6 (283.4)	30.8 (–1.6 to 61.3)	354.8 (269.0)	11.3 (–20.6 to 42.9)	339.1 (274.5)	12.0 (–20.7 to 43.8)
RA-related characteristics							
Rheumatoid factor positive, n (%)	1333 (75.1)	334 (90.8)	14.2 (10.7 to 17.9)	221 (77.0)	0.6 (–4.8 to 6.1)	237 (78.0)	1.9 (–3.3 to 7.2)
Disease duration, years, mean (SD)	12.0 (10.6)	13.9 (11.4)	0.5 (–0.8 to 1.7)	12.8 (11.4)	–0.3 (–1.6 to 1.0)	10.8 (10.8)	–1.7 (–3.0 to –0.5)
HAQ, mean (SD)	1.05 (0.63)	1.20 (0.63)	0.11 (0.03 to 0.18)	1.21 (0.67)	0.12 (0.03 to 0.22)	1.17 (0.64)	0.09 (0.01 to 0.18)
DAS28, mean (SD)	4.37 (1.37)	4.88 (1.22)	0.44 (0.28 to 0.61)	4.82 (1.30)	0.38 (0.19 to 0.57)	5.03 (1.38)	0.64 (0.45 to 0.82)
Tender joints, 0–28, mean (SD)	5.6 (5.2)	6.3 (5.5)	0.8 (0.1 to 1.4)	6.5 (5.4)	0.7 (–0.0 to 1.5)	7.5 (6.7)	1.8 (1.0 to 2.6)
Swollen joints, 0–28, mean (SD)	4.7 (4.5)	5.6 (4.9)	0.9 (0.3 to 1.5)	5.1 (4.1)	0.3 (–0.3 to 0.8)	6.2 (5.3)	1.5 (0.8 to 2.1)
Global health, mean (SD)	53.6 (24.9)	56.7 (24.4)	2.9 (–0.2 to 5.9)	58.9 (24.2)	4.9 (1.5 to 8.4)	59.2 (25.1)	5.0 (1.6 to 8.3)
ESR, mean (SD)	24.5 (21.4)	33.8 (25.1)	7.2 (4.1 to 10.2)	29.9 (22.3)	4.0 (0.9 to 6.9)	33.9 (25.5)	9.2 (6.0 to 12.4)
CRP, mean (SD)	14.7 (22.1)	21.4 (25.8)	5.7 (2.6 to 8.7)	18.5 (25.0)	3.5 (–0.0 to 6.8)	24.5 (29.1)	9.4 (6.1 to 12.9)
VAS pain, mean (SD)	53.8 (25.4)	56.1 (25.3)	2.1 (–1.2 to 5.2)	59.3 (24.1)	4.9 (1.5 to 8.3)	59.8 (25.0)	5.3 (1.8 to 8.5)
Conc. use of MTX, n (%)	1343 (72.8)	292 (77.7)	4.9 (0.4 to 9.6)	220 (75.3)	4.1 (–1.2 to 9.8)	232 (73.7)	1.2 (–4.1 to 6.5)
Conc. use of non-MTX csDMARD, n (%)	409 (22.2)	90 (23.9)	2.0 (–2.9 to 6.3)	58 (19.9)	–1.8 (–7.0 to 3.3)	51 (16.2)	–5.5 (–10.1 to –0.8)
Conc. use of oral steroids, n (%)	1106 (59.9)	252 (67.0)	7.4 (2.2 to 12.4)	207 (70.9)	10.5 (4.7 to 16.5)	207 (65.7)	7.0 (1.0 to 12.9)
Conc. use of NSAIDs, n (%)	852 (46.2)	166 (44.1)	–0.1 (–5.7 to 5.4)	115 (39.4)	–4.6 (–10.9 to 1.4)	145 (46.0)	0.6 (–5.2 to 6.3)
Reason for switch, n (%)							
Adverse event	374 (20.6)	72 (19.5)	–1.5 (–6.0 to 3.0)	67 (23.8)	3.1 (–2.3 to 8.3)	49 (15.8)	–5.8 (–10.6 to –1.4)
Lack of effect	1170 (64.4)	235 (63.7)	–0.1 (–5.7 to 5.4)	165 (58.7)	–6.4 (–12.9 to 0.3)	235 (75.6)	10.9 (5.4 to 16.3)
Other	272 (15.0)	62 (16.8)	1.6 (–2.9 to 5.7)	49 (17.4)	3.4 (–1.6 to 8.1)	27 (8.7)	–5.1 (–8.6 to –1.7)

Mean differences are compared with TNFi in multivariable linear regression adjusted for age, sex and geographical region with bootstrapped CIs.
bDMARD, biological disease-modifying anti-rheumatic drug; RA, rheumatoid arthritis; TNFi, tumour necrosis factor inhibitor.

higher proportion with recent malignancy and with seropositive RA. Unlike the channelling at first bDMARD, all non-TNFi groups had higher disease activity at switch than the TNFi group. Tocilizumab was more common among those who discontinued the first TNFi due to lack of effect; abatacept was more common among those discontinuing due to adverse events.

In sensitivity analyses of specific TNFis, individual drugs were overall very similar, although several differences reached nominal significance (online supplementary table s5). Infliximab initiators had lower average education (38% had 9 years or less, vs 20% in other groups), more work loss, and less psoriasis/psoriatic arthritis (PsA). There was also a significant difference in the proportion female, ranging from 69% for infliximab to 81% for golimumab. Those starting etanercept had accrued lower healthcare costs.

Expected differences in safety and effectiveness due to confounding by indication

Modelling using observed outcomes of patients starting bDMARDs in 2006–2010 indicated that several of the factors associated with choice of therapy were also significant predictors of safety and treatment outcomes (associations in online supplementary table s6). Age and sex were strong predictors of all outcomes except remaining on drug less than 1 year. Components of baseline disease activity were predictive of all outcomes, although with varying magnitude (HAQ was associated with risk of MACE; DAS28 and its components with achieving good EULAR response). Concomitant therapy at baseline was also a predictor of most outcomes, for example,glucocorticoids at baseline were predictive of adverse events and decreased proportion with good EULAR response. Medical history also predicted treatment outcomes, for example, a history of infection or cardiovascular disease predicted future infections and cardiovascular disease, while history of malignancy significantly predicted drug retention and (weakly) new onset of malignancy.

Taken together, the observed baseline differences led to substantial differences in the predicted risk of all-cause mortality, MACE and serious infections; smaller differences in risk for malignancy and for achieving EULAR good response; and virtually no expected differences in 1-year drug survival (table 3). In summary, a crude comparison of the non-TNFi drugs with the TNFi group would be particularly biased against rituximab and abatacept regarding both safety and EULAR response. The predicted bias was much less at switch from first TNFi, reflecting the greater similarity in patient groups.

Age and sex standardisation greatly reduced predicted bias, in particular for safety outcomes (table 3). The expected risks were still inflated for all safety outcomes except malignancies, however, and this standardisation did not reduce the biased difference in EULAR response, reflecting that age was not a strong predictor of that outcome, and that the differences in sex were minor between drugs.

DISCUSSION

In this large, nationwide study of contemporary Swedish patients with RA, we found evidence of substantial differences in baseline characteristics among patients assigned to different bDMARDs. Many predictors of treatment assignment were also predictors of adverse treatment outcomes, and in quantifying the magnitude of this, we showed that a direct comparison across therapies would not give accurate estimates of the treatments' relative effect, but would be biased in favour of TNFi.

Those not starting 'standard' TNFi therapy were older, had lower socioeconomic status and had a higher burden of other diseases. There was similar, although slighter, channelling at switch from a first ever TNFi, where a higher RA disease activity was also predictive of receiving a non-TNFi. While there were limited differences between those starting individual TNFi, channelling to and between non-TNFi bDMARD was substantial. Rituximab initiators were oldest, dominantly RF-positive and had the highest burden of other diseases (in particular malignancy), while those starting tocilizumab differed less from those starting TNFi in terms of medical history, but had significantly higher disease activity.

These differences are partly expected based on the tentative recommendations in favour of specific drug choice for some risk groups, where for example, American College of Rheumatology (ACR) guidelines have listed 'very low' evidence to support preference of rituximab over TNFi among patients with a history of malignancy, and of abatacept over TNFi among patients with serious infections, and 'moderate to very low' evidence to prefer non-TNFi among patients with congestive heart disease.[1 2] It seems clear that these tentative recommendations have been followed for some, but not most, patients.

By modelling the expected risk for several treatment outcomes conferred by observed patient characteristics, we

Table 3 Potential for confounding by indication; predicted percentage with adverse events within 5 years, and treatment outcome after 1 year, based on observed baseline characteristics

Cohort	All-cause mortality		Malignancy		MACE		Serious infection		Drug survival <1 year		Good EULAR response at 1 year	
First bDMARD	Crude	STD	Crude	STD	Crude	STD	Crude	STD	Crude	STD	Crude	STD
TNFi	4.8	–	5.6	–	5.4	–	14.4	–	30.3	–	31.0	–
Rituximab	13.3	7.0	8.8	6.1	10.0	6.1	24.2	17.7	29.4	28.9	25.3	23.2
Abatacept	11.9	8.1	7.0	5.8	9.1	6.9	21.3	18.1	31.2	31.1	27.9	29.2
Tocilizumab	8.8	7.1	6.1	5.4	7.1	6.1	17.9	15.9	30.7	30.9	30.3	31.6
Switch from TNFi												
TNFi	5.3	5.3	4.8	4.7	6.1	6.1	16.9	16.7	36.2	36.1	17.6	17.6
Rituximab	8.1	6.3	5.7	4.9	7.6	6.3	21.2	19.0	35.1	34.8	18.2	19.1
Abatacept	7.3	6.8	5.3	4.8	7.0	6.9	19.5	18.2	37.9	37.8	18.0	18.3
Tocilizumab	6.8	6.4	5.1	5.0	6.8	6.4	18.1	17.6	37.1	37.7	18.3	18.2

Predicted observed percentage (crude) and age-sex standardised to TNFi as first bDMARD (STD).
bDMARD, biological disease-modifying anti-rheumatic drug; EULAR, European League Against Rheumatism; MACE, major acute cardiovascular event; TNFi, tumour necrosis factor inhibitor.

showed that even if there were no true differences in drug effect, confounding by indication will make the non-TNFi drugs appear less safe and effective than the TNFi as first bDMARD. For many of the perceived differences, a simple adjustment for age and sex reduced this confounding dramatically. Residual confounding is, however, expected to give higher rates of adverse events and less treatment response, such that comparisons should be adjusted for medical history and disease activity when possible. As expected, the predicted bias was less when studying those switching from an initial TNFi, reflecting the reduced patient heterogeneity in this specific clinical situation.

We believe that the predictive modelling approach is helpful in combining the multitude of observed baseline differences in a metric comparable across cohorts, but several limitations should be noted. The models were based on historical data, and will be incorrect if the strength of association with each risk factor has changed over calendar time. The models were also limited by the covariates we had available, and we lacked data on for instance body mass index and smoking. Unless some unknown predictors work in the opposite direction, it is likely that we underestimate the predicted bias. The prediction models were intended as a convenient way of illustrating the risk of confounding by indication, not as the best possible prediction model for these outcomes. For this reason, we used a simple model building strategy, and did not perform cross-validation to assess the models' general predictive value or construct confidence bounds on the predictions. In other limitations, it should be noted that we made a large number of statistical comparisons and present adjusted differences between groups with standard CIs; several significant differences are likely to reflect false positives. Finally, these data are by their nature relevant to the Swedish clinical setting, where the physician is free to prescribe any drug of their choosing and the state (single payer) has made recommendations (but not restrictions) based on therapy cost. The relative costs of therapy and payer restrictions may vary by country. Therefore, although the pattern of use (preferentially TNFi as first biologic) is commonplace and the Swedish national guidelines are similar to the EULAR and ACR guidelines, the generalisability to other countries may vary.

This study has several strengths. Through the Swedish nationwide registers we were able to describe patient medical history and other characteristics using prospectively collected data, avoiding the risk for recall bias, and with a completeness that would otherwise have been difficult. We could also include the entire Swedish population, avoiding the risk of selection bias. Our main limitation is the lack of data on the physician's and patient's reasoning about the choice of treatment, which may among other factors have been influenced by the route and frequency of administration.

In conclusion, we found significant channelling of older and less healthy patients with RA to non-TNFi bDMARDs, both as first bDMARD and at switch from a first TNFi. Future studies should examine whether this channelling is medically justified or, paradoxically, act to reduce the overall effectiveness and safety of bDMARD therapy. We also demonstrated the extent to which this channelling will compromise the safety and effectiveness profile of individual bDMARDs. Unless differences in age, medical history, and RA disease activity are taken into account in studies of the relative safety and effectiveness of bDMARDs, most results will be severely confounded.

Acknowledgements The ARTIS Study Group conducts scientific analyses using data from the Swedish Biologics Register ARTIS, run by the Swedish Society for Rheumatology. The following were members of the ARTIS Study Group during the completion of this study: JA, Sofia Ernestam, Lars Klareskog, Ralph Nisell and Ronald van Vollenhoven (Karolinska Institutet, Stockholm, Sweden), Nils Feltelius (Medical Products Agency, Uppsala, Sweden), EB (Uppsala University, Uppsala, Sweden), Alf Kastbom (Linköping University, Linköping, Sweden), Lennart Jacobsson (Sahlgrenska Academy, Göteborg, Sweden), Carl Turesson and Elisabet Lindqvist (Lund University, Malmö/Lund, Sweden), HF-D'E and Solbritt Rantapää-Dahlqvist (Umeå University, Umeå, Sweden).

Contributors TF had full access to all data and takes responsibility for the integrity of the data and the accuracy of the data analysis. TF performed all analyses and drafted the first version of the manuscript. All authors were involved in drafting the study protocol before analyses, and reviewed the final manuscript for important intellectual contents.

Funding This study was supported by the Swedish Foundation for Strategic Research, Swedish Research Council, Swedish Cancer Society, Swedish Heart Lung Foundation, ALF funding collaboration between Karolinska Institute and Stockholm County, and the IMI funded EU project Be The Cure.

Competing interests ARTIS has entered into agreements with Abbvie, BMS, MSD, Lilly, Pfizer, Roche, Samsung Bioepis and UCB. Companies with products mentioned in this manuscript were given a courtesy review of the manuscript before publication, but were not involved in planning the study, performing the analysis or interpreting the results.

Ethics approval The Regional Ethical Review Board in Stockholm granted ethical approval (DNR: 2016/1986-32).

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Access to the national register data used for this study is granted on a restrictive basis, and may not be shared without additional specific permissions from the Swedish register-holding authorities.

REFERENCES

1 Singh JA, Furst DE, Bharat A, *et al*. 2012 Update of the 2008 American college of rheumatology recommendations for the use of disease-modifying antirheumatic drugs and biologic agents in the treatment of rheumatoid arthritis. *Arthritis Care Res* 2012;64:625–39.
2 Singh JA, Saag KG, Bridges SL, *et al*. 2015 American college of rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Rheumatol* 20162016;68:1–26.
3 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.
4 Smolen JS, Landewé R, Breedveld FC, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs. *Ann Rheum Dis* 2010;69:964–75.
5 Neovius M, Arkema EV, Olsson H, *et al*. Drug survival on TNF inhibitors in patients with rheumatoid arthritis comparison of adalimumab, etanercept and infliximab. *Ann Rheum Dis* 2015;74:354–60.
6 Wolfe F, Flowers N, Burke TA, *et al*. Increase in lifetime adverse drug reactions, service utilization, and disease severity among patients who will start COX-2 specific inhibitors: quantitative assessment of channeling bias and confounding by indication in 6689 patients with rheumatoid arthritis and osteoarthritis. *J Rheumatol* 2002;29:1015–22.
7 Petri H, Urquhart J. Channeling bias in the interpretation of drug effects. *Stat Med* 1991;10:577–81.
8 Putrik P, Ramiro S, Chorus AM, *et al*. Socioeconomic inequities in perceived health among patients with musculoskeletal disorders compared with other chronic disorders: results from a cross-sectional Dutch study. *RMD Open* 2015;1:e000045.
9 Kyriacou DN, Lewis RJ. Confounding by indication in clinical research. *JAMA* 2016;316:1818–9.
10 Askling J, Fored CM, Geborek P, *et al*. Swedish registers to examine drug safety and clinical issues in RA. *Ann Rheum Dis* 2006;65:707–12.
11 Frisell T, Holmqvist M, Källberg H, *et al*. Familial risks and heritability of rheumatoid arthritis: role of rheumatoid factor/anti-citrullinated protein antibody status, number

and type of affected relatives, sex, and age. *Arthritis Rheum* 2013;65:2773–82.
12 Eriksson JK, Askling J, Arkema EV. The Swedish rheumatology quality register: optimisation of rheumatic disease assessments using register-enriched data. *Clin Exp Rheumatol* 2014;32:S147–149.
13 Wadström H, Eriksson JK, Neovius M, *et al*. How good is the coverage and how accurate are exposure data in the Swedish biologics register (ARTIS)? *Scand J Rheumatol* 2015;44:22–8.
14 Socialstyrelsen. *Inpatient diseases in Sweden 1987-2008 [in Swedish]*. Stockholm: Socialstyrelsen, 2008.
15 Ludvigsson JF, Andersson E, Ekbom A, *et al*. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011;11:450.
16 Wettermark B, Hammar N, Fored CM, *et al*. The new Swedish prescribed drug register-opportunities for pharmacoepidemiological research and experience from the first six months. *Pharmacoepidemiol Drug Saf* 2007;16:726–35.
17 Barlow L, Westergren K, Holmberg L, *et al*. The completeness of the Swedish cancer register: a sample survey for year 1998. *Acta Oncol* 2009;48:27–33.
18 Schneeweiss S, Maclure M. Use of comorbidity scores for control of confounding in studies using administrative databases. *Int J Epidemiol* 2000;29:891–8.
19 Canty A, Riple BD. *boot: bootstrap R (S-Plus) functions*. R package version 1.3-20 edn: McMaster University Hamilton, Ontario, Canada, 2017.
20 Davison AC, Hinkley DV. *Bootstrap methods and their applications*. Cambridge: Cambridge University Press, 1997.

EXTENDED REPORT

Efficacy and safety of monotherapy with sirukumab compared with adalimumab monotherapy in biologic-naïve patients with active rheumatoid arthritis (SIRROUND-H): a randomised, double-blind, parallel-group, multinational, 52-week, phase 3 study

Peter C Taylor,[1] Michael H Schiff,[2] Qingmin Wang,[3] Yusang Jiang,[3] Yanli Zhuang,[3] Regina Kurrasch,[4] Shruti Daga,[5] Ravi Rao,[6] Paul P Tak,[6] Benjamin Hsu[3]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212496).

[1]NDORMS, Botnar Research Centre, University of Oxford, Oxford, UK
[2]School of Medicine, University of Colorado, Greenwood Village, Colorado, USA
[3]Janssen Research & Development, Spring House, Pennsylvania, USA
[4]GlaxoSmithKline, Collegeville, Pennsylvania, USA
[5]GlaxoSmithKline, Uxbridge, UK
[6]GlaxoSmithKline, Stevenage, UK

Correspondence to
Dr Benjamin Hsu, Janssen Research & Development, Spring House, PA 19477, USA; BHsu@its.jnj.com

PPT and BH contributed equally.

Received 4 October 2017
Revised 8 January 2018
Accepted 9 January 2018
Published Online First 26 February 2018

To cite: Taylor PC, Schiff MH, Wang Q, *et al*. *Ann Rheum Dis* 2018;**77**:658–666.

ABSTRACT

Objective This randomised, double-blind, parallel-group, phase 3 study compared monotherapy with sirukumab, an anti–interleukin-6 cytokine monoclonal antibody, with adalimumab monotherapy in patients with rheumatoid arthritis (RA).

Methods Biologic-naïve patients with active RA who were inadequate responders or were intolerant to, or inappropriate for, methotrexate were randomised to subcutaneous sirukumab 100 mg every 2 weeks (n=187), sirukumab 50 mg every 4 weeks (n=186) or adalimumab 40 mg every 2 weeks (n=186). Primary endpoints at week 24 were change from baseline in Disease Activity Score in 28 joints (DAS28) using erythrocyte sedimentation rate (ESR) and proportion of patients achieving an American College of Rheumatology (ACR) 50 response; these endpoints were tested in sequential order. This study is registered at EudraCT (number: 2013-001417-32) and ClinicalTrials.gov (number: NCT02019472).

Results Significantly greater improvements from baseline in mean (SD) DAS28 (ESR) were observed at week 24 with sirukumab 100 mg every 2 weeks (−2.96 (1.580)) versus adalimumab 40 mg every 2 weeks (−2.19 (1.437); P<0.001). Sirukumab 50 mg every 4 weeks also showed significantly greater improvement from baseline at week 24 in DAS28 (ESR) (−2.58 (1.524)) compared with adalimumab (P=0.013). The ACR50 response rates with the 100 mg (35.3%) and 50 mg (26.9%) doses of sirukumab were comparable to that with adalimumab (31.7%) at week 24. The safety profile of sirukumab was consistent with that observed with anti–interleukin-6 receptor antibodies. A dose-related effect on the incidence of injection-site reactions was observed with sirukumab.

Conclusion Sirukumab monotherapy showed greater improvements in DAS28 (ESR), but similar ACR50 response rates, versus adalimumab monotherapy.

INTRODUCTION

Currently, in the treatment of established rheumatoid arthritis (RA), a combination of biological disease-modifying antirheumatic drugs (bDMARD) with methotrexate (MTX) is superior to bDMARD monotherapy.[1 2] However, a number of patients discontinue MTX, most commonly due to side effects.[3] For example, in a study of 157 patients with RA who were currently or had previously used MTX, 29.3% discontinued MTX therapy, most often due to gastrointestinal or hepatic side effects.[3] For patients who cannot use conventional synthetic DMARDs (csDMARD), monotherapy with interleukin (IL)-6 pathway or Janus kinase (JAK) inhibitors may have advantages compared with monotherapy with other bDMARDs.[2 4]

Elevated IL-6 levels are present in synovial tissue of patients with RA and correlate with disease activity.[5–7] Sirukumab is a fully human monoclonal antibody that binds to IL-6 with high affinity and specificity, preventing IL-6 from binding to membrane and soluble forms of the IL-6 receptor (IL-6R).[8] The two dose regimens chosen for the phase 3 pivotal studies, sirukumab 50 mg every 4 weeks and 100 mg every 2 weeks, significantly improved signs and symptoms of disease among patients with active RA refractory to csDMARDs and refractory to ≥1 anti–tumour necrosis factor (TNF) drug or intolerant to ≥2 anti-TNF drugs.[9 10] The majority of patients in these trials received sirukumab in combination with csDMARDs.[9 10]

The objective of this phase 3 study (SIRROUND-H; EudraCT number, 2013-001417-32; ClinicalTrials.gov number, NCT02019472), the primary results of which are presented here, was to demonstrate superior efficacy of sirukumab monotherapy compared with adalimumab monotherapy (the most commonly used bDMARD for the treatment of RA[11]) over 52 weeks in patients with active RA who had an inadequate response to MTX or were intolerant to or inappropriate for MTX.

METHODS

This phase 3, randomised, double-blind, parallel-group, active comparator study evaluated the superiority (in terms of efficacy) of subcutaneous sirukumab monotherapy compared with adalimumab monotherapy, along with safety, physical function, pharmacokinetic properties and immunogenicity, in biologic-naïve patients with active RA (online supplementary figure 1).

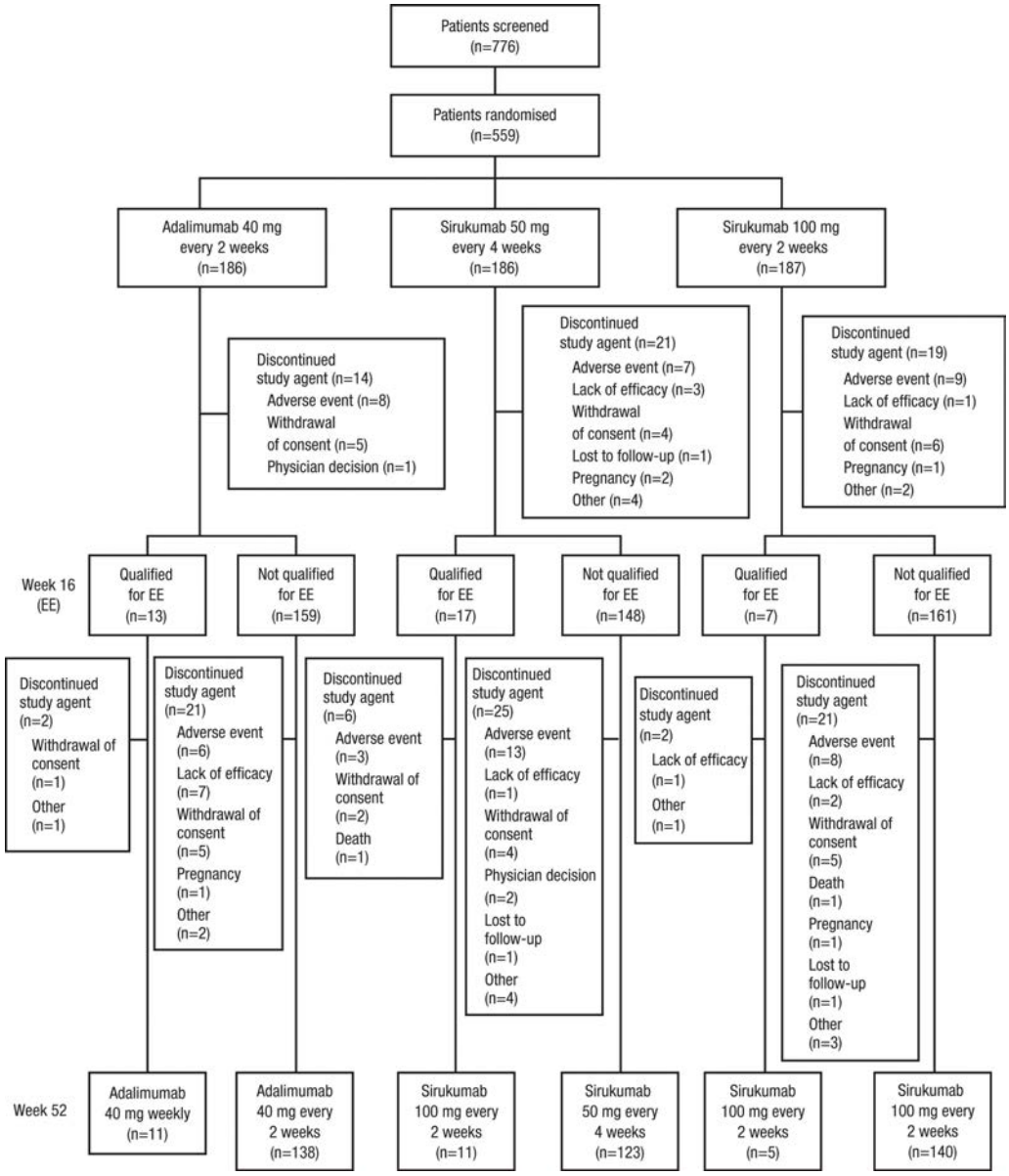


Figure 1 Patient distribution and disposition through week 52. EE, early escape.

Patients/study population

Eligible patients were ≥18 years of age with active RA (≥8 of 68 tender joints and ≥6 of 66 swollen joints at screening/baseline, and C-reactive protein (CRP) levels of ≥10 mg/L or erythrocyte sedimentation rate (ESR) of ≥28 mm/hour at screening) and were considered inadequate responders to MTX (after ≥12 weeks of MTX (dose of ≥15 mg/week)) or intolerant to or inappropriate for treatment with MTX for safety reasons (including MTX-naïve patients).

Study design

This 68-week study included a 52-week treatment period and a 16-week safety follow-up period.

Patients were randomised at 102 centres in the USA, Europe, Latin America and South Africa from April 2014 to May 2015. Eligible patients were randomised 1:1:1 to subcutaneous sirukumab 100 mg every 2 weeks, subcutaneous sirukumab 50 mg every 4 weeks or subcutaneous adalimumab 40 mg every 2 weeks. Details of randomisation and masking are provided in the online supplementary methods and results. Patients with <20% improvement from baseline in both swollen joint counts (SJC)/tender joint counts (TJC) at week 16 qualified for early escape (EE): patients receiving adalimumab 40 mg every 2 weeks changed to weekly dosing; patients receiving sirukumab 50 mg every 4 weeks changed to 100 mg every 2 weeks; and patients on sirukumab 100 mg every 2 weeks remained on their randomised dose. Patients receiving sirukumab who met EE criteria received weekly placebo injections in between to maintain blinding.

Study evaluations

All analyses were prespecified in the statistical analysis plan. The two primary efficacy endpoints were change from baseline in Disease Activity Score in 28 joints (DAS28) using ESR at week 24 and proportion of patients with an American College of Rheumatology (ACR) 50 response at week 24 (see details of hierarchical statistical testing in the Statistical Methods section). Major secondary efficacy endpoints included the proportion of patients with DAS28 (ESR) remission and the proportion with an ACR20 response at week 24. Additional efficacy endpoints included ACR70 response, Clinical Disease

Table 1 Baseline and demographic characteristics

		Sirukumab	
	Adalimumab 40 mg every 2 weeks (n=186)	50 mg every 4 weeks (n=186)	100 mg every 2 weeks (n=187)
Sex, n (%)			
Female	156 (83.9)	157 (84.4)	154 (82.4)
Male	30 (16.1)	29 (15.6)	33 (17.6)
Age (years)			
Mean (SD)	52.6 (12.15)	52.5 (12.46)	49.8 (12.31)
Median (IQR)	54.5 (46–60)	54.5 (46–60)	49.9 (40–59)
Race, n (%)			
White	173 (93.0)	166 (89.2)	174 (93.0)
Asian	1 (0.5)	2 (1.1)	5 (2.7)
Black or African American	2 (1.1)	5 (2.7)	3 (1.6)
Unknown	1 (0.5)	1 (0.5)	1 (0.5)
Other	9 (4.8)	12 (6.5)	4 (2.1)
Region, n (%)			
Europe	138 (74.2)	132 (71.0)	142 (75.9)
North America	25 (13.4)	32 (17.2)	31 (16.6)
Latin America	15 (8.1)	13 (7.0)	7 (3.7)
South Africa	8 (4.3)	9 (4.8)	7 (3.7)
BMI (kg/m^2), mean (SD)	27.86 (5.63)	27.77 (5.99)	27.60 (6.53)
Duration of RA, median (IQR)	4.00 (1.4–8.4)	4.24 (1.6–9.5)	4.60 (2.1–9.0)
Number of swollen joints, mean (SD)			
0–66	18.5 (10.06)	19.8 (11.91)	20.0 (11.93)
0–28	12.7 (5.65)	13.3 (6.47)	13.5 (6.01)
Number of tender joints, mean (SD)			
0–68	30.8 (14.36)	32.4 (15.83)	32.6 (14.93)
0–28	17.8 (6.37)	17.8 (7.22)	18.3 (6.57)
Patient's assessment of pain (VAS; 0–10)			
n	186	185	185
Mean (SD)	6.78 (1.96)	6.82 (1.89)	6.55 (2.09)
Patient's global assessment of disease activity (VAS; 0–10)			
n	186	185	185
Mean (SD)	6.85 (2.04)	6.80 (1.94)	6.70 (2.05)
Physician's global assessment of disease activity (VAS; 0–10), mean (SD)	6.79 (1.55)	6.78 (1.51)	6.83 (1.59)
HAQ-DI score, range: 0–3			
n	186	185	185
Mean (SD)	1.70 (0.63)	1.75 (0.55)	1.62 (0.61)
CRP (mg/dL), mean (SD)	2.07 (3.06)	2.11 (2.60)	1.79 (2.26)
ESR (mm/hour), mean (SD)	48.6 (23.17)	49.5 (23.50)	46.8 (21.91)
DAS28 (ESR)			
n	186	185	185
Mean (SD)	6.89 (0.85)	6.90 (0.88)	6.91 (0.86)
DAS28 (CRP)			
n	185	185	185
Mean (SD)	6.05 (0.96)	6.12 (0.96)	6.08 (0.97)
CDAI			
n	186	185	185
Mean (SD)	44.09 (12.17)	44.62 (13.39)	45.39 (12.84)
Anti-CCP positive, n (%)	142 (76.8)	138 (74.6)	141 (76.2)
RF positive, n (%)	130 (70.3)	140 (75.3)	134 (71.7)
SF-36			
PCS, mean (SD)	31.60 (6.92)	31.76 (5.97)	32.48 (6.77)
MCS, mean (SD)	41.08 (10.79)	40.86 (10.68)	40.93 (10.34)
FACIT-Fatigue, mean (SD)	26.8 (10.65)	25.7 (10.15)	25.0 (10.25)

BMI, body mass index; CCP, cyclic citrullinated peptide; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; DAS28 (CRP), Disease Activity Score in 28 joints using C-reactive protein; ESR, erythrocyte sedimentation rate; FACIT-Fatigue, Functional Assessment of Chronic Illness Therapy–Fatigue; HAQ-DI, Health Assessment Questionnaire–Disability Index; MCS, mental component summary score; PCS, physical component summary score; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, standard deviation; SF-36, 36-item Short Form Health Survey; VAS, visual analogue scale.

Activity Index (CDAI), the Health Assessment Questionnaire–Disability Index (HAQ-DI), the 36-item Short Form Health Survey (SF-36) and the Functional Assessment of Chronic Illness Therapy (FACIT)–Fatigue questionnaire (online supplementary methods and results). Efficacy endpoints were assessed through week 52. Safety was monitored throughout the 68-week study and included evaluations of treatment-emergent adverse events (TEAE) and clinical laboratory tests. Serum sirukumab or adalimumab concentrations and immunogenicity to sirukumab or adalimumab were assessed (online supplementary methods and results).

Statistical methods

Based on the results of a phase 4, active-controlled study of tocilizumab monotherapy (the ADACTA study[12]) and assuming a treatment difference of 0.6–0.8 for the change from baseline in DAS28 at week 24 (SD of 1.6–1.8) and an ACR50 response rate at week 24 of 45%–50% with sirukumab versus 30% with adalimumab, a sample size of 170 patients per treatment arm was needed to achieve a power of ≥81% for the primary endpoints to detect a treatment difference between sirukumab and adalimumab using an α of 0.05 (two sided). The primary hypotheses to be tested in this study, in sequential order, were: (1) sirukumab 100 mg every 2 weeks demonstrates superior efficacy versus adalimumab 40 mg every 2 weeks in change from baseline in DAS28 (ESR) at week 24, and (2) sirukumab 100 mg every 2 weeks demonstrates superior efficacy versus adalimumab 40 mg every 2 weeks in the proportion of patients with an ACR50 response at week 24. The change from baseline in DAS28 (ESR) was tested using an analysis of covariance model, controlling for treatment group, reason for MTX failure and baseline value; missing values were imputed using baseline observation carried forward methodology. ACR50 response was tested using a Cochran-Mantel-Haenszel test stratified by reason for MTX failure; missing values, EE or treatment failures (see online supplementary methods and results for definition) were imputed as non-responders. As prespecified, if the first (DAS28 (ESR) endpoint) comparison of sirukumab 100 mg every 2 weeks to adalimumab 40 mg every 2 weeks was statistically significant at a two-sided α level of 0.05, the study was considered positive. Differences in the change from baseline in DAS28 (ESR) at week 24 and proportion of patients with an ACR50 response at week 24 were evaluated between the sirukumab 50 mg every 4 weeks group and the adalimumab 40 mg every 2 weeks group as major secondary analyses using methodology similar to that used for sirukumab 100 mg every 2 weeks. Online supplementary figure 2 outlines the testing procedures for primary and secondary hypotheses and how they differed for global and USA-specific regulatory requirements.

RESULTS

Study population

Of 776 patients screened, 559 were randomised (figure 1). Demographic and baseline disease characteristics were well balanced across treatment groups (table 1). Among randomised patients, 57.1% (n=319) and 42.9% (n=240) failed MTX for efficacy and safety/tolerability reasons, respectively (online supplementary table 1). Overall, 97.9% (547/559) of patients had prior MTX use (online supplementary table 1). Treatment compliance was >95% and >93% across all groups through weeks 24 and 52, respectively. Through week 52, 131 patients discontinued study drug, most often due to adverse events (AE) (figure 1; online supplementary table 2).

Table 2 Primary, major secondary and other endpoints at week 24

	Adalimumab 40 mg every 2 weeks	Sirukumab 50 mg every 4 weeks	Sirukumab 100 mg every 2 weeks
Change from baseline in DAS28 (ESR) at week 24			
All evaluable patients			
n	186	185	185
Mean (SD)	−2.19 (1.437)	−2.58 (1.524)	−2.96 (1.580)
P value*		0.013	<0.001
Proportion of patients achieving ACR50 at week 24			
All evaluable patients			
n	186	186	187
Patients in response, n (%)	59 (31.7)	50 (26.9)	66 (35.3)
P value*		0.306	0.464
Proportion of patients achieving DAS28 (ESR) remission at week 24			
All evaluable patients			
n	186	186	187
Patients in remission, n (%)	14 (7.5)	24 (12.9)	38 (20.3)
Proportion of patients achieving ACR20 at week 24			
All evaluable patients			
n	186	186	187
Patients in response, n (%)	105 (56.5)	100 (53.8)	110 (58.8)
Proportion of patients achieving ACR70 at week 24			
All evaluable patients			
n	186	186	187
Patients in response, n (%)	24 (12.9)	22 (11.8)	29 (15.5)
Proportion of patients achieving SDAI-based ACR/EULAR remission at week 24			
All evaluable patients			
n	186	186	187
Patients in response, n (%)	12 (6.5)	14 (7.5)	15 (8.0)
Proportion of patients achieving Boolean-based ACR/EULAR remission at week 24			
All evaluable patients			
n	186	186	187
Patients in response, n (%)	7 (3.8)	7 (3.8)	7 (3.7)
SJC (0–66) at week 24			
All evaluable patients			
n	174	172	176
Mean (SD) % change from baseline	−69.2 (35.45)	−62.0 (39.40)	−68.9 (54.95)
TJC (0–68) at week 24			
All evaluable patients			
n	174	172	176
Mean (SD) % change from baseline	−61.4 (36.02)	−54.0 (35.87)	−59.0 (38.01)
Patient's global assessment of disease activity (VAS; 0–10) at week 24			
All evaluable patients			
n	174	172	176
Mean (SD) % change from baseline	−36.06 (44.17)	−32.08 (60.57)	−36.05 (54.06)
ESR (mm/hour) at week 24			
All evaluable patients			
n	175	173	176
Mean (SD) change from baseline	−13.7 (26.86)	−34.1 (28.56)	−34.7 (22.65)

Data presented are based on imputed values.
*P value compared with adalimumab 40 mg every 2 weeks.
ACR, American College of Rheumatology; DAS28 (ESR), Disease Activity Score in 28 joints using erythrocyte sedimentation rate; ESR, erythrocyte sedimentation rate; EULAR, European League Against Rheumatism; SD, standard deviation; SDAI, Simplified Disease Activity Index; SJC, swollen joint counts; TJC, tender joint counts; VAS, visual analogue scale.

Efficacy

For the first primary endpoint, the improvement from baseline in DAS28 (ESR) was significantly greater at week 24 for sirukumab 100 mg compared with adalimumab (P<0.001; table 2). For the second primary endpoint, the difference in ACR50 response rate at week 24 between patients receiving sirukumab 100 mg and those receiving adalimumab was not statistically significant (P=0.464; table 2). Following the prespecified testing procedure, the change from baseline in DAS28 (ESR) at week 24 was significantly greater for sirukumab 50 mg compared with adalimumab (P=0.013; table 2). The difference in ACR50 response rate at week 24 between

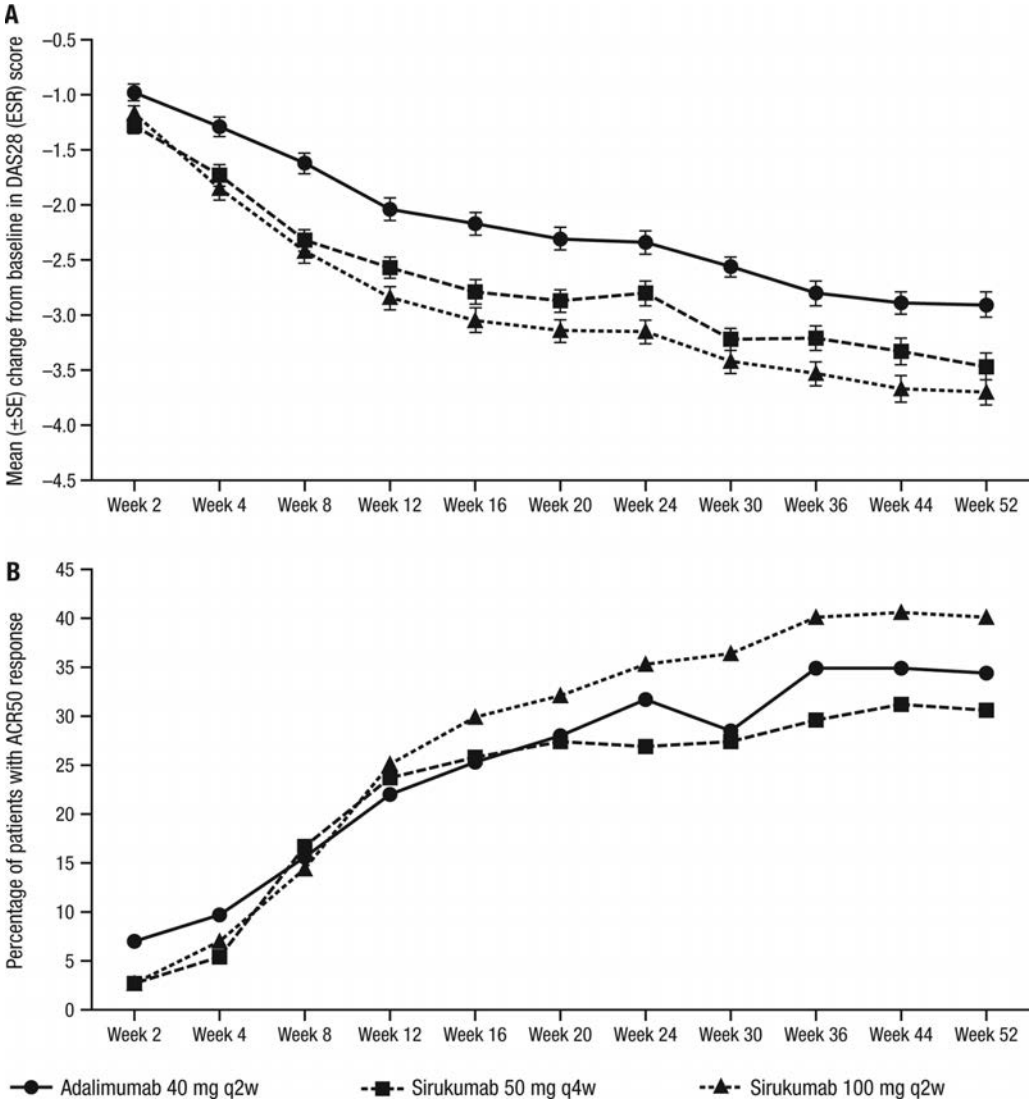


Figure 2 Primary endpoints: (A) Change from baseline in DAS28 (ESR)[a] and (B) proportion of patients achieving an ACR50 response by visit through week 52.[b,c] [a]Observed values; patients with missing baseline values were excluded from analysis. [b]Imputed values. [c]Data for A and B are included in online supplementary tables 9 and 10, respectively. ACR, American College of Rheumatology; DAS28 (ESR), Disease Activity Score in 28 joints, using erythrocyte sedimentation rate; q2w, every 2 weeks; q4w, every 4 weeks.

patients receiving sirukumab 50 mg and those receiving adalimumab was not statistically significant (P=0.306; table 2). Based on the testing hierarchy, no further hypothesis testing was performed. Results from sensitivity analyses to explore the impact of handling missing data on the primary endpoints were similar to the primary analysis (data not shown). For both doses of sirukumab and adalimumab, decreases (improvements) from baseline in DAS28 (ESR) were observed from as early as week 2 through week 52 (figure 2A). Across all three treatment groups, a clinically relevant proportion of patients achieved an ACR50 response through week 52 (figure 2B). At week 52, improvements from baseline in DAS28 (ESR) and the proportion of patients achieving ACR50 response were comparable to those at week 24 (online supplementary table 3). Improvements from baseline at week 24 in individual ACR components were similar for adalimumab and sirukumab 100 mg, and slightly lower for sirukumab 50 mg for some parameters (online supplementary table 4).

Major secondary endpoints and other efficacy endpoint analyses showed similar clinically meaningful improvements for both sirukumab groups and the adalimumab group and are summarised in table 2 for week 24 and online supplementary table 3 for week 52. Changes from baseline in the SJC, TJC, patient's global assessment of disease activity and ESR at week 24 are also summarised in table 2. DAS28 (ESR) remission rates were numerically higher across all treatment groups at week 52 compared with week 24; a numerically higher remission rate was observed in the sirukumab groups compared with the adalimumab group at weeks 24 and 52. ACR20 response rates at week 24 were similar across groups and remained generally comparable at week 52, while ACR70 response rates increased slightly from week 24 to week 52 across all groups. For the primary efficacy endpoints and major secondary endpoints, there was a trend for numerically greater improvements in patients randomised to sirukumab who had failed MTX for safety reasons compared with those who failed for efficacy reasons; however, this finding was not consistent for both doses of sirukumab across multiple endpoints and timepoints (online supplementary table 5).

Similar decreases (improvements) in CDAI (a disease activity index that includes clinical parameters and no acute phase reactants) and HAQ-DI scores from baseline were observed with

Table 3 Overall summary of safety through week 68

Adverse event* outcome, n (%)	Adalimumab 40 mg every 2 weeks (n=186)	Sirukumab 50 mg every 4 weeks (n=186)	Sirukumab 100 mg every 2 weeks (n=187)
Patients with ≥1 TEAE, n (%)	130 (69.9)	139 (74.7)	134 (71.7)
TEAEs (≥5% of patients in any sirukumab group)			
Injection-site erythema	13 (7.0)	17 (9.1)	33 (17.6)
Increased ALT	12 (6.5)	21 (11.3)	24 (12.8)
Rheumatoid arthritis	18 (9.7)	20 (10.8)	16 (8.6)
Increased AST	11 (5.9)	13 (7.0)	20 (10.7)
Neutropenia	4 (2.2)	17 (9.1)	11 (5.9)
Headache	11 (5.9)	11 (5.9)	13 (7.0)
Injection-site pruritus	8 (4.3)	6 (3.2)	17 (9.1)
Hypertension	10 (5.4)	12 (6.5)	8 (4.3)
Nasopharyngitis	16 (8.6)	10 (5.4)	9 (4.8)
Upper respiratory tract infection	10 (5.4)	10 (5.4)	9 (4.8)
Bronchitis	4 (2.2)	10 (5.4)	8 (4.3)
Injection-site swelling	4 (2.2)	4 (2.2)	11 (5.9)
Patients with ≥1 serious TEAE, n (%)	16 (8.6)	29 (15.6)	22 (11.8)
Patients with ≥1 TEAE that caused study agent discontinuation, n (%)	15 (8.1)	25 (13.4)	20 (10.7)
Patients with ≥1 infection, n (%)	58 (31.2)	63 (33.9)	59 (31.6)
Infections reported in ≥2% of patients in any group			
Nasopharyngitis	14 (7.5)	9 (4.8)	9 (4.8)
Upper respiratory tract infection	10 (5.4)	9 (4.8)	9 (4.8)
Bronchitis	4 (2.2)	9 (4.8)	8 (4.3)
Pharyngitis	3 (1.6)	3 (1.6)	6 (3.2)
Urinary tract infection	6 (3.2)	6 (3.2)	3 (1.6)
Influenza	2 (1.1)	5 (2.7)	2 (1.1)
Sinusitis	2 (1.1)	4 (2.2)	3 (1.6)
Pneumonia	1 (0.5)	4 (2.2)	2 (1.1)
Cellulitis	0	4 (2.2)	1 (0.5)
Cystitis	1 (0.5)	1 (0.5)	4 (2.1)
Respiratory tract infection, viral	2 (1.1)	1 (0.5)	4 (2.1)
Oral herpes	4 (2.2)	1 (0.5)	3 (1.6)
Respiratory tract infection	3 (1.6)	0	4 (2.1)
Patients with ≥1 serious infection, n (%)	4 (2.2)	14 (7.5)	5 (2.7)
Patients with ≥1 injection-site reaction†, n (%)	16 (8.6)	20 (10.8)	43 (23.0)
Patients with ≥1 MACE‡, n (%)	0	1 (0.5)	2 (1.1)
Patients with ≥1 hypersensitivity/serum sickness AE, n (%)	2 (1.1)	2 (1.1)	4 (2.1)
Patients with ≥1 malignancy, n (%)	1 (0.5)	3 (1.6)	2 (1.1)
Patients with ≥1 GI perforation, n (%)	0	1 (0.5)	1 (0.5)
Patients who died on study§, n (%)	0	3 (1.6)	1 (0.5)

*AEs were reported for the group to which the patient was initially randomised.
†All patients were observed by a blinded staff member for symptoms of injection-site reactions for ≥30 minutes after study drug administration through week 16; injection-site reactions included erythema, pain, pruritus and/or swelling.
‡The three MACEs that occurred in this study were all adjudicated as strokes (one in the sirukumab 50 mg every 4 weeks group and two in the sirukumab 100 mg every 2 weeks group).
§There were four deaths reported in the study through week 68 (three in the sirukumab 50 mg every 4 weeks group and one in the sirukumab 100 mg every 2 weeks group), all of which occurred after week 24. In the sirukumab 50 mg every 4 weeks group, one patient experienced an SAE of respiratory failure of severe intensity and subsequently died due to pneumonia (events considered not related to study agent); one patient had an SAE of metastatic adenocarcinoma with involvement of the brain, lungs, skeletal system and thoracolumbar lymph nodes and died as a result (considered not related to study agent); and one patient who had EE to 100 mg experienced an SAE 36 weeks later of erysipelas of severe intensity and died as a result of progressive respiratory and cardiovascular failure (SAE considered possibly related to study agent). In the sirukumab 100 mg every 2 weeks group, one patient experienced an SAE of haemorrhagic stroke and died as a result of circulatory arrest (events considered not related to study agent).
AE, adverse event; ALT, alanine aminotransferase; AST, aspartate aminotransferase; EE, early escaped; GI, gastrointestinal; MACE, major adverse cardiovascular event; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

sirukumab (both doses) and adalimumab treatment at weeks 24 and 52 (online supplementary table 6). Approximately 50%–60% of the patients in each treatment group achieved clinically meaningful improvements from baseline (≥5-point increase) in SF-36 physical component summary and mental component summary scores at weeks 24 and 52 (online supplementary table 6). High proportions of patients (≥60%) achieved clinically meaningful improvements from baseline (≥4-point increase) in FACIT-Fatigue score across all groups (online supplementary table 6).

Safety

All reported safety assessments are for the entire 68-week study, unless otherwise specified. Overall incidences of TEAEs for patients randomised to adalimumab, sirukumab 50 mg and

Table 4 Number of patients with NCI-CTCAE toxicity grades 3 and 4 postbaseline laboratory abnormalities through week 68*

NCI-CTCAE toxicity grades 3 and 4 abnormalities, n (%)	Adalimumab 40 mg every 2 weeks (n=186)	Sirukumab 50 mg every 4 weeks (n=186)	Sirukumab 100 mg every 2 weeks (n=187)
ALT (increased)			
n	186	186	187
Grade 3 (>5–20×ULN)	3 (1.6)	1 (0.5)	5 (2.7)
Grade 4 (>20×ULN)	0	0	1 (0.5)
AST (increased)			
n	186	186	187
Grade 3 (>5–20×ULN)	1 (0.5)	0	0
Grade 4 (>20×ULN)	0	0	1 (0.5)
Cholesterol (increased)			
n	186	182	183
Grade 3 (>10.36–12.95 mmol/L)	1 (0.5)	7 (3.8)	4 (2.2)
Grade 4 (>12.95 mmol/L)	0	1 (0.5)	0
Triglycerides (increased)			
n	185	182	182
Grade 3 (>5.65–11.30 mmol/L)	2 (1.1)	6 (3.3)	7 (3.8)
Grade 4 (>11.30 mmol/L)	1 (0.5)	2 (1.1)	1 (0.5)
Neutrophils (decreased)			
n	186	186	187
Grade 3 (<1–0.5×10^9/L)	1 (0.5)	6 (3.2)	7 (3.7)
Grade 4 (<0.5×10^9/L)	0	0	1 (0.5)
Platelets (decreased)			
n	186	186	187
Grade 3 (<50–25×10^9/L)	0	0	0
Grade 4 (<25×10^9/L)	0	1 (0.5)	0
Haemoglobin (decreased)			
n	186	186	187
Grade 3 (<8 g/dL)	3 (1.6)	0	0
Grade 4 (NA)†	0	0	0

*Laboratory abnormalities were reported for the group to which the patient was initially randomised.
†Life-threatening consequences; urgent intervention indicated.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; NA, not applicable; NCI-CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events; ULN, upper limit of normal.

sirukumab 100 mg were 69.9% (130/186), 74.7% (139/186) and 71.7% (134/187), respectively (table 3). The most frequently reported (>5%) individual TEAEs for sirukumab and adalimumab are summarised in table 3. TEAEs leading to treatment discontinuation and serious TEAEs occurred in more patients with sirukumab 50 mg compared with sirukumab 100 mg or adalimumab treatment (table 3); detailed listings of these TEAEs are provided in online supplementary tables 7 and 8.

The percentage of patients with injection-site reactions was approximately two-fold greater with sirukumab 100 mg compared with sirukumab 50 mg and adalimumab; none were considered serious (table 3). One patient each in the sirukumab 100 mg group (injection-site swelling) and adalimumab group (injection-site induration) discontinued treatment due to injection-site reactions. Rates of hypersensitivity reactions were low for all groups; no cases of anaphylaxis occurred (table 3).

The rate of infections was similar with sirukumab compared with adalimumab, with nasopharyngitis, upper respiratory tract infections and bronchitis being the most frequently reported (table 3). Among patients receiving adalimumab, sirukumab 50 mg and sirukumab 100 mg, rates of serious infections were 2.2% (4/186), 7.5% (14/186) and 2.7% (5/187), respectively. Two cases of reactivated pulmonary tuberculosis, one case of opportunistic infection, three major adverse cardiovascular events, six malignancies, seven pregnancies, two gastrointestinal perforations and four deaths were reported. Details of these events are summarised in the online supplementary methods and results.

Laboratory abnormalities of interest (associated with IL-6 inhibition) were more common with both sirukumab doses compared with adalimumab through week 52. All treatments were associated with liver enzyme increases; lipid level elevations and neutrophil count reductions were more frequently associated with sirukumab treatment (table 4). Additional details about laboratory abnormalities are included in the online supplementary methods and results.

Immunogenicity

The incidence of antibodies to sirukumab through week 68 was low (sirukumab 100 mg, 4.9% (9/183); sirukumab 50 mg, 3.8% (7/182)), while the incidence of antibodies to adalimumab was 91.9% (171/186). The presence of antibodies to sirukumab or adalimumab did not appear to markedly reduce response rates. More detailed pharmacokinetic and immunogenicity results are summarised in the online supplementary methods and results.

DISCUSSION

Some patients are unable to use csDMARDs, possibly due to tolerability issues.[3] For these patients, there may be advantages to using monotherapy with agents targeting the IL-6 pathway or JAK inhibitors.[2 4] Thus, the efficacy of sirukumab monotherapy was compared with that of adalimumab monotherapy, the most commonly used bDMARD for the treatment of RA.[11] For the first primary endpoint in this study, monotherapy with sirukumab 100 mg was superior to monotherapy with adalimumab 40 mg in biologic-naïve patients with active RA in terms of improvements in DAS28 (ESR) from baseline to week 24. However, for the second primary endpoint, ACR50 response rates were comparable for sirukumab 100 mg and adalimumab. It should be noted, however, that the proportion of patients achieving ACR50 response in the adalimumab group in this study (31.7%) was slightly higher than that reported for adalimumab monotherapy in other RA studies (22.1%–29.7%).[12–17] Similar results were observed for sirukumab 50 mg. Sirukumab's direct and greater effect on acute phase reactants (ESR and CRP) compared with adalimumab, coupled with the finding that sirukumab and adalimumab produced comparable improvements in the CDAI measure, may account for the superiority of sirukumab on the DAS28 (ESR) endpoint, but non-superiority on the ACR50 response endpoint in this study. The acute phase reactant component is weighted more heavily in the DAS28 (ESR) formula than in the ACR50 criteria, and not at all in the CDAI.[18–20] Both sirukumab and adalimumab showed early efficacy, with improvements in RA signs and symptoms as early as week 2. Overall, improvements in measures of signs and symptoms, physical function and patient-reported outcomes were generally similar across the sirukumab and adalimumab groups.

For certain endpoints that are generally harder to achieve (DAS28 remission, ACR50), a numerically greater treatment response was observed for sirukumab 100 mg compared with sirukumab 50 mg, suggesting a possible dose–response relationship for sirukumab monotherapy, although the study was not designed to compare the two doses. In contrast, no efficacy-

related dose response was identified when sirukumab was administered in combination with csDMARDs.[9 10]

In the ADACTA and MONARCH studies, both of which were direct comparative studies versus adalimumab, tocilizumab and sarilumab demonstrated significant improvements compared with adalimumab in CDAI and other measures, including ACR response and various patient-reported outcomes, when administered as monotherapy in patients with RA who were intolerant or inadequate responders to MTX.[12 17] In this study, improvements from baseline in signs and symptoms and patient-reported outcomes were generally comparable between sirukumab and adalimumab groups. There is no clear evidence-based mechanistic or scientific reason why the two anti–IL-6R antibody monotherapy regimens would be more efficacious than adalimumab, while sirukumab, which inhibits IL-6, and adalimumab were comparable in efficacy in the SIRROUND-H trial. In addition to the targeted mechanism of action, the studies differed in study design (eg, 52-week double-blind treatment period in this study vs 24 weeks in ADACTA and MONARCH), the geographical distribution of the study population (eg, >60% of patients were from Eastern Europe in this study, higher than ADACTA and MONARCH), as well as blinding and analysis methods.[12 17] The response rate in the adalimumab comparator groups varied across the three studies.[12 17]

The strengths of the study were that it was the only large study of sirukumab in bionaïve patients, and was a randomised, blinded, controlled monotherapy trial of 52 weeks' duration with an active comparator, which evaluated two doses of the investigational agent. The limitations were that adalimumab monotherapy control treatment yielded responses that were higher than in other adalimumab studies,[12–17] rendering indirect comparisons to other studies challenging. A direct comparative study of sirukumab against an anti–IL-6R antibody would be of interest but, at the time of study design, such a trial was not feasible for reasons of uncertain effect size, blinding and compound availability. Due to the effects of sirukumab on acute phase reactants, the use of DAS28 (ESR) as one of the two primary objectives may also have presented challenges for comparing efficacy between sirukumab and adalimumab, as discussed above. MTX carry-over effects may also have been present and differed between treatment groups; an MTX washout period of more than 2 weeks and/or balancing treatment groups based on this variable could have been useful.

The safety profile of sirukumab was generally consistent with the known safety profile of anti–IL-6R antibody treatment and previous sirukumab RA studies.[9 12 17 21 22] In this study, the rate of serious infections was numerically higher with sirukumab 50 mg than with adalimumab or sirukumab 100 mg. Two sirukumab-treated patients had gastrointestinal perforations, while none occurred with adalimumab. Four sirukumab-treated patients died, while no deaths were reported in the adalimumab group. All deaths occurred after week 24, lacked dose dependence, and the causes of death were diverse and typical of patients with RA. In the 24-week ADACTA and MONARCH studies, of the two deaths and one death reported, respectively, none occurred in the adalimumab groups.[12 17] Through week 68, no dose response was observed in AE or serious AE rates, except for injection-site reactions. Laboratory abnormalities commonly observed with sirukumab were liver transaminase increases, lipid level elevations and neutrophil count reductions. The safety and tolerability of adalimumab in this study were consistent with published data of adalimumab monotherapy in RA.[12 13 15 23 24] When determining which bDMARD monotherapy to use, individual patient comorbidities and risk profiles in relation to anti-TNF or IL-6 pathway inhibitor class effects should be taken into account.

The immunogenicity rate was low for sirukumab monotherapy (4.4%) through week 68, similar to that observed for sirukumab combined with csDMARD treatment in other studies.[9 10] The immunogenicity rate for adalimumab monotherapy through week 68 was high (91.9%) in this study, which could be related to the use of a validated, sensitive immunoassay and to administration of adalimumab as monotherapy. In this study, the presence of antibodies to either sirukumab or adalimumab did not appear to be associated with a notable reduction in efficacy. However, previous studies have shown that rates of anti-adalimumab antibodies are higher when adalimumab is used as monotherapy and appear to be associated with loss of response after prolonged treatment.[25–27]

In conclusion, treatment with sirukumab monotherapy demonstrated rapid and sustained improvement in signs and symptoms of RA, comparable to those achieved with adalimumab monotherapy, in a population of biologic-naïve patients with an inadequate response or intolerance to/inappropriateness for MTX. Unfortunately, because health authorities requested additional clinical data, which would have significantly delayed access to sirukumab in parts of the world, the sponsor company made the strategic decision to prioritise other therapies in development and to terminate the sirukumab RA programme.

Acknowledgements The authors thank all investigators and participating patients in the SIRROUND-H study. Writing and editorial support were provided by Lauren Fink, PhD, of MedErgy, and were funded by Janssen Global Services and GlaxoSmithKline.

Contributors PCT, YZ, RK, RR and PPT contributed to the design of the study and analysis and interpretation of the data. MHS, YJ and SD contributed to the analysis and interpretation of the data. QW contributed to the design and conduct of the study, recruitment and treatment of patients, and collection, analysis and interpretation of the data. BH contributed to the design and conduct of the study and the analysis and interpretation of the data. All authors contributed to drafting the work or revising it critically for important intellectual content, provided final approval of the version published and agreed to be accountable for all aspects of the work.

Funding This study was sponsored by Janssen Research & Development in collaboration with GlaxoSmithKline. PCT was supported in part by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre (BRC).

Disclaimer The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

Competing interests PCT has served as a consultant to AbbVie, Biogen, Bristol-Myers Squibb, Eli Lilly, Galapagos, GlaxoSmithKline, Janssen, Novartis, Pfizer, Roche, Sandoz and UCB Pharma, and has received research grant funding from Celgene, GlaxoSmithKline, Janssen and UCB Pharma. MHS has served as a consultant to AbbVie, Bristol-Myers Squibb, Johnson & Johnson, Eli Lilly and UCB Pharma, and as a speaker for AbbVie and Bristol-Myers Squibb. QW, YZ and BH are employees and shareholders of Janssen Research & Development. YJ is a contractor of Janssen Research & Development. RK, SD, RR and PPT are employees and shareholders of GlaxoSmithKline.

Patient consent Obtained.

Ethics approval The study protocol and amendments were reviewed by an Independent Ethics Committee or Institutional Review Board. This study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practices and applicable regulatory requirements.

Provenance and peer review Not commissioned; externally peer reviewed.

Author note Portions of the data in this manuscript have been previously presented as an oral presentation at the 2016 ACR/ARHP Annual Meeting (Taylor PC, Schiff M, Wang Q, Jiang Y, Kurrasch R, Daga S, Rao R, Hsu B, Tak PP. Efficacy and safety of monotherapy with sirukumab, an anti–IL-6 cytokine monoclonal antibody, compared with adalimumab monotherapy in biologic-naïve patients with active rheumatoid arthritis: results of a global, randomized, double-blind, parallel-group, phase 3 study [abstract]. *Arthritis Rheumatol*. 2016;68[suppl 10]. Available at: http://acrabstracts.org/abstract/efficacy-and-safety-of-monotherapy-with-sirukumab-an-anti-il-6-cytokine-monoclonal-antibody-compared-with-adalimumab-monotherapy-in-biologic-naive-patients-with-active-rheumatoid-arthritis/. Accessed August 2, 2017).

REFERENCES

1 Singh JA, Saag KG, Bridges SL, *et al*. 2015 American College of Rheumatology Guideline for the Treatment of Rheumatoid Arthritis. *Arthritis Rheumatol* 2016;68:1–26.
2 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.
3 El-Zorkany BK, Gamal SM, El-Mofty SA. Frequency and causes of discontinuation of methotrexate in a cohort of Egyptian patients. *The Egyptian Rheumatologist* 2013;35:53–7.
4 Tak PP. A personalized medicine approach to biologic treatment of rheumatoid arthritis: a preliminary treatment algorithm. *Rheumatology* 2012;51:600–9.
5 Gabay C. Interleukin-6 and chronic inflammation. *Arthritis Res Ther* 2006;8(Suppl 2):S3.
6 Ishihara K, Hirano T. IL-6 in autoimmune disease and chronic inflammatory proliferative disease. *Cytokine Growth Factor Rev* 2002;13:357–68.
7 Tak PP, Smeets TJ, Daha MR, *et al*. Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. *Arthritis Rheum* 1997;40:217–25.
8 Jones SA, Scheller J, Rose-John S. Therapeutic strategies for the clinical blockade of IL-6/gp130 signaling. *J Clin Invest* 2011;121:3375–83.
9 Aletaha D, Bingham CO, Tanaka Y, *et al*. Efficacy and safety of sirukumab in patients with active rheumatoid arthritis refractory to anti-TNF therapy (SIRROUND-T): a randomised, double-blind, placebo-controlled, parallel-group, multinational, phase 3 study. *Lancet* 2017;389:1206–17.
10 Takeuchi T, Thorne C, Karpouzas G, *et al*. Sirukumab for rheumatoid arthritis: the phase III SIRROUND-D study. *Ann Rheum Dis* 2017;76:2001–8.
11 Kaufmann J, Feist E, Roske AE, *et al*. Monotherapy with tocilizumab or TNF-alpha inhibitors in patients with rheumatoid arthritis: efficacy, treatment satisfaction, and persistence in routine clinical practice. *Clin Rheumatol* 2013;32:1347–55.
12 Gabay C, Emery P, van Vollenhoven R, *et al*. Tocilizumab monotherapy versus adalimumab monotherapy for treatment of rheumatoid arthritis (ADACTA): a randomised, double-blind, controlled phase 4 trial. *Lancet* 2013;381:1541–50.
13 Fleischmann R, Cutolo M, Genovese MC, *et al*. Phase IIb dose-ranging study of the oral JAK inhibitor tofacitinib (CP-690,550) or adalimumab monotherapy versus placebo in patients with active rheumatoid arthritis with an inadequate response to disease-modifying antirheumatic drugs. *Arthritis Rheum* 2012;64:617–29.
14 Heiberg MS, Rødevand E, Mikkelsen K, *et al*. Adalimumab and methotrexate is more effective than adalimumab alone in patients with established rheumatoid arthritis: results from a 6-month longitudinal, observational, multicentre study. *Ann Rheum Dis* 2006;65:1379–83.
15 van de Putte LB, Atkins C, Malaise M, *et al*. Efficacy and safety of adalimumab as monotherapy in patients with rheumatoid arthritis for whom previous disease modifying antirheumatic drug treatment has failed. *Ann Rheum Dis* 2004;63:508–16.
16 Felson DT. Tocilizumab versus adalimumab for rheumatoid arthritis. *The Lancet* 2013;382:394–5.
17 Burmester GR, Lin Y, Patel R, *et al*. Efficacy and safety of sarilumab monotherapy versus adalimumab monotherapy for the treatment of patients with active rheumatoid arthritis (MONARCH): a randomised, double-blind, parallel-group phase III trial. *Ann Rheum Dis* 2017;76:840–7.
18 Schoels M, Alasti F, Smolen JS, *et al*. Evaluation of newly proposed remission cut-points for disease activity score in 28 joints (DAS28) in rheumatoid arthritis patients upon IL-6 pathway inhibition. *Arthritis Res Ther* 2017;19:155.
19 Smolen JS, Aletaha D. Interleukin-6 receptor inhibition with tocilizumab and attainment of disease remission in rheumatoid arthritis: the role of acute-phase reactants. *Arthritis Rheum* 2011;63:43–52.
20 Smolen JS, Breedveld FC, Burmester GR, *et al*. Treating rheumatoid arthritis to target: 2014 update of the recommendations of an international task force. *Ann Rheum Dis* 2016;75:3–15.
21 Smolen JS, Weinblatt ME, Sheng S, *et al*. Sirukumab, a human anti-interleukin-6 monoclonal antibody: a randomised, 2-part (proof-of-concept and dose-finding), phase II study in patients with active rheumatoid arthritis despite methotrexate therapy. *Ann Rheum Dis* 2014;73:1616–25.
22 Genovese MC, Fleischmann R, Kivitz AJ, *et al*. Sarilumab plus methotrexate in patients with active rheumatoid arthritis and inadequate response to methotrexate: results of a phase III study. *Arthritis Rheumatol* 2015;67:1424–37.
23 Taylor PC, Genovese MC, Greenwood M, *et al*. OSKIRA-4: a phase IIb randomised, placebo-controlled study of the efficacy and safety of fostamatinib monotherapy. *Ann Rheum Dis* 2015;74:2123–9.
24 Breedveld FC, Weisman MH, Kavanaugh AF, *et al*. The PREMIER study: A multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. *Arthritis Rheum* 2006;54:26–37.
25 Bartelds GM, Krieckaert CL, Nurmohamed MT, *et al*. Development of antidrug antibodies against adalimumab and association with disease activity and treatment failure during long-term follow-up. *JAMA* 2011;305:1460–8.
26 Cludts I, Spinelli FR, Morello F, *et al*. Anti-therapeutic antibodies and their clinical impact in patients treated with the TNF antagonist adalimumab. *Cytokine* 2017;96:16–23.
27 Bartelds GM, Wijbrandts CA, Nurmohamed MT, *et al*. Clinical response to adalimumab: relationship to anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann Rheum Dis* 2007;66:921–6.

EXTENDED REPORT

Performance characteristics of rheumatoid factor and anti-cyclic citrullinated peptide antibody assays may impact ACR/EULAR classification of rheumatoid arthritis

Lieve Van Hoovels,[1] Julie Jacobs,[1] Bert Vander Cruyssen,[2] Stefanie Van den Bremt,[1] Patrick Verschueren,[3] Xavier Bossuyt[4]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212365).

[1]Department of Laboratory Medicine, Onze-Lieve-Vrouw Hospital, Aalst, Belgium
[2]Department of Rheumatology, Onze-Lieve-Vrouw Hospital, Aalst, Belgium
[3]Department of Rheumatology, University Hospital Leuven, Leuven, Belgium
[4]Department of Laboratory Medicine, University Hospital Leuven, Leuven, Belgium

Correspondence to
Lieve Van Hoovels, Department of Laboratory Medicine, Onze-Lieve Vrouw Hospital, Moorselbaan 164, Aalst 9300, Belgium;
lieve.Van.Hoovels@olvz-aalst.be

Received 10 September 2017
Revised 7 November 2017
Accepted 28 November 2017
Published Online First 23 January 2018

To cite: Van Hoovels L, Jacobs J, Vander Cruyssen B, *et al*. *Ann Rheum Dis* 2018;**77**:667–677.

ABSTRACT

Objectives Rheumatoid factor (RF) and anti-cyclic citrullinated protein/peptide antibodies (ACPA) are integrated in the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) classification criteria for rheumatoid arthritis (RA). The objectives of this study were to evaluate the technical and diagnostic performance of different RF and ACPA assays and to evaluate whether differences in performance impact RA classification.

Methods Samples from 594 consecutive patients who for the first time consulted a rheumatologist (44 of whom were diagnosed with RA) and 26 extra newly diagnosed patients with RA were analysed with six different RF assays (Menarini, Thermo Fisher, Inova, Roche, Abbott, Euroimmun) and seven different ACPA assays (Menarini, Thermo Fisher, Inova, Roche, Abbott, Euro Diagnostica, Euroimmun).

Results We found differences in analytical performance between assays. There was poor numerical agreement between the different RF and ACPA assays. For all assays, the likelihood ratio for RA increased with increasing antibody levels. The areas under the curve of receiver operating characteristic analysis of the RF (range 0.676–0.709) and ACPA assays (range 0.672–0.769) only differed between some ACPA assays. Nevertheless, using the cut-off proposed by the manufacturer, there was a large variation in sensitivity and specificity between assays (mainly for RF). Consequently, depending on the assay used, a subgroup of patients (13% for RF, 1% for ACPA and 9% for RF/ACPA) might or might not be classified as RA according to the 2010 ACR/EULAR criteria.

Conclusion Due to poor harmonisation of RF and ACPA assays and of test result interpretation, RA classification according to 2010 ACR/EULAR criteria may vary when different assays are used.

INTRODUCTION

Rheumatoid arthritis (RA) is the most common chronic inflammatory joint disease, affecting 0.5%–1% of the population in the industrialised world.[1] If left untreated, or undertreated, RA is associated with progressive and irreversible joint destruction leading to disability, reduction of quality of life and increased mortality.[2] The early start of aggressive therapy aiming to halt progression of disease is currently being emphasised as important strategic principle in view of the 'window of opportunity' theory.[3 4] However, in patients with early disease, diagnosis of RA is difficult.[5]

The American College of Rheumatology (ACR) criteria are widely used as the 'gold standard' for classification of RA. Due to the lack of sensitivity of the 1987 ACR criteria,[6] the ACR and the European League Against Rheumatism (EULAR) proposed new classification criteria in 2010.[7] Application of the 2010 classification criteria provides a score of 0–10, with a score ≥6 being indicative of definite RA. The presence of rheumatoid factor (RF) or anti-cyclic citrullinated protein/peptide antibodies (ACPA) contributes two points if detectable and three points if present at levels >3 times the upper limit of normal.[7]

RF is an antibody against the Fc portion of IgG. Despite its relatively low specificity (±80%), RF has historically been used as a serological marker for RA.[8] ACPA are antibodies to citrullinated peptides. ACPA are associated with a bad outcome and are more specific (±95%) for RA than RF.[9 10] Overall, sensitivities of RF and ACPA for RA are comparable (±60%).[2 11] In the 2010 ACR/EULAR criteria, RF and ACPA are regarded as equivalent.[7]

Over the past years, different assays for the detection of ACPA and RF have been introduced. Initially, ACPA were detected by ELISA using citrullinated recombinant rat filaggrin.[12] Subsequently, sensitivity of ACPA tests was enhanced without compromising specificity by using synthetic cyclic citrullinated peptides (CCP2) (second-generation ACPA).[13] More recently, a third-generation ACPA (CCP3) test has been designed.[14 15]

Most (not all) RF assays are calibrated against an international recognised standards, namely the WHO International standard W1066 or the British Standard of human RA serum 64/002 standard (National Institute of Biological Standards and Control (NIBSC)). Both standards are the same material.[16–18] ACPA assays, on the other hand, are not harmonised. There is a large variability between the different ACPA assays and numerical test results are not interchangeable.[19] Only recently, the Centers for Disease Control and Prevention (CDC) provided a reference human ACPA for in vitro immunodiagnostic use in solid phase enzyme immunoassays.[20]

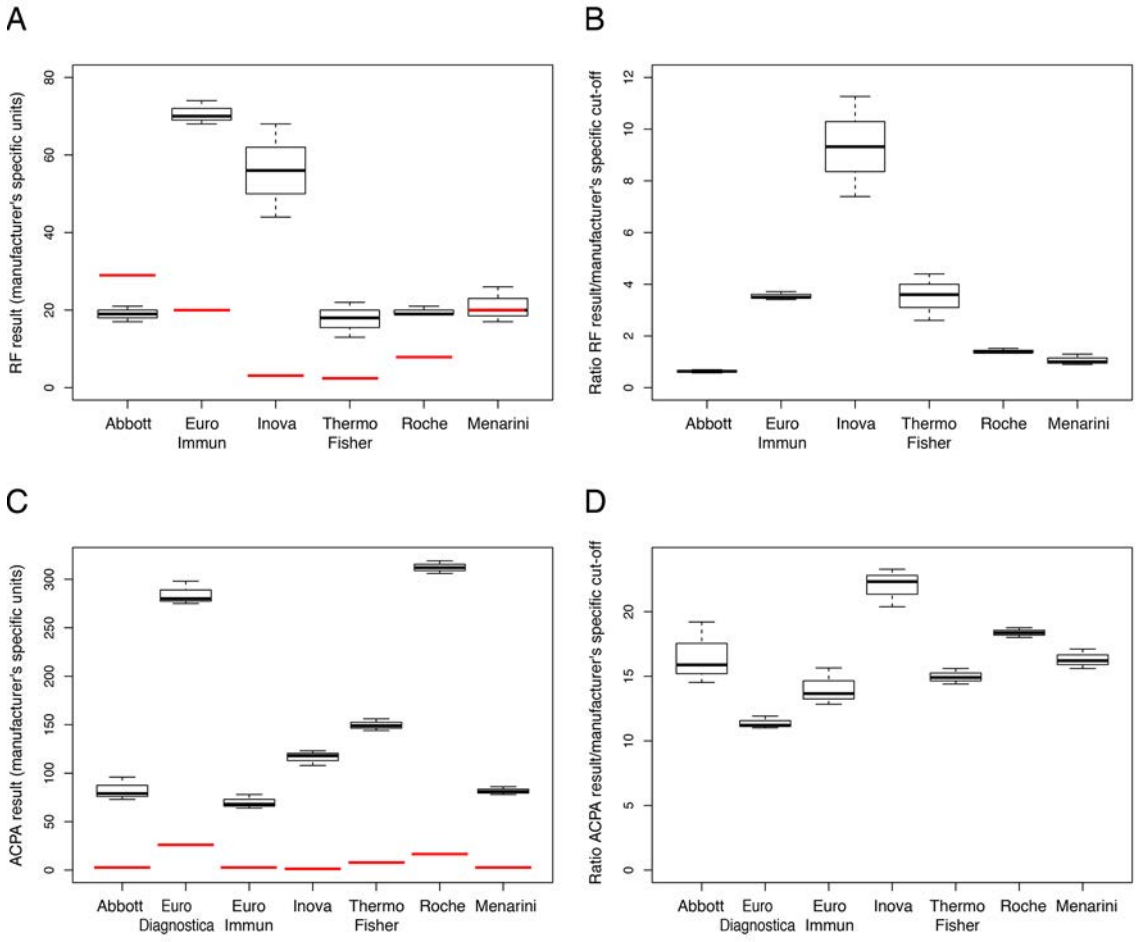


Figure 1 Quantification of WHO W1066 RF standard and Centers for Disease Control and Prevention (CDC) ACPA standard. The WHO W1066 RF standard (A and B) was measured for RF and the CDC ACPA international standard (C and D) was measured for ACPA. The measurements were done three times (in different runs) with assays from different manufacturers. (A and C) Box-whisker plots of the results obtained. The manufacturer's cut-offs are marked as red bars. The y-axis represents the manufacturer-specific units: manufacturer's RF units: IU/mL (Abbott, Thermo Fisher, Roche and Menarini), RU/mL (Euroimmun), U/mL (Inova); manufacturer's ACPA units: AU/mL (Menarini), U/mL (Thermo Fisher, Inova, Roche, Abbott, Euro Diagnostica), RU/mL (Euroimmun). (B and D) Box-whisker plots of the ratios obtained between the test result values and the manufacturer's specific cut-off value. ACPA, anti-cyclic citrullinated protein/peptide antibodies; RF, rheumatoid factor.

The objectives of this study were to evaluate the technical and diagnostic performance of different ACPA and RF assays and to study whether differences in test performance could impact the 2010 ACR/EULAR classification of RA.

MATERIALS AND METHODS

Patients and samples

Between January 2014 and June 2015, all unique patients (n=594) who for the first time underwent laboratory testing for a rheumatologic disease, requested by a rheumatologist of the Onze-Lieve-Vrouw Hospital in Aalst, Belgium (a secondary care hospital), were included. Patients for whom there was not enough serum to perform additional testing were excluded. All serum samples were stored at −20°C before analysis.

After review of the electronic medical records, the diagnosis was registered and reviewed by the consulting rheumatologist. Patients were categorised into three groups: RA, rheumatologic disease control group (RDCG) and disease control group (DCG). A patient with synovitis was considered to have RA (n=44) when the treating rheumatologist initiated methotrexate treatment (if no contraindication existed) and no alternative diagnosis could better explain the symptoms. These RA criteria were based on the criteria used for deriving the 2010 ACR/EULAR criteria.[7] The RA diagnosis was reviewed after 1 year of follow-up.

To enlarge the group of patients with RA, 26 additional patients with RA (recruited between June 2012 and April 2016) were included. These patients with RA were coded as described above.

For all patients coded as RA it was checked whether they fulfilled the 1987 ACR[6] and the 2010 ACR/EULAR criteria[7]: 53 patients fulfilled both criteria, 14 fulfilled only the 1987 criteria and 3 patients fulfilled only the 2010 ACR/EULAR criteria (online supplementary data S10).

Assays

Six commercial RF and seven commercial ACPA assays were included in this study.

For RF, the Quantia RF on the Abbott ARCHITECT c System (Abbott, Germany), QUANTA Lite RF IgM ELISA on a QUANTA-Lyser 2 (Inova Diagnostics, USA), RF EliA IgM on Phadia 250 (Thermo Fisher Scientific, Sweden), RF-II on a Cobas c502 analyser (Roche Diagnostics, Germany), Diagam RF on a ZENIT analyser (Menarini Diagnostics, Italy) and the RF IgM ELISA from Euroimmun (Euroimmun, Germany) were evaluated.

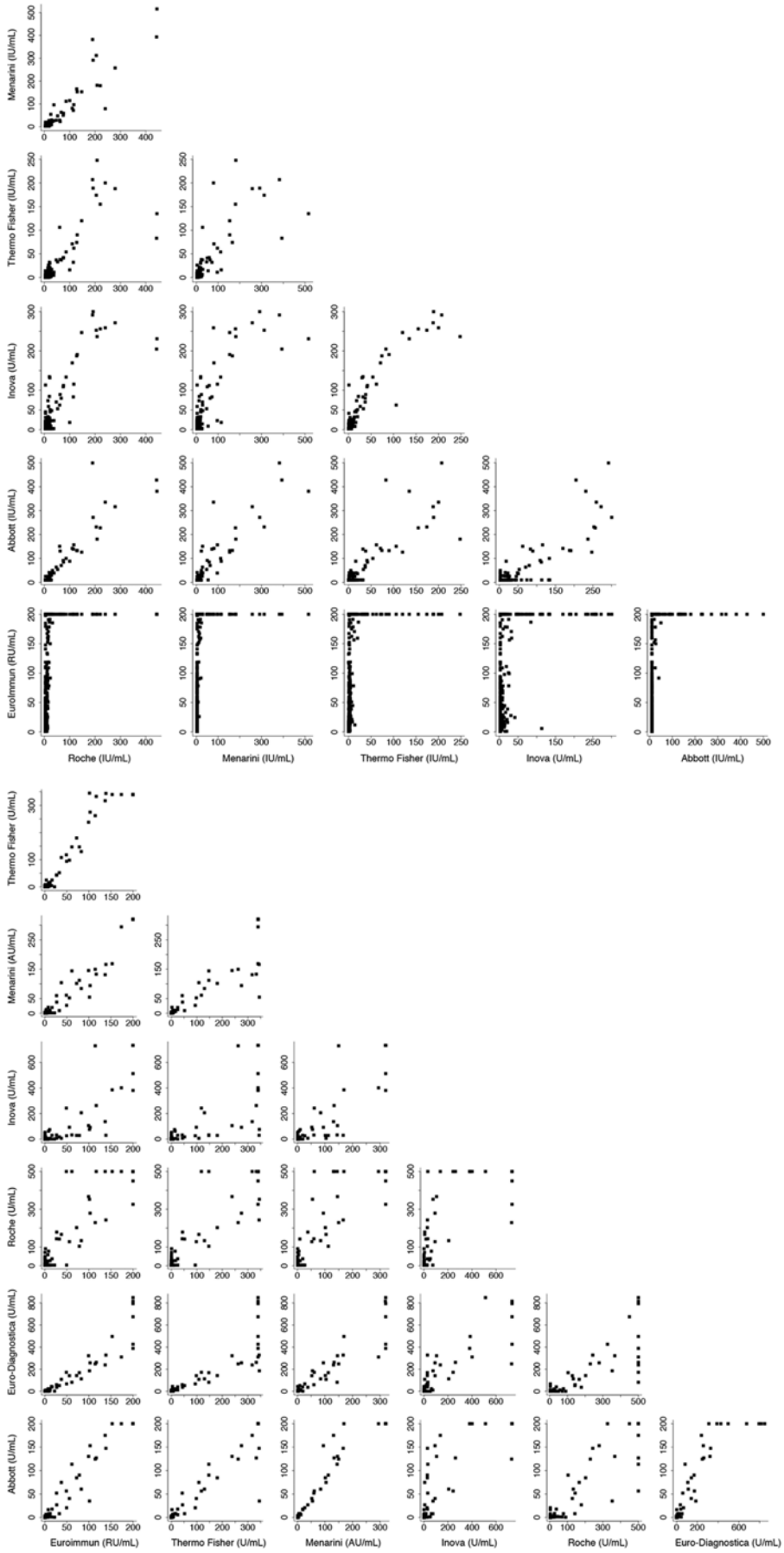


Figure 2 Spearman's rank (r) correlation plots of rheumatoid factor (RF) IgM (A) and anti-cyclic citrullinated protein/peptide antibodies (ACPA) IgG (B).

For ACPA, the ARCHITECT Anti-CCP assay on the ARCHITECT i System (Abbott), Immunoscan CCPlus (Euro Diagnostica, Sweden) on a QUANTA-Lyser 2 (Inova Diagnostics), QUANTA Flash CCP3 on the BIO-FLASH instrument (Inova Diagnostics), CCP EliA IgG on Phadia 250 (Thermo Fisher Scientific), Anti-CCP on a Cobas e601 analyser (Roche Diagnostics), ZENIT RA CCP on a ZENIT analyser (Menarini Diagnostics) and the Anti-CCP ELISA (IgG) from Euroimmun (Euroimmun) were included. With the exception of QUANTA Flash CCP3, all included ACPA assays were CCP2 tests.

Analytical performance

Imprecision was determined using the manufacturer's internal quality control (iQC) materials, a patient serum sample with a low and a patient sample with a high RF and/or ACPA concentration. All iQC samples were measured before and after every run during 10 runs.[21]

Linearity was assessed by diluting serum samples containing RF or ACPA with increasing amounts of a serum sample with very low levels of RF or ACPA.[22]

The *limit of quantification (LOQ)* was verified by analysing 10 times a serum sample with an RF/ACPA concentration around the LOQ provided by the manufacturer.[23]

The WHO W1066 international reference serum for RF (target value of 25 IU/mL) and the CDC ACPA standard (target value of 100 IU/mL) were analysed for, respectively, RF and ACPA with all assays. The standard material was reconstituted according to the guidelines, aliquoted and measured three times in different runs.[24]

To determine the amount of *carry-over*, a sample with a high concentration (H) of RF/ACPA and one with a low concentration (L) was measured two times (in the sequence HHLLL).[25]

For analytical *method comparison*, Bland-Altman plots (mean difference in U/mL), least squares regression analysis and Spearman's rank correlation coefficients (r) (and 95% CIs) were calculated for all assays.[26]

Diagnostic performance

Diagnostic performance was evaluated by sensitivity, specificity, likelihood ratio (LR) and receiver operating characteristic curve analysis.

Statistical analysis

Statistics were performed using MEDCALC (V.17.1, Ostend, Belgium).

RESULTS

Patients and samples

We included 594 unique consecutive patients for whom the rheumatologist considered the possibility of RA. Forty-four (7.4%) had RA, 247 (41.6%) were coded as RDCG and 225 (37.9%) as DCG. For 78 (13.1%) patients, the rheumatologic diagnosis remained undifferentiated. In addition, we included 26 extra newly diagnosed patients with RA. An overview of the demographic features of patients with RA and the controls is listed in online supplementary data table S1. Patients with RA were significantly older than the controls.

Analytical performance

Imprecision

Total CVs (online supplementary data table S2), except for Menarini RF, were within the manufacturer's specifications. The highest imprecision was found for Inova RF ELISA and Euro Diagnostica ACPA ELISA.

Carry-over

No significant *carry-over* was detected (<1% for all methods).

Linearity

For all assays, the Cusum test for linearity did not reveal significant deviation from linearity.

Limit of quantification

The LOQ was verified for every assay included (online supplementary data table S3). Not every manufacturer had a predefined criterion for LOQ available.

Quantification of WHO W1066 RF and CDC ACPA standards

With the exception of Euroimmun RF ELISA, all evaluated RF assays reported in their package insert traceability to an international RF standard (Menarini, Abbott, Thermo Fisher: W1066; Roche, Inova: British 64/002). W1066 was tested three times for RF with all assays. We found a good quantitative agreement between RF IgM assays from Menarini, Abbott, Thermo Fisher and Roche. The Inova RF IgM ELISA gave higher values (figure 1A). The ratio of the mean W1066 standard value over the manufacturer specific cut-off value varied from 0.6 to 9.3 (figure 1B). The W1066 standard was scored negative by one assay, borderline positive by one assay and strongly positive by three assays. For one assay the median W1066 value corresponded to the cut-off.

The CDC ACPA reference material was tested with all ACPA assays. We found large differences between numerical results obtained, with results from Euro Diagnostica and Roche being much higher than results from the other manufacturers (figure 1C). All assays scored the CDC ACPA reference material as 'strongly positive' according to the 2010 ACR/EULAR criteria, but ratios of median standard values over manufacturer's specific cut-off values varied from 11.2 to 22.3 (figure 1D).

Method comparison

For all methods, the median RF and ACPA titres were significantly higher in samples from patients with RA than in samples from controls (online supplementary data table S4), but the range and numerical values varied substantially among methods. Figure 2A,B and online supplementary data table S5 summarise the results of method comparison studies for RF and ACPA. Spearman's rank r varied between 0.400 and 0.783 and between 0.336 and 0.702 for RF and ACPA assays, respectively. Bland-Altman analysis and regression analysis revealed low quantitative agreement between assays. Poor numerical agreement was observed between assays, both for RF and ACPA assays, with large deviations away from the target values of 1.00 for slopes and 0.00 for intercepts. For RF, the best agreement was observed for results obtained with the Roche and Abbott methods. For ACPA, the best agreement was between Euroimmun and Abbott.

Diagnostic performance

Table 1 summarises RF and ACPA positivity in patients with RA and controls. The diagnostic performance characteristics are summarised in table 2.

Using the manufacturer's cut-off, RF positivity was found in 35.7%–60.0% of patients with RA and in 0.4%–27.5% of controls. The highest sensitivity (60.0%) and lowest specificity (71.6%) were found for Euroimmun RF. ACPA positivity was

Table 1 RF (A) and ACPA (B) positivity in patients with RA and control groups

(A) RF positive	Total n (% RA)	Menarini n (%)	Thermo Fisher n (%)	Inova n (%)	Roche n (%)	Abbott n (%)	Euroimmun n (%)
Patients with RA	70	28 (40.0)	29 (41.4)	33 (47.1)	32 (45.7)	25 (35.7)	42 (60.0)
Consecutive RA	44	16 (36.4)	17 (38.6)	20 (45.5)	20 (45.5)	15 (34.1)	25 (56.8)
Extra RA	26	12 (46.1)	12 (46.1)	13 (50.0)	12 (46.1)	10 (38.5)	17 (65.3)
Patients with RA aged <70 years	42 (60.0)	19 (45.2)	20 (47.6)	23 (54.8)	21 (50.0)	16 (38.1)	30 (71.4)
Patients with RA aged ≥70 years	28 (40.0)	9 (32.1)	9 (32.1)	10 (35.7)	11 (39.3)	9 (32.1)	12 (42.9)
Patients with early RA (of n=64 RA)	37 (57.8)	13 (35.1)	12 (32.4)	16 (43.2)	14 (37.8)	12 (32.4)	19 (51.4)
Patients with established RA (of n=64 RA)	27 (42.2)	10 (37.0)	12 (44.4)	12 (44.4)	13 (48.1)	8 (29.6)	18 (66.7)
1987 ACR compliant*	67 (95.7)	27 (40.3)	28 (41.8)	32 (47.8)	31 (46.3)	24 (35.8)	39 (58.2)
2010 ACR/EULAR compliant*	56 (80.0)	26 (46.4)	27 (48.2)	29 (51.8)	30 (53.6)	23 (41.1)	39 (69.6)
Erosive disease (of n=58 RA)	15 (25.9)	10 (66.7)	10 (66.7)	12 (80.0)	10 (66.7)	10 (66.7)	10 (66.7)
Disease control group†	225	3 (1.3)	12 (5.3)	36 (16.0)	12 (5.3)	1 (0.4)	58 (25.7)
Chondrocalcinosis	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fibromyalgia/psychological	31	0 (0.0)	2 (6.5)	6 (19.4)	1 (3.2)	0 (0.0)	11 (35.5)
Healthy	7	0 (0.0)	0 (0.0)	1 (14.3)	0 (0.0)	0 (0.0)	2 (28.6)
Mechanical pain	123	2 (1.6)	5 (4.1)	18 (14.6)	6 (4.9)	1 (0.8)	24 (19.5)
Neoplasia	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (100.0)
Osteoporosis	1	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0 (0.0)	1 (100.0)
Other	61	1 (1.6)	5 (8.2)	10 (16.4)	5 (8.2)	0 (0.0)	19 (31.1)
Rheumatological disease control group†	247	2 (0.8)	10 (4.0)	35 (14.2)	9 (3.6)	1 (0.4)	68 (27.5)
Erosive hand osteoarthritis	19	0 (0.0)	0 (0.0)	2 (10.5)	0 (0.0)	0 (0.0)	5 (26.3)
Gout	25	0 (0.0)	0 (0.0)	1 (4.0)	0 (0.0)	0 (0.0)	5 (20.0)
Lupus	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Osteoarthritis	53	0 (0.0)	1 (1.9)	6 (11.3)	1 (1.9)	0 (0.0)	14 (26.4)
Polymyalgia rheumatica	25	0 (0.0)	1 (4.0)	1 (4.0)	1 (4.0)	0 (0.0)	10 (40.0)
Postinfection	21	2 (9.1)	2 (9.1)	8 (36.4)	2 (9.1)	1 (4.5)	6 (28.6)
Psoriatic arthritis	43	0 (0.0)	3 (7.0)	6 (14.0)	2 (4.7)	0 (0.0)	11 (25.6)
Sjögren	5	0 (0.0)	3 (60.0)	3 (60.0)	3 (60.0)	0 (0.0)	4 (80.0)
Spondyloarthritis	50	0 (0.0)	0 (0.0)	7 (14.0)	0 (0.0)	0 (0.0)	13 (26.0)
Sarcoïdosis	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Systemic sclerosis	2	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)
Undifferentiated group†	78	6 (7.7)	9 (11.5)	16 (20.5)	14 (17.9)	4 (5.1)	8 (10.3)

(B) ACPA positive	Total n (% RA)	Menarini n (%)	Thermo Fisher n (%)	Inova n (%)	Roche n (%)	Abbott n (%)	Euro Diagnostica n (%)	Euroimmun n (%)
Patients with RA	70	27 (38.6)	26 (37.1)	26 (37.1)	25 (35.7)	27 (38.6)	24 (34.3)	29 (41.4)
Consecutive RA	44	16 (36.3)	15 (34.1)	14 (31.8)	14 (31.8)	16 (36.3)	14 (31.8)	18 (40.9)
Extra RA	26	11 (42.3)	11 (42.3)	12 (46.2)	11 (42.3)	11 (42.3)	10 (38.5)	11 (42.3)
Patients with RA aged <70 years	42 (60.0)	21 (50)	20 (47.6)	21 (50.0)	19 (45.2)	21 (50.0)	20 (47.6)	22 (52.4)
Patients with RA aged ≥70 years	28 (40.0)	6 (21.4)	6 (21.4)	5 (17.9)	6 (21.4)	6 (21.4)	4 (14.3)	7 (25)
Patients with early RA (of n=64 RA)	37 (57.8)	12 (32.4)	12 (32.4)	12 (32.4)	12 (32.4)	12 (32.4)	12 (32.4)	13 (35.1)
Patients with established RA (of n=64 RA)	27 (42.2)	12 (44.4)	11 (40.7)	11 (40,7)	10 (37.0)	12 (44.4)	9 (33.3)	13 (48.1)
1987 ACR compliant*	67 (95.7)	25 (37.3)	24 (35.8)	24 (35.8)	23 (34.3)	25 (37.3)	22 (32.8)	27 (40.2)
2010 ACR/EULAR compliant*	56 (78.6)	27 (48.2)	26 (46.4)	25 (44.6)	25 (44.6)	27 (48.2)	24 (42.9)	29 (51.8)
Erosive disease (of n=58 RA)	15 (25.9)	10 (66.7)	9 (60.0)	9 (60.0)	9 (60.0)	10 (66.7)	8 (53.3)	11 (73.3)
Disease control group†	225	1 (0.4)	1 (0.4)	4 (1.8)	5 (2.2)	1 (0.4)	1 (0.4)	2 (0.9)
Chondrocalcinosis	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fibromyalgia/psychological	31	0 (0.0)	0 (0.0)	1 (3.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Healthy	7	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mechanical pain	123	0 (0.0)	0 (0.0)	1 (0.8)	2 (1.6)	0 (0.0)	0 (0.0)	1 (0.8)
Neoplasia	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Osteoporosis	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Other	61	1 (1.6)	1 (1.6)	2 (3.3)	3 (4.9)	1 (1.6)	1 (1.6)	1 (1.6)

Continued

Table 1 Continued

(B) ACPA positive	Total n (% RA)	Menarini n (%)	Thermo Fisher n (%)	Inova n (%)	Roche n (%)	Abbott n (%)	Euro Diagnostica n (%)	Euroimmun n (%)
Rheumatological disease control group†	247	2 (0.8)	1 (0.4)	5 (2.0)	8 (3.2)	3 (1.2)	1 (0.4)	6 (2.4)
Erosive hand osteoarthritis	19	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.4)
Gout	25	0 (0.0)	0 (0.0)	1 (4.0)	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
Lupus	3	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Osteoarthritis	53	0 (0.0)	0 (0.0)	0 (0.0)	2 (3.8)	0 (0.0)	0 (0.0)	0 (0.0)
Polymyalgia rheumatica	25	0 (0.0)	0 (0.0)	2 (8.0)	1 (4.0)	0 (0.0)	0 (0.0)	0 (0.0)
Postinfection	21	1 (4.5)	1 (4.5)	1 (4.5)	2 (9.1)	1 (4.5)	1 (4.5)	1 (4.1)
Psoriatic arthritis	43	1 (2.3)	0 (0.0)	0 (0.0)	2 (4.7)	2 (4.7)	0 (0.0)	3 (1.2)
Sjögren	5	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Spondyloarthritis	50	0 (0.0)	0 (0.0)	1 (2.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (4.1)
Sarcoïdosis	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Systemic sclerosis	2	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Undifferentiated group†	78	3 (3.8)	3 (3.8)	6 (7.7)	2 (2.6)	3 (3.8)	2 (2.6)	5 (6.4)

*See online supplementary data S10 for a description of the clinical features of patients with RA only fulfilling 1987 ACR or 2010 ACR/EULAR RA classification criteria.
†See online supplementary data S11 for an overview of the 2010 ACR/EULAR RA classification criteria for controls that tested positive for RF and/or ACPA with all included assays.
ACPA, anti-cyclic citrullinated protein/peptide antibodies; ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; RA, rheumatoid arthritis; RF, rheumatoid factor.

comparable between the assays and varied between 34.3% and 41.4% in patients with RA and between 0.4% and 3.2% in controls. RF/ACPA prevalence and antibody level were higher in patients with RA aged <70 years compared with patients with RA aged ≥70 years. This was statistically significant for all included ACPA assays but not for the RF assays (online supplementary data table S6). For 64 of the 70 patients with RA included, the date of onset of symptoms could be retrieved. Prevalence of RF and ACPA tended to be lower in patients with early RA (n=37/64 with symptom onset <3 months before evaluation) than in patients with established RA, but this was not statistically significant (online supplementary data table S7). Prevalence of RF and ACPA was significantly higher in patients with RA with erosive RA disease than in patients without erosive disease ($P<0.05$ for all methods, except for Euroimmun RF ($P=0.4362$)).

The AUCs were 0.709, 0.687, 0.676, 0.709, 0.690 and 0.708 for, respectively, Menarini, Thermo Fisher, Inova, Roche, Abbott and Euroimmun RF assays, and 0.698, 0.769, 0.685, 0.672, 0.693, 0.734 and 0.709 for, respectively, Menarini, Thermo Fisher, Inova, Roche, Abbott, Euro Diagnostica and Euroimmun ACPA assays (online supplementary data table S8). For the RF assays, the AUCs were not statistically significantly different. For ACPA, the AUCs were significantly different between Roche and Euro Diagnostica ($P=0.0115$) and between Abbott, Inova, Euroimmun, Roche, Menarini on the one hand and Thermo Fisher on the other hand (respectively $P=0.0008$, $P=0.0057$, $P=0.0287$, $P=0.0011$ and $P=0.0037$).

At a cut-off that corresponded to a specificity of 95% for the RF assays, the sensitivity ranged from 37.1% to 44.3%, depending on the assay. The specificity of Abbott RF IgM assay at LOQ of 20 U/mL was already >97.5%. The specificity of the Euroimmun RF IgM ELISA never exceeded 95.5%. At a cut-off that corresponded to a specificity of 98.5% for the ACPA assays, sensitivity ranged from 32.9% to 40.0%.

Table 3 shows the LRs for RF and ACPA and a combination thereof according to the serological 2010 ACR/EULAR criteria. The LRs for RA were higher for strong positive results (>3 times cut-off) than for weak positive results (one to three times cut-off value). Strong positive RF or ACPA results had a high LR for RA (>10), except for two RF assays (from Inova and Euroimmun). The highest LRs were consistently found for double positivity for RF and ACPA. The differences in LR between assays were less pronounced when a cut-off that is based on a predefined specificity was applied.

As our study revealed differences in test results between different companies, we evaluated whether such differences could impact disease classification. All patients with RA were classified according to the 2010 ACR/EULAR criteria using RF and ACPA results obtained with assays from different manufacturers (table 4 and online supplementary data table S9). In 32 (of 70) patients with RA (46%), a 2010 ACR/EULAR criteria score ≥6 was obtained based solely on clinical data (ie, without lab data). Inflammation did not contribute to disease classification. Thus, in 38 patients (54%), RF and/or ACPA contributed to disease classification. When only RF was considered, then 49 or 58 patients fulfilled the criteria when, respectively, the least or most sensitive RF assay was considered. When only ACPA was considered, then 49 or 50 patients fulfilled the criteria when, respectively, the least or most sensitive assay was considered. When RF and ACPA were considered (by combining assays from the same manufacturer), then 53 or 59 patients fulfilled the criteria depending on the manufacturer. Thus, classification of patients using the 2010 ACR/EULAR criteria depended on the assays used.

DISCUSSION

In this study, we compared the analytical and diagnostic performance of six RF and seven ACPA assays in a secondary care hospital.

We found differences in analytical performance between assays. For example, some assays had a higher imprecision and poorer linearity than other assays. Several manufacturers did not specify the LOQ.

