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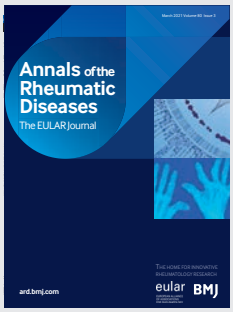

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Epidemiology

Letters

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Patient global assessment to define remission in rheumatoid arthritis: quo vadis?

Maarten Boers [1,2]

The American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) preliminary criteria for remission in rheumatoid arthritis (RA) have found widespread endorsement and adoption since their publication in 2011 (box 1).[1] Patients that fulfil the criteria are almost indistinguishable from healthy persons and live a normal life.[2] Nevertheless, the criteria are also seen as too strict,[3] as not including enough patient-important outcomes[4]; and their application in patient care, as advised by guideline committees, has problems. Most of the criticism focuses on the inclusion of patient global assessment (PGA) and its threshold.

In principle, these (perceived or real) limitations can be explained by re-examining the purpose and the development process of the criteria. And then solutions can be sought.

First of all, the committee was tasked with developing criteria that included, as a minimum, tender and swollen joint counts, and was strict, to counter existing criteria that failed to define remission.[1] So the strictness of the current criteria 'is not a bug, but a feature' as Bill Gates may have said when his problematic software was discussed. More seriously, patients close to, but not in remission, can be classified as having 'minimal disease activity' for which the Outcome Measurement in Rheumatology (OMERACT) initiative already formulated a set of definitions in 2005.[5] Briefly, patients can be considered in minimal disease activity if they meet five out of seven criteria (tender joint count ≤1; swollen joint count ≤1; health assessment questionnaire ≤0.5; pain ≤2; physician global assessment ≤1.5; PGA≤2.0; erythrocyte sedimentation rate (ESR≤20). Unfortunately, this concept has not gained much traction in guidelines, leaving a gap between 'low disease activity' and 'remission', even though it would arguably be a good target for treatment: more stringent than low disease activity and more feasible than remission.

Since then, several publications have pointed out that many patients fail to meet the remission criteria only because their PGA score is above 1 (on a scale of 0–10), see for example Ferreira *et al*,[6] and that of such patients, those with a PGA score of 2 closely resemble patients fully in remission.[3] Also, the Simplified Disease Activity Index definition of remission already is slightly less strict and includes such patients.[7] In addition, in many countries PGA is measured on a 0–100 scale, and it is unclear what the threshold should be on that scale: in theory patients scoring '1' on a scale from 0 to 10 could have a score of 14 or even 15 on a scale of 0–100 (depending on which rounding rule is favoured). Finally, our group is preparing a report that shows that patients themselves will frequently consider themselves in remission while scoring 2 on their PGA (Rasch *et al*, in preparation). All of these findings suggest the PGA threshold could be slightly relaxed, bringing more congruence to the two versions of the remission definition without changing the essence, a strict definition that identifies people who can live a normal life. In my view, it is important that this characteristic stays in place. Any amended definition would have to be very specific about the threshold on the 0–100 scale: that is, '20'. It is my understanding that ACR and EULAR will soon start an initiative in this direction.

A second and more principal point is the intended purpose of the criteria, and how they have been applied since. From the outset, the criteria were intended for use in research, especially clinical trials, to define a subgroup of patients that were doing optimally well. However, in the publication, this purpose was somewhat weakened by the suggestion (in the discussion) that the criteria could be of use in patient care. Initially, subsequent treatment guidelines suggested treat to target as the new standard, with remission the target. And it is exactly here that things have started to derail.

Most importantly: although ACR/EULAR remission rates are on the increase, both in recent trials and in cohorts, to date our treatments (including traditional disease-modifying antirheumatic drugs (DMARDs), glucocorticoids, biologics and synthetic targeted DMARDs) are simply not good enough to reach that target in the majority of patients. Second,

Box 1 American College of Rheumatology–European League Against Rheumatism remission criteria.[1]

Boolean-based definition

At any time point, patient must satisfy all of the following: Tender joint count ≤1*
Swollen joint count ≤1*
C reactive protein ≤1 mg/dL
Patient global assessment (PGA) ≤1 (on a 0–10 scale)†

Index-based definition

At any time point, patient must have a Simplified Disease Activity Index Score of ≤3.3‡

*For tender and swollen joint counts, use of a 28-joint count may miss actively involved joints, especially in the feet and ankles, and it is preferable to include feet and ankles also when evaluating remission.
†For the assessment of remission we suggest the following format and wording for the global assessment questions. Format: a horizontal 10 cm visual analogue or Likert Scale with the best anchor and lowest score on the left side and the worst anchor and highest score on the right side. Wording of question and anchors: For PGA, 'Considering all of the ways your arthritis has affected you, how do you feel your arthritis is today?' (anchors: very well–very poor). For physician/assessor global assessment, 'What is your assessment of the patient's current disease activity?' (anchors: none–extremely active).
‡Defined as the simple sum of the tender joint count (using 28 joints), swollen joint count (using 28 joints), PGA (0–10 scale), physician global assessment (0–10 scale) and C reactive protein level (mg/dL).

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in patients that are already in a state of minimal disease activity, there is little or no evidence to show that treatment intensification does more good than harm. Third, none of our current measurement tools are good enough to reliably detect or exclude residual disease activity that should be treated. Most current objections against the remission criteria are levelled at the validity of PGA (i.e., whether it measures disease activity or 'impact'), but the same goes for joint counts and acute phase reactants, as these also lack specificity and reliability in low disease activity states. Acute phase reactants are especially problematic in the assessment of treatments targeting interleukin 6 and pathway inhibitors.[8] More advanced tools such as sonography or MRI have not proven to be better in improving outcome in clinical trials, under optimum conditions,[9–11] so they cannot be recommended for this purpose in routine clinical practice.

Simply stated, we can now get most patients into a 'good' (minimal disease activity) state, but, once they are there:

- We do not know for sure whether that state is good enough.
- We do not have the treatments to move patients into a better state than 'good'.
- Even if we had such treatments, we do not have the tools to reliably measure success of treatment intensification in patients in a 'good' state.

The learned societies have taken note of the above considerations, and the most recent updates include 'low disease activity' as a target in addition to 'remission'.[12 13] As stated above, I hope in the next updates 'low disease activity' will be replaced by 'minimal disease activity'.

The validity of a state (such as remission) as defined by a committee of experts can be questioned: what does it really mean, and how important is it to be in that state? In the development, the committee decided to use follow-up data from clinical trials and assess two external anchors: good damage outcome (on X-rays of hands and feet), and good functional outcome (as measured by the Health Assessment Questionnaire). It then examined candidate definitions on their capability to predict these outcomes, and the current remission definition emerged as one of the top performers.[1] Although I am convinced that this was a good approach, it can and has been criticised. In any case, the current definition is an evidence-based agreement of experts.

Since then several studies have tried to replicate the results, with varying success. The main 'problem' of those studies and of most patients with RA currently under treatment is that treatment has improved so much. As a consequence, the rate and extent of damage progression has progressively decreased in the last 20 years; and because everyone is doing so well, it has become very difficult to detect contrasts between groups of patients. The same goes for functional decline. Thus, most patients will have good damage and functional outcome, at least for a couple of years, whether or not they are in 'full' remission, as long as they do not have really active disease over an extended period of time. In sum, our external anchors to detect the effects of being in different disease states (e.g., remission according to different definitions) have become less useful, mostly because the quality of treatment has gone up, and extended periods of high disease activity have become rare.

It is in this setting that Ferreira *et al* have embarked on a long journey to disentangle disease activity from impact, and to revise the remission criteria for use in patient care. Previously they have shown in the Etude et Suivi des Polyarthrites Indifférenciées Récentes (ESPOIR) cohort that the current definition has only numerical advantages over a definition without PGA to predict lack of damage progression.[14] They posit that disease activity should be measured with joint counts and acute phase reactants, and treated with DMARDs; impact should be measured with PGA and treated with other interventions. In this issue of the Annals, they present the results of an individual patient meta-analysis, where they compare the predictive performance (for good damage and functional outcome) of the original remission criteria with two alternatives that do not include PGA.[15] They conclude that PGA has little to add for damage outcome, but a lot for functional outcome. From their perspective, functional outcome is strongly related to impact independent of disease activity, so that this result is of little value to decide on a remission definition in practice.

I applaud Ferreira *et al* for so forcefully driving this important discussion, and all the beautifully conducted studies they have done to address these important issues. However, I mostly disagree with their conclusions, and with the idea that PGA should be taken out of considerations on disease activity. First of all, in the current study, the current remission definition performed better than their proposed alternatives, sometimes significantly, sometimes numerically. Second, in the ESPOIR study rapid damage progression was only seen in patients with a high PGA.[14] Third, the whole idea that an individual clinician and a patient would be completely bound by a remission definition to decide whether or not to escalate or switch treatment (in their words, causing 'overtreatment' of patients suffering only from impact, not disease activity) is overly simplistic. In our own clinic, treat-to-target trials ran into difficulties with physicians and patients not adhering to protocol-mandated intensification decisions when disease activity was low or minimal.[16] In clinical practice, several studies have shown that absence of 'objective' signs of inflammation, such as an isolated elevated PGA was the most frequent reason for deviations from advancing therapy in a treat to target setting.[17 18] In other words, rigid focus on remission as target is unlikely in routine clinical practice. In fact, lack of adherence to systematic measurement of disease status and treat to target schedules, that is, undertreatment, is probably more of a problem than the converse.[19] Finally, any disadvantages of the current definition can be repaired with less rigorous measures, as suggested above. I do agree that more efforts should be focused on understanding and addressing patient need in the area of impact: some impact may indeed be independent of disease activity, but I suspect there is still a part that reflects undetected residual disease activity, perhaps not detectable by other means.

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Use of multidimensional composite scores in rheumatology: parsimony versus subtlety

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ABSTRACT

Rheumatic and musculoskeletal diseases (RMDs) form a diverse group of diseases. Proper disease assessment is pivotal, for instance to make treatment choices and for optimising outcome in general. RMDs are multidimensional diseases, entrenching many, sometimes very different aspects. Composite outcome measures ('composites') have become very popular to assess RMDs, because of their claim to catch all relevant dimensions of the disease into one convenient measure. In this article we discuss dimensionality of RMDs in the context of the most popular conceptual framework of RMDs, being an inflammatory process leading to some sort of damage over time. We will argue that multidimensionality not only refers to heterogeneity in disease manifestations, but also to heterogeneity in possible outcomes. Unlike most unidimensional measures, multidimensional composites may include several disease manifestations as well as several outcome dimensions into one index. We will discuss fundamental problems of multidimensional composites in light of modern strategies such as treat-to-target and personalised medicine.

Finally, we will disentangle the use of multidimensional composites in clinical trials versus their use in clinical practice, and propose simple solutions in order to overcome problems of multidimensionality and to avoid harm to our patients due to overtreatment.

INTRODUCTION

Rheumatic and musculoskeletal diseases (RMDs) form a diverse group of diseases with different pathogeneses.[1] The prevalence of several RMDs is relatively high (>1%) and most RMDs are chronic diseases. Treatment options have recently expanded for some RMDs but are still sparse or absent for others. Treat-to-target strategies have become popular.[2] Proper disease assessment is pivotal for physicians to make choices about treatment start, intensification or tapering, and optimising outcome in general. The wish to choose the best and most (time) efficient instrument is understandable. Efficiency here implies capturing as much as possible by one 'simple' measure. This is why composite outcome measures have become so popular. Here we will investigate their rationale further, discuss the concept of dimensionality and warn against some misuses.

COMPOSITE INDICES

As a reflection of the wish to bring some order in a profusion of single outcome measures, composite indices have found their place in rheumatology. A 'composite' combines several measures into one quantifiable index, which is a rather generic principle,[3 4] that is visualised in figure 1. In theory, a composite index is better than the sum of its parts, but this assumption is hard to prove and sometimes not met.[5] If one single measure does not satisfactorily describe what is going on in most patients, if not in all, one could use multiple single measures that all reflect the same process to some extent. But multiple measures create multiple problems. If separate measures give diverse signals, which one then reflects the truth best? What potentially important aspect of a disease will be missed by making exclusive choices? What if among five single measures for improvement, three suggest improved disease activity and two do not? For a well-designed 'composite', developers must have thought critically about these problems. They must have achieved consensus on questions like what exactly to address, which variables to include and exclude (prioritisation), how do these variables correlate, how should variables be weighted, among others. It is not easy to design a 'good composite'. It is even more difficult, if not political, to obtain common support for a new index, so that it will be implemented.

Advocates of composites tend to believe that several instruments put together smartly give a better picture of the situation than only one instrument would do. Disease activity in rheumatoid arthritis (RA) can be measured by a plethora of different single measures. More pain (eg, on a visual analogue scale) may point to more active diseases, as does a higher swollen joint count, an increased C reactive protein (CRP) level and the patient's global impression of the disease. But not all patients with RA with active joints report similarly high levels of pain, while some with many swollen joints may have a normal CRP or no pain at all, and patients often rate their disease as being more active than their physicians do. The merging of different perspectives of the same domain into one index may sometimes add clarity and uniformity, and help clinical research and practice move forward as we have seen in the last three decades, but there are certainly also problems.

RMDS ARE MULTIDIMENSIONAL DISEASES

Patients with RMDs usually have musculoskeletal symptoms and sometimes extra-musculoskeletal manifestations. These latter can be organ specific or more diffuse, and may involve several internal organs. RMDs have many faces; they are multifaceted or multidimensional. Some RMDs, such as systemic lupus erythematosus (SLE) and psoriatic arthritis (PsA), are classical examples of multidimensionality. Phenotypically, they may express a multitude of manifestations, but infrequently in

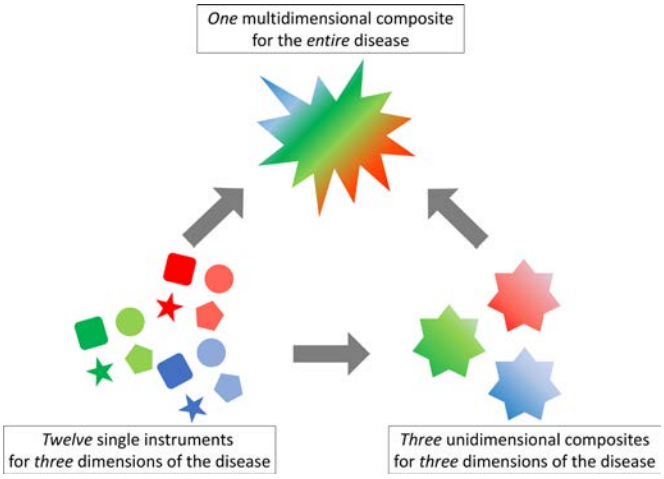

Figure 1 From single instruments to multidimensional composites; a reductionistic approach. The figure visualises how several single instruments, that are grouped to reflect different dimensions of the same disease (red–blue–green), may be aggregated into unidimensional composite indices or scores, by a data-driven process of prioritisation, exclusion and weighting (dark-grey arrows). Single instruments and unidimensional composite scores can further be aggregated into one multidimensional composite index or score by a similar process of prioritisation, exclusion and weighting. Different colours reflect different dimensions. Different tones reflect different perspectives (*bright*: patient's perspective, *dull*: physician's perspective). Increasing irregularity of the symbols reflects increasing versatility of measurements, increasing shades of colour reflects increased dimensionality.

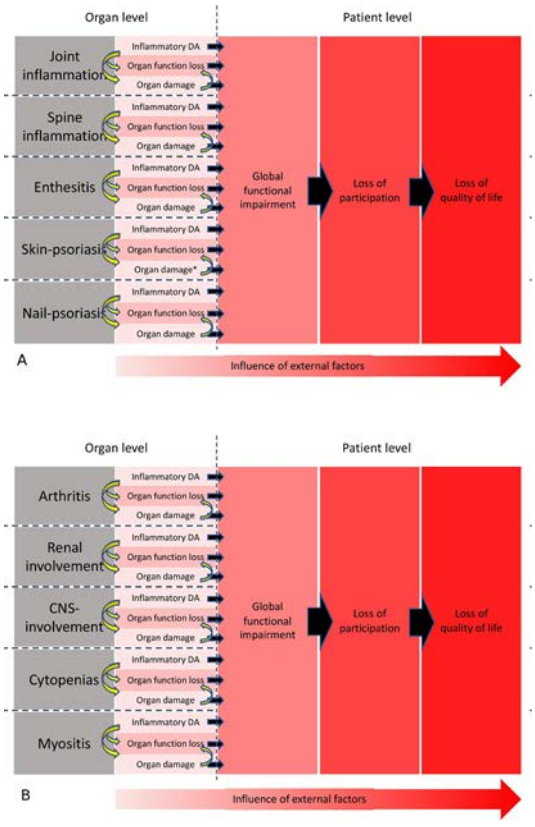

Figure 2 Multidimensionality of rheumatic musculoskeletal diseases (RMDs). The complexity of two multidimensional RMDs, psoriatic arthritis (PsA) (A) and systemic lupus erythematosus (SLE) (B). Note that, while disease manifestations of PsA and SLE are very different, both diseases share a large degree of multidimensionality. Phenotypical manifestations of the diseases are placed along the y-axis (in grey), and consequences of the disease are depicted along the x-axis (in red). The figure suggests two types of hierarchy, one with vertical orientation and one with horizontal orientation. The vertical hierarchy refers to organ (systems) and reflects the associations that exist between organ inflammation, organ damage and organ function loss (yellow arrows). The horizontal hierarchy reflects a natural gradient from 'organ' (left) to 'organism' (right). The larger coloured arrow outside the figure represents the multitude of external factors that may have an impact on the outcomes along the x-axis. The gradient of increasing 'redness' reflects increasing distance to the primary process underlying the RMD, and increasing sensibility to external, not necessarily disease-related factors. Examples are comorbidities, sensitivity to central sensitisation, personality traits, usage of coping mechanisms and illness perceptions, among others. The summing up of disease manifestations and outcomes in the figure is not intended to be complete, and any suggestion pointing to a hierarchy in severity of manifestations is unintentional. *Inflammatory psoriasis lesions will usually heal without damage (scars), but may lead to itching, scratching and scarring (indirect damage). CNS, central nervous system; DA, disease activity.

the same patient at the same time. Two patients diagnosed with the same disease may present very differently; there is marked *between-patient variability*, which has implications for properly assessing these patients. Good disease measures can discern this level of heterogeneity in all its possible extremes.

Most RMDs are chronic and rarely stable. They fluctuate in symptom intensity, naturally or under the influence of treatment; there is marked *within-patient variability*. Rheumatologists need to pick up these fluctuations in order to adjust treatment. Good disease measures can pick up these fluctuations reliably.

Multidimensionality does not only exist at the level of disease presentation, but expectedly also at the level of disease outcomes. Disease activity is an immediate outcome of many inflammatory RMDs. On top of that, patients with RMDs face a gradual accumulation of chronic and irreversible *consequences* of their disease (activity) over time. Examples are, among others, progressive joint destruction, increasing functional impairment or atherosclerosis. These consequences can be seen as dimensions too, but in a perpendicular orientation. Figure 2 provides a schematic representation of multidimensionality of RMDs in the opinion of the authors: phenotypical dimensions along the y-axis and dimensions of outcome along the x-axis.

Who wants to describe and understand the breadth of outcomes of an RMD must capture both the disease *process* and the consequences of that process, but we rather tend to simplify things. Categorising outcomes into analysable dichotomies, such as *responses* or *events*, which is often done in randomised trials, is an impoverishment, since most of the natural variability gets lost. The outcome of an RMD is usually not an event, such as a myocardial infarction or death, but rather a quantification of an ongoing disease process characterised by fluctuations that say a lot about the disease and the patient. Dichotomising outcomes into digestible binomial parcels provides statistical convenience and comprehension, but does not give sufficient credit to the complexity of RMDs. Still, we often do this, for reasons of simplicity, and obviously for buying time in a busy clinic.

FRAMEWORK: PROCESS AND DAMAGE

The conceptual framework underlying many of our RMDs is that immunological disturbances cause inflammation. The process of inflammation gives measurable clinical signs (eg, joint swelling) and symptoms (eg, pain, stiffness) instantaneously, and

irreversible structural organ destruction (damage) after a while. Many of our RMDs are not necessarily inflammatory RMDs. However, the conceptual framework, with inflammation as the *process* and damage as the *consequence*, has become so axiomatic that we have extended this label to all RMDs, even when inflammation as a cause is less clear. The degenerative disease osteoarthritis and the pain syndrome with the anachronistic name fibrositis owe their suffix *-itis* to this type of generalisation rather than to clear evidence that inflammation is key.

The inflammation-damage framework has been instrumental in the development of rheumatology as it stands today. First, the framework provided the insight that in order to avoid irreversible damage inflammation should be suppressed, an insight that has made way for successful drug development. Second, the framework stood model for the hypothesis of 'window-of-opportunity'; it appreciated the importance of 'time elapsed' which led to the paradigm of 'starting an intervention sooner rather than later'. *Time-is-joint*. Third, the framework has shaped the field of outcome assessment of RMDs. Both process and damage (note: damage in its broadest sense) can now be measured appropriately by a wealth of instruments. As in every cause–effect relationship, a proper interpretation of the temporal association between process and damage is essential. Simply stated, disease activity comes first and damage follows after some time. The interpretation of disease activity and damage at the same time, while ignoring the time elapsed as in a cause–effect relationship, conveys different signals. Disease activity happens *now*, damage is a remnant of a process in the *past*. As we will see later, some composites neglect the importance of time elapsed, and mix things up.

MULTIDIMENSIONALITY AND OUTCOME ASSESSMENT

The word dimension can be used to describe one *aspect* out of a spectrum. Myositis can be considered one dimension of the disease SLE, and cytopenia (haematological manifestation) another one. Skin psoriasis is one dimension of PsA, nail psoriasis another one and arthritis a musculoskeletal one. The word *dimension* can also be used to describe one *outcome* out of a spectrum of possible outcomes. Joint damage can be considered one dimension out of the spectrum of possible outcomes of PsA, and reduced quality of life another one. Not all patients with PsA and joint damage, however, will perceive and report reduced quality of life over time, or will lose their job due to the disease. External factors will largely determine to what extent proximal outcome variables measured at the organ level (eg, joint inflammation, joint damage) will ultimately impact quality of life and well-being (figure 2). The distinction between several dimensions is arbitrary and based on expert convention.

Rheumatologists have an irresistible desire to behold a multidimensional RMD as a whole, and to treat the patient with this RMD in its entirety; rheumatologists are 'lumpers' by soul and advertise the holistic view. No wonder that they have developed multidimensional composite measures that account for the whole patient, covering all aspects of the disease and its outcomes into one measure (see figure 1). Because of their presumed user-friendliness (*one size fits all)*, multidimensional composites enjoy significant popularity for use in trials and increasing attractiveness in clinical practice. Clinically relevant trade-offs allow categorisation into *disease states* and *response states*, which get an intuitive meaning among clinicians over time. One example of such a multidimensional composite is the Psoriatic Arthritis Disease Activity Score (PASDAS).[6] Minimal Disease Activity (MDA), a threshold, is conceptually a composite measure developed to be used as a treatment target in the same disease.[7] One of the many examples of multidimensional composites for SLE is the Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI), that captures many dimensions of the disease in one index.[8] Dichotomous derivatives of SELENA-SLEDAI include definitions for mild, moderate and severe flares. Those several multidimensional indices (such as Systemic Lupus Activity Measure, Lupus Activity Index, British Isles Lupus Assessment Group Index and the European Consensus Lupus Activity Measure, among others, (reviewed by Mikdashi and Nived)) that have seen the light over the years exemplify that multidimensionality of a disease does not necessarily add to consensus on how to measure the disease best.[9]

UNIDIMENSIONAL OUTCOME MEASURES

Unidimensional composites differ from multidimensional ones in that they cover only one aspect ('dimension') of the disease (figure 1). Many are in use for measuring the state or change of disease activity. Sometimes, it is not immediately clear whether a composite measure is unidimensional or multidimensional. A closer look at its history may give some resolution. A hallmark dimension of RMDs is joint inflammation (arthritis). The Disease Activity Score (DAS) was developed in 1990 for assessing disease activity in RA and clearly focused on arthritis.[10] It was obvious that one single measure (eg, a swollen joint count or an acute-phase reactant) would not suffice to appropriately describe disease activity in every patient with RA. The DAS has been set up as a composite index combining several measures covering the same dimension. DAS in its origin was a unidimensional index with a focus on (the immediate sequels of) arthritis. This does not imply, however, that once unidimensional means always unidimensional, as the following example may clarify. In the last decades, the *Gestalt* of RA has changed, due to earlier recognition, more effective treatment and better management.[11] As a consequence, among others, average inflammatory burden is assumed to be lower now than it was in the past. However, recent studies have suggested that the gradual decrease in swollen joint count and acute-phase reactants over time did not go hand in hand with less patient-reported pain, less joint tenderness and more well-being.[12 13] Part of this discrepancy is currently attributed to the existence of neuropathic pain mechanisms or central sensitisation.[14] Pain due to central sensitisation falls outside the conceptual inflammation-damage framework, although one may provocatively argue that central pain sensitisation is a long-term consequence of inflammation, and thus damage. Anyway, neuropathic pain constitutes a different dimension of RA than pain that accompanies inflammation. Indeed, this type of pain is rather insensitive to anti-inflammatory drug treatment, and does not correlate with CRP and swollen joint count. That means: DAS, once a unidimensional composite for disease activity in patients with active RA who had to start treatment,[10] may have gained dimensions over time, when used to monitor patients with RA in remission or in low disease activity. Exactly the same reasoning pertains to the DAS-lookalikes Simple Disease Activity Index[15] and Clinical Disease Activity.[16] That this may have implications for daily clinical care has been demonstrated by us and others recently in studies comparing DAS28 and the fully patient-reported index RAPID3 in all day practice.[12 13 17] Apparently, the context in which a measure has been developed versus the context in which it is used is relevant for a proper understanding of the measure's performance. Obviously, similar issues may happen with other measures in other diseases.

Table 1 Summary of advantages and disadvantages of single measures, unidimensional composites and multidimensional composites

	Advantages ('pros')	Disadvantages ('cons')
Single measures	Cost little time per measure, are easy to use and clearly interpretable (eg, 'VAS pain: 3/10')	Provide limited information per measure
	Many can be measured independently in the same patient	Prioritisation may lead to interpretational problems and *cherry picking*
	Recognise individual patients with extreme values and not-so-average problems	
Unidimensional composites	Provide an unambiguous picture of one dimension (eg, 'SDAI showing LDA')	'Dimension creep' may happen over time (eg, neuropathic pain impacts SDAI scores)
		May mix up process and damage variables (eg, HAQ score in the ACR response)
	Provide more statistical power by eliminating variability (smoothening)	Dampen the influence of extreme values that are not recognised as such anymore
	Provide feasible and consensual benchmarks for treat-to-target strategies	Benchmarks may have a different meaning in different stages of the disease
Multidimensional composites	Presume a holistic and unambiguous picture of the patient (eg, 'the patient is doing well')	Aggregate measures and composites based on statistical considerations ('lumping')
		May suffer from the index within the index fallacy which jeopardises feasibility (eg, HAQ score and PASI score in MDA)
		May mix up process and damage variables (eg, HAQ score in the MDA)
	Provide more statistical power by less variability (smoothening)	Assume different dimensions change always in the same direction (eg, skin and joints in PsA)
		Lose statistical (discriminatory) power if components of the composite or index are not correlated (eg, SLEDAI)
	Provide benchmarks for treat-to-target strategies (eg, 'the patient is not yet in MDA')	Benchmarks may have a different meaning in different patients, do not give resolution about which dimension to treat, and their use may lead to overtreatment
		Benchmarks may have a different meaning in different stages of the disease

Advantages and disadvantages are paired as much as possible.
ACR, American College of Rheumatology; HAQ, Health Assessment Questionnaire; LDA, low disease activity; MDA, minimal disease activity; PASI, Psoriasis Activity and Severity Index; PsA, psoriatic arthritis; SDAI, Simple Disease Activity Index; SLEDAI, Systemic Lupus Erythematosus Disease Activity Index; VAS, visual analogue scale.

DIMENSIONS, CORE DOMAINS AND INSTRUMENTS

Although there is certainly overlap, it is important to distinguish multidimensionality of RMDs from core domains, such as the ones operationalised by the Outcome Measures in Rheumatology Clinical Trials (OMERACT) organisation.[18] OMERACT has always aimed at clinical trials and has approached outcome assessment in rheumatology from the perspective of best (ie, feasible, discriminative and truthful) measurement. OMERACT makes a distinction between 'what to measure' (the core domains) and 'how to measure' (the best instruments). 'What to measure' refers to a conceptual framework that is accepted among all stakeholders as the 'truth'. Core domains can be very diverse and are supposed to represent so called core areas, such as death, life impact, resource use and economic impact, pathophysiological manifestations and adverse events. Certain aspects of the RMD (dimensions) may not pop up in OMERACT core-domain sets, for example because they cannot be measured well, or have a too low prevalence.

In summary, multidimensionality is a feature of RMDs. It requires a conceptual framework to explain the disease phenotypically, its pathogenetic causes and its longitudinal consequences. Whether these dimensions should be assessed or not in trials is the focus of OMERACT. OMERACT core sets increase the comparability across studies, which is pivotal, but do not aim at providing completeness.

Thus far, OMERACT has not taken an explicit stand with regard to the use of composite indices, but has allowed some composites as preferable instruments for assessing some of their core domains. Many of these composites, however, had been developed long before they were 'pulled through the OMERACT filter' and they have often been accepted under stakeholder pressure, since 'they are important to patients' or 'they work satisfactorily in the context of clinical trials'. Important limitations were either not realised or ignored. We will discuss a few.

IGNORING THE NATURAL ORDER OF CAUSE AND CONSEQUENCE

Composite indices should respect the natural order of cause and consequence, as argued above. Some indices used in rheumatology violate this principle. The American College of Rheumatology (ACR) response measure ACR20, endorsed by many regulatory bodies and OMERACT, was designed as a response measure for RA disease activity, but includes a measure of functional ability (the Health Assessment Questionnaire (HAQ)).[19] HAQ measures functional impairment as a *consequence* of RA disease activity, not disease activity itself. The PASDAS, a measure for disease activity, also includes the HAQ. Who looks at the content of the HAQ realises that all kinds of conditions, not only RA-related or PsA-related disease activity, may influence HAQ score. It is true that HAQ score correlates reasonably well with direct measures of disease activity in patients who have active disease, but we do not know how this works out in patients who are inactive or have only mild disease activity, nor in those that actually have pain without clinical signs of inflammation. Studies have shown that HAQ incorporates an irreversible component that proportionally increases over time,[20] which implies that an HAQ score in a patient with early active RA does not have the same meaning as the same HAQ score in a patient with quiescent but advanced disease. As such, the HAQ as a part of a response index should be considered methodologically inappropriate.

THE 'INDEX WITHIN AN INDEX' FALLACY

The aforementioned ACR20 response measure, an index, includes the HAQ, an index itself. The aforementioned MDA includes the HAQ and the Psoriasis Area Severity Index for skin involvement.[7] Indices tend to dampen the influence of extreme values and reduce variability. This helps increasing the signal-to-noise ratio, which is a statistical advantage, but goes at the cost of subtlety necessary to properly assess individual patients with non-classical presentations. This smoothening process is reinforced by dichotomising clinical

outcomes, as in ACR20 response (present or absent) and MDA state (present or absent), among others. While indices, with components that are indices themselves entrenched, may still work for groups of patients in randomised trials, they are essentially useless in describing and monitoring individual patients, unless these patients belong to the *typically averaged*. 'Useless' becomes 'potentially dangerous' if dichotomised multidimensional composites, such as MDA, are used as targets for intensifying treatment. Too many other factors than inflammatory disease activity alone may have impact on whether or not a patient meets a preset threshold. Overtreatment is the logical consequence of threshold medicine, when inappropriate measures to ascertain the threshold are used.

The dangers of threshold medicine also pertain to unidimensional indices, but to a lesser extent, and, besides, these unidimensional composites less often suffer from 'the index within an index' fallacy.

A PATIENT IS NOT THE SUM OF HIS DIMENSIONS

A patient with SLE who has active myositis does not necessarily have other manifestations of SLE. Change in the activity of myositis is not necessarily related to change in—for example—leucocyte count, skin rash or arthritis. Along similar lines, the relationship between psoriasis skin activity and musculoskeletal symptoms of PsA is modest at best.[21] That some drugs used for PsA may improve both skin and joints, and have indications for both, does not mean that skin and joint can be expected to always change in similar directions. Multidimensionality does not imply that separate dimensions, present at the same time, change at similar speed or in similar direction. Still, a multidimensional index pretends to allow a unidimensional (ie, linear) interpretation. Patients with scores above the threshold are 'not good'; only those with scores below the threshold are 'good'. Two patients with PsA, however, may have similar levels of PASDAS but very different manifestations and burden of disease (impact). Their response to treatment may also markedly differ. In groups of patients in randomised trials, this may work out to some extent, as long as experimental therapies have unidirectional positive effects on several dimensions. But in diseases like SLE, systemic sclerosis or primary Sjögren's, multidimensionality of outcome and response measures may obscure clinically relevant heterogeneity among patients. One of the potential explanations for failed trials with drugs that experienced physicians perceive as efficacious in patients with SLE, indeed pertains to this kind of heterogeneity that is inherent to the composite outcome measures. Multidimensionality can jeopardise sensitivity to change and discrimination.

IMPLICATIONS

Advocates of multidimensional composites will argue that these validated indices have sufficiently proven their value, but what does validation mean? Indeed, many of these composites have worked reasonably well in randomised trials, in that they can distinguish between groups on active treatment versus those on placebo. However, that is low-hanging fruit. As argued above, these indices tend to eliminate the outlier effects by statistical smoothening, resulting in better signal-to-noise ratios, more statistical power and better p values. Problems may arise, however, if results of randomised controlled trials are to be generalised to common clinical practice. It is uncertain whether the statistically significant result obtained with a multidimensional composite in a trial keeps up in patients with the same disease but a somewhat different, not-so-average clinical presentation or course. Problems may also arise if the trial has exploited a benchmark, for example, in the context of treat-to-target, and the strategy that includes the measure and the benchmark is implemented one-to-one in clinical practice. Patients that would benefit from intensification of treatment, since they have measurable residual inflammatory activity in one dimension, may not be picked up as such. More likely, though, benchmarks may falsely dictate further intensification of treatment in patients who have responded well to treatment in several dimensions, but fail to do so in a variable that reflects the consequence of a process (eg, function impairment) rather than the process itself (eg, inflammation). Further intensification of anti-inflammatory treatment may not necessarily improve these patients' lives meaningfully. Consequent overtreatment will make medicine unnecessarily costly and risky.[22] Benchmark medicine with suboptimal multidimensional instruments is pointless.

HOW CAN WE DO BETTER?

Composite indices have their value in clinical trials. A better signal-to-noise ratio adds to statistical power and limits the numbers of patients needed in the trial. However, composite indices should give credit to the complexity of the disease, not by trying to lump all dimensions into one index, but rather to respect the *time elapsed* between cause and consequence and avoid mixing both up. The versatility of an RMD is better valued by reporting different dimensions differently, as for example propagated by Schoels *et al* for PsA.[23] Multiple unidimensional indices likely are better tools for purpose than one single multidimensional index, but when the breadth of outcomes is relevant, it may be even better to describe the separate components of the composite as secondary outcomes, in conjunction with the unidimensional index itself.

When application of composites in clinical practice is in question, multidimensional composites lose their value, since interpretational mistakes are too easily made, and patients may fall victim to benchmark medicine. Unidimensional indices likely perform better, but also bear the risk of mixing up different perspectives into one index. The classic example is the patient with RA with high DAS but low inflammation. When decisions about treatment start, intensification or tapering are to be made, physicians should realise the rationale of (anti-inflammatory) treatment: to reduce the process of inflammation in order to avoid long-term consequences of the disease. This means, the measure to base such decisions on should linearly reflect the presence of (objective) inflammation. Swollen joint counts and acute-phase reactants, or a physician with real experience in detecting inflammation clinically, may do a better job here than composites. A summary of advantages and disadvantages of composite measures, unidimensional and multidimensional, versus single measures is provided in table 1.

Parsimony in outcome assessment can unintentionally lead to loss of subtlety and harm rather than benefit patients in clinical practice.

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EPIDEMIOLOGICAL SCIENCE

Burden and trajectory of multimorbidity in rheumatoid arthritis: a matched cohort study from 2006 to 2015

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ABSTRACT

Objectives To compare the onset and trajectory of multimorbidity between individuals with and without rheumatoid arthritis (RA).

Methods A matched, retrospective cohort study was completed in a large, US commercial insurance database (MarketScan) from 2006 to 2015. Using validated algorithms, patients with RA (overall and incident) were age-matched and sex-matched to patients without RA. Diagnostic codes for 44 preidentified chronic conditions were selected to determine the presence (≥2 conditions) and burden (count) of multimorbidity. Cross-sectional comparisons were completed using the overall RA cohort and conditional logistic and negative binomial regression models. Trajectories of multimorbidity were assessed within the incident RA subcohort using generalised estimating equations.

Results The overall cohort (n=277 782) and incident subcohort (n=61 124) were female predominant (76.5%, 74.1%) with a mean age of 55.6 years and 54.5 years, respectively. The cross-sectional prevalence (OR 2.29, 95% CI 2.25 to 2.34) and burden (ratio of conditions 1.68, 95% CI 1.66 to 1.70) of multimorbidity were significantly higher in RA than non-RA in the overall cohort. Within the incident RA cohort, patients with RA had more chronic conditions than non-RA (β 1.13, 95% CI 1.10 to 1.17), and the rate of accruing chronic conditions was significantly higher in RA compared with non-RA (RA × follow-up year, β 0.21, 95% CI 0.20 to 0.21, p<0.001). Results were similar when including the pre-RA period and in several sensitivity analyses.

Conclusions Multimorbidity is highly prevalent in RA and progresses more rapidly in patients with RA than in patients without RA during and immediately following RA onset. Therefore, multimorbidity should be aggressively identified and targeted early in the RA disease course.

Key messages

What is already known about this subject?

- Multimorbidity is a growing public health problem with an ageing population and rising rates of chronic conditions.
- While select comorbid conditions are known to complicate rheumatoid arthritis (RA), the timing of onset and rate of accruing multimorbidity in RA is unknown.

What does this study add?

- Using a large, commercial insurance database in the current treatment era, we have shown that multimorbidity is significantly more common in patients with RA.
- Multimorbidity occurs early in the RA disease course and progresses more rapidly than in patients without RA.

How might this impact on clinical practice or future developments?

- Multimorbidity should be targeted early in the RA disease course to prevent progression and achieve better long-term patient outcomes.

INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease that predisposes to physical impairment and premature mortality.[1–3] While extra-articular involvement is well recognised to complicate the RA disease course,[4] links between RA and other chronic diseases, including osteoporosis,[5] cardiovascular disease,[6] malignancy[7] and mental health disorders,[8] have also been identified. Additionally, therapies used to treat RA may have unintended consequences that predispose to the development of chronic diseases. For example, glucocorticoid use is associated with numerous adverse effects including bone loss, elevated blood glucose and blood pressure, and the development of cataracts and glaucoma, among other potential toxicities.[9]

The study of chronic conditions occurring in individuals with RA has primarily focused on select conditions co-occurring with RA, under the framework and terminology of 'comorbidity'. Multimorbidity, which considers the burden of conditions to the patient rather than to an index condition, has become the preferred terminology to describe the co-occurrence of multiple chronic conditions in the general population and rheumatic diseases.[10] Multimorbidity has been the subject of only limited investigation in RA to date. Initial studies in RA have shown lower rates of biological disease-modifying antirheumatic drug (DMARD) use and poorer response to treatment among those with multimorbidity,[11–13] as well as multimorbidity contributing to excess mortality occurring in RA.[14]

The world's population is ageing, with the WHO estimating the number of individuals aged 65 years or older to increase from 524 million in 2010 to 1.5 billion in 2050.[15] As a result of this

increased longevity and a rising frequency of chronic disease risk factors, chronic disease prevalence is projected to rise steadily with over 170 million individuals estimated to have at least one chronic condition by 2030 in the USA alone.[16] Accompanying the growing prevalence of chronic diseases is the development of multiple chronic conditions, constituting multimorbidity. In 2014, over 40% of US adults had multiple chronic conditions.[17] The consequences of multimorbidity include death and disability, reduced quality of life, and increased healthcare utilisation and costs.[18] Thus, multimorbidity is a critically important public health concern that needs to be aggressively targeted. This is especially true in RA, a disease perhaps of accelerated ageing[19 20] that portends poor long-term outcomes[1–3] and carries an enormous economic impact.[21]

Targeting multimorbidity with interventions requires understanding its onset and rate of progression.The purpose of this study was to compare the burden and trajectory of multimorbidity between individuals with and without RA. We hypothesised that the burden of multimorbidity and rate of accruing chronic conditions would be greater in RA.

METHODS

Study design and patient selection

We performed a matched, retrospective cohort study within the Truven MarketScan commercial claims and encounters database from 1 January 2006 to 30 September 2015. MarketScan is a US-wide database of commercially insured individuals with medical and pharmacy claims data that have been used extensively for rheumatic disease research.[22–25] Patients and the public were not involved in this study.

We constructed two RA cohorts (overall RA and incident RA) that were matched (1:1) to patients without RA from 1 January 2006 to 31 December 2014. We required patients to have 12 months of continuous enrolment during our study window to be eligible for analyses. We used validated RA algorithms that required at least two RA diagnostic codes (International Classification of Diseases ninth edition, clinical modification (ICD-9-CM): 714.0, 714.1, 714.2 and 714.8) between 30 and 365 days apart, including at least one diagnostic code from a rheumatology provider, and a DMARD prescription. Similar algorithms have a positive predictive value (PPV) for RA >90%.[26] Within this overall RA cohort, we identified a subcohort of incident patients with RA using an administrative algorithm requiring ≥12 months of continuous enrolment without RA diagnostic codes or DMARD prescription (PPV of 70%–80%).[27] The date patients fulfilled the algorithm was considered the RA index date. We then selected patients without diagnostic codes for RA and matched them 1:1 with patients with RA on sex, year of birth and year entering the database during our study window. We required controls to be enrolled on the index date of the accompanying patient with RA and assigned the same index date. Patients were followed until disenrolment, death or end of the study observation period (30 September 2015 due to transition to ICD-10).

Chronic conditions and multimorbidity

In addition to using established comorbidity indices (see below), we manually assembled a list of 44 chronic conditions based on their prevalence and importance in the general population and RA, informed by prior studies including systematic reviews of multimorbidity.[4 28–30] Diagnostic codes for these conditions were adapted from enhanced definitions for established comorbidity indices and the Healthcare Cost and Utilisation Project Clinical Classification Software codes (https://www.hcup-us.ahrq.gov/toolssoftware/ccs/ccs.jsp) (provided in online supplemental table S1). We queried these conditions from 1 January 2006 to 30 September 2015, a period using only ICD-9-CM codes, within inpatient and outpatient encounters. To minimise misclassification of these conditions (eg, unconfirmed or rule-out diagnoses), we required at least two diagnoses for these chronic conditions to be considered present, with the date of the second diagnostic code considered the date of onset. Once a condition occurred, we considered the condition prevalent throughout the remainder of follow-up.

We defined multimorbidity as the presence of at least two conditions from the aforementioned list. We did not include RA as one of the conditions, as this would inherently bias our results towards greater multimorbidity in RA. We also used a more stringent definition of multimorbidity, requiring the presence of at least three conditions from the list. The total count of chronic conditions present (possible range of 0–43, as two conditions were sex-specific) was considered to represent the burden of multimorbidity. To ensure results were not dependent on these definitions of multimorbidity, we also used established comorbidity indices. This included the Charlson-Deyo Comorbidity Index,[31] which has been extensively used in health services research, and the Rheumatic Disease Comorbidity Index (RDCI),[32] which has specifically been validated in individuals with rheumatic diseases.

Statistical analyses

We compared the cross-sectional prevalence of multimorbidity and individual chronic conditions between RA and non-RA in the overall cohort using conditional logistic regression models, conditioning on the matched pair. Comparisons of multimorbidity burden were completed using conditional negative binomial regression. In primary analyses, these comparisons were completed at the index date, while in secondary analyses we performed these comparisons at 1 year of follow-up to ensure all patients with RA had prevalent, rather than incident, disease.

The trajectory of multimorbidity burden in RA vs non-RA in the incident subcohort was assessed using generalised estimating equations with an interaction term between RA status and year of follow-up (to assess differences in the rate of accruing chronic conditions over time) and an autoregressive covariance matrix. The burden of multimorbidity (count of chronic conditions) was specified using a Gaussian distribution for clinical relevance. Skewness and residuals were similar to models generated using a negative binomial distribution (online supplemental figure 1), and observed means suggested a linear relationship between multimorbidity burden and RA status on the raw scale (online supplemental figure 2). In our primary approach, we censored individuals, but not the pair, who disenrolled from the insurance plan to maximise follow-up time. To account for differences that developed between patients with RA and patients without RA over follow-up periods, models included adjustments for age (updated at each year of follow-up) and sex. To investigate multimorbidity trajectory specifically during the period of RA onset, we performed secondary analyses restricting the sample to individuals with ≥3 years of observation before the index date (date classified as RA) and started follow-up at 2 years prior to the index date.

To ensure robustness of our results, we performed several sensitivity analyses. These were: (1) Removing chronic conditions that are known to be closely associated with RA or may be misclassified as RA (anaemia, osteoarthritis, fibromyalgia,

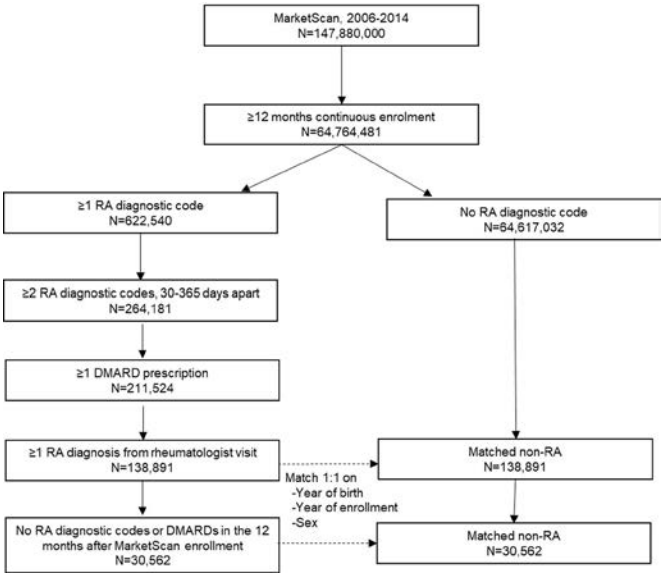

Figure 1 Study flow diagram. Overview of study cohort derivation. Patients with RA were identified within MarketScan commercial claims and encounters database between 2006 and 2014. A subcohort of incident RA was identified within the overall RA cohort. Patients with RA were matched 1:1 with patients without RA on sex, year of birth and year of enrolment. RA, rheumatoid arthritis; DMARD, disease-modifying antirheumatic drug.

interstitial lung disease, chronic back pain, gout, osteoporosis, inflammatory skin disorders), (2) Restricting our sample to patients with ≥1 year of follow-up, (3) Censoring the pair when one patient in the pair disenrolled, (4) Using a stricter 2-year period without RA diagnostic codes or DMARD prescription for incident RA,[27] (5) Requiring only ≥1 ICD-9 code to be present for a condition, (6) Adjusting for multimorbidity burden at the index date, and (7) Removing 'silent chronic conditions' that could be subject to surveillance bias. We assessed adjustment for geographical region but this did not confound results and was not included in the final models (data not shown). Analyses were completed using SAS V.9.4. Data are available on reasonable request and ethical approval.

RESULTS

Of the >147 million individuals enrolled in MarketScan between 2006 and 2014, we identified 138 891 who fulfilled our eligibility criteria and the RA algorithm, including 30 562 with incident RA (figure 1). After matching (1:1), there were 277 782 patients in the overall cohort and 61 124 patients in the incident subcohort. Baseline characteristics of these patients are shown in table 1. The study sample was female predominant (76.5% overall and 74.1% incident subcohort) with a mean age of 55.6 years and 54.5 years (overall and incident). Time elapsed from entering the database to the index date was 1.5 years (SD 1.7) in the overall cohort and 3.5 years (SD 1.8) in the incident subcohort. Biologic DMARD use was significantly less frequent in the incident cohort (10.1%) compared with the overall cohort (24.6%).

Multimorbidity prevalence and burden

At baseline, 57.4% of RA and 40.8% of non-RA had at least one chronic condition with 33.9% and 21.1% being multimorbid,

Table 1 Characteristics of patients with RA and patients without RA

	RA (overall)	Non-RA	RA (incident)	Non-RA
N	138 891	138 891	30 562	30 562
Age, years	55.6 (13.3)	55.6 (13.3)	54.5 (13.5)	54.5 (13.5)
Female sex, %	106 254 (76.5)	106 254 (76.5)	22 649 (74.1)	22 649 (74.1)
Year of entry,* %				
2006	43 543 (31.4)	43 543 (31.4)	12 636 (41.4)	12 636 (41.4)
2007	11 858 (8.5)	11 858 (8.5)	2916 (9.5)	2916 (9.5)
2008	25 187 (18.1)	25 187 (18.1)	5903 (19.3)	5903 (19.3)
2009	17 284 (12.4)	17 284 (12.4)	3596 (11.8)	3596 (11.8)
2010	16 569 (11.9)	16 569 (11.9)	2994 (9.8)	2994 (9.8)
2011	11 624 (8.4)	11 624 (8.4)	1653 (5.4)	1653 (5.4)
2012	7589 (5.5)	7589 (5.5)	616 (2.0)	616 (2.0)
2013	5237 (3.8)	5237 (3.8)	248 (0.8)	248 (0.8)
Time from entry to index date, years	1.5 (1.7)	1.5 (1.7)	3.5 (1.8)	3.5 (1.8)
US region, %				
Northeast	23 393 (16.8)	23 487 (16.9)	4806 (15.7)	4517 (14.8)
North central	29 559 (21.3)	31 941 (23.0)	6007 (19.7)	6435 (21.1)
South	62 618 (45.1)	53 884 (38.8)	14 732 (48.2)	12 854 (42.1)
West	21 280 (15.3)	25 669 (18.5)	4707 (15.4)	6488 (21.2)
Unknown	2041 (1.5)	3910 (2.8)	310 (1.0)	268 (0.9)
RA medications,† %				
Methotrexate	86 895 (62.6)	530 (0.4)	20 230 (66.2)	172 (0.6)
Hydroxychloroquine	42 288 (30.5)	629 (0.5)	11 252 (36.8)	234 (0.8)
Sulfasalazine	11 545 (8.3)	176 (0.1)	2879 (9.4)	69 (0.2)
Leflunomide	13 611 (9.8)	60 (0.04)	1892 (6.2)	19 (0.06)
b/tsDMARDs	34 177 (24.6)	265 (0.2)	3070 (10.1)	84 (0.3)

Values mean (SD) or n (%) of variables at the index date.
*Year entering the database during study window.
†RA medications received prior to, or on, the index date.
b/tsDMARDs, biologic or targeted synthetic disease-modifying anti-rheumatic drugs; RA, rheumatoid arthritis.

Table 2 Comparison of multimorbidity prevalence between RA and patients without RA

	Multimorbidity ≥2 conditions		Multimorbidity ≥3 conditions	
	N (%)	OR (95% CI)	N (%)	OR (95% CI)
Baseline (all patients, n=277 782)				
RA	47 083 (33.9)	2.29 (2.25 to 2.34)	29 229 (21.0)	2.42 (2.36 to 2.48)
Non-RA	29 311 (21.1)	1	16 083 (11.6)	1
With ≥1 year of follow-up for matched pair (n=226 850)				
RA	58 774 (51.8)	2.47 (2.42 to 2.51)	39 160 (34.5)	2.55 (2.50 to 2.61)
Non-RA	37 372 (33.0)	1	21 552 (19.0)	1

RA and non-RA matched on sex, year of birth and year of entry into the database.
All p<0.001.
RA, rheumatoid arthritis.;

respectively. The odds of multimorbidity were 2.3-fold higher in RA than non-RA at baseline (conditional OR 2.29, 95% CI 2.25 to 2.34) (table 2). Similar odds of multimorbidity for RA versus non-RA were observed when at least three conditions was used to define multimorbidity or when requiring ≥1 year of follow-up (table 2). The prevalence of multimorbidity in patients with RA was 51.8% when ≥1 year of follow-up was mandated. Of the 44 chronic conditions, 39 were over-represented in RA (online supplemental table 2). The most over-represented chronic conditions in RA were interstitial lung disease (OR 12.62, 95% CI 10.54 to 15.11), fibromyalgia (OR 5.86, 95% CI 5.50 to 6.25), osteoarthritis (OR 5.16, 95% CI 4.98 to 5.35) and osteoporosis (OR 4.54, 95% CI 4.19 to 4.92).

Multimorbidity burden (count of chronic conditions) was significantly higher in RA than non-RA (ratio 1.68, 95% CI 1.66 to 1.70) (table 3). Use of the Charlson-Deyo Comorbidity Index (ratio 1.32, 95% CI 1.29 to 1.35) and RDCI (ratio 1.39, 95% CI 1.37 to 1.41) to measure multimorbidity burden also showed a higher burden of multimorbidity in RA. Similar findings were obtained when requiring ≥1 year of follow-up after RA diagnosis.

Multimorbidity trajectory

In the trajectory analyses using the incident RA subcohort, the mean follow-up was 2.0 (SD 1.8) years in RA and 1.8 (SD 1.8) years in non-RA. Patients with RA had a greater burden of multimorbidity at diagnosis and throughout follow-up (table 4 and figure 2A). The rate of accrual of chronic conditions was significantly higher over time in patients with RA relative to patients without RA (table 4; RA × time (years) β 0.21, 95% CI 0.20 to 0.21, p<0.001). Other factors associated with greater multimorbidity burden were female sex, older age, and a longer duration of follow-up. The greater burden of multimorbidity throughout follow-up and higher rate of accruing chronic conditions persisted when removing conditions closely related to RA or that may be misclassified as RA (table 4 and figure 2B; RA × time β 0.12, 95% CI 0.11 to 0.13, p<0.001). The accelerated accrual of chronic conditions over time was greater in RA when restricting to individuals with pre-RA data (table 4 and figure 2C; RA × time β 0.33, 95% CI 0.32 to 0.35, p<0.001). Among those with pre-RA data, chronic conditions developed at a significantly higher rate in RA versus non-RA after RA onset (RA status × post/pre-RA period β 0.67, 95% CI 0.63 to 0.71, p<0.001). All sensitivity analyses confirmed a greater burden of multimorbidity and a higher rate of accruing conditions in RA (online supplemental figure 3).

DISCUSSION

Given an ageing population and growing prevalence of chronic conditions, multimorbidity represents a major public health concern.[15–17] In this study, we have evaluated the onset and trajectory of multimorbidity in individuals with RA in a large, US commercial claims database during the current treatment era with robust capture of medical care. We found a substantially higher prevalence and burden of multimorbidity in individuals with RA relative to those without RA. Importantly, we identified that the heightened burden of multimorbidity in RA appears to start early in the RA disease course or even during the pre-RA period. Our findings shed important light on the natural history of multimorbidity and will help inform the future development of preventive and/or therapeutic interventions aimed at reducing multimorbidity burden in this high-risk population.

RA is known to predispose to many chronic conditions and there are ongoing efforts to better understand multimorbidity in RA.[33–35] In this study, we have demonstrated that multimorbidity is highly prevalent in RA. When requiring ≥1 year of postdiagnosis follow-up in our overall cohort,

Table 3 Comparison of multimorbidity burden between RA and non-patients with RA

	Chronic conditions*		Charlson-Deyo Comorbidity Index†		RDCI	
	Mean (SD)	Ratio (95% CI)	Mean (SD)	Ratio (95% CI)	Mean (SD)	Ratio (95% CI)
Baseline (all patients, n=277 782)						
RA	1.47 (2.00)	1.68 (1.66 to 1.70)	0.23 (0.58)	1.32 (1.29 to 1.35)	0.54 (0.96)	1.39 (1.37 to 1.41)
Non-RA	0.88 (1.48)	Ref	0.18 (0.51)	Ref	0.39 (0.82)	Ref
With ≥1 year of follow-up (n=226 850)						
RA	2.20 (2.28)	1.66 (1.65 to 1.68)	0.37 (0.72)	1.37 (1.35 to 1.39)	0.85 (1.15)	1.42 (1.40 to 1.44)
Non-RA	1.33 (1.76)	Ref	0.27 (0.63)	Ref	0.60 (0.98)	Ref

RA and non-RA matched on sex, year of birth, and year of entry into the database.
All p<0.001.
*n=44 chronic conditions.
†Connective tissue disease was not included in scoring.
RA, rheumatoid arthritis; RDCI, Rheumatic Disease Comorbidity Index.

Table 4 Trajectory of multimorbidity in patients with incident rheumatoid arthritis (RA) compared with patients without RA

	A. Primary analysis (n=44 conditions)		B. Secondary analysis* (n=36 conditions)		C. 2 years preindex date (n=44 conditions)	
	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
RA vs non-RA	1.13 (1.10 to 1.17)	<0.001	0.62 (0.59 to 0.64)	<0.001	0.29 (0.27 to 0.31)	<0.001
Year of follow-up	0.24 (0.23 to 0.24)	<0.001	0.18 (0.18 to 0.19)	<0.001	0.48 (0.47 to 0.49)	<0.001
RA × time (years)	0.21 (0.20 to 0.21)	<0.001	0.12 (0.11 to 0.13)	<0.001	0.33 (0.32 to 0.35)	<0.001
Age (per year)	0.06 (0.06 to 0.06)	<0.001	0.05 (0.05 to 0.05)	<0.001	0.04 (0.04 to 0.04)	<0.001
Female sex	0.28 (0.24 to 0.32)	<0.001	0.15 (0.12 to 0.18)	<0.001	0.16 (0.12 to 0.20)	<0.001

A and B. n=61 528 patients and 173 469 observations.
C. N=33 202 patients and 153 121 observations.
*Excluded conditions known to be related to RA or could be misclassified as RA.
RA, rheumatoid arthritis.

>51% of patients with RA were multimorbid. Moreover, the odds of multimorbidity were 2.3-fold to 2.5-fold higher in RA relative to patients without RA. Similarly, the burden of multimorbidity, operationalised as the count of chronic conditions, was more than 60% higher in RA. To avoid overestimation resulting from cohort construction, we did not consider RA to be a condition contributing to the definition of multimorbidity. Therefore, our results underestimate the true prevalence and burden of multimorbidity affecting patients with RA. Notably, the list of 44 chronic conditions we compiled from prior studies of multimorbidity in the general population and known RA-related conditions was more sensitive for assessing the burden of multimorbidity in RA than either the Charlson-Deyo Index or the RDCI. While the focus of our study was on multimorbidity as a whole, most individual chronic conditions were over-represented in RA (39 of 44 conditions), as previously reported.[36] As expected, extra-articular manifestations (eg, interstitial lung disease) and other musculoskeletal conditions were the conditions most closely associated with RA.

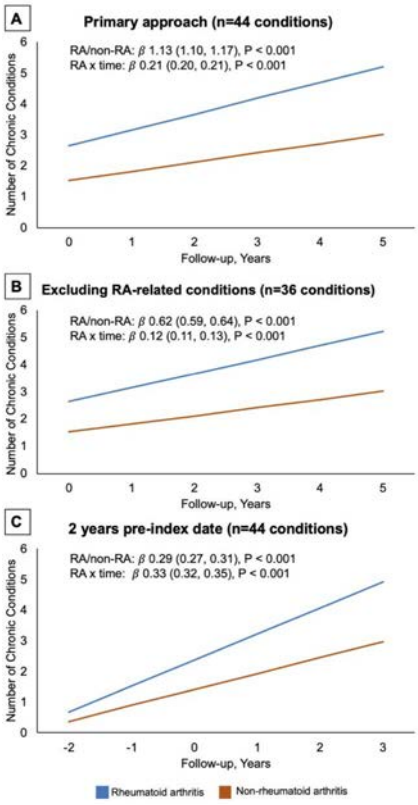

Figure 2 Predicted burden of multimorbidity in incident rheumatoid arthritis (RA) compared with patients without RA after diagnosis. Predicted burden of chronic conditions from generalised estimating equation models comparing patients with RA and patients without RA. Shown are the number of chronic conditions in RA and non-RA as well as the interaction term between RA status and follow-up time (years). Panel A, primary analytical approach requiring 1 year in the data set without RA diagnostic codes or medications and evaluating 44 chronic conditions. Panel B, similar analytical approach evaluating 36 chronic conditions after removing those known to be associated with RA or could be misclassified as RA. Panel C, restricting the population to individuals with at least 3 years of data prior to index date and beginning follow-up at 2 years before the index date (date fulfilling RA algorithm). CIs are shown but fall within the width of the predicted lines.

In addition to demonstrating a higher multimorbidity burden in RA, trajectory analyses in incident RA illustrate that the rate of acquiring chronic conditions increases disproportionately compared with persons without RA. This finding supports our proposed hypothesis and was robust to several sensitivity analyses, including analyses that excluded conditions that may be directly related to RA or misclassified as RA. It is also consistent with results from a recent study evaluating postdiagnosis conditions as predictors of mortality within the Nurses' Health Study where scores for the Multimorbidity Weighted Index increased more rapidly among women with RA than controls.[14] A novel finding from our national study of both women and men is that even during a treatment era characterised by earlier RA diagnosis and DMARD initiation, progression of multimorbidity in RA outpaced the rate in patients without RA during the pre-RA period. There are many potential mechanisms for this accelerated progression of multimorbidity in RA. In addition to some chronic conditions being well-established extra-articular features of RA, others may result from the inflammatory processes (eg, cardiovascular disease) and/or disease burden (eg, mental health disorders) accompanying RA.[37] Medications used to treat RA or manage RA symptoms may also contribute to the development of chronic conditions.[9] Finally, the onset of RA results in an increase in healthcare encounters and utilisation that may contribute to increased chronic disease screening and identification.[38] Because chronic conditions were frequent in our patients without RA and results were similar with adjustment for the number of chronic conditions at baseline as well as with the exclusion of 'silent chronic conditions', it is unlikely that heightened surveillance accounts for our findings.

The observation that multimorbidity occurs and progresses early in the disease course, or even preceding disease onset,

has important implications for future strategies targeting multimorbidity in RA to improve long-term outcomes. The early RA period is typically characterised by establishing the diagnosis of RA, initiating DMARDs, monitoring disease activity and adjusting DMARD regimen following a treat-to-target approach.[39 40] Other management considerations during this time include administration of vaccinations, adjunctive treatment modalities (physical and occupational therapy) and symptom management. While the rheumatologist may be focused on these important tasks, our findings illustrate the need for the early RA period to also include aggressive screening for, and management of, multimorbidity. Optimal care models for screening and managing multimorbidity in RA are not known and should be a focus of future research. Specifically, studies are needed to assess the existing patterns of screening for multimorbidity in patients with RA, which providers are performing these screenings and whether such methods are effectively identifying chronic conditions. In RA, co-management with a primary care physician improves screening for hyperlipidaemia.[41] Alternative care delivery models that use case managers and multidisciplinary teams have been tested with heterogenous results in the general population.[42]

Limitations of this study include the inability to adjust for health behaviours and sociodemographics, which may result in unmeasured confounding. There may be misclassification of RA status, incident versus prevalent RA and chronic condition development. However, we used validated algorithms for RA and required the presence of at least two diagnostic codes for chronic conditions.[26 27] The sample consisted of US individuals with commercial insurance and may not be generalisable outside of this setting. Because of the frequency of disenrolment from the commercial health plans, follow-up time was limited. Chronic conditions were considered independent, and future work will be needed to precisely characterise the interconnectedness of chronic conditions that defines multimorbidity in RA. The chosen 'silent conditions' may cause symptoms and are not exhaustive, but were selected as those most likely to be influenced by surveillance bias. Finally, while multimorbidity differentiates itself from comorbidity by not specifying an index condition, the study of multimorbidity in a specific population, such as RA, requires anchoring on the characteristic of that population.

In conclusion, in this large cohort study using a national commercial insurance database, we found a significantly higher burden of multimorbidity in RA compared with non-RA individuals. Our trajectory analyses demonstrate that multimorbidity onset occurs early in the RA disease course, or even precedes RA onset, and patients with RA experience an accelerated rate of accruing chronic conditions. Strategies aimed at managing multimorbidity to prevent its progression and complications will need to be delivered early in the RA disease course.

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Contributors BRE, TRM and JRC designed the study. FX and JRC were responsible for acquisition of data. BRE, PR, YY and HS analysed the data. All authors were responsible for interpretation of the data and for drafting, revising and approving the final submitted manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

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CLINICAL SCIENCE

Revisiting the use of remission criteria for rheumatoid arthritis by excluding patient global assessment: an individual meta-analysis of 5792 patients

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ABSTRACT

Objectives To determine the impact of excluding patient global assessment (PGA) from the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) Boolean remission criteria, on prediction of radiographic and functional outcome of rheumatoid arthritis (RA).

Methods Meta-analyses using individual patient data from randomised controlled trials testing the efficacy of biological agents on radiographic and functional outcomes at ≥2 years. Remission states were defined by 4 variants of the ACR/EULAR Boolean definition: (i) tender and swollen 28-joint counts (TJC28/SJC28), C reactive protein (CRP, mg/dL) and PGA (0–10=worst) all ≤1 (4V-remission); (ii) the same, except PGA >1 (4V-near-remission); (iii) 3V-remission (i and ii combined; similar to 4V, but without PGA); (iv) non-remission (TJC28 >1 and/or SJC28 >1 and/or CRP >1). The most stringent class achieved at 6 or 12 months was considered. Good radiographic (GRO) and functional outcome (GFO) were defined as no worsening (ie, change in modified total Sharp score (ΔmTSS) ≤0.5 units and ≤0.0 Health Assessment Questionnaire–Disability Index points, respectively, during the second year). The pooled probabilities of GRO and GFO for the different definitions of remission were estimated and compared.

Results Individual patient data (n=5792) from 11 trials were analysed. 4V-remission was achieved by 23% of patients and 4V-near-remission by 19%. The probability of GRO in the 4V-near-remission group was numerically, but non-significantly, lower than that in the 4V-remission (78 vs 81%) and significantly higher than that for non-remission (72%; difference=6%, 95% CI 2% to 10%). Applying 3V-remission could have prevented therapy escalation in 19% of all participants, at the cost of an additional 6.1%, 4.0% and 0.7% of patients having ΔmTSS >0.0, >0.5 and >5 units over 2 years, respectively. The probability of GFO (assessed in 8 trials) in 4V-near-remission (67%, 95% CI 63% to 71%) was significantly lower than in 4V-remission (78%, 74% to 81%) and similar to non-remission (69%, 66% to 72%).

Conclusion 4V-near-remission and 3V-remission have similar validity as the original 4V-remission definition in predicting GRO, despite expected worse prediction of GFO, while potentially reducing the risk of overtreatment. This supports further exploration of 3V-remission as the target for immunosuppressive therapy complemented by patient-oriented targets.

Key messages

What is already known about this subject?

- Few previous studies compared the prediction of good structural and functional outcomes between patients who fulfilled all four criteria of the current American College of Rheumatology/European League Against Rheumatism Boolean-based definition of remission ('4V-remission') versus those who attained only three ('3V-remission'), that is, excluding patient global assessment (PGA). No significant differences were found, but the two groups of patients evaluated significantly overlap.

What does this study add?

- This was the first study comparing these outcomes between patients achieving 4V-remission (23%) and those missing this status due solely to PGA above 1/10 (4V-near-remission) (19%). It is based on individual patient data meta-analysis of 11 recent clinical trials in rheumatoid arthritis (5792 patients).
- The rate of good radiographic outcome (≤0.5 units progression over the second year) was numerically higher in patients in 4V-remission (81%; 95% CI 74% to 87%) than in those in 4V-near-remission (78%; 95% CI 69% to 86%), but the difference is not statistically significant.
- In this population, if a 'treat-to-remission' strategy had been applied, the 3V-remission definition would have prevented therapy escalation in 19% of all patients, at the cost of an additional 6.1%, 4.0% and 0.7% of patients having a change in modified total Sharp score >0.0, >0.5 and >5 units over 2 years, respectively.

How might this impact on clinical practice or future developments?

- These results suggest that the use of 3V-remission as the target for immunosuppressive therapy, together with a separate assessment of disease impact on patient's lives, a dual target approach, deserves further consideration and research.

INTRODUCTION

Disease remission has become the guiding target in the management of rheumatoid arthritis (RA), as it conveys the best possible outcomes.[1] Current treatment recommendations advise that remission (or at least low disease activity) should be attained as soon and as consistently as possible, and changes in treatment should be considered when this does not happen.[2 3]

The most influential and authoritative definition of remission was published in 2011 under the auspices of the American College of Rheumatology (ACR), the European League Against Rheumatism (EULAR) and the Outcome Measures in Rheumatology (OMERACT) groups.[4] A Boolean-based definition was endorsed, and requires that scores of tender and swollen 28-joint counts (TJC28 and SJC28), C reactive protein (CRP, mg/dL) and patient global assessment of disease activity (PGA, 0–10 scale) are all ≤1.[4]

The inclusion of PGA in the definitions of remission in RA was justified because it added predictive value for later good radiographic and functional outcomes while conveying the much-needed patient's perspective.[4]

Despite this, the inclusion of PGA remains controversial.[5–9] Using the definitions mentioned previously, studies in different clinical practice cohorts[10–15] have reported that as many as 10%[13] to 38%[14] of all patients with RA do not reach remission solely due to a PGA score >1, a state that has become designated as '4V-near-remission'.[14 16] Moreover, it has been demonstrated that PGA bears little relationship with markers of the disease process, which drives structural damage, rather reflecting pain, fatigue and function.[9 17 18] This is especially evident when analyses are restricted to the lower levels of disease activity, in the range where the definition of remission has a decisive impact on whether to maintain or to escalate immunosuppressive treatment. According to this perspective, patients in 4V-near-remission would not benefit from additional immunosuppression, as this cannot be expected to improve their condition or foster remission,[9 17] and are exposed by current recommendations to the risk of overtreatment and unjustified side effects.[19]

These observations have led to the suggestion that the patients' interest would be better served by the adoption of two separate complementary targets: the first focused on remission of the inflammatory process, guided by an instrument without PGA; the second focused only on patient-reported impact measures.[9 16 20] However, this proposal would not be sustainable if, as suggested in the original ACR/EULAR/OMERACT paper, removing PGA from the Boolean-based remission significantly diminishes its ability to predict good radiographic and functional outcome.[4] A systematic literature review indicated that, among the individual components included in the definitions of remission, only swollen joints and acute phase reactants are associated with radiographic progression.[21] Two other studies, using data from a clinical cohort[13] and from clinical trials,[22] compared the prediction of good radiographic outcome by '4V-remission' versus '3V-remission' (without PGA) achieved in patients with RA: no significant differences were observed, but the two groups were not mutually exclusive. No study has ever compared the radiographic outcomes between the 4V-remission and 4V-near-remission groups.

The primary aim of this study was to compare 4V-near-remission and 4V-remission regarding their association with radiographic damage progression. Secondarily, we aimed to explore the impact of using 3V-remission instead of 4V-remission in patients with RA, both in terms of prevalence of remission and association with structural damage progression and functional impairment.

METHODS

Design and study selection

This was an individual patient data meta-analysis of published randomised controlled trials (RCTs) selected through a systematic literature review. The study protocol was registered in PROSPERO with the number CRD42017057099[23] and published elsewhere.[24]

RCTs were included if they tested the efficacy of biological disease-modifying antirheumatic drugs (bDMARDs) on ≥2-year radiographic outcomes in patients fulfilling the 1987 ACR or the 2010 ACR–EULAR criteria for RA.[25 26] Information on the processes of identifying and selecting studies, as well collecting data are reported in the protocol.[24]

Risk of bias assessment of individual studies

Studies selected for retrieval were assessed by two independent reviewers (RJOF and MN) for methodological validity prior to inclusion in this review, using the 'Risk of Bias 2' tool.[27] Any disagreements between the reviewers were resolved through discussion, or with a third reviewer (JAPS). The full protocols of the studies were consulted, and their authors contacted to request missing or additional data for clarification, where required.

Specification of outcomes

Primary outcome

The primary outcome of this study was the percentage of individuals with a good radiographic outcome (GRO) during the second year of the trial (ie, between month 12 and month 24), defined as a change (Δ) ≤0.5 units in the van der Heijde modified total Sharp score (mTSS).[28]

This ≤0.5 cut-off is preferred[29–31] over the one used in the ACR/EULAR pivotal publication (≤0 cut-off) because 0.5 is the optimal cut-off if the average of two readers is used,[32] as it allows to the very minimum difference of 1 unit out of 448 between the two readers.

Secondary outcomes

Two secondary endpoint cut-offs were used to define good radiographic outcome during the second year of the trial:

1. ΔmTSS ≤5 units, a higher, frequently used rate (sometimes referred to as clinically non-relevant radiographic progression);
2. ΔmTSS ≤0 units, to allow comparisons with the results obtained in the ACR/EULAR study.[4]

Also as secondary outcome, we studied the percentage of individuals with a good functional outcome (GFO) during the second year of the trial (ie, between month 12 and month 24), defined as no worsening, that is, a change (Δ) ≤0.0 units in the Health Assessment Questionnaire–Disability Index (HAQ-DI). This definition has been preferred over the one used in the ACR/EULAR pivotal publication (ΔHAQ ≤0.0 and HAQ ≤0.5 at both time points) because this is believed to be too strict, representing a better outcome even than expected for general population.[4 33] Despite this consideration, this definition of GFO was also tested to allow comparison with the original ACR/EULAR paper.

Comparisons: mutually and non-mutually exclusive definitions of remission

Analyses were based on different definitions of remission states, assessed at two time points, 6 months and 12 months, following

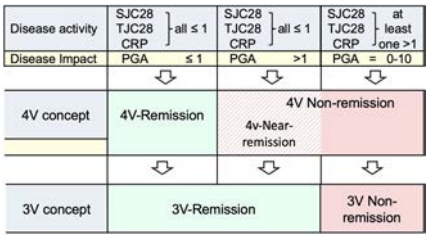

Figure 1 Definitions of remission tested in the study. *Legend*: CRP, C reactive protein, mg/dL; PGA, patient global assessment, range 0–10=worst; SJC28, swollen 28-joint count, range 0–28; TJC28, tender 28-joint count, range 0–28. *Footnote*: In general, in no remission states, disease-modifying antirheumatic drug (DMARD) therapy will be intensified, while at remission states, DMARD therapy will be unchanged or tapered. The no remission/4V-near-remission state (hatched) has a risk of overtreatment if DMARD therapy is intensified.

the methodology adopted by the ACR/EULAR committee,[4] as follows:

1. ACR/EULAR Boolean-based remission,[4] also designated in this study as '4V-Remission' (ie, TJC28 ≤1, SJC28 ≤1, CRP ≤1 mg/dL and PGA ≤1/10).
2. '4V-near-remission',[11 14] defined as TJC28 ≤1, SJC28 ≤1, CRP ≤1 mg/dL and PGA >1.
3. 'Non-remission' defined as TJC28 >1 and/or SJC28 >1 and/or CRP >1 mg/dL, irrespective of PGA value.

The three definitions are mutually exclusive, that is, each patient was categorised in one group only.

4. '3V-remission' defined as TJC28 ≤1, SJC28 ≤1 and CRP ≤1 mg/dL. This is a combination of 4V-remission and 4V-near-remission—patients classified in 4V-remission also meet the 3V-remission criteria (figure 1).

All definitions of remission were considered fulfilled if they were achieved at 6 or 12 months' follow-up and patients were classified according to the most stringent definition they satisfied (for instance, if a patient was in 4V-near-remission at 6 months and in 4V-remission at 12 months, he/she was classified as in 4V-remission).

Data analysis and synthesis

Data analysis

All 'primary' analyses were performed with SAS software (V.9.3), within the online secure platforms. For each trial, we determined the number of patients with GRO in each definition group (4V-remission, 4V-near-remission, 3V-remission and non-remission). The rates of true positive (TP), that is, remission and GRO; true negative (TN), that is, non-remission and not-GRO; false negative (FN), that is, non-remission and GRO; and false positive (FP), that is, remission and not-GRO, cases were also determined for all definitions. The percentage of patients with accurate prediction of having and not having GRO were also determined (sum of TP and TN) for the 4V-remission and 3V-remission. Missing data were not substituted. Similar analyses were performed for the secondary outcomes.

Meta-analysis

Frequency of remission status and outcomes

The frequency/proportion of each remission state observed in each of the trials were meta-analysed, irrespective of the treatment arm. The same procedure was used to determine the pooled prevalence of GRO and GFO according to remission status.

Primary analysis

Likelihood of achieving GRO for 4V-near-remission compared with 4V-remission and with non-remission

From our hypothesis that PGA might lead to false-negative rating of remission when using the 4V-remission definition, we aimed to analyse the value of 3V-remission definition, excluding PGA. Direct comparison of 4V-remission and 3V-remission however is not possible, given the overlap between the two states (see figure 1). Therefore, for each trial, we determined the differences in the proportion/chance (Δ proportion) of GRO (ΔmTSS ≤0.5) between 4V-near-remission and 4V-remission, mutually exclusive states, and then pooled these differences with the random-effects model to obtain an overall estimate of the difference (with 95% CI). We also compared this between 4V-near-remission and non-remission states. The risk ratio or relative risk (RR, 95% CI) for GRO between these groups were also calculated.

Secondary analyses

The likelihood of achieving each of the secondary outcomes for 4V-near-remission compared with 4V-remission and with non-remission was assessed using similar methods for the different definitions.

Sensitivity analyses

Different sensitivity analyses were performed regarding radiographic progression. The first was to explore the likelihood of GRO between remission states after excluding the seemingly outlier trials.

The second was a multivariate analysis. Multivariate logistic regressions were performed in each trial to explain GRO (dependent variable) using the mutually exclusive remission states as independent variables, adjusted for important covariates at baseline: gender, age, disease duration (except for three trials due to >50% of missing data in this covariate), rheumatoid factor status, level of radiographic damage and treatment arm. The OR obtained in each trial and its 95% CI and SE were meta-analysed to obtain the pooled OR of GRO comparing different mutually exclusive remission states. However, we hypothesise that this covariate adjustment may constitute an overcorrection because patients in remission are 'naturally' different from patients not in remission regarding these prognostic factors. For this reason, these sensitivity analyses are presented cautiously and only in online supplemental material.

The third was to clarify the value of PGA as a predictor of radiographic damage progression, selecting only the patients in 4V-near-remission (in 8 of the 11 trials, 796 patients, due to restrictions in accessing the data). We used Poisson regression models with 2y mTSS as dependent variable and PGA as independent variable. To assess the specific, independent impact of PGA, we corrected for SJC28, TJC28 and CRP, determined as the mean of the observation at 6 and 12 months, by also introducing them as independent variables, together with baseline mTSS. To allow the combined analysis the different variables, we standardised their values using z-scores. A meta-analysis was then performed to obtain pooled rate ratios (RR with 95% CI) per variable.

The last was to explore the proportion of patients in 3V-remission (8 trials; 1937 patients) who have radiographic damage progression ≥0.5 and those who have radiographic progression ≥5 during year 2, according to PGA score ≤1 versus >1 at 6 and 12 months.

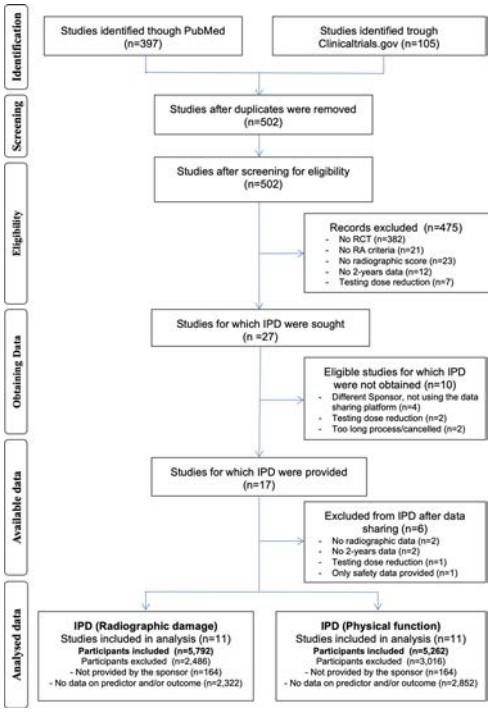

Figure 2 Flowchart with the process of study identification and data access. IPD, individual patient data; RA, rheumatoid arthritis; RCT, randomised controlled trial.

Likelihood of reaching good radiographic and functional outcomes with 4V-remission compared with 3V-remission

If the null hypothesis of this study (the chance of GRO in 4V-near-remission group are similar to the 4V-remission group) is not rejected, the current 4V-remission and the proposed 3V-remission can be compared in terms of their positive (LR+) and negative likelihood ratios (LR−) of GRO per remission group. The TP, TN, FN and FP values were used to synthesise these measures. Similar procedures were performed regarding GFO.

All meta-analyses were performed with the OpenMeta[Analyst] software,[34] using the DerSimonian-Laird random-effects method[35] and the arcsine-transformed proportion.[36] STATA software (V.14) was used only to determine OR adjusted to covariates (sensitivity analyses). The I^2 of Higgins and Thompson was calculated to quantify heterogeneity.[37]

RESULTS

Studies and participants

From a total of 27 identified studies, we were granted access to 17 through secure online platforms, but only 11 trials reported radiographic damage progression during the second year, thus allowing inclusion in the final analyses. Reasons for the non-inclusion of 16 out of the 27 trials initially identified are described in figure 2 and online supplemental table S1. The critical appraisal results for each of the 11 RCTs are summarised in online supplemental figure S1 (low risk of bias in all items assessed for all the trials). We had access to data from 100% of the randomised patients in 9 out of the 11 trials and from 93% of patients in the remaining two, resulting in a total sample of 8114 patients. Most trials tested anti-TNFα therapies (n=9), and included patients with insufficient response to methotrexate (n=7) and with established disease (>2 years) (n=9)—online supplemental table S2. The mean (SD) DAS28CRP3v ranged from 4.7 (0.9) to 5.3 (0.8) at baseline. The van der Heijde mTSS was used as the scoring method of radiographic damage progression in 10 of the trials. The remaining used the Genant method. The mean mTSS at baseline ranged from 5.9 (14.5) to 69.0 (55.8) (online supplemental table S2).

Altogether, 2322 patients (29%) were excluded from the final analyses (online supplemental table S3). The main reason for exclusion was the lack of data on radiographic outcome (71% of all cases). Those excluded from these analyses were older (1.3 years on average), reported higher PGA and HAQ, and had more active disease according to physician's global assessment. Regarding disease status at 6 or 12 months, 305 of the excluded patients had no data and the remaining 2017 had lower rates of 4V-remission and higher rates of non-remission, compared with those included.

Frequency of remission status, radiographic and functional outcomes

A total of 5792 (71%) patients had information on both the remission definition and on the primary outcome (radiographic progression) (table 1). Pooled meta-analytic frequency (95% CI) of 4V-remission at 6 or 12 months was 23.0% (18.0% to 28.0%), while for 4V-near-remission, it was 18.9% (15.4% to 22.1%), considering all treatment arms together (table 1).

Good radiographic outcome was observed in 74.1% (66.2% to 82.0%) of all patients using the primary cut-off (ΔmTSS ≤0.5) and by 94.6% (92.9% to 96.4%) using ΔmTSS ≤5 (table 1). Good functional outcome, which could only be assessed in eight RCTs (3904 patients), was observed in 70.6% (66.7% to 73.5%) of all patients using the elected cut-off (ΔHAQ-DI ≤0.0), and by 31.1% (24.9 to 37.2%) using ΔHAQ-DI ≤0.0 and HAQ-DI ≤0.5 (table 1).

Likelihood of reaching good radiographic outcome for patients in 4V-near-remission compared with patients in 4V-remission and with patients in non-remission

Overall, the proportion of GRO for the primary score (ΔmTSS ≤0.5) was high (71.8% to 81.1%) for the three mutually exclusive remission categories (table 2). The proportion of patients with GRO did not differ significantly between those in 4V-near-remission and 4V-remission: −2.9% (95% CI −7.3% to +1.5%). Patients in 4V-near-remission had a significantly higher chance of achieving GRO compared with patients in non-remission (+6.2%; 95% CI 2.3% to 10.1%). Results for these comparisons are shown in table 2 and figure 3. Similar observations were made for GRO defined as ΔmTSS ≤5 (table 2). None of the differences was statistically significant when ΔmTSS ≤0 was used (table 2).

We performed a sensitivity analysis by excluding the three apparent outliers in figure 3 (the DE019, GO-FURTHER and TEMPO trials) which confirmed no significant difference in the meta-analytic RRs (ΔmTSS ≤0.5) between 4V-remission and 4V-near-remission (RR 0.99; 95% CI 0.95 to 1.03).

