# AUTOIMMUNE AND INFLAMMATORY RHEUMATIC DISEASES: IDENTIFYING BIOMARKERS OF ESPONSE TO THERAPY WITH BIOLOGICS

EDITED BY: Anna Lisa Giuliani, Alessandra Bortoluzzi, Francesca Oliviero and Maria Efthymiou

PUBLISHED IN: Frontiers in Pharmacology

frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88974-224-0
DOI 10.3389/978-2-88974-224-0

## About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

## Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

## Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

## What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

# AUTOIMMUNE AND INFLAMMATORY RHEUMATIC DISEASES: IDENTIFYING BIOMARKERS OF RESPONSE TO THERAPY WITH BIOLOGICS

Topic Editors:
**Anna Lisa Giuliani,** University of Ferrara, Italy
**Alessandra Bortoluzzi,** University of Ferrara, Italy
**Francesca Oliviero,** University of Padua, Italy
**Maria Efthymiou,** University College London, United Kingdom

**Citation:** Giuliani, A. L., Bortoluzzi, A., Oliviero, F., Efthymiou, M., eds. (2022). Autoimmune and Inflammatory Rheumatic Diseases: Identifying Biomarkers of Response to Therapy With Biologics. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88974-224-0

# Table of Contents

frontiers in Pharmacology

EDITORIAL
published: 10 December 2021
doi: 10.3389/fphar.2021.815656

# Editorial: Autoimmune and Inflammatory Rheumatic Diseases: Identifying Biomarkers of Response to Therapy With Biologics

*Anna Lisa Giuliani*[1]*, *Alessandra Bortoluzzi*[1], *Maria Efthymiou*[2] and *Francesca Oliviero*[3]

[1]Department of Medical Sciences, University of Ferrara, Ferrara, Italy, [2]City of London College, London, United Kingdom, [3]Department of Medicine - DIMED, University of Padua, Padua, Italy

**Keywords: autoimmune diseases, inflammatory rheumatic diseases, biomarkers, therapies, biologic drugs**

## Editorial on the Research Topic

### Autoimmune and Inflammatory Rheumatic Diseases: Identifying Biomarkers of Response to Therapy With Biologics

The scope of this research topic was to provide an updated overview of the advances in the identification of new biomarkers of response to biologic drugs in autoimmune and inflammatory rheumatic diseases.

The use of biologic agents in autoimmune and inflammatory rheumatic diseases, has greatly increased in recent years, providing new treatment options to conventional anti-inflammatory and immunosuppressive strategies. Biologic drugs very often show selectivity of action towards specific targets and are currently used to induce disease remission or treat specific organ involvements in various inflammatory and autoimmune diseases. Given the variability in biological and clinical response between patients and the lack of knowledge in the mechanisms influencing individual responses, it has become urgent to identify biomarkers of response to therapy, especially to individuate patients who may potentially benefit from these therapies. This also in view of the possible role of biomarkers as indicators of personalized treatment. This research topic contains description of various diseases treated with biological drugs in 11 articles: 4 reviews, 1 systematic review, 5 original research papers, and 1 clinical trial.

Systemic lupus erythematosus (SLE) is one of the most severe among autoimmune rheumatic diseases characterized by complex and not well known pathogenesis and involvement of a high number of body organs. A study included in this research topic from Piantoni et al. (Front. Pharmacol. 2021 May 21; 12:666971) has reported the characterization of circulating peripheral B and T lymphocytes and the evaluation of soluble B-cell related factors belonging to the TNF/TNFR superfamily in a cohort of clinically active SLE patients treated with belimumab. It was found that the baseline BAFF serum levels are the strongest predictor of response to belimumab after 12 months of therapy.

Rheumatoid arthritis (RA) is another common autoimmune rheumatic disease characterized by involvement at joints and, in more severe cases, of different organs with increased mortality. RA has been dealt with in four papers of our research topic. A phase Ib/IIa clinical trial from Tang et al., has investigated the pharmacokinetics and pharmacodynamics of WBP216, a long half-life fully human monoclonal antibody against interleukin (IL)-6, in a limited number of Chinese RA patients to optimize the dosage regimen for future clinical trials (Front Pharmacol. 2021 Feb 18; 12:617265). Another study from Li et al. aimed to illustrate the regulation of Treg cells by arsenic trioxide ($As_2O_3$) in the pathogenesis of early RA. The researchers identified significantly modulated pathways and/or