We quantified the WHO W1066 RF standard with all RF assays. Of note, NIBSC 64/002 is the same material as WHO W1066.[16–18] Assays from Thermo Fisher, Menarini, Abbott RF

Table 2 Performance characteristics of RF and ACPA assays

	Assay					
(A) RF	**Menarini**	**Thermo Fisher**	**Inova**	**Roche**	**Abbott**	**Euroimmun**
AUC (95% CI)	0.709 (0.671 to 0.744)	0.687 (0.649 to 0.724)	0.676 (0.638 to 0.713)	0.709 (0.671 to 0.744)	0.690 (0.652 to 0.726)	0.708 (0.670 to 0.743)
Manufacturer's cut-off (U/mL)	20.0	5.0	6.0	14.0	30.0	20.0
Specificity (95% CI)	98.0% (96.4 to 99.0)	94.4% (92.1 to 96.1)	84.4% (81.1 to 87.3)	93.8% (91.5 to 95.7)	98.9% (97.6 to 99.6)	71.6% (67.7 to 75.4)
Sensitivity (95% CI)	40.0% (28.5 to 52.4)	40.0% (28.5 to 52.4)	47.1% (35.1 to 59.4)	45.7% (33.7 to 58.1)	35.7% (24.6 to 48.1)	60.0% (47.6 to 71.5)
LR(+) (95% CI)	20.0 (10.4 to 38.4)	7.1 (4.5 to 11.1)	3.0 (2.2 to 4.1)	7.4 (4.9 to 11.2)	32.7 (13.9 to 77.0)	2.1 (1.7 to 2.7)
LR(–) (95% CI)	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.8)	0.6 (0.5 to 0.8)	0.6 (0.5 to 0.7)	0.7 (0.5 to 0.8)	0.6 (0.4 to 0.7)
3× Manufacturer's cut-off (U/mL)	60.0	15.0	18.0	42.0	90.0	60.0
Specificity (95% CI)	99.6% (98.7 to 100)	98.2% (96.7 to 99.1)	95.3% (93.1 to 96.9)	99.5% (98.4 to 99.9)	99.5% (98.4 to 99.9)	84.6% (81.2 to 87.5)
Sensitivity (95% CI)	24.3% (14.8 to 36.0)	32.9% (22.1 to 45.1)	38.6% (27.2 to 51.0)	30.0% (19.6 to 42.1)	22.9% (13.7 to 34.4)	48.6% (36.4 to 60.8)
LR(+) (95% CI)	66.8 (15.8 to 283.0)	18.1 (9.0 to 36.4)	8.2 (5.1 to 13.2)	55.0 (16.8 to 179.7)	41.9(12.5 to 140.2)	3.1 (2.3 to 4.3)
LR(–) (95% CI)	0.8 (0.7 to 0.9)	0.7 (0.6 to 0.8)	0.6 (0.5 to 0.8)	0.7 (0.6 to 0.8)	0.8 (0.7 to 0.9)	0.6 (0.5 to 0.8)
Cut-off at 98.5% specificity (U/mL)	21.5	18.0	59.0	23.2	27.2	NA
Sensitivity (95% CI)	37.1% (25.9 to 49.5)	30.0% (19.6 to 42.1)	27.1% (17.2 to 39.1)	37.1% (25.9 to 49.5)	35.7% (24.6 to 48.1)	
LR(+) (95% CI)	25.5 (12.0 to 54.2)	20.6 (9.5 to 44.3)	18.7 (8.5 to 41.0)	25.5 (12.0 to 54.2)	24.6 (11.5 to 52.3)	
LR(–) (95% CI)	0.6 (0.5 to 0.8)	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.9)	0.6 (0.5 to 0.8)	0.7 (0.5 to 0.8)	
Cut-off at 95% specificity (U/mL)	10.3	6.5	15.7	15.1	NA	190.7
Sensitivity (95% CI)	42.9% (31.1 to 55.3)	37.1% (25.9 to 49.5)	38.6% (27.2 to 51.0)	44.3% (32.4 to 56.7)		41.4% (29.8 to 53.8)
LR(+) (95% CI)	8.7 (5.5 to 13.8)	7.6 (4.7 to 12.2)	7.6 (4.8 to 12.1)	9.0 (5.7 to 14.2)		8.4(5.3 to 13.4)
LR(–) (95% CI)	0.6 (0.5 to 0.7)	0.7 (0.6 to 0.8)	0.7 (0.5 to 0.8)	0.6 (0.5 to 0.7)		0.6 (0.5 to 0.8)

	Assay						
(B) ACPA	**Menarini**	**Thermo Fisher**	**Inova**	**Roche**	**Abbott**	**Euro Diagnostica**	**Euroimmun**
AUC (95% CI)	0.698 (0.660 to 0.734)	0.769 (0.734 to 0.802)	0.685 (0.647 to 0.722)	0.672 (0.634 to 0.709)	0.693 (0.655 to 0.729)	0.734 (0.698 to 0.769)	0.709 (0.671 to 0.744)
Manufacturer's cut-off (U/mL)	5.0	10.0	5.3	17.0	5.0	25.0	5.0
Specificity (95% CI)	98.9% (97.6 to 99.6)	99.1% (97.9 to 99.7)	97.1% (95.3 to 98.3)	97.3% (95.5 to 98.5)	98.7% (97.4 to 99.5)	99.3% (98.1 to 99.8)	97.6% (96.0 to 98.7)
Sensitivity (95% CI)	38.6% (27.2 to 51.0)	37.1% (25.9 to 49.5)	37.1% (25.9 to 49.5)	35.7% (24.6 to 48.1)	38.6% (27.2 to 51.0)	34.3% (23.3 to 46.6)	41.4% (29.8 to 53.8)
LR(+) (95% CI)	35.4 (15.1 to 82.6)	40.9 (16.2 to 103.0)	12.8 (7.2 to 22.6)	13.1 (7.3 to 23.6)	30.3 (13.7 to 67.0)	47.1 (16.8 to 131.9)	17.5 (9.6 to 32.1)
LR(–) (95% CI)	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.8)	0.7 (0.5 to 0.8)	0.7 (0.6 to 0.8)	0.6 (0.5 to 0.7)	0.7 (0.6 to 0.8)	0.6 (0.5 to 0.7)
3× Manufacturer's cut-off (U/mL)	15.0	30.0	15.9	51.0	15.0	75.0	15.0
Specificity (95% CI)	99.5% (98.4 to 99.9)	99.5% (98.4 to 99.9)	98.7% (97.4 to 99.5)	98.9% (97.6 to 99.6)	99.5% (98.4 to 99.9)	99.5% (98.4 to 99.9)	99.1% (97.9 to 99.7)
Sensitivity (95% CI)	34.3% (23.3 to 46.6)	32.9% (22.1 to 45.1)	34.3% (23.3 to 46.6)	32.9% (22.1 to 45.1)	34.3% (23.3 to 46.6)	27.1% (17.2 to 39.1)	34.3% (23.3 to 46.6)
LR(+) (95% CI)	62.9 (19.4 to 203.4)	60.2 (18.6 to 195.5)	26.9 (12.1 to 60.2)	30.1 (12.7 to 71.4)	62.9 (19.4 to 203.4)	49.8 (15.1 to 163.9)	37.7 (14.9 to 95.7)
LR(–) (95% CI)	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.8)
Cut-off at 98.5% specificity (U/mL)	3.1	4.9	15.1	38.1	2.9	10.4	7.6
Sensitivity (95% CI)	38.6% (27.2 to 51.0)	38.6% (27.2 to 51.0)	34.3% (23.3 to 46.6)	32.9% (22.1 to 45.1)	40.0% (28.5 to 52.4)	37.1% (25.9 to 49.5)	38.6% (27.2 to 51.0)
LR(+) (95% CI)	26.5 (12.5 to 56.1)	26.5 (12.5 to 56.1)	23.6 (11.0 to 50.4)	22.6 (10.5 to 48.5)	27.5 (13.1 to 57.9)	25.5 (12.0 to 54.2)	26.5 (11.3 to 55.2)
LR(–) (95% CI)	0.6 (0.5 to 0.8)	0.6 (0.5 to 0.8)	0.7 (0.6 to 0.8)	0.7 (0.6 to 0.8)	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.8)	0.6 (0.5 to 0.8)

AUC, sensitivity, specificity, positive (LR(+)) and negative likelihood ratios (LR(–)) with their 95% CI are given for the different RF (A) and ACPA (B) assays.
ACPA, anti-cyclic citrullinated protein/peptide antibodies; AUC, area under the ROC curve; NA, not applicable; RF, rheumatoid factor.

Table 3 Likelihood ratios for RF and ACPA and a combination thereof according to the serological 2010 ACR/EULAR RA classification criteria

LR	Menarini	Thermo Fisher	Inova	Roche	Abbott	Euroimmun	Euro Diagnostica
Manufacturer's cut-off RF/ACPA							
RF and ACPA negative (95% CI)	0.5 (0.4 to 0.6)	0.6 (0.5 to 0.7)	0.5 (0.4 to 0.7)	0.5 (0.4 to 0.7)	0.6 (0.4 to 0.7)	0.5 (0.4 to 0.7)	
RF or ACPA weakly positive (95% CI)	3.6 (1.3 to 10.0)	0.8 (0.2 to 3.1)	0.7 (0.3 to 1.6)	1.5 (0.7 to 3.3)	3.4 (0.9 to 12.8)	1.0 (0.5 to 1.8)	39.4
RF weakly positive (95% CI)	8.8 (3.7 to 20.8)	1.9 (0.7 to 4.8)	0.8 (0.4 to 1.8)	2.8 (1.5 to 5.3)	23.7 (6.6 to 85.3)	0.9 (0.4 to 1.8)	
ACPA weakly positive (95% CI)	7.9 (1.6 to 38.3)	11.8 (2.0 to 69.6)	1.8 (0.4 to 7.9)	1.8 (0.4 to 7.9)	5.9 (1.4 to 25.9)	4.9 (1.7 to 14.6)	39.4 (4.7 to 332.6)
RF or ACPA strongly positive (95% CI)	57.2 (20.7 to 157.8)	21.5 (11.3 to 41.0)	8.7 (5.7 to 13.3)	27.6 (13.1 to 58.2)	45.7 (18.3 to 114.3)	3.3 (2.5 to 4.5)	
RF and ACPA strongly positive (95% CI)	94.6 (12.5 to 716.7)	63.1 (14.8 to 268.6)	47.3 (14.3 to 156.6)	126.2 (17.0 to 936.9)	86.7 (11.4 to 661.8)	34.6 (13.5 to 88.4)	
RF strongly positive (95% CI)	67.0 (15.8 to 284.0)	18.1 (9.0 to 36.5)	8.2 (5.1 to 13.2)	55.2 (16.9 to 180.3)	42.1 (12.6 to 140.7)	3.1 (2.3 to 4.3)	
ACPA strongly positive (95% CI)	63.1 (19.5 to 204.1)	60.5 (18.6 to 196.2)	27.0 (12.1 to 60.4)	30.2 (12.7 to 71.7)	63.1 (19.5 to 204.1)	37.7 (14.9 to 95.7)	50.0 (15.2 to 164.5)
Cut-off at 98.5% specificity RF/ACPA							
RF and ACPA negative (95% CI)	0.5 (0.4 to 0.6)	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.7)	0.6 (0.5 to 0.7)	0.5 (0.4 to 0.7)	NA	
RF or ACPA weakly positive (95% CI)	6.8 (2.3 to 19.5)	0.8 (0.1 to 6.1)	6.3 (2.6 to 15.5)	3.0 (1.1 to 8.3)	3.0 (0.8 to 10.9)	NA	
RF weakly positive (95% CI)	13.1 (4.9 to 35.1)	10.5 (3.8 to 29.4)	9.0 (3.4 to 24.1)	9.0 (3.4 to 24.1)	11.0 (3.6 to 33.8)	NA	
ACPA weakly positive (95% CI)	15.8 (2.9 to 84.5)	2.0 (0.2 to 17.4)	15.8 (4.9 to 51.0)	2.6 (0.5 to 12.8)	6.3 (1.7 to 22.9)	6.3 (1.7 to 22.9)	3.2 (0.6 to 16.0)
RF or ACPA strongly positive (95% CI)	32.7 (14.9 to 71.8)	44.2 (17.6 to 110.6)	27.6 (11.5 to 66.0)	71.0 (22.1 to 227.9)	47.3 (19.0 to 118.0)	NA	
RF and ACPA strongly positive (95% CI)	86.7 (11.4 to 661.8)	86.7 (11.4 to 661.8)	NA	94.6 (12.5 to 716.8)	94.6 (12.5 to 716.8)	NA	
RF strongly positive (95% CI)	63.1 (14.8 to 268.6)	51.3 (11.8 to 222.4)	86.7 (11.4 to 661.8)	141.9 (19.2 to 1047.0)	47.3 (14.3 to 156.6)	NA	
ACPA strongly positive (95% CI)	31.5 (13.4 to 74.5)	51.3 (18.4 to 142.6)	25.2 (9.5 to 66.8)	55.2 (16.9 to 180.3)	63.1 (19.5 to 204.1)	60.0 (18.5 to 194.8)	47.3 (17.0 to 132.4)
Cut-off at 95.0% specificity RF/ACPA							
RF and ACPA negative (95% CI)	NA	0.5 (0.4 to 0.7)	0.6 (0.4 to 0.7)	NA	NA	0.5 (0.4 to 0.7)	
RF or ACPA weakly positive (95% CI)	NA	1.4 (0.6 to 3.2)	0.9 (0.3 to 2.5)	NA	NA	2.1 (1.2 to 3.8)	
RF weakly positive (95% CI)	2.2 1.0 to 4.9	2.1 (0.8 to 5.4)	2.1 (0.8 to 5.4)	9.0 (3.4 to 24.1)	NA NA	8.1 5.2 to 12.8)	
ACPA weakly positive (95% CI)	NA	1.1 (0.3 to 3.7)	1.8 (0.6 to 5.0)	NA	6.3 (1.7 to 22.9)	1.9 (0.7 to 4.8)	0.4 (0.1 to 3.1)
RF or ACPA strongly positive (95% CI)	NA	16.3 (9.1 to 29.4)	13.1 (7.7 to 22.3)	NA	NA	28.1 (12.6 to 62.5)	
RF and ACPA strongly positive (95% CI)	NA	149.8 (20.4 to 1102.1)	126.2 (17.0 to 936.9)	NA	NA	NA	
RF strongly positive (95% CI)	60.5 (18.6 to 196.2)	20.7 (9.5 to 45.0)	19.3 (9.3 to 40.2)	141.9 (19.2 to 1047.0)	NA	NA	
ACPA strongly positive (95% CI)	NA	30.3 (13.7 to 67.0)	18.9 (9.4 to 38.0)	NA	63.1 (19.5 to 204.1)	28.1 (12.6 to 62.5)	23.7 (11.6 to 48.2)

ACPA, anti-cyclic citrullinated protein/peptide antibodies; ACR, American College of Rheumatology; AUC, area under the ROC curve; EULAR, European League Against Rheumatism; LR, likelihood ratio; NA, not applicable; RA, rheumatoid arthritis; RF, rheumatoid factor.

Table 4 Overview of 2010 ACR/EULAR score of the included patients with RA in function of the laboratory test results

Score 2010 ACR/EULAR RA classification criteria	Total ≥6, n (% RA)	10	9	8	7	6	5	4	3	2
Clinical score (without laboratory findings)	32 (45.7)	0	0	0	0	32	0	29	6	3
Clinical score including inflammation*	32 (45.7)	0	0	0	24	8	26	8	4	0
Clinical score including RF test result										
Menarini RF	52 (74.3)	3	2	13	26	8	10	6	2	0
Thermo Fisher RF	53 (75.7)	4	3	16	20	10	9	6	2	0
Inova RF	54 (77.1)	7	2	17	19	9	9	5	2	0
Roche RF	53 (75.7)	4	4	16	21	8	9	6	2	0
Abbott RF	49 (70.0)	3	2	12	25	7	12	7	2	0
Euroimmun RF	58 (82.9)	6	7	21	17	7	8	3	1	0
Clinical score including ACPA test result										
Menarini ACPA	50 (71.4)	6	3	12	21	8	12	6	2	0
Thermo Fisher ACPA	50 (71.4)	5	3	12	22	8	12	6	2	0
Inova ACPA	50 (71.4)	6	2	12	22	8	12	6	2	0
Roche ACPA	50 (71.4)	5	2	12	23	8	12	6	2	0
Abbott ACPA	50 (71.4)	6	3	12	21	8	12	6	2	0
Euroimmun ACPA	50 (71.4)	5	5	13	19	8	12	6	2	0
Euro Diagnostica ACPA	49 (70.0)	3	4	10	24	8	13	6	2	0
Clinical score including RF and ACPA test result										
Menarini RF/ACPA	55 (78.6)	7	2	16	22	8	9	5	1	0
Thermo Fisher RF/ACPA	56 (80.0)	6	2	17	22	9	8	5	1	0
Inova RF/ACPA	58 (82.9)	8	3	19	19	9	7	4	1	0
Roche RF/ACPA	56 (80.0)	5	4	17	22	8	6	5	1	0
Abbott RF/ACPA	53 (75.7)	7	2	16	20	8	11	5	1	0
Euroimmun RF/ACPA	59 (84.3)	7	8	21	15	8	7	3	1	0

*C-reactive protein analysis (Cobas 6000 (Roche, Mannheim, Germany); positive test result >5 mg/L); sedimentation rate (Sedivette S 2200 (Desaga, Wiesloch, Germany); positive test result female >8 mm, male >11 mm).
ACPA, anti-cyclic citrullinated protein/peptide antibodies; ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; RA, rheumatoid arthritis; RF, rheumatoid factor.

and Roche are traceable to either W1066 or NIBSC 64/002 and gave comparable results close to the target value of 25 IU/mL (confirming good analytical accuracy). Despite traceability to NIBSC 64/002, the Inova RF assay revealed higher results and poor agreement with the other RF IgM assays (indicating poor analytical accuracy). Euroimmun did not mention traceability and results obtained with this assay differed from results obtained with the other assays.

Differences in numerical values between results obtained with different assays were further stressed by Bland-Altman analysis and regression analysis. Even assays calibrated against the same reference material do not give comparable results. These data illustrate a lack of harmonisation in RF testing with quantitative differences between assays. Test results cannot be used interchangeably. Although a reference serum for RF has been available since 1968,[18] standardisation of RF determination across companies has not yet been achieved.

Even though RF assays from Thermo Fisher, Roche, Menarini and Abbott are traceable to the same reference material and give comparable results for W1066 (around 20 IU/mL), they apply totally different cut-off values, respectively, below (5 and 14 IU/mL), on (20 IU/mL) or above (30 IU/mL) the value for W1066. Consequently, sensitivity, specificity and LRs differed among assays. For example, Abbott RF had a particularly high specificity, but a low sensitivity. This again highlights a lack of harmonisation of test result interpretation. Since the 2010 ACR/EULAR criteria take RF and ACPA positivity into account, cut-off values should be aligned among companies, for example, by defining cut-offs based on a predefined specificity in disease controls (eg, 95%).

None of the ACPA assays tested were traceable to an international standard. This lack of standardisation between the different assays obviously led to substantial dispersion in numerical test results when the CDC reference serum was tested. Such differences have previously been reported.[19] Euro Diagnostica and Roche ACPA assays gave much higher numerical values than the other assays, which is related to the fact that Roche calibrated its assay against Euro Diagnostica. Alignment of cut-off values across companies could be improved.

As our study revealed differences in clinical performance between different RF and ACPA assays, we evaluated whether such differences have an impact on disease classification based on the 2010 ACR/EULAR criteria. Indeed, we found that for some patients disease classification depended on the RF and/or ACPA assay used. This further illustrates the need to align clinical interpretation of test results between companies. Correct classification and diagnosis is important to initiate adequate treatment and to exclude self-limiting arthritis and avoid inappropriate treatment.[5 27]

The 2010 RA classification criteria give a score of 2 for a low-positive RF or ACPA and of 3 for a high-positive RF or ACPA. Our study revealed that the LR for RA of a high positive RF or ACPA test result (varying between 3.3 and 57, depending on the assay) was clearly higher than the LR of a low-positive RF or ACPA result (varying between 0.7 and 3.7). As previously pointed out,[28] future improvements of the RA classification

criteria should consider to give a higher relative weight to a high-positive RF or ACPA result compared with a low-positive RF or ACPA result.

In our study, LR for a negative test result was high (ranging from 0.5 to 0.6), indicating that a negative test result for both RF and ACPA does not exclude RA. By contrast, a strong positive RF or ACPA test had an LR >10 for most (but not all) assays. Such result has a significant effect on post-test probability.[29]

The prevalence of RF (36%–57%) and ACPA (32%–41%) in the RA population was lower than 60%, which is widely considered the sensitivity of RF and ACPA for RA.[2] We hypothesise that the low seropositivity in our study is related to the inclusion of older patients and of patients with early arthritis. First, 56% of the patients with RA included were >70 years. Elderly patients with RA are typically seronegative,[30 31] have a milder disease course and are referred to a secondary care hospital setting. If patients >65 years old were excluded, seropositivity increased to 45%–50% for RF and 45%–52% for ACPA, which is comparable to previous reports (48.5% for RF and 49% for ACPA).[32] Second, 57.8% of the patients with RA included were patients with early RA (less than 3 months' symptom duration before RA diagnosis). Although not statistically significant, there was a trend to lower RF/ACPA positivity in patients with early RA compared with the patients with established RA, as previously reported.[11 32 33] A higher prevalence of ACPA was found in patients with RA with erosive disease, confirming the prognostic value of ACPA.[34 35] We could not confirm the better diagnostic performance of CCP3 in early arthritis.[15]

All included patients with RA fulfilled either the 1987 or the 2010 ACR/EULAR criteria, with a higher proportion of patients fulfilling the 1987 criteria (95.7%) rather than the 2010 criteria (80.0%). This was an unexpected finding as the 2010 ACR/EULAR criteria intended to increase diagnostic sensitivity.[7 27] This could be explained by the specific characteristics of the study population including many patients with oligoarthritis, given the fact that a seronegative patient can only fulfil the 2010 ACR/EULAR criteria when >10 joints are involved.[7]

In conclusion, we illustrated differences in technical and diagnostic performance between RF and ACPA assays from different manufacturers. There is a lack of harmonisation of RF and ACPA assays in terms of numerical values and diagnostic performance (sensitivity, specificity, LR). The differences in diagnostic performance can have an impact on 2010 ACR/EULAR criteria classification, which must be confirmed on a larger RA population.

Acknowledgements We thank Abbott, Euro Diagnostica, Euroimmun, Inova, Menarini, Thermo Fisher and Roche for the donation of the assays. We are very thankful to the laboratory technicians for their most appreciated efforts.

Competing interests XB has received lecture fees from Thermo Fisher, Inova and Menarini and has been a consultant for Inova.

Ethics approval OLV Hospital Ethics Committee (Belgian registration number of ethical approval B126601627018).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet* 2010;376:1094–108.
2 Smolen JS, Redlich K. Rheumatoid arthritis. In: Rose NR, Mackey IR, eds. *The Autoimmune Diseases*. 5 edn. USA: Elsevier, 2014:511–9.
3 van Dongen H, van Aken J, Lard LR, *et al*. Efficacy of methotrexate treatment in patients with probable rheumatoid arthritis: a double-blind, randomized, placebo-controlled trial. *Arthritis Rheum* 2007;56:1424–32.
4 van Nies JA, Krabben A, Schoones JW, *et al*. What is the evidence for the presence of a therapeutic window of opportunity in rheumatoid arthritis? A systematic literature review. *Ann Rheum Dis* 2014;73:861–70.
5 van Gaalen FA, Linn-Rasker SP, van Venrooij WJ, *et al*. Autoantibodies to cyclic citrullinated peptides predict progression to rheumatoid arthritis in patients with undifferentiated arthritis: a prospective cohort study. *Arthritis Rheum* 2004;50:709–15.
6 Arnett FC, Edworthy SM, Bloch DA, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315–24.
7 Aletaha D, Neogi T, Silman AJ, *et al*. Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis Rheum* 2010;2010:2569–81.
8 Shmerling RH, Delbanco TL. The rheumatoid factor: an analysis of clinical utility. *Am J Med* 1991;91:528–34.
9 Vander Cruyssen B, Peene I, Cantaert T, *et al*. Anti-citrullinated protein/peptide antibodies (ACPA) in rheumatoid arthritis: specificity and relation with rheumatoid factor. *Autoimmun Rev* 2005;4:468–74.
10 Avouac J, Gossec L, Dougados M. Diagnostic and predictive value of anti-cyclic citrullinated protein antibodies in rheumatoid arthritis: a systematic literature review. *Ann Rheum Dis* 2006;65:845–51.
11 van Venrooij WJ, Zendman AJ, Pruijn GJ. Autoantibodies to citrullinated antigens in (early) rheumatoid arthritis. *Autoimmun Rev* 2006;6:37–41.
12 Vincent C, Nogueira L, Sebbag M, *et al*. Detection of antibodies to deiminated recombinant rat filaggrin by enzyme-linked immunosorbent assay: a highly effective test for the diagnosis of rheumatoid arthritis. *Arthritis Rheum* 2002;46:2051–8.
13 Schellekens GA, Visser H, de Jong BA, *et al*. The diagnostic properties of rheumatoid arthritis antibodies recognizing a cyclic citrullinated peptide. *Arthritis Rheum* 2000;43:155–63.
14 Trouw LA, Mahler M. Closing the serological gap: promising novel biomarkers for the early diagnosis of rheumatoid arthritis. *Autoimmun Rev* 2012;12:318–22.
15 Swart A, Burlingame RW, Gürtler I, *et al*. Third generation anti-citrullinated peptide antibody assay is a sensitive marker in rheumatoid factor negative rheumatoid arthritis. *Clin Chim Acta* 2012;414:266–72.
16 National Institute for Biological Standards and Control (NIBSC). *Non WHO reference material rheumatoid arthritis serum, 1st British standard NIBSC code: 64/002 Version 6.0*. Potters Bar, Hertfordshire: EN6 3QG WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory (Dated 04 Apr 2008).
17 National Institute for Biological Standards and Control (NIBSC). *WHO Human Reference Reagent Rheumatoid Arthritis Serum, Standard NIBSC code: W1066 Version 2.0*. Potters Bar, Hertfordshire: EN6 3QG WHO International Laboratory for Biological Standards, UK Official Medicines Control Laboratory (Dated 04 Apr 2008).
18 Anderson SG, Bentzon MW, Houba V, *et al*. International reference preparation of rheumatoid arthritis serum. *Bull World Health Organ* 1970;42:311–8.
19 Coenen D, Verschueren P, Westhovens R, *et al*. Technical and diagnostic performance of 6 assays for the measurement of citrullinated protein/peptide antibodies in the diagnosis of rheumatoid arthritis. *Clin Chem* 2007;53:498–504.
20 Centers for disease control. *Human ACPA serum, Centers for disease control reference reagent for human reference serum for citrullinated peptide/protein antibodies*, 2008. Product package insert catalogue insert catalogue IS 2723 DIL:06/2008.
21 CLSI. *Evaluation of precision performance of quantitative measurement methods; approved guideline-second edition. CLSI document EP5-A2*. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute, 2004.
22 CLSI. *Evaluation of the linearity of quantitative measurement procedures: a statistical approach; Approved guideline. CLSI document EP6-A*. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute, 2003.
23 CLSI. *Protocols for determination of limits of detection and limits of quantification; Approved guideline. CLSI document EP17-A2*. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute, 2012.
24 CLSI. *User verification of precision and estimation of bias; approved guideline-third edition. CLSI document EP15-A3*. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute, 2014.
25 Haeckel R. Recommendations for definition and determination of carry-over effects. *J Automat Chem* 1988;10:181–3.
26 CLSI. *Method comparison and bias estimation using patient samples; approved guideline-Second edition. CLSI document EP9-A3*. Wayne, Pennsylvania: Clinical and Laboratory Standards Institute, 2013.
27 Vonkeman HE, van de Laar MA. The new European League Against Rheumatism/American College of Rheumatology diagnostic criteria for rheumatoid arthritis: how are they performing? *Curr Opin Rheumatol* 2013;25:354–9.
28 Bossuyt X. Anticitrullinated protein antibodies: taking into account antibody levels improves interpretation. *Ann Rheum Dis* 2017;76:e33.

29 Bossuyt X. Clinical performance characteristics of a laboratory test. A practical approach in the autoimmune laboratory. *Autoimmun Rev* 2009;8:543–8.
30 Deal CL, Meenan RF, Goldenberg DL, *et al*. The clinical features of elderly-onset rheumatoid arthritis. A comparison with younger-onset disease of similar duration. *Arthritis Rheum* 1985;28:987–94.
31 Tan TC, Gao X, Thong BY, *et al*. Comparison of elderly- and young-onset rheumatoid arthritis in an Asian cohort. *Int J Rheum Dis* 2017;20:737–45.
32 Nielen MM, van Schaardenburg D, Reesink HW, *et al*. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004;50:380–6.
33 Nordberg LB, Lillegraven S, Lie E, *et al*. Patients with seronegative RA have more inflammatory activity compared with patients with seropositive RA in an inception cohort of DMARD-naïve patients classified according to the 2010 ACR/EULAR criteria. *Ann Rheum Dis* 2017;76:341–5.
34 Meyer O, Labarre C, Dougados M, *et al*. Anticitrullinated protein/peptide antibody assays in early rheumatoid arthritis for predicting five year radiographic damage. *Ann Rheum Dis* 2003;62:120–6.
35 van der Heijde D, van der Helm-van Mil AH, Aletaha D, *et al*. EULAR definition of erosive disease in light of the 2010 ACR/EULAR rheumatoid arthritis classification criteria. *Ann Rheum Dis* 2013;72:479–81.

EXTENDED REPORT

Preference phenotypes to facilitate shared decision-making in rheumatoid arthritis

Liana Fraenkel,[1,2] W Benjamin Nowell,[3] George Michel,[1,2] Carole Wiedmeyer[3]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212407).

[1]School of Medicine, Yale University, New Haven, Connecticut, USA
[2]VA Connecticut Healthcare System, West Haven, Connecticut, USA
[3]CreakyJoints, Global Healthy Living Foundation, New York City, New York, USA

Correspondence to
Liana Fraenkel, Section of Rheumatology, Yale University School of Medicine, New Haven, CT 06510, USA;
liana.fraenkel@yale.edu

Received 18 September 2017
Revised 22 November 2017
Accepted 27 November 2017
Published Online First 15 December 2017

To cite: Fraenkel L, Nowell WB, Michel G, *et al*. *Ann Rheum Dis* 2018;**77**:678–683.

ABSTRACT

Objective Implementing treat-to-target (TTT) strategies requires that patients with rheumatoid arthritis (RA) and their rheumatologists decide on how best to escalate care when indicated. The objective of this study was to develop preference phenotypes to facilitate shared decision-making at the point of care for patients failing methotrexate monotherapy.

Methods We developed a conjoint analysis survey to measure the preferences of patient with RA for triple therapy, biologics and Janus kinase (JAK) inhibitors. The survey included seven attributes: administration, onset, bothersome side effects, serious infection, very rare side effects, amount of information and cost. Each choice set (n=12) included three hypothetical profiles. Preference phenotypes were identified by applying latent class analysis to the conjoint data.

Results 1273 participants completed the survey. A five-group solution was chosen based on progressively lower values of the Akaike and Bayesian information criteria. Members of the largest group (group 3: 38.4%) were most strongly impacted by the cost of the medication. The next largest group (group 1: 25.8%) was most strongly influenced by the risk of bothersome side effects. Members of group 2 (11.2%) were also risk averse, but were most concerned with the risk of very rare side effects. Group 4 (6.6%) strongly preferred oral over parenteral medications. Members of group 5 (18.0%) were most strongly and equally influenced by onset of action and the risk of serious infections.

Conclusions Treatment preferences of patients with RA can be measured and represented by distinct phenotypes. Our results underscore the variability in patients' values and the importance of using a shared decision-making approach to implement TTT.

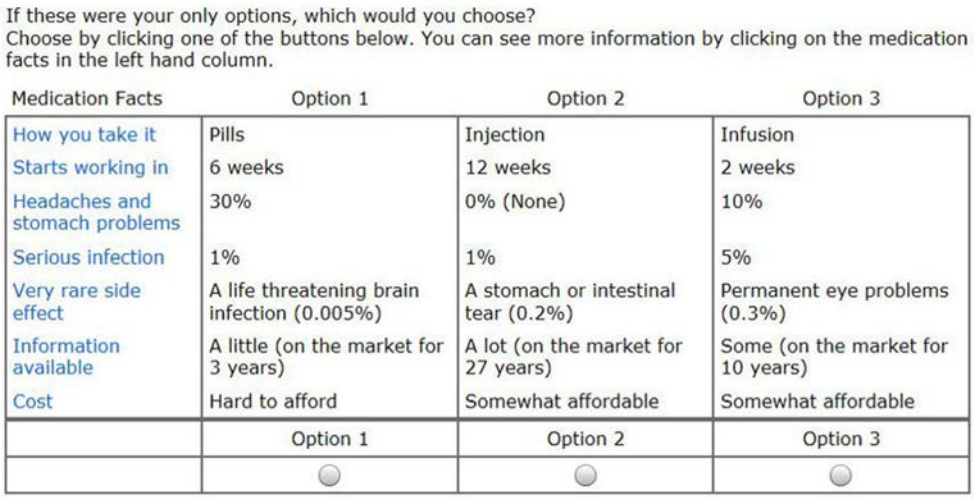
INTRODUCTION

Best practices for patients with rheumatoid arthritis (RA) call for patients to be treated to target (TTT). Adherence to this strategy requires ongoing disease activity monitoring and adjustments in treatment plans to attain, and subsequently maintain, a state of low disease activity or remission. TTT strategies are in large part possible because of the numerous effective treatment options currently available for patients with inflammatory arthritis. However, having many available options also paradoxically increases the difficulty of choosing how to adjust treatment.[1] Several studies have shown that increasing the number of options in a choice set significantly increases the difficulty of making a decision and increases the likelihood of deferral.[2 3] Indeed, asking physicians to help patients compare and contrast triple therapy, different biologics and JAK inhibitors, and to subsequently determine which option best fits with each patient's values and goals at the point of care is challenging. Consequently, patients are rarely effectively engaged in the decision-making process.[4]

Decision aids have been developed for several preference sensitive decisions in order to facilitate shared decision-making, and randomised controlled trials have proven them to be consistently effective in improving patients' knowledge, decreasing decisional conflict and, in some cases, improving patient participation in decision-making.[5] Despite these proven benefits, however, decision aids have not been effectively integrated into clinical practice, in large part due to time constraints.[6] To address this gap, we sought to develop a decision aid which rather than asking each physician–patient dyad to consider the numerous trade-offs involved in comparing all available options, presents a set of (rigorously derived and transparent) distinct preference phenotypes and asks patients to consider which best fits with their own values and goals. Asking patients to perform a matching task is a simpler cognitive task that may be better suited to decision-making at the point of care.

Conjoint analysis is a well-validated and widely used method to measure preferences. Originally developed to understand consumer preferences and predict market shares of innovative products, this approach is now recognised as a valuable means of assessing patient preferences for healthcare.[7–11] When faced with multiple alternatives, people make decisions by making trade-offs between the specific features of competing products. Conjoint analysis (CA) evaluates these trade-offs to determine which combination of attributes is most preferred by consumers. This approach assumes that each option is a composite of different characteristics, and that each characteristic represents one of a number of levels. Levels refer to the range of estimates for each characteristic. Respondents do not evaluate treatment alternatives directly. Rather, preferences are calculated based on how participants value differences between competing options. Answers to respondent-specific questions (see figure 1) allow the investigator to calculate values for specific treatment characteristics and to predict which option most closely suits each participant's individual preferences.

Shared decision-making is a key element of TTT because patients with the same level of disease activity have varying treatment preferences. Preference heterogeneity can be systematically examined via stratification or segmentation. Stratification


If these were your only options, which would you choose?
Choose by clicking one of the buttons below. You can see more information by clicking on the medication facts in the left hand column.

Medication Facts	Option 1	Option 2	Option 3
How you take it	Pills	Injection	Infusion
Starts working in	6 weeks	12 weeks	2 weeks
Headaches and stomach problems	30%	0% (None)	10%
Serious infection	1%	1%	5%
Very rare side effect	A life threatening brain infection (0.005%)	A stomach or intestinal tear (0.2%)	Permanent eye problems (0.3%)
Information available	A little (on the market for 3 years)	A lot (on the market for 27 years)	Some (on the market for 10 years)
Cost	Hard to afford	Somewhat affordable	Somewhat affordable
	Option 1	Option 2	Option 3
	○	○	○

Figure 1 Example of a choice task.

separates study participants into homogeneous groups based on observed characteristics (eg, demographics) and estimates either separate models or separate sets of coefficients for each strata. Stratification assumes that preference heterogeneity can be accurately determined a priori by observed variables; however, little empirical data support this assumption.[12 13] In contrast, segmentation clusters respondents into groups based on unobserved/latent characteristics. Segmentation of conjoint data allows one to subdivide a large population into meaningful groups that are similar within themselves but statistically different from other groups. This approach has been successfully used to reveal varying patterns of preferences for public health interventions and drug development.[14–18]

The objective of this study was to develop a set of distinct preference phenotypes for use at the point of care by applying latent class analysis to preference data collected in a large group of patients with RA in order to identify groups of patients whose values and preferences are similar to each other but distinct from other groups.

Table 1 Attributes and levels

Attributes	Levels
Route of administration	Pills
	Injection
	Infusion
Onset of action	2 weeks
	6 weeks
	12 weeks
Bothersome side effects	0%
	10%
	30%
Serious infection	1%
	3%
	5%
Very rare side effects	Stomach or intestinal tear (0.2%)
	Neurological disease like multiple sclerosis (0.05%)
	Permanent eye problems (0.3%)
	Life-threatening brain infection (0.005%)
Amount of information available	A lot (on the market for 27 years)
	Some (on the market for 10 years
	A little (on the market for 3 years)
Cost	Easy to afford
	Somewhat affordable
	Hard to afford

METHODS

Participants and recruitment procedures

To be eligible, participants had to be at least 18 years of age (21 in Puerto Rico), speak English or Spanish, live in the USA or Puerto Rico, report having a diagnosis of RA made by a physician, and be taking one or more disease-modifying antirheumatic drugs (DMARDs) and/or a biologic or JAK inhibitor. Those reporting being employed by pharmaceutical or insurance industries were excluded from the English language sample.

English-speaking participants were recruited via email invitations to CreakyJoints (https://creakyjoints.org/) members who had previously identified themselves as having a diagnosis of RA. CreakyJoints is a large arthritis patient network of approximately 55 000 patients in all 50 states. At the time of enrolment (approximately January 2016), about two-thirds of CreakyJoints members had RA. Among the CreakyJoints population who received the survey invitation and for whom demographic information was known, 90% were female, 80% were white and the average age was 51 (SD 12). The most common conditions in the CreakyJoint community at the time of the survey were RA (67%), osteoarthritis (41%), osteoporosis (13%), psoriatic arthritis (11%) and ankylosing spondylitis (9%).

Spanish-speaking participants were recruited in Spanish through a combination of Facebook ads on RA-related Facebook pages and email invitations to Spanish-speaking patients with RA through third-party respondent panel providers. Two research survey companies (Research Now and Market Cube) targeted US residents of the 50 states plus Washington D.C. and Puerto Rico who had previously reported receiving a diagnosis of RA and were Spanish speakers. They also targeted Spanish speakers in the same regions with no known history of arthritis, and screened for diagnosis of RA. All CreakyJoints members and panellists agreeing to participate in the study were provided a unique survey link that allowed them to take the survey one time. Respondents recruited via Facebook were directed to a sign-up page on CreakyJoints.org and then emailed a unique link to participate in the survey. Since it was possible that an individual could sign up with multiple email addresses to receive

more than one invitation/link, we de-duplicated by name and address. If someone took the survey twice, which occurred in two instances, we removed the duplicate response.

Survey development

The survey was designed to enable patients to differentiate between triple therapy, biologics and JAK inhibitors (ie, options commonly considered after failing methotrexate monotherapy). An initial set of attributes and levels were developed by a rheumatologist (LF) in consultation with a patient partner (CW). The preliminary list of attributes and levels was subsequently revised based on feedback obtained during a focus group with 10 patients with RA. We ultimately included seven attributes: route of administration, onset of action, bothersome side effects, serious infection, very rare side effects, amount of information and cost. Magnitude of benefit was not included as an attribute because efficacy was assumed to be equal across all options and thus not influence choice. Levels are listed in table 1, and detailed descriptions of each level provided to participants at the beginning of the survey are included in the online supplementary appendix. We also included an instructional video which demonstrated how to complete the conjoint questions. We performed cognitive interviews with 10 patients with RA and revised the survey wording and instructions based on their feedback prior to study launch. After the initial English version was fielded, the survey was translated into Spanish and then backtranslated into English. The survey was programmed and administered using Sawtooth Software.

We used the software's complete enumeration strategy to construct random choice sets. The complete enumeration method ensures that (1) each level is shown as few times as possible in a single task, (2) each level is shown approximately an equal number of timesacross the choice tasks and (3) the level of one characteristic is chosen independently of the levels of other characteristics. Each subject answered 12 random choice sets. An example of one of the choice sets is provided in figure 1. In addition, we included a fixed task in which the investigators defined the options in the choice set in order to gauge respondents' attention to the task. We also collected demographic and clinical characteristics. Participants were offered $25.00 after completing the survey.

Analyses

To examine the impact of each attribute on respondents' preferences, we divided the range of utilities for each attribute by the sum of the ranges and multiplied by 100. Latent class analysis was used to classify subjects into mutually exclusive categories based on how they valued each medication characteristic.[19] Class solutions were replicated five times from random starting seeds. A five-group solution was chosen based on progressively lower values of the Akaike and Bayesian information criteria.

Table 2 Aggregate relative importances

Attribute	Mean (SD) relative importances
Cost	24.66 (13.46)
Bothersome side effects	20.73 (10.35)
Very rare side effects	13.66 (9.03)
Onset of action	11.50 (7.16)
Serious infection	11.01 (6.68)
Route of administration	10.66 (8.60)
Time on the market	7.78 (4.79)

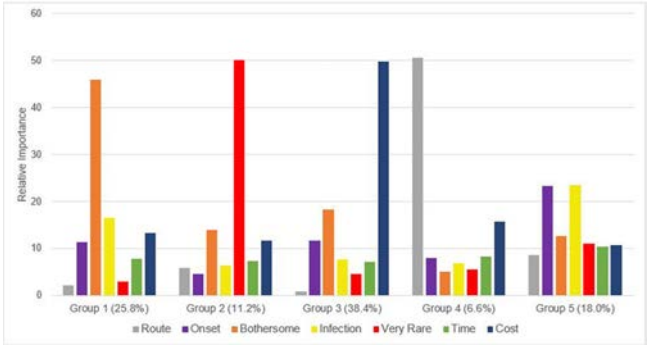


Figure 2 Relative importances per cluster. Grey: route of administration. Purple: onset of action. Orange: bothersome side effects. Yellow: risk of serious infection. Red: risk of serious, but rare side effect. Green: time available on the market. Dark blue: cost (affordability).

We used Sawtooth Software's simulator to estimate preferences for four illustrative sets of treatment choices:

1. Triple therapy (onset of action 12 weeks, 30% risk of bothersome side effects, 1% risk of serious infection, risk of permanent eye problems (0.3%), easy to afford) versus a subcutaneous anti-tumor necrosis inhibitor (TNF) biologic (onset of action 12 weeks, 10% risk of bothersome side effects, 3% risk of a serious infection, very rare risk of a neurological disease like multiple sclerosis (0.05%), hard to afford). Both options were described as having a lot of information available about them.
2. We then reran the preceding model but decreased the risk of bothersome side effects associated with triple therapy to 20%.
3. In a third simulation, we decreased the cost of the anti-TNF biologic from 'hard' to 'somewhat' affordable.
4. Lastly, we estimated preference for an infusion (no risk of bothersome side effects, 3% risk of serious infection, risk of a life-threatening brain infection (0.005%), on the market for 10 years) versus a JAK Inhibitor (pills, 10% risk of bothersome side effects, 5% risk of serious infection, risk of stomach or intestinal tear (0.2%), on the market for 5 years).

Treatment preferences were generated using the randomised first choice model in which utilities are summed across the levels corresponding to each option and then exponentiated and rescaled so that they sum to 100. This model is based on the assumption that participants prefer the option with the highest utility (or value). The randomised first choice model accounts for the error in the point estimates of the utilities as well as the variation in each respondent's total utility for each option and has been shown to have better predictive ability than other models.[20]

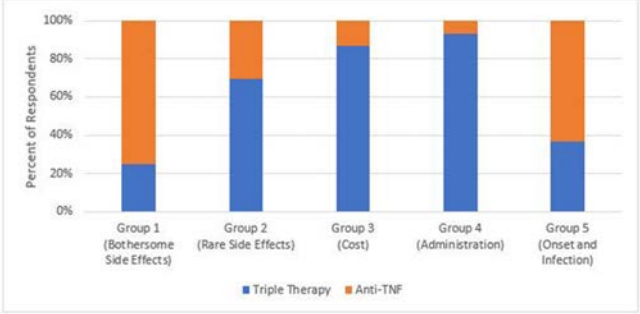


Figure 3 Preferences for triple therapy versus subcutaneous anti-TNF. Blue: preference for triple therapy. Orange: preference for anti-TNF.

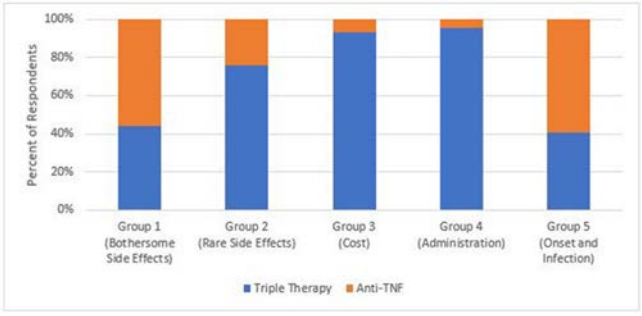


Figure 4 Preferences for lower risk triple therapy versus subcutaneous anti-TNF. Blue: preference for triple therapy. Orange: preference for anti-TNF.

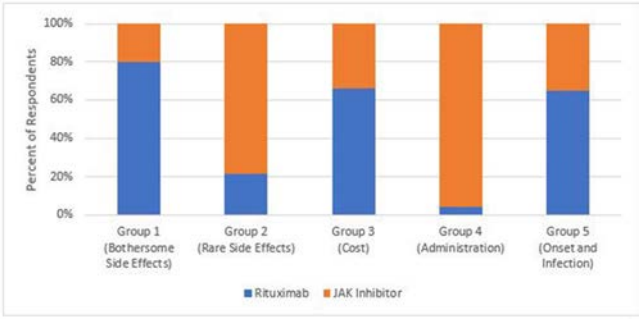


Figure 6 Preferences for rituximab versus JAK inhibitor. Blue: preference for rituximab. Orange: preference for JAK inhibitor.

We examined associations between patient characteristics with group membership using analysis of variance and χ^2 tests for continuous and categorical variable respectively. The study was approved by the Yale Human Studies Research Program.

RESULTS

Participants

A total of 1101 participants completed the survey in English. Of these, 42 were eliminated because they completed the survey in under 10 min and an additional 52 people were excluded because they did not respond correctly to the attention check task. Four hundred and twenty-one participants completed the survey in Spanish. Of these, 66 were eliminated because they completed the survey too quickly and an additional 89 people were excluded because they did not respond correctly to the attention check task.

The mean (SD) age of the study population (n=1273) was 50.7 (11.7). Participants' age ranged from 18 to 87 years. The majority were female (89.6%) and Caucasian (91.5%). Twenty-four per cent were Hispanic. About half (51.9%) were college graduates, 46.3% reported an annual household income of $60K or greater, 50.5% were employed and 22.6% reported being on disability. All but 3.4% reported having either private or government insurance. The mean (SD) patient global score was 4.6 (2.3), and 46.3% reported having a fair or poor overall health status.

Relative importance of each attribute

Table 2 lists the aggregate relative importance of each attribute. Given the levels included in the survey, affordability had the greatest impact on decision-making followed by the probability of bothersome side effects. Variability in relative importances across clusters is illustrated in figure 2. Members of the largest group (group 3: 38.4%) were most strongly impacted by the cost of the medication. The next largest group (group 1: 25.8%) was most strongly influenced by the risk of bothersome side effects. Members of group 2 (11.2%) were also risk averse, but were most concerned with the risk of very rare side effects. Members of the smallest group (group 4: 6.6%) strongly prioritised avoiding parenteral medications over the other medication characteristics included in the survey. Members of group 5 (18.0%) were most strongly and equally influenced by onset of action and the risk of serious infections.

Illustrative examples of how the five preference phenotypes are related to treatment preference are provided in figures 3–6. Preferences for triple therapy versus a subcutaneous anti-TNF biologic are described in figure 3. Triple therapy is preferred by the majority of participants in groups 2, 3 and 4. In contrast, those prioritising avoiding bothersome side effects (group 1) and rapid onset of action (group 5) prefer the subcutaneous anti-TNF. Biologics remain the preferred option for groups 1 and 5 even when the risk of bothersome side effects associated with triple therapy is decreased (figure 4). If, however, the cost of anti-TNFs is assumed to be 'somewhat' instead of 'hard to afford', biologics become the preferred option in members of group 3 (who are most concerned with cost) (figure 5). The impact of varying patient values on treatment preference is further illustrated in figure 6 which describes preferences for rituximab versus a JAK Inhibitor. Members of group 4 who prioritise route of administration (specifically strongly preferring oral vs parenteral medications) and those of group 2 who are most concerned with the risk of very rare side effects (specifically are much less worried about the risk of an intestinal tear compared with the remote risk of progressive multifocal leukoencephalopathy) strongly prefer a JAK Inhibitor, whereas the remainder would choose rituximab.

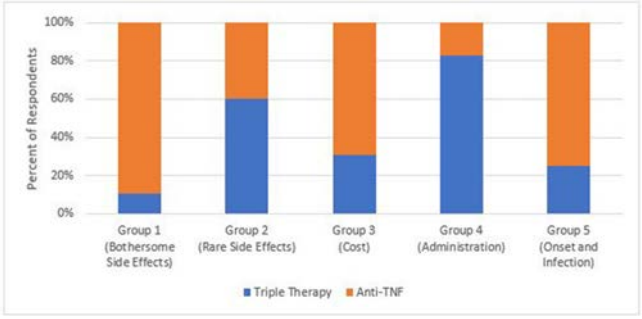


Figure 5 Preferences for triple therapy versus left costly subcutaneous anti-TNF. Blue: preference for triple therapy. Orange: preference for anti-TNF.

Associations between group membership and participant characteristics

We found no significant differences in age, education, employment status, income, overall self-reported health status or current biologic use across groups (data not shown)(table 3). Caucasians were less likely than non-Caucasians (6% vs 15%) while Hispanic subjects were more likely than non-Hispanics (12% vs 5%) to belong to group 4 (prioritised oral over parenteral treatment). Female participants were more likely than males to belong to group 3 (40% vs 29%) which prioritised cost, and less likely to belong to group 1 (25% vs 36%) which prioritised avoiding bothersome side effects. The patient global score was significantly higher in group 3 (prioritising cost) than in group 4 (prioritised oral treatment).

Table 3 Patient characteristics by group

Characteristic	Group 1 (n=329) n (%)	Group 2 (n=142) n (%)	Group 3 (n=489) n (%)	Group 4 (n=84) n (%)	Group 5 (n=229) n (%)	P value
Female (n=1140)	281 (85)	129 (91)	451 (92)	80 (95)	199 (87)	0.006
Caucasian (n=1165)	307 (93)	130 (92)	455 (93)	68 (81)	205 (90)	0.003
Hispanic (n=311)	86 (26)	49 (35)	74 (15)	36 (43)	66 (29)	<0.0001
Private insurance (n=873)	245 (75)	86 (61)	328 (67)	57 (68)	157 (69)	0.04
Patient global (mean, SD)*	4.6 (2.3)	4.4 (2.3)	4.8 (2.1)	4.0 (2.2)	4.6 (2.4)	0.02

*Tukey's procedure demonstrated that the only paired comparison reaching statistical significance (P<0.05) was between groups 3 and 4.

DISCUSSION

In this study, we found that treatment preferences of patients with RA can be measured and represented by distinct phenotypes. Our results underscore the variability in patients' values and the importance of using a shared decision-making approach to implement TTT. Presenting patients with a range of phenotypes can facilitate shared decision-making by (1) emphasising that there is no single best option for patients with RA who continue to have moderate-to-high disease activity despite adequate trials of methotrexate, and (2) assisting them to clarify their concerns and preferences. For example, patients identifying with group 1 are most concerned with bothersome side effects. They were much less concerned about the route of administration and the risk of very rare complications. Thus, how best to escalate care for these patients should focus on options which differ in the probability, and type, of bothersome side effects. In contrast, patients identifying with group 2 are most concerned with very rare side effects, and for them sufficient time to differentiate between rare black box warnings and underscoring the low probability of these events would best address patients' specific information needs. As previously reported,[16 21] we found that a small group of patients are reluctant to consider parenteral treatment. For some patients identifying with group 4, a specific educational session with a nurse regarding parenteral therapy may be helpful prior to making a treatment decision whereas for others focusing on triple therapy or a JAK Inhibitor would be most appropriate. Patients with concerns matching those of group 5 are those most concerned with onset of action and the risk of serious infections. An efficient personalised approach to shared decision-making for these patients could focus on the how triple therapy, biologics and JAK Inhibitors differ across these two characteristics.

Despite being a mostly insured population, cost had the strongest influence on treatment preferences, with group 3 being the largest cluster. Concerns over deductibles and expectations related to future cost increases are pervasive among patients with RA. The importance attributed to cost highlights the need for rheumatologists to present comparative cost data to patients when discussing therapeutic alternatives. Unfortunately, out-of-pocket expenses differ from patient to patient (even among those with the same insurance plans) and obtaining these estimates at the point of care is generally not feasible.

While we used robust methods to measure preferences and elicited input from a large RA population (including representation of Spanish-speaking patients), there are also important limitations to this study. Patients recruited through a research panel and an online arthritis community do not represent a population-based sample. Moreover, diagnosis was ascertained based on self-report of RA and current use of a DMARD and/or biologic, and was not confirmed by medical record or claims data. In addition, our sample included few African American patients with RA. The attributes and levels included in the study were chosen to reflect a broad range of medication characteristics; still, the results can only be generalised to those included in the survey. The impact of cost, for example, would not be expected to be relevant to patients whose co-pays are affordable and do not differ across options. In addition, cost is likely to be of much less importance to patients outside of the USA with effective and stable drug coverage.

In summary, we developed distinct RA preference phenotypes by applying latent class analyses to conjoint data generated by a large number of English and Spanish-speaking patients with RA. Preferences examined in this study include those available to patients with RA who have failed methotrexate monotherapy and are eligible for escalation to triple therapy, biologics or JAK Inhibitors. Future research will examine the feasibility of implementing a decision aid incorporating these phenotypes at the point of care.

Acknowledgements We would like to thank all the patients who took the time to participate in this research project. We would also like to thank Seth Ginsberg and David Curtis for their continuous support.

Contributors All authors meet criteria for authorship.

Funding Research reported in this publication was supported by the Rheumatology Research Foundation Innovative Research Grant program and the National Institute of Arthritis and Musculoskeletal and Skin Diseases, part of the National Institutes of Health, under Award Number AR060231-06 (Fraenkel).

Competing interests None declared.

Ethics approval Yale Human Research Protection Program.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Raw deidentified will be shared once resulting manuscripts from this grant have been published.

REFERENCES

1 Schwartz B. *The paradox of choice why more is less. Revised edition*. New York: Harper Collins, 2016.
2 Iyengar SS, Lepper MR. When choice is demotivating: can one desire too much of a good thing? *J Pers Soc Psychol* 2000;79:995–1006.
3 Shah AM, Wolford G. Buying behavior as a function of parametric variation of number of choices. *Psychol Sci* 2007;18:369–70.
4 Oshima Lee E, Emanuel EJ. Shared decision making to improve care and reduce costs. *N Engl J Med* 2013;368:6–8.
5 Stacey D, Légaré F, Lewis K, *et al*. Decision aids for people facing health treatment or screening decisions. *Cochrane Database Syst Rev* 2017;4:Cd001431.
6 Gravel K, Légaré F, Graham ID. Barriers and facilitators to implementing shared decision-making in clinical practice: a systematic review of health professionals' perceptions. *Implement Sci* 2006;1:16.
7 Brett Hauber A, Fairchild AO, Reed Johnson F. Quantifying benefit-risk preferences for medical interventions: an overview of a growing empirical literature. *Appl Health Econ Health Policy* 2013;11:319–29.
8 Bridges JF. Stated preference methods in health care evaluation: an emerging methodological paradigm in health economics. *Appl Health Econ Health Policy* 2003;2:213–24.
9 Bridges JFP, Onukwugha E, Johnson FR, *et al*. Patient preference methods - A patient-centered evaluation paradigm. *ISPOR Connections* 2007;13:4–7.
10 Fraenkel L, Bodardus S, Wittnik DR, *et al*. Understanding patient preferences for the treatment of lupus nephritis with adaptive conjoint analysis. *Med Care* 2001;39:1203–16.

11 Ryan M, Farrar S. Using conjoint analysis to elicit preferences for health care. *BMJ* 2000;320:1530–3.
12 Iragüen P, de Dios Ortúzar J. Willingness-to-pay for reducing fatal accident risk in urban areas: an Internet-based Web page stated preference survey. *Accid Anal Prev* 2004;36:513–24.
13 Morey E, Greer Rossmann K. Using stated-preference questions to investigate variations in willingness to pay for preserving marble monuments: Classic heterogeneity, random parameters, and mixture models. *J Cult Econ* 2003;27:215–29.
14 Cunningham CE, Bruce BS, Snowdon AW, *et al*. Modeling improvements in booster seat use: a discrete choice conjoint experiment. *Accid Anal Prev* 2011;43:1999–2009.
15 Deal K. Segmenting patients and physicians using preferences from discrete choice experiments. *Patient* 2014;7:5–21.
16 Fraenkel L, Suter L, Cunningham CE, *et al*. Understanding preferences for disease-modifying drugs in osteoarthritis. *Arthritis Care Res* 2014;66:1186–92.
17 Waschbusch DA, Cunningham CE, Pelham WE, *et al*. A discrete choice conjoint experiment to evaluate parent preferences for treatment of young, medication naive children with ADHD. *J Clin Child Adolesc Psychol* 2011;40:546–61.
18 Fraenkel L, Lim J, Garcia-Tsao G, *et al*. Variation in treatment priorities for chronic hepatitis c: a latent class analysis. *Patient* 2016;9:241–9.
19 *CBC latent class technical paper sequim*. WA: Sawtooth Software, 2004. https://www.sawtoothsoftware.com/support/technical-papers/sawtooth-software-products/cbc-latent-class-technical-paper-2004 (accessed 8 Aug 2017).
20 Huber J, Orme B, Miller R. The value of choice simulators. In: Gustafsson A, Herrmann AF, eds. *Conjoint measurement*. 4th edn. New York: Springer, 2007:347–62.
21 Constantinescu F, Goucher S, Weinstein A, *et al*. Understanding why rheumatoid arthritis patient treatment preferences differ by race. *Arthritis Rheum* 2009;61:413–8.

EXTENDED REPORT

Incidence of hip and knee replacement in patients with rheumatoid arthritis following the introduction of biological DMARDs: an interrupted time-series analysis using nationwide Danish healthcare registers

René Lindholm Cordtz,[1,2] Samuel Hawley,[3] Daniel Prieto-Alhambra,[3,4] Pil Højgaard,[1,2] Kristian Zobbe,[1,2] Søren Overgaard,[5,6] Anders Odgaard,[7,8] Lars Erik Kristensen,[2] Lene Dreyer[1,2,8]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212424).

For numbered affiliations see end of article.

Correspondence to
Dr René Lindholm Cordtz, Center for Rheumatology and Spine Diseases, Rigshospitalet-Gentofte, Kildegaardsvej 28, Copenhagen, DK-2900, Denmark;
rene.lindholm.cordtz.03@regionh.dk

Received 22 September 2017
Revised 27 November 2017
Accepted 27 November 2017
Published Online First 15 December 2017

ABSTRACT

Objectives To study the impact of the introduction of biological disease-modifying anti-rheumatic drugs (bDMARDs) and associated rheumatoid arthritis (RA) management guidelines on the incidence of total hip (THR) and knee replacements (TKR) in Denmark.

Methods Nationwide register-based cohort and interrupted time-series analysis. Patients with incident RA between 1996 and 2011 were identified in the Danish National Patient Register. Patients with RA were matched on age, sex and municipality with up to 10 general population comparators (GPCs). Standardised 5-year incidence rates of THR and TKR per 1000 person-years were calculated for patients with RA and GPCs in 6-month periods. Levels and trends in the pre-bDMARD (1996–2001) were compared with the bDMARD era (2003–2016) using segmented linear regression interrupted by a 1-year lag period (2002).

Results We identified 30 404 patients with incident RA and 297 916 GPCs. In 1996, the incidence rate of THR and TKR was 8.72 and 5.87, respectively, among patients with RA, and 2.89 and 0.42 in GPCs. From 1996 to 2016, the incidence rate of THR decreased among patients with RA, but increased among GPCs. Among patients with RA, the incidence rate of TKR increased from 1996 to 2001, but started to decrease from 2003 and throughout the bDMARD era. The incidence of TKR increased among GPCs from 1996 to 2016.

Conclusion We report that the incidence rate of THR and TKR was 3-fold and 14-fold higher, respectively among patients with RA compared with GPCs in 1996. In patients with RA, introduction of bDMARDs was associated with a decreasing incidence rate of TKR, whereas the incidence of THR had started to decrease before bDMARD introduction.

INTRODUCTION

In uncontrolled or severe rheumatoid arthritis (RA), inflammation can lead to irreversible joint damage.[1–3] In end-stage joint damage, prosthetic replacement of the damaged joint is the only available treatment. A recent study from the UK showed that the accumulated burden of disease activity measured by the disease activity score with 28-joint count (DAS28) within the first 5 years following diagnosis predicted the need for major joint surgery, and the highest incidence rates of surgery were observed among patients with moderate and high disease activity.[3] These findings lend further support to the importance of early and aggressive treatment emphasised in current RA treatment guidelines.[4 5] In non-contemporary RA cohorts, it was found that more than 50% of patients required joint surgery during the course of their disease.[6 7]

The introduction of tumour necrosis factor-alpha inhibitors (TNFi) as the mainstay of biological DMARDs (bDMARDs) in the late 1990s has improved the treatment of RA and shown to halt radiographic progression and development of joint erosions,[1 8] but it is unclear if these properties translate into a decreased need for total joint replacements. Most studies have suggested a decrease in the incidence of joint surgery following introduction of bDMARDs,[9–15] but studies showing no changes or increased number of joint surgeries have also been published.[9 10 16 17]

If treatment with bDMARD reduces the need for joint replacements, this will likely change the cost-effectiveness of these drugs. Joint replacements are expensive procedures and carry the risk of potential adverse events; and risk estimates for complications are increased in patients with RA.[18]

Using data from national Danish healthcare registers, we aimed at investigating the possible impact of the introduction of bDMARDs and associated guidelines for TNFi treatment in Denmark in 2002 on the 5-year incidence rate of total hip replacements (THR) and total knee replacements (TKR) in patients with incident RA compared with general population comparators (GPCs).

PATIENTS AND METHODS

Study design

This is a register-based, nationwide interrupted time-series analysis,[19 20] investigating the impact of introduction of bDMARDs in Denmark for the treatment of RA on the 5-year incidence rate of THR and TKR. Study methods and results are reported in accordance with Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[21]

Setting

All Danish residents have a personal identification number consistent throughout all registers

To cite: Cordtz RL, Hawley S, Prieto-Alhambra D, *et al*. *Ann Rheum Dis* 2018;**77**:684–689.

making register-linkage possible. The study period was from 1 January 1996 to 31 May 2016.

Data sources

The Civil Registration System (CRS) captures all deaths and migrations among Danish residents.[22] From CRS, we obtained dates of birth, emigration and death for all patients. Furthermore, CRS was used for the procedure of matching patients with RA with GPCs.

The Danish National Patient Register (DNPR) includes information on all inpatient (1977) and outpatient (1995) visits at Danish hospitals and private clinics.[23] Discharge diagnoses have been registered in accordance with the International Classification of Diseases (ICD) 10th edition since 1994, and from 1996, surgeries have been coded in accordance with the Nordic Medico-Statistical Committee (NOMESCO) Classification. With every discharge, information is provided on up to 20 diagnoses. For descriptive purposes, we obtained information on comorbidities in DNPR for both study populations (see online supplementary table S1 for ICD-10 codes).

DANBIO is a nationwide Danish rheumatology register established in 2000 to monitor the development in use and efficacy of bDMARDs.[24] The DANBIO steering committee publishes annual reports available online.[25]

Study population

Patients with RA

Patients with RA were identified using DNPR. We included all incident patients who received a diagnosis of RA (ICD-10: M05-06) at a hospital department or private clinic specialised in rheumatology or general internal medicine in the period of 1 January 1996 to 31 May 2011. A study by Thygesen *et al* found a high positive predictive value of DNPR diagnoses for conditions included in the Charlson Comorbidity Index with the positive predictive value of 98% for connective tissue diseases, including RA.[26]

General population comparator

For each patient with RA identified, we matched with up to 10 persons from the general population of Denmark. Matching criteria were sex, year of birth and municipality. Matching was carried out only once and thus no replacement matching took place following subsequent patient exclusions. The date of RA diagnosis and corresponding matching date for GPCs is termed 'index date' throughout the remainder of this paper.

Exclusion criteria

Individuals aged <18 years at the index date were excluded. Patients and GPCs who had received a THR prior to index date were excluded for the THR analysis; likewise, individuals with previous TKR surgery were excluded in the TKR analysis.

Outcomes

Outcomes of interest were primary THR and TKR within 5 years of index date in patients with incident RA and GPCs. NOMESCO codes were used for identification of the procedures in DNPR (THR: KNFB; TKR: KNGB). As THR and TKR were analysed separately, patients and GPCs could potentially have both outcomes.

To allow patients diagnosed near the end of the study period to contribute with the same amount of follow-up time as those diagnosed in the first years of the study period, we focused on the first 5 years after diagnosis for all patients and GPCs. Patients with moderate-to-high disease activity score during the first year(s) of RA are at increased risk of major joint replacement surgery.[3]

Follow-up

Separate analyses for THR and TKR were undertaken. In analyses of THR, follow-up started at index date and ended at date of THR, death, emigration or 5 years of follow-up, whichever came first. For analyses of TKR, follow-up started at index date and ended at first occurrence of TKR, death, emigration or 5 years of follow-up.

Intervention

The time of the intervention—introduction of bDMARDs—was set to a 1-year period from 1 January 2002 to 31 December 2002 representing the time at which TNFi treatment was introduced for the treatment of RA in Denmark. Infliximab was available for treatment of RA in Denmark in 2000, but there were three main reasons for choosing 2002 as the time of intervention. Figures from the annual DANBIO report showed that the use of TNFi dramatically started to increase in 2002.[25 27] Second, in 2002, three different TNFis were available for the treatment of RA, and according to the DANBIO figures, the use of each of these drugs increased.[25] Third, the Danish Institute for Rational Pharmacotherapy published their first national guideline for TNFi treatment in November 2002.[28]

We introduced the 1-year lag period in 2002 as changes in prescription patterns and guideline implementation were likely 'phased in' during this period rather than abruptly changed overnight.

Statistical analyses

Demographics and descriptive data are presented by means and SD. Groups were compared by independent t test and χ^2 test as appropriate.

We calculated the 5-year age and sex standardised incidence rates for THR and TKR separately among incident RA and GPCs, respectively, within each 6-month period from 1996 to 2011. An interrupted time-series analysis was carried out using biannual incidence rates of THR and TKR in two time segments: pre-bDMARD era (1996–2001) and bDMARD era (2003–2016) interrupted by the lag period in 2002.

We estimated the change in level (incidence rate/1000 person-years (pyrs)) and trend (Δ incidence rate/1000 pyrs per each 6-month period) in THR/TKR following the 1-year lag period in 2002. Using a backward stepwise procedure, the most parsimonious models were specified (P entry <0.05; P exit ≥0.20).[20] Results are presented as 1996 baseline incidence rates; pre-bDMARD era trend; change in incidence rate at start of bDMARD era; and trend in bDMARD era. It is not uncommon for residuals from ordinary least-squares regression of time-series data to be temporally correlated. We therefore tested for first-order autocorrelation (not present) using Durbin-Watson tests, with all values of the test statistics being close to 2.0.[20] Statistical analyses were performed using Stata V.13.1 (Stata, Texas, USA).

Sensitivity analyses

We performed a sensitivity analysis using data derived from incident patients and GPCs within 3-month periods instead of 6-month periods allowing us to inspect if results differed when the balance between the number of time points (2 vs 4 per year) and number of patients and events per time point changed.

Table 1 Baseline characteristics of rheumatoid arthritis (RA) and general population comparators (GPCs) included in the interrupted time-series analysis for total hip replacement (THR) and total knee replacement (TKR), respectively

	Eligible for THR analyses		Eligible for TKR analyses		
	RA	GPC	RA	GPC	P value *
Number of individuals	29427	290816	29703	294813	
Age in years, mean±SD	58.3±15.7	53.9±15.4	58.5±15.7	54.1±15.4	<0.001
Female sex, n (%)	20612 (70.0)	203232 (69.9)	20760 (69.9)	205979 (69.9)	0.566/0.936
Chronic obstructive pulmonary disease, n (%)	1591 (5.4)	11920 (4.1)	1638 (5.5)	12382 (4.2)	<0.001
Diabetes mellitus, n (%)	1539 (5.2)	11738 (4.0)	1525 (5.1)	12000 (4.1)	<0.001
Ischaemic heart disease, n (%)	3954 (13.4)	32567 (11.2)	4049 (13.6)	32429 (11.0)	<0.001
Obesity, n (%)	1061 (3.6)	7552 (2.6)	1039 (3.5)	7542 (2.6)	<0.001

*P values were the same in both the THR and TKR populations, except for sex (THR: P= value 0.566; TKR: P value=0.936).

Furthermore, we tested models based on quarterly data for seasonality, which was not present.

RESULTS

Baseline characteristics

We identified 30404 patients with incident RA diagnosed between 1996 and May 2011 (online supplementary table S2) and 297 916 GPCs (online supplementary table S2 and figure S1). Following exclusion of patients and GPCs who prior to index date had received a THR or TKR, 29 427 patients with RA were eligible for comparison with 290 778 GPCs in THR analyses, and 29 703 patients with RA and 294806 in TKR analyses (table 1). A higher proportion of patients with RA suffered from comorbidities (table 1 and online supplementary table S5).

Total hip replacements

In patients with RA, the 5-year incidence rate of THR was 8.72/1000 pyrs (95% CI 7.48 to 9.95) at the start of 1996 compared with 2.89/1000 pyrs (95% CI 2.64 to 3.14) in GPCs (table 2 and figure 1). In the pre-bDMARD era from 1996 to end of 2001, there was a decreasing trend in the incidence rate of THR among patients with RA. Following the lag period in 2002, there was a borderline significant step change increase in incidence rate (+2.23 THR surgeries/1000 pyrs, P=0.075). Following the lag period, the incidence rate of THR continued to decrease at the same rate as observed in the pre-bDMARD era. In parallel, incidence rates of THR increased among GPCs throughout the whole study period, but to a lesser extent from 2003 and onwards (table 2 and figure 1). Overall, results did not differ from the primary analysis when using quarterly instead of biannually derived data (online supplementary table S3).

Table 2 Changes in 5-year incidence rate of total hip replacement (THR) in patients with incident rheumatoid arthritis (RA) following introduction of biological DMARDs (bDMARDs) compared with secular trends in age, sex and municipality-matched general population comparators (GPCs)

Cohort	THR, n	Baseline incidence rate/1000 person years (95% CI) in 1996	Δ per year* 1996–2001 (pre-bDMARD era)	Δ in level 1 January 2003	Δ per year 2003-2016 (bDMARD era)
RA	935	8.72 (7.48 to 9.95)	−0.36 (P=0.004)	+2.23 (P=0.075)	−0.36 (P=0.004)
GPC	4744	2.89 (2.64 to 3.14)	+0.11 (P>0.001)	None	+0.02 (P=0.040)

Stepwise backward elimination to produce most parsimonious model: P entry <0.05 and P exit >0.2.
*Δ per year based on biannual data.

Total knee replacements

At the start of 1996, the age and sex standardised incidence rate of TKR was 14 times higher among patients with RA compared with matched GPCs: 5.87 vs 0.42/1000 pyrs (table 3). In the pre-bDMARD era, the incidence rate of TKR increased with +0.19 per year (P=0.173), but started to decrease with −0.20 per year (P=0.083) in the bDMARD era (figure 2). None of these trends were statistically significant. However, when applying quarterly instead of biannually derived data, the decreasing trend of TKR surgeries among patients with RA in the bDMARD era became statistically significant (−0.21 TKR surgeries/1000 pyrs per year, P=0.03) (online supplementary table S4 and figure S3).

Among GPCs, the incidence rate of TKR increased from 1996 to 2001 but less so from 2003 and onwards (table 3). This pattern was also observed in when applying quarterly derived data (online supplementary table S4).

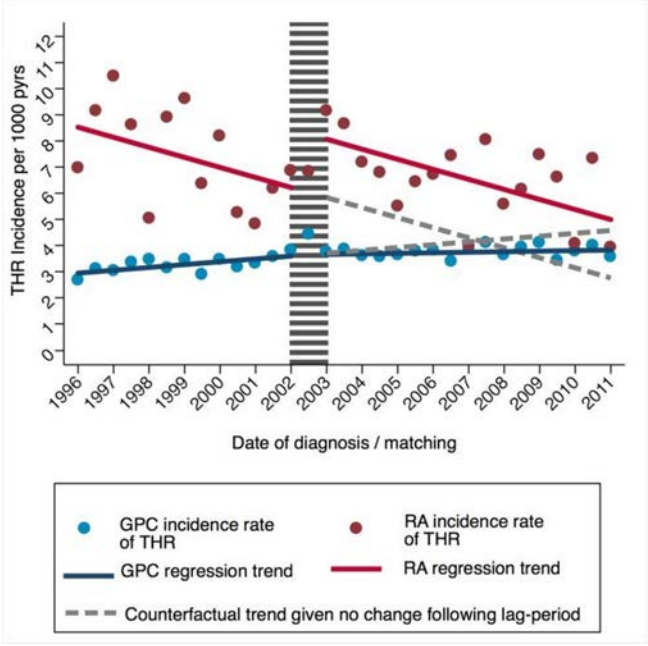


Figure 1 Results from interrupted time-series analysis of changes in 5-year incidence rates (per 1000 person years (pyrs)) of total hip replacement (THR) in patients with rheumatoid arthritis (RA) compared with general population comparators (GPCs) following introduction of biological DMARDs in 2002.

Table 3 Changes in 5-year incidence rate of total knee replacement (TKR) among patients with incident rheumatoid arthritis (RA) following introduction of biological DMARDs (bDMARDs) and age, sex and municipality-matched general population comparators (GPCs)

Cohort	TKR, n	Baseline incidence rate/1000 person years (95% CI) in 1996	Δ per year* 1996–2001 (pre-bDMARD era)	Δ in level 1 January 2003	Δ per year* 2003-2016 (bDMARD era)
RA	865	5.87 (4.52 to 7.22)	+0.19 (P=0.173)	None	−0.20 (P=0.083)
GPC	2438	0.42 (0.17 to 0.66)	+0.21 (P>0.001)	None	+0.08 (P=0.003)

Stepwise backward elimination to produce most parsimonious model: P entry <0.05 and P exit >0.2.
*Δ per year based on biannual data.

DISCUSSION

In this nationwide study, we set out to estimate the impact of the introduction of bDMARDs along with the publication of associated treatment guidelines on the 5-year incidence of THR and TKR among patients with incident RA compared with matched GPCs. We found that the incidence of TKR started to decrease among patients with RA following introduction of bDMARDs, whereas the incidence rate of THR had already started decreasing prior to bDMARD introduction. Incidence rates of THR and TKR among GPCs increased throughout the entire study but less so in the later years.

Our observed incidence rates and trends of THR and TKR among patients with RA are very similar to those observed among English and Welsh patients with RA.[29] Using data from primary care in England and Wales, we found that introduction of TNFi and publication of NICE Technology Appraisal 36 concerning TNFi treatment in patients with RA was associated with a significant decline in rates of TKR, but not THR.[29] By applying the same method on two different RA populations from different countries with different healthcare systems and reaching the same overall conclusion allows us to have greater confidence in our results. In patients with RA, knees are more often affected by synovitis than hips, and knee but not hip joints are routinely investigated in the widely used DAS28,[30–34] which could be an explanation for an impact of bDMARD introduction on TKR but not THR. However, it can also be speculated that the changes observed for THR are due to the more aggressive treat-to-target and tight disease-control strategies with conventional synthetic DMARDs implemented in the mid-1990s, resulting in a gradual decline in the incidence of THR due to RA-related synovitis and erosions, followed by a continued decline due to less frequent occurrence of secondary osteoarthritis of the hip.

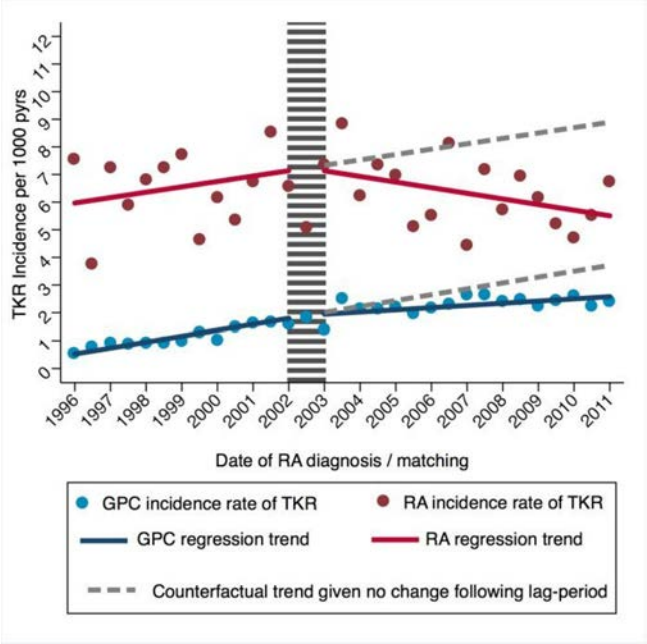


Figure 2 Results from interrupted time-series analysis of changes in 5-year incidence rates (per 1000 person years (pyrs)) of total knee replacement (TKR) in patients with rheumatoid arthritis (RA) compared with general population comparators (GPCs) following introduction of biological DMARDs in 2002.

Surprisingly, we observed an increase in the incidence of THR among patients with RA in 2003. We have no clear explanation for this finding, but a change in government in late 2001 and a subsequent political focus on bringing down the waiting lists for elective surgeries offer a potential explanation. Another explanation could be that patients with RA were considered more fit for surgery due to improved treatment options. Unfortunately, we are not able to verify these theories. Interestingly, the same increase in level for THR was observed in an English and Welsh RA population.[29] Hekmat *et al* investigated incidence rates in a regional RA population from Malmo, Sweden, and found that the incidence rate of THR had decreased from 1997 to 2007 in agreement with our findings. However, in that study, the incidence of TKR increased.[9] Possible explanations for the different findings could be that the Swedish cohort included both incident and prevalent RA patients, and a less widespread use of TNFi at the time in south Sweden. Nikiphorou *et al* used two RA inception cohorts from UK to investigate changes in the use of major orthopaedic surgery from 1986 to 2011. Whereas intermediate and minor surgical interventions decreased during this period, there were no changes in use of major joint surgery.[16] Studies from the USA have suggested a decline in joint surgery among patients with RA, but because of differences in healthcare access/systems, those results are more difficult to compare with ours.[14 15]

We decided to use biannually derived data for our primary analysis and quarterly data for the sensitivity analysis. At the time where this decision was made, there was no published guidance regarding the relative importance of number of time points in the pre-intervention and postintervention periods contra the number of individuals/outcomes occurring per time point. However, the results of a recent simulation study (unpublished) would suggest that given our large sample size, the use of quarterly derived data is the more appropriate in terms of providing greater statistical power. Indeed, while the overall findings did not change in sensitivity analyses using quarterly data, the results of the TKR analysis became statistical significant in patients with RA.

All patients and GPCs were followed up for the first 5 years following diagnosis, thus only allowing us to capture joint replacements performed within the first years after disease onset. Although this could underestimate the true long-term impact of bDMARDs on the outcomes, it allowed for all patients to have an equal amount of follow-up time regardless if they entered the study in the pre-bDMARD or the bDMARD era. Also, as observed in our study and a recent study by Nikiphorou *et al*, a non-negligible proportion of patients with RA go on to require joint replacement surgery within 5 years with the highest proportions among patients with moderate or severe disease activity.[3]

Thus, the 5-year incidence of joint replacement does not serve as a long-term outcome, but rather as a surrogate marker of the inflammatory burden suffered by the patient in the early years of the disease.

Our study has some important limitations. There is an inherent risk of misclassification of patients with RA using healthcare registers,[35] but our extraction criteria are likely to have minimised this as we only included patients with RA diagnosed at an inpatient or outpatient facility specialised in rheumatology or general internal medicine according to the DNPR. Another important limitation is one inherent to all correlational studies. We acknowledge that our findings of decreased need for TKR among patients with RA could at least partly be due to increased use of treat-to-target strategies and more aggressive conventional synthetic DMARD combination therapy.[36] Changes in diagnostic criteria for RA introduced in 2010 are not likely to have affected our results, but we cannot rule out that referral patterns of patients with RA to orthopaedic surgery have changed. Increased focus on and changes in non-treatment factors such as obesity along with changes in prevalence of certain comorbidities that would affect the use of or willingness to perform joint replacement surgery around the time of bDMARD introduction could theoretically also play a role in our findings. However, we found no changes in prevalence of comorbidities nor lifestyle-related diagnoses such as obesity and chronic obstructive pulmonary disease (online supplementary table S5), and no major changes were introduced to treatment guidelines except for those directly aimed at bDMARD treatment around the time of the intervention. To investigate the true impact of bDMARDs on the need for joint surgery, studies using individual-level based information on DMARD treatment are needed.

However, this study has also several strengths, including the nationwide population-based design with access to complete follow-up in a large sample of patients with RA and matched GPCs in a universal, tax-funded healthcare system. We compared our findings with trends among GPCs, thereby gaining insight into secular trends and enabling us to review our findings for the RA population in that context. The interrupted time-series analysis as choice of method is another strength. Had we not been able to identify the upward going/constant trend in incidence of TKR among patients with RA in the pre-bDMARD era but rather just compared the overall incidence rate in the two separate eras or used a survival analysis design comparing the risk of THR and TKR in each era, we would have concluded that the incidence rates and HRs of TKR were similar as the trends in the pre-bDMARD and the bDMARD era were equal but opposite.

In conclusion, we report that the incidence rate of THR and TKR was 3-fold and 14-fold higher, respectively among patients with RA compared with GPCs in 1996; that in patients with RA, but not in matched GPCs, introduction of bDMARDs was associated with a decreasing incidence rate of TKR, but not THR.

Author affiliations
[1]Center for Rheumatology and Spine Diseases, Rigshospitalet-Gentofte, Copenhagen, Denmark
[2]The Parker Institute, Bispebjerg and Frederiksberg Hospital, Frederiksberg, Denmark
[3]Musculoeskeletal Pharmaco- and Device Epidemiology, Centre for Statistics in Medicine, Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK
[4]GREMPAL Research Group, Idiap Jordi Gol and CIBERFes, Universitat Autònoma de Barcelona and Instituto de Salud Carlos III, Barcelona, Spain
[5]Department of Orthopaedic Surgery and Traumatology, Odense University Hospital, Odense, Denmark
[6]Department of Clinical Research, University of Southern Denmark, Odense, Denmark
[7]Department of Orthopaedic Surgery, Copenhagen University Hospital Herlev-Gentofte, Copenhagen, Denmark
[8]Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

Acknowledgements We wish to thank Christian Cato Holm at The Parker Institute for his invaluable help with data management.

Contributors RLC and LD had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. RLC, DPA, LD, SH: study concept and design. RC and LD: acquisition of data. RC, SH, DPA: statistical analysis. All authors: interpretation of data; critical revision of the manuscript for important intellectual content. RLC: drafting of the manuscript. LD, AO, LEK, DPA and SO: study supervision.

Funding The study was funded by The Danish Rheumatism Association, The Bjarne Jensen Foundation, The Oak Foundation and The Danish Council for Independent Research. This work has been supported by the National Institute for Health Research (NIHR) Biomedical Research Centre, Oxford. DPA is funded by a National Institute for Health Research Clinician Scientist award (CS-2013-13-012). This paper presents independent research funded by the NIHR. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health.

Competing interests DPA's research group has received research grants from Servier, UCB and Amgen; consultancy fees from UCB outside the present work. PH has received speaking fees from Celgene and UCB outside the present work. LEK has received fees for speaking and/or consultancy from Pfizer, AbbVie, Amgen, UCB, Celgene, BMS, Biogen, Sanofi, MSD, Novartis, Eli Lilly and Janssen Pharmaceuticals. LD has received speaking fees from MSD and UCB outside the present work.

Ethics approval Approval was given by the Danish Data Protection Agency (GEH-2014-043, I-Suite: 03166).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Smolen JS, Aletaha D, McInnes IB. Rheumatoid arthritis. *Lancet* 2016;388:2023–38.
2 Schett G, Gravallese E. Bone erosion in rheumatoid arthritis: mechanisms, diagnosis and treatment. *Nat Rev Rheumatol* 2012;8:656–64.
3 Nikiphorou E, Norton S, Young A, *et al*. Association between rheumatoid arthritis disease activity, progression of functional limitation and long-term risk of orthopaedic surgery: combined analysis of two prospective cohorts supports EULAR treat to target DAS thresholds. *Ann Rheum Dis* 2016;75:2080–6.
4 Smolen JS, Landewé R, Bijlsma J, *et al*. EULAR recommendations for the management of rheumatoid arthritis with synthetic and biological disease-modifying antirheumatic drugs: 2016 update. *Ann Rheum Dis* 2017;76:960–77.
5 Singh JA, Saag KG, Bridges SL, *et al*. 2015 American college of rheumatology guideline for the treatment of rheumatoid arthritis. *Arthritis Care Res* 2016;68:1–25.
6 Massardo L, Gabriel SE, Crowson CS, *et al*. A population based assessment of the use of orthopedic surgery in patients with rheumatoid arthritis. *J Rheumatol* 2002;29:52–6.
7 Ringen HO, Dagfinrud H, Mowinckel P, *et al*. Patients with rheumatoid arthritis report greater physical functional deterioration in lower limbs compared to upper limbs over 10 years. *Scand J Rheumatol* 2008;37:255–9.
8 Nam JL, Takase-Minegishi K, Ramiro S, *et al*. Efficacy of biological disease-modifying antirheumatic drugs: a systematic literature review informing the 2016 update of the EULAR recommendations for the management of rheumatoid arthritis. *Ann Rheum Dis* 2017;76:1113–36.
9 Hekmat K, Jacobsson L, Nilsson JÅ, *et al*. Decrease in the incidence of total hip arthroplasties in patients with rheumatoid arthritis--results from a well defined population in south Sweden. *Arthritis Res Ther* 2011;13:R67.
10 Pedersen AB, Mor A, Mehnert F, *et al*. Rheumatoid arthritis: trends in antirheumatic drug use, C-reactive protein levels, and surgical burden. *J Rheumatol* 2015;42:2247–54.
11 Jämsen E, Virta LJ, Hakala M, *et al*. The decline in joint replacement surgery in rheumatoid arthritis is associated with a concomitant increase in the intensity of anti-rheumatic therapy: a nationwide register-based study from 1995 through 2010. *Acta Orthop* 2013;84:331–7.
12 Danish Knee Arthroplasty Register - annual report. 2013 https://www.knee.dk/groups/dkr/pdf/DKR_2013.pdf.
13 Overgaard S, Pedersen AB, Hjelm AH, *et al*. Danish Hip Arthroplasty Register - annual report. 2013http://www.dhr.dk/Ny mappe/rapporter/DHR %25C3%25A5rsrapport 2013_full versionfinal_.pdf
14 Louie GH, Ward MM. Changes in the rates of joint surgery among patients with rheumatoid arthritis in California, 1983-2007. *Ann Rheum Dis* 2010;69:868–71.
15 Shourt CA, Crowson CS, Gabriel SE, *et al*. Orthopedic surgery among patients with rheumatoid arthritis 1980-2007: a population-based study focused on surgery rates, sex, and mortality. *J Rheumatol* 2012;39:481–5.

16 Nikiphorou E, Carpenter L, Morris S, *et al*. Hand and foot surgery rates in rheumatoid arthritis have declined from 1986 to 2011, but large-joint replacement rates remain unchanged: results from two UK inception cohorts. *Arthritis Rheumatol* 2014;66:1081–9.
17 Mertelsmann-Voss C, Lyman S, Pan TJ, *et al*. US trends in rates of arthroplasty for inflammatory arthritis including rheumatoid arthritis, juvenile idiopathic arthritis, and spondyloarthritis. *Arthritis Rheumatol* 2014;66:1432–9.
18 Cordtz RL, Zobbe K, Højgaard P, *et al*. Predictors of revision, prosthetic joint infection and mortality following total hip or total knee arthroplasty in patients with rheumatoid arthritis: a nationwide cohort study using Danish healthcare registers. *Ann Rheum Dis* 2017. doi:10.1136/ annrheumdis-2017-212339. [Epub ahead of print 2 Nov 2017].
19 Kontopantelis E, Doran T, Springate DA, *et al*. Regression based quasi-experimental approach when randomisation is not an option: interrupted time series analysis. *BMJ* 2015;350:h2750.
20 Wagner AK, Soumerai SB, Zhang F, *et al*. Segmented regression analysis of interrupted time series studies in medication use research. *J Clin Pharm Ther* 2002;27:299–309.
21 Von EE, Altman DG, Egger M, *et al*. Strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Br Med J* 2007;335:19–22.
22 Schmidt M, Pedersen L, Sørensen HT. The Danish civil registration system as a tool in epidemiology. *Eur J Epidemiol* 2014;29:541–9.
23 Lynge E, Sandegaard JL, Rebolj M. The Danish national patient register. *Scand J Public Health* 2011;39:30–3.
24 Ibfelt EH, Jensen DV, Hetland ML. The Danish nationwide clinical register for patients with rheumatoid arthritis: DANBIO. *Clin Epidemiol* 2016;8:737–42.
25 Annual reports from DANBIO. https://danbio-online.dk/formidling
26 Thygesen SK, Christiansen CF, Christensen S, *et al*. The predictive value of ICD-10 diagnostic coding used to assess Charlson comorbidity index conditions in the population-based Danish National Registry of Patients. *BMC Med Res Methodol* 2011;11:83.
27 Hetland ML. DANBIO--powerful research database and electronic patient record. *Rheumatology* 2011;50:69–77.
28 Institute for Rational Pharmacotherpy. Treatment with TNF-inhibitors guidelines. 2002 http://www.irf.dk/dk/publikationer/vejledninger/tnf-alfa_haemmende_behandling_02.htm
29 Hawley S, Cordtz R, Dreyer L, *et al*. Association between NICE guidance on biologic therapies with rates of hip and knee replacement among rheumatoid arthritis patients in England and Wales: an interrupted time-series analysis. *Semin Arthritis Rheum* 2017.
30 Listing J, Kekow J, Manger B, *et al*. Mortality in rheumatoid arthritis: the impact of disease activity, treatment with glucocorticoids, TNFα inhibitors and rituximab. *Ann Rheum Dis* 2015;74:415–21.
31 Duthie RB, Harris CM. A radiographic and clinical survey of the hip joint in sero-positive rheumatoid arthritis. *Acta Orthop Scand* 1969;40:346–64.
32 Eberhardt K, Fex E, Johnsson K, *et al*. Hip involvement in early rheumatoid arthritis. *Ann Rheum Dis* 1995;54:45–8.
33 Lehtimäki MY, Kaarela K, Hämäläinen MM. Incidence of hip involvement and need for total hip replacement in rheumatoid arthritis. An eight-year follow-up study. *Scand J Rheumatol* 1986;15:387–91.
34 Prevoo ML, van 't Hof MA, Kuper HH, *et al*. Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. *Arthritis Rheum* 1995;38:44–8.
35 Pedersen M, Klarlund M, Jacobsen S, *et al*. Validity of rheumatoid arthritis diagnoses in the Danish national patient registry. *Eur J Epidemiol* 2004;19:1097–103.
36 Moura CS, Abrahamowicz M, Beauchamp ME, *et al*. Early medication use in new-onset rheumatoid arthritis may delay joint replacement: results of a large population-based study. *Arthritis Res Ther* 2015;17:197.

EXTENDED REPORT

Early and sustained efficacy with apremilast monotherapy in biological-naïve patients with psoriatic arthritis: a phase IIIB, randomised controlled trial (ACTIVE)

Peter Nash,[1] Kamal Ohson,[2] Jessica Walsh,[3] Nikolay Delev,[4] Dianne Nguyen,[4] Lichen Teng,[4] Juan J Gómez-Reino,[5] Jacob A Aelion,[6] on behalf of the ACTIVE investigators

Handling editor Tore K Kvien

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-211568).

[1]Department of Medicine, University of Queensland, Brisbane, Queensland, Australia
[2]Memorial University of Newfoundland, St. John's, Newfoundland and Labrador, Canada
[3]Department of Internal Medicine, Division of Rheumatology, University of Utah School of Medicine, Salt Lake City, Utah, USA
[4]Celgene Corporation, Summit, New Jersey, USA
[5]Hospital Clínico Universitario, Santiago, Spain
[6]West Tennessee Research Institute, Jackson, Tennessee, USA

Correspondence to
Professor Peter Nash, Department of Medicine, University of Queensland, Brisbane, QLD 4072, Australia; drpnash@tpg.com.au

Received 30 March 2017
Revised 13 December 2017
Accepted 18 December 2017
Published Online First 17 January 2018

To cite: Nash P, Ohson K, Walsh J, *et al*. *Ann Rheum Dis* 2018;**77**:690–698.

ABSTRACT

Objective Evaluate apremilast efficacy across various psoriatic arthritis (PsA) manifestations beginning at week 2 in biological-naïve patients with PsA.

Methods Patients were randomised (1:1) to apremilast 30 mg twice daily or placebo. At week 16, patients whose swollen and tender joint counts had not improved by ≥10% were eligible for early escape. At week 24, all patients received apremilast through week 52.

Results Among 219 randomised patients (apremilast: n=110; placebo: n=109), a significantly greater American College of Rheumatology 20 response at week 16 (primary outcome) was observed with apremilast versus placebo (38.2% (42/110) vs 20.2% (22/109); P=0.004); response rates at week 2 (first assessment) were 16.4% (18/110) versus 6.4% (7/109) (P=0.025). Improvements in other efficacy outcomes, including 28-joint count Disease Activity Score (DAS-28) using C reactive protein (CRP), swollen joint count, Health Assessment Questionnaire-Disability Index (HAQ-DI), enthesitis and morning stiffness severity, were observed with apremilast at week 2. At week 16, apremilast significantly reduced PsA disease activity versus placebo, with changes in DAS-28 (CRP) (P<0.0001), HAQ-DI (P=0.023) and Gladman Enthesitis Index (P=0.001). Improvements were maintained with continued treatment through week 52. Over 52 weeks, apremilast's safety profile was consistent with prior phase 3 studies in psoriasis and PsA. During weeks 0–24, the incidence of protocol-defined diarrhoea was 11.0% (apremilast) and 8.3% (placebo); serious adverse event rates were 2.8% (apremilast) and 4.6% (placebo).

Conclusions In biological-naïve patients with PsA, onset of effect with apremilast was observed at week 2 and continued through week 52. The safety profile was consistent with previous reports.

Trial registration number NCT01925768; Results.

INTRODUCTION

Psoriatic arthritis (PsA) is heterogeneous, with patients exhibiting varied clinical symptoms, severity and disease course. Treatment goals include controlling disease activity, optimising functional status and minimising side effects to therapy.[1] Biologicals are commonly used after or in conjunction with conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), but safety monitoring and risks may limit their long-term use.[2 3]

The efficacy and safety of apremilast, an oral phosphodiesterase 4 inhibitor, were demonstrated in patients with active PsA in four phase III, placebo-controlled studies as part of the Psoriatic Arthritis Long-term Assessment of Clinical Efficacy (PALACE) clinical trial programme.[4–7] The PALACE 1, 2 and 3 studies evaluated apremilast in patients with prior exposure to csDMARDs and/or biologicals and allowed concomitant csDMARD use.[4–6] PALACE 4 evaluated apremilast monotherapy in csDMARD-naïve and biological-naïve populations.[7] Data demonstrating apremilast's efficacy across disease manifestations have been reported at week 16[4–6 8] and up to 4 years of treatment.[9] However, time to onset of therapeutic effect has not been reported before week 16.

Assessing Apremilast Monotherapy in a Clinical Trial of BIologic-NaïVE Patients With Psoriatic Arthritis (ACTIVE) aimed to evaluate apremilast monotherapy in biological-naïve PsA patients who may have had one prior csDMARD. ACTIVE also aimed to determine the onset of apremilast efficacy, with assessments beginning at week 2, and to examine additional outcome measures, including morning stiffness and enthesitis using the Gladman Enthesitis Index (GEI).[10] Diarrhoea adverse events (AEs) were further characterised using a protocol definition.

This report describes the early onset and overall efficacy and safety of apremilast monotherapy through week 52.

METHODS

Patients

Enrolled adults (≥18 years of age) had a documented diagnosis of active PsA for ≥3 months and met Classification Criteria for Psoriatic Arthritis.[11] At screening, patients were required to have at least three swollen and three tender joints, C reactive protein (CRP) of ≥0.2 mg/dL and be biological DMARD-naïve. No csDMARD washout before the study was required (except 4 weeks for cyclosporine and 12 weeks for leflunomide); however, patients had to discontinue their current csDMARD ≥1 day before baseline assessments. Patients were excluded if they had prior treatment

with more than one csDMARD; used prohibited systemic therapies, including cyclosporine or other calcineurin inhibitors, within 4 weeks of randomisation, corticosteroids >10 mg daily (prednisone or equivalent), oral agents such as retinoids, mycophenolate, thioguanine, hydroxyurea, sirolimus and tacrolimus; and inflammatory joint disease other than PsA. Also excluded were patients with active or incompletely treated tuberculosis, significant infection within 4 weeks of screening and current or history of malignancy (except for treated basal cell or squamous cell skin carcinoma or early forms of cervical carcinoma with no recurrence within 5 years).

All patients provided written informed consent before any study procedures were initiated.

Study design

This phase IIIB, multicentre, randomised, double-blind, placebo-controlled, parallel-group study evaluated the efficacy and safety of apremilast monotherapy in patients with active PsA.

Patients were randomised (1:1) to apremilast 30 mg twice daily or placebo for 24 weeks, stratified by previous csDMARD and baseline prednisone (or equivalent) use. Patients who did not improve by ≥10% in swollen joint count (SJC) and tender joint count (TJC) at week 16 were eligible for early escape at the investigator's discretion. Early escape patients initially randomised to placebo were switched to apremilast in blinded fashion, with dose titration during the first week of treatment; patients initially randomised to apremilast remained on apremilast. At week 24, all remaining patients receiving placebo switched to apremilast for the active treatment phase through week 52, when all patients were eligible to continue apremilast treatment in an open-label extension phase through week 104.

Concomitant medications

Patients could receive concurrent treatment with stable doses of oral corticosteroids (prednisone ≤10 mg/day or equivalent), non-steroidal anti-inflammatory drugs or opioid analgesics. Changes in corticosteroid doses and/or discontinuations were not allowed from day 0 to week 24 except for safety reasons or lack of availability. After week 24, the corticosteroid dose could be adjusted as clinically required. Patients could use low-potency topical corticosteroids for face, axillae and groin psoriatic lesions.

Outcomes

The primary outcome was 20% improvement in modified American College of Rheumatology response criteria (ACR20) at week 16. Other efficacy outcomes included 28-joint count Disease Activity Score (DAS-28) using CRP, SJC, TJC, six-point GEI score (0=no enthesitis, 6=all six sites active (ie, bilateral tibial tuberosity, plantar fascia and Achilles tendon insertion)) for patients with enthesitis at baseline, morning stiffness duration and severity, ACR50 and ACR70 and physical function assessments, including the Health Assessment Questionnaire-Disability Index (HAQ-DI), 36-item Short-Form Health Survey version 2 (SF-36v2) Physical Functioning (PF) domain and physical component summary (PCS) scores. Safety and tolerability evaluations included collection of AEs, vital signs, laboratory evaluations, physical examinations, electrocardiograms, chest X-rays and further characterisation of diarrhoea AEs using a protocol definition (two or more watery or liquid stools/day).

Efficacy outcomes were assessed starting at week 2 and at scheduled visits through week 52; SF-36v2 assessments started at week 4.

Statistical analysis

Efficacy analyses were based on the full analysis set, which included all randomised patients. The safety population included all randomised patients who received at least one dose of study medication. Sample size estimation was based on results from earlier phase III studies. A two-group χ^2 (continuity-corrected) test with a two-sided 0.05 significance level would have ≈90% power to detect a true 20% difference (35% vs 15%) between apremilast and placebo for the proportion of patients achieving ACR20 response at week 16, when the sample size in each group was 107.

Baseline patient demographics and disease characteristics were compared descriptively between the treatment groups.

For the placebo-controlled period, two-sided tests for efficacy outcomes were performed sequentially according to a prespecified hierarchical order to control the overall type I error rate (online supplementary table 1). P values <0.05 were considered statistically significant; if the P value did not reach the threshold of 0.05 during the hierarchical testing, the nominal P value was reported onwards. Therefore, P values <0.05 should be interpreted with caution for the secondary outcomes if a testing in a higher order of the hierarchy did not reach the threshold of 0.05.

Dichotomous variables such as ACR20 response were analysed using the generalised Cochran-Mantel-Haenszel test,[12] controlling for baseline prednisone (or equivalent) use (yes/no) and previous csDMARD use (yes/no). Patients escaping at week 16 were primarily treated as non-responders at the subsequent time points during the placebo-controlled period. Missing data were handled using non-responder imputation. Mixed-effect model for repeated measures was generally used for analyses of continuous variables such as HAQ-DI, where change or per cent change from baseline was the dependent variable and treatment group, time (ie, study week), treatment-by-time interaction, baseline prednisone (or equivalent) use (yes or no) and previous DMARD use (yes or no) were factors and baseline value was a covariate. Time was treated as a categorical variable in the mixed-effect model for repeated measures. Data obtained after early escape were excluded from the model.

Week 52 efficacy data descriptions were as-observed analyses when no placebo data were available for comparison.

Safety analyses were conducted for the placebo-controlled phase (weeks 0–24) and overall apremilast-exposure period, which includes all available safety data among patients who received at least one dose of apremilast at any time up to the data cut-off, at which time all patients remaining in the study had completed their week 52 visit. AEs were classified using MedDRA V.14.0.

RESULTS

Patients

A total of 219 patients were randomised (apremilast: n=110; placebo: n=109), and 84.5% completed week 24 (online supplementary figure 1). Of the 180 patients entering the long-term active treatment phase, 88.9% completed week 52. Treatment groups were comparable for baseline patient demographics and disease characteristics (table 1).

Efficacy

Primary outcome: ACR20 response

The ACR20 response rate at week 16 was significantly greater in patients receiving apremilast versus placebo (38.2% (42/110) vs 20.2% (22/109); P=0.004) (table 2), with response observed

Table 1 Baseline patient characteristics (full analysis set)

	Placebo n=109	Apremilast 30 mg twice daily n=110
Age, mean (SD), years	48.0 (13.8)	50.7 (12.2)
Female, n (%)	65 (59.6)	58 (52.7)
White, n (%)	105 (96.3)	109 (99.1)
Region, n (%)		
North America	42 (38.5)	42 (38.2)
Europe	38 (34.9)	47 (42.7)
Rest of world	29 (26.6)	21 (19.1)
Weight, mean (SD), kg	90.1 (21.1)	92.6 (24.0)
Body mass index, mean (SD), kg/m^2	31.8 (7.8)	32.0 (7.9)
PsA duration, mean (SD), years	3.6 (5.5)	4.0 (4.5)
SJC (0–76), mean (SD)	10.0 (5.9)	9.0 (4.9)
TJC (0–78), mean (SD)	18.4 (14.2)	17.2 (12.7)
High-sensitivity CRP, mg/dL, mean (SD)	1.25 (1.6)	1.44 (1.6)
Erythrocyte sedimentation rate, mm/h	30.3 (17.5)	33.1 (19.0)
Enthesitis*, n (%)	51 (46.8)	56 (50.9)
GEI score (0–6)†, mean (SD)	2.4 (1.6)	2.3 (1.3)
HAQ-DI sore (0–3), mean (SD)	1.20 (0.59)	1.25 (0.61)
Use of PsA-related medications		
Prior use of csDMARDs, n (%)	78 (71.6)	74 (67.3)
Prior use of methotrexate, n (%)	66 (60.6)	61 (55.5)
Baseline corticosteroid use‡ (mean dose, 4.4 mg/day), n (%)	14 (12.8)	13 (11.8)
Baseline non-steroidal anti-inflammatory drug use, n (%)	74 (67.9)	76 (69.1)

Note: the n reflects the number of patients who were randomised; actual number of patients available for each parameter may vary.
*Pre-existing enthesopathy is defined as having a baseline GEI score greater than 0.
†Provided for patients with pre-existing enthesopathy.
‡All converted to oral prednisone dose.
CRP, C reactive protein; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs; GEI, Gladman Enthesitis Index; HAQ-DI, Health Assessment Questionnaire-Disability Index; PsA, psoriatic arthritis; SJC, swollen joint count; TJC, tender joint count.

at week 2 (16.4% (18/110) vs 6.4% (7/109)); P=0.025). At week 24, greater improvements in ACR20 response rate were observed with apremilast versus placebo (43.6% (48/110) vs 24.8% (27/109); P=0.004).