Likelihood of reaching good functional outcome for patients in 4V-near-remission compared with patients in 4V-remission and with patients in non-remission

Overall, the proportion of GFO for the elected outcome (ΔHAQ-DI ≤0.0) was high (68.8% to 77.6%) for the three mutually exclusive remission categories (table 2). The proportion of patients with GFO was significantly lower in 4V-near-remission than 4V-remission: −11.0% (95% CI −16.3% to −5.7%). Patients in 4V-near-remission had a similar chance of achieving GFO compared with patients in non-remission (−2.2%; 95% CI

Table 1 Frequency of remission and good radiographic outcome in the included studies

Trial (year)	n*	Remission at 6 OR 12 months, n (%)			Good radiographic outcome from 12 to 24 months†, n (%)			Good functional outcome from 12 to 24 months, n (%)		
		4V-remission	4V-near-remission	Non-remission	ΔmTSS ≤0	ΔmTSS ≤0.5	ΔmTSS ≤5	n total	ΔHAQ-DI ≤0‡	ΔHAQ-DI ≤0 HAQ-DI ≤0.5
DE019 (2004)	425	68 (16.0)	45 (10.6)	312 (73.4)	245 (57.6)	297 (69.9)	397 (93.4)	398	281 (70.6)	114 (28.6)
TEMPO (2004)	442	113 (25.6)	91 (20.6)	238 (53.8)	282 (63.8)	330 (74.7)	423 (95.7)	421	300 (71.3)	152 (36.1)
COMET (2008)	344	102 (29.7)	107 (31.1)	135 (39.2)	250 (72.7)	289 (84.0)	329 (95.6)	324	237 (73.1)	138 (42.6)
RAPID 1 (2008)	650	177 (27.2)	143 (22.0)	330 (50.8)	424 (65.2)	508 (78.2)	636 (97.7)	642	420 (65.4)	135 (21.0)
RAPID 2 (2009)	417	51 (12.2)	81 (19.4)	285 (68.4)	286 (68.6)	324 (77.7)	398 (95.4)	435	290 (66.7)	79 (18.2)
GO-FORWARD (2010)	352	86 (24.4)	74 (21.0)	192 (54.6)	200 (56.8)	228 (64.8)	304 (86.4)	358	na	105 (29.3)
GO-BEFORE (2011)	499	117 (23.5)	80 (16.0)	302 (60.5)	403 (80.8)	446 (89.4)	493 (98.8)	507	na	187 (36.9)
LITHE (2011)	796	146 (18.3)	174 (21.9)	476 (59.8)	558 (70.1)	640 (80.4)	790 (99.2)	550	369 (67.1)	123 (22.4)
DE013 (2013)	540	156 (28.9)	50 (9.3)	334 (61.8)	286 (53.0)	351 (65.0)	483 (89.4)	518	383 (73.9)	249 (48.1)
GO-FURTHER (2014)	483	54 (11.2)	89 (18.4)	340 (70.4)	151 (31.3)	191 (39.5)	405 (83.9)	493	na	94 (19.1)
FUNCTION (2016)	844	308 (36.5)	151 (17.9)	385 (45.6)	713 (84.5)	766 (90.8)	840 (99.5)	616	470 (76.3)	250 (40.6)
Total n	5792	1378	1085	3329	3798	4370	5498	5262‡	2750	1626
Meta-analytic %		23.0	18.9	58.1	64.1	74.1	94.6		70.6	31.1
(95% CI)		(18.0 to 28.0)	(15.4 to 22.1)	(52.0 to 64.1)	(54.9 to 73.2)	(66.2 to 82.0)	(92.9 to 96.4)		(67.7 to 73.5)	(24.9 to 37.2)

4V-remission=SJC28, TJC28, CRP (mg/dL) and PGA (0–10), all ≤1; 4V-near-remission=SJC28, TJC28, CRP (mg/dL) ≤1 and PGA (0–10) >1; non-remission=SJC28 >1 or TJC28 >1 or CRP (mg/dL) >1 at 6 or 12 months of follow-up in all cases.
*Number of patients with information both on remission status and on radiographic outcome.
†All trials used van der Heijde mTSS (0 to 448) except the LITHE trial, in which the Genant mTSS (0 to 202) was used instead.
‡Not possible to be determined in the three golimumab trials due to changes that occurred in the research environment and statistical software available since the initial data analyses (thus, n=3904).
CRP, C reactive protein; HAQ-DI, Health Assessment Questionnaire–Disability Index; ΔmTSS, change in the modified total Sharp score during the second year of follow-up; PGA, patient global assessment; SJC28, swollen 28-joint count; TJC28, tender 28-joint count.

−6.8% to +2.4%). The differences between 4V-near-remission and 4V-remission were more striking for the GFO defined as ΔHAQ-DI ≤0 and HAQ-DI ≤0.5: −39.6% (95% CI −48.4% to −30.9%). The difference between 4V-near-remission and non-remission was non-significant (+1.7%; 95% CI −7.4 to +10.8).

Comparison of the 4V-remission and the proposed 3V-remission regarding prediction accuracy for radiographic and functional outcome

Having shown that the difference in the probability of GRO between 4V-remission and 4V-near-remission was neither statistically nor clinically relevant,[38] we were allowed to evaluate the difference between the 4V-remission and 3V-remission (the latter combining the 4V-near-remission and 4V-remission) groups (table 3). The results indicated that the likelihood ratio of having GRO (ΔmTSS ≤0.5) was higher for patients in 4V-remission compared with 4V-non-remission (LR+=1.36, 1.15 to 1.61) than between patients in 3V-remission versus 3V-non-remission (LR+=1.26; 1.13 to 1.41), although there was a large overlap in 95% CIs. Conversely, the likelihood of having GRO in the absence of remission was significantly smaller for the 3V-remission (LR−=0.86; 0.79 to 0.94) and non-significant for the 4V-remission (LR−=0.92; 0.81 to 1.04) versus their counterparts (table 3).

The same comparisons were made regarding functional outcomes (table 3). The likelihood ratio of having GFO (ΔHAQ≤0.0) was significantly higher for patients in 4V-remission compared with in 4V-non-remission (LR+=1.34; 1.16 to 1.54), while it was not significantly different between patients in 3V-remission versus 3V-non-remission (LR+=1.08; 0.99 to 1.17). Contrariwise, the likelihood of having GFO in the absence of remission was not significantly different from that for either the 3V-remission (LR−=0.94; 0.88 to 1.02) or the 4V-remission (LR−=0.90; 0.79 to 1.02) versus their comparator groups (table 3).

The proportion of patients whose prediction of GRO was accurate (=TP+TN) was, overall, quite low for both definitions of remission (≤53%). It was, however, higher for the 3V-remission definition than for the 4V-remission definition: 6.5%, 10.6% and17.2% higher at ΔmTSS ≤0.0, ≤0.5 and ΔmTSS ≤5, respectively (see figure 4). As expected, the improved accuracy of the 3V-remission is a result of a substantially lower percentage of FN, that is, patients without remission who do not have radiographic progression, at the cost of a much smaller increase in the percentage of FP, that is, the patients with remission who do have progression.

Regarding the elected definition of GFO, the proportion accurately predicted with the 3V definition (50.3%; 46.0 to 54.6) was significantly higher than with the 4V definition (43.8%; 40.9 to 46.6). The percentage accurately predicted was much higher for the alternative definition of GFO, the statistically significant difference being favourable for the 4V definition.

Figure 5 presents a 'clinical eye's' summary of good/bad radiographic outcomes observed according to the current and the proposed (3V) Boolean-based definitions of remission (95% CI and I^2 statistics are presented in online supplemental table S4). Overall, 73.3% (95% CI 63.9% to 81.8%) of the patients in non-4V-remission still had GRO (ΔmTSS≤0.5), and the same was observed for 71.8% (95% CI 62.1% to 80.5%) of those in non-3V-remission. The percentages of GRO increase to 81.1% (95% CI 74.4% to 86.9%) and 79.6% (95% CI 72.2% to 86.1%) among those in 4V-remission and 3V-remission, respectively. None of these differences were statistically significant.

Table 2 Pooled outcomes* and measures of association between remission categories and good radiographic and good functional outcomes, during the second year of follow-up

Good radiographic outcome (GRO) defined as ΔmTSS ≤0.5			
	4V-remission (n=1378)	**4V-near-remission (n=1085)**	**Non-remission (n=3329)**
Percentage GRO (95% CI)	81.1 (74.4 to 86.9)	78.2 (69.5 to 85.8)	71.8 (62.1 to 80.5)
	4V-near-remission vs 4V-remission	**4V-near-remission vs Non-remission**	
Δ percentage GRO (95% CI)	−2.9 (−7.3 to 1.5)	6.2 (2.3 to 10.1)	
Relative risk GRO (95% CI)	0.98 (0.94 to 1.02)	1.07 (1.02 to 1.12)	
Good radiographic outcome (GRO) defined as ΔmTSS ≤0			
	4V-remission	**4V-near-remission**	**Non-remission**
Percentage GRO (95% CI)	71.5 (63.5 to 78.8)	64.1 (54.6 to 73.2)	62.2 (51.5 to 72.4)
	4V-near-remission vs 4V-remission	**4V-near-remission vs non-remission**	
Δ percentage GRO (95% CI)	−7.7 (−16.6 to 1.1)	1.7 (−8.1 to 11.5)	
Relative risk GRO (95% CI)	0.91 (0.82 to 1.02)	1.04 (0.94 to 1.16)	
Good radiographic outcome (GRO) defined as ΔmTSS ≤5			
	4V-remission	**4V-near-remission**	**Non-remission**
Percentage GRO (95% CI)	97.5 (95.4 to 98.9)	96.1 (92.5 to 98.5)	94.2 (90.2 to 97.2)
	4V-near-remission vs 4V-remission	**4V-near-remission vs Non-remission**	
Δ percentage GRO (95% CI)	−2.5 (−7.5 to 2.6)	4.1 (0.7 to 7.6)	
Relative risk GRO (95% CI)	99.9 (0.97 to 1.01)	1.01 (1.00 to 1.02)	
Good functional outcome (GFO) defined as ΔHAQ-DI ≤0			
	4V-remission (n=1041)	**4V-near-remission (n=758)**	**Non-remission (n=2105)**
Percentage GFO (95% CI)	77.6 (74.3 to 80.8)	66.9 (62.6 to 71.2)	68.8 (66.0 to 71.7)
	4V-near-remission vs 4V-remission	**4V-near-remission vs Non-remission**	
Δ percentage GFO (95% CI)	−11.0 (−16.3 to −5.7)	−2.2 (−6.8 to 2.4)	
Relative risk GFO (95% CI)	0.87 (0.81 to 0.94)	0.98 (0.92 to 1.04)	
Good functional outcome (GFO) defined as ΔHAQ-DI ≤0 and HAQ-DI ≤0.5			
	4V-remission (n=1305)	**4V-near-remission (n=1003)**	**Non-remission (n=2954)**
Percentage GFO (95% CI)	60.2 (53.3 to 67.0)	22.5 (15.9 to 29.1)	21.2 (16.1 to 26.3)
	4V-near-remission vs 4V-remission	**4V-near-remission vs Non-remission**	
Δ percentage GFO (95% CI)	−39.6 (−48.4 to −30.9)	1.7 (−7.4 to 10.8)	
Relative risk GFO (95% CI)	0.37 (0.30 to 0.46)	1.12 (0.82 to 1.53)	

4V-remission=SJC28, TJC28, CRP (mg/dL) and PGA (0–10), all ≤1; 4V-near-remission=SJC28, TJC28, CRP (mg/dL) ≤1 and PGA (0–10) >1; non-remission=SJC28 >1 or TJC28 >1 or CRP (mg/dL) >1, irrespective of PGA value; at 6 or 12 months of follow-up in all cases.
*Determined by meta-analyses: for each trial, we calculated the differences in the proportion/change (Δ proportion) of GRO or GFO between 4V-near-remission and 4V-remission states and between 4V-near-remission and non-remission states; then, we pooled these differences with a random-effects model to obtain an overall estimate of the difference (with 95% CI).
CRP, C reactive protein; ΔHAQ-DI, change in Health Assessment Questionnaire–Disability Index; PGA, patient global assessment; SJC28, swollen 28-joint count; TJC28, tender 28-joint count.

The overall proportion of patients achieving 3V-remission was almost double of those reaching 4V-remission (41.9% vs 23.0%).

Sensitivity analyses

Adjustment to co-factors

The models adjusted for co-factors for the same comparisons showed even smaller differences between 4V-near-remission and 4V-remission categories regarding the prediction of good radiographic outcomes (online supplemental tables S5 and S6).

Exploration of radiographic damage in 4V-near-remission

Within the subgroup of patients in 4V-near-remission, PGA (at 6 and 12 months) is not a statistically significant predictor of radiographic progression over 2 years (RR 1.05 per SD unit increase, 95% CI 0.93 to 1.16); similarly, non-significant results were obtained for SJC28 and TJC28 (both 0 vs 1 in this subgroup): RR 1.09; 95% CI 0.90 to 1.27, and RR 0.86; 95% CI 0.68 to 1.04, respectively. Only CRP was a (borderline) statistically significant predictor of radiological progression (RR 1.06, 95% CI 1.00 to 1.12).

Radiographic damage progression according to PGA

In the subgroup of patients reaching 3V-remission, a ΔmTSS >5 units was observed in 2.3% (95% CI 1.0% to 4.3%) of patients scoring PGA >1 and in 1.3% (0.6 to 2.3%) of those with PGA <1. The corresponding values for ΔmTSS >0.5 units were 18.4% (13.8% to 23.5%) and 15.2% (9.9% to 21.4%), respectively (online supplemental table S7).

DISCUSSION

This is the first study assessing the prevalence of 4V-near-remission in RCTs and the first comparing radiographic damage

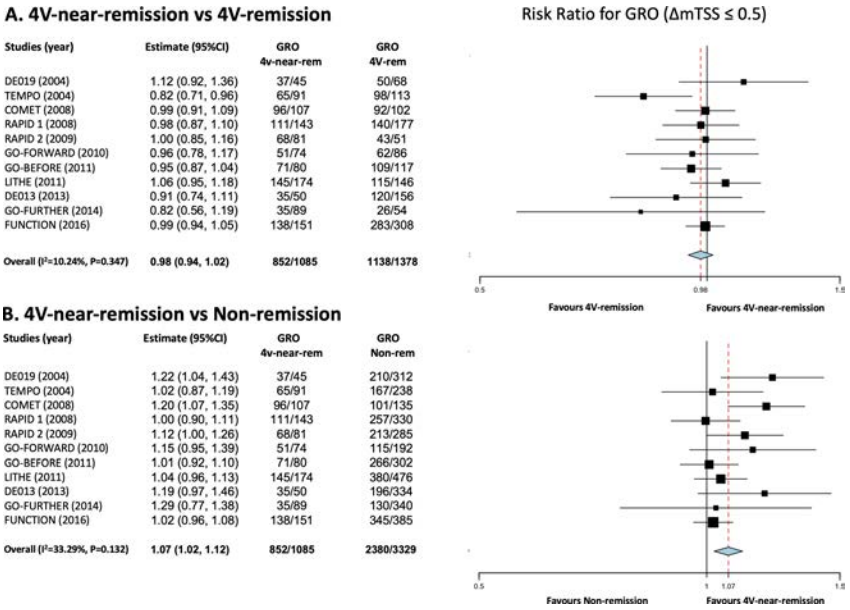

Figure 3 Meta-analyses of risk ratio of obtaining good radiographic outcome (ΔmTSS ≤0.5 units); 4V-near-remission vs 4V-remission and vs non-remission. *Legend*: 4V-remission=SJC28, TJC28, CRP (mg/dL) and PGA (0–10), all ≤1; 4V-near-remission=SJC28, TJC28 and CRP (mg/dL) ≤1 and PGA (0–10) >1; non-remission=SJC28 >1 and/or TJC28 >1 and/or CRP (mg/dL) >1, irrespective of PGA value; at 6 or 12 months of follow-up in all cases. CRP, C reactive protein; ΔmTSS, change in the modified total Sharp score during the second year of follow-up; GRO, good radiographic outcome; PGA, patient global assessment; SJC28/TJC28, swollen/tender 28-joint counts.

progression between patients in 4V-near-remission and in 4V-remission. The pooled rate of 4V-near-remission was almost the same of 4V-remission (19% vs 23%). These mutually exclusive groups did not differ significantly in terms of subsequent radiographic damage accrual. Patients in 4V-near-remission had a significantly better radiographic outcome than those in non-remission.

These observations legitimised the next step in our analyses: to explore the implications of choosing between the 3V and the 4V definitions of remission. The odds of good structural outcome were slightly higher for the 4V-remission, but without statistical or, in our view, clinical significance. The 3V-remission showed a better performance in terms of true estimations of significant damage (ie, sum of TP and TN estimations). If a 'treat-to-remission' strategy had been applied in this population, the 3V-remission definition would have prevented therapy escalation in 19% of all participants when compared with the 4V-remission. This would occur at the cost of having an excess of 6.1% of patients having a ΔmTSS >0.0, 4.0% of patients having a ΔmTSS >0.5 and of 0.7% having ΔmTSS >5 units. These trade-offs may be differently valued by different observers. Our proposal to use the 3V-remission definition is also rooted in solid clinical common sense: a (major) part of patients who fail remission solely because of PGA is not be expected to benefit from additional immunosuppressive therapy, as PGA does not reflect disease activity in these patients. However, clinical judgement is needed as to decide in individual patients whether the PGA level >1 indicates residual disease activity that might be successfully treated with more intensive RA treatment, or reflects another cause, for which more intensive RA treatment would be unnecessary and potentially harmful. Guiding definitions and recommendations should always be aligned with good clinical wisdom.

The data also emphasises that all remission concepts have a relatively poor predictive value regarding radiographic damage, as shown by low LRs (although better in 4V-remission) and

Table 3 Meta-analyses of good outcomes likelihood ratios for the 4V-remission and 3V-remission status

	4V-Remission (vs non-4V)		I^2 LR+	3V-Remission (vs non-3V)		I^2 LR+
Good outcome*	LR+ (95% CI)	LR− (95% CI)	LR−	LR+ (95% CI)	LR− (95% CI)	LR−
ΔmTSS ≤0.5	1.36 (1.15 to 1.61)	0.92 (0.81 to 1.04)	38% 0%	1.26 (1.13 to 1.41)	0.86 (0.79 to 0.94)	40% 3%
ΔmTSS ≤0	1.32 (1.17 to 1.50)	0.91 (0.82 to 1.02)	19% 0%	1.20 (1.12 to 1.29)	0.87 (0.81 to 0.93)	0% 0%
ΔmTSS ≤5	1.40 (0.88 to 2.23)	1.01 (0.76 to 1.33)	56% 0%	1.33 (1.03 to 1.71)	0.92 (0.77 to 1.10)	40% 0%
ΔHAQ-DI ≤0	1.34 (1.16 to 1.54)	0.90 (0.79 to 1.02)	18% 0%	1.08 (0.99 to 1.17)	0.94 (0.88 to 1.02)	17% 0%
ΔHAQ-DI ≤0 and HAQ-DI ≤0.5	3.35 (2.78 to 4.03)	0.60 (0.52 to 0.68)	72% 45%	1.82 (1.59 to 2.07)	0.55 (0.47 to 0.65)	80% 87%

4V-remission=SJC28, TJC28, CRP (mg/dL) and PGA (0–10), all ≤1; 3V-remission=SJC28, TJC28 and CRP (mg/dL) ≤1; non-remission=SJC28 >1 or TJC28 >1 or CRP (mg/dL) >1, irrespective of PGA value; at 6 or 12 months of follow-up in all cases.
*n=5792 for ΔmTSS, n=3904 for ΔHAQ-DI ≤0 and n=5262 for ΔHAQ-DI ≤0 and HAQ-DI ≤0.5.
CRP, C reactive protein; ΔHAQ-DI, change in Health Assessment Questionnaire–Disability Index; LR+, positive likelihood ratio; LR−, negative likelihood ratio; ΔmTSS, change in the modified total Sharp score during the second year of follow-up; PGA, patient global assessment; SJC28, swollen 28-joint count; TJC28, tender 28-joint count.

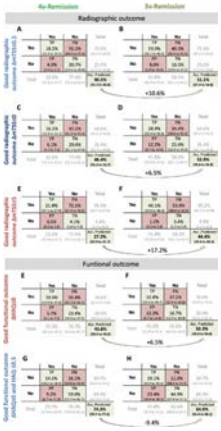

Figure 4 Pooled meta-analytic prediction accuracy of 4V-remission and 3V-remission status for the good radiographic and functional outcomes. *Footnote*: The sum of the meta-analytic percentages of TP, FN, FP and TN is slightly less than 100% due to error estimation when multi-category (k>2) prevalence is estimated.[35] All meta-analyses used double arcsine transformation as the preferred method to correct this situation.[35] The panels from A to F include 5792 analysed patients (11 randomised controlled trials (RCTs)), E and F include 3904 (8 RCTs), and G and H 5262 analysed patients (11 RCTs). *Legend*: 4V-remission=SJC28, TJC28, CRP (mg/dL) and PGA (0–10), all ≤1; 3V-remission=SJC28, TJC28 and CRP (mg/dL) ≤1; ΔHAQ, change in Health Assessment Questionnaire score; ΔmTSS, change in the modified total Sharp score from 12 months to 24 months; CRP, C reactive protein; FN, false negative; FP, false positive; PGA, patient global assessment; SJC28, swollen 28-joint count; TJC28, tender 28-joint count; TN, true negative; TP, true positive; accurately predicted=TP+TN. Between brackets is the pooled 95% CI.

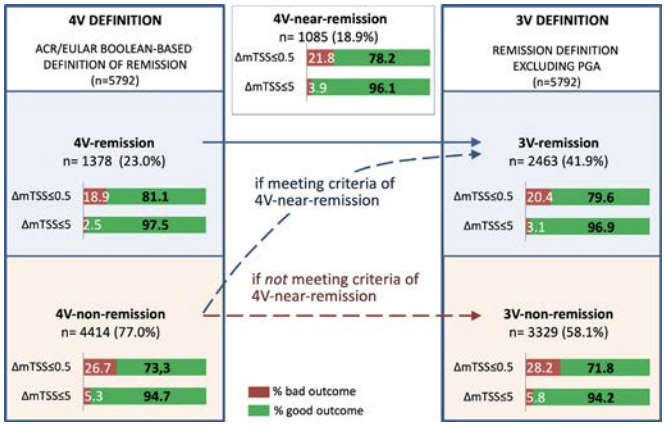

Figure 5 Reclassification of remission status and respective radiographic outcomes (n=5792). Percentages were calculated through meta-analyses. *Footnote*: Excluding PGA from the remission of remission (3V-remission) almost duplicated the percentage of patients in remission but showed only a slight increase in the rate of bad outcome when compared with 4V-remission. The radiographic outcome in the group of patients who had no overt signs of inflammation but who presented with high PGA (4V-near-remission) was also not statistically different from patient in 4V-remission. *Legend*: 4V-remission=SJC28, TJC28, CRP (mg/dL) and PGA (0–10), all ≤1; 4V-near-remission=SJC28, TJC28, CRP (mg/dL) ≤1 and PGA (0–10) >1; non-remission=SJC28 >1 and/or TJC28 >1 and/or CRP (mg/dL) >1, irrespective of PGA value; 3V-remission=SJC28, TJC28, CRP (mg/dL) ≤1. All definitions as observed at 6 or 12 months. *Note*: CIs and I^2 statistics of pooled radiographic outcomes can be found in online supplemental table S4. ΔmTSS, change in the modified total Sharp score during the second year of follow-up; CRP, C reactive protein; PGA, patient global assessment; SJC28/TJC28, swollen/tender 28-joint counts.

predictive accuracies below 53% (better in 3V-remission). This reflects the fact that 73% of patients in non-4V-remission had good radiographic outcomes and 19% of those in 4V-remission still presented radiographic progression (ΔmTSS >0.5).

4V-remission was associated with significantly higher rates of GFO (77.6%) compared with 4V-near-remission (66.9%); this latter rate is similar to that observed in non-remission (68.8%). The differences were more marked in favour of a 4V-remisision if the definition of GFO adopted by the ACR/EULAR committee was used (4V-remission=60.5%, 4V-near-remission=22.5%, non-remission=21.2%). Positive likelihood ratios also favoured 4V-remission, while negative LRs did not reach significance in favour of 4V-near-remission. The predictive accuracy of 3V-remission for the elected functional outcome was numerically better than for 4V-remission, nearly reaching statistical significance.

The results regarding functional outcome demand a critical appraisal. Overall, PGA and HAQ-DI are correlated to the level r=0.5 to 0.7. In higher disease activity states, both PGA and HAQ-DI predominantly reflect disease activity. In remission, they are expected to remain correlated, even if one assumes (as we do) that neither of them substantially reflects inflammation at this stage, because they are essentially determined by similar subjective factors and comorbidities.[9 14 17 39] It follows that, irrespective of disease activity, PGA is bound to predict HAQ-DI, and this obviously questions the use of HAQ-DI to assess the use of PGA, especially in a definition of remission, if it is intended to guide decisions on immunosuppressive therapy. The current results confirm this interpretation: How else could we coherently explain that, also in our study, 4V-remission is associated with significantly higher prevalence of GFO than 4V-near-remission if these two conditions share similar levels of SJC28, TJC28 and CRP (all ≤1) and similar levels of radiographic progression? The only difference is PGA.

The robustness of this work is supported by (1) the use of individual patient data, allowing uniform analyses procedures, (2) the availability of data collected under stringent RCT conditions, (3) the inclusion of over 5700 patients and (4) the use of both crude and adjusted statistical analyses. This study also has potential limitations and biases. The definition of remission was based only on two independent time-points (6 or 12 months) and used to predict radiographic progression over the following year. Although this was also the methodology used by the ACR/EULAR group,[4] it is recognised that alternative ways exist to quantify sustained remission, which might be useful both in understanding the construct of remission and investigating its relationship with structural damage accrual.[4] Good outcome was assessed only within the second year after randomisation. Although this is the efficacy endpoint used in most trials, longer follow-up assessment could provide different results.[40] When 3V-remission is agreed to be an acceptable endpoint for evaluating disease-modifying treatment in RA, the ability of the 3V-remission definition to detect differences between (effective) treatments, that is, its responsiveness, should be established and compared with that of 4V-remission and other established trial endpoints in RA. Patients with missing data, excluded from the analysis, had higher PGA and HAQ-DI scores and more active disease at 6 and 12 months, but they were not significantly different with regards to other factors recognised as relevant for radiographic outcome. The exclusion of these patients might

have changed the relationship between disease activity status and the outcomes under consideration in an unknown direction. It should be noted that we did not analyse within-trial arms and used the data of clinical trials as in observational studies, therefore discarding the effects of randomisation. As patients fulfilled inclusion criteria for RCTs, generalisability of our results is limited to patients with high disease activity starting treatment. In 7 out of the 11 RCTs, joint assessments were performed by independent assessors, and the 4 other studies did not use an independent joint assessor. We do not know whether this may have affected the (interpretation of the) results of our study in any way. Finally, some changes to the published protocol for this study need to be disclosed, namely the use of ΔmTSS ≤0.5 units as the primary outcome instead of the ≤0 cut-off, for the reasons outlined in the methods section.

The most relevant implications of this study for clinical practice and research relate to the most appropriate definition of remission and its use as the guiding target for therapy. Our results demonstrate that patients in 4V-near-remission do not differ significantly from those in 4V-remission in terms of radiographic damage accrual, while they can be clearly separated from those in non-remission. This supports the aggregation of the first two groups, that is, the proposed 3V-remission definition. Contrary to ACR/EULAR,[4] but in line with previous and current evidence,[13 21 22 41] our results demonstrated that the 3V-remission definition does not significantly diminish the ability to predict structural damage, while it may significantly reduce the risk of overtreatment, but this should be validated in clinical settings.[19 20] The implications of these observations should be further tested in the remission definitions based on composite indices Simplified Disease Activity Index and Clinical Disease Activity Index, as also endorsed by ACR/EULAR.

The ACR/EULAR committee also addressed the 3V-definition and reached the opposite conclusion.[4] This may be explained by differences in methodology and reasoning. First, ACR/EULAR tested one single and very strict cut-off to define good radiographic outcome (ΔmTSS ≤0), which is, in our view, excessively stringent, as it does not even allow for a difference of one unit in change score in the total of 448 joints assessed by the two radiograph assessors, which is averaged to 0.5. Both cut-offs are well below the smallest detectable change within one subject: 2–3 units according to an OMERACT expert panel.[38] However, in our study, the ΔmTSS ≤0 was the one with more favourable results for the 4V compared with the 3V-remission in terms of GRO prediction, predictive accuracy and rate of FN, but not in LR, for which the ΔmTSS ≤0.5 was more favourable. While considering these issues, one should take into account that ΔmTSS=1 has been estimated to justify a decrease of the HAQ score of only 0.01.[42] Second, the ACR/EULAR committee limited their analysis to 4V versus 3V, which significantly overlap, thus 'diluting' the characteristics of a very unique group of patients: 4V-near-remission. Also, the number of patients analysed by ACR/EULAR was much lower. Furthermore, the decision of the ACR/EULAR committee was, seemingly, strongly influenced by the much better prediction of good functional and 'overall' good outcomes for the 4V-remission versus the 3V-remission. This position was recently reaffirmed.[22] The reasons why we disagree with this approach are presented previously. Furthermore, the ACR/EULAR study analysed primarily the methotrexate-alone treatment groups of 3 trials, while we included all arms in each of 11 trials. This may explain why our likelihood ratios of GRO between 4V-remission and non-remission are much lower than the ACR/EULAR study, given that inhibition of radiographic damage by bDMARDs has been demonstrated even in the absence of remission, thus reducing the predictive accuracy of disease activity for radiographic damage.[43–45] However, we performed a sensitivity analysis, using data from patients in the monotherapy bDMARD arms (in nine RCTs), which showed that bDMARDs indeed reduce structural damage, and result in GRO in the majority, but not universally. Altogether, 28% of all patients exposed to bDMARDs monotherapy presented ΔmTSS ≥0.5 (11% to 57% in the individual trials; data not shown). In summary, we believe that our approach is valid and provides a better representation of current clinical practice. However, it will not fit contexts where access to bDMARDs is severely limited. Finally, the selection of tools by the ACR/EULAR committee was "based (…) on the need to include patient-reported outcomes", among other factors.[4] PGA was selected because it is associated with better prediction of the combination of radiographic and functional outcome.[4] While this is valid in the overall spectrum of disease activity, this argument is no longer true when the disease process is under control (SJC28, TJC28 and CRP ≤1) as demonstrated in this study and elsewhere.[17] It has been proposed to raise the cut-off value of PGA,[22 46 47] but this is at best a partial solution: we previously found that among 4381 international patients in 3V-remission, 63% scored PGA >1, but still 44% scored it >2, 32% >3 and 0.6% scored PGA as high as 10.[17] In addition, PGA at low disease activity states is essentially determined by subjective factors and comorbidities,[9 17 18] in contrast to, for example, swollen joint counts and CRP. The current study shows that PGA has no significant relationship with radiographic damage progression, both by comparing the 4V and 3V remission groups and by analysing the relationship between the two parameters within the specific group of patients in 4V-near-remission. These observations support our view to leave it out of the treatment target definition used to control inflammation (biological remission).

It has been recognised that treating to target often leaves room for improvement.[48] For patients with active disease, there is little doubt that controlling the disease is the most important means to improve the patient's condition, both at short and long term. Once low disease activity or remission is achieved, a persistently high disease impact should become the guiding target: after a diligent search for remaining (undetected) disease activity, it needs to be analysed and understood so as to choose the best adjunctive intervention, such as analgesia, rehabilitation or anti-depressive therapy, among other pharmacological and non-pharmacological therapies.[49] PGA score is not appropriate for this purpose, and more analytic instruments, such as the Patient Reported Outcome Measurement Information System (PROMIS),[50] the RA Impact of Disease (RAID) score[51 52] or the RA Flare Questionnaire,[53] are required.

Overall, these results support the proposal that the 3V definition of remission in parallel with a separate evaluation of the patient's perspective, that is, the dual target strategy, deserves consideration. The first target aims to control inflammation (biological remission) and the other one to control disease impact (symptom remission), guided by clinically informative PROMs.[9 16 20] Pursuing and achieving the first is an important contribution, but no guarantee that the second will be fulfilled. Further research, specifically regarding adjuvant interventions required to achieve effective control of disease impact endured by patients in biological remission designed to bring patients from 4V-near-remission into full remission, is warranted to validate the concept of dual target. Improving symptoms and signs of RA, both short and long term, is the major goal of treatment and it deserves being highlighted by an independent treatment target.

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Contributors All authors designed the study and protocol, which was firstly drafted by RJOF and JAPS. RJOF and PMJW performed the data analyses. RJOF and JAPS wrote the initial draft of the manuscript, which was critically revised and refined by all authors. All authors formally approved the final manuscript.

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CLINICAL SCIENCE

Safety profile of upadacitinib in rheumatoid arthritis: integrated analysis from the SELECT phase III clinical programme

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ABSTRACT

Objectives This integrated analysis presents the safety profile of upadacitinib, a Janus kinase inhibitor, at 15 mg and 30 mg once daily in patients with moderately to severely active rheumatoid arthritis (RA).

Methods Treatment-emergent adverse events (TEAEs) and laboratory data from five randomised, placebo- or active-controlled phase III trials of upadacitinib for patients with RA were analysed and summarised. Exposure-adjusted event rates are shown for placebo (three trials; 12/14 weeks), methotrexate (two trials; mean exposure: 36 weeks), adalimumab (one trial; mean exposure: 42 weeks), upadacitinib 15 mg (five trials; mean exposure: 53 weeks) and upadacitinib 30 mg (four trials; mean exposure: 59 weeks).

Results 3834 patients received one or more doses of upadacitinib 15 mg (n=2630) or 30 mg (n=1204), for a total of 4020.1 patient-years of exposure. Upper respiratory tract infection, nasopharyngitis and urinary tract infection were the most commonly reported TEAEs with upadacitinib. Rates of serious infection were similar between upadacitinib 15 mg and adalimumab but higher compared with methotrexate. Rates of herpes zoster and creatine phosphokinase (CPK) elevations were higher in both upadacitinib groups versus methotrexate and adalimumab, and rates of gastrointestinal perforations were higher with upadacitinib 30 mg. Rates of deaths, malignancies, adjudicated major adverse cardiovascular events (MACEs) and venous thromboembolic events (VTEs) were similar across treatment groups.

Conclusion In the phase III clinical programme for RA, patients receiving upadacitinib had an increased risk of herpes zoster and CPK elevation versus adalimumab. Rates of malignancies, MACEs and VTEs were similar among patients receiving upadacitinib, methotrexate or adalimumab.

Trial registration numbers SELECT-EARLY: NCT02706873; SELECT-NEXT: NCT02675426; SELECT-COMPARE: NCT02629159; SELECT-MONOTHERAPY: NCT02706951; SELECT-BEYOND: NCT02706847.

Key messages

What is already known about this subject?

- Upadacitinib is a Janus kinase (JAK) inhibitor which has been studied across a spectrum of patients with moderately to severely active rheumatoid arthritis (RA); the efficacy of upadacitinib has been reported from the five randomised controlled trials (RCTs) which comprise the phase III SELECT clinical programme.
- JAK inhibitors have been associated with several safety risks, including herpes zoster, serious and opportunistic infections, thromboembolic events and changes in laboratory parameters.

What does this study add?

- This integrated safety analysis of upadacitinib, based on more than 3500 patients and 4000 patient-years of exposure, supports an acceptable safety profile for treatment of patients with RA and reports no new safety risks compared with other JAK inhibitors.
- Upadacitinib 15 mg once daily had a similar safety profile to that of adalimumab for rates of serious infections, malignancies, major adverse cardiovascular events and venous thromboembolic events but higher rates of herpes zoster and creatine phosphokinase elevations.

How might this impact on clinical practice or future developments?

- The results of this integrated safety analysis of five RCTs suggest that upadacitinib has a similar safety profile to other JAK inhibitors as demonstrated in their clinical development programmes.

INTRODUCTION

Oral targeted synthetic disease-modifying antirheumatic drugs, such as Janus kinase inhibitors (JAKis), have demonstrated at least similar efficacy to biologic disease-modifying antirheumatic drugs (bDMARDs) in randomised controlled trials (RCTs) as treatment for rheumatoid arthritis (RA). Shared decision-making between physicians and patients regarding treatment selection requires understanding benefits and risks, including the safety profiles of treatment options.

Upadacitinib is a JAKi engineered for increased selectivity for JAK1 over JAK2, JAK3 and tyrosine kinase 2.[1] Upadacitinib 15 mg once daily was recently approved in the USA and Europe for

patients with moderately to severely active RA who are intolerant of or have had an inadequate response to methotrexate (MTX).[2 3] Efficacy and safety of upadacitinib were studied in patients with moderately to severely active RA in five pivotal phase III RCTs: SELECT-NEXT,[4] SELECT-BEYOND,[5] SELECT-MONOTHERAPY,[6] SELECT-COMPARE[7] and SELECT-EARLY.[8] Here, we report an integrated analysis of the safety profile of upadacitinib 15 and 30 mg once daily from these trials.

METHODS

Studies

Data were pooled from the five SELECT trials (online supplemental table S1), which evaluated upadacitinib administered with or without background conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) in patients with moderately to severely active RA, including MTX-naive patients and those with an inadequate response or intolerance to one or more csDMARDs or bDMARDs.

Patients aged ≥18 years with active RA (≥6 swollen and ≥6 tender joints and high-sensitivity C-reactive protein ≥3 mg/L (≥5 mg/L in SELECT-EARLY[8] and SELECT-COMPARE[7] at screening) who met the 2010 American College of Rheumatology (ACR)/European League Against Rheumatism classification criteria were enrolled.[9] Additional inclusion criteria in SELECT-EARLY and SELECT-COMPARE were erosive joint damage and/or autoantibody seropositivity.[7 8] Exclusion criteria are listed in the online supplemental material. Patients were tested for tuberculosis (TB) at screening; those with latent TB could enrol after initiating appropriate prophylactic treatment.

Patient and public involvement

Patients and the public were not involved in the design or analysis of this study.

Dosing

Depending on the study, patients received extended-release upadacitinib (15 or 30 mg once daily), placebo, MTX or subcutaneous adalimumab (40 mg every other week), as monotherapy or in combination with background csDMARDs. Patients were not permitted to switch between upadacitinib doses. MTX-naive patients randomised to MTX started oral medication at 10 mg/week (7.5 mg/week in China and Japan) and were titrated to a maximum of 20 mg/week (15 mg/week in Japan) through week 8, as tolerated.

Safety assessments

Data from patients who received one or more doses of study drug were integrated into five analysis sets (online supplemental table 1). The placebo-controlled analysis set included short-term data from patients who remained on stable doses of their current csDMARDs through week 12 (SELECT-NEXT[4] and SELECT-BEYOND)[5] or week 14 (SELECT-COMPARE).[7] The remaining four analysis sets included longer-term data up to 2.5 years. The MTX-controlled analysis set included pooled data from SELECT-EARLY[8 10] and SELECT-MONOTHERAPY,[6] censored at rescue. The adalimumab-controlled analysis set included patients randomised or rescued to adalimumab in SELECT-COMPARE.[7] Upadacitinib 15 mg data were pooled from all five studies; and upadacitinib 30 mg data were pooled from four studies (all except SELECT-COMPARE).

Adverse events (AEs) were assessed based on Outcome Measures in Rheumatology (OMERACT) criteria. Potentially clinically significant laboratory values (grades 2, 3 or 4) were determined by OMERACT criteria, except for creatine phosphokinase (CPK) and serum creatinine, which were based on the National Cancer Institute's Common Toxicity Criteria v4.03. Potentially clinically significant outliers were based on patient laboratory values meeting the criteria on one or more occasions.

Adverse events of special interest (AESIs) were selected due to their higher prevalence among RA populations, as a customary concern for immunomodulators, or because they were labelled/emerging risks with other JAKis. AEs were identified using the standardised Medical Dictionary for Regulatory Activities (MedDRA) query or company MedDRA query search criteria. A treatment-emergent adverse event (TEAE) was defined as an AE with onset on or after the first dose of study drug and no more than 30 days (70 days for adalimumab) after the last dose of study drug.

An independent external Data Monitoring Committee monitored unblinded clinical trial data. An independent Cardiovascular Adjudication Committee blindly adjudicated all deaths and potential cardiovascular (CV) events, including potential arterial and venous thromboembolic events (VTEs). Major adverse cardiovascular events (MACEs) included CV death, non-fatal myocardial infarction and non-fatal stroke. VTEs included deep vein thrombosis and pulmonary embolism (PE). Active/latent TB events and potential gastrointestinal (GI) perforations were assessed by the sponsor.

Statistical analyses

Baseline characteristics and exposure (last dose date minus first dose date plus 1, 7 and 14 days for upadacitinib, MTX and adalimumab) were summarised descriptively. TEAEs were summarised using the MedDRA version 19.1 system organ class and preferred term.

Exposure-adjusted event rates (EAERs) per 100 patient-years (PY) were summarised as events based on the treatment received at the time of each AE; multiple events occurring in the same patient were included in the numerator. 95% CIs were calculated using the Cochran-Mantel-Haenszel test (adjusted for each study). Exposure-adjusted incidence rates (EAIRs) per 100 PY were summarised as the number of patients with ≥1 event/100 PY (E/100 PY), with exposure calculated up to onset of the first event; 95% CIs were calculated using the exact method for the Poisson mean. Mean changes from baseline in laboratory parameters and vital signs were summarised.

HRs (95% CIs) for upadacitinib versus comparators were calculated using a Cox proportional hazards model including the prognostic factors of the treatment group and baseline covariates. Risk factors for herpes zoster (HZ) in upadacitinib-treated patients were identified using a univariate Cox regression model.

The standardised incidence ratio (SIR) for malignancy excluding non-melanoma skin cancer (NMSC) was calculated using age- and gender-specific malignancy data from the US National Cancer Institute Surveillance and Epidemiology and End Results database, 18 Registry Research Data 2000–2015; 95% CIs were calculated following a Poisson distribution. The standardised mortality ratio (SMR) used the WHO country-specific, age-specific and gender-specific death data for the general population; 95% CIs were calculated using Byar's approximation.

RESULTS

Patients and exposure

Across studies, 3834 patients received one or more doses of upadacitinib (15 mg once daily, n=2630; 30 mg once daily, n=1204)

Table 1 Demographics and baseline disease characteristics

Mean (SD) or n (%), unless specified	PBO pooled, n=1042	MTX pooled, n=530	ADA 40 mg EOW, n=579	UPA all phase III long term	
				Any UPA 15 mg once daily, n=2630	Any UPA 30 mg once daily, n=1204
	Short-term data up to 12/14 weeks	Long-term MTX monotherapy Mean exposure: 36 weeks (data censored at rescue)	Long-term ADA Mean exposure: 42 weeks (includes UPA→ADA post-switch)	Long-term UPA (monotherapy or in combination with MTX/other csDMARDs) Mean exposures: 53 weeks (UPA 15 mg) and 59 weeks (UPA 30 mg)	
Female	822 (78.9%)	419 (79.1%)	470 (81.2%)	2102 (79.9%)	948 (78.7%)
Age, years	54.8 (12.2)	54.1 (12.2)	54.1 (11.7)	54.1 (12.1)	55.3 (11.9)
Geographic region					
North America	321 (30.8%)	110 (20.8%)	122 (21.1%)	689 (26.2%)	429 (35.6%)
South/Central America	181 (17.4%)	121 (22.8%)	126 (21.8%)	529 (20.1%)	153 (12.7%)
Western Europe	92 (8.8%)	45 (8.5%)	29 (5.0%)	200 (7.6%)	129 (10.7%)
Eastern Europe	360 (34.5%)	164 (30.9%)	249 (43.0%)	934 (35.5%)	351 (29.2%)
Asia	37 (3.6%)	54 (10.2%)	18 (3.1%)	135 (5.1%)	85 (7.1%)
Other	51 (4.9%)	36 (6.8%)	35 (6.0%)	143 (5.4%)	57 (4.7%)
Time since RA diagnosis, years	9.0 (8.5)	3.9 (6.0)	8.2 (8.0)	7.7 (8.1)	7.0 (8.3)
Median (range)	6.4 (0.3 to 49.8)	1.2 (0.03 to 38.0)	5.5 (0.3 to 51.1)	4.8 (0.04 to 54.2)	3.7 (0.03 to 51.3)
DAS28-CRP	5.8 (0.9)	5.8 (1.0)	5.2 (1.3)	5.3 (1.3)	5.4 (1.2)
CRP, mg/L	16.5 (20.2)	18.5 (20.5)	14.2 (20.5)	17.0 (21.5)	15.9 (19.8)
Concomitant csDMARD at baseline					
MTX alone	914 (87.9%)	NA	576 (99.5%)	1769 (67.3%)	380 (31.6%)
MTX plus other csDMARD	68 (6.5%)	NA	0	103 (3.9%)	81 (6.7%)
csDMARD other than MTX	58 (5.6%)	NA	0	105 (4.0%)	100 (8.3%)
Prior bDMARD use	261 (25.0%)	0	57 (9.8%)	406 (15.4%)	281 (23.3%)
Concomitant steroids	573 (55.0%)	279 (52.6%)	349 (60.3%)*	1446 (55.0%)†	570 (47.3%)†
Seropositive (RF or ACPA)	880 (84.5%)	424 (80.0%)	497 (85.8%)	2237 (85.1%)	948 (78.7%)
Prior history of herpes zoster	58 (5.6%)	20 (3.8%)	22 (3.8%)	110 (4.2%)	87 (7.2%)
Prior history of herpes zoster vaccination	52 (5.1%)	17 (3.2%)	15 (2.6%)	80 (3.0%)	72 (6.0%)
Positive TB test at screening	124 (12.0%)	66 (12.5%)	77 (13.3%)	299 (11.4%)	119 (9.9%)
CV risk factors at baseline					
Medical history of hypertension	425 (40.8%)	203 (38.3%)	248 (42.8%)	1043 (39.7%)	481 (40.0%)
Diabetes mellitus	77 (7.4%)	36 (6.8%)	41 (7.1%)	212 (8.1%)	90 (7.5%)
History of tobacco/nicotine use (current+former)	371 (35.6%)	207 (39.1%)	199 (34.4%)	998 (37.9%)	509 (42.3%)
Elevated LDL-C (≥3.36 mmol/L)	275 (26.6%)	163 (30.9%)	200 (34.5%)	723 (27.5%)	318 (26.5%)
Lowered HDL-C (≤1.55 mmol/L)	594 (57.0%)	301 (56.8%)	283 (48.9%)	1504 (57.2%)	705 (58.6%)
Statin use at baseline	128 (12.3%)	60 (11.3%)	58 (10.0%)	300 (11.4%)	168 (14.0%)

All percentages calculated are on non-missing values.
*Baseline is redefined as start of ADA.
†Baseline is redefined as start of UPA.
ACPA, anti-citrullinated protein antibody; ADA, adalimumab; bDMARD, biologic disease-modifying antirheumatic drug; CRP, C-reactive protein; csDMARD, conventional synthetic disease-modifying antirheumatic drug; CV, cardiovascular; DAS28-CRP, Disease Activity Score for 28 joints-CRP; EOW, every other week; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MTX, methotrexate; NA, not applicable; PBO, placebo; RA, rheumatoid arthritis; RF, rheumatoid factor; TB, tuberculosis; UPA, upadacitinib.

for a mean duration of approximately 1 year and 4020.1 PY of exposure. Sixty-six per cent (15 mg, 61%; 30 mg, 75%) and 4% (15 mg, 4%; 30 mg, 5%) of patients received ≥48 and ≥96 weeks of upadacitinib treatment, respectively, with a maximum exposure of 2.5 years. Most patients were female and had been diagnosed with RA for a median of 1.2–6.4 years (table 1).

Overview of AEs

The most common TEAEs (≥10 E/100 PY) with upadacitinib were upper respiratory tract infection, nasopharyngitis, urinary tract infection and, for upadacitinib 30 mg only, increased blood CPK (online supplemental table S2). The EAER of serious TEAEs (SAEs) with upadacitinib 15 mg was comparable with adalimumab but higher than MTX (table 2). SAE rates were higher with upadacitinib 30 versus 15 mg. Pneumonia was the most common SAE reported with both upadacitinib doses.

There were 22 treatment-emergent deaths reported with upadacitinib (n=11 each for upadacitinib 15 and 30 mg): 10 adjudicated CV deaths and 12 non-CV deaths (online supplemental material). Compared with the general population, the SMR for treatment-emergent deaths in the upadacitinib groups was 0.58 (95% CI: 0.37 to 0.85). There were two, one and four deaths among the placebo, MTX and adalimumab groups, respectively.

Rates of AEs, AESIs and laboratory abnormalities were generally similar between the upadacitinib monotherapy population (online supplemental table S3) and the overall upadacitinib population.

Table 2 TEAEs in patients with upadacitinib compared with placebo and active controls*

				UPA all phase III long term	
	PBO pooled, n=1042	MTX pooled, n=530	ADA 40 mg EOW, n=579	UPA 15 mg once daily, n=2630	UPA 30 mg once daily, n=1204
E/100 PY (95% CI), unless stated otherwise	Short-term data up to 12/14 weeks	Long-term MTX monotherapy Mean exposure: 36 weeks (data censored at rescue)	Long-term ADA Mean exposure: 42 weeks (includes UPA→ADA post-switch)	Long-term UPA (monotherapy or in combination with MTX/other csDMARDs) Mean exposures: 53 weeks (UPA 15 mg) and 59 weeks (UPA 30 mg)	
Total PY of exposure, years	256.8	368.7	467.8	2655.1	1365.0
Median exposure, days (range)	97.0 (1 to 128)	179.5 (7 to 865)	257.0 (14 to 894)	375.0 (2 to 898)	431.0 (1 to 857)
Any AE	447.4 (421.9 to 474.1)	321.7 (303.6 to 340.5)	294.8 (279.4 to 310.8)	295.7 (289.2 to 302.3)	368.7 (358.6 to 379.0)
Any SAE	9.3 (6.0 to 13.9)	11.9 (8.7 to 16.0)	15.6 (12.2 to 19.6)	15.0 (13.6 to 16.6)	21.3 (18.9 to 23.9)
Any AE leading to discontinuation	10.9 (7.2 to 15.8)	9.5 (6.6 to 13.2)	11.1 (8.3 to 14.6)	8.4 (7.4 to 9.6)	13.3 (11.5 to 15.4)
Deaths†	0.8 (0.1 to 2.8)	0.3 (0.0 to 1.5)	0.9 (0.2 to 2.2)	0.5 (0.3 to 0.8)	1.0 (0.5 to 1.7)

*Patients who switched from PBO, ADA or MTX to UPA were included in the UPA analysis set from the start of UPA treatment, while those who switched from UPA to ADA were included in the ADA dataset from the start of ADA. There was no switch between UPA doses in any study.
†Deaths included non-treatment-emergent deaths that occurred >30 days after the last dose of study drug (UPA 15 mg, 3; UPA 30 mg, 3; and ADA, 1). When non-treatment deaths are included, the exposures are 2925.0 PY for UPA 15 mg and 1410.3 PY for UPA 30 mg.
ADA, adalimumab; AE, adverse event; csDMARD, conventional synthetic disease-modifying antirheumatic drug; EOW, every other week; MTX, methotrexate; PBO, placebo; E/100 PY, event per 100 patient-years; SAE, serious adverse event; TEAE, treatment-emergent adverse event; UPA, upadacitinib.

AEs of special interest

EAERs (figure 1) and EAIRs (online supplemental figure S1) of AESIs are summarised by treatment.

Serious infection EAERs were similar between the upadacitinib 15 mg and adalimumab groups, both of which were higher versus MTX; the EAER was higher for upadacitinib 30 mg versus 15 mg (figure 1). Cox regression analyses showed that upadacitinib 30 mg, but not 15 mg, was associated with an increased risk of serious infections versus placebo and adalimumab (online supplemental table S4). The serious infection EAER in the upadacitinib 15 mg group did not increase over time, although some increases were observed in the upadacitinib 30 mg group between 6 and 12 months on treatment (online supplemental figure S2).

EAERs of opportunistic infections were similar across treatment groups, with the highest rate observed in the upadacitinib 30 mg group (figure 1). The majority of opportunistic infections observed with upadacitinib were mucosal candida infections. There were three events (0.1 E/100 PY) of serious opportunistic infections among patients receiving upadacitinib 15 mg

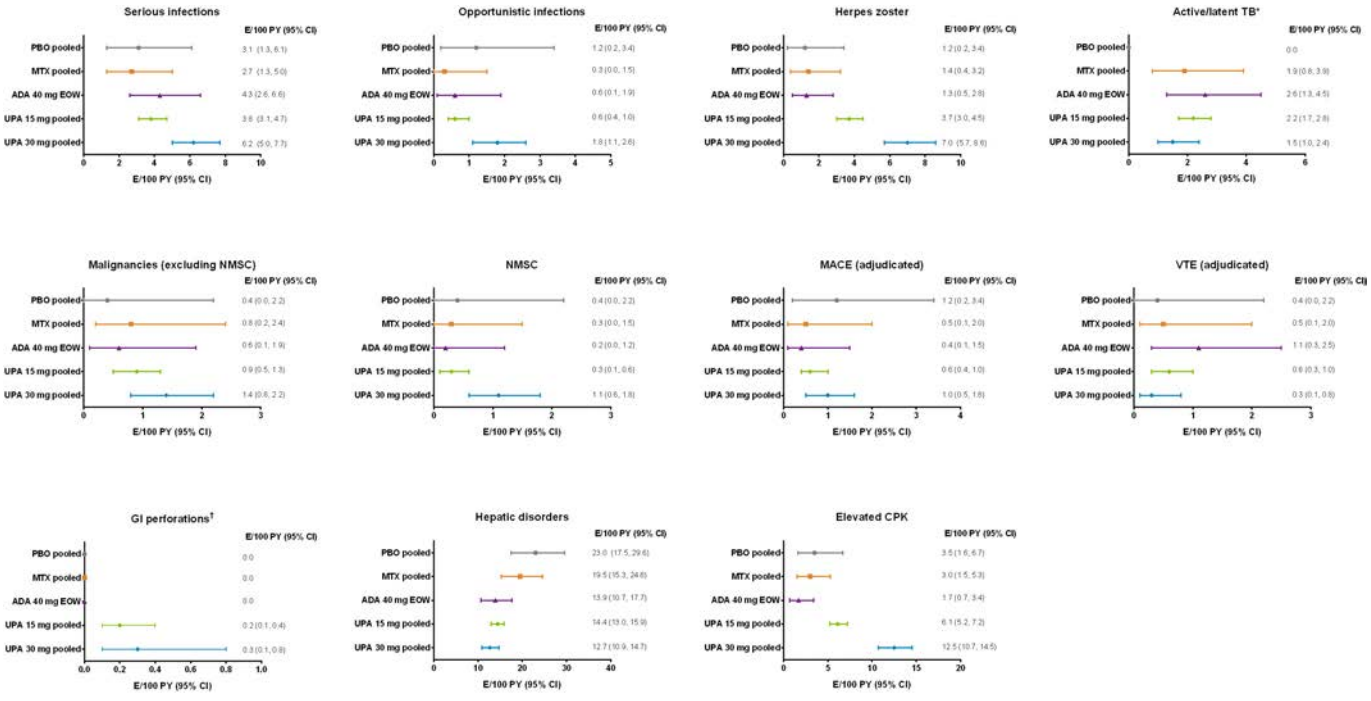

Figure 1 Event rates for AESIs. Additional details on AESIs are included in the online supplemental material. Incidence rates are shown in online supplemental figure S1.
*EAERs for active TB in E/100 PY: PBO, 0; MTX, 0; ADA, 0.2; UPA 15 mg, 0.1; UPA 30 mg, 0.1.
†Including all potential GI perforations; EAERs for confirmed GI perforations in E/100 PY: PBO, 0; MTX, 0; ADA, 0; UPA 15 mg, <0.1; UPA 30 mg, 0.3.
ADA, adalimumab; AESI, adverse event of special interest; CPK, creatine phosphokinase; E/100 PY, event per 100 patient-years; EAER, exposure-adjusted event rate; EOW, every other week; GI, gastrointestinal; MACE, major adverse cardiovascular event; MTX, methotrexate; NMSC, non-melanoma skin cancer; PBO, placebo; TB, tuberculosis; UPA, upadacitinib; VTE, venous thromboembolic event.

(bronchopulmonary aspergillosis, HZ disseminated and cryptococcal pneumonia) and none in the upadacitinib 30 mg group.

EAERs of HZ were greater with upadacitinib versus placebo, adalimumab and MTX (figure 1). Upadacitinib was associated with a higher risk of HZ than comparator groups (online supplemental table S4). Most HZ cases in the upadacitinib 15 and 30 mg groups were non-serious (96% and 93%) and involved a single dermatome (74% and 76%). There was one serious event of disseminated HZ, two non-serious ophthalmic HZ events and five non-serious postherpetic neuralgia events with upadacitinib 15 mg; and one non-serious event of disseminated HZ, one serious ophthalmic HZ event and six non-serious postherpetic neuralgia events with upadacitinib 30 mg. Both events of disseminated HZ had cutaneous involvement only. No deaths occurred as a result of HZ. Among patients treated with upadacitinib 15 mg, those who were Asian, aged ≥50 years or had a history of HZ had a higher risk of HZ (online supplemental table S5). At baseline, 2.6%–6.0% of patients across treatment groups reported a history of HZ vaccination. However, there was no evidence that prior HZ vaccination decreased HZ risk in upadacitinib-treated patients in this analysis.

EAERs of active/latent TB were similar between the upadacitinib, adalimumab and MTX groups; and no active/latent TB was reported in the placebo group (figure 1). Six patients had non-fatal active TB: three with upadacitinib 15 mg, two with 30 mg and one with adalimumab (online supplemental material). The overall rate of active TB was 0.1 E/100 PY (five events; exposure: 4020.1 PY) with upadacitinib.

The EAERs of NMSC and malignancies excluding NMSC were generally comparable across treatment groups, with the highest rates observed with upadacitinib 30 mg (figure 1). The age- and gender-adjusted SIR (95% CI) for non-NMSC malignancies with upadacitinib 15 mg, 1.05 (0.66 to 1.60), was within the expected range for the general US population. The observed types of non-NMSC malignancies reflected those expected in patients with RA (online supplemental material).

Nine potential GI perforations were identified with upadacitinib, occurring between 73 and 341 days after treatment initiation, and no events with placebo, MTX or adalimumab. Two of the five events (<0.1 E/100 PY) in the upadacitinib 15 mg group and all four events (0.3 E/100 PY) in the 30 mg group were assessed as GI perforations by the sponsor (online supplemental material).

EAERs of adjudicated MACE were comparable across treatment groups and did not increase over time with upadacitinib treatment (figure 1; online supplemental table S6; online supplemental figure S3). Dose-dependent increases in total cholesterol and low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were observed with upadacitinib treatment. LDL-C/HDL-C ratios remained constant throughout, with no apparent association of LDL-C levels with occurrence of MACE.

EAERs of adjudicated VTE were comparable across treatment groups (figure 1; online supplemental table S6). There was one fatal PE in the upadacitinib 15 mg group in a woman aged 55 years who developed PE after prolonged driving. There was no evidence of a dose relationship in VTE rate with upadacitinib nor a pattern of time-to-VTE-onset (23–1127 days of upadacitinib treatment). VTE in upadacitinib groups did not appear to be associated with increased platelet count (online supplemental material). There were two events of arterial thrombosis in the upadacitinib 30 mg group and none in the 15 mg group (online supplemental material).

Laboratory abnormalities

Slight decreases in haemoglobin were observed with upadacitinib 30 mg but not 15 mg (mean change from baseline at week 12 of −3.9 and −0.5 g/L, respectively, vs −1.5 g/L with placebo; online supplemental figure S4). The proportion of patients with grade 3/4 decreases in haemoglobin were generally similar between MTX and upadacitinib 15 mg and were highest with upadacitinib 30 mg (table 3).

The proportions of patients with grade 3 decreases in neutrophils were similar across treatment groups, with a greater proportion with upadacitinib 30 mg. Grade 4 decreases in neutrophils were rare. Mean lymphocyte counts increased over the first 36 weeks of treatment, followed by slight decreases afterwards. The proportions of patients with grade 3 decreases in lymphocytes were comparable between MTX and both upadacitinib groups and were higher than those for placebo and adalimumab. Grade 4 decreases were most frequent in the upadacitinib 30 mg group. There was no clear association between infectious events, including HZ, and decreased neutrophil or lymphocyte counts.

The proportions of patients experiencing grade 3 elevations in transaminases were similar between MTX and both upadacitinib groups and were greater than those in the placebo and adalimumab groups. Grade 4 increases occurred in few patients across treatment groups. Most transaminase elevations did not result in treatment discontinuation and resolved or were resolving regardless of whether upadacitinib was discontinued. There were no cases of probable drug-induced liver injury attributable to upadacitinib.

CPK elevations, including grade 3/4 increases, were more frequent with upadacitinib versus placebo, MTX or adalimumab (figure 1; table 3; online supplemental table S4). The greatest rise in CPK levels occurred for both upadacitinib doses at week 4 (50.1 and 74.3 U/L with 15 and 30 mg), after which CPK levels rose less markedly before plateauing around weeks 36 to 48. CPK elevations were typically asymptomatic; few led to discontinuation (two with upadacitinib 15 mg; three with 30 mg). One patient who received upadacitinib 30 mg had a serious event of rhabdomyolysis, with an alternative aetiology of influenza, which resulted in treatment interruption.

DISCUSSION

Based on an integrated analysis of the SELECT clinical trial programme, the overall safety profile of upadacitinib appeared comparable with other JAKis,[11–13] with no new or unexpected safety risks identified.

Treatment with upadacitinib was associated with an increased risk of HZ and CPK elevations versus placebo, MTX and adalimumab according to Cox regression analyses. Rates of deaths and malignancies with upadacitinib appeared consistent with expected rates from the general population. The serious infection rate observed with upadacitinib 15 mg was similar to that reported for other marketed RA therapies.[14–16] The rates of serious infections, HZ, CPK elevations and neutropaenia were higher for the unapproved upadacitinib 30 mg dose compared with the approved upadacitinib 15 mg dose.

Consistent with previously reported data for other JAKis,[11 12 17 18] HZ rates were higher with upadacitinib versus placebo, MTX and adalimumab, and higher HZ rates among upadacitinib were observed in older patients and those in Asia. The majority of HZ cases reported with upadacitinib were non-serious and involved a single dermatome. Few patients enrolled in the SELECT programme received HZ vaccination (limited to Zostavax) prior to randomisation. Information about the impact

Table 3 Proportion of patients with potentially clinically significant haematological and clinical chemistry values

				UPA all phase III long term	
	PBO pooled, n=1042	MTX pooled, n=530	ADA 40 mg EOW, n=579	Any UPA 15 mg once daily, n=2630	Any UPA 30 mg once daily, n=1204
n/N Obs (%)	Short-term data up to 12/14 weeks	Long-term MTX monotherapy Mean exposure: 36 weeks (data censored at rescue)	Long-term ADA Mean exposure: 42 weeks (includes UPA → ADA post-switch)	Long-term UPA (monotherapy or in combination with MTX/other csDMARDs) Mean exposures: 53 weeks (UPA 15 mg) and 59 weeks (UPA 30 mg)	
Haemoglobin (g/L)					
Grade 3 (70 to <80 or decreased 21 to <30)	23/1036 (2.2)	28/526 (5.3)	18/576 (3.1)	150/2622 (5.7)	133/1193 (11.1)
Grade 4 (<70 or decreased ≥30)	8/1036 (0.8)	12/526 (2.3)	6/576 (1.0)	39/2622 (1.5)	49/1193 (4.1)
Platelets ($\times 10^9$/L)					
Grade 3 (20 to <50)	0/1032	0/525	0/576	1/2619 (<0.1)	1/1192 (<0.1)
Grade 4 (<20)	0/1032	0/525	0/576	1/2619 (<0.1)	1/1192 (<0.1)
Neutrophils ($\times 10^9$/L)					
Grade 3 (0.5 to <1.0)	1/1036 (<0.1)	2/526 (0.4)	2/576 (0.3)	22/2622 (0.8)	28/1192 (2.3)
Grade 4 (<0.5)	0/1036	0/526	1/576 (0.2)	7/2622 (0.3)	2/1192 (0.2)
Lymphocytes ($\times 10^9$/L)					
Grade 3 (0.5 to <1.0)	119/1036 (11.5)	79/526 (15.0)	44/576 (7.6)	451/2622 (17.2)	250/1192 (21.0)
Grade 4 (<0.5)	7/1036 (0.7)	5/526 (1.0)	2/576 (0.3)	30/2622 (1.1)	29/1192 (2.4)
Leucocytes ($\times 10^9$/L)					
Grade 3 (1.0 to <2.0)	0/1036	0/526	1/576 (0.2)	9/2622 (0.3)	7/1193 (0.6)
Grade 4 (<1.0)	0/1036	0/526	0/576	0/2622	2/1193 (0.2)
ALT (U/L)					
Grade 3 (3.0 to <8.0× ULN)	13/1037 (1.3)	23/527 (4.4)	9/577 (1.6)	76/2620 (2.9)	37/1195 (3.1)
Grade 4 (>8.0× ULN)	2/1037 (0.2)	5/527 (0.9)	3/577 (0.5)	11/2620 (0.4)	6/1195 (0.5)
AST (U/L)					
Grade 3 (3.0 to <8.0× ULN)	6/1036 (0.6)	13/527 (2.5)	6/577 (1.0)	46/2620 (1.8)	17/1195 (1.4)
Grade 4 (>8.0× ULN)	1/1036 (<0.1)	1/527 (0.2)	4/577 (0.7)	7/2620 (0.3)	5/1195 (0.4)
CPK (U/L)					
Grade 3 (>5.0 to 10.0× ULN)	3/1037 (0.3)	2/527 (0.4)	1/577 (0.2)	38/2620 (1.5)	22/1196 (1.8)
Grade 4 (>10.0× ULN)	0/1037	0/527	1/577 (0.2)	10/2620 (0.4)	11/1196 (0.9)

N Obs indicates the number of patients with baseline and post-baseline values for the respective parameters.
ADA, adalimumab; ALT, alanine transaminase; AST, aspartate transaminase; CPK, creatine phosphokinase; csDMARD, conventional synthetic disease-modifying antirheumatic drug; EOW, every other week; MTX, methotrexate; PBO, placebo; ULN, upper limit of normal; UPA, upadacitinib.

of newer inactivated HZ vaccines (although not yet available worldwide) on the risk of HZ among patients receiving upadacitinib and other JAKis is necessary to inform clinical practice.

VTE is an emerging AESI among patients receiving JAKis,[2 3 19–22] but longer-term data are needed to characterise the risk of VTE with JAKi therapy. Patients with RA are at increased risk of VTE (incidence rates 0.3–0.8/100 PY)[23 24] compared with the general population, with a 2.4-fold increased rate.[25] In this analysis, the rates of adjudicated VTE were similar across both doses of upadacitinib, placebo, adalimumab and MTX, with no evidence of a dose relationship with upadacitinib treatment. In view of the increased risk of VTE and underlying VTE risk factors among patients with RA, patients should be promptly evaluated for signs and symptoms of possible thrombosis and appropriately treated during JAKi therapy.

Patients with RA receiving anti-interleukin 6 (IL-6) receptor therapy are at increased risk of GI perforation, with one study reporting a lower GI perforation rate of 0.27 E/100 PY with the IL-6 receptor inhibitor tocilizumab.[26 27] Although JAKis also inhibit IL-6 signalling,[28 29] GI perforations with upadacitinib 15 mg (0.08 E/100 PY) were observed at similar rates to tumour necrosis factor inhibitors (0.05 E/100 PY) and other JAKis (0.04–0.10 E/100 PY).[13 27 29] Upadacitinib 30 mg had higher rates of GI perforations (0.29 E/100 PY), although this was based on a limited number of events.

Decreases in haemoglobin and neutrophils, and increases in transaminase and CPK, observed with upadacitinib, were consistent with laboratory changes observed with other JAKis.[12 13] In vitro data suggest that JAKi-associated increase in CPK may represent restoration of myoblast differentiation.[30] Most laboratory abnormalities were resolved, and most patients experiencing them were able to remain on the study drug. Although engineered for increased JAK1 selectivity,[1] the effects of upadacitinib on parameters such as haemoglobin suggest that upadacitinib (particularly the unapproved 30 mg dose) may have some effects on JAK2. However, maximal efficacy of upadacitinib was achieved at the 15 mg dose with comparable safety to the approved doses of other JAKis, with no additional efficacy benefit observed with the 30 mg dose.[2 3 31] In contrast, the use of less selective JAKis at higher doses is associated with improved efficacy but is limited due to increased safety risks.[21 32–35]

The limited placebo exposure time prevented the placebo-controlled analysis of longer-term safety. However, longer-term controlled data versus MTX monotherapy (SELECT-EARLY) and adalimumab (SELECT-COMPARE) offer the opportunity to compare the safety profile of upadacitinib to other RA therapies.

As patients were not allowed to change upadacitinib doses, this allowed an unadulterated comparison of the safety profile of the upadacitinib 15 and 30 mg doses. While upadacitinib monotherapy was well tolerated with comparable safety to the overall upadacitinib population, further analyses are required to identify any differences in long-term safety between upadacitinib administered as monotherapy and in combination with csDMARDs. Despite a robust trial programme, the data remain limited by exposures to date, with ongoing monitoring still underway. As these data are from RCTs with specific eligibility criteria and clear follow-up protocols, this may limit the generalisability of these results to clinical practice. Monitoring by a specialist is recommended for oral treatments such as upadacitinib, as with all antirheumatic therapies.

Based on integrated data from five phase III RCTs, with 3834 patients and 4020.1 PY of exposure, no new safety risks emerged with upadacitinib compared with other approved JAKis. These results support an acceptable safety profile of upadacitinib 15 mg once daily for the treatment of moderately to severely active RA. Follow-up of patients receiving upadacitinib will continue in long-term extensions of clinical trials and postmarketing studies.

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Ethics approval Studies were conducted in compliance with the Declaration of Helsinki, International Conference on Harmonisation of Technical Regulations for Pharmaceuticals for Human Use guidelines, and applicable local country regulations. All study-related documents were approved by independent ethics committees and institutional review boards. All patients provided written, informed consent.

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Data availability statement Data are available on reasonable request. AbbVie is committed to responsible data sharing regarding the clinical trials we sponsor. This includes access to anonymised, individual and trial-level data (analysis datasets), as well as other information (eg, protocols and Clinical Study Reports), provided the trials are not part of an ongoing or planned regulatory submission. This includes requests for clinical trial data for unlicensed products and indications. These clinical trial data can be requested by any qualified researchers who engage in rigorous, independent scientific research and will be provided following review and approval of a research proposal and statistical analysis plan and execution of a Data Sharing Agreement. Data requests can be submitted at any time, and the data will be accessible for 12 months, with possible extensions considered. For more information on the process or to submit a request, visit https://www.abbvie.com/ourscience/clinicaltrials/clinicaltrialsdataandinformationsharing/dataandinformationsharingwithqualifiedresearchers.html.

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CLINICAL SCIENCE

Upadacitinib for psoriatic arthritis refractory to biologics: SELECT-PsA 2

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ABSTRACT

Background Upadacitinib is a Janus kinase inhibitor under evaluation for the treatment of psoriatic arthritis (PsA). We evaluated upadacitinib in patients with PsA and prior inadequate response or intolerance to at least one biologic disease-modifying antirheumatic drug (DMARD).

Methods In this 24-week randomised, placebo-controlled, double-blind, phase 3 trial, 642 patients were randomised (2:2:1:1) to once per day upadacitinib 15 mg or 30 mg, placebo followed by upadacitinib 15 mg or placebo followed by upadacitinib 30 mg at week 24. The primary endpoint was the proportion of patients achieving American College of Rheumatology (ACR) 20 response at week 12. Achievement of minimal disease activity (MDA) was assessed at week 24. Treatment-emergent adverse events are reported for all patients who received at least one dose of trial drug.

Results At week 12, significantly more patients receiving upadacitinib 15 mg and 30 mg versus placebo achieved ACR20 (56.9% and 63.8% vs 24.1%; p<0.001 for both comparisons). At week 24, MDA was achieved by more upadacitinib 15 mg-treated (25.1%) and 30 mg-treated patients (28.9%) versus placebo (2.8%; p<0.001 for both comparisons). Generally, the rates of treatment-emergent adverse events were similar with placebo and upadacitinib 15 mg and higher with upadacitinib 30 mg at week 24. Rates of serious infections were 0.5%, 0.5% and 2.8% with placebo, upadacitinib 15 mg and upadacitinib 30 mg, respectively.

Conclusion In this trial of patients with active PsA who had inadequate response or intolerance to at least one biologic DMARD, upadacitinib 15 mg and 30 mg was more effective than placebo over 24 weeks in improving signs and symptoms of PsA.

Clinical trial registration number NCT03104374

Key messages

What is already known about this subject?

- Despite the availability of biologic disease-modifying antirheumatic drugs (DMARDs) in psoriatic arthritis, only a small proportion of patients achieve the recommended target of minimal disease activity; therefore, additional treatment options are needed.

What does this study add?

- In this phase 3 trial of patients with psoriatic arthritis refractory or intolerant to biologic DMARDs, greater efficacy was demonstrated for once per day upadacitinib 15 mg and 30 mg versus placebo for clinical manifestations of psoriatic arthritis including musculoskeletal symptoms (peripheral arthritis, enthesitis, dactylitis and spondylitis), psoriasis, physical function, pain, fatigue and quality of life.

How might this impact on clinical practice or future developments?

- Once per day upadacitinib 15 mg and 30 mg demonstrated significant efficacy in patients with psoriatic arthritis refractory or intolerant to prior biologic DMARD therapy in the 24-week placebo-controlled period of this study.
- Efficacy was observed as early as week 2. Efficacy was demonstrated in all measures of the various core clinical domains of psoriatic arthritis. More upadacitinib-treated patients achieved a state of minimal disease activity.
- The safety findings are consistent with the known safety profile of upadacitinib observed in rheumatoid arthritis; no new safety risks have been identified.

INTRODUCTION

Psoriatic arthritis (PsA) is a systemic inflammatory disease with heterogeneous clinical manifestations such as plaque psoriasis, arthritis, dactylitis and enthesitis. Current treatment guidelines for PsA vary, recommending conventional synthetic disease-modifying antirheumatic drugs (DMARDs) such as methotrexate as initial therapy, followed by biologic DMARDs (tumour necrosis factor inhibitors (TNFi), interleukin-12/23 or interleukin-17 inhibitors) or targeted synthetic DMARDs, such as apremilast or tofacitinib, or TNFi initially, followed by other approved therapies.[1–3] While multiple therapeutic choices are now available, additional options are needed as under one-third achieving minimal disease activity (MDA) in most placebo-controlled trials.[4–9]

Upadacitinib is an oral, reversible Janus kinase inhibitor (JAKi) with selectivity for JAK1 over JAK2, JAK3 and tyrosine kinase 2,[10] approved for the treatment of rheumatoid arthritis based on five phase 3 studies.[11–15] Improvements in multiple composite measures, including stringent measures

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of low disease activity and remission, as well as patient-reported outcomes such as morning stiffness and pain, after treatment with upadacitinib 15 mg once per day, in patients with rheumatoid arthritis who failed biologic DMARDs were similar to those in patients who had failed conventional synthetic DMARDs or methotrexate.[11–15] We report the results of the SELECT-PsA 2 trial, a randomised phase 3 trial of upadacitinib in patients with active PsA who have had an inadequate response or intolerance to at least one biologic DMARD.

PATIENTS AND METHODS

Patients

Eligible patients were 18 years of age or older with active PsA, had a diagnosis of PsA with symptom onset for ≥6 months, fulfilled the Classification Criteria for Psoriatic Arthritis (CASPAR),[16] had historical or current plaque psoriasis, ≥3 swollen joints (of 66) and ≥3 tender joints (of 68) at screening and at baseline, and an inadequate response or intolerance to at least one biologic DMARD. Patients were excluded if they had previous exposure to a JAKi, had a history of fibromyalgia, had arthritis with onset prior to age 17 years or had diagnosis of inflammatory joint disease other than PsA. Online supplemental section 2 provides a complete list of eligibility criteria.

Trial design

A multicentre, randomised, double-blind, phase 3 placebo-controlled trial at 123 sites in 17 countries has been ongoing since April 2017, conducted per the International Conference on Harmonization guidelines, applicable regulations and guidelines governing clinical trial conduct, and the Declaration of Helsinki. All patients provided written informed consent.

Randomisation and treatments

An Interactive Response Technology system was used to assign patients, in a 2:2:1:1 ratio, to one of the following regimens: upadacitinib 15 mg once per day, upadacitinib 30 mg once per day or placebo switched to either upadacitinib 15 mg or 30 mg once per day at week 24. Stable background treatment of non-steroidal anti-inflammatory drugs, corticosteroids (equivalent to ≤10 mg/day prednisone) and ≤2 non-biologic DMARDs were permitted; background therapy was not required. Concomitant biologic therapies were prohibited. Concomitant treatments specifically for psoriasis (eg, topicals, light therapy, retinoids) were not permitted until after week 16.

Starting at week 16, patients who did not achieve ≥20% improvement in tender and swollen joint counts compared with baseline at weeks 12 and 16 had background medication(s) adjusted or initiated. Starting at week 36, patients who did not achieve ≥20% improvement in tender and swollen joint counts compared with baseline at two consecutive visits were discontinued from the study. All patients who completed week 56 were eligible to remain in the extension period of the trial for up to 3 years of trial participation in total (online supplemental figure 1).

Randomisation was stratified by extent of psoriasis (≥3%/<3% body surface area (BSA)), current use of at least 1 DMARD and number of prior biologic DMARDs failed (1 versus >1). The trial is ongoing; data presented include the 24-week placebo-controlled period during which investigators and the sponsor were blinded to treatment assignment.

Assessments

The primary endpoint was the proportion of patients achieving American College of Rheumatology (ACR) 20 response at week 12. Multiplicity-controlled secondary endpoints for each dose of upadacitinib versus placebo included: at week 12, change from baseline in Health Assessment Questionnaire-Disability Index (HAQ-DI)[17]; Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F) score[18] and Short Form Health Survey questionnaire (SF-36) Physical Component Summary (PCS) score[19]; at week 16, proportion of patients achieving a Static Investigator Global Assessment (sIGA) of Psoriasis of 0 or 1 and at least a 2 point improvement from baseline (sIGA 0/1) for patients with baseline sIGA ≥2[20]; Psoriasis Area Severity Index (PASI)75 response for patients with ≥3% BSA-psoriasis at baseline[21]; and change from baseline in Self-Assessment of Psoriasis Symptoms (SAPS) Questionnaire[22]; and at week 24, proportion of patients achieving MDA.[23] Additional key secondary efficacy endpoints included ACR50/70 response at week 12 and ACR20 response at week 2. Exploratory endpoints were proportion of patients achieving PASI90/100 response, resolution of enthesitis (defined by Leeds Enthesitis Index (LEI)=0) for patients with baseline LEI >0[24] and Spondyloarthritis Research Consortium of Canada Enthesitis Index ((SPARCC)=0) for patients with baseline SPARCC Enthesitis Index>0[25] and resolution of dactylitis (defined by Leeds Dactylitis Index (LDI)=0) for patients with baseline LDI>0,[26] and change from baseline in individual components of ACR response, Disease Activity in Psoriatic Arthritis (DAPSA) score,[27] and morning stiffness (mean of Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) questions 5 and 6). All outcomes are defined in online supplemental table S1.

Adverse events (AEs) and clinical laboratory testing are reported through week 24. An independent, external Cardiovascular Adjudication Committee blindly adjudicated deaths and cardiovascular events per predefined event definitions. An internal Gastrointestinal (GI) Perforation Adjudication Committee blindly adjudicated reported GI perforation events as stated in the GI perforation charter.

Statistical analysis

Efficacy analyses were conducted on all randomised patients who had received at least one dose of trial drug. A sample size of 630 patients was planned to provide at least 90% power for a 20% difference in ACR20 response rate (assuming a placebo ACR20 response rate of 20%) and for most of the key secondary endpoints (online supplemental section 3).

The overall type I error rate of primary and ranked key secondary endpoints was strongly controlled using a graphical multiple testing procedure starting with the primary endpoint using $\alpha/2$ for each dose followed by a prespecified α transfer path, which included downstream transfer along the endpoint sequence within each dose as well as cross-dose transfer (online supplemental figure S2). Once an endpoint was claimed significant, its significance level was transferred to subsequent endpoint(s) following the prespecified order and weight. All other outcomes were prespecified in the protocol and statistical analysis plan without adjustment for multiplicity.

The Cochran-Mantel-Haenszel test adjusting for the stratification factor of current DMARD use (yes/no) was used to compare treatment binary endpoints. Non-responder imputation was used for missing data handling, where patients with missing data at the specified week or those who prematurely discontinued the trial drug were considered non-responders. For continuous endpoints, analyses were conducted using the mixed-effects model repeated measures analysis based on observed longitudinal data, which included the fixed effects of treatment, visit,

treatment-by-visit interaction, the stratification factor of current DMARD use (yes/no) and the continuous fixed covariate of baseline measurement. An unstructured variance covariance matrix was used. Patients who met the discontinuation criteria were considered non-responders.

RESULTS

Patients

Of the 642 patients randomised, 641 received at least one dose of trial drug (placebo, n=212; upadacitinib 15 mg, n=211; upadacitinib 30 mg, n=218; online supplemental figure S3). Overall,

Table 1 Demographics and characteristics at baseline

	Placebo N=212	Upadacitinib 15 mg QD N=211	Upadacitinib 30 mg QD N=218
Female, n (%)	120 (56.6)	113 (53.6)	115 (52.8)
Age (years)	54.1±11.5	53.0±12.0	53.0±11.9
Race, n (%)			
White	186 (87.7)	183 (86.7)	196 (89.9)
Black or African American	7 (3.3)	5 (2.4)	5 (2.3)
American Indian/Alaska Native	0	3 (1.4)	0
Native Hawaiian or other Pacific Islander	1 (0.5)	1 (0.5)	1 (0.5)
Asian	17 (8.0)	19 (9.0)	16 (7.3)
Multiple	1 (0.5)	0	0
Duration of PsA symptoms (years)	14.6±11.7	12.2±8.8	13.3±10.8
Duration since PsA diagnosis (years)	11.0±10.3	9.6±8.4	9.7±8.7
Number of prior failed biologic DMARDs, n (%)			
0*	18 (8.5)	16 (7.6)	17 (7.8)
1	135 (63.7)	126 (59.7)	130 (59.6)
2	35 (16.5)	35 (16.6)	46 (21.1)
≥3	24 (11.3)	34 (16.1)	25 (11.5)
Monotherapy, n (%)	112 (52.8)	113 (53.6)	120 (55.0)
Any non-biologic DMARD at baseline, n (%)			
MTX alone	75 (35.4)	74 (35.1)	73 (33.5)
MTX+another non-biologic DMARD	7 (3.3)	6 (2.8)	5 (2.3)
Non-biologic DMARD other than MTX	18 (8.5)	18 (8.5)	20 (9.2)
MTX dose for patients with concomitant MTX alone at baseline (mg/week)			
Mean	16.26	15.06	16.76
Median	17.5	15.0	17.5
Steroid use at baseline, n (%)	24 (11.3)	22 (10.4)	13 (6.0)
NSAID use at baseline, n (%)	125 (59.0)	124 (58.8)	129 (59.2)
RF status positive, n (%)	6 (2.8)	11 (5.2)	8 (3.7)
Anti-CCP status positive, n (%)	10 (4.7)	7 (3.3)	5 (2.3)
TJC68	25.3±17.6	24.9±17.3	24.2±15.9
SJC66	12.0±8.9	11.3±8.2	12.9±9.4
hs-CRP >ULN† (mg/L), n (%)	121 (57.1)	126 (59.7)	128 (58.7)
hs-CRP (mg/L)	10.4±18.5	11.2±18.5	10.5±17.2
HAQ-DI	1.23±0.7	1.10±0.6	1.19±0.7
Patient's assessment of pain (NRS 0–10)	6.6±2.1	6.4±2.1	6.2±2.2
BSA-psoriasis ≥3%, n (%)	131 (61.8)	130 (61.6)	131 (60.1)
PASI (for baseline BSA-Ps ≥3%)	11.7±11.4	10.1±9.2	8.9±9.1
BSA-psoriasis >0%, n (%)	198 (93.4)	202 (95.7)	202 (92.7)
BSA-psoriasis (for baseline >0%)	12.8±18.4	10.0±15.7	10.0±15.8
sIGA of psoriasis score, n (%)			
0	17 (8.0)	9 (4.3)	16 (7.3)
1	32 (15.1)	31 (14.7)	38 (17.4)
2	59 (27.8)	82 (38.9)	78 (35.8)
3	88 (41.5)	78 (37.0)	77 (35.3)
4	16 (7.5)	11 (5.2)	9 (4.1)
Presence of enthesitis			
LEI >0, n (%)	144 (67.9)	133 (63.0)	152 (69.7)
SPARCC Enthesitis Index >0, n (%)	173 (81.6)	172 (81.5)	179 (82.1)
Presence of dactylitis (defined as LDI >0), n (%)	64 (30.2)	55 (26.1)	50 (22.9)
Morning stiffness score‡	5.8±2.5	6.0±2.5	5.7±2.7

Values are mean±SD unless noted.
*Patients with intolerance but not inadequate response to a biologic DMARD.
†ULN=2.87 mg/L.
‡Morning stiffness score is the mean of BASDAI questions 5 and 6.
Anti-CCP, anti-cyclic citrullinated peptide; ASDAS, Ankylosing Spondylitis Disease Activity Score; BASDAI, Bath Ankylosing Spondylitis Disease Activity Index; BSA, body surface area; DMARD, disease-modifying antirheumatic drug; HAQ-DI, Health Assessment Questionnaire-Disability Index; hs-CRP, high-sensitivity C-reactive protein; LDI, Leeds Dactylitis Index; LEI, Leeds Enthesitis Index; MTX, methotrexate; NRS, Numeric Rating Scale; NSAID, non-steroidal anti-inflammatory drug; PASI, Psoriasis Area Severity Index; Ps, psoriasis; PsA, psoriatic arthritis; QD, once per day; RF, rheumatoid factor; sIGA, Static Investigator Global Assessment; SJC, swollen joint count; SPARCC, Spondyloarthritis Research Consortium of Canada; TJC, tender joint count; ULN, upper limit normal.