Evidence of treatment effect in various additional PsA manifestations was observed with apremilast at week 2 (first evaluation after baseline), as assessed by DAS-28 (CRP), HAQ-DI, GEI and morning stiffness severity (table 2).

Disease activity, joint count, enthesitis and morning stiffness outcomes

Efficacy was seen across a number of secondary measures assessing disease activity, joint inflammation, enthesitis and morning stiffness (table 2). At week 16, apremilast-treated patients demonstrated a significant reduction from baseline in DAS-28 (CRP) score versus placebo (P<0.0001) (table 2). Reductions continued through week 24 (−1.26 vs −0.76; P=0.005).

Significant improvement was demonstrated by mean per cent change in SJC with apremilast versus placebo at week 16 (P=0.0001) (table 2), with continued improvements detected at week 24 (−59.1% vs −29.0%; P=0.002). Mean per cent changes in TJC were significant with apremilast versus placebo at week 16 (P=0.002) (table 2) and week 24 (−49.6% vs −25.3%; P=0.009).

Among patients with enthesopathy at baseline (apremilast: n=56; placebo: n=51), significant improvements in enthesitis counts were observed at week 16 (P=0.001) with apremilast versus placebo. Improvements were observed at week 2 (P=0.035) and continued to week 24 (−1.5 vs −0.5; P=0.003). Numerically greater proportions of apremilast patients achieved a GEI score of 0 through week 24 (44.6% (25/56) vs 33.3% (17/51)).

Improvements in morning stiffness duration were observed with apremilast versus placebo at week 16 (P=0.005) (table 2) and week 24 (median per cent change: −33.3% vs 0.0%; P=0.001). More apremilast-treated patients showed improvement in morning stiffness severity at week 16 (P=0.015) (table 2) continuing to week 24 (40.0% vs 20.2%; P=0.002).

Functional ability

Apremilast-treated patients experienced improvements in physical disability, as assessed by various outcomes for physical function. Clinically meaningful and significant improvements were observed in physical function, as indicated by decreases in HAQ-DI score at week 16 with apremilast versus placebo (−0.21 vs −0.06; P=0.023). Decreases were observed beginning at week 2 (P=0.040) (table 2). The improvements seen with apremilast continued through week 24, with a mean reduction of −0.27; however, the mean change did not reach statistical significance versus placebo due to an unexpected shift in mean improvement in the placebo group between weeks 16 and 24 (−0.27 vs −0.17; P=0.168).

Notably, mean changes in HAQ-DI score with apremilast met or exceeded the minimal clinically important difference (MCID) of −0.13 (prespecified analysis)[13] at weeks 2, 16 and 24. The proportion of patients achieving an MCID ≥0.35 (post hoc analysis)[14] was numerically higher with apremilast versus placebo at week 16 (table 2) and significantly higher with apremilast versus placebo at week 24 (40.9% vs 24.8%, P=0.014).

Significant improvement in physical function was demonstrated by improvements from baseline in SF-36v2 PF score with apremilast versus placebo at week 16 (P=0.004) (table 2). Continued SF-36v2 PF improvement was observed at week 24 with apremilast versus placebo (3.94 vs 1.26; P=0.017), with least-squares mean improvement exceeding the MCID of 2.5.[15] Similarly, significant improvements in the SF-36v2 PCS score were observed with apremilast versus placebo at week 16 (P=0.0001) (table 2) and at week 24 (5.00 vs 1.60; P=0.004), and the least-squares mean improvement at each time point with apremilast exceeded the MCID of 2.5.[15]

Subset analysis

In a subset of patients (69% of overall population) who had one prior csDMARD, significant ACR20 response rates were observed with apremilast versus placebo (39.2% (29/74) vs 20.5% (16/78); P=0.013) at week 16. These rates were similar to those observed in the overall population. Improvements in joint and enthesitis outcomes in the subset were also similar to those observed in the overall population. In the subset, the week 16 mean per cent change with apremilast versus placebo was −40.7% versus 3.1% (P=0.003) for SJC and −26.8% versus 5.4% (P=0.014) for TJC; mean change in GEI score was −1.51 versus −0.18 (P=0.001) (online supplementary table 2). Similar results were observed in the subset (58% of overall population) with prior methotrexate use.

Table 2 Efficacy outcome measures at week 2, week 16 and week 52†

	Week 2		Week 16		Week 52	
	Placebo n=109	Apremilast n=110	Placebo n=109	Apremilast n=110	Placebo/Apremilast n=91	Apremilast n=80
ACR20, n/m (%)	7/109 (6.4)	18/110 (16.4)*	22/109 (20.2)	42/110 (38.2)‡	54/90 (60.0)	53/79 (67.1)
ACR50, n/m (%)	2/109 (1.8)	3/110 (2.7)	5/109 (4.6)	20/110 (18.2)‡	26/91 (28.6)	29/79 (36.7)
ACR70, n/m (%)	0/109 (0.0)	0/110 (0.0)	0/109 (0.0)	7/110 (6.4)*	7/91 (7.7)	17/80 (21.3)
DAS-28 (CRP), mean change	−0.31	−0.59*	−0.39	−1.07§	−1.46	−1.71
SJC, mean % change	−17.5	−27.7	4.2	−46.4§	−71.9	−77.5
TJC, mean % change	−16.2	−14.8	2.5	−32.3‡	−61.4	−70.4
GEI (0–6), mean change¶	−0.4	−1.1*	−0.4	−1.5‡	−1.4	−1.6
GEI=0¶, n/m (%)	10/51 (19.6)	20/56 (35.7)	17/51 (33.3)	26/56 (46.4)	24/43 (55.8)	30/43 (69.8)
HAQ-DI score (0–3), mean change	−0.05	−0.13*	−0.06	−0.21*	−0.32	−0.40
HAQ-DI MCID ≥0.35, n/m (%)	13/109 (11.9)	24/110 (21.8)	30/109 (27.5)	39/110 (35.5)	38/91 (41.8)	40/80 (50.0)
SF-36v2 PF, mean change	NA	NA	−1.04	2.43‡	5.11	6.00
SF-36v2 PCS, mean change	NA	NA	−0.31	4.03§	5.64	6.49
Improvement in morning stiffness severity, n/m (%)	23/109 (21.1)	47/110 (42.7)‡	28/109 (25.7)	51/110 (46.4)‡	52/91 (57.1)	46/80 (57.5)
Morning stiffness duration (minutes), median % change	0.00	0.00*	0.00	−33.33‡	−41.67	−55.00

*P<0.05 versus placebo; based on a Cochran-Mantel-Haenszel test for binary parameters and mixed-effects model for repeated measures for continuous parameters (except using stratified Van Elteren test for morning stiffness duration, with last-observation-carried-forward approach for missing data).
†Full analysis set was used for weeks 2 and 16; for response parameters, patients without sufficient data (observed or imputed) for the determination of response status were categorised as non-responders. Week 52 analyses were as observed; actual number of patients may vary for each outcome depending on availability of data.
‡P<0.005; §P≤0.0001 versus placebo; based on a Cochran-Mantel-Haenszel test for binary parameters and mixed-effects model for repeated measures for continuous parameters (except using stratified Van Elteren test for morning stiffness duration, with last-observation-carried-forward approach for missing data).
¶Evaluated in patients with enthesitis at baseline (GEI >0).
ACR20, 20% improvement in modified American College of Rheumatology response criteria; DAS-28 (CRP), 28-joint count Disease Activity Score using C reactive protein; GEI, Gladman Enthesitis Index; HAQ-DI, Health Assessment Questionnaire-Disability Index; MCID, minimal clinically important differences; NA, not assessed at time point; n/m, number of responders/number of patients with sufficient data for evaluation; PCS, physical component summary; PF, Physical Functioning domain; SF-36v2, 36-item Short-Form Health Survey version 2.

Long-term durability

Clinical improvements across outcomes, including swollen and tender joints, enthesitis, morning stiffness and functional ability, were sustained through week 52 (table 2; figures 1–3; online supplementary figure 2); for individuals who received apremilast from baseline, mean per cent change in SJC was −77.5%, with 55.0% (44/80) achieving SJC ≤1, and mean per cent change in TJC was −70.4%, with 42.5% (34/80) achieving TJC≤1.

Safety

During the placebo-controlled phase (weeks 0–24), mean total exposure duration was 20.03 weeks (41.8 patient-years) for placebo patients and 20.93 weeks (43.7 patient-years) for apremilast patients. During the apremilast-exposure period, mean total duration of apremilast exposure was 52.1 weeks (205.6 patient-years).

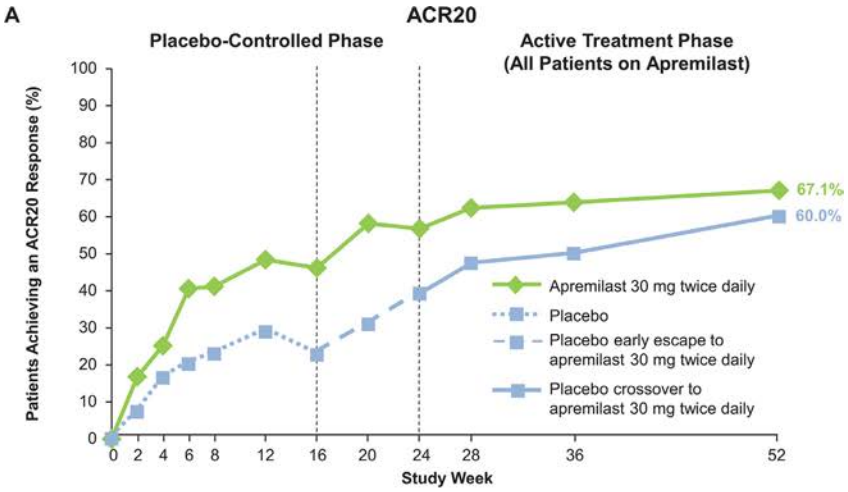
Overall AE incidence through week 24 was generally similar between the apremilast and placebo groups (table 3). The most commonly reported AEs (≥5% of either treatment group) during the placebo-controlled phase were diarrhoea, nasopharyngitis, nausea, headache, hypertension and upper respiratory tract infection (table 3). During weeks 0–24, a total of 15 patients (apremilast: n=10; placebo: n=5) discontinued because of AEs. The nature, incidence and severity of AEs were comparable with longer apremilast exposure. Six patients (five randomised to placebo at baseline; one randomised to apremilast at baseline) discontinued after week 24 because of AEs (online supplementary figure 1).

Serious AEs were low for both groups (apremilast: 2.8%; placebo: 4.6%) during the placebo-controlled phase; none were considered drug related. No serious opportunistic infections, including new or reactivated tuberculosis, were reported during the study. One death occurring after week 52 was due to atherosclerotic cardiovascular disease in a patient with a pre-existing history of hypertension and alcoholic cardiomyopathy (discovered at autopsy).

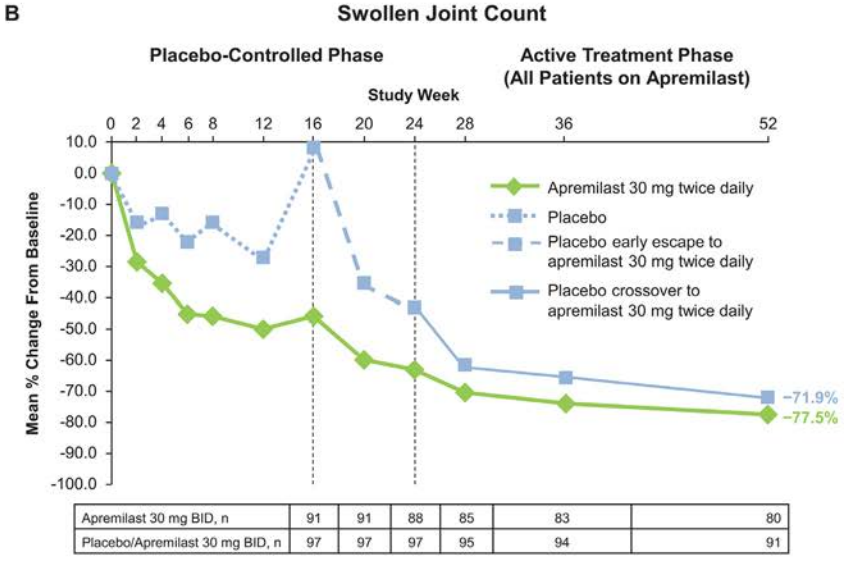
Diarrhoea was the most frequently reported AE during the placebo-controlled phase (apremilast: 14.7%; placebo: 11.0%); all cases were mild to moderate in severity. A protocol definition of diarrhoea was applied to further characterise the diarrhoea events. Using the criteria of two or more watery/liquid stools/day, 21 patients had diarrhoea (apremilast: n=12 (11.0%); placebo: n=9 (8.3%)) during the placebo-controlled phase. Four of these diarrhoea events led to study discontinuation in apremilast-treated patients. Three (apremilast: n=1; placebo: n=2) of the 21 patients took antidiarrhoeal medications. From week 24 to week 52, 10 new patients experienced protocol-defined diarrhoea AEs. Onset of diarrhoea (including protocol-defined diarrhoea AEs) was most frequently observed during the first 4 weeks of dosing. No evidence of increased gastrointestinal events was observed during the longer apremilast-exposure period versus the placebo-controlled phase.

No cases of suicidal ideation or behaviour occurred during the placebo-controlled phase or apremilast-exposure period. During the placebo-controlled phase, two apremilast patients experienced an AE of depression; one had a history of depression and the other had dysthymia. Two additional AEs of depression were reported in the apremilast-exposure period; one patient had a history of depression. All four AEs of depression were not serious.

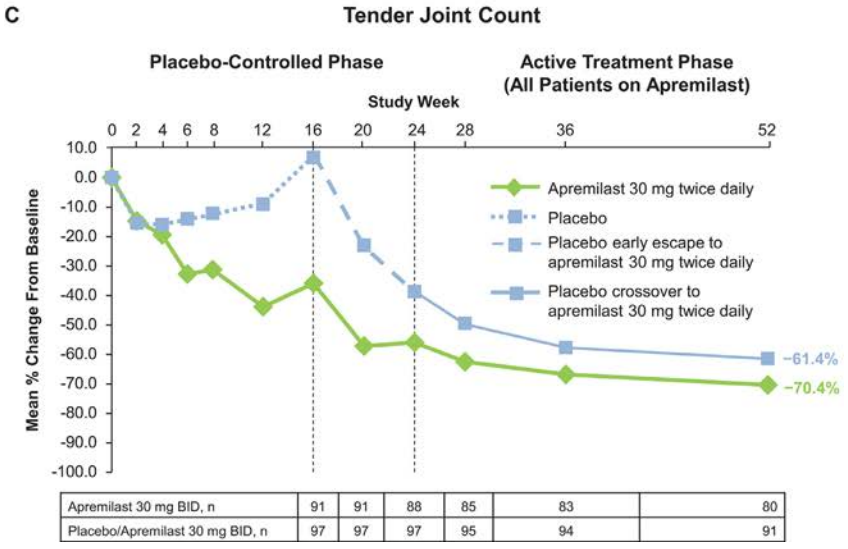
Throughout the study, markedly abnormal clinical laboratory values were infrequent and generally the result of single values outside the normal range (table 3).



Apremilast 30 mg BID, n/m	42/91	53/91	50/88	53/85	53/83	53/79
Placebo/Apremilast 30 mg BID, n/m	22/97	30/97	38/97	45/95	47/94	54/90

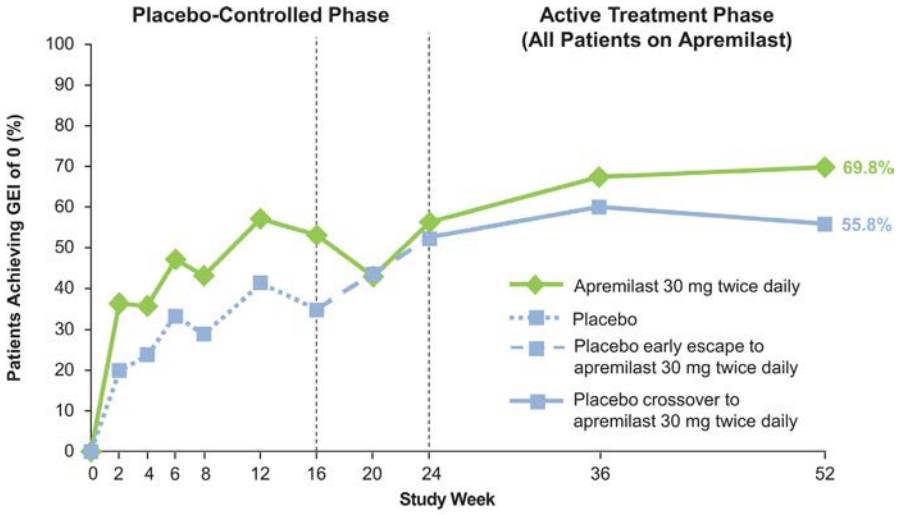


Apremilast 30 mg BID, n	91	91	88	85	83	80
Placebo/Apremilast 30 mg BID, n	97	97	97	95	94	91



Apremilast 30 mg BID, n	91	91	88	85	83	80
Placebo/Apremilast 30 mg BID, n	97	97	97	95	94	91

Figure 1 (A) ACR20 response, (B) mean per cent change in SJC and (C) mean per cent change in TJC through week 52. All data shown are as observed among patients as randomised at baseline and receiving at least one dose of apremilast. ACR20, 20% improvement in modified American College of Rheumatology response criteria; n/m, number of responders/number of patients with sufficient data for evaluation; SJC, swollen joint count; TJC, tender joint count.



Apremilast 30 mg BID, n/m	26/49	21/49	27/48	31/46	30/43
Placebo/Apremilast 30 mg BID, n/m	16/46	20/46	24/46	27/45	24/43

Figure 2 Proportion of patients achieving a GEI of 0* through week 52. All data shown are as observed among patients as randomised at baseline, receiving at least one dose of apremilast and having pre-existing enthesopathy at baseline (eg, GEI score >0, n=102). GEI, Gladman Enthesitis Index; n/m, number of responders/number of patients with sufficient data for evaluation.

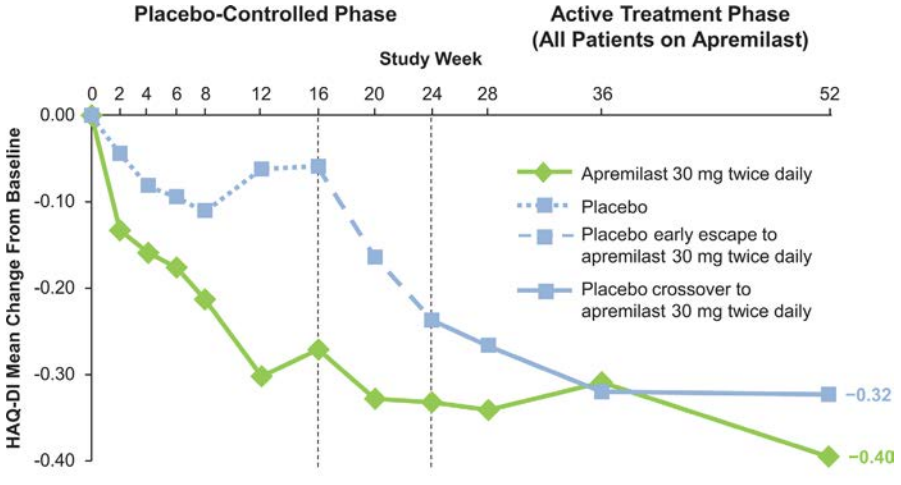
No patients reported weight decrease as an AE during the study; 78.9% of apremilast patients remained within ±5% of their baseline weight. At the end of the 52-week period, mean weight loss for apremilast patients was −1.20 kg and 15.7% of apremilast patients had experienced >5% weight loss.

DISCUSSION

ACTIVE was the first randomised controlled study to assess the onset of response to apremilast monotherapy in biological-naïve patients with active PsA. This study demonstrated that at week 2, many patients had clinical improvements across several PsA manifestations, including swollen and tender joints, enthesitis (among those with enthesitis at baseline), physical impairment and improvement in morning stiffness severity. Likewise, significant improvements in PsA measures at weeks 16 and 24 were observed with apremilast. Treatment response was maintained up to week 52 across measures for patients continuing apremilast and for placebo patients who switched to apremilast at week 16 or week 24.

These findings provide new data for apremilast, demonstrating that a proportion of patients experienced improvements in common symptoms of PsA at week 2. Additionally, the use of the GEI to assess peripheral entheseal sites in ACTIVE adds to our current knowledge of its effect on other entheseal sites, as measured by the Maastricht Ankylosing Spondylitis Enthesitis Score.

The PALACE studies evaluated apremilast in patients with several treatment failures (PALACE 1–3) and as a first-line treatment for DMARD-naïve patients (PALACE 4).[4–7] Most (~70%) of the ACTIVE patient population had exposure to one csDMARD. Efficacy in this subpopulation was similar to that of the overall population in ACTIVE. These findings further support apremilast as a treatment option for patients with PsA across the spectrum of treatment experiences.



Apremilast 30 mg BID, n	91	91	88	85	83	80
Placebo/Apremilast 30 mg BID, n	97	97	97	95	94	91

Figure 3 Mean change from baseline in HAQ-DI score through week 52. All data shown are as observed among patients as randomised at baseline and receiving at least one dose of apremilast. HAQ-DI, Health Assessment Questionnaire-Disability Index.

Table 3 Nature, incidence and severity of AEs

	Placebo-controlled phase (weeks 0–24*)		Cumulative apremilast exposure†
Patients, n (%)	Placebo n=109	Apremilast 30 mg twice daily n=109	Apremilast 30 mg twice daily n=206
Any AE	69 (63.3)	73 (67.0)	144 (69.9)
Any serious AE‡	5 (4.6)	3 (2.8)	10 (4.9)
Any AE leading to study drug withdrawal	5 (4.6)	10 (9.2)	17 (8.3)
Any AE leading to death	0 (0.0)	0 (0.0)	1 (0.5)
AEs with incidence ≥5% in any treatment group			
Diarrhoea§	12 (11.0)	16 (14.7)	33 (16.0)
Nausea	2 (1.8)	9 (8.3)	16 (7.8)
Nasopharyngitis	7 (6.4)	9 (8.3)	16 (7.8)
Headache	4 (3.7)	8 (7.3)	12 (5.8)
Hypertension	7 (6.4)	7 (6.4)	13 (6.3)
Upper respiratory tract infection	11 (10.1)	5 (4.6)	14 (6.8)
Select laboratory assessments, n/m (%)			
ALT >3 × ULN, U/L	1/108 (0.9)	1/108 (0.9)	4/205 (2.0)
Creatinine >1.7 × ULN, µmol/L	0/108 (0.0)	0/108 (0.0)	1/205 (0.5)
Haemoglobin value,<10.5 g/dL (male) or <8.5 g/dL (female)	2/108 (1.9)	0/109 (0.0)	2/205 (1.0)
Leucocytes <1.5, 10^9/L	0/108 (0.0)	0/109 (0.0)	0/205 (0.0)
Neutrophils <1.0, 10^9/L	1/108 (0.9)	1/109 (0.9)	1/205 (0.5)
Platelets <75, 10^9/L	1/107 (0.9)	0/109 (0.0)	0/204 (0.0)

*Includes the data through week 16 for placebo patients who escaped, and the data through week 24 for all other patients.
†Includes all available apremilast-exposure data up to the data cut of 5 November 2015 (including data beyond 52 weeks); patients with multiple reports are only counted once.
‡During the placebo-controlled phase, serious AEs reported by patients on placebo (n=5) were iron deficiency anaemia, angina pectoris, chest pain, cervical vertebral fracture, spinal column injury, acute myeloid leukaemia and respiratory papilloma; serious AEs reported by patients on apremilast 30 mg twice daily (n=3) were biliary colic, head injury and joint dislocation. New serious AEs of atrial fibrillation, coronary artery disease, alcoholic cardiomyopathy, hypertensive heart disease, cholelithiasis, infective arthritis, bladder transitional cell carcinoma, anxiety, ureteric obstruction and arteriosclerosis were reported by seven patients in the cumulative apremilast-exposure period.
§When using protocol-defined characterisation of diarrhoea of two or more watery or liquid stools/day, incidence rates were 8.3% for placebo and 11.0% for apremilast 30 mg twice daily during the placebo-controlled phase.
AEs, adverse events; ALT, alanine aminotransferase; n/m, number of patients with at least one occurrence of the abnormality/number of patients with at least one post-baseline value; ULN, upper limit of normal.

Apremilast was well tolerated in this biological-naïve PsA patient population; additionally, the overall safety profile in ACTIVE was found to be consistent with that observed in the PALACE studies.[4–7] An important study objective was to further characterise the gastrointestinal AE of diarrhoea. Overall, fewer cases of protocol-defined diarrhoea (two or more watery stools/day) were observed versus non-defined reported events. This criterion is more inclusive than the WHO's definition of diarrhoea of at least three loose or liquid stools/day. Diarrhoea AEs typically occurred within the first 4 weeks of treatment, were self-limiting, resolving within 15 days and usually did not require any major medical treatment.

Apremilast has a unique mechanism of action in modulating the expression of both pro-inflammatory and anti-inflammatory cytokines[16]; in ACTIVE, no evidence of increased incidence of serious or opportunistic infections and no cases of active tuberculosis with 52-week apremilast exposure were observed. Laboratory abnormalities were infrequent and showed no evidence of organ toxicity requiring specific monitoring. Safety results were consistent with the previous PALACE studies and provide additional characterisation of AEs of diarrhoea experienced during the placebo-controlled phase. The study design for ACTIVE allowed for immediate stopping of methotrexate without washout, which may be a desired option for some patients in routine clinical practice settings. Switching such as this happened seamlessly without any significant disease worsening/flares or tolerability issues.

Limitations

Several limitations should be considered when interpreting the study findings and comparing them with other apremilast clinical studies. The ACTIVE patient population had baseline heterogeneity regarding disease duration. Moreover, early escape was at the investigator's discretion, which may be biased with apremilast availability on the market. Longer term findings may be biased because patients who did not respond to or tolerate treatment may be more likely to discontinue. ACTIVE did not evaluate dactylitis, skin and nail outcomes; however, apremilast's impact on such outcomes has been assessed in the PALACE[6 17] and Efficacy and Safety Trial Evaluating the Effects of Apremilast in Psoriasis (ESTEEM) studies.[18 19] Additionally, this study did not include imaging to evaluate structural damage. Morning stiffness findings should be interpreted cautiously, as understanding of morning stiffness and PsA disease activity is limited.

CONCLUSIONS

For biological-naïve patients with active PsA, apremilast monotherapy resulted in early and sustained improvements across PsA manifestations, including swollen and tender joints, enthesitis and morning stiffness. No new safety concerns were observed. These results support the use of apremilast monotherapy in biological-naïve patients with PsA.

Acknowledgements The authors would like to thank Thomas Rano, PhD, Suktae Choi, PhD, and Marla Hochfeld, MD, of Celgene Corporation for their contributions

to the study. The authors received editorial support in the preparation of this report from Kristin Carlin, RPh, MBA, of Peloton Advantage, LLC, funded by Celgene Corporation. The authors, however, directed and are fully responsible for all content and editorial decisions.

Collaborators The ACTIVE study investigators: Australia – Christopher Fong (Boxhill Hospital, Camberwell, VIC); Jane Zochling (Southern Clinical Research, Hobart, TAS); Peter Yousseff (Royal Prince Alfred Hospital, Camperdown, NSW); Paul Bird (St. George Private Hospital, Miranda, NSW); Nicholas Manolios (Westmead Hospital, Sydney, NSW); and Rob Will (Colin Bayliss Research/Teaching Unit, Victoria Park, WA). Canada – Ben Lasko and Melanie Mason (Manna Research, Toronto, ON); Jude Rodrigues (private practice, Windsor, ON); Alfred Cividino (private practice, Hamilton, ON); Majed Khraishi (Nexus Clinical Research, St. John's, NL); Arthur Karasik (Arthur Karasik Medicine Professional Corp, Etobicoke, ON); and Louis Bessette (Groupe de Recherche en Maladies Osseuses Inc., Quebec, QC). Czech Republic – Petr Vitek (PV Medical Sro, Zlin); Zuzana Stejfova and Alena Ticha (Revmatologicka Ambulance, Praha); and Katerina Jarosova (Revmatologicky Ustav, Praha). Estonia – Airi Poder (Clinical Research Centre Ltd, Tartu); Andres Pille (East Tallinn Central Hospital, Tallinn); and Jaak Tälli (Innomedica, Tallinn). Hungary – László Sámson (MÁV Kórház és Rendelőintézet, Budapest); Istvan Szombati (Qualiclinic Kft, Budapest); Kiss Csaba (Csolnoky Ferenc Kórház, Veszprém); and Szántó Sándor (Debreceni Egyetem Orvos, Debreceni). New Zealand – Daniel Ching (Timaru Hospital, Timaru); Rajiv Gupta (Middlemore Hospital, Auckland); and Doug White (Rheumatology Clinic, Auckland). Romania – Mariana Pavel (Sf. Apostol Andrei Emergency Clinical County Hospital, Galați); Violeta Bojinca (Sf. Maria Clinical Hospital, Bucharest); Silvia Bojin (Covamed Serv SRL, Sfântu Gheorghe); and Simona Rednic (Cluj-Napoca Emergency Clinical County Hospital, Cluj-Napoca). Russia – Valentin Oleynikov (Penza Regional Clinical Hospital, Penza); Leysan Myasoutova (City Clinical Hospital #7, Kazan); Nikolay Korshunov (Yaroslavl Reg. Clinical Hospital, Yaroslavl); and Diana Krechikova (Dept. Hospital at Smolensk, Smolensk). Spain – Francisco Blanco (Complejo Hospitalario Universitario, Coruña); Antonio Fernandez-Nebro (Hospital General de Málaga, Málaga); Federico Díaz-Gonzalez (Hospital Universitario de Canarias, Santa Cruz de Tenerife); Eduardo Angulo (Hospital de Basurto – Osakidetza, Basurto); Emilio Mola (Hospital Universitario La Paz, Madrid); Jordi Gratacos Masmitja (Corporació Sanitària Parc Taulí, Barcelona); Jesús Rodriguez (Hospital Universitari de Bellvitge, l'Hospitalet de Llobregat); and Agusti Sellas Fernandez (Hospital Universitari Vall d'Hebron, Barcelona). United States – Monika Mohan (Advanced Rheumatology, Lansing, MI); Richard Olson (Rockford Orthopedic Associates, Ltd, Rockford, IL); Carmen Perez-Masuelli (The Clinical Research Institute of Houston, Houston, TX); Jeffrey Alloway (Physicians East, PA, Greenville, NC); Jeffrey Alper (private practice, Naples, FL); Alan J. Kivitz (Altoona Center For Clinical Research, Altoona, PA); Miriam Lara (Palmetto Medical Research, Hialeah, FL); Vipul Joshi (Bay Area Arthritis and Osteoporosis, Brandon, FL); Roger Diegel (Glacier View Research Institute, Kalispell, MT); Tina Bunch (Austin Regional Clinic, Austin, TX); Alfred Felber (Heartland Clinical Research Inc., Augusta, KS); Michael Calmes (Arthritis and Osteoporosis Associates, LLP, Lubbock, TX); David McLain (Achieve Clinical Research, LLC, Birmingham, AL); Robert Holmes (Piedmont Medical Research, LLC, Winston-Salem, NC); John Carter (University of South Florida, Tampa, FL); Farrukh Zaidi (Suncoast Clinical Research, New Port Richey, FL); Maria Greenwald (Desert Medical Advances, Palm Desert, CA); Michael Burnette (Health Point Medical Group, Brandon, FL); Ramesh Gupta (private practice, Memphis, TN); Charles Bradley Franz (Mountain State Clinical Research, Bridgeport, WV); John Cush (Baylor Research Institute, Dallas, TX); Craig Wiesenhutter (Coeur d'Alene Arthritis Clinic, Coeur d'Alene, ID).

Contributors All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. PN had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study conception and design: PN and ND. Acquisition of data: ND and DN. Analysis and interpretation of data: all authors. Statistical expertise: LT.

Funding This study was sponsored by Celgene Corporation.

Competing interests PN has received grant/research support and honoraria from Celgene Corporation. KO has received grant/research support from Celgene Corporation. JW has received non-financial support from Amgen Inc, Pfizer and UCB and has received personal fees from Celgene Corporation and Novartis. ND, DN and LT are employees of Celgene Corporation. JJG-R has received grant/research support from Roche and Schering-Plough and has served as a consultant to Bristol-Myers Squibb, Pfizer, Roche, Schering-Plough and UCB. JAA has received grant/research support from AbbVie Inc, Ardea Biosciences, Inc, AstraZeneca, Bristol-Myers Squibb, Celgene Corporation, Centocor, Galápagos, Genentech Inc, GlaxoSmithKline, Human Genome Sciences, Janssen, Eli Lilly and Company, Merck & Co, Mesoblast, Novartis Pharmaceuticals Corporation, Novo Nordisk, Pfizer, Roche, Sanofi-Aventis, Takeda Pharmaceuticals, UCB and Vertex Pharmaceuticals.

Patient consent Obtained.

Ethics approval Institutional review boards of the participating centres.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement All authors had full access to study data and had final responsibility for the decision to submit.

Author note The results from this study have been published in abstract form: Nash P *et al*. Early onset of efficacy with apremilast monotherapy in biologic-naïve patients with active psoriatic arthritis: a phase IIIB, randomized, controlled trial [abstract 1703]. *Arthritis Rheumatol* 2016;68(Suppl 10):2133–4, and Nash P *et al*. Early onset of efficacy with apremilast monotherapy in biologic-naïve patients with active psoriatic arthritis: a phase IIIB, randomized, controlled trial [abstract OP0219]. *Ann Rheum Dis* 2017;76(Suppl 2):143.

REFERENCES

1 Coates LC, Kavanaugh A, Mease PJ, *et al*. Group for research and assessment of psoriasis and psoriatic arthritis 2015 treatment recommendations for psoriatic arthritis. *Arthritis Rheumatol* 2016;68:1060–71.
2 Kalb RE, Fiorentino DF, Lebwohl MG, *et al*. Risk of serious infection with biologic and systemic treatment of psoriasis: results from the Psoriasis Longitudinal Assessment and Registry (PSOLAR). *JAMA Dermatol* 2015;151:961–9.
3 Nast A, Boehncke WH, Mrowietz U, *et al*. German S3-guidelines on the treatment of psoriasis vulgaris (short version). *Arch Dermatol Res* 2012;304:87–113.
4 Kavanaugh A, Mease PJ, Gomez-Reino JJ, *et al*. Longterm (52-week) results of a phase III randomized, controlled trial of apremilast in patients with psoriatic arthritis. *J Rheumatol* 2015;42:479–88.
5 Cutolo M, Myerson GE, Fleischmann RM, *et al*. A phase III, randomized, controlled trial of apremilast in patients with psoriatic arthritis: results of the PALACE 2 trial. *J Rheumatol* 2016;43:1724–34.
6 Edwards CJ, Blanco FJ, Crowley J, *et al*. Apremilast, an oral phosphodiesterase 4 inhibitor, in patients with psoriatic arthritis and current skin involvement: a phase III, randomised, controlled trial (PALACE 3). *Ann Rheum Dis* 2016;75:1065–73.
7 Wells AF, Edwards CJ, Kivitz AJ, *et al*. *Apremilast monotherapy as the first systemic treatment in DMARD-naive patients with active psoriatic arthritis: 3-year treatment results [abstract THU0422]*. London, UK: Presented at: the Annual Congress of the European League Against Rheumatism, 2016.
8 Edwards CJ, Adebajo AO, Kivitz AJ, *et al*. *Apremilast, an oral phosphodiesterase 4 inhibitor, is associated with long-term (52-week) improvements in enthesitis or dactylitis in patients with psoriatic arthritis: results from the PALACE 4 phase 3, randomized, controlled trial [poster 1572]*. Boston, MA: Presented at: the Annual Meeting of the American College of Rheumatology/Association of Rheumatology Health Professionals, 2014.
9 Edwards CJ, Blanco FJ, Crowley JJ, *et al*. Apremilast is associated with long-term DAS-28 (CRP) remission and improvements in skin disease: results from a phase III study in DMARD/biologic-experienced active psoriatic arthritis patients [abstract 1734]. *Arthritis Rheumatol* 2016;68(Suppl 10().
10 Healy PJ, Helliwell PS. Measuring clinical enthesitis in psoriatic arthritis: assessment of existing measures and development of an instrument specific to psoriatic arthritis. *Arthritis Rheum* 2008;59:686–91.
11 Taylor W, Gladman D, Helliwell P, *et al*. Classification criteria for psoriatic arthritis: development of new criteria from a large international study. *Arthritis Rheum* 2006;54:2665–73.
12 Landis JR, Heyman ER, Koch GG. Average partial association in three-way contingency tables: a review and discussion of alternative tests. *International Statistical Review / Revue Internationale de Statistique* 1978;46:237–54.
13 Kwok T, Pope JE. Minimally important difference for patient-reported outcomes in psoriatic arthritis: health assessment questionnaire and pain, fatigue, and global visual analog scales. *J Rheumatol* 2010;37:1024–8.
14 Mease PJ, Woolley JM, Bitman B, *et al*. Minimally important difference of health assessment questionnaire in psoriatic arthritis: relating thresholds of improvement in functional ability to patient-rated importance and satisfaction. *J Rheumatol* 2011;38:2461–5.
15 Revicki DA, Menter A, Feldman S, *et al*. Adalimumab improves health-related quality of life in patients with moderate to severe plaque psoriasis compared with the United States general population norms: results from a randomized, controlled Phase III study. *Health Qual Life Outcomes* 2008;6:75.
16 Schafer P. Apremilast mechanism of action and application to psoriasis and psoriatic arthritis. *Biochem Pharmacol* 2012;83:1583–90.

17 Kavanaugh A, Mease PJ, Gomez-Reino JJ, *et al*. Treatment of psoriatic arthritis in a phase 3 randomised, placebo-controlled trial with apremilast, an oral phosphodiesterase 4 inhibitor. *Ann Rheum Dis* 2014;73:1020–6.
18 Papp K, Reich K, Leonardi CL, *et al*. Apremilast, an oral phosphodiesterase 4 (PDE4) inhibitor, in patients with moderate to severe plaque psoriasis: results of a phase III, randomized, controlled trial (Efficacy and Safety Trial Evaluating the Effects of Apremilast in Psoriasis [ESTEEM] 1). *J Am Acad Dermatol* 2015;73:37–49.
19 Paul C, Cather J, Gooderham M, *et al*. Efficacy and safety of apremilast, an oral phosphodiesterase 4 inhibitor, in patients with moderate-to-severe plaque psoriasis over 52 weeks: a phase III, randomized controlled trial (ESTEEM 2). *Br J Dermatol* 2015;173:1387–99.

EXTENDED REPORT

Limited radiographic progression and sustained reductions in MRI inflammation in patients with axial spondyloarthritis: 4-year imaging outcomes from the RAPID-axSpA phase III randomised trial

Désirée van der Heijde,[1] Xenofon Baraliakos,[2] Kay-Geert A Hermann,[3] Robert B M Landewé,[4] Pedro M Machado,[5] Walter P Maksymowych,[6] Owen R Davies,[7] Natasha de Peyrecave,[7] Bengt Hoepken,[8] Lars Bauer,[8] Tommi Nurminen,[8] Juergen Braun[9]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212377).

For numbered affiliations see end of article.

Correspondence to
Professor Désirée van der Heijde, Department of Rheumatology, Leiden University Medical Center, Leiden 2300 RC, The Netherlands; mail@dvanderheijde.nl

Data from this study have previously been presented at the ACR/ARHP Annual Meeting 2016 and the European League Against Rheumatism Annual Meeting 2017.

Received 12 September 2017
Revised 20 December 2017
Accepted 29 December 2017
Published Online First 17 January 2018

To cite: van der Heijde D, Baraliakos X, Hermann K-GA, *et al*. *Ann Rheum Dis* 2018;**77**:699–705.

ABSTRACT

Objectives To report 4-year imaging outcomes in the RAPID-axSpA (NCT01087762) study of patients with ankylosing spondylitis (AS) and non-radiographic axial spondyloarthritis (nr-axSpA), treated with certolizumab pegol (CZP).

Methods This phase III, randomised trial was placebo-controlled and double-blind to week 24, dose-blind to week 48 and open-label to week 204. Patients fulfilling the Assessment of Spondyloarthritis International Society (ASAS) axSpA criteria with active disease were stratified (AS/nr-axSpA) according to the modified New York (mNY) criteria at randomisation. Spinal radiographs were assessed using the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS). MRI inflammation used the Spondyloarthritis Research Consortium of Canada (SPARCC) score for sacroiliac joints (SIJ) and the Berlin spinal score (remission defined as SPARCC <2 and Berlin ≤2, respectively).

Results MRI improvements from baseline (BL) to week 12 were maintained to week 204 (SPARCC BL: AS=8.5, nr-axSpA=7.5; SPARCC week 204: AS=1.3, nr-axSpA=2.4; Berlin BL: AS=7.4, nr-axSpA=4.4; Berlin week 204: AS=2.6, nr-axSpA=1.9). 66.7% of patients with AS and 69.6% of patients with nr-axSpA with BL SPARCC scores ≥2, and 65.4% of patients with AS and 57.3% of patients with nr-axSpA with BL Berlin score >2, achieved remission at week 204. Mean mSASSS change in AS from BL to week 204 was 0.98 (95% CI 0.34, 1.63); 0.67 (95% CI 0.21,1.13) from BL to week 96; and 0.31 (95% CI 0.02,0.60) from week 96 to week 204. Corresponding nr-axSpA changes were 0.06 (95% CI −0.17,0.28), −0.01 (95% CI −0.19,0.17) and 0.07 (95% CI −0.07,0.20). 4.5% of patients with nr-axSpA fulfilled the mNY criteria at week 204, while 4.3% of patients with AS no longer did so.

Conclusions In patients with CZP-treated axSpA, rapid decreases in spinal and SIJ MRI inflammation were maintained to week 204. Overall, 4-year spinal progression was low, with less progression during years 2–4 than 0–2. Radiographic SIJ grading changes demonstrated limited progression.

Trial registration number NCT01087762; Post-results.

INTRODUCTION

Axial spondyloarthritis (axSpA) is a chronic inflammatory disease primarily characterised by inflammation of the axial skeleton (the spine and the sacroiliac (SI) joints). Patients with evidence of structural damage to the SI joints (radiographic sacroiliitis), which is identifiable using X-ray imaging and fulfils the modified New York (mNY) classification criteria, are considered to have ankylosing spondylitis (AS; also termed radiographic axSpA). However, many patients with axSpA do not fulfil the mNY criteria; this is termed non-radiographic axSpA (nr-axSpA) and has the potential to develop into AS.[1 2] Importantly, the disease burden and clinical features are similar in both subpopulations, representing a spectrum of the same disease.[3 4]

In contrast to clinical outcomes, long-term imaging data in tumour necrosis factor (TNF) inhibitor-treated patients are limited. There are currently no long-term modified Stoke Ankylosing Spondylitis Spine Score (mSASSS)[5] data available for patients with nr-axSpA.

RAPID-axSpA is the only large trial to include both patients with AS and nr-axSpA and previously demonstrated that certolizumab pegol (CZP), a PEGylated fragment-crystallisable (Fc)-free anti-TNF agent, improved the signs and symptoms of axSpA from as early as 12 weeks of treatment, which were maintained over 4 years.[4 6–8]

Here, we report the imaging outcomes over 4 years of CZP treatment. This represents the longest term MRI imaging study in patients with anti-TNF-treated axSpA to date, and the only data addressing X-ray and MRI imaging of both SI joints and spine in AS and nr-axSpA subpopulations.

METHODS

Study design

RAPID-axSpA (NCT01087762) was a 204-week, phase III, randomised, parallel-group, multicentre trial, conducted at 83 centres in Europe, North America and Latin America. The study was placebo-controlled and double-blind until week 24, dose-blind to week 48 and open-label to week 204.

Full details have been published previously.[4] Patients were randomised 1:1:1 to placebo or CZP 400 mg at weeks 0, 2 and 4 (loading dose),

followed by either CZP 200 mg every 2 weeks or CZP 400 mg every 4 weeks (online supplementary figure 1).

Patients

Full inclusion and exclusion criteria have been reported previously.[4] Eligible participants were aged ≥18 years at screening and fulfilled the ASAS axSpA classification criteria, with a clinical diagnosis of adult-onset axSpA of ≥3 months' duration and active disease defined by Bath Ankylosing Spondylitis Activity Index ≥4, spinal pain ≥4 on a 0–10 Numerical Rating Scale, and either elevated C-reactive protein (>7.9 mg/L) or a positive SI joint MRI assessment.

To define AS and nr-axSpA subpopulations, the most recent SI joint X-rays (performed ≤12 months prior to screening) were locally read to determine the presence/absence of radiographic sacroiliitis.

Study procedures and evaluations

The primary outcome (ASAS20 response at week 12) has been reported previously,[4] as have clinical data to week 204[6 7] and imaging data to week 96.[4 6 8–10] Here we report the long-term imaging results (radiographs and MRI of both SI joints and spine) from the complete 4-year study period.

SI joint X-rays were conducted at baseline and week 204/early withdrawal (if after week 104). Lateral radiographs of the lumbar/cervical spine were performed at baseline, week 96 and week 204. MRI assessments of both the spine and SI joints were conducted at baseline and weeks 12, 48, 96 and 204. MRI and radiograph assessments were each performed independently by two central readers blinded to timepoint, treatment group and clinical data. In the event of disagreement between central readers when grading SI joint radiographs, an additional third reader assessed the radiographs from the patient in question. A third reader was not used for MRI or spinal radiographs.

The short tau inversion recovery sequence of MRI scans was assessed for disease activity using the Spondyloarthritis Research Consortium of Canada (SPARCC) scoring method for SI joints (0–72 scale)[11] and the Berlin modification of the Ankylosing Spondylitis spine MRI-activity scoring system for the spine (0–69 scale).[12] Spinal radiographs were assessed using the mSASSS scoring method.

Data are reported from the week 204 reading campaign, which included all available images from baseline to week 204 with the exception of SI joint radiographs; only SI joint images from patients with both baseline and week 204/early withdrawal radiographs were included.

Statistical analysis

Data are presented for patients who received ≥1 dose of CZP (200 mg every 2 weeks (Q2W) and 400 mg every 4 weeks (Q4W) groups combined) at any timepoint to week 204, including rerandomised placebo-treated patients. Statistical analyses were conducted assuming data were missing at random.[13] The number of images available for each imaging modality is presented in online supplementary table 1.

The MRI set included all randomised patients with valid MRI assessments (either spine or SI joint) at baseline and ≥1 other timepoint during the trial (n=158). Week 12 MRI data were not used from patients randomised to placebo. Average MRI scores of the two readers were considered for statistical analyses, and group least squares (LS) mean Berlin and SPARCC scores were estimated post hoc by mixed-model repeated measures (MMRM) analysis on observed data using 'visit' as a fixed factor with an unstructured within-patient covariance matrix. The proportions achieving MRI remission (SPARCC <2 or Berlin ≤2) were estimated by multiple imputation: estimated proportions of patients in MRI remission were pooled from 50 multiply imputed data sets, where missing actual scores were imputed via predicted mean matching, with the predicted value at a visit based on linear regression of values from other visits.[14 15] Results were summarised for patients with MRI baseline inflammation (Berlin score >2 or SPARCC score ≥2).

Radiographic data were examined for all CZP-treated patients with ≥1 mSASSS assessment (X-ray set), including those rerandomised from placebo. Based on average scores of the two readers, LS mean mSASSS and changes between visits were estimated using MMRM analyses on observed data, as described above. The online supplementary material includes observed mean changes for subjects with a complete sequence of images at baseline, week 96 and week 204. Radiographic progression rates (an increase of ≥2 points from baseline) at weeks 96 and 204 were estimated using multiple imputation, as described above. Within-patient correlation coefficients were calculated between change from baseline to week 96, and change from week 96 to week 204. Plausibility of the missing-at-random assumption was evaluated by comparing disease activity outcomes of patients with no mSASSS data at week 96/week 204 and those with data at all relevant timepoints. In particular, Ankylosing Spondylitis Disease Activity Score (ASDAS) levels were compared using observed data and last observation carried forward-imputed data. For plots of individual patient mSASSS results, observed data are presented for all patients with ≥2 valid mSASSS assessments. Patients with ≥1 non-bridging or bridging syndesmophyte (defined as a score of 2 or 3, respectively) and syndesmophyte formation (defined as a shift in score from 0 or 1 to 2 or 3) were considered when reported by both readers for a given vertebral edge.

Presence of definitive sacroiliitis (grade ≥2 bilateral or grade 3–4 unilateral) was based on the judgement of two central readers. In the event of disagreement, a third reader's results were used to provide a majority. Agreement between central readers was calculated using simple kappa (κ) statistics. Statistical analyses were performed with SAS V.9.3 and V.9.4.

RESULTS

Patient disposition and baseline characteristics

Three hundred and twenty-five patients were randomised at week 0, 107 to placebo and 218 to CZP (111 to CZP 200 mg Q2W and 107 to CZP 400 mg Q4W). Of 315 patients (174 AS and 141 nr-axSpA) who received ≥1 dose CZP at any point in the trial, 199 (63.2%) completed the study to week 204. One hundred and fifty-eight patients had valid MRI assessments at baseline and ≥1 other timepoint (MRI set). The baseline characteristics of the MRI set and overall population were similar (table 1, and data not shown).

One hundred and ninety-six patients had ≥1 mSASSS assessment and were included in the MMRM and multiple imputation analyses of radiograph parameters (X-ray set; online supplementary table 2); this included 45 patients who received 1 mSASSS reading at baseline with no further mSASSS assessments during the study. No major differences in disease activity were observed between those with and without complete mSASSS data (online supplementary table 3). One hundred and thirty-seven patients with SI joint radiographs at baseline and week 204/early withdrawal were assessed for radiographic progression based on the mNY criteria.

Table 1 Baseline characteristics of all CZP patients

	Overall axSpA N=315	AS n=174	nr-axSpA n=141
Mean age, years (SD)	39.7 (12.0)	41.5 (11.7)	37.5 (11.9)
Male, %	62.2	73.0	48.9
Symptom duration, years, median (min, max)	7.8 (0.3, 50.9)	9.1 (0.3, 50.9)	5.8 (0.3, 41.5)
CRP, mg/L, median (min, max)	13.4 (0.1, 174.8)	14.2 (0.1, 174.8)	12.0 (0.1, 156.2)
Patients with elevated CRP (>15 mg/L), %	40.6	43.7	36.9
BASDAI, mean (SD)	6.4 (1.5)	6.4 (1.6)	6.5 (1.5)
BASFI, mean (SD)	5.4 (2.3) n=314	5.7 (2.2)	4.9 (2.3) n=140
BASMI linear, mean (SD)	3.8 (1.7)	4.4 (1.7)	3.1 (1.5)
ASDAS, mean (SD)	3.9 (0.9) n=313	3.9 (0.9)	3.8 (0.8) n=139
Spinal radiographs			
mSASSS			
Mean (SD)*	9.5 (16.1)	13.2 (18.2)	4.4 (11.0)
Median	1.5 n=190	3.0 n=110	0.0 n=80
Patients with ≥1 bridging or non-bridging syndesmophyte at baseline, n (%)	63 (33.2)	47 (42.7)	16 (20.0)
MRI set	n=158	n=92	n=66
SPARCC (SI joints)			
Mean (95% CI)*	8.1 (6.1, 10.2)	8.5 (5.6, 11.4)	7.5 (4.4, 10.6)
Median	2.0 n=151	1.0 n=91	3.0 n=60
Patients with MRI inflammation (SPARCC ≥2), estimate, %	51.2	47.0	57.3
Berlin score (spine)			
Mean (95% CI)*	6.2 (4.8, 7.5), n=157	7.4 (5.6, 9.2)	4.4 (2.4, 6.5), n=65
Median	2.0 n=153	4.3	0.5 n=61
Patients with MRI inflammation (Berlin >2) estimate, %	50.4 n=157	57.6	40.1 n=65

*Least squares mean scores were estimated using mixed-model for repeated measures analyses. Inflammation was defined as Berlin >2 or SPARCC ≥2. Data are presented for all patients who received ≥1 dose CZP at any point in the trial. To define AS and nr-axSpA subpopulations, the most recent SI joint X-rays (performed ≤12 months prior to screening) were locally read to determine the presence/absence of radiographic sacroiliitis.

AS, ankylosing spondylitis; ASDAS, Ankylosing Spondylitis Disease Activity Score; axSpA, axial spondyloarthritis; BASDAI, Bath Ankylosing Spondylitis Activity Index; BASFI, Bath Ankylosing Spondylitis Functional Index; BASMI, Bath Ankylosing Spondylitis Metrology Index; CRP, C-reactive protein; CZP, certolizumab pegol; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score; nr, non-radiographic; SI, sacroiliac; SPARCC, Spondyloarthritis Research Consortium of Canada.

MRI data

Spinal and SI joint MRI assessments showed reduction of inflammation with rapid improvements from baseline to week 12 maintained to week 204 (figure 1). At baseline, the LS mean spinal inflammation assessed by Berlin score (standard error; SE) for AS and nr-axSpA subpopulations was 7.4 (0.92) and 4.4 (1.03), respectively, which reduced to 2.5 (0.50) and 1.7 (0.40) at week 12, and 2.6 (0.56) and 1.9 (0.44) at week 204. Similarly, the LS mean SPARCC scores for patients with AS and nr-axSpA at baseline were 8.5 (1.45) and 7.5 (1.53), respectively, which were reduced to 1.6 (0.66) and 2.6 (0.67) at week 12, and were maintained at 1.3 (0.46) and 2.4 (0.85) at week 204.

Of patients with respective inflammation at baseline, 66.7% (AS) and 69.6% (nr-axSpA) achieved SI joint MRI remission at week 204, and 65.4% of patients with AS and 57.3% of patients with nr-axSpA achieved spinal MRI remission.

Radiographic progression

Limited changes in SI joint grading were observed to week 204: 2/44 (4.5%) patients with nr-axSpA fulfilled the mNY criteria, while 4/93 (4.3%) patients with AS no longer did so at week 204. Agreement between the two central readers regarding the absence/presence of radiographic sacroiliitis was moderate, with disagreement occurring in 39/158 cases assessed at baseline (κ=0.49). In total, 113/158 (71.5%) images were read by a third reader due to grading disagreements between the main two readers.

Mean baseline mSASSS scores of 13.2 and 4.4 were observed in patients with AS and nr-axSpA, respectively. Limited spinal radiographic progression occurred in CZP-treated patients, with most progression seen in the AS cohort. In patients with AS, the mean mSASSS change between baseline and week 204 was 0.98 (95% CI 0.34 to 1.63), with the majority of progression observed during the first 2-year period (0.67 (95% CI 0.21 to 1.13)) compared with years 2–4 (0.31 (95% CI 0.02 to 0.60); figure 2). Patients with nr-axSpA exhibited a mean mSASSS change of 0.06 (95% CI −0.17 to 0.28) over 204 weeks. Observed changes in patients with complete mSASSS readings available at baseline, week 96 and week 204 are summarised in online supplementary figure 2; observed mean mSASSS changes between baseline and week 204 were 1.12 and 0.04 for patients with AS and nr-axSpA, respectively. Patients with AS who progressed during years 1 and 2 were more likely to progress in the second 2-year period: within-patient correlation coefficients between change from baseline to week 96, and change from week 96 to week 204, were 0.53 (AS) and 0.05 (nr-axSpA).

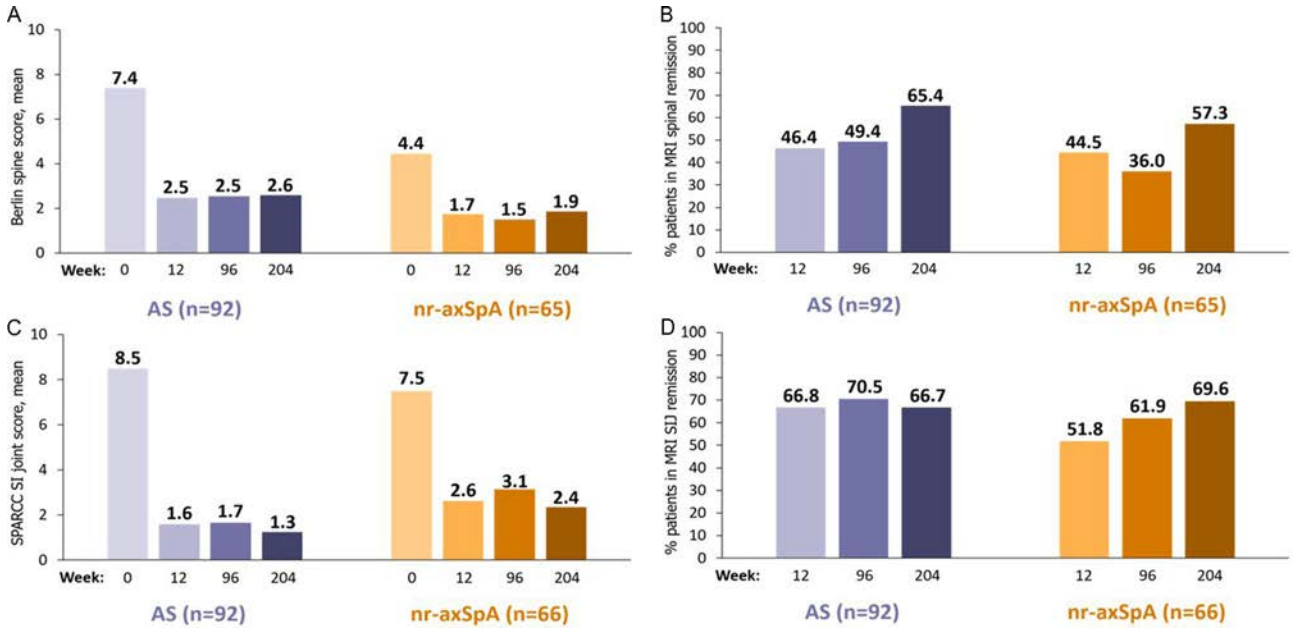


Figure 1 MRI imaging results to week 204. Sustained improvement in (A) LS mean Berlin score (MMRM), (B) percentage of patients in MRI spinal remission (Berlin score ≤2) to week 204 (missing at random (MAR)) in the subgroup of patients with MRI spinal inflammation at baseline (Berlin score >2), (C) LS mean SPARCC SIJ score (MMRM), and (D) percentage of patients in MRI SIJ remission (SPARCC score <2) to week 204 (MAR) in the subgroup of patients with inflammation at baseline (SPARCC ≥2). AS, ankylosing spondylitis; LS, least squares; MMRM, mixed-model repeated measures; nr-axSpA, non-radiographic axial spondyloarthritis; SIJ, sacroiliac joints; SPARCC, Spondyloarthritis Research Consortium of Canada.

Based on the multiple imputation analysis, 84.2% of patients with AS did not progress (progression was defined as an mSASSS increase of ≥2 points) from baseline to week 96. By week 204, 80.6% of patients with AS had not progressed. Progression was observed in only two patients with nr-axSpA, and therefore multiple imputation analysis was not performed. Of 85 patients with AS, 5 (5.9%) developed ≥1 syndesmophyte by week 96 and only 1 patient with nr-axSpA demonstrated one new syndesmophyte during the study. Sixty-one AS patients were assessed at week 204; at that time, no additional patients were observed to develop syndesmophytes. No patients with an absence of syndesmophytes at baseline developed syndesmophytes at 4 years. Individual patient mSASSS results are presented in figure 3.

DISCUSSION

RAPID-axSpA is the first long-term, large study to investigate imaging results in AS and nr-axSpA subpopulations when treated with a TNF-inhibitor. Here, CZP treatment rapidly improved axSpA inflammation of the spine and SI joints as observed using MRI in patients with AS and nr-axSpA. These improvements

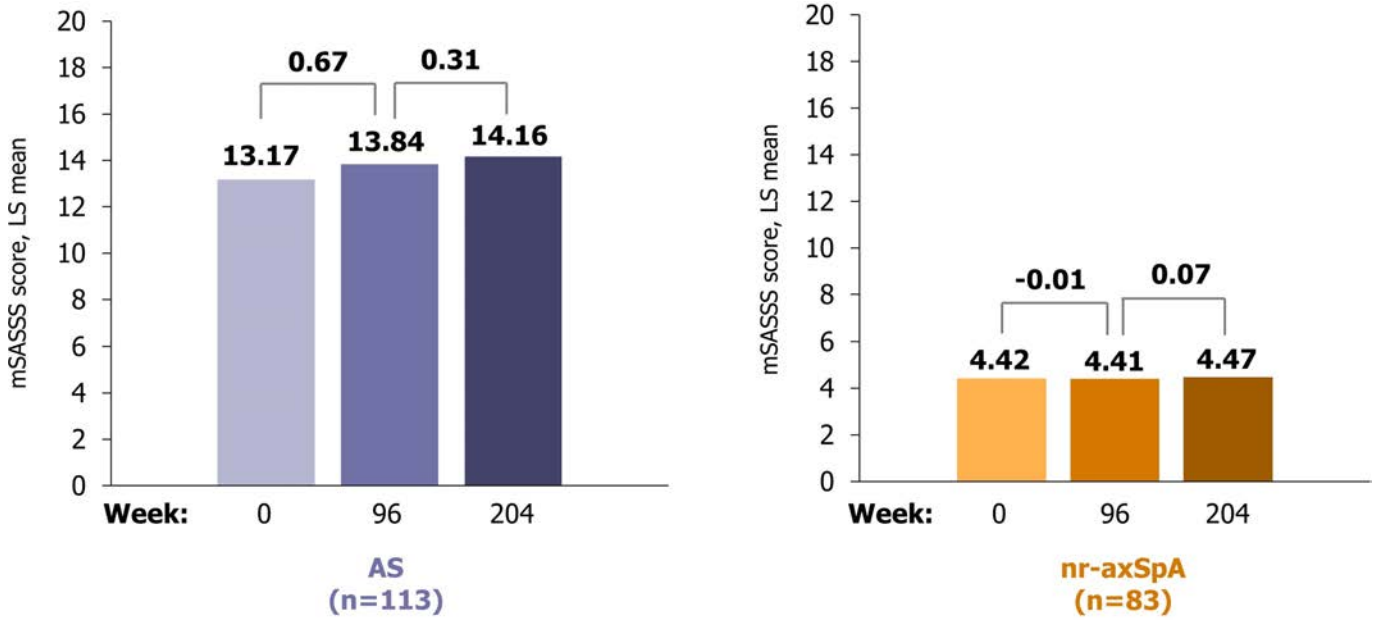
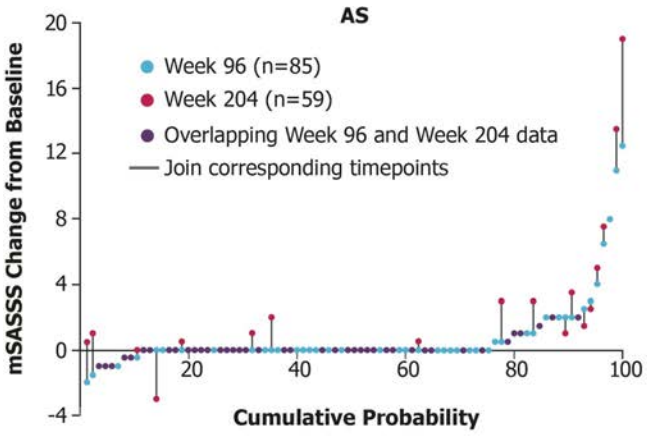
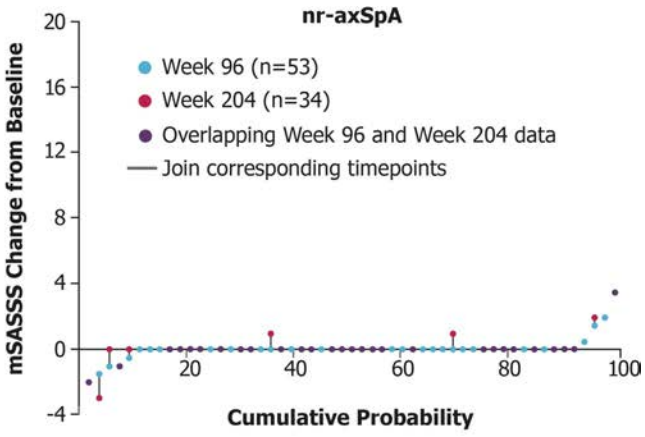


Figure 2 Radiographic imaging results of the spine to week 204. AS, ankylosing spondylitis; LS, least squares; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score; nr-axSpA, non-radiographic axial spondyloarthritis.



	Week 96 (%)	Week 204 (%)
mSASSS CFB >0	24.7	37.7
mSASSS CFB ≥2	15.3	19.7



	Week 96 (%)	Week 204 (%)
mSASSS CFB >0	7.5	10.3
mSASSS CFB ≥2	3.8	5.1

Figure 3 Proportion of patients with spinal progression at years 2 and 4. AS, ankylosing spondylitis; CFB, change from baseline; mSASSS, modified Stoke Ankylosing Spondylitis Spine Score; nr-axSpA, non-radiographic axial spondyloarthritis.

were maintained to week 204, with similar responses observed in both populations.

Changes from baseline in Berlin spine and SPARCC SI joint scores were comparable with other AS and nr-axSpA trials.[16 17] However, none of the longer term MRI studies (spanning up to 3 years of therapy) have reported results of both patients with AS and nr-axSpA in parallel.[16 18–21] It is important to note that comparisons between clinical trials should be treated with caution, as differences in population, study design and the years in which the trial was conducted can lead to variation between study outcomes. Comparisons between trials commencing at largely different timepoints may introduce chronology bias caused by differences in standard medical practice at the time of each investigation.

Many patients with MRI inflammation at baseline achieved spinal and SI joint MRI remission by week 204, with improvements in both scores seen as early as week 12 in both AS and nr-axSpA cohorts. Spinal MRI inflammation has been shown to be associated with radiographic progression, as vertebral edge inflammation contributes to the development of new syndesmophytes, although it remains to be proven that by reducing MRI inflammation in early axSpA, future structural damage may be prevented.

Limited changes from mNY negative to mNY positive were observed. Net progression to week 204 was minimal (−1.5%); similar proportions of patients 'progressed' from nr-axSpA to AS (4.5%) as 'regressed' from AS to nr-axSpA (4.3%). Given the low numbers of patients whose disease was reclassified, and the similar movement in both directions between the two populations, this is likely to represent intrareader variability, with little true progression. Previous follow-ups of untreated axSpA cohorts have reported progression rates (from nr-axSpA to AS) between 10% and 12% over 2 years.[22–24] In the DEvenir des Spondyloarthrites Indifférenciées Récentes (DESIR) cohort, the net progression from nr-axSpA to radiographic axSpA (AS) was 5.1% over 5 years.[25] However, a direct comparison between DESIR and RAPID-axSpA is not recommended since DESIR was a prevalence cohort of early axSpA. RAPID-axSpA also included patients with longer disease duration and higher disease activity. During the ESTHER trial, radiographic progression from nr-axSpA to AS was observed mainly between baseline and year 2 with no patients progressing to AS between years 2 and 4. In EMBARK, in which 161 patients had X-rays available at baseline and week 104, one patient (0.6%) satisfied the mNY criteria at baseline. Of 160 patients with mNY negative scores at baseline, none became mNY positive at week 104.[19 26]

Recognition of sacroiliitis on pelvic radiographs is generally considered to be difficult, due to both the complexity of the SI joints and the poor visualisation associated with plain radiographs. Previous radiographic studies have observed large intraobserver/interobserver variability in reading SI joint radiographs,[27–29] with significant variability reported between central and local readers.[22] In RAPID-axSpA, determination of mNY status, used as a stratification factor, was based on local SI X-ray reads, which are more reflective of daily clinical practice. This approach has been used in a number of previous AS trials investigating anti-TNFs, as well as the ABILITY-1 nr-axSpA study.

The rates of spinal radiographic progression in patients with axSpA are variable[2]; however, in the majority of patients with axSpA, several years may elapse before new bone formation in radiographs can be assessed. Therefore, a minimum 2-year follow-up is required to investigate radiographic progression. Structural spinal damage and inflammation in axSpA have an impact on patient quality of life, especially through reduction of mobility and function.[30–32] Recently Poddubnyy *et al*[33] investigated the effect of radiographic spinal progression and disease activity on function and spinal mobility in anti-TNF-treated patients with established AS. Both functional status and spinal mobility remained stable during 10 years of anti-TNF therapy despite radiographic progression, suggesting that reduction and control of inflammation may counteract the effects of radiographic spinal progression at a group level.

Interestingly, no patients with an absence of syndesmophytes at baseline developed syndesmophytes during 4 years of CZP treatment. Several studies showed that syndesmophyte prevalence predisposes to more rapid radiographic progression, and therefore could be used as a predictor for future radiographic damage despite the variability of progression rates in patients with axSpA.[34–36]

Here, we observed limited spinal radiographic progression, with a decrease in progression rate with long-term CZP therapy. After 4 years, 80.6% of patients with AS did not progress (<2 mSASSS points change from baseline) and the mean change was 0.98. As expected, patients with AS in RAPID-axSpA were generally more progressive than patients with nr-axSpA. The limited progression over 4 years observed in this study in patients with AS (80.3% non-progression defined as mSASSS change from baseline <2) is consistent with recent reports from the MEASURE 1 trial, in which 79% of patients with AS treated with secukinumab did not progress (<2 mSASSS points change from baseline) over 4 years.[37] However, these findings cannot be used in isolation to confirm an impact on disease progression since in both cases a control was absent. In the absence of a control arm, further data are required to elucidate the natural history of AS to better understand the impact of biologic treatment on disease progression. Long-term spinal X-ray data have also been reported for patients with AS in the GO-RAISE study[38] (4 years) and in an 8-year follow-up to a randomised controlled trial investigating the effects of infliximab, although this was conducted using a limited number of patients (n=69).[39] In GO-RAISE, patients with AS treated with 50 mg or 100 mg golimumab every 4 weeks demonstrated a mean mSASSS change from baseline (SD) of 1.3 (4.1) and 2.0 (5.6), respectively, at week 208[38] (although it should be noted that the GO-RAISE study should not be directly compared with RAPID-axSpA due to differing trial designs and study populations).

Two-year radiographic results from RAPID-axSpA exhibited a mean mSASSS change of 0.67 observed in patients with AS to week 96. The decrease in progression rate observed in RAPID-axSpA between years 2 and 4, and the diminished progression observed in long-term anti-TNF studies,[40 41] support earlier observations that prolonged use of TNF inhibitors may be associated with reduction of progression. Further evidence suggests a link between disease activity and radiographic progression. Results from the OASIS study, in which patients with AS were followed up for 12 years, found that disease activity was longitudinally associated with radiographic progression. In an AS prospective cohort study, the observed reduction in radiographic progression during anti-TNF treatment appeared to be mediated by a decrease in disease activity.[42] Consequently, long-term anti-TNF treatment could have the potential to inhibit structural progression by suppressing disease activity.

The analyses reported here are not without limitation. Patient withdrawal introduces bias, since patients whose symptoms do not improve sufficiently or those who suffer side effects are less likely to continue the trial. In long-term studies, the cumulative impact of missing data is more pronounced. Notably, there was a high proportion of missing MRI and radiographic data in this study. Since disease activity is likely to be associated with radiographic progression, ASDAS outcomes were compared between those with and without complete mSASSS assessments. Given that no major differences were observed, it is unlikely that study dropouts or otherwise missed assessments would have caused major bias to the study results.

Use of the mSASSS scoring system for quantification of radiographic progression could also be considered a limitation. The mSASSS system captures changes at the anterior vertebral corners of both the cervical and lumbar spine,[5] but does not evaluate other elements of the axial skeleton, for example, the thoracic spine or facet joints.[43] Subsequently, changes in these regions may have gone undetected. Nevertheless, at present, the mSASSS is the preferred scoring method for use in AS and is endorsed by the ASAS and Outcome Measures in Rheumatology (OMERACT).[44]

As two readers were used to evaluate the presence and formation of syndesmophytes, it was important to establish the level of agreement required. Here, agreement between readers was required at vertebral edge level; this approach is likely to underestimate the prevalence and incidence of syndesmophytes, but has been used in previous trials such as the ASSERT study.[32]

In conclusion, early improvements in MRI inflammation observed in a CZP-treated axSpA population, including both patients with AS and nr-axSpA, were maintained to week 204. MRI assessments demonstrated a rapid reduction of inflammation and sustained rates of remission in both SI joint and spinal examinations. Radiograph assessments revealed a low rate of spinal progression during the first 2 years of RAPID-axSpA with a decrease in the rate of spinal progression observed between years 2 and 4, and limited SI joint progression during 4 years of study.

Author affiliations
[1]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[2]Ruhr-University Bochum, Herne, Germany
[3]Department of Radiology, Charité Medical School, Berlin, Germany
[4]Academic Medical Center, Amsterdam and Atrium Medical Center, Heerlen, The Netherlands
[5]Centre for Rheumatology and MRC Centre for Neuromuscular Diseases, University College London, London, UK
[6]Department of Medicine, University of Alberta, Edmonton, Alberta, Canada
[7]UCB Celltech, Slough, UK
[8]UCB Pharma, Monheim, Germany
[9]Rheumazentrum Ruhrgebiet, Herne, Germany

Acknowledgements The authors thank the patients and their caregivers in addition to the investigators and their teams who contributed to this study. The authors also acknowledge Alvaro Arjona, PhD, UCB Pharma, Brussels, Belgium, and Helen Chambers, PhD, CMPP, Costello Medical, Cambridge, UK, for publication coordination, and Eleanor Thurtle, MChem, Costello Medical, Cambridge, UK, for medical writing and editorial assistance.

Contributors Substantial contributions to study conception/design, or acquisition/analysis/interpretation of data: DvdH, XB, K-GAH, RBML, PMM, WPM, ORD, NdP, BH, LB, TN and JB. Drafting of the publication or revising it critically for important intellectual content: DvdH, XB, K-GAH, RBML, PMM, WPM, ORD, NdP, BH, LB, TN and JB. Final approval of the publication: DvdH, XB, K-GAH, RBML, PMM, WPM, ORD, NdP, BH, LB, TN and JB.

Funding UCB Pharma sponsored the study and the development of this manuscript, and reviewed the text to ensure that from UCB perspective, the data presented in the publication are scientifically, technically and medically supportable, that they do not contain any information that has the potential to damage the intellectual property of UCB, and that the publication complies with applicable laws, regulations, guidelines and good industry practice. The authors approved the final version to be published after critically revising the manuscript for important intellectual content.

Competing interests DvdH has received consulting fees from AbbVie, Amgen, Astellas, AstraZeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Celgene, Daiichi, Eli Lilly, Galapagos, Gilead, Janssen, Merck, Novartis, Pfizer, Regeneron, Roche, Sanofi and UCB Pharma, and is the director of Imaging Rheumatology BV. XB has received consulting and/or speaker's fees and/or research grants from AbbVie, Bristol-Myers Squibb, Boehringer Ingelheim, Celgene, Chugai, Janssen, MSD, Novartis, Pfizer and UCB Pharma. K-GAH has received speaker's fees for AbbVie, MSD, Pfizer and UCB Pharma. RBML has received consulting fees and/or research grants and/or speaker's bureau from Abbott, Ablynx, Amgen, AstraZeneca, Bristol-Myers Squibb, Centocor, GlaxoSmithKline, Merck, Novartis, Pfizer, Roche, Schering-Plough, UCB Pharma and Wyeth. PMM has received consulting/speaker's fees from AbbVie, Centocor, Janssen, MSD, Novartis, Pfizer and UCB Pharma. WPM has received consulting and/or speaker's fees and/or grants from AbbVie, Amgen, Eli Lilly, Janssen, Merck, Novartis, Pfizer, Sanofi and UCB Pharma, and is the Chief Medical Officer of Canadian Research Education (CaRE) Arthritis. ORD, NdP, BH, LB and TN are employees of UCB Pharma. JB has received consulting fees/research grants from Abbott, Bristol-Myers Squibb, Celgene, Celltrion, Chugai, Johnson & Johnson, MSD, Novartis, Pfizer, Roche and UCB Pharma.

Patient consent Obtained.

Ethics approval The study protocol, amendments and subject informed consent were reviewed by a national, regional or independent ethics committee (IEC) or institutional review board (IRB).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Rudwaleit M, van der Heijde D, Landewé R, *et al*. The development of assessment of spondyloarthritis international society classification criteria for axial spondyloarthritis (part II): validation and final selection. *Ann Rheum Dis* 2009;68:777–83.
2 Baraliakos X, Braun J. Non-radiographic axial spondyloarthritis and ankylosing spondylitis: what are the similarities and differences? *RMD Open* 2015;1:e000053.
3 Rudwaleit M, Haibel H, Baraliakos X, *et al*. The early disease stage in axial spondylarthritis: results from the German Spondyloarthritis Inception Cohort. *Arthritis Rheum* 2009;60:717–27.
4 Landewé R, Braun J, Deodhar A, *et al*. Efficacy of certolizumab pegol on signs and symptoms of axial spondyloarthritis including ankylosing spondylitis: 24-week results of a double-blind randomised placebo-controlled Phase 3 study. *Ann Rheum Dis* 2014;73:39–47.
5 Creemers MC, Franssen MJ, van't Hof MA, *et al*. Assessment of outcome in ankylosing spondylitis: an extended radiographic scoring system. *Ann Rheum Dis* 2005;64:127–9.
6 Sieper J, Landewé R, Rudwaleit M, *et al*. Effect of certolizumab pegol over ninety-six weeks in patients with axial spondyloarthritis: results from a phase III randomized trial. *Arthritis Rheumatol* 2015;67:668–77.
7 van der Heijde D, Dougados M, Landewé R, *et al*. Sustained efficacy, safety and patient-reported outcomes of certolizumab pegol in axial spondyloarthritis: 4-year outcomes from RAPID-axSpA. *Rheumatology* 2017;56:1498–509.
8 Braun J, Baraliakos X, Hermann KG, *et al*. Effect of certolizumab pegol over 96 weeks of treatment on inflammation of the spine and sacroiliac joints, as measured by MRI, and the association between clinical and MRI outcomes in patients with axial spondyloarthritis. *RMD Open* 2017;3:e000430.
9 van der Heijde D, Maksymowych W, Landewé R, *et al*. Structural progression of the spine measure by X-ray in patients with axial spondyloarthritis treated with certolizumab pegol over 96 weeks, including ankylosing spondylitis and non-radiographic axial spondyloarthritis. *Arthritis Rheumatol* 2014;66:S248.
10 Deodhar AA, Dougados M, Landewé R, *et al*. Safety and Efficacy of Certolizumab Pegol over 204 Weeks in Patients with Axial Spondyloarthritis, Including Ankylosing Spondylitis and Non-Radiographic Axial Spondyloarthritis (abstract). *Arthritis Rheumatol* 2016;68(suppl 10(). Abstract number: 687.
11 Maksymowych WP, Inman RD, Salonen D, *et al*. Spondyloarthritis Research Consortium of Canada magnetic resonance imaging index for assessment of spinal inflammation in ankylosing spondylitis. *Arthritis Rheum* 2005;53:502–9.
12 Braun J, Baraliakos X, Golder W, *et al*. Magnetic resonance imaging examinations of the spine in patients with ankylosing spondylitis, before and after successful therapy with infliximab: evaluation of a new scoring system. *Arthritis Rheum* 2003;48:1126–36.
13 National Research Council. *The prevention and treatment of missing data in clinical trials*. Washington, DC: The National Academies Press, 2010.
14 van Buuren S. Multiple imputation of discrete and continuous data by fully conditional specification. *Stat Methods Med Res* 2007;16:219–42.
15 Vink G, Frank LE, Pannekoek J, *et al*. Predictive mean matching imputation of semicontinuous variables. *Stat Neerl* 2014;68:61–90.
16 Braun J, Baraliakos X, Hermann KG, *et al*. Golimumab reduces spinal inflammation in ankylosing spondylitis: MRI results of the randomised, placebo- controlled GO-RAISE study. *Ann Rheum Dis* 2012;71:878–84.
17 Maksymowych WP, Dougados M, van der Heijde D, *et al*. Clinical and MRI responses to etanercept in early non-radiographic axial spondyloarthritis: 48-week results from the EMBARK study. *Ann Rheum Dis* 2016;75:1328–35.
18 Cantarini L, Fabbroni M, Talarico R, *et al*. Effectiveness of adalimumab in non-radiographic axial spondyloarthritis: evaluation of clinical and magnetic resonance imaging outcomes in a monocentric cohort. *Medicine* 2015;94:e1170.
19 Dougados M, Maksymowych WP, van der Heijde D, *et al*. SAT0405 No Radiological Sacroiliac Joint Progression after 2 Years of Etanercept Treatment in Non-Radiographic Axial Spondyloarthritis: Data from The Embark Study. *Ann Rheum Dis* 2016;75:816.
20 Braun J, Landewé R, Hermann KG, *et al*. Major reduction in spinal inflammation in patients with ankylosing spondylitis after treatment with infliximab: results of a multicenter, randomized, double-blind, placebo-controlled magnetic resonance imaging study. *Arthritis Rheum* 2006;54:1646–52.
21 Song IH, Hermann KG, Haibel H, *et al*. Consistently good clinical response in patients with early axial spondyloarthritis after 3 years of continuous treatment with etanercept: longterm data of the ESTHER trial. *J Rheumatol* 2014;41:2034–40.
22 Poddubnyy D, Rudwaleit M, Haibel H, *et al*. Rates and predictors of radiographic sacroiliitis progression over 2 years in patients with axial spondyloarthritis. *Ann Rheum Dis* 2011;70:1369–74.
23 Poddubnyy D, Sieper J. Radiographic progression in ankylosing spondylitis/axial spondyloarthritis: how fast and how clinically meaningful? *Curr Opin Rheumatol* 2012;24:363–9.
24 Sampaio-Barros PD, Bertolo MB, Kraemer MH, *et al*. Undifferentiated spondyloarthropathies: a 2-year follow-up study. *Clin Rheumatol* 2001;20:201–6.
25 Dougados M, Sepriano A, Molto A, *et al*. Sacroiliac radiographic progression in recent onset axial spondyloarthritis: the 5-year data of the DESIR cohort. *Ann Rheum Dis* 2017;76:1823–8.
26 Dougados M, Maksymowych W, Landewe R, *et al*. THU0382 Change in sacroiliac joint structural radiographic damage after two years of etanercept therapy in comparison to a contemporary control cohort in non-radiographic axial spondyloarthritis [abstract]. *Ann Rheum Dis* 2017;76:S350–1.
27 van Tubergen A, Heuft-Dorenbosch L, Schulpen G, *et al*. Radiographic assessment of sacroiliitis by radiologists and rheumatologists: does training improve quality? *Ann Rheum Dis* 2003;62:519–25.
28 Yazici H, Turunç M, Ozdoğan H, *et al*. Observer variation in grading sacroiliac radiographs might be a cause of 'sacroiliitis' reported in certain disease states. *Ann Rheum Dis* 1987;46:139–45.
29 Hollingsworth PN, Cheah PS, Dawkins RL, *et al*. Observer variation in grading sacroiliac radiographs in HLA-B27 positive individuals. *J Rheumatol* 1983;10:247–54.
30 Landewé R, Dougados M, Mielants H, *et al*. Physical function in ankylosing spondylitis is independently determined by both disease activity and radiographic damage of the spine. *Ann Rheum Dis* 2009;68:863–7.
31 Wanders A, Landewé R, Dougados M, *et al*. Association between radiographic damage of the spine and spinal mobility for individual patients with ankylosing spondylitis: can assessment of spinal mobility be a proxy for radiographic evaluation? *Ann Rheum Dis* 2005;64:988–94.
32 Machado P, Landewé R, Braun J, *et al*. Both structural damage and inflammation of the spine contribute to impairment of spinal mobility in patients with ankylosing spondylitis. *Ann Rheum Dis* 2010;69:1465–70.
33 Poddubnyy D, Fedorova A, Listing J, *et al*. Physical function and spinal mobility remain stable despite radiographic spinal progression in patients with ankylosing spondylitis treated with TNF-α inhibitors for up to 10 years. *J Rheumatol* 2016;43:2142–8.
34 Baraliakos X, Listing J, Rudwaleit M, *et al*. Progression of radiographic damage in patients with ankylosing spondylitis: defining the central role of syndesmophytes. *Ann Rheum Dis* 2007;66:910–5.
35 Baraliakos X, Listing J, von der Recke A, *et al*. The natural course of radiographic progression in ankylosing spondylitis--evidence for major individual variations in a large proportion of patients. *J Rheumatol* 2009;36:997–1002.
36 Maas F, Spoorenberg A, Brouwer E, *et al*. Spinal radiographic progression in patients with ankylosing spondylitis treated with TNF-α blocking therapy: a prospective longitudinal observational cohort study. *PLoS One* 2015;10:e0122693.
37 Braun J, Baraliakos X, Deodhar A, *et al*. Secukinumab Demonstrates Low Radiographic Progression and Sustained Efficacy through 4 Years in Patients with Active Ankylosing Spondylitis [abstract]. *Arthritis Rheumatol* 2017;69. Abstract number: 3L.
38 Braun J, Baraliakos X, Hermann KG, *et al*. The effect of two golimumab doses on radiographic progression in ankylosing spondylitis: results through 4 years of the GO-RAISE trial. *Ann Rheum Dis* 2014;73:1107–13.
39 Baraliakos X, Listing J, Fritz C, *et al*. Persistent clinical efficacy and safety of infliximab in ankylosing spondylitis after 8 years--early clinical response predicts long-term outcome. *Rheumatology* 2011;50:1690–9.
40 Baraliakos X, Haibel H, Listing J, *et al*. Continuous long-term anti-TNF therapy does not lead to an increase in the rate of new bone formation over 8 years in patients with ankylosing spondylitis. *Ann Rheum Dis* 2014;73:710–5.
41 Maas F, Arends S, Brouwer E, *et al*. Reduction in spinal radiographic progression in ankylosing spondylitis patients receiving prolonged treatment with tumor necrosis factor inhibitors. *Arthritis Care Res* 2017;69:1011–9.
42 Molnar C, Scherer A, Baraliakos X, *et al*. TNF blockers inhibit spinal radiographic progression in ankylosing spondylitis by reducing disease activity: results from the Swiss Clinical Quality Management cohort. *Ann Rheum Dis* 2018;77:63–9.
43 Ramiro S, van Tubergen A, Stolwijk C, *et al*. Scoring radiographic progression in ankylosing spondylitis: should we use the modified Stoke Ankylosing Spondylitis Spine Score (mSASSS) or the Radiographic Ankylosing Spondylitis Spinal Score (RASSS)? *Arthritis Res Ther* 2013;15:R14.
44 van der Heijde D, Landewé R. Selection of a method for scoring radiographs for ankylosing spondylitis clinical trials, by the Assessment in Ankylosing Spondylitis Working Group and OMERACT. *J Rheumatol* 2005;32:2048–9.

EXTENDED REPORT

Lupus Low Disease Activity State (LLDAS) attainment discriminates responders in a systemic lupus erythematosus trial: *post-hoc* analysis of the Phase IIb MUSE trial of anifrolumab

Eric F Morand,[1] Teodora Trasieva,[2] Anna Berglind,[2] Gabor G Illei,[3] Raj Tummala[4]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212504).

[1]Centre for Inflammatory Diseases, Monash University, Melbourne, Victoria, Australia
[2]AstraZeneca, Gothenburg, Sweden
[3]MedImmune LLC, Gaithersburg, MD, USA
[4]AstraZeneca, Gaithersburg, MD, USA

Correspondence to
Dr Eric F Morand, Centre for Inflammatory Diseases, Monash University, Melbourne, Victoria, Australia;
eric.morand@monash.edu

Received 6 October 2017
Revised 7 December 2017
Accepted 3 January 2018
Published Online First
2 February 2018

To cite: Morand EF, Trasieva T, Berglind A, *et al*. *Ann Rheum Dis* 2018;**77**:706–713.

ABSTRACT

Objectives In a *post-hoc* analysis, we aimed to validate the Lupus Low Disease Activity State (LLDAS) definition as an endpoint in an systemic lupus erythematosus (SLE) Phase IIb randomised controlled trial (RCT) (MUSE [NCT01438489]) and then utilize LLDAS to discriminate between anifrolumab and placebo.

Methods Patients received intravenous placebo (n=102) or anifrolumab (300 mg, n=99; 1,000 mg, n=104) Q4W plus standard of care for 48 weeks. LLDAS attainment (SLE Disease Activity Index 2000 ≤4 without major organ activity, no new disease activity, Physician's Global Assessment ≤1, prednisolone ≤7.5 mg/d and standard immunosuppressant dosage tolerance) was assessed. Associations with endpoints and LLDAS attainment differences between treatments were explored.

Results LLDAS attainment at Week 52 was associated with SLE Responder Index 4 (SRI[4]) and British Isles Lupus Assessment Group–based Composite Lupus Assessment (BICLA) (74/85[87%] and 62/84[74%] were also SRI[4] and BICLA responders, respectively; both nominal p<0.001). Only 74/159 (47%) of SRI(4) and 62/121 (51%) of BICLA responders reached LLDAS. Anifrolumab-treated patients achieved earlier LLDAS, and more spent at least half their observed time in LLDAS (OR vs. placebo; 300 mg: 3.04, 95% CI 1.34 to 6.92, nominal p=0.008; 1,000 mg: 2.17, 95% CI 0.93 to 5.03, nominal p=0.072) vs placebo-treated patients. At Week 52, 17/102 (17%), 39/99 (39%) and 29/104 (28%) of patients on placebo, anifrolumab 300 and 1,000 mg, respectively, attained LLDAS (OR vs. placebo; 300 mg: 3.41, 95% CI 1.73 to 6.76, p<0.001; 1,000 mg: 2.03, 95% CI 1.01 to 4.07, nominal p=0.046).

Conclusions LLDAS attainment represents a clinically meaningful SLE outcome measure, and anifrolumab is associated with more patients who met LLDAS criteria versus placebo. These data support LLDAS as an SLE RCT endpoint.

Trial registration number NCT1438489; Post-results.

INTRODUCTION

Attainment of low disease activity (LDA) is a standard of care in rheumatoid arthritis (RA), supported by empirical evidence of validity (i.e., association with improved long-term outcomes) and utility (discrimination of treatment response).[1 2] In contrast, a well-defined LDA definition in systemic lupus erythematosus (SLE) was only recently identified as a key research goal.[3 4] In response to this unmet need, increasing evidence suggests that the Lupus Low Disease Activity State (LLDAS) represents a clinically meaningful state with potential utility in both research and clinical settings.[5]

Patients with SLE who spend the majority of their time in LLDAS are protected from damage accrual, and LLDAS is also associated with better health-related quality of life (HRQOL) and is more stringent than expert opinion.[5–8] Validation in a clinical trial setting is necessary to demonstrate the utility of LLDAS as a response measure in SLE randomised controlled trials (RCTs). Recently, rates of LLDAS attainment were demonstrated to differentiate treatments in a trial comparing azathioprine and mycophenolate in nonrenal SLE.[9] Utility of a novel endpoint such as LLDAS in trials of novel therapies requires it to be attainable and to align with existing response measures, but also to offer additional information, and to allow for discrimination between active treatment and placebo. Here, we present a *post-hoc* analysis of a large Phase IIb SLE RCT dataset and demonstrate LLDAS utility.

METHODS

MUSE trial design

LLDAS was evaluated in a *post-hoc* analysis of data from the 52-week MUSE RCT (NCT01438489) of anifrolumab in SLE.[10] Patients (≥18–65 years old) with moderate to severe SLE were randomised 1:1:1 to receive intravenous placebo or anifrolumab 300 or 1,000 mg every 4 weeks for 48 weeks plus standard therapy. Patients met the American College of Rheumatology SLE classification criteria at screening, including positive antinuclear antibody ≥1:80 or elevated anti–double-stranded DNA (anti-dsDNA) or anti-Smith antibodies.[11] Other inclusion criteria at screening were SLE Disease Activity Index 2000 (SLEDAI-2K) ≥6 (excluding points attributed to SLE headache or organic brain syndrome), 'Clinical' SLEDAI-2K≥4, a British Isles Lupus Assessment Group (BILAG) 2004 organ domain score of ≥1A or ≥2B and a Physician's Global Assessment (PhGA; 0–3) score ≥1.0.[12 13] Patients with active severe or unstable neuropsychiatric SLE or lupus nephritis were excluded. Randomisation stratification factors were SLEDAI-2K (<10 vs. ≥10), baseline oral corticosteroid (OCS) dosage (<10 vs. ≥10 mg/d prednisone-equivalent), and type I interferon (IFN) gene signature (IFNGS) based on a four-gene expression

assay (test–high vs. test–low).[10] A total of 305 patients received placebo (n=102) or anifrolumab (300 mg: n=99; 1,000 mg: n=104).

The MUSE primary endpoint was the difference from placebo in the percentage of responders at Week 24, defined as SLE Responder Index 4 (SRI[4]), with patients who withdrew or were unable to taper Day 85–Week 24 OCS dosage to <10 mg/d and ≤day 1 dosage considered to be nonresponders.[14] Additional endpoints included BILAG-based Composite Lupus Assessment (BICLA), Major Clinical Response (MCR), BILAG flares (defined as either one or more new BILAG-2004 A items or two or more new BILAG-2004 B items compared with the previous visit) and patient-reported outcomes (PROs) including Lupus Quality of Life (LupusQOL) and Patient's Global Assessment (PaGA).[10 15 16] Nonresponse imputation of missing data was used for the binary outcomes and baseline-observation-carried-forward approach for continuous data following withdrawal from study or discontinuation of treatment, whereas intermittently missing data were imputed using the last-observation-carried-forward approach. The study was completed in accordance with the Declaration of Helsinki and the Good Clinical Practice guidelines. Written informed consent was obtained from all patients. Further details on MUSE design and endpoints have been published.[10]

Post-hoc validation of LLDAS as an outcome measure

LLDAS was conceptually defined as 'a state which, if sustained, is associated with a low likelihood of adverse outcome, considering disease activity and medication safety'.[5] Subsequently defined using consensus methodology, LLDAS is attained if all of the following items are met: (1) SLEDAI-2K ≤4, with no activity in major organ systems (renal, central nervous system, cardiopulmonary, vasculitis and fever) and no haemolytic anaemia or gastrointestinal activity; (2) no new features of lupus disease activity compared with the previous assessment; (3) PhGA (0–3) ≤1; (4) current prednisolone-equivalent dosage ≤7.5 mg/d; and (5) well-tolerated standard maintenance dosages of immunosuppressive drugs and approved biologics.

The published definition of LLDAS[5] was applied *post-hoc* programmatically as a binary measure for each visit based on the collected and unblinded MUSE data. Details of the derivation of LLDAS are presented in the online supplement (online supplementary table S1). Results from statistical analyses are presented using point estimates, 95% CI where appropriate and nominal p-values. We first assessed the prevalence of LLDAS and then examined the association of LLDAS with SRI(4) responders with OCS taper at Week 24, and SRI(4), BICLA and MCR responders at Week 52. We then assessed the association between the number of flares throughout the study and LLDAS attainment at Week 52. Relationships between LLDAS attainment and PaGA scores and LupusQOL domains were explored. Details of the statistical methods used for these analyses are provided in the online supplementary appendix.

Post-hoc application of LLDAS to discriminate between placebo and anifrolumab

A detailed description of the statistical methods and application of LLDAS to discriminate between placebo and anifrolumab treatment groups is provided in the online supplementary appendix. We compared the percentages of patients who attained LLDAS over time in placebo and anifrolumab treatment groups. We also compared the percentage of patients who spent more than 20%, 50% and 70% of their time in LLDAS, and who managed to sustain LLDAS across four, five, six or seven consecutive visits either during the whole study or after Week 12. Time to first LLDAS attainment also was compared between treatment groups. By using the approach recently described by van der Heijde *et al*,[17] we generated heat maps of LLDAS and SRI(4) attainment across the entire study, sorted by treatment, SLEDAI-2K and IFNGS at screening.

RESULTS

Patient characteristics

Key MUSE demographics and baseline characteristics are presented in the online supplementary table 2. A total of 305 patients with active SLE were enrolled, the majority of whom were anti-dsDNA–positive (Farr assay) and IFNGS test–high. Details have been published.[10]

MUSE efficacy endpoints

As reported, patients in both anifrolumab treatment arms were more likely to reach a range of prespecified endpoints compared with placebo.[10] A greater percentage of patients receiving anifrolumab treatment achieved SRI(4) with OCS taper at Week 24, SRI(4) and BICLA at Week 52, and MCR (online supplementary figure S1).

Post-hoc validation of LLDAS as an outcome measure

To test the association of LLDAS with other measures, we first assessed LLDAS attainment, using data pooled from all treatment arms. LLDAS attainment was positively associated with, but more stringent than, standard endpoints. LLDAS was attained by 51 of 305 patients (16.7%) at Week 24 (figure 1A). At Week 24, 41 of 51 patients in LLDAS (80.4%) achieved the primary endpoint (SRI[4] with OCS taper; figure 1A). However, only 41 of 82 primary endpoint responders (50.0%) at Week 24 met the definition of LLDAS at the same time point (Cochran-Mantel-Haenszel (CMH) test χ^2=68.06, p<0.001). Similar results were observed for a comparison of LLDAS with SRI(4) and BICLA at Week 52. At Week 52, 85 of 305 patients (27.9%) attained LLDAS, compared with 159 of 305 patients (52.1%) and 121 of 301 patients (40.2%) attaining SRI(4) or BICLA, respectively (figure 1B and C); 74 of 85 LLDAS responders (87.1%) were SRI(4) responders, but only 74 of 159 SRI(4) responders (46.5%) attained LLDAS (CMH χ^2=49.20, p<0.001). Furthermore, 62 of 84 LLDAS responders (73.8%) met BICLA criteria, but only 62 of 121 BICLA responders (51.2%) attained LLDAS (CMH χ^2=39.74, p<0.001). During the study, 44 of 305 patients (14.4%) met MCR criteria; of these, 29 of 44 (65.9%) also were in LLDAS at Week 52; correspondingly, 29 of 85 patients in LLDAS (34.1%) at Week 52 met MCR criteria (CMH χ^2=25.62, p<0.001; figure 1D). Patients who attained LLDAS at Week 52 had a 75.2% lower BILAG flare rate during the study compared with those who did not attain LLDAS at the same time point. The annualised BILAG flare rate during the study for patients who met LLDAS criteria at Week 52 was estimated as 0.15 flares per patient-year (95% CI 0.08 to 0.27) compared with 0.61 (95% CI 0.45 to 0.83) for patients not meeting the LLDAS criteria (p<0.001).

LLDAS attainment was also associated with improved PROs. Patients who did or did not attain LLDAS at Week 52 had decreased PaGA from baseline of 23.0 and 9.1 mm on a 100-mm visual analogue scale, respectively (Wilcoxon signed rank test S=–1264 and S=–2,441, both p<0.001; figure 2A). At Week 52, patients in LLDAS had lower PaGA compared with patients not in LLDAS (F[1, 297]=38.93, p<0.001). Patients in LLDAS

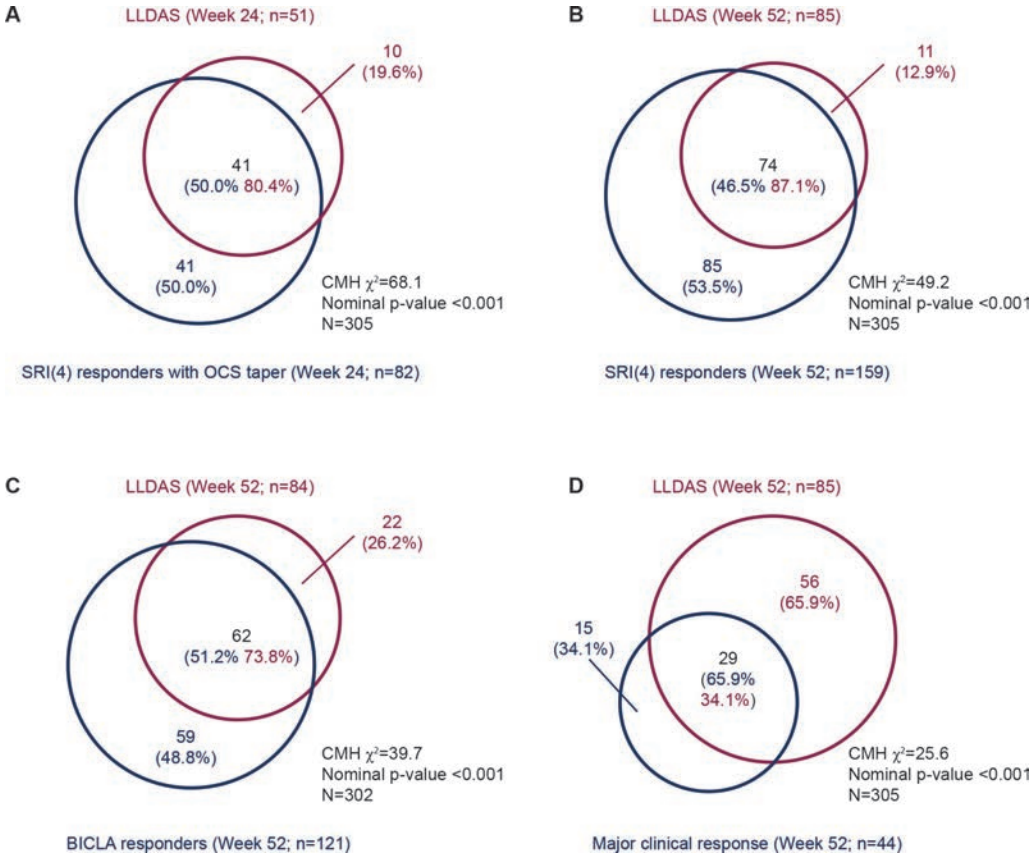


Figure 1 Association of LLDAS with other endpoints for pooled patients with active SLE treated with placebo or anifrolumab. Percentages of patients meeting LLDAS (pink) and other endpoints (blue); (A) SRI(4) with OCS taper, at Week 24; (B) SRI(4) at Week 52; (C) BICLA at Week 52; (D) MCR. Nominal p-values were based on CMH test of independence, adjusting for treatment and randomisation stratification factors. Patients without BILAG A or B at baseline were excluded from the BICLA analysis. BICLA, British Isles Lupus Assessment Group (BILAG)-based Composite Lupus Assessment; CMH, Cochran-Mantel-Haenszel; LLDAS, Lupus Low Disease Activity State; MCR, Major Clinical Response; OCS, oral corticosteroid; SLE, systemic lupus erythematosus; SRI(4), SLE Responder Index 4.

at Week 52 also had greater LupusQOL scores than did patients who did not attain LLDAS (figure 2B).

Post-hoc application of LLDAS to discriminate between placebo and anifrolumab

LLDAS criteria were met at least once by 36 of 102 (35.3%), 51 of 99 (51.5%) and 48 of 104 (46.2%) patients receiving placebo, anifrolumab 300 mg or anifrolumab 1,000 mg, respectively (OR vs. placebo; 300 mg: 1.97, 95% CI 1.08 to 3.58, p=0.027; 1000 mg: 1.63, 95% CI 0.90 to 2.95, p=0.103; table 1). Differentiation of LLDAS attainment in favour of anifrolumab over placebo was detected as early as Week 12 for anifrolumab 300 mg, with a range of ORs for subsequent visits from 1.71 at Week 24 (p=0.175) to 3.59 at Week 32 (p=0.001); this benefit of anifrolumab 300 mg was observed consistently after Week 24 with ORs >2 and CIs excluding 1 at all but one time point (figure 3). Differentiation was less pronounced for anifrolumab 1,000 mg, and was first detected at Week 28, with subsequent ORs ranging from 1.68 at Week 44 (p=0.136) to 2.49 at Week 28 (p=0.025). At Week 52, 17 of 102 (16.7%), 39 of 99 (39.4%) and 29 of 104 (27.9%) of patients on placebo, anifrolumab 300 mg and anifrolumab 1,000 mg, respectively, attained LLDAS (OR vs. placebo; 300 mg: 3.41, 95% CI 1.73 to 6.76, p<0.001; 1,000 mg: 2.03, 95% CI 1.01 to 4.07, p=0.046; figure 3). Anifrolumab-treated patients achieved LLDAS earlier than did placebo-treated patients (300 mg: χ^2=6.39, p=0.012; 1,000 mg: χ^2=2.44, 0.119; figure 4A). Patients receiving anifrolumab 300 or 1,000 mg spent greater total percentages of observed time in LLDAS than did patients receiving placebo (table 1). Patients receiving anifrolumab were also more likely to achieve LLDAS for longer periods of time (figure 4B and C). Greater percentages of anifrolumab-treated patients spent at least half of their observed time in LLDAS (OR vs. placebo; 300 mg: 3.04, 95% CI 1.34 to 6.92, p=0.008; 1,000 mg: 2.17, 95% CI 0.93 to 5.03, p=0.072; figure 4B). Furthermore, only 3 of 102 patients receiving placebo (2.9%) sustained LLDAS for seven consecutive visits, compared with 13 of 99 recipients of anifrolumab 300 mg (13.1%) and 11 of 104 patients receiving anifrolumab 1,000 mg (10.6%; figure 4C). Similar results were observed when analysis was restricted to the period after 12 weeks, when the onset of action of anifrolumab is assumed to have occurred (figure 4D).

We performed *pro-forma* power calculations to estimate sample sizes needed to detect differences to placebo in SRI(4) responders and LLDAS attainment at Week 52, assuming identical treatment effects to those observed in MUSE and this *post-hoc* analysis of MUSE data. Seventy-seven patients per group would be required for 80% power to detect a treatment effect for SRI(4) at a significance level of 5%. Fewer patients (n=61) per group would be necessary to achieve the same power for LLDAS attainment.

Graphical depiction of both attainment and retention of study endpoints across individual patients in clinical trials has recently been improved through the use of heat maps,[17] and this approach may have particular utility in a relapsing-remitting disease such

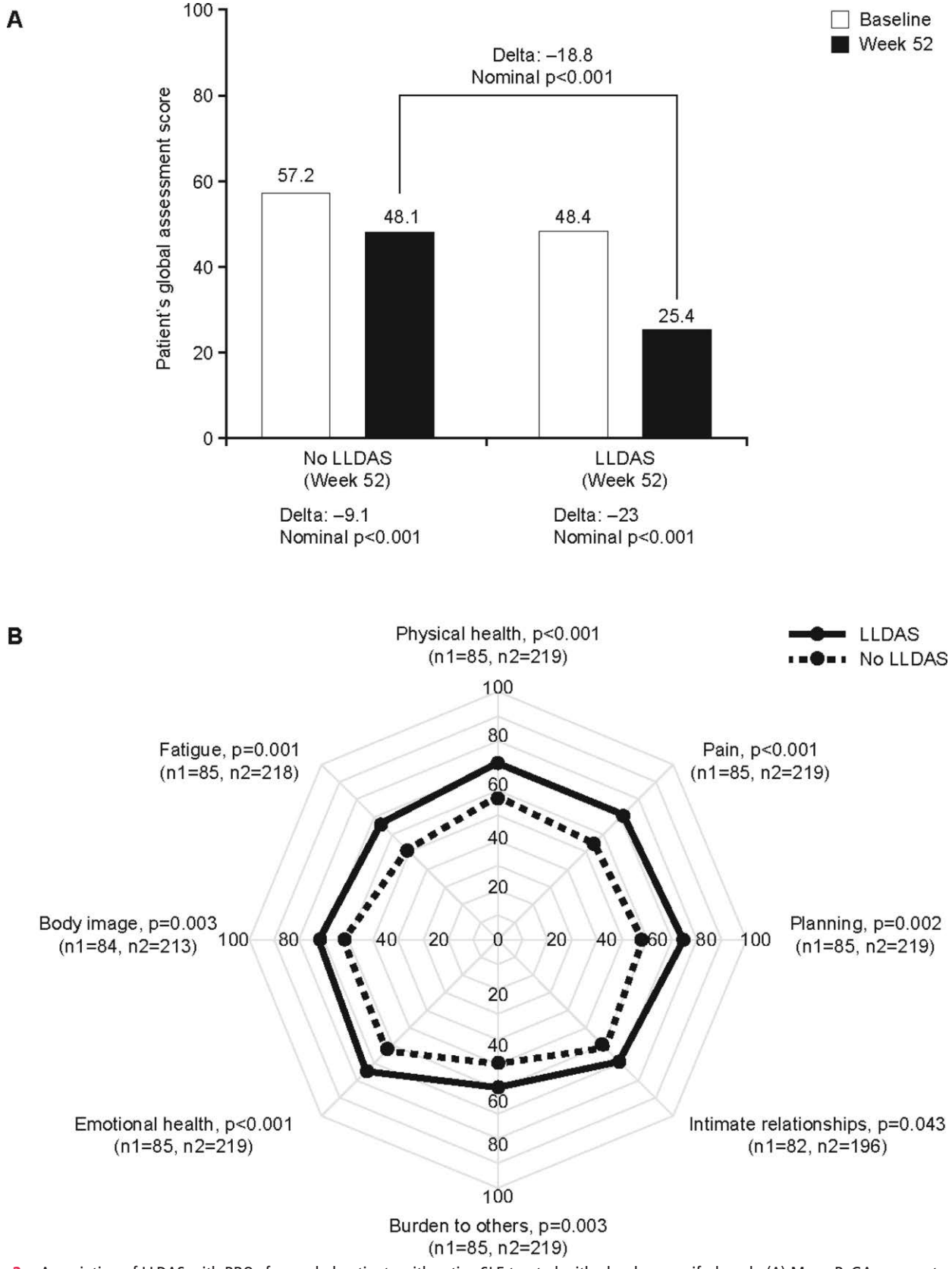


Figure 2 Association of LLDAS with PROs for pooled patients with active SLE treated with placebo or anifrolumab. (A) Mean PaGA scores at baseline and Week 52 by LLDAS attainment at Week 52. (B) Mean LupusQOL domain scores at Week 52 by LLDAS attainment at Week 52. The nominal p-values and delta for comparing the difference in mean scores between patients in LLDAS and those who did not attain LLDAS at Week 52 were based on an ANCOVA test adjusted for treatment, randomisation stratification factors and respective baseline domain scores. Nominal p-values for comparing baseline with Week 52 PaGA scores were based on a Wilcoxon signed rank test. ANCOVA, analysis of covariance; LLDAS, Lupus Low Disease Activity State; PaGA, Patient 's Global Assessment; PROs, patient-reported outcome; QOL, Quality of Life.

Table 1 Prevalence of LLDAS

	Placebo (n=102)	Anifrolumab 300 mg (n=99)	Anifrolumab 1,000 mg (n=104)
Duration of observed study time per patient (years), mean (SD)	0.84 (0.29)	0.95 (0.20)	0.89 (0.25)
Patients with at least one episode of LLDAS, n (%)	36 (35.3)	51 (51.5)	48 (46.2)
Cumulative LLDAS duration per patient (years), mean (SD)	0.12 (0.22)	0.24 (0.29)	0.19 (0.27)
Percentage of observed study time in LLDAS per patient (years), mean (SD)	12.4 (22.0)	24.0 (28.7)	19.4 (27.1)

LLDAS, Lupus Low Disease Activity State.

as SLE. Attainment and retention of LLDAS and SRI(4) across the duration of the trial, stratified according to screening SLEDAI-2K and IFNGS test status and treatment, are shown in figure 5. Both attainment and retention were numerically greater for SRI(4) than for LLDAS. LLDAS attainment occurred more often for anifrolumab-treated versus placebo-treated patients and was more frequent for patients with lower baseline disease activity. In placebo-treated patients, the likelihood of LLDAS attainment at Week 52 was lower for IFNGS test–high patients versus IFNGS test–low patients (at screening) (8/76 vs. 9/26, respectively; CMH χ^2=4.19, p=0.041; figure 5).

DISCUSSION

LLDAS was originally developed as a definition to use in treat-to-target pragmatic studies, and initial validation studies focused on the association of LLDAS with improved outcome in SLE.[5 6] In RA, LDA is also used as a clinical trial endpoint, wherein the percentage of patients who attain LDA is used to compare treatments.[2] Confirmation of the utility of LLDAS in SLE RCTs would provide a much-needed additional measure of treatment response. The potential for differences in rates of LLDAS attainment to permit discrimination between treatments is supported by the recently reported findings of Ordi-Ros *et al*[9] in their trial comparing mycophenolate and azathioprine in active nonrenal SLE; the study demonstrated that mycophenolate was superior to azathioprine in rates of LLDAS attainment. In our analysis, we provide novel evidence suggesting LLDAS utility as an endpoint in SLE RCTs. Partly because of the way LLDAS was defined, it was associated with existing response measures, including PROs, but was more stringent than other commonly used composite endpoints (SRI[4], BICLA), providing additional and complementary information. Our findings show that (1) LLDAS attainment and persistence were clearly differentiated between active treatment and placebo, indicating that the application of LLDAS can separate treatments and (2) anifrolumab treatment was associated with earlier, more frequent and more sustained LLDAS compared with placebo.

The definition of LLDAS was reached using a consensus methodology in response to the unmet need for such a measure, which was outlined in major reviews and by an international task force.[3–5] Initial validation in a single-centre cohort demonstrated that a considerable percentage of patients attained LLDAS, distinguishing LLDAS from stringent definitions of remission, which are very seldom attained.[18] Moreover, being in LLDAS for longer cumulative periods of time was associated with significant protection from damage accrual in two independent cohorts.[5 6] Use of the operational definition of LLDAS was also recently found to be more stringent than expert opinion in assigning patients to LDA, and importantly, in a large prospective multinational study, that patients meeting the LLDAS definition had better HRQOL.[7 8]

As opposed to established trial endpoints such as SRI(4) and BICLA, which measure change from baseline, LLDAS represents a prespecified desirable outcome state.[14 15] In analysis disregarding treatment, LLDAS attainment was associated with the MUSE primary endpoint of SRI(4) with OCS taper at Week 24, as well as with SRI(4) and BICLA at Week 52. However, although LLDAS was attainable, it was a more stringent endpoint—only approximately half of the patients who were SRI(4) or BICLA responders also met LLDAS criteria. This finding suggests that

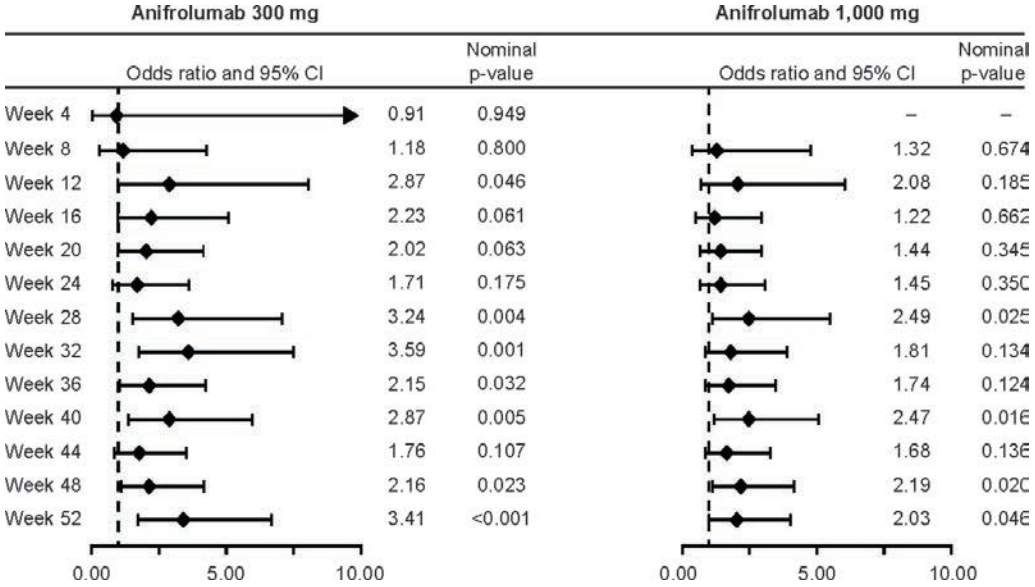


Figure 3 Forest plot of LLDAS attainment comparing anifrolumab 300 mg (left) or 1,000 mg (right) at each time point during 52 weeks. ORs, 95% CIs and nominal p-values are based on a logistic regression model adjusted for randomisation stratification factors. LLDAS, Lupus Low Disease Activity State.

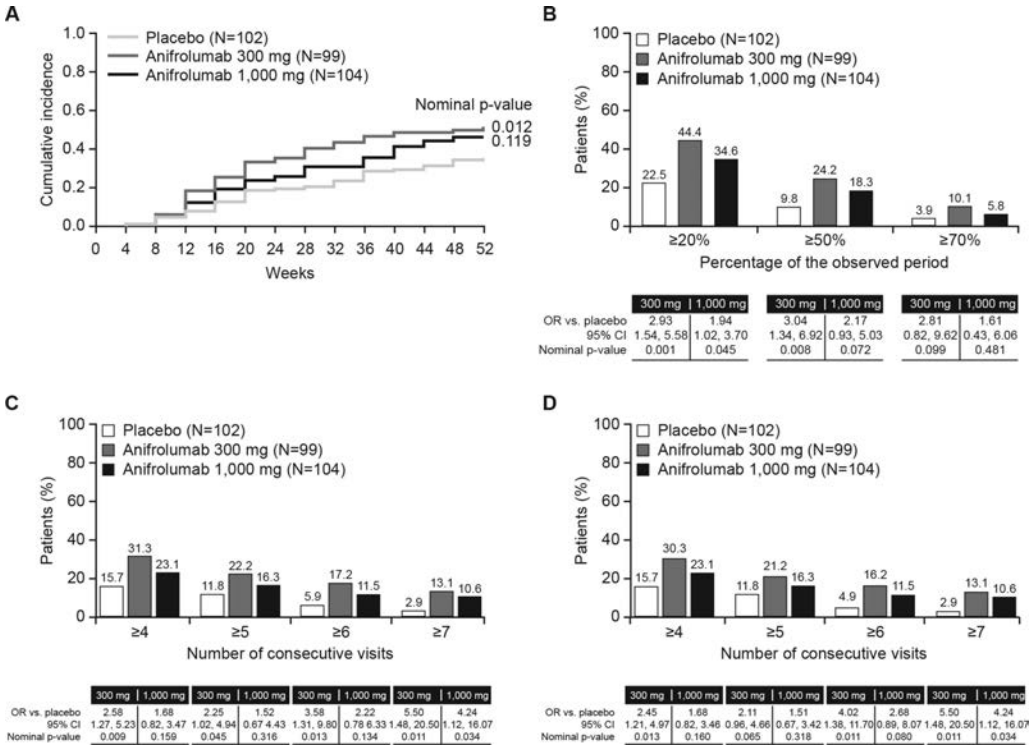


Figure 4 Time course of LLDAS attainment for patients with active SLE treated with placebo or anifrolumab. (A) Time to first attainment of LLDAS. (B) Percentages of patients attaining LLDAS for at least 20%, 50% and 70% of the observed period. (C) Percentages of patients sustaining LLDAS for at least 4, 5, 6 or 7 consecutive visits during the observed period. (D) Percentages of patients sustaining LLDAS for at least 4, 5, 6 or 7 consecutive visits during the period after Week 12. Nominal p-values were based on Grey's test for each anifrolumab group versus placebo, or logistic regression models, adjusted for randomisation stratification factors. LLDAS, Lupus Low Disease Activity State.

measuring LLDAS attainment provides additional information, complementary to that obtained using the previously established endpoints. Consistent with findings of a recent large multinational cohort study, LLDAS was associated with improvements in HRQOL compared with results for patients not achieving LLDAS, as measured by both the LupusQOL and PaGA measures.[7] Together this suggests that in addition to change measures such as SRI(4) or BICLA, a stringent target state measure such as LLDAS has potential value in clinical trials in SLE.

Anifrolumab is a novel monoclonal antibody directed at the type I IFN receptor (IFNAR1) subunit, thereby blocking the actions of all IFN-α, IFN-β and IFN-ω cytokines.[19] In the MUSE RCT, anifrolumab treatment was associated with greater percentages of patients who achieved the primary endpoint, SRI(4) with OCS taper at Week 24, as well as secondary endpoints, including SRI(4) and BICLA at Week 52, compared with placebo. Increasing the dosage from 300 to 1,000 mg did not lead to an increase in efficacy in MUSE. A greater rate of *herpes zoster* infection, as well as drop-out rate, in the anifrolumab 1,000 mg compared with the 300 mg group indicates a more favourable risk–benefit profile for the 300 mg dosage, which is the focus of the pivotal studies of anifrolumab.[10] In the results presented here, an effect of anifrolumab on LLDAS was consistently seen across the different analyses, including greater percentages of patients attaining LLDAS at any time, as well as earlier and more sustained LLDAS attainment with more pronounced differentiation between anifrolumab 300 mg versus placebo. Our findings are consistent with the MUSE study results. These data suggest that LLDAS has utility to discriminate between treatment arms in an SLE RCT.

The SRI(4) endpoint was developed from an analysis of factors contributing to the ability to show the benefit of belimumab treatment versus placebo, and it has been used in several trials since.[14] However, poor discrimination between active treatments and placebo is one of several issues that has plagued the evaluation of novel therapies for SLE, even when using endpoints derived from this measure and drugs addressing the same target.[20] Clinically meaningful and more stringent endpoints could potentially allow for smaller trials, thereby permitting more agents to be studied. Also, though not intended to supplant measures of change such as SRI(4), endpoints that provide evidence of more pronounced therapeutic responses provide complementary information.

Illustration of drug trial outcomes by heat maps[17] allows a unique oversight of overall patient outcomes over time, including the comparative time course of attainment and persistence of these outcomes. This method allows the comparison of endpoints, as well as the comparison of treatment arms. As provided in figure 5, LLDAS attainment was less frequent overall than SRI(4), consistent with its greater stringency as an endpoint, and not unexpectedly, was more often attained for patients with lower baseline disease activity. However, compared with placebo, treatment with anifrolumab was associated with increased LLDAS attainment and persistence overall, including patients with high baseline disease activity or IFNGS test–high status. Interestingly, for placebo-treated patients (receiving standard of care), LLDAS attainment was less likely for patients with a baseline IFNGS test–high score, suggesting that IFNGS status may be informative about patient outcomes receiving standard SLE therapy.

Limitations of this study include that it is a *post-hoc* analysis, although of prospectively acquired and adjudicated data. Additional studies of LLDAS utility in independent clinical trial datasets, and ultimately prospectively in RCTs, are needed to confirm

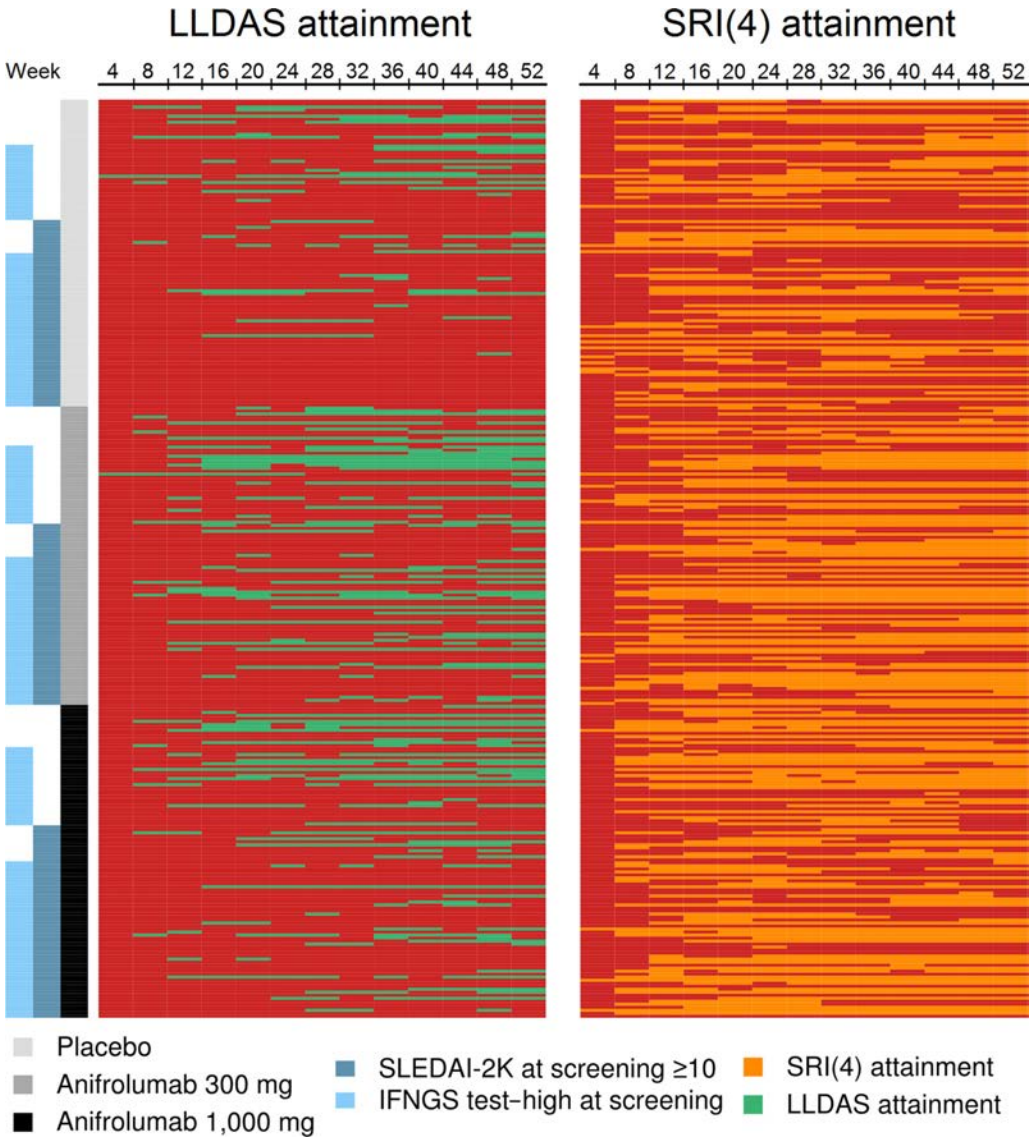


Figure 5 Heat map of LLDAS and SRI(4) attainment. Responses according to LLDAS or SRI(4) over 52 weeks of study, stratified by treatment and baseline characteristics. LLDAS, Lupus Low Disease Activity State; IFNGS, type I interferon gene signature; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SRI(4), SLE Responder Index.

our conclusions. A consensus also needs to emerge regarding operationalising LLDAS in clinical trials. For example, LLDAS is designed to be measured at a single point in time. Using a 30-day SLEDAI-2K,[21] the disease activity domains refer to the preceding 30 days; fortunately, visit intervals in typical SLE clinical trials are 1 month. The assumption that gastrointestinal activity, which is not measured in the SLEDAI-2K or BILAG, is captured sufficiently in the PhGA also needs to be tested. A consensus on whether data on glucocorticoid and immunosuppressive drug treatment should be analysed similarly has not been reached. In the current study, several ways of handling these data were assessed, with little effect on the outcomes (data not shown), suggesting the pragmatic approach to recording treatment as of the day of assessment is sufficient.

In conclusion, we have evaluated the utility of LLDAS as an endpoint in a placebo-controlled randomised trial of a novel SLE therapy. The findings suggest that LLDAS is readily deployed in a trial setting, aligns with but is more stringent that existing measures of response thereby adding information complementary to these measures, is associated with HRQOL and is sensitive to detect an effect of an active treatment. The findings also suggest superiority of anifrolumab relative to placebo with respect to LLDAS attainment and persistence in patients with active SLE. The fact that LLDAS has been independently associated with improved long-term outcomes in SLE suggests the potential for clinically meaningful extrapolation of LLDAS attainment via the use of a novel therapy such as anifrolumab to the clinical context. Our findings support the inclusion of LLDAS as a measure of response in clinical trials of new treatments for SLE.

Acknowledgements Editorial support was provided by Francis J Golder, BVSc, PhD, of Endpoint Medical Communications, Inc and Michael A Nissen, ELS, of AstraZeneca.

Contributors EFM: proposed the study design and wrote the manuscript. TT and AB: co-proposed the study design, undertook the statistical analysis and contributed to the manuscript. GI and RT: co-proposed the study design and oversaw the analysis and writing.

Funding AstraZeneca funded the MUSE study.

Competing interests EFM received research support, consultancy or honoraria from AstraZeneca, Bristol Myers Squibb, Glaxo Smith Kline, Janssen and UCB (Union

Chimique Belge). TT, AB and RT are employees of AstraZeneca. GI was an employee of Medimmune during the conduct of this analysis; he is currently an employee of Regenxbio.

Ethics approval Independent Ethics Committee or independent Institutional Review Board approvals were obtained from all 101 clinical sites and all patients provided written informed consent in accordance with local requirements.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Smolen JS, Aletaha D, Bijlsma JW, *et al*. Treating rheumatoid arthritis to target: recommendations of an international task force. *Ann Rheum Dis* 2010;69:631–7.
2 Smolen JS, Burmester GR, Combe B, *et al*. Head-to-head comparison of certolizumab pegol versus adalimumab in rheumatoid arthritis: 2-year efficacy and safety results from the randomised EXXELERATE study. *Lancet* 2016;388:2763–74.
3 van Vollenhoven RF, Mosca M, Bertsias G, *et al*. Treat-to-target in systemic lupus erythematosus: recommendations from an international task force. *Ann Rheum Dis* 2014;73:958–67.
4 Franklyn K, Hoi A, Nikpour M, *et al*. The need to define treatment goals for systemic lupus erythematosus. *Nat Rev Rheumatol* 2014;10:567–71.
5 Franklyn K, Lau CS, Navarra SV, *et al*. Definition and initial validation of a Lupus Low Disease Activity State (LLDAS). *Ann Rheum Dis* 2016;75:1615–21.
6 Tsang-A-Sjoe MW, Bultink IE, Heslinga M, *et al*. Both prolonged remission and Lupus Low Disease Activity State are associated with reduced damage accrual in systemic lupus erythematosus. *Rheumatology* 2017;56:121–8.
7 Golder V, Kandane-Rathnayake R, Hoi AY, *et al*. Association of the Lupus Low Disease Activity State (LLDAS) with health-related quality of life in a multinational prospective study. *Arthritis Res Ther* 2017;19:62.
8 Golder V, Huq M, Franklyn K, *et al*. Does expert opinion match the operational definition of the Lupus Low Disease Activity State (LLDAS)? A case-based construct validity study. *Semin Arthritis Rheum* 2017;46:798–803.
9 Ordi-Ros J, Sáez-Comet L, Pérez-Conesa M, *et al*. Enteric-coated mycophenolate sodium versus azathioprine in patients with active systemic lupus erythematosus: a randomised clinical trial. *Ann Rheum Dis* 2017;76:1575–82.
10 Furie R, Khamashta M, Merrill JT, *et al*. Anifrolumab, an anti-interferon-alpha receptor monoclonal antibody, in moderate to severe SLE. *Arthritis Rheumatol* 2017;69:376–86.
11 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997;40:1725.
12 Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol* 2002;29:288–91.
13 Isenberg DA, Rahman A, Allen E, *et al*. BILAG 2004. Development and initial validation of an updated version of the British Isles Lupus Assessment Group's disease activity index for patients with systemic lupus erythematosus. *Rheumatology* 2005;44:902–6.
14 Furie RA, Petri MA, Wallace DJ, *et al*. Novel evidence-based systemic lupus erythematosus responder index. *Arthritis Rheum* 2009;61:1143–51.
15 Wallace DJ, Kalunian K, Petri MA, *et al*. Efficacy and safety of epratuzumab in patients with moderate/severe active systemic lupus erythematosus: results from EMBLEM, a phase IIb, randomised, double-blind, placebo-controlled, multicentre study. *Ann Rheum Dis* 2014;73:183–90.
16 McElhone K, Castelino M, Abbott J, *et al*. The LupusQoL and associations with demographics and clinical measurements in patients with systemic lupus erythematosus. *J Rheumatol* 2010;37:2273–9.
17 van der Heijde D, Dougados M, Landewé R, *et al*. Sustained efficacy, safety and patient-reported outcomes of certolizumab pegol in axial spondyloarthritis: 4-year outcomes from RAPID-axSpA. *Rheumatology* 2017;56:1498–509.
18 Urowitz MB, Feletar M, Bruce IN, *et al*. Prolonged remission in systemic lupus erythematosus. *J Rheumatol* 2005;32:1467–72.
19 Peng L, Oganesyan V, Wu H, *et al*. Molecular basis for antagonistic activity of anifrolumab, an anti-interferon-α receptor 1 antibody. *MAbs* 2015;7:428–39.
20 Merrill JT, van Vollenhoven RF, Buyon JP, *et al*. Efficacy and safety of subcutaneous tabalumab, a monoclonal antibody to B-cell activating factor, in patients with SLE. *Ann Rheum Dis* 2016;75:332–40.
21 Touma Z, Urowitz MB, Ibañez D, *et al*. SLEDAI-2K 10 days versus SLEDAI-2K 30 days in a longitudinal evaluation. *Lupus* 2011;20:67–70.

EXTENDED REPORT

Anti-NT5C1A autoantibodies are associated with more severe disease in patients with juvenile myositis

Richard M Yeker,[1] Iago Pinal-Fernandez,[1,2] Takayuki Kishi,[3] Katherine Pak,[1] Ira N Targoff,[4,5] Frederick W Miller,[3] Lisa G Rider,[3] Andrew L Mammen,[1,2,6] for the Childhood Myositis Heterogeneity Collaborative Study Group

Handling editor Josef S Smolen

[1]Muscle Disease Unit, Laboratory of Muscle Stem Cells and Gene Regulation, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health (NIH), Bethesda, Maryland, USA
[2]Department of Neurology, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA
[3]Environmental Autoimmunity Group, National Institute of Environmental Health Sciences, Bethesda, Maryland, USA
[4]US Department of Veterans Affairs Medical Center, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, USA
[5]Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, USA
[6]Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland, USA

Correspondence to
Dr Andrew L Mammen, National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health (NIH), Bethesda, MD 20892, USA;
andrew.mammen@nih.gov

LGR and ALM contributed equally.
RMY and IP-F contributed equally.

Received 25 September 2017
Revised 5 December 2017
Accepted 3 January 2018
Published Online First
23 January 2018

To cite: Yeker RM, Pinal-Fernandez I, Kishi T, *et al*. *Ann Rheum Dis* 2018;**77**:714–719.

ABSTRACT

Objectives Autoantibodies recognising cytosolic 5′-nucleotidase 1A (NT5C1A) are found in adult patients with myositis and other autoimmune diseases. They are especially prevalent in adults with inclusion body myositis (IBM), in which they are associated with more severe weakness and higher mortality. This study was undertaken to define the prevalence and clinical features associated with anti-NT5C1A autoantibodies in juvenile myositis.

Methods We screened sera from 380 patients with juvenile myositis, 30 patients with juvenile idiopathic arthritis (JIA) and 92 healthy control children for anti-NT5C1A autoantibodies. Clinical characteristics were compared between patients with myositis with and without anti-NT5C1A autoantibodies.

Results Anti-NT5C1A autoantibodies were present in 102 of 380 (27%) patients with juvenile myositis and in 11 of 92 (12%) healthy control children (P=0.002) and 27% of children with JIA (P=0.05 vs controls). Sera of 83 of 307 (27%) patients with juvenile dermatomyositis and 16 of 46 (35%) patients with juvenile overlap myositis were anti-NT5C1A autoantibody-positive (P<0.01 vs healthy controls for each), but sera of only 3 of 27 (11%) patients with juvenile polymyositis were anti-NT5C1A-positive. Patients with juvenile myositis with and without anti-NT5C1A autoantibodies had similar clinical phenotypes. However, patients with anti-NT5C1A autoantibody-positive myositis had greater pulmonary symptoms at diagnosis (P=0.005), more frequent hospitalisations (P=0.01) and required a larger number of medications (P<0.001).

Conclusion Anti-NT5C1A autoantibodies are present in more than one-quarter of children with juvenile myositis and JIA compared with only 12% of healthy children, suggesting they are myositis-associated in children. As in adults with IBM, patients with juvenile myositis with anti-NT5C1A autoantibodies have more severe disease.

INTRODUCTION

Myositis is a diverse group of autoimmune diseases that includes polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM).[1] Patients with myositis frequently have autoantibodies associated with distinct clinical phenotypes.[2] Autoantibodies found exclusively in patients with myositis are known as myositis-specific autoantibodies (MSAs), whereas those that are also found in other autoimmune conditions are known as myositis-associated autoantibodies (MAAs). Interestingly, while the same autoantibodies found in adult myositis are also common in juvenile myositis, they are not always seen in the same frequency or associated with the same clinical features in both age groups. For example, autoantibodies against p155/140 (transcriptional intermediary factor1 (TIF-1)) are highly associated with malignancy in adults, but not in children.[3]

Autoantibodies recognising cytosolic 5′-nucleotidase 1A (NT5C1A) were initially described in adults with IBM.[4 5] In these and subsequent studies, the reported prevalence of anti-NT5C1A autoantibodies in patients with IBM has ranged from 33% to 80%, depending on the patient population, type of assay used and the cut-offs chosen to define a positive result.[4–12] Importantly, since all studies have shown that <10% of adults with PM are positive for anti-NT5C1A, detection of this autoantibody may be a potentially useful biomarker for distinguishing these two forms of myositis. Subsequent studies demonstrated that anti-NT5C1A autoantibodies are also found in about 10%–15% of adult patients with DM and in 4%–36% of adult patients with lupus or Sjogren syndrome.[8 9 12] Given that they are found in myositis as well as in other autoimmune diseases, anti-NT5C1A can be defined as an adult MAA.

In adults with DM, no distinguishing clinical features have been identified for patients with anti-NT5C1A autoantibodies.[8 9] However, one study has shown that patients with anti-NT5C1A autoantibody-positive IBM are more likely to have dysphagia, facial weakness, reduced forced vital capacity and require assistive devices than those without these autoantibodies.[7] Consistent with the possibility that anti-NT5C1A autoantibodies confer a more severe disease phenotype, another recent paper showed that patients with IBM with this serological feature had a higher adjusted mortality risk than patients with autoantibody-negative IBM.[13]

As anti-NT5C1A autoantibodies have not been previously described in children with myositis, the purpose of the present study was to define the prevalence and clinical features of anti-NT5C1A autoantibodies in a large cohort of patients with juvenile myositis. We also examined whether anti-NT5C1A autoantibodies are present in another paediatric rheumatological condition, juvenile idiopathic arthritis (JIA), towards determining if they are myositis-specific or myositis-associated autoantibodies in children.

PATIENTS AND METHODS

Patients and serum samples

Patients from the Childhood Myositis Heterogeneity Collaborative Study with probable or definite myositis by Bohan and Peter criteria[14] with a serum sample available for anti-NT5C1A autoantibody testing were included in the study. Serum samples, stored at −80°C from 1 to 27 years, were available from 380 children with myositis, 30 with JIA and 92 healthy control children. Patients with myositis included 307 (81%) with juvenile DM (JDM), 27 (7%) with juvenile PM (JPM) and 46 (12%) with juvenile connective tissue disease–myositis overlap (JCTM) syndromes. The JCTM subgroup included 7 patients with JIA, 14 with juvenile systemic lupus, 11 with systemic sclerosis and 14 with various other rheumatic conditions. Healthy control children were enrolled in the same studies and were often age-matched, gender-matched and race-matched to patients with myositis. They had no family history of autoimmune disease in first-degree relatives, no history of infections or immunisations within the 2 months prior to enrolment, and no history of chronic inflammatory diseases.

All subjects were enrolled in investigational review board-approved natural history studies from 1990 to 2016, as previously described,[15] and all provided informed consent. A standardised physician questionnaire captured demographics, clinical features, laboratory features, environmental exposures at illness onset or diagnosis, as well as therapeutic usage and responses.[15] Seven organ system symptom scores at diagnosis, defined as the number of symptoms present divided by the number of symptoms assessed, and an overall clinical symptom score as the average of the seven individual organ symptom scores were calculated as previously described.[16 17] Complete clinical response and remission were defined as at least 6 months of inactive disease on or off therapy, respectively.[17] A course of treatment was defined as a single episode from beginning of administration of a given medication to the termination of treatment with that medication, or combination of medications, in each patient. The majority of patients had verification of the data via medical record review. Human leucocyte antigen (HLA) typing of DRB1 and DQA1 alleles was performed as previously described.[18] Sera from a control group of healthy children was obtained in the same protocols, and sera from 39 patients with JIA were obtained from the NIEHS Twin Sibling study.[19]

Myositis autoantibody assays

Anti-NT5C1A autoantibody detection

As previously described, lysates of human embryonic kidney (HEK) 293 cells transfected with NT5C1A and non-transfected HEK 293 cell lysates were electrophoresed on sodium dodecyl sulfate–polyacrylamide gels, transferred to nitrocellulose membranes and immunoblotted with either a positive control rabbit polyclonal antibody recognising NT5C1A (Applied Biological Materials) or human sera diluted 1:1000 for 1 hour. To achieve uniformity between assays, each assay run included a positive control (ie, rabbit anti-NT5C1A immunoblotting non-transfected vs NT5C1A-transfected HEK 293 lysates).[8] Exposures of immunoblots in which the positive control lanes were of equivalent intensity were used for scoring. Human sera that recognised the 43-kd NT5C1A protein in NT5C1A transfected cells but not in untransfected cells were considered to be positive for anti-NT5C1A autoantibodies. All blots were independently scored by two readers (RY and ALM, who were blinded to sample identity) as being positive or negative for anti-NT5C1A reactivity. The inter-rater reproducibility for the positivity of this study was excellent, with an agreement of 96.1% and a Cohen's kappa coefficient of 0.90. In the few cases where there was disagreement, a third blinded reader (IPF) adjudicated.

Other myositis autoantibodies were tested by validated methods, including protein and RNA immunoprecipitation (IP) using radiolabelled HeLa or K562 cell extracts and double immunodiffusion.[15] For anti-p155/140, anti-MJ (Nuclear Matrix Protein 2, NXP2) and anti-melanoma differentiation-associated gene 5 (MDA5) autoantibodies, serum samples were screened by IP, with confirmation testing by IP immunoblotting.[15] Anti-3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) autoantibodies were screened by ELISA and confirmed by immunoprecipitation using a ^{35}S-methionine-labelled HMGCR protein produced by in vitro transcription and translation as previously described.[20]

Analysis

Dichotomous variables were expressed as percentages and absolute frequencies, and continuous features were reported as means and SD. Pairwise comparisons for categorical variables between groups were made using χ^2 test or Fisher's exact test, as appropriate, while continuous variables were compared using Student t-test. Logistic and linear regression were used to adjust the comparisons for possible confounding variables, including the year of diagnosis, length of follow-up and myositis autoantibodies. Creatine kinase, a highly positively skewed variable, was expressed as median, first and third quartile for descriptive purposes and transformed through a base-10 logarithm for analysis. All statistical analyses were performed using Stata/MP V.14.1. As this was an exploratory study, a two-sided P value of ≤ 0.05 was considered statistically significant except for the HLA analyses, in which the Benjamini and Hochberg method to correct for multiple comparisons was performed.

RESULTS

Anti-NT5C1A autoantibodies were more prevalent in patients with juvenile myositis than in healthy control children (27% vs 12%; P=0.002) (table 1). Sera from 27% of patients with JDM, 11% with JPM and 35% with JCTM had anti-NT5C1A

Table 1 Prevalence of anti-NT5C1A in the sera of paediatric patients

	Total (n=502) % (N)
Juvenile myositis (n=380)	27 (102)**
Juvenile dermatomyositis (n=307)	27 (83)**
Juvenile PM (n=27)	11 (3)
Juvenile myositis overlap syndromes (n=46)	35 (16)**
Juvenile myositis overlapping with juvenile systemic lupus erythematosus (n=14)	36 (5)*
Juvenile myositis overlapping with juvenile systemic sclerosis (n=11)	27 (3)
Juvenile myositis overlapping with JIA (n=7)	14 (1)
Juvenile myositis overlapping with other paediatric autoimmune diagnosis (n=14)	50 (7)**
JIA (n=30)	27 (8)*
Healthy paediatric controls (n=92)	12 (11)

*P<0.05, **P<0.01, ***P<0.001.
%, percentage positive for anti-NT5C1A autoantibodies; JIA, juvenile idiopathic arthritis; n, number of sera testing positive for anti-NT5C1A autoantibodies; NT5C1A, cytosolic 5′ -nucleotidase 1A; PM, polymyositis.

Table 2 Demographic and myositis autoantibody features of patients with juvenile myositis according to anti-NT5C1A autoantibody status

	Anti-NT5C1A Ab+ (n=102)	Anti-NT5C1A Ab− (n=278)	P value
Age at diagnosis (years)	9.5 (4.4)	8.8 (4.3)	0.2
Age at enrolment (years)	11.8 (6.0)	12.7 (7.4)	0.3
Delay to diagnosis (years)	0.69 (1.00)	0.71 (1.23)	0.9
Follow-up (years)	4.4 (4.6)	6.2 (6.8)	0.01
Girl	70% (71/102)	72% (200/278)	0.7
Race			
White	65% (66/102)	65% (182/278)	0.9
Black	12% (12/102)	17% (47/278)	0.2
Hispanic	7% (7/102)	6% (17/278)	0.8
Other races	17% (17/102)	12% (32/278)	0.2
Myositis autoantibodies			
Anti-p155/140	42% (42/100)	31% (82/268)	0.04
Anti-NXP2	22% (22/101)	21% (58/274)	0.9
Anti-MDA5	10% (10/102)	8% (22/275)	0.6
Anti-synthetase autoantibodies	4% (4/96)	4% (11/273)	1.0
Anti-SRP	0% (096)	3% (7/273)	0.2
Anti-Mi2	4% (4/96)	3% (9/267)	0.8
Anti-HMGCR	1% (1/102)	1% (3/278)	1.0
MSA negative	19% (19/102)	29% (77/269)	0.05

Dichotomous variables were represented as percentage (count/total) and continuous variables as mean (SD).
Ab, autoantibody; HMGCR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; MDA5, melanoma differentiation protein 5; MSA, myositis-specific autoantibody; NT5C1A, cytosolic 5′-nucleotidase 1A; NXP2, nuclear matrix protein; SRP, signal recognition particle.

autoantibodies. Among these clinical subgroups, the prevalence of anti-NT5C1A autoantibodies was significantly increased in patients with JDM (P=0.003) and JCTM (P=0.001), including those with lupus–myositis overlap (P=0.02), compared with healthy controls. Patients with JCTM had a higher prevalence of anti-NT5C1A autoantibodies than those with JPM (P=0.03); otherwise there were no significant differences in autoantibody prevalences among the JDM, JPM and JCTM subgroups. Of note, the prevalence of anti-NT5C1A autoantibodies was significantly increased in the children with JIA compared with healthy controls (27% vs 12%; P=0.02) (table 1).

There were no significant differences in gender or race between patients with juvenile myositis with and without anti-NT5C1A autoantibodies (table 2). There was an association between the presence of anti-NT5C1A and anti-p155/140 autoantibodies, and fewer of the patients positive for anti-NT5C1A autoantibodies were negative for other MSAs. Specifically, 34% of the patients positive for anti-p155/140 autoantibodies were also positive for anti-NT5C1A autoantibodies compared with just 24% of patients negative for anti-p155/140 (P=0.04). Conversely, patients with anti-NT5C1A autoantibodies more frequently had coexisting anti-p155/140 autoantibodies than those who were negative for anti-NT5C1A autoantibodies (42% vs 31%, P=0.04). Of note, patients positive for anti-NT5C1A autoantibodies were more likely to have shorter follow-up times (4.4 vs 6.2 years, P=0.01) compared with patients negative for anti-NT5C1A autoantibodies (table 2). Given these differences, subsequent multivariate analyses were adjusted for follow-up duration and the presence of myositis autoantibodies (including anti-p155/140 autoantibodies) as well as the year of diagnosis (considering that treatment strategies may have changed over time).

In the multivariate analysis comparing their clinical features, patients positive for anti-NT5C1A autoantibodies were more likely to have Raynaud's phenomenon and V-sign or shawl rashes compared with patients negative for anti-NT5C1A autoantibodies (table 3). The prevalences of dysphonia and photosensitivity were increased among patients positive for anti-NT5C1A autoantibodies in the univariate analysis but not in the multivariate analysis, and there was a trend towards more frequent interstitial lung disease in those with anti-NT5C1A autoantibodies (table 3). There were no other significant differences in the prevalences of the main muscle, lung, gastrointestinal, constitutional involvement or other cutaneous manifestations between these two groups in either univariate or multivariate analysis (table 3).

A number of differences between patients positive and negative for the anti-NT5C1A autoantibodies suggested that patients with these autoantibodies had more severe disease. In the multivariate analysis, patients positive for anti-NT5C1A autoantibodies had higher pulmonary symptom scores (P=0.003) and showed a trend towards higher total symptom score (P=0.09) at the time of diagnosis (table 3). Patients positive for anti-NT5C1A autoantibodies had a higher mean number of hospitalisations (1.6 vs 1.1, P=0.01) than those without thieseautoantibodies. Furthermore, patients positive for the anti-NT5C1A autoantibodies required a greater number of medication treatments per year (4.8 vs 3.6, P≤0.001) with a higher percentage of patients requiring intravenous pulse steroids (78% vs 47%, P<0.001), intravenous immunoglobulin (67% vs 24%, P<0.001) and use of other immunosuppressive medications (33% vs 20%, P=0.04) (table 4). Patients positive for anti-NT5C1A autoantibodies had a trend for a more severe functional class at last assessment, but this did not reach statistical significance in the multivariate analysis (29% vs 18% functional class 2, P=0.08).

The multivariate analyses were adjusted for the presence or absence of each MSA. Consequently, our finding that anti-NT5C1A autoantibodies are associated with more severe disease is independent of the MSA status of the patient. Furthermore, when analysed separately, all patients negative for MSA, positive for anti-p155/140 autoantibodies and positive for anti-NXP2 autoantibodies had evidence of more severe disease when they were also positive for anti-NT5C1A autoantibodies (data not shown).

To determine whether there is an immunogenetic association with anti-NT5C1A autoantibodies, we compared the prevalence of HLA DRB1 and DQA1 alleles between Caucasian patients with juvenile myositis with and without this autoantibody. We also compared Caucasian patients with anti-NT5C1A autoantibodies with healthy Caucasian subjects and all patients positive for anti-NT5C1A with those negative for anti-NT5C1A autoantibodies. However, after multiple correction adjustment, no statistically significant associations between anti-NT5C1A autoantibodies and these class II MHC alleles were found in any of these comparisons.

DISCUSSION

In adults, autoantibodies recognising NT5C1A are considered to be MAAs rather than MSAs because they are found not only in patients with myositis, but also in those with lupus and Sjogren syndrome.[8 9] In this study, we show that anti-NT5C1A autoantibodies are also MAAs in children since they are found not only in patients with juvenile myositis, but also in those with JIA. As expected for a MAA, we found that anti-NT5C1A autoantibodies

Table 3 Clinical features according to anti-NT5C1A autoantibody status

	Ever present			
	Anti-NT5C1A Ab+ (n=102)	Anti-NT5C1A Ab– (n=278)	Univariate P value	Multivariate P value
Muscle involvement				
Proximal weakness	99% (101/102)	100% (277/278)	0.5	0.6
Distal weakness	50% (51/101)	45% (122/271)	0.3	0.4
Muscle atrophy	35% (36/102)	38% (105/274)	0.6	0.8
Myalgia	66% (67/101)	63% (172/271)	0.6	0.5
Falling	44% (45/102)	45% (122/274)	0.9	0.8
Dysphonia	40% (40/101)	29% (80/275)	0.05	0.2
Lung involvement				
Interstitial lung disease	13% (13/102)	7% (20/276)	0.09	0.06
Dyspnoea on exertion	35% (36/102)	27% (74/273)	0.1	0.2
Joint involvement				
Arthritis	57% (58/102)	49% (137/277)	0.2	0.5
Arthralgia	66% (67/102)	64% (177/276)	0.8	0.7
Joint contractures	68% (69/102)	57% (159/277)	0.07	0.4
Skin involvement				
Heliotrope rash	84% (85/101)	77% (214/277)	0.1	0.6
Gottron's papules	87% (89/102)	81% (223/277)	0.1	0.5
Calcinosis	27% (28/102)	31% (86/278)	0.5	0.5
Raynaud's phenomenon	17% (17/102)	14% (39/276)	0.5	0.03
Mechanic's hands	9% (9/100)	7% (18/275)	0.4	0.2
V or Shawl sign rash	43% (44/102)	26% (72/276)	0.001	0.02
Malar rash	81% (83/102)	66% (184/278)	0.004	0.1
Photosensitivity	62% (61/99)	43% (116/272)	0.001	0.09
Linear extensor erythema	42% (42/101)	34% (91/271)	0.2	0.5
Gastrointestinal involvement				
Dysphagia	44% (45/102)	39% (109/277)	0.4	0.5
Regurgitation	25% (26/102)	19% (52/277)	0.2	0.4
Systemic involvement				
Weight loss	44% (44/101)	42% (115/277)	0.7	0.9
Fever	30% (30/100)	31% (83/267)	0.8	0.6
Early symptom scores				
Total	0.27 (0.12)	0.23 (0.11)	0.005	0.1
Muscle	0.38 (0.19)	0.38 (0.20)	0.8	0.9
Joint	0.52 (0.41)	0.43 (0.42)	0.06	0.4
Cutaneous	0.29 (0.15)	0.24 (0.13)	0.002	0.3
Gastrointestinal	0.09 (0.13)	0.07 (0.10)	0.03	0.06
Pulmonary	0.13 (0.17)	0.08 (0.15)	0.004	0.005
Cardiac	0.03 (0.08)	0.03 (0.08)	0.8	0.6
Constitutional	0.41 (0.26)	0.39 (0.27)	0.4	0.8

Dichotomous variables were represented as percentage (count/total), continuous variables as mean (SD) and the creatine kinase was presented as median (Q1–Q3).
Ab, autoantibody; NT5C1A, cytosolic 5′-nucleotidase 1A.

were frequently found in association with other MSA, especially with anti-p155/140 autoantibodies.

The prevalence of anti-NT5C1A autoantibodies varies among adults with different myositis subtypes. They occur most frequently in those with IBM, less frequently in DM and least frequently in those with PM. Here, we demonstrate that the prevalence of these autoantibodies in children also varies among myositis subtypes, with 27% of JDM but only 11% of patients with JPM testing positive for anti-NT5C1A autoantibodies. In the current study, we also found that anti-NT5C1A autoantibodies are present in 35% of children with myositis-overlap syndromes. Although the number of patients in each myositis overlap subgroup was small, the autoantibodies were found most commonly in those with myositis associated with juvenile lupus (36%), less frequently in myositis associated with juvenile systemic sclerosis (27%) and least frequently in those with myositis associated with JIA (14%). In adults, the prevalence of anti-NT5C1A autoantibodies was <5% in adults with myositis–scleroderma overlap,[9] but future studies will be required to define the prevalence of anti-NT5C1A autoantibodies in adults with other forms of myositis-overlap.

In adults with IBM, anti-NT5C1A autoantibodies have been associated with more severe muscle disease[7] and increased mortality.[13] However, no evidence for an association between anti-NT5C1A autoantibodies and more severe disease in adults with other forms of myositis has been reported. Here, we demonstrate that in patients with juvenile myositis, these autoantibodies are associated with more frequent hospitalisations, higher pulmonary symptoms and increased number of medications used. The underlying reasons for the association of

Table 4 Disease outcomes and medications received according to anti-NT5C1A autoantibody status

	Anti-NT5C1A Ab+ (n=102)	Anti-NT5C1A Ab– (n=278)	Univariate P value	Multivariate P value
Disease course				
Monocyclic course	16% (12/76)	23% (53/229)	0.2	0.2
Polycyclic course	18% (14/76)	24% (56/229)	0.3	0.6
Chronic continuous course	66% (50/76)	52% (120/229)	0.04	0.2
Steinbrocker functional class at final assessment (mean)	1.5 (0.8)	1.4 (0.8)	0.6	0.6
Muscle enzymes				
Peak creatine kinase (IU/L)	1010 (296–3971)	672 (252–5460)	0.7	0.2
Peak aldolase (IU/L)	16.9 (23.9)	20.8 (37.2)	0.3	0.7
Severity at onset (mean, 0–4)	2.1 (1.4)	2.2 (0.9)	0.4	0.3
Mortality	4% (4/102)	3% (9/278)	0.8	0.3
Hospitalised	65% (62/96)	55% (148/268)	0.1	0.1
Number of hospitalisations	1.6 (2.3)	1.1 (1.7)	0.04	0.01
Wheelchair use	21% (21/100)	18% (47/268)	0.4	0.1
Response to treatment				
Complete clinical response	22% (19/87)	33% (74/225)	0.06	0.6
Remission	15% (13/88)	27% (63/232)	0.02	0.5
Total number of medications used	4.8 (2.0)	3.6 (2.0)	<0.001	<0.001
Medications received				
Oral steroids	99% (87/88)	99% (230/232)	1.0	0.6
Intravenous pulsed steroids	78% (69/88)	47% (110/232)	<0.001	<0.001
Methotrexate	83% (73/88)	71% (164/232)	0.03	0.4
Intravenous immunoglobulin	67% (59/88)	24% (55/232)	<0.001	<0.001
Other DMARDs	33% (29/88)	20% (46/232)	0.01	0.04

Dichotomous variables were represented as percentage (count/total), continuous variables as mean (SD) and the creatine kinase was presented as median (Q1–Q3).
Ab, autoantibody; DMARDs, disease-modifying anti-rheumatic drugs.; NT5C1A, cytosolic 5′-nucleotidase 1A.

anti-NT5C1A autoantibodies with more severe disease remain unclear, but may relate to a direct effect of the autoantibodies on myofibre protein degradation, as demonstrated in a study of passive transfer of anti-NT5C1A autoantibodies into mice.[21] Another possibility is that a more severe inflammatory response in juvenile myositis predisposes patients to the development of additional immunoreactivities such as that against NT5C1A.

In some instances, individual autoantibodies have significant associations with specific HLA alleles. However, in this study, we did not find an association between the presence of anti-NT5C1A autoantibodies and any class II HLA alleles. This is consistent with a recent report by Limaye, showing no HLA associations in adult patients with IBM with anti-NT5C1A autoantibodies.[11]

In the current study, we were surprised to find that 11 out of 94 (12%) healthy children were positive for anti-NT5C1A autoantibodies detected by a previously validated immunoblotting assay.[7] We have demonstrated that 61% of adult patients with IBM and only 5% of adult patients with PM were anti-NT5C1A autoantibody-positive using the immunoblotting method, findings which are in agreement with an established dot blot technique[4] and an established immunoprecipitation technique.[5] In our previous study, we also found that 5% of healthy adult controls were anti-NT5C1A-positive by immunoblotting.[7] While another study reported that none of 32 adult healthy controls were anti-NT5C1A-positive by immunoprecipitation,[5] the prevalence of anti-NT5C1A autoantibodies in healthy adult controls has not been reported for the dot blot assay[4] or either of two different ELISA.[6] Nonetheless, taken together, these comparisons indicate that the anti-NT5C1A immunoblot assay performs as well as other detection methods and does not have an unacceptably high false positive rate. Of note, autoantibodies against Ro52, another common target of the immune system in various systemic autoimmune diseases, are also found in healthy controls as well as in patients with more severe disease manifestations.[22]

When comparing the prevalence of anti-NT5C1A autoantibodies in children and adults within the same clinical groups using the same immunoblotting detection method, younger subjects consistently are more likely to have reactivity to these autoantibodies. For example, 27% of JDM but only 15% of adult patients with DM[7] are anti-NT5C1A autoantibody-positive (P=0.03). Similarly, 11% of JPM but only 5% of adult patients with PM[7] have these autoantibodies. Given that the same pattern is also observed among healthy children and healthy adults, we hypothesise that in both diseased and healthy groups, immunoreactivity against NT5C1A may decrease with age.

The current study has several limitations. First, the cohort of patients with juvenile myositis had some data collected retrospectively, resulting in some missing data, and was collected over >20 years, with potential chronology bias. However, we adjusted the variables of this study for the year of diagnosis and tested the distribution of missing values across groups and did not find evidence of a significant bias (data not shown). Second, we only screened a small number of patients with one other systemic autoimmune disease, JIA; the small sample size in this group of patients did not allow us to study differences in severity between patients with and without anti-NT5C1A autoantibodies in a reliable way. Future studies will be needed to determine the full range of paediatric rheumatological conditions in which anti-NT5C1A autoantibodies are found and to determine whether their presence correlates with disease severity or other clinical features. Third, there is no widely accepted ‘gold standard’ for detecting anti-NT5C1A autoantibodies. However, we have developed and validated an immunoblotting detection method. This technique has a sensitivity (61%) and specificity (95%) for

detecting anti-NT5C1A autoantibodies in adult patients with IBM that is consistent with most other methods that utilise a full-length NT5C1A protein (sensitivity range 47%–80% and specificity range 95%–100%).[4–12] Of note, one published study utilising an ELISA method detected anti-NT5C1A autoantibodies in only 37% of patients with IBM. However, this assay used three short peptides rather than the full-length NT5C1A protein and the authors of the study acknowledged that it may have poor sensitivity since it cannot detect reactivity to conformational epitopes.[9]

These limitations notwithstanding, this study shows that anti-NT5C1A autoantibodies are present in approximately one-quarter of patients with juvenile myositis as well as JIA. Furthermore, as shown for adults with IBM, patients with juvenile myositis with anti-NT5C1A autoantibodies have more severe disease than those without these autoantibodies. Additional studies will be required to confirm the association with disease severity in JM and to determine whether anti-NT5C1A autoantibodies are a result or a cause of the more severe clinical manifestations seen in the patients with juvenile myositis and adult patients with IBM who have them.

Acknowledgements The authors thank Mariana Kaplan, David Amici, Cassie Parks, Rebecca de Lorenzo, Maria Casal-Dominguez, Assia Derfoul and Jose Milisenda for their critical reading and suggestions on the manuscript. This manuscript is based on work previously presented and published as a conference abstract at (a) The Global Conference on Myositis in Potomac, Maryland, USA (5-8 May 2017), (b) The 2017 American College of Rheumatology Annual Meeting in San Diego, California, USA (19 October 2017) and (c) The European League Against Rheumatism Annual European Congress of Rheumatology, Madrid, Spain (14-17 June 2017).

Collaborators Daniel A Albert, Bita Arabshahi, Imelda M Balboni, John F Bohnsack, Michael S Borzy, Suzanne L Bowyer (postmortem), Chun Peng T Chao, Randy Q Cron, Marietta M DeGuzman, Ellen A Goldmuntz, Thomas A Griffin, William Hannan, Melissa Hawkins-Holt, J Roger Hollister, Russell J Hopp, Jerry C Jacobs (postmortem), Anna Jansen, Olcay Y Jones, Lawrence K Jung, Ankur Kamdar, Steven J Klein, Bianca A Lang, Katherine L Madson, Paul L McCarthy, Gulnara Mamyrova, Stephen R Mitchell, Frederick T Murphy, Kabita Nanda, Terrance P O'Hanlon, Elif A Oral, Lauren M Pachman, Murray H Passo, Maria D Perez, Donald A Person, Linda I Ray, Robert M Rennebohm, Rafael F Rivas-Chacon, Tova Ronis, Adam Schiffenbauer, Bracha Shaham, David D Sherry, Sara H Sinal, Abigail Smukler, Sangeeta H Sule, Scott A Vogelgesang, Jennifer C Wargula and Patience H White.

Contributors All authors: involved in drafting the article or revising it critically for important intellectual content and approved the final manuscript to be submitted and agreed to be accountable for all aspects of the work. RMY, IP-F, FWM, LGR and ALM: conception and design of the study. RMY, IP-F, TK, KP, INT and LGR: acquisition of data. RMY, IP-F, TK, INT, FWM, LGR and ALM: analysis and interpretation of data.

Funding This work was supported in part by the National Institute of Environmental Health Sciences and the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health. IP-F and TK were supported by research fellowships from The Myositis Association.

Disclaimer IT is a consultant to the Oklahoma Medical Research Foundation Clinical Immunology Laboratory regarding myositis autoantibody testing.

Competing interests None declared.

Patient consent Obtained.

Ethics approval All subjects were enrolled in investigational review board-approved natural history studies from 1990 to 2016. The parents of the pediatric patients in this study signed a consent form approved by the Institutional Review Board at the National Institutes of Health.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement No unpublished data related to this study are publicly available.

REFERENCES

1 Dalakas MC. Inflammatory Muscle Diseases. *N Engl J Med Overseas Ed* 2015;372:1734–47.
2 Rider LG, Miller FW. Deciphering the clinical presentations, pathogenesis, and treatment of the idiopathic inflammatory myopathies. *JAMA* 2011;305:183–90.
3 Gunawardena H, Wedderburn LR, North J, *et al*. Clinical associations of autoantibodies to a p155/140 kDa doublet protein in juvenile dermatomyositis. *Rheumatology* 2008;47:324–8.
4 Larman HB, Salajegheh M, Nazareno R, *et al*. Cytosolic 5′-nucleotidase 1A autoimmunity in sporadic inclusion body myositis. *Ann Neurol* 2013;73:408–18.
5 Pluk H, van Hoeve BJ, van Dooren SH, *et al*. Autoantibodies to cytosolic 5′-nucleotidase 1A in inclusion body myositis. *Ann Neurol* 2013;73:397–407.
6 Greenberg SA. Cytoplasmic 5′-nucleotidase autoantibodies in inclusion body myositis: Isotypes and diagnostic utility. *Muscle Nerve* 2014;50:488–92.
7 Goyal NA, Cash TM, Alam U, *et al*. Seropositivity for NT5c1A antibody in sporadic inclusion body myositis predicts more severe motor, bulbar and respiratory involvement. *J Neurol Neurosurg Psychiatry* 2016;87:373–8.
8 Lloyd TE, Christopher-Stine L, Pinal-Fernandez I, *et al*. Cytosolic 5′-Nucleotidase 1A As a Target of Circulating Autoantibodies in Autoimmune Diseases. *Arthritis Care Res* 2016;68:66–71.
9 Herbert MK, Stammen-Vogelzangs J, Verbeek MM, *et al*. Disease specificity of autoantibodies to cytosolic 5′-nucleotidase 1A in sporadic inclusion body myositis versus known autoimmune diseases. *Ann Rheum Dis* 2016;75:696–701.
10 Kramp SL, Karayev D, Shen G, *et al*. Development and evaluation of a standardized ELISA for the determination of autoantibodies against cN-1A (Mup44, NT5C1A) in sporadic inclusion body myositis. *Auto Immun Highlights* 2016;7:16.
11 Limaye VS, Lester S, Blumbergs P, *et al*. Anti- C N1A antibodies in South Australian patients with inclusion body myositis. *Muscle Nerve* 2016;53:654–5.
12 Muro Y, Nakanishi H, Katsuno M, *et al*. Prevalence of anti-NT5C1A antibodies in Japanese patients with autoimmune rheumatic diseases in comparison with other patient cohorts. *Clin Chim Acta* 2017;472:1–4.
13 Lilleker JB, Rietveld A, Pye SR, *et al*. Cytosolic 5′-nucleotidase 1A autoantibody profile and clinical characteristics in inclusion body myositis. *Ann Rheum Dis* 2017;76:862–8.
14 Bohan A, Peter JB. Polymyositis and dermatomyositis (second of two parts). *N Engl J Med* 1975;292:403–7.
15 Shah M, Mamyrova G, Targoff IN, *et al*. The clinical phenotypes of the juvenile idiopathic inflammatory myopathies. *Medicine* 2013;92:25–41.
16 Habers GE, Huber AM, Mamyrova G, *et al*. Brief report: association of myositis autoantibodies, clinical features, and environmental exposures at illness onset with disease course in juvenile myositis. *Arthritis Rheumatol* 2016;68:761–8.
17 Oddis CV, Rider LG, Reed AM, *et al*. International consensus guidelines for trials of therapies in the idiopathic inflammatory myopathies. *Arthritis Rheum* 2005;52:2607–15.
18 Mamyrova G, O'Hanlon TP, Monroe JB, *et al*. Immunogenetic risk and protective factors for juvenile dermatomyositis in Caucasians. *Arthritis Rheum* 2006;54:3979–87.
19 Gan L, O'Hanlon TP, Lai Z, *et al*. Gene expression profiles from disease discordant twins suggest shared antiviral pathways and viral exposures among multiple systemic autoimmune diseases. *PLoS One* 2015;10:e0142486.
20 Kishi T, Rider LG, Pak K, *et al*. Association of anti-3-hydroxy-3-methylglutaryl-coenzyme a reductase autoantibodies with drb1*07:01 and severe myositis in juvenile myositis patients. *Arthritis Care Res* 2017;69:1088–94.
21 Tawara N, Yamashita S, Zhang X, *et al*. Pathomechanisms of anti-cytosolic 5′ -nucleotidase 1A autoantibodies in sporadic inclusion body myositis. *Ann Neurol* 2017;81:512–25.
22 Burbelo PD, Ching KH, Han BL, *et al*. Extraordinary antigenicity of the human Ro52 autoantigen. *Am J Transl Res* 2010;2:145–55.

EXTENDED REPORT

Efficacy and safety of adrenocorticotropic hormone gel in refractory dermatomyositis and polymyositis

Rohit Aggarwal,[1] Galina Marder,[2] Diane Carol Koontz,[1] Preeya Nandkumar,[2] Zengbiao Qi,[1] Chester V Oddis[1]

Handling editor Tore K Kvien

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212047).

[1]Department of Medicine, Division of Rheumatology and Clinical Immunology, University of Pittsburgh, Pittsburgh, Pennsylvania, USA
[2]Northwell Health, Formerly North Shore–Long Island Jewish Medical Center, Great Neck, New York, USA

Correspondence to
Dr Rohit Aggarwal, Department of Medicine, Division of Rheumatology and Clinical Immunology, University of Pittsburgh, Pittsburgh, PA 15217, USA;
aggarwalr@upmc.edu

Received 10 July 2017
Revised 27 November 2017
Accepted 29 November 2017
Published Online First 13 December 2017

To cite: Aggarwal R, Marder G, Koontz DC, *et al*. *Ann Rheum Dis* 2018;**77**:720–727.

ABSTRACT

Aim To evaluate the efficacy, safety, tolerability and steroid-sparing effect of repository corticotropin injection (RCI), in an open-label clinical trial, in refractory adult polymyositis (PM) and dermatomyositis (DM).

Methods Adults with refractory PM and DM were enrolled by two centres. Inclusion criteria included refractory disease defined as failing glucocorticoid and/or ≥1 immunosuppressive agent, as well as active disease defined as significant muscle weakness and >2 additional abnormal core set measures (CSMs) or a cutaneous 10 cm Visual Analogue Scale score of ≥3 cm *and* at least three other abnormal CSMs. All patients received RCI of 80 units subcutaneously twice weekly for 24 weeks. The primary end point for the trial was the International Myositis Assessment and Clinical Studies definition of improvement. Secondary end points included safety, tolerability, steroid-sparing as well as the 2016 American College of Rheumatology (ACR)/European League Against Rheumatism myositis response criteria (EULAR)

Results Ten of the 11 enrolled subjects (6 DM, 4 PM) completed the study. Seven of 10 met the primary end point of efficacy at a median of 8 weeks. There was a significant decrease in prednisone dose from baseline to conclusion (18.5 (15.7) vs 2.3 (3.2); P<0.01). Most individual CSMs improved at week 24 compared with the baseline, with the muscle strength improving by >10% and the physician global by >40%. RCI was considered safe and tolerable. No patient developed significant weight gain or an increase of haemoglobin A1c or cushingoid features.

Conclusion Treatment with RCI was effective in 70% of patients, safe and tolerable, and led to a steroid dose reduction in patients with adult myositis refractory to glucocorticoid and traditional immunosuppressive drugs.

Trial registration number NCT01906372; Results.

INTRODUCTION

Idiopathic inflammatory myopathies (commonly referred to as myositis) are a group of systemic autoimmune muscle diseases characterised by inflammation of skeletal muscle, the most common of which include dermatomyositis (DM) and polymyositis (PM). The treatment of PM and DM is unsatisfactory as many patients either require high doses of glucocorticoids with significant side effects or are refractory to conventional immunosuppressive drugs.[1 2] Neither glucocorticoids or immunosuppressive agents are Food and Drug Administration (FDA)-approved for myositis, but H.P. Acthar Gel (repository corticotropin injection (RCI)) has been an FDA-approved treatment for myositis since 1952, and in 2010 the FDA retained this indication. RCI is a long-acting full-sequence adrenocorticotropic hormone ($ACTH_{1–39}$) and includes other pro-opiomelanocortin peptides thought to have anti-inflammatory and immunomodulatory effects mediated through melanocortin (MC) receptors. It is indicated for other immune-mediated disorders including multiple sclerosis, nephrotic syndrome and infantile spasm syndromes. Despite its FDA approval for PM and DM, as well as possible glucocorticoid-independent immune-mediated effects, there are limited data on its clinical utility in myositis. A recent retrospective review of five patients with refractory myositis demonstrated improved muscle strength after RCI,[3] but was limited due to its retrospective design, lack of long-term follow-up and evaluation of validated outcome measures. These reports led to a prospective pilot clinical trial of RCI in patients with refractory myositis using the six validated core set measures (CSMs) and outcome measures proposed by the International Myositis Assessment and Clinical Studies (IMACS) group. Herein we assess the efficacy, safety, tolerability and steroid-sparing effect of RCI in adult patients with refractory PM and DM.

PATIENTS AND METHODS

Study population

This study was conducted at two sites (University of Pittsburgh Medical Center and Northwell Health, formerly North Shore Long Island Jewish Medical Center) with an expected enrolment of 10 patients with PM/DM. Written informed consent was obtained from each study subject.

Eligible patients included adults at least 18 years of age or older with a diagnosis of definite or probable DM or PM according to the criteria of Bohan *et al*.[4] Patients with PM either possessed a myositis-associated autoantibody or underwent adjudication for confirmation of the PM diagnosis by another myositis expert (RA or CVO) to eliminate the enrolment of mimics of PM.[5] Patients had refractory and active disease defined as failing an adequate glucocorticoid trial (≥2 months of high doses (0.75–1 mg/kg) or intolerance to such therapy) and/or ≥1 conventional immunosuppressive agent (eg, methotrexate (MTX), azathioprine (AZA), tacrolimus (TAC), ciclosporin, mycophenolate mofetil (MMF), intravenous immunoglobulin (IVIG), antitumour necrosis factor agent or rituximab) at near maximal doses for ≥3 months. It was recommended to enrol refractory patients failing

(or intolerant to) both glucocorticoids *and* at least one conventional immunosuppressive agent. Concomitant immunosuppressive agents or glucocorticoids were allowed, but subjects should have been on these therapies at least 8 weeks (and at least 4 weeks for glucocorticoids) and on a stable dose for ≥4 weeks and ≥2 weeks, respectively, prior to the start of the trial. No dose changes other than glucocorticoid tapering were permitted during the trial except for rescue medication or changes related to patient safety or adverse events. IVIG or biological agents were not allowed during the course of the trial.

Active myositis was defined by baseline Manual Muscle Testing (MMT-8) no greater than 125/150 and at least two additional abnormal CSMs (ie, ≥2 cm on 10 cm Visual Analogue Scale (VAS) of patient global,[6] physician global and extramuscular disease activity, Health Assessment Questionnaire Disability Index, minimum score of 0.25, and elevated muscle enzymes >1.3 × the upper limit of normal). To allow the enrolment of patients with active DM with a moderate to severe rash who may not meet the MMT-8 criterion noted above, patients with DM could be enrolled if their cutaneous VAS score on the Myositis Disease Activity Assessment Tool (MDAAT) was ≥3 cm on the 10 cm VAS scale *and* at least three of the above five CSMs were abnormal (excluding the MMT-8).

If patients discontinued immunosuppressive therapy before enrolment, a 4-week washout was required for MTX, AZA, TAC, MMF, ciclosporin and leflunomide, and an 8-week washout for infliximab or adalimumab, 2 weeks for etanercept, 6 months for rituximab, and 2 months for IVIG and cyclophosphamide. To minimise confounding, patients with the following conditions were excluded: juvenile DM or PM, myositis in overlap with another systemic autoimmune rheumatic disorder, cancer-associated myositis, inclusion body myositis or any other non-immune-mediated myopathy. Also patients with hypersensitivity to study drug, pregnant or lactating women, and any concomitant illness including severe cardiac, pulmonary disease and active infections that precluded an accurate treatment response during the trial or posed an added risk for participants were excluded. Patients with malignancy within 3 years of screening (except basal cell cancer or squamous cell cancer of skin) were excluded. We excluded patients with severe muscle damage defined as a baseline global muscle damage score on the Myositis Damage Index of ≥5 cm on a 10 cm VAS. Patients were allowed to continue an exercise programme that had been initiated before the 4-week screening period. However, no new exercise programme for muscle strengthening during the trial was permitted. The definition of worsening was the same employed in previous myositis trials.[7]

METHODS

RCI is a highly purified sterile preparation of full-length ACTH (39 amino acid peptide) and other pro-opiomelanocortin peptides in 16% gelatin to provide a prolonged release after intramuscular or subcutaneous injection. RCI was supplied as a 5 mL multidose phial containing 80 units/mL.

Study design

This was a proof-of-concept study to evaluate efficacy, safety, tolerability and the steroid-sparing effect of RCI in patients with refractory PM and DM using a prospective, open-label design for 24 weeks. We enrolled 10 patients with active and refractory PM/DM with evaluations every 4 weeks for 24 weeks (seven total visits including baseline). Study subjects subcutaneously self-administered RCI 80 units (1 mL) twice weekly for 24 weeks. The glucocorticoid dose could be increased ≤10 mg (prednisone equivalent) daily as a rescue medication without constituting the subject as a treatment failure. However, any subject requiring rescue medication exceeding 10 mg equivalent of prednisone or an immunosuppressive agent above their baseline dose or the addition of any new immunosuppressive drug or new glucocorticoid after the 8-week time point was considered a treatment failure even though they remained in the trial to receive study drug and scheduled assessments.

Adverse events

Adverse events (AEs) and serious adverse events (SAEs) along with infusion reactions were monitored and reported in a standardised manner using the Common Terminology Criteria of the National Cancer Institute V.4.03, with clinical site investigators determining their relatedness to the study drug. An AE or SAE was regarded as possibly related to the study drug if the investigator believed (1) there was a clinically plausible time sequence between onset of the AE and the administration of RCI, and/or (2) there was a biologically plausible mechanism by which RCI could cause or contribute to the AE, and (3) the AE could not be attributed solely to the concurrent/underlying illness, other drugs or procedures. Study investigators reported each AE and SAE as one of the following: definitely related, probably related, possibly related, unlikely to be related or unrelated. For purposes of analysis, only AEs and SAEs deemed to have a definite, probable or possible relationship to the study drug were considered to be related.

Primary and secondary end points

The primary end point for the trial was the IMACS definition of improvement (DOI): three of any of the six CSMs improved by ≥20%, with no more than 2 CSMs worsening by ≥25% (worsening measure cannot include the MMT). Patients meeting DOI at any visit should continue to meet DOI on subsequent visits until study completion. Primary end points were also separately evaluated on a subset of patients with severe muscle weakness (≤125/150 of MMT at baseline) as well as moderate to severe cutaneous DM rashes (≥2.5/10 of cutaneous VAS score at baseline). Secondary safety and tolerability end points were measured by frequency and type of AEs and SAEs. AEs and SAEs were measured by detailed questionnaires, patient report and study withdrawal due to study drug side effects or tolerability problems. Additional secondary end points included (1) median change in individual CSM from baseline to end of study, (2) median time to DOI from baseline, (3) *2016 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) myositis response criteria* and (d) mean change in glucocorticoid dose (equivalent prednisone dose) at 24 weeks compared with baseline.[8–11]

Statistical analysis

Descriptive statistics were evaluated for baseline demographic, clinical and laboratory variables. The frequency of patients meeting primary and secondary outcome criteria at 24 weeks was evaluated based on a modified intention-to-treat analysis if the subject received at least 8 weeks of study drug. The median score of each CSM at last study visit was compared with baseline values using the Wilcoxon rank-sum test. The median time to DOI was determined by Wilcoxon rank-sum test. The frequency and severity of AEs and SAEs related/unrelated to study drug were reported. The mean change in the glucocorticoid dose (prednisone equivalent) and changes in safety labs (HbA1C,

weight, hemoglobin, etc) from baseline to final evaluation was compared using paired t-test.

RESULTS

Ten patients completed the study, each receiving RCI 80 units (1 mL) twice weekly for 24 weeks without dose modification. One patient dropped out due to heart block unrelated to the study drug and was not included in the analysis as he did not complete the minimum 8 weeks of study drug required for outcome assessment as per study protocol (patient 11 in table 1). Table 1 summarises the baseline clinical features of all 11 enrolled patients. Briefly, there were five patients with PM

Table 1 Baseline characteristics of study cohort

Patient no.	Age/Gender/Ethnicity	Diagnosis/Myositis autoantibody	Disease duration	Baseline: muscle enzyme*	Baseline: MMT (0–150)	Baseline: physician global disease activity VAS (0–10)	Baseline: cutaneous VAS (0–10)
All	Mean (SD) age: 49.2 (14.6) Gender: 90.91% female Ethnicity: 45.5% Caucasian	PM: 5; DM: 6 Autoantibodies: anti-Mi-2: 4; anti-SRP: 2; Jo-1: 1; SSA: 1; EJ: 1; none: 2	Mean (SD): 1.8 (1.9) Median (IQR): 1.4 (1–1.7)	Mean (SD): 13.8 Median (IQR): 2.9 (1.4–8.4)	Mean (SD): 120.5 (18.4) Median (IQR): 118.5 (111–130.5)	Mean (SD): 5.1 (2.0) Median (IQR): 5.3 (4.4–6.5)	Mean (SD): 1.5 (1.8) Median (IQR): 0.3 (0–2.75)
1	37.9 years Caucasian female	DM; none	27 years	1.22	138	4.5	3
2	47.9 years Caucasian female	DM; none	7.3 years	1.2	149	3.2	3
3	27.5 years AA male	PM (NM); anti-SRP	1.4 years	30.6	93	8	0
4	58.7 years Caucasian female	PM; anti-Mi-2	1.4 years	1.45	116	6.5	0
5	75.0 years Caucasian female	DM; anti-Mi-2	1 year	1.38	118	5	2.5
6	53.2 years Caucasian female	DM; anti-Mi-2	1.3 years	1.77	146	5.5	5
7	54.1 years AA female	DM; anti-Mi-2	1.6 years	5.62	119	4.5	2.5
8	51.0 years Asian female	PM (NM); anti-SRP	1 year	11.12	123	0.8	0
9	45.4 years AA female	PM; anti-SSA	1.7 years	91.65	106	6.5	0
10	64.0 years	DM; anti-Jo-1	0.35 years	2.84	96	7.2	0.5
11	27.0 years AA female	PM; anti-EJ	0.58 years	3.04	121	4.3	0

Patient no.	Previously failed IS	Concomitant IS	Baseline prednisone dose	Key clinical feature
All	2.6 (1.1) IS failed+all failed pred	Mean (SD): 2.4 (0.8) Pred: 11; MTX: 5; AZA: 3; TAC: 1; MMF: 5; HCQZ: 2	Mean (SD) dose: 19.5 (15.3)	Severe muscle weakness: 8 (one dropped out at week 6) Moderate-severe DM rash: 5
1	Pred (30 months, 60 mg taper), MTX (30 months, 15 mg), MMF (15 months, 2 g)	Pred, MTX (15 mg), MMF (2 g)	7.5 mg	Moderate-severe rashes and mild muscle weakness
2	Pred (>12 months, 60 mg taper), TAC (39 months, 4 mg), AZA (unknown, 200 mg), MMF (unknown, 2 g), MTX (4 months, 15 mg)	Pred, HCQZ (400 mg)	20 mg	Moderate-severe rashes, no muscle weakness
3	Pred (17 months, 60 mg taper), MTX (16 months, 20 mg), AZA (14 months, 100 mg)	Pred, AZA (100 mg), MTX (15 mg)	42.5 mg	Severe muscle weakness
4	Pred (14 months, 80 mg taper), MTX (14 months, 25 mg), MMF (2 months, 2 g), AZA (6 months, 100 mg)	Pred, AZA (100 mg), MMF (2 g)	10 mg	Severe muscle weakness
5	Pred (11 months, 60 mg taper), MTX (5 months, 20 mg), MMF (3 months, 3 g), AZA (3 months, 100 mg)	Pred, AZA (100 mg), MMF (3 g)	50 mg	Moderate-severe rashes and severe muscle weakness
6	Pred (15 months, 60 mg taper), TAC (3 months, 3 mg), AZA (3 months, 100 mg), MMF (5 months, 3 g), MTX (9 months, 25 mg)	Pred, TAC (3 mg)	7.5 mg	Severe rashes and mild muscle weakness
7	Pred (18 months, 60 mg taper), IVIG (6 months), MMF (7 months, 3 g), MTX (18 months, 22.5 mg)	Pred, MTX (22.5 mg), MMF (3 g), HCQZ (400)	10 mg	Moderate-severe rashes and severe muscle weakness
8	Pred (24 months, 40 mg taper), MTX (8 months, 17.5 mg)	Pred, MTX (17.5 mg)	2.5 mg	Severe muscle weakness; dysphagia
9	Pred (24 months, 60 mg taper), IVIG (8 months, 2 g/kg), rituximab (12 months ago, 2 g), MTX (18 months, unknown)	Pred	20 mg	Severe muscle weakness; dysphagia
10	Pred (6 months, 60 mg taper), MTX (5 months, 25 mg)	Pred, MTX	15 mg	Severe muscle weakness; mild arthritis
11	Pred (8 months, 50 mg taper), IVIG (8 months, 1 g/kg), MMF (5 months, 3 g)	Pred, MMF (3 g)	30 mg	Severe muscle weakness

All medication doses are daily doses except MTX which is weekly.
*Muscle enzymes are represented as times the upper limit of normal of the most abnormal enzyme (ie, CK, aldolase, LDH, AST, ALT) at baseline.
AA, African–American; ALT, alanine transaminase; AST, aspartate transaminase; AZA, azathioprine; DK, creatine kinase; DM, dermatomyositis; HCQZ, hydroxychloroquine; IS, immunosuppression; IVIG, intravenous immunoglobulin; LDH, lactate dehydrogenase; MMF, mycophenolate; MMT, Manual Muscle Testing; MTX, methotrexate; NM, necrotising myopathy; PM, polymyositis; pred, prednisone; TAC, tacrolimus; VAS, Visual Analogue Scale.

and six patients with DM with a mean (SD) age of 49.2 (14.6); 91% were female and 46% Caucasian, with a mean (SD) disease duration of 1.8 (1.9) years. Seven of the 10 subjects had significant muscle weakness (MMT ≤125/150), and 5 of the 10 had active and moderate to severe DM rashes (≥2.5/10 cutaneous VAS score). Overall, disease was considered active in all patients, as evidenced by the mean (SD) physician global assessment of disease activity VAS of 5.17 cm (2.1) at the study entry. All patients were refractory having failed glucocorticoids and a mean of 2.6 additional immunosuppressive agents before trial entry. Concomitant therapy remained stable from the 8-week pretrial period throughout the 24-week trial period except for decreases in prednisone dosing and the discontinuation of MMF in one patient due to safety concerns (herpes zoster). Concomitant therapy included all subjects on prednisone (19.5 (15.3) mg), as well as MTX (46%), AZA (27%), MMF (46%), TAC (9%) and hydroxychloroquine (18%). No patient received IVIG or rituximab or any other biological agent during the study, but patients 3 and 1 had failed IVIG and rituximab before the clinical trial, respectively. Autoantibody subsets were well represented, with 82% of the cohort possessing at least one myositis-associated autoantibody as determined by immunoprecipitation. This included anti-Mi-2 (n=4), anti-SRP (n=2), anti-Jo-1 (n=1), anti-EJ (n=1) and anti-SSA (n=1). Other than muscle weakness and rash, additional clinical features included four patients with dysphagia, two with arthralgia, one with calcinosis, six with myalgias, and none with ILD, Raynaud phenomenon or fever.

Primary and secondary outcomes

Seven of the 10 patients completing the study met the DOI by a median (IQR) of 8 (4–20) weeks (figure 1A). Two additional subjects met the DOI initially, but their improvement was not sustained through the end of the trial; therefore, a total of three patients (table 1, patients 2, 9 and 10) did not meet the primary end point. Ninety per cent of subjects met the secondary outcome measure of minimal improvement using the new 2016 ACR-EULAR myositis response criteria, but similar to the primary outcome two patients had significant worsening before the 24-week period (figure 1B). The median (IQR) total improvement score (a metric derived from the 2016 ACR-EULAR myositis response criteria which corresponds to magnitude of improvement) was 52.5 (30–65) at 24 weeks,[8] with 40%, 30% and 20% of patients achieving minimal, moderate and major improvement, respectively (figure 1B). PM and DM subjects did not differ in their response to study drug. Among the seven patients with significant muscle disease, five (71%) met primary outcome and showed a median (IQR) MMT improvement of 19.3% (11.5%–25.4%), with a 12% (−19% to 76%) improvement in the serum muscle enzyme. Similarly, among the five patients with significant DM-related cutaneous disease, four (80%) met the primary outcome and showed an 88% (83.3%–100%) improvement in the cutaneous VAS score on the MDAAT. Figure 2 depicts the improvement in rash observed in patient 6, a subject who had failed TAC, MMF, MTX and AZA before initiation of RCI. No subjects met the criteria of worsening or required glucocorticoid rescue therapy during the trial.

Changes in six CSMs and steroid dose reduction

Details of changes in all CSMs are summarised in online supplementary tables 1 and 2. In addition, trends of changes in the MMT and extramuscular global score in each patient are shown in figure 3. Note that the changes in extramuscular global score are predominantly due to changes in cutaneous disease activity.

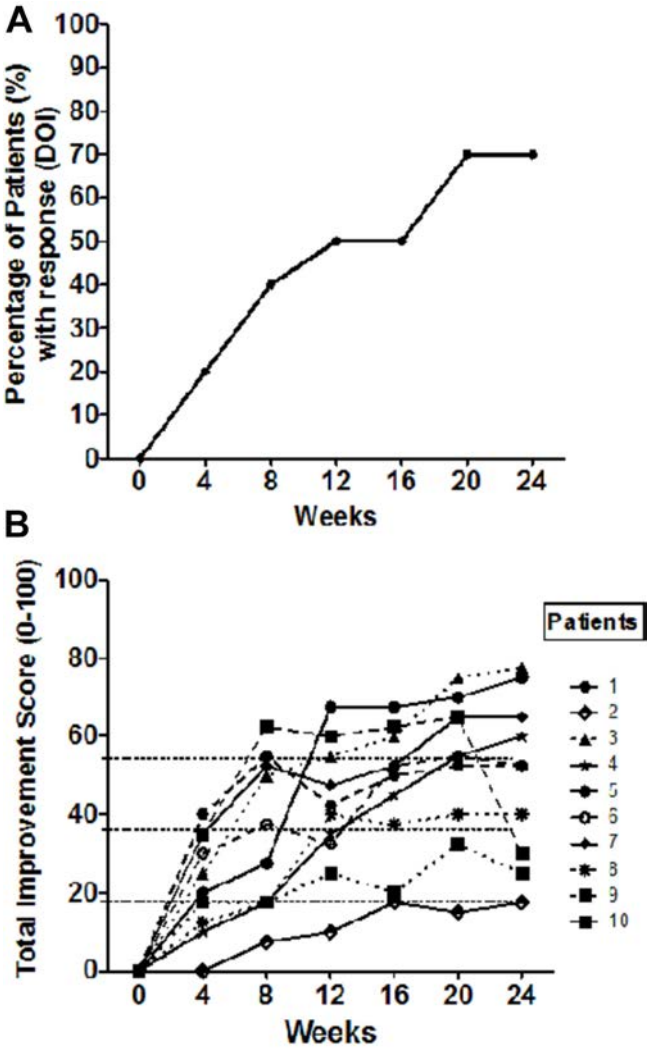


Figure 1 Primary outcome criteria as DOI (A) and secondary outcome criteria as 2016 American College of Rheumatology-European League Against Rheumatism myositis response criteria (B). DOI, definition of improvement.

Overall, there were significant reductions in physician global, patient global and extramuscular global, and a significant increase in MMT scores. The key CSM of MMT improved by a median of >10% in all patients and >15% in patients (n=7) with baseline severe muscle weakness. The physician global improved by a median of >40% in all patients. The extramuscular global, primarily driven by skin rash in our study, showed a median of 10% improvement in all patients and 20% in patients with baseline moderate to severe rash. There was a significant reduction in mean (SD) prednisone dose from baseline (18.5 (15.7)) to last follow-up (2.3 (3.2), P<0.01) (figure 4), with 50% off prednisone.

Safety and tolerability

RCI at 80 units twice weekly was generally safe and well-tolerated over the 24-week study period. There were a total of 5 SAEs in three patients and 22 AEs in eight patients during the study period, of which 3 SAEs and 22 AEs were related to study drug. Among five SAEs, two were herpes zoster infections, which were considered related to the study drug and required hospitalisation, including one with disseminated herpes zoster causing herpes pneumonitis. Both patients were treated with

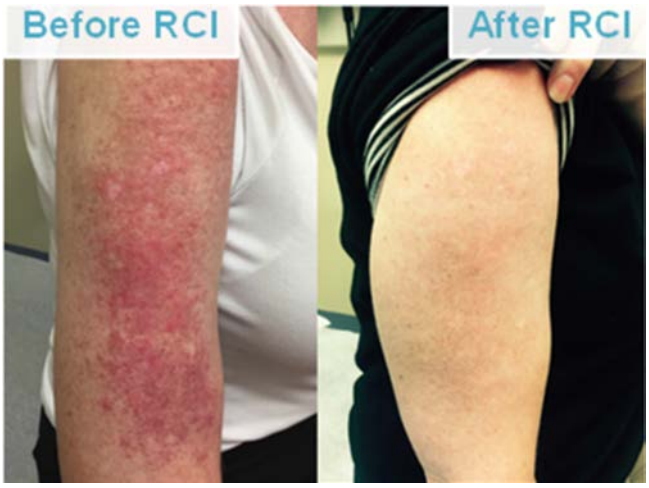


Figure 2 Cutaneous rash improvement in a patient with dermatomyositis before and after repository corticotropin injection (RCI).

antiviral medications and were continued on the study drug after temporary discontinuation for antiviral treatment. The subject with disseminated zoster was admitted to hospital with chest pain, which was felt to be related to herpes pneumonitis. Both

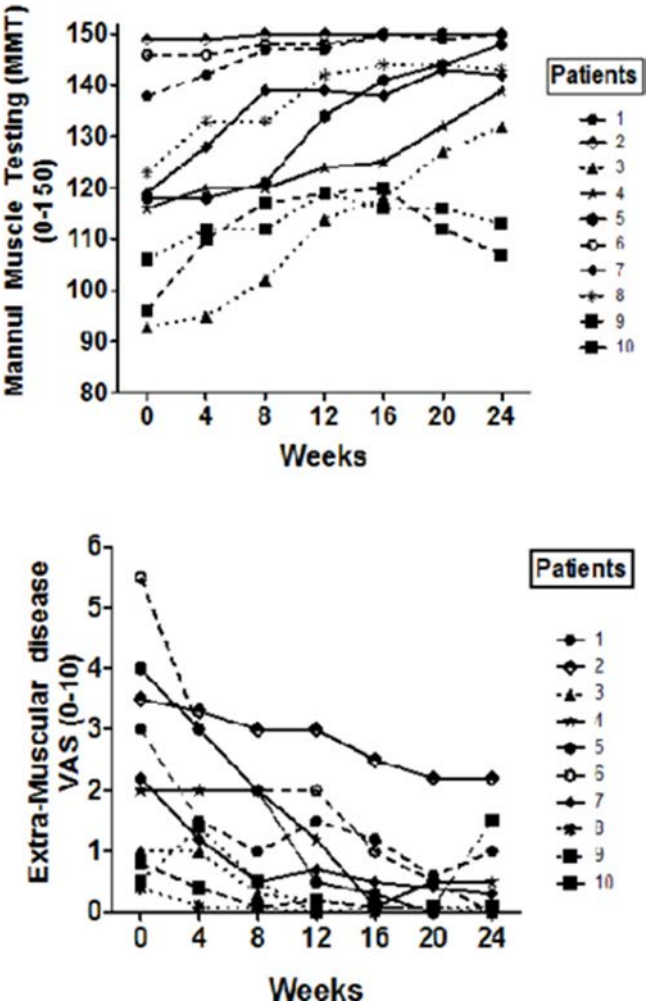


Figure 3 Longitudinal changes in Manual Muscle Testing and extramuscular disease activity in all patients over 24 weeks. VAS, Visual Analogue Scale.

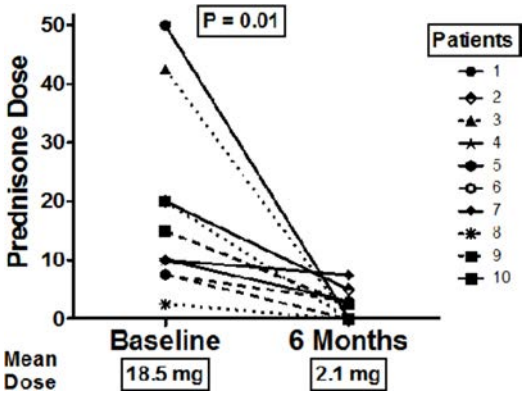


Figure 4 Changes in prednisone dose at baseline and 6 months last follow-up.

patients were on MMF, glucocorticoid as well as another immunosuppressive agent (AZA or MTX) at the time of zoster infection. One patient developed avascular necrosis (AVN) leading to total hip arthroplasty (post study) and was continued on study drug without discontinuation for AVN. This patient was being tapered down from high-dose glucocorticoid therapy (50 mg at baseline). Another subject developed third-degree atrio-ventricular (AV) heart block 6 weeks after initiating study drug and received a transvenous pacemaker and withdrew from the study. The investigator reported this event as not related to study drug, but possibly related to pre-existing history of similar conduction abnormalities. Among non-serious AEs, the most notable were worsening calcinosis, transient hyperglycaemia, transient hypertension, anxiety, insomnia and injection site bruising (table 2). However, none of the AEs required long-term dose interruption or dose reduction and were generally considered mild. There was no significant increase in mean (SD) weight from baseline (66.0 (8.7) kg) to 24 weeks (68.6 (9.7) kg; P=0.53). Only three patients gained over 5 kg (<10 kg) and all were on a high prednisone dose at baseline (42.5, 50, 15 mg, respectively). Also, the mean (SD) glycosylated haemoglobin A1c (HbA1c) did not change over 24 weeks (5.8 (0.27) to 5.6 (0.17); P=0.2), and no patient developed microalbuminuria or cushingoid features. One patient on metformin due to steroid-induced diabetes prior to enrolment was able to discontinue it due to improvement in diabetes during the trial. Further, there were no significant changes in white blood cell count, haemoglobin, platelet count, sedimentation rates, serum creatinine or blood glucose over the 24-week trial period (online supplementary table 3).

DISCUSSION

This prospective, open-label clinical trial with validated end points demonstrated a clinically significant response to RCI in 70% of patients with refractory myositis. RCI was generally well-tolerated with a reasonable safety profile. This is the first clinical trial of RCI in adult DM and PM using rigorous methodology, where all six validated myositis CSMs were prospectively measured and predetermined validated outcome measures were used to determine efficacy and safety. Responders met both the IMACS DOI as well as the new ACR/EULAR myositis response criteria, which supports a more robust response. Enrolled subjects represented a generally refractory cohort who had failed glucocorticoids and, on average, 2.6 additional immunosuppressive agents. The addition of RCI led to a reduction in prednisone dose from an average of 18.5 mg at baseline to 2.3 mg at 24 weeks, with half of the patients discontinuing prednisone completely. This suggests that RCI may provide novel anti-inflammatory or

Table 2 Summary of adverse events

Event	Events and patients (n)	Related to study drug	Severity	Effect of study drug	Resolution	Comments
Serious adverse events						
Herpes zoster	1	Yes	Moderate	None	Resolved	
Disseminated herpes zoster	1	Yes	Severe	Interrupted	Resolved	
Avascular necrosis	1	Yes	Severe	N/A	Resolved	Total left hip arthroplasty
Chest pain	1	Yes	Mild	None	Resolved	
Heart block	1	No	Severe	Withdrew	Resolved	Transvenous pacemaker insertion
Non-serious adverse events						
Injection site bruising and rash	4	Yes	Mild	None	Resolved	
Diarrhoea	1	Yes	Mild	None	Resolved	
Anxiety	1	Yes	Mild	None	Resolved	
Insomnia	2	Yes	Mild	None	Resolved	
Calcinosis	2	Yes	Moderate	None	Continuing	
Depression	1	Yes	Mild	None	Resolved	
Agitation	1	Yes	Mild	None	Resolved	
Herpes pneumonitis	1	Yes	Moderate	Interrupted	Controlled	
Sinus tachycardia	1	Yes	Moderate	N/A	Resolved	
High cholesterol	1	Yes	Mild	N/A	Resolved	
Hyperglycaemia	3	Yes	Mild	N/A	Controlled	
Infection (sinusitis and upper respiratory tract infection (URI)	2	Yes	Mild	None	Resolved	
Hypertension	2	Yes	Mild	None	Controlled	

N/A, not applicable.

immunomodulatory effects distinct from glucocorticoids that include non-steroid-dependent immune mechanisms.[12] Given that all enrolled subjects had failed high doses of glucocorticoids, it is likely that a non-steroid-dependent mechanism contributed to clinical improvement in some patients. Although many of the observed AEs in this trial were similar to those seen with glucocorticoids, we did not observe significant weight gain, diabetes or cushingoid features, which are typically associated with high steroid doses given for an extended period.

There were no differences noted in PM versus DM or muscle weakness versus skin rash response rates, but the numbers studied were too small to make meaningful conclusions. Both muscle and skin disease seemed to respond to RCI as noted by the 70% and 80% response rates among patients with severe muscle disease and skin disease, respectively. Two of three patients with refractory cutaneous disease but minimal muscle involvement also improved. The median time to response was 2 months, suggesting a rather rapid onset of action, and two subjects were wheelchair users at study entry, and both were ambulating independently without assistive devices by the end of the trial.

Although RCI has an FDA-approved designation for PM and DM, there were no prospective studies demonstrating its efficacy and/or safety profile. A previous retrospective case series using the same dosing regimen employed in our trial noted similar response rates, and a follow-up retrospective study of 24 patients with myositis treated with RCI at different clinical centres showed 58.3% response rates.[3 13] Another small retrospective case series demonstrated a steroid dose reduction and similar efficacy in three of four adult patients with refractory DM/PM including one patient with the anti-SRP autoantibody who failed IVIG and rituximab.[14] Again no differences were noted in the response rates of DM versus PM or rates of response to muscle weakness versus cutaneous rashes in previous retrospective studies.

RCI is an injectable formulation containing porcine ACTH purified from pituitary extracts. The full-sequence ACTH1-39 hormone is one of a number of peptides produced from pro-opiomelanocortin, a family of peptides that bind to MC receptors found in a wide variety of cells.[15] Despite FDA approval for various rheumatic diseases, it was primarily being used for the treatment of infantile spasm, nephrotic syndrome and acute exacerbations of multiple sclerosis.[16 17] FDA first approved ACTH for human use in 1952 after it was tested in rheumatoid arthritis (RA) in 1949.[18] In the 1950s it was used for several rheumatic conditions, including RA, gout, lupus, rheumatic fever, psoriasis and others, as well as non-rheumatic autoimmune conditions such as ulcerative colitis and multiple sclerosis.[17 19 20] However, after the discovery that cortisone suppressed inflammation, ACTH use became negligible. Half a century after the discovery and approval of ACTH, it again emerged with the seminal observation that the anti-inflammatory actions of ACTH were *retained* in an adrenalectomised mouse model of gout.[21]

ACTH and α-melanocyte-stimulating hormones (MSH), β-MSH and γ-MSH are the four endogenous MC peptides derived from the precursor pro-opiomelanocortin protein. MCs are produced during inflammation acting to mitigate the inflammatory process by engagement of the MC receptors (MC1–MC5). MC2 is only found in the adrenal cortex, while the remaining four MC receptors (MC1, MC3, MC4 and MC5) are expressed on a variety of immune cells.[12] Thus, ACTH exerts its anti-inflammatory action via two independent mechanisms: a steroid-dependent effect and a broader, steroid-independent, anti-inflammatory effect.[22] The former effect is through activation of MC2 receptor on adrenal glands leading to cortisol synthesis—this accounts for both the known anti-inflammatory effects as well as the adverse sequelae similar to steroids. The novel, steroid-independent effects of ACTH mediated through activation of MC receptors 1, 3, 4 and 5 induce a broad range of immunomodulatory effects,[22–25] likely responsible for the unique

effects of RCI not explained by cortisol synthesis,[15] for example its efficacy in steroid-refractory infantile spasms, nephrotic syndrome and acute exacerbations of multiple sclerosis. It is the latter proposed mechanism that has led to renewed interest in ACTH and other MC for treating various diseases.[26–30] The use of RCI is currently being explored in various other rheumatic diseases including sarcoidosis, lupus, RA and gout.[31–36] A recent study of 181 patients with gout reported a 78% response rates within 1 day of ACTH injection with similar efficacy in pseudogout.[37 38] It is not surprising that specific MC peptides are being developed for various indications to target the MC system through non-steroidogenic mechanisms.[39]

The MC receptors are expressed on immune cells including macrophages, mast cells, neutrophils and lymphocytes, as well as osteoclasts, osteoblasts, chondrocytes and fibroblasts.[40–44] Activation of the MC receptors results in the inhibition of proinflammatory transcription factors at the molecular level, ultimately decreasing the production of cytokines, chemokines, growth factors and adhesion molecules.[12 22 45] Interestingly, MC can be locally synthesised by immune cells at sites of inflammation (eg, RA synovium),[46 47] suggesting a 'local' anti-inflammatory circuit independent of the hypothalamic–pituitary–adrenal axis. α-MSH may also play a role in energy homeostasis in skeletal muscle through the MC5 receptor, as suggested by its increased expression in regenerating and dystrophic skeletal muscle.[48 49] More specifically, ACTH has a known trophic effect on skeletal muscle development in mouse model.[6 50–52]

In our trial, RCI was well-tolerated, with no patient requiring prolonged discontinuation from RCI adverse effects. Injection site reactions were very mild. Infections should be considered as a potential risk of RCI similar to glucocorticoids. Calcinosis occurred in one responder without previous clinically known calcinosis and worsened in one non-responder. AVN was seen in one patient also taking concomitant glucocorticoids, perhaps implicating the known steroidogenesis effect discussed above. In contrast ACTH through its steroid independent effect is thought to have protective actions on bone and joints due to a reduction in osteoclastogenesis and metalloproteases produced by chondrocytes.[53 54] Although two patients had hypertension and three had hyperglycaemia during the trial, these were transient and resolved spontaneously. Contrary to common side effects seen with high doses of glucocorticoids, no patient developed significant weight gain (≥10 kg), cushingoid features, diabetes, persistent hypertension or hyperglycaemia, or an increase of HbA1c (≥1). This is plausible given that many of the metabolic side effects of glucocorticoids are mediated through transcription of glucocorticoid responsive elements which is coupled to the anti-inflammatory effects primarily through nuclear factor kappa B (NF-kB)mediated transcription.[30] In contrast, α-MSH directly inhibits NF-kB activation, perhaps leading to the beneficial anti-inflammatory effect of RCI without a similar degree of metabolic side effects associated with glucocorticoids.[45] We did not measure bone density before and after administration of study drug. The safety and tolerability results seen in this trial are similar to what has been observed in myositis and non-myositis studies.[3 14]

Despite reasonable biological plausibility, the lack of a control group and randomisation in this trial does dampen the enthusiasm regarding efficacy of RCI for PM and DM. Also, the dose and interval of administration of RCI therapy in myositis are unclear and also were not addressed in this pilot trial. However, we employed the standard dosing regimen used in previously reported retrospective studies. It may be possible to use RCI at higher doses to induce disease remission of disease with subsequent tapering similar to the clinical strategy currently used for glucocorticoids. Given the small sample size, it is difficult to evaluate clinical predictors of response to Acthar in PM and DM. Moreover, long-term outcome studies of efficacy and safety with comparison to high doses of glucocorticoids are required to better delineate the role of RCI in myositis beyond its use in refractory cases.

To summarise, the results from this prospective, open-label pilot trial are encouraging and, perhaps, support the concept of RCI as a novel immunomodulatory therapy for myositis beyond the steroidogenesis effect.[22] Treatment with RCI may provide an alternative to glucocorticoids and other immunosuppressive agents, especially in patients who are refractory or intolerant to conventional agents.[1 2] However, given the cost of RCI, it is unlikely to be used as first-line therapy in myositis. Perhaps, a future cost benefit analysis will be helpful in defining the proper place of RCI in the treatment algorithm of myositis. While this is the largest prospective trial of RCI in myositis providing excellent data on efficacy and safety profile, a larger, randomised control trial is necessary to conclusively establish the efficacy of RCI in myositis. Nevertheless, this study has demonstrated that treatment with RCI was effective in 70% of refractory cases, safe and led to a reduction in concomitant glucocorticoid dosing in myositis.