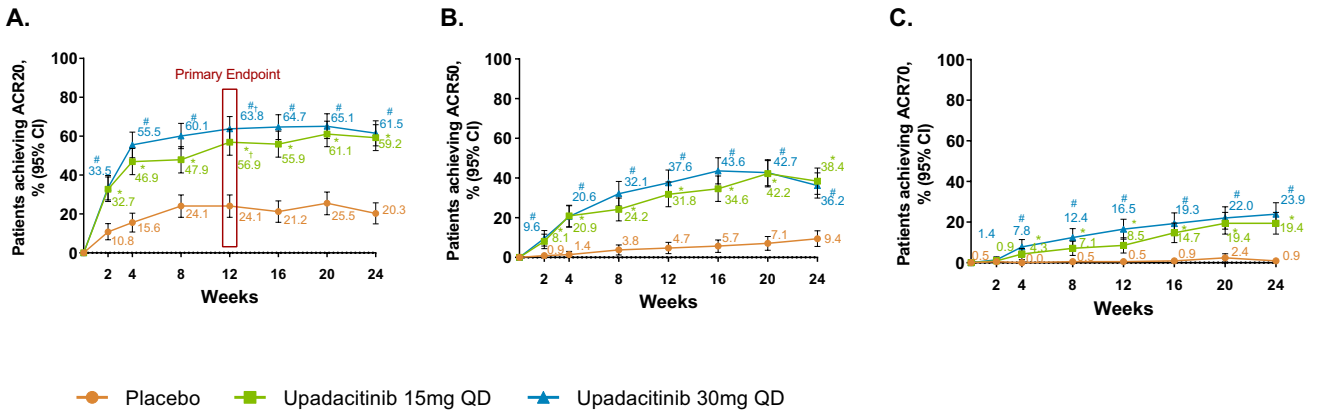

Figure 1 Proportions of patients achieving (A) ACR20 (B) ACR50 and (C) ACR70 response over 24 weeks (NRI). *p≤0.05 for comparison of upadacitinib 15 mg once per day versus placebo; #p≤0.05 for comparison of upadacitinib 30 mg once per day versus placebo; †Significant in the multiplicity-controlled analysis. ACR20/50/70, 20%/50%/70% improvement in American College of Rheumatology criteria. Results are based on non-responder imputation. 95% CIs for response rate were calculated based on normal approximation to the binominal distribution. 95% CIs for response rate difference were calculated based on normal approximation. Nominal p value was constructed using Cochran-Mantel-Haenszel test adjusted for the main stratification factor of current disease-modifying antirheumatic drug use (yes/no).

543 (84.6%) patients completed week 24 on trial drug. Baseline demographics, disease characteristics and disease severity were generally balanced across treatment arms (table 1).

Efficacy

At week 12, significantly more patients achieved an ACR20 response in the upadacitinib 15 mg and 30 mg arms versus the placebo arm (56.9%, 63.8% and 24.1%, respectively; p<0.001 for both upadacitinib arms vs placebo; figure 1, tables 2 and 3). By week 2, ACR20 response was achieved by more upadacitinib 15 mg-treated and 30 mg-treated patients (nominal p<0.001). The proportion of patients with ACR20 response continued to increase over time in both treatment groups with the plateau of response observed at week 12 for the upadacitinib 30 mg group, whereas the proportion of patients with ACR20 response in the upadacitinib 15 mg group increased through week 20, approximating the response rate in the 30 mg dose group by the end of the placebo-controlled period. Subgroup analyses for ACR20 based on demographic and baseline disease characteristics are shown in online supplemental figure S4. Response rates for upadacitinib 15 mg and upadacitinib 30 mg were 44.9% and 64.8% in the subgroup of patients who had failed >1 biologic DMARD and 55.8% and 66.7% in the subgroup of patients that were on monotherapy; these responses were similar to results in the overall population. Additionally, improvements in ACR50 and ACR70 were observed with both upadacitinib doses versus placebo at week 12 (figure 1 and table 3). From week 2 through week 24, improvement from baseline in all components of ACR response was observed with upadacitinib 15 mg or 30 mg versus placebo (online supplemental figure S5).

The 15 mg and 30 mg doses of upadacitinib showed greater improvement versus placebo with respect to all key secondary endpoints (table 2 and online supplementary material).

By week 12 and through week 24, improvement in psoriasis was observed with both upadacitinib doses versus placebo as measured by PASI75/90/100 (at week 16, p<0.001 for PASI75 and nominal p<0.001 for PASI90/100; nominal p<0.001 for all the other time points; figure 2) and sIGA 0/1 (p<0.001 at week 16; nominal p<0.001 for weeks 12 and 24; online supplemental figure S6). The changes from baseline in SAPS were greater for both upadacitinib arms versus placebo at weeks 16 (p<0.001) and 24 (nominal p<0.001; online supplemental figure S7).

Improvements in physical function were observed in patients on both doses of upadacitinib versus placebo based on the mean change from baseline in HAQ-DI from week 2 through week 24 (p<0.001 at week 12) and SF-36 PCS at weeks 12 (p<0.001) and 24 (nominal p<0.001; online supplemental figure S8). Patients on both doses of upadacitinib reported improvements in fatigue as assessed by FACIT-F versus placebo at weeks 12 (p<0.001) and 24 (nominal p<0.001; online supplemental figure S9). Mean improvements from baseline in morning stiffness were observed at weeks 12 and 24 (nominal p<0.001; online supplemental figure S10).

Resolution of enthesitis using both the LEI and the SPARCC enthesitis index and of dactylitis was reported in a higher proportion of patients on either dose of upadacitinib versus placebo from week 12 to week 24 (nominal p<0.001; table 3 and online supplemental figure S11).

A higher proportion of patients receiving either dose of upadacitinib achieved MDA through week 24 versus placebo (p<0.001 at week 24; nominal p<0.001 for weeks 12 and 16; figure 3).

Mean changes from baseline in the DAPSA score were greater with both upadacitinib doses versus placebo through week 24 (nominal p<0.001 for all time points; figure 4).

Safety

Through week 24, the rate of overall treatment-emergent AEs (TEAEs) was higher in the upadacitinib 30 mg arm and rates of serious AEs (SAEs) and TEAEs leading to discontinuation of trial drug were higher with both upadacitinib doses versus placebo (table 4).

The most commonly reported TEAEs were upper respiratory tract infection and nasopharyngitis in upadacitinib-treated patients (online supplemental table S3). SAEs were reported in 4 (1.9%) patients on placebo, 12 (5.7%) on upadacitinib 15 mg and 18 (8.3%) on upadacitinib 30 mg. Serious infections occurred in one patient each (0.5%) on placebo and upadacitinib 15 mg and six (2.8%) patients on upadacitinib 30 mg. Pneumonia was the most frequently reported serious infection (one patient on

Table 2 Primary and multiplicity-controlled efficacy endpoints

	Placebo	Upadacitinib 15 mg QD	Upadacitinib 30 mg QD
ACR20 response at week 12			
N	212	211	218
n (%)	51 (24.1)	120 (56.9)	139 (63.8)
Response rate mean difference vs placebo (95% CI)		32.8 (24.0 to 41.6)	39.7 (31.1 to 48.3)
P value		<0.001	<0.001
HAQ-DI change from baseline at week 12			
N	180	199	204
LS mean (95% CI)	−0.10 (−0.16 to −0.03)	−0.30 (−0.37 to −0.24)	−0.41 (−0.47 to −0.35)
LS mean difference (95% CI)		−0.21 (−0.30 to −0.12)	−0.31 (−0.40 to −0.22)
P value		<0.001	<0.001
FACIT-F score change from baseline at week 12			
N	184	201	206
LS mean (95% CI)	1.3 (0.1 to 2.5)	5.0 (3.8 to 6.1)	6.1 (4.9 to 7.2)
LS mean difference (95% CI)		3.7 (2.0 to 5.4)	4.8 (3.1 to 6.4)
P value		<0.001	<0.001
SF-36 PCS score change from baseline at week 12			
N	185	201	206
LS mean (95% CI)	1.6 (0.6 to 2.7)	5.2 (4.1 to 6.2)	7.1 (6.1 to 8.1)
LS mean difference (95% CI)		3.5 (2.1 to 5.0)	5.4 (4.0 to 6.9)
P value		<0.001	<0.001
Proportion of patients achieving sIGA of psoriasis score of 0 or 1 and at least a 2-point improvement from baseline at week 16 (for patients with baseline sIGA ≥2)			
N	163	171	164
n (%)	15 (9.2)	63 (36.8)	66 (40.2)
Response rate mean difference (95% CI)		27.6 (19.2 to 36.1)	31.0 (22.3 to 39.8)
P value		<0.001	<0.001
PASI75 response at week 16 (for patients with ≥3% BSA-psoriasis at baseline)			
N	131	130	131
n (%)	21 (16.0)	68 (52.3)	74 (56.5)
Response rate mean difference (95% CI)		36.3 (25.6 to 46.9)	40.5 (29.9 to 51.0)
P value		<0.001	<0.001
Self-Assessment of Psoriasis Symptoms score change from baseline at week 16			
N	182	191	200
LS mean (95% CI)	−1.5 (−4.7 to 1.8)	−24.4 (−27.5 to −21.2)	−29.7 (−32.8 to −26.6)
LS mean difference (95% CI)		−22.9 (−27.4 to −18.4)	−28.2 (−32.7 to −23.8)
P value		<0.001	<0.001
Proportion of patients achieving minimal disease activity at week 24			
N	212	211	218
n (%)	6 (2.8)	53 (25.1)	63 (28.9)
Response rate mean difference (95% CI)		22.3 (16.0 to 28.6)	26.1 (19.7 to 32.5)
P value		<0.001	<0.001

ACR20, 20% improvement in American College of Rheumatolog criteria; BSA, body surface area; FACIT-F, Functional Assessment of Chronic Illness Therapy-Fatigue; HAQ-DI, Health Assessment Questionnaire-Disability Index; LS, least squares; PASI75, 75% improvement in Psoriasis Area and Severity Index ; QD, once per day; SF36-PCS, 36-Item Short Form Health Survey Physical Component Summary score; sIGA, Static Investigator Global Assessment.

Table 3 Additional secondary efficacy endpoints

	Placebo	Upadacitinib 15 mg QD	Upadacitinib 30 mg QD
ACR50 response rate at week 12			
N	212	211	218
n (%)	10 (4.7)	67 (31.8)	82 (37.6)
Response rate mean difference (95% CI)		27.0 (20.1 to 33.9)	32.9 (25.9 to 39.9)
Nominal p value		<0.001	<0.001
ACR70 response rate at week 12			
N	212	211	218
n (%)	1 (0.5)	18 (8.5)	36 (16.5)
Response rate mean difference (95% CI)		8.1 (4.2 to 11.9)	16.0 (11.0 to 21.1)
Nominal p value		<0.001	<0.001
ACR20 response rate at week 2			
N	212	211	218
n (%)	23 (10.8)	69 (32.7)	73 (33.5)
Response rate mean difference (95% CI)		21.9 (14.3 to 29.4)	22.6 (15.1 to 30.2)
Nominal p value		<0.001	<0.001
Exploratory endpoints			
Resolution of enthesitis at week 12 (defined as LEI=0)			
N	144	133	152
n (%)	29 (20.1)	52 (39.1)	73 (48.0)
Response rate difference (95% CI)		19.0 (8.4 to 29.5)	27.9 (17.6 to 38.2)
Nominal p value		<0.001	<0.001
Resolution of dactylitis at week 12 (defined as LDI=0)			
N	64	55	50
n (%)	23 (35.9)	35 (63.6)	38 (76.0)
Response rate difference (95% CI)		27.7 (10.4 to 45.0)	40.1 (23.4 to 56.7)
Nominal p-value		<0.001	<0.001

ACR20/50/70, 20%/50%/70% improvement in American College of Rheumatology criteria; LDI, Leeds Dactylitis Index; LEI, Leeds Enthesitis Index; QD, once per day.

upadacitinib 15 mg and three patients on upadacitinib 30 mg). Up to week 24, treatment-emergent opportunistic infections, excluding tuberculosis and herpes zoster, included one event each of candidiasis of the trachea and oropharyngeal candidiasis, both with upadacitinib 30 mg. Herpes zoster was reported in two, three and eight patients in the placebo, upadacitinib 15 mg and 30 mg arms, respectively; none of the cases were serious. One patient on upadacitinib 15 mg and two patients on upadacitinib 30 mg had cutaneous disseminated herpes zoster. No cases of herpes zoster with central nervous system involvement were observed. Hepatic disorders were reported in 3 (1.4%) patients on placebo, 4 (1.9%) on upadacitinib 15 mg and 18 (8.3%) on upadacitinib 30 mg; most were asymptomatic liver enzyme elevations.

Malignancies were reported in three patients in each upadacitinib arm (upadacitinib 15 mg: one basal cell carcinoma, one prostate cancer, one rectal cancer; upadacitinib 30 mg: one rectal adenocarcinoma, one ovarian and endometrial cancer, and one basal cell carcinoma) and none in the placebo arm. The time to event onset for these malignant events was <6 months.

There were no adjudicated gastrointestinal perforations reported through week 24. One case of major adverse cardiovascular event (MACE; 0.5%, non-fatal myocardial infarction) and one case of venous thromboembolic event (VTE; 0.5%; pulmonary embolism) were reported in the upadacitinib 15 mg arm; both patients had at least one risk factor (eg, obesity, hypertension or hypercholesterolaemia) for MACE or VTE, respectively.

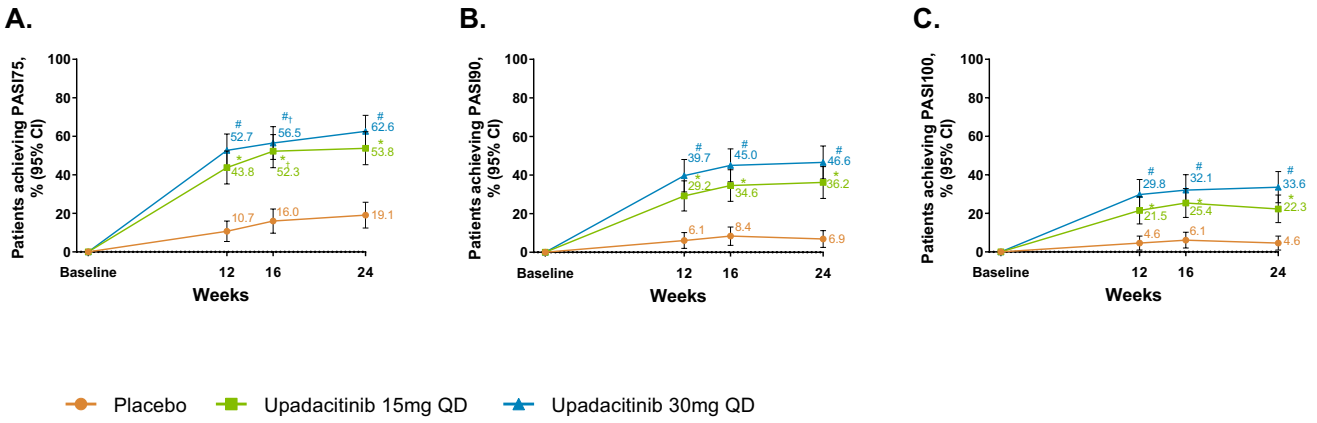

Figure 2 Proportion of patients achieving (A) PASI75, (B) PASI90 and (C) PASI100. response over 24 weeks. *p≤0.05; for upadacitinib 15 mg QD versus placebo; #p≤0.05; for upadacitinib 30 mg QD versus placebo; †significant in the multiplicity-controlled analysis. After week 16, assessments have been performed. Patients may use concomitant treatments specifically for psoriasis per investigator judgement. Results are based on non-responder imputation. 95% CIs for response rate were calculated based on normal approximation to the binominal distribution. 95% CIs for response rate difference were calculated based on normal approximation. Nominal p value was constructed using Cochran-Mantel-Haenszel test adjusted for the main stratification factor of current disease-modifying antirheumatic drug use (yes/no). PASI75/90/100, 75%/90%/100% improvement in Psoriasis Area Severity Index; QD, once per day.

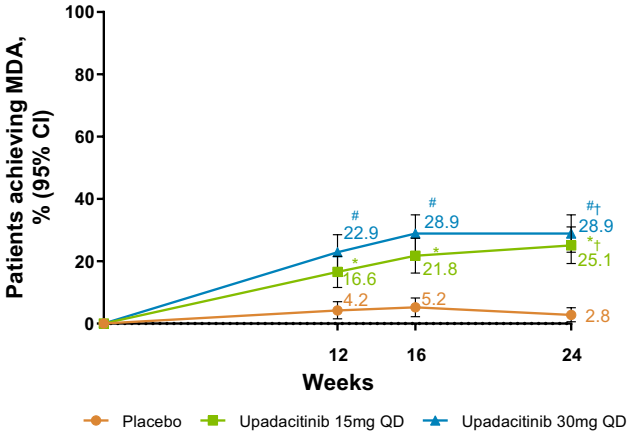

Figure 3 Proportion of patients achieving minimal disease activity (MDA) over 24 weeks. *p≤0.05; for upadacitinib 15 mg QD versus placebo; #p≤0.05; for upadacitinib 30 mg QD versus placebo; †significant in the multiplicity-controlled analysis. Results for MDA at week 24 are based on non-responder imputation with additional rescue handling, where MDA at week 24 for patients rescued at week 16 is imputed as non-responder. 95% CIs for response rate were calculated based on normal approximation to the binominal distribution. 95% CIs for response rate difference were calculated based on normal approximation. Nominal p value was constructed using Cochran-Mantel-Haenszel test adjusted for the main stratification factor of current disease-modifying antirheumatic drug use (yes/no). QD, once per day.

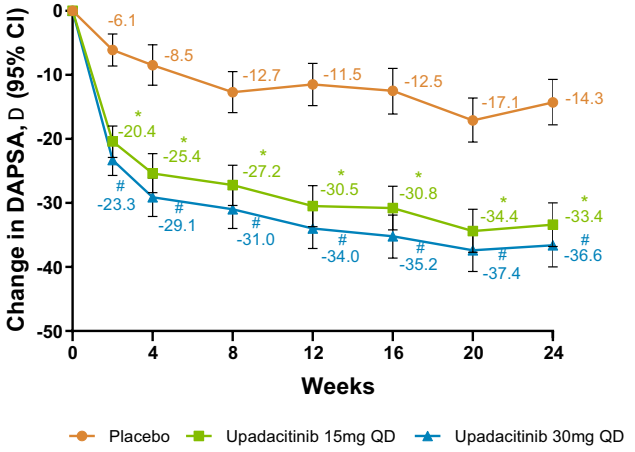

Figure 4 Change from baseline in Disease Activity in Psoriatic Arthritis (DAPSA) score. *p≤0.05; for upadacitinib 15 mg QD versus placebo; #p≤0.05; for upadacitinib 30 mg QD versus placebo. Within group least square mean and 95% CI, and between group least square mean, 95% CI and nominal p value are based on mixed-effect model repeated measurement (MMRM) analysis with unstructured variance-covariance matrix, including treatment, visit, treatment-by-visit interaction, the stratification factor current disease-modifying antirheumatic drug use (yes/no) as fixed factors and the continuous fixed covariate of baseline measurement. QD, once per day.

Over the 24-week period, one death was reported in the placebo arm related to a motor vehicle accident.

Generally, mean haemoglobin, neutrophil, lymphocyte and platelet levels remained within normal limits from baseline through week 24 in all treatment arms (online supplemental figure S12 and online supplemental table S5). There were two patients with grade 3 decreases in haemoglobin values in the upadacitinib 30 mg arm (online supplemental table S4). Grade 3 decreases in neutrophils were reported in one patient on placebo (0.5%), two patients on upadacitinib 15 mg (1.0%) and four patients on upadacitinib 30 mg (1.8%). No patients had grade 4 decreases in platelets, leucocytes, neutrophils or lymphocytes.

Isolated grade 3 increases in alanine aminotransferase or aspartate aminotransferase were observed in ≤1% of the patients among the treatment arms, and no grade 4 increases were observed (online supplemental table S4). No Hy's law cases were reported. Grade 3 increases in creatine phosphokinase (CPK) values were reported in one (0.5%), one (0.5%) and five (2.3%) patients in the placebo, and upadacitinib 15 mg and 30 mg arms, respectively. Grade 4 increases in CPK values were reported in

Table 4 Safety summary through week 24

	Placebo N=212	Upadacitinib 15 mg QD N=211	Upadacitinib 30 mg QD N=218
Patients with adverse events (AE), n (%)			
Any AE	139 (65.6)	135 (64.0)	170 (78.0)
Serious AE	4 (1.9)	12 (5.7)	18 (8.3)
AE leading to discontinuation of trial drug	11 (5.2)	15 (7.1)	20 (9.2)
Deaths	1 (0.5)	0	0
Infection	73 (34.4)	71 (33.6)	108 (49.5)
Serious infection	1 (0.5)	1 (0.5)	6 (2.8)
Opportunistic infection excl. tuberculosis and herpes zoster	0	0	2 (0.9)
Herpes zoster	2 (0.9)	3 (1.4)	8 (3.7)
Active tuberculosis	0	0	0
Hepatic disorder	3 (1.4)	4 (1.9)	18 (8.3)
Malignancy	0	3 (1.4)	3 (1.4)
Non-melanoma skin cancer	0	1 (0.5)	1 (0.5)
Malignancy other than NMSC	0	2 (0.9)	2 (0.9)
Lymphoma*	0	1 (0.5)	0
Anaemia	2 (0.9)	4 (1.9)	14 (6.4)
Neutropenia	1 (0.5)	2 (0.9)	6 (2.8)
Lymphopenia	0	2 (0.9)	2 (0.9)
Creatine phosphokinase elevation	4 (1.9)	4 (1.9)	12 (5.5)
Renal dysfunction	1 (0.5)	0	1 (0.5)
MACE (adjudicated)	0	1 (0.5)	0
VTE (adjudicated)	0	1 (0.5)	0
Laboratory data (LS mean change from baseline to week 24±SD)			
Haemoglobin, g/L	−0.7±7.44	−3.6±9.45	−5.5±10.78
Neutrophils, 10^9/L	−0.056±1.6435	−0.286±1.9578	−0.610±2.0242
Lymphocytes, 10^9/L	−0.076±0.5484	−0.028±0.5460	−0.057±0.5403
Platelets, 10^9/L	1.7±59.35	8.4±51.59	18.3±72.08
LDL-C, mmol/L	0.003±0.6839	0.219±0.6567	0.453±0.9283
HDL-C, mmol/L	−0.008±0.2278	0.199±0.2599	0.243±0.3451
ALT, U/L	−0.7±10.28	6.8±16.05	9.1±16.45
AST, U/L	−0.1±8.41	6.5±22.17	8.3±13.29
Creatinine, umol/L	2.2±10.87	4.7±9.19	5.3±9.48
Creatine phosphokinase, U/L	−19.9±140.87	166.8±1198.70	138.7±165.85

AEs were coded per the Medical Dictionary for Regulatory Activities. Laboratory data was graded using the Common Toxicity Criteria of the National Cancer Institute 4.03.
*In the once per day upadacitinib 15 mg arm, one event of treatment-emergent lymphocyte morphology abnormal was identified; per the investigator, no further diagnosis was made.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LS, least squares; MACE, major adverse cardiovascular events (defined as non-fatal myocardial infarction, non-fatal stroke and cardiovascular death); NMSC, non-melanoma skin cancer; QD, once per day; VTE, venous thromboembolic event (defined as deep vein thrombosis and pulmonary embolism).

two patients with placebo and one patient with upadacitinib 15 mg. None led to discontinuation of trial drug, and there were no events of rhabdomyolysis. Slight mean elevations in low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were observed in the upadacitinib arms versus the placebo arm (online supplemental figure S13). The ratios of LDL-C:HDL-C and total cholesterol:HDL-C generally remained constant through week 24.

DISCUSSION

In this phase 3 trial of patients refractory or intolerant to biologic DMARDs, greater efficacy was demonstrated for upadacitinib 15 mg and 30 mg once per day versus placebo for clinical manifestations of PsA including musculoskeletal symptoms (peripheral arthritis, enthesitis, dactylitis and spondylitis), psoriasis, physical function, pain, fatigue and quality of life.

Despite the advent of biologic DMARDs in PsA, many patients are either refractory or develop refractoriness to such treatment, underscoring the need for new therapy options. Both upadacitinib doses demonstrated efficacy in this particularly refractory population, wherein approximately 31% of the patients had failed ≥2 biologic DMARDs. Furthermore, treatment with both upadacitinib doses resulted in improvements over placebo in more rigorous measures of disease control, as demonstrated by the ACR70, PASI100, sIGA 0/1, resolution of enthesitis and dactylitis, and MDA. Notably, efficacy was achieved with both upadacitinib doses as monotherapy and in combination with non-biologic DMARDs. Both upadacitinib doses also provided rapid efficacy on arthritis signs/symptoms, as evidenced by greater improvement of ACR20 compared with placebo at week 2.

Upadacitinib 30 mg resulted in numerically greater efficacy when compared with 15 mg for the primary and key secondary endpoints. Upadacitinib showed improvement in psoriasis similar to that observed in recent studies of biologics and small molecules in patients with PsA and previous inadequate response

to biologic DMARDs.[28–30] However, the efficacy differences in musculoskeletal manifestations between the upadacitinib doses appear to decrease by week 24. Dose-dependent efficacy will be further evaluated with long-term data.

The safety profile of upadacitinib was generally consistent with results reported previously in rheumatoid arthritis trials.[11–14] More serious infections, opportunistic infections and herpes zoster events were reported with upadacitinib 30 mg compared with upadacitinib 15 mg and placebo; however, percentages of malignancy and lymphopenia were the same in the upadacitinib arms. Although the sample size and trial duration may not be enough to make a determination from this study, there was a lack of MACE and VTE reports in the upadacitinib 30 mg arm, suggesting no dose-dependent increased risk of these cardiovascular events with upadacitinib therapy. Few grade 3 or 4 laboratory abnormalities were seen in either upadacitinib arm.

Due to the 24-week duration of the placebo-controlled portion of this trial, limited safety conclusions may be made for events with longer latency or rare events. Long-term safety and efficacy of upadacitinib in patients with PsA are continuing to be evaluated in the ongoing extension phase. Further, this trial did not assess the effect of upadacitinib on radiographic progression compared with that of placebo. However, radiographic progression was evaluated in a parallel trial (NCT03104400) registered on clinicaltrials.gov.

In summary, in a PsA population refractory or intolerant to prior biologic DMARD therapy, upadacitinib 15 mg and 30 mg once per day, with or without concomitant non-biologic DMARD therapy, showed rapid improvements versus placebo as measured by ACR20 response and efficacy across all clinical domains of PsA, including rigorous levels of efficacy in musculoskeletal and psoriatic skin disease measures as well as of comprehensive disease control. No new safety signals were identified compared with what has been observed with upadacitinib in rheumatoid arthritis.

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Collaborators JA, AP participated in the design of the study. PM, AL, JA, FB, ST, ED, MK, WT participated in the acquisition and interpretation of data. AL, JA, RM, PZ, AP participated in the analysis and interpretation of data. SZ and XW participated in the analysis of data. AL, JA, and PM contributed to the drafting of the manuscript. All authors contributed to the critical revision of the manuscript for important intellectual content.AbbVie and the authors thank the patients who participated in the trial and all trial investigators for their contributions. The authors thank Dr. Mark C. Genovese for his support in the conduct of the trial. Ramona Vladea, PhD of AbbVie Inc. provided medical writing support.

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TRANSLATIONAL SCIENCE

Combined genetic analysis of juvenile idiopathic arthritis clinical subtypes identifies novel risk loci, target genes and key regulatory mechanisms

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ABSTRACT

Objectives Juvenile idiopathic arthritis (JIA) is the most prevalent form of juvenile rheumatic disease. Our understanding of the genetic risk factors for this disease is limited due to low disease prevalence and extensive clinical heterogeneity. The objective of this research is to identify novel JIA susceptibility variants and link these variants to target genes, which is essential to facilitate the translation of genetic discoveries to clinical benefit.

Methods We performed a genome-wide association study (GWAS) in 3305 patients and 9196 healthy controls, and used a Bayesian model selection approach to systematically investigate specificity and sharing of associated loci across JIA clinical subtypes. Suggestive signals were followed-up for meta-analysis with a previous GWAS (2751 cases/15 886 controls). We tested for enrichment of association signals in a broad range of functional annotations, and integrated statistical fine-mapping and experimental data to identify target genes.

Results Our analysis provides evidence to support joint analysis of all JIA subtypes with the identification of five novel significant loci. Fine-mapping nominated causal single nucleotide polymorphisms with posterior inclusion probabilities ≥50% in five JIA loci. Enrichment analysis identified RELA and EBF1 as key transcription factors contributing to disease risk. Our integrative approach provided compelling evidence to prioritise target genes at six loci, highlighting mechanistic insights for the disease biology and *IL6ST* as a potential drug target.

Conclusions In a large JIA GWAS, we identify five novel risk loci and describe potential function of JIA association signals that will be informative for future experimental works and therapeutic strategies.

Key messages

What is already known about this subject?

- Juvenile idiopathic arthritis (JIA) is the most common form of childhood arthritis. However, our understanding of the genetic basis of JIA is hampered by low disease prevalence and extensive clinical heterogeneity represented by seven disease subtypes, with only 17 known susceptibility loci to date.

What does this study add?

- Although JIA is a heterogeneous disease, we show that most susceptibility loci are shared across multiple clinical subtypes, enabling joint analysis of clinically related subtypes, both for this study and future projects, increasing the power of our study leading to the identification of five novel susceptibility loci in the largest genome-wide genetic study to date.
- By linking susceptibility genetic variants to target genes, integrating functional annotations, statistical fine mapping, expression data from 15 immunological cell types and chromatin interaction data (HiChIP and Hi-C) from human T and B cell types, we identify putative causal (i) single nucleotide polymorphisms; (ii) genes and (iii) cell types while also highlighting key regulatory mechanisms underlying disease.

INTRODUCTION

The contribution of large-scale genetic studies to the understanding of pathogenesis and management of complex traits has been widely documented over the last decade with the identification of thousands of genetic associations and their subsequent implications for biological pathways, drug discovery and repurposing.[1 2] However, progress in low-prevalence diseases has not been as rapid owing to hindrances in the recruitment of well-powered cohorts. This is well illustrated by considering the distinct and heterogeneous forms of childhood arthritis that are clinically encompassed under the term of juvenile idiopathic arthritis (JIA). JIA comprises childhood rheumatic conditions characterised by inflammatory arthritis of unknown origin that persists for at least 6 weeks and begins before the age of 16 years.[3] The International League of Associations for Rheumatology (ILAR) distinguishes seven JIA subtypes: oligoarticular arthritis (oligoJIA); rheumatoid factor (RF)-negative polyarthritis (RF–polyJIA); RF-positive polyarthritis (RF+polyJIA); juvenile psoriatic arthritis (JPsA); enthesitis-related arthritis childhood spondyloarthropathy (ERA); systemic arthritis (sJIA); and undifferentiated arthritis.[4]

To date genetic studies in JIA susceptibility have identified 17 genome-wide significant associations

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Key messages

How might this impact on clinical practice or future developments?

- The results of this study demonstrate that clinically heterogeneous subtypes can be analysed in a combined approach to identify novel shared susceptibility loci which is an approach that will be informative for genetic studies of other clinically heterogeneous diseases.
- We identify causal genes at JIA susceptibility loci which is an essential step in the translational of genetic discoveries to clinical benefit by highlighting potential therapeutic targets.

highlighting a number of key findings[5–7] including, first, that there is overlap of susceptibility loci between two of the most common JIA subtypes, oligoJIA and RF–polyJIA; specifically, in the human leucocyte antigen (HLA) region, these two subtypes share the presence of a glycine at amino acid position 13 of HLA-DRB1 as their highest risk factor, resembling the findings in adult seronegative rheumatoid arthritis (RA).[6 7] Together these two JIA subtypes define a genetically homogeneous cluster sometimes referred to with the term 'polygo'.[7–9] Second, this high genetic correlation is not as evident in the remaining subtypes, especially considering their divergent associations observed across the HLA region. For example, the presence of a histidine at the same HLA-DRB1 position confers the highest risk for RF+polyJIA, consistent with the association reported in adult seropositive RA.[7] In addition, the amino acid at position 58 of HLA-DRB1 has been shown to be a specific risk factor for sJIA.[10]

The clinical heterogeneity of JIA remains a challenging issue in deciphering its genetic architecture by balancing the need to focus on more clinically/genetically homogeneous subtypes against potentially sacrificing sample size. As a result, there has been a tendency to address the genetics of JIA in a subtype-based manner.[6–9] However, multinomial approaches have recently been developed to overcome the heterogeneity problem by allowing exploration of the genetic relationships between multiphenotype categories.[11] In this study, we hypothesised that a genome-wide association study (GWAS) combining all JIA subtypes would optimise the success rate in locus discovery. We, therefore, performed a new genome-wide scan of ~7.5 million single nucleotide polymorphisms (SNPs) in the largest JIA GWAS cohort recruited to date, and implemented a novel approach to systematically investigate specificity and sharing of associated loci across ILAR subtypes to support our strategy.

METHODS

Study cohort and GWAS quality control

A total of 4520 UK JIA samples and 9965 healthy individuals were recruited for the present study. JIA DNA samples were genotyped on the Illumina Infinium CoreExome and Infinium OmniExpress genotyping arrays. Sample-level quality control (QC) was applied based on the following exclusion criteria: call rate <0.98 and discrepancy between genetically inferred sex and database records. SNPs that were non-autosomal, had a call rate <0.98 or a minor allele frequency (MAF) <0.01 were excluded. Healthy controls were genotyped using the Illumina Infinium CoreExome genotyping array. QC was consistent with that described above for JIA samples.

Identity-by-descent was used to identify related individuals across all study samples. For each related pair, the sample with the highest call rate was retained. Outliers were identified and excluded based on ancestry using principal component (PC) analysis performed with the flashpca software package (V.2.0) where outliers were identified using aberrant R library (V.1.0).[12 13]

The total number of individuals that remained in the final QC-filtered data set was 12 501 (3305 cases and 9196 healthy controls) (online supplemental table 1).

Imputation

The QC-filtered GWAS data set was subjected to whole-genome genotype imputation. Haplotype phasing and imputation were performed in the Michigan Imputation server using SHAPEIT2[14] and Minimac3,[15] respectively, and the Haplotype Reference Consortium reference panel. Following imputation, SNPs were excluded based on MAF <0.01 and imputation quality (r^2) <0.4.

Association testing and meta-analysis

Case-control association testing was performed by SNPTEST software package (V.2.5.2). Three PCs were included as covariates to account for any residual population substructure. Any SNP with a p value $<5 \times 10^{-6}$ was selected for validation in GWAS summary statistics from an independent data set of 2751 JIA cases (oligoJIA and RF–polyJIA) and 15 886 controls of European ancestry.[8] An inverse variance weighted fixed effects meta-analysis was performed using the software package GWAMA (V.2.2.2).[16] The presence of heterogeneity of ORs across data sets was evaluated with the test statistics I^2 and Q.

Clinical subtype specificity

The specificity and sharing of JIA susceptibility SNPs across ILAR subtypes was interrogated using Bayesian multinomial logistic regression assuming an additive model implemented in the software package Trinculo (V.0.96).[11] Model selection for specificity or sharing was based on comparison of log-Bayes factors (logBFs) where a positive logBF was interpreted as evidence that a particular association is specific to an ILAR subtypes, and vice versa.

Statistical fine-mapping of JIA-associated loci

Statistical fine-mapping of the association signal within each locus was performed using the FINEMAP software package (V.1.3.1).[17] The method estimates the posterior inclusion probabilities (PIPs) for SNPs to be causal, which in turn were used to generate 95% credible SNP sets for each locus (the smallest list of variants that jointly have a probability of including the causal variant ≥95%).

Functional annotation enrichment analysis

Summary statistics from the GWAS including all ILAR subtypes were tested for enrichment in four categories of annotations based on experimental genomic data including gene structure (coding sequence (CDS), 3'UTR and 5'UTR) from the GENCODE Project, binding sites for 165 transcription factors (TFs) from the ENCODE Project, and enhancers and active promoters for 98 cell types derived from the Roadmap Epigenomics Project.[18–20] Enrichment of JIA associations were tested separately in each annotation using fgwas (V.0.3.6).[21] A joint model of independent enrichments was further identified using the cross-validation likelihood option implemented in fgwas.

Gene prioritisation

Expression quantitative trait locus (eQTL) data for 15 immune cell types was downloaded from the DICE (Database of Immune Cell Expression, eQTLs and Epigenomics) project website.[22]

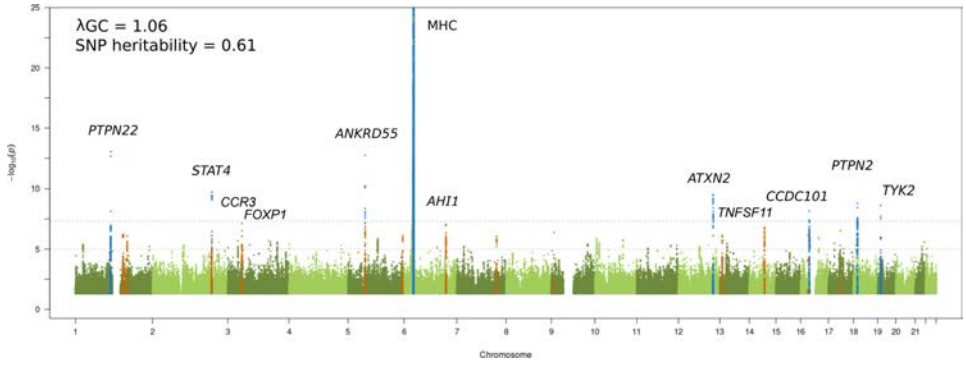

Figure 1 Manhattan plot representing the JIA GWAS results. The $-\log_{10}$ of the p values are plotted against their physical chromosomal position. The upper and lower lines represent the genome-wide significance level ($p \leq 5 \times 10^{-8}$) and p value threshold at $p \leq 1 \times 10^{-6}$, respectively. The plot has been truncated at $p \leq 1 \times 10^{-25}$. Genome-wide significant associations are coloured blue and suggestive significance are coloured orange. Genome-wide significant loci and the suggestive signals that reached $p \leq 5 \times 10^{-8}$ after the replication step are labelled. The genomic inflation factor (λ_{GC}) estimated on the complete data set was 1.06, with a rescaled λ_{1000} of 1.01 indicating minimal residual population stratification based on inflation of test statistics. The SNP-based heritability for JIA susceptibility was estimated to be 0.61 (SE 0.04). JIA, juvenile idiopathic arthritis; GWAS, genome-wide association study; SNP, single nucleotide polymorphism.

Correlation of susceptibility association signals and gene expression were identified by selecting the top eQTL SNP for each gene and retaining those that were also present in the combined list of all credible SNPs. This analysis was further supported by statistical colocalisation of association and eQTL signals. The identification of the target genes of JIA-associated regions was further complemented by the interrogation of high-resolution maps of chromatin interactions for SNPs correlated with eQTL signals using H3K27ac HiChIP data in B and T cells.[23] We also explored chromatin interaction maps obtained by capture Hi-C.[24]

Additional details of the Methods are available in the online supplemental material.

RESULTS

Five novel susceptibility loci for JIA

We performed a JIA GWAS comprising 12 501 individuals (3305 cases and 9196 healthy controls) and a high-density SNP panel with 7 461 861 variants. The combined analysis of all available JIA cases identified eight loci reaching genome-wide significance ($p \leq 5 \times 10^{-8}$), of which seven have previously been reported and recognised by the notable gene at each locus: MHC (6p25-p34), *PTPN22* (1p13.2), *STAT4* (2q32.2-q32.3), *ANKRD55* (5q11.2), *ATXN2* (12q24.12), *PTPN2* (18p11.21), and *TYK2* (19p13.2) (figure 1 and table 1).[6] The strongest association was found to SNPs within the extended MHC region (chr6: 28 477 797 to 33 448 354). An in-depth analysis of this region has previously been reported for JIA, including a subset of samples from the current study; therefore this study will focus on non-MHC associations.[7] The novel genome-wide significant association was represented by the lead SNP rs497523 ($p = 7.12 \times 10^{-9}$), which is intronic to *CCDC101* (16p11.2), also known as *SGF29*. A further 37 lead SNPs from independent loci, based on linkage disequilibrium, reached the suggestive significance threshold ($p \leq 5 \times 10^{-6}$). This included previously reported and potentially novel JIA loci (table 1). Summary statistics for 22 of these variants were available from a previously published JIA GWAS comprising 2751 cases and 15 886 controls[8] and meta-analysed with the current GWAS data. The meta-analysis identified a further four novel SNPs exceeding genome-wide significance in the proximity of the genes *AHI1* (6q23.3), *CCR3* (3p21.31), *TNFSF11* (13q14.11) and *FOXP1* (3p13) (table 2 and online supplemental table 2). Hence, a total of five new signals associated with JIA were identified. In addition, three SNPs showed evidence for replication in the independent data set although the meta-analysis test statistic did not exceed genome-wide significance (table 2): *TNFSF8* (rs7043505) ($_{meta}p = 8.27 \times 10^{-8}$), *AFF3* (rs11692867) ($_{meta}p = 9.26 \times 10^{-8}$), and *RUNX3* (rs72657048) ($_{meta}p = 3.51 \times 10^{-7}$). There was no evidence of between-study heterogeneity at any of these loci.

Evidence for shared non-HLA loci across JIA clinical subtypes

We systematically addressed the genetic relationship across ILAR subtypes in a Bayesian framework. We performed a Bayesian model selection between the best subgroup-specific model and the best sharing model and estimated the logBFs for specificity of effects at each locus. The analysis included the 44 non-HLA index SNPs passing study suggestive significance threshold (5×10^{-6}) (table 1). The results revealed evidence for sharing of JIA susceptibility loci across multiple ILAR subtypes since most of the analysed SNPs showed negative logBFs for specificity. This pattern was also evident for previously reported JIA susceptibility SNPs based on a combined cohort of oligoJIA and RF-polyJIA subtypes (online supplemental table 3, online supplemental figure 1). Moreover, the vast majority of the strongest logBFs (values between −4.5 and −9) were observed for the sharing model that comprised all JIA subtypes. Only seven loci (16%) showed weak evidence in favour of them being specific to the polygo subgroup (logBF of 0.06 to 0.5). Overall, these findings support our approach of performing a joint analysis of all available JIA cases to maximise power to detect novel susceptibility loci.

Enrichment of JIA susceptibility SNPs in TFBS and cell-type specific regulatory regions

We investigated the over-representation of JIA susceptibility SNPs in functional categories including gene structure (CDS, 3'UTR and 5'UTR), transcription factor binding sites (TFBSs) and enhancers and active promoters in 98 cell/tissue types. Our results showed no evidence for significant enrichment of JIA susceptibility SNPs in any of the gene structure annotations (p values>0.1) (figure 2). The most significant enrichment was found to binding sites for the TF RELA (p value=2.66×10^{-8}) (online supplemental table 4 and online supplemental figure 2). Additionally 52 out of the 165 TFBSs interrogated showed significant over-representation, including EBF1 (p value=6.00 x

Table 1 Non-HLA index SNPs passing study suggestive significance threshold (5×10^{-6}) and genome-wide significance threshold

SNP	Chr.	Position (bp)	Notable genes	Risk/non-risk allele	RAF	HWE (cases)	HWE (controls)	P value	OR	95% CI
rs6679677	**1**	114 303 808	***RSBN1; PTPN22***	**A/C**	**0.1**	**0.19**	**0.35**	**9.18E-14**	**1.36**	**1.24 to 1.48**
rs7731626	**5**	55 444 683	***ANKRD55***	**G/A**	**0.63**	**0.27**	**0.38**	**1.76E-13**	**1.22**	**1.15 to 1.3**
rs11889341	**2**	191 943 742	***STAT4***	**T/C**	**0.22**	**0.96**	**1**	**1.83E-10**	**1.24**	**1.16 to 1.32**
rs4766578	**12**	111 904 371	***ATXN2***	**T/A**	**0.49**	**0.21**	**0.13**	**3.03E-10**	**1.22**	**1.15 to 1.29**
rs9960807	**18**	12 770 851	***RP11-973H7.1;PTPN2***	**G/A**	**0.13**	**0.74**	**0.46**	**1.58E-09**	**1.26**	**1.16 to 1.36**
rs34536443	**19**	10 463 118	***TYK2***	**G/C**	**0.95**	**0.77**	**0.56**	**2.32E-09**	**1.53**	**1.31 to 1.79**
rs497523	**16**	28 577 931	***CLN3; CCDC101***	**T/C**	**0.65**	**0.94**	**0.03**	**7.12E-09**	**1.17**	**1.11 to 1.25**
rs13160933	5	55 545 859	*NA*	C/T	0.88	0.84	1	6.49E-08	1.26	1.15 to 1.38
rs79815064	3	46 277 577	*CCR3*	A/G	0.87	0.45	0.6	7.61E-08	1.25	1.14 to 1.37
rs2614258	6	135 677 202	*AHI1*	A/G	0.38	0.2	0.22	9.17E-08	1.15	1.08 to 1.22
rs1051533	14	69 259 662	*ZFP36L1*	A/C	0.21	0.63	0.85	1.62E-07	1.2	1.12 to 1.28
rs113171555	17	38 296 272	*CASC3*	A/G	0.02	0.73	0.14	2.92E-07	1.46	1.22 to 1.75
rs72704368	9	8 894 396	*PTPRD*	A/G	0.05	0.76	0.64	4.14E-07	1.3	1.15 to 1.47
rs2481065	1	154 311 911	*ATP8B2; IL6R*	G/A	0.11	0.32	0.87	6.11E-07	1.24	1.14 to 1.35
rs77011494	16	24 333 566	*CACNG3*	A/G	0.04	0.86	0.89	7.04E-07	1.41	1.23 to 1.6
rs7320806	13	27 684 929	*USP12*	C/A	0.09	0.86	0.12	7.36E-07	1.25	1.14 to 1.37
rs6434390	2	191 262 762	*INPP1; MFSD6*	G/C	0.48	0.05	0.54	7.42E-07	1.16	1.1 to 1.23
rs12654812	5	176 794 191	*RGS14*	A/G	0.34	0.22	0.64	7.61E-07	1.17	1.1 to 1.24
rs840012	1	167 414 872	*CD247*	C/T	0.59	0.46	0.95	8.21E-07	1.15	1.08 to 1.22
rs12706860	7	128 570 026	*NA*	C/G	0.65	0.6	0.13	8.78E-07	1.18	1.11 to 1.25
rs7204355	16	58 951 694	*RP11-410D17.2*	G/T	0.79	0.26	0.07	1.04E-06	1.19	1.1 to 1.28
rs706778	10	6 098 949	*IL2RA*	T/C	0.4	0.34	0.78	1.28E-06	1.15	1.09 to 1.22
rs4869314	5	96 229 225	*ERAP2*	G/T	0.49	0.58	0.88	1.35E-06	1.14	1.08 to 1.21
rs7082720	10	90 742 049	*ACTA2*	T/C	0.45	0.6	0.66	1.67E-06	1.15	1.09 to 1.21
rs2222138	18	12 889 217	*PTPN2*	G/T	0.68	0.42	0.98	2.02E-06	1.17	1.1 to 1.24
rs1521088	3	132 815 094	*TMEM108*	T/C	0.02	0.74	0.06	2.08E-06	1.41	1.18 to 1.68
rs34173901	3	33 087 914	*GLB1*	C/G	0.15	0.3	0.65	2.13E-06	1.2	1.12 to 1.3
rs76870128	3	138 211 845	*CEP70*	C/T	0.97	0.57	0.53	2.66E-06	1.61	1.31 to 2
rs58923164	21	44 158 451	*PDE9A*	T/G	0.04	1	1	2.68E-06	1.34	1.17 to 1.53
rs13433914	3	159 902 148	*IL12A-AS1*	C/G	0.22	0.71	0.63	2.74E-06	1.17	1.09 to 1.25
rs2371887	2	214 085 179	*NA*	G/A	0.43	0.33	0.97	2.79E-06	1.15	1.08 to 1.21
rs1717501	10	14 354 673	*FRMD4A*	C/A	0.12	0.57	0.55	3.07E-06	1.23	1.13 to 1.34
rs138815617	17	19 445 425	*SLC47A1*	A/G	0.01	0.6	1	3.28E-06	1.54	1.22 to 1.94
rs12430303	13	43 032 027	*TNFSF11*	C/T	0.45	0.4	0.57	3.61E-06	1.13	1.07 to 1.2
rs186715000	4	1 589 324	*NA*	G/A	0.01	1	0.27	3.72E-06	1.52	1.24 to 1.87
rs7043505	9	117 628 528	*TNFSF8*	A/G	0.55	0.47	0.25	3.74E-06	1.15	1.08 to 1.21
rs72657048	1	25 289 734	*RUNX3*	G/C	0.5	0.65	0.77	3.90E-06	1.14	1.08 to 1.21
rs7647909	3	71 200 157	*FOXP1*	G/T	0.24	0.13	0.6	4.56E-06	1.16	1.09 to 1.23
rs11692867	2	100 759 477	*AFF3*	G/A	0.64	0.53	0.6	4.57E-06	1.13	1.07 to 1.2
rs80136777	3	45 931 005	*CCR9*	T/A	0.88	0.57	0.2	4.68E-06	1.2	1.1 to 1.32
rs139529714	4	169 369 671	*DDX60L*	C/T	0.01	1	0.27	4.78E-06	1.52	1.24 to 1.87
rs521786	11	129 607 371	*NA*	C/A	0.11	0.65	0.11	4.94E-06	1.19	1.09 to 1.3
rs661171	11	110 016 519	*ZC3H12C*	G/T	0.72	0.82	0	4.95E-06	1.16	1.09 to 1.24
rs6506561	18	8 233 559	*PTPRM*	T/C	0.55	0.86	0.1	5.00E-06	1.13	1.07 to 1.19

Genome-wide significant loci for juvenile idiopathic arthritis are highlighted in bold.
bp, base pair; Chr., chromosome; HLA, human leucocyte antigen; HWE, Hardy-Weinberg equilibrium; RAF, risk allele frequency; SNP, single nucleotide polymorphism.

10^{-6}), BATF (p value=1.51 x 10^{-4}) and FOXA2 (p value=9.06 x 10^{-4}). Enrichment of JIA susceptibility SNPs was also identified to cell-type specific enhancers in three broad tissue types: blood, thymus, and gastrointestinal tract (online supplemental table 5, online supplemental figure 3). Specially, JIA SNPs showed over-representation of enhancers in different subsets of T cells, pointing to primary effector/memory T cells, primary T helper memory cells, primary T helper 17, primary natural killer and primary T regulatory cells as key players for JIA pathogenesis. Enrichment in active promoters was observed in a wider range of tissue/cell types, with GM12878 lymphoblastoid B cells showing the strongest over-representation (p value=1.06 x 10^{-3}) (online supplemental table 6, online supplemental figure 4).

Given the expected correlation between the analysed annotations, we then proceeded to perform a stepwise selection process to select a subset of non-redundant annotations. A combined model derived from all categories of annotations consisted of binding sites for RELA and EBF1, and enhancers in primary T helper memory cells and the T cell leukaemia cell line DND-41. The maximum likelihood of this cross-category model exceeded that from any of the single-annotation models thus identifying the most statistically relevant regulatory elements

Table 2 SNP showing genome-wide significant or suggestive associations with juvenile idiopathic arthritis in the meta-analysis

SNP	Chr.	Position (bp)	Notable genes	Risk/non-risk allele	UK GWAS P value	USA GWAS P value	META P value	META OR	META 95% CI	Q statistic	Q P value	I2
rs2614258	6	135 677 202	*AHI1*	A/G	9.17E-08	6.50E-06	9.47E-12	1.17	1.12–1.22	0.18	0.67	0
rs79815064	3	46 277 577	*CCR3*	A/G	7.61E-08	8.43E-05	3.31E-11	1.25	1.17–1.34	0.87	0.35	0
rs12430303	13	43 032 027	*TNFSF11*	C/T	3.61E-06	9.23E-04	1.88E-09	1.14	1.09–1.19	0.56	0.45	0
rs7647909	3	71 200 157	*FOXP1*	G/T	4.56E-06	5.27E-05	2.02E-09	1.17	1.11–1.23	0	0.96	0
rs7043505	9	117 628 528	*TNFSF8*	A/G	3.74E-06	0.008333	8.27E-08	1.12	1.07–1.17	1.09	0.3	0.08
rs11692867	2	100 759 477	*AFF3*	G/A	4.57E-06	0.004152	9.26E-08	1.13	1.08–1.19	0.59	0.44	0
rs72657048	1	25 289 734	*RUNX3*	G/C	3.90E-06	0.008138	3.51E-07	1.13	1.08–1.18	0.69	0.41	0

bp, base pair; Chr., chromosome; GWAS, genome-wide association study; SNP, single nucleotide polymorphism.

(figure 2). No annotations in the final model were excluded with cross-validation.

Prioritising potential causal SNPs

Using our high-density SNP panel, we aimed to identify the putative causal SNPs driving the association signals. For this purpose, we applied a Bayesian fine-mapping approach[17] to define the PIP of each variant being causal given all other variants in the region. We fine-mapped each of the five newly discovered loci and 12 previously reported non-MHC susceptibility loci (p value$<5 \times 10^{-6}$ in the present study) to identify 95% credible SNP sets. There was no evidence to support multiple distinct association signals at any locus. For 5 (29%) and 10 (59%) of the 17 loci, fine-mapping resolved the association signal to 95% credible sets of ≤10 and ≤30 causal variants, respectively (online supplemental tables 7 and 8). Moreover, we identified five SNPs with PIPs of at least 0.5 for the following loci: *RSBN1-PTPN22* (1p13.2; rs6679677), *FOXP1* (3p13; rs7647909), *CCR3* (3p21.31; rs79815064), *ANKRD55* (5q11.2; rs7731626) and *TYK2* (19p13.2; rs34536443) (online supplemental table 7). Interestingly, the method was able to identify rs34536443, a well-characterised non-synonymous variant in autoimmunity,[25] as the likely causal variant for *TYK2* locus with a PIP of 80%.

Prioritising target genes

The identification of the target genes of the disease-associated variants is a crucial step towards describing the biological impact of a statistical association. To address this question, we first used eQTL data derived from 15 disease relevant immune cell types to correlate the identified credible SNPs with genes in each locus. The credible SNP sets captured the lead eQTL SNP for 15 genes (eGenes) at nine loci (figure 3 and online supplemental table 9). These observations were supported by statistical colocalisation (online supplemental table 10). Subsequently, we complemented the identification of the putative target genes of JIA SNPs by analysing high-resolution maps of enhancer-promoter interactions in human B and T cells. We observed HiChIP interactions for the promoters of 6 out of the 15 JIA eGenes: *IL2RA*, *CLN3*, *ATP2A1*, *IL6ST*, *CCDC101* (*SGF29*) and *ERAP2* (online supplemental table 11). In addition, *SULT1A2*, *SULT1A1*, *ACTA2*, *FAS* and *AHI1* promoters were located within 1 kb windows of JIA credible SNPs that overlapped an H3K27ac peak as identified from HiChIP data. We also observed promoter interactions for JIA credible SNPs and the promoters of *IL2RA*, *CLN3*, *IL6ST*, *CCDC101* and *ERAP2* through chromatin interaction maps obtained by capture Hi-C experiments (online supplemental table 12).

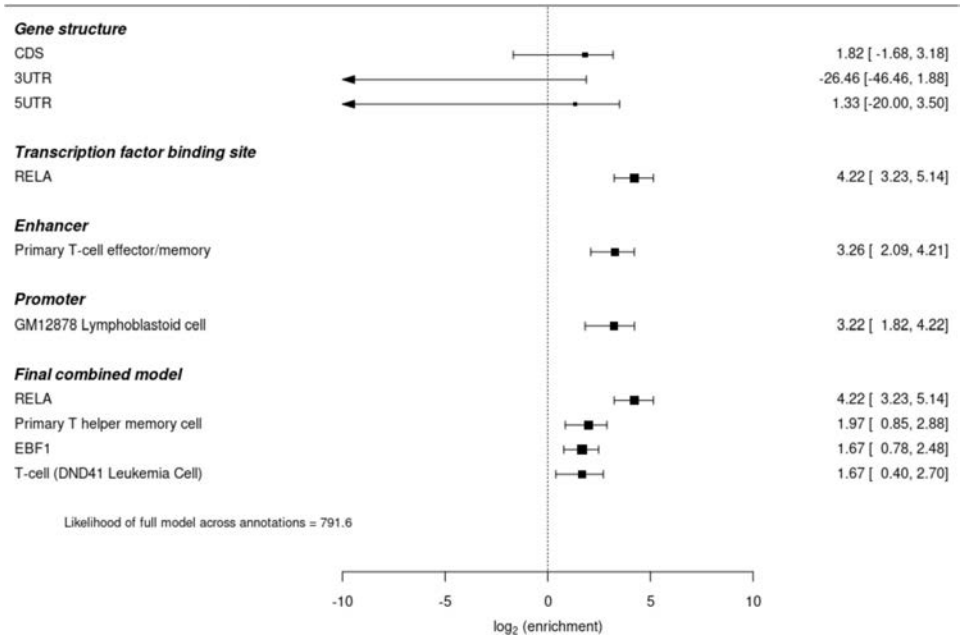

Figure 2 Functional enrichment analysis for JIA associations. Forest plot representing enrichment analysis results across four annotation categories based on experimental functional genomic data, and the final statistical annotation model. JIA, juvenile idiopathic arthritis.

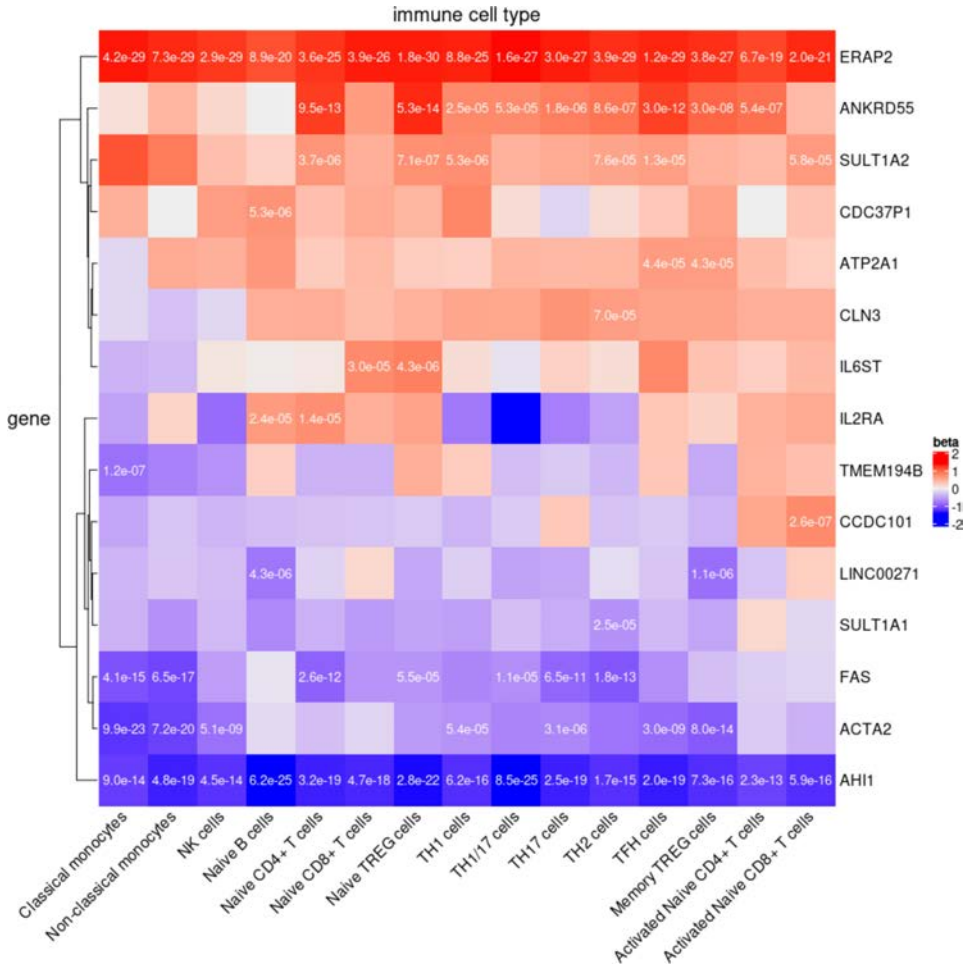

Figure 3 eQTL analysis. Significant eQTL from 15 disease relevant cell types of the DICE database including three innate immune cell types (classical monocytes, non-classical monocytes and natural killer cells), four adaptive immune cell types that have not encountered cognate antigen in the periphery (naive B cells, naive CD4$^+$ T cells, naive CD8$^+$ T cells and naive regulatory T cells (Treg)), six CD4$^+$ memory or more differentiated T cell subsets (Th1, Th1/17, Th17, Th2, follicular helper T cell (Tfh) and memory Treg), and two activated cell types (naive CD4$^+$ and CD8$^+$ T cells that were stimulated ex vivo). The p value for significant correlations are reported in each cell for credible SNPs that capture the most significant eQTL. Beta coefficients to illustrate direction and magnitude as determined by risk allele. eQTL, expression quantitative trait locus; SNPs,single nucleotide polymorphisms.

Interestingly, our analysis allowed us to refine the target gene of the association signal at 5q11.2 to *IL6ST*, since the credible SNP (rs7731626) showed chromatin contacts to the promoter of this gene but we did not observe interactions to the classically reported gene *ANKRD55* (figure 4). This exemplifies the potential of integrative analyses in deciphering the plausible mechanistic effect of association signals.

In total, we found 11 JIA targets genes showing both significant eQTL and H3K27ac HiChIP evidence.

DISCUSSION

We used a Bayesian model selection approach to demonstrate extensive sharing of JIA susceptibility loci across the ILAR subtypes and subsequent joint analysis of subtypes led to the identification of five novel risk loci, bringing the total of genome-wide significant regions for JIA to 22. We were able to prioritise causal genes at six loci integrating Bayesian fine-mapped credible SNPs, transcriptomics and chromatin interaction maps derived from disease-relevant cells.

A key challenge for studies investigating JIA susceptibility is how to account for the clinical heterogeneity across the ILAR clinical subtypes. Previous studies have focussed on the more frequent ILAR subtypes in an attempt to mitigate the loss of power due a non-specific phenotype definition.[26] However, in the present study this would have resulted in the exclusion of 30% of the available cases. Guided by the Bayesian model selection, we chose to perform a combined analysis across all ILAR subtypes, which we show maximises power to detect novel loci. However, it is important to recognise that this approach will only increase power to detect loci that underlie biological pathways shared by multiple ILAR subtypes and does not exclude the existence of subtype specific risk factors, which are known to exist.[7 9 27]

Enrichment of JIA susceptibility loci in functional annotations highlighted that most association signals affect disease risk through regulatory effects on gene expression and in a cell-type specific manner. Our analysis pointed to the TFBS of RELA and EBF1 as two main non-redundant regulatory elements suggesting a crucial contribution of them in JIA risk. Interestingly, RELA and EBF1 are known to regulate Treg-induced tolerance[28] and B cell specification and commitment,[29] respectively.

Identifying target genes of the association signals is a crucial step to translate statistical findings to biological meaning and, in turn, for the development of new therapeutic strategies. Applying

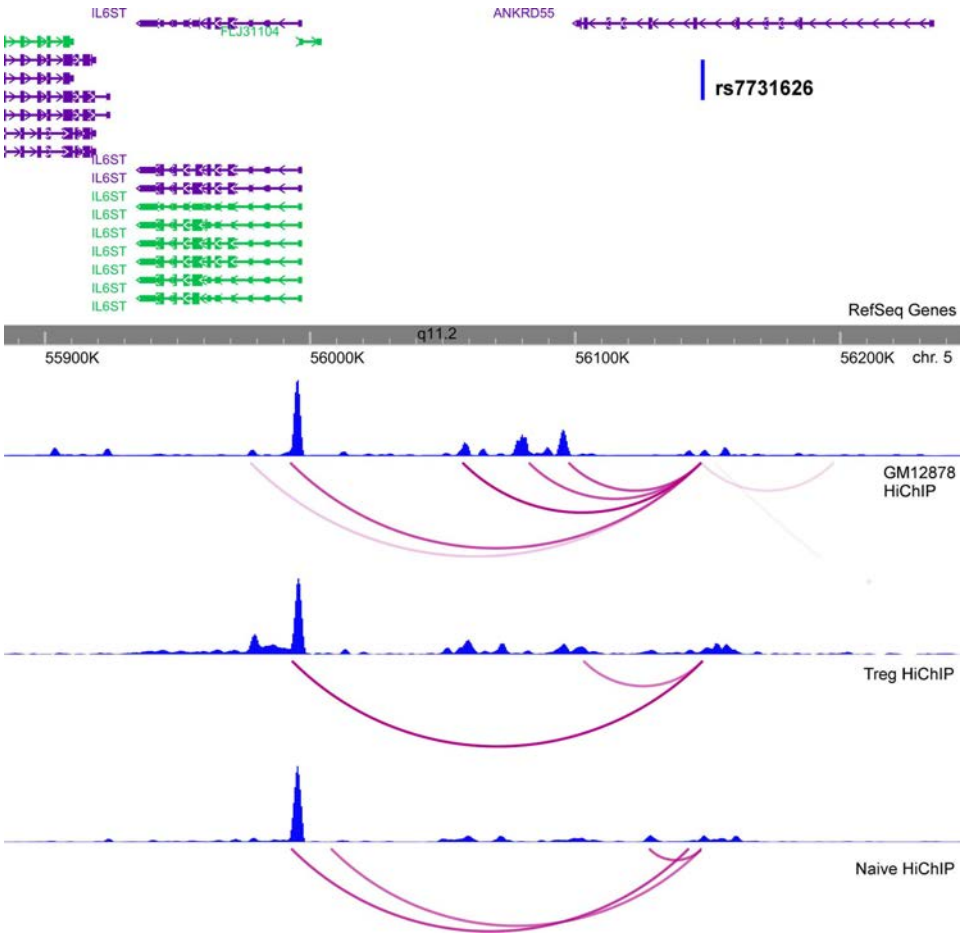

Figure 4 Chromatin interaction analysis at the *ANKRD55* locus. H3K27ac HiChIP signal at 5q11.2 showing enhancer-promoter chromatin interaction of rs7731626 to *IL6ST*. Blue graphs represent overlap with H3K27ac ChIP-seq peaks.

an integrative approach, we provide robust evidence to nominate target genes at the novel locus at 16p11.2. This is a known susceptibility locus for multiple chronic inflammatory diseases and includes attractive biological candidate genes such as *IL27*. However, complementary evidence from eQTL and chromatin data implicates the genes *CLN3* and *SULT1A2*. *CLN3* encodes a protein that is involved in lysosomal function suggesting a role for lysosome-mediated degradative pathways via autophagy and phagocytosis. Interestingly, Peeters *et al* reported that synovial fluid T cells derived from JIA patients showed enhanced autophagy.[30] *SULT1A2* encodes a catalytic enzyme that sulfonates different molecular components like thyroid hormones. Therefore, this target gene may establish a link for the comorbidity observed between rheumatic conditions and thyroid disorders. A second example of successful refinement is the association signal at 5q11.2 to *IL6ST*, instead of the classically reported gene *ANKRD55*.[6] We found that the *ANKRD55* intronic SNP, rs7731626, interacts with the promoter of *IL6ST*, and that its risk allele increases the expression of the gene. IL6ST is the interleukin 6 (IL-6) signal transducer and is the drug target of satralizumab, a biological drug that is currently in Phase III of a clinical trial for neuromyelitis optica, a rare autoimmune disease of the nervous system.[31] Considering that other biological drugs targeting the IL-6 pathway, such as tocilizumab, are currently in use for the treatment of JIA, our findings provide genetic support for the study of satralizumab as a new therapeutic target for JIA.

In conclusion, our results highlight the utility of joint analysis considering all JIA subtypes to maximise discovery, shifting the classical paradigm on which previous JIA genetic studies were based, and illustrate the potential of integrative approaches to gain further insights into the genetic susceptibility of the disease, which may in turn inform future therapeutic drug targets and pathways.

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CLINICAL SCIENCE

Interleukin 6 receptor inhibition in primary Sjögren syndrome: a multicentre double-blind randomised placebo-controlled trial

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ABSTRACT

Objectives No immunomodulatory drug has been approved for primary Sjögren's syndrome, a systemic autoimmune disease affecting 0.1% of the population. To demonstrate the efficacy of targeting interleukin 6 receptor in patients with Sjögren's syndrome-related systemic complications.

Methods Multicentre double-blind randomised placebo-controlled trial between 24 July 2013 and 16 July 2018, with a follow-up of 44 weeks, involving 17 referral centres. Inclusion criteria were primary Sjögren's syndrome according to American European Consensus Group criteria and score ≥5 for the EULAR Sjögren's Syndrome Disease activity Index (ESSDAI, score of systemic complications). Patients were randomised to receive either 6 monthly infusions of tocilizumab or placebo. The primary endpoint was response to treatment at week 24. Response to treatment was defined by the combination of (1) a decrease of at least 3 points in the ESSDAI, (2) no occurrence of moderate or severe activity in any new domain of the ESSDAI and (3) lack of worsening in physician's global assessment on a Visual Numeric Scale ≥1/10, all as compared with enrolment.

Results 110 patients were randomised, 55 patients to tocilizumab (mean (SD) age: 50.9 (12.4) years; women: 98.2%) and 55 patients to placebo (54.8 (10.7) years; 90.9%). At 24 weeks, the proportion of patients meeting the primary endpoint was 52.7% (29/55) in the tocilizumab group and 63.6% (35/55) in the placebo group, for a difference of −11.4% (95% credible interval −30.6 to 9.0) (Pr[Toc >Pla]=0.14).

Conclusion Among patients with primary Sjögren's syndrome, the use of tocilizumab did not improve systemic involvement and symptoms over 24 weeks of treatment compared with placebo.

Trial registration number NCT01782235.

Key messages

What is already known about this subject?

- No immunomodulatory drug has been approved for primary Sjögren's syndrome, a systemic autoimmune disease affecting 0.1% of the population.
- What is the efficacy of targeting interleukin 6 (IL-6) receptor in primary Sjögren's syndrome?

What does this study add?

- In this randomised clinical trial that enrolled 110 patients, the proportion of patients who had a decrease in the systemic disease activity was 52.7% in the tocilizumab group and 63.6% in the placebo group, a non-significant difference.
- In primary Sjögren's syndrome, inhibition of the IL-6 receptor did not improve the systemic disease activity.

How might this impact on clinical practice or future developments?

- These negative results for clinical outcome, patient-reported outcomes and immunological outcomes indicate that IL-6 does not represent a relevant therapeutic target in primary Sjogren's syndrome.

INTRODUCTION

Primary Sjögren syndrome (pSS) is a systemic autoimmune disease affecting 0.1% of the general population[1] that mainly targets the exocrine system, such as salivary and lachrymal glands. The clinical presentation is highly heterogeneous. Fatigue, dryness and pain are hallmarks of the disease; one-third to one-half of patients show systemic involvement (notably, articular, lung involvement, peripheral neuropathy, vasculitis) and 5%–10% mucosa-associated lymphoid tissue lymphoma.[2]

To date, no specific immunomodulatory drug has demonstrated efficacy for this disease. Hydroxychloroquine, prescribed for symptomatic relief of fatigue and pain for a long time, did not prove efficacy despite the inhibition of interferon α.[3 4] Drugs first developed for rheumatoid arthritis (RA), such as tumour necrosis factor inhibitors,[5 6] or rituximab, also failed to demonstrate effectiveness, except in one of four trials for rituximab.[7–10]

New insights into understanding the pathogenesis of the disease, including the role of T helper cell subsets,[11] salivary gland lymphoid neogenesis[12] and kinases involved in the B-cell receptor transduction pathway,[13] have not translated into positive randomised trials.[14–17] Reasons for these numerous failures include the clinical heterogeneity of patients and challenges in clinical trial design, recruitment and outcome in such a complex autoimmune disease. Our national network of clinicians dedicated to pSS, and involved in the present clinical trial, previously designed some of these negative trials.[3 5 9] In these previous trials, most patients did not have systemic complications and were mainly evaluated with patient-reported outcomes. An internationally validated score for systemic disease activity, the European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI),[18] now allows for defining a threshold for moderate systemic disease activity (ESSDAI≥5) and a clinically relevant improvement in systemic disease activity (a 3-point decrease in ESSDAI).[19]

Interleukin 6 (IL-6) is suspected to play an important pathogenic role in pSS with its crucial roles in B-cell activation and T-cell polarisation.[11 20] IL-6 deficiency corrected features of SS in a mouse model of the disease.[21] In addition, the safety profile of IL-6 receptor inhibitors has been studied for 10 years in RA.[22] Therefore, we investigated the interest of targeting IL-6 in patients with pSS with moderate or high systemic disease activity in a randomised placebo-controlled trial.

METHODS

Design

The randomisation used a centralised website based on a randomisation list using size-six block generated by an independent statistician. Patients were randomly assigned at a 1:1 ratio stratified by centre to receive 6 monthly infusions of tocilizumab (8 mg/kg) or a placebo between week 0 (W0) and W20. The drug and placebo were indistinguishable in appearance. Treatments were assigned after electronic verification of the correctness of inclusion criteria. Neither the investigators in charge of the study nor the participants were aware of the treatment assignments. A triple-blind procedure was applied, the statistician being unaware of the allocated treatment group during the analyses.

The primary endpoint was assessed at W24. After drug discontinuation, patients had two additional follow-up visits at W32 and W44.

Participants

In total, 17 referral clinical centres in France enrolled patients in the Efficacy of TocilizumAb in Primary Sjögren's syndrome (ETAP) trial between 24 July 2013 and 29 June 2017. The last follow-up date for the last participant was 16 July 2018. To be included, participants had to fulfil the following criteria: American European Consensus Group (AECG) criteria for pSS, ESSDAI≥5, anti-SSA antibody-positive, >18 years old, signed informed consent, no contraindication to tocilizumab and receiving stable doses of non-steroidal anti-inflammatory drugs, oral corticosteroids (prednisone ≤15 mg/day), or pilocarpine, cevimeline, or topical cyclosporine for at least 2 weeks before enrolment, and stable doses of methotrexate, hydroxychloroquine, chloroquine, quinacrine, leflunomide, azathioprine, mycophenolate mofetil or a psychoactive drug for at least 8 weeks before enrolment. We excluded patients who received a biologic, intravenous immunoglobulins, cyclophosphamide or plasmapheresis therapy within 6 months before enrolment and those with severe systemic complications related to pSS at enrolment (vasculitis with renal neurologic, digestive or cardiac involvement, severe interstitial lung disease, symptomatic cryoglobulinemia with severe neurologic involvement, severe renal function impairment, severe myositis).

On 3 October 2015, after the inclusion of 41 patients, because of recruitment difficulties, the protocol was amended to allow the inclusion of anti-SSA antibody-negative patients fulfilling AECG criteria (online supplemental file 2).

Table 1 Baseline characteristics of patients with primary Sjögren syndrome by treatment with tocilizumab or placebo

	Tocilizumab n=55	Placebo n=55
Age, years, mean (SD)	50.9 (12.4)	54.8 (10.7)
Female	54 (98.2%)	50 (90.9%)
Weight, kg, mean (SD)	69.1 (16.8)	69.3 (14.5)
Time/first symptoms, years, median (IQR)	7.1 (3.3–13.5)	6.9 (4.6–13.8)
Time/diagnosis, years, median (IQR)	4.4 (1.6–9.0)	4.9 (1.7–7.3)
Anti-SSA antibodies	48/53 (90.6%)	43/53 (81.1%)
Anti-SSB antibodies	29/53 (54.7%)	19/51 (37.3%)
Rheumatoid factor	37/51 (72.5%)	27/54 (50.0%)
IgG, mean (SD)	16.2 (6.2)	15.1 (6.2)
IgA, mean (SD)	2.9 (1.2)	2.3 (1.3)
IgM, mean (SD)	1.2 (0.71)	1.3 (1.0)
ESR, median (IQR)	20 (12–4)	20 (10–28)
CRP, median (IQR)	4.4 (1.6–9.0)	4 (2–5.2)
Abnormal Schirmer test (≤5 mm in 5 min)	29 (56.9%)	34 (68.0%)
Decreased unstimulated salivary flow (≤0.1 mL/min)	28 (62.2%)	31 (68.9%)
ESSDAI, median (IQR)	11 (8–13.5)	10 (8–14.8)
ClinESSDAI, median (IQR)	9(6–13)	9(6–12)
Physician's global evaluation of systemic disease activity, mean (SD)	5.2 (1.7)	5.1 (1.5)
Prednisone	9 (16.4%)	5 (9.1%)
Other immunomodulatory drugs	7 (12.7%)	6 (10.9%)
Number of tender joints, median (IQR)	4 (0–11)	5.5(1–12)
Number of swollen joints, median (IQR)	0.5 (0–4)	0.5 (0–3.5)
ESSPRI, mean (SD)	6.4 (1.8)	6.4 (1.9)
NAS score for dryness (0–10), mean (SD)	6.7 (2.2)	6.6 (2.2)
NAS score for pain (0–10), mean (SD)	6.9 (2.2)	7.0 (2.3)
NAS score for fatigue (0–10), mean (SD)	5.9 (2.6)	5.6 (2.4)
FACIT, mean (SD)	21.2 (12.0)	25.0 (11.2)
SF-36 PCS, mean (SD)	166.0 (75.3)	174.9 (76.9)
SF-36 MCS, mean (SD)	164.7 (81.0)	183.8 (85.3)
HAD score D, mean (SD)	9.0 (4.7)	7.6 (4.7)
HAD score A, mean (SD)	10.2 (3.7)	9.1 (4.4)

Data are no (%) unless otherwise indicated.
A, anxiety; ClinESSDAI, clinical ESSDAI; CRP, C reactive protein; D, depression; ESR, erythrocyte sedimentation rate; ESSDAI, European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; FACIT, Functional Assessment of Chronic Illness Therapy; HAD, Hospital Anxiety and Depression scale; MCS, Mental Component Score; NAS, Numeric Analogue Scale; PCS, Physical Component Score; SF-36, Medical Outcomes Survey Short-form 36.

Endpoints

The primary endpoint was response to treatment evaluated at week 24. Response to treatment was defined by the combination of (1) a decrease of at least 3 points in ESSDAI, (2) no occurrence

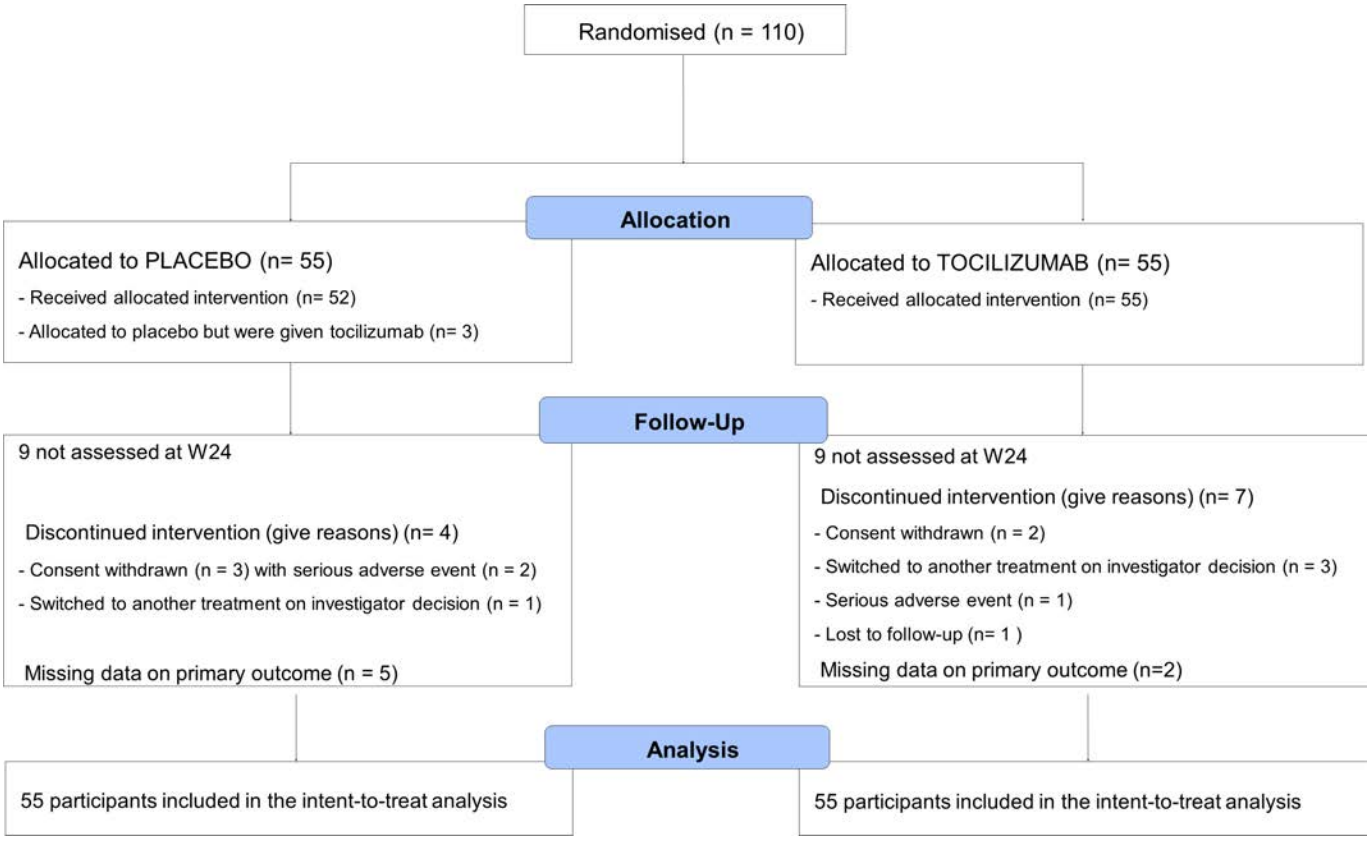

Figure 1 Flow chart of the trial.

of moderate or severe activity in any new domain of the ESSDAI and (3) no worsening in physician's global assessment on a visual numeric scale ≥1/10, all when compared with enrolment. We chose this composite primary outcome, which is close to the Systemic Lupus Erythematosus (SLE) Response Index in SLE,[23] because a 3-point decrease in ESSDAI corresponds to the minimally clinically relevant improvement for individuals with systemic complications[19] and to avoid observing a new systemic complication or a worsening of the physician's global evaluation despite a global improvement in ESSDAI.

Secondary endpoints were each of the three components of the composite primary endpoint; the ESSDAI[24]; number of tender and swollen joints; the EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI)[25]; the Schirmer test score; unstimulated salivary flow; serum IgG, IgA and IgM levels; the Medical Outcomes Survey Short-form 36 (SF-36) quality-of-life index; the Functional Assessment of Chronic Illness Therapy (FACIT)—fatigue scale and Hospital Anxiety and Depression (HAD) Scale score.

Statistical analysis

Sample size

With the Casagrande and Pike method,[26] we determined that we needed 48 participants in each group to achieve 80% power to detect a difference of 30% between group proportions. The proportion was assumed to be 25% in the placebo group and 55% in the tocilizumab group. The alpha level was set at 5%. The global sample size was increased to 110 to take into account potential missing data and lost to follow-up.

Statistics

Categorical data are described with number (%) and continuous data with mean (SD) or median (IQR).

Data were analysed with Bayesian methods. Beta distributions Beta(alpha,beta) were used to estimate binary or categorical data and means were estimated with normal distribution ($N(\mu,\sigma^2)$), with specific parameter values depending on the variables (see table 1). The variances were given weakly informative priors.

From a probabilistic point of view, the goal of the study was to compute the probability that the proportion of participants meeting the primary outcome was larger in the tocilizumab than placebo group.

The main outcome was analysed by comparing the proportion of participants meeting the primary outcome with Beta(alpha,beta) distributions. All analyses of the primary outcome were on an intent-to-treat (ITT) basis. For each analysis of the main outcome, we computed the proportion difference (%) (95% credible interval (CrI)) and the probability that the difference was >0 in favour of the tocilizumab group, that is, Pr[Toc >Pla]. This probability must not be confused with the classical p value. Within the ITT analysis, missing data were modelled under the missing at random assumption and managed by using multiple imputation based on treatment group.