Contributors RA and CVO planned the study and wrote the protocol. RA, CVO and GM enrolled patients and evaluated outcome measures. DCK and PN executed the study from patient enrolment to data collection, to data management. ZQ planned the biospecimen and laboratory data collection, and executed the biospecimen sample collection and laboratory results. All authors participated in data analysis and manuscript write-up.

Funding This was an investigator-initiated clinical trial funded by Mallinckrodt.

Competing interests RA, CVO and GM received an honorarium from Mallinckrodt for an advisory board unrelated to this trial.

Ethics approval University of Pittsburgh IRB. The protocol was approved by the Institutional Review Board at each location.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Data used for the publication and additional unpublished data from the study can be shared with experienced myositis researchers upon request to PI.

REFERENCES

1 Dalakas MC. Immunotherapy of myositis: issues, concerns and future prospects. *Nat Rev Rheumatol* 2010;6:129–37.
2 Marie I, Mouthon L. Therapy of polymyositis and dermatomyositis. *Autoimmun Rev* 2011;11:6–13.
3 Levine T. Treating refractory dermatomyositis or polymyositis with adrenocorticotropic hormone gel: a retrospective case series. *Drug Des Devel Ther* 2012;6:133–9.
4 Bohan A, Peter JB, Bowman RL, *et al*. Computer-assisted analysis of 153 patients with polymyositis and dermatomyositis. *Medicine* 1977;56:255–86.
5 van der Meulen MF, Bronner IM, Hoogendijk JE, *et al*. Polymyositis: an overdiagnosed entity. *Neurology* 2003;61:316–21.
6 Labbé O, Desarnaud F, Eggerickx D, *et al*. Molecular cloning of a mouse melanocortin 5 receptor gene widely expressed in peripheral tissues. *Biochemistry* 1994;33:4543–9.
7 Oddis CV, Reed AM, Aggarwal R, *et al*. Rituximab in the treatment of refractory adult and juvenile dermatomyositis and adult polymyositis: a randomized, placebo-phase trial. *Arthritis Rheum* 2013;65:314–24.
8 Aggarwal R, Rider LG, Ruperto N, *et al*. 2016 American college of rheumatology/European league against rheumatism criteria for minimal, moderate, and major clinical response in adult dermatomyositis and polymyositis: an international myositis assessment and clinical studies group/paediatric rheumatology international trials organisation collaborative initiative. *Ann Rheum Dis* 2017;76:792–801.
9 Aggarwal R, Rider LG, Ruperto N, *et al*. 2016 American college of rheumatology/European league against rheumatism criteria for minimal, moderate, and major clinical response in adult dermatomyositis and polymyositis: an international myositis assessment and clinical studies group/paediatric rheumatology international trials organisation collaborative initiative. *Arthritis Rheumatol* 2017;69:898–910.

10 Rider LG, Aggarwal R, Pistorio A, *et al*. 2016 American college of rheumatology/European league against rheumatism criteria for minimal, moderate, and major clinical response in juvenile dermatomyositis: an international myositis assessment and clinical studies group/paediatric rheumatology international trials organisation collaborative initiative. *Arthritis Rheumatol* 2017;69:911–23.
11 Rider LG, Aggarwal R, Pistorio A, *et al*. 2016 American college of rheumatology/European league against rheumatism criteria for minimal, moderate, and major clinical response in juvenile dermatomyositis: an international myositis assessment and clinical studies group/paediatric rheumatology international trials organisation collaborative initiative. *Ann Rheum Dis* 2017;76:782–91.
12 Montero-Melendez T. ACTH: the forgotten therapy. *Semin Immunol* 2015;27:216–26.
13 Levine T, Malone J, *et al*. H.P. Acthar® gel in dermatomyositis and polymyositis treatment registry: an interim analysis. *J Neurol Disord* 2016;4.
14 Patel A, Seely G, Aggarwal R. Repository corticotropin injection for treatment of idiopathic inflammatory myopathies. *Case Rep Rheumatol* 2016;2016:1–4.
15 Gong R. The renaissance of corticotropin therapy in proteinuric nephropathies. *Nat Rev Nephrol* 2011;8:122–8.
16 Gettig J, Cummings JP, Matuszewski K. H.p. Acthar gel and cosyntropin review: clinical and financial implications. *P T* 2009;34:250–7.
17 Miller H, Newell DJ, Ridley A. Multiple sclerosis. Treatment of acute exacerbations with corticotrophin (A.C.T.H.). *Lancet* 1961;2:1120–2.
18 Hench PS, Kendall EC. The effect of a hormone of the adrenal cortex (17-hydroxy-11-dehydrocorticosterone; compound E) and of pituitary adrenocorticotropic hormone on rheumatoid arthritis. *Proc Staff Meet Mayo Clin* 1949;24:181–97.
19 Gutman AB, YU TF. Effects of adrenocorticotropic hormone (ACTH) in gout. *Am J Med* 1950;9:24–30.
20 Hench PS, Kendall EC, Slocumb CH, *et al*. Effects of cortisone acetate and pituitary ACTH on rheumatoid arthritis, rheumatic fever and certain other conditions. *Arch Intern Med* 1950;85:545–666.
21 Getting SJ, Christian HC, Flower RJ, *et al*. Activation of melanocortin type 3 receptor as a molecular mechanism for adrenocorticotropic hormone efficacy in gouty arthritis. *Arthritis Rheum* 2002;46:2765–75.
22 Catania A, Lonati C, Sordi A, *et al*. The melanocortin system in control of inflammation. *ScientificWorldJournal* 2010;10:1840–53.
23 Catania A, Gatti S, Colombo G, *et al*. Targeting melanocortin receptors as a novel strategy to control inflammation. *Pharmacol Rev* 2004;56:1–29.
24 Dores RM. Adrenocorticotropic hormone, melanocyte-stimulating hormone, and the melanocortin receptors: revisiting the work of Robert Schwyzer: a thirty-year retrospective. *Ann N Y Acad Sci* 2009;1163:93–100.
25 Xing Y, Parker CR, Edwards M, *et al*. ACTH is a potent regulator of gene expression in human adrenal cells. *J Mol Endocrinol* 2010;45:59–68.
26 Bomback AS, Tumlin JA, Baranski J, *et al*. Treatment of nephrotic syndrome with adrenocorticotropic hormone (ACTH) gel. *Drug Des Devel Ther* 2011;5:147–53.
27 Baram TZ, Mitchell WG, Tournay A, *et al*. High-dose corticotropin (ACTH) versus prednisone for infantile spasms: a prospective, randomized, blinded study. *Pediatrics* 1996;97:375–9.
28 Simsarian JP, Saunders C, Smith DM. Five-day regimen of intramuscular or subcutaneous self-administered adrenocorticotropic hormone gel for acute exacerbations of multiple sclerosis: a prospective, randomized, open-label pilot trial. *Drug Des Devel Ther* 2011;5:381–9.
29 Cone RD. Studies on the physiological functions of the melanocortin system. *Endocr Rev* 2006;27:736–49.
30 Liem EB, Lin CM, Suleman MI, *et al*. Anesthetic requirement is increased in redheads. *Anesthesiology* 2004;101:279–83.
31 Baughman RP, Barney JB, O'Hare L, *et al*. A retrospective pilot study examining the use of Acthar gel in sarcoidosis patients. *Respir Med* 2016;110:66–72.
32 Decker DA, Grant C, Oh L, *et al*. Immunomodulatory effects of H.P. Acthar Gel on B cell development in the NZB/W F1 mouse model of systemic lupus erythematosus. *Lupus* 2014;23:802–12.
33 Fiechtner JJ, Montroy T. Treatment of moderately to severely active systemic lupus erythematosus with adrenocorticotropic hormone: a single-site, open-label trial. *Lupus* 2014;23:905–12.
34 Li X, Golubovsky J, Hui-Yuen J, *et al*. Adrenocorticotropic hormone gel in the treatment of systemic lupus erythematosus: a retrospective study of patients. *F1000Res* 2015;4:1103.
35 Zhou Y, Lower EE, Li H, *et al*. Sarcoidosis patient with lupus pernio and infliximab-induced myositis: Response to Acthar gel. *Respir Med Case Rep* 2016;17:5–7.
36 Khanna D, Khanna PP, Fitzgerald JD, *et al*. 2012 American college of rheumatology guidelines for management of gout. part 2: therapy and antiinflammatory prophylaxis of acute gouty arthritis. *Arthritis Care Res* 2012;64:1447–61.
37 Daoussis D, Antonopoulos I, Yiannopoulos G, *et al*. ACTH as first line treatment for acute gout in 181 hospitalized patients. *Joint Bone Spine* 2013;80:291–4.
38 Daoussis D, Antonopoulos I, Yiannopoulos G, *et al*. ACTH as first line treatment for acute calcium pyrophosphate crystal arthritis in 14 hospitalized patients. *Joint Bone Spine* 2014;81:98–100.
39 Doi K, Hu X, Yuen PS, *et al*. AP214, an analogue of alpha-melanocyte-stimulating hormone, ameliorates sepsis-induced acute kidney injury and mortality. *Kidney Int* 2008;73:1266–74.
40 Böhm M, Grässel S. Role of proopiomelanocortin-derived peptides and their receptors in the osteoarticular system: from basic to translational research. *Endocr Rev* 2012;33:623–51.
41 Böhm M, Raghunath M, Sunderkötter C, *et al*. Collagen metabolism is a novel target of the neuropeptide alpha-melanocyte-stimulating hormone. *J Biol Chem* 2004;279:6959–66.
42 Cornish J, Callon KE, Mountjoy KG, *et al*. Alpha -melanocyte-stimulating hormone is a novel regulator of bone. *Am J Physiol Endocrinol Metab* 2003;284:E1181–E1190.
43 Grässel S, Opolka A, Anders S, *et al*. The melanocortin system in articular chondrocytes: melanocortin receptors, pro-opiomelanocortin, precursor proteases, and a regulatory effect of alpha-melanocyte-stimulating hormone on proinflammatory cytokines and extracellular matrix components. *Arthritis Rheum* 2009;60:3017–27.
44 Zaidi M, Sun L, Robinson LJ, *et al*. ACTH protects against glucocorticoid-induced osteonecrosis of bone. *Proc Natl Acad Sci U S A* 2010;107:8782–7.
45 Manna SK, Aggarwal BB. Alpha-melanocyte-stimulating hormone inhibits the nuclear transcription factor NF-kappa B activation induced by various inflammatory agents. *J Immunol* 1998;161:2873–80.
46 Star RA, Rajora N, Huang J, *et al*. Evidence of autocrine modulation of macrophage nitric oxide synthase by alpha-melanocyte-stimulating hormone. *Proc Natl Acad Sci U S A* 1995;92:8016–20.
47 Catania A, Gerloni V, Procaccia S, *et al*. The anticytokine neuropeptide alpha-melanocyte-stimulating hormone in synovial fluid of patients with rheumatic diseases: comparisons with other anticytokine molecules. *Neuroimmunomodulation* 1994;1:321–8.
48 Hughes S, Smith ME. Upregulation of the pro-opiomelanocortin gene in spinal motoneurones in muscular dystrophy in mice. *Neurosci Lett* 1993;163:205–7.
49 Smith ME, Hughes S. POMC neuropeptides and their receptors in the neuromuscular system of wobbler mice. *J Neurol Sci* 1994;124 Suppl:56–8.
50 An JJ, Rhee Y, Kim SH, *et al*. Peripheral effect of alpha-melanocyte-stimulating hormone on fatty acid oxidation in skeletal muscle. *J Biol Chem* 2007;282:2862–70.
51 Cossu G, Cusella-De Angelis MG, Senni MI, *et al*. Adrenocorticotropin is a specific mitogen for mammalian myogenic cells. *Dev Biol* 1989;131:331–6.
52 De Angelis L, Cusella-De Angelis MG, Bouchè M, *et al*. ACTH-like peptides in postimplantation mouse embryos: a possible role in myoblast proliferation and muscle histogenesis. *Dev Biol* 1992;151:446–58.
53 Patel HB, Bombardieri M, Sampaio AL, *et al*. Anti-inflammatory and antiosteoclastogenesis properties of endogenous melanocortin receptor type 3 in experimental arthritis. *Faseb J* 2010;24:4835–43.
54 Kaneva MK, Kerrigan MJ, Grieco P, *et al*. Chondroprotective and anti-inflammatory role of melanocortin peptides in TNF-α activated human C-20/A4 chondrocytes. *Br J Pharmacol* 2012;167:67–79.

EXTENDED REPORT

A20 haploinsufficiency (HA20): clinical phenotypes and disease course of patients with a newly recognised NF-kB-mediated autoinflammatory disease

Florence A Aeschlimann,[1] Ezgi D Batu,[2] Scott W Canna,[3] Ellen Go,[4] Ahmet Gül,[5] Patrycja Hoffmann,[6] Helen L Leavis,[7] Seza Ozen,[2] Daniella M Schwartz,[8] Deborah L Stone,[6] Annet van Royen-Kerkof,[9] Daniel L Kastner,[6] Ivona Aksentijevich,[6] Ronald M Laxer[1,10]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212403).

For numbered affiliations see end of article.

Correspondence to
Dr Ronald M Laxer, Division of Rheumatology, The Hospital for Sick Children, Department of Paediatrics University of Toronto, Toronto, Ontario, Canada; ronald.laxer@sickkids.ca

Received 18 September 2017
Revised 15 December 2017
Accepted 23 December 2017
Published Online First
9 January 2018

ABSTRACT

Objectives The association between mutations in *TNFAIP3*, encoding the NF-kB regulatory protein A20, and a new autoinflammatory disease has recently been recognised. This study aims at describing the clinical phenotypes and disease course of patients with A20 haploinsufficiency (HA20).

Methods Data for all cases from the initial publication, and additional cases identified through collaborations since, were collected using standardised data collection forms.

Results A total of 16 patients (13 female) from seven families with a genetic diagnosis of HA20 were included. The disease commonly manifested in early childhood (range: first week of life to 29 years of age). The main clinical symptoms were recurrent oral, genital and/or gastrointestinal ulcers (16/16), musculoskeletal (9/16) and gastrointestinal complaints (9/16), cutaneous lesions (8/16), episodic fever (7/16), and recurrent infections (7/16). Clinical phenotypes varied considerably, even within families. Relapsing-remitting disease course was most common, and one patient died. Laboratory abnormalities included elevated acute-phase reactants and fluctuating presence of various autoantibodies such as antinuclear antibodies (4/10 patients tested) and anti-dsDNA (2/5). Tissue biopsy of different sites revealed non-specific chronic inflammation (6/12 patients tested), findings consistent with class V lupus nephritis in one patient, and pustules and normal results in two patients each. All patients were treated: 4/16 received colchicine and 12/16 various immunosuppressive agents. Cytokine inhibitors effectively suppressed systemic inflammation in 7/9 patients.

Conclusions Early-onset recurrent oral, genital and/or gastrointestinal ulcers are the hallmark feature of HA20. Frequency and intensity of other clinical manifestations varied highly. Treatment regimens should be based on disease severity, and cytokine inhibitors are often required to control relapses.

INTRODUCTION

The protein A20, also known as TNAP3, encoded by *TNFAIP3*, plays a crucial role in the negative regulation of inflammation and immunity.[1] TNFAIP3 is an ubiquitin-editing (deubiquitinase; DUB) enzyme with a critical function in the inhibition of key proinflammatory molecules, including inhibitor of nuclear factor kappa B kinase subunit gamma (IKKγ (NEMO)) and receptor-interacting protein kinase 1 (RIPK1), in the canonical NF-kB and other signalling pathways.[2] Zhou and colleagues[3] recently described a new autoinflammatory disease caused by heterozygous loss-of-function mutations in *TNFAIP3*, leading to haploinsufficiency of A20 (HA20). These mutations cause insufficient DUB activity of A20 and lead to increased NF-κB signalling and phosphorylation of c-Jun N-terminal kinase and p38 mitogen-activated protein kinases (MAPKs). HA20-associated mutations were found in six unrelated families who presented with mostly childhood-onset systemic inflammation and a 'Behçet-like' disorder that may lead to end-organ damage and death. Since the initial description, a few additional cases of HA20 have been reported in the literature.[4–7] To date, the clinical manifestations, severity of symptoms, disease course and complications of this newly described disorder are not well described and appreciated. Early recognition and diagnosis are crucial, as targeted therapies may alter disease course and improve outcome.

Therefore, the aims of the study were (1) to describe the disease features and course, treatment regimens, complications and outcomes of patients; and (2) to improve clinical recognition of this poorly defined disorder.

PATIENTS AND METHODS

Sixteen patients from six unrelated families previously identified and diagnosed with HA20 by Zhou and colleagues[3] at the National Institutes of Health in the USA were initially included in the study. The patients were followed at various centres worldwide and their charts were retrospectively reviewed by their primary care physicians. Because the spectrum of clinical phenotypes associated with HA20 is still widely unknown, these patients' histories were used to prepare a comprehensive data collection form including all signs and symptoms thought to be related to HA20. In the second step, patient charts were again reviewed by the primary care physicians using the newly created

To cite: Aeschlimann FA, Batu ED, Canna SW, *et al*. *Ann Rheum Dis* 2018;**77**:728–735.

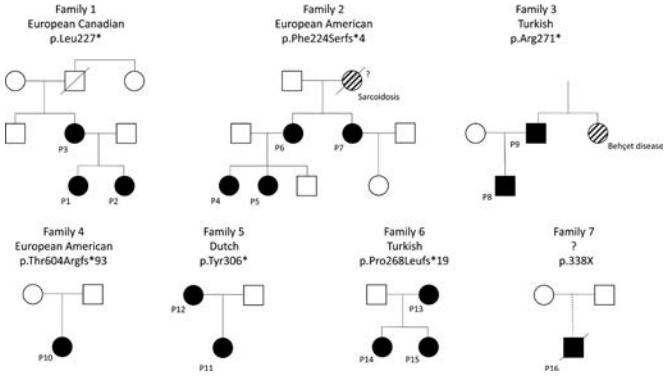


Figure 1 Pedigrees of the seven families with mutations in *TNFAIP3* leading to haploinsufficiency of A20 (HA20). The grandmother of family 2 was diagnosed with sarcoidosis and is thought to be affected by HA20, however died years prior to availability of genotyping. Patient 16 was adopted, and the ethnic origin is unknown.

data collection forms in order to collect detailed information. Demographic, clinical, laboratory, imaging and histological features were recorded. Disease course, treatment regimens, and disease- and treatment-related complications were captured. Additional cases were sought and one identified subsequently through collaborations.

All data were compiled and analysed using descriptive statistics. Patient consent, or consent from a parent in the case of children, was obtained by the responsible physician in the respective institutions.

RESULTS

A total of 16 patients (81% female) from seven families were included (figure 1 and table 1). All patients were Caucasian, while the ethnic origin of patient 16 is unknown (figure 1). No consanguinity was reported within the families. Prior to the recognition of HA20, patients were diagnosed with various conditions including Behçet disease (BD), systemic lupus erythematosus (SLE), juvenile idiopathic arthritis (JIA), periodic fever, aphthous stomatitis, pharyngitis and adenitis (PFAPA), and inflammatory bowel disease (IBD). The grandmother of family 2 was diagnosed with sarcoidosis and succumbed to the complications of the disease. Given her medical history she is presumed to carry the F224Sfs*4 mutation present in four affected family members; however, her DNA sample was not available for genotyping. Thus, the molecular diagnosis of HA20 has not been confirmed and this patient was not included in this analysis.

The demographic features of the patients are summarised in table 1.

Disease presentation

All patients were symptomatic and reduced penetrance was not reported in any family members. First symptoms occurred early, before 10 years of age in 11/16 (69%) patients, and disease onset ranged from the first week of life to 29 years of age. Clinical presentation was heterogeneous between families and between family members with the same mutation (figure 1 and table 2). In 10/16 (63%) patients, recurrent oral and/or genital ulcers were the symptoms leading to initial specialised medical consultation.

Disease course

During disease course, symptoms and severity were highly variable. Clinical features emerged over a period of several years. All patients developed recurrent painful oral, genital and/or gastrointestinal ulcers. Other common symptoms that occurred at various time points during disease course included gastrointestinal complaints (9/16, 56%), polyarthritis and/or arthralgia (9/16, 56%), skin involvement (8/16, 50%) and recurrent fever

Table 1 Characteristics of patients with A20 haploinsufficiency

Patient no	Family	Sex	Current age	Age at onset	Previous diagnosis	Previous treatment	Current treatment
1	Family 1	F	25 years	10 months	JIA, Behçet disease	CS, MTX, CYS	MTX, thalidomide
2	Family 1	F	23 years	15 months	JIA, Behçet disease	CS, MTX, CYS, AZA, thalidomide, IVIG, ETN	IFX
3	Family 1	F	51 years	Early 20s	Rheumatoid arthritis (RF–), Behçet disease	None	Colchicine
4	Family 2	F	25 years	10 years	JIA, undifferentiated connective tissue disease/cutaneous vasculitis, SLE with CNS vasculitis	CS, MMF, ETN, thalidomide, RTX, IFX, MTX, ADA, cyclo, IVIG, autologous haematopoietic stem cell transplant	Anakinra, AZA, CS
5	Family 2	F	29 years	Around 8 years	Behçet disease, lupus nephritis	CS, colchicine, ETN, hydroxychloroquine	Anakinra
6	Family 2	F	51 years	Around 6 years	Rheumatic fever, arthritis	None	Anakinra
7	Family 2	F	56 years	Around 6 years		None	Anakinra
8	Family 3	M	9 years	9 months	Behçet disease	CS, colchicine, AZA	Colchicine
9	Family 3	M	46 years	?	Behçet disease		On treatment (not specified)
10	Family 4	F	15 years	8 weeks	Suspicion of PFAPA	CS, colchicine, ETN, anakinra, ADA, MTX	Tofacitinib
11	Family 5	F	17 years	Around 4 years	Suspicion of Behçet disease	CYS, dapsone, CS, AZA, IVIG for Ig deficiency	IFX
12	Family 5	F	47 years	Infancy	Suspicion of Behçet disease	CS, colchicine, AZA, IFX, IVIG for Ig deficiency	None
13	Family 6	F	38 years	29 years	Behçet disease	None	Colchicine
14	Family 6	F	19 years	15 years	Suspicion of Behçet disease	None	Colchicine
15	Family 6	F	15 years	13 years	Suspicion of Behçet disease	None	Colchicine
16	Family 7	M	(8 years)†	1 week	Crohn's disease, Behçet disease	CS, mesalamine, dapsone, MTX, AZA, colchicine, IFX, ADA, certolizumab, anakinra, canakinumab, tacrolimus, IVIG, tocilizumab	NA

ADA, adalimumab; AZA, azathioprine; CNS, central nervous system; CS, systemic corticosteroids; cyclo, cyclophosphamide; CYS, ciclosporin; ETN, etanercept; F, female; IFX, infliximab; IVIG, intravenous immunoglobulin; JIA, juvenile idiopathic arthritis; M, male; MTX, methotrexate; NA, not applicable; PFAPA, periodic fever, aphthous stomatitis, pharyngitis, adenitis; RF, rheumatoid factor; SLE, systemic lupus erythematosus.

Table 2 Clinical features of the patients with HA20 haploinsufficiency

Patient no	Fever	Ulcers	Musculoskeletal	Ocular	Skin	Cardiovascular	Intestinal	Other	Disease-related or treatment-related complications	Other symptoms possibly related to HA20
1	With flares	Oral, genital and perineal	Oligoarthritis, then polyarthritis*	Bilateral anterior uveitis	None	None	Bloody diarrhoea	Recurrent URTI and tonsillitis during childhood resulting in tonsillectomy; sometimes LAD (axillary, inguinal) with flares	Severe Cushing syndrome growth retardation, cataract and glaucoma requiring multiple ocular surgeries	Premature ovarian failure, geographic tongue, dental crowding secondary to small jaw
2	None	Oral, genital and perineal	Polyarthritis with dactylitis*	Bilateral anterior uveitis	None	None	Bloody diarrhoea		Cushing syndrome, cataract and glaucoma requiring multiple ocular surgeries	Acne during adolescence, eczema, pityriasis rosea, geographic tongue, dental crowding secondary to small jaw
3	None	Oral and genital*	Polyarthritis	None	Painful pustules in the genital region, breasts, axillae and sacrum, dermal abscess	None	None	Recurrent RTI during childhood		Acne during adolescence, thyroid goitre treated with radiation
4	Intermittent	Oral, genital and intestinal	Polyarthritis*	Retinal vasculitis, anterior uveitis	Stevens-Johnson syndrome, malar rash, painful subcutaneous rash, purpura, pustular, folliculitis-like rash on the extremities	None	Diarrhoea, secondary malabsorption syndrome	CNS vasculitis, idiopathic thrombocytopaenic purpura	Steroid-induced diabetes mellitus	
5	None	Oral and genital*	Polyarthritis	None	Pustular, folliculitis-like rash on the extremities	None	None	Suspicion of pre-eclampsia during pregnancies due to arterial hypertension and proteinuria, later diagnosis of membranous nephropathy		Irregular menses, late menarche (at age 18 years)
6	Intermittent	Oral and genital*	Polyarthritis	None	Folliculitis, palmoplantar pustules	None	Diarrhoea, once bloody	Recurrent URTI and tonsillitis during childhood, occasional LAD (neck), intermittent arterial hypertension		Eczema, periodontal disease since childhood, recurrent unintentional weight loss
7	With flares	Oral and genital*	Polyarthritis	None	Folliculitis	None	None	Recurrent RTI resulting in tonsillectomy	Removal of fibroids complicated by severe staphylococcus infection	Asthma, enamel loss, uterine fibroids, status postgastric bypass for obesity
8	With flares	Oral, genital and intestinal	Arthralgia	None	None	Pericardial effusion*	Bloody diarrhoea		Recurrent URTI under azathioprine according to parents	
9		Oral and genital*	None	None	None	None				
10	Recurrent*	Oral, rectal and perineal	None	None	None	Pericardial effusion	Abdominal pain, anorexia, nausea, vomiting, weight loss	Recurrent RTI during childhood (starting at 8 weeks of age) resulting in adenoidectomy and tonsillectomy, cervical LAD with fevers		Scoliosis requiring brace and subsequent surgical fusion therapy, vesicoureteral reflux surgery (pyelonephritis), attention deficit hyperactivity disorder
11	None	Oral and genital*	None	None	None	None	Abdominal pain, weight loss	Recurrent URTI and otitis media during childhood, requiring repeated tympanostomy, recurrent urinary tract infections		Cerebral palsy due to perinatal lacunar infarction, autism, severe foot pain associated with neurological monoplegia due to cerebral palsy requiring gabapentin and amitriptyline, recurrent headaches

Continued

Table 2 Continued

Patient no	Fever	Ulcers	Musculoskeletal	Ocular	Skin	Cardiovascular	Intestinal	Other	Disease-related or treatment-related complications	Other symptoms possibly related to HA20
12	None	Oral and genital*	Arthralgia	None	Severe acne in adulthood in the face, inguinal region and buttocks	None	Abdominal pain, diarrhoea, rectal bleeding	Recurrent RTI especially during childhood, asthmatic bronchitis	Bilateral pneumonia complicated by empyema, drainage and 3× streptokinase treatment and decortication (right lung) with debridement (pus evacuation), azathioprine-induced severe lymphopaenia and neutropaenia	Dysmenorrhoea resulting in hysterectomy, rosacea
13	With flares (low-grade)	Oral and genital*	Myalgia	None	Acne-like rash on the extremities	None	None			Hypothyroidism, arthralgia and myalgia (diagnosis of fibromyalgia), 'Dental problems'
14	None	Oral and genital*	None	None	None	None	None			
15	None	Oral and genital*	None	None	None	None	None			
16	With flares*	Oral, intestinal and perineal	None	None	Non-specific rash	Pulmonary embolism	Poor feeding, abdominal pain*, bloody diarrhoea, bowel perforation	Small vessel CNS vasculitis, inflammatory fibroepithelial polyp on the arytenoid	Posterior reversible encephalopathy syndrome, steroid-induced vertebral compression fractures, cataracts, hyperglycaemia and arterial hypertension	

*First symptom/finding leading to medical consultation.
CNS, central nervous system; HA20, A20 haploinsufficiency; LAD, lymphadenopathy; RTI, respiratory tract infection; URTI, upper respiratory tract infection.

Table 3 Overview of clinical and laboratory characteristics observed in patients with A20 haploinsufficiency

Features	Patients, n (%)
Mucous membrane	16 (100)
Musculoskeletal	9 (56)
Arthritis	7 (44)
Gastrointestinal	9 (56)
(Bloody) diarrhoea	5 (31)
Recurrent fever	8 (50)
Cutaneous	8 (50)
Ocular	3 (19)
Cardiovascular	3 (19)
Pericarditis	2 (13)
Increased acute-phase reactants during disease flare	10/10
Pathergy positive	3/10
Autoantibodies positive	8/14

(8/16, 50%). Less frequently, ocular (3/16, 19%) and cardiovascular involvement (3/16, 19%) was observed.

Seven patients (44%) suffered from recurrent, predominantly respiratory tract and otorhinolaryngological infections, especially during childhood. Infections were of viral and/or bacterial origin; only one of these patients was concomitantly treated with immunosuppressive agents. An overview of clinical symptoms occurring during the disease course is presented in tables 2 and 3.

Ulcers

Recurrent painful oral, genital and/or gastrointestinal ulcers were the hallmark feature of the disease. All 16 (100%) patients developed oral ulcers, whereas genital ulcers were observed in 15/16 (94%) and gastrointestinal ulcers in 6/9 patients with gastrointestinal complaints. Ulcerations recurred frequently (every month to every few months, at various intervals), isolated or in association with other symptoms such as fever, abdominal pain and arthralgia. Singular or multiple ulcers of various sizes (0.5–2 cm) lasted about 7–10 days and some healed with scarring (oral, genital or gastrointestinal location). Oral ulceration sites included the lips, tongue, cheeks, gums and hard palate, genital ulcers developed on vulva, vagina or the scrotum, and intestinal ulcerations were observed from the oesophagus to the rectum and perineum. Most patients could not identify an underlying trigger; in one patient, oral ulcers were exacerbated by acidic food.

Other clinical manifestations

Gastrointestinal symptoms were documented in 9/16 (56%) patients and ranged from isolated abdominal pain to severe intestinal inflammation with bowel perforation. Six (38%) patients suffered from recurrent, intermittently bloody diarrhoea. Musculoskeletal symptoms were frequent (9/16, 56%): polyarthritis was documented in seven patients, and in three of them arthritis was the initial disease manifestation. Cutaneous involvement (8/16, 50%) varied considerably and included pustular, folliculitis-like rashes, acne and dermal abscesses (figure 2). Ocular findings included severe, treatment-refractory anterior uveitis in two sisters (patients 1 and 2), as well as retinal vasculitis with chorioretinal scarring and macular fibrosis and anterior uveitis in another young girl (patient 4). Cardiovascular involvement was noted in three (19%) patients. Patient 8 was presented at age 9 months with fever and a pericardial

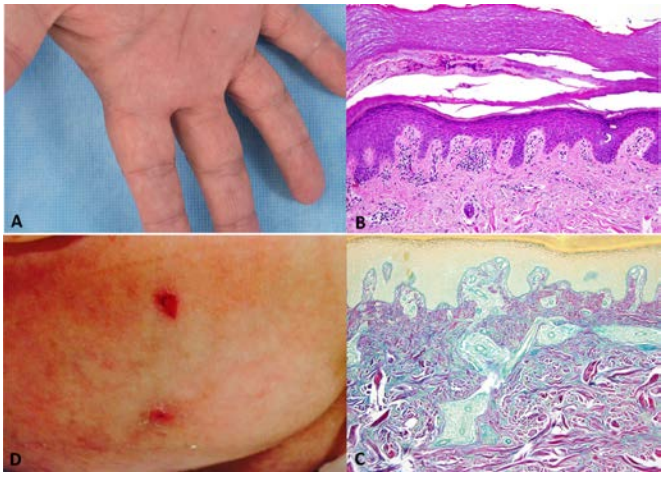


Figure 2 Clinical and histological manifestations in patients with A20 haploinsufficiency (HA20). (A) Pustules and vesicles at various stages of development are seen. There is a mild desquamation and some hyperkeratosis. (B) Magnification image (20×) of the palmar pustule seen in (A), stained with H&E. An infiltrate containing lymphocytes and neutrophils is noted in the stratum corneum. (C) The colloidal iron staining for mucin (blue, magnification image 20×) shows extensive intradermal mucin accumulation. Mucin accumulation is a feature of connective tissue diseases, such as systemic lupus erythematosus, but is not characteristically seen in palmoplantar pustulosis or pustular psoriasis. This is an unusual finding and fits with the HA20 phenotype of both autoimmune and autoinflammatory manifestation. (D) Cutaneous ulcers on the buttock.

effusion, which responded well to wide-spectrum antibiotic treatment. Another patient was diagnosed with pericarditis with a large effusion possibly related to *Mycoplasma pneumoniae* infection, which relapsed after discontinuation of corticosteroids but responded to colchicine (patient 10). In addition, patient 16, an 8-year-old boy with negative antiphospholipid antibodies and unremarkable hypercoagulability screen, presented with venous thrombi at sites of previous indwelling catheters. Two months later he was diagnosed with pulmonary arterial embolisms while he had no apparent venous thrombi. It is unclear whether these were true pulmonary embolisms or thromboses resulting from pulmonary artery vasculitis. Notably, none of the other patients developed thromboses or emboli. Neurological manifestations were reported in two (13%) patients, and both were diagnosed with central nervous system (CNS) vasculitis (patient 4 based on brain imaging and patient 16 based on a frontal lobe punctate). The clinical manifestations of the individual patients are shown in table 2.

Laboratory and histology findings

Acute-phase reactants were elevated, especially during relapses. Most patients had normal acute-phase reactants in between flares; in three patients, C reactive protein and erythrocyte sedimentation rate were persistently elevated prior to treatment response. We observed fluctuating levels of various low-titre antibodies, including antinuclear antibodies (4/10 patients tested), anti-dsDNA (2/6) and anti-Sm/RNP (2/4) antibodies. Lupus anticoagulant was positive in 6/7 patients tested and anti-cardiolipin antibody in 2/5 patients. Patients 11 and 12 (family 5), both of whom suffered from recurrent viral and bacterial infections, were diagnosed with unclassified immunodeficiency with IgG subclass deficiency, absent polysaccharide vaccination response and lymphopaenia. None of the eight patients investigated for recurrent genital ulcers had evidence of herpes simplex infection.

Given clinical similarity with BD, we reviewed pathergy results and found they were variable among the 10 patients tested: positive in three patients, negative in six patients and inconclusive in one patient. HLA-B51 was positive in 2/5 patients tested. Tissue biopsy of at least one site was performed in 12/16 (75%) patients; pathological findings on histology included non-specific chronic inflammation in six, findings consistent with pustules and normal results in two patients each (bone marrow aspirate and intestinal mucosa in one, and lymph node in another) (figure 2). A kidney biopsy performed in patient 5 was consistent with class V lupus nephritis with glomerular basement membrane thickening, minimal inflammatory cell infiltrate, and extensive deposition of complement and immunoglobulins. Except for the frontal lobe punctate performed in patient 16, none of the tissue samples showed evidence of vasculitis. Online supplementary table 1 summarises the laboratory and histopathological features of patients with HA20.

Treatment

All patients required treatment. Four patients responded well to monotherapy with colchicine. The other patients were treated with a monotherapy or a combination of immunosuppressive drugs, including systemic corticosteroids, disease-modifying drugs and cytokine inhibitors (anti-tumour necrosis factor (anti-TNF), anti-interleukin-1 (IL-1), anti-IL-6). After treatment-refractory disease courses, 12 patients eventually improved on either infliximab, anakinra, tofacitinib, colchicine or methotrexate in combination with thalidomide. More recently, therapeutic approaches were based on functional cytokine studies; cytokine inhibitors such as infliximab and anakinra proved effective in suppressing systemic inflammation in 7/9 patients. Most patients responded to high-dose corticosteroids but also suffered from major side effects. Patient 4 eventually underwent autologous haematopoietic stem cell transplantation for CNS vasculitis associated with a severe, SLE-resembling condition. She went into remission for 18 months, but subsequently developed a CNS vasculitis flare, anterior uveitis, idiopathic thrombocytopaenic purpura and recurrence of orogenital ulcers, for which various immunosuppressive agents were reinitiated. Previous and current treatment regimens of the individual patients are presented in table 1.

Three out of seven patients with recurrent respiratory tract and otorhinolaryngological infections underwent tonsillectomy; two patients (mother and daughter of family 5) received immunoglobulin replacement for low IgG subclass and the daughter also had repeat tympanostomy.

Outcome/complications

HA20 disease was characterised by unprovoked episodes of inflammatory symptoms or chronic inflammation. None of the patients developed lymphoma or malignancy. One patient died from HA20. Patient 16, who presented with severe intestinal involvement and presumed small vessel CNS vasculitis, died from uncontrollable disease and upper airway obstruction due to haemorrhage following erosion of the carotid artery from extension of bilateral tonsillar ulcerations. He was anticoagulated for pulmonary arterial embolisms.

Treatment-associated complications included corticosteroid-induced side effects such as Cushing syndrome, growth retardation, vertebral compression fractures, diabetes mellitus

and arterial hypertension in five patients, and severe lymphopaenia and neutropaenia under azathioprine in another patient. Disease-associated and treatment-associated complications are shown in table 2.

DISCUSSION

Herein, we describe the clinical manifestations and disease course of 16 patients with HA20, the largest cohort to date. The disease was characterised by early-onset systemic inflammation accompanied with recurrent oral, genital and/or gastrointestinal ulcers. Other clinical manifestations and disease course varied considerably even among patients with the same mutation, and ranged from severe or fatal multisystemic inflammation to mild disease with recurrent orogenital ulcers, arthralgia and cutaneous lesions. This suggests a role of modifying alleles in the disease progression and possible contribution of environmental factors such as diet.

Recurrent painful oral, genital and/or gastrointestinal ulcers were the hallmark feature in all subjects. Besides ulcers, various other, BD-like clinical manifestations such as articular, gastrointestinal, cutaneous and ocular symptoms were notable.[8–13] As a consequence, the majority of patients (>70%) were initially diagnosed or suspected of having BD. HA20 is considered a monogenic type of BD due to highly penetrant novel germline mutations in *TNFAIP3* and earlier onset symptoms. Polygenic and common BD, on the other hand, typically manifests in early adulthood and does not have clear dominant inheritance. Large case series of patients with paediatric BD reported a symptom onset in later childhood, between 6.9 and 12.3 years of age[14–18]; however, it is not clear how many of these patients carry mutations in *TNFAIP3*. Despite some similarities with BD, we recognised several important characteristics that help differentiate HA20 from polygenic BD, as shown in table 4. These other distinguishing observations in patients with HA20 included scarring oral ulcers, isolated anterior uveitis or retinal vasculitis with necrotising inflammation, recurrent fever, severe intestinal inflammation, elevated acute-phase reactants, the fluctuating presence of various autoantibodies, and a disease course refractory to standard treatment, all of which are unusual findings in typical BD.[13 19]

Although BD was the most common initial diagnosis, the heterogeneity of clinical phenotypes, variable temporal occurrence of symptoms, presence of various autoantibodies and histology resulted in the diagnosis of other inflammatory and autoimmune diseases. Family 1 presented with a clinical picture resembling JIA and/or rheumatoid arthritis (RA), patient 10 with symptoms compatible with PFAPA and patient 16 with features resembling Crohn's disease. Half of the patients were found to have fluctuating autoantibodies and two sisters (patients 4 and 5) were initially diagnosed with SLE. Thus, it is likely that some patients with early-onset SLE might have mutations in *TNFAIP3*.

In addition to the 16 patients described in this study, four case reports including a total of 11 patients from four unrelated families were published in the literature since the initial publication by Zhou and colleagues.[4–7] HA20 was recently reported in two Japanese families with an early-onset BD-like clinical picture.[4 5] Similar to most patients in our cohort, these two families presented with recurrent orogenital ulcers and fevers; some family members also suffered from intestinal involvement, cutaneous lesions, nephrotic syndrome, polyarthritis or uveitis.[4 5] Furthermore, HA20 was reported in a Japanese patient diagnosed with autoimmune lymphoproliferative syndrome (ALPS) and in a British boy with complex autoimmunity.[6 7] The Japanese patient had presented with early onset, recurrent fever, bilateral cervical lymphadenopathy, hepatosplenomegaly and an extensive cutaneous rash suggestive of Kawasaki disease. Consistent with the diagnosis of ALPS, immunophenotyping revealed an increased percentage of double-negative T cells and a decrease in IgM memory B cells. However, unlike in patients with ALPS, the central memory, naïve, terminally differentiated effector memory T cells re-expressing CD45RA+, and effector memory subpopulations of $CD3^+$ $CD8^+$ T cells were normal in this patient.[6] The boy of British ancestry was investigated for a complex, treatment-refractory autoimmune syndrome characterised by insulin-dependent diabetes, cytopaenias, hepatitis, enteropathy and interstitial lung disease.[7] Prior to his molecular diagnosis of HA20, he underwent haematopoietic stem cell transplantation and is in complete remission (except for diabetes). He was diagnosed with a novel de novo heterozygous 2 bp deletion in *TNFAIP3*, p.V489Afs*7 in the second zinc finger domain (ZnF2). His disease-associated variant resides in a different domain of A20 from pathogenic mutations reported in all other patients and the ZnF4 domain may have other unappreciated functions.[3 7] Most patients reported in our study are carriers for loss-of function protein truncating mutations in the ovarian tumour domain. This resulted in decreased deubiquitination of

Table 4 Clinical and laboratory features that are helpful to differentiate between A20 haploinsufficiency (HA20) and Behçet disease.

Features	HA20	Behçet disease[9 13 16–19]
Disease onset	Mostly early childhood	Early adulthood
Inheritance	Autosomal dominant	Complex inheritance pattern with familial aggregation in up to 20% of cases
Fever	Recurrent	Usually absent
Ulcers	May heal with scarring	Usually no scarring of oral ulcers
Eyes	Severe ocular disease ► Anterior uveitis ► Retinal vasculitis and choroiditis with necrotising inflammation	► Posterior or panuveitis ► Recurrent superficial retinal infiltrates resolving within days without chorioretinal scarring ► Peripheral retinal occlusive periphlebitis
Gastrointestinal	(Bloody) diarrhoea	Isolated abdominal pain*
Musculoskeletal	Mostly polyarthritis	Usually oligoarthritis
Erythrocyte sedimentation rate/C reactive protein	Elevated, especially during disease relapses	Often normal
Autoantibodies Autoimmune features	Low titre, fluctuating presence Systemic lupus erythematosus-like disease and other autoimmune features possible	Usually absent

*Gastrointestinal involvement in Behçet disease is usually mild and consists essentially of abdominal pain or discomfort except for patients from Japan and Korea.

key signalling molecules, such as RIP1 and NEMO, increased activity of the NF-kB pathway, and high expression of proinflammatory cytokines.[3 20] Although the molecular aetiologies of the British boy's disease and our patients with HA20 are the same, his distinct clinical symptoms could be related to the presence of other unknown modifying gene alleles. Identification of additional HA20-associated mutations is necessary to better understand the full spectrum of phenotypes associated with the distribution of pathogenic mutations in A20.

Polymorphisms or mutations in *TNFAIP3* have been associated with many autoimmune diseases, among them JIA, RA, IBD, SLE, type 1 diabetes, psoriasis, coeliac disease and coronary artery disease.[20–27] In murine models, cell-specific ablation of A20 causes clinical features characteristic of these human diseases.[1 20] Mice with A20 deficiency in myeloid cells develop polyarthritis mimicking human RA,[28] while enterocyte-specific deficiency of A20 increases the susceptibility of mice to intestinal inflammation.[29] Tissue-specific deletion of A20 in B cells or dendritic cells leads to the production of autoantibodies and an autoimmune syndrome resembling SLE.[30 31] Mice deficient for A20 ($A20^{-/-}$) develop severe multiorgan inflammation, cachexia and early death.[32] Although A20 was initially described as required for termination of TNF-induced signals, the excessive inflammation observed in double-deficient mice, A20-TNF or A20-TNFR1, suggested that A20 might be critical for the regulation of TNF-independent signals. In addition, A20 has been shown to downregulate the activity of NLRP3 inflammasome and patients with HA20 had increased activity of NLRP3.[3] A20 functions as a tumour suppressive gene and somatic mutations have been identified in B cell lymphomas.[33] None of the patients in this cohort developed lymphoma or malignancy.

In clinical practice, HA20 may be considered in patients with an early-onset, dominantly inherited inflammatory disease who present with recurrent oral and genital ulcerations, fluctuating autoantibodies, and a treatment-refractory disease course.

There was no standardised treatment in this HA20 cohort. The patients received various immunosuppressive drugs prior to the diagnosis of HA20; more recently, therapeutic approaches were guided by functional cytokine studies. Elevated levels of many proinflammatory cytokines (IL-1, TNF, IL-6, IL-18, IFNγ, IP-10) have been documented in patients with HA20.[3 20] Anticytokine agents such as anti-TNF or anti-IL-1 have been effective in suppressing the systemic inflammation in most of our patients. Haematopoietic stem cell transplant might be considered in patients with severe and treatment-refractory disease.

This study is limited by its retrospective design and other biases related to which patients received testing.[3] Recurrent ulcers and a Behçet-like disease were characteristic features in this cohort, likely reflecting bias in which patients are screened for HA20. However, ulcers of variable extension and severity were documented in index cases and in all affected family members, suggesting that ulcers may be the hallmark feature of the disease. Given the retrospective study design and the disease pleiotropy, it is unlikely that HA20 is the unique underlying cause for all disease manifestations. The increased use of diagnostic whole exome sequencing will help identify other contributing disease modifying rare or common variants.[34]

In conclusion, HA20 disease in this cohort was characterised by early-onset inflammation and recurrent oral, genital and/or gastrointestinal ulcers and often positive family history. Other disease features and disease course varied considerably with an overall high morbidity and mortality. Treatment regimens should be based on severity of inflammatory manifestations, and often consist of targeted therapy with cytokine inhibitors to control the inflammation. In the future, identification of patients with monogenic inflammatory diseases will be important to understand the pathophysiology and guide treatments for common rheumatological diseases.

Author affiliations
[1]Division of Rheumatology, The Hospital for Sick Children, Department of Paediatrics, University of Toronto, Toronto, Ontario, Canada
[2]Department of Pediatric Rheumatology, Hacettepe University, Ankara, Turkey
[3]Department of Pediatric Rheumatology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, Pennsylvania, USA
[4]Division of Pediatric Rheumatology, Riley Hospital for Children, Indiana University, Indianapolis, Indiana, USA
[5]Department of Internal Medicine, Istanbul University, Istanbul, Turkey
[6]Inflammatory Disease Section, National Human Genome Research Institute, Bethesda, Maryland, USA
[7]Department of Rheumatology and Clinical Immunology, University Medical Center Utrecht, Utrecht, The Netherlands
[8]Molecular Immunology and Inflammation Branch, National Institute of Arthritis and Musculoskeletal and Skin Diseases, Bethesda, Maryland, USA
[9]Department of Pediatric Immunology, University Medical Center Utrecht, Utrecht, The Netherlands
[10]Department of Medicine, University of Toronto, Toronto, Ontario, Canada

Acknowledgements We thank the patients and their families for participating in this study. We thank Ingrid Goh for the technical support. The authors acknowledge Dres Chyi-Chia R Lee and Ed Cowen for their valuable assistance in the management of the cases.

Contributors FAA and RML: coordination of the study, data collection and analysis, manuscript preparation. EDB, SWC, EG, AG, PH, HLL, SO, DMS, DLS, AvR-K, IA and DLK: data collection and analysis, revision of the manuscript for important intellectual content. All authors approved the final version to be published.

Funding FAA was supported by grants from the Rhyner-Bangerter Foundation, Starr-Foundation, Swiss League against Rheumatism, Foundation W!, Alberta Children's Hospital Research Institute Foundation and SickKids Foundation.

Competing interests None declared.

Ethics approval The study was approved by the Institutional Research Ethics Board (REB 1000053697).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Catrysse L, Vereecke L, Beyaert R, *et al*. A20 in inflammation and autoimmunity. *Trends Immunol* 2014;35:22–31.
2 Wertz IE, O'Rourke KM, Zhou H, *et al*. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. *Nature* 2004;430:694–9.
3 Zhou Q, Wang H, Schwartz DM, *et al*. Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. *Nat Genet* 2016;48:67–73.
4 Ohnishi H, Kawamoto N, Seishima M, *et al*. A Japanese family case with juvenile onset Behçet's disease caused by TNFAIP3 mutation. *Allergol Int* 2017;66:146–8.
5 Shigemura T, Kaneko N, Kobayashi N, *et al*. Novel heterozygous C243Y A20/TNFAIP3 gene mutation is responsible for chronic inflammation in autosomal-dominant Behçet's disease. *RMD Open* 2016;2:e000223.
6 Takagi M, Ogata S, Ueno H, *et al*. Haploinsufficiency of TNFAIP3 (A20) by germline mutation is involved in autoimmune lymphoproliferative syndrome. *J Allergy Clin Immunol* 2017;139:1914–22.
7 Duncan CJA, Dinnigan E, Theobald R, *et al*. Early-onset autoimmune disease due to a heterozygous loss-of-function mutation in TNFAIP3 (A20). *Ann Rheum Dis* 2017:annrheumdis-2016-210944.
8 Koné-Paut I, Darce-Bello M, Shahram F, *et al*. Registries in rheumatological and musculoskeletal conditions. Paediatric Behçet's disease: an international cohort study of 110 patients. One-year follow-up data. *Rheumatology* 2011;50:184–8.
9 Nanthapisal S, Klein NJ, Ambrose N, *et al*. Paediatric Behçet's disease: a UK tertiary centre experience. *Clin Rheumatol* 2016;35:2509–16.
10 Koné-Paut I, Gorchakoff-Molinas A, Weschler B, *et al*. Paediatric Behçet's disease in France. *Ann Rheum Dis* 2002;61:655–6.
11 Mora P, Menozzi C, Orsoni JG, *et al*. Neuro-Behçet's disease in childhood: a focus on the neuro-ophthalmological features. *Orphanet J Rare Dis* 2013;8:18.
12 Uluduz D, Kürtüncü M, Yapıcı Z, *et al*. Clinical characteristics of pediatric-onset neuro-Behçet disease. *Neurology* 2011;77:1900–5.
13 Saadoun D, Wechsler B. Behçet's disease. *Orphanet J Rare Dis* 2012;7:20.

14 Atmaca L, Boyvat A, Yalçındağ FN, *et al*. Behçet disease in children. *Ocul Immunol Inflamm* 2011;19:103–7.
15 Karincaoglu Y, Borlu M, Toker SC, *et al*. Demographic and clinical properties of juvenile-onset Behçet's disease: A controlled multicenter study. *J Am Acad Dermatol* 2008;58:579–84.
16 Koné-Paut I, Shahram F, Darce-Bello M, *et al*. Consensus classification criteria for paediatric Behçet's disease from a prospective observational cohort: PEDBD. *Ann Rheum Dis* 2016;75:958–64.
17 Koné-Paut I, Yurdakul S, Bahabri SA, *et al*. Clinical features of Behçet's disease in children: an international collaborative study of 86 cases. *J Pediatr* 1998;132:721–5.
18 Krause I, Uziel Y, Guedj D, *et al*. Childhood Behçet's disease: clinical features and comparison with adult-onset disease. *Rheumatology* 1999;38:457–62.
19 Koné-Paut I. Behçet's disease in children, an overview. *Pediatr Rheumatol Online J* 2016;14:10.
20 Aksentijevich I, Zhou Q. NF-κB Pathway in autoinflammatory diseases: dysregulation of protein modifications by ubiquitin defines a new category of autoinflammatory diseases. *Front Immunol* 2017;8:399.
21 Plenge RM, Cotsapas C, Davies L, *et al*. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. *Nat Genet* 2007;39:1477–82.
22 Thomson W, Barton A, Ke X, *et al*. Rheumatoid arthritis association at 6q23. *Nat Genet* 2007;39:1431–3.
23 Wellcome Trust Case Control Consortium. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007;447:661–78.
24 Fung EY, Smyth DJ, Howson JM, *et al*. Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. *Genes Immun* 2009;10:188–91.
25 Nair RP, Duffin KC, Helms C, *et al*. Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* 2009;41:199–204.
26 Boonyasrisawat W, Eberle D, Bacci S, *et al*. Tag polymorphisms at the A20 (TNFAIP3) locus are associated with lower gene expression and increased risk of coronary artery disease in type 2 diabetes. *Diabetes* 2007;56:499–505.
27 Trynka G, Zhernakova A, Romanos J, *et al*. Coeliac disease-associated risk variants in TNFAIP3 and REL implicate altered NF-kappaB signalling. *Gut* 2009;58:1078–83.
28 Matmati M, Jacques P, Maelfait J, *et al*. A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. *Nat Genet* 2011;43:908–12.
29 Vereecke L, Sze M, Mc Guire C, *et al*. Enterocyte-specific A20 deficiency sensitizes to tumor necrosis factor-induced toxicity and experimental colitis. *J Exp Med* 2010;207:1513–23.
30 Chu Y, Vahl JC, Kumar D, *et al*. B cells lacking the tumor suppressor TNFAIP3/A20 display impaired differentiation and hyperactivation and cause inflammation and autoimmunity in aged mice. *Blood* 2011;117:2227–36.
31 Hövelmeyer N, Reissig S, Xuan NT, *et al*. A20 deficiency in B cells enhances B-cell proliferation and results in the development of autoantibodies. *Eur J Immunol* 2011;41:595–601.
32 Lee EG, Boone DL, Chai S, *et al*. Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 2000;289:2350–4.
33 Ma A, Malynn BA. A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat Rev Immunol* 2012;12:774–85.
34 Demirkaya E, Zhou Q, Smith CK, *et al*. Brief report: deficiency of complement 1r subcomponent in early-onset systemic lupus erythematosus: the role of disease-modifying alleles in a monogenic disease. *Arthritis Rheumatol* 2017;69:1832–9.

EXTENDED REPORT

DNA methylation mapping identifies gene regulatory effects in patients with systemic lupus erythematosus

Juliana Imgenberg-Kreuz,[1,2] Jonas Carlsson Almlöf,[1] Dag Leonard,[2] Andrei Alexsson,[2] Gunnel Nordmark,[2] Maija-Leena Eloranta,[2] Solbritt Rantapää-Dahlqvist,[3] Anders A Bengtsson,[4] Andreas Jönsen,[4] Leonid Padyukov,[5] Iva Gunnarsson,[5] Elisabet Svenungsson,[5] Christopher Sjöwall,[6] Lars Rönnblom,[2] Ann-Christine Syvänen,[1] Johanna K Sandling[1,2]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212379).

[1]Department of Medical Sciences, Molecular Medicine and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[2]Section of Rheumatology, Department of Medical Sciences, Uppsala University, Upssala, Sweden
[3]Department of Public Health and Clinical Medicine/Rheumatology, Umeå University, Umeå, Sweden
[4]Department of Clinical Sciences, Section of Rheumatology, Lund University, Skane University Hospital, Lund, Sweden
[5]Rheumatology Unit, Department of Medicine Solna, Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden
[6]Department of Clinical and Experimental Medicine, Rheumatology/Division of Neuro and Inflammation Sciences, Linköping University, Linköping, Sweden

Correspondence to
Dr Johanna K Sandling, Department of Medical Sciences, Uppsala University, Rudbeck Laboratory, Uppsala 751 85, Sweden; johanna.sandling@medsci.uu.se

Received 12 September 2017
Revised 11 January 2018
Accepted 15 January 2018
Published Online First 1 February 2018

To cite: Imgenberg-Kreuz J, Carlsson Almlöf J, Leonard D, *et al*. *Ann Rheum Dis* 2018;**77**:736–743.

ABSTRACT

Objectives Systemic lupus erythematosus (SLE) is a chronic autoimmune condition with heterogeneous presentation and complex aetiology where DNA methylation changes are emerging as a contributing factor. In order to discover novel epigenetic associations and investigate their relationship to genetic risk for SLE, we analysed DNA methylation profiles in a large collection of patients with SLE and healthy individuals.

Methods DNA extracted from blood from 548 patients with SLE and 587 healthy controls were analysed on the Illumina HumanMethylation 450 k BeadChip, which targets 485 000 CpG sites across the genome. Single nucleotide polymorphism (SNP) genotype data for 196 524 SNPs on the Illumina ImmunoChip from the same individuals were utilised for methylation quantitative trait loci (*cis*-meQTLs) analyses.

Results We identified and replicated differentially methylated CpGs (DMCs) in SLE at 7245 CpG sites in the genome. The largest methylation differences were observed at type I interferon-regulated genes which exhibited decreased methylation in SLE. We mapped *cis*-meQTLs and identified genetic regulation of methylation levels at 466 of the DMCs in SLE. The meQTLs for DMCs in SLE were enriched for genetic association to SLE, and included seven SLE genome-wide association study (GWAS) loci: *PTPRC* (CD45), *MHC-class III*, *UHRF1BP1*, *IRF5*, *IRF7*, *IKZF3* and *UBE2L3*. In addition, we observed association between genotype and variance of methylation at 20 DMCs in SLE, including at the *HLA-DQB2* locus.

Conclusions Our results suggest that several of the genetic risk variants for SLE may exert their influence on the phenotype through alteration of DNA methylation levels at regulatory regions of target genes.

INTRODUCTION

Systemic lupus erythematosus (SLE, MIM 152700) is an autoimmune disease characterised by defective clearance of apoptotic cells, production of a large number of different autoantibodies and activation of the type I interferon (IFN) system.[1 2] So far, more than 80 genetic loci that contribute to SLE susceptibility have been identified.[3 4] Both candidate gene and genome-wide association studies (GWAS) have provided insights into the affected signalling pathways, but do not fully explain the genetic susceptibility to SLE.[5 6]

Epigenetic regulation is emerging as an important contributing factor in SLE. Promoter demethylation in lymphocytes leading to overexpression has been reported for several SLE candidate genes, as has global DNA hypomethylation in lymphocytes in patients with SLE.[7–9] In addition, demethylating agents are known to cause drug-induced lupus.[10] Using the Illumina HM450k BeadChip to analyse fractionated blood cells from patients with SLE and healthy controls, Absher *et al* and Coit *et al* report hypomethylation at type I IFN system genes across all tested blood cell types.[11 12] These studies indicate a role for DNA methylation in regulating the type I IFN system in SLE. Associations between DNA methylation and different manifestations of SLE have also been reported, and they include autoantibody production, nephritis and skin rash.[13–16] However, these findings are yet to be independently replicated.

In order to discover novel epigenetic associations in SLE, we generated genome-wide methylation profiles from a large collection of Swedish patients with SLE and healthy controls. To date, there have been no large-scale studies that investigate the role of genetics in regulating DNA methylation levels and variance of DNA methylation in SLE and the effect of these measures on SLE susceptibility. Therefore, we intersected our genome-wide DNA methylation data with genetic data on the same cohorts to identify gene regulatory effects on DNA methylation in SLE.

METHODS

For full details of methods see online Supplementary file 1.

Subjects and samples

In the discovery phase, patients with SLE visiting university hospitals in Uppsala and Linköping,[17] Sweden (n=400), and control individuals from the Uppsala BioResource (n=400) of healthy blood donors were included. As replication, patients with SLE (n=201) and controls (n=187) from the Karolinska University hospital in Stockholm, Sweden, were included. All subjects provided informed consent to participate in the study. Five hundred and forty-eight patients fulfilling at least four of the eleven 1982 American College for Rheumatology (ACR) criteria for SLE[18] were included in the subsequent analyses.

DNA methylation analysis

DNA methylation levels of 485 577 CpG (C-phosphate-G) sites were determined using the HM450k BeadChip (Illumina, San Diego, California, USA).[19] Signal intensities were parsed into the Minfi R package for quality control (QC) and Subset-quantile Within Array Normalisation.[20–22] The post-QC dataset comprised 385 851 CpG sites, 347 patients with SLE and 400 controls for the discovery phase and 201 patients and 188 controls for the replication phase. The aggregate of methylation beta values for all CpG sites followed identical bimodal distributions in both cases and controls (see figure S1 in the online Supplementary file 2).

Genotyping

Quality controlled genotype data for 133 838 SNPs generated on the Infinium ImmunoChip (Illumina)[23] were available for 527 patients with SLE and 567 of the healthy control individuals with HM450k data. The SLE case–control genetic association analysis included a larger set of 1135 Swedish patients with SLE and 2931 Swedish control individuals from the university hospital rheumatology clinics at Uppsala, Stockholm Karolinska Solna, Linköping, Lund, and the four northernmost counties of Sweden.

Epigenome-wide association analysis

Relative blood cell composition of the samples was determined using the method by Houseman *et al*[24] (see figure S2 in the online Supplementary file 3). To determine differential methylation between patients with SLE and controls, a linear regression model was fitted. Differentially methylated CpG sites (DMCs) were called in the discovery phase if they had a $P<1.3\times10^{-7}$ for association based on Bonferroni correction and an absolute average difference in methylation beta values between cases and controls of >0.05. Significance in the replication phase was determined as $P<0.05$ divided by the number of tested CpG sites and same direction of effect. Similarly, the role of methylation in different disease manifestations was investigated in a case–case analysis as was the association between different medications and methylation.

Methylation quantitative trait loci (meQTL) analysis

Methylation levels were tested in PLINK for genotype association separately in patients and controls assuming an additive model.[25] A Bonferroni corrected $\alpha<0.05$ was considered significant. Methylation variance was calculated as the difference between a subject's methylation value and the genotype-specific mean.

RESULTS

Genome-wide DNA methylation patterns in SLE

We used the Illumina HumanMethylation 450k BeadChip[19] and analysed methylation levels at 385 851 CpG sites across the human genome in an epigenome-wide association study (EWAS) for SLE in genomic DNA from whole blood. The study included a total of 548 patients with SLE and 588 age-matched and gender-matched controls, and we employed a discovery and replication phase study design (see table S1 in the online Supplementary file 4). In the discovery phase, we identified 7625 DMCs using logistic regression in patients with SLE compared with controls at a Bonferroni corrected P value $<1.3\times10^{-7}$ and average methylation difference $|\Delta\beta|>0.05$ (figure 1; see table S2 in the online Supplementary file 5). The vast majority of the DMCs identified in the discovery cohort exhibited decreased DNA methylation levels in patients with SLE compared with controls (75%; 5717 of 7625 CpG sites). As many as 7245 DMCs (95%) replicated in the second cohort (Bonferroni corrected P value $<6.6\times10^{-6}$) (see table S2 in the online Supplementary file 5). A noteworthy result from the genome-wide DNA methylation analysis is that we observed large differential methylation of $|\Delta\beta|>0.1$ almost exclusively at IFN-regulated genes (table 1). This epigenetic IFN pattern was observed both in patients with active and inactive disease, although the effect was more prominent in active SLE (see table S3 in the online Supplementary file 6). The CpG site with the largest increased methylation in SLE was cg08450017 in *CXCR6*, which is involved in C–X–C chemokine signalling and whose ligand CXCL16 is elevated in SLE serum and has been suggested as a biomarker in SLE (figure 2; see table S2 in the online Supplementary file 5).[26 27]

A total of 4034 of the replicated DMCs in SLE that we identify in blood cells in patients with SLE are novel and are annotated to 1638 unique genes that have to our knowledge not previously been linked with DNA methylation in SLE.[11 12 28 29] Among the most significant novel DMCs in SLE we note cg03889044 in *PDCD1*, which is a confirmed SLE susceptibility locus.[30 31] *PDCD1* encodes the Programmed Cell Death 1 (PD-1) protein that functions in preventing autoimmunity by inhibiting activation of self-reactive lymphocytes.[32] Another example of a previously unreported DMC in SLE is cg24414363 in centromere protein M (CENPM). CENPM is involved in regulating cell division processes and is preferentially expressed in activated lymphocytes.[33] We further identified highly significant novel DMCs in SLE at the genes adenylate kinase 2 (*AK2*) playing a role in apoptotic processes and activating signal cointegrator 1 complex subunit 2 (*ASCC2*) involved in transcriptional regulation.

To further characterise our most significant DMCs in SLE, we performed gene ontology enrichment analysis for the most significant and replicated DMCs annotated to genes. We found that genes which had DMCs in SLE were highly enriched in the molecular functions enzyme binding, regulatory region DNA binding and transcription factor activity, as well as in biological processes related to leucocyte activation (table 2, see table S4 in the online Supplementary file 7). Additionally, we found that the replicated DMCs in SLE were depleted in CpG islands, but were enriched in regions with a histone mark for active enhancers (H3K4me1) in B and T cells (see figure S3 in the online Supplementary file 8).

To avoid confounding due to gender differences in DNA methylation patterns, CpG sites located on the sex chromosomes were analysed separately in females and males. In females, we replicated 27 X-chromosomal DMCs in SLE (see table S5 in the online Supplementary file 9). These DMCs were annotated to several genes implicated in immune cell function, such as *TLR8* involved in pathogen recognition and *VSIG4*, a negative regulator of T-cell proliferation. In males, there were three replicated X-chromosomal DMCs in SLE; these were annotated to the *SH2D1A* and *SEPT6* genes and an intergenic region, respectively (see table S6 in the online Supplementary file 10). SH2D1A plays a role in stimulation of T and B cells and septin 6 is required for cytokinesis.

Methylation changes associated with SLE disease manifestations

As SLE is a clinically heterogeneous disease, we compared the DNA methylation levels between patients that display a specific disease manifestation defined in the ACR 1982 classification

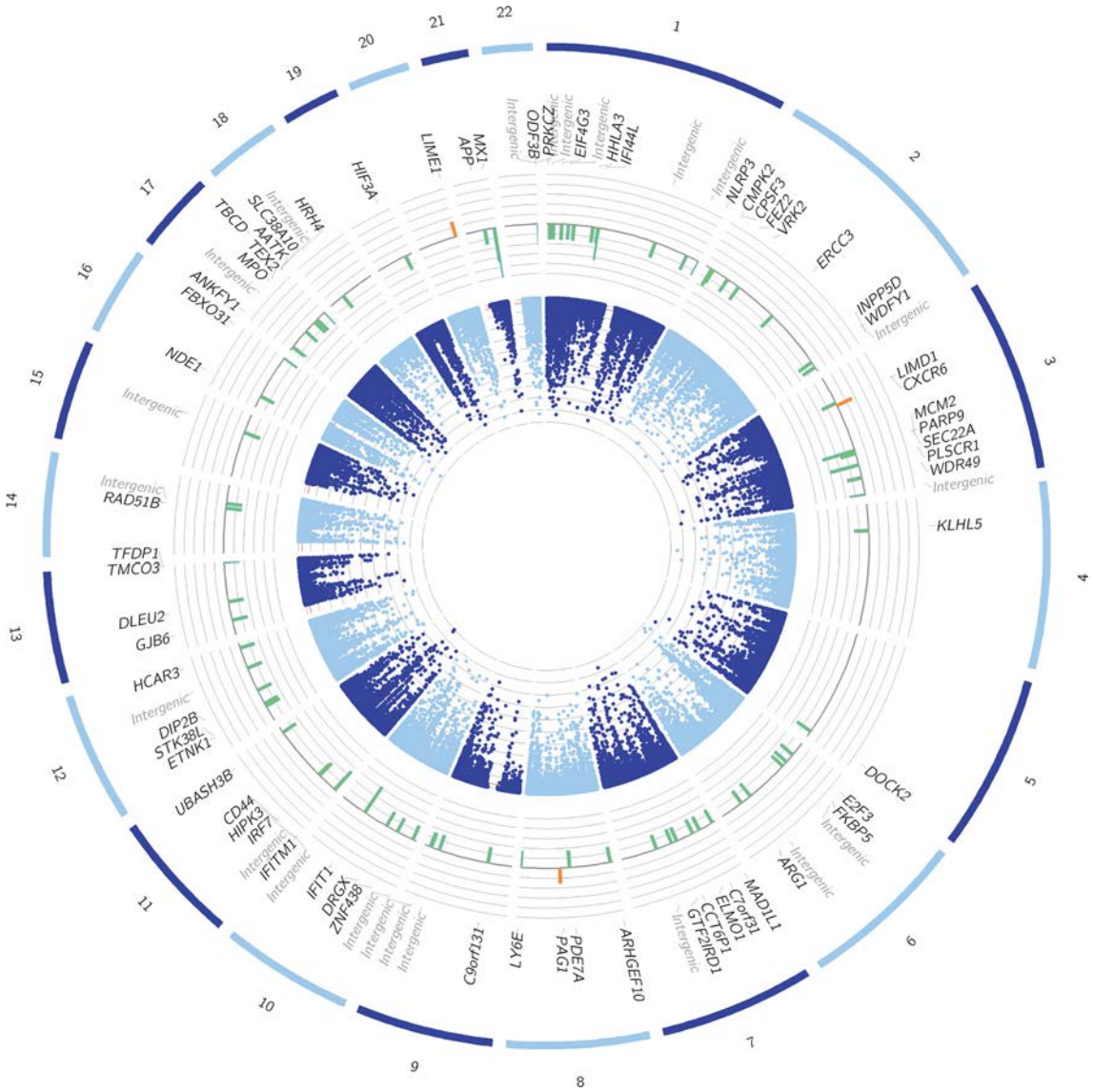


Figure 1 Results from the case–control epigenome-wide association study (EWAS) in systemic lupus erythematosus (SLE) in the discovery cohort. Inner circle: circular Manhattan plot of the results of the SLE case–control association analysis. P values are presented on the –log10 scale where the innermost scale line represents 10^{-214}. Middle circle: average methylation difference (Δβ) between patients with SLE and controls for the top 100 differentially methylated CpG sites in the EWAS (scale –0.4 to 0.4). Green bars indicate decreased methylation and orange bars represent increased methylation levels in patients compared with controls. The outer circle represents chromosomes 1–22.

criteria for SLE[18] against the remaining patients lacking this disease manifestation (see table S1 in the online Supplementary file 4). We were only able to identify a total of 49 DMCs associated with ACR criteria for SLE in the discovery cohort (P value $<1.3\times10^{-7}$, $|\Delta\beta|>0.05$) (see table S7 in the online Supplementary file 11). None of these 49 DMCs reached the corrected significance threshold in the replication cohort.

Methylation changes associated with SLE treatment

As a majority of the patients with SLE received treatment to control their disease at the time of blood sampling, we investigated whether methylation levels were associated with the most commonly prescribed medications. By comparing patients that received a specific medication at blood sampling to those who did not, we identified and replicated 5321 DMCs for medication in SLE when correcting for disease activity (see table S8 in the online Supplementary file 12). The overwhelming majority of the DMCs for medication were observed for glucocorticoid treatment (n=5196), which typically was associated with decreased methylation.

Due to the large number of CpG sites associated with glucocorticoid treatment, we repeated the SLE case–control methylation analyses in the subsets of patients who were not receiving glucocorticoid treatment at the time of blood sampling (discovery n=132 and replication n=89). Of the 7245 replicated DMCs in SLE, 3295 were also significant in this analysis applying Bonferroni correction for multiple testing, and 6411 reached nominal significance (P<0.05) in both cohorts with the same direction of the effect (see table S9 in the online Supplementary file 13 and figure S4 in the online Supplementary file 14).

Genetic regulation of DNA methylation in SLE

To search for *cis*-acting genetic variants that regulate DNA methylation in SLE, we analysed DNA methylation levels against the genotypes of single nucleotide polymorphisms (SNPs) in risk loci for autoimmune diseases in a *cis*-meQTL analysis (see figure S5

Table 1 Top differentially methylated CpG sites (DMCs) in the systemic lupus erythematosus case–control association analysis

Chromosome	Position	CpG site	Gene	Interferon induced*	Discovery		Replication	
					P value†	Methylation Δβ‡	P value†	Methylation Δβ‡
21	42799141	cg21549285	*MX1*	Yes	3.5E−139	−0.42	6.4E− 83	−0.47
3	122281881	cg22930808	*PARP9*	Yes	1.4E−105	−0.27	1.2E−74	−0.33
21	42797588	cg22862003	*MX1*	Yes	2.5E−126	−0.27	1.8E−77	−0.30
1	79088769	cg05696877	*IFI44L*	Yes	1.9E−120	−0.26	4.1E−86	−0.32
1	79085586	cg03607951	*IFI44L*	Yes	3.0E−141	−0.25	4.8E−83	−0.27
10	91153143	cg05552874	*IFIT1*	Yes	2.5E−128	−0.25	1.8E−73	−0.27
3	146260954	cg06981309	*PLSCR1*	Yes	4.9E− 157	−0.24	7.6E−91	−0.25
3	122281975	cg00959259	*PARP9*	Yes	9.3E−105	−0.23	6.1E−71	−0.27
11	315102	cg23570810	*IFITM1*	Yes	1.6E−5	−0.20	3.4E−60	−0.24
21	42797847	cg26312951	*MX1*	Yes	1.3E−82	−0.18	1.2E−59	−0.22
22	50971140	cg20098015	*ODF3B*	Yes	7.1E−96	−0.15	1.1E−52	−0.16
2	7004578	cg01028142	*CMPK2*	Yes	1.2E−64	−0.15	4.5E−47	−0.19
1	79085713	cg17980508	*IFI44L*	Yes	3.8E−179	−0.14	1.1E−79	−0.13
8	66751182	cg14864167	*PDE7A*	Yes	3.6E−41	−0.14	4.0E−37	−0.20
8	144099482	cg17052170	*LOC100133669,LY6E*	Yes	1.5E−58	−0.13	2.6E−35	−0.15
11	319667	cg09122035	*Intergenic*	NA	3.1E−72	−0.13	9.9E−41	−0.13
11	319555	cg20045320	*Intergenic*	NA	1.0E−63	−0.13	8.3E−38	−0.13
11	614761	cg08926253	*IRF7*	Yes	6.8E−81	−0.13	1.5E−54	−0.14
1	79085162	cg13304609	*IFI44L*	Yes	5.0E−63	−0.13	3.3E−47	−0.16
6	35654363	cg03546163	*FKBP5*	Yes	1.5E−66	−0.13	1.8E−24	−0.11
22	50973101	cg05523603	*Intergenic*	NA	4.7E−71	−0.13	2.0E−50	−0.14
16	87371097	cg01787084	*FBXO31*	NA	1.4E−126	−0.13	8.3E−61	−0.10
11	315262	cg03038262	*IFITM1*	Yes	1.6E−50	−0.12	2.7E−48	−0.17
1	79118191	cg01079652	*IFI44*	Yes	3.6E−43	−0.12	1.7E−25	−0.13
7	2444534	cg10152449	*CHST12*	Yes	3.6E−102	−0.11	3.5E−49	−0.10
6	29911550	cg17608381	*HLA-A*	Yes	4.8E−25	−0.11	4.1E−15	−0.12
2	7018020	cg10959651	*RSAD2*	Yes	2.9E−110	−0.11	3.1E−66	−0.12
11	319718	cg17990365	*IFITM3*	Yes	9.5E−56	−0.11	1.5E−35	−0.11
1	79085765	cg00855901	*IFI44L*	Yes	1.0E−135	−0.11	2.6E−70	−0.11
2	7016509	cg23213327	*RSAD2*	Yes	4.7E−92	−0.10	8.0E−58	−0.10
20	62204908	cg01190666	*PRIC285 (HELZ2)*	Yes	1.2E−111	−0.10	5.7E−61	−0.11
3	45984838	cg08450017	*CXCR6,FYCO1 (RUFY3)*	Yes	1.4E− 130	0.13	1.8E−51	0.10

DMCs with $|\Delta\beta| > 0.1$ in both the discovery and replication cohorts are listed.
*Database of interferon-regulated genes http://interferome.org.
†P value for case–control comparison using a linear regression model containing cell count estimates, age and sex as covariates.
‡Difference in average methylation beta value between patients and control individuals.

in the online Supplementary file 15). To increase the power to detect meQTLs for low frequency variants, the patients with SLE in the discovery and replication cohorts were combined for this analysis, as were the controls.

At 466 CpG sites of the 7245 replicated DMCs in SLE, we observed evidence of genetic control in the form of meQTLs in patients with SLE or controls ($P<6.5\times10^{-9}$) (see table S10 in the online Supplementary file 16). To investigate whether the meQTL SNPs could inform genetic associations from studies on SLE, we compared their P values for association with SLE to the P values for all SNPs on the ImmunoChip in a case–control genetic association analysis in a larger set of Swedish patients with SLE and controls ($n_{SLE}=1135$; $n_{ctrl}=2931$). We found that SNPs which are meQTLs for SLE-associated methylation changes were enriched for low P values in the genetic association analysis for SLE in our Swedish cohorts (figure 3). Among the SLE-associated meQTLs, we note seven GWAS risk loci for SLE[34–36]: *PTPRC* (CD45), *MHC-class III*, *UHRF1BP1*, *IRF5*, *IRF7*, *IKZF3* and *UBE2L3* (see table S11 in the online Supplementary file 16). This suggests that variants at SLE risk loci may in part exert their influence on the phenotype through alteration of DNA methylation levels at regulatory regions of target genes. For example, at the *UBE2L3* locus, the tested GWAS SNP is located downstream of the gene, but acts as a meQTL for an SLE associated DMC in the promoter of *UBE2L3* (figure 4). For some of the SLE GWAS loci, the meQTL effect was observed in both patients and controls and in others exclusively in the patient or control group (see figure S6 in the online Supplementary file 18).

Lastly, we investigated whether SNPs affected the methylation variance at DMCs in SLE. We found that a small fraction of the 7245 DMCs in SLE had SNPs associated with variation in DNA methylation levels (var-meQTLs; 20 unique CpG sites, see table S12 in the online Supplementary file 19). The most significant var-meQTLs in both patients and control individuals were observed for one CpG site (cg07180897) in the major histocompatibility complex (MHC) class II gene *HLA-DQB2*, which is a known SLE risk locus. Nineteen of the 20 var-meQTL CpG sites also had meQTLs, that is, the genotype affected both the mean DNA methylation and variance of DNA methylation at these sites.

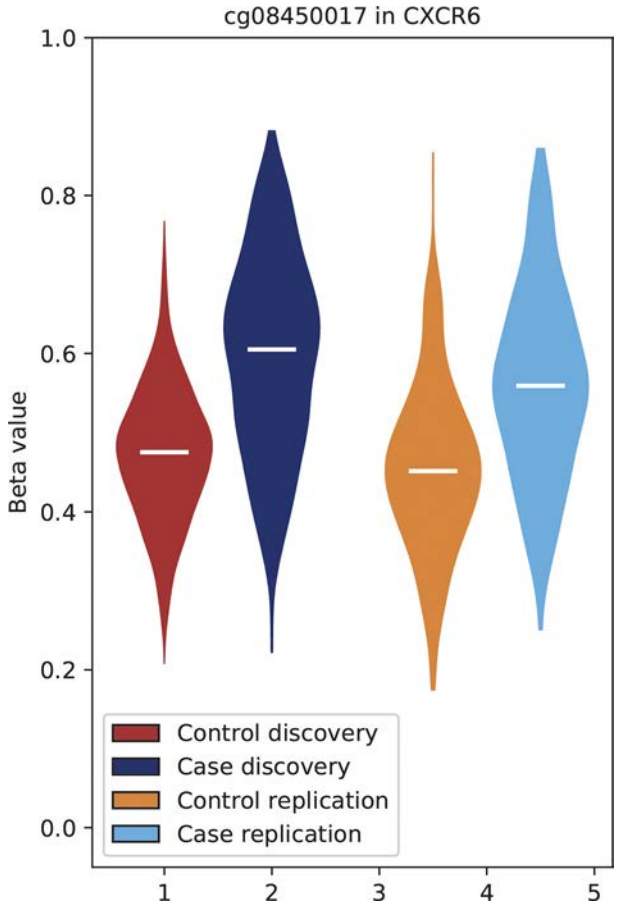


Figure 2 Violin plot of the DNA methylation levels at the *CXCR6* gene in patients with systemic lupus erythematosus (SLE) and control individuals. Methylation levels at the CpG site cg08450017 in *CXCR6* were increased in patients with SLE compared with controls in both the discovery and replication cohorts (P discovery=1.4×10^{-130} and P replication=1.8×10^{-51}). Median methylation beta values are represented by the white horizontal lines in the violin plots.

DISCUSSION

We find wide-spread DNA methylation changes in SLE, the majority of which exhibit decreased methylation levels in patients compared with healthy controls. The top signals replicate previously reported associations in fractionated blood cells from patients with SLE, and we identify a large number of novel associations. Previous SLE methylation studies have been performed in smaller numbers of samples, which most likely is the reason for the large number of novel signals that we observe. Among CpG sites that have to our knowledge not previously been reported as epigenetically associated to SLE, we note multiple DMCs with increased methylation levels in SLE located in the promoter region of *PDCD1* which encodes the PD-1 protein. PDCD1 acts an immune checkpoint receptor with a primary role in regulating T cell responses in order to maintain immune tolerance. Functional enrichment analyses indicate that the set of most significant DMCs in SLE are located in genes which play a role in regulating transcription in immune cells.

We observe a striking pattern of hypomethylation at IFN-signature genes, despite the fact that the majority of patients were inactive or under treatment at time of blood sampling. However, this IFN-pattern was more pronounced in patients with active disease. We have previously reported decreased methylation at IFN-induced genes also for primary Sjögren's syndrome.[37] We note that the pattern of hypomethylation at IFN-signature genes in blood is more pronounced in SLE, with patients with Sjögren's syndrome exhibiting average methylation levels which are intermediary to those of healthy individuals and patients with SLE. This is in line with gene expression studies showing an increased expression of IFN-induced genes in the vast majority of patients with SLE,[38] while the IFN-signature is less prevalent in primary Sjögren's syndrome.[39]

The study was conducted on whole blood samples and we corrected our analysis for major blood cell types. To analyse the systemic components of autoimmunity, blood is thought to be the most appropriate sample type, while mechanisms of local inflammation at specific target organs would require analysis of additional tissue types.[40] DNA extracted from whole blood is more readily available for analysis, but to fully decipher the contribution of DNA methylation variation in SLE, additional analyses of fractionated cells are needed. Such studies would have the ability to detect DNA methylation changes in SLE that are restricted to smaller cell subsets.

Despite previous reports of DMCs for ACR criteria, we were unable to formally replicate any of the associations with ACR criteria we observed in the discovery cohort. Reasons for the difference between this and previous studies could be the different cell types and different study designs that were used in the analyses.[14 16] Factors that complicate the analysis of altered DNA methylation in relation to the clinical criteria are that the SLE ACR criteria are collected cumulatively over a patient's disease course and that individual patients fulfil multiple criteria.

Table 2 Enrichment analyses of gene ontology (GO) terms based on the top 500 replicated differentially methylated CpG sites (according to association P value in the discovery cohort) with gene name annotation and the most significant GO terms are shown*

	Molecules	P value
Molecular function		
Enzyme binding GO:0019899	*PDE4D;ACACA;TBC1D2;MCM2;RXRA;TBC1D16;ADORA2A;CLU;MAP3K11;FXYD1;PLSCR1;CUL1;STX8;VRK2;ANKFY1;POR;AMBRA1;CBX4;APP;YWHAG;PRDX6;ERLIN1;CRY2;NCOR2;STC2;PRKAA1;PDE4DIP;PRKAG1;CSTA;EIF3A;PRKCZ;SLC12A7;MAP2K6;ATP2A2;RAB11FIP3;SP1;HDAC4;SPTBN1;MAML1;NCK1;NDUFS2;LAX1;KSR1;RCOR1;TBC1D1;RALBP1;KCNQ1;MMS19;RAB13;SMG6;DNMT3B;CACNA1C;ATF7;CALR;GRK5;CAST;RDX;HNRNPUL1;LCK;RUNX2;RUNX3;CCND2;SCARB2;CD44;LRP4;ELANE;PPP1R18;LRPAP1;TNFRSF1B;CDH1;ENO1;SMAD3;EXOC4*	4.6E−06
Regulatory region DNA binding GO:0000975	*BACH2;ZMYND8;ETS2;ACTN4;BCL11A;RREB1;RFX8;RXRA;GATAD2B;PRDX5;MNT;CBX4;CRY2;NCOR2;ZNF335;ZNF516;CUX1;GABPB1;SP1;ZBTB16;ZNF148;HDAC4;IRF5;RCOR1;NR1I2;NFE2;NFIL3;EHF;ATF7;TCF12;ARID3A;NRF1;AKNA;NR1H3;E2F3;RUNX2;RUNX1;RUNX3;IKZF4;LMO2;SMAD3*	4.7E−06
Biological process		
Leucocyte activation GO:0045321	*PDCD1;HLA DMB;TUSC2;HLX;BCL11A;CD83;ADORA2A;PRAM1;FCER1G;CLU;FCGR3B;MAD1L1;FES;AIF1;PLSCR1;PILRB;SFTPD;ZBTB32;PIK3R6;PRF1;ZNF335;PRKCZ;ZBTB16;IMPDH1;HDAC4;TNFSF13;AZU1;NCK1;LAX1;PTPRE;ZFP36L1;DOCK2;LST1;RIPK3;NR1H3;LCK;RUNX2;LFNG;NLRP3;CORO1A;CD247;CD44;LY9;SMAD3;CDK6*	3.8E−08

*Functional gene-set enrichment analysis was performed using the ToppGene Suite database https://toppgene.cchmc.org.

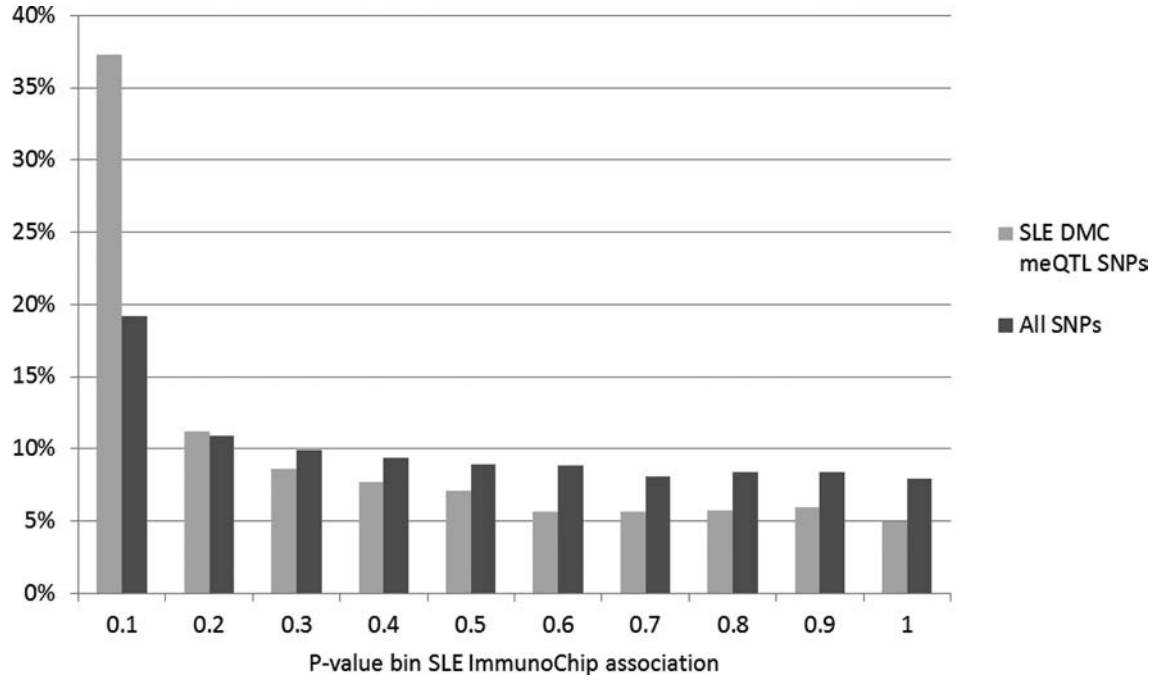


Figure 3 Enrichment of associated genetic variants in systemic lupus erythematosus (SLE) to methylation quantitative trait loci (meQTL) single nucleotide polymorphisms (SNPs) for differentially methylated CpG sites (DMCs) in SLE. The x-axis represents bins of P values from an SLE case–control genetic association analysis including 1135 Swedish patients with SLE and 2931 control individuals. Light grey bars represent SNPs which are significant meQTLs for CpG sites differentially methylated in SLE (466 CpG sites; 5307 SNPs). Bars in darker grey represent all SNPs on the ImmunoChip (133 838 quality controlled SNPs).

Longitudinal studies of DNA methylation would be useful in disentangling its role in clinical presentation of SLE.

Association with prescribed medications revealed a large number of affected CpG sites in patients treated with glucocorticoids. However, the majority of the observed DMCs in SLE were nominally significant also in the group of patients not treated with glucocorticoids. This indicates that the replicated SLE DMCs are not mainly driven by treatment effects. A previous study on the effects of systemic glucocorticoid exposure in patients with chronic obstructive pulmonary disease revealed that the majority of associated CpG sites had decreased methylation levels in treated patients,[41] which is in line with the results presented here. Association of DNA methylation patterns with treatment may be confounded by the underlying cause for prescribing the drug and analyses of treatment effects on DNA methylation are hampered by high rates of medication non-adherence in SLE.[42]

It has previously been suggested for rheumatoid arthritis that DNA methylation could be a mediator of genetic risk in the disease,[43] and we have recently reported genetic regulation of methylation at GWAS risk loci for Sjögren's syndrome.[37] Similarly, we here observe evidence of genetic regulation of DNA

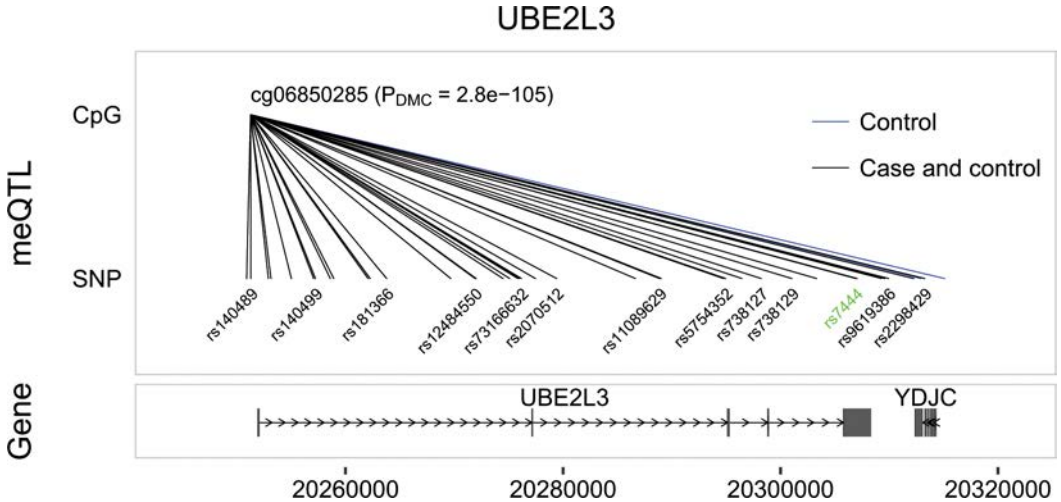


Figure 4 Illustration of genetic regulation of DNA methylation at the *UBE2L3* genetic susceptibility locus for systemic lupus erythematosus (SLE) from a genome-wide association study (GWAS). The *UBE2L3* locus on chromosome 22 with the differentially methylated CpG site (DMC) cg06850285 from the epigenome-wide association study is indicated at the top panel. The middle panel represents significant methylation quantitative trait loci (meQTLs) in controls only (illustrated by blue lines) or shared in both cases and controls (illustrated by black lines). The GWAS index single nucleotide polymorphism (SNP) is indicated in green. The bottom panel illustrates the RefSeq genes in the region.

methylation at DMCs in SLE. Notably, we find GWAS variants associated with risk for SLE among the significant meQTLs, suggesting a functional mechanism for these genetic variants. However, since the coverage of CpG sites at SLE GWAS loci was low for the HM450k BeadChip, we have limited possibilities of fine-mapping the association signals. The fact that some meQTLs are observed exclusively in either the patient or control group suggests that a subset of the meQTLs that we detect are context dependent. These contexts could, for example, be differences in cell type composition as previously reported for eQTLs.[44] The majority of meQTLs that we report are, however, shared between patients and controls. In contrast to genetic regulation of mean methylation levels which was observed for hundreds of CpG sites, we only observed genetic regulation of methylation variance at 20 DMCs in SLE. This suggests that genetic regulation of DNA methylation in SLE mainly is operating via effects on DNA methylation levels means, but that a smaller set of variants also have the ability to influence phenotype plasticity.

A main limitation of these data is that it is not possible to infer whether the methylation differences in SLE are causes or effects of the disease. Longitudinal studies will be required to completely elucidate the role of DNA methylation in SLE disease aetiology. In addition, it is possible that differences in proportions of cell subtypes affected the results. Another limitation is that only methylation at a defined fraction of all CpG sites in the genome was analysed. Alternative approaches such as whole genome bisulfite sequencing of fractionated cells have the potential to fully characterise the epigenetic landscape in SLE. Epigenetic variants could be the starting point for developing novel epigenetic biomarkers to improve diagnosis in SLE, and the reversible nature of epigenetic marks suggests them as potential targets for therapeutic interventions.

Acknowledgements We thank Rezvan Kiani, Marianne Petersson and Karolina Tandre for collecting samples from patients and controls and medicinska Biobanken Norr for providing samples from control individuals. Genotyping and DNA methylation analyses were performed at the SNP&SEQ Technology Platform at the National Genomics Infrastructure (NGI) hosted by Science for Life Laboratory in Uppsala, Sweden (www.genotyping.se; www.sequencing.se). We thank Tomas Axelsson, Anna Haukkala and Anders Lundmark for excellent technical assistance. We especially thank all patients and blood donors who donated samples to this study.

Contributors JI-K, A-CS, JKS designed the study and drafted the manuscript. DL, GN, M-LE, SR-D, AAB, AJ, LP, IG, ES, CS and LR collected patient and control material and clinical data. JI-K and JKS performed the experiments. JI-K, JKS, AA and JCA analysed the data. All authors read and provided critical review and accepted the final version of the manuscript.

Funding This study was supported by grants from the Knut and Alice Wallenberg Foundation (KAW 2011.0073), the Swedish Research Council for Medicine and Health (VR-MH Dnr 521-2014-2263 to ACS, Dnr 521-2013-2830 to LR, Dnr 521-2014-3954 to ES and Dnr 2016-01982 to GN), an AstraZeneca-Science for Life Laboratory research collaboration grant (to LR), the Swedish Society for Medical Research (to CS), the Swedish Rheumatism Association (to CS and DL), King Gustaf V's 80th Birthday Fund (to DL) and a Swedish Research Council postdoc grant (VR Dnr 350-2012-256 to JKS). Funding to collect samples and characterise patients/controls from the Karolinska University hospital was provided by the Swedish Heart-Lung foundation (ES), Stockholm County Council (ALF) (ES and IG), King Gustaf V's 80th Birthday Fund (ES and IG) and the Swedish Rheumatism Association (ES and IG). The SNP&SEQ Technology Platform is supported by Science for Life Laboratory, the Knut and Alice Wallenberg Foundation and the Swedish Research Council (VR-RFI).

Competing interests None declared.

Patient consent Obtained.

Ethics approval The study was approved by the Regional Ethics board in Uppsala with Dnr 00-227 and 2016/155.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Normalised or raw intensity data of the HM450k BeadChips are available upon request from the authors on a collaborative basis.

REFERENCES

1 Bengtsson AA, Rönnblom L. Systemic lupus erythematosus: still a challenge for physicians. *J Intern Med* 2017;281:52–64.
2 Hagberg N, Rönnblom L. Systemic lupus erythematosus – a disease with a dysregulated type I interferon system. *Scand J Immunol* 2015;82:199–207.
3 Chen L, Morris DL, Vyse TJ. Genetic advances in systemic lupus erythematosus: an update. *Curr Opin Rheumatol* 2017;29:423–33.
4 Langefeld CD, Ainsworth HC, Cunninghame Graham DS, *et al*. Transancestral mapping and genetic load in systemic lupus erythematosus. *Nat Commun* 2017;8:16021.
5 Deng Y, Tsao BP. Advances in lupus genetics and epigenetics. *Curr Opin Rheumatol* 2014;26:482–92.
6 Teruel M, Alarcón-Riquelme ME. Genetics of systemic lupus erythematosus and Sjögren's syndrome: an update. *Curr Opin Rheumatol* 2016;28:506–14.
7 Lu Q, Qiu X, Hu N, *et al*. Epigenetics, disease, and therapeutic interventions. *Ageing Res Rev* 2006;5:449–67.
8 Javierre BM, Fernandez AF, Richter J, *et al*. Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. *Genome Res* 2010;20:170–9.
9 Richardson B, Scheinbart L, Strahler J, *et al*. Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum* 1990;33:1665–73.
10 Rubin RL. Drug-induced lupus. *Expert Opin Drug Saf* 2015;14:361–78.
11 Absher DM, Li X, Waite LL, *et al*. Genome-wide DNA methylation analysis of systemic lupus erythematosus reveals persistent hypomethylation of interferon genes and compositional changes to CD4+ T-cell populations. *PLoS Genet* 2013;9:e1003678.
12 Coit P, Jeffries M, Altorok N, *et al*. Genome-wide DNA methylation study suggests epigenetic accessibility and transcriptional poising of interferon-regulated genes in naïve CD4+ T cells from lupus patients. *J Autoimmun* 2013;43:78–84.
13 Chung SA, Nititham J, Elboudwarej E, *et al*. Genome-Wide Assessment of Differential DNA Methylation Associated with Autoantibody Production in Systemic Lupus Erythematosus. *PLoS One* 2015;10:e0129813.
14 Coit P, Renauer P, Jeffries MA, *et al*. Renal involvement in lupus is characterized by unique DNA methylation changes in naïve CD4+ T cells. *J Autoimmun* 2015;61:29–35.
15 Renauer P, Coit P, Jeffries MA, *et al*. DNA methylation patterns in naïve CD4+ T cells identify epigenetic susceptibility loci for malar rash and discoid rash in systemic lupus erythematosus. *Lupus Sci Med* 2015;2:e000101.
16 Mok A, Solomon O, Nayak RR, *et al*. Genome-wide profiling identifies associations between lupus nephritis and differential methylation of genes regulating tissue hypoxia and type 1 interferon responses. *Lupus Sci Med* 2016;3:e000183.
17 Ighe A, Dahlström Ö, Skogh T, *et al*. Application of the 2012 Systemic Lupus International Collaborating Clinics classification criteria to patients in a regional Swedish systemic lupus erythematosus register. *Arthritis Res Ther* 2015;17:3.
18 Tan EM, Cohen AS, Fries JF, *et al*. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–7.
19 Bibikova M, Barnes B, Tsan C, *et al*. High density DNA methylation array with single CpG site resolution. *Genomics* 2011;98:288–95.
20 R Core Team. *R: a language and environment for statistical computing: R Foundation for Statistical Computing*. Vienna, Austria: R Core Team, 2016.
21 Aryee MJ, Jaffe AE, Corrada-Bravo H, *et al*. Minfi: a flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics* 2014;30:1363–9.
22 Maksimovic J, Gordon L, Oshlack A. SWAN: Subset-quantile within array normalization for illumina infinium HumanMethylation450 BeadChips. *Genome Biol* 2012;13:R44.
23 Cortes A, Brown MA. Promise and pitfalls of the Immunochip. *Arthritis Res Ther* 2011;13:101.
24 Houseman EA, Accomando WP, Koestler DC, *et al*. DNA methylation arrays as surrogate measures of cell mixture distribution. *BMC Bioinformatics* 2012;13:86.
25 Purcell S, Neale B, Todd-Brown K, *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;81:559–75.
26 Qin M, Guo Y, Jiang L, *et al*. Elevated levels of serum sCXCL16 in systemic lupus erythematosus; potential involvement in cutaneous and renal manifestations. *Clin Rheumatol* 2014;33:1595–601.
27 Wen S, He F, Zhu X, *et al*. IFN-γ, CXCL16, uPAR: potential biomarkers for systemic lupus erythematosus. *Clin Exp Rheumatol* 2017 [Epub ahead of print 16 Jun 2017].

28 Coit P, Yalavarthi S, Ognenovski M, *et al*. Epigenome profiling reveals significant DNA demethylation of interferon signature genes in lupus neutrophils. *J Autoimmun* 2015;58:59–66.
29 Zhu H, Mi W, Luo H, *et al*. Whole-genome transcription and DNA methylation analysis of peripheral blood mononuclear cells identified aberrant gene regulation pathways in systemic lupus erythematosus. *Arthritis Res Ther* 2016;18:162.
30 Prokunina L, Castillejo-López C, Oberg F, *et al*. A regulatory polymorphism in PDCD1 is associated with susceptibility to systemic lupus erythematosus in humans. *Nat Genet* 2002;32:666–9.
31 Gao J, Gai N, Wang L, *et al*. Meta-analysis of programmed cell death 1 polymorphisms with systemic lupus erythematosus risk. *Oncotarget* 2017;8:36885–97.
32 Okazaki T, Wang J. PD-1/PD-L pathway and autoimmunity. *Autoimmunity* 2005;38:353–7.
33 Bierie B, Edwin M, Melenhorst JJ, *et al*. The proliferation associated nuclear element (PANE1) is conserved between mammals and fish and preferentially expressed in activated lymphoid cells. *Gene Expr Patterns* 2004;4:389–95.
34 Morris DL, Sheng Y, Zhang Y, *et al*. Genome-wide association meta-analysis in Chinese and European individuals identifies ten new loci associated with systemic lupus erythematosus. *Nat Genet* 2016;48:940–6.
35 Bentham J, Morris DL, Graham DSC, *et al*. Genetic association analyses implicate aberrant regulation of innate and adaptive immunity genes in the pathogenesis of systemic lupus erythematosus. *Nat Genet* 2015;47:1457–64.
36 Welter D, MacArthur J, Morales J, *et al*. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* 2014;42:D1001–6.
37 Imgenberg-Kreuz J, Sandling JK, Almlöf JC, *et al*. Genome-wide DNA methylation analysis in multiple tissues in primary Sjögren's syndrome reveals regulatory effects at interferon-induced genes. *Ann Rheum Dis* 2016;75:2029–36.
38 Chiche L, Jourde-Chiche N, Whalen E, *et al*. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol* 2014;66:1583–95.
39 Brkic Z, Maria NI, van Helden-Meeuwsen CG, *et al*. Prevalence of interferon type I signature in CD14 monocytes of patients with Sjogren's syndrome and association with disease activity and BAFF gene expression. *Ann Rheum Dis* 2013;72:728–35.
40 Cole MB, Quach H, Quach D, *et al*. Epigenetic signatures of salivary gland inflammation in sjögren's syndrome. *Arthritis Rheumatol* 2016;68:2936–44.
41 Wan ES, Qiu W, Baccarelli A, *et al*. Systemic steroid exposure is associated with differential methylation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2012;186:1248–55.
42 Costedoat-Chalumeau N, Pouchot J, Guettrot-Imbert G, *et al*. Adherence to treatment in systemic lupus erythematosus patients. *Best Pract Res Clin Rheumatol* 2013;27:329–40.
43 Liu Y, Aryee MJ, Padyukov L, *et al*. Epigenome-wide association data implicate DNA methylation as an intermediary of genetic risk in rheumatoid arthritis. *Nat Biotechnol* 2013;31:142–7.
44 Zhernakova DV, Deelen P, Vermaat M, *et al*. Identification of context-dependent expression quantitative trait loci in whole blood. *Nat Genet* 2017;49:139–45.

EXTENDED REPORT

Poly(ADP-ribose) polymerase-1 regulates fibroblast activation in systemic sclerosis

Yun Zhang,[1] Sebastian Pötter,[1] Chih-Wei Chen,[1] Ruifang Liang,[1] Kolja Gelse,[2] Ingo Ludolph,[3] Raymund E Horch,[3] Oliver Distler,[4] Georg Schett,[1] Jörg H W Distler,[1] Clara Dees[1]

Handling editor Tore K Kvien

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212265).

[1]Department of Internal Medicine 3 for Rheumatology and Immunology, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), University Hospital of Erlangen, Erlangen, Germany
[2]Department of Trauma Surgery, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), University Hospital of Erlangen, Erlangen, Germany
[3]Department of Plastic and Hand Surgery, Laboratory for Tissue Engineering and Regenerative Medicine, Friedrich-Alexander University of Erlangen-Nürnberg (FAU), University Hospital of Erlangen, Erlangen, Germany
[4]Research of Systemic Autoimmune Diseases, University Hospital Zurich, Zurich, Switzerland

Correspondence to
Clara Dees, Department of Internal Medicine 3 for Rheumatology and Immunology, Friedrich-Alexander-University Erlangen-Nürnberg (FAU), University Hospital of Erlangen, Erlangen 91054, Germany; clara.dees@uk-erlangen.de

JHWD and CD contributed equally.