A sensitivity analysis was performed on complete data by using different informative priors from previous studies.[3] The different priors used in the sensitivity analyses of the main outcome are in online supplemental table 1. The sensitivity analyses of the primary outcome also considered the missing not at random (MNAR) assumption. For this, missing data were imputed under one of two maximum bias patterns: with all missing data replaced by success in the placebo group and failure in the tocilizumab group (MNAR1) or vice versa (MNAR2). A non-responder imputation was also used.

For the secondary outcomes and post hoc analyses, no data imputation was performed for incomplete data. For the secondary outcomes, lowly informative priors were specified for

each variable. Posterior estimation of the parameters (difference of means or proportions, OR) were provided with their median and 95% posterior CrI. The different scores, such as ESSDAI, are bounded (ie, with a minimal and maximal possible value) and were modelled first by using beta regression. Comparisons of these models with Gaussian approximations showed no major differences; therefore, results are expressed, based on Gaussian models, as mean (SD) for simplicity. Count data, such as the number of serious adverse events (SAEs), swollen and tender joints were compared by Poisson regression.

Repeated data were analysed by using mixed models with a random effect to take into account the intraindividual correlation. For these repeated measures, results are expressed as after–before mean difference (95% CI) within each group with the addition of the interaction term (with 95% CI) to quantify the mean between-group variation difference that expresses the treatment effect.

For each analysis, a burn-in of 5000 iterations, followed by 100 000 iterations was used for a single Markov chain Monte Carlo (McMC) chain. Convergence of the McMC sample chain was checked graphically and if required, with the Brooks-Gelman-Rubin test. Convergence was observed in each case. Autocorrelation was negligible in each case. All computations involved using R V.3.5.1 and JAGS V.4.3.0 with all the required additional packages.

Patient and public involvement

This research was done without patient involvement. Patients were not invited to comment on the study design and were not consulted to develop patient relevant outcomes or interpret the results. We have invited patients to help us develop our dissemination strategy.

Dissemination declaration

We plan to disseminate the results to study participants and patient organisations.

RESULTS

Efficacy

A total of 110 patients were randomised: 55 to tocilizumab and 55 to placebo. The flow chart of the participants in the study is shown in figure 1. Mean age (SD) was 50.9 (12.4) and 54.8 (10.7) years in the tocilizumab and placebo group, respectively. Median (IQR) disease duration from diagnosis was 4.4 (1.6–9.0) and 4.9 (1.7–7.3) years and median ESSDAI was 11 (8–13.5) and 10 (8–14.8). Baseline characteristics of patients are reported in table 1 and baseline systemic complications in table 2.

At 24 weeks, the proportion of patients meeting the primary endpoint was 52.7% (29/55) in the tocilizumab group and 63.6% (35/55) in the placebo group, for a difference of −11.4% (95% CrI −30.6 to 9.0), (Pr[Toc >Pla]=0.14) after missing data multiple imputation (table 3). In sensitivity analyses, the difference between groups was −24.5 (−41.4 to −6.5), Pr[Toc >Pla]<0.001 (MNAR1) and 7.0% (−11.1 to 24.8), Pr[Toc >Pla]=0.78 (MNAR2).

Mean (SD) ESSDAI at week 24 was 8.3 (5.7) and 7.2 (5.3) in the tocilizumab and placebo groups, respectively, with a similar difference in changes from baseline between groups: 2% (95% CrI −1.2 to 5.2), (Pr[Toc >Pla]=0.89) (figure 2). In a post hoc analysis, at week 24, the mean (SD) clinical ESSDAI (not taking into account the biological domain of the ESSDAI))[27] was 5.6 (6.3) and 4.8 (5.9) in the tocilizumab and placebo groups, respectively, with a similar difference in changes from baseline between groups: 1.6% (95% CrI −2.0 to 5.3), (Pr[Toc >Pla]=0.81).

A post hoc analysis restricted to anti-SSA-positive patients showed a similar proportion of patients meeting the primary endpoint (47.5% (19/40) in the tocilizumab group and 64.9%

Table 2 Domains of the European League Against Rheumatism Sjögren's Syndrome Disease Activity Index (ESSDAI) at enrolment by treatment

		Tocilizumab n=55	Placebo n=55
Skin	0	48 (87.3%)	48 (88.9%)
	1	3 (5.5%)	3 (5.6%)
	2	4 (7.3%)	1 (1.9%)
	3	0 (0%)	2 (3.7%)
Pulmonary	0	44 (80%)	33 (61.1%)
	1	8 (14.6%)	12 (22.2%)
	2	2 (3.6%)	9 (16.7%)
	3	1 (1.8%)	0 (0%)
Renal	0	54 (98.2%)	54 (100%)
	1	0 (0%)	0 (0%)
	2	1 (1.8%)	0 (0%)
	3	0 (0%)	0 (0%)
Articular	0	16 (29.1%)	13 (24.1%)
	1	15 (27.3%)	19 (35.2%)
	2	16 (29.1%)	13 (24.1%)
	3	8 (14.6%)	9 (16.7%)
Muscular	0	51 (92.7%)	52 (96.3%)
	1	2 (3.6%)	1 (1.9%)
	2	1 (1.8%)	1 (1.9%)
	3	1 (1.8%)	0 (0%)
Peripheral neuropathy	0	46 (83.6%)	45 (83.3%)
	1	7 (12.7%)	2 (3.7%)
	2	2 (3.6%)	7 (13.0%)
	3	0 (0%)	0 (0%)
Central nervous system	0	54 (98.2%)	54 (100%)
	2	1 (1.8%)	0 (0%)
	3	0 (0%)	0 (0%)
Glandular	0	26 (47.3%)	26 (48.2%)
	1	19 (34.6%)	16 (29.6%)
	2	10 (18.2%)	12 (22.2%)
Constitutional	0	40 (72.7%)	39 (72.2%)
	1	15 (27.3%)	14 (25.9%)
	2	0 (0%)	1 (1.9%)
Haematological	0	33 (60%)	30 (55.6%)
	1	20 (36.4%)	21 (38.9%)
	2	2 (3.6%)	3 (5.6%)
	3	0 (0%)	0 (0%)
Lymphadenopathy	0	47 (87.0%)	50 (92.6%)
	1	6 (11.1%)	4 (7.41%)
	2	1 (1.9%)	0 (0%)
	3	0 (0%)	0 (0%)
Biological	0	19 (35.2%)	25 (46.3%)
	1	12 (22.2%)	12 (22.2%)
	2	23 (42.6%)	17 (31.5%)

Clinical involvement corresponding to each of the 12 domains is defined according to the ESSDAI. 0: no activity; 1: low activity; 2: moderate activity; 3: high disease activity. Sum of all frequencies do not add up to 55 in each group because of missing data. Percentages are computed on non-missing data.

Table 3 Patients meeting the primary endpoint and/or each of its components (decrease of at least 3 points in the ESSDAI and no occurrence of moderate or severe activity in any new domain of the ESSDAI compared with enrolment and no worsening in physician's global assessment on a Visual Numeric Scale ≥1/10)

	Tocilizumab n/N (%)	Placebo n/N (%)	Difference between placebo and tocilizumab % difference (95% CrI)	Pr(diff >0)
Primary endpoint				
W12	20/44 (45.5)	25/44 (56.8)	−10.9 (−30.7 to 9.4)	0.15
W24 (ITT)	29/55 (52.7)	35/55 (63.6)	11 (−9.0 to 30.6)	0.86
W24	24/46 (52.2)	29/46 (63.0)	10.4 (−9.2 to 29.7)	0.85
W32	24/43 (55.8)	19/43 (44.2)	−11.1 (−31.2 to 9.4)	0.14
W44	21/41 (51.2)	21/38 (55.3)	3.8 (−17.4 to 24.9)	0.64
3-point decrease in ESSDAI				
W12	29/49 (59.2)	29/49 (59.2)	0.0 (−14.9 to 14.9)	0.50
W24	28/49 (57.1)	35/50 (70)	12.4 (−6.1 to 30.5)	0.91
W32	30/46 (65.2)	29/47 (61.7)	−3.4 (−22.3 to 15.7)	0.36
W44	26/43 (60.5)	28/44 (63.6)	3.0 (−16.7 to 22.7)	0.62
No new systemic complication				
W12	51/51 (100)	48/50 (96)	1.0 (−12.7 to 14.6)	0.56
W24	38/49 (77.6)	42/50 (84)	6.2 (−9.2 to 21.7)	0.79
W32	40/47 (85.1)	42/49 (85.7)	0.7 (−13.6 to 15.1)	0.53
W44	40/47 (85.1)	44/49 (89.8)	4.6 (−8.9 to 18.3)	0.75
No worsening according to the physician				
W12	36/47 (76.6)	42/45 (93.3)	11.49 (−1.4 to 24.3)	0.96
W24	44/46 (95.7)	39/46 (84.8)	−10.4 (−23.5 to 1.7)	0.05
W32	41/46 (89.1)	34/45 (75.6)	−13.0 (−28.6 to 2.3)	0.05
W44	36/44 (81.8)	34/42 (81.0)	−0.9 (−17.4 to 15.5)	0.46

CrI, credible interval; ESSDAI, European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; ITT, intent to treat ; W, week.

(24/37) in the placebo group; % difference=−16.48% (−37.1 to 4.9) [Pr(diff >0)=0.07]).

In a post hoc analysis, the improvement between W0 and W24 in each of the 12 domains of the ESSDAI, defined as a change from high to moderate or low or no activity, from moderate to low or no activity or from low to no activity, was similar between the tocilizumab and the placebo groups (table 4).

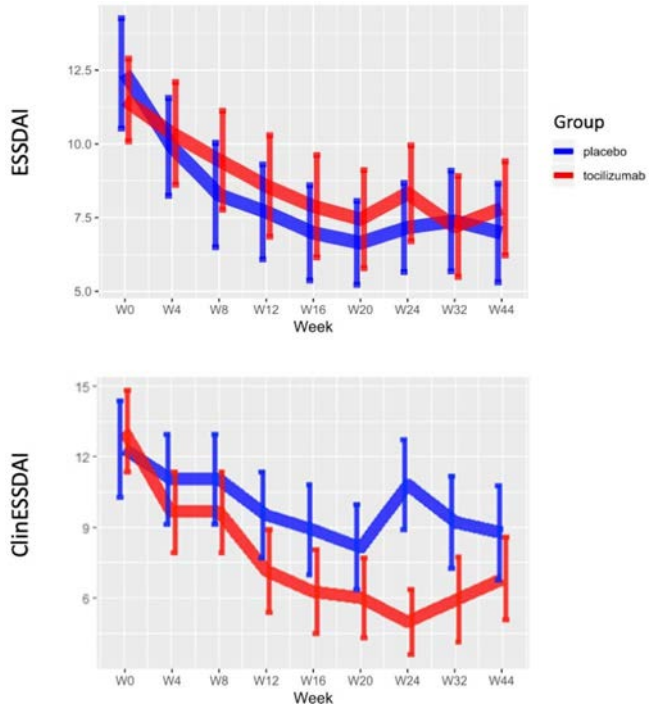

Figure 2 Change in ESSDAI and in ClinESSDAI data for tocilizumab (red) and placebo (blue) are percentage (95% credible interval). ClinESSDAI, clinical ESSDAI; ESSDAI, European League Against Rheumatism Sjögren's Syndrome Disease Activity Index.

In post hoc analyses of each domain of the ESSDAI, tocilizumab did not improve systemic involvement in patients with moderate or high systemic disease activity compared with placebo (data not shown). In addition, changes were similar between the two groups in all the secondary clinical endpoints (table 5). Mean (SD) tender joint count at W24 was 4.4 (6.2) and 4.6 (7.5) in the tocilizumab and placebo groups (relative risk (RR): 1.1 (95% CI 0.5 to 2.1), (Pr[RR >1]=0.56), respectively. Mean swollen joint count at W24 was 0.5 (1.1) and 1.2 (3.8), respectively (RR=1.2 (0.4 to 2.6), (Pr[RR >1]=0.55). Mean ESSPRI at week 24 was 5.8 (2.0) and 6.2 (2.1), respectively, with a similar difference in changes from baseline between groups (−0.4 (−1.0 to 0.3)), (Pr[Toc >Pla]=0.12) (figure 3).

Changes in ocular and oral dryness assessed by the Schirmer test and unstimulated salivary flow are reported in table 5. Changes were similar between the two groups in fatigue assessed by the FACIT —Fatigue scale, quality of life assessed by the SF-36 and psychological discomfort assessed by the HAD scale (table 5).

A post hoc analysis showed a similar proportion of patients at W24 with a decrease in ESSPRI from baseline ≥1 point (47.9% (23/48) in the tocilizumab group and 42.2% (19/45) in the placebo group; % difference=5.45% (95% CrI −14.2 to 24.9) (Pr[Toc >Pla]=0.71)) and a similar proportion of patients at W24 with a decrease from baseline in ESSDAI≥3 points or a decrease from baseline in ESSPRI≥1 point (tocilizumab: 72% (35/48) vs 77.8% (35/45), % difference=−4.6% (95% CrI −21.7 to 12.7), (Pr[Toc >Pla]=0.3).

Changes were similar in the two groups in serum immunoglobulin and complement levels at W12 (table 5).

Table 4 Change in disease activity in each domain of the ESSDAI

Domains	Tocilizumab W0 % of no/low/moderate/high	Tocilizumab W24 % of no/low/moderate/high	% improvement in tocilizumab group	Placebo W0 % of no/low/moderate/high	Placebo W24 % of no/low/moderate/high	% improvement in placebo group	Comparison of % improvement % difference (95% CI)
Skin	n=55 87.3/5.5/7.3/0	n=49 80.0/1.8/7.3/0	8.2	n=54 87.3/5.5/1.8/3.6	n=50 85.5/1.8/3.6/0	8.0	−0.2 (−11.7 to 11.3), Pr(diff >0)=0.49
Pulmonary	n=55 80.0/14.5/3.6/1.8	n=49 80.0/5.5/3.6/0	12.2	n=54 60.0/21.8/16.4/0	n=50 78.2/5.5/5.5/1.8	24.0	12.3 (−2.9 to 27.7), Pr(diff >0)=0.94
Renal	n=55 98.2/0/1.8/0	n=49 87.3/0/1.8/0	NA	n=54 98.2/0/0/0	n=50 90.9/0/0/0	NA	0 (−5.8 to 5.6), Pr(diff >0)=0.5
Articular	n=55 29.1/27.3/29.1/14.5	n=49 45.5/25.5/18.2/0	49.0	n=54 23.6/34.5/23.6/16.4	n=50 49.1/16.4/20.0/5.5	46.0	−2.9 (−21.8 to 16.3), Pr(diff >0)=0.38
Muscular	n=55 92.7/3.6/1.8/1.8	n=49 85.5/1.8/0/1.8	2.0	n=54 94.5/1.8/1.8/0	n=50 89.1/1.8/0/0	2.0	−0.1 (−7.8 to 7.6), Pr(diff >0)=0.49
Peripheral neuropathy	n=55 83.6/12.7/3.6/0	n=49 78.2/7.3/3.68/0	2.0	n=54 81.8/3.6/12.7/0	n=50 81.8/3.6/5.5/0	12.0	9.5 (−0.6 to 20.8), Pr(diff >0)=0.97
CNS	n=55 98.2/0/1.8/0	n=49 87.3/0/1.8/0	NA	n=54 98.2/0/0/0	n=50 90.9/0/0/0	NA	0 (−5.8 to 5.6), Pr(diff >0)=0.5
Glandular	n=55 47.3/34.5/18.2	n=49 63.6/20.0/5.56	34.7	n=54 47.3/29.1/21.8	n=50 63.6/16.4/10.9	34.0	−0.7 (−18.9 to 17.5), Pr(diff >0)=0.47
Constitutional	n=55 72.7/27.3/0	n=49 70.9/16.4/1.8	14.3	n=54 70.9/25.5/1.8	n=50 80.0/10.9/0	20.0	5.5 (−9.3 to 20.3), Pr(diff >0)=0.77
Haematological	n=54 60.0/36.4/3.6/0	n=49 40.0/40/9.1/0	8.2	n=54 54.5/38.2/5.5/0	n=50 49.1/38.2/1.8/1.8	18.0	9.4 (−3.8 to 23,0), Pr(diff >0)=0.92
Lymphadenopathy	n=54 85.5/10.9/1.8/0	n=49 87.3/1.8/0/0	14.6	n=54 90.9/7.3/0/0	n=50 89.1/1.8/0/0	8.0	−6.4 (−19.5 to 6.3), Pr(diff >0)=0.16
Biological	n=54 34.5/21.8/41.8	n=49 14.5/41.8/32.7	10.4	n=54 45.5/21.8/30.9	n=50 43.6/20.0/27.3	8.0	−2.4 (−14.6 to 9.6), Pr(diff >0)=0.34

CNS, central nervous system; ESSDAI, European League Against Rheumatism Sjögren's Syndrome Disease Activity Index; NA, not assessed.

Table 5 EULAR Sjögren's Syndrome Patient-Reported Index (ESSPRI), patient-related outcome and biological variables between weeks 0 and 24

	Tocilizumab			Placebo				
Variables	N	Mean	SD	N	Mean	SD	Time × group interaction (percentiles 2.5%; 97.5%)	Pr[Toc >Pla]
ESSPRI W0	55	6.4	1.8	52	6.4	1.9		
ESSPRI W12	49	5.9	1.8	51	6.0	1.8	−0.3 (−0.9; 0.4)	0.217
ESSPRI W24	49	5.8	2.0	48	6.2	2.1	−0.4 (−1.0; 0.3)	0.125
Dryness NAS W0	55	6.7	2.2	52	6.6	2.2		
Dryness NAS W12	49	6.4	2.1	51	6.5	2.4	−0.2 (−1.0; 0.6)	0.327
Dryness NAS W24	49	6.1	2.4	48	6.4	2.9	−0.4 (−1.2; 0.5)	0.209
Fatigue NAS W0	55	6.9	2.2	52	7.0	2.3		
Fatigue NAS W12	49	6.6	2.4	51	6.2	2.2	0.5 (−0.3; 1.3)	0.896
Fatigue NAS W24	49	6.5	2.5	48	6.6	2.6	0.1 (−0.7; 0.9)	0.617
Pain NAS W0	55	5.9	2.6	52	5.6	2.4		
Pain NAS W12	49	4.7	2.5	51	5.4	2.4	−1.1 (−1.9; −0.3)	0.006
Pain NAS W24	49	4.9	2.4	48	5.3	2.4	−0.8 (−1.7; 0.1)	0.035
FACIT score W0	55	21.2	12.0	52	25.0	11.2		
FACIT score W12	46	25.3	11.8	50	27.1	10.4	1.9 (−1,5; 5,2)	0.866
FACIT score W24	49	25.2	12.9	48	27.6	13.5	0.8 (−2,6; 4,1)	0.676
SF36 PCS W0	46	166.0	75.3	42	174.9	76.9		
SF36 PCS W24	41	212.0	80.4	40	203.1	86.9	4.5 (−23.6; 31.6)	0.631
SF36 MCS W0	46	164.7	80.9	42	183.8	85.3		
SF36 MCS W24	41	208.3	82.3	40	200.3	93.6	12.3 (−12.6; 36.3)	0.840
HAD-A W0	55	10.1	3.7	52	9.1	4.4		
HAD-A W24	49	9.4	4.6	48	8.6	4.9	−0.09 (−1.1; 1.0)	0.433
HAD-D W0	55	9.0	4.7	52	7.6	4.6		
HAD-D W24	49	8.1	4.6	48	7.7	5.1	−0.8 (−1.9; 0.3)	0.071
Schirmer W0	51	9.4	10.7	50	9.1	11.2		
Schirmer W24	46	7.8	8.3	41	9.5	11.7	−0.9 (−4.2; 2.4)	0.297
Salivary flow W0	51	1.0	1.2	51	0.6	1.2		
Salivary flow W24	47	0.9	1.1	43	0.6	0.8	−0.1 (−0.5; 0.4)	0.439
ESR W0	41	26.6	22.4	43	23.4	17.9		
ESR W12	44	9.9	11.6	41	24.0	20.0	−16.8 (−23.5; −9.9)	<0.001
CRP W0	44	6.0	5.4	47	5.0	5.1		
CRP W24	47	3.6	9.0	49	4.1	3.0	−2.0 (−4.5; 0.6)	0.06
IgG W0	29	16.4	6.2	33	15.1	6.2		
IgG W12	39	14.5	6.6	44	14.3	6.6	−2.5 (−4.2; −0.8)	0.002
IgA W0	29	2.9	1.2	32	2.4	1.3		
IgA W12	38	3.1	2.8	44	2.5	1.2	0.1 (−0.9; 1.2)	0.596
IgM W0	29	1.2	0.7	32	1.3	1.0		
IgM W12	39	1.5	2.2	44	1.2	0.6	−0.1 (−0.2; −0.0)	0.017
C3 W0	30	1.1	0.3	28	1.1	0.2		
C3 W24	47	0.9	0.2	47	1.1	0.2	−0.2 (−0.3; −0.1)	<0.001
C4 W0	30	0.2	0.1	27	0.2	0.1		
C4 W24	47	0.1	0.1	47	0.2	0.1	−0.1 (−0.1; −0.0)	0.001

Data are mean (SD) unless specified.
Interaction: inclusion—subsequent timepoint difference of the tocilizumab–placebo difference.
*The ESSPRI corresponds to the mean of a patient's Numeric Analogue Scale score for dryness, pain and fatigue.
A, anxiety; CRP, C reactive protein; D, depression; ESR, erythrocyte sedimentation rate; FACIT, Functional Assessment of Chronic Illness Therapy – Fatigue; HAD, Hospital Anxiety and Depression scale; Ig, immunoglobulin; MCS, mental component score; NAS, Numeric Analogue Scale; NR, not relevant; PCS, Physical Component Score; SF-36, Medical Outcomes Survey Short-form 36.

Tolerance

Over the first 24 weeks, 14 SAEs occurred in the tocilizumab group and 6 in the placebo group (RR=2.81 (95% CI 0.98 to 6.92)). In the last 24 weeks, 1 SAE occurred in the tocilizumab group and 5 SAEs in the placebo group (table 6). Between W0 and W44, 15 SAEs occurred in the tocilizumab group and 11 in the placebo group (RR=1.53 (0.64 to 3.14)). Three lymphomas occurred in the tocilizumab group and one in the placebo group. In the tocilizumab group, one lymphoma was diagnosed fortuitously thanks to a protocolised salivary gland biopsy before the first infusion of tocilizumab; one lymphoma was diagnosed after the first infusion, but after chart review, could have been suspected before randomisation; one lymphoma was diagnosed 1 month after the last infusion.

DISCUSSION

The present randomised double-blind placebo-controlled trial did not demonstrate a superiority of tocilizumab over placebo in

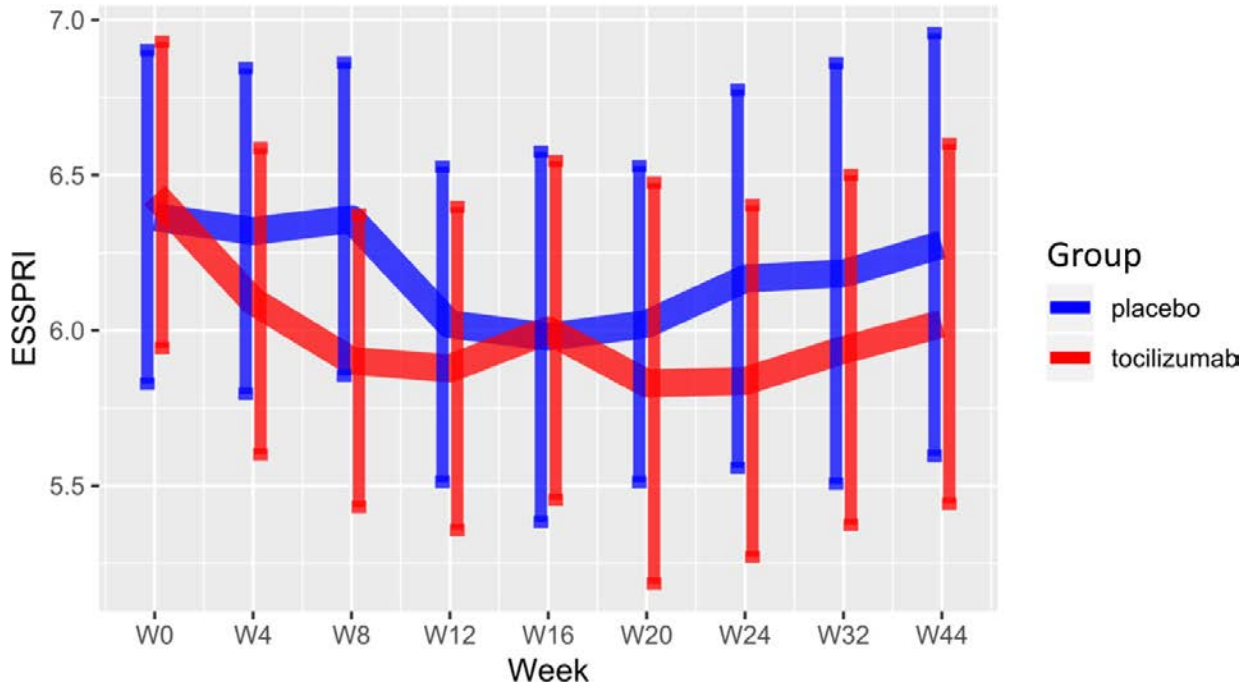

Figure 3 Change in ESSPRI Data are percentage (95% credible interval). ESSPRI, EULAR Sjögren's Syndrome Patient-Reported Index.

patients with pSS and moderate or high systemic disease activity. Ineffectiveness was observed on primary and secondary endpoints.

Tocilizumab was prescribed as monthly infusions. Thus, concerns about patients' adherence to treatment cannot explain the lack of efficacy of tocilizumab. In RA or giant cell arteritis, clinical efficacy is usually observed rapidly, within a few weeks of treatment. In pSS, anti-CD40 antibody treatment and the combination of leflunomide and hydroxychloroquine could improve disease manifestations at weeks 12 and 24, respectively.[28 29] Therefore, a longer-term evaluation of the primary endpoint (after 6 months) would not have changed the overall results.

Inhibition of the IL-6 receptor did not improve the systemic disease activity even articular involvement or the main symptoms, fatigue, pain and dryness, of participants. We found no change in objective assessments of dryness, but missing data on Schirmer test and unstimulated salivary flow limit the interpretation of these results.

Moreover, the immunological impact of systemic IL-6 inhibition was unexpectedly low. Before this trial, IL-6 was considered one of the cytokines driving B-cell activation in pSS, along with B-cell activating factor (BAFF) and IL-21.[11 30] IL-6 receptor inhibition did not improve serum levels of immunoglobulins or complement at week 12, or the biological domain of the ESSDAI at week 24, in contrast with the effect of other biologics such as rituximab or abatacept.[8 31] These results suggest that systemic IL-6 is not a main contributor to peripheral B-cell activation in pSS. Altogether, these negative results for clinical outcome, patient-reported outcomes and immunological outcomes indicate that IL-6 does not represent a relevant therapeutic target in pSS, regardless of the concern regarding the high placebo effect observed, which we discuss below. Of note, pSS and systemic sclerosis are diseases in which systemic inflammation is much less prominent than RA and giant cell arteritis. Tocilizumab did not reach its primary outcome in systemic sclerosis either,[32] although some secondary endpoints were reached in that trial.

Limitations of the study mainly include the inclusion criteria, restricted to patients with systemic disease activity and the high placebo effect (decrease of at least 3 points in the ESSDAI observed in more than 60% of the placebo-treated patients).

We included only patients with systemic disease activity. This choice was justified by the preference to use a biologic, with potential adverse events, in patients with more active and severe disease. Such inclusion criteria worsened difficulties in recruitment, already well reported in pSS,[33] and thus study duration. In addition, as expected, given that disease activity increases the risk of lymphoma,[2] lymphoma was diagnosed in four patients during the study. The present study was the first randomised trial registered in ClinicalTrials.gov to evaluate clinical response according to the ESSDAI. Since then, 16 randomised trials have used the ESSDAI as a primary outcome[14 16 28 29 34–45] and similar inclusion criteria (ESSDAI≥5 or 6).[46] Of note, the placebo response was very high and concerned all domains of the ESSDAI. The high placebo response was concordant with that observed recently in a negative phase III abatacept trial (51% of patients treated with placebo had a decrease of at least 3 points in the ESSDAI at 24 weeks)[47] and in two positive phase IIb trials evaluating ianalumab, an B-cell–depleting BAFF-receptor inhibitor (61.2% of patients treated with placebo had a decrease of at least 3 points in the ESSDAI at 24 weeks)[48] and iscalimab, an anti-CD40 antibody (55% of patients treated with placebo had a decrease of at least 3 points in the ESSDAI at 12 weeks).[49] Remaining to be determined is whether a time-varying decrease in ESSDAI is related to a natural history of the disease (eg, spontaneous improvement of arthralgias/synovitis, purpura or parotid swelling), heterogeneous clinical assessment in multicentre trials, difficulties to discriminate disease activity from damage or the scoring system itself. Using the ESSDAI, disease activity can be very difficult to differentiate from damage. This might lead to inadequately high ESSDAI scores at enrolment. Moreover, during follow-up, if disease activity is considered as stable, it must not be scored as persistently active after 12 months, according to the ESSDAI scoring system.[50] This results in a decrease of the ESSDAI in these patients with high ESSDAI score at enrolment. Given the large number of ongoing or future trials that have based their primary

Table 6 Serious adverse events in patients with pSS by treatment

Serious adverse event	Tocilizumab (n=55)	Placebo (n=55)
Between W0 and W24	14 (25.5%)	6 (10.9%)
Type of serious adverse event		
Prolonged hospitalisation for headache and nausea		1
Infectious parotitis	1	1
Mononeuritis		1
Sjögren's flare with purpura		1
Wrist fracture		1
CPK increase		1
MALT lymphoma	1	
Nodal marginal zone B-cell lymphoma	1	
Sinus tachycardia	1	
Cotunnius disease	1	
Normal pregnancy	1	
Neutropenia	1	
Gastroenteritis	1	
Sjögren flare with arthralgia	1	
Abdominal pain	1	
Cutaneous lupus erythematosus flare	1	
Hepatic cytolysis	1	
Epiretinal membrane	1	
Miscarriage	1	
Between W24 and W48	1 (1.8%)	5 (9.1%)
Type of serious adverse event		
Pneumopathy and hemoptysis		1
Retinal detachment		1
Non-Hodgkin's lymphoma		1
Chest pain		1
Ulnar nerve transposition		1
MALT lymphoma	1	
Total events	15 (27.3%)	11 (20.0%)

Data are number unless specified.
CPK, creatine phosphokinase; MALT, mucosa-associated lymphoid tissue; pSS, primary Sjögren syndrome.

outcome on ESSDAI, re-evaluating the use of this score as a primary outcome criterion might be important. NECESSITY, a European initiative, will combine the data from the present trial with those of previous randomised trials to determine new clinical outcomes in pSS. An initiative from OMERACT on clinical outcomes in pSS is also ongoing to progress on this crucial topic.

CONCLUSION

Among patients with pSS, the use of tocilizumab did not improve systemic involvement or symptoms over 24 weeks of treatment compared with placebo.

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CLINICAL SCIENCE

Efficacy and safety of abatacept in active primary Sjögren's syndrome: results of a phase III, randomised, placebo-controlled trial

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ABSTRACT

Objectives To evaluate efficacy and safety of abatacept in adults with active primary Sjögren's syndrome (pSS) in a phase III, randomised, double-blind, placebo-controlled trial.

Methods Eligible patients (moderate-to-severe pSS [2016 ACR/European League Against Rheumatism (EULAR) criteria], EULAR Sjögren's Syndrome Disease Activity Index [ESSDAI] ≥5, anti-SS-related antigen A/anti-Ro antibody positive) received weekly subcutaneous abatacept 125 mg or placebo for 169 days followed by an open-label extension to day 365. Primary endpoint was mean change from baseline in ESSDAI at day 169. Key secondary endpoints were mean change from baseline in EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) and stimulated whole salivary flow (SWSF) at day 169. Other secondary clinical endpoints included glandular functions and patient-reported outcomes. Selected biomarkers and immune cell phenotypes were examined. Safety was monitored.

Results Of 187 patients randomised, 168 completed double-blind period and 165 continued into open-label period. Mean (SD) baseline ESSDAI and ESSPRI total scores were 9.4 (4.3) and 6.5 (2.0), respectively. Statistical significance was not reached for primary (ESSDAI −3.2 abatacept vs −3.7 placebo, p=0.442) or key secondary endpoints (ESSPRI, p=0.337; SWSF, p=0.584). No clinical benefit of abatacept over placebo at day 169 was seen with other clinical and PRO endpoints. Relative to baseline, abatacept was associated with significant differences vs placebo in some disease-relevant biomarkers (including IgG, IgA, IgM-rheumatoid factor) and pathogenic cell subpopulations (post hoc analyses). No new safety signals were identified.

Conclusions Abatacept treatment did not result in significant clinical efficacy compared with placebo in patients with moderate-to-severe pSS, despite evidence of biological activity.

Key messages

What is already known about this subject?

- In patients with primary Sjögren's syndrome (pSS), open-label uncontrolled studies of various therapeutic agents with efficacy in other autoimmune diseases have shown some promising results based on different outcome measures, but large controlled studies have so far been unable to demonstrate a meaningful treatment benefit.

What does this study add?

- Abatacept treatment did not result in significant clinical efficacy versus placebo in this randomised controlled trial, but it showed evidence of disease-relevant biological activity.
- The lack of clinical benefit of abatacept treatment for patients with pSS in the face of an apparent biological effect is not understood.

How might this impact on clinical practice or future developments?

- Although this study does not support the use of abatacept in pSS, further studies would be needed to assess the impact of factors such as the heterogeneity of pSS.
- In highlighting the clinical heterogeneity of pSS and the major challenges in designing efficacy studies of novel therapies targeting systemic disease, the results from this study can be used to inform the development of new composite endpoints—which are sensitive to change and reflect clinical and biological effects—to aid future clinical development in pSS.

INTRODUCTION

Primary Sjögren's syndrome (pSS) is a chronic, systemic autoimmune disease typically affecting the salivary and lacrimal glands and producing symptoms of dry mouth, dry eyes, fatigue and pain.[1] The estimated prevalence of pSS in the general population is 0.01%–0.1%; pSS is associated with a high burden of disease and diminished quality of life.[2 3]

Treatment recommendations for patients with pSS focus mainly on symptomatic agents.[4] Available symptomatic therapies include artificial tears and saliva, cholinergic agonists such as pilocarpine[5] and cevimeline,[6] cyclosporine[7] and lifitegrast eye drops.[8] There are currently no approved disease-modifying treatments for pSS. Small, open-label, uncontrolled and controlled clinical efficacy studies of methotrexate,[9] leflunomide,[10] hydroxychloroquine,[11] rituximab,[12–14] epratuzumab (B-cell-targeted agents),[15] belimumab (B-cell-activating factor-blocking agent)[16] and infliximab (tumour

necrosis factor-α-blocking agent)[17] have shown mixed results using a variety of outcome measures. Additionally, randomised placebo-controlled trials of hydroxychloroquine[18] and rituximab[12 13] for pSS have been negative.

Abatacept is a selective costimulation modulator that blocks the interaction between CD80/CD86 on antigen-presenting cells and CD28 on T cells,[19 20] thereby disrupting T-cell activation, a likely key step in pSS pathogenesis.[21] Proven efficacy of abatacept for treatment of patients with rheumatoid arthritis (RA),[22 23] a T-cell-driven systemic autoimmune disease,[19 24–27] supports the rationale that blocking this co-stimulatory pathway can produce clinical efficacy in an autoimmune disease.

Early studies of abatacept in pSS showed promising results. In two small, open-label pilot studies with pSS, a 24-week course of intravenous abatacept treatment was associated with a beneficial effect on disease activity and an acceptable safety profile.[28 29] Additionally, a small, prospective observational study of 11 patients with pSS from Brazil recently reported a significant reduction in European League Against Rheumatism (EULAR) Sjögren's Syndrome Disease Activity Index (ESSDAI) and improved salivary flow following treatment with intravenous abatacept.[30] An open-label study of secondary SS (associated with RA) in Japanese patients demonstrated efficacy of intravenous abatacept for RA-related and SS-related manifestations.[31]

Here, we present the results of a double-blind (day 169) phase III, randomised, placebo-controlled trial with an extended open-label (day 365) treatment period to assess efficacy and safety of subcutaneous (SC) abatacept in patients with moderate-to-severe pSS.

METHODS

Study design

In this phase III, double-blind, placebo-controlled trial (ClinicalTrials.gov: NCT02915159), eligible patients with active pSS were randomised 1:1 to receive either weekly SC abatacept 125 mg or SC matching placebo for 169 days. A subsequent 197-day (365-day in Japan) open-label extension followed the initial double-blind period, when all eligible patients received SC abatacept 125 mg/week (those receiving placebo switched to abatacept). Post-treatment safety follow-up lasted an additional 168 days.

Patients were recruited from December 2016 to January 2018 from 60 centres in 13 countries. Random assignment of study treatment was performed by a central system. Randomisation schedules were generated by the Randomisation Group within Drug Supply Management of Bristol Myers Squibb Company. Randomisation was stratified globally by current corticosteroid use, current hydroxychloroquine use, enrolment in Japan (yes/no) and level of stimulated whole salivary flow (SWSF; $</\geq 0.1$ mL/min). A block size of 2 was applied.

This study was conducted in accordance with the Declaration of Helsinki[32] and the International Conference on Harmonisation Good Clinical Practice guidelines.[33] All patients enrolled provided written informed consent in accordance with local laws.

Patients

Patients aged ≥18 years with pSS defined by 2016 American College of Rheumatology/EULAR criteria[34] and moderate-to-severe disease activity with an ESSDAI score ≥5,[35] who were refractory to symptomatic or local therapy (eg, non-steroidal anti-inflammatory drugs) and anti-SS-related antigen A/anti-Ro antibody positive, were included. Patients were excluded if: they had another systemic autoimmune disease, inflammatory conditions, severe fibromyalgia or other medical conditions associated with clinical features of pSS that could interfere with assessment of treatment response; or they had received intravenous, intramuscular, SC or intra-articular corticosteroids within 4 weeks prior to randomisation, rituximab within 12 months or belimumab, other biological therapy or methotrexate within 12 weeks. Additional information regarding exclusion criteria can be found in the online supplemental appendix.

Primary and key secondary endpoints

The primary endpoint was mean change from baseline (day 1) in ESSDAI at day 169 for abatacept versus placebo. The two key secondary endpoints were mean changes from baseline in EULAR Sjögren's Syndrome Patient Reported Index (ESSPRI) and in SWSF (among patients with SWSF ≥0.1 mL/min at screening and baseline) of abatacept versus placebo at day 169. ESSDAI includes 12 domains (cutaneous, respiratory, renal, articular, muscular, peripheral nervous system, central nervous system, haematological, glandular, constitutional, lymphadenopathy and lymphoma, and biological)[36 37] and ESSPRI is a patient-reported symptom index for dryness, fatigue and limb (joint/muscular) pain.[38] SWSF was determined by vigorously chewing (one chew/second) a piece of preweighed sterile gauze for 2 min and determining difference in weight.

Other efficacy and exploratory endpoints

Other secondary clinical endpoints included mean change from baseline in 28-joint Disease Activity Score based on C reactive protein (DAS28 [CRP]) at day 169, ESSDAI score according to hydroxychloroquine and corticosteroid use (both were stratification variables for randomisation), Physician Global Assessment score and proportion of patients with minimally clinically important improvement in both ESSDAI score (decrease ≥3)[35] and in ESSPRI score (decrease ≥1).[35] Secondary patient-reported outcome endpoints included mean changes from baseline in Patient Global Assessment score, Patient-Reported Outcomes Measurement Information System fatigue score and Female Sexual Function Index score, which measures sexual function in six subdomains. Other glandular function endpoints were also evaluated and included mean changes from baseline in SWSF, unstimulated WSF (UWSF; expectorated unstimulated saliva for 15 min), numeric rating scale for eye and mouth dryness, Schirmer's test (measure of aqueous tear production over 5 min), tear break-up time (TBUT; seconds between patient's last blink and first appearance of a random dry spot on the cornea) and ocular staining scores[39] (OSS; cornea and conjunctiva staining pattern). All ocular assessments were performed by a trained ophthalmologist. Exploratory endpoints included mean changes from baseline in biomarkers of B-cell hyperactivity and immune cell phenotypes.

Assessments

Patient demographics and disease characteristics were assessed at baseline; clinical disease activity and safety were assessed regularly during the double-blind and open-label periods. The endpoint assessments were conducted at various intervals throughout the study along with tender and swollen joint counts.

For the post hoc analysis of biomarkers and laboratory parameters, changes from baseline were determined for erythrocyte sedimentation rate, high-sensitivity CRP, CH50 complement, C3 complement, C4 complement, IgG, IgA, IgM, IgM-rheumatoid factor (RF), kappa light chain, lambda light chain and beta-2

microglobulin. Serum biomarker chemokine ligand 13 (CXCL13) analyte was measured using the SIMOA assay from Myriad RBM. Immune cell phenotyping of whole blood samples was assessed in a subpopulation of patients from sites that participated in the flow cytometry analysis with data analysed using BD FACSDiva software.

Adverse events (AEs) were monitored throughout the study.

Statistical analysis

A hierarchical testing procedure was applied to the primary and key secondary endpoints to preserve the overall type I error of 5%. The first key secondary endpoint would only be tested (at significance level 5%) if the test for the primary endpoint was statistically significant (significance level 5%). If both the test for the primary endpoint and the first key secondary endpoint were statistically significant (both at significance level 5%), the second key secondary endpoint would be tested (at significance level 5%). The primary and key secondary endpoints, along with selected biomarkers, were analysed by a longitudinal repeated measures model. Power and sample size calculations are included in the online supplemental appendix.

Baseline demographics and disease characteristics of the study population were summarised descriptively. All efficacy analyses used the modified intent-to-treat (mITT) population, which comprised all randomised patients who received ≥1 dose of study medication. Missing data for responders were imputed as non-responders. Estimates of adjusted mean change were derived from a repeated measures mixed model; model analysis details are included in the online supplemental appendix.

Safety was summarised descriptively throughout the trial up to 56 days after last study drug dose.

Patient and public involvement

In addition to implementation of the intervention, patient-reported outcomes were key components of the study clinical efficacy outcomes. Independently of the study and through a patient engagement network, patients with pSS provided input towards key aspects of the final study design (such as the desired concomitant use of stable-dose hydroxychloroquine). Patients and patient advocacy groups were not involved in the data interpretation, writing or editing of this manuscript.

RESULTS

Patient disposition and baseline characteristics

Of 187 patients randomised (abatacept n=92; placebo n=95), 168 completed the double-blind period and 165 continued into the open-label period (online supplemental figure S1). A total of 19 patients discontinued treatment during the double-blind period; reasons for discontinuation were generally balanced between treatment arms (online supplemental figure S1). Patient baseline characteristics were similar between treatment groups (table 1). For the overall study population, mean age was 52 years, 95% of patients were female and 64% were white; mean disease duration was 5 years. Mean baseline ESSDAI and ESSPRI total scores were 9.4 and 6.5 and were similar between treatment groups. At baseline, 39% of patients received concomitant stable-dose hydroxychloroquine and 24% received oral corticosteroids (≤10 mg/day prednisone equivalent). Mean baseline SWSF (mL/min) was 1.0 and similar between treatment groups.

Table 1 Baseline patient demographics and disease characteristics

Characteristic	Abatacept (n=92)	Placebo (n=95)	Total (n=187)
Age, years	51.2 (12.3)	52.9 (13.5)	52.0 (12.9)
Weight, kg	71.4 (18.6)	67.5 (17.3)	69.4 (18.0)
Female, n (%)	85 (92.4)	92 (96.8)	177 (94.7)
Race, white, n (%)	60 (65.2)	60 (63.2)	120 (64.2)
Disease duration, years	5.0 (5.0)	5.1 (5.3)	5.0 (5.2)
ESSDAI total score	8.7 (3.4)	10.1 (5.0)	9.4 (4.3)
ESSPRI total score	6.6 (2.1)	6.5 (1.9)	6.5 (2.0)
SWSF, mL/min	1.1 (0.9)	0.9 (0.9)	1.0 (0.9)
SWSF ≥0.1 mL/min, n (%)	84 (91.3)	86 (90.5)	170 (90.9)
Concomitant treatment at day 1, n (%)			
Non-steroidal anti-inflammatory drugs	44 (47.8)	31 (32.6)	75 (40.1)
Topical eye preparation	13 (14.1)	14 (14.7)	27 (14.4)
Parasympathomimetics	15 (16.3)	21 (22.1)	36 (19.3)
Hydroxychloroquine	37 (40.2)	36 (37.9)	73 (39.0)
Oral corticosteroids*	22 (23.9)	22 (23.2)	44 (23.5)
Concomitant treatment prior to day 1, n (%)			
Non-steroidal anti-inflammatory drugs	49 (53.3)	37 (38.9)	86 (46.0)
Topical eye preparation	17 (18.5)	16 (16.8)	33 (17.6)
Parasympathomimetics	17 (18.5)	22 (23.2)	39 (20.9)
Hydroxychloroquine	48 (52.2)	45 (47.4)	93 (49.7)
Methotrexate	20 (21.7)	15 (15.8)	35 (18.7)
Oral corticosteroids	32 (34.8)	27 (28.4)	59 (31.6)

Data are mean (SD) unless otherwise stated.
*≤10mg/day prednisone equivalent.
ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; SWSF, stimulated whole salivary flow.

Primary and key secondary endpoints

At day 169, adjusted mean change from baseline in ESSDAI score (primary endpoint) was not statistically different between treatment groups: −3.2 for abatacept vs −3.7 for placebo (p=0.442; figure 1A). At day 365 (end of open-label period), adjusted mean change from baseline in ESSDAI score was −3.8 for abatacept vs −4.4 for placebo (switched to abatacept at day 169; figure 1A). At days 169 and 365, proportions of patients with minimally clinically important improvements from baseline in ESSDAI total score (decrease ≥3) were 55% and 48% for abatacept, and 58% and 56% for placebo (switched to abatacept at day 169), respectively. In the stratified subgroups, patients not receiving corticosteroids or hydroxychloroquine at baseline had similar mean changes in ESSDAI score in both treatment groups (adjusted mean differences from placebo [95% CI] 0.1 [−1.3 to 1.4] and 0.4 [−1.1 to 1.9], respectively). In patients who received concomitant stable-dose oral corticosteroids during the double-blind period, adjusted mean difference from placebo (95% CI) in ESSDAI at day 169 was 2.7 (0.2 to 5.1).

Due to non-statistically significant primary endpoint results, the two key secondary endpoints, ESSPRI and SWSF, could not be tested for significance; nominal p values are presented. For ESSPRI score, adjusted mean changes from baseline at day 169 were −1.3 and −1.5 in the abatacept and placebo groups, respectively (nominal p=0.337; figure 1B). At day 365, the adjusted mean change from baseline in ESSPRI score was −1.4 and −1.5 with abatacept and placebo (switched to abatacept at day 169), respectively (figure 1B). Proportions of patients with minimally clinically important improvement from baseline in ESSPRI total score (≥1) at days 169 and 365 were 41% and 41% for abatacept, and 53% and 51% for placebo (switched to abatacept at day 169), respectively. Among patients with SWSF ≥0.1 mL/min

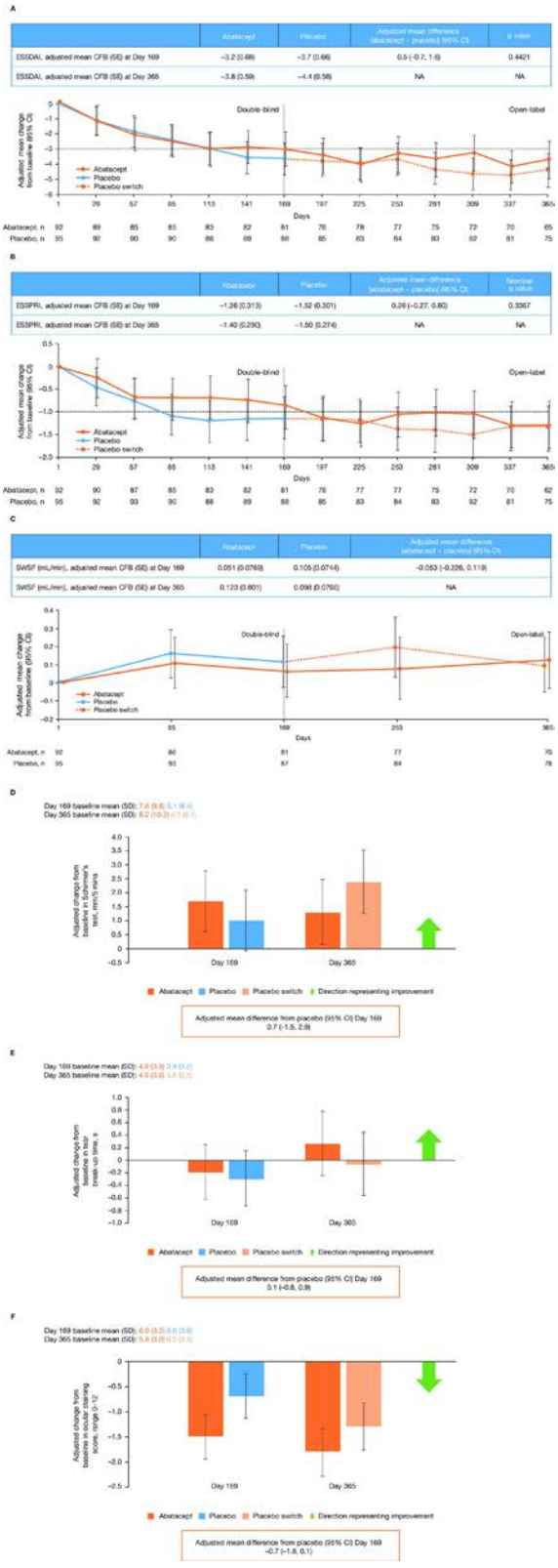

Figure 1 Adjusted mean changes from baseline in clinical efficacy outcomes over time for (A) total ESSDAI score, (B) total ESSPRI score, (C) SWSF (mITT population), (D) Schirmer's test, (E) tear break-up time and (F) ocular staining scores. (A–C) The results for day 169 in the table are from the primary analysis and the data in the plot are based on the 1-year analysis. (D–F) The adjusted mean differences from placebo (95% CI) at day 169 in the text boxes are from the primary analysis and the data in the plot are based on the 1-year analysis. Study eye is defined as the eye with the higher total score for ocular surface staining at baseline. If both eyes have the same total score for ocular surface staining at baseline, the eye with the lower Schirmer's test time (STT) at baseline will be selected. If both eyes have equal STT at baseline, then the eye with the lower tear break-up time will be selected. If all of the parameters above are equal, then the right eye will be selected as the study eye. CFB, change from baseline; ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; mITT, modified intent to treat; NA, not applicable; SWSF, stimulated whole salivary flow.

Table 2 Summary of change from baseline in primary and secondary clinical, glandular and patient-reported outcome measures at days 169 and 365

	Day 1 (baseline scores)		Day 169 (adjusted mean change from baseline [SE] scores)			Day 365 (adjusted mean change from baseline [SE] scores)	
	Abatacept	Placebo	Abatacept	Placebo	Adjusted mean treatment difference for abatacept versus placebo (95% CI)	Abatacept	Placebo
Disease activity							
ESSDAI score	8.7 (3.4)	10.1 (5.0)	−3.2 (0.7)	−3.7 (0.7)	0.5 (−0.7 to 1.6)	−3.8 (0.6)	−4.4 (0.6)
ESSDAI responders*, n/N (%)	NA	NA	51/92 (55.4)	55/95 (57.9)	−2.7 (−17.2 to 11.7)**	44/92 (47.8)	53/95 (55.8)
DAS28 (CRP)	3.5 (1.3)	3.6 (1.3)	−0.9 (0.1)	−1.1 (0.1)	0.3 (0.0 to 0.5)	−0.9 (0.1)	−1.1 (0.1)
Physician GDA	47.8 (17.3)	47.8 (19.3)	−23.0 (2.4)	−23.7 (2.4)	0.6 (−4.3 to 5.6)	ND	ND
Patient-reported outcomes							
ESSPRI score							
Total	6.6 (2.1)	6.5 (1.9)	−1.3 (0.3)	−1.5 (0.3)	0.3 (−0.3 to 0.8)	−1.4 (0.3)	−1.5 (0.3)
Dryness	7.0 (2.4)	7.0 (2.3)	−0.8 (0.3)	−1.0 (0.3)	0.2 (−0.5 to 0.8)	−1.2 (0.3)	−1.4 (0.3)
Fatigue	6.6 (2.4)	6.6 (2.5)	−1.3 (0.3)	−1.6 (0.3)	0.3 (−0.4 to 0.9)	−1.9 (0.4)	−2.0 (0.3)
Pain	6.1 (2.7)	6.0 (2.7)	−1.1 (0.3)	−1.5 (0.3)	0.3 (−0.3 to 1.0)	−1.3 (0.4)	−1.4 (0.3)
ESSPRI responders, n/N (%)‡	NA	NA	38/92 (41.3)	50/95 (52.6)	−11.2 (−25.6 to 3.2)**	38/92 (41.3)	48/95 (50.5)
Ocular dryness, NRS§	6.8 (2.4)	6.6 (2.5)	−0.9 (0.3)	−1.0 (0.3)	ND	−1.3 (0.4)	−1.4 (0.3)
Oral dryness, NRS§	7.3 (2.3)	6.9 (2.5)	−1.3 (0.3)	−1.2 (0.3)	ND	−1.7 (0.3)	−1.6 (0.3)
Patient GDA	58.6 (22.4)	58.0 (21.1)	−10.1 (3.1)	−9.0 (3.0)	−1.1 (−7.4 to 5.1)	−12.9 (3.4)	−12.6 (3.2)
PROMIS-Fatigue	61.2 (8.8)	59.5 (8.6)	−5.6 (1.2)	−5.6 (1.1)	0.04 (−2.3 to 2.4)	−6.5 (1.2)	−6.3 (1.2)
FSFI	13.9 (8.7)††	17.3 (9.7)††	−2.3 (1.7)	−1.9 (1.8)	−0.5 (−3.5 to 2.6)	−0.3 (1.0)	2.3 (1.0)
Glandular function							
Schirmer's test, mm	7.4 (9.4)††	5.0 (8.0)††	1.7 (1.1)	1.0 (1.1)	0.7 (−1.5 to 2.9)	1.3 (1.2)	2.4 (1.1)
TBUT, s	4.7 (3.8)††	3.7 (3.1)††	−0.2 (0.4)	−0.3 (0.4)	0.1 (−0.8 to 0.9)	0.3 (0.5)	−0.1 (0.5)
OSS	6.1 (3.2)††	6.5 (3.5)††	−1.5 (0.4)	−0.7 (0.4)	−0.7 (−1.6 to 0.1)	−1.8 (0.5)	−1.3 (0.2)
SWSF, mL/min	1.1 (0.9)	0.9 (0.8)	0.1 (0.1)	0.1 (0.1)	−0.1 (−0.2 to 0.1)	0.1 (0.1)	0.1 (0.1)
UWSF, mL/min	0.1 (0.1)††	0.1 (0.1)††	0.02 (0.01)	0.03 (0.01)	−0.004 (−0.03 to 0.03)	0.02 (0.01)	0.03 (0.01)

Values are mean (SD) unless otherwise noted. Ocular assessments are for study eye. The primary and key secondary endpoints (except those marked §) were analysed by a longitudinal repeated measures model, which included randomisation stratification factors of current corticosteroid use (yes/no), current hydroxychloroquine use (yes/no), enrolment in Japan (yes/no) and SWSF </≥0.1 mL/min. Data at day 169, including adjusted mean treatment differences, are based on the primary analysis, while data at day 365 are based on the 1-year analysis. The change in outcome measures was equal to the difference between the values at baseline (day 1) and day 169 or day 365, as shown. The adjusted mean treatment difference was equal to the adjusted change in the abatacept group minus the adjusted change in the placebo group. Baseline data are for all randomised patients, except where marked with †, which were based on those patients included at day 29 or ††, which were based on day 85 (earliest post-baseline analysis) of the primary analysis. SWSF data at baseline and day 169 are for patients in the mITT population with SWSF of at least 0.1 mL/min at baseline and data at day 365 are for the overall mITT population; baseline measurements for this endpoint were from those patients included at day 169.

*Patients with minimally clinically important improvement from baseline (≥3 points) in ESSDAI total score.

**Estimate of difference (rather than adjusted mean treatment difference).

‡Patients with minimally clinically important improvement from baseline (≥1 point) in ESSPRI total score.

DAS28 (CRP), 28-joint Disease Activity Score based on C reactive protein; ESSDAI, EULAR Sjögren's Syndrome Disease Activity Index; ESSPRI, EULAR Sjögren's Syndrome Patient Reported Index; FSFI, Female Sexual Function Index; GDA, global disease assessment; NA, not available; ND, not determined; NRS, numeric rating scale; OSS, ocular staining scores; PROMIS-Fatigue, Patient-Reported Outcomes Measurement Information System Fatigue Score; SWSF, stimulated whole salivary flow; TBUT, tear break-up time; UWSF, unstimulated whole salivary flow.

at screening and baseline, adjusted mean change from baseline at day 169 was 0.06 for abatacept vs 0.11 for placebo (p=0.5841).

The study was terminated prematurely by the sponsor after the primary analysis failed to show a statistically significant difference in the primary endpoint, and analyses of secondary endpoints failed to demonstrate clinically meaningful differences between abatacept and placebo groups.

Other efficacy endpoints

For SWSF score, adjusted mean changes from baseline at days 169 and 365 were 0.05 and 0.12 for abatacept vs 0.11 and 0.10 for placebo (switched to abatacept at day 169), respectively (figure 1C) in the overall mITT population. The adjusted mean treatment difference (95% CI) for UWSF at day 169 was −0.004 (−0.03, 0.03) (table 2). We observed no significant differences between treatment groups in mean change in DAS28 (CRP) from baseline (table 2). Mean changes from baseline in Schirmer's test, TBUT and OSS were all similar between treatment groups (figure 1D–F).

Changes from baseline in other clinical, glandular and patient-reported outcome measures at days 169 and 365 are summarised in table 2.

Post hoc analyses

Numerical differences between ESSDAI domains were observed. For example, in patients with an ESSDAI biological domain involvement at baseline, the proportion of those with improvements (moderate to low/no activity and low to no activity) in this domain was higher (statistical significance was not tested) with abatacept (12/40; 30%) vs placebo

(6/41; 15%) at day 169; this was maintained up to day 365 (data not shown). Additionally, proportions of patients with improvements in haematological and pulmonary domains of ESSDAI were numerically higher with abatacept (7/16; 44% and 3/6; 50%) vs placebo (6/29; 21% and 1/14; 7%) at day 169, respectively, among those with involvement of the corresponding domain at baseline (data not shown). Proportions of patients by ESSDAI domain activity at baseline and day 169 are shown (online supplemental figure S2). A high placebo effect was seen in several ESSDAI domains such as lymphadenopathy and articular.

Of 12 selected disease-relevant laboratory parameters and biomarkers, mean change in IgG, IgA, IgM-RF, kappa light chain and C4 complement serum levels was significantly different between the abatacept and placebo treatment groups at day 169 (figure 2A–E; Benjamini–Hochberg procedure). At baseline, based on patients with data available at day 85, mean serum CXCL13 levels were similar between treatment arms (abatacept 90.4; placebo 97.0); however, by day 169, these levels were significantly reduced in the abatacept vs placebo group (nominal $p<0.0001$; figure 2F). At day 365, adjusted mean changes from baseline in IgG, IgA, IgM-RF, kappa light chain, C4 complement and CXCL13 serum levels were similar for the abatacept and placebo (switched to abatacept at day 169) treatment groups (figure 2). The numbers (%) of patients at baseline and day 169 with abnormally elevated IgG levels were 38 (41.3) and 31 (33.7) with abatacept, and 45 (47.4) and 50 (52.6) with placebo; those with elevated kappa light chains were 54 (58.7) and 41 (44.6) with abatacept, and 68 (71.6) and 61 (64.2) with placebo, respectively.

The subset of patients included for the immune cell phenotyping analysis (n=78) had similar baseline demographics and disease characteristics to the study population. In this subset, abatacept-treated patients had numerically greater decreases at day 169 (year 1 analysis) in the proportions of blood CD4+ effector memory T cells (TEM) (adjusted mean difference [95% CI] –9.1 [–14.2 to –4.0]), T helper type 1 cells (Th1) (adjusted mean difference [95% CI]–2.6 [–4.2 to –1.0]), regulatory T cells (Treg) (adjusted mean difference [95% CI] –1.8 [–3.0 to –0.6]), T follicular helper cells (Tfh) (adjusted mean difference [95% CI] –1.4 [–2.2 to –0.5]) and ICOS-positive Tfh ($ICOS^+$ Tfh) (adjusted mean difference [95% CI] –14.4 [–19.3 to –9.5]) cells vs placebo (figure 3A–E). After switch to abatacept at day 169, mean changes from baseline in Treg, Tfh and $ICOS^+$ Tfh cellular subsets were similar for abatacept and placebo treatment groups (figure 3C–E); for CD4+TEM and Th1, mean differences seen at day 169 were less pronounced by day 365 (figure 3A,B).

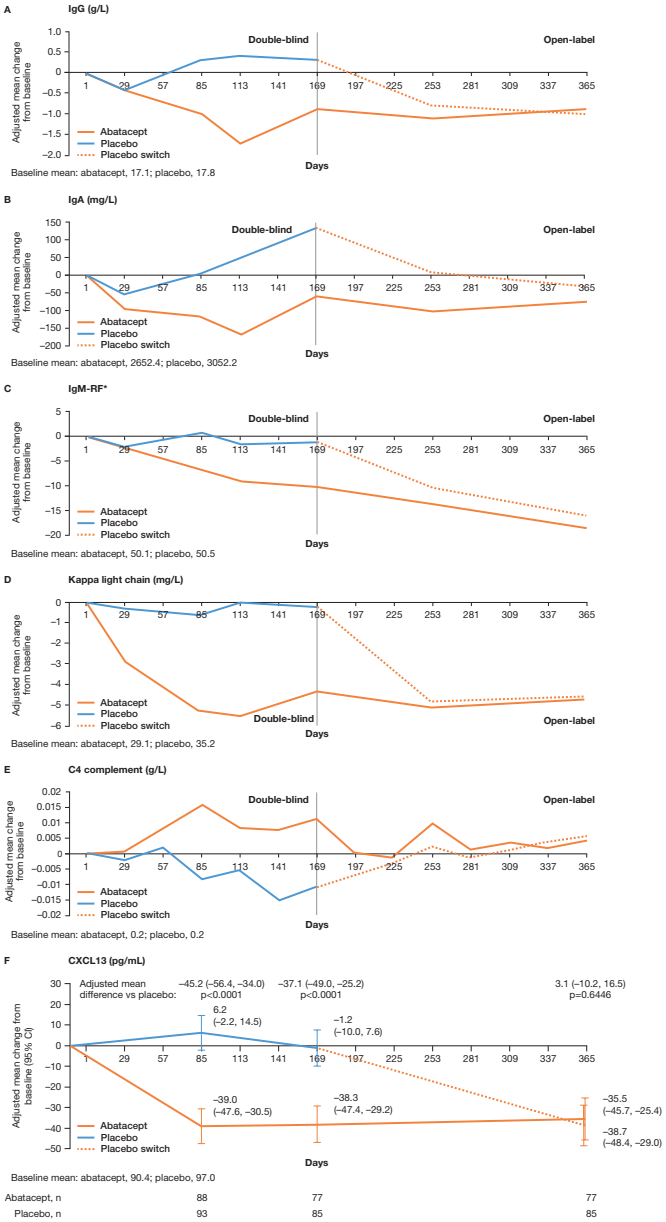

Figure 2 Adjusted mean change from baseline over time for selected biomarkers to day 365: (A) IgG, (B) IgA, (C) IgM-RF, (D) kappa light chain, (E) C4 complement and (F) CXCL13. P values were nominal. Adjusted mean differences at day 365 are versus the placebo arm switched to abatacept (rather than vs placebo). Biomarker assessments up to 56 days post-dose are included. Estimates of adjusted mean change are from a repeated measure mixed model that includes baseline biomarker result, treatment group, randomisation stratification factors (baseline oral corticosteroid use [yes/no], baseline hydroxychloroquine use [yes/no]), time, time-by-treatment group interaction and time-by-baseline biomarker result interaction. Baseline values were based on those patients included at day 29 (day 85 for CXCL13). *Units are calibrated against standard curves derived from a WHO international reference. CXCL13, chemokine ligand 13; RF, rheumatoid factor.

Safety

A summary of AEs in the double-blind and open-label treatment periods is shown in table 3. In the double-blind period, serious AEs (SAEs) were reported in 12 patients. Among patients treated with abatacept, 20 had SAEs: 9 in the double-blind period and 11 in the open-label period with follow-up to 56 days after the last treatment. Reported SAEs included two deaths (one placebo-treated patient [septic shock] and one abatacept-treated patient [cardiac event; patient had a history of pulmonary embolism]) and one neoplasm (plasma cell myeloma) in one abatacept-treated patient. SAEs related to study drug occurred during the double-blind treatment period in 3% (pneumonia bacterial, anaphylactoid reaction and drug hypersensitivity) of abatacept-treated and 1% (septic shock) of placebo-treated patients. Related AEs occurred during the double-blind treatment period in 46% and 25% of abatacept-treated and placebo-treated patients, respectively, but this difference was not driven by any specific AE. No new safety signals were identified compared with the known abatacept safety profile.

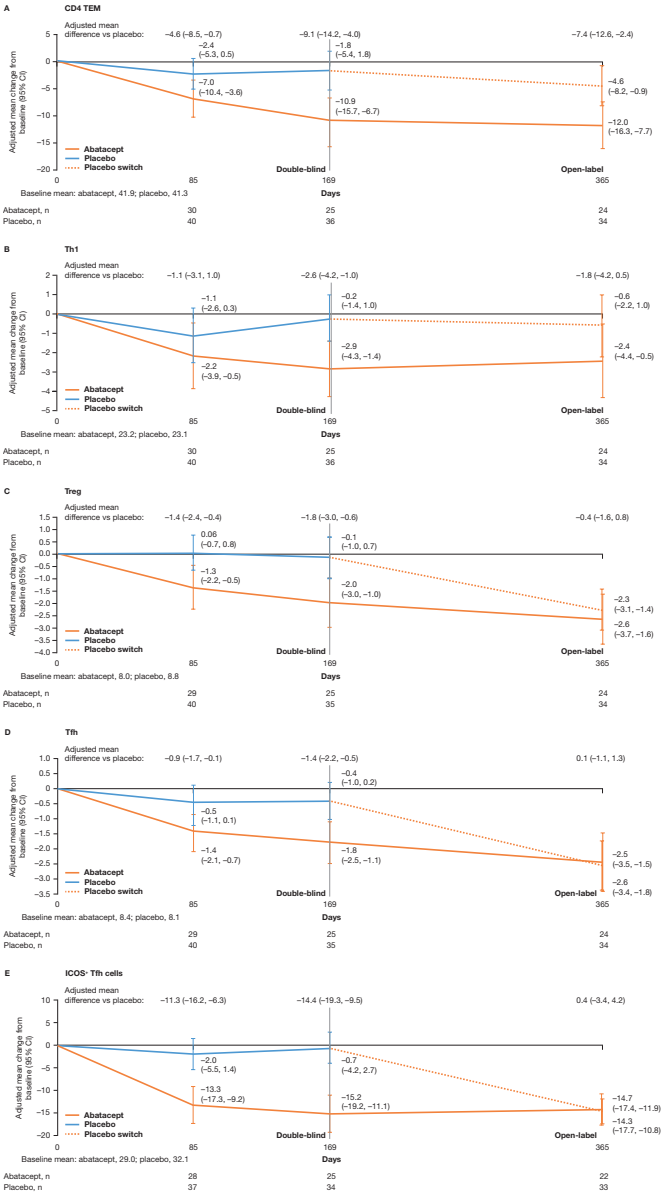

Figure 3 Adjusted mean change over time from baseline to day 365 in circulating T-cell subtypes: (A) CD4 TEM, (B) Th1, (C) Treg, (D) Tfh and (E) ICOS$^+$ Tfh cells. Adjusted mean differences at day 365 are versus the placebo arm switched to abatacept (rather than vs placebo). (A) CD4+TEM expressed as a percentage of CD4+ cells. Markers for CD4 TEM cells=CD3+CD4+CD45RA−CCR7−. (B) Markers for Th1 cells=CD3+CD4+CXCR3+CCR6−. (C) Treg expressed as a percentage of CD4 T cells. Markers for Treg cells=CD3+CD4+CD25+CD127−/LO. (D) Tfh is expressed as a percentage of CD4 +T cells (CXCR5+PD1+). Markers for Tfh cells=CD3+CD4+CD185+CD279+. (E) ICOS$^+$ Tfh expressed as a percentage of Tfh cells. Markers for ICOS$^+$ Tfh cells=CD3+CD4+CD185+CD279+CD278+. ICOS$^+$ Tfh, ICOS-positive Tfh costimulator; TEM, effector memory T cells; Tfh, T follicular helper cells; Th1, T helper type 1 cells; Treg, regulatory T cells.

DISCUSSION

This large, randomised, double-blind study evaluated efficacy and safety of treatment with abatacept versus placebo in patients with active, moderate-to-severe pSS. The treatment effect did not reach statistical significance for the primary or two key secondary endpoints, and showed no clinical benefit of abatacept over placebo in other clinical efficacy and patient-reported outcome endpoints at the end of the double-blind period at day 169, or at the end of the open-label extended follow-up period at day 365. The safety profile of abatacept in patients with pSS was similar to that in other diseases treated with abatacept.[40] Notably, abatacept therapy did have a clear impact on selected disease-relevant markers of biological activity likely related to central mechanisms of pSS pathogenesis.

In a recent randomised, placebo-controlled, investigator-initiated, single-centre study of SC abatacept in patients with early active pSS in the Netherlands (ASAP III NCT02067910; n=80), results for the primary endpoint (ESSDAI at 24 weeks) were similar to the present study.[41] In contrast to the current study, the secondary ESSPRI endpoint (ESSPRI responders at weeks 12 and 24) was significantly different, in favour of abatacept versus placebo. Differences in results between ASAP III and the current study may be due to variations between study populations and designs, including the single-centre versus multiple-centre nature of the two studies. For instance, in ASAP III the use of hydroxychloroquine was not allowed and corticosteroids were used by fewer patients than in the current study. Additionally, at study entry all patients in ASAP III had positive biopsies, a ≤7 year disease duration and higher baseline activity (mean ESSDAI baseline score 13.5) than the current study.

Despite no detectable clinical effect in the current study, favourable improvements were observed in disease-relevant laboratory parameters and biomarkers. Some of these findings suggested an effect of therapy on T-cell-induced, B-cell hyperactivity. For example, CXCL13, the serum levels of which were significantly reduced by abatacept treatment,[21] is a chemokine secreted by Tfh cells, which play a pivotal role in the migration and activation of B cells in salivary gland ectopic lymphoid structures[42 43]; in pSS, its serum levels correlate with disease activity and histomorphological parameters.[21 44 45] In previous open-label pilot studies,[28 29] 24 week intravenous abatacept treatment reduced glandular inflammation, induced cellular changes (lymphocytic foci and B and T cell subtypes) and increased salivary production in 11 patients with pSS.[29] Additionally, a study of 15 patients with early pSS found that 24-week intravenous abatacept treatment significantly reduced ESSDAI, ESSPRI, RF and IgG at 24 weeks.[28] More recently, it has been reported that 24-week intravenous abatacept treatment decreased the number of germinal centres in parotid glands of patients with pSS.[46] While abatacept has been proven effective for treatment of RA, polyarticular juvenile idiopathic arthritis and psoriatic arthritis, it has not shown significant therapeutic efficacy in systemic lupus erythematosus and multiple sclerosis.[47 48] The mechanistic underpinnings across the autoimmune spectrum are complex and incompletely understood. A partial overlap in the clinical and serological features of different autoimmune diseases does not necessarily extrapolate to mutually shared treatment efficacy. Further explanation for why a detectable clinical effect was not observed with abatacept in this study, despite evidence of biological activity, may be due to limitations in the design of pSS studies. The variable characteristics and heterogeneity seen within the pSS patient population raise major challenges for study design.[49] In addition, some pSS outcome measures can be subjective or difficult to standardise (eg, salivary flow has high intervariability and intravariability). Furthermore, there is a need for the development of composite study endpoints with improved cut-off and assessment time points. For example, although ESSDAI score reflects all domains of disease activity, its value in detecting small changes has been debated; as a result, there is a minimum ESSDAI score threshold required for trial entry, effectively excluding a large proportion of patients.[13 50]

Table 3 Summary of patients with adverse events* reported in the double-blind period and in the cumulative abatacept-treated population

	Double-blind treatment period		Cumulative abatacept-treated population† (n=178)
	Abatacept (n=92)	Placebo (n=95)	
Deaths	0 (0)	1 (1.1)	1 (0.6)
Serious adverse events	9 (9.8)	3 (3.2)	20 (11.2)
Cardiac disorders	1 (1.1)	1 (1.1)	2 (1.1)
Gastrointestinal disorders	1 (1.1)	1 (1.1)	1 (0.6)
Immune system disorders	2 (2.2)	0	2 (1.1)
Infections and infestations	1 (1.1)	1 (1.1)	3 (1.7)
Musculoskeletal and connective tissue disorders	2 (2.2)	0	4 (2.2)
Hepatobiliary disorders	1 (1.1)	0	2 (1.1)
Neoplasms	1 (1.1)	0	3 (1.7)
General disorders	0	0	2 (1.1)
Blood and lymphatic system disorders	0	0	1 (0.6)
Product issues	0	0	1 (0.6)
Respiratory, thoracic and mediastinal disorders	0	0	1 (0.6)
Study drug-related serious adverse events	3 (3.3)	1 (1.1)	6 (3.4)
Discontinuations due to serious adverse events	2 (2.2)	1 (1.1)	4 (2.2)
Adverse events	79 (85.9)	68 (71.6)	127 (71.3)
Study drug-related adverse events‡	42 (45.7)	24 (25.3)	67 (37.6)
Discontinuations due to adverse events	3 (3.3)	2 (2.1)	5 (2.8)

Data are n, %.
*Adverse events reported up to 56 days post-last abatacept dose. Serious adverse events include hospitalisations for elective surgical procedures. Study drug-related adverse event or serious adverse event is defined as an adverse event or serious adverse event with a related or missing relationship to study medication.
†The cumulative abatacept-treated population were followed from the first day of abatacept treatment in the study up to 56 days after the last abatacept treatment in the study.
‡Adverse events related to abatacept were not driven by any specific system organ class.

The current trial did not confirm the promising early results from open-label studies of abatacept in pSS; this disparity has also been seen in the development of other biologics for treatment of pSS.[12 13] In the TEARS[13] and TRACTISS[12] randomised controlled trials, rituximab demonstrated no significant improvement in ESSDAI score,[12 13] despite promising early results from a previous smaller study.[14] Potential explanations for the disparate findings in these rituximab trials include lack of patient stratification, insufficient tissue depletion of B cells, and the choice and timing of primary outcome evaluation.[50] A study of leniolisib (a P13Kδ inhibitor), which had outcome measures and a patient population similar to the current study, showed no significant improvement in clinical outcome measures despite a significant decrease in CXCL13 serum levels, similar to our study.[51] Other randomised controlled trials in pSS, like the current study, show evidence of a strong placebo effect.[12 13] Considering the large placebo effect seen in this study, a reduction of at least −6.7 in ESSDAI score (placebo effect +≥3) from a baseline value of 8.7 would have been required to demonstrate therapeutic benefit over placebo.

CONCLUSION

No significant clinical effect was seen with abatacept versus placebo in this randomised controlled trial in patients with active, moderate-to-severe pSS. However, abatacept therapy had a positive effect on disease-relevant biomarkers, providing evidence of biological activity. No new safety signals were identified for abatacept.

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TT: Grant/Research: AbbVie, Asahi Kasei, Astellas, AYUMI, Chugai, Daiichi Sankyo, Eisai, Mitsubishi Tanabe, Nipponkayaku, Novartis, Pfizer Japan, Takeda; Consultant: AbbVie, Astellas, Astra Zeneca, Chugai, Eli Lilly Japan, GlaxoSmithKline, Janssen, Mitsubishi Tanabe, Nipponkayaku, Novartis, Taiho, Taisho Toyama, UCB Japan; Speakers' bureau: AbbVie, Astellas, Bristol Myers Squibb Company, Chugai, Daiichi Sankyo, Eisai, Mitsubishi Tanabe, Novartis, Pfizer Japan, Sanofi, Takeda, Teijin. RS: Grant/research support: Pfizer; Consultant: Amgen, Bristol Myers Squibb Company, Celgene, GlaxoSmithKline, Lilly, Pfizer, Roche. GF: Consultant: Aldeyra, Allysta, Aurinia, Bristol Myers Squibb Company, Clemencia, Hovione, Kala, Lexitas PharmaServices, Nicox, Noveome, Sight Sciences, Tarsus, Tear Solutions; Stock: TearLab. MN: Employee: Bristol Myers Squibb Company; Shareholder: Bristol Myers Squibb Company. SM: Employee: Bristol Myers Squibb Company; Shareholder: Bristol Myers Squibb Company. RW: Employee: Bristol Myers Squibb Company; Shareholder: Bristol Myers Squibb Company. NR: Employee: Bristol Myers Squibb Company; Shareholder: Bristol Myers Squibb Company. HB: Unrestricted grant: Bristol Myers Squibb Company, Roche; Consultant: Speakers bureau: Bristol Myers Squibb Company, Novartis.

Patient consent for publication Not required.

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CLINICAL SCIENCE

Efficacy of tocilizumab in patients with hand osteoarthritis: double blind, randomised, placebo-controlled, multicentre trial

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ABSTRACT

Objective To evaluate the efficacy of tocilizumab, an antibody against IL-6 receptor, in patients with hand osteoarthritis.

Methods This was a multicentre, 12-week, randomised, double-blind, placebo-controlled study from November 2015 to October 2018. Patients with symptomatic hand osteoarthritis (pain ≥40 on a 0–100 mm visual analogue scale (VAS) despite analgesics and non-steroidal anti-inflammatory drugs; at least three painful joints, Kellgren-Lawrence grade ≥2) were randomised to receive two infusions 4 weeks apart (weeks 0 and 4) of tocilizumab (8 mg/kg intravenous) or placebo. The primary endpoint was changed in VAS pain at week 6. Secondary outcomes included the number of painful and swollen joints, duration of morning stiffness, patients' and physicians' global assessment and function scores.

Results Of 104 patients screened, 91 (45 to tocilizumab and 46 to placebo; 82% women; mean age 64.4 (SD 8.7) years) were randomly assigned and 79 completed the 12-week study visit. The mean change between baseline and week 6 on the VAS for pain (primary outcome) was −7.9 (SD 19.4) and −9.9 (SD 20.1) in the tocilizumab and placebo groups (p=0.7). The groups did not differ for any secondary outcomes at weeks 4, 6, 8 or 12. Overall, adverse events were slightly more frequent in the tocilizumab than placebo group.

Conclusion Tocilizumab was no more effective than placebo for pain relief in patients with hand osteoarthritis.

Key messages

What is already known about this subject?

- Synovitis is a common finding in hand osteoarthritis (OA) and is associated with clinical symptoms.
- Preclinical and epidemiological studies suggested that IL-6 is involved in OA structural damage.
- Whether IL-6 blockade can modulate OA pain is unknown.

What does this study add?

- In this double-blind randomised controlled trial, tocilizumab, an antibody against IL-6 receptor, did not significantly improved pain or function in patients with hand OA.

How might this impact on clinical practice or future developments?

- Our findings do not support a role for an IL-6 signalling pathway in OA-related hand pain. Long-term studies to investigate the benefit of IL-6 blockade on joint structure are warranted.

INTRODUCTION

Symptomatic hand osteoarthritis (OA) is a highly prevalent disease affecting about 10% of the general population.[1–3] It causes pain, stiffness, impaired physical function and quality of life. In some patients, the global burden of disease can be as severe as in rheumatoid arthritis.[4]

Guidelines recommend a combination of pharmacological and non-pharmacological approaches for managing hand OA.[5–7] Nevertheless, despite optimal treatment, some patients often experience no relief, which highlights an unmet need in the field.

In the past decade, data from ultrasonography and MRI studies allowed us to better understand the mechanisms of pain in hand OA. Cross-sectional studies showed that both synovitis and bone-marrow lesions were common findings in hand OA and were associated with clinical symptoms and structural damage.[8–10] In addition, longitudinal studies found change in severity of synovitis correlated with change in pain.[11 12] Thus, local inflammation may be a major therapeutic target in patients with hand OA.[13 14]

Randomised controlled trials of IL-1[15] or tumour necrosis factor (TNF) blockers[16–19] failed to demonstrate their efficacy for pain, which suggests that those cytokines were not deeply involved in the hand OA pain process.

IL-6 is a pleiotropic inflammatory cytokine involved in many diseases and particularly OA, where it acts as a mediator of hypoxia-inducible factor 2α to upregulate matrix metalloproteinase 3 and 13 levels.[20] High IL-6 serum level is an independent predictor of incident radiographic knee OA,[21] and within the joint, high IL-6 level is found in OA synovial fluid.[22] Conversely, low innate capacity to produce IL-6 is associated with absence of OA in

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old age.[23] We and others showed that targeting IL-6 or the signal transducer and activator of transcription 3 signalling pathway slowed the progression of experimental OA in mice.[24–26] Altogether, these data suggest a pivotal role for IL-6 in OA structural damage.

Whether IL-6 blockade can modulate OA pain is unknown. We aimed to evaluate the efficacy of tocilizumab, an antibody against IL-6 receptor (IL-6R), in patients with symptomatic hand OA.

METHODS

Study design

This was a randomised, double-blind, placebo-controlled, multicentre, 3-month, parallel-group study of patients with hand OA (NCT02477059). The study was conducted in 11 centres in France from November 2015 to October 2018.The study was conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki.

Study population

Patients were adults with painful hand OA meeting the classification criteria of the American College of Rheumatology for hand OA. Inclusion criteria were age 40–85 years; at least three painful proximal interphalangeal (PIP) or distal interphalangeal (DIP) joints for more than 3 months; pain level ≥40 mm (global pain in the last 24 hours on a visual analogue scale (VAS) 0–100 mm) at screening and baseline; pain not responding to acetaminophen or weak opioids and to non-steroidal anti-inflammatory drugs (NSAIDs) taken for at least 15 days in the past 3 months; recent X-rays of the hand (<6 months) with at least three OA joints (DIP or PIP; Kellgren and Lawrence grade ≥2). Main exclusion criteria were previous therapies with anti-TNFα or IL-6 blockers; psoriasis; hand OA secondary to previous inflammatory diseases and any painful syndrome of the upper limb that may interfere with evaluation of hand pain; inflammatory rheumatic diseases; overall contraindications to IL-6 blockers such as acute or chronic infectious states (latent tuberculosis or risk of reactivation of silent tuberculosis; HIV or hepatitis virus (C and B) infection; conditions with increased risk factors for infectious disease such as chronic cutaneous ulcers, urinary catheters and previous infection from any medical device); cancer or malignant blood disease; history of sigmoiditis; heart failure (New York Heart Association classification 3–4); leucopenia (white cell count <3.0 10^9/L); thrombopenia (platelet count <100 10^9/L); elevated liver enzymes (>3 times upper limit of normal); chronic kidney disease >stage 3; dyslipidaemia; scheduled surgery for hand OA or any surgical procedure anticipated within the next 6 months; intra-articular injections of corticosteroids in the past month or hyaluronic acid intra-articular injection in IP joints in the 6 months before joining the study; slow-acting drugs for OA (soybean and avocado extracts, glucosamine, chondroitin, diacerhein) started <3 months before the study; and other treatments for hand OA such as methotrexate, hydroxychloroquine, colchicine and sulfasalazine.

Intervention

Patients were randomised 1:1 by use of a computer-generated randomisation code to receive two intravenous infusions of placebo or tocilizumab (8 mg/kg) at a 4-week interval. Randomisation was stratified by centre and by block size of 4. The placebo was identical in volume to tocilizumab, and all infusion bags and intravenous lines prepared in the pharmacy unit of each centre were opaque to ensure blinding. The tocilizumab dose (8 mg/kg), route and scheme of administration were determined in reference to trials of rheumatoid arthritis,[27] which showed a rapid response and statistical difference versus placebo on the primary outcome with two infusions.[28–31] Patients, physicians and nurses were blinded to treatments. Each infusion was performed by a nurse, independently of the medical investigators and outcome measures. All investigators, staff and participants were kept unaware of the outcome measurements and trial results. Permitted concomitant treatments were restricted to acetaminophen (up to 4 g/day), which had to be stopped within 24 hours of a study visit. Oral NSAIDs were not authorised until week 6 and were allowed thereafter.

Outcomes

The primary outcome was pain (0–100 mm VAS) at week 6 (ie, 2 weeks after the second infusion). The question asked was: 'What is the global level of pain in your hands in the past 24 hours?". Secondary efficacy outcomes were evaluated at weeks 4, 6, 8 and 12: morning stiffness (minutes), patient global assessment (0–100 mm VAS), physician global assessment (0–100 mm VAS), number of painful joints (IP joints; (0–30) (spontaneous or under pressure, enough to blanch the tip of the examiner's fingernail (0–30)), number of swollen joints (IP joints; (0–30)), Functional Index for Hand Osteoarthritis (0–30) and Cochin Hand Functional Scale score (0–90).

Statistical analysis

A sample size of 90 patients (45 per group) was calculated to detect a between-group difference of 12 mm in pain (0–100 mm VAS) at week 6, with an estimated SD of 20 mm. This sample size provides 80% power to detect an effect size of 0.6 with an alpha level of 0.05. All outcomes were analysed in the intent-to-treat population including all randomised patients who received at least one infusion and who underwent at least one outcome assessment. The primary outcome was assessed by analysis of covariance models with baseline measurement as covariate. Last observation carried forward and imputation was used for missing values for the primary outcome. The primary outcome was also analysed in the per-protocol population including only completers (patients who received the two infusions and who completed all study visits). A post hoc analysis of the primary and secondary outcomes involved patients with at least one swollen joint at baseline. For secondary outcomes, we used the mixed effects model for repeated measurements to compare adjusted mean changes from baseline to weeks 4, 6, 8 and 12. Fixed effects included treatment, time and treatment-by-time interaction. The baseline value was included as a covariable. No adjustment for multiplicity was considered for secondary criteria since they were considered as exploratory. $p<0.05$ was considered statistically significant.

Safety

Adverse events were assessed and physical examination was performed throughout the study.

RESULTS

Participants

From November 2015 to October 2018, 104 patients were screened, and 91 were randomly assigned to receive tocilizumab (n=45) or placebo (n=46) (figure 1). Of these, 42 and 41 received the first infusion of tocilizumab or placebo, respectively. Finally, 40 (88%) patients in the tocilizumab group and 39 (84%) in the placebo group completed the 12-week study. The

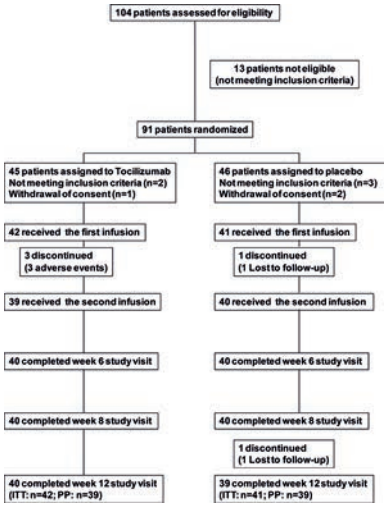

Figure 1 Flow of patients with hand osteoarthritis through the study. Five weeks were allowed between the screening visit and baseline, and 1 week separated the randomisation from the first infusion.

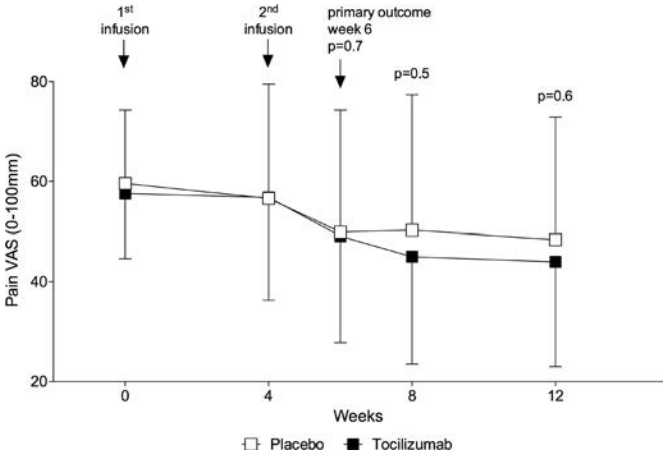

Figure 2 Pain (VAS) of patients receiving tocilizumab versus placebo (in the intention to treat (ITT) population). VAS, visual analogue scale.

mean age of participants was 64.4 years (SD 8.7) and 68 (82%) were women. Except for BMI and morning stiffness, which were higher in the placebo group, baseline characteristics were similar between groups (table 1).

Primary and secondary clinical outcomes

The mean change between baseline and week 6 in VAS pain (primary outcome) was −7.9 (SD 19.4) and −9.9 (SD 20.1) in the tocilizumab and placebo groups (p=0.7) (figure 2). Change in VAS pain from baseline at other times was similar between the groups (figure 2; table 2). The two groups did not differ in all secondary outcomes (stiffness, painful joints, swollen joints, function) at any time (table 2). In the per-protocol analysis, the mean change on VAS pain at week 6 was −8.2 (SD 20.1) and −9.6 (SD 20.3) in the tocilizumab and placebo groups (p=0.8). On sensitivity analysis, when excluding eight patients who took NSAIDs during the first 8 weeks of the study, the between-group difference on VAS pain was still non-significant (p=0.9).

Table 1 Baseline characteristics of participants with hand osteoarthritis (OA) who received tocilizumab or placebo in the intent-to-treat analysis

	Tocilizumab (n=42)	Placebo (n=41)
Age, years	64.1±8.9	64.7±8.6
Sex, female, n (%)	34 (81.0%)	34 (82.9%)
BMI (kg/m²)	23.1±3.9	25.7±4.9
Duration of disease, years	9.1±6.3	10.7±9.8
Family history of hand OA, n (%)	26 (69%)	25 (61%)
Knee OA (self-reported)	14 (33.3%)	20 (48.8%)
Manual activities>4 hour/day, n (%)	11 (26.2%)	14 (34.1%)
Pain score (VAS; 0–100 mm)	57.6±13.0	59.6±14.7
Morning stiffness (min)	33.4±28.4	56.8±124.5
Painful joints (spontaneous; 0–30)	5.7±4.7	5.6±5.8
Painful joints (pressure; 0–30)	12.5±6.5	10.9±5.9
Swollen joints (0–30)	2.9±3.0	2.9±2.7
Patient global assessment (VAS; 0–100 mm)	60.3±14.0	62.1±17.6
Physician global assessment (VAS; 0–100 mm)	57.6±15.3	58.6±12.6
FIHOA (0–30)	13.2±5.8	13.7±5.1
CHFS (0–90)	29.8±15.3	32.6±17.8
Diabetes, n (%)	6 (14.3%)	2 (4.9%)
Hypertension, n (%)	13 (31.0%)	17 (41.5%)
Hypercholesterolaemia, n (%)	16 (38.1%)	4 (9.8%)
Hypertriglyceridaemia, n (%)	4 (9.5%)	1 (2.4%)
Coronary heart disease, n (%)	3 (7.1%)	2 (4.9%)

Data are mean (SD) or n (%).
BMI, body mass index; CHFS, Cochin Hand Function Scale score; FIHOA, Functional Index for Hand Osteoarthritis; VAS, visual analogue scale.

Subgroup analysis

Because synovitis has been found associated with pain and inflammation, we compared the change in VAS pain at week 6 in patients who had at least one swollen joint at baseline (n=29 and n=27 in the tocilizumab and placebo groups). There was no between-group difference in change in VAS pain (mean change −4.8 (SD 20.3) and −8.3 (SD 22.0) in the tocilizumab and placebo groups (p=0.6) or in any secondary outcomes. Similarly, we found no between-group difference for the change in VAS pain at week 6 in patients who self-reported knee OA (p=0.7).

Safety

Overall, adverse events were more common in the tocilizumab than placebo group (n=29, 69.0% and n=22, 53.7%) (table 3). Most frequent non-serious adverse events in the tocilizumab group were infections (n=12, 28.6%) and neutropenia (n=2, 4.9%). There was no death.

DISCUSSION

Our study failed to demonstrate the efficacy of IL-6 blockade over placebo to alleviate pain and improve function in patients with hand OA. These findings do not support a role for an IL-6 signalling pathway in OA-related hand pain.

The role played by IL-6 in OA is mainly suggested by preclinical or human studies showing the involvement of this cytokine in the joint degradation process, either radiological or histological. Longitudinal studies found elevated serum or synovial fluid level of IL-6 predicting the development or worsening of radiographic knee OA.[21 32 33] Within the joint, the source of IL-6 production varies and includes subchondral osteoblasts,[34] synoviocytes[35 36] and chondrocytes,[25] which likely explains the high level of IL-6 in synovial fluid from knee patients with OA.[37] In parallel, other studies found serum or synovial fluid level of IL-6 associated with OA pain.[38 39] Finally, administration of tocilizumab in an OA animal model decreased mechanical allodynia.[40]

Table 2 Secondary outcomes: least squares mean change for treatment groups

	Tocilizumab		Placebo			
	N	Mean (SD)	N	Mean (SD)	Mean difference (95% CI)	P value
VAS pain						
Week 4	42	−0.9±3.1	40	−3.0±3.2	2.0 (−6.8 to 10.9)	0.6
Week 6	41	−8.3±3.1	40	−9.7±3.2	1.4 (−7.4 to 10.3)	0.7
Week 8	39	−12.3±3.2	37	−9.4±3.2	−2.9 (−11.9 to - 6.1)	0.5
Week 12	41	−13.5±3.1	38	−11.6±3.2	−1.8 (−10.8 to 7.1)	0.6
Stiffness						
Week 4	41	15.9±10.5	38	−11.9±11.0	27.9 (−2.2 to 58.0)	0.06
Week 6	41	−2.3±10.6	38	−19.3±11.0	17.0 (−13.1 to 47.2)	0.2
Week 8	38	−8.6±10.9	36	−17.2±11.2	8.6 (−22.2 to 39.5)	0.5
Week 12	41	−8.5±10.6	35	−19.6±11.2	11.1 (−19.3 to 41.6)	0.4
Painful joints*						
Week 4	41	−0.3±0.6	40	−1.0±0.7	0.7 (−1.2 to 2.6)	0.4
Week 6	40	−0.7±0.7	40	−1.5±0.7	0.8 (−1.0 to 2.8)	0.3
Week 8	39	−1.0±0.7	37	−1.3±0.7	0.2 (−1.7 to 2.2)	0.7
Week 12	41	−1.6±0.6	37	−1.8±0.7	0.1 (−1.7 to 2.1)	0.8
Painful joints†						
Week 4	41	−0.5±0.7	40	−0.7±0.7	0.2 (−1.9 to 2.3)	0.8
Week 6	40	−2.0±0.7	40	−2.4±0.7	0.4 (−1.7 to 2.6)	0.7
Week 8	39	−3.0±0.7	37	−1.9±0.8	−1.0 (−3.3 to 1.1)	0.3
Week 12	41	−2.6±0.7	37	−1.6±0.8	−0.9 (−3.1 to 1.2)	0.3
Swollen joints						
Week 4	41	−0.2±0.3	40	−0.7±0.3	0.4 (−0.5 to 1.4)	0.3
Week 6	40	−1.1±0.3	40	−0.2±0.3	−0.8 (−1.8 to 0.1)	0.08
Week 8	39	−1.6±0.3	37	−0.8±0.3	−0.7 (−1.8 to 0.2)	0.1
Week 12	41	−1.4±0.3	37	−1.2±0.3	−0.1 (−1.1 to 0.8)	0.7
PGA (patients)						
Week 4	40	−1.7±3.0	38	−5.4±3.1	3.6 (−5.0 to 12.3)	0.4
Week 6	39	−8.3±3.1	38	−10.1±3.1	1.8 (−6.9 to 10.5)	0.6
Week 8	37	−10.4±3.1	36	−10.6±3.2	0.1 (−8.7 to 8.9)	0.9
Week 12	39	−13.4±3.1	36	−12.9±3.2	−0.5 (−9.3 to 8.2)	0.9
PGA (physicians)						
Week 4	35	−3.7±3.1	34	−4.2±3.2	0.5 (−8.2 to 9.3)	0.9
Week 6	36	−7.3±3.0	34	−8.0±3.2	0.6 (−8.0 to 9.4)	0.8
Week 8	32	−15.0±3.1	32	−7.4±3.2	−7.5 (−16.5 to 1.4)	0.09
Week 12	36	−14.2±3.0	30	−12.1±3.3	−2.1 (−11.1 to 6.8)	0.6
FIHOA						
Week 4	39	0.4±0.6	38	0.3±0.6	0.07 (−1.7 to 1.8)	0.9
Week 6	39	−0.04±0.6	38	0.2±0.6	−0.2 (−2.0 to 1.5	0.7
Week 8	39	−0.3±0.6	35	0.5±0.6	−0.8 (−2.6 to 0.9)	0.3
Week 12	39	−1.0±0.6	35	−0.1±0.6	−0.9 (−2.7 to 0.8)	0.2
CHFS						
Week 4	39	1.1±1.9	38	0.2±1.9	0.9 (−4.4 to 6.3)	0.7
Week 6	39	0.8±1.9	38	−0.2±1.9	1.0 (−4.3 to 6.4)	0.6
Week 8	38	0.3±1.9	35	0.4±1.9	−0.04 (−5.4 to 5.3)	0.9
Week 12	39	−0.8±1.9	35	- 0.8±1.9	0.04 (−5.3 to 5.4)	0.9

*Painful joints (spontaneous).
†Painful joints (pressure). P values are from ANCOVA adjusted for treatment group and baseline values as a covariate.
ANCOVA, analysis of covariance; CHFS, Cochin Hand Functional Scale score; FIHOA, Functional Index for Hand Osteoarthritis; PGA, patients/physicians global assessment; VAS, visual analogue scale.

Given these findings and because IL-6 might be involved in chronic pain,[41] we hypothesised that targeting the IL-6R in patients with severe hand OA, a disease often accompanying synovitis,[11 12 39] could be effective in reducing OA symptoms, through acting in the subchondral bone and in the synovium

We found no statistical difference between the tocilizumab and placebo groups in the 6-week primary endpoint, change in VAS pain. The baseline characteristics of participants were approximately similar and could not explain the lack of efficacy we observed. Similarly, the response to placebo was low and thus did not lower the ability to detect a between-group difference.

As compared with recent randomised controlled trials of hand OA,[15 17 18 42] we did not recruit our patients on the basis of swollen joints or synovitis determined by ultrasonography

Table 3 Reported adverse events in the intent-to-treat population

	Tocilizumab (n=42)	Placebo (n=41)
Serious adverse events		
Total	**3**	**1**
Neutropenia <1000/mm^3	1	0
Fracture	1	0
Suicide attempt	1	0
Road traffic injuries	0	1
Non-serious adverse events		
Total	**26**	**21**
Infections	12	6
Upper airways	8	3
Lower airways	1	1
Urinary tract	1	0
Skin	0	1
Gastrointestinal	1	1
Conjunctivitis	1	
Mild neutropenia	2	0
Other	12	15

*In the placebo group: abdominal pain (n=2); legs pain (n=1); asthenia (n=1); cutaneous allergic reaction (n=2); diarrhoea (n=1); OA flare (n=2), low back pain (n=2); migraine (n=2); dyslipidaemia (n=1); trauma (n=1). In the tocilizumab group: dental pain (n=2); vertigo (n=1); pancreas cyst (n=1); OA flare (n=1); low back pain/sciatica (n=2); oral aphthae (n=1); migraine (n=1); hypoglycaemia (n=1); fall (n=1); abdominal pain (n=1).

or MRI. In our post hoc analysis of patients with at least one swollen joint, we found no treatment effect for the primary or secondary outcomes. Nevertheless, we acknowledge that clinically determined synovitis is less relevant than an imaging assessment of the synovial membrane. In addition, we cannot rule out the possibility that tocilizumab was not able to enter the joint in sufficient concentrations to have an effect given the mild synovial inflammation in our patients. Of note, all trials that assessed other anti-cytokine agents (lutikizumab, adalimumab, etanercept)[15 17 18] or hydroxychloroquine[43] in patients with OA and synovitis were also negative for their primary outcome. Altogether, these data suggest that IL-1, TNF and IL-6 are not involved in the mechanism of hand OA pain, even with objective evidence of joint inflammation.

These negative trials might question the presumably pivotal role of inflammation related to proinflammatory cytokines in the complex mechanisms involved in hand OA pain. Yet, OA mice with TNF knockout are not protected against pain-related behaviours.[44] In a trial comparing 5 mg prednisolone and placebo, synovitis as determined by MRI at baseline was not correlated with baseline VAS for hand pain and did not predict the OMERACT-OARSI response of prednisolone.[45] in the HOPE trial, although effective on pain and function, 10 mg oral prednisolone was not superior to placebo for decreasing synovitis as assessed with MRI or power Doppler ultrasonography.[42] However, synovial thickening and bone marrow lesions were less severe in the prednisolone group, highlighting the multiple interactions between inflammation, pain and structure in hand OA.[44 45]

Pain in hand OA is complex[3 46] and is not solely due to excess nociception related to structural damage. As was seen for knee and hip OA,[47 48] central and peripheral sensitisation were independent contributors to pain in hand OA according to recent studies.[49] Apart from synovitis, other potential sources of nociceptive pain in hand OA include the presence of bone-marrow lesions, erosions and bone attrition.[8 10 50] Given the long duration and severity of OA in our patients, pain may mainly originate from the subchondral bone with involvement of a pain mediator other than IL-6. A growing body of evidence supports a role for the innate immune pathways in OA pain,[51 52] with the involvement of proteins such as C-C motif chemokine ligand 2 (CCL2)[53] and nerve growth factor (NGF) or aggrecan fragment that activates Toll-like receptor 2 on joint nociceptors.[52 54] Although IL-6 upregulates both CCL2 and NGF in different tissues,[55–57]it is capable of sensitising C fibres in the joint[58] and mitigated OA pain in a rodent model,[40] our results indicate that removing IL-6 signalling alone in the short term is not sufficient to inhibit OA pain in humans. However, because we assessed solely pain and function, we cannot rule out that tocilizumab, as was seen in animal models,[24] could slow the cartilage degradation in humans. Therefore, long-term studies to investigate the benefit of IL-6 blockade on joint structure are warranted.

Overall, tolerance of tocilizumab in our hand OA population was acceptable. Mild neutropenia and infections were expected and led to treatment discontinuation in only three patients after the first infusion.

Our study, which is the first to assess IL-6 blockade in OA, has not only strengths, particularly its design and rigorous recruitment, but also limitations. Our trial was powered to detect an effect size of 0.6, that is, a large analgesic effect of tocilizumab versus placebo. Our study was thus underpowered to detect a smaller effect size. Two infusions of tocilizumab (8 mg/kg) might be considered insufficient to block IL-6R. First, we chose this scheme of administration for safety reasons because the rate of side effects increases with duration of exposure to the drug. Second, data in rheumatoid arthritis showed that two infusions are enough to fully block the IL-6 signalling pathway. Two weeks after 2 monthly infusions of tocilizumab 8 mg/kg, more than 95% of the soluble IL-6R molecules are bound as an immune complex with tocilizumab.[59] Moreover, tocilizumab effectively inhibits IL-6 signalling at serum concentrations ≥1 µg/mL, which is two times lower than levels obtained up to day 42 after a single 8 mg/kg infusion in patients with rheumatoid arthritis.[59 60] Finally, phase 3 trials in rheumatoid arthritis found tocilizumab (8 mg/kg) superior to placebo after two infusions,[30 31] and in the OPTION study, C reactive protein level normalised by week 2 of treatment.[30] Taken together, these data strongly suggest that our scheme of administration did not likely explain our negative results.

In conclusion, tocilizumab was not more effective than placebo in reducing pain in patients with hand OA, and targeting IL-6 signalling may be ineffective to improve symptoms in hand OA.

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Contributors PR, XC and EV designed the study. PR, AL, JS, DW, MP, PG, Y-MP, FE, SO, PO, R-MF, OP and XC collected the data. PR, XC and EV analysed the data. PR, AL, JS, DW, MP, PG, Y-MP, FE, SO, PO, R-MF, BF, JPB and XC interpreted the data and wrote the manuscript.

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TRANSLATIONAL SCIENCE

Precise targeting of miR-141/200c cluster in chondrocytes attenuates osteoarthritis development

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ABSTRACT

Objectives Despite preclinical studies involving miRNA therapeutics conducted in osteoarthritis (OA) over the years, none of these miRNAs have yet translated to clinical applications, owing largely to the lack of efficient intra-articular (IA) delivery systems. Here, we investigated therapeutic efficacy of the chondrocyte-specific aptamer-decorated PEGylated polyamidoamine nanoparticles (NPs)-based miRNAs delivery for OA.

Methods The role of miR-141/200c cluster during skeletal and OA development was examined by miR-141/200c$^{flox/flox}$ mice and Col2a1-CreER^{T2}; miR-141/200c$^{flox/flox}$ mice. Histological analysis was performed in mouse joints and human cartilage specimens. Chondrocyte-specific aptamer-decorated NPs was designed, and its penetration, stability and safety were evaluated. OA progression was assessed by micro-CT analysis, X-ray and Osteoarthritis Research Society International scores after destabilising the medial meniscus surgery with miR-141/200c manipulation by NPs IA injection. Mass spectrometry analysis, molecular docking and molecular dynamics simulations were performed to investigate the interaction between aptamer and receptor.

Results Increased retention of NPs inside joint space is observed. The NPs are freely and deeply penetrant to mice and human cartilage, and unexpectedly persist in chondrocytes for at least 5 weeks. OA chondrocytes microenviroment improves endo/lysosomal escape of microRNAs (miRNAs). Therapeutically, IA injection of miR-141/200c inhibitors provides strong chondroprotection, whereas ectopic expression of miR-141/200c exacerbates OA. Mechanistically, miR-141/200c promotes OA by targeting SIRT1, which acetylates histone in the promoters of interleukin 6 (IL-6), thereby activating IL-6/STAT3 pathway.

Conclusions Our findings indicate that this nanocarrier can optimise the transport kinetics of miR-141/200c into chondrocytes, fostering miRNA-specific disease-modifying OA drugs development.

Key messages

What is already known about this subject?

- It has been well documented that microRNAs (miRNAs) play crucial roles in cartilage development and osteoarthritis (OA) pathogenesis. To date, no miRNA has entered into clinical trial for modulating OA.

What does this study add?

- Dysregulation in miR-141/200c cluster profile disturbs cartilage development and contributes to OA progression.
- The aptamer (tgg2)-PEG2000-PEGylated polyamidoamine (PAMAM)6.0-cy5.5 nanocarrier keeps stable in human OA synovial fluid, resides in human OA cartilage at high level up to day 35 days and penetrates cartilage up to a depth of at least 1600 µm.
- The membrane protein bound by chondrocyte-specific aptamer, tgg2, is FGFR1.
- Silencing of miR-141/200c in chondrocytes can maintain its normal phenotype and even reverse cartilage degradation by targeting SIRT1/interleukin-6/STAT3 pathway.

How might this impact on clinical practice or future developments?

- This study suggests that chondrocyte-specific aptamer (tgg2)-functionalised PEGylated PAMAM nanocarrier could be used as an efficient delivery system for promoting various miRNAs into chondrocytes, providing a promising nanotechnology-based precision-targeting strategy for OA.

INTRODUCTION

Osteoarthritis (OA) is a highly prevalent and debilitating whole-joint disorder, which is primarily characterised by cartilage destruction, ultimately resulting in pain and physical disability.[1–3] The substantial individual and socioeconomic burden of OA will continue to grow as the progressive ageing of the general population, with the number of people affected projected to double by 2030.[4–6] Despite this enormous unmet medical need, no drugs have been approved for OA modification, and currently, available interventions are limited to pain relief, which leads to the inevitable referral for arthroplasty.[7–9] This lack of effective disease-modifying OA drugs (DMOADs) mainly results from our poor understanding of the mechanisms responsible for initiation and progression of OA.[10–13] Unravelling novel molecular mechanisms underpinning the maintenance and destruction of cartilage is thus likely to yield new therapeutic strategies.

Intriguingly, microRNAs (miRNAs) are small evolutionarily conserved non-coding RNAs (18-25nt in length), which maintain cellular function by fine-tuning multiple genes expression and are increasingly implicated in the pathogenesis of human diseases.[14–17] Of note, the critical roles

of miRNAs in many aspects of cartilage homeostasis and OA pathophysiology have been well documented.[18–21] Therapeutic approaches to modulate miRNAs have progressed from bench to bedside, with some successful phase I trials and ongoing phase II/III trials in some diseases.[22–24] However, no miRNA-targeting therapeutics for OA have so far moved into clinical development owing largely to the lack of efficient intra-articular (IA) delivery system.

In recent years, cationic nanocarriers have been widely used for sustained delivery in highly negatively charged avascular cartilage tissue, providing prolonged drug retention before being rapidly cleared from the joint space via subsynovial capillaries and lymphatics, respectively.[25–27] However, these approaches do not guarantee sufficient drug penetration into the dense cartilage to exert therapeutic effects on chondrocytes due to the fact that cartilage extracellular matrix (ECM) has an approximate pore size of 60 nm.[28] Moreover, using the tight binding mechanisms (ECM-targeting nanocarriers) for increasing therapeutic cargo retention inside cartilage matrix may be appreciated, whereas such strong or irreversible binding-mechanisms would dramatically slow down transport of nanocarriers as they get trapped in the surface layers of cartilage, preventing them from penetrating further to reach chondrocytes.[29–31] Ideally, chondrocyte-homing nanocarriers with optimal size and positive charge may provide a unique opportunity for therapeutics delivery to chondrocytes, especially in the middle and deep zones of cartilage, helping to shape the future therapeutic landscape.

Here, we equipped optimally charged PEGylated polyamidoamine (PAMAM) dendrimers with a chondrocyte-specific aptamer, in which cartilage ECM could be ingeniously converted from a barrier into an accomplice for sustained IA delivery, thereby accelerating transport of nanocarriers to chondrocytes. This is the first study reporting chondrocyte-specific aptamer (tgg2)-functionalised PEGylated PAMAM nanocarriers encapsulating miR-141/200c for OA therapy, which updates IA delivery systems from a tissue level to a cellular level and improve the targeting specificity and pharmacokinetic profile of the nanocarrier. Moreover, our findings open up new therapeutic perspectives with the evidence that pharmacological inhibition of miR-141/200c could ameliorate cartilage degradation, offering a promising nanotechnology-based precision-targeting strategy for OA.

MATERIALS AND METHODS

See online supplemental materials and methods.

RESULTS

Identifying key miRNAs relevant to osteoarthritic phenotype

To systematically identify miRNAs that play key roles in OA, microarray was employed for miRNA profiling of cartilage tissues from OA patients and controls (figure 1A,B). Unsupervised clustering analysis with these significantly dysregulated miRNAs was able to distinguish OA patients from controls (figure 1C–E). We first tested these candidate miRNAs using an independent cohort of 22 controls and 36 OA patients. Only miRNAs with a mean fold change >8 or <0.125 and a $p<0.01$ were selected for further analysis. Using the above-mentioned criteria, miR-217, miR-150, miR-421, miR-141/200c, miR-588, miR-497/195 and 218-5 p were observed to be significantly dysregulated (see online supplemental table S1). These miRNAs were further evaluated by RT-qPCR using additional independent cohort comprising of 30 controls and 57 OA patients. Of these miRNAs, miR-150, miR-218–5 p, miR-141/200c and miR-497/195 were found to be significantly upregulated in OA patients compared with controls (see online supplemental table S1). Because of the critical role of miR-141 in nucleus pulposus cell phenotype, extremely similar to chondrocytes, which is reported in our previous study,[32] we, therefore, selected miR-141/200c for further investigation. Quantitative data from cartilage tissues and chondrocytes showed a significant increase in miRNA-141/200c in OA patients (figure 1F), which was further confirmed by fluorescence in situ hybridisation (FISH) (figure 1G). Using the online tool MethPrimer with the default criteria,[33] we found that miR-141/200c presented one clear CpG islands in their upstream chromosomal regions, respectively. Quantitative methylation analysis indicated that the miR-141/200c up-stream promoter regions was hypomethylated in osteoarthritic cartilage tissues, which may explain why miR-141/200c levels were high in OA (figure 1H). As expected, increased cartilage degradation and synovitis were noted in OA patients (figure 1I and J), and a positive correlation between miR-141/200c expression and the modified Mankin scale was observed (see online supplemental figure S1). Gain-of-function and loss-of-function experiments indicated that upregulation or downregulation of miR-141/200c significantly affects chondrocytes proliferation and apoptosis, anabolic and catabolic markers (figure 1K–N and see online supplemental figure S2–S4). These findings imply the possibility that miR-141/200c cluster has disease-specific effects in OA.

INDUCIBLE CARTILAGE-SPECIFIC DELETION OF MIR-141/200C ATTENUATES OA PATHOGENESIS

Pregnant mice with embryos at E12.5 were injected with tamoxifen (TM). The knockdown efficiency of miR-141/200c was confirmed in Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ mice (figure 2A). Whole skeletal alizarin red and alcian blue staining and histological examinations were performed in E16.5, E18.5 and P0 embryos (figure 2B–E). Notably, Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ mice exhibited significantly shorter whole skeletons and extremities when compared with miR-141/200c$^{flox/flox}$ littermates. Moreover, increased percentages of the proliferative and hypertrophic zones were observed in the limb of Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ mice (figure 2E and see online supplemental figure S5). In addition, Col II and Aggrecan expressions were decreased, whereas Col X expression was increased in Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ mice (figure 2F). We also performed in situ TUNEL assays to detect apoptotic changes in the cartilage growth plate of Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ mice (figure 2G).

To further investigate whether miR-141/200c deficiency affects cartilage degradation in ageing mice, we observed spontaneously developed OA in miR-141/200c cKO mice with ageing. MiR-141/200c$^{flox/flox}$,Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ and Col2a1-CreER^{T2}/miR-141/200c$^{flox/+}$ mice were obtained (figure 2H1). Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ and miR-141/200c$^{flox/flox}$ mice (8 weeks old) were injected intraperitoneally with TM daily for 5 days, in which TM-treated Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ and miR-141/200c$^{flox/flox}$ mice were referred as miR-141/200c cKO and control mice, respectively. The knockout efficiency was examined by RT-qPCR and FISH in the 10-week-old mice (see online supplemental figure S6). Histological analysis for postnatal month 3 control and miR-141/200c cKO mice indicated intact articular cartilage surfaces and vigorous proteoglycan staining (figure 2J and K). By P6M and P9M, mild and moderate cartilage degradation was noted in control mice. By P12M, the OA-like features of control

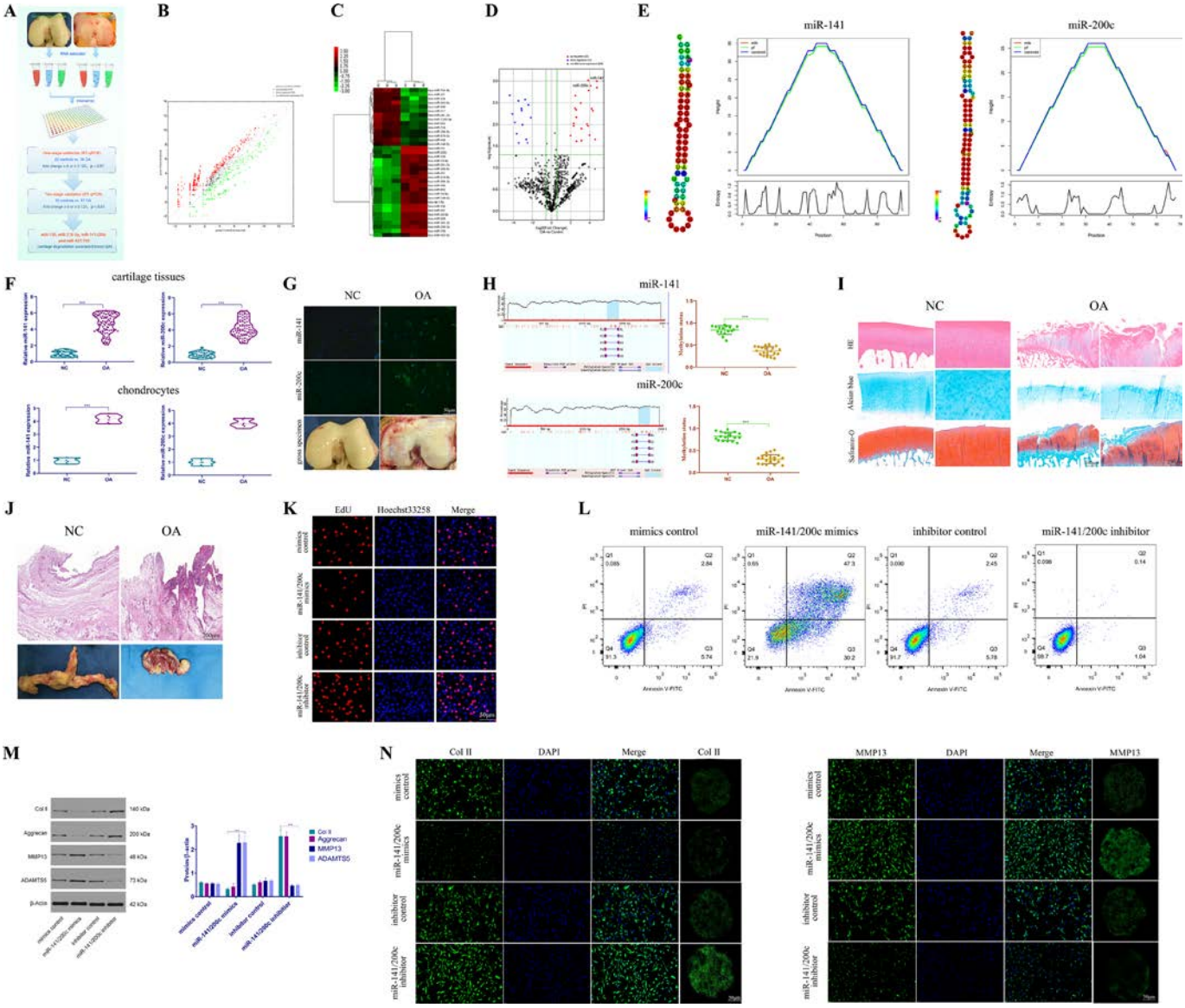

Figure 1 Identification of differentially expressed miRNAs in cartilage tissues from OA patients. (A) Selection strategy of miRNAs in cartilage tissues derived from microarray-based profiling. (B) Scatter plot of miRNA expression profile between OA patients and controls. (C) Heat map depicting 36 differentially expressed miRNAs (fold change >4 or <0.25, Benjamini-Hochberg-corrected p). (D) Volcano plot illustrating the biological and statistical significance of differential miRNA expression levels between OA patients and controls. miR-141/200c is indicated. (E) The secondary structure and the positional entropy for each position of miR-141/200c. (F) Compared with controls (n=52), miR-141/200c expression levels were upregulated in OA patients (n=93). Higher levels of miR-141/200c were observed in chondrocytes of OA patients when compared with controls (n=6). ***P<0.001 by Mann-Whitney U test. (G) FISH analysis of cartilage tissues from OA patients demonstrated increased level of miR-141/200c. Scale bar, 50 µm. (H) The percentage of C+G nucleotides (CG %) and the density of CpG dinucleotides are shown for a region spanning 2-kbp upstream of miR-141/200c. CpG islands located upstream of the miR-141/200c cluster were hypomethylated in OA (n=22) compared with controls (n=18). (I) Representative histopathological staining of normal and OA cartilage tissues. Scale bar, left, 500 µm; right, 200 µm. (J) Representative HE images of synovium from human OA patients and controls. Scale bar, 200 µm. (K) Cell proliferation was analysed using EdU assays. n=3 biological replicates per group, Scale bar, 50 µm. (L) Chondrocytes apoptosis was assayed by FCM. n=3 biological replicates per group. (M) The expression levels of Col II, aggrecan, MMP13 and ADAMTS5 were detected by western blot. n=3 biological replicates per group, ***P<0.001 by one-way ANOVA test followed by Tukey's post hoc. (N) Immunofluorescence analysis of Col II and MMP13. Data are shown as the mean±SD. ANOVA, analysis of variance; FCM, flow cytometry; FISH fluorescence in situ hybridisation; miRNAs, microRNAs; OA, osteoarthritis.

mice were aggravated compared with miR-141/200c cKO mice (figure 2J and K).

To explore the role of miR-141/200c in other forms of OA, we subjected the 10-week-old male control and miR-141/200c cKO mice to destabilised medial meniscus (DMM) model of OA or sham surgery. At 8 weeks after DMM surgery, histological evaluations of miR-141/200c cKO mice knee joints showed some loss of proteoglycans and chondrocyte cellularity. However, control mice displayed a severe OA(figure 2L,M and see online supplemental figure S7). Moreover, decreased chondrocyte apoptosis in cartilage of miR-141/200c cKO mice was detected (figure 2N). The percentages of MMP13 and ADAMTS5-positive chondrocytes were markedly higher, whereas Col II and Aggrecan were significantly lower in control mice than miR-141/200c cKO mice at 8 weeks after DMM surgery (figure 2O and see online supplemental figure S8).

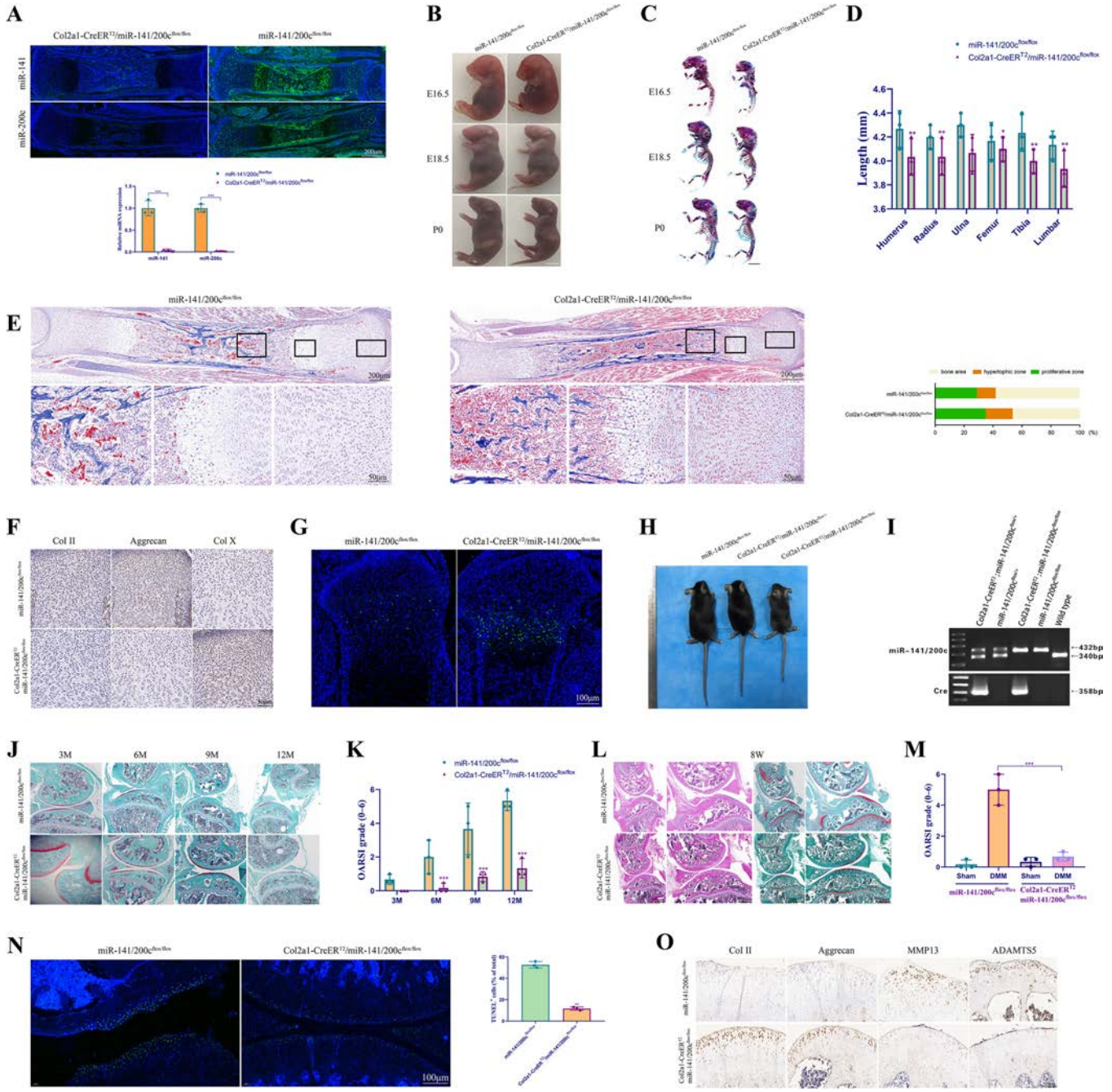

Figure 2 The critical role of miR-141/200c cluster in skeletal development and OA progression. (A) FISH on tibial sections of mouse embryos (E13.5). RT-qPCR analysis of miR-141/200c expression in miR-141/200c$^{flox/flox}$ or Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ mice (n=3). ***P<0.001 by two-tailed unpaired Student's t-test. Scale bar, 200 µm. (B) Gross appearance of miR-141/200c$^{flox/flox}$ and Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ littermate embryos (E16.5, E18.5 and P0). Scale bars, 1 mm. (C) Double staining with alizarin red and alcian blue of the whole skeleton of miR-141/200c$^{flox/flox}$ and Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ littermate embryos (E16.5, E18.5 and P0). Scale bars, 1 mm. (D) Length of long bones and vertebra (first to fifth lumbar spines) of miR-141/200c$^{flox/flox}$ and Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ littermate embryos (E16.5). n=3 per group, *P<0.05, **p<0.01 by two-tailed unpaired Student's t-test. (E) Masson trichrome staining of whole tibias. Scale bar, upper, 200 µm; lower, 50 µm. n=6 per group (P0). (F) Representative immunohistochemistry of Col II, Aggrecan and Col X in the tibia of the miR-141/200c$^{flox/flox}$ and Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ embryos (E18.5). Scale bar, 50 µm. (G) TUNEL assays in tibia sections of miR-141/200c$^{flox/flox}$ and Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ mice at E16.5. (H) Representative images of miR-141/200c$^{flox/flox}$, Col2a1-CreER^{T2}/miR-141/200c$^{flox/+}$ and Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ male mice at 4 weeks old. (I) Representative images of PCR genotyping. (J) HE and Safranin O staining of knee joints from 3, 6, 9 and 12 months miR-141/200c$^{flox/flox}$ or Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ mice. Scale bars, 500 µm. (K) The histological grades (OARSI) was evaluated (n=3 per group). ***P<0.001 by two-tailed unpaired Student's t test. (L) miR-141/200c$^{flox/flox}$ and Col2a1-CreER^{T2}/miR-141/200c$^{flox/flox}$ littermates were subjected to DMM surgery. Representative images of 8 weeks post-OA surgery knee joint sections. Scale bar, 1000 µm, 500 µm. (M) OARSI scores was quantified (n=3). ***P<0.001 by two-tailed unpaired Student's t test. (N) TUNEL staining in mouse knee joints (8 weeks post-OA surgery). Scale bars, 100 µm. n=3 **p<0.01 by two-tailed unpaired Student's t-test. (O) Immunohistochemistry assay with the indicated antibodies in mice 8 weeks after DMM. Scale bar, 50 µm. Data are shown as the mean±SD. DMM, destabilising the medial meniscus; FISH, fluorescence in situ hybridisation; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International.

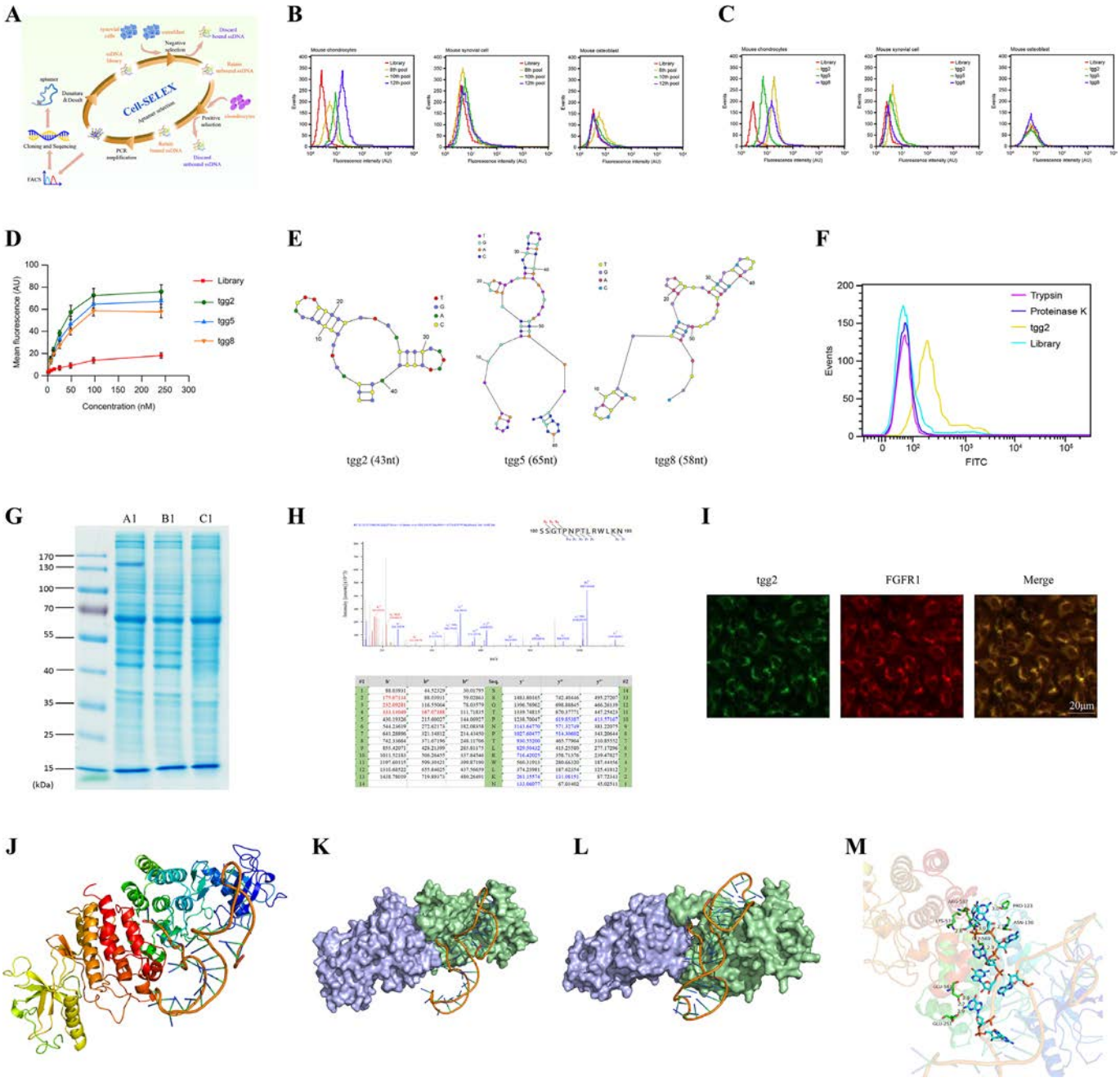

Figure 3 Identification of chondrocyte-specific aptamers using cell-SELEX. (A) Schematic illustration of aptamers selection in chondrocytes. (B) Binding ability of the enriched pools to target cells (mouse chondrocytes) and non-target cells (mouse synovial cell and osteoblast) as determined by flow cytometry. (C) Binding ability of the FAM-labelled aptamer candidates (tgg2, tgg5 and tgg8) to target cells and non-target cells as determined by flow cytometry. (D) Flow cytometry to determine the binding affinity of the aptamer candidates for the target cells. Kd (tgg2)=23.6±2.9 nM; Kd (tgg5)=29.8±3.2 nM; kD (tgg8)=32.3±4.2 nM. The concentrations of the aptamer candidates ranged from 5nM to 250nM. (E) Proposed secondary structure of tgg2. (F) The binding of tgg2 to chondrocytes, treated with trypsin and proteinase K, was analysed by flow cytometry. (G) Coomassie blue-stained SDS-PAGE was employed to analyse aptamer-assisted target purification. A1, protein captured with tgg2; B1, protein captured with the library sequences; C1, magnetic beads only. (H) Annotated MS/MS spectrum assigned to the FGFR1 peptide. Data acquired from analysis of samples by high-sensitivity LC-MS/MS on a Q Exactive mass spectrometer. (I) The subcellular localisation of tgg2 and FGFR1 in chondrocytes was visualised using FITC-labelled tgg2 and PE-labelled anti-FGFR1 antibody. (J–L) Interaction model between tgg2 and FGFR1. (M) The key residues are as follows: GLU-251, GLU-561, GLY-569, LYS-574, ARG-587, PRO-123 and ASN-130. SELEX, systematic evolution of ligands by exponential enrichment.

Selection of chondrocyte-specific aptamers and identification of its binding proteins

To construct a chondrocyte-specific delivery system for miRNA treatment, cell-based systematic evolution of ligands by exponential enrichment was employed to select chondrocyte-specific aptamers[34] (figure 3A). Flow cytometry analysis showed that three aptamer candidates had good binding ability to chondrocytes (figure 3B,C) with equilibrium dissociation constants in the nanomolar-to-picomolar range (figure 3). We chose the tgg2 aptamer for further investigation due to its satisfactory secondary structure and shorter nucleotide sequences (figure 3E), making it easier to synthesise and conjugate to nanoparticles (NPs).

To explore what type of target molecules are bound by tgg2, chondrocytes were treated with Proteinase K or trypsin for 1 hour, followed by incubation with tgg2. Interestingly, tgg2 completely lost its ability to bind chondrocytes after enzymatic treatment compared with control group (figure 3F). We subsequently focused on the identification of membrane proteins bound by tgg2. To this end, total membrane proteins of chondrocytes were extracted and incubated with biotinylated tgg2 or library, respectively. Of note, one protein band with relative molecular mass of ~145 kDa was obviously present from the tgg2 column, but not from the library or the control column (figure 3G), which was further analysed by LC-MS/MS (figure 3H). Among these candidates, FGFR1 ranked first with the highest score and the maximum content. Moreover, FGFR1 has been reported to be frequently high expression in OA.[35 36] To validate the result from the above-mentioned procedures, the location of tgg2 and FGFR1 on chondrocytes was examined (figure 3I and see online supplementary figure S9) .

To provide molecular insights into the dynamic behaviour of FGFR1-tgg2 complexes, a total of 10ns simulation time was performed and molecular dynamics trajectory was employed for extracting the refined binding model (figure 3J–L and see online supplemental figure S10). Seven amino acids of protein FGFR1 that formed important interactions with tgg2 were identified as key residues (figure 3M and see online supplemental figure S11). The interaction between FGFR1 and tgg2 mainly depended on hydrogen bond. Four nucleic acid bases participated in the formation of an H-bond network with 7 residues of FGFR1 (see online supplemental figure S12). Importantly, the binding free energy between tgg2 and FGFR1 was −102.0859kcal/mol (see online supplemental figure S13).

Synthesis and characterisation of NPs

Historically, the conjugation of therapeutic cargo to polymeric carriers has been a mainstay of the drug delivery field, with several conjugates being tested in clinical trials or successfully translated into clinical practice.[25] We developed an aptamer (tgg2)-PEG2000-PAMAM6.0-cy5.5 (TPPC) nanoplatform for delivering miR-141/200c(figure 4A,B). Of note, the bright band resulting from free miRNA completely disappeared at the TPPC:miRNA weight ratio of 16:1, implying that all miRNA molecules have been completely complexed and retarded by TPPC (figure 4C). Furthermore, the NPs were observable for at least 24 hours in synovial fluid from OA patients, whereas most of the free miR-141/200c degraded within 1.5 hours and no miR-141/200c was discernible after 2 hours in synovial fluid (figure 4D). Dynamic light scattering (DLS) measurements demonstrated that the surface zeta potential of the NPs was 4.9±0.38 mV (figure 4E). Additionally, transmission electron microscopy (TEM) evealed that the NPs had a spherical morphology with a diameter of around 38.2±1.6 nm, further confirmed by DLS (figure 4F).

Besides long-term retention in joint space, any DMOADs targeting chondrocytes must penetrate 1000–2000 µm of cartilage in humans to access all resident chondrocytes.[29] However, many studies in the cartilage delivery field focus solely on mouse or rat cartilage, which is about 10 times thinner. In our study, mice treated with tgg2-PEG-PAMAM-cy5.5 showed a persistent bright signal at 35 days, whereas the knees treated with free cy5.5 lost most of the signal in 24 hours and fluorescence of PEG-PAMAM-cy5.5 was not detectable in the joint at 7 days (figure 4G). Unexpectedly, cy5.5 could still be detected in human OA explants at high level up to day 35 (figure 4H). As a proof of concept that our non-viral-based vector could be eventually translated into clinical use, we evaluated the efficiency of tgg2-PEG-PAMAM-cy5.5 penetration in human OA and normal cartilage. The NPs can be found penetrating human OA cartilage up to a depth of at least 1600 µm (figure 4I), implying that they may reach all resident chondrocytes requiring treatment for therapeutic gain.

The NPs (ranging from 0.01 to 50 µm) exhibited no cytotoxicity to human chondrocytes (figure 4J). Further, chondrocytes apoptosis was investigated using human normal cartilage tissues obtained from amputation (figure 4K). Normal histopathology of liver, kidney and lungs was observed in mice at 3 months after IA injection (see online supplemental figure S14). To examine the cellular internalisation mechanism of NPs, cellular uptake and intracellular trafficking were analysed. Both PEG-PAMAM-cy5.5 and tgg2-PEG-PAMAM-cy5.5 displayed increased fluorescence intensity in chondrocytes with increasing incubation time (figure 4L). As expected, the red fluorescence intensity of chondrocytes treated with tgg2-PEG-PAMAM-cy5.5 was markedly higher than those treated with PEG-PAMAM-cy5.5, suggesting that the introduction of tgg2 results in high cellular uptake. Aside from delivering miRNA into chondrocytes, various intracellular barriers needed to be overcome.[37] Of these barriers, the escape of miRNA from endosomal compartments to cytosol has been considered as the most important challenge. In human normal chondrocytes treated using PEG-PAMAM-cy5.5, the red fluorescence of Cy5.5 overlapped with the green fluorescence of LysoTracker Green, which stained the endo/lysosomes at 1 hour and 3 hours (figure 4M). At 6 hours, a few red fluorescence dots separated from the green fluorescence were observed. In contrast, the overlap between the green and red fluorescence was reduced in chondrocytes treated by tgg2-PEG-PAMAM-cy5.5 at all three time points (particularly 3 hours and 6 hours) (figure 4M), implicating successful escape of the tgg2-PEG-PAMAM-cy5.5/miR-141/200c from endo/lysosomes.

Administration of miR-141/200c alleviates OA progression in mice

NPs-miR-141/200c mimics/inhibitor or negative control was IA administered to the affected joint beginning 1 week later (figure 5A). The delivery of miR-141/200c mediated by NPs demonstrated an ideal delivery effect, documented by in vivo imaging system and histological examinations (figure 5B,C). Exacerbated OA pathology was found in DMM-operated mice injected with NPs-miR-141/200c mimics or negative control, whereas injection of NPs-miR-141/200c inhibitor into joint cavity clearly ameliorated DMM-induced OA pathology, as indicated by gross appearance, radiographic and histopathological findings (figure 5D–H and see online supplemental figure S15). Moreover, the expression of MMP13 and ADAMTS5 was significantly decreased by NPs-miR-141/200c inhibitor treatment, whereas an increase in collagen II and Aggrecan expressions was noted (figure 5I and see online supplemental figure S16). TUNEL staining showed remarkably decreased chondrocytes apoptosis in mice treated with NPs-miR-141/200c inhibitor (figure 5J). In pain-related behavioural tests, mice receiving NPs-miR-141/200c inhibitor injection exhibited higher pain thresholds (figure 5K), indicating that silencing of miR-141/200c in OA knee joints not only ameliorated histological features, but also reduced pain, a prominent symptom affecting OA patients. These results collectively suggest therapeutic effects of silencing of miR-141/200c on protecting cartilage from destruction,

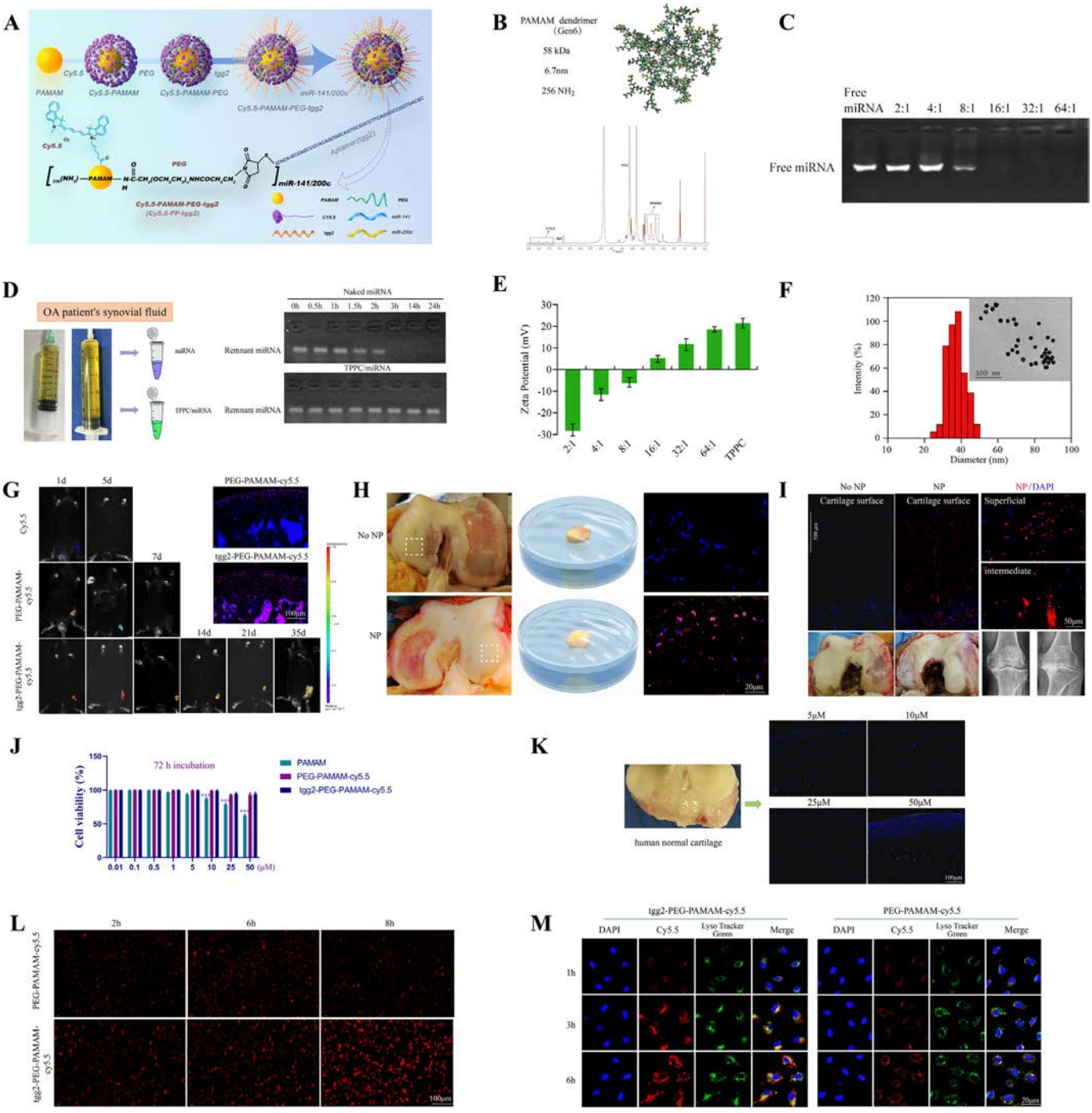

Figure 4 The nanoparticles (NPs) facilitate miRNA delivery into chondrocytes. (A) Schematic of tgg2-PEG2000-PAMAM6.0-cy5.5 synthesis. (B) ^{1}H NMR spectrum of NPs. (C) Electrophoretic mobility of miRNA in an agarose gel. (D) Agarose gel results of the remnant miRNA from naked miRNA and tgg2-PEG2000-PAMAM6.0-cy5.5 (TPPC)/miRNA. Both of them were first incubated in synovial fluid from OA patients. (E) Monitoring the zeta potential change of the resulting nanocomplexes at different weight ratios. (F) TEM image of the TPPC/miRNA NPs at 16:1 wt ratio. DLS result demonstrated the size distribution of the TPPC/miRNA NPs at 16:1 wt ratio. (G) In vivo imaging showing preferential accumulation of TPPC within knee joint cavity. Obviously, tgg2 can extend the retention time and effectively increase its penetration. Scale bar, 100 μm. (H) Human OA cartilage explants were incubated with fluorescent NPs for 48 hours, the excess NP was then washed off, and explants were kept in complete culture medium for up to 35 days. Scale bar, 20 μm. (I) Sagittal cartilage from OA patients sections were examined for depth of NPs penetration scale bars, 500 μm, 50 μm. (J) In vitro viability of chondrocytes treated with PAMAM, PEG-PAMAM-cy5.5 and tgg2-PEG-PAMAM-cy5.5. n=3 biological replicates per group, ***p<0.001 by one-way ANOVA test followed by Tukey's post hoc. (K) TUNEL assays in cartilage sections of human cartilage at different concentrations of tgg2-PEG-PAMAM-cy5.5. Scale bar, 100 μm. (L) Cellular uptake of PEG-PAMAM-cy5.5 and tgg2-PEG-PAMAM-cy5.5 after 2, 6 and 8 hours of incubation, respectively. Scale bar, 100 μm. (M) Intracellular distribution of PEG-PAMAM-cy5.5 and tgg2-PEG-PAMAM-cy5.5 in chondrocytes. Scale bar, 20 μm. Data are shown as the mean±SD. ANOVA, analysis of variance; DLS, dynamic light scattering; miRNAs, microRNAs; OA, osteoarthritis; PAMAM, PEGylated polyamidoamine.

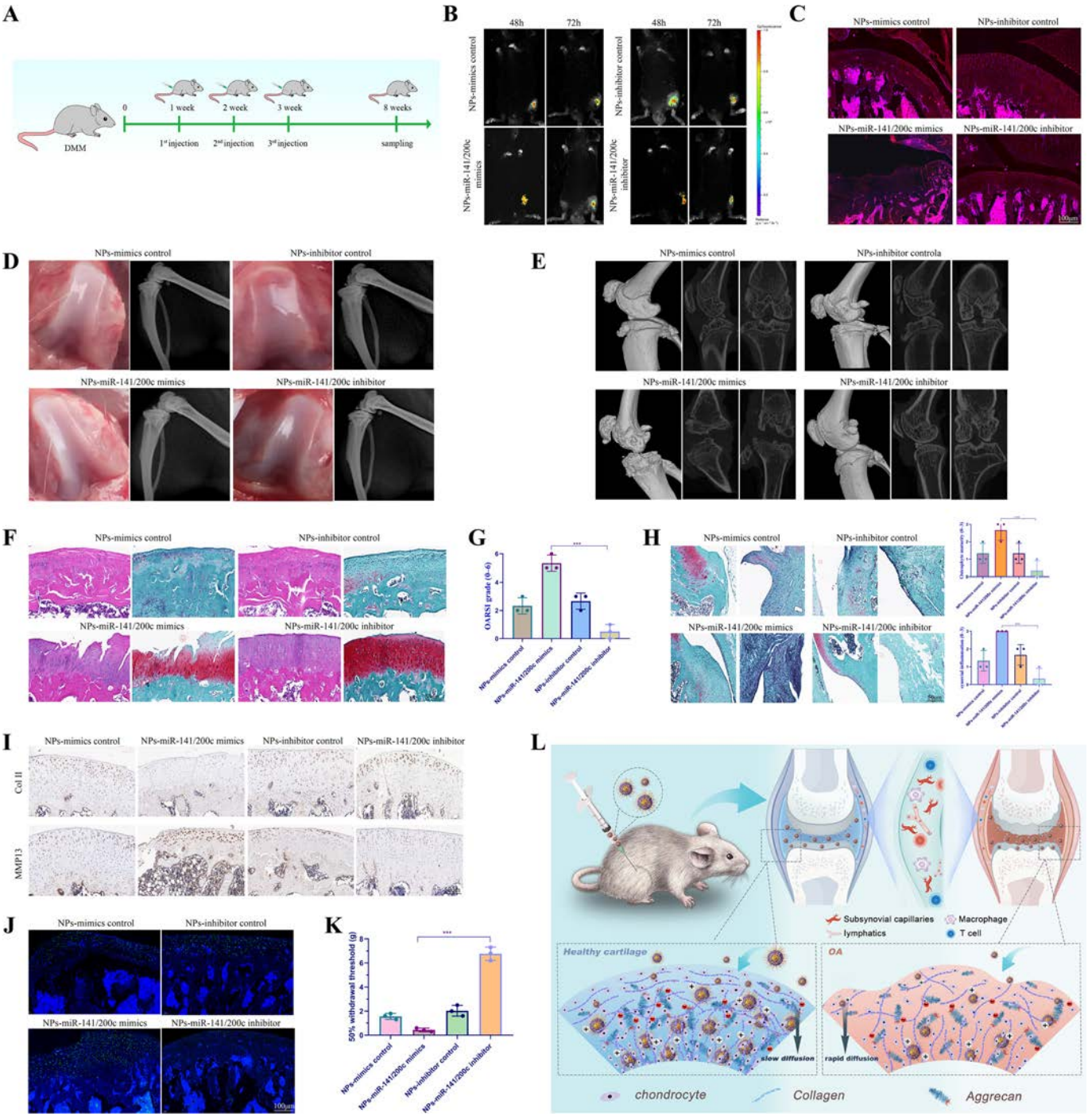

Figure 5 IA delivery of miR-141/200c inhibitor NPs attenuated OA development. (A) Overview of the experimental set-up with injections of miR-141/200c mimics, miR-141/200c inhibitor or their negative control NPs. (B) In vivo time-dependent fluorescence image in mice at 48 and 72 hours after the administration of Cy5.5-miR-141/200c NPs. (C) Histological analysis of NPs in cartilage tissues. Scale bar, 100 µm. (D–G) The cartilage degradation evaluated by gross appearance, X-ray, micro-CT, HE and Safranin O staining. Histological score (OARSI) showed a significant decrease in mice treated by miR-141/200c inhibitor NPs (8 weeks post-surgery). Scale bar, 50 µm. ***P<0.001 by two-tailed unpaired Student's t-test. n=3 per group. (H) Representative images of osteophyte formation and synovial inflammation determined by safranin-O staining in different treatment groups. Scale bar, 50 µm. ***P<0.001 by two-tailed unpaired Student's t-test. n=3 per group. (I) Immunohistochemistry assay with the indicated antibodies in mice 8 weeks after treatment. Scale bar, 50 µm. (J) TUNEL staining in mouse knee joints after IA administration of miR-141/200c NPs. Scale bars, 100 µm. n=3 biologically independent experiments. (K) IA injection of miR-141/200c NPs reduces pain sensitivity induced by OA. The Von Frey test was performed in the 6-month-old mice receiving miR-141/200c NPs injection at the age of 12 weeks. n=3 per group. ***P<0.001 by two-tailed unpaired Student's t-test. (L) The different diffusion model of cationic nanocarrier binding to negatively charged chondrocyte ECM via electrostatic interaction and then delivered into chondrocytes in normal and OA cartilage. Data are shown as the mean±SD. ECM, extracellular matrix; IA, intra-articular; NPs, nanoparticles; OA, osteoarthritis; OARSI, Osteoarthritis Research Society International.

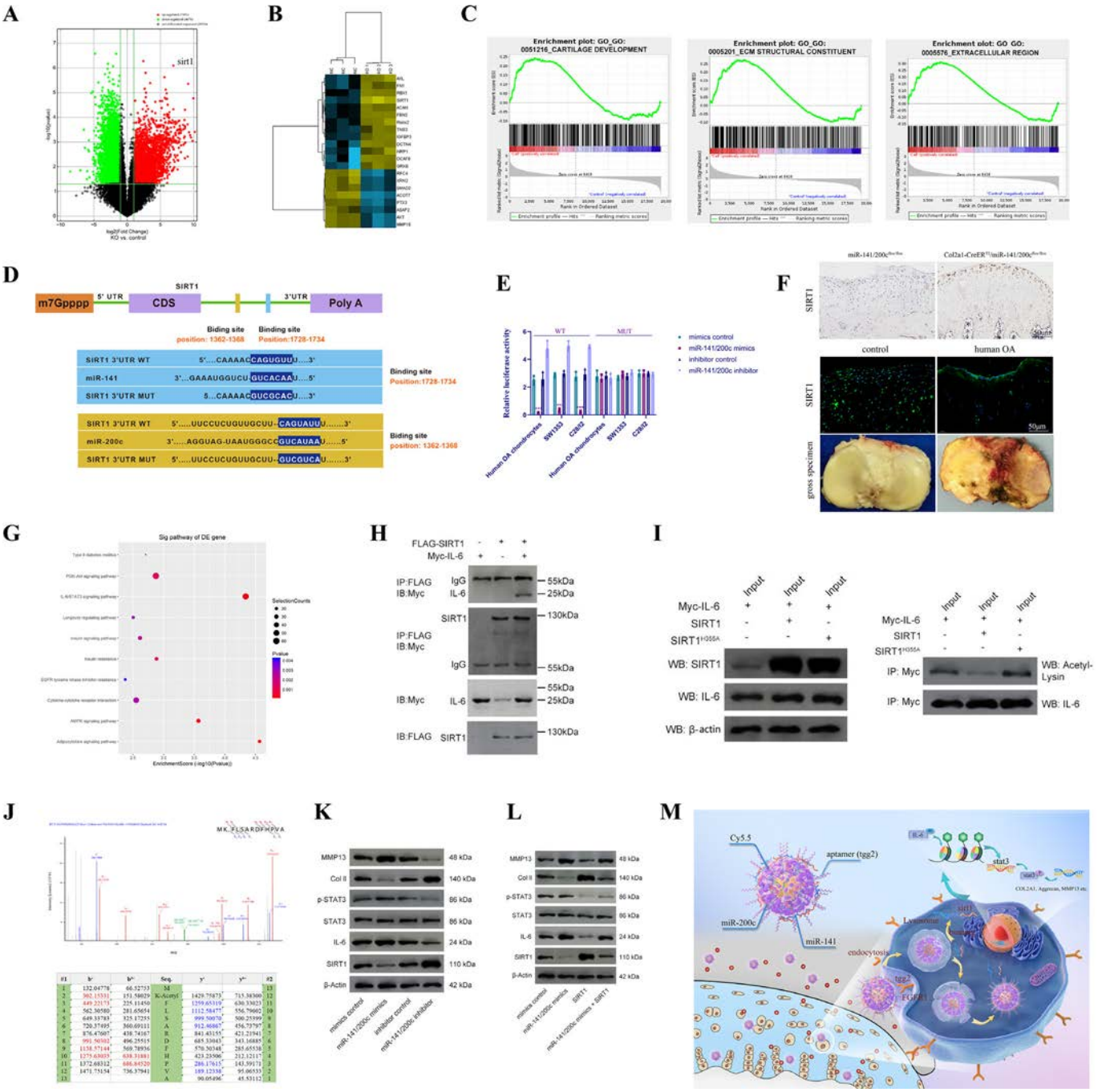

Figure 6 The modulation of miR-141/200c on SIRT1/IL-6/STAT3 signaling pathway. (A) Volcano plot showing the biological and statistical significance of differential genes expression levels between OA patients and controls. SIRT1 is indicated. (B) Heat map demonstrating differentially expressed genes (fold change >2 or <0.5, Benjamini-Hochberg-corrected p). (C) Upregulated GO terms with the most significant p values for biological processes, molecular function and cellular component. (D) Sequence alignment of a putative miR-141/200c-binding site within the 3'UTR of SIRT1 mRNA. (E) The wild-type or mutant-type SIRT1 3'UTR reporter plasmid was cotransfected with miR-141/200c mimics or inhibitor into cultured primary human OA chondrocytes, SW1353 and C28/I2. Forty-eight hours after transfection, luciferase activity was measured. n=3 replicates per group, ***p<0.001 by one-way ANOVA test followed by Tukey's post hoc. (F) SIRT1 expression level was analysed in cartilage from Col2a1-CreERT2/miR-141/200cflox/flox mice and human OA. Scale bar, 50 μm. (G) KEGG analysis demonstrating IL-6/STAT3 pathway enriched in OA. (H, I) CoIP of SIRT1 and IL-6. Further, chondrocytes were cotransfected with Myc-tagged IL-6, SIRT1 and SIRT1H355A to detect IL-6 deacetylation. (J) LC-MS/MS spectrum of acetylated IL-6 peptides. (K) Cultured primary human chondrocytes were transfected with miR-141/200c mimics, miR-141/200c inhibitor and their negative control for 72 hours and then the levels of the related genes were measured. (L) The rescue experiments were established in cultured primary human chondrocytes to validate the relationship between miR-141/200c and SIRT1. (M) Schematic representation of mechanisms by which miR-141/200c mediates OA development. ANOVA, analysis of variance; IL-6; interleukin 6; OA, osteoarthritis.

highlighting miR-141/200c as a promising therapeutic target for OA (figure 5L).

MiR-141/200c promotes OA development by modulating SIRT1/interleukin-6/STAT3 signaling axis

To comprehensively elucidate the underlying mechanisms of miR-141/200c in cartilage degradation, we performed RNA microarray in miR-141/200c KO and the control chondrocytes (see online supplemental figure S17). SIRT1 was significantly up-regulated in miR-141/200c KO chondrocytes (figure 6). Upregulated gene GO terms with the most significant p values for biological processes, molecular function and cellular component were related to cartilage development (GO:0051216), ECM structural constituent (GO:0005201) and extracellular region (GO:0005576) (figure 6). Importantly, SIRT1 was identified as the putative target of miR-141/200c (figure 6). To further confirm the functional interaction between miR-141/200c and SIRT1, we performed luciferase reporter assay analysis (figure 6), which was further supported by gene expression (figure 6).

Furthermore, KEGG pathway analysis showed that interleukin 6 (IL-6)/STAT3 signalling pathway was markedly enriched in miR-141/200c KO chondrocytes (figure 6). Of note, SIRT1 is known to regulate protein activity through deacetylation on lysine residues.[38] We speculate that SIRT1 could directly deacetylate IL-6, thus suppressing IL-6-dependent gene transcription. Our coimmunoprecipitation and LC-MS/MS results confirmed this hypothesis (figure 6). The findings that miR-141 promotes OA mediated by SIRT1 prompted us to investigate the potential association between miR-141/200c and SIRT1/IL-6/STAT3 pathway (figure 6). Rescue experiments were further performed to validate the relationship between miR-141/200c and SIRT1/IL-6/STAT3 (figure 6). Taken together, these results indicate that miR-141/200c-mediated protection in OA is primarily through the SIRT1/IL-6/STAT3 pathway (figure 6).

DISCUSSION

We rigorously document here the pivotal role of miR-141/200c cluster in cartilage development and OA pathogenesis using an integration of microarray bioinformatics coupled with large-scale patient data sets and experimental models. In a more therapeutically oriented approach, our results reveal that local IA administration of miR-141/200c into chondrocytes can maintain its normal phenotype and even reverse cartilage degradation.

Importantly, we found that increased miR-141/200c levels were accompanied by the decreased expression of ECM anabolic markers in OA patients, and their levels were correlated with cartilage degradation. Moreover, data from our gain and loss-of-function studies showed that miR-141/200c was a novel catabolic regulator of the process driving OA pathology. These observations provide the first clinical insight into the contribution of miR-141/200c to the molecular regulation of cartilage destruction during the progression of OA. Furthermore, our experimental evidence from in vivo studies demonstrated that OA phenotype induced by ageing and DMM surgery was attenuated by miR-141/200c cKO. Intriguingly, chondrocyte senescence has been proven to be a common molecular mechanism underlying both age-related and post-traumatic OA.[10 11] Mounting evidence strongly suggests that accumulated senescent chondrocytes promote chronic inflammatory response that transitions OA from an indolent to an aggravating phase, which can be modulated by miRNAs.[10 11 18 39 40] Notably, our data showed that miR-141/200c cKO mice displayed decreased levels of inflammatory markers and increased SIRT1 mRNA level, the gene that has been suggested to play key roles in ageing and age-related diseases, implying an essential role of miR-141/200c cluster in modulating cellular senescence. Accordingly, our study may pave, at least in part, the way for comprehensively investigating how miR-141/200c cluster critically regulates the fate of senescent chondrocytes and what molecular pathway involved in future studies, providing rational therapeutic strategies that safely interfere with the detrimental effects of chondrocyte senescence.

A growing body of evidence indicates that IL-6/STAT3 signalling is a key mediator of pleiotropic proinflammatory cytokine involved OA.[41 42] Tocilizumab, a humanised anti-IL-6R monoclonal neutralising antibody, is currently in phase III trials for treating human OA (NCT02477059), further suggesting the pivotal role of IL-6/STAT3 pathway in OA. Strikingly, the clinically relevant, dysregulated miR-141/200c/IL-6/STAT3 signalling axis identified here might be responsible for chronic, comparatively low-grade inflammation observed in human OA. Therapeutic targeting of this wellestablished low-grade inflammation in OA through silencing miR-141/200c cluster could form the new basis of such muchneeded DMOADs.

Notably, the small size and adaptable positive charge are particularly attractive for our nanocarrier as it can substantially extend miR-141/200c IA half-life and enable full penetration of mice and human cartilage, thereby maintaining therapeutic amounts of miRNAs in cartilage tissue. More importantly, by taking advantage of the high affinity and specificity of aptamer (tgg2), therapeutic compounds can be targeted to chondrocytes, which improves their local concentration and therapeutic efficacy. In the setting of OA, synovial oxygen tensions can fall further to very low levels, with subsequent effects on oxygen tension in the cartilage matrix. The matrix pH is considerably more acidic in this context due to substantially compromised acid extrusion.[43–45] Under this acidic environment, primary and secondary amines of polyamidoamine (PAMAM) exhibit enhanced protonated effect, which promotes proton sponge effect, significantly contributing to a successful endo/lysosomal escape of the encapsulated miRNA. Given the unique chondrocyte microenvironment, our model offers the possibility of gaining novel insights into appropriate design of a pH-responsive NP with clinical efficacy against OA in future investigations.

It should be noted that PEG, PAMAM dendrimers, aptamer and miRNA are produced commercially at large scale and can be thoroughly characterised by well-established methods.[46 47] Furthermore, PAMAM has been used safely in clinical trials (NCT 01577537, NCT 03500627 and EUDRACT 2016-000877-19). The tgg2-PEG-PAMAM-141/200c can, therefore, be characterised and purified by standard chemical techniques. The IA delivery system reported here is particularly suitable for charged matrices with small mesh size, such as cartilage, due to its unique physicochemical properties compared with other nanocarriers. NPs transport kinetics must be further investigated using larger animal models with thicker cartilage more like human before being translated to the clinic.

In summary, the findings presented here expands our knowledge on the mechanisms by which dysregulation in miR-141/200c cluster contributes to the progression of OA. Particularly, our study establishes a proof-of-concept platform technology to facilitate cell-type-specific, sustained IA delivery, which substantially enhances miRNAs into chondrocytes, fueling hope for rejuvenating the field of OA drug treatment and accelerating the discovery of miRNA-specific DMOADs.

Contributors All authors were involved in drafting or critically revising the manuscript for important intellectual content, and all authors approved the final

version for publication. JL, XTW, M-LJ and HJ conceived the ideas and designed the experiments. M-LJ, FW, RG, LKY, YCL and JHX conducted experiments and analySed the data. JL and M-LJ interpreted the data and wrote the manuscript.

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EPIDEMIOLOGICAL SCIENCE

Genome-wide association of phenotypes based on clustering patterns of hand osteoarthritis identify *WNT9A* as novel osteoarthritis gene

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ABSTRACT

Background Despite recent advances in the understanding of the genetic architecture of osteoarthritis (OA), only two genetic loci have been identified for OA of the hand, in part explained by the complexity of the different hand joints and heterogeneity of OA pathology.

Methods We used data from the Rotterdam Study (RSI, RSII and RSIII) to create three hand OA phenotypes based on clustering patterns of radiographic OA severity to increase power in our modest discovery genome-wide association studies in the RS (n=8700), and sought replication in an independent cohort, the Framingham Heart Study (n=1203). We used multiple approaches that leverage different levels of information and functional data to further investigate the underlying biological mechanisms and candidate genes for replicated loci. We also attempted to replicate known OA loci at other joint sites, including the hips and knees.

Results We found two novel genome-wide significant loci for OA in the thumb joints. We identified *WNT9A* as a possible novel causal gene involved in OA pathogenesis. Furthermore, several previously identified genetic loci for OA seem to confer risk for OA across multiple joints: *TGFa*, *RUNX2*, *COL27A1*, *ASTN2*, *IL11* and *GDF5* loci.

Conclusions We identified a robust novel genetic locus for hand OA on chromosome 1, of which *WNT9A* is the most likely causal gene. In addition, multiple genetic loci were identified to be associated with OA across multiple joints. Our study confirms the potential for novel insight into the genetic architecture of OA by using biologically meaningful stratified phenotypes.

Key messages

What is already known about this subject?

- Hand osteoarthritis (OA) is one of the most prevalent forms of OA and has a large genetic component, yet only two common genetic loci have been found.
- Lack of findings may be attributed to the modest samples sizes in previous genome-wide association studies (GWAS) and the high disease heterogeneity, which can negatively affect statistical power to robustly identify genetic loci.

What does this study add?

- Using three distinct hand OA phenotypes (hand, finger, thumb OA), based on clustering patterns of radiographic OA severity, we have increased the power of our GWAS (n~10 000), and robustly identified a novel genetic locus for thumb OA.
- Functional genomic data from OA disease relevant tissue identified a potential causal variant, predicted to be located in a gene regulatory element, which through chromatin looping interacts with the *WNT9A* promoter to influence *WNT9A* expression.

How might this impact on clinical practice or future developments?

- Our results provide the first evidence for WNT9A, a non-canonical Wnt ligand, in human thumb OA.

INTRODUCTION

Osteoarthritis (OA) is a serious destructive joint disorder and the third most rapidly rising condition associated with disability.[1] Despite this, no effective treatments that target OA are available. Current treatments only manage pain, not the underlying mechanisms of disease aetiology. An estimated 5% of the world population is affected by OA, with hand OA as one of its most prevalent forms. Given the high prevalence with age and high estimated lifetime risk rates for symptomatic hand OA (39.8%), the number of individuals affected will only continue to increase.[2 3] Hand OA has a high clinical burden, involving considerable pain, deformity and impaired function.[4–6] In addition, hand joints are non-weight bearing, and therefore may reflect the systemic aspects of the disease more than knee and/or hip OA, where mechanical loading is a dominant risk factor.[7] A better understanding of hand OA, its causes and pathophysiological mechanisms is therefore urgently needed.

Hand OA is a complex multifactorial disorder. It shares risk factors, such as repetitive movements

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and obesity, with OA at other joint sites.[8 9] Hand OA also has a strong genetic component, with heritability estimates ranging from 39% to 84% depending on the hand joint affected.[10 11] Recently, major advances were made in elucidating the genetic background of hip and knee OA, using large (n>400 000 individuals) genome-wide association studies (GWAS).[12–14] Yet, for hand OA only two common genetic loci have been found near the *MGP* and *ALDH1A2* genes.[15 16] The lack of findings for hand OA may be attributed to the modest samples sizes (n<9000 individuals) in previous GWAS and disease heterogeneity,[17] which is known to reduce power to robustly detect genetic loci.[18]

OA in the hand may be present in any joint, but is most prevalent in the distal interphalangeal joints (DIP), first carpometacarpal (CMC1) and trapezioscaphoid (TS) joints, followed by the proximal interphalangeal joints (PIP). OA is least prevalent in metacarpophalangeal joints (MCP).[19 20] Diverse definitions of hand OA have been used including nodal hand OA (interphalangeal (IP) joints), thumb base OA (CMC1/TS) and generalised hand OA (DIP/PIP/CMC1)[19 20] with varying results, suggesting that disease aetiology may differ between the joints. Moreover, OA affects multiple tissues within the joint including cartilage and bone. This further contributes to disease heterogeneity and warrants the assessment of hand OA by endophenotypes such as joint space narrowing (JSN) and osteophytes (OST) that may capture separate biological processes underlying OA pathology. The use of endophenotypes, quantifiable biological phenotypes intermediate to the genes and the disease, has been successfully used in OA for the detection of novel genetic loci[16 21 22] in knee and hip OA, and may provide new insights into hand OA. For heterogeneous diseases such as OA, stratification of OA phenotypes into different dimensions of disease is one way of increasing power in GWAS.[18]

There are few GWAS of hand OA and none has examined hierarchically defined clusters of OA joint presentation in the hands or hand OA endophenotypes that may provide new insights into hand OA pathogenesis. We therefore set out to examine the occurrence of OA across hand joints and conduct a GWAS stratified by hand OA patterns[23] to identify novel genetic loci for hand OA.