Received 21 August 2017
Revised 4 January 2018
Accepted 16 January 2018
Published Online First 3 February 2018

To cite: Zhang Y, Pötter S, Chen C-W, *et al*. *Ann Rheum Dis* 2018;**77**:744–751.

ABSTRACT

Objectives The enzyme poly(ADP-ribose) polymerase-1 (PARP-1) transfers negatively charged ADP-ribose units to target proteins. This modification can have pronounced regulatory effects on target proteins. Recent studies showed that PARP-1 can poly(ADP-ribosyl)ate (PARylate) Smad proteins. However, the role of PARP-1 in the pathogenesis of systemic sclerosis (SSc) has not been investigated.

Methods The expression of PARP-1 was determined by quantitative PCR and immunohistochemistry. DNA methylation was analysed by methylated DNA immunoprecipitation assays. Transforming growth factor-β (TGFβ) signalling was assessed using reporter assays, chromatin immunoprecipitation assays and target gene analysis. The effect of PARP-1 inactivation was investigated in bleomycin-induced and topoisomerase-induced fibrosis as well as in tight-skin-1 (Tsk-1) mice.

Results The expression of PARP-1 was decreased in patients with SSc, particularly in fibroblasts. The promoter of *PARP-1* was hypermethylated in SSc fibroblasts and in TGFβ-stimulated normal fibroblasts. Inhibition of DNA methyltransferases (DNMTs) reduced the promoter methylation and reactivated the expression of PARP-1. Inactivation of PARP-1 promoted accumulation of phosphorylated Smad3, enhanced Smad-dependent transcription and upregulated the expression of TGFβ/Smad target genes. Inhibition of PARP-1 enhanced the effect of TGFβ on collagen release and myofibroblast differentiation in vitro and exacerbated experimental fibrosis in vivo. PARP-1 deficiency induced a more severe fibrotic response to bleomycin with increased dermal thickening, hydroxyproline content and myofibroblast counts. Inhibition of PARylation also exacerbated fibrosis in Tsk-1 mice and in mice with topoisomerase-induced fibrosis.

Conclusion PARP-1 negatively regulates canonical TGFβ signalling in experimental skin fibrosis. The downregulation of PARP-1 in SSc fibroblasts may thus directly contribute to hyperactive TGFβ signalling and to persistent fibroblast activation in SSc.

INTRODUCTION

Systemic sclerosis (SSc) is a chronic fibrosing connective tissue disease of unknown aetiology that affects the skin and various internal organs. A major hallmark of SSc is the uncontrolled release of excessive amounts of extracellular matrix by persistently activated fibroblasts, which perturbs the normal architecture of the affected tissues and thus leading to progressive organ dysfunction.[1 2] Transforming growth factor-β (TGFβ) has been characterised as a key mediator of fibroblast activation in fibrotic diseases.[3] Numerous studies demonstrate increased TGFβ signalling in SSc and other fibrotic diseases and highlight that persistent activation of TGFβ is sufficient to induce fibrosis,[4 5] although the underlying molecular mechanisms that lead to the uncontrolled activation of TGFβ signalling remain incompletely understood. However, fibroblasts isolated from patients with SSc retain their activated phenotype over several passages in culture,[6] suggesting that cell endogenous mechanisms play a key role in fibroblast activation. These endogenous mechanisms may be epigenetic modifications. The best studied epigenetic modification to date is DNA methylation, which leads to the formation of densely packed heterochromatin and thus to gene silencing.[7] Indeed, several studies showed elevated methylation levels in the promoter regions of antifibrotic genes.[8–10] In contrast to changes in the nucleotide sequence, epigenetic changes are reversible. DNA methyltransferases (DNMTs) can be targeted by small molecule inhibitors like 5-azacytidine and 5-aza-2′-deoxycytidine that are already approved for clinical use in different haematological diseases.

Poly(ADP-ribose) polymerases (PARPs) are enzymes that transfer ADP-ribose groups onto various substrate proteins either as monomeric or oligomeric moieties or as linear or branched poly-ADP-ribose (PAR) chains.[11 12] As the so-called PARylation modulates protein half-life or subcellular localisation, PARP-1 has profound regulatory effects on various processes.[13] Of the 18 members of the PARP family in humans, PARP-1 is by far most well characterised.[11] PARP-1 is involved in multiple aspects of cellular metabolism, such as transcription, chromatin remodelling, apoptosis and DNA repair.[14] Of particular interest for fibrotic diseases, PARP-1 has recently been shown to PARylate Smad3 and to either positively or negatively regulate Smad-mediated transcription, depending on the cellular context.[15 16] Moreover, PARP-1 can contribute to carcinogenesis by promoting cancer cell survival in response to genotoxic insults, which may allow cells to survive and accumulate DNA damage. The involvement of PARP proteins in the cellular response to DNA damage or cellular stress responses suggests that PARPs are attractive candidates for novel therapies for the treatment of cancer

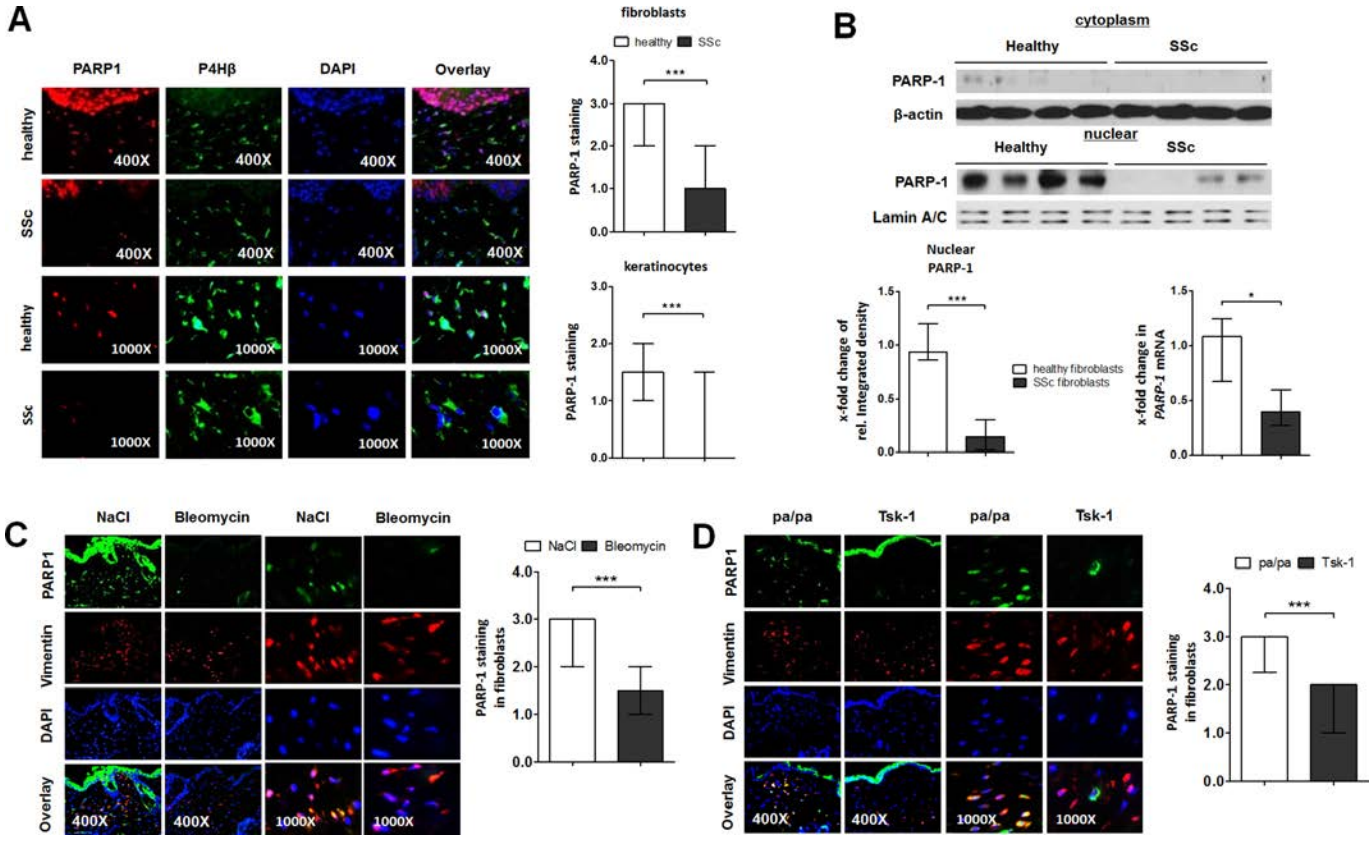


Figure 1 The expression of PARP-1 is decreased in SSc. (A) PARP-1 immunofluorescence staining in the skin of patients with SSc and matched healthy volunteers with costaining for the fibroblast marker prolyl-4-hydroxylase-β (P4Hβ) and 4',6-diamidino-2-phenylindole (DAPI) are shown at 400-fold and 1000-fold magnification. Semiquantitative analysis of PARP-1 staining in fibroblasts and keratinocytes in the skin of patients with SSc and healthy volunteers (n=12 patients with SSc and 10 controls). (B) Protein levels (left) and mRNA levels (right) of PARP-1 in fibroblasts of healthy individuals and patients with SSc. (C and D) Immunofluorescence staining for PARP-1 with costaining for vimentin and DAPI at 400-fold and 1000-fold magnification and semiquantitative analyses of PARP-1 expression in the skin of mice challenged with bleomycin and non-fibrotic control mice (n=6) (C) and in the skin samples of control pa/pa mice and Tsk-1 mice (n=6) (D). *P < 0.05, **P< 0.01, ***P < 0.001. PARP-1, poly(ADP-ribose) polymerase-1; SSc, systemic sclerosis; Tsk-1, tight-skin-1.

and for autoimmune diseases.[13 17 18] Indeed, numerous PARP inhibitors have been developed, some of which have recently entered clinical trials for stroke or cancer (http://clinicaltrials.gov; NCT01983358 and NCT01351909).

Here, we aimed to investigate the role of PARP-1 in the pathogenesis of SSc. We demonstrate that PARP-1 is downregulated in SSc by increased DNA methylation in the PARP-1 promoter region. The decreased expression of PARP-1 may directly contribute to hyperactive TGFβ signalling in SSc, as overexpression of PARP-1 ameliorated the stimulatory effects of TGFβ on fibroblasts, pharmacological or genetic inactivation of PARP-1 promoted TGFβ-induced fibroblast activation in vitro and exacerbated experimental fibrosis in vivo. Our data thus reveal the downregulation of PARP-1 in SSc fibroblasts as a potential novel mechanism for the persistent activation of fibroblast in SSc.

MATERIAL AND METHODS

Patient samples and fibroblast culture

Skin biopsies were obtained from the volar aspect of the forearm of 23 patients with SSc. All patients fulfilled the American College of Rheumatology/European League Against Rheumatism criteria for SSc.[19] The study included 16 female and 7 male patients with SSc. The median age was 53 years, ranging from 20 years to 71 years, and median disease duration was 7 years, ranging from 1 year to 15 years. Seven patients had limited cutaneous disease, while 13 patients had diffuse cutaneous disease. Patients did not receive any disease-modifying antirheumatic drug treatment at the time of biopsy. Active disease was defined according to the European Scleroderma Trials and Research group (EUSTAR) criteria for disease activity.[20] Twenty age-matched and sex-matched healthy volunteers served as controls.

Pharmacological inhibition of PARP-1, TGFβ signalling and DNMTs

The experimental procedure about inhibition of PARP-1, TGFβ and DNMTs is summarised in the online supplementary methods.

Quantitative real-time PCR

Gene expression was quantified by real-time PCR using the MxPro 3005P QPCR System (Agilent Technologies, Santa Clara, California, USA) as previously described.[21 22] The primer sequences are summarised in online supplementary table 1.

Immunohistochemical analyses

Immunohistochemical analysis of paraffin-embedded sections was performed as previously described.[23 24] The experimental procedure is summarised in the online supplementary methods.

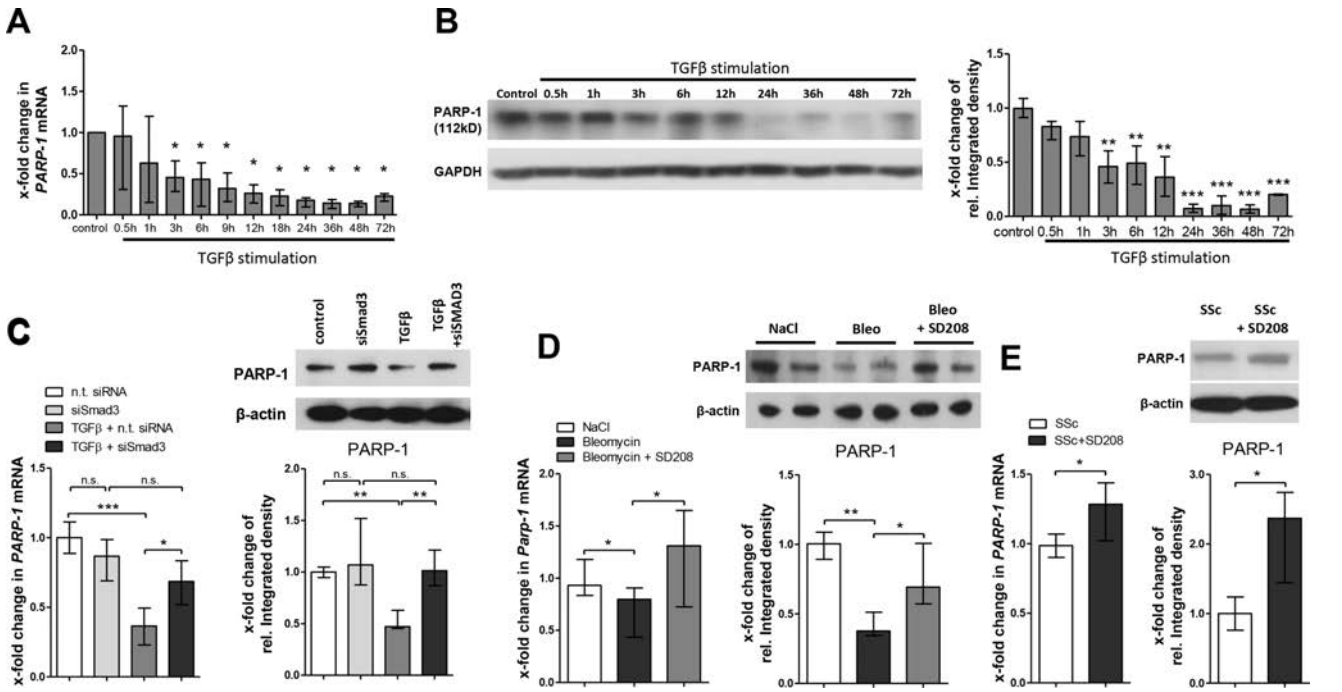


Figure 2 TGFβ downregulates the expression of PARP-1 in healthy fibroblasts. (A) mRNA levels of PARP-1 in human fibroblasts stimulated with TGFβ (n=4 biological replicates with ≥2 technical replicates). (B) Western blot for the protein levels of PARP-1 in human fibroblasts at different time points after stimulation with TGFβ (n=4 biological replicates with ≥2 technical replicates). (C) Effects of siRNA-mediated knockdown of Smad3 on the mRNA and protein levels of PARP-1 (n=4 biological replicates with ≥2 technical replicates). (D) mRNA and protein levels of PARP-1 in the skin of control mice, mice challenged with bleomycin and bleomycin-challenged mice treated with TGFβ receptor type I kinase inhibitor SD208. (E) mRNA and protein levels of PARP-1 in SSc fibroblasts after incubation with SD208 (n=6 biological replicates with ≥2 technical replicates for in vitro and in vivo experiments). *P< 0.05, **P< 0.01, ***P < 0.001. PARP-1, poly(ADP-ribose) polymerase-1; SSc, systemic sclerosis; TGFβ, transforming growth factor-β.

Methylated DNA immunoprecipitation (MeDIP) and chromatin immunoprecipitation (ChIP) assays

Details about MeDIP and ChIP assays are provided in the online supplementary.

PARP-1 overexpression

Human PARP-1 was amplified from whole blood and cloned into the pDNOR221 plasmid (Invitrogen). Empty pDNOR221 plasmid served as control. Human dermal fibroblasts were transfected using the Lonza 4D-Nucleofector (Lonza, Verviers, Belgium).[25]

Western blot and coimmunoprecipitation

The experimental procedure is summarised in the online supplementary methods.

Smad-binding sequences (CAGA) reporter assays

One thousand infectious units (ifu) CAGA viruses per fibroblast were added in 1% serum supernatant for 48 hours.[25] The infections were conducted in triplicate for different treatments of each cell line. The luciferase activities of fibroblast lysates were evaluated using Microwin software.

Mouse models of fibrosis

Three different mouse models of SSc were used: bleomycin-induced or topoisomerase-induced skin fibrosis and the Tsk-1 model. A detailed description of the experiments is given in the online supplementary methods.

Statistical analysis

Data are expressed as median with IQR, and differences between the groups were tested for their statistical significance by non-parametric Mann-Whitney U test. P values less than 0.05 were considered significant.

Additional experimental details are provided in the online supplementary.

RESULTS

The expression of PARP-1 is decreased in SSc

We first analysed the expression of PARP-1 in the skin of patients with SSc and matched healthy volunteers by immunofluorescence and immunohistochemistry. SSc skin showed less intensive staining of PARP-1 compared with healthy skin (figure 1A and online supplementary figure 1). Semiquantitative analysis of the costaining of PARP-1 and the fibroblast marker prolyl-4-hydroxylase-β demonstrated reduced expression of PARP-1 in SSc fibroblasts as compared with healthy skin (figure 1A) and showed trends towards more pronounced decreases in patients with diffuse cutaneous SSc and active disease as compared with limited cutaneous SSc and stable disease. Although there was a trend towards an inverse correlation of PARP-1 staining with the modified Rodnan skin score, no statistical significance was achieved. PARP-1 mRNA levels as well as the nuclear levels of PARP-1 protein were also significantly decreased in cultured fibroblasts from patients with SSc even after several passages in vitro (figure 1B). The findings in human SSc skin were mimicked by murine models of SSc with reduced Parp-1 levels in fibroblasts in bleomycin-induced fibrosis (figure 1C) and in Tsk-1 mice (figure 1D) as compared with non-fibrotic control mice.

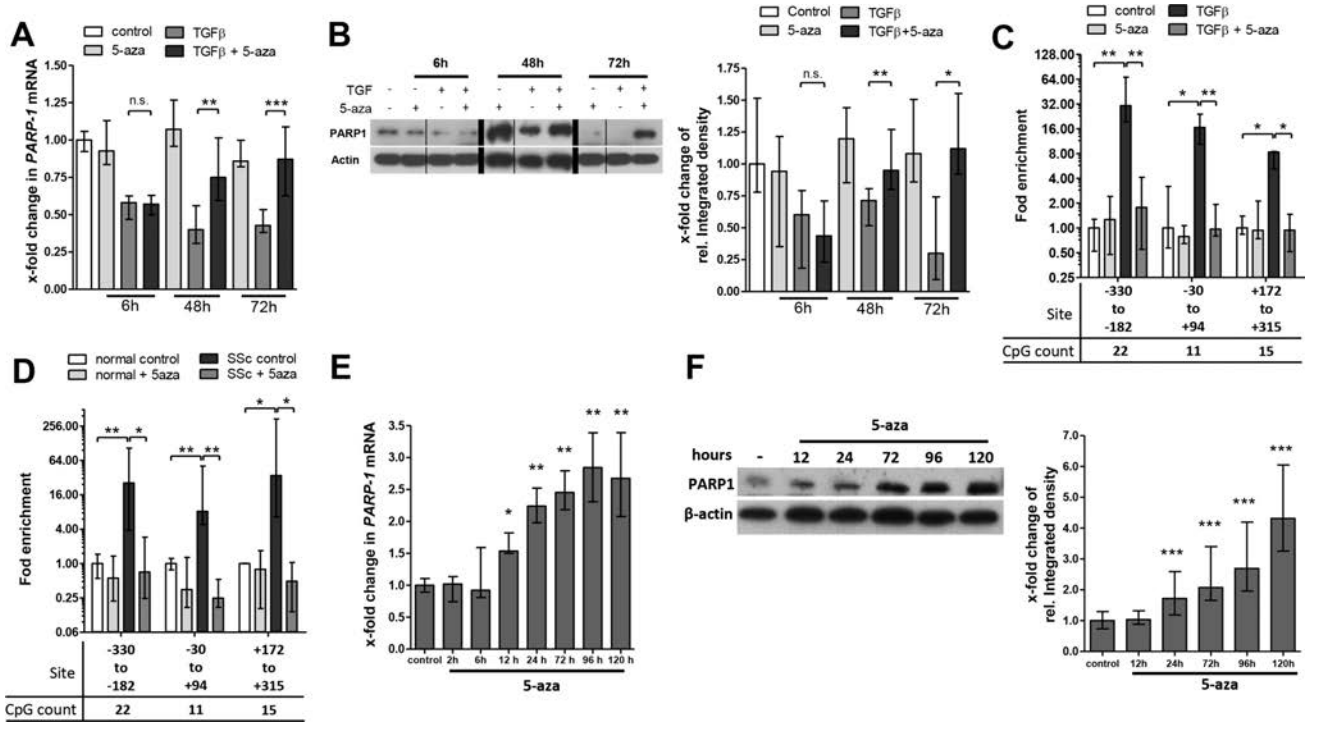


Figure 3 PARP-1 expression is regulated by DNA methylation. (A) mRNA levels of PARP-1 in human fibroblasts stimulated with TGFβ and incubated with 5-aza for 6, 48 and 72 hours (n=5 biological replicates with three technical replicates). (B) Protein levels of PARP-1 in human fibroblasts stimulated with TGFβ and incubated with 5-aza for 6, 48 and 72 hours (n=3 biological replicates with three technical replicates). (C) MeDIP analyses of the PARP-1 promoter in normal human fibroblasts stimulated with TGFβ and incubated with 5-aza. Sites are given with respect to the transcription start site (n=5 biological replicates with two technical replicates). (D) MeDIP analyses of the PARP-1 promoter in normal and SSc fibroblasts in the absence of external stimulation. Sites are given with respect to the transcription start site (n=5 biological replicates with two technical replicates). (E) mRNA levels of PARP-1 in SSc fibroblasts on incubation with 5-aza (n=5 biological replicates with two technical replicates). (F) Protein levels of PARP-1 in SSc fibroblasts on incubation with 5-aza at different time points (n=6 biological replicates with two technical replicates). *P< 0.05, **P< 0.01, ***P < 0.001. MeDIP, methylated DNA immunoprecipitation; PARP-1, poly(ADP-ribose) polymerase-1; SSc, systemic sclerosis; TGFβ, transforming growth factor-β.

The levels of PARP-1 are reduced by TGFβ stimulation

Given the central role of TGFβ in fibrotic diseases, we wondered whether the expression of PARP-1 might be regulated by TGFβ. Indeed, we observed a time-dependent decrease of PARP-1 mRNA and protein on stimulation with TGFβ in cultured fibroblasts (figure 2A,B). Knockdown of SMAD3 abrogated the TGFβ-induced downregulation of PARP-1 demonstrating that TGFβ-induced repression of PARP-1 is dependent on canonical Smad signalling (figure 2C and online supplementary figure 2). Moreover, selective inhibition of TGFβ signalling in bleomycin-induced fibrosis by SD-208 prevented the downregulation of Parp-1, and inhibition of TGFβ signalling in SSc fibroblasts upregulated PARP-1 expression (figure 2D,E), further highlighting the central role of TGFβ in regulating PARP-1 expression in fibrosis.

PARP-1 is silenced by TGFβ-induced promoter hypermethylation

Given accumulating evidence for a central role of DNA-hypermethylation in the pathogenesis of SSc,[8–10 26–31] we hypothesised that TGFβ may induce DNA methylation of the PARP-1 promoter to induce chronic silencing of PARP-1 expression. Indeed, incubation with the DNMT inhibitor 5-aza strongly blocked the inhibitory effects of TGFβ on PARP-1 mRNA and protein in normal dermal fibroblasts at later timepoints (48 and 72 hours) (figure 3A,B). Consistent with the proposed model, 5-aza did not interfere with the early effects of TGFβ on PARP-1 expression as analysed after 6 hours (figure 3A,B). To directly demonstrate TGFβ-induced hypermethylation of the PARP-1 promoter, we performed MeDIP assays. DNA methylation was induced in normal dermal fibroblasts by prolonged TGFβ stimulation at three out of four sites within the CpG island with particularly pronounced effects at site −330 to −182 bp with respect to the transcription start site (TSS) (figure 3C). Coincubation with 5-aza prevented the TGFβ-induced promoter hypermethylation, thus confirming the specificity of our finding. Comparison of the methylation status between SSc fibroblasts, and normal fibroblasts demonstrated increased promoter methylation at sites −330 to −182, –30 to +94 and +172 to +315 bp referred to the TSS in SSc fibroblasts (figure 3D). Moreover, prolonged incubation of SSc fibroblasts with 5-aza upregulated the mRNA and protein levels of PARP-1 (figure 3E,F), providing further evidence that DNA-hypermethylation contributes to the downregulation of PARP-1 in SSc fibroblasts. Consistently, treatment with 5-aza decreased collagen and αSMA expression and stress fibre formation in SSc fibroblasts (online supplementary figure 3).

Inhibition of PARP-1 enhances fibroblast activation and collagen release

We next investigated whether PARP-1 in turn might regulate TGFβ-induced fibroblast activation. Inhibition of PARP-1 by 3AB in fibroblasts from healthy individuals enhanced the stimulatory effects of TGFβ on myofibroblast differentiation. Incubation

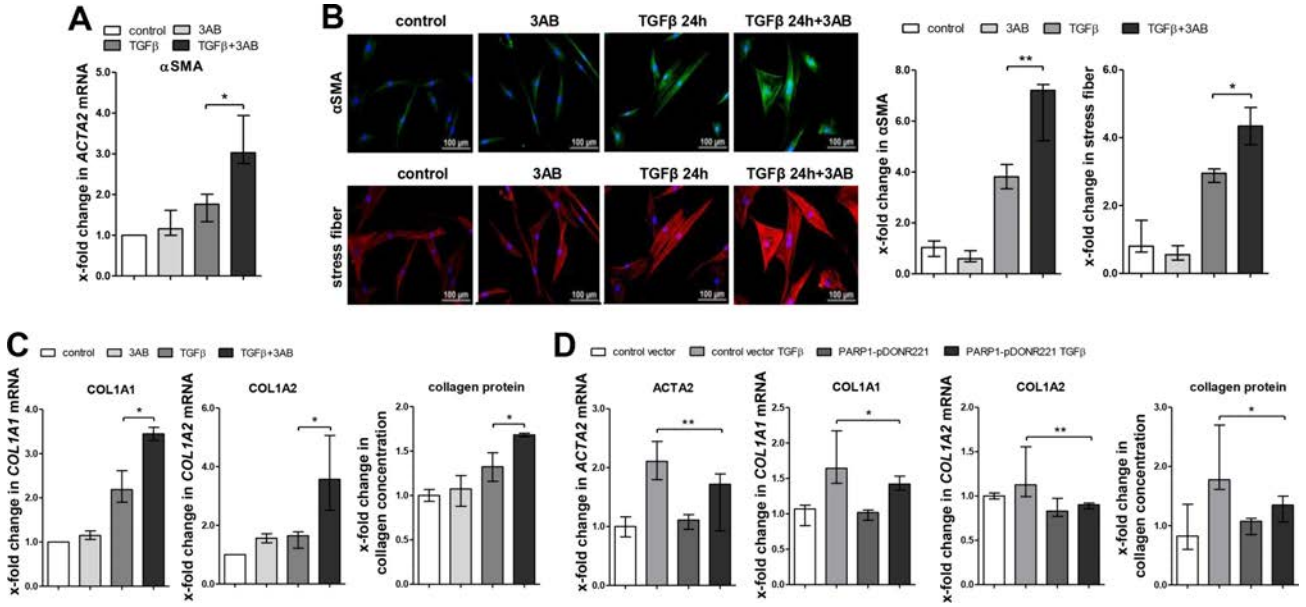


Figure 4 Inhibition of PARP-1 promotes fibroblasts differentiation and collagen release. (A–C) Inhibition of PARP-1: (A) mRNA levels of ACTA2 in TGFβ stimulated human fibroblasts with or without PARP-1 inhibition. (B) Immunofluorescence staining for αSMA and stress fibres in fibroblasts incubated with TGFβ and 3AB at 200-fold magnification. (C) mRNA and protein levels of collagen in TGFβ-stimulated healthy human fibroblasts after inhibition of PARP-1 by 3AB. (D) Overexpression of PARP-1: mRNA levels of ACTA2 and collagen, protein levels of collagen in TGFβ-stimulated human fibroblasts overexpressing PARP-1 (n≥4 biological replicates with ≥2 technical replicates for all experiments and readouts). *P< 0.05, **P< 0.01, ***P < 0.001. PARP-1, poly(ADP-ribose) polymerase-1; TGFβ, transforming growth factor-β.

with 3AB further increased the mRNA level of ACTA2 encoding for αSMA (figure 4A), the expression of αSMA protein and stress fibre formation (figure 4B) as compared with fibroblasts stimulated with TGFβ alone. Inhibition of PARP-1 also fostered TGFβ-induced collagen release and increased the mRNA levels of COL1A1 and COL1A2, the levels of type I collagen protein as well as the release of collagen protein into the supernatant in normal fibroblasts (figure 4C and online supplementary figure 4A). Consistent with the low expression levels of PARP-1 in SSc, incubation with 3AB had only mild effects on the expression levels of collagen and contractile proteins in SSc fibroblasts (online supplementary figure 5A).

In contrast, overexpression of PARP-1 inhibited TGFβ-induced fibroblast activation and reduced the mRNA levels of ACTA2, COL1A1 and COL1A2, type I collagen and inhibited the release of collagen protein (figure 4D and online supplementary figure 4B).

PARP-1 binds to Smad3 to enhance TGFβ signalling

To further characterise the effect of PARP-1 on TGFβ signalling, we analysed the levels of pSmad3 after inhibition of PARP-1 in TGFβ-stimulated fibroblasts. Treatment with 3AB further enhanced the accumulation of pSmad3 in TGFβ-stimulated fibroblasts (figure 5A,B). Reporter assays showed increased Smad-dependent transcription on inhibition of PARP-1 (figure 5C). Consistently, the mRNA levels of the classical TGFβ/Smad target genes PAI-1, Smad7 and CTGF were increased further by inhibition of PARP-1 in TGFβ-stimulated normal fibroblasts, whereas the effects of 3AB were less pronounced in SSc fibroblasts (figure 5C and online supplementary figure 5B). ChIP assays demonstrated that inhibition of PARP-1 enhances TGFβ-dependent binding of Smad3 to SBE in the ACTA2 promoter (figure 5D). Costaining of fibroblasts for PARylation, pSmad2/3 and Smad2/3 showed that the staining for PARylation colocalises with the staining for pSmad2/3 and Smad2/3 in TGFβ-stimulated fibroblasts and that this PARylation is effectively blocked by 3AB (figure 5E and online supplementary figure 6). Coimmunoprecipitation assays with antibodies against Smad3 or PARylation demonstrated that stimulation with TGFβ promoted direct binding of PARP-1 to Smad3 and PARylation of Smad3, which was inhibited by coincubation with 3AB, thus confirming the TGFβ-induced PARylation of Smad3 and of pSmad3 (figure 5F).

Inactivation of Parp-1 exacerbates bleomycin-induced fibrosis

We next aimed to analyse the role of PARP-1 in experimental fibrosis. We first evaluated the outcome of Parp-1-deficient mice in bleomycin-induced fibrosis. In the absence of bleomycin, Parp-$1^{-/-}$ mice demonstrated a normal skin phenotype (online supplementary figure 7A). However, Parp-$1^{-/-}$ mice developed more severe fibrosis on injection of bleomycin as compared with Parp-$1^{+/+}$ littermates (online supplementary figure 7A). Skin thickening, hydroxyproline content and differentiation of resting fibroblasts into myofibroblasts were more pronounced in bleomycin-injected Parp-$1^{-/-}$ mice compared with Parp-$1^{+/+}$ mice (online supplementary figure 7B-D). We also observed increased mRNA levels of Pai-1, Smad7 and Ctgf compared with Parp-$1^{+/+}$ mice (online supplementary figure 8).

Consistent with the findings in Parp-$1^{-/-}$ mice, pharmacological inactivation of Parp-1 by two structurally non-related inhibitors, 3AB and PJ34, also exacerbated bleomycin-induced skin fibrosis (online supplementary figure 9) with enhanced skin thickening, collagen accumulation and myofibroblast differentiation as compared with vehicle-treated, bleomycin-challenged mice.

Pharmacological inhibition of Parp-1 exaggerates topoisomerase-induced fibrosis

The effects of pharmacological inactivation of Parp-1 via 3AB could also be observed in topoisomerase-induced skin fibrosis

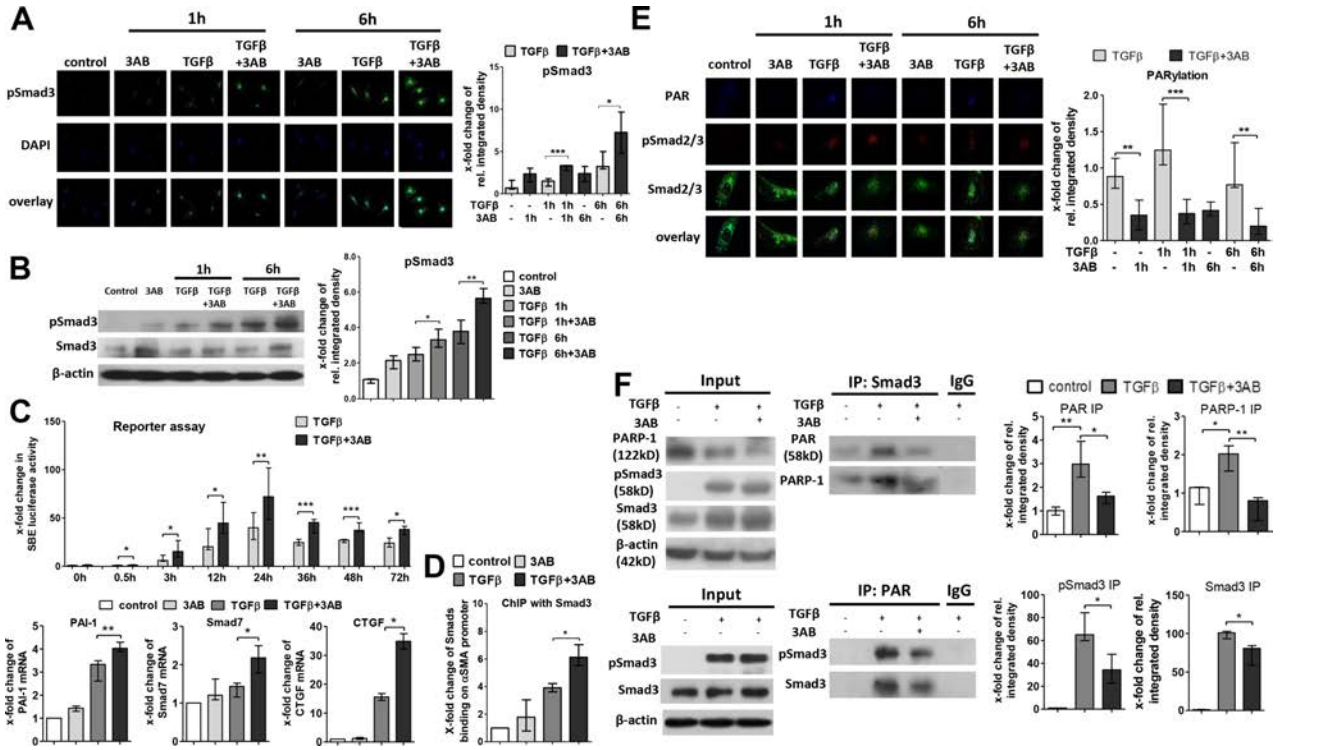


Figure 5 Inhibition of PARP-1 enhances TGFβ signalling in a Smad3-dependent manner. (A) Immunofluorescence staining for pSmad3 in TGFβ-stimulated fibroblasts incubated with 3AB at 200-fold magnification and quantification of the relative fluorescence intensity of pSmad3. (B) Western blot for pSmad3 in TGFβ-stimulated fibroblasts incubated with 3AB and quantification of the relative integrated density of pSmad3 (n=3 biological replicates with two technical replicates). (C) Transcriptional activity of Smad in Smad-binding elements (SBEs) reporter assays in fibroblasts stimulated with TGFβ and coincubated with 3AB; mRNA levels of the TGFβ target genes PAI-1, Smad7 and CTGF after inhibition of PARP-1 (n=4 biological replicates with two technical replicates). (D) PARP-1 inhibition enhanced the effects of TGFβ stimulation on binding of Smad3 to SBEs of the human *ACTA2* promoter in ChIP assays (n=4 biological replicates with two technical replicates). (E) Representative immunofluorescence pictures of PAR, pSmad2/3 and Smad2/3 at 1000-fold magnification and quantification of related integrated density of PARylated protein. (F) Coimmunoprecipitation using antibodies against Smad3 or PARylation in lysates of TGFβ-stimulated fibroblasts with or without 3AB treatment (n=3 biological replicates with two technical replicates). *P< 0.05, **P< 0.01, ***P < 0.001. ChIP, chromatin immunoprecipitation; PARP-1m, poly(ADP-ribose) polymerase-1; TGFβ, transforming growth factor-β.

(figure 6A–D). Repeated injections of recombinant topoisomerase I induced dermal fibrosis with dermal thickening, increased hydroxyproline content and higher myofibroblast counts. Inhibition of Parp-1 by 3AB in topoisomerase-challenged mice further increased the fibrotic changes and nearly doubled dermal thickness and hydroxyproline content in lesional skin compared with vehicle-treated fibrotic control mice (figure 6B,C). Cotreatment with 3AB also further enhanced topoisomerase-induced myofibroblast differentiation (figure 6D).

Inhibition of Parp-1 promotes fibrosis in Tsk-1 mice

Bleomycin-induced and topoisomerase-induced dermal fibrosis serve as models for early, inflammatory stages of SSc but is less appropriate for less inflammatory subgroups of patients with SSc. To overcome this limitation, we aimed to study the effects of Parp-1 inactivation in the Tsk-1 model. Inhibition of Parp-1 by 3AB enhanced the Tsk-1 phenotype (figure 6E). Hypodermal thickening, accumulation of collagen and myofibroblast counts were all increased in Tsk-1 mice treated with 3AB as compared with vehicle-treated Tsk-1 mice (figure 6F-H).

DISCUSSION

We demonstrate in the present study that the expression of PARP-1 is decreased in patients with SSc and in murine models of skin fibrosis. We provide evidence that the downregulation of PARP-1 is due to endogenous activation of TGFβ signalling in SSc fibroblasts. Of note, the downregulation of PARP-1 persisted in vitro and resulted in reduced PARylation in cultured SSc fibroblasts even after several passages. This chronic repression of PARP-1 expression is mediated by a TGFβ-induced hypermethylation of the PARP-1 promoter and the downregulation of PARP-1 expression in SSc fibroblasts can be reversed by inhibition of DNA methyltransferases, highlighting the crucial role of epigenetic alterations for the aberrant fibroblast activation in SSc.[8–10 26–31] Pathological activation of epithelial keratinocytes may also play a role in fibrogenesis. Takahashi *et al* demonstrated that knockout of the transcription factor FLI1 in keratinocytes results in autoimmunity and fibrosis.[32] Our immunohistochemical data suggest that PARP-1 expression is also decreased in keratinocytes of patients with SSc, and this mechanism may also be relevant for the pathogenesis of SSc. However, additional studies are necessary to confirm a potential contribution of altered PARP-1 expression in keratinocytes to fibrotic tissue remodelling.

The impaired PARylation in SSc has profound effects on canonical TGFβ signalling. Under physiological conditions, PARylation induces a negative feedback-loop to limit canonical TGFβ signalling. TGFβ induces binding of PARP-1 to Smad3 with subsequent PARylation of Smad3. The PARylation of Smad3 has been shown to induce dissociation of Smad3 from DNA,

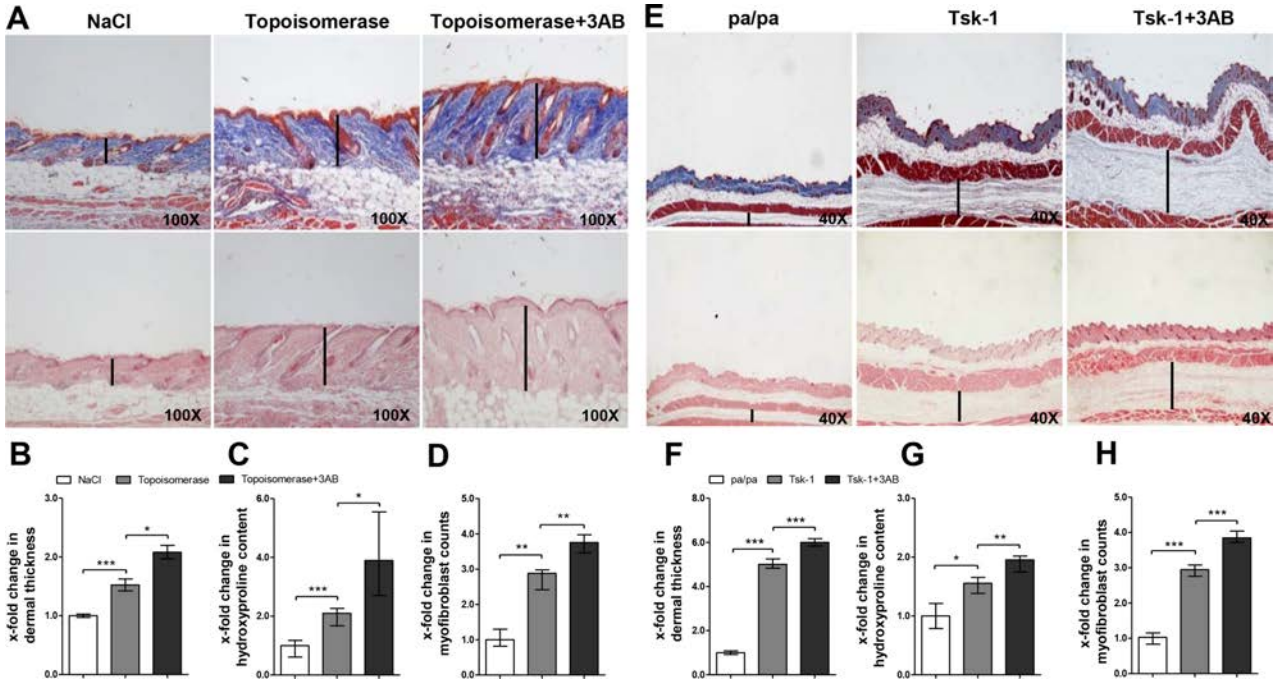


Figure 6 Inhibition of PARP-1 by 3AB exacerbates topoisomerase I-induced skin fibrosis and promotes fibrosis in Tsk-1 mice. (A) Representative trichrome-stained and H&E-stained skin sections are shown at 100-fold magnification. (B–D) Inhibition of PARP-1 enhanced topoisomerase I-induced dermal thickening (B), increased the hydroxyproline content (C) and stimulated myofibroblast differentiation (D) (n=6 for each group in all readouts). (E) Representative trichrome-stained and H&E-stained skin sections at 40-fold magnification. (F–H) Hypodermal thickening (F), hydroxyproline content (G) and myofibroblast counts (H) in control mice, Tsk-1 mice and Tsk-1 mice treated with 3AB (n=6 mice for all groups and outcomes). *P< 0.05, **P< 0.01, ***P < 0.001. PARP-1, poly(ADP-ribose) polymerase-1; Tsk-1, tight-skin-1.

thereby abrogating Smad-dependent transcription.[16] However, our data demonstrate that chronically activated TGFβ signalling as in SSc interferes with PARP-1-induced inhibition of TGFβ/Smad signaling. Persistently high levels of TGFβ decrease the expression of PARP-1 in fibroblasts, leading to impaired PARylation-induced inactivation of Smad3. Given the roles of both pathways in the pathogenesis of neoplastic diseases, one might speculate whether deregulation of PARP-1 with subsequent alterations of TGFβ signalling may have an impact on carcinogenesis in SSc. Whereas the decreased expression of the oncogene PARP-1 in SSc does not favour an increased risk to carcinogenesis, the prolonged activation of TGFβ-Smad3 signalling may theoretically favour metastasis and decrease responsiveness to therapy.[2]

Besides regulation by TGFβ, enhanced oxidative stress may also contribute to the deregulation of PARP-1 expression in SSc, given that PARP-1 is a DNA nick sensor enzyme that is activated by DNA breaks.[14 33]

The reduced PARP-1 activity in turn further promotes canonical TGFβ/Smad signaling in SSc. Inhibition of PARylation stimulated Smad-dependent transcription with enhanced Smad reporter activity and increased levels of classical Smad target genes. Consistent with the central role of TGFβ signalling in fibrogenesis, the enhanced TGFβ/Smad signaling in skin fibrosis translates directly into hyperactivation of fibroblasts. Inactivation of PARP-1 enhanced the stimulatory effects of TGFβ on myofibroblast differentiation and the release of collagen in vitro. Moreover, inhibition of Parp-1 also exacerbated skin fibrosis in inflammation-dependent and non-inflammation-dependent models. Of note, the role of PARP-1 in fibrosis may differ depending on the affected organ systems. In internal organs such as liver, lungs and kidneys, most publications report that PARP-1 promotes epithelial-to-mesenchymal transition (EMT) and fibrosis,[34–38] while other studies report exacerbation of EMT by inhibition of PARP-1.[39] Consistent with the different outcomes in fibrosis, inhibitory as well as stimulatory effects of PARP-1 on TGFβ/Smad signalling have been reported in vitro.[15 16 37 39–41] Although cell-type and context-specific effects may contribute to the different outcomes in some cases, the underlying reasons for those different effects warrant further studies.

Our data also provide evidence that different members of the PARP family have opposing effects on fibroblast activation and tissue fibrosis. PARP-5a and PARP-5b, also known as tankyrases, can PARylate axin, a central component of the β-catenin destruction complex.[42] PARylation of axin decreases its stability, thereby fostering the nuclear translocation of β-catenin, which increases canonical Wnt signalling.[42 43] Consistent with the potent stimulatory effects of Wnt signalling on fibroblast activation and collagen synthesis,[44–49] selective inactivation of PARP-5a and PARP-5b demonstrated potent antifibrotic effects in preclinical models of fibrosis.[50] Indeed, tankyrase inhibitors are currently considered as candidates for clinical trials in fibrotic diseases.[51] In contrast to the antifibrotic effects of an inhibition of tankyrases, profibrotic as well as antifibrotic effects of inactivation of PARP-1 have been reported.[15 16 34–41] Those findings have direct implications on the developmental programme with tankyrase inhibitors in fibrotic diseases, because they warrant the use of highly selective tankyrase inhibitors.

In summary, our study identifies PARP-1 as a central regulator of skin fibrosis. Stimulation with TGFβ recruits PARP-1 to Smad3, resulting in PARylation-induced inactivation of Smad3 (summarised in online supplementary figure 10). However, in SSc, this regulatory feedback loop is inactivated by downregulation of PARP-1. Inactivation of PARP-1 fosters canonical TGFβ signalling, stimulates fibroblast activation and exacerbates

experimental skin fibrosis and may thus contribute to persistent fibroblast activation and progression of fibrosis in SSc.

Acknowledgements We would like to thank Katja Dreißigacker, Regina Kleinlein, Christiane Bliß, Rosella Mancuso and Mónica Pascual Maté for excellent technical assistance.

Contributors YZ, JHWD and CD designed the study. YZ, SP, C-WC, RL and CD were involved in acquisition of data. YZ, SP, C-WC, RL, OD, GS, JHWD and CD were involved in interpretation of data. YZ, SP, JHWD and CD prepared the manuscript. KG, IL and REH provided essential material.

Funding DE 2414/2-1, DI 1537/5-1, DI 1537/7-1, DI 1537/8-1, DI 1537/9-1 and AK 144/1-1 of the Deutsche Forschungsgemeinschaft, grants J39 and A57 of the IZKF in Erlangen, the ELAN-Program of the University of Erlangen-Nuremberg and the Career Support Award of Medicine of the Ernst Jung Foundation.

Competing interests OD has consultancy relationships and/or has received research funding from Actelion, Pfizer, Ergonex, BMS, Sanofi-Aventis, United BioSource Corporation, medac, Biovitrium, Boehringer Ingelheim, Novartis, 4D Science and Active Biotech in the area of potential treatments of SSc; JHWD has consultancy relationships and/or has received research funding from Actelion, Pfizer, Ergonex, BMS, Celgene, Bayer Pharma, Boehringer Ingelheim, JB Therapeutics, Sanofi-Aventis, Novartis, UCB, GSK, Array Biopharma and Active Biotech in the area of potential treatments of SSc and is stock owner of 4D Science GmbH.

Ethics approval Local ethical review board.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Gabrielli A, Avvedimento EV, Krieg T. Scleroderma. *N Engl J Med* 2009;360:1989–2003.
2 Tyndall AJ, Bannert B, Vonk M, *et al*. Causes and risk factors for death in systemic sclerosis: a study from the EULAR Scleroderma Trials and Research (EUSTAR) database. *Ann Rheum Dis* 2010;69:1809–15.
3 Samarakoon R, Overstreet JM, Higgins PJ. TGF-β signaling in tissue fibrosis: redox controls, target genes and therapeutic opportunities. *Cell Signal* 2013;25:264–8.
4 Sonnylal S, Denton CP, Zheng B, *et al*. Postnatal induction of transforming growth factor beta signaling in fibroblasts of mice recapitulates clinical, histologic, and biochemical features of scleroderma. *Arthritis Rheum* 2007;56:334–44.
5 Varga J, Abraham D. Systemic sclerosis: a prototypic multisystem fibrotic disorder. *J Clin Invest* 2007;117:557–67.
6 Leroy EC. Connective tissue synthesis by scleroderma skin fibroblasts in cell culture. *J Exp Med* 1972;135:1351–62.
7 Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* 2012;13:484–92.
8 Dees C, Schlottmann I, Funke R, *et al*. The Wnt antagonists DKK1 and SFRP1 are downregulated by promoter hypermethylation in systemic sclerosis. *Ann Rheum Dis* 2014;73:1232–9.
9 Wang Y, Fan PS, Kahaleh B. Association between enhanced type I collagen expression and epigenetic repression of the FLI1 gene in scleroderma fibroblasts. *Arthritis Rheum* 2006;54:2271–9.
10 Zeisberg EM, Zeisberg M. The role of promoter hypermethylation in fibroblast activation and fibrogenesis. *J Pathol* 2013;229:264–73.
11 Kim MY, Zhang T, Kraus WL. Poly(ADP-ribosyl)ation by PARP-1: 'PAR-laying' NAD+ into a nuclear signal. *Genes Dev* 2005;19:1951–67.
12 Schreiber V, Dantzer F, Ame JC, *et al*. Poly(ADP-ribose): novel functions for an old molecule. *Nat Rev Mol Cell Biol* 2006;7:517–28.
13 Gibson BA, Kraus WL. New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nat Rev Mol Cell Biol* 2012;13:411–24.
14 Woodhouse BC, Dianov GL. Poly ADP-ribose polymerase-1: an international molecule of mystery. *DNA Repair* 2008;7:1077–86.
15 Huang D, Wang Y, Wang L, *et al*. Poly(ADP-ribose) polymerase 1 is indispensable for transforming growth factor-β Induced Smad3 activation in vascular smooth muscle cell. *PLoS One* 2011;6:e27123.
16 Lönn P, van der Heide LP, Dahl M, *et al*. PARP-1 attenuates Smad-mediated transcription. *Mol Cell* 2010;40:521–32.
17 Pacher P, Szabó C. Role of poly(ADP-ribose) polymerase 1 (PARP-1) in cardiovascular diseases: the therapeutic potential of PARP inhibitors. *Cardiovasc Drug Rev* 2007;25:235–60.
18 Shevalye H, Stavniichuk R, Xu W, *et al*. Poly(ADP-ribose) polymerase (PARP) inhibition counteracts multiple manifestations of kidney disease in long-term streptozotocin-diabetic rat model. *Biochem Pharmacol* 2010;79:1007–14.
19 van den Hoogen F, Khanna D, Fransen J, *et al*. 2013 classification criteria for systemic sclerosis: an American College of Rheumatology/European League against Rheumatism collaborative initiative. *Arthritis Rheum* 2013;65:2737–47.
20 Valentini G, Iudici M, Walker UA, *et al*. The European Scleroderma Trials and Research group (EUSTAR) task force for the development of revised activity criteria for systemic sclerosis: derivation and validation of a preliminarily revised EUSTAR activity index. *Ann Rheum Dis* 2017;76:270–6.
21 Dees C, Tomcik M, Zerr P, *et al*. Notch signalling regulates fibroblast activation and collagen release in systemic sclerosis. *Ann Rheum Dis* 2011;70:1304–10.
22 Reich N, Maurer B, Akhmetshina A, *et al*. The transcription factor Fra-2 regulates the production of extracellular matrix in systemic sclerosis. *Arthritis Rheum* 2010;62:280–90.
23 Dees C, Akhmetshina A, Zerr P, *et al*. Platelet-derived serotonin links vascular disease and tissue fibrosis. *J Exp Med* 2011;208:961–72.
24 Palumbo K, Zerr P, Tomcik M, *et al*. The transcription factor JunD mediates transforming growth factor {beta}-induced fibroblast activation and fibrosis in systemic sclerosis. *Ann Rheum Dis* 2011;70:1320–6.
25 Palumbo-Zerr K, Zerr P, Distler A, *et al*. Orphan nuclear receptor NR4A1 regulates transforming growth factor-β signaling and fibrosis. *Nat Med* 2015;21:150–8.
26 Altorok N, Kahaleh B. Epigenetics and systemic sclerosis. *Semin Immunopathol* 2015;37:453–62.
27 Altorok N, Tsou PS, Coit P, *et al*. Genome-wide DNA methylation analysis in dermal fibroblasts from patients with diffuse and limited systemic sclerosis reveals common and subset-specific DNA methylation aberrancies. *Ann Rheum Dis* 2015;74:1612–20.
28 Huang SK, Fisher AS, Scruggs AM, *et al*. Hypermethylation of PTGER2 confers prostaglandin E2 resistance in fibrotic fibroblasts from humans and mice. *Am J Pathol* 2010;177:2245–55.
29 Jüngel A, Distler JH, Gay S, *et al*. Epigenetic modifications: novel therapeutic strategies for systemic sclerosis? *Expert Rev Clin Immunol* 2011;7:475–80.
30 Mann J, Chu DC, Maxwell A, *et al*. MeCP2 controls an epigenetic pathway that promotes myofibroblast transdifferentiation and fibrosis. *Gastroenterology* 2010;138:705–14.
31 Mann J, Mann DA. Epigenetic regulation of wound healing and fibrosis. *Curr Opin Rheumatol* 2013;25:101–7.
32 Takahashi T, Asano Y, Sugawara K, *et al*. Epithelial Fli1 deficiency drives systemic autoimmunity and fibrosis: Possible roles in scleroderma. *J Exp Med* 2017;214:1129–51.
33 Kim MY, Mauro S, Gévry N, *et al*. NAD+-dependent modulation of chromatin structure and transcription by nucleosome binding properties of PARP-1. *Cell* 2004;119:803–14.
34 Hu B, Wu Z, Hergert P, *et al*. Regulation of myofibroblast differentiation by poly(ADP-ribose) polymerase 1. *Am J Pathol* 2013;182:71–83.
35 Mukhopadhyay P, Rajesh M, Cao Z, *et al*. Poly (ADP-ribose) polymerase-1 is a key mediator of liver inflammation and fibrosis. *Hepatology* 2014;59:1998–2009.
36 Okada H, Inoue T, Kikuta T, *et al*. Poly(ADP-ribose) polymerase-1 enhances transcription of the profibrotic CCN2 gene. *J Am Soc Nephrol* 2008;19:933–42.
37 Wang Y, Wang L, Zhang F, *et al*. Inhibition of PARP prevents angiotensin II-induced aortic fibrosis in rats. *Int J Cardiol* 2013;167:2285–93.
38 Yoon SP, Kim J, Poly KJ. Poly(ADP-ribose) polymerase 1 activation links ischemic acute kidney injury to interstitial fibrosis. *J Physiol Sci* 2015;65:105–11.
39 Pu H, Horbinski C, Hensley PJ, *et al*. PARP-1 regulates epithelial-mesenchymal transition (EMT) in prostate tumorigenesis. *Carcinogenesis* 2014;35:2592–601.
40 Dahl M, Maturi V, Lönn P, *et al*. Fine-tuning of Smad protein function by poly(ADP-ribose) polymerases and poly(ADP-ribose) glycohydrolase during transforming growth factor β signaling. *PLoS One* 2014;9:e103651.
41 Zhang P, Nakatsukasa H, Tu E, *et al*. PARP-1 regulates expression of TGF-β receptors in T cells. *Blood* 2013;122:2224–32.
42 Riffell JL, Lord CJ, Ashworth A. Tankyrase-targeted therapeutics: expanding opportunities in the PARP family. *Nat Rev Drug Discov* 2012;11:923–36.
43 Huang SM, Mishina YM, Liu S, *et al*. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* 2009;461:614–20.
44 Akhmetshina A, Palumbo K, Dees C, *et al*. Activation of canonical Wnt signalling is required for TGF-β-mediated fibrosis. *Nat Commun* 2012;3:735.
45 Beyer C, Reichert H, Akan H, *et al*. Blockade of canonical Wnt signalling ameliorates experimental dermal fibrosis. *Ann Rheum Dis* 2013;72:1255–8.
46 Beyer C, Schramm A, Akhmetshina A, *et al*. β-catenin is a central mediator of pro-fibrotic Wnt signaling in systemic sclerosis. *Ann Rheum Dis* 2012;71:761–7.
47 Distler A, Ziemer C, Beyer C, *et al*. Inactivation of evenness interrupted (EVI) reduces experimental fibrosis by combined inhibition of canonical and non-canonical Wnt signalling. *Ann Rheum Dis* 2014;73:624–7.
48 Wei J, Fang F, Lam AP, *et al*. Wnt/β-catenin signaling is hyperactivated in systemic sclerosis and induces Smad-dependent fibrotic responses in mesenchymal cells. *Arthritis Rheum* 2012;64:2734–45.
49 Wei J, Melichian D, Komura K, *et al*. Canonical Wnt signaling induces skin fibrosis and subcutaneous lipoatrophy: a novel mouse model for scleroderma? *Arthritis Rheum* 2011;63:1707–17.
50 Distler A, Deloch L, Huang J, *et al*. Inactivation of tankyrases reduces experimental fibrosis by inhibiting canonical Wnt signalling. *Ann Rheum Dis* 2013;72:1575–80.
51 Ulsamer A, Wei Y, Kim KK, *et al*. Axin pathway activity regulates in vivo pY654-β-catenin accumulation and pulmonary fibrosis. *J Biol Chem* 2012;287:5164–72.

EXTENDED REPORT

Methotrexate limits inflammation through an A20-dependent cross-tolerance mechanism

Cristina Municio,[1] Ángeles Dominguez-Soto,[2] Sara Fuentelsaz-Romero,[1] Amalia Lamana,[3] Nuria Montes,[3] Víctor D Cuevas,[2] Raquel García Campos,[1] José L Pablos,[4] Isidoro González-Álvaro,[3] Amaya Puig-Kröger[1]

Handling editor Josef S Smolen

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212537).

[1]Laboratorio de Inmuno-Metabolismo, Hospital General Universitario Gregorio Marañón, Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain
[2]Centro de Investigaciones Biologicas, Madrid, Spain
[3]Servicio de Reumatología, Hospital Universitario La Princesa, Instituto de Investigación Sanitaria Hospital Universitario La Princesa, Madrid, Spain
[4]Servicio de Reumatología, Instituto de Investigación Hospital Universitario 12 de Octubre, Universidad Complutense de Madrid, Madrid, Spain

Correspondence to
Dr Amaya Puig-Kröger, Laboratorio de Inmuno-Metabolismo, Instituto de Investigación Sanitaria Gregorio Marañón, Hospital General Universitario Gregorio Marañón, 28007 Madrid, Spain; amaya.puig@salud.madrid.org

CM and ÁD-S contributed equally.

Received 15 October 2017
Revised 21 December 2017
Accepted 15 January 2018
Published Online First 3 February 2018

ABSTRACT

Objectives Methotrexate (MTX) is the anchor drug for treatment of rheumatoid arthritis (RA), but the mechanism of its anti-inflammatory action is not fully understood. In RA, macrophages display a proinflammatory polarisation profile that resembles granulocyte-macrophage colony-stimulating factor (GM-CSF)-differentiated macrophages and the response to MTX is only observed in thymidylate synthase$^+$ GM-CSF-dependent macrophages. To determine the molecular basis for the MTX anti-inflammatory action, we explored toll-like receptor (TLR), RA synovial fluid (RASF) and tumour necrosis factor receptor (TNFR)-initiated signalling in MTX-exposed GM-CSF-primed macrophages.

Methods Intracellular responses to TLR ligands, TNFα or RASF stimulation in long-term low-dose MTX-exposed human macrophages were determined through quantitative real-time PCR, western blot, ELISA and siRNA-mediated knockdown approaches. The role of MTX in vivo was assessed in patients with arthritis under MTX monotherapy and in a murine sepsis model.

Results MTX conditioned macrophages towards a tolerant state, diminishing interleukin (IL)-6 and IL-1β production in LPS, LTA, TNFα or RASF-challenged macrophages. MTX attenuated LPS-induced MAPK and NF-κB activation, and toll/IL-1R domain-containing adaptor inducing IFN-beta (TRIF1)-dependent signalling. Conversely, MTX increased the expression of the NF-κB suppressor A20 (*TNFAIP3*), itself a RA-susceptibility gene. Mechanistically, MTX-induced macrophage tolerance was dependent on A20, as siRNA-mediated knockdown of A20 reversed the MTX-induced reduction of IL-6 expression. In vivo, *TNFAIP3* expression was significantly higher in peripheral blood cells of MTX-responsive individuals from a cohort of patients with arthritis under MTX monotherapy, whereas MTX-treated mice exhibited reduced inflammatory responses to LPS.

Conclusions MTX impairs macrophage proinflammatory responses through upregulation of A20 expression. The A20-mediated MTX-induced innate tolerance might limit inflammation in the RA synovial context, and positions A20 as a potential MTX-response biomarker.

To cite: Municio C, Dominguez-Soto Á, Fuentelsaz-Romero S, *et al*. *Ann Rheum Dis* 2018;**77**:752–759.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that primarily affects synovial joints. Predominant cytokines in RA pathogenesis are TNFα, interleukin (IL)-6 and IL-1β, which are mainly produced by macrophages and act locally and systemically.[1–3] In fact, macrophages accumulate in the synovium of RA joints, where they exhibit destructive and remodelling potential and contribute considerably to inflammation and joint destruction.[4] Importantly, reduction in the number of macrophages in the synovium constitutes a biomarker for response to treatment in patients with RA.[5]

GM-CSF induces macrophage differentiation from haematopoietic progenitor cells and is a key driver of tissue inflammation.[6] GM-CSF was one of the first cytokines detected in human synovial fluid from inflamed joints and several lines of data suggest that GM-CSF strongly influences the development and pathogenesis of RA.[6] GM-CSF-deficient mice are protected from developing collagen-induced arthritis and blockade of GM-CSF reduces the severity of established disease in wild-type mice, thus supporting a key role for GM-CSF in the initiation and development of inflammatory arthritis. Moreover, overexpression or injection of GM-CSF is associated with flares of arthritis and patients receiving GM-CSF after chemotherapy showed exacerbation of established RA.[7] In line with all the above findings, macrophages from patients with active RA display a transcriptomic and phenotypical proinflammatory polarisation profile that resembles GM-CSF-differentiated macrophages.[8] According to the role of GM-CSF in RA, phase I and II clinical trials targeting GM-CSF or GM-CSF receptor-α in RA have shown rapid and sustained clinical responses without major safety concerns.[9–11]

The folic acid antagonist methotrexate (MTX) is the disease modifying anti-rheumatic drug of first choice in the treatment of early and established RA, and shows good efficacy, with 40% of patients with RA achieving an American College of Rheumatology score 50 (ACR50) response.[12 13] MTX blocks different enzymes of the folate or one-carbon metabolism. While MTX blocks dihydrofolate reductase, polyglutamated MTX potently inhibits thymidylate synthase (TS), and both enzymes are crucial for the de novo biosynthesis of purines and pyrimidines required for DNA replication and cellular proliferation.[13] In patients with RA, MTX treatment is associated with a significant decrease in serum IL-6 and IL-8.[14] However, despite the beneficial effect of MTX in current RA therapies, its mechanism of action as an anti-inflammatory drug remains inconclusive. Different cellular-specific mechanisms have been proposed to explain the inhibition of NF-κB,

the central regulator of proinflammatory cytokine gene expression. Previous studies on T lymphocytes and fibroblast-like synoviocytes have found that MTX inhibits NF-κB activity via tetrahydrobiopterin (BH_4) depletion, lincRNA-p21 increase or adenosine receptor activation, respectively.[15 16] Although MTX restores NF-κB activity in peripheral blood mononuclear cells (PBMCs) from patients with RA,[15] no information is available on the effect of clinical doses of MTX on NF-κB in human macrophages, whose transcriptome is significantly modulated after low-dose MTX exposure.[17]

We have also previously described that MTX exclusively targets proinflammatory TS^+ GM-CSF-primed macrophages.[17] In an attempt to determine the molecular mechanism underlying the anti-inflammatory actions of MTX, we have explored the inflammatory response of MTX-exposed GM-CSF-primed macrophages. We now report that long-term low-dose MTX treatment modifies macrophage response to TLR ligands or TNFα stimulation by increasing *TNFAIP3* (A20) expression in macrophages, and that MTX conditions macrophages for impaired responses towards pathogen agents like LPS, TNFα or RA synovial fluid (RASF).

METHODS

Detailed methods are supplied in the online supplementary file.

Human PBMCs were isolated from buffy coats from normal donors. Monocytes were purified from PBMCs by magnetic cell sorting using CD14 microbeads (Miltenyi) and were cultured at 0.5×10^6 cells/mL for 7 days in Roswell Park Memorial Institute (RPMI) supplemented with 10% fetal calf serum, and containing GM-CSF (1000 U/mL) to generate GM-CSF-polarised macrophages (GM-MØ). Independent preparations of monocytes were unexposed or exposed once to MTX (50 nM)[18 19] and differentiated to GM-MØ for 7 days. MTX is a drug given weekly to patients with RA and we followed in vitro the patient schedule.[12] LPS (10 ng/mL, 01111:B4 strain), LTA (5 µg/mL), TNFα (20 ng/mL) and RASF were added for the indicated time points to 7-day fully differentiated GM-MØ and were analysed by quantitative real-time RT-PCR, immunobloting or ELISA. RASF were obtained from patients with active knee arthritis, confirmed by a highly cellular synovial fluid, and were heterogeneous regarding demographic, disease characteristics and previous RA therapy. For in vivo MTX-cross tolerance, C57BL/6J mice between 6 and 8 weeks of age (n=6 mice/group) received phosphate-buffered saline (PBS) or MTX intraperitoneally (2 mg/kg). Seven days later, intraperitoneal LPS (9 mg/kg) was administered and serum collected after 4 hours for IL-6 and TNFα determination by ELISA. Patients with early arthritis in MTX monotherapy were recruited from the Princesa Early Arthritis Register Longitudinal study. Informed consent is received from the patient.[20] Statistical analysis was performed using paired Student's t-test and a P value <0.05 was considered significant (*P<0.05, **P<0.01, ***P<0.001).

RESULTS

Long-term low-dose MTX treatment diminishes LPS, LTA, TNFα and RASF-induced proinflammatory cytokine production in human macrophages

A variety of TLR ligands, either endogenous or of microbial origin, are present within RA synovia.[21] To determine the role of MTX on macrophage TLR4 activation, monocytes were exposed to a single dose of MTX before initiation of the GM-CSF-driven differentiation process, and later challenged with LPS at the 7-day culture (figure 1A). MTX pretreatment diminished LPS-induced IL-6 expression at the mRNA and protein levels (figure 1B). Similarly, MTX pretreatment reduced LPS-induced TNFα, IL-12p40 and IL-1β production although with different kinetics (figure 1C and online supplementary figure S1). By contrast, the expression of other LPS-responsive macrophage genes[22] was either unaltered (*IFIT2*, *PKIG*) or increased (*CCR7*) on MTX pretreatment (online supplementary figure S1), thus indicating that proinflammatory cytokine production is selectively inhibited in MTX-exposed macrophages. The specificity of MTX to attenuate LPS-dependent proinflammatory cytokine production in macrophages was analysed in the presence of folinic acid (FA). FA restored LPS-induced IL-6 secretion in MTX-treated macrophages, indicating that MTX-induced effects are mediated through blocking the one-carbon metabolism (figure 1D). Similar to TLR4 activation, MTX-treated macrophages exhibited a lower level of LTA-dependent IL-6 and IL-1β gene expression and protein secretion (figure 1E). More importantly, analogous findings were observed when macrophages were challenged with TNFα, the predominant inflammatory cytokine found in RA joints[1]: MTX-treated macrophages exhibited a lower expression of TNFα-induced *IL6* and *IL1B* mRNA and IL-6 and IL-12p40 secretion than untreated macrophages (figure 1F), thus indicating that MTX attenuates proinflammatory cytokine expression in response to TLR4 and TLR2 ligands as well as after TNFα stimulation.

Finally, to determine whether MTX-exposed macrophages are also refractory to the effect of the RA synovial environment, MTX-treated macrophages were exposed to RASF from patients with active disease. RASF-induced *IL6* mRNA was attenuated in MTX-treated macrophages although the effect of MTX differed among tested RASF (figure 2). Altogether, these results indicate that MTX diminishes the production of RA-associated cytokines in human macrophages exposed to stimuli known to be present in RA joints (TLR2 and TLR4 ligands, TNFα and RASF).

TNFAIP3 is an MTX response gene and is involved in MTX-induced tolerance in macrophages

The above results suggested that low-dose MTX renders macrophages less responsive to a subsequent stimulation by proinflammatory stimuli and that MTX might impose a state of desensitisation in macrophages that resembles the 'LPS tolerance' phenomenon.[23] Interestingly, and in line with the potential acquisition of a tolerance state in MTX-treated macrophages, Gene Set Enrichment Analysis (GSEA) on the transcriptome of MTX-treated macrophages (GSE71253) revealed that long-term MTX treatment promotes a significant upregulation of the 'TNFα signalling via NF-κB' (false discovery rate (FDR) q value=0.0001) and 'inflammatory response' (FDR q value=0.0001) gene sets (figure 3A).[17 24] In agreement with the GSEA prediction, MTX-treated monocytes led to the generation of macrophages with a significantly higher *IL1B*, *IL1A* and *IL6* mRNA expression (figure 3B).

To address whether MTX promotes 'bona fide' innate tolerance in macrophages, and since tolerant cells exhibit a lower level of MAPK and NF-κB activation on TLR stimulation,[23 24] we first determined the levels of LPS and TNFα-induced MAPK and IκBα activation in MTX-treated macrophages. MTX pretreatment reduced the activation of p38 and jun amino-terminal kinase (JNK) in response to LPS and TNFα (figure 3C). Moreover, a higher level of IκBα was detected in LPS and TNFα-exposed MTX-pretreated macrophages (figure 3C), indicating that the LPS and TNFα-induced activation of both MAPK and NF-κB was impaired in MTX-treated macrophages. These results were

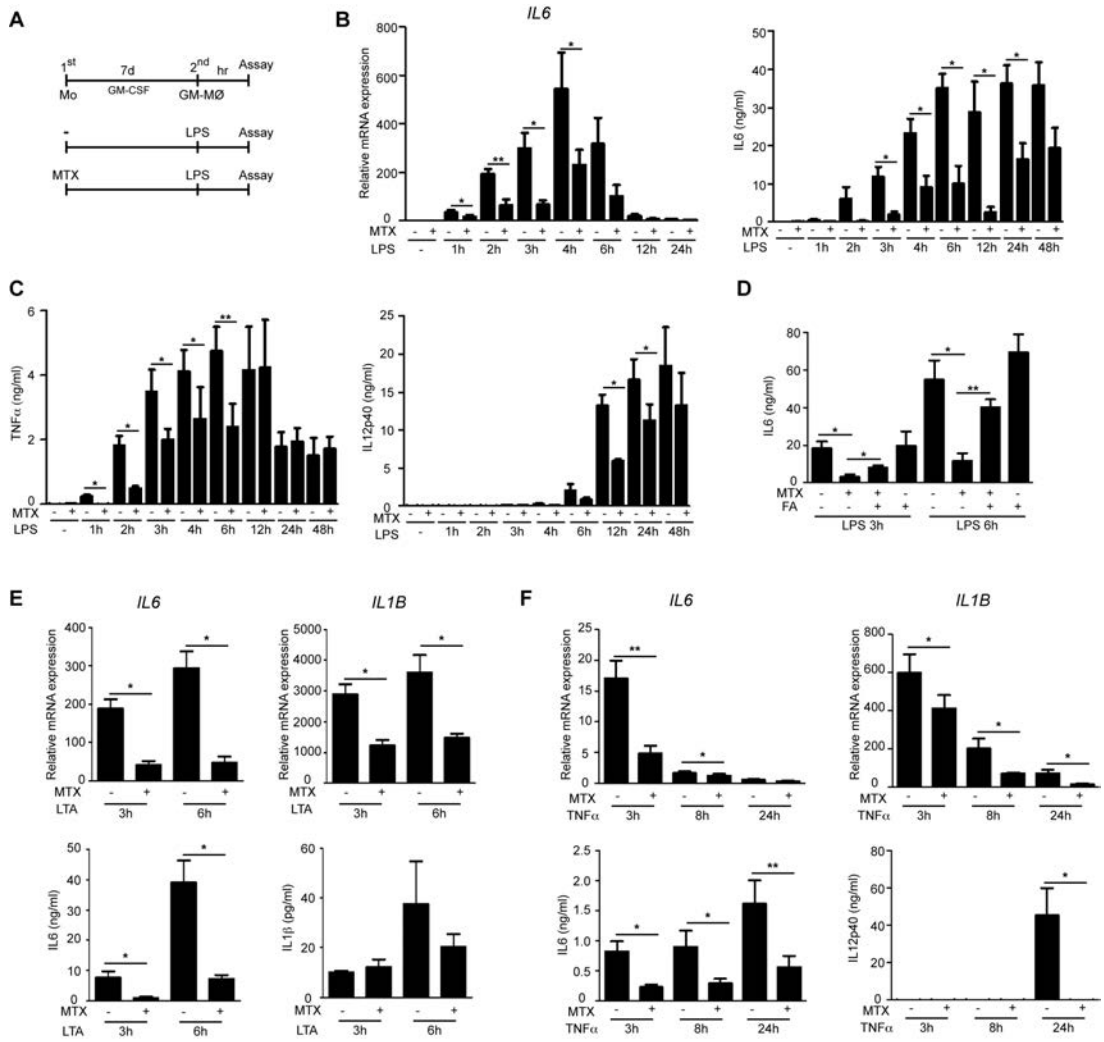


Figure 1 MTX alters TLR4, TLR2 and TNFα responsiveness in GM-CSF-primed macrophages. (A) Schematic representation of the experiments. Monocytes were exposed to 50 nM MTX at the beginning of the 7-day macrophage differentiation process with GM-CSF and challenged with LPS (10 ng/mL), LTA (5 µg/mL) or TNFα (20 ng/mL) on day 7. Cells (GM-MØ) were assayed at time points poststimulation. (B) Expression of IL-6 by qRT-PCR (left) or ELISA (right) by monocytes differentiated with GM-CSF in the absence or presence of MTX and challenged with LPS for 48 hours. Mean±SEM of four independent donors are shown (*P<0.05, **P<0.01). (C) Production of TNFα or IL-12p40 by monocytes differentiated with GM-CSF in the absence or presence of MTX and challenged with LPS for 48 hours. Mean±SEM of four independent donors are shown (*P<0.05, **P<0.01). (D) Production of IL-6 by monocytes differentiated with GM-CSF in the absence or presence of MTX or FA (1 µM) and challenged with LPS for the indicated time points, as determined by ELISA. Mean±SEM of three independent donors are shown (*P<0.05, **P<0.01). (E–F) Expression of IL-6, IL-1β and IL-12p40 by qRT-PCR (upper panels) or ELISA (lower panels) by monocytes differentiated with GM-CSF in the absence or presence of MTX and challenged with LTA (E) or TNFα (F) for the indicated time points. Mean±SEM of three (E) and five (F) independent donors are shown (*P<0.05). FA, folinic acid; IL, interleukin; Mo, monocytes; MTX, methotrexate; qRT-PCR, quantitative real-time PCR.

further confirmed after analysis of the LPS-induced NF-κB-dependent transcription (figure 3D), as MTX-treated macrophages exhibited lower LPS-induced NF-κB-dependent transcriptional activity than untreated GM-MØ. Therefore, MTX treatment conditions macrophages for an impaired NF-κB transcriptional activity after LPS stimulation. The TRIF-dependent pathway, implicated in mediating the type I interferon activation of the LPS signalling,[25] was also explored. Since MTX-treated cells exhibited lower C-X-C motif chemokine ligand 10 (CXCL10) secretion and phosphorylated signal transducer and activator of transcription 1 (p-Stat1) activation in response to LPS than untreated cells (figure 3E), MTX appears to exert its effects also via the TRIF pathway.

Next, we determined the expression of regulators of TLR-induced cytokine production that have been previously implicated in LPS tolerance.[23] Long-term MTX treatment significantly increased the expression of *TNFAIP3*, decreased *TLR4* and *TLR2* and did not modulate *IRAK3* (IRAK-M), *INPP5D* (SHIP1), *SOCS1*, *SOCS3* and *PELI3* mRNA expression in GM-MØ (figure 3F).[23 24 26 27] Reduction of *TLR2* and *TLR4* by MTX was modest at the mRNA level and not observed in all donors at the protein level (online supplementary figure S2). By contrast, *TNFAIP3* induction by MTX was observed in all donors examined. *TNFAIP3* codes the A20 protein, a ubiquitin-modifying enzyme that acts as a pivotal NF-κB suppressor after TLRs or TNFR stimulation.[28] Kinetic studies revealed that *TNFAIP3* expression increased 5 days after MTX addition in GM-CSF-primed macrophages (figure 3G), thus suggesting a role for A20 in MTX-induced tolerance. To explore whether this is the case, we determined the effect of silencing *TNFAIP3* expression. siRNA-mediated TNFAIP3 knockdown in MTX-treated GM-MØ significantly restored IL-6 secretion in response to

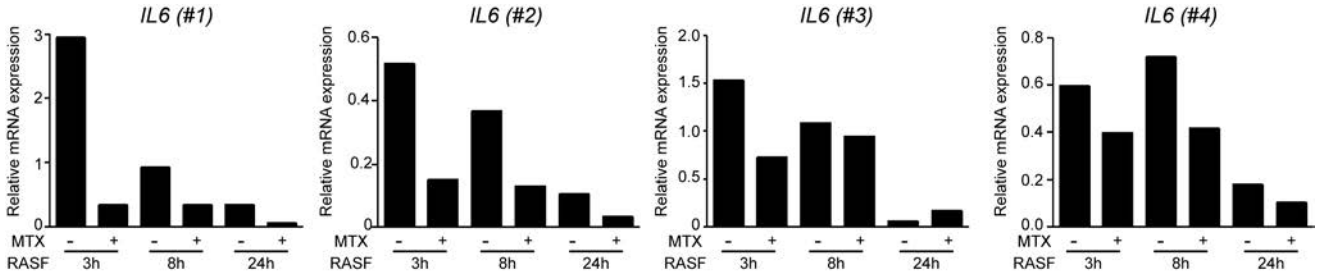


Figure 2 MTX diminishes RASF IL6 induction in GM-CSF-primed macrophages. Expression of *IL6* by quantitative real-time PCR by monocytes differentiated with GM-CSF in the absence or presence of MTX and challenged with 25% synovial fluid from patients with active RA (RASF) on day 7 for the indicated time points (*P<0.05). Independent macrophage donors (nos 1–4) were challenged with RASF from two different patients with RA (donor no. 1 with RASF-A and donors no. 2, no. 3 and no. 4 with RASF-B). IL, interleukin; MTX, methotrexate; RA, rheumatoid arthritis; RASF, RA synovial fluid.

LPS, as macrophages with lower A20 expression yielded significantly higher levels of LPS-induced IL-6 (figure 3H). Therefore, MTX-induced A20 contributes to the reduced LPS-induced IL-6 expression seen in MTX-treated macrophages, suggesting its involvement in the MTX-induced tolerance.

MTX-dependent expression of TNFAIP3 involves TS inhibition and TP53 activation

To explore the role of MTX on *TNFAIP3* expression, we first determined the sensitivity of the MTX-dependent *TNFAIP3* upregulation to FA. *TNFAIP3* induction by MTX was inhibited by the simultaneous addition of FA, indicating that MTX-triggered *TNFAIP3* induction relies on blocking one-carbon metabolism (figure 4A). However, FA in the clinic is usually prescribed 24 hours after MTX treatment.[29] As expected, FA did not reversed MTX-induced *TNFAIP3* expression when added 24 hours after MTX, showing that FA does not block the potential beneficial clinical effects of *TNFAIP3* induction by MTX in RA (figure 4B).

Unlike the rapid induction of A20 in response to inflammatory stimuli that takes place 30–60 min after NF-κB activation,[28] a slow induction of A20 was observed in MTX-exposed macrophages (figure 3G). We have previously demonstrated that the mechanism involved in MTX response in proinflammatory macrophages relies on TS inhibition and p53 activation, what led us to assess whether the TS/p53 axis is involved in MTX-induced A20 expression.[17] Assessment of the underlying mechanism revealed that MTX-triggered *TNFAIP3* induction in GM-MØ was significantly diminished (40%) after TS silencing with two different small interfering RNA (figure 4C), thus indicating that MTX-triggered *TNFAIP3* upregulation is partly dependent on TS expression. Moreover, knockdown of TS sufficed to increase *TNFAIP3* mRNA expression in GM-MØ, an effect that was prevented in the presence of the p53 inhibitor pifithrin-α (figure 4D). Therefore, MTX induces a tolerant state in macrophages by upregulating the expression of *TNFAIP3* in a TS/p53-dependent pathway.

MTX-induced tolerance in vivo

To address the relevance of the MTX-induced tolerance in vivo, mice were treated with MTX for 7 days, mimicking the patient schedule therapy currently in use,[12] and challenged with an intraperitoneal injection of LPS before determination of the serum concentrations of IL-6 and TNFα (figure 5A). LPS-induced serum IL-6 and TNFα significantly diminished in MTX-pretreated mice (figure 5B, C), whose white blood cell counts did not differ significantly from those of untreated mice (not shown). The same results were obtained when MTX was injected once a week for 4 weeks (not shown). Therefore, and in agreement with its ability to limit macrophage responses to LPS in vitro, MTX is capable of inducing a state of tolerance in mice in vivo.

TNFAIP3 mRNA expression increases in early arthritis patients responders to MTX

To evaluate the association between MTX treatment and *TNFAIP3* expression in patients with arthritis, we determined *TNFAIP3* mRNA levels in PBMCs from patients with early arthritis at baseline and during 1 year of MTX monotherapy (see online supplementary table S1). We found that *TNFAIP3* mRNA expression levels tend to increase along the follow-up (figure 6A, model 1 in online supplementary table S2). Furthermore, *TNFAIP3* mRNA expression significantly increased only in those patients who respond to MTX therapy (figure 6B,C, model 2 and model 3 in online supplementary table S2). In fact, being or not an MTX responder explained 20% of the variability of *TNFAIP3* expression (R^2 m=0.196, model 3 in online supplementary table S2). Altogether, these results indicate that *TNFAIP3* expression is higher in MTX responder patients with arthritis, suggesting that TNFAIP3 might be an MTX responsiveness biomarker, although these findings require confirmation in larger cohorts.

DISCUSSION

Weekly administered MTX is the main starting therapy and the anchor drug for the treatment of RA, cutaneous psoriasis and psoriatic arthritis.[30] However, the exact mechanism underlying the anti-inflammatory actions of MTX remains not fully understood.[13] We have previously shown that MTX triggers the acquisition of a proinflammatory gene profile in human GM-CSF-primed macrophages.[17] We now report that long-term low-dose MTX conditions GM-CSF-primed macrophages towards a tolerance state that renders them less responsive to TLR ligands, TNFα and RASF stimulation. Mechanistically, MTX reduces LPS and TNFα-dependent MAPK activation, IκBα degradation, NF-κB activity and proinflammatory cytokine production in human macrophages. In addition, MTX increases A20 expression, a pivotal NF-κB suppressor whose knockdown impairs the MTX-induced tolerance effect. In line with these findings, MTX-treated mice exhibit a reduced inflammatory response to LPS. Altogether, these results indicate that the global anti-inflammatory activity of MTX relies on its ability to induce a proinflammatory profile in GM-CSF-primed macrophages, making MTX-conditioned macrophages less responsive to proinflammatory stimuli. Our results reconcile the transcriptional[17] and functional effects of MTX on human

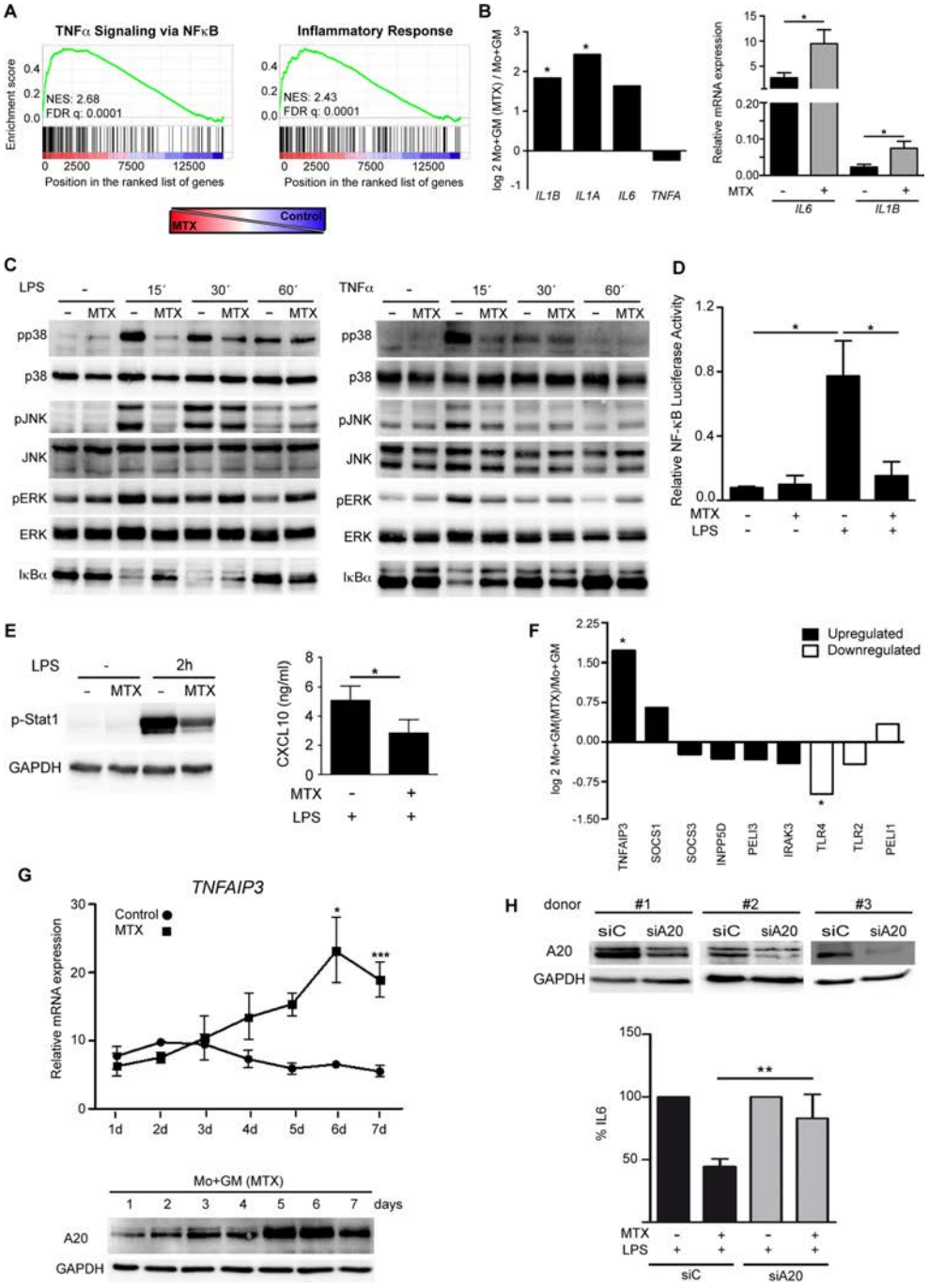


Figure 3 MTX suppresses TLR4 and TNFR signalling and induces *TNFAIP3* (A20) in macrophages. (A) Gene Set Enrichment Analysis results for MTX-treated versus untreated macrophages for 7 days indicating the normalised enrichment score and the false discovery rate. (B) Relative expression of *IL1B*, *IL1A*, *IL6* and *TNFA* in MTX-treated (Mo+GM(MTX)) and untreated (Mo+GM) GM-CSF-primed macrophages (*P<0.05, adjusted P value), as determined by microarray (left) and qRT-PCR (right). (C) Immunoblot analysis of pp38, pJNK and pERK, p38, JNK, extracellular signal-regulated kinase (ERK) and IκBα by monocytes differentiated with GM-CSF in the absence or presence of MTX for 7 days and challenged with LPS (left) or TNFα (right) for the indicated time points. A representative experiment of four independent donors is shown. (D) Basal and LPS-induced NF-κB-dependent transcriptional activity in MTX-treated and untreated GM-MØ. Mean±SEM of the relative NF-κB luciferase activity (compared with Renilla luciferase activity) of five independent experiments are shown (*P<0.05). (E) Immunoblot analysis of pStat1 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (left), and CXCL10 secretion (right) by monocytes differentiated with GM-CSF in the absence or presence of MTX for 7 days and challenged with LPS for 2 hours (left) or 24 hours (right). (F) Relative expression of the genes encoding molecules involved in tolerance (black, upregulated in LPS tolerance; white, downregulated in LPS tolerance) in MTX-treated (Mo+GM(MTX)) and untreated (Mo+GM) GM-CSF-primed macrophages (*P<0.05, adjusted P value). (G) Expression of *TNFAIP3* by monocytes differentiated with GM-CSF in the absence or presence of MTX, as determined by qRT-PCR. Mean±SEM of three independent donors are shown (*P<0.05, ***P<0.001). Immunoblot analysis of A20 and GAPDH in monocytes differentiated with GM-CSF in the presence of MTX at the indicated time points. (H) Monocytes differentiated with GM-CSF in the absence or presence of MTX for 5 days were transfected with control siRNA (siC) or siRNA for A20 (siA20) and 48 hours later, cells were then stimulated with LPS for 3 hours, and expression levels of A20, GAPDH (immunoblot) and IL-6 (ELISA) were detected. IL, interleukin; Mo, monocytes; MTX, methotrexate; qRT-PCR, quantitative real-time PCR.

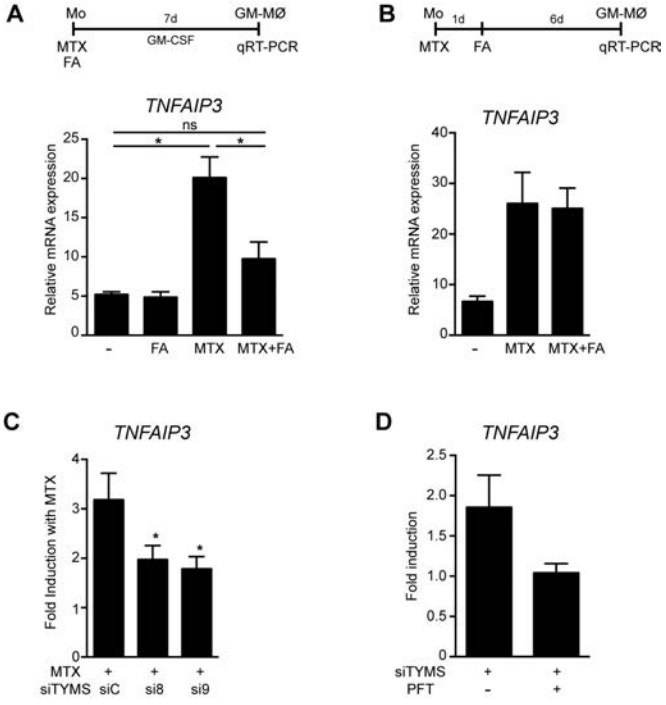


Figure 4 MTX-dependent expression of *TNFAIP3*. *TNFAIP3* expression by monocytes differentiated with GM-CSF in the absence or presence of MTX (50 nM), FA or both, as determined by qRT-PCR. In (A), FA (1 µM) was added simultaneously with MTX and in (B) FA (50 nM, concentration of FA found in the serum of patients with RA) was added 24 hours after MTX treatment, as indicated. Mean±SEM of three independent donors are shown (*P<0.05). (C) *TNFAIP3* mRNA expression on GM-MØ transfected with control siRNA (siC) or siRNA for TYMS (si8, si9) and exposed to MTX for 48 hours, as determined by qRT-PCR. Results are expressed as fold induction with MTX. Mean and SEM of six independent donors are shown (*P<0.05). (D) *TNFAIP3* expression on GM-MØ transfected with control siRNA and siRNA for TYMS and exposed to PFT (50 µM) for 48 hours, as determined by qRT-PCR. Results are expressed as fold induction, which indicates the expression of *TNFAIP3* in siRNA TYMS-transfected relative to siRNA control cells, and untreated or treated with PFT. Mean and SEM of six independent donors are shown. FA, folinic acid; IL, interleukin; Mo, monocytes; MTX, methotrexate; PFT, pifithrin-α; qRT-PCR, quantitative real-time PCR; RA, rheumatoid arthritis; TYMS, thymidylate synthetase.

macrophages, and clarify the molecular basis for the anti-inflammatory activity of low-dose MTX.

We also found that *TNFAIP3* mRNA expression is significantly higher in PBMCs of MTX-responsive individuals from a cohort of patients with early arthritis under MTX monotherapy. In the context of RA, A20 is a susceptibility gene because (1) polymorphisms of the A20-coding gene *TNFAIP3* are associated with RA[31–34] and other inflammatory diseases such as systemic lupus erythematosus and psoriasis[34]; (2) myeloid cell-specific deletion of the *Tnfaip3* gene in mice leads to enhanced NF-κB and inflammasome signalling and triggers a spontaneous erosive polyarthritis that resembles human RA[35]; and (3) A20 mRNA expression in PBMCs is lower in patients with RA compared with healthy individuals.[33] The modulation of A20 by MTX that we now report supports the existence of a myeloid-specific effect of the main anchor drug in the treatment of RA and positions A20 as a potential biomarker of responsiveness to MTX, an urgently required parameter to identify those patients with early arthritis who achieve an ACR50 response to MTX monotherapy (usually around 40%).[13] Therefore, the analysis of *TNFAIP3* polymorphism in MTX-treated patients with RA might be of interest to predict MTX responsiveness in patients with RA and could become a useful MTX response biomarker. Supporting this suggestion, a single nucleotide polymorphism within the *OLIG3/TNFAIP3* locus is associated with reduced likelihood to remain on MTX therapy in patients with early RA.[36]

MTX is considered a prodrug, a compound that undergoes a biochemical modification to become its active form. MTX is polyglutamated once taken up by the cells and MTX polyglutamates (MTX-Pgl) constitute the active form of the drug. MTX-Pgl potently inhibits TS and aminoimidazole-carboxamide-ribonucleoside transformylase. In human macrophages, proinflammatory TS^{+} GM-CSF-primed macrophages retain higher levels of $MTX\text{-}Glu_2$ and $MTX\text{-}Glu_3$ than anti-inflammatory $TS^{low/-}$ M-CSF-polarised macrophages and are more susceptible to MTX.[17] Importantly, retention of MTX-Pgl in cells exceeds its half-life in plasma, suggesting that MTX metabolites persist in tissues. In fact, long-lived MTX-Pgl remains in the liver and in bone marrow myeloid precursors for a long period of time.[37] The accumulation of MTX in myeloid precursors correlates with the tolerance mechanism that we now describe because macrophages in the inflamed synovial tissue of patients with RA are continuously replaced by circulating monocytes,[38 39] and because myeloid precursors appear to contribute to trained innate immunity.[40] We hypothesise that monocytes from MTX-treated patients would exhibit impaired responsiveness to danger signals (TNFα, RASF) and that they would display a lower proinflammatory profile than resident macrophages within the inflamed synovia. Therefore, the entry of macrophages with a lower proinflammatory potential into the inflamed synovia would contribute to the beneficial effect of MTX in RA. In any event, the involvement of A20 in MTX response provides a new mechanism of action for MTX, an old drug with low cost and a good safety record.

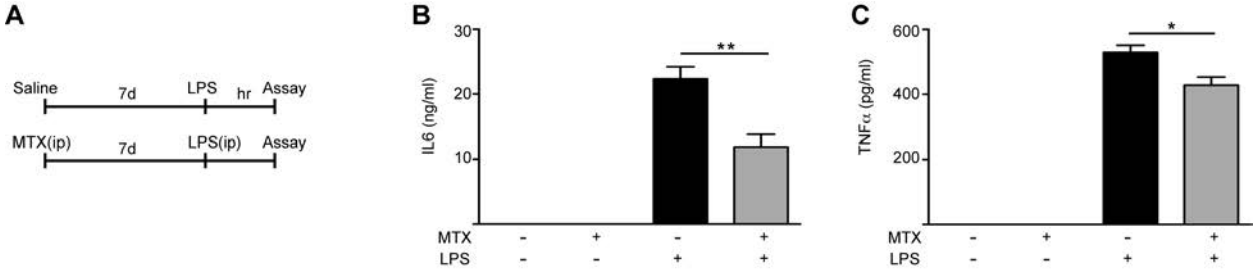


Figure 5 Effect of low-dose MTX in LPS tolerance in vivo. (A) Schematic representation of the experiment. C57BL/6J mice received an intraperitoneal injection of either saline or MTX (2 mg/kg). Seven days later, intraperitoneal LPS (9 mg/kg) was administered and serum collected after 4 hours for IL-6 (B) and TNFα (C) determination by ELISA. Data represent the results of two independent experiments using a total of 12 mice per group. Mean±SEM of 12 mice per group are shown (*P<0.05, **P<0.01). MTX, methotrexate.

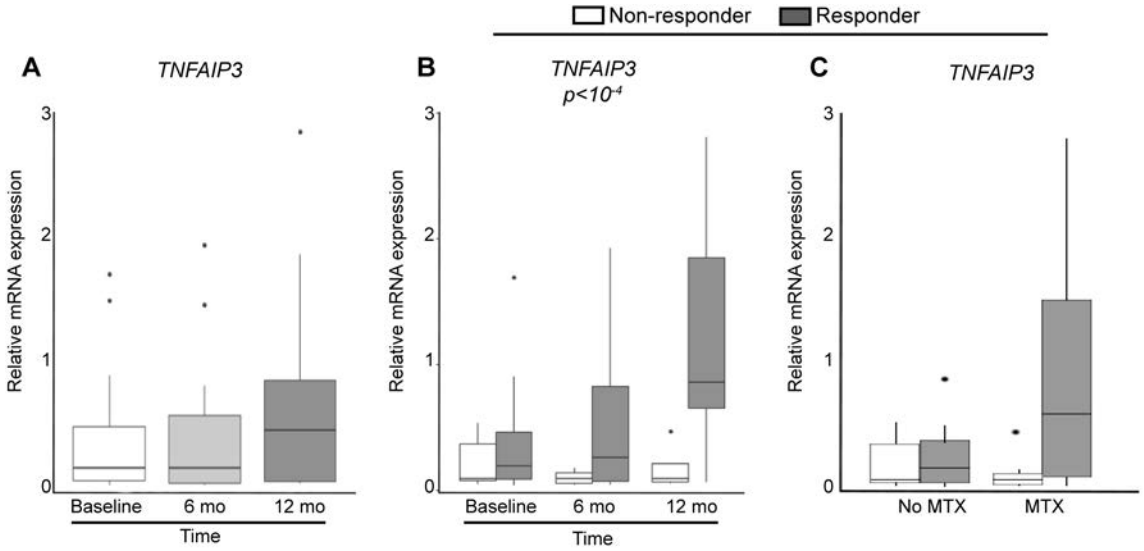


Figure 6 Effect of MTX on *TNFAIP3* mRNA expression of peripheral blood mononuclear cells from patients with early arthritis. (A) *TNFAIP3* expression along the follow-up (n=17 patients; online supplementary table S2). (B) *TNFAIP3* expression between responder and non-responder patients along the follow-up (n=17 patients). The variable that explained *TNFAIP3* expression was being responder to MTX ($P<10^{-4}$, model 3 in online supplementary table S2). (C) *TNFAIP3* expression between responder and non-responder patients untreated or treated with MTX (n=15 visits in non-responder and n=35 visits in responder patients). MTX, methotrexate.

Acknowledgements The authors acknowledge Carlota Chiralt and Julia Villarejo for technical help in the sepsis experiments and Dr Angel L. Corbí, Dr Juan D. Cañete and Dr Paloma Sánchez-Mateos for helpful discussions.

Contributors CM and AD-S designed research, performed research and analysed data; SF-R, AL, NM, VDC and RGC performed research and analysed data; JLP participated in the research; IG-A designed research and analysed data; AP-K conceived the study, designed research, performed some research, analysed data and wrote the paper. All authors had final approval of the version.

Funding This work was supported by grants from Instituto de Salud Carlos III/FEDER (PI14/00075 and PI17/00037) to AP-K, PI14/00422 to IG-A, RIER RD16 to JLP, IG-A and AP-K, Ministerio de Economía y Competitividad SAF2014-54423-R to ALC. FEDER, Fondo Europeo de Desarrollo Regional: una manera de hacer Europa. SF-R and AP-K are supported by FIBHGM.

Competing interests None declared.

Patient consent Obtained.