METHODS

GWAS, discovery, replication and meta-analysis

We conducted GWAS on radiographic structural phenotypes for OA of the hand using data from the Rotterdam Study (RS) (n∼8700).[24] For a detailed description of the RS, subcohorts and GWAS methods, see online supplemental text and table 1. Briefly, genotypes were imputed to the Haplotype Reference Consortium reference panel (V.1.0) using the Michigan Imputation Server.[25] We assessed genetic associations in each RS sub-cohort using linear regression models adjusted for age, sex and the first four genetic principal components. RVtests[26] was used for the GWAS analyses and results were quality controlled using EasyQC.[27] Variants with an imputation quality <0.3, minor allele frequency <0.05 or effective allele count <5 were excluded and genomic control correction was applied to all SE and p values. Meta-analysis between the discovery cohorts was performed using fixed-effects inverse variance weighting with METAL.[28] Manhattan plots and QQ plots were generated using R and R package qqman.[29] Independent variants with a $p<1\times10^{-6}$ and a χ^2 statistic test of heterogeneity $p>1\times10^{-6}$ were selected for replication in the Framingham Heart Study (FHS) (n=1203).[30] For a detailed description of the FHS, see the online supplemental text. Summary level data from the discovery and replication stage were combined in a joint meta-analysis (METAL).[28] Variants met criteria for replication if the association reached a p<0.05, had the same direction of effect as the discovery sample and reached a joint meta-analysis $p<5\times10^{-8}$. Replicated variants were also examined for association with clinical OA (ie, hospital diagnosed OA) based on GWAS summary statistics from a large-scale OA meta-analysis of data from the UK Biobank and Icelandic deCODE populations.[14 15] For a detailed description of the UK Biobank and deCODE, see the online supplemental text. Associations that reached a p value <0.01 were considered statistically significant.

Table 1 General characteristics of the study population

Cohort	RSI	RSII	RSIII
Hand			
n (OA cases)	4829 (1830)	1791 (688)	2071 (526)
Female (%)	2773 (0.57)	964 (0.54)	1180 (0.57)
Age (SD)	67.6 (7.9)	64.6 (7.9)	57.1 (7.0)
KLsum (aver. (SD))	8.4 (9.9)	6.9 (4.0)	4.7 (6.5)
Osteophytes (aver. (SD))	7.1 (7.8)	6.8 (8.2)	4.6 (6.3)
JSN (aver. (SD))	0.84 (2.4)	0.34 (1.3)	0.2 (0.8)
Finger			
n (OA cases)	4839 (1244)	1803 (474)	2072 (298)
Female (%)	2779 (0.57)	972 (0.54)	1181 (0.57)
Age (SD)	67.6 (7.9)	64.6 (7.9)	57.1 (7.0)
KLsum (aver. (SD))	5.8 (7.4)	4.5 (6.1)	3.0 (4.7)
Osteophytes (aver. (SD))	4.7 (5.5)	4.3 (5.8)	2.9 (4.5)
JSN (aver. (SD))	0.6 (1.9)	0.25 (1.1)	0.1 (0.7)
Thumb			
n (OA cases)	4882 (916)	1813 (255)	2083 (166)
Female (%)	2785 (0.57)	972 (0.54)	1184 (0.57)
Age (SD)	67.6 (7.9)	64.6 (7.9)	57.1 (7.0)
KLsum (aver. (SD))	2.1 (3.3)	1.2 (2.3)	0.8 (1.7)
Osteophytes (aver. (SD))	1.3 (2.1)	0.93 (1.7)	0.6 (1.3)
JSN (aver. (SD))	0.4 (1.0)	0.18 (0.63)	0.1 (0.4)

aver., average; JSN, joint space narrowing; KL, Kellgren-Lawerence; OA, osteoarthritis; RS, Rotterdam Study.

Detailed phenotype descriptions

For each participant, all hand joints (16 joints per hand, 32 joints per individual) were scored for Kellgren and Lawrence (KL) grade[31] based on hand radiographs. KL grade is a semi-quantitative score ranging from 0 to 4, where higher scores indicate more severe disease. Radiographic OA was defined as KL grade ≥2 (definite JSN and definite OST). Each joint was also scored for individual radiographic features including JSN and OST.[31] The JSN and OST scores are semi-quantitative scores ranging from 0 to 3, where 0=none, 1=possible, 2=definite and 3=marked.

We conducted hierarchical clustering of KL grade across all hand joints to identify patterns of disease occurrence defined by location and disease severity (see online supplemental text). This yielded three semi-quantitative hand OA phenotypes for analysis: (1) hand KLsum=sum of KL grades across all DIP, PIP, MCP, IP and CMC1 joints in both hands (15 joints per hand, 30 joints per individual, hand KLsum score range: 0–120), (2) finger KLsum=sum of KL grades across all DIP and PIP joints in both hands (8 joints per hand, 16 joints per individual, KLsum score range: 0–64) and (3) thumb KLsum=sum of KL grades across the CMC1 and TS joint (2 joints per hand, 4 joints per individual, KLsum score range: 0–16). Individuals with a missing

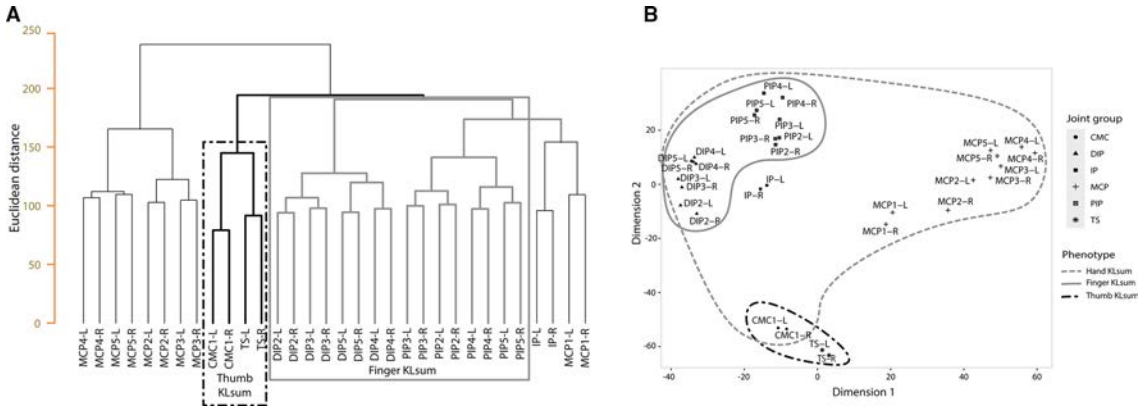

Figure 1 Tree-dendrogram and multidimensional scaling (MDS) plot of KL scores in the joints of the hand. (A) Tree-dendogram of complete hierarchical clustering of Euclidean distance matrix of KL scores of all hand joints left (L) and right (R). (B) MDS plot of KL scores for all hand joints left (L) and right (R). Data consisted of all RSI, RSII and RSIII (n=8691) participants of whom radiographic X-rays of the hands were made. Selected phenotypes are depicted by the different (dashed) lines. TS, trapezioscaphoid joints; CMC, carpometacarpal joints; DIP, distal interphalangeal joints; IP, interphalangeal joints; KL, Kellgren-Lawrence OA severity score; L/R, left or right joint; MCP, metacarpophalangeal joints; OA, osteoarthritis; PIP, proximal interphalangeal joints; IP, interphalangeal joints; number denotes which joint, ie, PIP2-L, the second PIP joint at the left hand. See online supplemental figures 2 and 3 for tree dendrogram and MDS plots for joint space narrowing and osteophytosis scores.

KL grade in one or more hand joints were excluded from the analysis of phenotypes that required scoring of the missing joint(s). Also, individuals with missing age, sex or genetic principal components were excluded from all analyses (table 1).

Post-GWAS analysis

Post-GWAS analysis consisted of multiple bioinformatic and functional analyses (online supplemental text). Briefly, all GWAS variants were annotated using HaploReg (V.4.1) and FUMA.[32 33] Intersection of variants with epigenetic markers, proteins, transcription factor (TF) motifs and binding and chromatin interactions was done using data from ROADMAP, ENCODE, HaploReg (V.4.1) and the three-dimensional (3D) genome browser.[34–37] Functional studies included expression quantitative trait loci (eQTL) analysis, methylation expression quantitative trait loci (meQTL) analysis, ATAC-seq analysis and differential gene expression analysis. All functional analyses were performed in human articular cartilage. Details are provided in the online supplemental text.

RESULTS

Patterns of osteoarthritis severity in joints of the hand

We used hierarchical cluster analysis on the KL grades of all 32 hand joints of the left hand and right hand to identify clusters (figure 1). Tight symmetric clustering between the left and right joints was seen, in addition to clustering based on joint group, which is in line with previous findings[19 38 39] (figure 1A,B). Clustering based on individual radiographic features, JSN and number of OST produced similar symmetric and joint group clusters (online supplemental figures S1 and 2). We observed consistent clustering of the PIP with the DIP joints and the TS with the CMC joints (figure 1, online supplemental figures S1 and 2). Based on these analyses, we created three semi-quantitative hand OA phenotypes: hand KLsum, thumb KLsum and finger KLsum (table 1).

Identification of genetic hand osteoarthritis loci

We conducted GWAS on each of the three identified hand OA phenotypes in a discovery sample that included RSI, RSII and RSIII cohorts (n~8700) (online supplemental table S1). In total, we identified seven independent signals with genome-wide suggestive association ($p<1\times10^{-6}$), which were taken forward for replication in the FHS (n=1203) (figure 2 and table 2). In total, four independent signals were genome-wide significant in the meta-analysis ($p\leq5\times10^{-8}$), of which three were significantly replicated ($p<0.05$) (table 2). Two of these signals were novel OA associated loci. The first and most significant novel locus was located on chromosome 1 near the *ZNF678, WNT3A and WNT9A* genes, with rs10916199 as the lead single nucleotide variant (SNV). This signal is replicated ($p<0.05$) and genome-wide significantly associated with thumb KLsum (beta=−0.31, $p=2.36\times10^{-13}$). The second replicated novel locus is located on chromosome 11 containing the *F2, LRP4* and *CREB3L1* genes, and is associated with thumb KLsum (beta=−0.19, $p=4.7\times10^{-8}$). We also identified two known OA associated loci. The first locus was located near the *MGP* gene, with rs4767133 as the lead SNV. This locus was previously found to be associated with hand KLsum.[16] The second known OA locus, also located on chromosome 12, is the *CCDC91* locus, which was also previously found to be associated with hand KLsum, although it did not reach genome-wide significance.[16] Here, the lead variant rs12049916, was genome-wide significantly associated with hand KLsum (beta=0.78, $p=1.5\times10^{-8}$) and finger KLsum (beta=0.58, $p=2.0\times10^{-8}$), but did not reach nominal significance in the replication cohort, though the direction of effect was the same between discovery and replication cohorts (table 2).

To examine if replicated loci were also associated with clinically defined OA, we looked up findings in GWAS summary statistics from a recent large-scale OA meta-analysis that included the UK Biobank and deCODE populations[14 15] (table 3). Of the novel signals, only rs10916199 (thumb KLsum) was significantly associated with its matching clinical OA phenotype (thumb OA: OR=0.9, 95% CI=0.86 to 0.95, $p=5.7\times10^{-5}$). Of the known signals, rs4764133 was significantly associated with multiple clinical OA phenotypes: thumb OA (OR=1.07, 95% CI=1.03 to 1.12, $p=6.3\times10^{-4}$), finger OA (OR=1.12, 95% CI=1.07 to 1.17, $p=5.7\times10^{-7}$), hand OA (OR=1.09, 95% CI=1.04 to 1.13, $p=6.7\times10^{-5}$) and nominal significantly with knee OA (OR=0.99, 95% CI=0.96 to 1.00, $p=1.8\times10^{-2}$) (table 3). In addition, we also performed a sensitivity analysis in our discovery cohorts to see if the association of rs10916199 with thumb

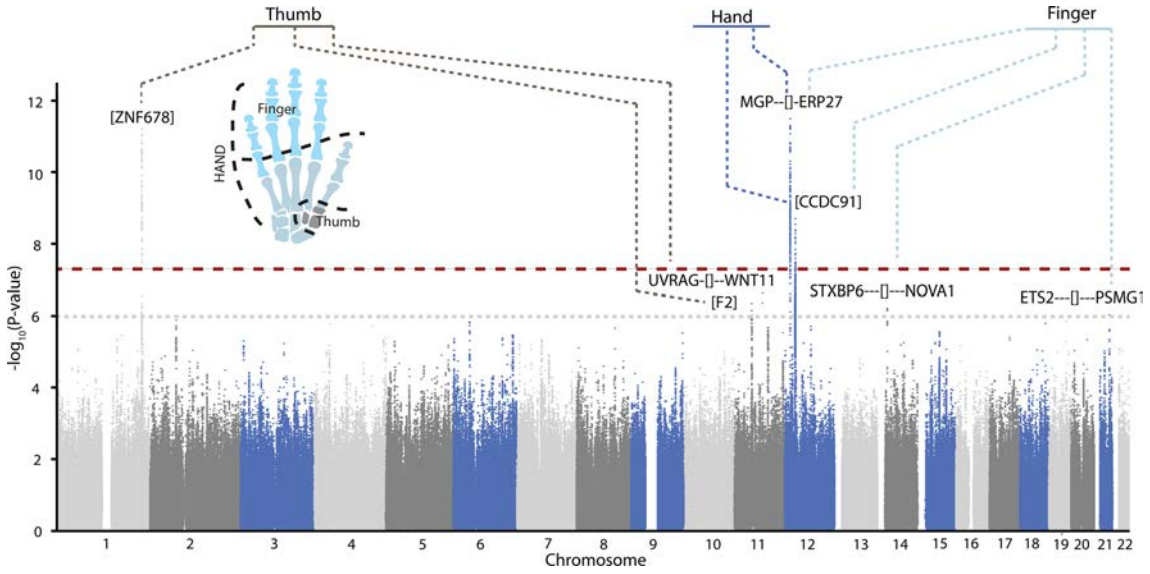

Figure 2 Combined Manhattan plot of genome-wide association studies (GWAS) discovery results of all radiographic hand OA structural phenotypes. Thumb=thumb KLsum score, finger=finger KLsum score and hand=hand KLsum score. GWAS discovery consists of RSI, RSII and RSIII, and was adjusted for age, sex and the first four genetic principle components. The $-\log_{10}$ p values, for each of the ~11 million single nucleotide polymorphisms (SNPs) analysed (remaining after EASYQC quality control) from the association studies is plotted against their position per chromosome. The dotted red horizontal line corresponds to the genome-wide significant threshold ($p=5\times10^{-8}$). The dotted grey line corresponds to the selection for replication threshold ($p=1\times10^{-6}$). Lead SNP location is represented by [] (intronic), if the SNP is localised intergenic the dashes denotes the distance, - ≤10 kb, -- ≤100 kb, ---- ≤1 Mkb, ---- ≥1 Mkb. For plots of the individual GWAS, see online supplemental figures 3–5.

KLsum was driven by body mass index (BMI. After additional adjustment for BMI, rs10916199 remained strongly associated with thumb KLsum (beta=−0.37, SE=0.05, $p=5.8\times10^{-13}$).

WNT9A as potential causal gene for thumb OA

We examined the rs10916199 locus in more detail given the strong and consistent association with thumb OA and thumb KLsum. Different levels of information were leveraged for all genes within 1 Mb surrounding rs10916199 in order to prioritise a putative causal gene (figure 3 and online supplemental text). First, we meta-analysed two human osteoarthritic cartilage expression quantitative trait loci (eQTL) datasets (n=116, hip/knee joints, online supplemental table S1)[40 41] and found no significant effect of rs10916199 on gene expression in these datasets. Next, there were significant methylation quantitative trait loci (meQTL) associated with rs10916109 and two CpG sites in human osteoarthritic cartilage (knee/hip joints): CpG09796739 (beta=0.39, FDR $p=8.3\times10^{-7}$) and CpG11520395 (beta=0.21, FDR $p=5.5\times10^{-3}$) (figure 3E, zone 2). These methylation sites are located in a region flanking an active transcriptional start site in primary osteoblasts and chondrogenic cells (figure 3B).

To further assess co-localisation of the identified genetic loci with regulatory function during cartilage differentiation, as many OA loci haven been linked to skeletal development,[42] we intersected the GWAS signals in the locus with accessible chromatin regions (ATAC-seq peaks) in rare human fetal cartilage acquired

Table 2 Summary of radiographic hand OA structural phenotypes GWAS results

			Discovery*						Replication†			Meta-analysis‡			
rsID	Chr	Pos (hg19)	EA	NEA	EAF	Beta	SE	P value	Beta	SE	P value	Beta	SE	P value	Locus§
Thumb KLsum															
rs10916199	1	227 902 472	A	G	0.81	−0.31	0.04	2.1×10^{-12}	−0.27	0.13	3.8×10^{-2}	−0.31	0.04	2.4×10^{-13}	(ZNF678)
rs2070852	11	46 744 925	C	G	0.69	−0.19	0.04	4.5×10^{-7}	−0.22	0.11	3.4×10^{-2}	−0.19	0.04	4.7×10^{-8}	(F2)
rs621457	11	75 858 695	A	G	0.52	−0.18	0.03	2.4×10^{-7}	−0.07	0.11	5.2×10^{-1}	−0.17	0.03	3.3×10^{-7}	UVRAG-[]--WNT11
Finger KLsum															
rs4764133	12	15 064 363	T	C	0.38	0.61	0.09	5.7×10^{-12}	1.38	0.38	2.7×10^{-4}	0.65	0.09	4.8×10^{-14}	MGP—[]-ERP27
rs12049916	12	28 359 985	G	A	0.22	0.57	0.11	7.5×10^{-8}	0.73	0.44	9.9×10^{-2}	0.58	0.10	2.0×10^{-8}	(CCDC91)
rs1950427	14	25 955 502	T	C	0.12	0.66	0.13	6.2×10^{-7}	0.63	0.53	2.4×10^{-1}	0.66	0.13	3.0×0^{-7}	STXBP6 ---[]---NOVA1
rs1029003	21	40 309 122	A	G	0.46	0.43	0.09	9.3×10^{-7}	0.35	0.37	3.4×10^{-1}	0.42	0.09	5.9×10^{-7}	ETS2---[]---PSMG1
Hand KLsum															
rs4764133	12	15 064 363	T	C	0.38	0.75	0.12	3.0×10^{-10}	1.76	0.49	2.9×10^{-4}	0.81	0.12	2.9×10^{-12}	MGP—[]-ERP27
rs12049916	12	28 359 985	A	G	0.77	0.77	0.14	5.3×10^{-8}	0.90	0.56	0.11	0.78	0.14	1.5×10^{-8}	(CCDC91)

*Discovery consists of RSI, RSII, RSIII, samples sizes per phenotype are: thumb KLsum n=8778; finger KLsum n=8714; hand KLsum n=8629.
†Replication cohorts consist of Framingham Heart Study n=1203.
‡Meta-analysis is discovery and replication cohorts using inverse variance weighted meta-analysis using METAL.
§SNP location represented by [], if the SNP is localised intergenic the dashes denotes the distance, - ≤10 kb, -- ≤100 kb, --- ≤1 Mkb, ----- >1 Mkb.
EA, effect allele; EAF, effect allele frequency; GWAS, genome-wide association studies; NEA, non-effect allele; RS, Rotterdam Study; SNP, single nucleotide polymorphism.

Table 3 Association with hip, knee, hand, finger and thumb OA

rsID	EA	EAF	Thumb OA (7280 cases; 605 132 controls)			Finger OA (7037 cases; 222 772 controls)			Hand OA (8591 cases; 224 326 controls)			Hip OA (17 151 cases, 613 790)			Knee OA (24 919 cases, 613 702 controls		
			OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P value
rs10916199	A	0.81	0.91	0.86 to 0.95	5.7×10^{-5}	0.98	0.93 to 1.04	0.57	1.03	0.98 to 1.08	0.32	1.00	0.98 to 1.03	0.95	0.99	0.97 to 1.02	0.59
rs2070852	C	0.69	0.97	0.89 to 1.05	0.47	0.99	0.94 to 103	0.58	0.99	0.96 to 103	0.80	0.98	0.96 to 1.01	0.22	0.99	0.97 to 1.01	0.40
rs4764133	T	0.39	1.07	1.03 to 1.12	6.3×10^{-4}	1.12	1.07 to 1.17	5.7×10^{-7}	1.09	1.04 to 1.13	6.7×10^{-4}	0.99	0.96 to 1.01	0.30	0.98	0.96 to 1.00	0.018
rs12049916	A	0.77	1.00	0.96 to 1.05	0.87	0.99	0.94 to 1.04	0.67	0.99	0.94 to 1.04	0.68	0.96	0.93 to 0.99	2.9×10^{-3}	0.99	0.97 to 1.01	0.27

Thumb OA, finger OA, hand OA GWAS summary statistics are from the deCODE cohort. Hip OA and knee OA summary statistics are from a meta-analysis of deCODE and UK Biobank cohorts.
EA, effect allele; EAF, effect allele frequency; NEA, non-effect allele; OA, osteoarthritis.

from proximal and distal long bones from gestational day(E) 59 of development.[43] Two of the SNVs in high LD ($r^2 \geq 0.8$, figure 3A) with rs10916199 (rs74140304 and rs11588850) intersected with accessible chromatin regions across multiple human long bone cartilage (figure 3C and 3D). In addition, rs74140304 also intersected with an active transcription start site in osteoblast and chondrogenic cells (figure 3B).[28] Next, we examined 3D chromatin conformation in the locus. Since no chromatin conformation capture data were available for chondrogenic or bone cells, we examined data from human mesenchymal stem cells, which are stem cell progenitors for chondrocytes and osteoblasts (figure 3G).[28] The genomic location of rs1158850 (figure 3G, zone 2) appears to come into close proximity with the promoter region of *WNT9A* (figure 3G, zone 3). In addition, CTCF binding peaks in osteoblasts also intersect with the *WNT9A* promoter region (figure 3F, zone 3), and are located near rs11588850 (figure 3F, zone 2).

Next, differential expression analysis between OA lesioned and preserved cartilage (hip/knee joints) identified *WNT9A* as the most significant result, with increased expression in OA lesioned cartilage (n=21, fold change=2.42, $p=9.4\times10^{-8}$, online supplemental table S2). Lastly, we examined whether rs11588850 may significantly affect ($p<4.0\times10^{-8}$) the regulatory TF binding motifs[37] located within the *WNT9A* promoter.[35] The minor allele (G) of rs11588850 is in high linkage disequilibrium ($r^2 > 0.8$) with the OA risk increasing allele (G) of rs10916199, which significantly increases the binding affinity of the TF binding motif for RAD21 (G-allele logarithm of odds (LOD)=11.2, A-allele LOD=9.8). The RAD21 protein has been previously shown to bind to the *WNT9A* promoter region (online supplemental table S3). Thus, our results indicate *WNT9A* as novel OA associated gene, where rs1158850 is a potential regulatory variant for *WNT9A* (figure 3, zones 2–3).

Additional hand osteoarthritis associated loci

OA is highly heritable and co-occurrence of OA in multiple joint sites is well recognised[44] . As the hand joints are non-weight bearing, causes of OA in these joints may reflect effects of systemic risk factors, unlike the hip and knee joint where mechanical loading is a dominant risk factor.[7] Thus, we examined whether other known OA loci may also confer risk for hand OA (figure 4). For 29 of the previously reported OA associated SNVs,[13 14] nominally significant associations ($p<0.05$) were observed for one or more hand OA phenotypes. Strong associations were seen for known hand OA loci: *ALDH1A2-locus* (rs3204689), *MGP-locus* (rs4767133) (figure 4A,B) and *COG5* (rs3815148),[45] an SNV identified from a candidate gene study for hand OA. Interestingly, the *MGP*-loci and *ALDH1A2*-loci, were only associated with finger and/or hand OA phenotypes, but not with thumb OA. In contrast, the *BCL7A*-locus (rs11059094), was only associated with thumb OA. Strikingly, several reported knee and hip OA loci were also associated with hand OA phenotypes in our study (Bonferroni, $p<5.8\times10^{-4}$): *RUNX2-locus* (rs12154055), *COL27A1-locus* (rs919642), *ASTN2-locus* (rs13253416), *IL11-locus* (rs4252548), *TGFa-locus (rs3771501)* and *GDF5-locus* (rs143384) (figure 4B). Since some of these known loci were previously found to be associated with knee and/or hip OA, they may reflect common mechanisms across all joints in OA.

DISCUSSION

We identified four genome-wide significant loci associated with hand OA phenotypes, of which two were novel and specific for thumb OA. Integration of multiple lines of data provided

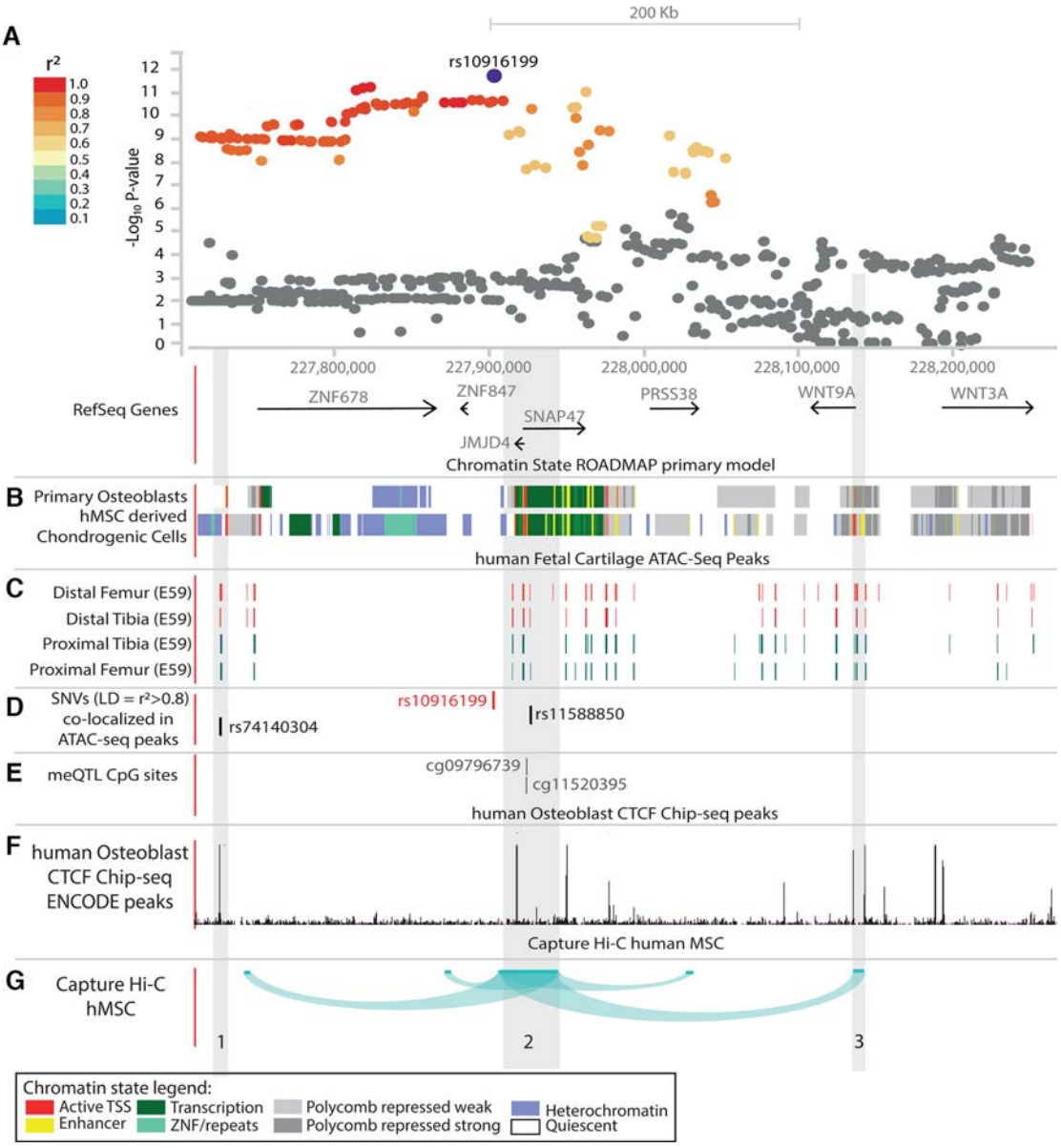

Figure 3 Schematic overview of part of the rs10916199 locus. (A) LocusZoom plot of rs10916199 locus, the Y-axis depicts the $-\log_{10}$ p value of the single nucleotide variant (SNV) from the thumb Kellgren-Lawrence (KL)sum genome-wide association studies (GWAS). Colours depict the linkage disequilibrium (LD) (r^2) between the variant and the LD SNV rs10916199. The X-axis depicts the relative genomic location, depict are the protein coding genes at those genomic locations. For this genomic region depicted in figure (B–G) are several epigenetic annotations are plotted. (B) Chromatin state, as predicted by the ROADMAP 15-state model on histone modifications, for primary osteoblasts and human mesenchymal stem cell-derived chondrocytes. Colours depict chromatin states, legend at bottom of full figure. (C). ATAC-seq peaks from human embryonic cartilage at different bone development sites at gestation day(E) 59. (D) Location of the lead SNV, rs10916199 and two putative causal SNVs which co-locative with ATAC-seq peaks. (E) Genomic location of rs10916199 meQTL CpG sites. (F) Chip-seq CTCF protein binding peaks from human primary osteoblasts from ENCODE. (G) Capture Hi-C chromatin interactions from three-dimensional (3D) genome browser for human mesenchymal stem cells (hMSC) and mesoderm. Depicted are the chromatin interactions from the promoters of the queried gene to the genomic location of interaction, this was done for the JMJD4/SNAP47 transcription start site (TSS), WNT9A TSS and the WNT3A TSS. For details on methods and underlying data, see online supplemental methods.

cumulative evidence that *WNT9A* may be a causal gene for thumb OA. We first conducted a cluster analysis of hand joints to identify less heterogeneous clusters of joints that served as the basis of the hand OA phenotypes assessed in this GWAS. With this approach of using radiographically defined biologically relevant OA phenotypes to reduce phenotype heterogeneity and increase statistical power, we were able to robustly identify known and novel OA loci despite our modest sample size (n~9900).[18 22] This indicates that assessment of stratified phenotypes in OA may be warranted to improve GWAS statistical power and provide novel insight into OA biology.

Using bioinformatic analysis and functional genomics datasets, we were able to identify rs1158850 as potential causal variant. This SNV is nearby meQTL CpGs and the G allele of this variant is predicted to increase RAD21 (RAD21 Cohesin Complex Component) binding affinity in a region that has chromatin interactions with the *WNT9A* promoter. RAD21 is a part of the cohesion complex, involved in the formation of chromatin

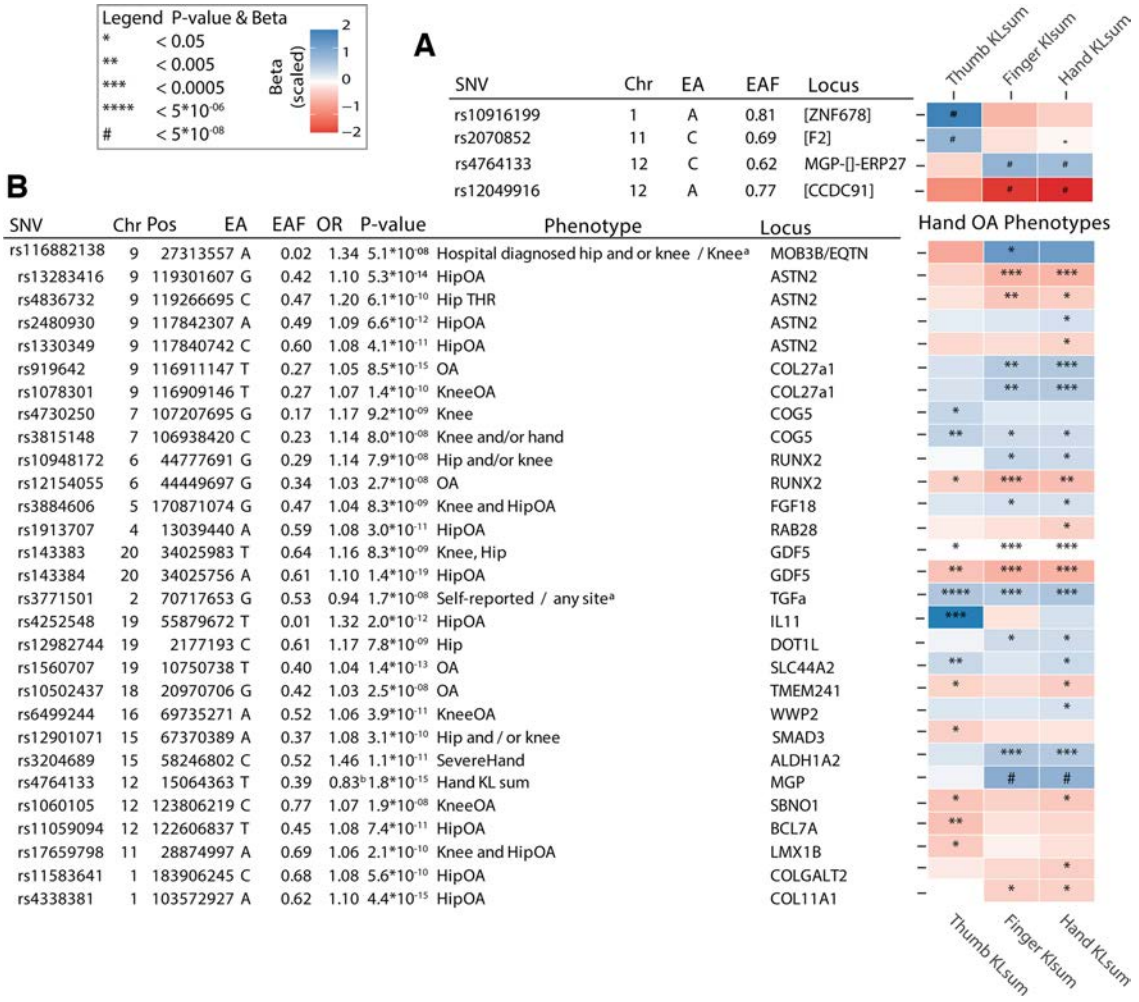

Figure 4 Heatmap depicting the effect of osteoarthritis (OA) associated single nucleotide variants (SNVs) in each hand OA phenotype. (A) The found associated lead SNVs of the hand OA phenotypes and their beta and p value in the other phenotypes. (B) Depicted are all known OA SNVs which had a nominal significant effect in at least one stratified hand OA phenotype. All betas were calculated for the reported effect allele and scaled. Colours represent the scaled beta of the effect allele, which is here the minor allele. P values are represented by * in the box. Chr, chromosome; EA, effect allele; EAF, effect allele frequency; Gene, reported gene from the GWAs study; KL, Kellgren-Lawrence score; Pos, base pair position on the chromosome Hg19.

loops with CTCF.[46] Both RAD21 and CTCF bind to the *WNT9A* promoter region. In line with these findings, *WNT9A* expression was significantly increased in OA lesioned cartilage compared with preserved OA cartilage. Combining all results, we postulate that rs1158850 is located in a gene regulatory element, increases RAD21 binding, and mediated by CTCF, interacts with the *WNT9A* promoter to influence *WNT9A* expression.

WNT9A (wingless-type MMTV integration site family, member 9A) previously known as *WNT14*, is a member of the *WNT* gene family, and has been shown to play a central role in synovial joint formation.[47 48] Knockout *WNT9A* mice have severe skeletal developmental defects, and are neonatal lethal.[49] Expression of *WNT* members by chondrocytes leads to the destruction of the cartilage matrix by the upregulation of Wnt/β-catenin signalling. Inhibition of WNT members has been suggested as a plausible OA therapeutic strategy, with recent success in a murine model of OA.[50 51] However, this is the first evidence for WNT9A, a non-canonical Wnt ligand, in human OA.

In addition, to identifying novel OA loci, we also provide evidence for a subset of generalised OA genetic risk loci: *GDF5* (rs143384), *TGFα* (rs2862851/rs3771501), *RUNX2* (rs12154055), *ASTN2* (rs2480930, rs13823416), *COL27A1* (rs919642) and *IL11* (rs4252548). These loci should be given priority as potential therapeutic targets since genetically supported drug targets have been shown to double the success rate of therapeutics in clinical development and intervention at these target loci may be beneficial regardless of which joint site is affected by OA.[52]

Although collectively our findings implicate *WNT9A* in thumb OA, there are several limitations. First, age is the most predominant risk factor for OA, yet the genetic background may determine the age of onset, rather than the lifetime risk for OA. Therefore, future genetic studies may benefit from examining the age of onset rather than adjust for age.[53] Second, our functional findings are based on chondrogenic data sourced from several different tissues that did not include tissues from the hand joints. However, consistent results were found across the available chondrogenic source material from several different origins (primary, cell culture, developmental), indicating a more general role for the *WNT9A* locus in chondrocyte functional pathways. Given the complex nature of OA susceptibility and the fact that pathophysiological causes are not uniform across skeletal sites, alterations in *WNT9A* expression may be seen in other

joints, but may have a more marked detrimental effect on the thumb joints. The lack of genetic association of WNT9A variants for knee and hip OA might be due to the phenotype definition of these GWAS studies: self-reported OA and 10th revision of the International Statistical Classification of Diseases codes, which do not necessarily correspond or have enough statistical power to identify genetic variants associated to radiographic phenotypes.

In summary, by examining the distribution of radiographic OA features in the hand joints, we identified three distinct hand OA phenotypes that provided the basis for identification of a novel locus for thumb OA despite our modest sample size. We identified *WNT9A* as a plausible causal gene for thumb OA, providing new insights into the genetic architecture of hand OA and a new candidate for OA therapeutic development.

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Contributors CGB designed the study, performed the analyses, made the figures and tables and wrote the manuscript. MSY performed the replication analysis. SJR, RCdA, KC and LS performed functional analyses and look-ups (eQTL, meQTL and differential expression), MY and TDC performed ATAC-seq analysis, US provided GWAS data of deCODE and UK Biobank, LB provided analysis help. AGU provided access to the Rotterdam study dataset, DF provided replication data, EZ, JL, TDC and IM provided functional data. JBJvM designed the study and supervised this work. All authors critically assessed the manuscript.

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Data availability statement Data are available on reasonable request. GWAS summary statistics are available through the GWAS catalogue. All data relevant to the study are included in the article or uploaded as supplementary information. All relevant data supporting the key findings of this study are available within the article and its supplementary information files. Other data are available from the corresponding author on reasonable request. Due to ethical and legal restrictions, individual-level data of the Rotterdam Study (RS) cannot be made publicly available. Data are available on request to the data manager of the Rotterdam Study Frank van Rooij (f.vanrooij@erasmusmc.nl) and subject to local rules and regulations. This includes submitting a proposal to the management team of RS, where upon approval, analysis needs to be done on a local server with protected access, complying with GDPR regulations.

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TRANSLATIONAL SCIENCE

OATargets: a knowledge base of genes associated with osteoarthritis joint damage in animals

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ABSTRACT

Objectives To collate the genes experimentally modulated in animal models of osteoarthritis (OA) and compare these data with OA transcriptomics data to identify potential therapeutic targets.

Methods PubMed searches were conducted to identify publications describing gene modulations in animal models. Analysed gene expression data were retrieved from the SkeletalVis database of analysed skeletal microarray and RNA-Seq expression data. A network diffusion approach was used to predict new genes associated with OA joint damage.

Results A total of 459 genes were identified as having been modulated in animal models of OA, with ageing and post-traumatic (surgical) models the most prominent. Ninety-eight of the 143 genes (69%) genetically modulated more than once had a consistent effect on OA joint damage severity. Several discrepancies between different studies were identified, providing lessons on interpretation of these data. We used the data collected along with OA gene expression data to expand existing annotations and prioritise the most promising therapeutic targets, which we validated using the latest reported associations. We constructed an online database OATargets to allow researchers to explore the collated data and integrate it with existing OA and skeletal gene expression data.

Conclusions We present a comprehensive survey and online resource for understanding gene regulation of animal model OA pathogenesis.

Key messages

What is already known about this subject?

- Animal models are commonly used as preclinical discovery tools to study osteoarthritis (OA).
- Genes are often modulated in these animal models to understand pathogenic signalling or recapitulate a disease modifying treatment scenario.

What does this study add?

- A knowledge base of all genes modulated in animal models of OA and integration with all publicly available OA transcriptomics data.
- Prioritisation approach for expanding known functional OA genes—validated using the latest reported research findings.

How might this impact on clinical practice or future developments?

- The knowledge base provides a roadmap to pinpoint druggable functional OA candidates for future therapies.

INTRODUCTION

Animal models have been used widely in the study of osteoarthritis (OA) as preclinical discovery tools to identify key molecular mechanisms contributing to OA pathophysiology.[1 2] Animal models are a powerful research tool allowing the controlled study of the earliest time points of OA initiation through disease progression, assessing joint-wide pathology and omics analysis which is not possible in human tissues.[3 4] There are a lack of validated *in vitro* models for OA with these models primarily consisting of cell or tissue-based systems, usually from a single-joint tissue, with supraphysiological levels of cytokines under glucose rich and normoxic conditions, that have uncertain relevance to the *in vivo* disease.[5] The use of animal models overcomes some of the limitations of human *ex vivo* OA culture models, potentially allowing more translatable research not only with modelling of pathology of the whole joint but also clinically-relevant pain outcomes.[6]

Previous publications have reviewed the range of OA animal models with regard to species, and mode of OA initiation, and described their relative advantages and limitations.[1 7–9] These animal models fall into broad categories of: (1) post-traumatic OA through surgical and mechanical (injurious load, excessive exercise) induction, with varying severity depending on the injury target (eg, meniscus, cruciate ligament, intra-articular fracture), (2) mouse strains with increased genetic susceptibility (eg, *Col9a1* or *Col2a1* mutant, STR/ort mice), (3) metabolic/obesity induced by high-fat diet, (4) hormonal induced by ovariectomy, (5) chemically induced (eg, monosodium iodoacetate, collagenase) and (6) spontaneous/age-associated OA.[6 10] These animal models are often genetically tractable allowing knockout, transgenic overexpression or knock-in mutation of genes, to investigate and define the key regulators of pathogenic joint signalling. In addition, these models have been used with interventions in the form of treatment with drugs, antibodies, transient gene/protein overexpression or knock-down which may better recapitulate the effect of gene modulation in a disease modifying treatment scenario.

There is no up-to-date database describing what genes have been manipulated in OA animal models and the effect on the resulting OA phenotypes.[2]

The results of these interventions in animal models are primarily available in fragmented publications, hindering efforts to learn from previous work. This study aimed to bring together this knowledge to gain an overview of the use of genetic manipulation in animal model OA research to investigate OA pathophysiology. We compare the OA-associated genes with OA transcriptomic data, prioritise yet unstudied genes, and for the first time provide an updatable resource for rapidly exploring evidence for candidate gene involvement in OA and target tractability.

MATERIALS AND METHODS

Literature search

A systematic search for publications describing animal models of OA was performed in PubMed, searching for English-language articles published between 1 January 2000 and 29 July 2020 using the following terms in combination with 'osteoarthritis'; 'mouse', 'mice', 'rat', '*in vivo*', 'animal model'. Papers were curated to retain reports of genetic (knockout/in, overexpression) or exogenous (virus, protein, antibody, drugs with defined structure and targets) interventions and the resulting effect (or lack of effect) on incidence/severity of OA in animal models including any one of cartilage degradation, proteoglycan loss, subchondral bone remodelling/sclerosis, osteophytes and synovitis, but excluding solely pain. Reports with both increased and decreased observed severity in different tissue types were recorded as having a mixed effect. Models of inflammatory arthritis such as interleukin-1b (IL1B), tumour necrosis factor α (TNF), collagen-induced or antibody-induced arthritis were excluded.

Labelling the effects of gene modulations on OA severity

The types of gene modulation (increase or decrease in gene activity) and the observed effects on OA phenotypes were used to label the 459 unique modulated genes as 'protective', 'detrimental' or 'no effect' for each individual experiment. For both the gene expression comparison and network expansion, non-protein coding genes were removed and the individual experiment inferred effects for each of the 425 protein-coding genes were combined. Genes were labelled 'ambiguous' if there was disagreement in direction of effect between experiments (ie, both protective and detrimental effects reported). Observations of no effect were considered superseded by any observation of a significant effect (positive or detrimental) on OA phenotypes for that modulated gene.

Gene expression analysis

All available animal model and human OA transcriptomic datasets (cartilage, bone, synovium, whole joint) were downloaded from SkeletalVis (http://skeletalvis.ncl.ac.uk/skeletal) on 9 June 2020.[11] Differentially expressed genes (absolute ≥1.5 fold change and adjusted p value ≤0.05) were used to find enriched Reactome pathways with goseq (adjusted p value ≤0.05).[12 13] Gene identifiers were mapped to human gene symbols via Ensembl orthologs. miRNA entries were removed for the comparison as small RNA expression datasets are not included in SkeletalVis. The Fishers exact test with Benjamini-Hochberg multiple testing correction was used to test gene set overlaps. χ^2 tests with Benjamini-Hochberg correction were used to test the proportions of protective and detrimental genes or surgical and spontaneous model genes in the overlaps.

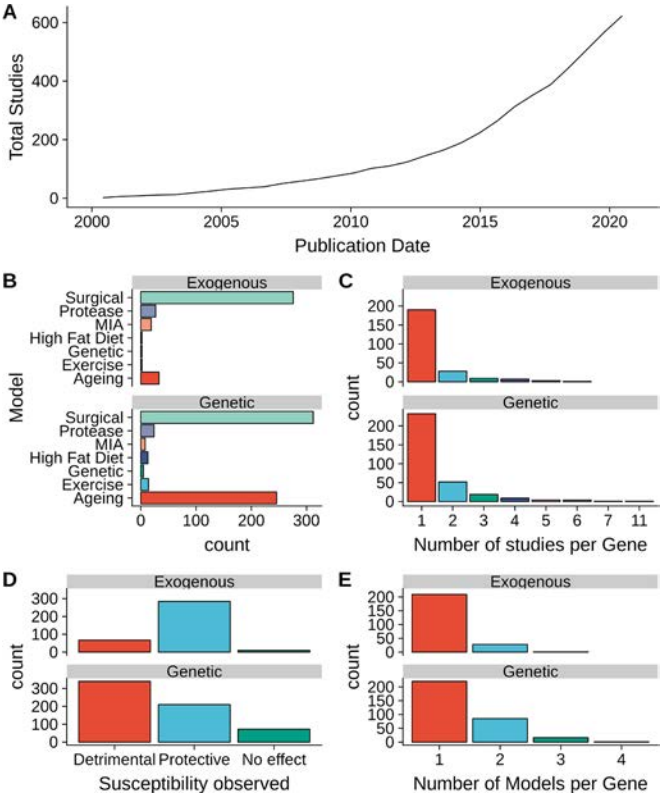

Figure 1 Summary of studies examining susceptibility to osteoarthritis (OA) after gene modulation. (A) Cumulative number of studies by published date. (B) Number of individual gene modulations per type of OA animal model. (C) Number of individual studies reporting each modulated gene. (D) Total number of observations by susceptibility observed. (E) Number of OA animal models used per gene studied. MIA, monosodium iodoacetate.

Network expansion of OA-associated genes

A network diffusion algorithm was used to rank genes based on network proximity to OA genes with an effect on OA severity, and repeated cross validation was used to test the predictive performance of this approach (online supplemental methods).[14] Newly reported animal model OA associations from the 2020 Osteoarthritis Research Society International (OARSI) conference abstracts were used to test prioritisation performance on new data. The Wilcox test was used to test the expected and the observed gene ranks. OA genome-wide association study (GWAS) signal variants were retrieved from a recent review.[15] Target drug tractability information was obtained from the OpenTargets platform.[16]

Data availability

Data and code to reproduce the analysis are available at www.github.com/soulj/OATargets.

RESULTS

Summary of genes modulated in animal models of OA

Search and curation of the literature for reports of OA susceptibility or progression in animal models after gene modulation identified 623 publications with 459 unique modulated genes (termed "OA genes" henceforth) with an increase in the rate of publications from 2000 to 2020 (figure 1A). Observations from these studies were grouped into genetic modulations (eg, overexpression, knockout, knockin) or exogenous modulations (eg, transient knockdown, drug treatment). In total, 415 publications reported 622

Table 1 Examples of gene modulations in osteoarthritis (OA) models with discrepancies

Human gene	PMID	Intervention	Effect on protein product	OA model	Observed effect	Inferred gene effect	Specificity	Species
Mixed effects between models and tissue specificity								
EZH2	30327434	Knockout	Removal	Spontaneous	No effect	No effect	Cartilage	Mouse
	31910305	Knockout	Removal	Surgical – MM	Detrimental	Protective	Cartilage inducible	Mouse
	27539752	EPZ005687	Inhibition	Surgical – ACLT	Protective	Detrimental	Articular cavity	Mouse
Opposite effects between models								
MINK1	31647983	Knockout	Removal	Spontaneous	Protective	Detrimental	Global	Mouse
		Knockout	Removal	Surgical – DMM	Detrimental	Protective	Global	Mouse
Detrimental effects with any gene modulation								
TTR	28941045	Knockout	Removal	Surgical – DMM	Detrimental	Detrimental	Global	Mouse
		Knockout	Removal	Spontaneous	Detrimental	Detrimental	Global	Mouse
		Overexpression	Overexpression	Surgical – DMM	Detrimental	Protective	Global	Mouse
Effect observed in only one type of model								
CD9	27784871	Knockout	Removal	Surgical - MML+ MCL	No effect	No effect	Global	Mouse
		Knockout	Removal	Spontaneous	Protective	Detrimental	Global	Mouse
TLR4	31044181	Knockout	Removal	High fat diet	Protective	Detrimental	Global	Mouse
	26245312	Knockout	Removal	Surgical – DMM	No effect	No effect	Global	Mouse
	24703622	Knockout	Removal	Surgical – DMM + MM	No effect	No effect	Global	Mouse
Potential effect of direction of gene modulation, model or tissue specificity								
RHEB	29991473	Knockout	Removal	Collagenase	Protective	Detrimental	Macrophage	Mouse
	31229684	Overexpression	Overexpression	Surgical – DMM	Protective	Protective	Articular cartilage	Mouse

Examples of gene perturbations in animal models of OA with disagreements in the inferred gene effect are shown.
ACLT, anterior cruciate ligament transection; DMM, destabilisation of the medial meniscus; MCL, medial collateral ligament; MM, medial meniscectomy; MML, medial meniscotibial ligament.

observations of genetic modulations of 322 unique genes, and 266 publications described 361 exogenous modulations of 238 unique genes (online supplemental table 1). Post-traumatic (surgical) and spontaneous/ageing models were found to be the most prevalent models of OA in genetic interventions, while the exogenous modulations were primarily performed in surgical models (figure 1B). Most of the studied genes, in both genetic and exogenous interventions, were reported in a single study and in a single type of OA model (figure 1C,E). The majority of genetic manipulation studies reported detrimental outcomes while exogenous interventions primarily reported improvement of OA phenotypes (figure 1D). All genetic studies identified were performed in mice, while greater diversity of species was used in exogenous modulations, including studies in rat and rabbit models.

To facilitate assessment of consistency between experiments, the types of gene modulation (increase or decrease in gene activity) and the observed effects on OA phenotypes were used to label the modulated genes as protective, detrimental or no effect OA genes in each experiment, akin to the idea of an oncogene versus a tumour suppressor in cancer. For instance, a protective label was inferred if an increase in OA severity was observed on inhibition of a gene, while inhibition of a detrimental gene would attenuate OA progression. Using this approach, 98/143 genes (69%) genetically modulated more than once had a consistent inferred effect on OA (online supplemental table 1). Similarly, 61/74 (82%) genes studied multiple times in the exogenous model had the same inferred effect. Examples of genes with inconsistent results are shown in table 1. A total of 101 genes were studied through both genetic and exogenous approaches, of these 71 (70%) had consistent results, although several of these findings are reported from within the same publication or research group. Interestingly, 68/82 (83%) genes with unambiguous effects within the same OA model were consistent in their inferred effect between spontaneous and surgical models. Examples of genes confirmed in multiple models both through genetic and pharmacological means in separate studies are shown in table 2.

Integration with OA transcriptomics data

To investigate the regulation of these 459 unique genes modulated in animal models (OA genes), 57 expression profiles identifying differential gene expression in human OA and animal model OA were examined (online supplemental table 2).[3 17–39] Enrichment analysis showed statistically significant overlap between the 425 protein coding OA genes and the sets of differentially expressed genes, regardless of species and OA model (online supplemental figure 1). A total of 70% (298/425) and 80% (340/425) of the protein-coding OA genes were found to be differentially expressed in at least one human OA and animal model expression dataset, respectively. However, this observation is confounded by the use of existing knowledge of gene differential regulation to choose which genes to modulate in animal models.

The individual observations for each OA gene were combined to label each gene with a consensus inferred effect (see methods). Both protective and detrimental OA genes were found to be differentially expressed in datasets across species and tissues in generally similar proportions, suggesting the direction of OA expression changes is not typically indicative of protective or detrimental effects of OA genes on modulation in induced OA (figure 2). Genes with solely no effect observations were also often differentially expressed, suggesting disease-associated regulation is not necessarily indicative of functional effects on modulation in induced OA. Interestingly, in the genes upregulated in human intact OA cartilage compared with non-OA cartilage, there was a statistically significant proportion of protective compared with detrimental OA genes (online supplemental table 2). These protective OA genes include extracellular matrix genes and growth factors that are upregulated in the human intact OA versus non-OA cartilage suggesting a protective anabolic response in the intact OA cartilage. These results suggest that the curated OA genes from mixed animal models are consistently differentially regulated across a range of tissues and species.

Table 2 Examples of gene modulations in osteoarthritis (OA) models with both genetic and exogenous evidence

Human gene	PMID	Intervention	Effect on protein product	OA model	Observed effect	Inferred gene effect	Specificity	Species
SIRT1	23723318	Knockout	Removal	Surgical – DMM +MM	Detrimental	Protective	Cartilage	Mouse
	32665267	Knockout	Removal	Surgical – DMM	Detrimental	Protective	Cartilage	Mouse
	32499111	Knockout	Removal	Surgical – DMM	Detrimental	Protective	Cartilage Inducible	Mouse
	32499111	Knockout	Removal	Spontaneous	Detrimental	Protective	Cartilage Inducible	Mouse
	23723318	Knockout	Removal	Spontaneous	Detrimental	Protective	Cartilage	Mouse
	23587642	Knockout	Removal	Spontaneous	Detrimental	Protective	Global	Mouse
	23124828	Mutation	Inhibition	Spontaneous	Detrimental	Protective	Global	Mouse
	22258484	Haploinsufficiency	Deficiency	Spontaneous	Detrimental	Protective	Global	Mouse
	29922443	SRT1720	Activation	Surgical – DMM	Protective	Protective	Systemic	Mouse
	31989845	SRT2104	Activation	Surgical – DMM	Protective	Protective	Articular cavity	Mouse
FYN	29555825	Knockout	Removal	Spontaneous	Protective	Detrimental	Global	Mouse
	29555825	Knockout	Removal	Surgical – DMM	Protective	Detrimental	Global	Mouse
	31534047	Knockout	Removal	Surgical – DMM	Protective	Detrimental	Global	Mouse
	29555825	PP1	Inhibition	Surgical – DMM	Protective	Detrimental	Systemic	Mouse
	29555825	AZD0530	Inhibition	Surgical – DMM	Protective	Detrimental	Systemic	Mouse
TNFRSF11B	30623241	Knockout	Removal	Surgical – TMJ	Detrimental	Protective	Global	Mouse
	27541035	Knockout	Removal	Spontaneous	Detrimental	Protective	Global	Mouse
	26018435	Knockout	Removal	Spontaneous	Detrimental	Protective	Global	Mouse
	17907189	Haploinsufficiency	Deficiency	Spontaneous	Detrimental	Protective	Global	Mouse
	17907189	Haploinsufficiency	Deficiency	Surgical – DMM	Detrimental	Protective	Global	Mouse
	17907189	Protein	Increase	Surgical – DMM	Protective	Protective	Articular cavity	Mouse
	18668550	Protein	Increase	Surgical – DMM + MM	Protective	Protective	Articular cavity	Mouse
	23723320	Protein	Increase	MIA	Protective	Protective	Systemic	Rat
ADAMTS5	21337391	Knockout	Removal	Surgical – DMM	Protective	Detrimental	Global	Mouse
	21337391	Knockout	Removal	Treadmill + TGFB	Protective	Detrimental	Global	Mouse
	19010693	Knockout	Removal	Surgical – DMM	Protective	Detrimental	Global	Mouse
	17968948	Knockout	Removal	Surgical – DMM	Protective	Detrimental	Global	Mouse
	15800624	Knockout	Removal	Surgical – DMM	Protective	Detrimental	Global	Mouse
	23954517	Antibody	Inhibition	STR/ort	Protective	Detrimental	Articular cavity	Mouse
	26410555	Antibody	Inhibition	Surgical – DMM	Protective	Detrimental	Systemic	Mouse
	28120109	siRNA	Knockdown	Surgical – DMM	Protective	Detrimental	Articular cavity	Mouse

Examples of gene perturbations in animal models of OA with data from both genetic and exogenous interventions are shown.
DMM, destabilisation of the medial meniscus; MIA, monosodium Iodoacetate; MM, medial meniscectomy; TMJ, temporomandibular joint hyperocclusion.

Network expansion of OA-associated genes

The collated data allow a genome-wide view of the pathways that when altered enhance or protect against induced OA. Pathway analysis identified 128 pathways significantly enriched in the OA genes (online supplemental table 3). Of the human Reactome pathways, 44% (961/2203) are covered by at least one OA gene, suggesting a large coverage of known signalling pathways (online supplemental table 3).

Analysis of associated genes from human diseases has suggested the presence of protein–protein interaction (PPI) network disease modules where groups of disease-related genes in the same signalling pathways occur.[40] To examine if OA genes can be predicted based on pathways, we tested the ability of a network diffusion algorithm to successfully recover hidden (held-out) OA genes (figure 3A). Across 100 random samples of OA genes with an effect on OA severity (ie, not labelled as no effect), the median rank of the held-out OA genes was 1575/17557 compared with 8965 for unlabelled (not known to be associated) genes, suggesting the held-out OA genes can be successfully recovered (figure 3B). This network approach allows identification of highly ranked unlabelled genes which are potential OA genes, therefore enabling expansion of OA signalling pathways.

To further prioritise genes, all OA genes with an effect on OA severity were input into the diffusion algorithm. This approach significantly prioritised the separate validation dataset of the latest potential associations from newly published conference abstracts (p value 0.001953) (figure 3C, online supplemental table 4). Interestingly, several yet unstudied genes nearest (upstream or downstream) to OA GWAS variants were also highly ranked, allowing prioritisation of these candidates. These resulting predictions were combined with differential expression in human OA expression datasets to provide orthogonal evidence of relevance to human OA (table 3). For example, the highly ranked *ACKR2* is differentially expressed in multiple human OA datasets and is a receptor for several chemokines known to affect OA in animal models, making it a potential candidate for future study and illustrating how relevant pathways can be systematically expanded using previously studied genes and available expression data (figure 3D).

Knowledgebase of OA modulations in animal models

To facilitate the use of this work as a resource for OA researchers, a website (OATargets) was constructed to allow searching of the curated data, prioritisation of targets from the network algorithm and visualisation of PPI interactions between OA genes (figure 4A). The database provides an PPI network coloured by the inferred effect on the OA phenotype,

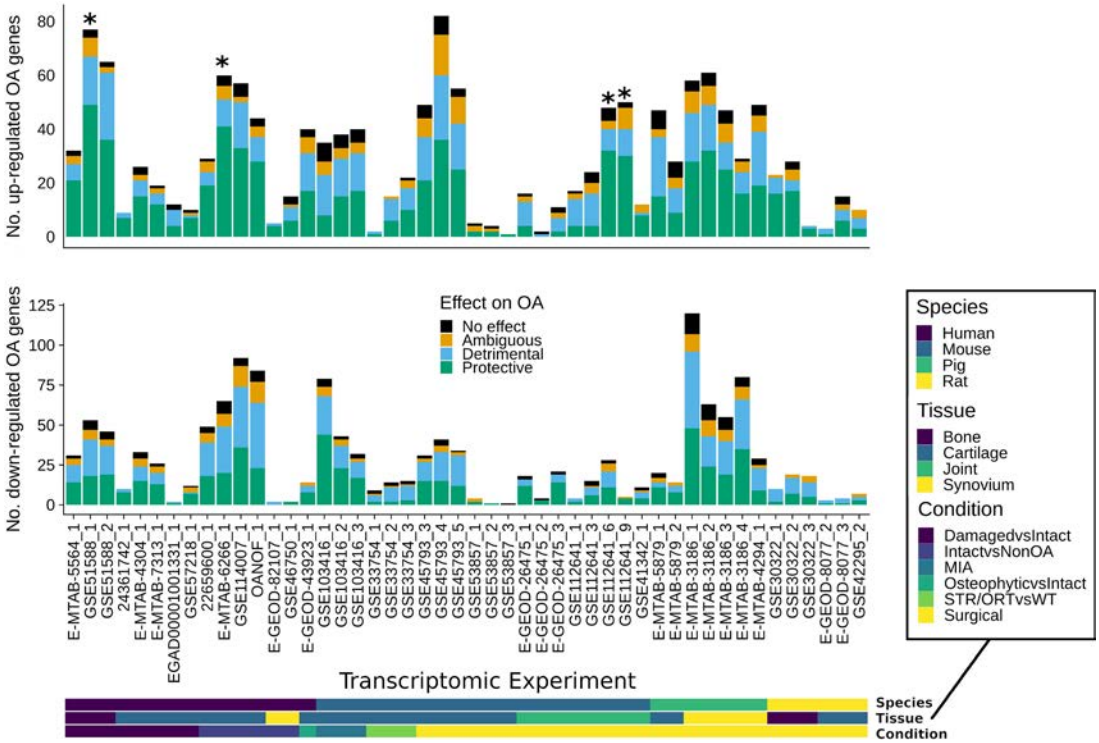

Figure 2 Differential expression of the osteoarthritis (OA) genes. The overlaps of upregulated and downregulated animal model or human OA differentially expressed genes with protective or detrimental OA genes are shown. Stars indicate statistically significant (Benjamini-Hochberg p value ≤0.05) differences in the proportions of differentially expressed protective and detrimental OA genes. The species, condition and tissue of the gene expression studies are indicated in the bottom bars. MIA, monosodium iodoacetate.

enabling exploration of the signalling neighbourhood of a gene. For example, *EZH2* interacts with several other OA genes illustrating the concept of OA pathways (figure 4B). This database is linked to the existing resource SkeletalVis to provide integration with over 700 skeletal gene expression profiles. Experiments with differential expression of a selected gene can be identified to assess tissue localisation or to find transcriptional regulators of that gene (figure 4C). Again, using *EZH2* as an example, it is dysregulated in several post-traumatic gene expression datasets. The knowledge base is publicly available at http://skeletalvis.ncl.ac.uk/OATargets/.

DISCUSSION

We have curated two decades of OA research to identify the large number of genes studied in OA animal models and have produced a database for future research. The generally consistent results between heterogeneous OA models support the robustness of the findings from these models. Several genes have complementary evidence from genetic and exogenous modulations that make promising putative human drug targets for further study. For instance, cartilage specific knockout of *Sirt1* increases susceptibility to both ageing and surgically-induced OA, while pharmacological intra-articular activation of *Sirt1* protects against surgically-induced OA.[41 42] Collection of these data also highlights several cases with discrepancies between studies, providing important cautionary lessons in interpreting these data. Knockout of *Mink1* showed protective effects in an ageing model, but detrimental effects in a surgical model, within the same publication, indicating different models can give divergent conclusions.[43] Several gene perturbations showed a phenotype in one model, but no effect in another suggesting that molecular regulation of OA is disease-phenotype-dependent, for example, knockout of *Tlr4* protects against high-fat diet induced OA, but not post-traumatic OA.[44–46]

OA is a joint-wide disease, so a range of tissues are examined for phenotypes in the identified studies, but most of the genetic perturbations are global/systemic or cartilage specific. The cell types targeted and timing of interventions between acute exogenous and global genetic or inducible genetic modulation may be responsible for some of the observed differences in studies examining the same gene. *Rheb* overexpression is protective in articular cartilage, but *Rheb* knockout in macrophages is also protective, suggesting caution should be employed when interpreting global knockouts or systemic drug treatments.[47 48] Different cells are known to be targeted in *Col2-Cre* and tamoxifen-inducible *Col2-CreER* genetic modulations.[49] Furthermore, it is often unclear what cells are most affected by exogenous interventions. Recent studies using surgical models reported inducible cartilage knockout of *Ezh2* to be detrimental, but treatment with an *Ezh2* inhibitor in the articular cavity was protective.[50 51] Drugs may have off-target effects and many studies do not assess if the drug at the selected dose was on target, which may contribute to results of a drug-based intervention against a designated target differing from an inducible genetic manipulation. These data suggest the need to look back at older results more critically, with the possibility of repeating gene modulations in other models.

Many genes have been studied in only one model, so it is unclear how generalisable results from such studies are. However, generally consistent findings were found between those genes that were studied in both spontaneous and surgical models, suggesting a core set of genes may be involved in both disease phenotypes. Subgroups of OA have previously been

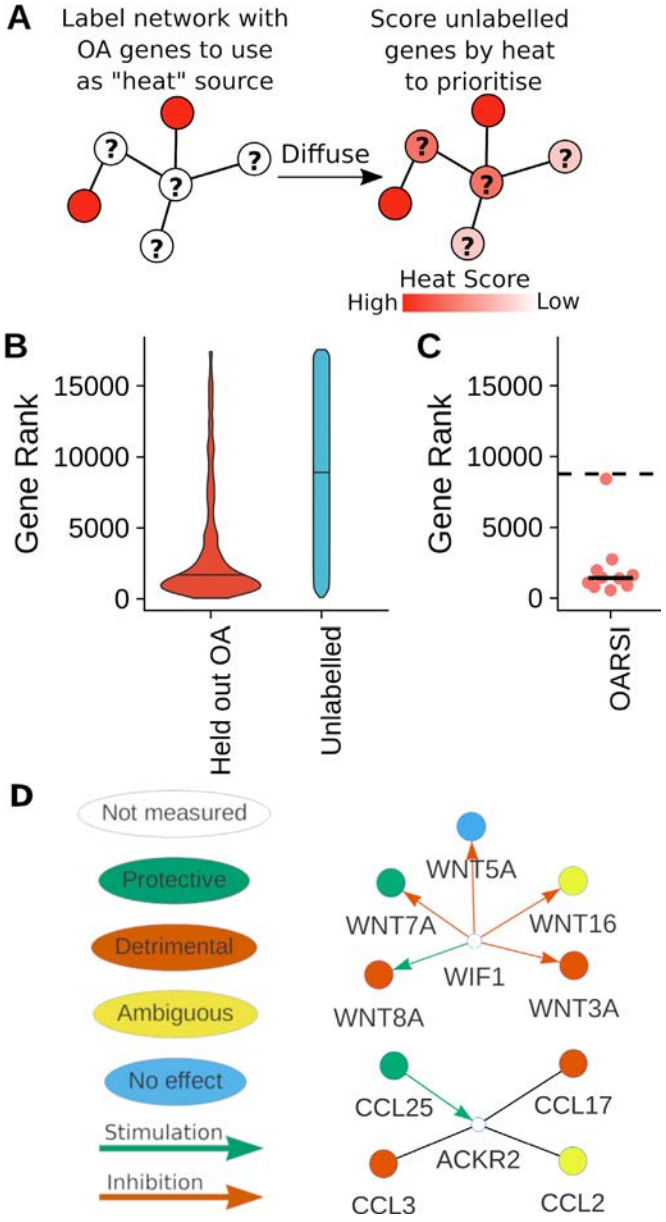

Figure 3 Expansion of known osteoarthritis (OA) genes. (A) Schematic of network diffusion algorithm used to expand known OA genes. (B) Violin plots demonstrating the ability to recover held-out known OA genes on the basis of network topology. Network diffusion-based ranks of held out known OA genes and unlabelled genes from 100 repeats of fivefold cross validation. (C) Network ranks of the latest reported OA genes from conference abstracts. Expected mean random rank showed by dashed line. (D) Example networks of a highly ranked genes (white), showing interactions to OA genes. The inferred effect of the OA genes and known regulatory interactions are indicated.

identified from cartilage genome-wide expression analysis of 'end-stage disease' (joint replacement) demonstrating the heterogeneity of the human disease.[17] We therefore suggest it is advisable to examine genes in multiple models of OA, and at multiple time points or stages of progression, potentially representing different subpopulations of human OA patients. Furthermore, where possible the use of tissue-specific genetic modification will enable a clearer understanding of the potential origin of OA phenotypes.

While bringing these studies together is useful for understanding OA pathways, combining the results from these variable

Table 3 Potential regulators of osteoarthritis (OA) severity

Gene name	No of human studies differentially expressed	No. interactions with OA genes	Rank
ACKR2	4	4	241
SAT2	3	5	263
NOG	4	4	311
FBLN2	4	4	356
FZD9	5	4	361
MMP14	4	4	362
WIF1	3	5	363
FZD8	6	6	381
ITGA11	6	4	387
CD36	3	11	395

The top predicted regulators using network-based expansion of the OA genes are shown. Regulators were filtered to be differentially expressed in at least three human OA expression datasets, to have at least four interactions with known OA genes and to exclude known OA genes. The rank of the network-based score is shown out of 17 557 total genes in the network.

studies has limitations. We present an inclusive list of findings using different scoring systems and variable statistical power to detect differences between conditions. Additionally, we do not record the sex of the animals studied, but the majority of DMM studies use only male mice. It is challenging to quantify the relative severity of the induced OA between studies due to differences in scoring systems which are usually semiquantitative and subjective. The OA models examined are heterogeneous, variations of surgical models have differences in OA severity and the severity induced within a given model may differ between surgeons.[10] The approach of labelling genes as protective or detrimental is a simplification as genes may have a homeostatic role requiring calibrated expression for joint health or have a differential function during the early to late disease process. For example, either overexpression or knockout of *Ttr* in a surgical

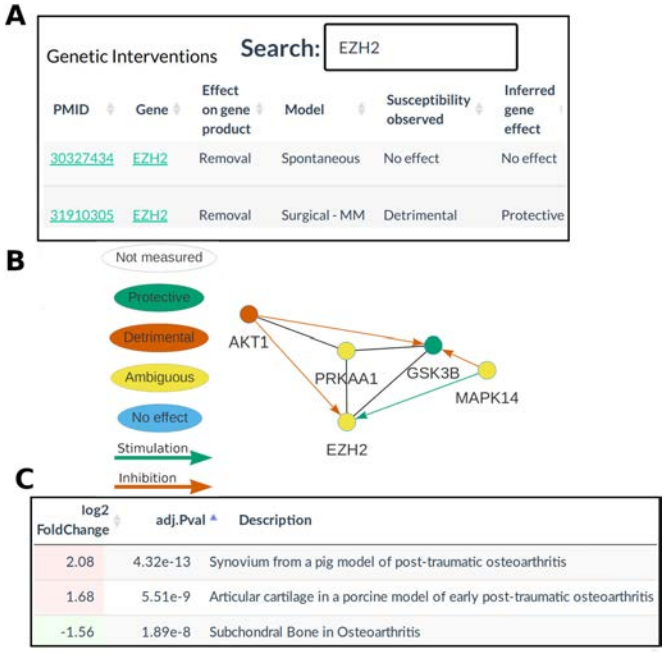

Figure 4 Database of osteoarthritis (OA) models and targets. Analysis of genes modulated in OA animal models (OA genes) with the OATargets database. (A) The database provides searchable tables of curated data. (B) interactive protein interaction networks with (C) links to an existing gene expression database. MM, medial meniscectomy.

model is detrimental to joint function.[52] A more granular, tissue level annotation of the OA phenotypes would be interesting to explore in the future as gene modulations may vary in the tissues they affect. However, this is currently challenging to perform meaningfully given the above caveats and as most studies do not evaluate all individual tissue phenotypes. The idea of using human omics data to prioritise animal model research (ie, 'the bedside-to-bench' approach) is attractive as inclusion aids relevance to human disease and potential translatability. Future comparison to proteomics and protein activity data would add an important layer of evidence, particularly as the latter may correlate poorly with transcriptomics data due to post-translational regulation, that is, phosphorylation, or protease activation/inhibition.

OA is a polygenic disease and the network analysis suggests the close network proximity of many of the experimentally perturbed genes. Modulation of many individual genes can give rise to the same phenotype.[40] The observed network proximity in OA is likely influenced by both bias in publishing of tested genes by researchers, as well as the presence of disease pathways responsible for the OA phenotypes. Despite the bias in the data collected, the prediction of genes that are OA relevant allows inference of gaps in knowledge and prioritisation of research. The top ranked genes are potential candidates for future studies in animal or *in vitro* models. For example, the extracellular Wnt antagonist *WIF1* interacts with several Wnt proteins known to affect OA, is dysregulated in human OA transcriptomic datasets, has small-molecule tractability and has been reported to correlate with histological cartilage grade.[53] Understanding the redundancy and relatedness of genes within the same pathway in terms of OA phenotypes could be useful for reducing, essentially reiterative, animal model use. There is only limited negative data published and future access to such information would be very useful in better predicting genes that are likely to be functional. The current network prioritisation does not account for functional redundancy so is likely to include false positives, for example, *ADAMTS4* is highly ranked, but knockout in mice does not affect spontaneous or surgically induced OA.[54]

The next step of finding key drivers of the pathogenic processes that occur in human OA over a much longer time scale and that can be therapeutically targeted in humans to improve joint function at time points amenable to intervention is a major challenge. There is a prevalence of surgical models used in the exogenous studies. Interventional studies of the most promising targets, perhaps identified in post-traumatic OA models, in longer time course, ageing based models would be beneficial in understanding the impact of intervention timing and the long-term benefits of treatment. Target druggability and benefit-to-risk ratio for OA treatment must also be considered. We believe that providing a resource with multiple layers of evidence and tractability data will aid future work towards better OA target selection. For instance, evaluation of past clinical trial failures IL1 and TNF using OATargets shows they have mixed effects in the animal models, no genetic and limited transcriptomics support for their use in OA structural disease modification.[55] Future inclusion of gene modulation effects on pain phenotypes would be useful for critical symptom-modifying drug target selection for OA.

This study has provided a resource for researchers to contextualise new data or explore existing publications. The OA genes can be used in tools to combine new differential expression datasets with the prior knowledge of OA joint damage-associated genes.[56] This database provides researchers with means to mine new targets for evidence of interactions with known OA genes and examine cross-species and cross-model gene expression dysregulation. Ultimately, we hope ongoing addition to and use of the database will improve understanding of the molecular pathophysiology of OA joint damage and lead to the development of disease modifying therapies for this currently intractable condition.

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CLINICAL SCIENCE

Prevalence and clinical outcomes of COVID-19 in patients with autoimmune diseases: a systematic review and meta-analysis

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ABSTRACT

Objectives The prevalence and clinical outcomes of COVID-19 in patients with autoimmune diseases who are frequently treated with disease modifying therapies remains poorly understood. This meta-analysis aims to assess the prevalence and clinical outcomes of COVID-19 in autoimmune diseases.

Methods Electronic databases were searched for observational and case–controlled studies. We sorted medications into glucocorticoids, conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and biologic or targeted synthetic DMARDs (b/tsDMARDs), which was also divided into monotherapy and b/tsDMARDs–csDMARDs combination therapy.

Results We analysed 62 observational studies with a total of 319 025 patients with autoimmune diseases. The prevalence of COVID-19 was 0.011 (95% CI: 0.005 to 0.025). Meta-analysis of seven case–controlled studies demonstrated that the risk of COVID-19 in autoimmune diseases was significantly higher than in control patients (OR: 2.19, 95% CI: 1.05 to 4.58, p=0.038). Meta-regression analysis showed glucocorticoids were significantly associated with the risk of COVID-19. For clinical outcomes, we assessed 65 studies with 2766 patients with autoimmune diseases diagnosed with COVID-19. The rates of hospitalisation and mortality were 0.35 (95% CI: 0.23 to 0.50) and 0.066 (95% CI: 0.036 to 0.12), respectively. Glucocorticoids, csDMARDs and b/tsDMARDs–csDMARDs combination therapy increased the risk of these outcomes, whereas b/tsDMARDs monotherapy, particularly antitumour necrosis factor agents, were associated with a lower risk of hospitalisation and death.

Conclusions Our meta-analysis demonstrated that patients with autoimmune diseases had an increased risk of COVID-19, primarily attributed to glucocorticoid use. b/tsDMARDs monotherapy was associated with a lower risk of severe COVID-19 suggesting its safety in the COVID-19 pandemic.

Key messages

What is already known about this subject?

- The prevalence and clinical outcomes of COVID-19 in patients with autoimmune diseases who are frequently treated with immunosuppressive or anticytokine drugs remains poorly understood.

What does this study add?

- The prevalence of COVID-19 in autoimmune diseases was 0.011 (95% CI: 0.005 to 0.025) which was significantly higher than in the comparator population.
- Glucocorticoids increased the risk of COVID-19 and its severe outcomes.
- Conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and biologic or targeted synthetic DMARDs (b/tsDMARDs)–csDMARDs combination therapy significantly increased the risk of severe outcomes, whereas b/tsDMARDs monotherapy, in particular anti-tumour necrosis factor therapy, reduced the risk of severe COVID-19.

How might this impact on clinical practice or future developments?

- Unlike glucocorticoids, csDMARDs and b/tsDMARDs–csDMARDs combination therapy, b/tsDMARDs monotherapy can be safely used during COVID-19 pandemic.

INTRODUCTION

The outbreak of COVID-19 caused by the novel SARS-CoV-2 has spread worldwide leading to large number of infections and deaths.[1] Patients with autoimmune diseases (ADs) are frequently treated with immunosuppressive or anticytokine drugs, which raises concern for infectious complications, placing patients and physicians at a crossroads with respect to continuation or cessation of these disease modifying therapies.

To understand the incidence and prognosis of COVID-19 in ADs, international registries of patients with inflammatory bowel disease (SECURE-IBD registry[2]) or rheumatic diseases (C19-GRA[3]) diagnosed with COVID-19 have been developed and analysed their COVID-19 outcomes. These data have demonstrated that similar to the general population, age and underlying comorbidities are poor prognostic factors of COVID-19 in ADs.[4] In terms of treatments, both registries demonstrated that patients treated with glucocorticoids (GCs) had poor clinical outcomes of COVID-19, whereas those treated with antitumour necrosis factor (TNF) therapies, particularly when used as a monotherapy, had a decreased risk of hospitalisation due to COVID-19.[2 3] These findings suggest that anti-TNF monotherapy may be protective against severe COVID-19. However, each study or registry has a limited sample size. Therefore, there

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is a need to integrate findings across studies to better understand the risk of COVID-19 in ADs.

This systematic review and meta-analysis aimed to determine the prevalence of COVID-19 and investigate its clinical outcomes in ADs. We also assessed how individual risk factors, including comorbidities and medical therapies, influence the prevalence and clinical outcomes in ADs.

METHODS

Search strategy and study selection

This meta-analysis was conducted according to a priori defined protocol that is in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses guideline.[5] The protocol of this meta-analysis has been submitted to the International Prospective Register of Systematic Reviews.[6] We searched PubMed/MEDLINE, Scopus, EMBASE, medRxiv (https://www.medrxiv.org/) from inception to 31 July 2020 to identify studies assessing the prevalence and clinical outcomes of COVID-19 in ADs.

As for inclusion criteria, we considered observational or case–controlled studies reporting the prevalence and clinical outcomes of COVID-19 in ADs. There were no restrictions regarding age, sex or duration of the study. We imposed no geographic or language restrictions. Three authors (SA, SH and AS) independently screened each of the potential studies to determine whether they were eligible for inclusion. Areas of disagreement or uncertainty were resolved by consensus among the authors. Studies were identified with the following terms: 'COVID-19', 'inflammatory bowel disease', 'psoriasis', 'rheumatic diseases', 'systemic lupus erythematosus' and 'autoimmune diseases'.

Single case reports were excluded. Given several studies used initial data from C19-GRA registry, we included a study with data of the first 600 patients submitted to C19-GRA registry[3] and excluded other studies with preliminary data.[7–9] For an analysis for the prevalence of COVID-19, studies in which all of included patients were COVID-19 were excluded. As for clinical outcomes of COVID-19, studies that included only hospitalised or deceased patients were excluded. The search strategy is described in figure 1.

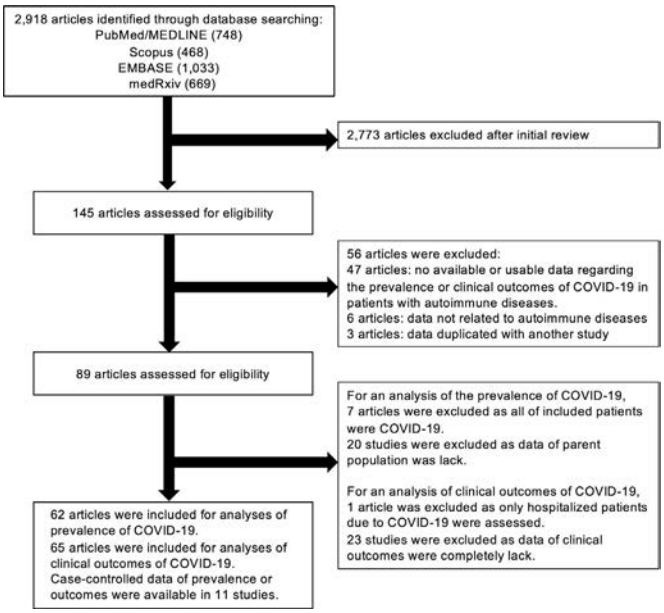

Figure 1 Flow chart of the assessment of the studies identified in the meta-analysis.

Data extraction and quality assessment

All data were independently abstracted in duplicate by two authors (SA and AS) by using a data extraction form. Data on the study characteristics, such as author name, year of publication, study design, duration, study location, sample size, diagnosis of ADs, type of medications, age and gender of patients, comorbidities including hypertension, diabetes and obesity, prevalence and clinical outcomes of COVID-19 were collected. We rated the quality of evidence according to the Grades of Recommendation, Assessment, Development and Evaluation (GRADE) approach to assess the certainty of evidence obtained from the present meta-analysis.[10]

Outcome assessment

The primary outcome was the prevalence of suspected or confirmed COVID-19 with a positive PCR test for SARS-CoV-2 in ADs. The numbers of patients with COVID-19 and confirmed cases in each of studies are shown in online supplemental table S1. To conduct subgroup analyses with each diagnosis, we classified ADs based on the digestive, musculoskeletal and integumentary systems. Diseases of the digestive system were categorised into IBD and autoimmune hepatic diseases (AHD). Rheumatic diseases (RD) included rheumatoid arthritis, systemic lupus erythematosus (SLE), psoriatic arthritis, spondyloarthritis, ankylosing spondylitis, vasculitis, polymyalgia rheumatica, Sjögren's syndrome (SjS), systemic sclerosis (SSc) and other autoimmune-mediated diseases (including Behcet's syndrome, sarcoidosis and inflammatory myopathies). Given that several studies of RD focused only on patients with SLE, SjS or SSc, these studies were categorised into 'SLE/SjS/SSc'. Diseases of the skin were categorised as 'psoriasis/autoimmune skin diseases (AISD)'. Two studies included various ADs and were classified as 'immune-mediated inflammatory disease (IMID)'.[11 12]

Secondary outcomes included the following COVID-19 clinical outcomes: (1) hospitalisation, (2) intensive care unit (ICU) admission, (3) mechanical or non-invasive ventilation and (4) death. Subgroup analyses evaluating individual comorbidities[13] and medication use prior to COVID-19 diagnosis were conducted. We divided medication use into the following three categories: (1) GCs, (2) conventional synthetic disease-modifying antirheumatic drugs (csDMARDs), (3) biologic or targeted synthetic DMARDs (b/tsDMARDs). Budesonide, which is used as an ileal release form in IBD, was not included in the GCs when data were available. csDMARDs included hydroxychloroquine, chloroquine, thiopurines, cyclophosphamide, cyclosporine, tacrolimus, leflunomide, methotrexate, mycophenolate mofetil/mycophenolic acid and sulfasalazine. b/tsDMARDs included abatacept, belimumab, CD-20, interleukin (IL)-1, IL-6, IL-12/23, IL-23, IL-17, TNF, α4β7 integrin and Janus kinase inhibitors.[3] We also divided b/tsDMARDs into monotherapy and b/tsDMARDs–csDMARDs combination therapy if studies separately presented the data. If not, we considered b/tsDMARDs as utilised as a monotherapy.

Statistical analysis

We undertook a meta-analysis of the prevalence and clinical outcomes of COVID-19 among individuals with ADs from observational or case–control studies by using a random effects model. We evaluated the presence of heterogeneity across studies by using the I^2 statistic. An I^2 value of <25% indicates

low heterogeneity, 25%–75% as moderate heterogeneity and >75% as considerable heterogeneity.[14] Heterogeneity was evaluated by using Cochran's Q-statistics with a significance level of p<0.10.[15] Begg's and Egger's tests were performed to access publication bias and funnel plots were constructed to visualise possible asymmetry when three or more studies were available.[16 17] A random effects meta-regression model was used to assess the contributions of each of potential risk factors and medication class to the prevalence and adverse clinical outcomes. If the number of available studies for each analysis was less than 10, we did not perform meta-regression analysis due to its low reliability.

Statistical analyses were performed using the Comprehensive Meta Analysis Software (V.3.0; Biostat, Englewood, NJ, USA). All statistical tests except for the Q-statistics used a two-sided p-value of 0.05 for significance.

RESULTS

Study characteristics

We identified 2918 citations through the literature search, excluded 2773 titles and abstracts after initial screening and assessed 145 studies for eligibility. A final number 89 full-text articles met all eligibility criteria. For the analysis of COVID-19 prevalence, we included 62 observational studies with a total of 319 025 patients with ADs. For clinical outcomes, we included 65 studies with 2766 patients with ADs diagnosed with COVID-19. Among these studies, we identified 11 studies with case–controlled data which compared the prevalence or clinical outcomes of COVID-19 in patients with ADs to those without ADs or the general population (figure 1). The characteristics and outcomes of the included studies are summarised in online supplemental table S1.

Prevalence of COVID-19 in autoimmune diseases

Meta-analysis of 62 observational studies including 319 025 patients with ADs from 15 countries showed that the prevalence of COVID-19 was 0.011 (95% CI: 0.005 to 0.025) (figure 2A). In the subgroup analyses, the prevalence of COVID-19 in AHD, IBD, psoriasis/AISD, RD and SLE/SjS/SSc were 0.036 (95% CI: 0.004 to 0.258), 0.003 (95% CI: 0.001 to 0.006), 0.011 (95% CI: 0.006 to 0.021), 0.009 (95% CI: 0.005 to 0.014), 0.034 (95% CI: 0.014 to 0.080), respectively, with IBD having the lowest prevalence (figure 2A). SLE/SjS/SSc showed a higher prevalence (0.034) when compared with the other disease groups, which is likely due to a higher proportion of GC use (60.3%) in the SLE/SjS/SSc subgroup (online supplemental table S1). Heterogeneity was considerable in overall (I^2=96.8%) and most subgroup analyses, which was primarily due to the difference in study sizes. The funnel plot was not asymmetric, indicating no publication bias, which was supported by Egger's test (p=0.083) but not Begg's test (p=0.002) (online supplemental figure S1). The subgroup analysis according to country showed that the prevalence range of COVID-19 was 0.002–0.012, with European countries having the highest prevalence (online supplemental figure S2).

Meta-analysis of seven case–controlled studies showed that the risk of COVID-19 in ADs was significantly higher than in control patients (OR: 2.19, 95% CI: 1.05 to 4.58, p=0.038). These studies only included individuals with psoriasis and RD, and both diseases demonstrated an elevated risk of COVID-19 as compared with controls (OR: 3.43, 95% CI: 1.68 to 7.01, p=0.001, OR: 1.60, 95% CI: 1.13 to 2.25, p=0.008, respectively) (figure 2B). There was low to considerable heterogeneity in overall (I^2=78.0%) and in each subgroup analysis (I^2=0% with psoriasis, and I^2=53.1% with RD). No publication bias was detected by Begg's and Egger's tests (Begg: p=1.00, Egger: p=0.25) (online supplemental figure S3).

Meta-regression analysis of the variables potentially associated with the risk of COVID-19 showed that studies with a higher proportion of GC use in patients with ADs had a higher prevalence of COVID-19 (regression coefficient: 0.020, 95% CI: 0.001 to 0.040, p=0.042). Meanwhile, age, proportion of males, hypertension, diabetes or therapies including csDMARDs and b/tsDMARDs did not contribute to the risk of COVID-19 (table 1).

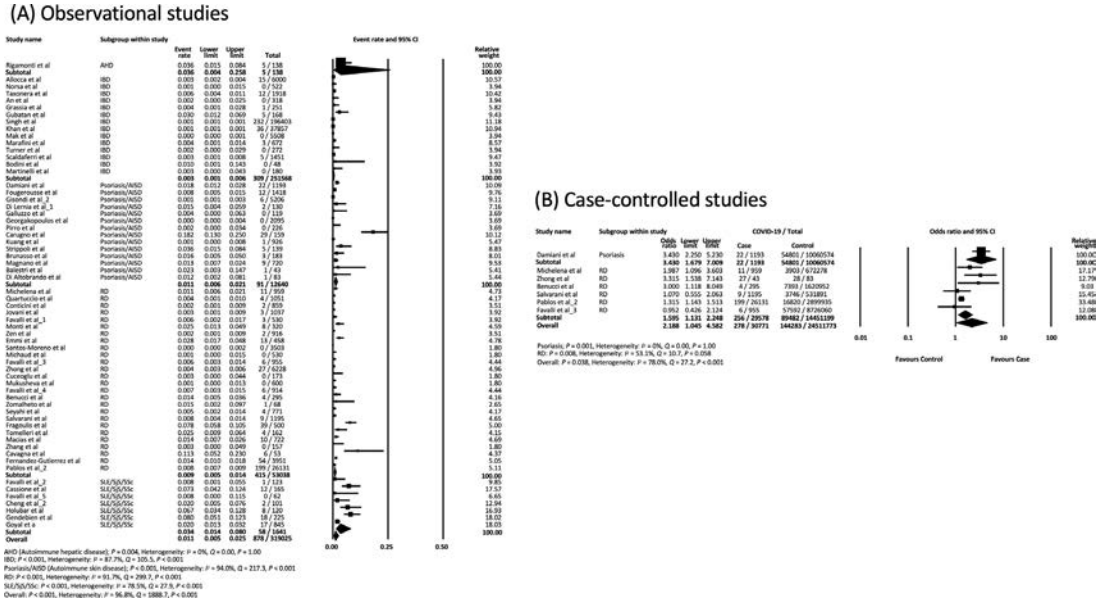

Figure 2 (A) Meta-analysis of observational studies to determine the prevalence of COVID-19 in patients with autoimmune diseases. (B) Meta-analysis of case–controlled studies to compare the prevalence of COVID-19 in autoimmune diseases with those without autoimmune diseases or general population.

Table 1 Meta-regression of the variables potentially associated with the prevalence of COVID-19

Variables	Number of studies	Coefficient	SE	Lower 95% CI	Upper 95% CI	Z value	P value
Age (mean/median)	42	0.043	0.024	−0.003	0.089	1.82	0.069
Male (%)	44	−0.018	0.011	−0.040	0.004	−1.64	0.101
HTN (%)	12	0.025	0.029	−0.031	0.081	0.88	0.377
DM (%)	13	0.060	0.099	−0.134	0.253	0.60	0.546
Obesity (%)	<10	NA	NA	NA	NA	NA	NA
Comorbidities (≥1) (%)	<10	NA	NA	NA	NA	NA	NA
Glucocorticoids (%)	26	0.020	0.010	0.001	0.040	2.04	**0.042**
csDMARDs (%)	24	0.005	0.010	−0.015	0.025	0.47	0.637
b/tsDMARDs (%, mono)	31	−0.006	0.008	−0.021	0.010	−0.72	0.469
b/tsDMARDs (%, combo)	<10	NA	NA	NA	NA	NA	NA
b/tsDMARDs (%, mono/combo)	34	−0.004	0.007	−0.019	0.010	−0.56	0.574
TNF antagonists (%, mono/combo)	30	−0.020	0.013	−0.045	0.004	−1.63	0.104
Non-TNF antagonists (% mono/combo)	29	−0.006	0.012	−0.029	0.018	−0.47	0.641

b/tsDMARDs, biologic or targeted synthetic DMARDs (abatacept, belimumab, CD-20, IL-1, IL-6, IL-12/23, IL-23, IL-17, α4β7 integrin, TNF and Janus kinase (JAK) inhibitors); combo, combination therapy with csDMARDs; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs (hydroxychloroquine, chloroquine, thiopurines, cyclophosphamide, cyclosporine, tacrolimus, leflunomide, methotrexate, mycophenolate mofetil/mycophenolic acid and sulfasalazine); DM, diabetes; HTN, hypertension; mono, monotherapy; NA, not available; TNF, tumour necrosis factor.

Clinical outcomes of COVID-19 in autoimmune diseases

Meta-analysis of 65 observational studies including 2766 patients with ADs diagnosed with COVID-19 showed that the hospitalisation rate due to COVID-19 was 0.35 (95% CI: 0.23 to 0.50) (figure 3A). Hospitalisation rates of AHD, IBD, IMID, psoriasis/AISD, RD and SLE/SjS/SSc were 0.52 (95% CI: 0.23 to 0.80), 0.29 (95% CI: 0.21 to 0.38), 0.24 (95% CI: 0.12 to 0.43), 0.26 (95% CI: 0.15 to 0.41), 0.54 (95% CI: 0.46 to 0.63) and 0.33 (95% CI: 0.20 to 0.49), respectively, with RD having the highest hospitalisation rate. Studies of RD included more elderly patients and patients with comorbidities (online supplemental table S1). Heterogeneity was considerable in overall (I^2=81.8%) and moderate to considerable in subgroup analyses (I^2=28.1%–79.9%) except for AHD (I^2=0%). Funnel plot demonstrated no asymmetry, therefore suggesting there was no small-study effects or publication bias, which was supported by Begg's and Egger's tests (online supplemental figure S4A).

The mortality due to COVID-19 in patients with ADs was 0.066 (95% CI: 0.036 to 0.12) (figure 3B). Mortality of AHD, IBD, IMID, psoriasis/AISD, RD and SLE/SjS/SSc were 0.094 (95% CI: 0.019 to 0.36), 0.045 (95% CI: 0.032 to 0.063), 0.017 (95% CI: 0.004 to 0.065), 0.097 (95% CI: 0.042 to 0.21), 0.113 (95% CI: 0.098 to 0.13) and 0.069 (95% CI: 0.032 to 0.14), respectively. Patients with RD had the highest mortality rate, which was consistent with the analysis of the hospitalisation rate. Heterogeneity was moderate in overall (I^2=26.6%) and absent in subgroup analyses (I^2=0%) except for IBD (I^2=49%). Begg's (p=0.003), but not Egger's (p=0.093), test was suggestive of

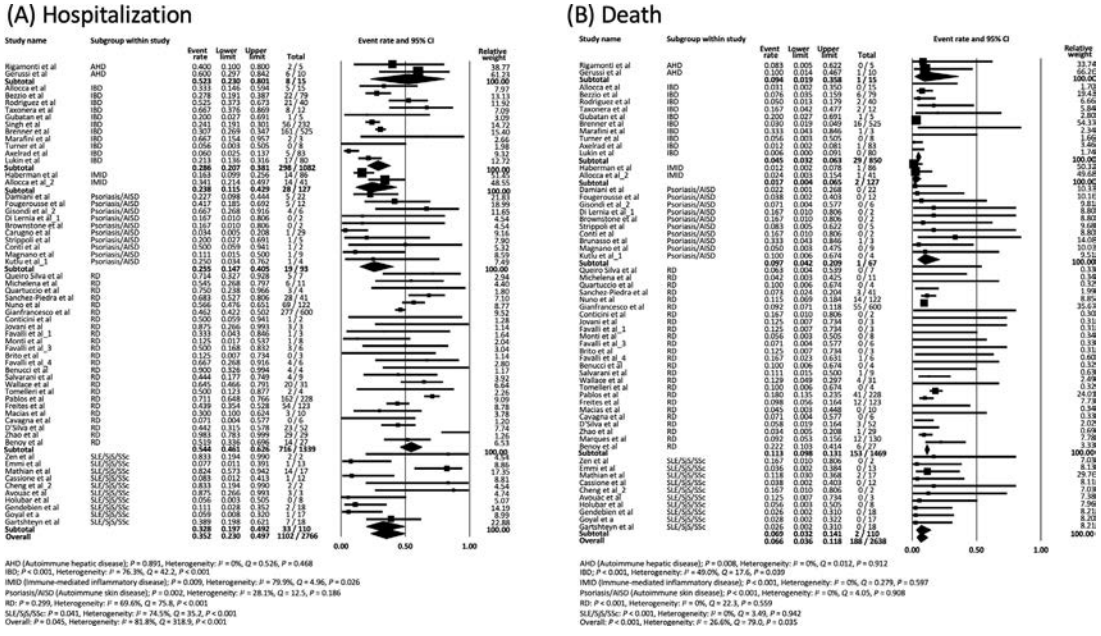

Figure 3 (A) Meta-analysis of observational studies to assess the hospitalisation rate of COVID-19 in patients with autoimmune diseases. (B) Meta-analysis of observational studies to assess the mortality rate of COVID-19 in patients with autoimmune diseases.

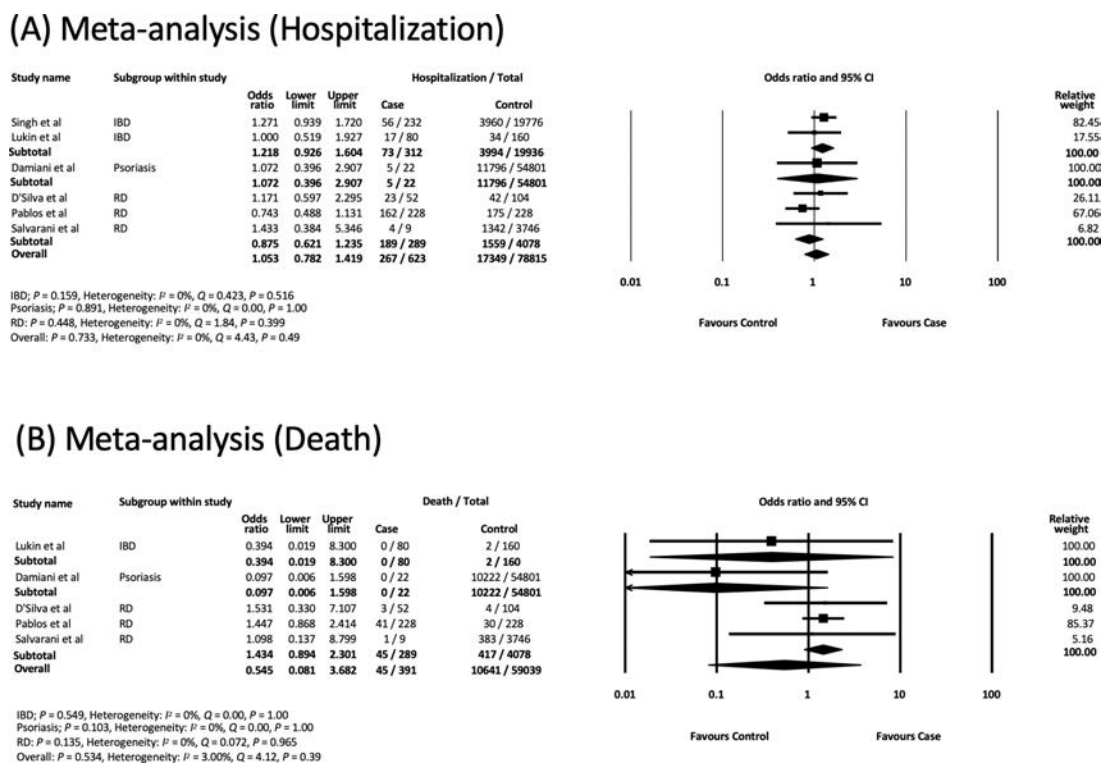

Figure 4 (A) Meta-analysis of case–controlled studies to assess the hospitalisation rate of COVID-19 in patients with autoimmune diseases. (B) Meta-analysis of case–controlled studies to assess the mortality rate of COVID-19 in patients with autoimmune diseases.

publication bias, but the funnel plot was not asymmetric (online supplemental figure S4B). Overall rates of ICU admission and mechanical or non-invasive ventilation were 0.087 (95% CI: 0.045 to 0.16) (online supplemental figure S5A) and 0.11 (95% CI: 0.063 to 0.18) (online supplemental figure S5B), respectively.

Meta-analysis of six case–controlled studies showed no differences in hospitalisations (OR: 1.05, 95% CI: 0.78 to 1.42, p=0.73) (figure 4A), death (OR: 0.55, 95% CI: 0.081 to 3.68, p=0.53) (figure 4B), ICU admission (OR: 1.22, 95% CI: 0.42 to 3.60, p=0.72) (online supplemental figure S6A) or mechanical/non-invasive ventilation (OR: 1.03, 95% CI: 0.22 to 4.81, p=0.97) when compared with the control population (online supplemental figure S6B). Each disease subgroup did not show any remarkable differences in these clinical outcomes. All analyses showed low to moderate heterogeneity (I^2=0%–73.5%) and no publication bias (online supplemental figure S6C,D and S7).

Subgroup analyses according to comorbidities showed that patients with age ≥64 years old, male gender, hypertension, diabetes, BMI ≥30 and at least one comorbidity had higher rates of hospitalisation, ICU admission, ventilation and death due to COVID-19 when compared with those without these comorbidities (online supplemental table S2). Subgroup analyses according to medical therapies showed that patients treated with GCs, csDMARDs or b/tsDMARDs–csDMARDs combination therapy had a 2–3 times higher event rate of each clinical outcome when compared with those treated with b/tsDMARDs monotherapy (online supplemental table S3). Importantly, patients with anti-TNF monotherapy use tended to have a lower rate of hospitalisation and mortality when compared with those with non-TNF-targeted monotherapy (online supplemental table S3). Analysis of hospitalisation rates showed moderate heterogeneity, but most other analyses had low heterogeneity (online supplemental tables S2 and S3).

Meta-regression analysis showed that older age (regression coefficient: 0.070, 95% CI: 0.046 to 0.095, p<0.001), a higher proportion of patients with hypertension (regression coefficient: 0.017, 95% CI: 0.002 to 0.032, p=0.024), or at least one comorbidity (regression coefficient: 0.024, 95% CI: 0.007 to 0.040, p=0.004) in patients with ADs and COVID-19 had a higher risk of hospitalisation due to COVID-19. Older age (regression coefficient: 0.068, 95% CI: 0.048 to 0.089, p<0.001), a higher proportion of hypertension (regression coefficient: 0.034, 95% CI: 0.022 to 0.045, p<0.001) and diabetes (regression coefficient: 0.038, 95% CI: 0.012 to 0.064, p=0.004) were associated with a higher mortality rate due to COVID-19 (table 2). In terms of treatments, studies with a greater proportion of patients on csDMARDs or b/tsDMARDs–csDMARDs combination therapy showing a higher rate of hospitalisation or death and conversely, studies with a higher proportion of patients on b/tsDMARDs monotherapy, particularly anti-TNF monotherapy, had a lower rate of hospitalisation and mortality due to COVID-19. A higher proportion of GC use tended to be associated with a higher rate of hospitalisation and death, although this result was not statistically significant (table 2).

Grading the quality of evidence

Based on the GRADE approach, an overall quality of evidence for this analysis was moderate as the heterogeneity was considerable (online supplemental table S4).

DISCUSSION

Our meta-analysis showed that although patients with ADs have a higher prevalence of COVID-19, their clinical outcomes were not considerably worse when compared with individuals without ADs. Meta-regression analysis demonstrated that prior GC use was associated with the increased risk of SARS-CoV-2 infection.

Table 2 Meta-regression of the variables potentially associated with clinical outcomes of COVID-19

Variables	Number of studies	Coefficient	SE	Lower 95% CI	Upper 95% CI	Z value	P value
Hospitalisation							
Age (mean/median)	50	0.070	0.013	0.046	0.095	5.61	**<0.001**
Male (%)	50	−0.012	0.008	−0.028	0.004	−1.52	0.129
HTN (%)	38	0.017	0.008	0.002	0.032	2.26	**0.024**
DM (%)	36	0.024	0.014	−0.004	0.052	1.67	0.095
Obesity (%)	24	0.012	0.009	−0.006	0.030	1.32	0.187
Comorbidities (≥1) (%)	27	0.024	0.008	0.007	0.040	2.85	**0.004**
Glucocorticoids (%)	44	0.011	0.006	−0.0003	0.022	1.91	0.056
csDMARDs (%)	40	0.014	0.005	0.005	0.023	2.94	**0.003**
b/tsDMARDs (%, mono)	49	−0.014	0.004	−0.022	−0.005	−3.13	**0.002**
b/tsDMARDs (%, combo)	26	0.016	0.007	0.001	0.030	2.11	**0.035**
b/tsDMARDs (%, mono/combo)	49	−0.005	0.004	−0.013	0.003	−1.18	0.237
TNF antagonists (%, mono)	44	−0.019	0.007	−0.032	−0.005	−2.66	**0.008**
TNF antagonists (%, combo)	22	0.028	0.017	−0.006	0.062	1.59	0.111
TNF antagonists (%, mono/combo)	46	−0.015	0.007	−0.027	−0.002	−2.24	**0.025**
Non-TNF antagonists (%, mono)	44	−0.012	0.008	−0.027	0.002	−1.64	0.102
Non-TNF antagonists (%, combo)	21	0.039	0.019	0.003	0.076	2.09	**0.036**
Non-TNF antagonists (%, mono/combo)	47	−0.002	0.007	−0.015	0.011	−0.33	0.739
Death							
Age (mean/median)	48	0.068	0.010	0.048	0.089	6.54	**<0.001**
Male (%)	48	−0.006	0.008	−0.023	0.010	−0.76	0.449
HTN (%)	37	0.034	0.006	0.022	0.045	5.84	**<0.001**
DM (%)	35	0.038	0.013	0.012	0.064	2.86	**0.004**
Obesity (%)	24	0.013	0.007	−0.001	0.027	1.87	0.062
Comorbidities (≥1) (%)	26	0.013	0.008	−0.004	0.029	1.53	0.127
Glucocorticoids (%)	43	0.011	0.006	−0.001	0.022	1.78	0.075
csDMARDs (%)	40	0.012	0.004	0.004	0.020	2.99	**0.003**
b/tsDMARDs (%, mono)	49	−0.011	0.005	−0.020	−0.002	−2.31	**0.021**
b/tsDMARDs (%, combo)	26	0.013	0.009	−0.004	0.030	1.52	0.128
b/tsDMARDs (%, mono/combo)	49	−0.010	0.004	−0.018	−0.002	−2.48	**0.013**
TNF antagonists (%, mono)	44	−0.018	0.008	−0.033	−0.003	−2.29	**0.022**
TNF antagonists (%, combo)	22	0.009	0.019	−0.029	0.047	0.47	0.642
TNF antagonists (%, mono/combo)	46	−0.017	0.007	−0.030	−0.004	−2.55	**0.011**
Non-TNF antagonists (%, mono)	45	−0.006	0.008	−0.022	0.010	−0.78	0.438
Non-TNF antagonists (%, combo)	21	0.030	0.019	−0.007	0.066	1.57	0.115
Non-TNF antagonists (%, mono/combo)	48	−0.006	0.007	−0.019	0.007	−0.87	0.387

b/tsDMARDs, biologic or targeted synthetic DMARDs (abatacept, belimumab, CD-20, IL-1, IL-6, IL-12/23, IL-23, IL-17, TNF, α4β7 integrin and Janus kinase (JAK) inhibitors); combo, combination therapy with csDMARDs; csDMARDs, conventional synthetic disease-modifying antirheumatic drugs (hydroxychloroquine, chloroquine, thiopurines, cyclophosphamide, cyclosporine, tacrolimus, leflunomide, methotrexate, mycophenolate mofetil/mycophenolic acid and sulfasalazine); DM, diabetes; HTN, hypertension; mono, monotherapy; TNF, tumour necrosis factor.

We also found that the following factors associated with severe COVID-19 outcomes: (1) GC use, (2) older age, (3) comorbidities such as hypertension or diabetes, (4) csDMARDs and (5) b/tsDMARDs–csDMARDs combination therapy. However, b/tsDMARDs monotherapy, particularly anti-TNF therapy, was associated with reduced risk of hospitalisation and mortality due to COVID-19.

Our data showed that the prevalence of COVID-19 in ADs was 0.011 (95% CI: 0.005 to 0.025) and subgroup analysis revealed the prevalence in IBD was lower than that in RD or

SLE/SjS/SSc. Previous studies have also reported differences in the prevalence of COVID-19 in patients with IBD (0.4%[18]) and RD (0.76%).[19] Our meta-regression analysis demonstrated that GC use prior to COVID-19 significantly contributed to the disease prevalence. Indeed, the mean percentage of GC use in studies of IBD (12.6%) was lower than in RD (37.8%) and SLE/SjS/SSc (60.3%), suggesting that the differential infectious risk among diseases might be attributed to GC use prior to developing COVID-19. Recent studies showed that active disease and GC use were associated with higher risk of SARS-CoV-2 infection[20] or severe COVID-19[21] in patients with ADs. Another study reported on the beneficial effect of dexamethasone in reducing mortality among those hospitalised with COVID-19.[22] Further investigations into the use of GCs in patients with ADs and the risk of COVID-19 in patients with active disease requiring GCs are needed.

In terms of the clinical outcomes, we found that the subgroup of RD had the highest rate of hospitalisation and mortality due to COVID-19. Our meta-regression analysis demonstrated that older age, comorbidities, csDMARDs and b/tsDMARDs–csDMARDs combination therapy contributed to severe COVID-19 outcomes. Supporting this result, the mean age (58.3 years), proportion of individuals with underlying comorbidities (71.8%) and b/tsDMARDs–csDMARDs combination therapy use (33.1%) was highest in the RD subgroup when compared with all other disease subgroups. Meanwhile, our data showed that b/tsDMARDs monotherapy, particularly anti-TNF therapy, might be protective against severe COVID-19. This finding was consistent with the C19-GRA registry which reported that the hospitalisation rate of RD patients treated with csDMARDs and b/tsDMARDs–csDMARDs combination therapy was 55% and 36%, respectively, whereas those with b/tsDMARDs monotherapy had a lower hospitalisation rate (29%).[3] A recent study which assessed associations between serum levels of cytokines including IL-1β, IL-6 and TNF and COVID-19 outcomes demonstrated that an increased level of TNF can be a predictor of poor outcomes in patients under 70 years.[23] These findings suggested that anti-TNF therapies might prevent severe COVID-19, however, further investigations are needed because anti-TNF drugs are associated with increased risk of serious infections in ADs.[24 25]

Limitations

Meta-analyses of observational studies regarding the prevalence of COVID-19 and hospitalisation rate had considerable heterogeneities. The cause of this heterogeneity could be potentially explained by the differences in study size, inclusion of different diseases and study location. Thus, we undertook subgroup analyses and performed meta-regression to assess the effect of each potential risk factor on the individual outcomes. Subgroup analyses regarding the hospitalisation outcome revealed low-moderate heterogeneities, which suggested that the difference among subgroups contributed to the initial heterogeneity. Second, although we assessed the effect of b/tsDMARDs monotherapy and b/tsDMARDs–csDMARDs combination therapy on the outcomes separately, not all studies presented data in these two groups. In a situation where csDMARDs were stopped for fear of COVID-19 in patients on combination therapies, washout periods of csDMARDs could not be considered. Third, the sensitivity of RT-PCR for SARS-CoV-2 from nasopharyngeal swab is roughly 70%.[26 27] Meanwhile, although there was no guideline regarding COVID-19 testing in patients starting immunosuppressants,[28] patients with ADs might have been tested earlier and more frequently compared with the general population due to their concern of infectious risk of SARS-CoV-2. Hence, these issues might affect the result of the prevalence data in our meta-analysis.

CONCLUSION

This study is the first comprehensive meta-analysis which determined the prevalence and clinical outcomes of COVID-19 in ADs. Our study suggests that GC use increases the risk of SARS-CoV2 infection and might contribute to the higher prevalence of COVID-19 in ADs. Although GCs, csDMARDs and b/tsDMARDs–csDMARDs combination therapy contributed to disease severity in COVID-19, b/tsDMARDs monotherapy, especially anti-TNF monotherapy, was associated with reduced risk of severe disease. Our meta-analysis provides evidence that b/tsDMARDs monotherapy can be safely used during the pandemic.

Contributors Literature search: SA, SH and AS. Figures creation: SA. Study design: SA and AS. Data collection: SA and AS. Data analysis: SA. Data interpretation: SA and AS. Drafting of manuscript: SA, DM and AS. Full responsibility for the integrity of the work as a whole, from inception to published article: AS.

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CLINICAL SCIENCE

High frequency of variants in genes associated with primary immunodeficiencies in patients with rheumatic diseases with secondary hypogammaglobulinaemia

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ABSTRACT

Objectives Treatment of rheumatic diseases requires immunomodulatory agents which can compromise antibody production. However, even in case of agents directly targeting B cells, a minority of patients develop hypogammaglobulinaemia, suggesting a genetic predisposition, which has not been investigated so far. The phenotypic overlap between primary immunodeficiency disorders (PIDs) and rheumatic diseases suggests a shared genetic basis, especially in case of patients with rheumatic diseases with hypogammaglobulinaemia.

Methods 1008 patients with rheumatic diseases visiting the outpatient clinics of the Hannover University Hospital were screened for hypogammaglobulinaemia. Those with persistent hypogammaglobulinaemia and an equal number of patients without it underwent targeted next-generation sequencing, searching for variations in genes linked with hypogammaglobulinaemia in the context of PIDs.

Results We identified 33 predicted pathogenic variants in 30/64 (46.9%) patients with persistent secondary hypogammaglobulinaemia. All 33 variants were monoallelic and 10 of them in 10/64 (15.6%) patients were found in genes associated with autosomal dominant PIDs. 2/64 (3.1%) patients harboured variants which were previously reported to cause PIDs. In the group without hypogammaglobulinaemia we identified seven monoallelic variants in 7/64 (10.9%), including a variant in a gene associated with an autosomal dominant PID.

Conclusions Approximately half of patients with persistent secondary hypogammaglobulinaemia harboured at least a variant in a PID gene. Despite the fact that previous immunomodulatory treatment is an exclusion criterion in the diagnosis of PIDs, we identified genetic variants that can account for PID in patients with clear rheumatic phenotypes who developed hypogammaglobulinaemia after the introduction of immunomodulatory treatment. Our data suggest the common genetic causes of primary and secondary hypogammaglobulinaemia.

Key messages

What is already known about this subject?

- Immunomodulatory agents for the treatment of rheumatic diseases induce hypogammaglobulinaemia in a minority of treated, suggesting a likely genetic predisposition.

What does this study add?

- This is the first study evaluating the genetic background of secondary hypogammaglobulinaemia.
- Despite the fact that primary immunodeficiency is most often conceived as susceptibility to infections, identification of variants in primary immunodeficiency disorder (PID) genes in a cohort of patients with rheumatic diseases, most of whom had no history of severe or recurrent infections, suggests that rheumatic disease may be the dominant phenotypic aspect of PID.

How might this impact on clinical practice or future developments?

- This study questions the classification of hypogammaglobulinaemia into primary and secondary, especially in patients with rheumatic diseases, alerting treating physicians for considering PID in patients with rheumatic diseases with hypogammaglobulinaemia.
- The latter may lead to re-evaluation of treatment of hypogammaglobulinaemia in patients with rheumatic diseases and consideration of precision-directed therapies, which are employed to treat autoimmune manifestations of monogenic PIDs.

INTRODUCTION

To diagnose primary hypogammaglobulinaemia, and especially common variable immunodeficiency (CVID), secondary causes of hypogammaglobulinaemia need to be excluded.[1] These include protein-losing conditions, haematological malignancies and certain medications, such as anticonvulsive and immunomodulatory agents.[2] Some of the latter target B cells directly, whereas others have a broader impact on the immune system, which nonetheless affects B cells and antibody production. Treatment with immunomodulatory agents accounts for secondary immunodeficiency and hypogammaglobulinaemia in patients with rheumatic diseases. In addition to

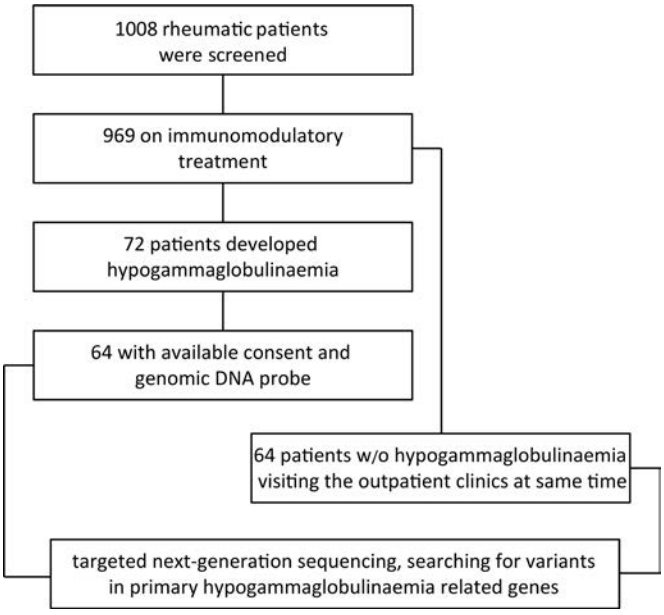

Figure 1 Study design.

recurrent infections, the phenotype of CVID includes autoimmunity, also in the form of a rheumatic disease, such as rheumatoid arthritis (RA), systemic lupus erythematosus and Sjögren's syndrome (SjS).[3–5] As autoimmune manifestations in the context of CVID usually necessitate treatment with immunosuppressive agents, the discrimination between primary and secondary hypogammaglobulinaemia can become challenging, especially when immunoglobulin (Ig) levels before treatment introduction have not been controlled.[6 7]

Expanding evidence, and especially the discovery and phenotypic characterisation of monogenic primary immunodeficiency disorder (PID), suggests that primary immunodeficiency and autoimmunity share common pathogenic pathways.[8] The advent of next-generation sequencing (NGS) aided the identification of PID-causing genetic defects. The spectrum of genetic variants underlying PID is expanding and currently, defects in more than 400 genes have been linked to PID.[9] The most common symptomatic adult-onset PID, CVID,[10] is largely a polygenic disease, though considering the more recent reports, the proportion of monogenic forms appears to expand, exceeding 20% of cases.[11 12] However, incomplete penetrance and variable expressivity of genetic variants reported as PID-causing, question the exact division between monogenic and polygenic forms and suggest the influence of additional genetic modifiers, epigenetic regulation and/or environmental factors.[13] The phenotypes of relatively common monogenic PIDs, such as due to *NFKB1* loss-of-function variants, *STAT3* gain-of-function (GOF) variants, CTLA4 insufficiency, LRBA deficiency and activated PI3K delta syndrome include features of rheumatic diseases such as arthritis, enthesiopathy and vasculitis.[14–20]

As discussed above, immunomodulatory regimens for rheumatic diseases can lead to hypogammaglobulinaemia.[2] However, even after introduction of rituximab, which directly targets B cells and reduces plasma cell precursors, only a minority of treated patients develop hypogammaglobulinaemia,[21 22] suggesting a genetic vulnerability or even a genetic cause of secondary hypogammaglobulinaemia. Despite recent advances in understanding the genetic basis of primary immunodeficiency, evidence on the genetics of secondary immunodeficiency is scarce. The phenotypic overlap between adult-onset primary antibody deficiencies and secondary hypogammaglobulinaemia in the context of rheumatic diseases suggests shared genetic aetiologies. Hence, we employed a panel NGS-approach searching for primary hypogammaglobinaemia-associated variants in a cohort of patients with rheumatic diseases and persistent hypogammaglobulinaemia, developing after introduction of treatment with immunomodulatory agents.

PATIENTS AND METHODS

Study cohort

This single-centre study included all patients with rheumatic disease visiting our rheumatology outpatient clinic between November 2018 and March 2019 (N=1008, figure 1). Visits of patients were scheduled approximately every 3–6 months and serum Ig levels were measured at every visit. The normal range of serum IgG values for adults lies between 7 g/L and 16 g/L. Secondary hypogammaglobulinaemia has been defined as persistently reduced IgG (<7 g/L) at time of the study and in follow-up visits during at least the year before the study, developing after the introduction of immunomodulatory regimens including prednisolone, diverse synthetic and/or biological disease-modifying antirheumatic drugs (DMARD), known to cause hypogammaglobulinaemia,[2] in patients who previously had normal or high IgG levels.

Targeted NGS

Blood samples were collected in the outpatient clinics of the department of Rheumatology and Immunology of Hannover University Hospital. Genomic DNA (gDNA) was isolated from peripheral whole blood using QIAamp DNA Blood Midi Kit, according to the manufacturer's protocol (Qiagen). Targeted NGS was performed with a gene panel (Agilent Technologies), comprising known and candidate genes associated with primary antibody deficiencies (online supplemental table 1), using a MiSeq desktop sequencer (Illumina) as described previously.[23] The detected genetic alterations were validated by Sanger sequencing using a service from Eurofins. We analysed the original NGS data with Agilent SureCall software (Agilent Technologies). Genome Reference Consortium Human Build 37 was employed as reference genome. Allele frequency, variant annotation and potential functional effect were considered for variant selection. Variants with an allele frequency in the general population higher that 1% according to the Genome Aggregation Database were not considered. The functional effect of nonsense, frameshift, splice site affecting or start/stop codon introducing variants was evaluated with the following bioinformatics tools: Combined Annotation-Dependent Depletion (CADD) Score, Mutation Taster, Protein Variation Effect Analyser and Polymorphism Phenotyping v2.

Statistical analysis

For statistical calculation we used GraphPad prism 8 (GraphPad, La Jolla, USA). Descriptive statistics are reported as median and IQR in case of continuous variables and as counts and percentages for dichotomous variables. Categorical variables were compared by the X^2 test. Non-categorical variables were compared with the Mann-Whitney U test. To correct for multiple testing, p values were adjusted for Benjamini-Hochberg false discovery rate (FDR). P values were considered significant if they were lower than a threshold selected to control an FDR of 5%.

Table 1 Patients' characteristics and immunomodulatory regimes

Characteristic	All (N=1008)	Hypogammaglobulinaemia (N=64)	W/o hypogammaglobulinaemia (N=64)
Median age (IQR)—years	56 (47–66)	58 (52–68)	53 (42–60)
Median age at diagnosis of rheumatic disease (IQR)—years	42 (31–53)	46 (32–52)	38 (30–48)
Male sex—no (%)	229 (22.7)	14 (21.9)	13 (20.3)
Diagnosis			
RA—no (%)	274 (27.2)	18 (28.1)	16 (25)
SpA—no (%)	250 (24.8)	14 (21.9)	16 (25)
SLE—no (%)	216 (21.4)	15 (23.4)	16 (25)
SjS—no (%)	203 (20.1)	8 (12.5)	8 (12.5)
Other—no (%)	65 (6.4)	9 (14)	8 (12.5)
Immunomodulatory agents			
RA			
MTX—no (%)	153/274 (55.8)	15/18 (83.3)	6/16 (37.5)
Other csDMARD—no (%)	71/274 (25.9)	4/18 (22.2)	2/16 (12.5)
RTX—no (%)	64/274 (23.4)	2/18 (11.1)	2/16 (12.5)
TNFi—no (%)	59/274 (21.5)	3/18 (16.7)	7/16 (43.8)
Other bDMARD—no (%)	28/274 (10.2)	2/18 (11.1)	1/16 (6.3)
SpA			
MTX—no (%)	109/250 (43.6)	8/14 (57.1)	6/16 (37.5)
SSZ—no (%)	40/250 (16)	3/14 (21.4)	3/16 (18.8)
Other csDMARD—no (%)	27/250 (10.8)	1/14 (7.1)	2/16 (12.5)
TNFi—no (%)	106/250 (42.4)	4/14 (28.6)	8/16 (50)
Secukinumab—no (%)	13/250 (5.2)	1/14 (7.1)	0/16 (0)
SLE			
HCQ—no (%)	149/216 (69)	6/15 (40)	9/16 (56.3)
MMF—no (%)	64/216 (29.6)	8/15 (53.3)	8/16 (50)
AZA—no (%)	57/216 (26.4)	3/15 (20)	3/16 (18.8)
Other csDMARD—no (%)	28/216 (13)	4/15 (26.7)	1/16 (6.3)
bDMARD*—no (%)	18/216 (8.3)	1/15 (6.7)	2/16 (12.5)
SjS			
HCQ—no (%)	94/203 (46.3)	3/8 (37.5)	3/8 (37.5)
MTX—no (%)	41/203 (20.2)	3/8 (37.5)	1/8 (12.5)
AZA—no (%)	41/203 (20.2)	1/8 (12.5)	1/8 (12.5)
Other csDMARD—no (%)	34/203 (16.7)	2/8 (25)	3/8 (37.5)
RTX—no (%)	8/203 (3.9)	1/8 (12.5)	0/8 (0)
Other			
csDMARD—no (%)	48/65 (73.8)	7/9 (77.8)	7/8 (87.5)
bDMARD—no (%)	8/65 (12.3)	2/9 (22.2)	2/8 (25)

*RTX or belimumab.
.AZA, azathioprine; bDMARD, biological DMARD; csDMARD, conventional synthetic DMARD; DMARD, disease-modifying antirheumatic drug; HCQ, hydroxychloroquine; MMF, mycophenolate mofetil; MTX, methotrexate; no, number; RA, rheumatoid arthritis; RTX, rituximab; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; SSZ, sulfasalazine; TNFi, tumour necrosis factor inhibitor.

RESULTS

Characteristics of patients with rheumatic diseases with hypogammaglobulinaemia

Out of 72 identified patients with rheumatic diseases with persistent hypogammaglobulinaemia, secondary to treatment with immunomodulatory agents, 64 were enrolled in the study (figure 1). The rest (ie, 8/72 patients) were excluded, due to lack of consent or gDNA. The 64 patients with secondary hypogammaglobulinaemia and an equal number of randomly selected patients with rheumatic diseases with normal serum IgG levels underwent targeted NGS. The characteristics of all 128, who underwent NGS as well as those of patients from the original cohort, are summarised in table 1. Most patients (52/64, 81.3%) had an isolated reduction of IgG. In addition to reduced IgG, 6/64 (9.4%) patients had low IgA, 6/64 (9.4%) had low IgM and one patient displayed panhypogammaglobulinaemia. Most patients were diagnosed with hypogammaglobulinaemia while receiving conventional synthetic DMARDs (online supplemental table 2). Retrospective evaluation of medical records of studied patients with hypogammaglobulinaemia revealed recurrent or severe infections in 15/64 (23.4%) of them. These were mostly recurrent upper respiratory tract infections. One patient had recurrent skin abscesses and 2/64 (3.1%) had a history of recurrent herpes reactivations necessitating antiviral prophylaxis. Further, 2/64 (3.1%) patients had a history of candida esophagitis but none of them had recurrent candida or other fungal infections and none was receiving prophylactic antifungals. Six of sixty-four (9.4%) patients were receiving prophylactic antibiotics and 8/64 (12.5%) of them were on Ig replacement therapy.

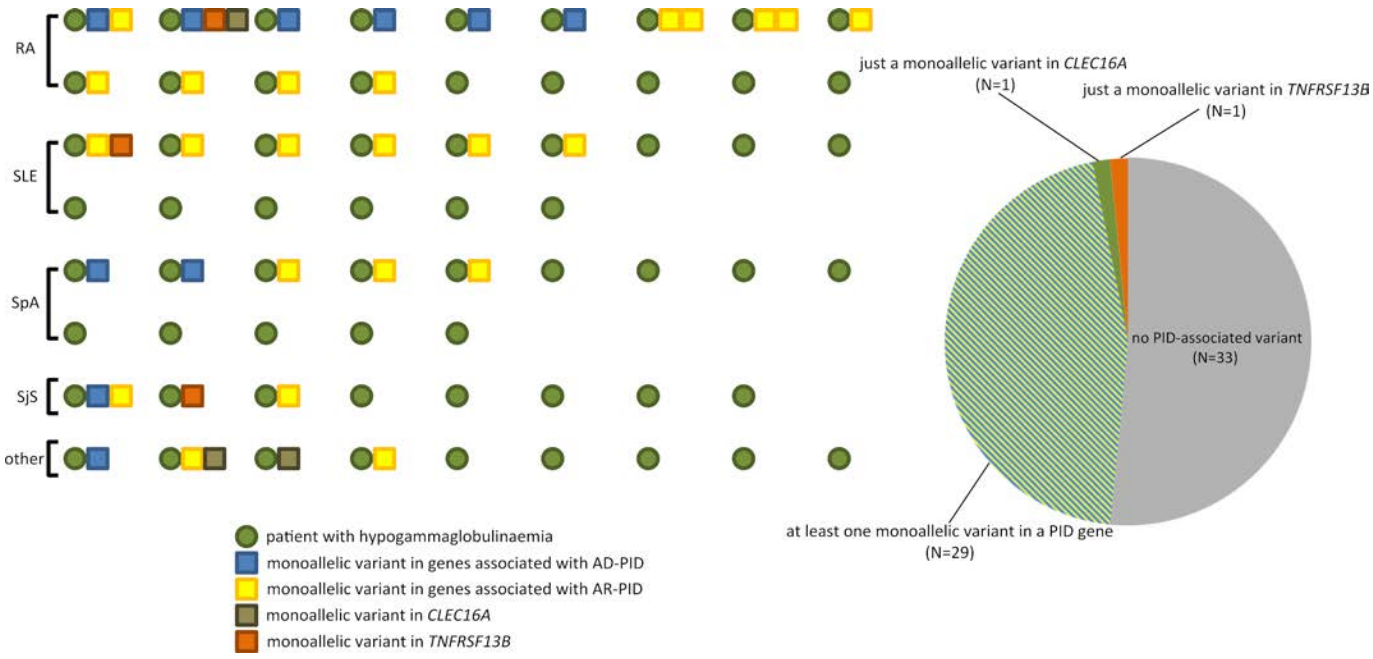

Figure 2 Summary of genetic findings in n=64 patients with rheumatic diseases with secondary hypogammaglobulinaemia; each green cycle matches a studied patient. Boxes indicate genetic findings for each patient classified as monoallelic variants in genes associated with autosomal dominant (AD) primary immunodeficiency disorders (PID) (marked with blue colour) or autosomal recessive (AR) primary PID (marked with yellow colour). Monoallelic variants in *TNFRSF13B* are indicated with orange boxes and those in *CLEC16A* with green ones. Diagnosis of rheumatic disease is indicated in the left side of the graph. RA, rheumatoid arthritis; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SpA, spondyloarthritis.

Variants in autosomal dominant PID-causing genes

The employed targeted NGS approach included a panel of genes linked to predominantly antibody deficiencies (online supplemental table 1). Considering allele frequency as well as the CADD and Mutation Significance Cut-off scores of each identified variant, as described above, we ended up with 35 rare and likely deleterious variations in 31/64 patients (48.4%), all of which were monoallelic (figure 2).

Ten patients had a variant in a gene linked to autosomal dominant (AD) PID (figure 3). In particular, five patients harboured variants in *NFKB1*, the gene encoding the p105 subunit of the transcription factor NF-κB1. Three of four identified *NFKB1*

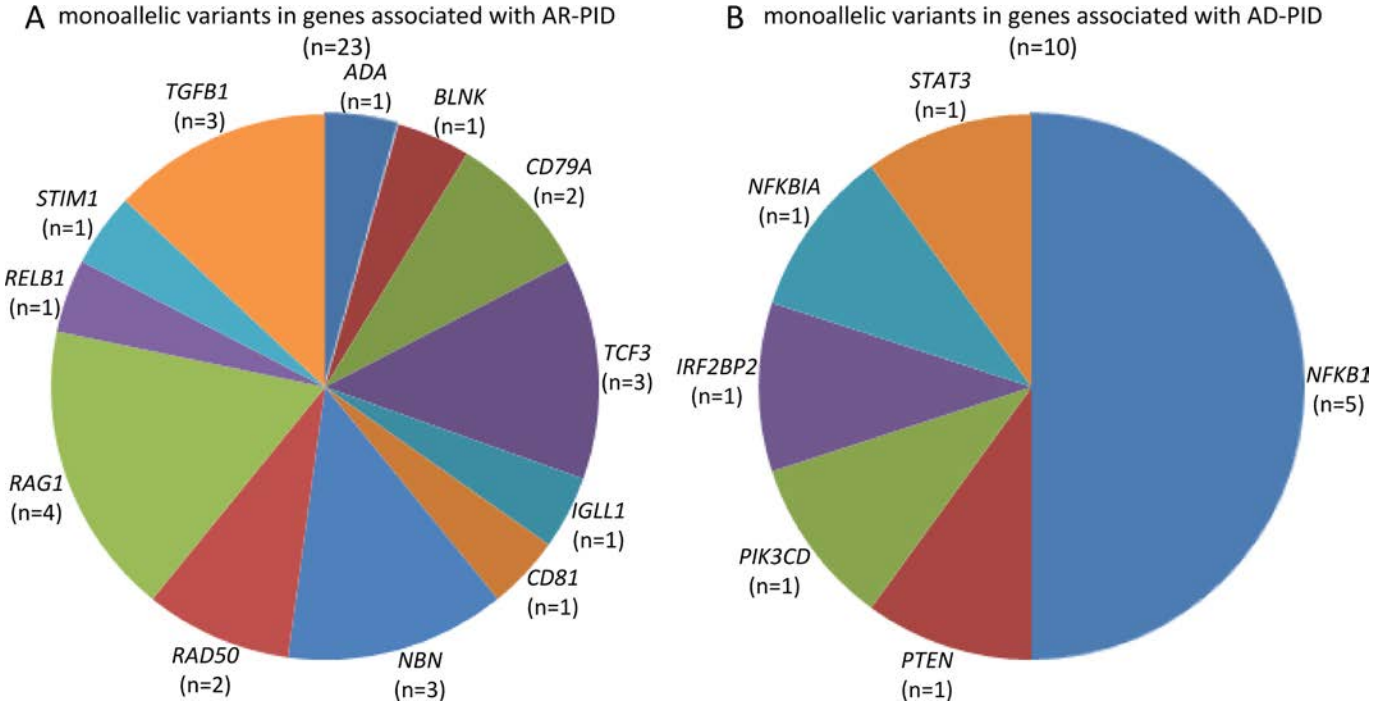

Figure 3 Summary of genes, whose variations were detected in a cohort of c patients with rheumatic diseases with hypogammaglobulinaemia developing after introduction of immunomodulatory regimens, (A) genes linked to AR-PID and (B) genes linked to AD-PID. AD-PID, autosomal dominant primary immunodeficiency disorder; AR-PID, autosomal recessive PID.

Table 2 Monoallelic variants in genes associated with autosomal dominant PIDs, identified in patients with rheumatic diseases with persistent hypogammaglobulinaemia and those without

Pat. ID	Gene	Ref. seq. transcript	Coding change	Protein change	gnomAD allele freq.	RS-ID	CADD score	MSC–CADD score	PolyPhen2 score	PolyPhen2 pred.	SIFT score	SIFT pred.
A: Patients with persistent hypogammaglobulinaemia												
4	*NFKB1*	NM_001165412.ex.15	c.1601G>A	p.R534H	0.0004455	RS150281816	29.000	3.313	0.960	Probably damaging	0.000	D
14	*PIK3CD*	NM_005026.ex.22	c.2799C>A	p.H909Q	–	–	28.500	23.800	0.995	Probably damaging	0.000	D
25	*PTEN*	NM_001304718.ex.8	c.1021T>G	p.F341V	–	–	27.900	2.182	0.998	Probably damaging	NA	NA
29	*NFKB1*	NM_001165412.ex.24	c.2793G>C	p.E930D	–	–	23.800	3.313	0.990	Probably damaging	0.000	D
33	*NFKB1*	NM_003998.ex.10	c.865G>T	p.E289*	–	–	41.000	3.313	NA	NA	0.000	D
42	*NFKBIA*	NM_020529.ex.5	c.682C>T	Q228*	–	–	39.000	24.800	NA	NA	0.040	D
57	*NFKB1*	NM_003998.ex.16	c.1736G>A	p.R579K	0.001	RS4648086	18.740	3.313	0.911	Probably damaging	0.830	T
59	*STAT3*	NM_003151.ex.22	c.2144C>T	p.T715M	–	–	21.400	15.29	0.719	Possibly damaging	0.100	T
60	*IRF2BP2*	NM_181524.ex.5	c.1282C>A	p.L428M	–	–	24.000	3.313	0.999	Probably damaging	0.090	T
62	*NFKB1*	NM_003998.ex.10	c.865G>T	p.E289*	–	–	41.000	3.313	NA	NA	0.000	D
B: Patients without hypogammaglobulinaemia												
121	*IRF2BP2*	NM_001077397.ex.1	c.958C>A	p.P320T	–	–	23.600	3.313	0.961	Probably damaging	0.010	D

*substitution - nonsense.
*, substitution-nonsense; CADD, Combined Annotation Dependent Depletion; D, damaging; freq., frequency; gnomAD, Genome Aggregation Database; Pat. ID, patient identification number; MSC, Mutation Significance Cut-off; NA, not applicable; PIDs, primary immunodeficiency disorders; PolyPhen2, Polymorphism Phenotyping v2; pred., prediction; ref. seq., reference sequence; RS-ID, reference-single nucleotide polymorphism identity number; SIFT, Sorting Intolerance From Tolerance; T, tolerated.

variants (table 2) were missense variants and the c.865G>T variant, which was identified in two unrelated patients, is predicted to be a stop codon-gain variant. Among the identified *NFKB1* variants, the c.1601G>A (p.R534H) substitution has been previously reported to cause monogenic CVID.[13] Further, we identified a rare missense variant in *PIK3CD* in patient with RA, a *PTEN* missense variant in a patient with spondyloarthritis (SpA), one nonsense variant in *NFKBIA* in a patient with RA and a missense variant in *STAT3* in a patient with late-onset RA. The latter variant has been previously reported to be a GOF, resulting in PID.[15] Clinical and immunological characteristics of patients harbouring variants in AD PID-causing genes are summarised in online supplemental table 3 and online supplemental table 4, respectively.

Half of patients with secondary hypogammaglobulinaemia had a predominantly articular rheumatic disease (32/64), diagnosed as either RA (18/64) or SpA (14/64). Articular disease was more common among patients with variants in AD PID-causing genes (8/10 vs 2/54, p=0.0389, q=0.0648). The prevalence of infectious manifestations was similar in patients with variants in AD PID-causing genes and those without (3/10 vs 13/54, p=0.691, q=0.838). With respect to the immunological parameters of studied patients with hypogammaglobulinaemia, concomitant reduction of IgA and/or IgM appeared with same frequency among patients with hypogammaglobulinaemia with or without variants in AD-PID genes (2/10 vs 11/54, p=1, q=1). Further, retrospective evaluation of available peripheral lymphocyte subset counts revealed that patients with a variant in AD-PID genes displayed significantly lower proportions of class-switched (online supplemental text).

Monoallelic variants in *TNFRSF13B*, ***CLEC16A*** and autosomal recessive PID-causing genes

In addition, we detected 23 variants in genes associated with autosomal recessive (AR) PID in 21/64 patients with secondary hypogammaglobulinaemia, which only in the context of homozygosity or compound heterozygosity would cause a PID. These included variants in genes linked to AR-PIDs, including agammaglobulinaemia (*CD79A*, *TCF3*, *BLNK* and *IGLL1*) and severe combined immunodeficiency genes (*ADA*, *RAG1*, *STIM1*) (figure 3, table 3). Variants in *TNFRSF13B*, the gene encoding the transmembrane activator and calcium-modulating cyclophilin ligand interactor, have been reported in a considerable proportion of patients with CVID and are rather predisposing but do not solely cause hypogammaglobulinaemia.[24 25] Among the 64 tested patients, 3 (4.7%) had a rare monoallelic variant in *TNFRSF13B* (table 3). Considering the previously reported association of *CLEC16A* single-nucleotide polymorphism with CVID and the reported B cell dysfunction in Clec16 knockdown mice,[26] which both suggest the pathogenic relevance of *CLEC16A* in PID, we tested our patients for *CLEC16A* variants and identified three different monoallelic missense variants in 3/64 patients with secondary hypogammaglobulinaemia (table 3).

Targeted NGS in patients with rheumatic diseases without hypogammaglobulinaemia

To evaluate the association of the above described genetic findings with secondary hypogammaglobulinaemia in the context of rheumatic disease rather than with the rheumatic disease itself, in parallel to the 64 patients with hypogammaglobulinaemia we tested an equal number of patients with rheumatic diseases without hypogammaglobulinaemia. Seeking for variants in the same PID-related genes, we identified 6 AR-PID variants (table 3) and a single AD-PID variant (table 2) in 7/64 (10.9%) patients with rheumatic diseases without hypogammaglobulinaemia. Both AR-PID and AD-PID variants were more commonly detected among patients with rheumatic diseases with hypogammaglobulinaemia than those without (patients with at least one AR-PID variant: 21/64 vs 6/64, p=0.0012, q=0.007; patients with at least one AD-PID variant: 10/64 vs 1/64, p=0.0045, q=0.015). The AD-PID variant was found in *IRF2BP2* (c.958C>A, p.P320T), in a woman with SjS and recurrent herpes infections (table 2, online supplemental file 1 and online supplemental table 4). Considering the fact that immunodeficiency due to heterozygous *IRF2BP2* mutations does not necessarily cause hypogammaglobulinaemia,[27] the identified variant may account for recurrent herpes infections in this patient.

Retrospective evaluation of medical records of the 128 sequenced patients revealed that 47/64 with persistent hypogammaglobulinaemia and 27/64 without hypogammaglobulinaemia

Table 3 Monoallelic variants in genes associated with autosomal recessive PIDs as well as in *CLEC16A* and *TNFRSF13B*, identified in patients with rheumatic diseases with persistent hypogammaglobulinaemia and those without

Pat. ID	Age	Sex	Rheumatic disease	Gene	Ref. seq. transcript	Coding change	Protein change	gnomAD allele freq.	RS-ID	CADD score	MSC–CADD score	PolyPhen2 score	PolyPhen2 pred.	SIFT score	SIFT pred.
A: Patients with persistent hypogammaglobulinaemia															
1	57	F	SpA	*RAG1*	NM_000448.ex.2	c.1346G>A	p.R449K	–	RS4151031	18.720	1.118	0.900	Possibly damaging	0.190	T
2	58	F	SpA	*CD79A*	NM_001783.ex.2	c.371G>A	p.R124H	0.00091180	–	33.000	22.900	0.984	Probably damaging	NA	NA
3	63	F	SpA	*TCF3*	NM_001136139.ex.9	c.709G>T	p.G237W	–	–	32.000	3.313	0.986	Probably damaging	0.000	D
6	51	F	RA	*RAD50*	NM_005732.ex.23	c.3596G>T	p.G1199V	–	–	32.000	0.001	1.000	Possibly damaging	0.000	D
				NBN	NM_001024688.ex.4	c.37G>A	p.D13N	0.001858	RS61753720	26.300	0.003	1.000	Probably damaging	0.030	D
10	80	F	GPA	*CLEC16A*	NM_001243403.ex.21	c.2572C>A	p.Q840K	–	–	22.100	3.313	0.318	Benign	0.000	D
14	53	F	RA	*TCF3*	NM_001136139.ex.5	c.302A>G	p.K101R	–	RS41275842	18.400	3.313	0.052	Benign	0.040	D
16	59	M	PAN	*CLEC16A*	NM_015226.ex.22	c.2675G>A	p.S892N	–	RS72650687	19.680	3.313	0.001	Benign	0.000	D
				RAG1	NM_000448.ex.2	c.1537C>A	p.L513I	–	–	25.600	1.118	0.991	Probably damaging	0.000	D
19	68	F	RA	*ADA*	NM_000022.ex.8	c.700G>T	p.E234*	–	–	40.000	11.150	NA	NA	0.010	D
				NBN	NM_001024688.ex.10	c.1077C>A	p.N277K	–	–	19.570	0.003	0.576	Possibly damaging	0.010	D
20	66	F	SjS	*TNFRSF13B*	NM_012452.ex.4	c.542C>A	p.A181E	0.005360	rs72553883	22.800	0.027	0.395	Benign	0.050	D
21	64	M	SLE	*TGFB1*	NM_000660.ex.5	c.832C>T	p.R278*	–	–	36.000	6.330	NA	NA	1.000	T
22	53	F	RA	*STIM1*	NM_003156.ex.12	c.1928G>A	p.R643H	0.00097200	RS140080199	29.900	14.110	0.980	Probably damaging	0.000	D
26	69	F	RA	*NBN*	NM_001024688.ex.12	c.1840_1840delA	I614Yfs*43	–	–	23.700	0.003	NA	NA	NA	NA
28	52	M	RA	*RAG1*	NM_000448.ex.2	c.2824A>C	p.T942P	–	–	23.200	1.118	0.997	Probably damaging	NA	NA
29	53	F	RA	*CLEC16A*	NM_001243403.ex.21	c.2430C>A	p.D810E	0.00002000	RS200908373	23.900	3.313	0.292	Benign	0.000	D
				TNFRSF13B	NM_012452.ex.4	c.542C>A	p.A181E	0.005360	RS72553883	22.800	0.027	0.395	Benign	0.050	D
30	69	F	RA	*BLNK*	NM_001114094.ex.2	c.88G>A	p.G30R	0.0007476	RS143109144	32.000	3.313	0.973	Probably damaging	0.200	T
31	58	F	SLE	*RAD50*	NM_005732.ex.16	c.2548C>T	p.R850C	0.00028850	RS181961360	35.000	0.001	0.724	Possibly damaging	0.050	D
32	62	F	SLE	*RELB*	NM_006509.ex.1	c.56C>G	p.P19R	–	–	24.500	3.313	0.993	Probably damaging	0.000	D
				TNFRSF13B	NM_012452.ex.2	c.198C>A	p.C66*	0.000008258	RS144718007	36.000	0.027	NA	NA	1.000	T
41	72	F	SLE	*IGLL1*	NM_020070.ex.3	c.421_421delT	Y141Ifs*5	0.0003378	–	24.200	3.313	NA	NA	NA	NA
43	47	F	SpA	*TCF3*	NM_001136139.ex.5	c.302A>G	p.K101R	0.00964	RS41275842	18.400	3.313	0.052	Benign	0.040	D
51	61	F	SjS	*CD81*	–	IVS5-1	–	–	–	24.400	12.063	NA	NA	NA	NA
56	28	M	SLE	*TGFB1*	NM_000660.ex.3	c.593T>C	p.F198S	–	–	32.000	6.330	1.000	Probably damaging	0.000	D
58	40	F	RA	*RAG1*	NM_000448.ex.2	c.1346G>A	p.R449K	0.0036	–	18.720	1.118	0.900	Possibly damaging	0.190	T
62	60	F	SjS	*CD79A*	NM_021601.ex.5	c.499G>T	p.G167C	–	–	22.900	22.900	NA	Possibly damaging	0.239	NA
63	74	M	SLE	*TGFB1*	NM_000660.ex.7	c.1147A>T	p.I383F	–	–	28.200	6.330	0.000	Possibly damaging	0.027	D
B: Patients without hypogammaglobulinaemia															
65	73	F	SjS	*RLTPR*	NM_001013838.ex.20	c.1783_1783delA	p.K595Sfs*11	0.000008381	–	34.000	3.313	NA	NA	NA	NA
72	48	F	SLE	*RLTPR*	NM_001317026.ex.36	c.3914G>T	p.G1305V	–	–	23.500	3.313	0.767	Possibly damaging	0.030	D
84	52	F	SLE	*NBN*	NM_152309.ex.2	c.84G>T	p.R28S	–	–	29.000	0.003	1.000	Probably damaging	0.000	D
85	33	F	SLE	*NBN*	NM_002485.ex.2	c.79G>T	p.G27*	–	–	38.000	0.003	NA	NA	0.000	D
89	51	F	SLE	*CLEC16A*	NM_001243403.ex.21	c.2524C>T	p.R842C	0.0002983	RS199513229	35.000	3.313	0.997	Probably damaging	0.000	D
120	52	F	SjS	*CR2*	NM_001877.ex.14	c.2659G>A	p.V887M	0.0003997	RS147451324	25.600	3.832	0.951	Probably damaging	0.100	T

*substitution - nonsense.
.*, substitution-nonsense; AR, autosomal recessive; CADD, Combined Annotation Dependent Depletion; D, damaging; F, female; freq., frequency; gnomAD, Genome Aggregation Database; GPA, granulomatosis with polyangiitis; Pat. ID, patient identification number; M, male; MSC, Mutation Significance Cut-off; NA, not applicable; PAN, polyarteritis nodosa; PIDs, primary immunodeficiency disorders; PolyPhen2, Polymorphism Phenotyping v2; pred., prediction; RA, rheumatoid arthritis; ref. seq., reference sequence; SIFT, Sorting Intolerance From Tolerance; SjS, Sjögren's syndrome; SLE, systemic lupus erythematosus; SpA, spondyloarthritis; T, tolerated.

had received no corticosteroids or other immunomodulatory agent at first presentation in our outpatient clinic. Evaluation of IgG values at first presentation of those treatment-naive patients revealed similar IgG levels between patients harbouring at least one genetic variant in a PID-related gene and those without any PID variant (see online supplemental text and online supplemental figure 1).

DISCUSSION

While the spectrum of genetic defects underlying CVID is expanding,[8] the genetic basis of secondary hypogammaglobulinaemia remained unknown. Here, in a cohort of patients with diverse rheumatic diseases, who developed hypogammaglobulinaemia after introduction of an immunomodulatory therapy, we identified at least a variant in a PID-associated gene in approximately half (48.4%) of studied patients. This finding suggests an at least partially shared genetic background for primary and secondary hypogammaglobulinaemia. Further, we show that a sizeable minority of patients with predominantly articular rheumatic diseases, that is, RA and SpA, harboured genetic variants, which could account for hypogammaglobulinaemia in the context of PID, even leading to reclassification of physician-diagnosed rheumatic disease into PID. It is noteworthy that the identified variants, especially in AD-PID genes, are predicted to be deleterious but were not functionally tested to evaluate their pathogenicity. However, two variants in AD-PID genes were previously reported to cause CVID-like immunodeficiency. Identification of variants in AR PID-related genes does not explain hypogammaglobulinaemia in patients with rheumatic diseases. Nonetheless, the fact that such variants were more often detected among patients with rheumatic diseases with hypogammaglobulinaemia, suggests their representing risk factors for hypogammaglobulinaemia, which needs to be further investigated in larger cohorts of patients with rheumatic disease.

PID may manifest as autoimmune disease, necessitating treatment with immunomodulatory agents that can induce hypogammaglobulinaemia, independently of the underlying PID. Immunological investigations and especially the measurement of Ig levels as well as the documentation of infections before and after starting an immunomodulatory treatment can aid differentiating a pre-existing hypogammaglobulinaemia or susceptibility to infections, falling under PID, from a secondary immunodeficiency. However, considering the natural history of primary hypogammaglobulinaemia and its likely progressive course,[28] clinically evident immunodeficiency may follow the onset of autoimmunity and therefore, the introduction of a hypogammaglobulinaemia-inducing immunomodulatory treatment. In that case, the differentiation of primary from secondary hypogammaglobulinaemia can be challenging or even impossible. The identification of genetic variants that could account for PID in a cohort of patients with rheumatic diseases and per se secondary hypogammaglobulinaemia, developing after introduction of immunomodulatory regimens, suggests that genetic testing, may be of diagnostic value in resolving the above-presented diagnostic dilemma between primary and secondary hypogammaglobulinaemia.

Timely distinguishing of PID from secondary hypogammaglobulinaemia may be relevant in clinical practice. Diagnosis of an underlying PID results in a higher degree of vigilance for the identification of infections, malignancies, such as gastric cancer and lymphoproliferative diseases,[29 30] which may be relevant for patients with rheumatic diseases harbouring variants in PID-associated genes. Identification of the overlapping genetic background of primary and secondary hypogammaglobulinaemia in rheumatic disease may lead to the expansion of the indication for Ig replacement, which is currently limited to PID,[31] also for patients with rheumatic diseases with recurrent secondary hypogammaglobulinaemia-associated infections. Further, detection of genetic variants conferring risk for hypogammaglobulinaemia in patients with rheumatic diseases may result in extra caution before introducing immunomodulatory regimens with a relatively stronger immunosuppressive or hypogammaglobulinaemia-inducing effect, such as glucocorticoids or rituximab.[21 32 33] Finally, patients with rheumatic diseases harbouring particular defects such as *STAT3* or *PIC3CD* GOF variants or *CTLA-4* insufficiency may benefit from individualised therapeutic approaches, already employed to treat autoimmune manifestations in the context of PID.[34–37] However, parameters such as the penetrance of disease-causing variants and the natural history of each monogenic condition, as well as the availability and the cost of genetic testing, need to be determined before launching routine screening of patients with rheumatic diseases for PID variants prior to starting immunomodulatory therapies.

Despite the fact that immunodeficiency is most often conceived as susceptibility to infectious diseases, identification of PID-causing mutations in patients with rheumatic diseases, highlights the fact that PID may manifest with autoimmunity. The term inborn errors of immunity (IEI), is a synonym for 'primary immune deficiency disorders', which highlights the increasingly identified genetic background of PIDs.[8 38] These conditions are monogenic defects, whose phenotypic description is largely based on cohorts of patients with clinically evident immunodeficiency, that is, infectious manifestations. Our identification of PID-causing variants in patients with rheumatic diseases without noticeable infection records, suggests that autoimmunity and immune dysregulation can be the dominant phenotypic aspect of IEI. Especially the identification of monoallelic variants in AD PID-associated genes in a cohort of patients with rheumatic diseases, suggests that phenotypic characterisation of IEI based on cohorts of patients with clinically evident immunodeficiency may overestimate the prevalence of infectious manifestations at the expense of autoimmune phenotypes.

Our study has several limitations. As discussed above, despite using stringent selection criteria, including the rarity and pathogenicity prediction scores, we did not demonstrate the pathogenicity of identified variants, which may lead to an overestimation of the incidence of PID-related hypogammaglobulinaemia among patients with rheumatic diseases. In addition, despite testing for variations in genes most commonly accounting for IEI, such as *NFKB1*, *STAT3*, *CTLA4* and *PIK3CD*, the employed panel did not include all genes previously associated with primary hypogammaglobulinaemia, which would be possible through whole exome sequencing. Considering the expanding number of gene defects reported to be involved in PID, it is likely that a subset of tested patients with rheumatic diseases may harbour hypogammaglobulinaemia-causing variants in genes which were not included in our gene panel.

In summary, the identification genetic variants that can account for PID in patients with clear rheumatic phenotypes who developed hypogammaglobulinaemia after the introduction of immunomodulatory agents provides evidence on the overlapping genetic aetiology of primary and secondary hypogammaglobulinaemia in the context of rheumatic diseases. Our data suggest that primary immunodeficiency and autoimmune rheumatic diseases are not mutually exclusive entities, but rather related pathophysiological processes.

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Contributors GS and FA conceived and planned the study. GS took the lead in writing the manuscript. TW and RES significantly contributed to drafting and revision of the paper. GS and FA contributed substantially to data acquisition and interpretation, and performed the statistical analysis. ND, IRA and MA collected DNA samples and performed targeted NGS. All authors approved the final version.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication All studied patients signed an informed consent form.

Ethics approval This study was conducted in accordance with the Declaration of Helsinki and was also approved from the Ethical Committee of the Hannover Medical School (approval number: 8875).

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COVID-19 and how evidence of a new disease evolves

In 7 months' time, COVID-19 has developed from a single case to a pandemic affecting more than nine million people worldwide, and the outlook of many more to come. While the majority fare a mild disease course, the world has seen large numbers of critically ill and deaths. These are unprecedented times, in modern history only comparable to the 1918 Spanish influenza,[1] as we are faced by the worldwide spread of a disease that was non-existent less than a year ago.

'Evidence-based medicine' is proudly rooted in our practice nowadays and also expected to provide us with guidance on how to respond to COVID-19. As a result, the number of studies on COVID-19 is increasing exponentially. The accumulating data are widely available owing to the 'digital era' we live in, which, despite obvious advantages of public availability of information, also poses risks of 'information overload' or 'fake news'.

The rapid increase in research on COVID-19 is encouraging, yet, it is important to realise what these published data entail. For example, the discussion whether hydroxychloroquine is effective and safe in the treatment of COVID-19, nicely outlined by Kim *et al*,[2 3] and the recent retraction by major journals of two of their papers that were based on large but unreliable data repositories, illustrate why it is important to carefully interpret literature that is being published and the large societal consequences this interpretation may have. To accommodate the demand for guidance from patients and clinicians, also within the field of rheumatology, recommendations are issued by groups of the so-called experts and (inter)national societies, such as, among others, American College of Rheumatology[4] and European League Against Rheumatism[5] have done. Traditionally, such recommendations are evidence based, but what evidence can recommendations for COVID-19 be based on?

A PubMed search for available evidence on COVID-19 in the context of rheumatic and musculoskeletal diseases (RMDs) from 1 January 2019 until 24 June 2020, using search terms encompassing COVID-19, RMDs and drugs used in RMDs, generates 1725 hits. The exponential increase in publications over time is evident (figure 1). However, the majority (60%) are viewpoints or (narrative) literature reviews, and only a small proportion actually presents original data in the form of case reports or case series (15%), observational cohort studies (10%) or clinical trials (<1%). Moreover, most studies focus on drugs used in the field of rheumatology and their putative ability to treat COVID-19; roughly 10% is specific to COVID-19 in the context of RMDs. Of these, again, only 20% presents any original data (case reports, case series or observational cohort studies).

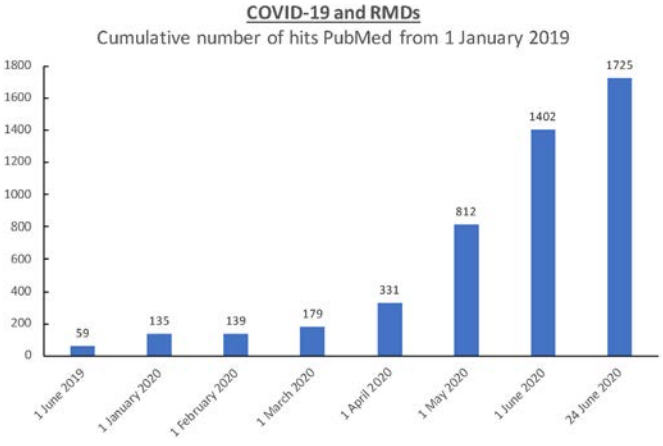

Figure 1 Cumulative number of hits in PubMed from 1 January 2019, using search terms encompassing COVID-19, rheumatic and musculoskeletal diseases (RMDs) and drugs used to treat RMDs.

While case reports are generally appreciated to yield only low levels of evidence, limitations of cohort studies on COVID-19 in RMDs should not be overlooked. Even in well-established registries or large cohorts with extensive correction for confounders, selection bias can hardly be eliminated and may lead to spurious associations. While traditionally seen as conveying the highest level of evidence, systematic literature reviews (SLRs) or meta-analyses of these studies, which will undoubtedly appear more frequently in the next few months in response to requests by users who feel overwhelmed by a multitude of data, will not eliminate the internal bias present in individual studies (an SLR does not whitewash the biases in inferior studies).

In conclusion, while evidence on COVID-19 evolves at an enormously rapid pace, the sheer *number* of studies is no measure for the *quality* of the data presented. To date, no robust evidence is available to allow strong conclusions on the effects of COVID-19 in patients with RMDs or whether RMDs or its treatment impact incidence of infection or outcomes. As researchers and clinicians, it is our responsibility to carefully interpret study results that emerge, even more so in this 'digital era', in which published data can quickly have a large societal impact.

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High oligoclonality of immunoglobulins in SARS-CoV2 positive patients

SARS-CoV2 virus affects the immune system at multiple sites.[1] Severe cases with a cytokine storm show massive defects of all T cell subsets with lymphopenia. B cells are rather hyper-reactive, suggesting defective regulation from T cells and patients with severe forms produce higher levels of antivirus antibodies.[2] However, the production of high affinity and protective antibodies requires a fine tuning of the interactions between viral-specific T and B cells. Defects of such regulation can lead to changes in immunoglobulin (Ig) production, with reduction of their protective properties, and induction of autoreactive and possibly pathogenic antibodies through tolerance loss. Serum protein electrophoresis is a simple way to look at Ig heterogeneity. Profiles can show polyclonal hypergammaglobulinaemia, oligoclonality with several small spikes or monoclonality with single M-spike.

From 3 March to 30 April 2020, 136 patients tested PCR-positive for SARS-CoV2 and 258 negative patients admitted at the same time for another diagnosis and systematically tested for SARS-CoV2, were included. All had serum protein electrophoresis at entry. C reactive protein (CRP) and neutrophil lymphocyte ratio were used as markers of severity.[3] PCR-positive patients were older and 51/136 (37.5%) in intensive care units (table 1).

Table 1 Analysis of serum protein electrophoresis in SARS-CoV2 positive patients

SARS-CoV2 PCR	Positive	Negative	P value
N	136	258	
Age (years)	74.5±16.8	66.2±19.8	<0.0001
Sex ratio (F/M)	1.06	0.83	0.28
Intensive care unit	51	3	<0.0001
Normal electrophoresis	21 (15.4%)	45 (17.4%)	0.67
Inflammatory pattern	83 (61.0%)	116 (44.9%)	0.003
Albumin decrease	41 (30.1%)	53 (20.5%)	0.02
Oligoclonality	29 (21.3%)	6 (2.3%)	<0.0001
Monoclonal peak	10 (7.4%)	20 (7.7%)	0.99
Increased gamma globulins	15 (11%)	35 (13.6%)	0.53
Decreased gammaglobulins	12 (8.8%)	36 (14%)	0.15

Fisher's exact test was used to analyse qualitative differences.

Comparison of electrophoresis patterns showed differences. As expected,there was an increased frequency of inflammatory profiles (83/136, 61% in positive vs 116/258, 45% in negative patients, p=0.003). The key difference was the high frequency of oligoclonal profiles with a few small spikes, in 29/136, 21.3% in positive vs 6/258, 2.3% negative patients, p<0.0001. There was no difference for the presence of a single monoclonal M-spike (all but two in SARS-CoV2 patients were already known). Frequency of increased (11% for positive vs 13.6% for negative patients, p=0.53) or decreased (8.8% vs 14%, respectively, p=0.15) gammaglobulin concentrations was not different between the two groups.

Such 10-fold-increase of oligoclonality could results from the virus, inflammation or both. In the 136 PCR-positive patients, CRP was not different between patients with and without oligoclonality (48±52 mg/L vs 61±69 mg/L, p=0.65), as for WCC count (8.3±3.6 10^9/L vs 7.8±8.4 10^9/L, p=0.13). The neutrophil/lymphocyte ratio was modestly higher in patients with oligoclonal profiles (5.9±4.3 vs 4.2±4.3, p=0.08), with higher neutrophil count (5.8±2.7 vs 4.5±2.6 G/L, p=0.03 g/L). Frequency of oligoclonal profiles in positive patients admitted to intensive care units was not different than in those that did not require such admission (19.6% vs 22.3%, p=0.8).

These results are in favour of defects in the regulation of Ig synthesis during COVID-19, and suggest a contribution from both the virus and inflammation.[3 4] Similar increase of Ig oligoclonality is commonly seen in autoimmunity, typically in Sjogren's syndrome, but also in other infections.[5] Such profile in COVID-19 further indicates defects in the crosstalk between T and B cells. They add to the concerns regarding the quality of the immune response. In COVID-19, various autoantibodies have been described, such asanti-phospholipid antibodies that can contribute to massive immune-mediated thrombosis and emboli.[6] Long-term studies are needed to evaluate the duration and pathogenicity of these changes. These results with possible induction of autoreactivity by the virus are also important to consider for current vaccine development.

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Increased risk of systemic lupus erythematosus in patients with autoimmune haemolytic anaemia: a nationwide population-based cohort study

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterised by immune inflammation.[1] Autoimmune haemolytic anaemia (AIHA) is a pathological state in which antibodies attack red blood cells. AIHA and SLE shared genetic and environmental risk factors and pathophysiological mechanisms.[2] AIHA is clearly over-represented in patients with SLE and often occurs before a diagnosis of SLE.[3] However, at present, studies on the incidence of SLE in patients with AIHA are scarce. Therefore, we explored the correlation between AIHA and SLE risk in a nationwide, population-based, matched cohort study.

In the 2003–2013 Taiwanese National Health Insurance Database, we identified patients newly diagnosed with AIHA between 2005 and 2012 (online supplemental figure S1). We selected age-matched and sex-matched (1:20) non-AIHA cohort from one million representative populations. From this cohort, we further selected a comparison group via propensity-score matching (PSM, 1:2) for age, sex, comorbidities and possible confounders using the greedy algorithm (online supplemental methods). Ultimately, we identified 713 patients with AIHA and 1416 PSM-matched individuals without AIHA, with balanced baseline characteristics between groups (online supplemental table S1). Before PSM, we examined the risk of SLE associated with AIHA using the Cox proportional regression analysis after adjusting for demographics, medical utilisation and comorbidities at baseline shown as HR with 95% CIs. Sensitivity analyses were conducted using various definitions of SLE based on SLE treatment or after exclusion of patients with secondary AIHA. After PSM, we estimated the association between AIHA and SLE incidence was estimated using the conditional Cox model. The cumulative incidence of SLE was significantly higher in the AIHA group than in the control group ($p<0.001$) at the end of the follow-up period before PSM (online supplemental figure 2A) and after PSM (online supplemental figure 2B). Before PSM, the incidence of SLE in the AIHA cohort was 172.92 times higher than that in the non-AIHA group (403.13 vs 2.33 per 100 000 person-months), and the risk of SLE was increased in the patients with AIHA (HR, 155.38; 95% CI, 95.42 to 253.00, table 1). In different Cox regression models, the risk of SLE was consistently increased in patients with AIHA (online supplemental table S2). Women and younger age groups were also associated with an increased risk of SLE (online supplemental table S2). Sensitivity analyses revealed consistent results (online supplemental table S3). After PSM, the incidence of SLE in the AIHA cohort was 64.76 times higher than that in the non-AIHA cohort (409.07 vs 6.32 per 100 000 person-months) and patients with AIHA had an increased risk of SLE (HR, 54.67; 95% CI, 22.33 to 133.89, table 1).

SLE may affect the blood system, leading to one or more lineages of haemocytopaenia. Early recognition of SLE features in patients with haemocytopaenia may lead to a different management strategy.[4] Recently, Zhu *et al* showed a significant association between idiopathic thrombocytopaenia and SLE risk (HR=17.4) in PSM-matched populations.[5] The present study used a similar method to assess the risk of SLE in patients with AIHA and demonstrated a high association (HR=54.7) between AIHA and SLE risk

Table 1 Incidence of SLE in the study groups before and after PSM

	Before PSM (1:20 age–sex matching)		1:2 PSM	
	Non-AIHA	AIHA	Non-AIHA	AIHA
n	14 620	731	1416	713
Follow-up person-months	857 910	29 023	79 155	28 357
SLE	20	117	5	116
Incidence rate* (95% CI)	2.331 (2.328 to 2.335)	403.13 (402.90 to 403.36)	6.32 (6.30 to 6.33)	409.07 (408.84 to 409.31)
Crude relative risk (95% CI)	Reference	172.92 (107.62 to 277.86)	Reference	64.76 (26.46 to 158.53)
Adjusted hazard ratio (95% CI)	Reference	155.38 (95.42 to 253.00)†	Reference	54.67 (22.33 to 133.89)‡

*Incidence rate, cases per 100 000 person-months.
†Cox proportional hazard regressions for estimation of HR on SLE with AIHA exposure adjusted for demographic variables, medical utilisation and comorbidities at baseline.
‡Conditional Cox model for estimation of HR on SLE with AIHA exposure alone.
AIHA, autoimmune haemolytic anaemia; PSM, propensity-score matching; SLE, systemic lupus erythematosus.

using PSM-matched populations. A study has reported that the incidence of anaemia in patients with SLE with active disease is relatively high.[6] The mechanisms of SLE anaemia mainly include immunological and non-immunological factors, of which AIHA is the most common immunological factor.[3]

In conclusion, patients with AIHA had a significantly higher risk of SLE than non-AIHA individuals. Clinicians should conduct early monitoring of SLE in patients with AIHA and provide relevant education for patients with AIHA. Further research is needed in the future to clarify the possible mechanisms of these correlations.

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Toll-like receptors revisited; a possible role for TLR1 in lupus nephritis

Several studies in systemic lupus erythematosus (SLE) have shown a possible role of endosomal toll-like receptors (TLRs) in lupus nephritis (LN), but the role of those interacting with ligands in the plasma membrane remains unclear.[1] Herein, we revisit the genetic contribution of TLRs in SLE inspired by a patient with LN who carries a rare TLR1 variant.

We analysed coding and regulatory regions of the TLR1–10 genes in 855 patients with SLE (online supplemental data 1). Six variants (rs142003616, rs76600635, rs72493538, rs41305843, rs113706342, rs41311400) within TLR1, one (rs10006364) within TLR2, one (rs79088436) within TLR5 and two (rs55695972, rs117985012) within TLR6 were significantly enriched in LN but only rs142003616 (TLR1) remained significant after Bonferroni correction ($p<0.039$, online supplemental table 1). To assess its biological significance, we employed in-silico functional annotation. The calculated deleteriousness score, CADD PHRED, for rs142003616 (5.56) points at the variant's potential functional importance. The rare risk allele is predicted to create a strong binding site for the core binding factor (CBF).[2] CBF, also known as runt-related transcription factors (RUNX), are also associated with SLE, psoriasis and rheumatoid arthritis.[3] To evaluate rs142003616 functional potential, we lastly performed a reporter assay that demonstrated a significantly higher expression of the reporter with the G allele in Jurkat ($p<0.0001$) and Daudi cells ($p<0.001$), and a strong enhancer potential without allelic difference in THP-1 (figure 1).