Ethics approval This study was approved by Research Ethics Committee of Hospital Universitario La Princesa (PI-518).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Choy EH, Panayi GS. Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med* 2001;344:907–16.
2 Scott DL, Wolfe F, Huizinga TW. Rheumatoid arthritis. *Lancet* 2010;376:1094–108.
3 McInnes IB, Schett G. The pathogenesis of rheumatoid arthritis. *N Engl J Med* 2011;365:2205–19.
4 Hamilton JA, Tak PP. The dynamics of macrophage lineage populations in inflammatory and autoimmune diseases. *Arthritis Rheum* 2009;60:1210–21.
5 Haringman JJ, Gerlag DM, Zwinderman AH, *et al*. Synovial tissue macrophages: a sensitive biomarker for response to treatment in patients with rheumatoid arthritis. *Ann Rheum Dis* 2005;64:834–8.
6 Wicks IP, Roberts AW. Targeting GM-CSF in inflammatory diseases. *Nat Rev Rheumatol* 2016;12:37–48.
7 de Vries EG, Willemse PH, Biesma B, *et al*. Flare-up of rheumatoid arthritis during GM-CSF treatment after chemotherapy. *Lancet* 1991;338:517–8.
8 Soler Palacios B, Estrada-Capetillo L, Izquierdo E, *et al*. Macrophages from the synovium of active rheumatoid arthritis exhibit an activin A-dependent pro-inflammatory profile. *J Pathol* 2015;235:515–26.
9 Behrens F, Tak PP, Østergaard M, *et al*. MOR103, a human monoclonal antibody to granulocyte-macrophage colony-stimulating factor, in the treatment of patients with moderate rheumatoid arthritis: results of a phase Ib/IIa randomised, double-blind, placebo-controlled, dose-escalation trial. *Ann Rheum Dis* 2015;74:1058–64.
10 Burmester GR, Weinblatt ME, McInnes IB, *et al*. Efficacy and safety of mavrilimumab in subjects with rheumatoid arthritis. *Ann Rheum Dis* 2013;72:1445–52.
11 Burmester GR, McInnes IB, Kremer J, *et al*. A randomised phase IIb study of mavrilimumab, a novel GM-CSF receptor alpha monoclonal antibody, in the treatment of rheumatoid arthritis. *Ann Rheum Dis* 2017;76:1020–30.
12 Visser K, Katchamart W, Loza E, *et al*. Multinational evidence-based recommendations for the use of methotrexate in rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative. *Ann Rheum Dis* 2009;68:1086–93.
13 Brown PM, Pratt AG, Isaacs JD. Mechanism of action of methotrexate in rheumatoid arthritis, and the search for biomarkers. *Nat Rev Rheumatol* 2016;12:731–42.
14 Kremer JM, Lawrence DA, Hamilton R, *et al*. Long-term study of the impact of methotrexate on serum cytokines and lymphocyte subsets in patients with active rheumatoid arthritis: correlation with pharmacokinetic measures. *RMD Open* 2016;2:e000287.
15 Spurlock CF, Tossberg JT, Matlock BK, *et al*. Methotrexate inhibits NF-κB activity via long intergenic (noncoding) RNA-p21 induction. *Arthritis Rheumatol* 2014;66:2947–57.
16 Spurlock CFMethotrexate-mediated inhibition of nuclear factor kappaB activation by distinct pathways in T cells and fibroblast-like synoviocytes*Rheumatology* 2015;54:178–87.
17 Municio C, Soler Palacios B, Estrada-Capetillo L, *et al*. Methotrexate selectively targets human proinflammatory macrophages through a thymidylate synthase/p53 axis. *Ann Rheum Dis* 2016;75:2157–65.
18 Hoekstra M, Haagsma C, Neef C, *et al*. Bioavailability of higher dose methotrexate comparing oral and subcutaneous administration in patients with rheumatoid arthritis. *J Rheumatol* 2004;31:645–8.
19 Godfrey C, Sweeney K, Miller K, *et al*. The population pharmacokinetics of long-term methotrexate in rheumatoid arthritis. *Br J Clin Pharmacol* 1998;46:369–76.
20 González-Álvaro I, Ortiz AM, Alvaro-Gracia JM, *et al*. Interleukin 15 levels in serum may predict a severe disease course in patients with early arthritis. *PLoS One* 2011;6:e29492.
21 Joosten LA, Abdollahi-Roodsaz S, Dinarello CA, *et al*. Toll-like receptors and chronic inflammation in rheumatic diseases: new developments. *Nat Rev Rheumatol* 2016;12:344–57.
22 Riera-Borrull M, Cuevas VD, Alonso B, *et al*. Palmitate Conditions Macrophages for Enhanced Responses toward Inflammatory Stimuli via JNK Activation. *J Immunol* 2017;199:3858–69.

23 Biswas SK, Lopez-Collazo E. Endotoxin tolerance: new mechanisms, molecules and clinical significance. *Trends Immunol* 2009;30:475–87.
24 Park SH, Park-Min KH, Chen J, *et al*. Tumor necrosis factor induces GSK3 kinase-mediated cross-tolerance to endotoxin in macrophages. *Nat Immunol* 2011;12:607–15.
25 Häcker H, Redecke V, Blagoev B, *et al*. Specificity in Toll-like receptor signalling through distinct effector functions of TRAF3 and TRAF6. *Nature* 2006;439:204–7.
26 Nomura F, Akashi S, Sakao Y, *et al*. Cutting edge: endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. *J Immunol* 2000;164:3476–9.
27 Murphy MB, Xiong Y, Pattabiraman G, *et al*. Pellino-3 promotes endotoxin tolerance and acts as a negative regulator of TLR2 and TLR4 signaling. *J Leukoc Biol* 2015;98:963–74.
28 Shembade N, Harhaj EW. Regulation of NF-κB signaling by the A20 deubiquitinase. *Cell Mol Immunol* 2012;9:123–30.
29 Whittle SL, Hughes RA. Folate supplementation and methotrexate treatment in rheumatoid arthritis: a review. *Rheumatology* 2004;43:267–71.
30 Gossec L, Smolen JS, Ramiro S, *et al*. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. *Ann Rheum Dis* 2016;75:499–510.
31 Shen N, Ruan Y, Lu Y, *et al*. Three single nucleotide polymorphisms of TNFAIP3 gene increase the risk of rheumatoid arthritis. *Oncotarget* 2017;8:20784–93.
32 Dieguez-Gonzalez R, Calaza M, Perez-Pampin E, *et al*. Analysis of TNFAIP3, a feedback inhibitor of nuclear factor-kappaB and the neighbor intergenic 6q23 region in rheumatoid arthritis susceptibility. *Arthritis Res Ther* 2009;11:R42.
33 Zhu L, Wang L, Wang X, *et al*. Characteristics of A20 gene polymorphisms and clinical significance in patients with rheumatoid arthritis. *J Transl Med* 2015;13:215.
34 Ma A, Malynn BA. A20: linking a complex regulator of ubiquitylation to immunity and human disease. *Nat Rev Immunol* 2012;12:774–85.
35 Matmati M, Jacques P, Maelfait J, *et al*. A20 (TNFAIP3) deficiency in myeloid cells triggers erosive polyarthritis resembling rheumatoid arthritis. *Nat Genet* 2011;43:908–12.
36 Plant D, Farragher T, Flynn E, *et al*. A genetic marker at the OLIG3/TNFAIP3 locus associates with methotrexate continuation in early inflammatory polyarthritis: results from the Norfolk Arthritis Register. *Pharmacogenomics J* 2012;12:128–33.
37 Koizumi S, Curt GA, Fine RL, *et al*. Formation of methotrexate polyglutamates in purified myeloid precursor cells from normal human bone marrow. *J Clin Invest* 1985;75:1008–14.
38 Thurlings RM, Wijbrandts CA, Bennink RJ, *et al*. Monocyte scintigraphy in rheumatoid arthritis: the dynamics of monocyte migration in immune-mediated inflammatory disease. *PLoS One* 2009;4:e7865.
39 Issekutz AC, Issekutz TB. Monocyte migration to arthritis in the rat utilizes both CD11/CD18 and very late activation antigen 4 integrin mechanisms. *J Exp Med* 1995;181:1197–203.
40 Netea MG, Joosten LA, Latz E, *et al*. Trained immunity: A program of innate immune memory in health and disease. *Science* 2016;352:aaf1098.

EXTENDED REPORT

Drug-induced modulation of gp130 signalling prevents articular cartilage degeneration and promotes repair

Ruzanna Shkhyan,[1] Ben Van Handel,[1,2] Jacob Bogdanov,[1] Siyoung Lee,[1] Yifan Yu,[1,3] Mila Scheinberg,[1] Nicholas W Banks,[1] Sean Limfat,[1] Arthur Chernostrik,[1] Carlos Eduardo Franciozi,[1,4] Mohammad Parvez Alam,[5] Varghese John,[5] Ling Wu,[1] Gabriel B Ferguson,[1] Ali Nsair,[6] Frank A Petrigliano,[7] C Thomas Vangsness,[1] Kanagasabai Vadivel,[7] Paul Bajaj,[7] Liming Wang,[8] Nancy Q Liu,[1] Denis Evseenko[1,2,7]

Handling editor Tore K Kvien

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212037).

For numbered affiliations see end of article.

Correspondence to
Denis Evseenko, Stem Cell Research and Regeneration Medicine USC, 1450 Biggy St, Nrt 4509, Los Angeles, CA 90033, USA; evseenko@usc.edu

RS, BVH and JB contributed equally.

Received 7 July 2017
Revised 3 January 2018
Accepted 16 January 2018
Published Online First
7 February 2018

ABSTRACT

Objective Human adult articular cartilage (AC) has little capacity for repair, and joint surface injuries often result in osteoarthritis (OA), characterised by loss of matrix, hypertrophy and chondrocyte apoptosis. Inflammation mediated by interleukin (IL)-6 family cytokines has been identified as a critical driver of proarthritic changes in mouse and human joints, resulting in a feed-forward process driving expression of matrix degrading enzymes and IL-6 itself. Here we show that signalling through glycoprotein 130 (gp130), the common receptor for IL-6 family cytokines, can have both context-specific and cytokine-specific effects on articular chondrocytes and that a small molecule gp130 modulator can bias signalling towards anti-inflammatory and antidegenerative outputs.

Methods High throughput screening of 170 000 compounds identified a small molecule gp130 modulator termed regulator of cartilage growth and differentiation (RCGD 423) that promotes atypical homodimeric signalling in the absence of cytokine ligands, driving transient increases in MYC and pSTAT3 while suppressing oncostatin M- and IL-6-mediated activation of ERK and NF-κB via direct competition for gp130 occupancy.

Results This small molecule increased proliferation while reducing apoptosis and hypertrophic responses in adult chondrocytes in vitro. In a rat partial meniscectomy model, RCGD 423 greatly reduced chondrocyte hypertrophy, loss and degeneration while increasing chondrocyte proliferation beyond that observed in response to injury. Moreover, RCGD 423 improved cartilage healing in a rat full-thickness osteochondral defect model, increasing proliferation of mesenchymal cells in the defect and also inhibiting breakdown of cartilage matrix in de novo generated cartilage.

Conclusion These results identify a novel strategy for AC remediation via small molecule-mediated modulation of gp130 signalling.

INTRODUCTION

Articular cartilage (AC) is an avascular, specialised tissue found in diarthrodial joints and acts as a substrate to enable fluid motion of joint surfaces. Adult AC is comprised of mostly extracellular matrix (ECM) and water, with chondrocytes constituting only 2%–5% of total tissue volume.[1] Cartilaginous ECM consists mostly of collagens, with collagen II being the most abundant, and proteoglycans including aggrecan. Osteoarthritis (OA) is a degenerative joint disease whose hallmarks include degradation of ECM by proteases including matrix metalloproteinases (MMPs) and members of the disintegrin-like and a metalloproteinase with thrombospondin motif (ADAMTS) family, expression of developmental hypertrophy genes, apoptosis and localised compensatory proliferation termed chondrocyte cloning (reviewed in reference 2[2]). During homoeostasis, articular chondrocytes do not undergo hypertrophy; however, in some pathological conditions, changes mimicking developmental hypertrophy can occur and drive OA.[3 4] In addition to expression of matrix-degrading enzymes, chondrocytes upregulate collagen 10 (*COL10A1*), *RUNX2* and alkaline phosphatase while downregulating articular chondrocyte genes including *COL2A1*, lubricin (*PRG4*) and *SOX9*.[2] Eventually, AC undergoes calcification and chondrocytes are lost to apoptosis. Although the regenerative potential of mature AC is minimal, chondrocytes closest to injured regions on the joint surface in the superficial zone have been shown to proliferate;[2 5 6] however, the frequency of cells that can divide and deposit large amounts of matrix is low and insufficient to enact repair.

The pathogenesis of OA often begins from an injury to AC, which establishes chronic, low-grade inflammation mediated by interleukin-6/glycoprotein 130 (IL-6/gp130) and other factors that promote hypertrophy, matrix degradation and eventual destruction of cartilage (reviewed in reference 7[7]). The IL-6 family of cytokines share a common co-receptor, IL-6RST (gp130; signal transducer (ST)), and includes IL-6, leukaemia inhibitory factor (LIF), oncostatin M (OSM) and others.[8] Signalling downstream of these cytokines involves activation of proteins including MAPKs, JAK/STAT proteins, AKT and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB);[9] both activation of MAPKs (ERK1/2) and NF-κB have been linked to hypertrophy during OA.[10 11] OSM promotes matrix loss and disease progression.[12 13] IL-6 suppresses chondrocyte

To cite: Shkhyan R, Van Handel B, Bogdanov J, *et al*. *Ann Rheum Dis* 2018;**77**:760–769.

proliferation,[14] promotes mineralisation in AC,[15] downregulates matrix proteins[16] and increases expression of proteases.[17 18] Moreover, blockade of IL-6 in mouse models of OA is chondroprotective.[17 18] Importantly, higher serum levels of IL-6 have been correlated with the development of OA in humans,[19] and an antibody against IL-6R is currently in phase III trials for treatment of hand OA (NCT02477059). The downstream mediator of IL-6 signalling STAT3 has been demonstrated to have pleiotropic effects during chondrogenesis and in articular chondrocytes. During chondrogenic differentiation of multipotent mesenchymal stem cells, IL-6/STAT3 promote chondrocyte commitment and matrix production.[20] Similarly, loss of STAT3 during limb formation results in increased hypertrophy, premature ossification and decreased SOX9 expression.[21] This could potentially be regulated in part by the STAT3 target gene *Myc*, which both promotes proliferation and inhibits hypertrophy in developing chondrocytes.[22] In contrast, inhibition of STAT3 downstream of exogenous IL-6 is chondroprotective, reducing the severity of OA-like pathology in a mouse model.[17] These data implicate IL-6 family cytokine signalling as a major regulator of articular chondrocyte biology with potentially context-specific effects.

Here we define the molecular and functional outcomes downstream of gp130 signalling in articular chondrocytes and unveil a small molecule that selectively shifts the output of this pathway to achieve disease-modifying activity in two rat models of cartilage pathology.

RESULTS

Identification of RCGD 423 as a small molecule inhibitor of hypertrophy

Based on the literature defining the relationship between IL-6 family cytokines, hypertrophy and catabolism,[12 13 23–26] we hypothesised that a small molecule regulating this pathway could prevent cartilage loss. To identify compounds that could inhibit hypertrophy, we isolated total limb cells from *Col10a1*-mCherry mice[27] and cultured them in the presence of bone morphogenetic protein (BMP)-4, a driver of developmental chondrocyte hypertrophy;[28] 170 000 compounds were assayed for their ability

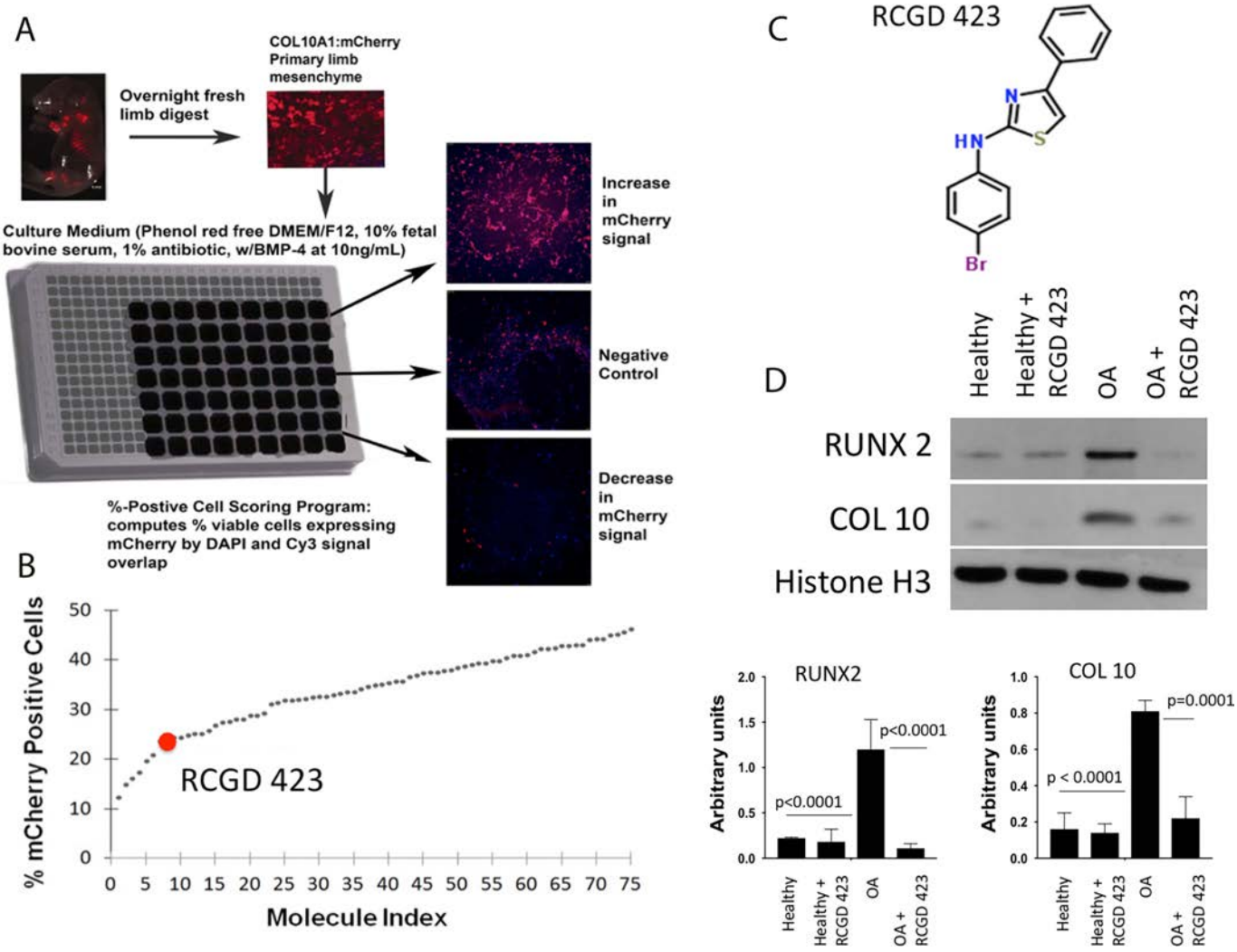


Figure 1 Small molecule screen to identify regulators of cartilage hypertrophy and differentiation. (A) Schematic representation of the high throughput screen performed to identify putative small molecule regulators of chondrocyte differentiation state. Limb mesenchymal cells were isolated from E13.5 mouse embryos carrying a Col10a1-mCherry transgene. Compounds were considered positive hits if they reduced mCherry signal after induction with the prodifferentiation factor BMP-4. (B) Quantitation of top 75 positive hits. (C) Structure of regulator of cartilage growth and differentiation (RCGD) 423. (D) RCGD 423 decreases levels of RUNX2 and COL 10 protein in articular chondrocytes from osteoarthritic donors (n=3). DMEM, Dulbecco's Modified Eagle Media; OA, osteoarthritis.

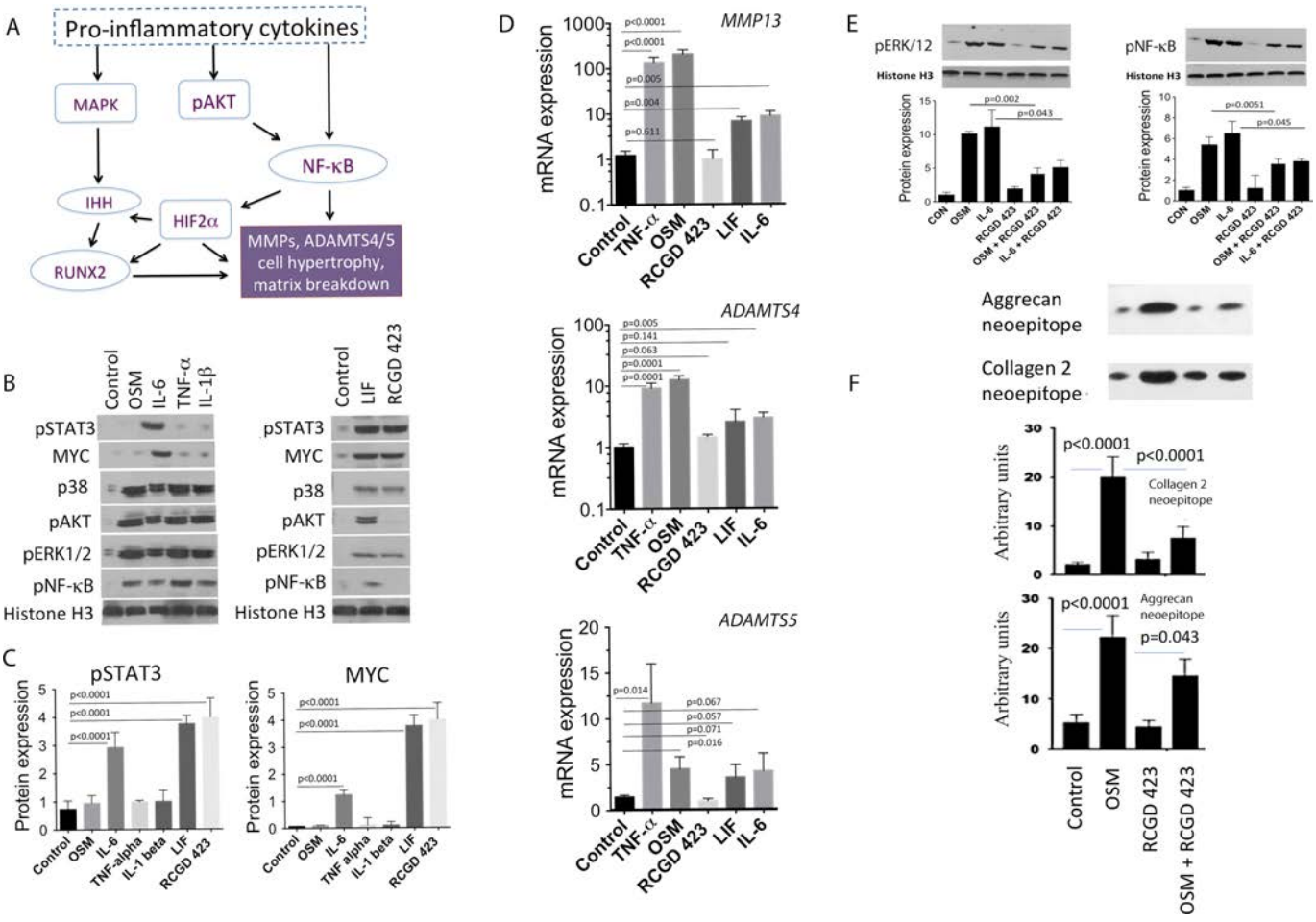


Figure 2 IL-6 family cytokines induce proinflammatory, catabolic signalling that can be directly competed by regulator of cartilage growth and differentiation (RCGD) 423. (A) Schematic of the proinflammatory signalling pathways that cause cell hypertrophy and matrix degradation in chondrocytes. (B) Adult pig articular chondrocytes were cultured for 24 hours with the indicated cytokines or RCGD 423 and the levels of pSTAT3 and MYC were quantified (C; n=3) with respect to histone H3. Representative data for other proteins in MAPK (p38 and pERK1/2), AKT (phospho-AKT; pAKT) or NF-κB (pNF-κB) are also shown. (D) Transcription of catabolic genes was determined via qPCR in adult pig articular chondrocytes treated with oncostatin M (OSM), IL-6, TNF-α, leukaemia inhibitory factor (LIF) or RCGD 423. Data are represented at mean±SD. (E) Adult human chondrocytes were incubated with the indicated cytokines in the presence or absence of RCGD 423 and the levels of downstream proteins quantitated with respect to histone H3. (F) Pig articular cartilage explants were incubated with OSM, RCGD 423 or both; levels of cleaved aggrecan and collagen epitopes in the supernatant are normalised to the wet weight of the explant. For all panels, n=3.

to decrease the mCherry signal (figure 1A). Seventy-five were chosen for follow-up (figure 1B), and additional vetting based on reproducibility and magnitude of *Col10a1* inhibition led to the selection of regulator of cartilage growth and differentiation (RCGD) 423 for continued characterisation (figure 1C). The ability of RCGD 423 to inhibit hypertrophy in human cells was confirmed by assessing RUNX2 and COL 10 levels in healthy and osteoarthritic articular chondrocytes following incubation with the compound (figure 1D); these results demonstrated that RCGD 423 could attenuate hypertrophy in vitro.

RCGD 423 elicits different signalling in chondrocytes than IL-6 family cytokines and can inhibit their catabolic effects

Cartilage degeneration is a feed-forward process, as micro-environmental stresses including inflammation can interact with chondrocyte hypertrophy and loss to promote structural damage.[29] In turn, these alterations promote matrix loss via MMPs (collagenases) and aggrecanases (ADAMTS4/5; figure 2A). To elucidate the mechanisms of action of IL-6 family cytokines, RCGD 423 and known proinflammatory cytokines in the promotion of degeneration, we stimulated pig articular chondrocytes and quantitated the levels of activated downstream signalling proteins (Figure 2B,C) as well as assessed the expression levels of *MMP13*, *ADAMTS4* and *ADAMTS5* (Figure 2D). Treatment with tumor necrosis factor (TNF)-α, a classic proinflammatory cytokine, resulted in strong activation of ERK1/2 and AKT, culminating in increased pNF-κB and upregulation of all catabolic enzymes. Within the IL-6 family cytokines, OSM acted in a similar fashion to TNF-α, while both LIF and IL-6 stimulated the activation of ERK1/2 and AKT to varying degrees and resulted in low-level upregulation of matrix proteases. In parallel with this catabolic signalling, LIF and IL-6 also increased levels of pSTAT3 and MYC, suggesting that both cytokines may have both procatabolic and antihypertrophic effects. RCGD 423 elicited a unique signalling profile, driving strong increases in pSTAT3 and MYC levels without activating AKT or NF-κB, resulting in a signalling milieu downstream of gp130 similar to actively proliferating and anabolic fetal chondrocytes (online supplementary figure 1A–C); concordantly, no upregulation of *MMP13*, *ADAMTS4* and *ADAMTS5* occurred.

In fetal cartilage both STAT3 and MYC signalling are critically important for chondrocyte survival and proliferation, and activation of these pathways appears to be primarily driven by LIF (online supplementary figure 1C–E). We then assessed whether RCGD 423 could inhibit the catabolic effects of these cytokines. Incubation of human articular chondrocytes with IL-6, OSM and RCGD 423 decreased levels of pNF-κB and pERK1/2 when RCGD 423 was included (figure 2E) and reduced catabolic gene expression (online supplementary figure 2A). Moreover, this effect was not limited to chondrocytes, as RCGD 423 reduced both IL-6- and OSM-induced increases in pNF-κB and pERK 1/2 in human synoviocytes and peripheral blood mononuclear cells (PBMCs; online supplementary figure 2B,C). To assess if RCGD 423 could prevent matrix loss stimulated by IL-6 or OSM, we incubated explants of pig AC with either cytokine and the compound and measured neoepitopes of collagen and aggrecan released into the media;[30] inclusion of RCGD 423 strongly reduced the production of cleavage products of both proteins (figure 2F). Together, these data demonstrate that IL-6 family cytokines have developmental stage-specific effects and elicit varying degrees of procatabolic, prohypertrophic responses in adult chondrocytes which can be inhibited by RCGD 423.

RCGD 423 stimulates proliferation and prevents apoptosis in adult articular chondrocytes

To assess the functional effects of RCGD 423 on adult human articular chondrocytes, we first validated that RCGD 423 significantly increased pSTAT3 and MYC (figure 3A) and in a dose-dependent and time-dependent manner (figure 3B). RCGD 423 also increased the proliferative potential and decreased apoptosis in articular chondrocytes (figure 3C,D) as well as increased proliferation in pig AC explants (figure 3E). We next evaluated the effects of RCGD 423 at the transcriptional level on human adult articular chondrocytes (n=7). As expected, the variability between these samples was high, so we focused our analysis on genes that increased more than 1.5-fold in four of seven replicates. GO analysis of these 1244 enriched genes revealed categories related to cell cycle, secretion and migration (figure 3F), while untreated cells were enriched for categories related to IL-6-mediated inflammation (online supplementary figure 3A); we confirmed RCGD 423 upregulated three of these genes with roles in cartilage biology (*HAS3*[31], *FGF18*[32] and *CDK6*[33]; online supplementary figure 3B). We hypothesised that RCGD 423 may promote proliferation through MYC and therefore focused on the 31 genes in the M phase Gene Ontology (GO) category enriched in drug treated cells. We compared these with MYC targets defined by ChIP-Seq,[34] which gave a statistically significant overlap (P=0.0062; hypergeometric test). We then incubated pig articular chondrocytes with RCGD 423 and inhibitors of either STAT3 or MYC to directly assess the roles of these proteins in promoting proliferation. Blockade of STAT3 or MYC reduced proliferation downstream of RCGD 423 (figure 3G). Together, these data demonstrate that RCGD 423 stimulates increases in adult chondrocyte proliferation and survival and suggest that pSTAT3 and MYC may mediate these effects.

RCGD 423 is a direct modulator of gp130 and acts via promoting homodimerisation

Given the molecular and functional effects of RCGD 423, we hypothesised that it may act within the gp130 signalling cascade. To evaluate this, we incubated pig articular chondrocytes with inhibitors of various proteins in this pathway in the presence of either LIF, a canonical activator of gp130, or RCGD 423 (figure 4A,B) and measured levels of pSTAT3 and MYC. Inhibitors of both JAKs and gp130 (SC144)[35] greatly reduced pSTAT3 and MYC activation induced by both LIF and RCGD 423, while the compound induced gp130 activation in a dose-dependent manner (figure 4C), suggesting that RCGD 423 may directly interact with gp130 to induce signalling in the absence of ligand.

To gain better understanding of how RCGD 423 could interact with gp130, we modelled its binding to gp130 using the Swissdock and Gold programmes; this revealed a potential high affinity binding site in domain 2 of the gp130 extracellular region (figure 4D). Notably, deletion of four residues proposed to interact with the drug (K151–R154) has been shown to occur in hepatocellular lesions, and overexpression of this gp130 mutant induced constitutive gp130/STAT3 signalling,[36] while deletion of adjacent residues promoted unbridled gp130/STAT3 activation due to induction of stable homodimerisation of gp130. Based on these findings, we hypothesised that RCGD 423 may increase pSTAT3 by binding to domain 2 of gp130 and stabilising homodimers. To address this, we first transfected $gp130^{-/-}$ Ba/F3 cells (figure 4E)[37] with plasmids encoding either full-length gp130 (WTgp130) or gp130 lacking domain 2 (gp130ΔD2) and cultured them with or without RCGD 423; in gp130ΔD2 cells, the compound did not induce increases in pSTAT3 and MYC (figure 4E,F). We then transfected cells with both Flag-tagged and Myc-tagged gp130 (figure 4G) and cultured them with or without RCGD 423. Cellular extracts were immunoprecipitated with an anti-Flag antibody and then subjected to western blot using an anti-Myc-tag antibody. Only in cells treated with RCGD 423 could we detect complexes containing both forms of gp130, demonstrating that RCGD 423 induced homodimers of gp130. Finally, to demonstrate that gp130 homodimerisation increases levels of pSTAT3 and MYC, we transfected pig articular chondrocytes with a mutant gp130 plasmid shown to form homodimers (ΔS[36]) and performed western blots (figure 4H).

To confirm that gp130 mediates the effects of RCGD 423 in vivo, we co-injected the compound into rat knee joints with either the gp130 inhibitor SC144 or a gp130 domain 2-specific blocking antibody;[38] both abolished the effects of RCGD 423 (online supplementary figure 4A,B). These data demonstrate RCGD 423 acts via gp130 and support the hypothesis that the compound could inhibit IL-6 family cytokines by sequestering gp130 into homodimeric complexes. To directly address receptor competition, we transfected cells with gp130-Flag and treated them with IL-6, RCGD 423 or both and quantitated gp130/IL-6R interactions; inclusion of RCGD 423 dramatically reduced levels of this complex (figure 4I). Determination of the dissociation constant (K_d), a measure of the affinity between gp130 and its binding partners OSMR and IL-6R, in the presence of RCGD 423 and either OSM or IL-6 demonstrated a strong ability of the compound to interfere with ligand-mediated gp130 heterodimerisation (figure 4J,K). Overall, these data indicate that RCGD 423 promotes the formation of ligand-independent homodimers of gp130 that can prevent heterodimerisation with IL-6 family cytokine receptors. Although direct physical interaction of RCGD 423 with gp130 has not been experimentally demonstrated, the homodimer formation required the integrity of domain 2, which, based on in silico studies, is predicted to bind gp130.

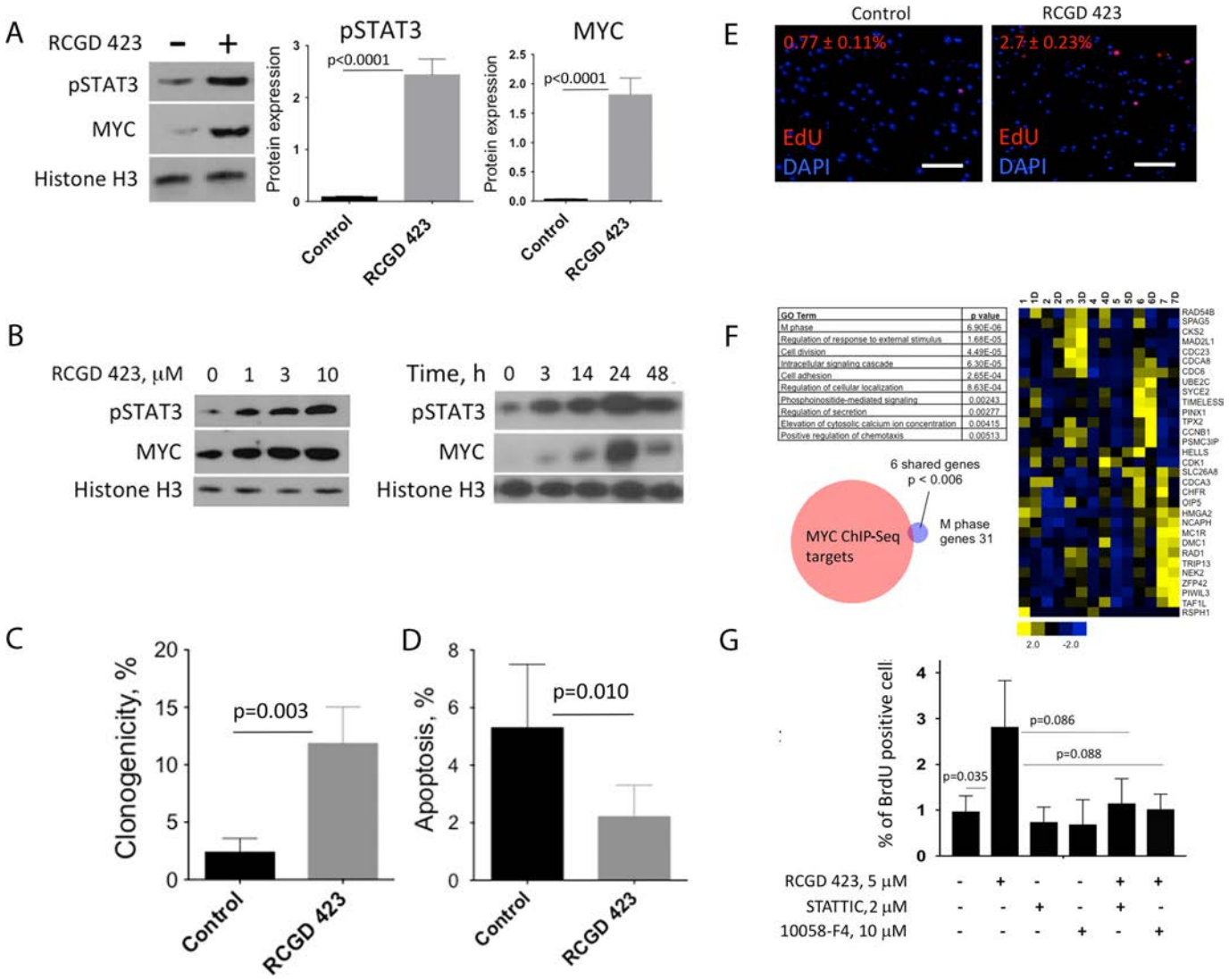


Figure 3 Regulator of cartilage growth and differentiation (RCGD) 423 inhibits apoptosis and promotes proliferation via induction of pSTAT3 and MYC in adult human chondrocytes. (A) Adult human articular chondrocytes were incubated with or without RCGD 423 and levels of MYC and pSTAT3 were quantified relative to histone H3 after 24 hours. (B) Increases in pSTAT3 and MYC proteins occurred in both a dose-dependent and time-dependent fashion following stimulation with RCGD 423. (C) Single human adult articular chondrocytes were cultured for 5 weeks with or without stimulation with RCGD 423 and assessed for colony formation. (D) Adult human articular chondrocytes were incubated with or without RCGD 423 in Mebiol hydrogel for 24 hours and then apoptotic cells were quantitated via flow cytometry for annexin V. (E) Proliferation in explants of adult pig articular cartilage in the absence or presence of RCGD 423 as shown by EdU incorporation. Scale bars represent 25 μm. P<0.0001. n=5. (F) Seven independent samples of human adult articular chondrocytes were cultured with or without RCGD 423 and then subjected to RNA-Seq. Genes that were significantly enriched in four of seven drug-treated samples ('D') when compared with their untreated controls were analysed using GO. Selected categories and their respective P values are shown. Heat map depicting the 31 genes in the 'M phase' GO category. Relative expression for all 14 matched samples are shown. Venn diagram depicting the overlap of the 31 genes from the 'M phase' GO category and a gene set defined by Zeller *et al*[34] comprised of direct MYC target genes. (G) Adult human articular chondrocytes were cultured with RCGD 423 and inhibitors of either STAT3 (STATTIC) or MYC (10058-F4) for 48 hours; 5-bromo-2'-deoxyuridine (BrdU) incorporation was measured by flow cytometry. Unless noted, n=3. DAPI, 4'-6-diamidino-2-phenylindole.

RCGD 423 prevents AC degeneration in vivo

Based on these data, we hypothesised that RCGD 423 could promote cartilage retention following injury by inhibiting inflammation and hypertrophy and inducing proliferation. We first defined the half maximal effective concentration (EC_{50}) of RCGD 423 on human articular chondrocytes by quantifying phosphorylation of gp130 and STAT3 as well as MYC protein levels; these results demonstrated an EC_{50} in the range of 4.5–7.2 μM (online supplementary figure 5). To address whether RCGD 423 could ameliorate cartilage degeneration, we adopted a rat partial meniscectomy model,[39] in which 30%–50% of the meniscus is removed (online supplementary figure 7A). As a delivery vehicle, we employed US Food and Drug Administration-approved poly(lactic-co-glycolic) acid (PLGA) microspheres loaded with RCGD 423 (online supplementary figure 6A). Direct evaluation of offloading kinetics of RCGD 423 from PLGA microspheres showed that 20 μg of the compound could sustain a 0.1–0.3 μM concentration for at least 12 days (online supplementary figure 6B) in vitro, which would be fivefold to sevenfold higher in the context of a 150–200 μL rat joint.[40] Based on these data, we designed a 6-week experiment in which operated animals

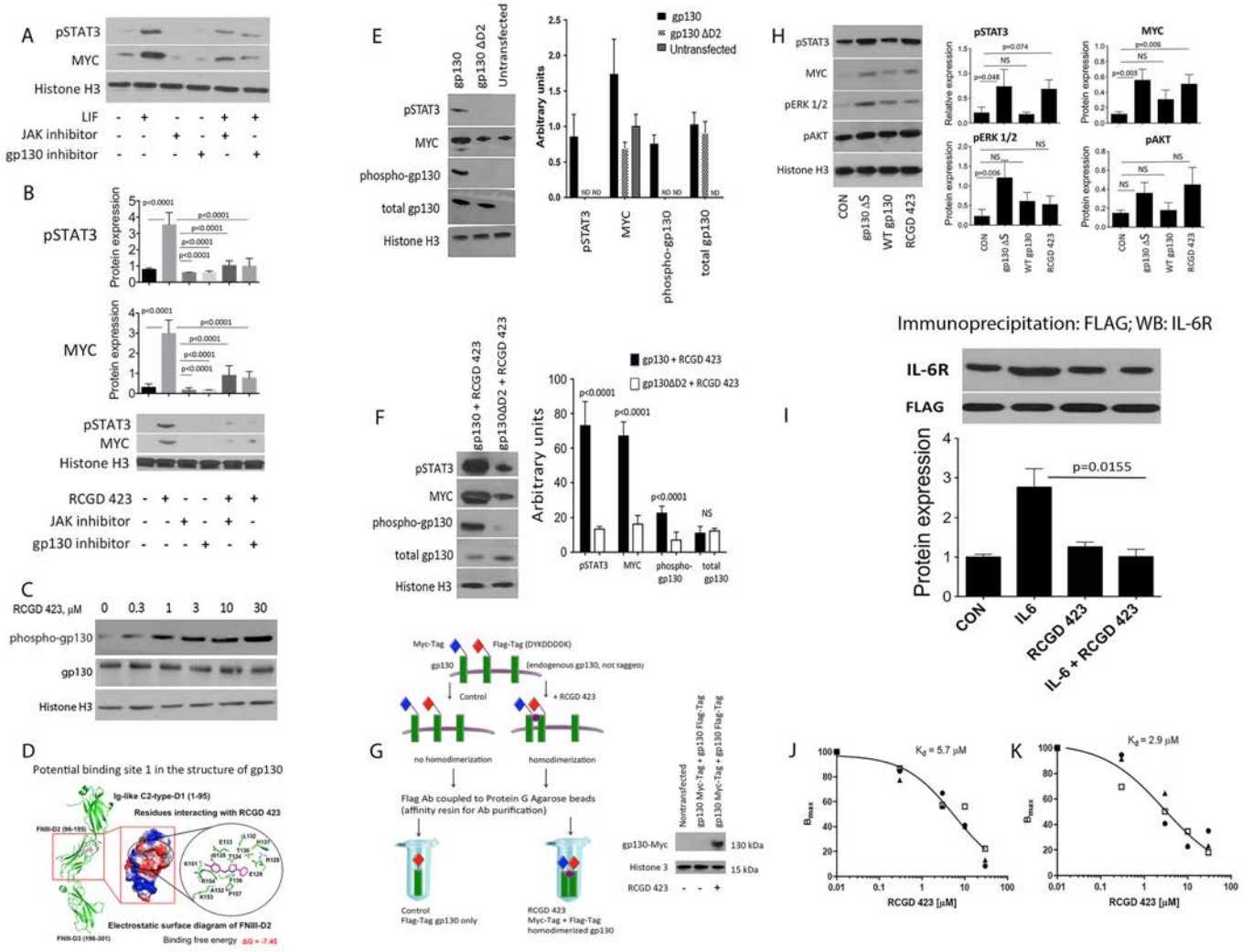


Figure 4 Regulator of cartilage growth and differentiation (RCGD) 423 binds to domain 2 of glycoprotein 130 (gp130) and induces stable homodimerisation, thereby competing out IL-6 family cytokine receptors. (A) Levels of MYC and pSTAT3 protein after 24 hours in adult pig articular chondrocytes in the presence or absence of leukaemia inhibitory factor (LIF) and JAK or gp130 inhibitors. Histone H3 was used as a loading control. (B) Adult pig articular chondrocytes were cultured in the presence of the indicated combinations of RCGD 423, JAK and/or gp130 inhibitors and the levels of MYC and pSTAT3 proteins were quantitated relative to histone H3 after 24 hours. (C) Phosphorylation of gp130 (Tyr905) occurred in a dose-dependent manner after stimulation with RCGD 423. (D) Predicted binding site of RCGD 423 in the extracellular domain of gp130. The structure of the indicated gp130 domains is shown in ribbon diagram representation (left) as well as with electrostatic potential (blue, positive charge; red, negative charge; white, neutral) mapped onto the molecular surface (right). RCGD 423 and gp130 residues within 4Å surrounding it are shown in stick representation in the expanded views. Carbon atoms are shown in green for gp130 and pink for RCGD 423. Oxygen and nitrogen atoms are in red and blue, respectively; the bromine atom in RCGD 423 is shown in red, while the sulfur atom is in yellow. The electrostatic potential surfaces are drawn at ±3 kT/e. D1, domain 1; FNIII, fibronectin type-III. (E) Protein was isolated from cultures of Ba/F3 cells 24 hours after transfection with either full-length gp130 or gp130 lacking domain 2 (ΔD2). (F) BaF3 cells were transfected with the indicated variants of gp130. Three independent clones were used for transfection. (G) Schematic representation of experimental design to assess RCGD 423 interaction with gp130. Protein complexes were immunoprecipitated with an anti-Flag antibody; western blotting was performed with an anti-Myc-tag antibody. Representative results are shown from four independent experiments. (H) Pig articular chondrocytes were transfected with wild type (WT) gp130 or gp130 lacking four amino acids (S187–Y190; ΔS) and the levels of indicated proteins quantified by western blot. (I) Adult human chondrocytes were transfected with gp130-Flag and then incubated with IL-6, RCGD 423 or both; an anti-Flag antibody was used to immunoprecipitate gp130-associated proteins. Western blots for IL-6R and gp130-Flag were used to quantitate gp130/IL-6R interactions and normalise immunoprecipitated protein levels, respectively. Adult human chondrocytes transfected with gp130-Flag were incubated with various concentrations of RCGD 423 in the presence of oncostatin M (OSM) or IL-6; levels of immunoprecipitated (J) oncostatin M receptor (OSMR) or (K) IL-6R were used to calculate the dissociation constant (K_d) for each complex. For all panels, n=3.

would receive an intra-articular injection of 4 μg RCGD 423 loaded onto microspheres at the time of surgery and another 3 weeks later. This dosing strategy was projected to produce an average intra-articular concentration of ~0.3 μM RCGD 423, as this concentration consistently resulted in increased gp130 pathway activity in vitro (figure 4 and data not shown). Micro CT verified loss of cut meniscus (online supplementary figure 7B). We then used the Osteoarthritis Research Society International (OARSI) histological scoring system[41] to quantify the extent of cartilage damage in all animals. Tissue sections were

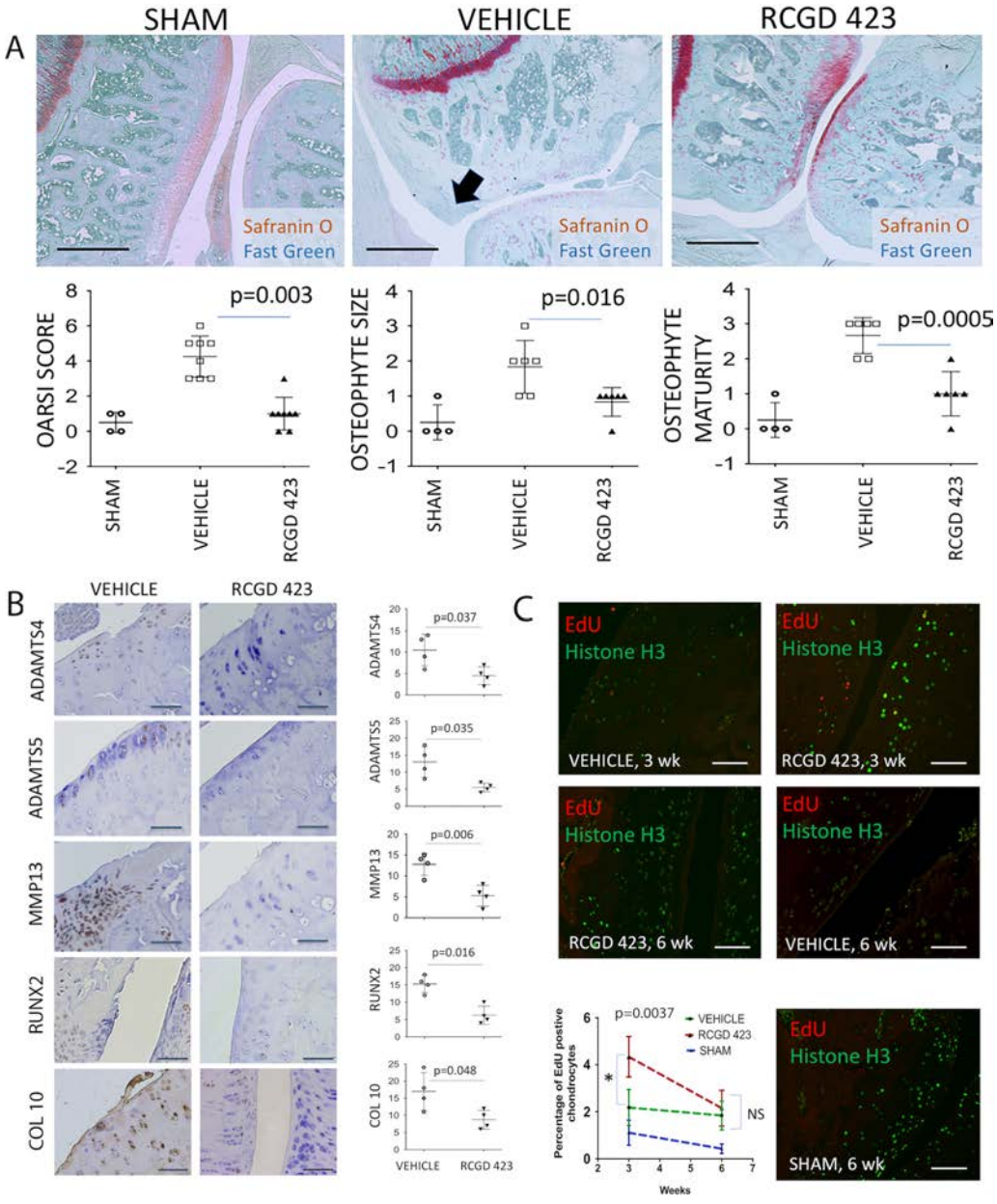


Figure 5 Regulator of cartilage growth and differentiation (RCGD) 423 prevents articular cartilage degeneration in vivo. (A) Histological staining and quantitative assessment of cartilage degradation and changes in joint morphology of rat knee joints 6 weeks after partial meniscectomy surgery. RCGD 423-loaded or empty microspheres were injected intra-articularly at the time of surgery and 3 weeks later (online supplementary figure 7). Safranin O delineates proteoglycans; arrow indicates a representative osteophyte. Sham=joint capsule exposure but no meniscectomy. Scale bars represent 100 µm, n=8. (B) Sections of RCGD 423-treated or control joints were stained for matrix degrading enzymes and markers of hypertrophy. Representative images are shown; scale bars=25 µm. n=4. (C) Partial meniscectomies were performed on rats and RCGD 423 or saline injected intra-articularly immediately and at weeks 1 and 2 or at weeks 3, 4 and 5 (online supplementary figure 7); EdU was injected intraperitoneally each day for 4 days after each articular injection. EdU$^+$ cells in articular cartilage were scored in four animals for each condition and time point; histone H3 was used to stain nuclei. Scale bars=25 µm.

stained with Safranin O to detect proteoglycans (figure 5A); these results demonstrated dramatic loss of cartilage on the tibial plateau in control animals, while in RCGD 423-treated animals there was little to no cartilage degradation, structural damage or generation of osteophytes (figure 5A). Control animals showed increased levels of cartilage-degrading proteins and markers of hypertrophy, while these were mostly absent in RCGD 423-treated rats (figure 5B). To evaluate proliferation, animals were injected with RCGD 423 once a week and administered 5-ethynyl-2'-deoxyuridine (EdU) for 4 days after each injection; animals were sacrificed at 3 weeks and 6 weeks after meniscectomy (online supplementary figure 7C). These results demonstrated RCGD 423 enhanced the minimal chondrocyte proliferation occurring soon after surgery; later, the ability of the compound to induce proliferation was limited (figure 5C). Together, these data indicate that RCGD 423 can prevent cartilage degeneration in vivo, associated with decreasing levels of catabolic enzymes and an early proliferative response.

RCGD 423 promotes cartilage repair in vivo

We then evaluated the ability of RCGD 423 to promote cartilage repair in a rat osteochondral defect model

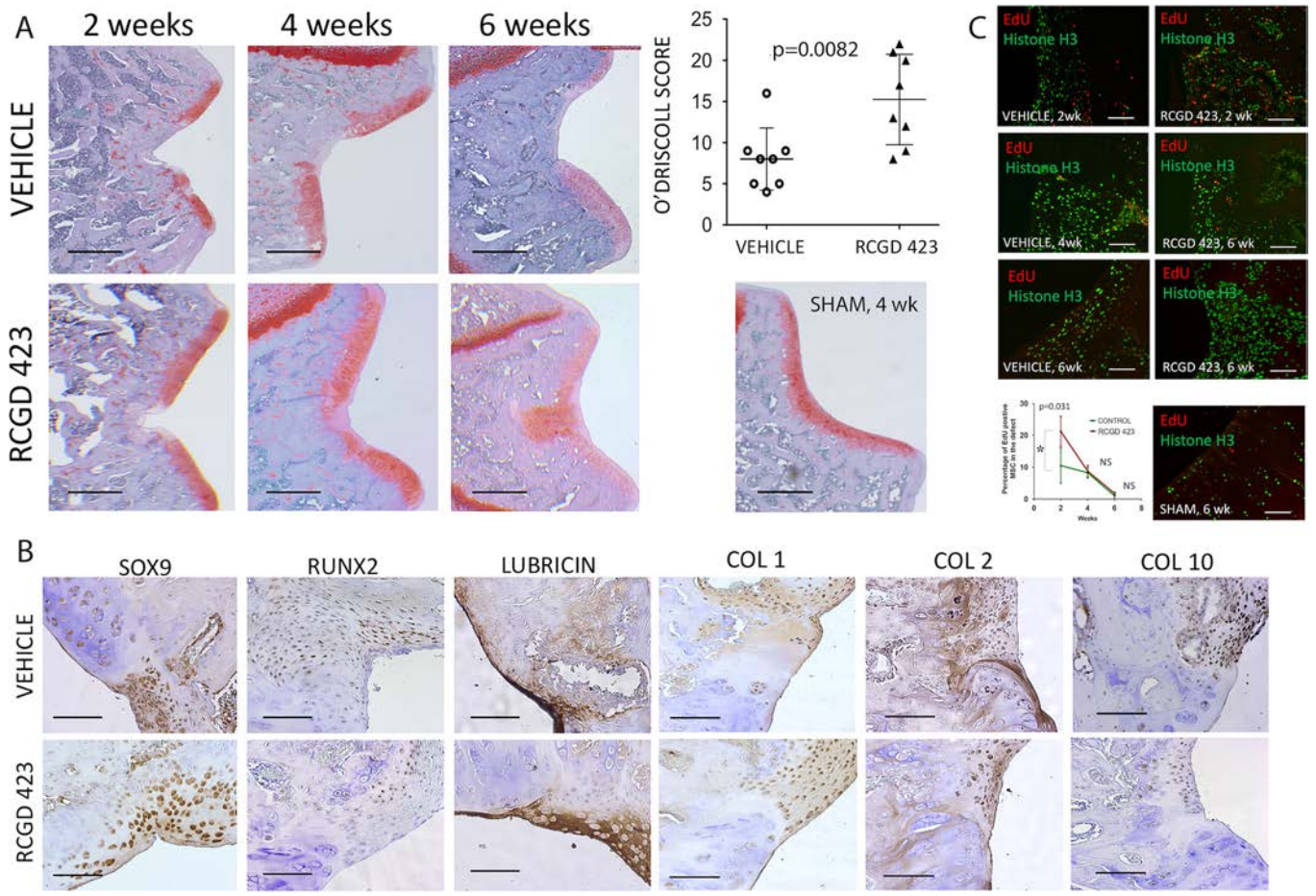


Figure 6 Regulator of cartilage growth and differentiation (RCGD) 423 promotes cartilage repair following osteochondral injury. (A) Full-thickness osteochondral defects were created in the patellar grooves of rats. Saline (vehicle) or RCGD 423 were injected intra-articularly at the time of surgery and weekly afterwards. Animals were sacrificed at the indicated time points for Safranin O staining and histological scoring (4 weeks; n=8). Scale bars=100 µm. (B) Markers of chondrocyte identity, hypertrophy and fibrosis were assessed on the cells present in the defects of RCGD 423-treated and saline-treated animals. n=4. Scale bars=25 µm. (C) Osteochondral defects and treatments were conducted as in (A); EdU was injected intraperitoneally for 4 days after each treatment (online supplementary figure 8). EdU$^+$ cells in the defects were scored in four animals for each condition and time point; histone H3 was used to stain nuclei. Scale bars=25 µm.

(online supplementary figure 8), in which new cartilage is generated by both synovial and bone marrow stromal cells.[42 43] These defects spontaneously heal in 4 weeks, and cartilage repair was assessed at this time point;[44] these results demonstrated a highly significant improvement in cartilage resurfacing in the presence of RCGD 423 (figure 6A). This result was further verified using traditional markers of chondrocyte identity (SOX9, COL 2) or hypertrophy (RUNX2, COL 10; figure 6B). In vitro experiments demonstrated the compound significantly decreased matrix degradation in cartilage pellets generated using pig synovial stromal cells (online supplementary figure 8A) in the presence of OSM, consistent with effects of RCGD 423 on cartilage explants exposed to either IL-6 or OSM. We hypothesised that enhanced repair may also be due to proliferation induced by RCGD 423. Two weeks after injury, EdU incorporation by cells in the defect was significantly increased in RCGD 423-treated rats; this effect was temporary, as at 4 weeks and 6 weeks when defects were fully repaired no difference was observed (figure 6C). Taken together, these data define the gp130 modulator RCGD 423 as an agent that can potentially improve cartilage healing following full-thickness injury by reducing catabolism and enhancing the proliferative response.

DISCUSSION

Hypertrophy driven by IL-6 family cytokines has emerged as one driver of OA. Here we show that small molecule-mediated modulation of gp130 signalling can bias output downstream of this pleiotropic pathway against hypertrophic, procatabolic effects and promote chondrocyte proliferation in vivo. We identified RCGD 423 based on its ability to inhibit developmental hypertrophy in mouse limb mesenchymal cells, and these effects were conserved in preventing disease-based hypertrophy in chondrocytes from human patients with OA. Molecularly, RCGD 423 promotes formation of active homodimers signalling primarily via pSTAT3/MYC; this mechanism of action can actively compete against IL-6 family cytokine-mediated heterodimerisation, thereby inhibiting the hypertrophic and catabolic effects of this pathway mediated by ERK1/2 and NF-κB. Importantly, gp130 signal modulation occurs in chondrocytes, synoviocytes and PBMCs, thus providing a means to combat the proinflammatory, procatabolic milieu found in destabilised and full-thickness injured rat joints. Together, our results provide additional insight into IL-6 family cytokine signalling in chondrocytes and nominate gp130 signal modulation as a potential therapeutic strategy for OA.

The function of IL-6 family members in cartilage biology and pathogenesis has been the focus of much study (reviewed in reference 45[45]). IL-6, OSM and LIF have been shown to promote OA, either through acting as proinflammatory cytokines or directly regulating matrix destruction.[13 25 26] Consequently, all members of the IL-6 family are often considered to be detrimental to chondrocyte biology. However, IL-6 has been shown to be chondroprotective in ageing mice,[46] in agreement with our data that during human development and when output of gp130 is modulated this pathway can be beneficial. Our data show that individual cytokines activate MAPK and NF-κB differently and result in varied transcriptional responses of catabolic genes (figure 2). Moreover, OSM elicits minimal upregulation of pSTAT3 or MYC, while IL-6 and LIF both increase levels of these proteins. These data suggest that there is great diversity in response to IL-6 family members in chondrocytes and that individual cytokines (eg, IL-6 and LIF) can have both positive (induction of proliferation) and negative (increases in NF-κB and catabolic gene expression) effects. RCGD 423 represents an interesting new facet of this story, as it uncouples some of the effects downstream of gp130 signalling, such as MAPK/NF-κB activation and catabolic gene expression, from STAT3/MYC activation. This bimodal mechanism of action will need to be evaluated in the context of more advanced cartilage injury to assess the ability of RCGD 423 to reverse pre-existing damage. Moreover, the compound can compete against procatabolic signalling mediated by both IL-6 and OSM, presenting an advantage over existing anti-IL-6/IL-6R therapies.

Although proliferation of adult articular chondrocytes is minimal (figure 5), we found moderate upregulation of proliferation induced by RCGD 423 both in vitro and in vivo. In vivo, EdU^+ cells were found in both the most superficial as well as adjacent layers of cartilage, suggesting that a surprising number of resident chondrocytes are capable of responding to activation signals present in the injured joint. Chondrocyte cloning has been well documented in the past, but this is typically associated with later stages of disease progression;[2] RCGD 423 significantly increased rates of EdU incorporation, and it will be critical in future work to determine the molecular identity of these cells as well as to address the relative contributions of proliferation versus anticatabolic effects to the reduction in cartilage degeneration supported by RCGD 423. Moreover, given the decrease in proliferation elicited by the compound at 6 weeks postinjury, it will be important to ascertain if cells capable of responding are limited in the number of divisions they can undergo as this would suggest that other cell types such as synovium[43] may need to be harnessed for cartilage repair or that a therapeutic window exists for potential intervention.

LIF and other members of the IL-6 family have been shown to mediate proliferation and regeneration in a variety of cellular contexts. IL-6 and OSM have both been shown to be important for the regenerative response in the liver, acting upstream of STAT3 to promote proliferation.[47] As we demonstrate here, levels of pSTAT3 and MYC are high in rapidly growing fetal tissues; temporary and controlled upregulation by a more MYC-inducing RCGD 423 analogue, recently synthesised by our group, may be beneficial for tissue repair (online supplementary figure 9) as was recently shown by Flores *et al*[48] in the context of acceleration of the hair cycle. Intriguingly, Ocampo *et al*[49] recently demonstrated that repeated, transient increases in systemic MYC protein improved regeneration and did not result in tumorigenesis. These results suggest that both intra-articular, as well as systemic administration of RCGD 423 may represent potential strategies to promote tissue repair; however, close monitoring of proliferation in all tissues will be critical. Our work indicates that gp130 can function as a node whose modulation may tip the balance in pathological conditions away from tissue degeneration and towards repair. In summary, we have identified a novel small molecule modulator of gp130 signalling that demonstrates prominent disease-modifying activity in two rat models of cartilage injury or/and degeneration. Optimised analogues of this compound may represent attractive therapeutic candidates for patients with both degenerative and inflammatory forms of arthritis.

Author affiliations
[1]Department of Orthopaedic Surgery, University of Southern California (USC), Los Angeles, California, USA
[2]Department of Stem Cell Research and Regenerative Medicine, University of Southern California, Los Angeles, California, USA
[3]Department of Orthopaedic Surgery, Huai'an First People's Hospital, Nanjing Medical University, Huai'an, China
[4]Department of Orthoapedic Surgery, Federal University of São Paulo (UNIFESP), São Paulo, Brazil
[5]Drug Discovery Laboratory, Department of Neurology, University of California at Los Angeles, Los Angeles, California, USA
[6]Department of Medicine, Division of Cardiology, David Geffen School of Medicine (DGSOM), University of California, Los Angeles, California, USA
[7]Department of Orthopaedic Surgery, David Geffen School of Medicine (DGSOM), University of California at Los Angeles, Los Angeles, California, USA
[8]Department of Orthopaedic Surgery, Nanjing First Hospital, Nanjing Medical University, Nanjing, China

Correction notice This article has been corrected since it published Online First. The affiliation for Mohammad Parvez Alam has been corrected.

Acknowledgements The authors thank Ms Felicia Codrea and Ms Jessica Scholes of UCLA for their help with sample analysis and sorting. The authors also thank Dr Xinmin Li from UCLA CMC for his help with sequencing experiments and Dr Kenneth Dorshkind (UCLA) for critical reading of the manuscript, as well as Dr Zucman E Rossi for sharing reagents.

Contributors RS, BVH, JB and DE designed experiments; RS, BVH, JB, SL, YY, MS, NWB, SL, AC, CEF, MPA, LW, GBF, KV, NQL and DE conducted experiments; VJ, AN, FAP, CTV, LW and PB provided expertise and/or reagents; BVH and DE wrote the manuscript, all authors reviewed it.

Funding This work is supported by NIH grant K01AR061415, Department of Defense grant W81XWH-13-1-0465, California Institute for Regenerative Medicine grant RB5-07230, a USC Stevens Technology Advancement Grants award and a Wright Foundation award (all to DE) as well as a postdoctoral scholarship from Fundação de Amparoà Pesquisa do Estado de São Paulo E FAPESP (grant# 2015/08952E6) to CEF.

Competing interests DE, BVH and VJ are inventors onPCT/US16/20126. DE and BVH are cofounders and shareholdersof CarthroniX.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement Primer sequences are available upon request; please contact the corresponding authors.

REFERENCES

1 Sophia Fox AJ, Bedi A, Rodeo SA. The basic science of articular cartilage: structure, composition, and function. *Sports Health* 2009;1:461–8.
2 van der Kraan PM, van den Berg WB. Chondrocyte hypertrophy and osteoarthritis: role in initiation and progression of cartilage degeneration? *Osteoarthritis Cartilage* 2012;20:223–32.
3 Saito T, Fukai A, Mabuchi A, *et al*. Transcriptional regulation of endochondral ossification by HIF-2alpha during skeletal growth and osteoarthritis development. *Nat Med* 2010;16:678–86.
4 Yang S, Kim J, Ryu JH, *et al*. Hypoxia-inducible factor-2alpha is a catabolic regulator of osteoarthritic cartilage destruction. *Nat Med* 2010;16:687–93.
5 Yasuhara R, Ohta Y, Yuasa T, *et al*. Roles of β-catenin signaling in phenotypic expression and proliferation of articular cartilage superficial zone cells. *Lab Invest* 2011;91:1739–52.
6 Dowthwaite GP, Bishop JC, Redman SN, *et al*. The surface of articular cartilage contains a progenitor cell population. *J Cell Sci* 2004;117(Pt 6):889–97.

7 Sokolove J, Lepus CM. Role of inflammation in the pathogenesis of osteoarthritis: latest findings and interpretations. *Ther Adv Musculoskelet Dis* 2013;5:77–94.
8 Scheller J, Chalaris A, Schmidt-Arras D, *et al*. The pro- and anti-inflammatory properties of the cytokine interleukin-6. *Biochim Biophys Acta* 2011;1813:878–88.
9 Ogura H, Murakami M, Okuyama Y, *et al*. Interleukin-17 promotes autoimmunity by triggering a positive-feedback loop via interleukin-6 induction. *Immunity* 2008;29:628–36.
10 B. Marcu K, Otero M, Olivotto E, *et al*. Goldring MB: NF-κB Signaling: Multiple angles to target OA. *Current drug targets* 2010;11:599–613.
11 Prasadam I, van Gennip S, Friis T, *et al*. ERK-1/2 and p38 in the regulation of hypertrophic changes of normal articular cartilage chondrocytes induced by osteoarthritic subchondral osteoblasts. *Arthritis Rheum* 2010;62:1349–60.
12 Beekhuizen M, van Osch GJ, Bot AG, *et al*. Inhibition of oncostatin M in osteoarthritic synovial fluid enhances GAG production in osteoarthritic cartilage repair. *Eur Cell Mater* 2013;26:80–90.
13 Hui W, Rowan AD, Richards CD, *et al*. Oncostatin M in combination with tumor necrosis factor alpha induces cartilage damage and matrix metalloproteinase expression in vitro and in vivo. *Arthritis Rheum* 2003;48:3404–18.
14 Jikko A, Wakisaka T, Iwamoto M, *et al*. Effects of interleukin-6 on proliferation and proteoglycan metabolism in articular chondrocyte cultures. *Cell Biol Int* 1998;22:615–21.
15 Nasi S, So A, Combes C, *et al*. Interleukin-6 and chondrocyte mineralisation act in tandem to promote experimental osteoarthritis. *Ann Rheum Dis* 2016;75:1372–9.
16 Legendre F, Dudhia J, Pujol JP, *et al*. JAK/STAT but not ERK1/ERK2 pathway mediates interleukin (IL)-6/soluble IL-6R down-regulation of Type II collagen, aggrecan core, and link protein transcription in articular chondrocytes. Association with a down-regulation of SOX9 expression. *J Biol Chem* 2003;278:2903–12.
17 Latourte A, Cherifi C, Maillet J, *et al*. Systemic inhibition of IL-6/Stat3 signalling protects against experimental osteoarthritis. *Ann Rheum Dis* 2017;76:748–55.
18 Ryu JH, Yang S, Shin Y, *et al*. Interleukin-6 plays an essential role in hypoxia-inducible factor 2α-induced experimental osteoarthritic cartilage destruction in mice. *Arthritis Rheum* 2011;63:2732–43.
19 Livshits G, Zhai G, Hart DJ, *et al*. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: The Chingford Study. *Arthritis Rheum* 2009;60:2037–45.
20 Kondo M, Yamaoka K, Sakata K, *et al*. Contribution of the Interleukin-6/STAT-3 Signaling Pathway to Chondrogenic Differentiation of Human Mesenchymal Stem Cells. *Arthritis Rheumatol* 2015;67:1250–60.
21 Hall MD, Murray CA, Valdez MJ, *et al*. Mesoderm-specific Stat3 deletion affects expression of Sox9 yielding Sox9-dependent phenotypes. *PLoS Genet* 2017;13:e1006610.
22 Iwamoto M, Yagami K, Lu Valle P, *et al*. Expression and role of c-myc in chondrocytes undergoing endochondral ossification. *J Biol Chem* 1993;268:9645–52.
23 Moran EM, Mullan R, McCormick J, *et al*. Human rheumatoid arthritis tissue production of IL-17A drives matrix and cartilage degradation: synergy with tumour necrosis factor-alpha, Oncostatin M and response to biologic therapies. *Arthritis Res Ther* 2009;11:R113.
24 Lotz M, Moats T, Villiger PM. Leukemia inhibitory factor is expressed in cartilage and synovium and can contribute to the pathogenesis of arthritis. *J Clin Invest* 1992;90:888–96.
25 Henrotin YE, De Groote DD, Labasse AH, *et al*. Effects of exogenous IL-1 beta, TNF alpha, IL-6, IL-8 and LIF on cytokine production by human articular chondrocytes. *Osteoarthritis Cartilage* 1996;4:163–73.
26 Hui W, Bell M, Carroll G. Soluble glycoprotein 130 (gp130) attenuates OSM- and LIF-induced cartilage proteoglycan catabolism. *Cytokine* 2000;12:151–5.
27 Maye P, Fu Y, Butler DL, *et al*. Generation and characterization of Col10a1-mcherry reporter mice. *Genesis* 2011;49:410–8.
28 Wu L, Bluguermann C, Kyupelyan L, *et al*. Human developmental chondrogenesis as a basis for engineering chondrocytes from pluripotent stem cells. *Stem Cell Reports* 2013;1:575–89.
29 Hunziker EB. Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects. *Osteoarthritis Cartilage* 2002;10:432–63.
30 Janusz MJ, Little CB, King LE, *et al*. Detection of aggrecanase- and MMP-generated catabolic neoepitopes in the rat iodoacetate model of cartilage degeneration. *Osteoarthritis Cartilage* 2004;12:720–8.
31 Hiscock DR, Caterson B, Flannery CR. Expression of hyaluronan synthases in articular cartilage. *Osteoarthritis Cartilage* 2000;8:120–6.
32 Mori Y, Saito T, Chang SH, S-h C, *et al*. Identification of fibroblast growth factor-18 as a molecule to protect adult articular cartilage by gene expression profiling. *J Biol Chem* 2014;289:10192–200.
33 Ito K, Maruyama Z, Sakai A, *et al*. Overexpression of Cdk6 and Ccnd1 in chondrocytes inhibited chondrocyte maturation and caused p53-dependent apoptosis without enhancing proliferation. *Oncogene* 2014;33:1862–71.
34 Zeller KI, Jegga AG, Aronow BJ, *et al*. An integrated database of genes responsive to the Myc oncogenic transcription factor: identification of direct genomic targets. *Genome Biol* 2003;4:R69.
35 Xu S, Grande F, Garofalo A, *et al*. Discovery of a novel orally active small-molecule gp130 inhibitor for the treatment of ovarian cancer. *Mol Cancer Ther* 2013;12:937–49.
36 Rebouissou S, Amessou M, Couchy G, *et al*. Frequent in-frame somatic deletions activate gp130 in inflammatory hepatocellular tumours. *Nature* 2009;457:200–4.
37 Stuhlmann-Laeisz C, Lang S, Chalaris A, *et al*. Forced dimerization of gp130 leads to constitutive STAT3 activation, cytokine-independent growth, and blockade of differentiation of embryonic stem cells. *Mol Biol Cell* 2006;17:2986–95.
38 Chevalier S, Fourcin M, Robledo O, *et al*. Interleukin-6 family of cytokines induced activation of different functional sites expressed by gp130 transducing protein. *J Biol Chem* 1996;271:14764–72.
39 Lozoya KA, Flores JB. A novel rat osteoarthrosis model to assess apoptosis and matrix degradation. *Pathol Res Pract* 2000;196:729–45.
40 Dowd E, McQueen DS, Chessell IP, *et al*. P2X receptor-mediated excitation of nociceptive afferents in the normal and arthritic rat knee joint. *Br J Pharmacol* 1998;125:341–6.
41 Gerwin N, Bendele AM, Glasson S, *et al*. The OARSI histopathology initiative - recommendations for histological assessments of osteoarthritis in the rat. *Osteoarthritis Cartilage* 2010;18(Suppl 3):S24–34.
42 Eltawil NM, De Bari C, Achan P, *et al*. A novel in vivo murine model of cartilage regeneration. Age and strain-dependent outcome after joint surface injury. *Osteoarthritis Cartilage* 2009;17:695–704.
43 Roelofs AJ, Zupan J, Riemen AHK, *et al*. Joint morphogenetic cells in the adult mammalian synovium. *Nat Commun* 2017;8:15040.
44 O'Driscoll SW, Keeley FW, Salter RB. Durability of regenerated articular cartilage produced by free autogenous periosteal grafts in major full-thickness defects in joint surfaces under the influence of continuous passive motion. A follow-up report at one year. *J Bone Joint Surg Am* 1988;70:595–606.
45 Kapoor M, Martel-Pelletier J, Lajeunesse D, *et al*. Role of proinflammatory cytokines in the pathophysiology of osteoarthritis. *Nat Rev Rheumatol* 2011;7:33–42.
46 de Hooge AS, van de Loo FA, Bennink MB, *et al*. Male IL-6 gene knock out mice developed more advanced osteoarthritis upon aging. *Osteoarthritis Cartilage* 2005;13:66–73.
47 Li W, Liang X, Kellendonk C, *et al*. STAT3 contributes to the mitogenic response of hepatocytes during liver regeneration. *J Biol Chem* 2002;277:28411–7.
48 Flores A, Schell J, Krall AS, *et al*. Lactate dehydrogenase activity drives hair follicle stem cell activation. *Nat Cell Biol* 2017;19:1017–26.
49 Ocampo A, Reddy P, Martinez-Redondo P, *et al*. In vivo amelioration of age-associated hallmarks by partial reprogramming. *Cell* 2016;167:1719–33.

EXTENDED REPORT

Circular RNA VMA21 protects against intervertebral disc degeneration through targeting miR-200c and X linked inhibitor-of-apoptosis protein

Xiaofei Cheng,[1,2] Liang Zhang,[3] Kai Zhang,[1] Guoying Zhang,[4] Ying Hu,[5] Xiaojiang Sun,[1] Changqing Zhao,[1] Hua Li,[1] Yan Michael Li,[2] Jie Zhao[1]

Handling editor Tore K Kvien

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/annrheumdis-2017-212056).

[1]Shanghai Key Laboratory of Orthopaedic Implants, Department of Orthopaedic Surgery, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China
[2]Department of Neurosurgery, University of Rochester School of Medicine and Dentistry, Rochester, New York, USA
[3]Department of Orthopedics, Clinical Medical College of Yangzhou University, Jiangsu Subei People's Hospital, Yangzhou, China
[4]Department of Orthopedics, The General Hospital of Chinese People's Liberation Army, Beijing, China
[5]Department of Toxicity Evaluation, Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China

Correspondence to
Dr Jie Zhao, Department of Orthopaedic Surgery, Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine, Shanghai 200011, China; profzhaoj@sina.com

XC and LZ contributed equally.

Received 11 July 2017
Revised 27 December 2017
Accepted 2 January 2018
Published Online First 17 January 2018

To cite: Cheng X, Zhang L, Zhang K, *et al*. *Ann Rheum Dis* 2018;**77**:770–779.

ABSTRACT

Objectives Circular RNAs (circRNAs) have been proven to function as competing endogenous RNAs to interact with microRNAs (miRNAs) and influence the expression of miRNA target mRNAs. In this study, we investigated whether circRNAs could act as competing endogenous RNAs to regulate the pathological process of intervertebral disc degeneration (IVDD).

Methods The role and mechanism of a circRNA, circVMA21, in IVDD were explored in nucleus pulposus (NP) cells and degenerative NP tissues from patients and rat models. The interaction between circVMA21 and miR-200c as well as the target mRNA, X linked inhibitor-of-apoptosis protein (XIAP), was examined.

Results The decreased expression of XIAP in the inflammatory cytokines-treated NP cells and the degenerative NP tissues was directly associated with excessive apoptosis and imbalance between anabolic and catabolic factors of extracellular matrix. miR-200c regulated NP cell viability and functions through inhibiting XIAP. circVMA21 acted as a sponge of miR-200c and functioned in NP cells through targeting miR-200c and XIAP. Intradiscal injection of circVMA21 alleviated IVDD in the rat model.

Conclusions CircVMA21 could alleviate inflammatory cytokines-induced NP cell apoptosis and imbalance between anabolism and catabolism of extracellular matrix through miR-200c-XIAP pathway. It provides a potentially effective therapeutic strategy for IVDD.

INTRODUCTION

It is well documented that low back pain is a common condition and the leading cause of disability globally.[1] A widely recognised contributor to low back pain is intervertebral disc (IVD) degeneration, which is the major cause of a series of degenerative disc diseases. A disc is composed of the inner nucleus pulposus (NP) and surrounded by annulus fibrosus. Nucleus pulposus cells (NPCs) are the main type of cells residing in the NP and responsible for synthesising components of extracellular matrix (ECM) such as type II collagen (collagen II) and aggrecan. These proteins are the major functional compositions of the IVD to maintain disc height and confront diverse external mechanical compression. Multiple abnormal stimuli can increase the levels of inflammatory cytokines (eg, interleukin -1β (IL-1β) and tumour necrosis factor-α (TNF-α)) in the NP, and then induce an imbalance between anabolic and catabolic activities of NPCs, such as increased generation of catabolic factors (eg, matrix metalloproteinases (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS)) and inhibited expression of anabolic factors (eg, collagen II and aggrecan), as well as excessive NPC apoptosis. These adverse factors initiate or accelerate intervertebral disc degeneration (IVDD).[2–6] Thus, it is necessary to find an effective way to inhibit NPC apoptosis, attenuate inflammatory response, and reverse the imbalance between the anabolism and catabolism within the NP microenvironment.

MicroRNAs (miRNAs) are small, single-stranded, non-coding RNA molecules that impede protein production by directly interacting with the 3'untranslated region (UTR)of the target mRNAs. miR-200c is often associated with various cancers because it exhibits tumour suppressive behaviour by blocking epithelial to mesenchymal transition of cancer cells.[7] Moreover, recent studies have revealed its ability to induce cell apoptosis by targeting different mRNAs.[8 9] In addition, miRNAs have gained considerable attention as regulators of gene expression and play important roles in the prevention and treatment of IVDD.[10 11]

Circular RNAs (circRNAs) are another type of RNAs that form loop structures without 5′-3′ polarities and polyadenylated tails. Most of the circRNAs are endogenous non-coding RNAs, conserved between different species and showed a higher degree of stability than linear mRNAs.[12 13] They mainly arise from one or multiple exons by a back-splice mechanism. Although the specific biogenesis of circRNAs is not completely clear, many studies have found a reliable model of circularisation driven by paring between flanking intron sequences. For example, complementary Alu repetitive elements within the flanking introns prompt the formation of exon-derived circRNAs.[14 15] Lately, some circRNAs have been proven to be enriched with miRNA binding sites, and function as competing endogenous RNAs (ceRNAs) to interact with miRNAs and influence the expression of target mRNAs.[16–20] Until now, it remains unclear whether circRNAs can act as ceRNAs to regulate the viability and functions of NPCs, and the pathological process of IVDD. In this study, we identified a circRNA derived from vacuolar ATPase assembly factor (VMA21) gene (named circVMA21; also termed hsa_circ_0091702 in CircBase (http://www.circbase.org)) in the NP and systemically investigated its role in cell and animal model of IVDD.

MATERIALS AND METHODS

Construction of miRNA and circRNA vector

Vectors were constructed by amplified DNA fragments including the sequence of pre-miR-200c or third exon of VMA21 gene with flanking introns containing complementary Alu elements.

CircRNA inhibition

CircVMA21 was knocked down using specific small interfering RNAs (siRNAs) targeting the backsplice region.

Luciferase reporter assay

The 3'-UTR of X linked inhibitor-of-apoptosis protein (XIAP) gene or circVMA21 fragments were inserted into the luciferase vector. Cells were transfected with the vectors and miR-200c.

A rat model of IVDD

The circVMA21 vectors were injected into the IVDs of rat models. Radiographic and histological examinations were performed to evaluate the change in severity of IVDD (see online supplementary methods for details). All sequences are listed in online supplementary table S1.

RESULTS

miR-200c was upregulated in degenerative NP tissues and involved in the regulation of NPC viability and functions

Differential expression of miRNAs in degenerative NP tissues was investigated using microarray data obtained from National Center for Biotechnology Information Gene Expression Omnibus database (GSE45856). Of 2672 miRNAs detected by miRNA microarray, 14 miRNAs were upregulated in the degenerative NP tissues compared with the controls when using the criteria of mean fold change >2.0 and P values <0.01 (figure 1A). These candidate miRNAs were chosen to analyse the validation using quantitative real-time reverse transcription-PCR (qRT-PCR) assay. Using the above-mentioned criteria, miR-200c, miR-130b-5p and miR-2355–5 p were observed to be significantly upregulated in the degenerative NP tissues compared with the controls, and miR-200c had the highest level of upregulation (figure 1B). The expression pattern of these miRNAs in the rat model of IVDD was consistent with that in the patient samples (figure 1C). Northern blot also confirmed the increase in miR-200c levels in the degenerative NP tissues (figure 1D). Therefore, miR-200c was selected for further analysis. The effects of miR-200c in NPCs were investigated. miR-200c-overexpressing cells (figure 1E) demonstrated increased percentage of apoptotic cells (figure 1F), elevated caspase activation, increased expression of MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5, and decreased expression of collagen II and aggrecan (figure 1G). These results indicated the proapoptotic and procatabolic effect of miR-200c in NPCs. The treatment of TNF-α and IL-1β increased miR-200c levels in NPCs, which could be suppressed by miR-200c antagonist (figure 1H). The enhanced apoptosis and changes in ECM metabolism response to the treatment of TNF-α and IL-1β were markedly suppressed after miR-200c knockdown (figure 1I,J). Thus, the loss-of-function and gain-of-function experiments supported a critical role of miR-200c in negatively regulating NPC survival and functions.

miR-200c regulated NPC viability and functions through inhibiting its target, XIAP

As predicted by bioinformatic programs, XIAP, a well-known regulator of apoptotic pathway, is a potential target of miR-200c (figure 2A). Gene ontology (GO) analysis revealed a correlation between the upregulated miRNAs in the degenerative NP tissues and the regulation of apoptotic signalling pathway. The luciferase signal of the wild-type XIAP reporter was suppressed by miR-200c, whereas introduction of mutations abolished the inhibitory effect of miR-200c (figure 2B). A decrease in XIAP expression was observed in the degenerative NP tissues compared with the controls (figure 2C). Elevated levels of miR-200c reduced XIAP expression, whereas knockdown of endogenous miR-200c increased XIAP expression in NPCs (figure 2D, online supplementary figure S1A). The levels of XIAP pre-mRNA were not changed in the degenerative NP tissues or miR-200c-overexpressing NPCs (online supplementary figure S1B,C). The treatment of TNF-α and IL-1β induced a decrease in the levels of XIAP protein in NPCs, while the miR-200c antagonist attenuated this decrease (figure 2E). Further, we investigated whether miR-200c and XIAP were functionally related in NPCs. The results showed that XIAP knockdown induced NPC apoptosis, exacerbated catabolic response and reduced expression of ECM compositions (online supplementary figure S1D,E). Furthermore, the absence of XIAP markedly counteracted the effects of miR-200c antagonist in the NPCs treated with TNF-α and IL-1β (figure 2F,G), indicating that miR-200c exerted its functions through XIAP.

CCircVMA21 acted as a sponge of miR-200c. miR-200c is predicted to have binding sites for several circRNAs by starBase. We screened the top 20 predicted circRNAs according to their clipreadNum scores. The existence of the selected circRNAs in the NP samples was detected using specific divergent primers by qRT-PCR analysis. Predicted splice junction of circVMA21 (online supplementary table S2) was validated in the NP tissues (figure 3A, upper). The amplified product using divergent primers was confirmed in accordance with the sequence of circVMA21 by sequencing (figure 3A, lower). CircVMA21 levels were markedly reduced in the degenerative NP tissues compared with the controls by qRT-PCR analysis (figure 3B). This result was also confirmed by RNA fluorescence in situ hybridisation (FISH) (figure 3C, online supplementary figure S2A). The level of circVMA21 was positively correlated with XIAP level in the degenerative NP tissues (online supplementary figure S2B). To test whether circVMA21 can be bounded by miR-200c, we compared the sequence of circVMA21 with that of miR-200c using RNAhybrid and noticed that circVMA21 contains six putative target sites of miR-200c (figure 3D, online supplementary table S3). Of them, five sites were validated by the luciferase assay (figure 3E). This circRNA was abundant and resistant to RNase R treatment in contrast to mRNA (figure 3F). Northern blot analysis showed that linear VMA21 was detectable by a linear probe but not a circular probe within the splice sites (figure 3G). Pull-down assay revealed a more enrichment of circVMA21 in the miR-200c-captured fraction compared with the introduction of miR-200c mutation that disrupted the binding site of miR-200c for circVMA21 (figure 3H). CircVMA21 is derived from the last exon of VMA21 gene mainly transcribing the 3'UTR of mRNA. It has been found to own 69 binding sites of Argonaute 2 (AGO2) protein as shown in the CircInteractome (online supplementary table S4). Crosslinking and immunoprecipitation (CLIP) sequence revealed at least 30 AGO2-bound regions located in the 3'UTR of VMA21 mRNA (online supplementary table S5). Of them, five regions are overlapped with the binding sites of miR-200c (figure 3I, upper). AGO2 immunoprecipitation found that endogenous circVMA21 pulled down with AGO2 was enriched in NPCs transfected with miR-200c but not its mutant (figure 3I, lower), indicating that miR-200c facilitated the association between AGO2 and circVMA21. Northern blot analysis

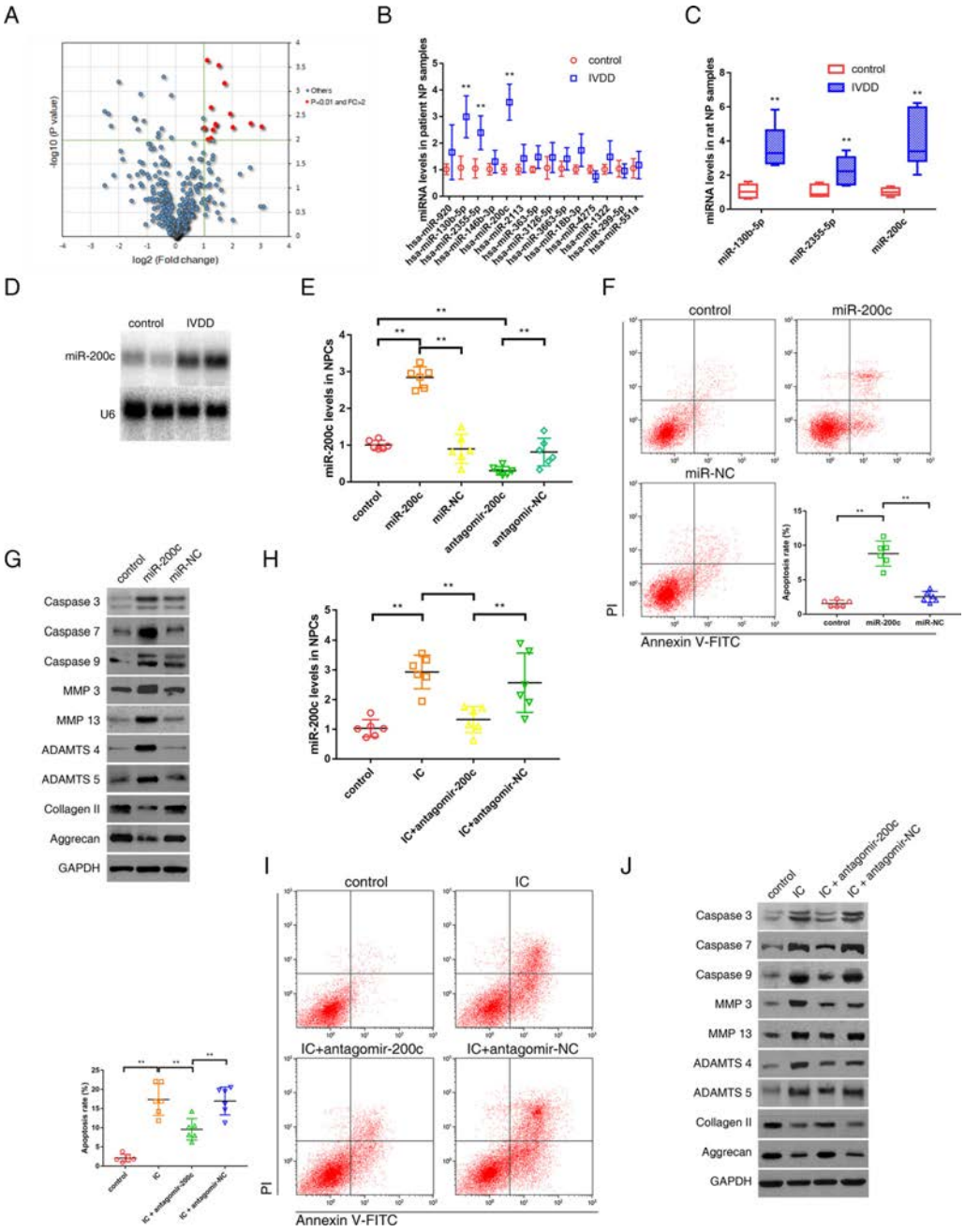


Figure 1 miR-200c was upregulated in the degenerative NP tissues and involved in the regulation of NPC viability and functions. (A) Differential upregulation of miRNAs detected by microarray in degenerative NP tissues compared with controls. Volcano plots were constructed using fold-change values and P values. The vertical green line corresponds to 2.0-fold upregulation between degenerative samples and controls, and the horizontal green line represents a P value of 0.01. The red point in the plot represents the differentially upregulated miRNAs with statistical significance. (B) qRT-PCR analysis confirmed the upregulated miRNAs in the degenerative NP samples from patients with IVDD. n=12; **P<0.01 compared with the controls. (C) qRT-PCR analysis confirmed the upregulated miRNAs in the degenerative NP samples from the rat model of IVDD. n=8; **P<0.01 compared with the controls. (D) Representative northern blots showing miR-200c levels in the NP samples from patients with or without IVDD. (E) NPCs were transfected with miR-200c, miR-negative control (NC), antagomir-200c or antagomir-NC. miR-200c levels in NPCs were analysed by qRT-PCR. **P<0.01. (F) Representative dot plots of apoptosis flow cytometry detection were shown after Annexin V-FITC/propidium iodide (PI) dual staining. The transfection of miR-200c increased apoptosis rate of NPCs. **P<0.01. (G) Western blot analysed protein expression of apoptotic effector caspases (caspase-3, caspase-7 and caspase-9), catabolic enzymes (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5) and extracellular matrix (ECM) compositions (collagen II, aggrecan) in NPCs after transfection of miR-200c. (H) NPCs were transfected with miR-200c antagonist or its NC, and then treated with inflammatory cytokines (IC; interleukin 1β plus tumour necrosis factor α). qRT-PCR showed increased miR-200c levels in NPCs treated with IC, which could be converted by transfection of miR-200c antagonist. **P<0.01. (I) Representative dot plots of apoptosis flow cytometry detection were shown after Annexin V-FITC/PI dual staining. The knockdown of miR-200c inhibited apoptosis induced by IC in NPCs. **P<0.01. (J) Western blot analysis showed that miR-200c knockdown attenuated the apoptotic and catabolic response and reversed the decreased expression of ECM compositions induced by IC treatment in NPCs. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IVDD, intervertebral disc degeneration; miRNA, microRNAs; MMP, matrix metalloproteinases; NP, nucleus pulposus; NPC, nucleus pulposus cells; qRT-PCR, quantitative real-time reverse transcription-PCR.

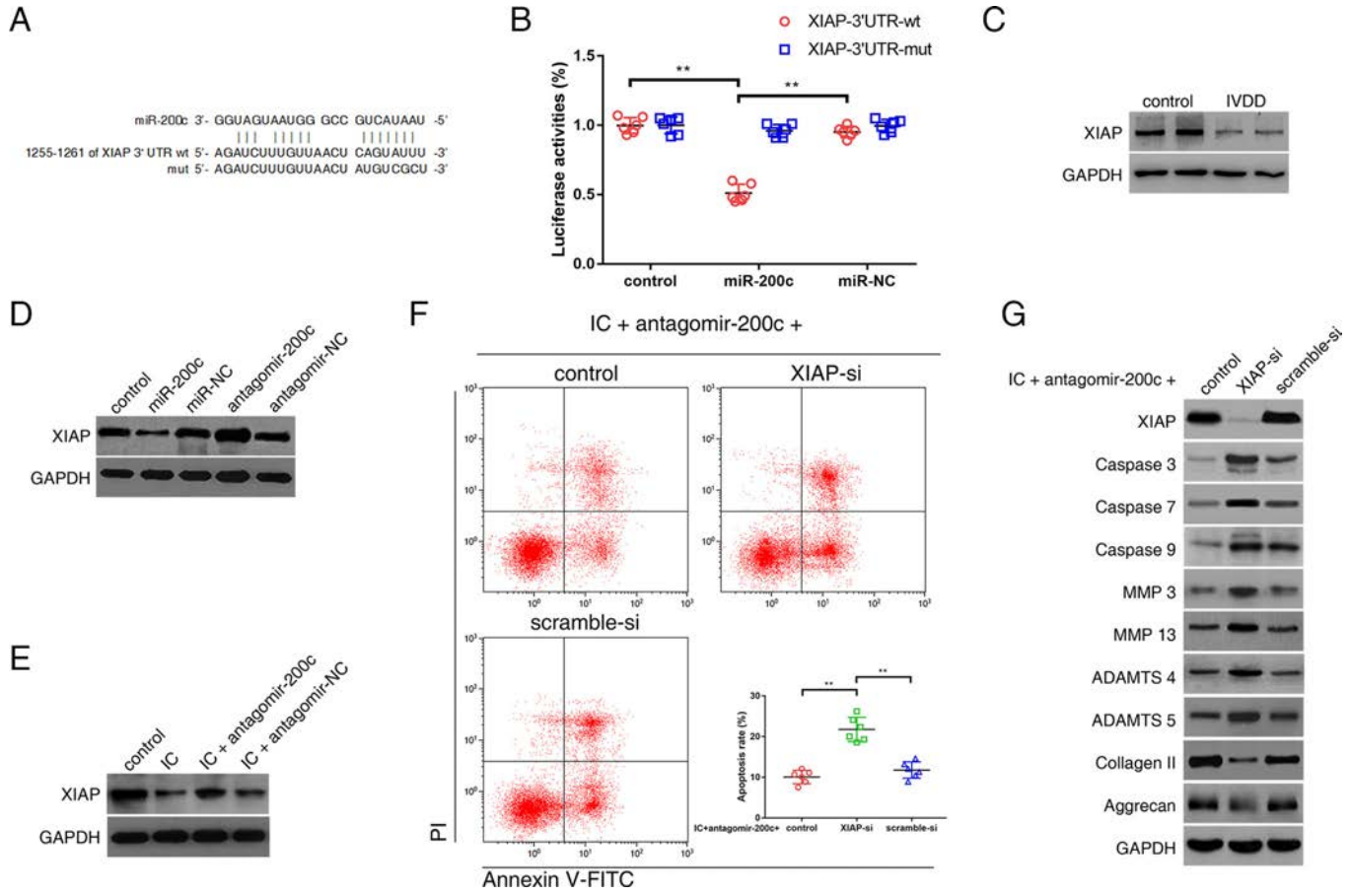


Figure 2 miR-200c-regulated NPC viability and functions through inhibiting its target mRNA, X chromosome-linked inhibitor-of-apoptosis protein (XIAP). (A) 3′-UTR region of XIAP mRNA was found to harbour a binding site for miR-200c. (B) NPCs were transfected with miR-200c or miR-negative control (NC), and then transfected with the luciferase constructs of the wild-type XIAP-3′UTR (3′UTR-wt) or the mutated XIAP-3′UTR (3′UTR-mut). Luciferase reporter assay found that miR-200c exclusively decreased luciferase activity of the wild-type reporter plasmids. n=6; **P<0.01. (C) Western blot analysis revealed lower expression of XIAP in the degenerative NP tissues compared with the controls. (D) NPCs were transfected with miR-200c, miR-NC, antagomir-200c or antagomir-NC. Western blot analysis showed that the expression of XIAP was suppressed by miR-200c upregulation and elevated by miR-200c knockdown. (E) NPCs were transfected with antagomir-200c or its NC, and then treated with inflammatory cytokines (IC; interleukin 1β and tumour necrosis factor-α). Western blot analysis showed decreased XIAP expression in NPCs treated with IC, which could be alleviated by transfection of miR-200c antagonist. (F, G) NPCs were cotransfected with antagomir-200c and XIAP siRNA (XIAP-si) or scramble siRNA (scramble-si), and then exposed to IC. (F) Representative dot plots of apoptosis flow cytometry detection were shown after Annexin V-FITC/propidium iodide (PI) dual staining. The knockdown of XIAP attenuated the inhibitory effects of miR-200c antagonist on apoptosis induced by IC in NPCs. **P<0.01. (G) Western blot analysed protein expression of apoptotic effector caspases (caspase-3, caspase-7 and caspase-9), catabolic enzymes (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5) and extracellular matrix compositions (collagen II, aggrecan) in NPCs. The knockdown of XIAP interfered with the effects of miR-200c antagonist on the expression of these functional proteins in IC-treated NPCs. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IVDD, intervertebral disc degeneration; MMP, matrix metalloproteinases; NP, nucleus pulposus; NPC, nucleus pulposus cell; UTR, untranslated region.

revealed that circVMA21 could in reverse pull down miR-200c (figure 3J). RNA FISH found colocalisation of circVMA21 and miR-200c in the cytoplasm of NPCs (figure 3K, online supplementary figure S2C). All things considered, our results indicated that circVMA21 was able to directly bind to miR-200c in NPCs.

circVMA21 functioned in NPCs through targeting miR-200c and XIAP

NPCs were infected by constructed adenovirus harbouring circVMA21, linear VMA21, circVMA21 siRNA or control siRNA. The results of qRT-PCR showed that the infection of adenovirus circVMA21 resulted in an overexpression of circVMA21 in the NPCs and that siRNA depressed the expression of circVMA21 (figure 4A). Knockdown of VMA21 mRNA did not affect circVMA21 levels (online supplementary figure S3A). Likewise, circVMA21 siRNA had no effect on mRNA levels (online supplementary figure S3B). Northern blot analysis also confirmed the elevated amount of circVMA21 after the intake of exogenous circVMA21 (figure 4B). Then, the effect of circVMA21 on XIAP expression was detected by western blot assay. Upregulation of circVMA21 increased the expression of XIAP, whereas circVMA21 knockdown induced a decrease in the XIAP expression (figure 4C). Similar changes were observed from a well-known target of miR-200c, zinc finger E-box binding homeobox transcription factor 1 (ZEB1; online supplementary figure S3C). Moreover, the upregulation of circVMA21 counteracted the inhibitory effect of miR-200c on XIAP expression (figure 4D) and activity (figure 4E). Taken together, these results suggested that circVMA21 acted as a functional sponge of miR-200c to regulate XIAP expression and activity. Next, we

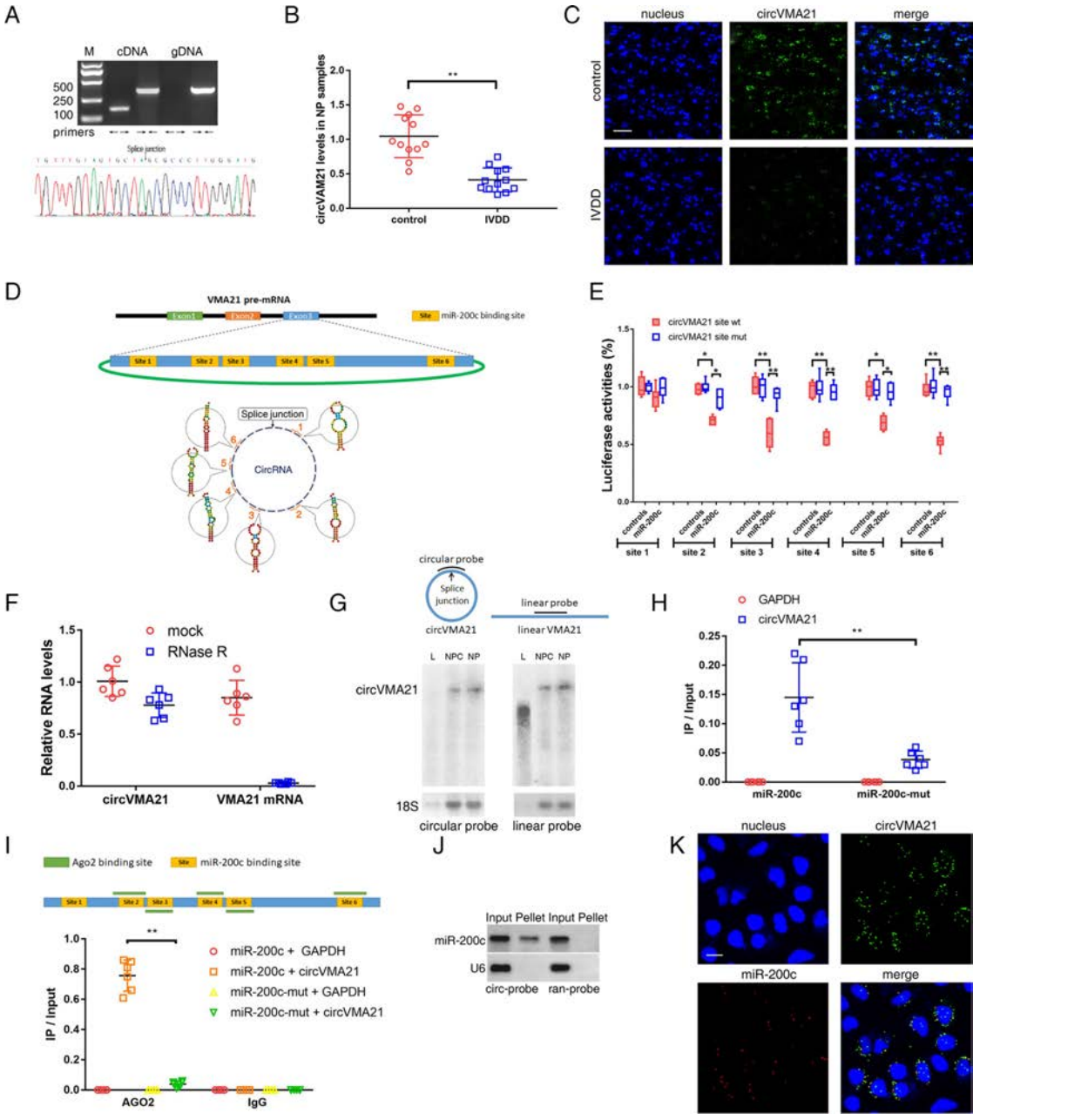


Figure 3 circVMA21 acted as a sponge of miR-200c. (A) Agarose gel electrophoresis found that divergent primers (←→) amplified circVMA21 in complementary DNA (cDNA) but not genomic DNA (gDNA) (upper). The amplified product of specific divergent primers was confirmed in accordance with the sequence of circVMA21 by sequencing (lower). (B) qRT-PCR analysis detected circVMA21 levels in the NP samples from patients with or without IVDD. n=12; **P<0.01. (C) The expression of circVMA21 was detected in the NP samples from patients with or without IVDD by RNA fluorescence in situ hybridisation (FISH). circVMA21 probe was labelled with Alexa 488. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Scale bar=50 µm. (D) circVMA21 is transcribed from the third exon of the VMA21 gene and contains six putative binding sites complementary to miR-200c. (E) NPCs were transfected with miR-200c and luciferase constructs of circVMA21 containing wild-type putative miR-200c binding sites (circVMA21 site wt) or mutated sites (circVMA21 site mut). n=6; *P<0.05, **P<0.01. (F) qRT-PCR analysis for the abundance of circVMA21 and VMA21 mRNA in NPCs with or without RNase R treatment. The amounts were normalised to the value of circVMA21 measured in the mock treatment. n=6. (G) Northern blot analysis showed that linear VMA21 was detectable by a linear but not circular probe. L, linear VMA21 transcribed in vitro; NPC, total RNAs extracted from NPCs; NP, total RNAs extracted from NP tissue samples; circular probe, probe within splice site; linear probe, head-to-tail probe. (H) The biotinylated miR-200c or its mutant (miR-200c-mut) was transfected into NPCs. The RNA levels of circVMA21 and GAPDH were quantified by qRT-PCR analysis, and the relative ratios of immunoprecipitate (IP) to input were plotted. **P<0.01. (I) CLIP sequence revealed five AGO2-bound regions overlapped with the binding sites of miR-200c within the circVMA21 sequence (upper). AGO2 RNA immunoprecipitation in NPCs transfected with miR-200c or its mutant. The levels of circVMA21 and GAPDH were quantified by qRT-PCR analysis, and the relative ratios of IP to input were plotted. **P<0.01 (lower). (J) miR-200c was pulled down by the circular probe for circVMA21 (circ-probe) but not random probe (ran-probe). The levels of miR-200c were detected by northern blot. Input, 20% samples were loaded; Pellet, all samples were loaded. (K) RNA FISH for colocalisation of circVMA21 and miR-200c in cytoplasm of NPCs. circVMA21 and miR-200c probes were labelled with Alexa 488 and Cy-5, respectively. Nuclei were stained with DAPI. Scale bar=10 µm. AGO2, Argonaute 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IVDD, intervertebral disc degeneration; NP, nucleus pulposus; NPC, nucleus pulposus cells; qRT-PCR, quantitative real-time reverse transcription-PCR.