Of interest, despite the higher prevalence of proliferative LN overall (215 of 292 LN, 75%), it was less associated with rs142003616 in comparison to membranous LN ($p=0.047$, Fisher's exact test). Table 1 summarises the characteristics of patients with LN carrying the minor allele of rs142003616. One of them was a 39-year-old woman (#6 table 1) admitted

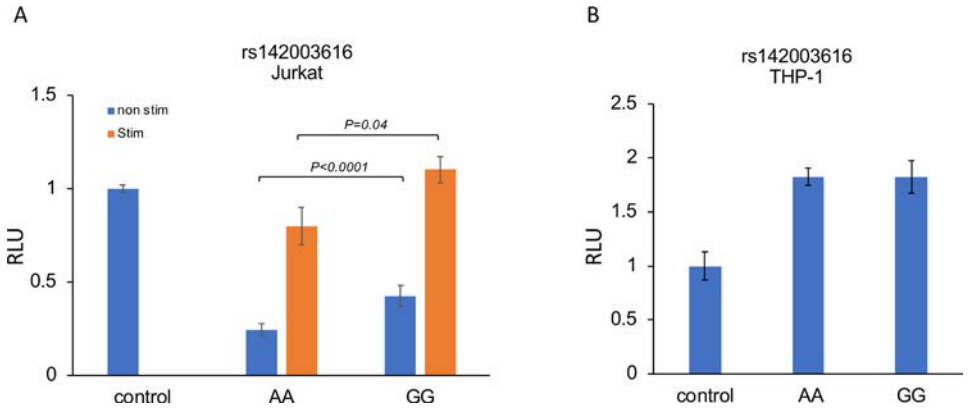

Figure 1 DNA fragments of 160 bp length with the variant in the middle were synthesised and cloned into pGL4.26 vector. Jurkat cells were transfected with reporter constructs containing different alleles of rs142003616 (A). Cells were left unstimulated for 48 hours or stimulated (stim) with PMA and ionomycin for 16 hours before harvesting. Protein lysates were assayed by the dual-reporter assay (Promega). Unpaired t-test was used for analysis of allelic difference. Similar low transcriptional levels and statistically significant allelic difference was detected upon transfection of Daudi cells (not shown). In THP-1 cells (B), the constructs show enhancer potential without allelic difference. Bars represent mean values +/-SEM. Non-stim, non-stimulated; RLU, relative light units.

to the hospital with prolonged fever (39°C), headache, non-productive cough, rash, leucopenia, high CRP (78 mg/L), microscopic haematuria and pyuria. Because of sinusitis and interstitial pneumonitis confirmed by chest tomography, the patient received doxycycline, and the fever and cough slowly disappeared, as did rash. Due to positive ANA (1/400), and persistence of haematuria, she was referred to the rheumatology department. The laboratory results showed dsDNA (1/40), decreased classical complement function (23% of normal; (N: 80%–120%)), low C4, anaemia, proteinuria (0.4gr/d) and haematuria. Her diagnosis was confirmed with SLE after immunofluorescent staining of her renal biopsy resulted in WHO Class Vb LN, although light microscopy result demonstrated postinfectious glomerulonephritis. She went into remission with hydroxychloroquine, prednisone and enalapril for 7 years. Due to uprising proteinuria (up to 1gr/d), rebiopsy was performed, which demonstrated WHO Class IIb. We calculated her polygenic risk score (PRS), which was normal, 8.27.[4] Our patient's history commencing with symptomatic infection, low PRS, which was against the general observation in LN, besides recovering to WHO class IIb without immunosuppressive therapy intrigued us and led to hypothesise that variants within TLR genes might contribute to the development or progression of LN.

Growing evidence highlights the role of podocytes in LN, not only as an integral part of kidney filtration barrier, but also their active involvement in immune-mediated kidney injury.[5] Podocytes constitutively express TLR1–6 and TLR8, respond to TLR ligands with proinflammatory cytokine release, activation of type I IFN signalling, and, ultimately, podocyte injury with proteinuria.[6] While innate immune responses play a central role in podocyte injury, evidence suggests that podocyte injury can initiate kidney damage in LN.[5] We identified a rare variant associated with LN, which affects TLR1 gene expression and might exert its effect via podocytes and immune cells. In conclusion, exogenous TLR ligands might contribute to the development of LN, rare polymorphisms in this locus might be considered when treating patients with LN triggered by exogenous agents.

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Table 1 Clinical characteristics of patients with lupus nephritis carrying TLR1 minor allele (rs142003616)

Patient	Gender	Age at diagnosis	European descent	ACR 1	ACR 2	ACR 3	ACR 4	ACR 5	ACR 6	ACR 7	ACR 8	ACR 9	ACR 10	ACR 11	AutoAbs*	Biopsy ever	WHO-class†
1	F	16	Yes	Yes	No	No	No	Yes	No	Yes	No	No	Yes	Yes	Y:N:N	No	–
2	F	26	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	Y:Y:N	No	–
3	F	16	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	No	No	Yes	N:N:N	Yes	Unknown
4	F	53	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	No	Yes	N:N:Y	Yes	V
5	F	15	Yes	Yes	No	No	No	Yes	No	Yes	No	Yes	Yes	Yes	Y:N:N	Yes	IV d
6‡	F	39	Yes	No	No	No	No	No	No	Yes	No	Yes	Yes	Yes	Y:N:Y	Yes	V b

ACR classification criteria,[7 8] ACR 1: malar rash, ACR 2: discoid rash, ACR 3: photosensitivity, ACR 4: oral ulcer, ACR 5: non-erosive arthritis, ACR 6: pleuritis or pericarditis, ACR 7: renal disorder, ACR 8: neurological disorder, ACR 9: haematological disorder, ACR 10: immunologic disorder, ACR 11: positive ANA.
*Autoantibodies, anti-ds-DNA: anti-Sm: anti-phospholipid antibodies, respectively.
†WHO-classification of lupus nephritis.
‡The case represented in the text. The detailed clinical characteristics of all patients with SLE (855 with SLE, of whom 292 had LN) can be found in Bolin K *et al*.[9]
AutoAbs, autoantibodies; LN, lupus nephritis; N, no; SLE, systemic lupus erythematosus; Y, yes.

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Janus kinase (JAK) inhibition with baricitinib in refractory juvenile dermatomyositis

Juvenile dermatomyositis (JDM) is a systemic vasculopathy with weakness and rash, frequently exhibiting a chronic/polycyclic course, and treated with broad immunosuppression. An interferon (IFN) signature correlates with disease activity.[1] Interferonopathies have been successfully targeted by janus kinase (JAK) inhibitors.[2 3] We report the first comprehensive prospective evaluation of JAK inhibition (baricitinib) in JDM.

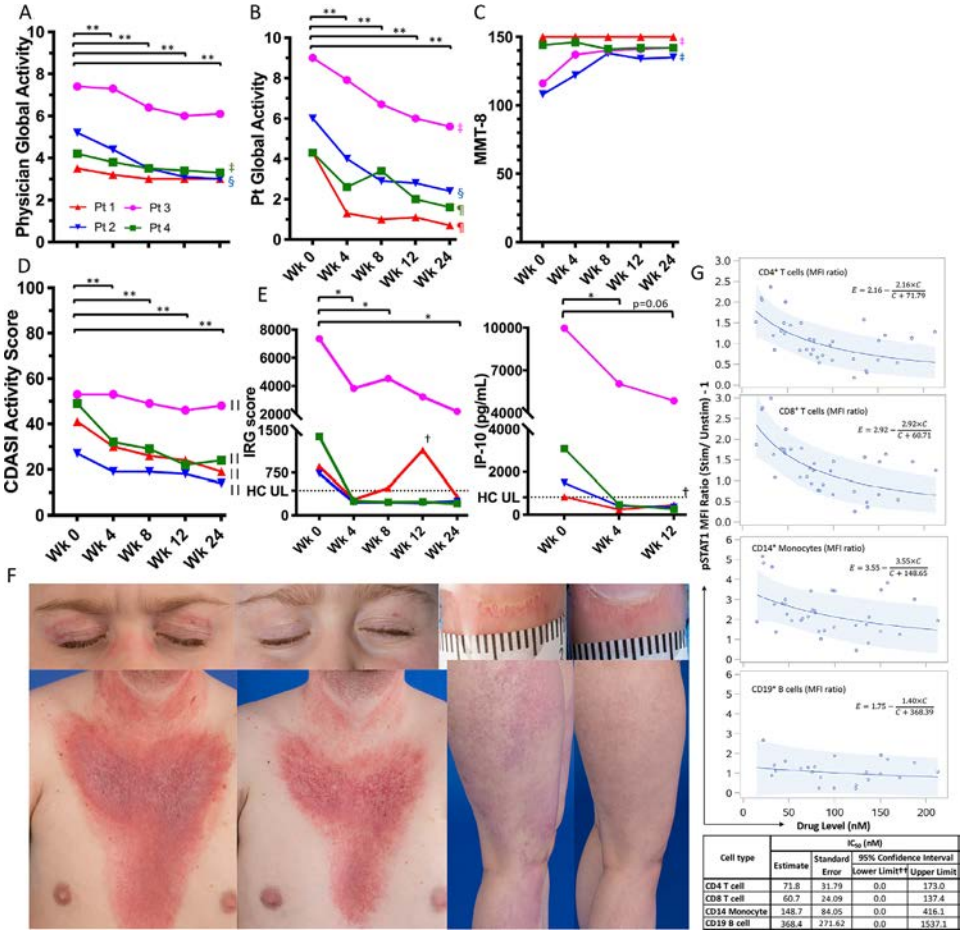
Four patients (5.8–20.7 years old), with chronically active JDM (≥3/6 core set measures)[4] who had failed three to six immunomodulatory medications, were enroled on compassionate use study NCT01724580 (online supplementary methods, online supplementary table 1). Biologics other than intravenous immunoglobulin were washed out and other medications continued.

Subjects were assessed before and 4, 8, 12 and 24 weeks after starting baricitinib (4–8 mg/day divided two times per day) dosed by weight and renal function.[3] Significant improvement was noted by week 4 in Physician Global Activity, Patient/Parent Global Activity, and Extramuscular Global Activity, and Cutaneous Dermatomyositis Disease Area and Severity Index (figure 1, online supplementary table 2). Two patients with baseline weakness improved by week 4 (ACR/EULAR Myositis Response Criteria) and showed clinically relevant improvement in Manual Muscle Testing-8 by week 8, confirmed by blinded MRI assessment (online supplementary figures 1 and 2). There was no significant change in muscle enzymes (online supplementary table 3), though some had variable elevations with stable/improved strength. Daily corticosteroids were decreased (0.28 to 0.18 mg/kg/day); other immunosuppressive medications were decreased/discontinued (online supplementary table 1). There were no flares/worsening requiring increased immunosuppression. There was no notable change in calcinosis (n=2).

Pharmacokinetic (PK) analysis revealed generally shorter half-life in the lower weight category, and longer half-life with lower renal function (online supplementary table 4). Dosing (mean 7.25 mg/day) resulted in ~50% higher exposure (Area Under of Curve (AUC) $_{0–24,\ ss}$: 1988 hours*nM) compared with adult rheumatoid arthritis (RA, 4 mg/day, 1304 hours*nM, data on file, Eli Lilly and Company). Dosing is likely justified by IFN targeting[3] distinct from RA targets, and increased clearance, with dose-normalised PK parameter estimates similar to paediatric interferonopathies.[3]

No serious adverse events (AEs) occurred and no subject discontinued baricitinib. There were 43 AEs by week 24 (online supplementary table 5), with infection (upper respiratory) the most common as expected.[2 5] BK virus was monitored due to concerns for opportunistic infection.[2] BK virus was detectable at baseline in one patient, with viraemia resolving and viruria decreasing by week 24, contemporaneous with tacrolimus discontinuation. Another patient developed BK viruria. Other expected AEs included haematologic abnormalities and elevated creatine kinase (CK)[2 5] (online supplementary tables 3 and 5).

STAT phosphorylation (pSTAT) assays were timed with PK samples to assess baricitinib concentrations required for 50% inhibition of stimulated pSTAT (IC_{50}). IFN-markers (interferon-regulated gene score, IP-10/CXCL10) decreased in all, with three



Cell type	IC_{50} (nM)			
	Estimate	Standard Error	95% Confidence Interval Lower Limit†¶	Upper Limit
CD4 T cell	71.8	31.79	0.0	173.0
CD8 T cell	60.7	24.09	0.0	137.4
CD14 Monocyte	148.7	84.05	0.0	416.1
CD19 B cell	368.4	271.62	0.0	1537.1

Figure 1 Change in disease activity and pharmacodynamic markers on baricitinib treatment. (A–D) Multiple clinical assessments are shown at baseline (WK 0), WKs 4, 8, 12 and 24 with the range of assessment values on the Y axis. Each colour represents a different patient. Clinically relevant improvement in core set measures was based on relative percent change from baseline or a five point decrease on CDASI at 24 weeks. P values were calculated based on linear mixed-model analysis of the repeated measures data. At 24 weeks, p values are FDR adjusted since the 24 week timepoint compared with the baseline is the main analysis of interest. At other timepoints, p values are not adjusted for multiplicity. (E) Left panel: 28 gene IRG score shown at baseline (Wk 0) and WKs 4, 8, 12 and 24. Right panel: serum IP-10 levels at baseline (Wk 0), Wk 4 and Wk 12. Log2 values were analysed via two-tailed paired t-test without correction versus baseline. (F) Example images of each of the four patients showing the same part of the body at baseline (left) and after 24 weeks (right) for heliotrope and malar rash, dilated and tortuous nailfold capillaries on right second finger, V-sign rash with significant erythema and scale, and violaceous erythema on the lateral thigh and proximal leg. (G) Scatter plots with model curves for pSTAT1 by cell type are shown with MFI ratio (IFN-α stimulated divided by unstimulated) minus 1 versus the peripheral blood drug level. The solid line and light blue band show best-fit curves with 95% predictive intervals, respectively. The table shows IC_{50} values by cell type calculated based on modelling (estimated) with SE and 95% CIs. *p<0.05; **p<0.01; ‡Clinically relevant minimal improvement; §Clinically relevant moderate improvement; ¶Clinically relevant major improvement; ‖Clinical significant improvement in three by week 4 and in the fourth (Patient 3) by week 12. †This patient had a suspected viral infection around the week 12 visit. ††: Calculated 95% lower limits which were negative are reset to 0 as drug levels are never negative. The dotted line represents the highest value from healthy controls. CDASI, cutaneous dermatomyositis area and severity index; HC, healthy controls; IRG, interferon-regulated gene; MFI, median fluorescence intensity; MMT-8, manual muscle testing 8; PGA, physician global activity; Pt: patient/parent; pSTAT, STAT phosphorylation; STIM, stimulated; UL, upper limit; Unstim, unstimulated; Wk, week.

reducing to normal by week 4 (figure 1E, online supplementary table 2). IFN-α stimulated pSTAT1 and interleukin-2 (IL-2) stimulated pSTAT5 IC_{50}s were lowest in $CD4^+$ and $CD8^+$ T cells, while IL-10-stimulated pSTAT3 IC_{50}s were lowest in $CD4^+$ T and $CD19^+$ B cells (figure 1G, online supplementary figure 3).

These results indicate baricitinib was clinically beneficial and safe in refractory patients, extending previous case reports.[6] The correlation of dose-dependent decrease in pharmacodynamic measures and clinical improvement provide proof-of-concept for JAK inhibition in JDM. One patient had IFN-marker elevation with suspected viral infection, which is reassuring when balancing pathogenic and physiologic IFN signalling with JAK inhibition. Infection monitoring including BK virus is recommended. As transaminitis and elevated CK (muscle enzymes) have been reported with baricitinib,[2 5] clinical assessment of strength and/or other assessments (ie, MRI) is important when using baricitinib for myositis. While increases in the number of patients and duration of treatment are needed, benefit in this open-label study is strongly supported by objective measures including blinded MRI scoring and photography. Baricitinib is an exciting therapeutic option that merits further study in JDM.

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Antimalarial use and arrhythmias in COVID-19 and rheumatic patients: a matter of dose and inflammation?

We read with great interest the paper by Graef and colleagues, 'Festina lente: hydroxychloroquine, covid-19 and the role of the rheumatologist'.[1] As the authors correctly point out, despite firm evidence that their efficacy and safety are lacking,[2] antimalarials are being widely prescribed for the treatment of patients with COVID-19. This, as also underlined with some concern by the European League Against Rheumatism President Iain McInness,[3] has rapidly led to antimalarial supply shortages worldwide, primarily affecting patients with rheumatic disease, such as those with systemic lupus erythematosus and rheumatoid arthritis (RA). In these groups, low-dose antimalarials (hydroxychloroquine up to 6 mg/kg/day and chloroquine up to 4 mg/kg/day) are the mainstay to control immunological response and to prevent flare in view of their favourable efficacy and safety profile.

However, electrophysiological experiments in isolated cardiac preparations and animal models, and some case reports in rheumatic patients, have reported a proarrhythmic effect of antimalarials. Arrhythmias, potentially triggered by hypoxia, metabolic/electrolyte derangement and viral myocarditis, have been reported in 16.7% of hospitalised patients with COVID-19.[4] While this suggests that antimalarial use may further augment the risk of fatal arrhythmias in patients with COVID-19, the evidence supporting this link is currently limited. In a recent retrospective study in 368 hospitalised patients with COVID-19, the use of high-dose hydroxychloroquine was associated with an excess of all-cause mortality compared with standard supportive measures (adjusted HR, 2.61).[5] Moreover, in a parallel phase II trial in severely ill patients with COVID-19, the use of high-dose chloroquine, especially in combination with antivirals and azithromycin, was associated with higher prevalence of QT in lead II corrected with Bazett's formula (QTc)>500 ms compared with low-dose chloroquine (18.9% vs 11.1%).[6] These data are in contrast with those of the WHO, which failed to show a higher risk of sudden death with antimalarials, despite the hundreds of millions of doses given for the treatment of malaria worldwide.[7]

The lack of consensus regarding the proarrhythmic effects of antimalarials in different patient groups, and whether there is a dose–effect relationship, mandates, as clearly stated by Graef and colleagues,[1] robust prospective studies that also account for relevant clinical and demographic characteristics.

Pending the conduct of such studies, we assessed the QT interval in a real-life consecutive series of patients with RA treated with low-dose hydroxychloroquine versus other disease-modifying antirheumatic drugs (DMARDs), with mild to moderate disease, low inflammatory burden and no previous cardiovascular events, enrolled in the Endothelial Dysfunction in Rheumatoid Arthritis study (ClinicalTrials.gov, NCT02341066).[8] Barring C reactive protein, there were no significant between-group differences in clinical and demographic characteristics. Patients treated with low-dose hydroxychloroquine (mean dose 331±95 mg/day) for more than 6 months had a longer QTc and a higher prevalence of prolonged QTc. However, their mean QTc, 420 ms, is within normal limits and, more importantly, the prevalence of QTc>500 ms was very low and not significantly different between patients taking low-dose hydroxychloroquine and those taking other DMARDs (table 1). This suggests that low-dose hydroxychloroquine is unlikely to be proarrhythmic 'per se' and that other factors might predispose severely ill patients, including patients with COVID-19, to malignant arrhythmias.

Table 1 Demographic, clinical and electrocardiographic parameters in patients with RA receiving low-dose hydroxychloroquine or other DMARDs

	RA treated with hydroxychloroquine (n=104)	RA treated with other DMARDs (n=541)	P value
Age (years)	61.0±9.2	60.6±9.5	0.715
Disease duration (months)	114±103	129±116	0.210
DAS-28ESR	3.61±1.38	3.66±1.38	0.743
HAQ	0.75±0.6	0.77±0.6	0.866
CRP (mg/dL)	1.08±2.4	2.02±6.1	0.009
ESR (mm/hour)	26.2±20	27.5±22	0.599
HR (beats/min)	70.1±8.1	69.2±9.9	0.438
QTc (ms)	420.3±30.8	410.6±28.7	0.002
QTc, prolonged, n (%)	10 (9.6)	24 (4.4)	0.030
QTc, >500 ms, n(%)*	0	2 (0.4)	1
K+† (mEq/L)	4.1±0.3	4.1±0.3	0.862
Ca++† (mg/dL)	9.2±0.4	9.1±0.4	0.752

*Fisher's exact test.
†Available in 150 subjects. Values are mean ±1 SD.
CRP, C reactive protein; DAS28-ESR, Disease Activity Score 28-joints measured with erythrocyte sedimentation rate; DMARD, disease-modifying antirheumatic drug; HAQ, Health Assessment Questionnaire; HR, heart rate; QTc, QT in lead II corrected with Bazett's formula; RA, rheumatoid arthritis.

Among such factors, the presence of high-grade systemic inflammation, through the release of the proinflammatory cytokine interleukin (IL)-6, has been shown to predispose to QTc prolongation via the inhibition of the rapidly activating repolarising K+ current.[9] Of note, the use of tocilizumab, an IL-6 receptor blockade approved for the treatment of cytokine release syndrome, has been shown to reverse QTc prolongation. High-grade systemic inflammation might lower the arrhythmic threshold both in rheumatic patients with high disease activity and in patients with severe COVID-19, increasing the risk of malignant arrhythmias with antimalarials, particularly at high doses.

Collectively taken, these observations suggest that (1) the risk of clinically relevant arrhythmias with antimalarials, although negligible at low, 'rheumatological' doses, may be different with higher dosages; (2) QTc screening and monitoring should be encouraged in patients taking high-dose antimalarials, particularly when combined with other QT-prolonging medications; and (3) the prompt and aggressive control of inflammation may be helpful to reduce the arrhythmic risk in severely ill patients.

In conclusion, pending the generation of robust evidence of efficacy and safety in patients with COVID-19 and given their acceptable safety profile at low doses, we strongly believe that every possible effort should be made to ensure sufficient supply of antimalarials to rheumatic patients, a large group that continues to depend on these agents for disease activity control.[1–3]

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Response to: 'Antimalarial use and arrhythmias in COVID-19 and rheumatic patients: a matter of dose and inflammation?' by Erre *et al*

We read the comment by Erre *et al* to our correspondence about hydroxychloroquine (HCQ) use during the COVID-19 with great interest.[1 2] As also highlighted by others, antimalarial use such as HCQ during the COVID-19 pandemic has the potential for cardiotoxicity.[3 4] Patients with COVID-19 and those with rheumatic disease represent distinct populations with different dosing strategies. We agree that the potential for cardiotoxicity from antimalarials may also be different related to these issues.

The authors present interesting data on QTc intervals in patients with rheumatoid arthritis (RA) on maintenance HCQ, drawn from an established cohort of patients without known significant underlying cardiovascular disease. While these patients had statistically significant higher QT intervals on HCQ compared with those on other disease-modifying antirheumatic drugs (DMARDs), the mean remained within normal limits so this difference is unlikely to have a large clinical impact. Therefore, it appears that the QTc interval is not pathologically prolonged among patients with RA on maintenance HCQ. While it is possible that HCQ may affect risk for a small proportion of patients with rheumatic diseases, perhaps with borderline or unrecognised QTc prolongation or other cardiac disorders, we find these data reassuring. Despite widespread use in rheumatic diseases, pathological QTc prolongation has not been recognised as a complication of HCQ.

Another group of investigators recently studied pre-QTc and post-QTc interval measurements among hospitalised patients with COVID-19 who received at least 1 day of HCQ, with or without azithromycin.[5] Their patients studied had a similar mean age to those reported by Erre *et al* (60 years), though 10% had coronary artery disease. Of the 37 who received HCQ monotherapy, 19% developed prolonged QTc of 500 ms or more. Of the 53 who received HCQ with concomitant azithromycin, incidence of prolonged QTc was 21%. Compared with baseline values, those receiving HCQ alone had a mean QTc increase of 5.5 ms, while those who received combination HCQ and azithromycin had a mean QTc increase of 23 ms. Among all 90 patients, 28% had elevated troponin levels consistent with acute cardiac injury. Therefore, patients infected with COVID-19 may be particularly susceptible to QTc prolongation with HCQ use related to several factors such as higher dose of HCQ, high levels of systemic inflammation, ongoing cardiac injury and concomitant use of other QTc prolonging medications such as azithromycin. A recent observational study among hospitalised patients with COVID-19 associated combined HCQ and azithromycin use with increased risk of cardiac arrest compared with use of neither drug, also highlighting this concerning possible cardiotoxicity of HCQ among that patient population.[6]

While we caution against strong inference, these data justify continued investigation regarding the cardiotoxicity of antimalarials specifically in COVID-19. We agree with the authors that reports of potential cardiotoxicity of HCQ in COVID-19 should not be extrapolated to patients with rheumatic disease where its safety is well established.

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Hydroxychloroquine shortages during the COVID-19 pandemic

Across the globe, concerns of hydroxychloroquine (HCQ) supply shortages for patients with rheumatic disease are growing,[1] in part as a consequence of the immense scientific and public enthusiasm for HCQ as a potential COVID-19 therapy.[2 3] However, published data on the occurrence of HCQ shortages during the COVID-19 pandemic are presently lacking. Therefore, we conducted a national survey of 531 Canadian rheumatologists between 14 and 24 April 2020. The 5-question electronic survey (French or English) included province of practice, whether respondents were concerned about HCQ shortages in their province, and whether they had been contacted by patients or pharmacies regarding difficulties renewing HCQ prescriptions during the COVID-19 pandemic. Physicians who answered 'yes' to the latter question were asked to estimate for how many patients this occurred. The McGill University Health Centre research ethics board approved this survey.

Of 134 rheumatologists who completed the survey (25% response rate), three quarters (n=102, 76%) were concerned about HCQ shortages, while 81 (60%) had been contacted by pharmacies or patients regarding difficulties accessing or renewing HCQ (see table 1). In the province of Quebec, 29/31 (94% (95% CI 79 to 98)) physicians had been contacted, compared with 52/103 (50% (95% CI 41 to 60)) in the rest of Canada. Among those contacted by patients/pharmacies, 71 (88%) provided a numerical (rather than qualitative) estimate of the patients affected, with a median of 50 patients per physician in Quebec (IQR 25–100), compared with a median of 4 (IQR 2–5) patients per physician in the rest of Canada ($p<0.0001$ for Mann-Whitney U test).

In his editorial, Dr McInnes notes the imperative to protect HCQ supply for patients with rheumatic diseases.[1] While Quebec has reported higher COVID-19 rates (256/100 000 population) than the rest of Canada combined (70/100 000 population), the substantially different experience of Quebec rheumatologists may furthermore be an unintended consequence of system-level mitigation strategies to proactively manage impending HCQ shortages. Following optimistic reports of the possible effectiveness of HCQ for COVID-19, Quebec health authorities determined that there was a significant risk of HCQ shortage in the province (only 2–3 weeks of estimated supply available) and made the unprecedented decision to restrict HCQ access for all indications except systemic lupus erythematosus (SLE) (as well as pregnant and paediatric patients) to reserve supply for these vulnerable groups.[4] Patients such as those with rheumatoid arthritis (1% of the Quebec population)[5] abruptly lost HCQ access. Pharmacies now contact rheumatologists to confirm diagnoses, potentially causing delayed access even for eligible patients. Whether these restrictions have succeeded in protecting specific groups, such as patients with SLE, from shortages, and to what extent HCQ cessation among all others will lead to disease flares, remains to be determined. In SLE, there is already ample evidence to indicate that HCQ discontinuation could lead to hospitalisation or even death.[6]

Due to the limited number of survey questions (to maximise response rate) we could not assess provider characteristics such as type of practice (academic vs community). Furthermore, physicians experiencing more HCQ access issues may have been more likely to complete the survey. Nevertheless, the consistent estimated number of affected patients per physician in most provinces (median five or less) and the drastically higher numbers reported in Quebec (10-fold more) lends validity to our observations.

Our survey establishes that HCQ shortages are reported by rheumatologists in most Canadian provinces during the COVID-19 pandemic, with over half of respondents receiving at least one notification of a HCQ access issue, now believed to stem from regional distribution problems rather than lack of

Table 1 Experiences with HCQ shortages among Canadian rheumatologists according to province of practice (n=134)

Province*	British Columbia	Alberta	Saskatchewan	Manitoba	Ontario	Quebec	New Brunswick	Nova Scotia	Newfoundland and Labrador
Respondents (% total)	14 (10)	22 (16)	3 (2)	8 (6)	48 (36)	31 (23)	3 (2)	3 (2)	2 (1)
COVID-19 cases per 100 000 population†	36	84	28	19	88	256	15	85	49
Concerned about HCQ shortage, n (%)									
Yes	11 (79)	16 (72)	0 (0)	5 (63)	40 (83)	27 (87)	2 (67)	0 (0)	1 (50)
No	0 (0)	4 (18)	0 (0)	1 (13)	4 (8)	2 (6)	1 (33)	1 (33)	0 (0)
Unsure	3 (21)	2 (9)	3 (100)	2 (25)	4 (8)	2 (6)	0 (0)	2 (67)	1 (50)
Contacted by pharmacies or patients for HCQ access issues, n (%)									
Yes	9 (64)	9 (41)	0 (0)	2 (25)	29 (60)	29 (94)	1 (33)	1 (33)	1 (50)
No	5 (36)	13 (59)	3 (100)	6 (75)	19 (40)	2 (6)	2 (67)	2 (67)	1 (50)
Number of patients per physician affected by HCQ access issues, median (IQR)‡	5 (4–10)	5 (3–13)	n/a	3 (3–4)	3 (2–6)	50 (25–100)	3§	1§	1§
Number of patients per physician affected by HCQ access issues, mean (SD) ‡	6.9 (5.7)	8.0 (8.3)	n/a	3.0 (1.4)	4.5 (3.8)	74.9 (73.7)	3§	1§	1

*No respondents from Prince Edward Island, Yukon, Northwest Territories or Nunavut.
†Estimates based on: Coronavirus Disease 2019 (COVID-19) Daily Epidemiology Update. Health Canada. https://www.canada.ca/content/dam/phac-aspc/documents/ services/diseases/2019-novel-coronavirus-infection/surv-covid19-epi-update-eng.pdf; Statistics Canada. Table 17-10-0009-01, Population estimates, quarterly. DOI: https://doi.org/10.25318/1710000901-eng. Both accessed 23 April 2020.
‡Includes only respondents providing a numerical estimate of patients affected (n=71).
§n=1 respondent; median (IQR) and mean (SD) not calculated.
HCQ, hydroxychloroquine.

supply. Monitoring the clinical impact of shortages for patients with rheumatic diseases and mobilising efforts to restore HCQ access are now critical.

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Response to: 'Hydroxychloroquine shortages during the COVID-19 pandemic' by Mendel *et al*

As the COVID-19 crisis moves to its next phase, from 'acute to chronic' as it were, it is important to note that a variety of immune modifier medicines are now being proposed or actively trialled in pursuit of a novel effective intervention for early through to poor prognosis coronavirus infection. Such imaginative and creative approaches are to be welcomed. Given the current mathematical models of COVID-19 pandemic resolution, it seems that this may be a lengthy scenario. In the meantime, some disease-modifying antirheumatic drugs (DMARD) are being used on a presumptive basis, for understandable compassionate reasons, though not always based on robust medical evidence. One consequence of widespread uptake of DMARD use in COVID-19 is that drug availability may be diminished and thereby hamper the management of existing immune-mediated inflammatory diseases and particularly rheumatic and musculoskeletal diseases (RMDs). Many people with RMDs are reliant on such DMARDs to retain their well-being and loss of therapeutics may lead potentially to flare, and disease progression. Mendel *et al* provide us with important evidence of hydroxychloroquine shortages in Canada provided via a survey of Canadian rheumatologists.[1] They are to be commended for seeking evidence of the same. This aligns well with numerous similar reports emerging across European countries. The European League Against Rheumatism is committed to ensuring equitable access to the medicines necessary to optimally treat RMDs during the COVID-19 pandemic.[2] This is especially the case when as yet, few or no randomised controlled data support the use of immune modifiers in the management of COVID-19. Thus, while we are supportive of high-quality clinical trial medicine and indeed are hopeful that successful outcomes will emerge in pursuit of a solution for COVID-19, we prefer that the use of agents such as hydroxychloroquine is reserved in the context of appropriate trials, be they of controlled or well-annotated cohort design.

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Correspondence on 'Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus under long-term treatment with hydroxychloroquine'

We thank Mathian *et al* for reporting the outcomes of COVID-19 disease in a series of 17 patients with systemic lupus erythematosus (SLE) from several hospitals across France.[1] In a context where there is substantial interest in the role of hydroxychloroquine (HCQ) as a potential preventive or therapeutic agent for severe acute respiratory syndrome–coronavirus-2 (SARS-CoV-2), these cases are noteworthy.

While the authors have correctly acknowledged the limitations of this case series report, we wish to emphasise several important points that impact the interpretation of the findings. The denominator of all patients with lupus on hydroxychloroquine who are 'at risk' of COVID-19 in this setting is unknown and may indeed be impossible to even estimate as it would need to be adjusted for risk of exposure to SARS-CoV-2. Notwithstanding occupation and travel, presently, the risk of exposure to the virus differs from one geographical location to another, even within each country.[2] For example, having 17 infections among several thousand individuals at risk may indeed be consistent with some protective effect of hydroxychloroquine. Moreover, as hydroxychloroquine is a staple maintenance treatment in the majority of patients with SLE, it may not be possible to source valid comparator groups who are not on hydroxychloroquine, and matching for other pertinent risk factors may not be possible across disease groups, such as rheumatoid arthritis and ankylosing spondylitis, as the demography of these diseases and their intrinsic impact on the immune system are distinctly different from those of SLE.

Second, we note the high burden of comorbidity and immunosuppressive medications among these patients with SLE, with 59% being obese, 47% having chronic kidney disease and 41% being treated with immunosuppressant drugs. Other comorbidities in this series of patients included cerebrovascular and cardiovascular disease, hypertension, malignancy and chronic lung disease. It is indeed possible that such high burden of comorbidities and concomitant immunosuppressive treatment may have overcome any protective effect of hydroxychloroquine. For example, while there are no head-to-head comparisons of antiviral response, immune-compromised patients with influenza have higher viral loads, higher frequency of viral mutation, prolonged viral shedding, and hence poorer treatment response and outcomes than immunocompetent hosts.[3] During the H1N1 influenza pandemic in 2009, despite treatment with oseltamivir, poor outcomes were reported in an immune-suppressed cancer population.[4]

In conclusion, we advise caution in relation to making any inferences regarding the preventive or therapeutic efficacy of HCQ for SARS-CoV-2 based on this case series alone. There may still be a place for HCQ in the prevention of SARC-CoV-2 in at-risk individuals without comorbid conditions who are not immune suppressed. Only well-designed randomised placebo-controlled trials will be able to shed light on this matter.

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Response to: 'Correspondence on 'Clinical course of coronavirus disease 2019 (COVID-19) in a series of 17 patients with systemic lupus under long-term treatment with hydroxychloroquine" by Nikpour *et al*

We thank Nikpour *et al* for their interest in our study reporting on the course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease 2019 (COVID-19 in a case series of 17 patients with systemic lupus erythematosus (SLE) under long-term treatment with hydroxychloroquine (HCQ).[1 2] As mentioned in our study, we did not intend to analyse the incidence rate and the severity of COVID-19 in SLE because we are aware that our cohort most likely over-represents the most symptomatic and severe cases, resulting from a selection bias. Our conclusion was rather that patients with SLE treated with HCQ are not universally protected from COVID-19, a finding recently confirmed in another observational study in which data collected through the COVID-19 Global Rheumatology Alliance registry were analysed.[3]

We agree with Nikpour *et al* that it is next to impossible to identify the denominator of patients with SLE treated with HCQ who are at risk of infection with SARS-CoV-2, apart from the difficulty to assign relevant control subjects, and that, for these reasons alone, one should be careful in the interpretation of the data as to the preventive effects of HCQ against SARS-CoV-2 infection.

Moreover, we also agree with Nikpour *et al* that the increased prevalence of comorbidities in the SLE population could lower the putative protective effect of HCQ against COVID-19 and that a protective effect of HCQ against viral infection cannot be ruled out based on the results from our observations alone.

However, there is no evidence as yet that HCQ has any preventive or curative efficacy on SARS-Cov-2 except in in vitro experimental settings and in a few clinical studies marked by numerous methodological flaws.[4 5] Conversely, several recent observational studies[6–8] and a multicentre, randomised controlled trial[9] have shown that administering HCQ to patients hospitalised for COVID-19 was associated with neither a lowered nor an increased risk of death,[7 8] death or intubation,[6] survival without transfer to an intensive care unit,[8] alleviation of symptoms or negative conversion.[9] Together, these studies do not support the notion of a therapeutic effect of HCQ in both mild to moderate and severe forms of COVID-19. HCQ is also under investigation in several clinical trials for prophylaxis of SARS-CoV-2 infection,[10] but their results have not yet been reported. Nevertheless, an irrefutable demonstration of the usefulness of HCQ for the treatment of COVID-19 will be very difficult to obtain, because its therapeutic effectiveness (or ineffectiveness) is very likely to depend on the administered dose, as well as its combined use with azithromycin. Indeed dosages of HCQ above 400 mg/day (ie, a dose rarely exceeded in the treatment of SLE), together with the administration of azithromycin, have been reported to be more effective than HCQ alone.[4] Physicians should also keep in mind that even if the cardiac safety at doses of HCQ at 200–400 mg/day is not compromised, the administration of larger doses of HCQ, or its combination with azithromycin, is much more problematic because of an enhanced risk of a significant QT interval prolongation.[11 12] Thus, rather than promoting an uncertain preventive role of HCQ in the protection against COVID-19 and given the lack of agents with clinically proven antiviral efficacy, we believe, like Favalli *et al*, that physical distancing and the adoption of strict rules for the prevention of contagion are the key elements of COVID-19 prophylaxis in patients with SLE, especially for those suffering from comorbidities and/or treated with immunosuppressants.[13]

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Patients with lupus with COVID-19: University of Michigan experience

We read with interest the recent report by Mathian *et al*[1] regarding the clinical course of COVID-19 infection in 17 patients with systemic lupus erythematosus (SLE) under long-term hydroxychloroquine treatment. We report supportive findings in five patients with SLE, contextualised in a larger cohort of patients with rheumatological conditions, from an academic, tertiary-care population.

Between 1 March and 20 April 2020, 31 patients followed at University of Michigan rheumatology clinics were diagnosed with COVID-19 (see online supplementary table 1). Five (16%) of these patients had SLE, four of whom were taking hydroxychloroquine with a median (range) duration of 7 (6–8) years. Compared with the overall cohort, patients with SLE appear more likely to be of black race (80% vs 42%), obese (80% vs 65%), have chronic obstructive pulmonary disease or asthma (60% vs 35%), use glucocorticoid therapy (80% vs 39%) and have a history of tobacco exposure (80% vs 45%).

Four patients with SLE (80%) were hospitalised for COVID-19; three (60%) required invasive ventilation; and one (20%) died of the disease. In the overall cohort, 20 (64%) were hospitalised; 6 (19%) required invasive ventilation; and 4 (13%) died. Among those patients with SLE, the median (range) SLE duration was 13 (9–36) years. At COVID-19 diagnosis, all four patients with SLE who were hospitalised with COVID-19 were in remission (Systemic Lupus Erythematosus Disease Activity Index score<3). The remaining patient, who had a mild COVID-19 course, had no laboratory evaluation when symptomatic.

In summary, our experience suggests that patients with SLE may develop more severe manifestations of COVID-19 infection, even relative to patients with other autoimmune diseases. Like Mathian *et al*, our SLE population had clinically quiescent lupus and long-term hydroxychloroquine exposure. Black race, respiratory comorbidities, and glucocorticoid and tobacco exposure were common in our cohort, and higher rates of these predisposing factors among patients with SLE may help explain the higher rate of severe disease from COVID-19.

Beth Wallace [1,2] **Laraine Washer,**[3] **Wendy Marder,**[1,4] **J Michelle Kahlenberg** [1]

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Response to: 'Patients with lupus with COVID-19: University of Michigan experience' by Wallace *et al*

We thank Wallace and Waher[1] for their interest in our study on the course of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) disease 2019 (COVID-19) in a case series of patients with systemic lupus erythematosus (SLE) under long-term treatment with hydroxychloroquine (HCQ) and the reporting of their own case series.[1 2] Their results corroborate those from our and other recently published observational studies in SARS-CoV-2-infected patients with SLE pointing to a lack of a preventive effect of HCQ,[3 4] and furthermore underscore the notion that a high percentage of these patients suffer from several comorbidities.[1 2] In their case series patients with SLE appeared to be more prone to obesity (80%), chronic obstructive pulmonary disease or asthma (60%), hypertension (20%), diabetes (20%), and chronic kidney disease (20%),[1] while in our cohort the main comorbidities were obesity or overweight (71%), chronic kidney disease (47%) and hypertension (35%).[2] These chronic medical conditions have all been reported to be associated with severe forms of COVID-19,[5–7] and the presence of a similar association with symptomatic or severe cases of COVID-19 in patients with SLE therefore does not come as a surprise.

While the presence of an underlying immunosuppressed condition has not yet been associated with an increased death rate during the course of COVID-19,[5] it is nevertheless important to note that, both in our case series and that reported by Wallace and Waher, 71% and 41%[2] and 80% and 60%[1] of the patients were treated with glucocorticoids or immunosuppressants, respectively. In a recent study on COVID-19 in immune-mediated inflammatory diseases such as psoriasis, rheumatoid arthritis, ankylosing spondylitis and inflammatory bowel diseases, the use of oral glucocorticoids and methotrexate was higher among patients for whom hospitalisation was warranted.[8] These drugs might therefore represent a risk factor for developing symptomatic or severe forms of COVID-19, although more data will be required to confirm a possible, causative, link between immunosuppressive therapy and COVID-19 severity.

Patients with SLE are possibly at risk to develop symptomatic or severe COVID-19, not because of their primary disease, glucocorticoid and/or immunosuppressive therapy, but as a consequence of associated comorbidities. Although patients with SLE have a greater burden of comorbidities such as hypertension, chronic kidney disease and hyperlipidaemia,[9] the prevalence of obesity and overweight is less documented in SLE and may vary depending on the country.[9 10] On the other hand, it is important to note that patients with lupus are mostly women of young age, two factors associated with a better prognosis of COVID-19.[5 6] Notwithstanding the similar conclusions that can be drawn from our case series and that of Wallace and Waher,[1] only larger cohort studies based on the detection of SARS-CoV-2, as well as the presence of specific anti-SARS-CoV-2 antibodies, will provide detailed information on the incidence and severity of COVID-19 in this fragile population. In this respect, several national and international registers have been launched at the beginning of the pandemic, and we expect that the forthcoming results will provide more insight into the complexity of risk factor involvement in COVID-19 severity in SLE and other autoimmune diseases.

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Severe COVID-19-associated pneumonia in 3 patients with systemic sclerosis treated with rituximab

The case reported by Guilpain *et al* attracted our attention. This case of granulomatosis with polyangiitis on immunosuppressants, including recent maintenance therapy with rituximab (RTX), developed a severe and life-threatening coronavirus-2 (SARS-CoV-2) disease 2019 (COVID-19). The particularity of this observation was the occurrence of a more progressive worsening than observed in most series.[1] Herein, we report the observation of three patients with systemic sclerosis (SSc) routinely treated with RTX who were affected by COVID-19 and who also experienced a late clinical worsening to severe pneumonia. RTX is often used off-label in patients with SSc mainly for refractory skin, musculoskeletal or interstitial lung disease. Observational studies reported a safety profile similar to that reported in rheumatoid arthritis.[2]

Their main disease characteristics are presented in table 1. Patient 1 had early diffuse cutaneous SSc, with positive RNA polymerase-3 antibodies, and severe cutaneous involvement (peak modified Rodnan Skin Score at 32/51) as the main clinical involvement. Patient 2 had long-lasting limited cutaneous SSc with recurrent digital ulcers and inflammatory arthritis as the main disease manifestations. Patient 3 had a limited cutaneous subset evolving from 2 years, with positive RNA polymerase-3 antibodies and persisting arthritis. None of these patients had interstitial lung disease and primary or secondary heart involvement. Regarding the main comorbidities, patient 1 was treated for high blood pressure by perindopril, furosemide and lercanidipine, and patient 2 had chronic renal insufficiency and history of pulmonary embolism (2002 and 2008).

All patients presented typical COVID-19 first symptoms (table 1). Patients 2 and 3 had confirmation of COVID-19 diagnosis by reverse transcription (RT)-PCR from nasopharyngeal swab specimens. Chest high-resolution CT was performed for all three patients and demonstrated typical bilateral interstitial pneumonia (figure 1). All patients experienced secondary clinical worsening and sudden respiratory failure requesting emergency hospitalisation. Due to acute respiratory distress syndrome, patients 1 and 3 were transferred to ICU and recovered after 7 and 15 days of non-invasive ventilation without other specific therapy, respectively, with withdrawal of oxygen support. Patient 2 also requested ventilatory support by high-flow nasal cannula. She received four subcutaneous daily injections of anakinra in association with lopinavir. Despite this treatment, rapid respiratory deterioration led to the use of intravenous corticosteroid pulses (120 mg) for 3 days and tocilizumab (1 infusion of 8 mg/kg). These treatments were associated with improved clinical outcome, characterised by decreased oxygen support requirement. No thromboembolism and bacterial secondary infection were observed in these three patients on heparin (prophylactic dosing: patients 1 and 3, therapeutic dosing: patient 2) and antibiotic therapies. Moreover, despite several recent descriptions of peripheral vascular manifestations in COVID+ patients, microangiopathy was not progressive in the three cases.

Some points regarding these observations are important to be considered and discussed. No specific disease subset specifically at risk of COVID-19 was identified. Indeed, the three patients had heterogeneous disease profiles in term of age, disease duration, cutaneous subset and disease manifestations. The World Scleroderma Foundation has recently proposed preliminary advices for the management of patients with SSc during the COVID-19 pandemic.[3] Given the frequent presence of interstitial lung disease (ILD) and concurrent immunosuppressive treatment, patients with SSc may be considered at risk for a more severe disease course and higher mortality when they develop SARS-CoV-2 virus infection. Importantly, these three patients had no pre-existing ILD that may have favoured the severity of the infection. On the other hand, the potential implication of age and comorbidities for patients 1 and 2 (table 1), as well as immunosuppressors including long-lasting RTX therapy, need to be taken in consideration.

As described by Guilpain *et al*, the COVID-19 course of these three patients was characterised by a late clinical worsening compared with what is classically described (days 19, 15 and 23, respectively).[1 4] RTX, but also methotrexate (patients 1 and 3) and/or long-term corticosteroid use (all three patients), may have initially but insufficiently limited the cytokine storm, leading to a delayed worsening. The impairment of antiviral humoral response, more specifically observed with RTX, might

Table 1 Clinical characteristics of the three patients with systemic sclerosis and COVID-19

	Patient 1	Patient 2	Patient 3
Age (years)	71	84	44
Gender	Male	Female	Female
Body mass index (kg/m^2)	24.5	20	29
Disease duration (years)	4	18	2
Cutaneous subset	Diffuse	Limited	Limited
Autoantibody profile	Anti-RNA polymerase 3	No specific autoantibody	Anti-RNA polymerase 3
Interstitial lung disease	No	No	No
Pulmonary hypertension	No	No	No
Other comorbidities	High blood pressure Dyslipidaemia	Chronic renal insufficiency Pulmonary embolism	Thyroidectomy for goitre
Rituximab			
Date of the first infusion	07/17	01/15	01/18
Dose	500 mg/6 months	500 mg/8 months	1 g/8 months
Last infusion	01/20	06/19	10/19
B-cell depletion	Complete (February 2020)	Complete (February 2020)	Complete (January 2020)
Gammaglobulin (g/L)	7.0 (February 2020)	12.9 (February 2020)	9.5 (January 2020)
Associated treatments			
csDMARDs	Methotrexate (20 mg/week)	None	Methotrexate (15 mg/week)
Prednisone (mg/day)	5	5	2.5
COVID-19			
Day 0	01/04/20	12/04/20	15/03/20
Confirmation by RT-PCR	Not done	Yes	Yes
Compatible chest CT scan	Yes	Yes	Yes
First symptoms	Fever, cough, dyspnoea	Fever, diarrhoea, cough	Fever, cough, sore throat, myalgia
Day of clinical worsening	Day 19	Day 15	Day 23
Hospitalisation	Yes	Yes	Yes
Hospitalisation in ICU	Yes	No	Yes
Duration in hospitalisation/ICU	7 days	Still in the general ward	Still in the general ward
Ventilatory support	NIV	HFNC	CPAP
Antibiotics	Yes	Yes	Yes
Other specific treatment	No	Yes (anakinra, corticosteroids, tocilizumab)	No
Outcome	Favourable	Favourable	Favourable

CPAP, continuous positive airway pressure; csDMARD, conventional synthetic disease-modifying antirheumatic drug; HFNC, high-flow nasal cannula; ICU, intensive care unit; NIV, non-invasive ventilation; RT-PCR, reverse transcription PCR.

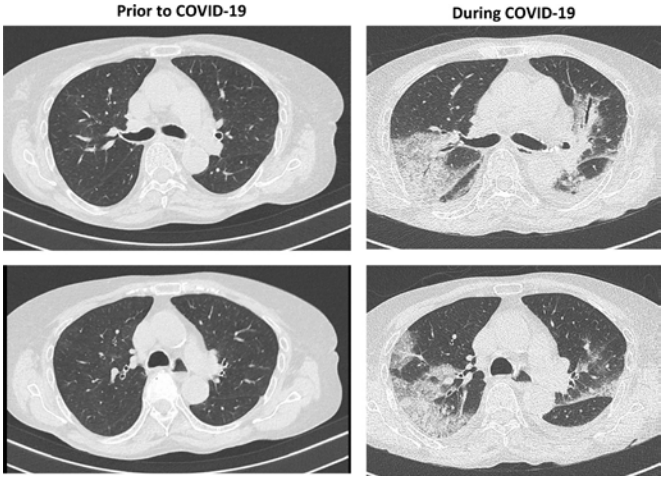

Figure 1 Representative images of chest high-resolution CT scan performed of patient 2, showing no pre-existing systemic sclerosis–associated interstitial lung disease and typical bilateral interstitial pneumonia related to COVID-19.

also have contributed to this secondary worsening. In these three patients, the routine RTX regimen with a complete B-cell depletion confirmed at least 2 months before COVID-19, but without severe hypogammaglobulinemia, might have been an additional risk of infection. Although the impact of RTX on infectious events remains to be clarified, two additional observations of COVID-19-related severe pneumonia on RTX maintenance therapy have been recently reported. The first case concerned a 32-year-old woman with SSc and pulmonary involvement treated with hydroxychloroquine and RTX. She developed a severe pattern of COVID-19 interstitial pneumonia requiring hospitalisation in intensive care, where, despite intubation and an attempt with tocilizumab, she died.[5] The second case, described by Guilpain *et al*, was a 52-year-old woman followed for granulomatosis with polyangiitis, who presented sudden COVID-19-related respiratory failure on day 18, requiring endotracheal intubation and mechanical ventilation, before its clinical condition secondarily improved. Therefore, altogether, these cases suggest that a careful follow-up is required for patients with autoimmune diseases treated by RTX. In particular, a specific attention should be given to the fact that these patients may experience a delayed progression, which need a careful monitoring.

Preliminary experience suggested that patients with chronic inflammatory rheumatic disorders receiving biologic or synthetic targeted disease-modifying anti-rheumatic drugs might not exhibit an increased risk of severe COVID-19.[6–8] However, these observations of severe and life-threatening form of COVID-19 support the continuous attention of patients with SSc under immunosuppressants. The launch of the EUSTAR COVID-19 registry (https://nettskjema.no/a/146481) will permit to obtain additional relevant information from a large number of patients and draw more robust conclusions.

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Response to: 'Severe COVID-19 associated pneumonia in 3 patients with systemic sclerosis treated with rituximab' by Avouac *et al*

We read with deep interest the comments by Avouac and colleagues[1] and their report of severe cases of COVID-19 in three patients with systemic sclerosis (SSc) under rituximab treatment. The heterogeneous profile of patients as well as the potential implication of comorbidities appear to be the hallmarks of this viral outbreak. Applied to the field of SSc, the absence of pre-existing interstitial pneumonia is an illustration of the viral ability to surprise and challenge our classic thinking. A singular profile of patients with both autoimmune disease and COVID-19 has not yet emerged, and each patient may be a special case when faced with COVID-19, considering the gathering and interplay of pathophysiological mechanisms and clinical features of the rheumatic disease, comorbidities,[2] viral aggression and immune response against coronavirus.[3] The weight of comorbidities is at least illustrated by the high number (until today: 323) of referenced papers on PubMed, while numerous risk factors are suspected and debated.[2] To date, large data concerning rituximab during the pandemic are lacking, and whether rituximab is associated with a specific risk of more severe COVID-19 is not yet established.

However, this is a reasonable possibility when considering the impairment of the numerous functions of B cells (particularly those related to humoral response) by rituximab, as commented by Monti *et al*.[4] While interesting data on T cell-specific responses are emerging,[5] antibody response remains crucial for neutralising virus, although higher antibody titres may be associated with bad outcome in some individuals,[3 4] possibly through the phenomenon of antibody-dependent enhancement (implicating non-neutralising virus-specific IgG). However, the delayed worsening (up to day 23) of COVID-19 in the rituximab-treated patients described by Avouac and colleagues[1] as well as in our patient[6] is intriguing and raises additional comments.

Indeed, the median duration from symptom onset to intensive care unit (ICU) is classically about 10 days.[7 8] The median time to ICU may depend on the cause of the worsening and varies from 8 to 15 days with a median of 12 days, in the series by Zhou *et al*.[7] In addition, heterogeneous presentation of COVID-19 as well as atypical symptoms (anosmia, ageusia, digestive, neurological, cutaneous manifestations and so on) make possibly difficult the dating of the very first symptom. Consequently, the date of worsening may be approximative in some patients from the general population published in literature studies, and thus delayed worsening might occur sometimes. In addition, our observations might be rather related to the specific recruitment of our departments, as a bias of selection. However, since B cells are essential in primary and secondary immune responses, the implication of rituximab should be further discussed.

First of all, it is noteworthy that the critical severity of COVID-19 is mainly related to the development of inflammatory cytokine storm, implicating interleukin (IL)-1, IL-6, tumour necrosis factor, interferons and many immune cells (monocytes, macrophages, T helper (Th) lymphocytes and antigen-presenting cells such as dendritic cells).[3] Notably, during a normal immune response and besides the production of autoantibodies, B lymphocytes also play the role of antigen-presenting cells, through the B cell receptor recognition and internalisation of antigens, and the processing and presentation of peptides to Th lymphocytes using major histocompatibility complex (MHC) class II molecules. So the presentation of coronavirus antigens might be impaired by rituximab and the activation of immune cells consequently delayed, holding up the onset of the cytokine storm. Furthermore, B cells also play key roles in cellular interactions. In their recent study, Wen and colleagues[9] observed that B cells could secrete IL-6 and thus initiate an inflammatory cascade involving T cells and monocytes, leading to the inflammatory cytokine production. Comparing early and late recovery states, the authors suggested that the interactions between immune competent cells may accelerate or delay the recovery from COVID-19.

Taken together, these elements suggest that the delayed worsening observed in our rituximab-treated patients may not occur by pure chance. Whether rituximab exhibits specific effects in COVID-19 (especially compared with other immunosuppressants) remains to be established. We can hope that future studies and national/international registries (in France, the French rheumatic and musculoskeletal diseases (RMD) COVID-19 cohort (FAI2R/SFR/SNFMI consortium) and its future contribution to the European League Against Rheumatism registry) will provide answers to the dramatic question of the tolerance for this immunosuppressive drug, as well as for the others.

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COVID-19 infection in a patient with FMF: does colchicine have a protective effect?

We read with great interest the report by Monti *et al* on the 320 rheumatic patients treated with various disease-modifying anti-rheumatic drugs (DMARDs) in the era of COVID-19 infection.[1] They suggest that patients with chronic arthritis receiving DMARDs may not have an increased risk of severe COVID-19. We agree that patients under DMARD treatment should be closely monitored since data are lacking. Also, we hypothesise that some DMARDs (especially colchicine) may protect rheumatic patients from COVID-19 or perhaps cause them to pass in a milder form of the disease. COVID-19 is not just a simple viral infection; it is an autoinflammatory/autoimmune process that develops as a result of immune system dysfunction, cytokine release syndrome and haemophagocytic lymphohistiocytosis.[2] Herein we reported COVID-19 infection in a patient with familial Mediterranean fever (FMF) under treatment with colchicine.

A 36-year-old male patient has been on follow-up with the diagnosis of FMF since 2008 and has been using colchicine. Obesity and hypertension are present as comorbid disease. He presented with complaints of widespread headache, back pain, muscle and joint pain, fatigue, and loss of taste and sensation, which started 5 days earlier. He did not describe fever, cough and sore throat. On physical examination, widespread tenderness was present in the joints and muscles, while systemic examination, fever and blood pressure were normal. Laboratory examinations revealed mild serum erythrocyte sedimentation rate, C reactive protein and ferritin elevation, and renal and liver function tests were normal. Leucopenia and lymphopenia on the complete blood count was detected. The patient who was a hospital staff and worked in a COVID-19 clinic was evaluated for a possible COVID-19 infection, and the real-time PCR test was positive. On radiological investigation, thorax CT was normal (figure 1). The patient was diagnosed with COVID-19 and treatment according to accepted protocol (hydroxychloroquine, azithromycin, oseltamivir) in our country was started. Colchicine was also continued. Marked regression in the patient's complaints after treatment was seen and control COVID-19 PCR test was negative.

COVID-19 is an acute viral infection that can involve predominantly the upper airway and lung. It acts by binding to ACE 2 (ACE2) receptors in target organs such as lung alveolar type 2 cells.[3] When COVID-19 is passed into the cell via ACE2, activation of NLRP3 inflammasome is triggered by immunological mechanisms. The presence of high NLRP3-induced pro-inflammatory cytokines (IL-1, IL-1β) in the serum of patients with COVID-19 supports this hypothesis.[4] Colchicine is an anti-inflammatory agent which inhibits the microtubule polymerisation on the cytoskeleton. Microtubules play an important role in cell migration, signal transduction and gene expression.[5] Colchicine acts on NLRP3 inflammasome resulting in inhibition of important signalling pathways involving intracellular secretion of cytokines and chemokines. It is estimated that one of the important pathogenic mechanisms of COVID-19 is through activation of NLRP3 inflammasome.[6] Considering the mechanism of action of colchicine, it would be rational to use it in patients with COVID-19 infection.[7] Our patient with FMF developed COVID-19 infection under treatment with colchicine. The patient was PCR positive for COVID-19 and has only mild symptoms of the disease (such as myalgia and arthralgia) but without fever or pneumonia development. Although we cannot draw any definitive conclusion from our observation, we hypothesise that colchicine may prevent a severe form of the disease. Prospective, randomised, placebo-controlled studies are needed in this regard.

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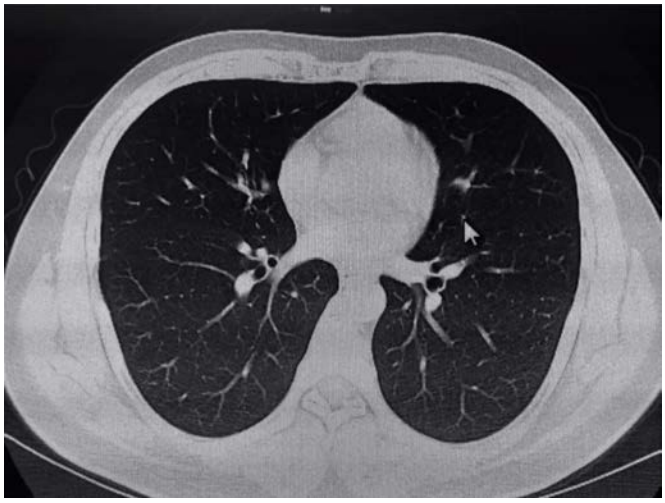

Figure 1 Thorax CT of patient with familial Mediterranean fever–COVID-19 showed normal finding.

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Candidate rheumatologic treatments for COVID-19. Response to: 'COVID-19 infection in a patient with FMF: does colchicine have a protective effect?' by Kobak

We appreciate the comment from Dr Kobak[1] to our previously published report on the clinical course and outcome of COVID-19 in a cohort of patients treated with biologic and targeted synthetic disease modifying antirheumatic drugs.[2] The author described the case of a patient with familial Mediterranean fever treated with colchicine who experienced a mild course of COVID-19. Although the effects of colchicine on the clinical course of COVID-19 on large-scale populations are still unknown, colchicine is one of the numerous rheumatologic treatments being tested against severe acute respiratory syndrome coronavirus-2, given its effects on the inflammasome and interleukin and cytokine activation.[3] To date, there are at least 11 studies registered on clinicaltrials.gov to test the effects of colchicine on COVID-19. While we await for the results of these trials, the report by Dr Kobak offers further reassuring impressions on the clinical course of COVID-19 in patients treated with various types of antirheumatic agents.

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COVID-19 and Behçet's disease: clinical case series

We read with interest the study of Monti *et al*,[1] the first rheumatic disease cases with COVID-19. In detail, the authors described the clinical course of COVID-19 in a series of 11 patients with rheumatoid arthritis, one with psoriatic arthritis and one with spondyloarthritis treated with immunosuppressive targeted therapies. Here, we describe the main characteristics of four patients with Behçet's disease (BD) with COVID-19.

Data on patients with systemic autoimmune diseases with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection are currently lacking. Data from the first 110 patients included in the COVID-19 Global Rheumatology Alliance and the European League Against Rheumatism (EULAR)–COVID-19 Database have been recently published.[2]

Here we describe, to our knowledge, the first single-centre experience of COVID-19 in patients who fulfilled the international criteria for BD,[3] including clinical characteristics, antiviral and immunomodulatory treatment, and outcomes. All patients gave informed consent for publishing their clinical data. We used nasopharyngeal swab samples for all diagnoses, amplifying the betacoronavirus E gene and the specific SARS-CoV-2 RdRp gene by PCR.

On 16 April 2020, 2135 consecutive patients with SARSCoV-2 infection had been admitted to Hospital Clínic de Barcelona, Barcelona, Catalonia, Spain. We admitted 238 (11%) into intensive care units and we discharged 1481 (67%) with supervised outpatient care. Of all patients, four (0.19%, 95% CI 0.05–0.48) had BD (table 1), of whom three were admitted to the hospital. Two of the patients were nurses and have had contact with patients with COVID-19. Only one of the patients with BD had comorbidities, and in all of them, disease activity, measured with Behçet's Disease Activity Index (BDAI)[4] at the time of first COVID-19 symptoms, was low (BDAI score of <3). Three patients had upper respiratory infection and one had viral pneumonia. No patient required admission to the intensive care unit or invasive and non-invasive mechanical ventilation. In other words, the severity of COVID-19 infection was mild in all cases.

Anti-SARS-CoV2 treatment (hydroxychloroquine, lopinavir-boosted ritonavir and azythromicin) was administered to the three patients admitted to the hospital on the day of diagnosis. Due to diarrhoea, protease inhibitors were discontinued in two of them. In all patients, COVID-19 resolved without complications. Regarding BD status, one patient presented with a flare during COVID-19 (patient 2) and another patient presented with it after 15 days of COVID-19 resolution (patient 4). In both cases, BD activity improved with colchicine.

Our case series of patients with BD deserves some comments. First of all, patients with BD accounted for 0.19% of patients with COVID-19 who required admission to Hospital Clinic de Barcelona. Of the first 110 patients included in the COVID-19 Global Rheumatology Alliance and the EULAR–COVID-19 Database, 7% had vasculitis.[2] Unfortunately, the authors have not described in detail the type of vasculitis. Second, people on immunosuppressive treatment are more prone to infections. However, no specific data exist to suggest that medication-induced immunosuppressed state predisposes patients to SARS-CoV-2 infections or to more severe forms of COVID-19. Of note, all patients with BD had a COVID-19 clinical picture resembling the general population, and the severity of COVID-19 infection was mild in all cases. Two of our patients were receiving immunosuppressive agents at COVID diagnosis. Third, colchicine is the drug of

Table 1 Demographics, clinical characteristics at admission, laboratory features, treatment and outcomes of four patients with BD and COVID-19

	Patient 1	Patient 2	Patient 3	Patient 4
Demographic and BD status				
Age (years)	40	51	37	47
Gender	Female	Female	Female	Female
Comorbidities	None	Breast cancer	None	None
BD manifestations				
Oral aphthosis	Yes	Yes	Yes	Yes
Genital aphthosis	Yes	Yes	Yes	Yes
Ocular lesions	Yes	No	Yes	No
Vascular manifestations	Yes	No	No	No
Neurological manifestations	No	No	Yes	No
IMT before admission	PDN 5 mg/day MTX 20 mg/week	COL 1 mg/day	PDN 7.5 mg/day AZA 100 mg/day COL 0.5 mg/day	Pentoxifiline 400 mg/day
Duration of symptoms (days)	6	4	2	7
Clinical manifestations (at admission)				
Temperature	Fever (39°C)	Fever (38.5°C)	Fever (38.5°C)	Fever (38.5°C)
Symptoms	Cough, malaise, diarrhoea, headache	Cough, malaise, sore throat, headache	cough	Cough, malaise, sore throat, anosmia, ageusia, headache
Thrombosis	No	No	No	No
O_2 saturation (in ambient air)	SpO_2 95%	SpO_2 95%	SpO2 96%	ND
Chest X-ray findings	Left basal ground-glass opacity	Normal	Normal	Normal
Laboratory results*				
White blood cell count (×10⁶/L)	10 000	2340	6250	ND
Lymphocyte (×10⁶/L)	3200	870	1380	ND
Platelets (×10⁶/L)	685 000	158 000	331 000	ND
LDH (U/L)	207	168	155	ND
C reactive protein (mg/dL)	2.7	0.4	0.91	ND
Ferritin (ng/mL)	295	152	130	ND
D-dimer (ng/mL)	357	400	800	ND
Procalcitonin (ng/mL)	0.05	<0.03	ND	ND
Treatments				
IMT during admission	MTX stopped	COL stopped	PDN, AZA, COL	None
Lopinavir/ritonavir	Yes	Yes	Yes	No
Hydroxychloroquine	Yes	Yes	Yes	No
Azithromycin	Yes	Yes	Yes	No
Corticosteroids	Same dose	No	Same dose	No
Tocilizumab	No	No	No	No
LMWH	No	No	No	No
Others	Ceftriaxone	No	Ceftriaxone	No
Outcomes				
Admitted to an intensive care unit	No	No	No	No
Invasive or non-invasive mechanical ventilation	No	No	No	No
Length of hospital stay (days)	8	6	6	No hospital admission
Length of home hospitalisation (days)†	18	–	8	15

Continued

Table 1 Continued

	Patient 1	Patient 2	Patient 3	Patient 4
Outcomes	Cured	Cured	Cured	Cured
Comments	No BD flare during or after COVID-19 infection MTX reinitiated at 3 weeks	BD flare during COVID-19 infection (oral aphthosis and erythema nodosum) COL reinitiated at 6 days	No BD flare during or after COVID-19 infection	BD flare 15 days after COVID-19 infection (oral and genital aphthosis) COL initiated at 15 days

Lopinavir-boosted ritonavir was given as 400 mg of ritonavir boosted with 100 mg of lopinavir twice a day for 14 days; azithromycin was given as 500 mg once a day, with a loading dose on the first day, and then 250 mg once a day for 4 days; hydroxychloroquine was given as 400 mg twice a day with a loading dose on the first day and then 200 mg twice a day for 4 days.
*Worst laboratory result.
†Discharged with a supervised home-care programme.
AZA, azathioprine; BD, Behçet's disease; COL, colchicine; IMT, immunomodulatory treatment; LDH, lactate dehydrogenase; LMWH, low-molecular-weight heparin; MTX, methotrexate; ND, not done; PDN, prednisone.

choice for the prevention of recurrent mucocutaneous lesions of BD.[5] Due to its anti-inflammatory properties by preventing the activation of pro-IL-1ß into active IL-1ß, it could be established as a treatment for patients with COVID-19.[6] Two of our patients had been treated with colchicine at COVID-19 diagnosis. The potential protective role of disease-modifying antirheumatic drugs and immunomodulatory agents in COVID-19 infection is unknown. By generating information such as what we have presented here, the management and prognosis of patients with BD and SARS-CoV-2 might be improved.

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Myositis as a manifestation of SARS-CoV-2

We read with great interest the paper from Monti *et al* describing clinical course of coronavirus disease 2019 (covid-19) in patients with chronic arthritis. We would like to emphasise that symptoms mimicking connective tissue disease can occur at the early phase of covid-19 infection.[1]

Despite the fact that myalgia has been already reported in several cohorts of patients with covid-19 infection,[2] myositis was not described in these studies. We report a case of a MRI-documented myositis secondary to covid-19 in a patient. The patient was not under medication prior to the illness. Symptoms appeared suddenly on waking with diffuse myalgias and proximal lower limb muscle weakness, causing him to fall. On arrival at the hospital, the patient was afebrile and did not present any upper or lower airway symptoms. Motor testing revealed a bilateral hip flexion deficit graded at 3/5 on the Medical Research Council (MRC) muscle scale. Initial blood work-up revealed creatine kinase (CK) at 25 384 IU/L (n <195 IU/L), C reactive protein at 54 mg/L and a lymphocytopaenia. Initial management consisted of administration of intravenous fluids.

On day 4 after the appearance of symptoms, the patient presented with fever at 39°C. Blood and urine cultures were negative, and nasopharyngeal swab multiplex PCR for respiratory viruses, not including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was also negative. A chest CT scan on day 5 showed bilateral lower lobe ground-glass opacities. On day 7, the patient desaturated and required oxygen at 1 L/min, and a SARS-CoV-2 nasopharyngeal swab was negative. A proximal lower limb MRI showed bilateral external obturator muscle and quadricipital oedema (figure 1), compatible with bilateral myositis. Specific overlap myositis, dermatomyositis, immune-mediated necrotising myositis and antinuclear antibody testing were negative. On day 10, the patient's respiratory status worsened and a ventral chest CT scan showed worsening of bilateral ground-glass opacities. A second specific SARS-CoV-2 nasopharyngeal swab still remained negative. The patient was transferred to the intensive care unit on day 11, where bronchoalveolar lavage fluid was finally positive for SARS-CoV-2. The patient still remains in critical condition.

The prevalence of myalgia varies between 11% and 50% in different studies[2–5] and muscle weakness related to covid-19 has been reported; however to our knowledge, this is the first MRI documentation of such myositis.

In the Guan *et al* study, two patients had rhabdomyolysis (0.2%) and the CK levels were elevated in 13.7% patients.[3] One study showed statistical association between elevated CK levels and mortality.[6] As observed in autoimmune myositis, an association between myositis and myocarditis could explain this excess in mortality. Indeed, some studies reported elevation of N-terminal pro-brain natriuretic peptide (NT-pro-BNP) and troponin.[7]

In our patient, the subsequent association of myositis followed by interstitial pneumonitis led to the hypothesis of autoimmune myositis but all the immunological tests looking for any forms of myositis were negative.

In conclusion, covid-19 manifestations, although frequently limited to upper and lower airways, can, as shown in our case, reveal itself by acute myositis. Since the association of muscle inflammation with interstitial pneumonia can be seen in either covid-19 or autoimmune myositis, this differential diagnosis should be known by clinicians.

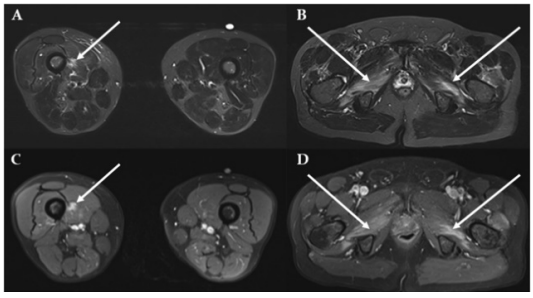

Figure 1 Pelvic and thigh MRI. (A) Thigh MRI in T2 STIR sequence showing oedema of the right vastus medialis (arrow). (B) Pelvic MRI in T2 STIR (short TI inversion recovery) sequence showing bilateral oedema of external obturator muscles (arrows). (C and D) T1 sequences revealing enhancement of muscle lesions after gadolinium infusion (arrows).

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MB and KC are co-first authors.

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Rheumatic disease and COVID-19

We appreciated the letter from Monti *et al*, which was the first dedicated report of patients with rheumatic disease who have been diagnosed with COVID-19.[1] We have also reviewed the response from Joob and Wiwanitkit,[2] which unfortunately perpetuates the notion that individuals with systemic lupus erythematosus (SLE) or other rheumatic disease may be protected from COVID-19 infection via hydroxychloroquine use. Although the origins of this claim are not entirely clear, they may arise from the fact that rheumatic or autoimmune diseases were not initially reported among other comorbidities in the first large Chinese case series.[3–5] Some of the subsequent publications have also not reported these conditions in their tables of baseline characteristics.[6–8]

However, as the aphorism goes, 'the absence of evidence is not evidence of absence'. Instead, we have to consider the following: Were these comorbidities searched for, but not found? Or were they not included in the search at all? The latter is understandable and should not automatically be discounted as a methodological oversight. While the prospective collection of granular data would have been ideal, this was likely precluded by the sheer volume and urgency of medical care delivered during the early stages of the pandemic. With the global medical and scientific community awaiting clinical data, those first publications were eagerly welcomed for the information that they could provide on the exploding crisis.

It is worth noting that this paucity of data on the rheumatic disease population ultimately prompted the rheumatology community to form a global case registry.[9] In a report of the initial 110 patients from this registry, there were 19 with SLE who had been diagnosed with COVID-19.[10] In the USA especially, widespread testing has been delayed and has likely resulted in lower counts of COVID-19 cases.[11] Disparities in healthcare access, which are well documented in US patients with SLE,[12] may have further potentiated this under-reporting. Further data from rheumatology-specific registries are forthcoming, especially as confirmed case numbers continue to rise. We expect that we will learn more about the impact of COVID-19 on people living with rheumatic disease in due course.

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Correction notice This article has been corrected since it published Online First. The second affiliation has been corrected.

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COVID-19 in rheumatology outpatient clinics: Dutch mirror image to Lombardy, Italy

In response to the article published by Monti *et al*[1] regarding the clinical course of COVID-19 in a series of patients with chronic arthritis treated with immunosuppressive targeted therapies, we started to collect similar data from our patients with chronic rheumatic disease as well, to be able to aid in providing preliminary data on how severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) impacts our patients with immunosuppressive therapies. The first officially reported case in the Netherlands originates from 27 February in the province of North Brabant.[2] A few weeks later, after community screening and case identification, it came apparent that the virus had to be present in the community already 2 weeks before the first official case was described. Two and a half million people inhabit North Brabant. In this province, Carnival was celebrated between 21 and 26 February. Carnival is a public celebration in regions of Catholic descent, involving parades and street parties in which at least 200 000 people participate in various cities and villages. It is thought that these public celebrations allowed SARS-CoV-2 to rapidly spread in communities, making North Brabant, together with Limburg (an adjacent province), the regions with the highest number of infections in the Netherlands. Our hospital is situated in Eindhoven and Veldhoven, in the province of North Brabant. The number of proven SARS-CoV-2 infections, COVID-19-associated hospital admissions and COVID-19-related deaths is recorded for the general population per municipality and made public by the Dutch National Institute For Public Health and Environment. During the period of 1 March 2020–25 April 2020, we recorded the data of all patients who contacted our clinic with positive reverse transcription (RT)-PCR tests and questions about treatment, as well as the patients with a chronic rheumatic disease who were admitted to the emergency room or clinical wards. This allows for a preliminary comparison between the number of patients from our outpatient clinic that are admitted for severe SARS-CoV-2 disease and the number of admissions in the general population surrounding our hospital.

In total, we identified 27 patients of which 19 tested positive by RT-PCR in our centre, and others were deemed to be positive on the basis of a family member with confirmed SARS-CoV-2 and typical symptoms (eg, bilateral pneumonia, dyspnoea and dry cough) or had a positive test result confirmed by their general practitioner. The characteristics of all the patients are provided

Table 1 Characteristics of patients with COVID-19 in perspective of the total outpatient population

	N total outpatient population	COVID+ patients (n) (% of COVID+, % of total care population)	Non surviving COVID+ patients (n) (% of total deaths)
Patients	7600	27 (100, 0.4)	6
Age (years), mean (SD)	65.5 (12.1)	68 (14)	75 (9)
Rheumatoid arthritis	3314	17 (63.0, 0.5)	6 (100)
Psoriatic arthritis and peripheral spondyloarthritis	1306	6 (22.0, 0.4)	0
Axial spondyloarthritis	581	0	0
Gout and CPPD	563	2 (7.0, 0.4)	0
Polymyalgia	501	5 (18, 1)	0
Osteoarthritis	262	0	0
Systemic autoimmune diseases (SSc, MCTD, SSj and myositis)	179	0	0
Systemic lupus erythematodes	160	0	0
Giant cell arteritis	45	0	0
Other	689	1 (4, 0.15)	0
Methotrexate	–	16 (60, –)	6 (100)
Prednisolone	–	6 (22, –)	1 (17)
Sulfasalazine	–	2 (7, –)	1 (17)
Leflunomide	–	1 (4, –)	0
Hydroxychloroquine	–	5 (18, –)	0
Anti-TNF alpha	–	4 (15, –)	1 (17)
Jak inhibitors	–	1 (4, –)	0
Other	–	1 (4, –)	6 (100)
Female	–	10 (37, –)	0
Male	–	17 (63, –)	6 (100)
Recovered	–	12 (45, –)	0
Not recovered, alive	–	9 (33, –)	0
Not recovered, dead	–	6 (22, –)	6 (100)
BMI>25	–	14 (52, –)	2 (33)
Hospital admission	–	14 (52, –)	4 (67)
Oxygen suppletion	–	11 (40, –)	3 (50)
Intensive care unit admission	–	2 (7, –)	0
Ventilator support	–	2 (7, –)	0
Comorbidity (malignancy, chronic lung disease, cardiovascular disease and diabetes)		12 (45, –)	6 (100)

BMI, Body Mass Index; CPPD, calcium pyrophophate disease; MCTD, mixed connective tissue disease; SSc, systemic sclerosis; SSj, Sjögrens syndrome; TNF, tumour necrosis factor.

in table 1. Since most patients are admitted to their local hospitals, the number of SARS-CoV-2 admissions is a reliable number for our population.

The number of SARS-CoV-2-related hospital admissions in the general population of the municipalities surrounding our hospital is between 60 and 325 per 100 000 inhabitants (0.06%–0.32%). In our population of 7600 patients with chronic rheumatic disease, 14 were admitted to the hospital due to SARS-CoV-2 infection (0.18% of the total outpatient population), which is very similar to the general population.

Of the 27 patients who were identified, 6 died; all of the patients were male and suffered from rheumatoid arthritis and were treated with methotrexate. Three of the deceased patients were not treated for COVID-19, out of their own personal beliefs of passing away at old age or their wish to stay at home. One of the patients who was admitted and died had pre-existent severe dilating cardiomyopathy and emphysema with underlying malignancy; one patient suffered from chronic pulmonary obstructive disease and pre-existent lung disease due to rheumatoid arthritis and sarcoidosis. The sixth patient had a previous diagnose of complicated diabetes and obesity.

The total number of 27 SARS-CoV-2-positive patients out of 7600 (0.4%) is similar to that of the general population in the municipalities of our region, 120–900 per 100 000 inhabitants (0.12%–1%). The same holds true for the number of deaths: 0.08% in our population vs 0.041%–0.195% in the general population. Due to the restricted testing policy in the Netherlands, only patients with severe symptoms or healthcare workers were tested initially; in addition, patients in nursing homes were not systematically tested initially. Hence, the numbers on deaths and prevalence of COVID-19 in the general population are thought to be underestimated, and the comparison with our outpatient population warrants caution.

In line with the findings from Monti *et al*, we do not see a clear increased risk of complications requiring hospital admission for patients undergoing immunosuppressive treatment as compared with the general population in the municipality, even when taking into account that the patients in our outpatient clinic have a higher age as compared with the general population at risk in the municipalities in our region. We have to note, however, that our data are very preliminary and underpowered, and no definite conclusions can be drawn from our findings. Especially the number of out-of-hospital deaths and the full scale of patients with light symptoms that do not contact the hospital are not fully known to us. It is nevertheless reassuring to see that similar observations are made in Italy and the Netherlands for hospital admissions. As mentioned by Monti *et al* and underscored by Professor Dr McInnes,[3] further large international efforts such as the EULAR-COVID-19 database are pivotal to provide further information of the impact of SARS-CoV-2 on our patient populations.

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3 McInnes IB. COVID-19 and rheumatology: first steps towards a different future? *Ann Rheum Dis* 2020;79:551–2.

Correction: *Pragmatic randomised controlled trial of very early etanercept and MTX versus MTX with delayed etanercept in RA: the VEDERA trial*

Emery P, Horton S, Dumitru RB, *et al*. Pragmatic randomised controlled trial of very early etanercept and MTX versus MTX with delayed etanercept in RA: the VEDERA trial. *Ann Rheum Dis* 2020;79:464–71.

Abstract: results section, line 6 should read as "PD was fully suppressed by week 48 in 74-87%…" as opposed to currently stated "PD was fully suppressed by week 48 in over 90%…"

Results and 'Imaging outcomes', para 2:

a. line 3: This should read "Over 60% in each arm…" as opposed to "Over 50% in each arm…"
b. line 4–5 should read, "….by week 12 to 24%–37% in each arm, further reduced to 13%–26% by week 48…" as opposed to "….by week 12% to 15% in each arm, maintained by week 48…"

Discussion, para 4, line two should read "…US PD suppressed in both arms to 13%–26%…" as opposed to "…US PD suppressed in both arms to <13%…"

Table 1 (baseline characteristics) and table 4 (Total grey scale and Power Doppler ultrasound scores) have been updated with the correct values.

Table 1 Baseline demographics and disease profile for the entire group, ETN+MTX and MTX-TT

Variable	All	ETN+MTX	MTX-TT
Demographics			
Age, years Mean (SD)	50.0 (12.8)	49.6 (12.5)	50.3 (13.2)
Female % (n/N)	71% (85)	65% (39)	77% (46)
RA presenting history, % (n/N) (unless otherwise stated)			
Symptom duration, weeks, median (Q1, Q3)	20.3 (13.1, 30.8)	19.2 (12.5, 28.1)	20.8 (15.9, 31.9)
Previous IM steroid	1% (1/120)	0% (0/60)	2% (1/60)
Previous IA steroid	0% (0/120)	0% (0/60)	0% (0/60)
Concomitant oral steroid	3% (3/120)	0% (0/60)	5% (3/60)
Concomitant NSAID	88% (105/120)	92% (55/60)	83% (50/60)
RA disease phenotype, % (n/N)			
RF positive	73% (87/120)	70% (42/60)	75% (45/60)
ACPA positive	84% (101/120)	82% (49/60)	87% (52/60)
ANA positive	15% (18/120)	18% (11/60)	12% (7/60)
RA disease activity components, Median (Q1, Q3) (unless otherwise stated)			
TJC28	11.0 (7.0, 17.0)	11.5 (6.0, 20.0)	10.0 (7.0, 16.0)
SJC28	5.0 (2.0, 9.0)	5.0 (3.0, 10.5)	5.0 (2.0, 9.0)
ESR, mm/hr	31.5 (18.5, 51.0)	30.5 (17.0, 51.5)	32.5 (20.5, 51.0)
CRP, mg/L	8.8 (2.3, 24.0)	10.2 (1.8, 28.0)	8.0 (2.7, 21.5)
Disease activity VAS, mm Mean (SD)	57.1 (22.3)	60.7 (21.6)	53.6 (22.6)
RA disease activity scores, Mean (SD)			
DAS28-ESR	5.7 (1.1)	5.8 (1.1)	5.6 (1.0)
DAS44-ESR	3.7 (0.8)	3.7 (0.9)	3.7 (0.7)
DAS28-CRP	5.1 (1.2)	5.2 (1.2)	4.9 (1.1)
DAS44-CRP	3.4 (0.8)	3.5 (0.9)	3.3 (0.8)
SDAI	31.6 (13.7)	34.2 (14.7)	29.0 (12.3)
CDAI	29.8 (12.7)	32.2 (13.6)	27.3 (11.2)
Patient-reported outcome measures, Mean (SD) (unless otherwise stated)			
Global pain VAS, mm	53.5 (24.5)	59.0 (23.4)	48.1 (24.6)
HAQ-DI	1.2 (0.5)	1.2 (0.5)	1.1 (0.5)
RAQoL	17.3 (7.3)	16.8 (7.4)	17.9 (7.2)
In paid work % (n/N)	73% (88/120)	82% (49/60)	65% (39/60)
EQ5D-3L index	0.5 (0.3)	0.4 (0.3)	0.5 (0.3)
RAWIS	18.2 (6.6)	19.0 (6.7)	17.3 (6.4)
Ultrasound scores Median (Q1, Q3)			
Total GS score	4.0 (2.0, 6.0)	34.0 (2.0, 7.0)	3. 5 (1.05, 6.0)
Total PD score	2.0 (0.0, 4.0)	2.0 (0.0, 4.05)	02.0 (0.0, 3.0)
Total erosion score	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)
Radiographic score Median (Q1, Q3)			
Total modified Sharp score	2.5 (0.5, 6.0)	2.0 (0.5, 5.0)	2.5 (0.5, 6.3)

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