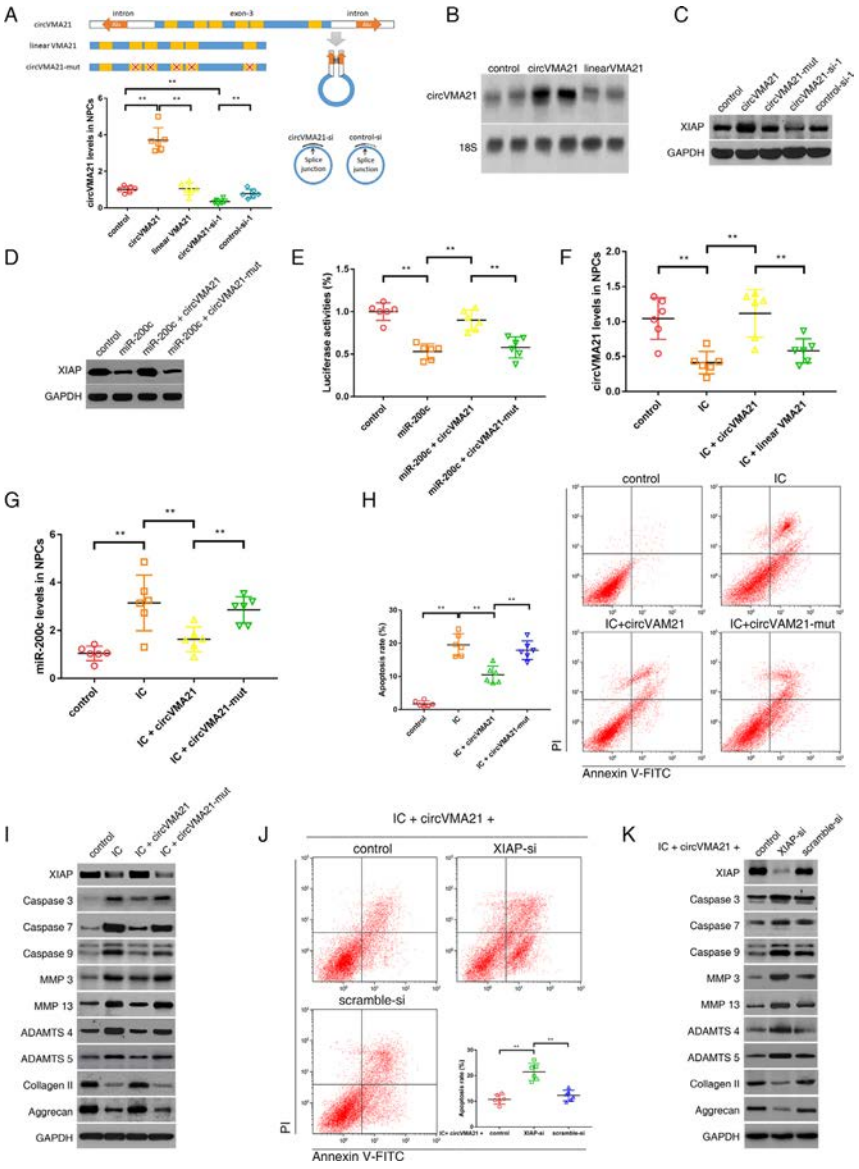


Figure 4 circVMA21 functioned in NPCs through targeting miR-200c and XIAP. (A) The third exon of VMA21 gene along with approximate 1 kb flanking intron sequences containing complementary Alu elements was amplified to construct circVMA21 vector. The exon with and without mutation was used as controls. NPCs were transfected with circVMA21, linear VMA21, circVMA21 siRNA-1 (circVMA21-si-1) or control siRNA-1 (control-si-1), and circVMA21 levels were analysed by qRT-PCR. **P<0.01 (lower). (B) NPCs were transfected with circVMA21 or linear VMA21 for northern blot analysis, and the blots were probed against circVMA21 with 18S ribosomal RNA as an internal control. (C) NPCs were transfected with circVMA21, its mutation (circVMA21-mut), circVMA21-si or scramble-si. XIAP expression was analysed by western blot assay. The expression of XIAP was enhanced after circVMA21 upregulation and reduced after circVMA21 knockdown. (D) NPCs were cotransfected with miR-200c and circVMA21 or circVMA21-mut. Western blot assay showed that circVMA21 blocked the inhibitory effect of miR-200c on XIAP expression. (E) NPCs were cotransfected with XIAP 3′UTR luciferase construct, miR-200c and circVMA21 or circVMA21-mut. Luciferase assay showed that circVMA21 blocked the inhibitory effects of miR-200c on XIAP activity. **P<0.01. (F,G,H,I) NPCs were transfected with circVMA21 or a control (linear VMA21 or circVMA21-mut), and then treated with inflammatory cytokines (IC; interleukin 1β and tumour necrosis factor-α). (F) qRT-PCR showed a decrease in circVMA21 expression in NPCs treated with IC, which could be converted by transfection of circVMA21. **P<0.01. (G) qRT-PCR showed an increase in miR-200c levels in NPCs treated with IC, which could be downregulated by transfection of circVMA21. **P<0.01. (H) Representative dot plots of apoptosis flow cytometry detection were shown after Annexin V-FITC/propidium iodide (PI) dual staining. The transfection of circVMA21 inhibited apoptosis induced by IC in NPCs. **P<0.01. (I) Western blot analysed expression of XIAP, apoptotic effector caspases (caspase-3, caspase-7 and caspase-9), catabolic enzymes (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5) and extracellular matrix (ECM) compositions (collagen II, aggrecan) in NPCs. The transfection of circVMA21 attenuated the apoptotic and catabolic response, and rescued the reduced expression of ECM compositions induced by IC treatment. (J,K) NPCs were cotransfected with circVMA21 and XIAP siRNA (XIAP-si) or scramble siRNA (scramble-si), and then exposed to IC. (J) Representative dot plots of apoptosis flow cytometry detection were shown after Annexin V-FITC/PI dual staining. The inhibitory effect of circVMA21 on NPC apoptosis was attenuated after the knockdown of XIAP. **P<0.01. (K) Western blot analysed the expression of XIAP, apoptotic effector caspases (caspase-3, caspase-7 and caspase-9), catabolic enzymes (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5) and ECM compositions (collagen II, aggrecan) in NPCs. The knockdown of XIAP impaired the protective effect of circVMA21 on NPC functions. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; MMP, matrix metalloproteinases; NPC, nucleus pulposus cells; qRT-PCR, quantitative real-time reverse transcription-PCR; XIAP, X linked inhibitor-of-apoptosis protein.

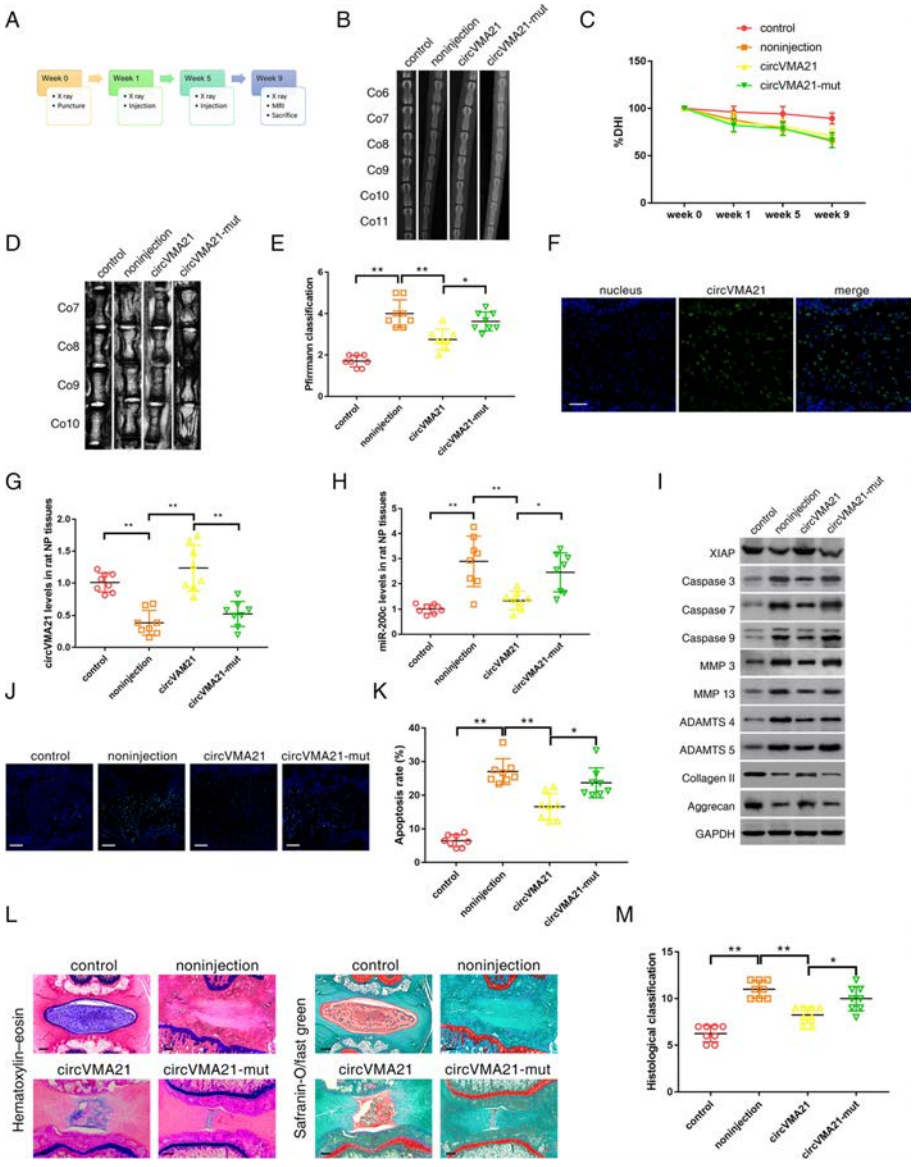


Figure 5 CircVMA21 alleviated IVDD in vivo. (A) A flow diagram of the experiments in vivo. A total of 32 rats were randomly divided into four groups: non-puncture group (control), non-injection with puncture group (non-injection), circVMA21 injection with puncture group (circVMA21), and circVMA21 mutant injection with puncture group (circVMA21-mut). (B) Radiographs of the indicated groups were obtained 9 weeks after needle puncture. Co6/7, Co8/9 and Co10/11 were punctured with Co7/8 and Co9/10 left intact. (C) Changes in disc height index (DHI) of the indicated groups after needle puncture. The DHI was measured at weeks 0, 1, 5 and 9 time point. A significant decrease of the %DHI was observed in all puncture groups at 1 week after surgery ($P<0.01$). At each time point after puncture, a significant decrease of %DHI was noted in all puncture groups compared with the control group ($P<0.01$). No significant difference was found in the %DHI between all puncture groups. (D) MRIs of the indicated groups were obtained 9 weeks after needle puncture. Co6/7, Co8/9 and Co10/11 were punctured with Co7/8 and Co9/10 left intact. (E) The MRI grade in the indicated groups at 9 weeks after needle puncture. The degree of disc degeneration by MRI grade was significantly lower in the circVMA21 group than in the non-injection group. *$P<0.05$, **$P<0.01$. (F) In vivo RNA fluorescence in situ hybridisation found circVMA21 located in the NP region. Blue fluorescence (4,6-diamidino-2-phenylindole, DAPI) indicating cell nucleus; green fluorescence (Alexa 488) indicating circVMA21. Scale bar=100 µm. (G) qRT-PCR showed that the decreased levels of circVMA21 in the punctured IVDs were rescued by the injection of circVMA21. **$P<0.01$. (H) qRT-PCR showed that the increased levels of miR-200c in the punctured IVDs were depressed by the injection of circVMA21. *$P<0.05$, **$P<0.01$. (I) Western blot analysed the expression of XIAP, apoptotic effector caspases (caspase-3, caspase-7 and caspase-9), catabolic enzymes (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5) and extracellular matrix (ECM) compositions (collagen II, aggrecan) in the rat NP tissues. The injection of circVMA21 alleviated the degenerative changes of the NP such as enhanced apoptotic and catabolic response, and reduced the expression of ECM compositions in the rat model of IVDD. (J) Terminal deoxynucleotidyl transferase-mediated dUTP nick end labelling (TUNEL) staining of the IVDs in the indicated groups at 9 weeks after needle puncture. Blue fluorescence (DAPI) indicating total cells; green fluorescence (fluorescein isothiocyanate) indicating TUNEL positive cells. Scale bar=100 µm. (K) A significant decrease in the apoptosis rate was noted in the circVMA21 group compared with the non-injection group. *$P<0.05$, **$P<0.01$. (L) H&E (left) and safranin-O/fast green (right) staining of the IVDs in the indicated groups at 9 weeks after needle puncture. Scale bar=100 µm. (M) A significant decrease in the grade of IVDD was noted in the circVMA21 group compared with the non-injection group. *$P<0.05$, **$P<0.01$. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IVD, intervertebral disc; IVDD, intervertebral disc degeneration; MMP, matrix metalloproteinases; NP, nucleus pulposus; qRT-PCR, quantitative real-time reverse transcription-PCR; XIAP, X linked inhibitor-of-apoptosis protein.

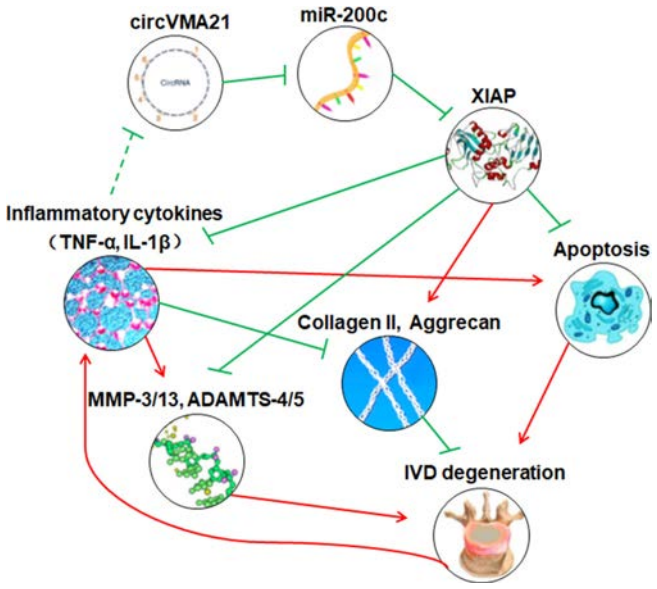


Figure 6 Schematic of the working hypothesis. The decreased expression of XIAP in the inflammatory cytokines-treated NPCs and the degenerative NP tissues is directly associated with excessive NPC apoptosis and imbalance between anabolism and catabolism of extracellular matrix. The treatment of circVMA21 could inhibit these adverse factors through binding miR-200c, and thus delay the progression of intervertebral disc degeneration. ADAMTS, a disintegrin and metalloproteinase with thrombospondin motifs; IL-1β, interleukin-1β; IVD, intervertebral disc; MMP, matrix metalloproteinases; NP, nucleus pulposus; NPC, nucleus pulposus cells; TNF-α, tumour necrosis factor-α; XIAP, X linked inhibitor-of-apoptosis protein.

investigated the function of circVMA21 in the TNF-α-treated and IL-1β-treated NPCs. After the treatment of the inflammatory cytokines, qRT-PCR analysis found a decrease in circVMA21 levels and an increase in miR-200c levels in NPCs, both of which could be reversed by the upregulation of circVMA21 (figure 4F,G). As a consequence, the enforced expression of circVMA21 restrained the apoptotic and catabolic effects of these cytokines, and promoted the expression of collagen II and aggrecan (figure 4H,I). Additionally, we explored whether XIAP was the downstream mediator of circVMA21 in the inflammatory cytokines-treated NPCs. We cotransfected circVMA21 and XIAP siRNA into NPCs, and observed that the positive effects of circVMA21 on NPC vitality and functions were attenuated in the absence of XIAP (figure 4J,K). Collectively, these data indicated that circVMA21 functioned in NPCs through modulating miR-200c and XIAP.

Intradiscal injection of circVMA21 alleviated IVDD in a rat model

We successfully established a rat model of IVDD by the needle puncture (figure 5A). At 1 and 5 weeks after the puncture, adenoviral human circVMA21 were injected into the punctured IVDs using a 33-gauge fine needle. X-rays obtained at time 1, 5 and 9 weeks demonstrated progressive disc space narrowing over time in all IVD punctured groups. At each time point after injection, no significant difference in the percentage of disc height index (%) was noted between the circVMA21 group and the non-injection or circVMA21-mut group (figure 5B,C, online supplementary figure S4A). At 9 weeks after injection, the MRI degeneration score of the IVDs was significantly lower in the circVMA21 group than in the non-injection group (figure 5D,E, online supplementary figure S4B). In vivo RNA FISH found circVMA21 located in the NP region (figure 5F, online supplementary figure S4C). After the injection of adenoviral circVMA21, the levels of circVMA21 in the degenerative NP tissues were remarkably elevated, while the levels of miR-200c were decreased (figure 5G,H). The injection of adenoviral circVMA21 alleviated the degenerative changes of the NP, such as enhanced apoptotic and catabolic response, and reduced ECM compositions in the rat model of IVDD (figure 5I,J,K, online supplementary figure S4D,E). The histological score was significantly higher in the non-injection group than in the circVMA21 group at 9 weeks (figure 5L,M). Taken together, these results revealed the positive effects of elevated circVMA21 levels on attenuating NPC apoptosis, inhibiting ECM catabolism, promoting anabolism in the NP and resultantly alleviating IVDD in vivo.

DISCUSSION

Multiple lines of evidence have shown that certain miRNAs could target distinct genes related to the development and progression of IVDD, and play roles in regulating the vitality and functions of NPCs.[10 11] In this study, we first identified miR-200c as a key miRNA involved in IVDD. miR-200c participated in the proapoptotic response and imbalanced expression of anabolic and catabolic factors. We then investigated the potential effects of circRNAs on the regulatory functions of miR-200c in the NPCs. The results revealed that circVMA21 markedly decreased the activity of miR-200c by capturing it and suppressed its functions. Therefore, a mechanism was proposed in which circVMA21 sponged miR-200c to inhibit NPC apoptosis, promote ECM anabolism and suppress ECM catabolism, and consequently delayed the progression of IVDD.

CircRNAs are a type of widespread, tissue-specific and conserved endogenous non-coding RNAs in mammalian cells. Although the specific functions of most circRNAs still remain unclear, accumulating evidence has revealed a role of circRNAs as miRNA sponges.[16–20] No free ends can render circRNAs to evade destabilisation and degradation mediated by miRNAs. Several recent studies have indicated the availability of circRNAs as miRNA sponges to take part in the occurrence and progression of various diseases.[21–23] Nevertheless, there are still arguments against miRNA sponges being a major function of circRNAs because of their low amount or lack of reiterated miRNA binding sites.[24 25] In the present study, qRT-PCR, northern blot and FISH assay revealed abundant circVMA21 in NPCs. VMA21 gene is located on the X chromosome and encodes an integral membrane protein to assist in the assembly of the V-ATPase.[26] circVMA21 is generated by back splicing of the third exon of VMA21 gene. It mainly comprises 3'UTR of VMA21 mRNA, so its sequence is relatively conserved between human and rat (online supplementary table S6). The bioinformatics analysis found circVMA21 containing multiple target sites of miR-200c, which was validated by luciferase, pull-down, RNA binding protein immunoprecipitation (RIP and FISH analyses. Furthermore, the expression of miR-200c target mRNA, XIAP, was positively modulated by circVMA21. Thus, the binding sites of circVMA21 for miR-200c were proven effective.

In this study, we selected TNF-α and IL-1β as the agents to induce a range of pathogenic responses in NPCs, because they have vital roles in the pathological process of IVDD.[2 6 27] Our results were consistent with previous findings that the

stimulation of NPCs with these cytokines caused a similar pattern of changes observed in patients with IVDD or animal models, including excessive NPC apoptosis, enhanced expression of the ECM-degrading enzymes (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5), and inhibited expression of the ECM proteins (collagen II and aggrecan).[28–30] Deletion of XIAP remarkably impaired the antiapoptotic and anti-inflammatory ability of circVMA21 or miR-200c antagonist, confirming that XIAP was the direct target of circVMA21 and miR-200c to suppress the effects of TNF-α and IL-1β in NPCs. XIAP belongs to the inhibitor-of-apoptosis proteins (IAP) that represent a family of endogenous caspase inhibitors. Accumulated TNF-α and IL-1β are thought to activate the effector caspases to exert apoptotic effects in NPCs via the extrinsic and intrinsic pathways.[4 5] Of note, XIAP is the only IAP that can bind and directly inhibit the activity of the three most important apoptosis effector caspases, caspase-3, caspase-7 and caspase-9.[31 32] The absence or inhibition of XIAP increases the formation and subsequent activation of the death inducing signalling complex, and renders the majority of cells to be sensitised to death receptor-induced apoptosis, such as with TNF-α.[33 34] Mehrkens *et al*[35] recently reported that notochordal cell-derived conditioned medium upregulates the genomic expression of XIAP and thus inhibits inflammatory cytokines-induced NPC apoptosis. Moreover, the blockage of caspase signalling attenuates IVDD by inhibiting NPC apoptosis and by regulating the expression of matrix metabolism enzymes. Yamada *et al*[36] find that caspase-3 knockdown reduces the production of matrix-degrading enzymes (MMP-3, MMP-13, ADAMTS-4 and ADAMTS-5) and increases the expression of proanabolic proteins (tissue inhibitor of metalloproteinases-1, collagen II and aggrecan). XIAP knockdown-induced activation of caspases could lead to consequent matrix metabolism imbalance as shown in the present study.

In addition to caspases inhibition, a growing body of evidence exists to support the modulatory role for XIAP in inflammation. Loss of XIAP facilitates the proinflammatory effect of TNF-α and causes severe sterile inflammation, which can be reduced by anti-TNF therapy.[34 37 38] Meanwhile, XIAP suppresses inappropriate or excess IL-1β activity, while absence of XIAP promotes excessive IL-1β secretion in different cell types.[39 40] During the process of IVDD, TNF-α and IL-1β are produced by both leucocytes and NPCs themselves.[41] Therefore, XIAP seemed to function in NPCs by blocking the effects of exogenous TNF-α and IL-1β, and by inhibiting endogenous generation of these cytokines.

The age gap between the patients with and without IVDD may lead to a bias in this study. Nevertheless, it is an inevitable confounding factor when detecting the samples from human NP tissues because of the clinicopathological characteristics of IVDD. The results of in vitro and in vivo experiments would help eliminate the influence of age. In addition to ceRNA, there may exist other potential mechanisms of circVMA21 to regulate the process of IVDD, for example, through interacting with RNA binding proteins (RBPs) other than AGO2. RBPs CLIP shows that the 3'UTR of VMA21 mRNA can bind to abundant human antigen R (HuR), an extensively studied RBP with substantial regulatory effects on the stability and translation of multiple mRNAs. The binding of some circRNAs to HuR has been identified to prevent HuR binding to mRNAs and thus lower their translation.[42] In addition, the mechanism of the decrease in circVMA21 levels during the degenerative process remains unclear. Further investigation is needed to completely understand the role of circVMA21 in IVDD.

In summary, circVMA21 could alleviate inflammatory cytokines-induced NPC apoptosis and imbalance between anabolism and catabolism of ECM through the miR-200c-XIAP pathway (figure 6). It provides a potentially effective therapeutic strategy for IVDD.

Contributors XC and JZ designed the experiments. XC, LZ, KZ, GZ, YH and XS performed the experiments and acquired the data. XC, LZ, CZ and HL analysed the data. XC, YML and JZ supervised the project and wrote the manuscript.

Funding This work was supported by grants from the National Natural Science Foundation of China (81572168, 81401830), and the Industry-Academy-Research Cooperation Project of Shanghai Science and Technology Committee (13DZ1940504, 13DZ1940505), the Natural Science Foundation of Shanghai, the Department Integration Foundation (160065), and the Fundamental Research Program Funding of the Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine.

Competing interests None declared.

Patient consent Obtained.

Ethics approval Ethics Committee of Shanghai Ninth People's Hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016;388:1545–602.
2 Risbud MV, Shapiro IM. Role of cytokines in intervertebral disc degeneration: pain and disc content. *Nat Rev Rheumatol* 2014;10:44–56.
3 Kepler CK, Markova DZ, Hilibrand AS, *et al*. Substance P stimulates production of inflammatory cytokines in human disc cells. *Spine* 2013;38:E1291–E1299.
4 Ding F, Shao ZW, Xiong LM. Cell death in intervertebral disc degeneration. *Apoptosis* 2013;18:777–85.
5 Johnson ZI, Schoepflin ZR, Choi H, *et al*. Disc in flames: roles of TNF-α and IL-1β in intervertebral disc degeneration. *Eur Cell Mater* 2015;30:104–17.
6 Dudek M, Yang N, Ruckshanthi JP, *et al*. The intervertebral disc contains intrinsic circadian clocks that are regulated by age and cytokines and linked to degeneration. *Ann Rheum Dis* 2017;76.
7 Kumar S, Nag A, Mandal CC. A Comprehensive Review on miR-200c, a promising cancer biomarker with therapeutic potential. *Curr Drug Targets* 2015;16:1381–403.
8 Magenta A, Cencioni C, Fasanaro P, *et al*. miR-200c is upregulated by oxidative stress and induces endothelial cell apoptosis and senescence via ZEB1 inhibition. *Cell Death Differ* 2011;18:1628–39.
9 Schickel R, Park SM, Murmann AE, *et al*. miR-200c regulates induction of apoptosis through CD95 by targeting FAP-1. *Mol Cell* 2010;38:908–15.
10 Wang HQ, Yu XD, Liu ZH, *et al*. Deregulated miR-155 promotes Fas-mediated apoptosis in human intervertebral disc degeneration by targeting FADD and caspase-3. *J Pathol* 2011;225:232–42.
11 Ji ML, Lu J, Shi PL, *et al*. Dysregulated miR-98 contributes to extracellular matrix degradation by targeting IL-6/STAT3 signaling pathway in human intervertebral disc degeneration. *J Bone Miner Res* 2016;31:900–9.
12 Meng X, Li X, Zhang P, *et al*. Circular RNA: an emerging key player in RNA world. *Brief Bioinform* 2016:bbw045.
13 Guo Y, Luo F, Liu Q, *et al*. Regulatory non-coding RNAs in acute myocardial infarction. *J Cell Mol Med* 2017;21:1013–23.
14 Jeck WR, Sorrentino JA, Wang K, *et al*. Circular RNAs are abundant, conserved, and associated with ALU repeats. *RNA* 2013;19:141–57.
15 Zhang XO, Wang HB, Zhang Y, *et al*. Complementary sequence-mediated exon circularization. *Cell* 2014;159:134–47.
16 Piwecka M, Glažar P, Hernandez-Miranda LR, *et al*. Loss of a mammalian circular RNA locus causes miRNA deregulation and affects brain function. *Science* 2017;357:eaam8526.
17 Memczak S, Jens M, Elefsinioti A, *et al*. Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 2013;495:333–8.
18 Hansen TB, Jensen TI, Clausen BH, *et al*. Natural RNA circles function as efficient microRNA sponges. *Nature* 2013;495:384–8.

19 Zheng Q, Bao C, Guo W, *et al*. Circular RNA profiling reveals an abundant circHIPK3 that regulates cell growth by sponging multiple miRNAs. *Nat Commun* 2016;7:11215.
20 Hansen TB, Kjems J, Damgaard CK. Circular RNA and miR-7 in cancer. *Cancer Res* 2013;73:5609–12.
21 Han D, Li J, Wang H, *et al*. Circular RNA circMTO1 acts as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression. *Hepatology* 2017;66:1151–64.
22 Wang K, Long B, Liu F, *et al*. A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223. *Eur Heart J* 2016;37:2602–11.
23 Chen L, Zhang S, Wu J, *et al*. circRNA_100290 plays a role in oral cancer by functioning as a sponge of the miR-29 family. *Oncogene* 2017;36:4551–61.
24 Guo JU, Agarwal V, Guo H, *et al*. Expanded identification and characterization of mammalian circular RNAs. *Genome Biol* 2014;15:409.
25 Conn SJ, Pillman KA, Toubia J, *et al*. The RNA binding protein quaking regulates formation of circRNAs. *Cell* 2015;160:1125–34.
26 Ramachandran N, Munteanu I, Wang P, *et al*. VMA21 deficiency prevents vacuolar ATPase assembly and causes autophagic vacuolar myopathy. *Acta Neuropathol* 2013;125:439–57.
27 Phillips KL, Cullen K, Chiverton N, *et al*. Potential roles of cytokines and chemokines in human intervertebral disc degeneration: interleukin-1 is a master regulator of catabolic processes. *Osteoarthritis Cartilage* 2015;23:1165–77.
28 Hiyama A, Skubutyte R, Markova D, *et al*. Hypoxia activates the notch signaling pathway in cells of the intervertebral disc: implications in degenerative disc disease. *Arthritis Rheum* 2011;63:1355–64.
29 Wang J, Tian Y, Phillips KL, *et al*. Tumor necrosis factor α- and interleukin-1β-dependent induction of CCL3 expression by nucleus pulposus cells promotes macrophage migration through CCR1. *Arthritis Rheum* 2013;65:832–42.
30 Wang J, Markova D, Anderson DG, *et al*. TNF-α and IL-1β promote a disintegrin-like and metalloprotease with thrombospondin type I motif-5-mediated aggrecan degradation through syndecan-4 in intervertebral disc. *J Biol Chem* 2011;286:39738–49.
31 Deveraux QL, Takahashi R, Salvesen GS, *et al*. X-linked IAP is a direct inhibitor of cell-death proteases. *Nature* 1997;388:300–4.
32 Eckelman BP, Salvesen GS. The human anti-apoptotic proteins cIAP1 and cIAP2 bind but do not inhibit caspases. *J Biol Chem* 2006;281:3254–60.
33 Vasilikos L, Spilgies LM, Knop J, *et al*. Regulating the balance between necroptosis, apoptosis and inflammation by inhibitors of apoptosis proteins. *Immunol Cell Biol* 2017;95:160–5.
34 Wicki S, Gurzeler U, Wei-Lynn Wong W, *et al*. Loss of XIAP facilitates switch to TNFα-induced necroptosis in mouse neutrophils. *Cell Death Dis* 2016;7:e2422.
35 Mehrkens A, Matta A, Karim MZ, *et al*. Notochordal cell-derived conditioned medium protects human nucleus pulposus cells from stress-induced apoptosis. *Spine J* 2017;17:579–88.
36 Yamada K, Sudo H, Iwasaki K, *et al*. Caspase 3 silencing inhibits biomechanical overload-induced intervertebral disk degeneration. *Am J Pathol* 2014;184:753–64.
37 Wong WW, Vince JE, Lalaoui N, *et al*. cIAPs and XIAP regulate myelopoiesis through cytokine production in an RIPK1- and RIPK3-dependent manner. *Blood* 2014;123:2562–72.
38 Lawlor KE, Khan N, Mildenhall A, *et al*. RIPK3 promotes cell death and NLRP3 inflammasome activation in the absence of MLKL. *Nat Commun* 2015;6:6282.
39 Vince JE, Wong WW, Gentle I, *et al*. Inhibitor of apoptosis proteins limit RIP3 kinase-dependent interleukin-1 activation. *Immunity* 2012;36:215–27.
40 Yabal M, Müller N, Adler H, *et al*. XIAP restricts TNF- and RIP3-dependent cell death and inflammasome activation. *Cell Rep* 2014;7:1796–808.
41 Le Maitre CL, Hoyland JA, Freemont AJ. Catabolic cytokine expression in degenerate and herniated human intervertebral discs: IL-1beta and TNFalpha expression profile. *Arthritis Res Ther* 2007;9:R77.
42 Abdelmohsen K, Panda AC, Munk R, *et al*. Identification of HuR target circular RNAs uncovers suppression of PABPN1 translation by CircPABPN1. *RNA Biol* 2017;14:361–9.

Reactivation of hepatitis B virus infection in patients with rheumatoid arthritis receiving tofacitinib: a real-world study

A recent study reported long-term safety outcomes of tofacitinib for rheumatoid arthritis (RA) in global clinical trials[1]; the safety profile of tofacitinib exposure through 8.5 years appeared stable over time, with no new detectable safety signals. However, since most tofacitinib studies excluded patients with chronic hepatitis B (HBV) infection,[1 2] the risk of HBV reactivation among tofacitinib-treated patients remains unknown.

More than two billion people globally have been infected by HBV,[3] and a substantial number of patients with RA outside North America and Western Europe have coexisting HBV infection.[4] HBV reactivation is a critical challenge in patients with RA receiving biological therapy[5]; consequently, HBV screening is recommended before initiating biologics.[3] Janus kinase inhibition may counteract the suppressive effects of interferon α on viral replication[6]; therefore, we assessed the risk of HBV reactivation in patients receiving tofacitinib.

We established a retrospective cohort of 116 Taiwanese patients with RA who received tofacitinib at a single medical centre between April 2015 and February 2017 (figure 1A). Eighty-one (69.8%) had HBV infection, based on positive screening results for IgG antibody to anti-HBV core antigen with/without anti-HBV surface antibody (anti-HBsAb) — commensurate with 68.5% HBV core antibody positivity in a recent population-based survey in Taiwan.[7] Among patients with prior HBV infection, six were defined as HBV carriers by HBV surface antigen (HBsAg) positivity and a normal alanine aminotransferase (ALT) level (table 1). Follow-up HBV DNA and ALT was done 3–6 months after tofacitinib treatment; the other 75 had resolved HBV infections, defined as prior HBV infection with normal ALT levels, but without detectable serum HBV DNA or HBsAg.[8] Patients 1 and 2 in table 1, who had low viral loads and did not receive pre-emptive nucleotide analogues (NUCs), developed HBV reactivation, defined by a 10-fold rise in HBV DNA.[5] Patient 1 had an elevated ALT level; rescue NUCs with entecavir diminished the HBV viral load and ALT level, and this patient continued conventional disease-modifying antirheumatic drugs and tofacitinib (figure 1B). Among four HBV carriers who did not develop HBV reactivation, two had received pre-emptive NUCs treatment. Conversely, none of the 75 patients with prior HBV infection received antiviral therapy. HBsAbs were detectable in 54 (72.0%) patients. Fifty-three (70.7%) had repeated HBV DNA after tofacitinib therapy; no HBV reactivation was observed.

This retrospective observational study is the first to report HBV reactivation in HBV carriers with RA who did not receive pre-emptive antiviral treatment during tofacitinib therapy, despite having low levels of HBV DNA. Furthermore, no patients with RA who received prophylactic therapy developed HBV reactivation, indicating the efficacy of antiviral prophylaxis in preventing HBV reactivation.[8] Although half of the HBV carrier patients without pre-emptive NUCs did not experience HBV reactivation, more evidence is needed to demonstrate whether stringent HBV DNA monitoring followed by rescue treatment could be a safe alternative to antiviral prophylaxis.[9] A recent report demonstrated HBV reactivation in rheumatic patients with resolved HBV infection,[10] whereas none of our HBsAg-negative patients

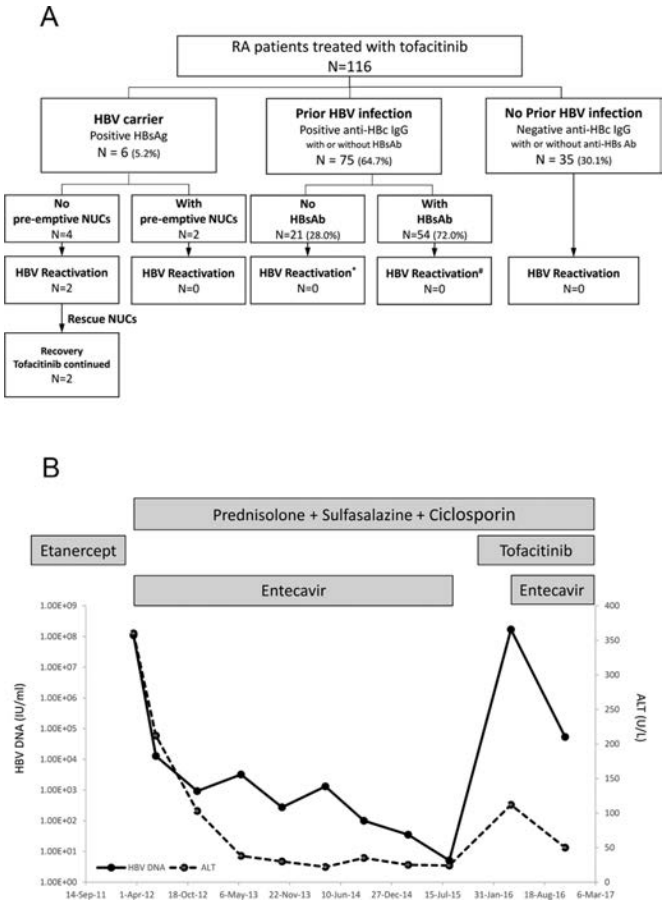


Figure 1 (A) Flow diagram of patients with RA and HBV infection during tofacitinib therapy. (B) Liver function tests and HBV viral loads following tofacitinib treatment in patient 1 of table 1 with HBV carrier. *Repeated HBV DNA available in 13 patients, #Repeated HBV DNA available in 40 patients. ALT, alanine aminotransferase; anti-HBc IgG, IgG antibody to antihepatitis B core antigen; HBsAg, HBV surface antigen; HBsAb, HBV surface antibody; HBV, hepatitis B virus; NUCs, nucleotide analogues; RA, rheumatoid arthritis.

with resolved HBV infection developed HBV reactivation. The presence of HBsAb may provide additional protection against viral reactivation.[9] Further studies are warranted to infer protection of HBsAb in patients with RA with tofacitinib treatment. Repeated viral load measurements may be necessary if overt hepatitis occurs.

In conclusion, prophylactic antiviral treatment and periodic HBV DNA follow-up are critical for chronic HBV carriers with RA receiving tofacitinib treatment. Tofacitinib therapy appears safe in patients with resolved HBV infection. It is clinically important for physicians to beware of the risk of HBV reactivation in HBV carriers with RA who do not receive pre-emptive NUCs treatment before tofacitinib therapy.

Yi-Ming Chen,[1,2,3,4] Wen-Nan Huang,[1,2] Yi-Da Wu,[1] Ching-Tsai Lin,[1] Yi-Hsing Chen,[1,2] Der-Yuan Chen,[1,2,3,4,5] Tsu-Yi Hsieh[1,6]

[1]Division of Allergy, Immunology and Rheumatology, Taichung Veterans General Hospital, Taichung, Taiwan
[2]Faculty of Medicine, National Yang-Ming University, Taipei, Taiwan
[3]Institute of Biomedical Science, National Chung Hsing University, Taichung, Taiwan
[4]Rong Hsing Research Center for Translational Medicine, National Chung Hsing University, Taichung, Taiwan
[5]Institute of Microbiology and Immunology, Chung Shan Medical University, Taichung, Taiwan

Table 1 Clinical characteristics of six tofacitinib-treated HBV carriers with rheumatoid arthritis

	Patient number					
Characteristics	**1**	**2**	**3**	**4**	**5**	**6**
Rheumatoid arthritis duration (years)	6.7	5.2	11.6	11.9	14.9	10.8
Rheumatoid factor (IU/mL)*	73.4	51.2	143	215	939.4	<14
Anti-CCP antibody (units)†	170.03	70.11	146.28	NA	NA	1.3
DAS28	6.59	6.74	5.52	6.22	6.03	5.73
HBsAg	+	+	+	+	+	+
HBsAb	–	–	–	–	–	–
HBeAg	+	NA	NA	NA	–	–
Anti-HCV antibody	–	–	–	–	–	–
Prior HBV flare	Yes	No	No	No	No	Yes
Baseline ALT (U/L)‡	24	16	25	19	25	30
Baseline HBV DNA (IU/mL)	5.09×10^{1}	2.62×10^{2}	3.43×10^{1}	$<2\times10^{1}$	1.14×10^{6}	$<2\times10^{1}$
Pre-emptive nucleotide analogues	–	–	–	–	Entecavir	Entecavir
Tofacitinib exposure (months)	14.2	19.6	5.2	5.9	7.4	6.3
Follow-up HBV DNA (IU/mL)§	1.07×10^{8}	4.32×10^{4}	1.74×10^{2}	$<2\times10^{1}$	$<2\times10^{1}$	$<2\times10^{1}$
Follow-up ALT (U/L)§	112	17	15	25	27	29
HBV flare status	Vir+Bio	Vir	–	–	–	–
Time to HBV reactivation (months)	6	12	–	–	–	–
Rescue nucleotide analogues	Entecavir	Entecavir	–	–	–	–
Concomitant medication(s)						
Average prednisolone (mg/day)	10	5	5	5	5	5
Methotrexate (mg/week)	–	15	10	10	7.5	–
Sulfasalazine (mg/day)	1000	–	–	1000	–	–
Hydroxychloroquine (mg/day)	–	400	–	–	400	400
Leflunomide (mg/day)	–	–	20	–	–	–
Ciclosporin (mg/day)	100	–	–	–	100	–
Previous biologics	Etanercept	–	Adalimumab	–	–	Abatacept
Outcome	Alive	Alive				
Tofacitinib	Continued	Continued	Continued	Continued	Continued	Continued

*Normal <10 IU/mL.
†Normal <14 units.
‡Normal <50 U/L.
§Follow-up HBV DNA and ALT at sixth (patient 1), 12th (patient 2), and third–sixth (patients 3–6) months after tofacitinib treatment.
ALT, alanine aminotransferase; Bio, biochemical flare, defined by elevation of ALT above twice the upper limit of normal; CCP, cyclic citrullinated peptide; DAS28, Disease Activity Score for 28 joints; HBeAg, HBV e antigen; HBsAb, HBV surface antibody; HBsAg, HBV surface antigen; HBV, hepatitis B virus; HCV, hepatitis C virus; NA, not available; Vir, virological flare, defined by a rise in viral load of ≧1 log.

[6]Ph D Program of Business, College of Business, Feng Chia University, Taichung, Taiwan

Correspondence to Dr Tsu-Yi Hsieh, Division of Allergy, Immunology and Rheumatology, Taichung Veterans General Hospital, No. 1650, Sec. 4, Taiwan Boulevard, Taichung 40705, Taiwan; zuyihsieh@gmail.com

Acknowledgements The authors would like to thank Ms Tzu-Yin Yen for her assistance with case management of patients with RA. Dr David Neil (PhD) of Content Ed Net (Taiwan) provided medical editing services on behalf of Taichung Veterans General Hospital.

Contributors All authors made substantial intellectual contributions to conception of the work, the interpretation of data and approval of the final manuscript. T-YH and Y-MC conceived of the study, generated the original hypothesis, designed the study, and drafted and revised the manuscript. W-NH, Y-DW, C-TL and Y-HC acquired clinical data and analysed data. D-YC conceived of the study, analysed data and revised the manuscript.

Funding This study was supported by a grant from Taichung Veterans General Hospital, Taiwan (TCVGH-1057309C).

Competing interests None declared.

Patient consent Obtained.

Ethics approval Institution Review Board of Taichung Veterans General Hospital.

Provenance and peer review Not commissioned; externally peer reviewed.

To cite Chen Y-M, Huang W-N, Wu Y-D, *et al*. *Ann Rheum Dis* 2018;**77**:780–782.

Received 16 February 2017
Revised 18 May 2017
Accepted 3 June 2017
Published Online First 29 June 2017

Ann Rheum Dis 2018;**77**:780–782. doi:10.1136/annrheumdis-2017-211322

REFERENCES

1 Cohen SB, Tanaka Y, Mariette X, *et al*. Long-term safety of tofacitinib for the treatment of rheumatoid arthritis up to 8.5 years: integrated analysis of data from the global clinical trials. *Ann Rheum Dis* 2017;76:1253–62.
2 Burmester GR, Blanco R, Charles-Schoeman C, *et al*. Tofacitinib (CP-690,550) in combination with methotrexate in patients with active rheumatoid arthritis with an inadequate response to tumour necrosis factor inhibitors: a randomised phase 3 trial. *Lancet* 2013;381:451–60.
3 Koutsianas C, Thomas K, Vassilopoulos D. Hepatitis B reactivation in rheumatic diseases: screening and Prevention. *Rheum Dis Clin North Am* 2017;43:133–49.
4 Zou CJ, Zhu LJ, Li YH, Yh L, *et al*. The association between hepatitis B virus infection and disease activity, Synovitis, or joint destruction in rheumatoid arthritis. *Clin Rheumatol* 2013;32:787–95.
5 Lan JL, Chen YM, Hsieh TY, *et al*. Kinetics of viral loads and risk of hepatitis B virus reactivation in hepatitis B core antibody-positive rheumatoid arthritis patients undergoing anti-tumour necrosis factor alpha therapy. *Ann Rheum Dis* 2011;70:1719–25.

6 Boor PP, de Ruiter PE, Asmawidjaja PS, *et al*. JAK-inhibitor tofacitinib suppresses interferon alfa production by plasmacytoid dendritic cells and inhibits arthrogenic and antiviral effects of interferon alfa. *Transl Res* 2016:S1931-5244(16)30385–1.
7 Chen CL, Yang JY, Lin SF, *et al*. Slow decline of hepatitis B burden in general population: results from a population-based survey and longitudinal follow-up study in Taiwan. *J Hepatol* 2015;63:354–63.
8 Sarin SK, Kumar M, Lau GK, *et al*. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. *Hepatol Int* 2016;10:1–98.
9 Reddy KR, Beavers KL, Hammond SP, *et al*. American Gastroenterological Association Institute guideline on the prevention and treatment of hepatitis B virus reactivation during immunosuppressive drug therapy. *Gastroenterology* 2015;148:215–9.
10 Fukuda W, Hanyu T, Katayama M, *et al*. Incidence of hepatitis B virus reactivation in patients with resolved infection on immunosuppressive therapy for rheumatic disease: a multicentre, prospective, observational study in Japan. *Ann Rheum Dis* 2017;76:1051–6.

How are enthesitis, dactylitis and nail involvement measured and reported in recent clinical trials of psoriatic arthritis? A systematic literature review

While enthesitis, dactylitis and nail involvement are recognised as important outcomes of psoriatic arthritis (PsA) in the core set of domains in PsA,[1 2] it is still unclear how these outcomes should best be measured.[1 2] We systematically reviewed the instruments and the cut-offs used to report state or improvement, for enthesitis, dactylitis and nail involvement in recent randomised controlled trials (RCTs) in PsA.

A systematic literature review of RCTs on any pharmacological intervention in patients with PsA was conducted to inform the European League Against Rheumatism (EULAR) recommendations for the management of PsA, by searching Medline, Embase and Cochrane datasets for the period 2010–2015.[3 4] Only published papers and only results of the placebo-controlled phases were analysed. The presence and type of all outcome measures reflecting enthesitis, dactylitis and nail involvement were collected. Cut-offs used for each measure (either as state or change, absolute or relative) were also collected. The proportion of trials in which each of the cut-offs for each measure was reported was calculated.

Of 2278 articles screened, 14 trials met the inclusion criteria: 4 (29%) reported on non-biologic drugs (included targeted synthetic disease-modifying antirheumatic drugs, one trial), 5 (36%) on tumour necrosis factor inhibitors, 4 (29%) on other biologic modes of action and there was one strategy trial. The trials included a total of 4744 patients. Four of the trials (29%) did not report any outcome on any of the three domains of interest (table 1). Enthesitis and dactylitis outcomes were reported in the remaining 10 trials, while nail involvement was only reported in three trials (21%). These three outcomes have been measured in several different ways, none of which having been used in more than three trials (21%), and the majority of them was actually employed in only one (7%) or two (14%) trials. Different instruments have been used, different cut-offs and different statistics reported (eg, mean and median improvement or resolution of the outcome, eg, enthesitis score of zero) (table 1). It was often the case that the same outcome measure

Table 1 Outcome measures used in 14 recent trials in PsA

Manifestation	Outcome measure	Level of measurement	n (%)
Any	No manifestation reported		4 (29)
Enthesitis	Absolute change in Enthesitis score	Change (mean) in Leeds Enthesitis Index	2 (14)
		Change (mean) in PsA modified MASES	3 (21)
		Change (median) in MASES	1 (7)
	Relative change (%) in Enthesitis score	% change in MASES	2 (14)
	Proportion of patients with enthesitis	MASES (0–13) ≥1	2 (14)
	Proportion of patients with change	% of patients with improvement in ≥1 tendon/ligament	1 (7)
	Resolution of enthesitis	MASES=0 (0–13)	1 (7)
		Leeds Enthesitis Index=0 (0–6)	1 (7)
		Enthesitis score=0 (0–4)*	1 (7)
Dactylitis	Absolute change in Dactylitis score	Change (mean) in Dactylitis score (0–20)†	2 (14)
		Change (median) in Dactylitis score (0–20)	1 (7)
		Change (median) in Leeds Dactylitis Index (0–60)	2 (14)
		Change (mean) in Leeds Dactylitis Index (0–60)	2 (14)
	Relative change (%) in Dactylitis score	% change in Leeds Dactylitis Index (0–60)	3 (21)
	Proportion of patients with Dactylitis	Leeds Dactylitis Index (0–60)≥1	2 (14)
	Resolution of Dactylitis	Dactylitis score=0 (0–20)	3 (21)
Nail involvement	Absolute change in score of nail involvement	Change (mean) in modified Nail Psoriasis Severity Index	1 (7)
		Change (median) in modified Nail Psoriasis Severity Index	1 (7)
		Change (median) in Nail Psoriasis Severity Index	1 (7)
	Relative change (%) in score of nail involvement	% change in Nail Psoriasis Severity Index	1 (7)

*Enthesitis score: 4-point enthesitis index to measure the presence (score of 1) or absence (score of 0) of tenderness at the lateral epicondyle humerus (left and right) and proximal Achilles (left and right).
†Dactylitis score: score of 1 for the presence of dactylitis and 0 for the absence in each digit (n=20), for an overall score ranging from 0 to 20.
MASES, Maastricht Ankylosing Spondylitis Enthesitis Score; PsA, psoriatic arthritis.

was used (eg, the Maastricht Ankylosing Spondylitis Enthesitis Score (MASES)), but then reported in such different ways (eg, percentage of change, percentage ≥1, percentage of improvement in one tendon/ligament, etc) that the potential uniformity in the measures used got diluted (table 1). There was also heterogeneity in the timing of report of the outcome measures across trials.

In summary, there is substantial lack of uniformity in the measurement of enthesitis, dactylitis and nail involvement in recent clinical trials of PsA. A similar lack of uniformity had previous been described for patient-reported outcomes in PsA,[5 6] and in what concerns enthesitis, dactylitis and nail involvement measurement is the heterogeneity even larger. This relates to both the instruments used and the evaluation and interpretation of the results. An important aspect that requires attention are the ways in which the data are reported, namely the cut-offs chosen or the different statistics reported, which make the heterogeneity larger, even when one single outcome measure (see the example of MASES) is being used. Assessment of dactylitis and enthesitis needs further development taking both their resolution and appearance into account. Another methodological aspect deserving attention is the fact that these outcomes are actually only investigated in patients with active involvement at baseline, which violates the principle of intention-to-treat analysis. Consensus is necessary and more elegant solutions should be considered. An update of the PsA Core Set of domains has just been published, however, without any indication of the instruments and cut-offs to be used.[2] Harmonisation of measures to be used in trials and possibly also clinical practice is desirable to allow for optimal assessment and better comparability of the efficacy of interventions.

Sofia Ramiro,[1] Josef S Smolen,[2,3] Robert Landewé,[4] Désirée van der Heijde,[1] Laure Gossec[5]

[1]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[2]Division of Rheumatology, Department of Medicine 3, Medical University of Vienna, Vienna, Austria
[3]Department of Medicine, Hietzing Hospital, Vienna, Austria
[4]Department of Clinical Immunology and Rheumatology, Amsterdam Rheumatology Center, Amsterdam and Zuyderland Hospital, Heerlen, The Netherlands
[5]Department of Rheumatology, Sorbonne Universités, UPMC Univ Paris 06, Institut Pierre Louis d'Epidémiologie et de Santé Publique, GRC-UPMC 08 (EEMOIS); AP-HP, Pitié Salpêtrière Hospital, Paris, France

Correspondence to Dr Sofia Ramiro, Department of Rheumatology, Leiden University Medical Center, 2333 ZA Leiden, The Netherlands; sofiaramiro@gmail.com

Contributors All authors contributed to this letter and approved its last version.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

To cite Ramiro S, Smolen JS, Landewé R, *et al*. *Ann Rheum Dis* 2018;**77**:782–783.

Received 9 March 2017
Revised 24 April 2017
Accepted 8 May 2017
Published Online First 5 June 2017

Ann Rheum Dis 2018;**77**:782–783. doi:10.1136/annrheumdis-2017-211447

REFERENCES

1 Gladman DD, Mease PJ, Strand V, *et al*. Consensus on a core set of domains for psoriatic arthritis. *J Rheumatol* 2007;34:1167–70.
2 Orbai A-M, de Wit M, Mease PJ, *et al*. Updating the Psoriatic Arthritis (PsA) core domain set: a report from the PsA workshop at OMERACT 2016. *J Rheumatol* 2017:jrheum.160904.
3 Ramiro S, Smolen JS, Landewé R, *et al*. Pharmacological treatment of psoriatic arthritis: a systematic literature review for the 2015 update of the EULAR recommendations for the management of psoriatic arthritis. *Ann Rheum Dis* 2016;75:490–8.
4 Gossec L, Smolen JS, Ramiro S, *et al*. European League Against Rheumatism (EULAR) recommendations for the management of psoriatic arthritis with pharmacological therapies: 2015 update. *Ann Rheum Dis* 2016;75:499–510.
5 Palominos PE, Gaujoux-Viala C, Fautrel B, *et al*. Clinical outcomes in psoriatic arthritis: a systematic literature review. *Arthritis Care Res* 2012;64:397–406.
6 Kalyoncu U, Ogdie A, Campbell W, *et al*. Systematic literature review of domains assessed in psoriatic arthritis to inform the update of the psoriatic arthritis core domain set. *RMD Open* 2016;2:e000217.

Early-onset autoimmune disease due to a heterozygous loss-of-function mutation in *TNFAIP3* (A20)

Rare Mendelian disorders increasingly contribute to our understanding of the genetic architecture of autoimmune disease and the key molecular pathways governing its pathogenesis. Early-onset autoimmune disease can arise through activating mutations in inflammatory signalling pathways or loss-of-function mutations in immunoregulatory proteins.

We investigated the molecular basis of complex autoimmunity—characterised by the onset of insulin-dependent diabetes, cytopaenias, hepatitis, enteropathy and interstitial lung disease at age 10—in a 14-year-old boy of healthy non-consanguineous British parents. Immunological analysis revealed lymphopaenia with no naive T cells and a high proportion of activated T cells (table 1). Pathogenic variants in *STAT3* and *FOXP3* were excluded. The clinical course was refractory to intensive immunosuppression with prednisolone, sirolimus, tacrolimus, infliximab or rituximab, necessitating haematopoietic stem cell transplantation. Twenty-one months post-transplant, he is thriving off all immunosuppressive medication with complete remission of autoimmune disease (except diabetes).

Ethical approval was granted (ref: 10/H0906/22) and written informed consent provided prior to study commencement. By whole exome sequencing of peripheral blood genomic DNA (Illumina MiSeq) and downstream bioinformatic filtering (Ingenuity Variant Analysis), we identified a single biologically plausible variant—a novel de novo heterozygous 2 bp deletion in tumour necrosis factor-alpha-induced protein 3 (*TNFAIP3*, figure 1A). *TNFAIP3* encodes the ubiquitin-editing enzyme A20, a negative regulator of the nuclear factor-κB (NF-κB) pathway.[1] A20 removes K63-linked ubiquitin chains from key adaptor proteins, replacing them with K48-linked polyubiquitin chains, to trigger proteasomal degradation and termination of the NF-κB activation cascade.[2] Polymorphisms in *TNFAIP3* have been linked to the development of several autoimmune diseases in genome-wide association studies.[3–7] A conditional knockout of A20 in immune cells leads to the development of autoimmunity in the mouse.[8] However, autoimmune phenomena were not prominent in a recently described cohort of patients with

Table 1 Immunological and clinical parameters

Parameters	Pretransplant	Post-transplant	Reference range
Laboratory			
Haemoglobin (g/dL)	9.8	12.4	13.5–17.5
Leucocytes (10^9/L)	1.88	3.47	150–450
Lymphocytes (10^9/L)	0.17	1.31	1.2–5.2
Neutrophils (10^9/L)	1.52*	1.79	1.8–8.0
Monocytes (10^9/L)	0.19	0.37	0.2–0.8
Platelets (10^9/L)	29	183	150–400
CD3+ (cells/µL)	800	1914	800–3500
CD8+ (cells/µL)	554	936	200–1200
CD4+ (cells/µL)	238	920	400–1200
CD56+ (cells/µL)	35	99	70–1200
CD19+ (cells/µL)	138	99	200–600
Activated T cells (HLA-DR+ %)	55	25	N/A
CD4+ naive (%)	Not detected	244	N/A
CD27− IgD+ (naive) (%)	87	93	75.2–86.7
CD27+ IgD+ (memory) (%)	9	4	4.6–10.2
CD27+ IgD− (class-switched) (%)	2	3	3.3–9.6
IgM (g/L)	0.55	0.25	0.50–1.90
IgG (g/L)	6.4	8.2	5.4–16.1
IgA (g/L)	0.92	0.33	0.80–2.80
Tetanus (IU/mL)	0.93	ND	0.1–10
Haemophilus influenzae b (mg/mL)	1.8	ND	1.0–20.0
Pneumococcal (mg/mL)	10	ND	20–200
Anti-GAD antibody (IU/mL)	>2000	>2000	0–9.9
Islet cell antibody	Detected	Detected	N/A
pANCA	Detected	Detected	N/A
Clinical			
FEV_1 (% predicted)	38	84	95–100

*Peripheral neutrophils were supported pretransplant by recombinant granulocyte colony stimulating factor. Post-transplant parameters were obtained at 18 months (FBC and T-cell indices, lung function) or 21 months post-HSCT (B cell and antibody indices). Post-HSCT antibody indices were measured during concomitant subcutaneous immunoglobulin supplementation. No other autoantibodies were detected pre-HSCT or post-HSCT.
FBC, full blood count; FEV_1, forced expiratory volume in 1 s; GAD, glutamic acid decarboxylase; HLA-DR, human leucocyte antigen–antigen D related; HSCT, haematopoietic stem cell transplantation; ND, not done; pANCA, perinuclear anti neutrophil cytoplasmic antibody.

germline A20 haploinsufficiency, who instead presented with an autoinflammatory phenotype resembling Behçet's disease.[9]

The c.1466_1467delTG variant—which we confirmed by capillary sequencing[10] (figure 1B)—introduces a frameshift substitution of alanine for valine at position 489, generating a downstream premature stop codon (p.V489Afs*7) in the zinc finger (ZnF)2 domain of A20. This variant is absent from public databases (ExAc/dbSNP) and distinct from disease-associated mutations affecting the ovarian tumour or ZnF4 domains of A20[9] (figure 1C). Immunoblotting[10] of patient and control dermal fibroblast lysates with an N-terminal antibody confirmed the reduced basal and TNF-α-induced expression of A20 (figure 1D).

To address the consequence of this reduced A20 expression, we performed functional experiments in patient and control dermal fibroblasts. Initially, we stimulated these cells with TNF-α (10 ng/mL) and analysed downstream signalling events by immunoblot (figure 1E). We observed exaggerated and prolonged phosphorylation of components of the NF-κB pathway, which would be expected to enhance NF-κB-dependent transcriptional effects. In keeping with this prediction, RNA sequencing (Illumina NextSeq-500) revealed a significant global increase in both the range and magnitude of TNF-α-stimulated differential gene expression (fold-change ≥2; false discovery rate-adjusted p≤0.01, figure 1F). We also confirmed enhanced expression of the key NF-κB target gene interleukin 6 (IL-6) at the protein level by ELISA (p=0.0015, figure 1G). In these respects, the molecular consequences of the p.V489Afs*7 variant were indistinguishable from reported pathogenic A20 mutations,[9] although owing to the lack of leucocyte material, we were not able to extend our analysis to inflammasome activation.

Here we provide novel validation of considerable existing evidence that implicates *TNFAIP3* in autoimmune pathogenesis. This case expands the clinical spectrum of A20 haploinsufficiency.[9] As A20 regulates multiple innate and adaptive signalling pathways,[1] it is logical that patients with inactivating mutations in A20 might manifest pathological features of autoimmunity and/or autoinflammation. Finally, we report that correction of the molecular defect within the haematopoietic cell compartment could represent a viable treatment option for severe clinical manifestations.

Christopher J A Duncan,[1] Emma Dinnigan,[1] Rachel Theobald,[1] Angela Grainger,[1] Andrew J Skelton,[2] Rafiqul Hussain,[3] Joseph D P Willet,[1] David J Swan,[1] Jonathan Coxhead,[3] Matthew F Thomas,[4] Julian Thomas,[5] Veena Zamvar,[6] Mary A Slatter,[7] Andrew J Cant,[7] Karin R Engelhardt,[1] Sophie Hambleton[1,7]

[1]Primary Immunodeficiency Group, Institute for Cellular Medicine, Newcastle University, UK
[2]Bioinformatics Support Unit, Institute for Cellular Medicine, Newcastle University, UK
[3]Genomic Core Facility, Institute for Genetic Medicine, Newcastle University, UK
[4]Department of Paediatric Respiratory Medicine, Great North Children's Hospital, Royal Victoria Infirmary, UK
[5]Department of Paediatric Gastroenterology, Great North Children's Hospital, Royal Victoria Infirmary, UK
[6]Department of Paediatric Gastroenterology, Leeds General Infirmary, UK
[7]Department of Paediatric Immunology and Stem Cell Transplant Unit, Great North Children's Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust, UK

Correspondence to Prof Sophie Hambleton, Primary Immunodeficiency Group, Institute for Cellular Medicine, Newcastle University Medical School, Newcastle upon Tyne NE2 4HH, UK; sophie.hambleton@ncl.ac.uk

Acknowledgements The authors thank the patient and their family for their trust and assistance. They are grateful to colleagues in St. James' Hospital, Leeds and Great North Children's Hospital, Newcastle for providing clinical care. They thank R. Harry, N. Maney, J. Isaacs and A. Pratt for the kind gift of reagents.

Contributors CJAD, KRE and SH: designed research. MFT, JT, VZ, MAS, AJC and SH: clinical investigation and phenotyping. CJAD, ED, RT, AG, JDPW and DJS: performed experiments. CJAD, ED, RT, AJS, KRE and SH: analysed and interpreted data. CJAD and AJS: performed statistical analysis; CJAD and SH: wrote the manuscript. All authors: reviewed the manuscript for intellectual content.

Funding CJAD was supported by the Academy of Medical Sciences (AMS-SGCL11), the British Infection Association and the UK National Institute for Health Research (NIHR); JDPW, DJS, KRE and SH were supported by the Sir Jules Thorn Trust (12/JTA); AG was supported by the Bubble Foundation; AJS was supported by the MRC-Arthritis Research UK Centre for Integrated research into Musculoskeletal Ageing (CIMA) and NIHR Newcastle Biomedical Research Centre.

Competing interests None declared.

Patient consent Obtained.

Ethics approval NHS North East-Newcastle and North Tyneside 1 REC.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement RNA-Seq data have been submitted to GEO (ref: GSE95078). Details of Sanger sequencing primers and the Ingenuity Variant Analysis bioinformatic filtering strategy are available on request.

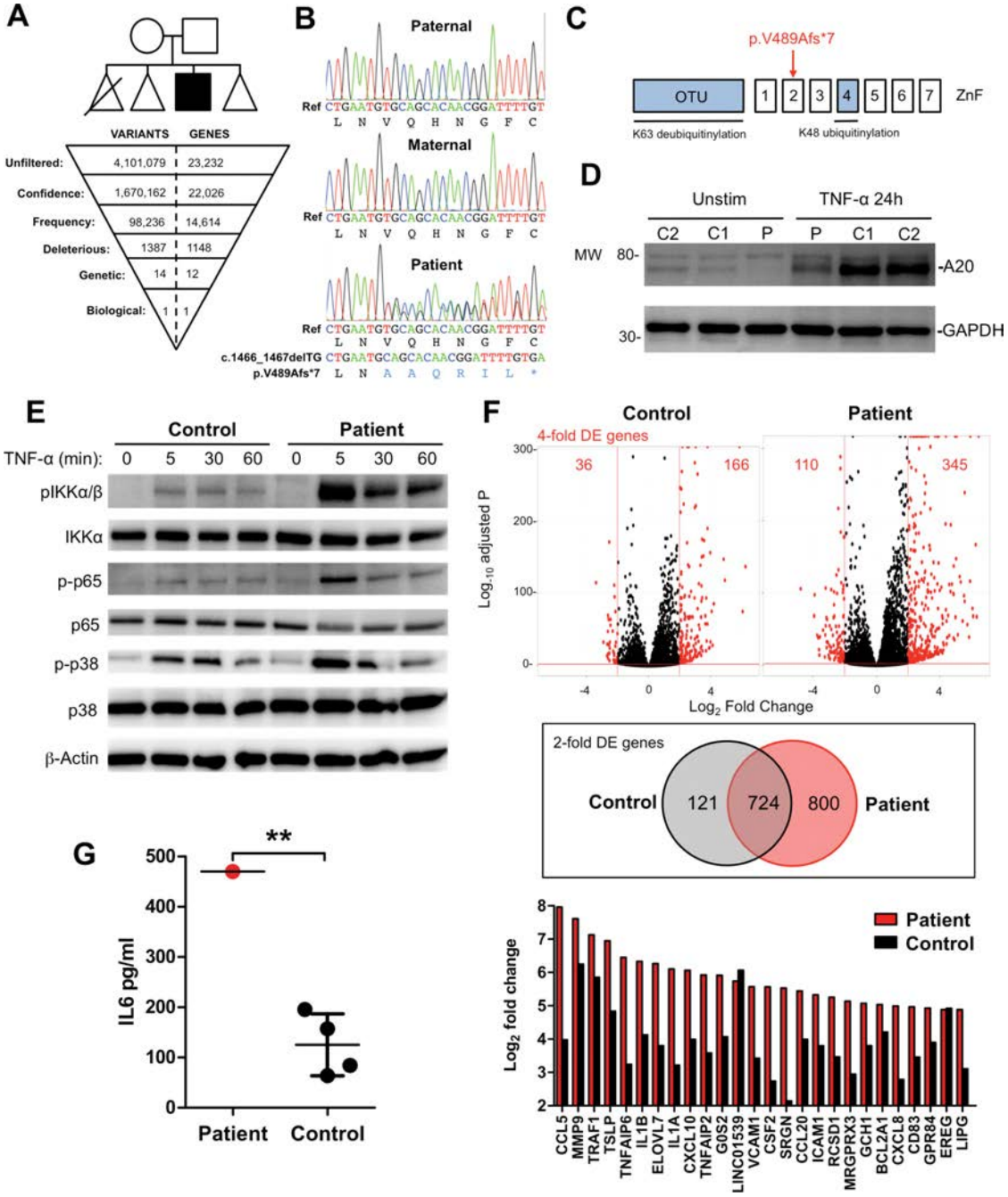


Figure 1 *TNFAIP3* variant identification and functional validation. (**A**) The family pedigree is shown (triangles are used to preserve the anonymity of healthy unaffected siblings). The first-born infant died as a result of prematurity. Whole exome sequencing data were filtered (Ingenuity Variant Analysis) by confidence (call quality ≥20; read depth ≥10; allele fraction ≥45%); frequency (ExAc allele frequency ≤0.01%); deleteriousness (nonsense/deleterious missense (SIFT/PolyPhen), splice-site disruption); genetic segregation (ie, present in patient and absent from 47 unrelated disease controls) and biological function (linked to phenotype), identifying a single heterozygous frameshift variant in *TNFAIP3* (c.1466_1467TGdel). (**B**) Variant confirmation by Sanger sequencing. (**C**) The c.1466_1467TGdel variant resulted in a frameshift and premature stop codon (V489Afs*7) in the second ZnF domain and is distinct from previously described mutations in the OTU and ZnF4 domains (blue). (**D**) V489Afs*7 reduced basal and TNF-induced A20 protein in patient (P) versus control (C1, C2) fibroblasts (immunoblot representative of n=4 independent experiments with n=4 controls). (**E**) Signalling responses downstream of TNF-α stimulation in patient fibroblasts were exaggerated and prolonged compared with control (immunoblot representative of n=4 independent experiments with n=4 controls). (**F**) RNA-seq analysis of transcriptional response to 6-hour TNF-α stimulation in patient and control fibroblasts (stimulations performed in triplicate in a single experiment). Top panel: displayed in red are significant (FDR-corrected p≤0.01) DE transcripts regulated ≥4 fold (≥2$\log_2$-fold); middle panel: Venn diagram displaying all overlapping DE transcripts ≥2 fold (≥$\log_2$-fold); Bottom panel: top 20 significant DE transcripts in patient (red bars) versus control (black bars), demonstrating many major NF-κB target genes. (**G**) Levels of IL-6 quantified by ELISA in supernatants from patient and control fibroblasts stimulated with TNF-α for 24 hours (mean±SD of average values from two independent experiments in patient and n=4 controls compared by one-sample t-test; **p=0.0015). DE, differentially expressed; FDR, false discovery rate; IL-6, interleukin 6; NF-κB, nuclear factor-κB; OTU, ovarian tumour; PolyPhen, polymorphism phenotyping; SIFT, Sorting Intolerant from Tolerant; TNF-α, tumour necrosis factor-alpha; TNFAIP3, tumour necrosis factor-alpha-induced protein 3; ZnF, zinc finger.

OPEN ACCESS

CJAD, ED and RT contributed equally.

To cite Duncan CJA, Dinnigan E, Theobald R, *et al*. *Ann Rheum Dis* 2018;**77**:783–786.

Received 3 January 2017
Revised 24 April 2017
Accepted 18 May 2017
Published Online First 28 June 2017

Ann Rheum Dis 2018;**77**:783–786. doi:10.1136/annrheumdis-2016-210944

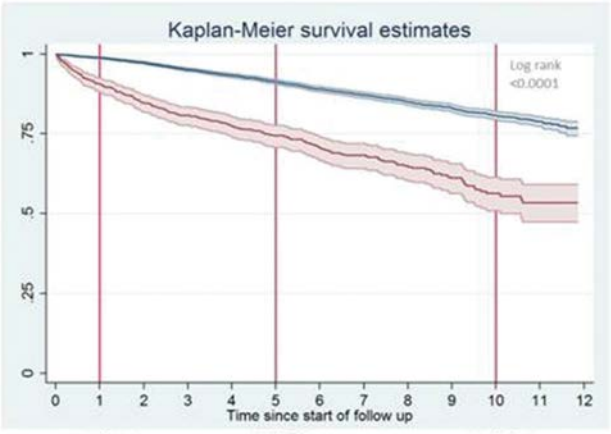
REFERENCES

1 Catrysse L, Vereecke L, Beyaert R, *et al*. A20 in inflammation and autoimmunity. *Trends Immunol* 2014;35:22–31.
2 Wertz IE, Newton K, Seshasayee D, *et al*. Phosphorylation and linear ubiquitin direct A20 inhibition of inflammation. *Nature* 2015;528:370–5.
3 Graham RR, Cotsapas C, Davies L, *et al*. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. *Nat Genet* 2008;40:1059–61.
4 Musone SL, Taylor KE, Lu TT, Tt L, *et al*. Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus. *Nat Genet* 2008;40:1062–4.
5 Fung EY, Smyth DJ, Howson JM, *et al*. Analysis of 17 autoimmune disease-associated variants in type 1 diabetes identifies 6q23/TNFAIP3 as a susceptibility locus. *Genes Immun* 2009;10:188–91.
6 Lodolce JP, Kolodziej LE, Rhee L, *et al*. African-derived genetic polymorphisms in TNFAIP3 mediate risk for autoimmunity. *J Immunol* 2010;184:7001–9.
7 Musone SL, Taylor KE, Nititham J, *et al*. Sequencing of TNFAIP3 and association of variants with multiple autoimmune diseases. *Genes Immun* 2011;12:176–82.
8 Tavares RM, Turer EE, Liu CL, *et al*. The ubiquitin modifying enzyme A20 restricts B cell survival and prevents autoimmunity. *Immunity* 2010;33:181–91.
9 Zhou Q, Wang H, Schwartz DM, *et al*. Loss-of-function mutations in TNFAIP3 leading to A20 haploinsufficiency cause an early-onset autoinflammatory disease. *Nat Genet* 2016;48:67–73.
10 Duncan CJ, Mohamad SM, Young DF, *et al*. Human IFNAR2 deficiency: Lessons for antiviral immunity. *Sci Transl Med* 2015;7:307ra154.

Correction: *Mortality in idiopathic inflammatory myopathy: results from a Swedish nationwide population-based cohort study*

Dobloug GC, Svensson J, Lunmdberg IE, *et al*. Mortality in idiopathic inflammatory myopathy: results from a Swedish nationwide population-based cohort study. *Ann of Rheum Dis* 2018;77:40–7.

Figure 2 had been inverted. The correct figure 2 should be as below:



Ann Rheum Dis 2018;**77**:786. doi:10.1136/annrheumdis-2017-211402corr1

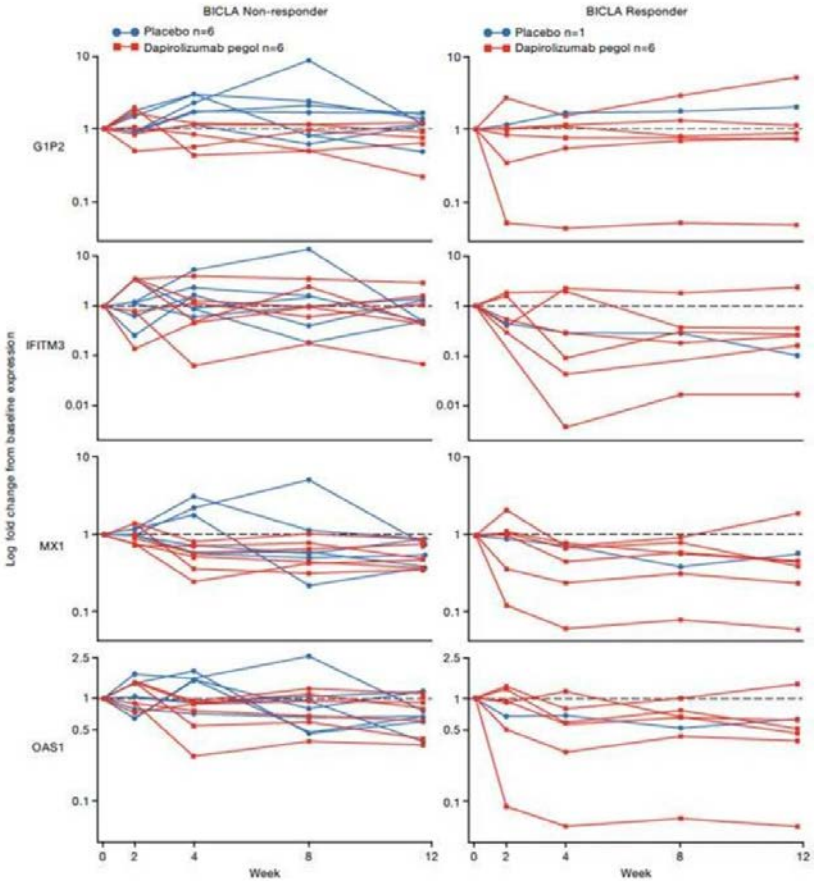
Correction: *Repeated administration of dapirolizumab pegol in a randomised phase I study is well tolerated and accompanied by improvements in several composite measures of systemic lupus erythematosus disease activity and changes in whole blood transcriptomic profiles*

Chamberlain C, Colman PJ, Ranger AM, *et al*. Repeated administration of dapirolizumab pegol in a randomised phase I study is well tolerated and accompanied by improvements in several composite measures of systemic lupus erythematosus disease activity and changes in whole blood transcriptomic profiles. *Ann of Rheum Dis* 2017;77:1837-44.

Since publishing the above article, an error in the programmatic analysis of the renal component of the exploratory BILAG endpoint was identified. Correction of this error results in the following updates to the reported BILAG, BICLA and type I interferon-response genes RNA transcript data. These updates see a small increase in the number of patients treated with dapirolizumab pegol achieving a clinical response, and in no way change our interpretation of these exploratory endpoints.

1. In Table 1 (Baseline patient demographics and characteristics), baseline BILAG median (range) total scores are corrected from 10.0 (2–21) to 10.0 (2–24) for placebo and from 13.0 (2–21) to 13.0 (2–24) for dapirolizumab pegol. The number of patients in the dapirolizumab pegol group with at least 1 BILAG Grade B is updated from 12 (75.0%) to 13 (81.3%).
2. The corrected BILAG analysis identified one additional BICLA responder. The BICLA responder rate in the dapirolizumab pegol group is revised from 5/11 (45.5%) to 6/12 (50.0%) (page 5, paragraph 1).
3. Data for the mean fold change in RNA transcript levels for the additional BICLA responder are added to Figure 4 as shown below. This does not alter the conclusions in the paper.

During this process an additional error was also identified. One patient who received concomitant systemic oral corticosteroids for 'arthritis' rather than 'SLE' was mistakenly



omitted from Table 1. The number of patients receiving concomitant corticosteroids in Table 1 is updated from 14 (87.5%) to 15 (93.8%).

Ann Rheum Dis 2018;**77**:787–788. doi:10.1136/annrheumdis-2017-211388corr1

GRAPPA-OMERACT initiative to standardise outcomes in psoriatic arthritis clinical trials and longitudinal observational studies

We read with interest the recent letter by Ramiro *et al*[1] reporting data from a systematic literature review on the measurement of enthesitis, dactylitis and nail disease in psoriatic arthritis (PsA) clinical trials. The authors highlight the great variety in the outcome measures chosen, cut points and the statistical analysis performed (percentage change, proportion resolved). We are pleased the authors have highlighted this problem and agree with their viewpoints on the clear lack of standardisation of domains and instruments in clinical trials evidenced by the data. Indeed this inconsistency of data reporting has led to significant heterogeneity in both physician-assessed and patient-reported outcomes particularly in the field of PsA. It is the domains of enthesitis, dactylitis, nail disease, as well as skin and axial disease, and the unique impact they subsequently have on physical function and quality of life for patients with PsA, that differentiate PsA from other types of inflammatory arthritis like rheumatoid arthritis. Therefore the accurate assessments of these disease manifestations are of vital importance in drug trials.

In an effort to standardise outcome assessment in PsA, the first PsA core domain set was developed in 2006 by the Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) Outcome Measures in Rheumatology (OMERACT) working group.[2] This PsA core domain set represented the minimum set of outcomes to be measured in randomised controlled trials (RCTs) and longitudinal observational studies (LOS). A systematic literature review demonstrated increased measurement of the complete PsA core domain set from 23.5% of RCTs in 2006–2010 to 59% of RCTs in 2010–2015.[3] The PsA core domain set was updated, with enhanced patient representation, in 2016 following an extensive programme of work.[4] As Ramiro *et al* state, the next step is to generate instruments and cut-offs for the measurement of these domains: a Core Outcome Measurement Set.

Several international work streams comprise the Core Outcome Measures for Psoriatic Arthritis Clinical Trials (COMPACT) study and have been underway since 2016 to address specifically this problem. The GRAPPA-OMERACT PsA core set working group is leading this work following OMERACT Filter 2.0 methodology.[5] This programme of work includes multiple systematic literature reviews, incorporating data up to 2017, in order to synthesise the existing evidence on PsA instrument properties (across RCT and LOS data sources as the authors suggest), a Delphi process with stakeholders (including patients, clinicians, triallists, methodologists and payers), and working group meetings and discussion and voting at OMERACT 2018. The resulting Core Outcome Measurement Set will synthesise the evidence and provide guidance for the use of PsA outcome instruments, including enthesitis, dactylitis and nail involvement, as discussed by Ramiro *et al*, and all pathophysiological manifestations, life impact and resource use defined in the PsA core domain set.

The OMERACT[6] and Core Outcomes Measurement for Effectiveness Trials methodology[7] we are following in the COMPACT study will provide evidence-based guidance with international consensus on the best instruments to measure the domains of psoriatic disease and equally importantly identify current gaps and a research agenda to generate the evidence. In the near future, this will facilitate standardisation of outcomes chosen in clinical trials while ensuring that key domains important to both patients and physicians are assessed.

The report by Ramiro *et al* highlights the importance of developing a framework of domains and valid instruments for the consistent assessment of PsA in RCTs and observational studies, and we suggest herein a robust framework, underway, to achieve this standardisation.

William Tillett,[1,2] Ana-Maria Orbai,[3] Alexis Ogdie,[4] Ying Ying Leung,[5] Vibeke Strand,[6] Dafna D Gladman,[7] Philip J Mease,[8] Laura C Coates,[9] On Behalf of the GRAPPA OMERACT Psoriatic Arthritis working group

[1]Royal National Hospital for Rheumatic Disease, Bath, UK
[2]Pharmacy and Pharmacology, University of Bath, Bath, UK
[3]Department of Medicine/Rheumatology, Johns Hopkins University, Baltimore, Maryland, USA
[4]Division of Rheumatology and Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, Pennsylvania, USA
[5]Department of Rheumatology and Immunology, Singapore General Hospital, Singapore, Singapore
[6]Department of Immunology/Rheumatology, Stanford University, Stanford, California, USA
[7]Krembil Research Institute, University Health Network,Toronto Western Hospital, Toronto, Ontario, Canada
[8]Department of Rheumatology Research, Swedish Medical Center, Seattle, Washington, USA
[9]Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, University of Oxford, Oxford, UK

Correspondence to Dr William Tillett, Royal National Hospital for Rheumatic Disease, University of Bath, BA11RL, Bath, UK; w.tillett@nhs.net

Competing interests None declared.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Tillett W, Orbai A-M, Ogdie A, *et al*. *Ann Rheum Dis* 2018;**77**:e23.

Received 30 June 2017
Accepted 30 June 2017
Published Online First 26 July 2017

Linked

► http://dx.doi.org/10.1136/annrheumdis-2017-212012

Ann Rheum Dis 2018;**77**:e23. doi:10.1136/annrheumdis-2017-212004

REFERENCES

1 Ramiro S, Smolen JS, Landewé R, *et al*. How are enthesitis, dactylitis and nail involvement measured and reported in recent clinical trials of psoriatic arthritis? A systematic literature review. *Ann Rheum Dis* 2018;77:782–3.
2 Gladman DD, Mease PJ, Strand V, *et al*. Consensus on a core set of domains for psoriatic arthritis. *J Rheumatol* 2007;34:1167–70.
3 Kalyoncu U, Ogdie A, Campbell W, *et al*. Systematic literature review of domains assessed in psoriatic arthritis to inform the update of the psoriatic arthritis core domain set. *RMD Open* 2016;2:e000217.
4 Orbai AM, de Wit M, Mease P, *et al*. International patient and physician consensus on a psoriatic arthritis core outcome set for clinical trials. *Ann Rheum Dis* 2016;76:673–80.
5 Boers M, Kirwan JR, Wells G, *et al*. Developing core outcome measurement sets for clinical trials: OMERACT filter 2.0. *J Clin Epidemiol* 2014;67:745–53.
6 Tugwell P, Boers M, D'Agostino MA, *et al*. Updating the OMERACT filter: implications of filter 2.0 to select outcome instruments through assessment of 'truth': content, face, and construct validity. *J Rheumatol* 2014;41:1000–4.
7 Prinsen CA, Vohra S, Rose MR, *et al*. Core Outcome Measures in Effectiveness Trials (COMET) initiative: protocol for an international Delphi study to achieve consensus on how to select outcome measurement instruments for outcomes included in a 'core outcome set'. *Trials* 2014;15:247.

Response to: 'The GRAPPA-OMERACT initiative to standardise outcomes in Psoriatic Arthritis clinical trials and longitudinal observational studies' by Tillet *et al*

We thank Tillet *et al* for their comments[1] on our letter to the editor entitled 'How are enthesitis, dactylitis and nail involvement measured and reported in recent clinical trials of psoriatic arthritis? A systematic literature review'.[2] We appreciate that the authors are in agreement with our view regarding the clear need for the harmonisation of outcome assessment in PsA.[1 2] We are aware of the work in this regard from the GRAPPA-OMERACT initiative,[3] as cited in our letter,[2] which has indeed already led to an update of the core set of domains for PsA. Hopefully, the next step that needs to be taken, namely the development of a core set of outcome measurements, will represent an important advance in standardisation. Being an OMERACT initiative, it will implicitly need to follow the OMERACT filter,[4] which means that 'For applicability, each instrument must prove to be truthful (valid), discriminative, and feasible'. For this we would like to highlight that feasibility is an important aspect that deserves appropriate attention, as otherwise the desired harmonisation of outcome measurement will not be achieved, even if the instrument may have good psychometric properties. We further hope that the GRAPPA-OMERACT initiative takes all the issues addressed in our letter, but also the various aspects of instrument development discussed during the generation of the updated treat-to-target recommendations for axial and peripheral spondyloarthritis into account when proposing the core outcome measurement set.[2 5] We look forward to the updated core set and especially to its implementation in clinical trials and in clinical practice.

Sofia Ramiro,[1] Josef S Smolen,[2,3] Robert B M Landewé,[4,5] Désirée van der Heijde,[1] Laure Gossec[6,7]

[1]Department of Rheumatology, Leiden University Medical Center, Leiden, The Netherlands
[2]Division of Rheumatology, Department of Medicine III, Medical University of Vienna, Vienna, Austria
[3]Department of Medicine, Hietzing Hospital, Vienna, Austria
[4]Department of Clinical Immunology and Rheumatology, Amsterdam Rheumatology Center, Amsterdam, The Netherlands
[5]Zuyderland Hospital, Heerlen, The Netherlands
[6]UPMC Univ Paris 06, Institut Pierre Louis d'Epidémiologie et de Santé Publique, GRC-UPMC 08 (EEMOIS), Sorbonne Universités, Paris, France
[7]Department of Rheumatology, AP-HP, Pitié Salpêtrière Hospital, Paris, France

Correspondence to Dr Sofia Ramiro, Department of Rheumatology, Leiden University Medical Center, PO Box 9600, Leiden, The Netherlands; sofiaramiro@gmail.com

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

To cite Ramiro S, Smolen JS, Landewé RBM, *et al*. *Ann Rheum Dis* 2018;**77**:e24.

Received 10 July 2017
Accepted 18 July 2017
Published Online First 16 August 2017

Linked

► http://dx.doi.org/10.1136/annrheumdis-2017-212004

Ann Rheum Dis 2018;**77**:e24. doi:10.1136/annrheumdis-2017-212012

REFERENCES

1 Tillett W, Orbai AM, Ogdie A, *et al*. GRAPPA OMERACT Psoriatic Arthritis working group. GRAPPA-OMERACT initiative to standardise outcomes in psoriatic arthritis clinical trials and longitudinal observational studies. *Ann Rheum Dis* 2018;77:e23.
2 Ramiro S, Smolen JS, Landewé R, *et al*. How are enthesitis, dactylitis and nail involvement measured and reported in recent clinical trials of psoriatic arthritis? A systematic literature review. *Ann Rheum Dis* 2018;77:782–3.
3 Gladman DD, Mease PJ, Strand V, *et al*. Consensus on a core set of domains for psoriatic arthritis. *J Rheumatol* 2007;34:1167–70.
4 Boers M, Kirwan JR, Wells G, *et al*. Developing core outcome measurement sets for clinical trials: omeract filter 2.0. *J Clin Epidemiol* 2014;67:745–53.
5 Smolen JS, Schöls M, Braun J, *et al*. Treating axial spondyloarthritis and peripheral spondyloarthritis, especially psoriatic arthritis, to target: 2017 update of recommendations by an international task force. *Ann Rheum Dis* 2018;77:3–17.

Spontaneous hypertensive rat exhibits bone and meniscus phenotypes of osteoarthritis: is it an appropriate control for MetS-associated OA?

The potential roles of metabolic syndrome (MetS) in the onset and progression of osteoarthritis (OA) have been a hot topic in the field since it may potentially open up to new non-surgical treatment regimens. To better study the relationship between MetS and OA, a suitable animal model would be a vital tool in understanding the pathomechanism and also for screening and testing various potential drug candidates.

We have recently read Deng and colleagues' letter entitled 'Eplerenone treatment alleviates the development of joint lesions in a new rat model of spontaneous metabolic-associated osteoarthritis' published online this May, which mentioned the use of 'obese spontaneously hypertensive heart failure' (SHHF$^{cp/cp}$) rat model to study MetS-associated OA and chronic administration of eplerenone, a mineralocorticoid receptor antagonist, as a treatment.[1] While we appreciate the authors' dedicated effort, we believe there are several issues concerning the novel animal model that are worth mentioning.

MetS is a cluster of at least three out of five of the following conditions: central obesity, hypertension, hyperglycaemia, high cholesterol levels and low high-density lipoprotein levels. Since it is a complex medical condition, we were very much intrigued by the authors' choice to study the mixed components of MetS in one model rather than studying the effect of the individual components. This study design does indeed allow the authors to look into potential synergistic effects of the MetS components; the major flip side is that up to this moment neither are the weights of the individual components contributing to MetS-associated OA known nor are all these components as well studied as obesity and also hyperglycaemia. One component in MetS that we would like to highlight here is hypertension. We believe that there is still a huge research gap in the relationship between hypertension and MetS-associated OA to warrant an independent study. To put this into context, in the latest Framingham osteoarthritis study, Niu and colleagues observed that after adjustment for weight or body mass index, all metabolic syndrome components except hypertension have no significant association with the occurrence of OA.[2] In other words, hypertension is highly likely a key factor in the pathogenesis of MetS-associated OA although little is known about the mechanism behind.

As we congratulate the authors' accomplishment in successfully developing the MetS strain reported in a previous publication which does indeed check three of the five boxes ("ie," dyslipidemia, hypertension and obesity), we are reserved about the use of spontaneously hypertensive rat (SHHF+/+) for the control group. While we understand the rationale behind choosing this strain, we think it is far from an optimal control since it is still unclear whether the hypertensive background itself may exert any effects on the joint structure and it has been previously suggested that hypertension on its own may play a role in the onset and progression of OA.[3 4] Indeed in our recent study, we did observe significant changes in bone and menisci by 9 months including the presence of subchondral cyst-like giant voids near the anterior cruciate ligament (ACL) enthesis and increased ossified tissue volume of the menisci in the spontaneously hypertensive rat with the Wistar Kyoto strain as the default normotensive control (figure 1).[5]

We cannot help but think that the authors seem to have regrettably overlooked the significance of hypertension in this study and we are very interested in learning from their response regarding the above issues.

PokMan Boris Chan,[1] Chunyi Wen[2]

[1]Department of Orthopaedics and Traumatology, The University of Hong Kong, Hong Kong, Hong Kong
[2]Interdisciplinary Division of Biomedical Engineering, The Hong Kong Polytechnic University

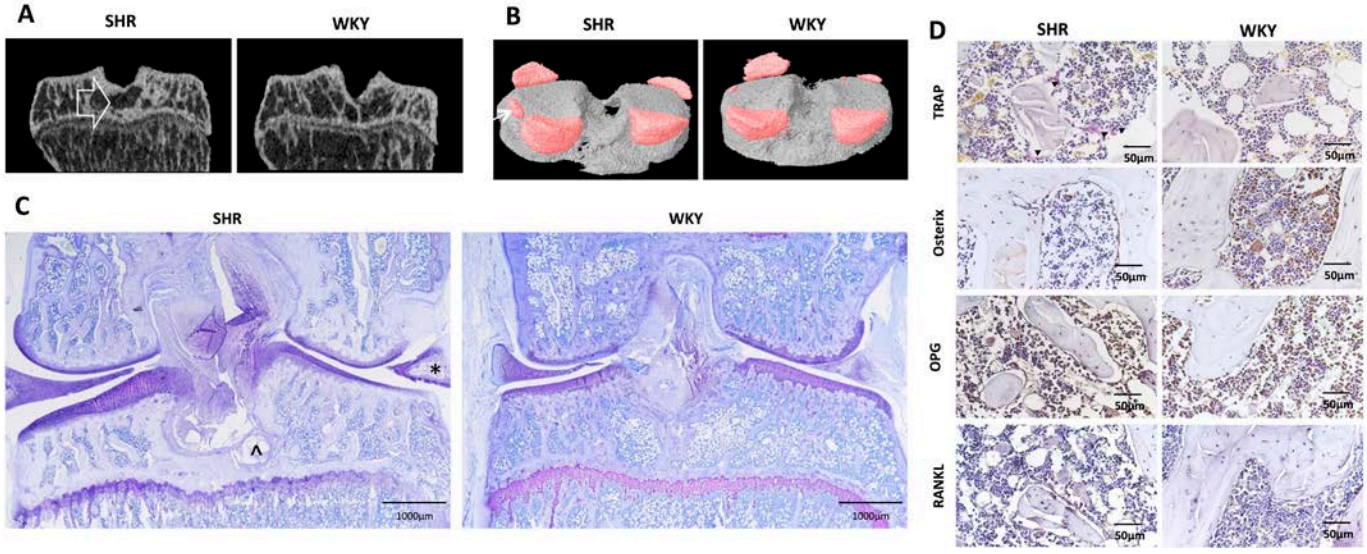


Figure 1 Osteoarthritis-like changes in 9-month-old spontaneously hypertensive rats (SHR). (A) SHR model exhibited subchondral bone cyst (block arrow) yet the control Wistar Kyoto strain (WKY) rats did not have it. (B) The menisci ossification was much more pronounced in the middle portion of the medial meniscus (arrow) in the SHR model. (C) All these micro-CT findings were echoed by histopathological examination (^, subchondral bone cyst; *, menisci ossification). (D) Uncoupled subchondral bone remodelling in SHR was characterised by increased tartrate-resistant acid phosphatase+ (TRAP+) osteoclasts but decreased Osterix+ osteoprogenitors. It was possibly due to elevated receptor activator of nuclear factor kappa-B ligand (RANKL) expression level relative to osteoprotegerin (OPG) expression level.

Correspondence to Dr Chunyi Wen, Hong Kong Polytechnic University, Biomedical Engineering, ST417, Hong Kong, Hong Kong; chunyi.wen@polyu.edu.hk

Contributors PBC and CW equally contributed to this manuscript.

Competing interests None declared.

Provenance and peer review Not commissioned; internally peer reviewed.

To cite Chan PMB, Wen C. *Ann Rheum Dis* 2018;**77**:e25.

Received 28 June 2017
Accepted 30 June 2017
Published Online First 10 August 2017

Linked

► http://dx.doi.org/10.1136/annrheumdis-2017-212003

Ann Rheum Dis 2018;**77**:e25. doi:10.1136/annrheumdis-2017-211995

REFERENCES

1 Deng C, Bianchi A, Presle N, *et al*. Eplerenone treatment alleviates the development of joint lesions in a new rat model of spontaneous metabolic-associated osteoarthritis. *Ann Rheum Dis* 2018;77:315–6.
2 Niu J, Clancy M, Aliabadi P, *et al*. Metabolic syndrome, its Components, and knee osteoarthritis: the Framingham Osteoarthritis Study. *Arthritis Rheumatol* 2017;69:1194–203.
3 Conaghan PG, Vanharanta H, Dieppe PA. Is progressive osteoarthritis an atheromatous vascular disease? *Ann Rheum Dis* 2005;64:1539–41.
4 Findlay DM. Vascular pathology and osteoarthritis. *Rheumatology* 2007;46:1763–8.
5 Chan PB, Yang W, Wen C, *et al*. Spontaneously hypertensive rat as a novel Model of Co-morbid knee osteoarthritis. *Osteoarthritis Cartilage* 2017;25:S319–S320.

Response to: 'Spontaneous hypertensive rat exhibits bone and meniscus phenotypes of osteoarthritis: is it an appropriate control for MetS-associated OA?' by Chan and Wen

We thank Dr Chan and Dr Wen for their interest in our report[1] and their resulting eLetter.[2] We fully agree that, among the different components of metabolic syndrome (MetS), hypertension has very recently been brought out as a critical feature in the development of osteoarthritis (OA) in humans.[3 4] In these reports using either data from the Framingham OA study[3] or the Osteoarthritis Initiative study,[4] it has been emphasised that high blood pressure (diastolic or systolic, respectively) was associated with increased incidence of radiographic knee OA.

In order to further experimentally investigate the actual role of hypertension in OA onset and development, Chan and colleagues describe in a yet unpublished study the development of OA features in the spontaneous hypertensive rat (SHR) model, a widely characterised model of systemic hypertension.[2 5] Although the spontaneous hypertensive heart failure (SHHF) rat strain we employed is deriving from the SHR strain[6] and suffers high blood pressure likewise, we were not able to identify OA-like lesions in our experimental model of SHHF$^{+/+}$ lean rats.[1] These seeming discrepancies obtained in two related rat strains raised the concerns expressed by Chan and Wen on our recent results.

First, we respectfully disagree on the reservations put forward by Chen and Wen regarding our choice 'to study the mixed components of MetS in one model'.[2] Indeed, in contrast to their study,[2 5] our goal was purposely to explore the contribution of MetS as a whole,[1] not to investigate a single and isolated MetS component, such as hypertension. Besides, since SHHF$^{+/+}$ and SHHF$^{cp/cp}$ are both hypertensive,[7 8] our results demonstrate the contribution of the metabolic components of MetS (obesity, dyslipidaemia and insulin resistance) in knee OA development after adjustment for both age and blood pressure. Interestingly enough, the ability of eplerenone to prevent OA development[1] without decreasing blood pressure[8] further support the role of MetS as a whole, but not that of hypertension, in the OA lesions we observed in SHHF rats.[1]

Second, Chan and Wen suggest the inadequate use of SHHF$^{+/+}$ as proper controls in our study.[2] Because both SHHF$^{+/+}$ and SHHF$^{cp/cp}$ share the very same genetic background, SHHF$^{+/+}$ rats are very appropriate controls to compare to their littermate SHHF$^{cp/cp}$ rats. By contrast, Wistar Kyoto (WK) used as control in Chan's study and SHR rats[2 5] are more distant genetically and may differ greatly besides the normal versus hypertensive status, which therefore may force caution in the proposed interpretations of the results. It stands, however, true that, in contrast to Chan and colleagues' results,[2 5] we did not observe OA-like lesions in 12.5-month-old SHHF$^{+/+}$ knee joints,[1] when they do report changes in bone and menisci, but no gross cartilage damage, in the knees of 9-month-old SHR rats.[2 5] The apparent discrepancies may be attributable to the difference in the parameters examined to evaluate OA in the two studies. Indeed, Chan *et al* mostly evaluated changes in subchondral bone and meniscus,[2 5] whereas we performed histopathological analysis of synovial and cartilage tissues.[1] Although subchondral remodelling is critical in OA, we have not looked closely at the subchondral bone phenotype as clearly stated in our Letter.[1] So, if as explained above we can exclude that hypertension does cause by itself OA-like phenotype in synovial and articular cartilage tissues of $^{12.5}$SHHF$^{+/+}$ rats, we cannot rule out that, similar to what can be observed in SHR rats, SHHF$^{+/+}$ rats (and SHHF$^{cp/cp}$ for that matter) do present significant remodelling of subchondral bone, a highly vascularised tissue, more prone to get affected by systemic pressure modification.

Based on Chan's eLetter comments[2] and forthcoming study on SHR rats,[5] we acknowledge that further investigation will be required to study and clarify the role of hypertension in SHHF rats as well. As a matter of fact, beside a comprehensive µCT analysis of the subchondral bone of both placebo-treated and eplerenone-treated SHHF$^{+/+}$ and SHHF$^{cp/cp}$ knees, we are also currently analysing the knee phenotype of 18-month-old SHHF$^{+/+}$ rats. Compared with age-matched Wistar Kyoto normotensive rats as controls, this would allow to answer more directly Chan and Wen concern regarding the independent role played by hypertension in the SHHF model and probably corroborate their findings obtained in the SHR rats.[2 5]

Again, we appreciate the interest Chan and Wen show in our work and hope we adequately answered their concerns.

Chaohua Deng,[1,2] Arnaud Bianchi,[1,2] Nathalie Presle,[1,2] David Moulin,[1,2,3] Meriem Koufany,[1,2] Cécile Guillaume,[1,2] Hervé Kempf,[1,2] Anne Pizard[2,3,4,5]

[1]UMR 7365 CNRS-Université de Lorraine, Ingénierie Moléculaire et Physiopathologie Articulaire, Vandoeuvre-lès-Nancy, Lorraine, France
[2]Fédération de Recherche 3209, Université de Lorraine, Vandoeuvre-lès-Nancy, Lorraine, France
[3]CHRU, Nancy, Vandoeuvre-lès-Nancy, Lorraine, France
[4]UMRS U1116 Inserm, Université de Lorraine, DCAC, Vandoeuvre-lès-Nancy, Lorraine, France
[5]CIC-P 1433 Inserm, CHU Nancy, Vandoeuvre-lès-Nancy, Lorraine, France

Correspondence to Hervé Kempf, UMR 7365 CNRS-UL, IMoPA, 9 avenue de la Forêt de Haye, Vandoeuvre-lès-Nancy 54500 France; herve.kempf@inserm.fr and Dr Anne Pizard, Inserm U1116, CHRU Nancy, 4 rue du Morvan, Vandoeuvre-lès-Nancy 54500, France; anne.pizard@inserm.fr

Contributors HK and AP: concept, writing; CD, AB, NP, DM, MK, CG, HK and AP: final approval.

Competing interests None declared.

Provenance and peer review Commissioned; internally peer reviewed.

HK and AP are joint senior authors

To cite Deng C, Bianchi A, Presle N, *et al*. *Ann Rheum Dis* 2018;**77**:e26.

Received 17 August 2017
Revised 11 September 2017
Accepted 15 September 2017
Published Online First 7 October 2017

► http://dx.doi.org/10.1136/annrheumdis-2017-211995

Ann Rheum Dis 2018;**77**:e26. doi:10.1136/annrheumdis-2017-212003

REFERENCES

1 Deng C, Bianchi A, Presle N, *et al*. Eplerenone treatment alleviates the development of joint lesions in a new rat model of spontaneous metabolic-associated osteoarthritis. *Ann Rheum Dis* 2018;77:315–6.
2 Chan PB, Wen C. Spontaneous hypertensive rat exhibits bone and meniscus phenotypes of osteoarthritis: is it an appropriate control for MetS-associated OA? *Ann Rheum Dis* 2018;77:e25.
3 Niu J, Clancy M, Aliabadi P, *et al*. Metabolic syndrome, its components, and knee osteoarthritis: the Framingham Osteoarthritis Study. *Arthritis Rheumatol* 2017;69:1194–203.

4 Lo GH, McAlindon TE, Katz JN, *et al*. Systolic and pulse pressure associate with incident knee osteoarthritis: data from the Osteoarthritis Initiative. *Clin Rheumatol* 2017. [Epub ahead of print 1 Jun 2017].
5 Chan PB, Yang W, Wen C, *et al*. Spontaneously hypertensive rat as a novel model of co-morbid knee osteoarthritis. *Osteoarthritis Cartilage* 2017;25:S319–S320.
6 McCune SA, Baker PB, Stills HF. SHHF/Mcc-cp rat: model of obesity, non-insulin-dependent diabetes, and congestive heart failure. *Ilar J* 1990;32:23–7.
7 Youcef G, Olivier A, L'Huillier CP, *et al*. Simultaneous characterization of metabolic, cardiac, vascular and renal phenotypes of lean and obese SHHF rats. *PLoS One* 2014;9:e96452.
8 Youcef G, Olivier A, Nicot N, *et al*. Preventive and chronic mineralocorticoid receptor antagonism is highly beneficial in obese SHHF rats. *Br J Pharmacol* 2016;173:1805–19.