***Edited and reviewed by:***
*Paola Patrignani,*
*University of Studies G.d'Annunzio Chieti and Pescara, Italy*

***Correspondence:**
*Anna Lisa Giuliani*
*a.giuliani@unife.it*

***Specialty section:***
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

***Received:*** *15 November 2021*
***Accepted:*** *25 November 2021*
***Published:*** *10 December 2021*

***Citation:***
*Giuliani AL, Bortoluzzi A, Efthymiou M and Oliviero F (2021) Editorial: Autoimmune and Inflammatory Rheumatic Diseases: Identifying Biomarkers of Response to Therapy With Biologics. Front. Pharmacol. 12:815656. doi: 10.3389/fphar.2021.815656*

functional categories of genes regulating metabolism in Treg cells, a finding that might be important in the treatment-naïve RA (Front Pharmacol. 2021 May 24; 12:656124). Alternative approach with respect to conventional anti-inflammatory and immunomodulatory drugs is presented in the review from Cai et al. which summarized the roles of the main enzymes and their derivatives involved in the pathogenesis of RA and explored the explicit and potential targeted actions of herbal medicinal products with anti-RA activity (Front Pharmacol. 2021 Mar 16; 12:626342). A new perspective in RA treatment can be inferred by the data reported in the paper from Xiao et al. In this study, an animal model of RA, the collagen-induced arthritis (CIA) model, has been used to evaluate alterations in the gut microbial communities in the ileum and cecum of CIA and control rats including microbial richness, diversity and taxa as well as the expression of IL-1β and IL-17A in the ileum and cecum of CIA rats. The ileal microbiota of CIA rats presented main alterations together with significant increase in IL-1β and IL-17A mRNA expression, consistently with the immune-mediated inflammatory features of CIA (Fron Pharmacol. 2020 Nov 24; 11:587,534).

A review from Choida et al. (Front Pharmacol 2021 Feb 2; 11: 635,823.) presented the results of a comprehensive search in the literature to identify clinical, serological, genetic, cellular, and imaging biomarkers that can assist clinicians in efforts to personalize disease-modifying anti-rheumatic drugs (DMARDs) prescription and adjust treatment strategies for Juvenile idiopathic arthritis (JIA) patients, with particular attention to studies investigating etanercept treatment in the most severe JIA phenotype. The conclusion of this study is that, although there are no ideal biomarkers in JIA, serological biomarkers with potential clinical utility have been identified and strategies of combining biomarkers of response to biologics in JIA is suggested.

A narrative review by Silvagni et al. (Front Pharmacol. 2021 Jun 15; 12:672515) provided an up-to-date overview of targeted therapies and treatment response biomarkers in psoriatic arthritis. This review focused on TNF-α-, IL-23/IL-17-, and JAK/STAT-dependent signal transduction axes, in the optics to define the relationship among different activated proinflammatory processes suitable for targeting by different currently available drugs.

Dermatomyositis (DM) is a rare autoimmune disease defined as an idiopathic inflammatory myopathy characterised by cutaneous manifestations. The study from Li et al. (Front Pharmacol. 2021 Sep 17; 12:727901), identified the profiles of noncoding RNAs, both lncRNAs and miRNAs, carried in exosomes (EXOs) from peripheral neutrophils of DM patients. This revealed a high number of upregulated and downregulated noncoding RNAs in DM neutrophil EXOs. The study also explored their potential functional roles and utility as new biomarkers and therapeutic targets.

A study from Lu et al. (Front Pharmacol. 2021 Mar 16; 12: 635654) has evaluated the efficacy of tocilizumab (TCZ) in adult patients with refractory immune-mediated necrotizing myopathies and investigated possible predictive biomarkers of the response to treatment with TCZ. Baseline serum IL-6 and muscle IL-6 mRNA levels and the percentage of CD56-positive muscle fibres have been found useful to predict the response to TCZ treatment in these patients.

Non-infectious uveitis (NIU) is believed to be an immune-mediated ocular inflammation frequently associated to systemic autoimmune diseases such as JIA. The systematic review from Li et al. (Front Pharmacol. 2021 Apr 26; 12:673984) summarised the current evidence from randomised controlled trials regarding the efficacy and safety of adalimumab (ADA) treatment in NIU. Metanalysis performed on seven randomized controlled trials (RCTs) confirmed that ADA considerably lowered the risk of treatment failure or visual loss as compared to placebo, leading to the conclusion that ADA is both effective and safe in treating NIU.

Biologic therapies are employed in many inflammatory and immune mediated diseases, such as psoriasis, a chronic inflammatory disease characterised by erythematous scaly plaques frequently accompanied by systemic damages. The review from Shi et al. explored the most life-threatening associations between psoriasis and cardiometabolic comorbidities (cardiovascular diseases, obesity, diabetes mellitus, and metabolic syndrome), emphasizing benefits and precautions of biologic therapy in the management of psoriatic patients with cardiometabolic comorbidities and outlining the positive effects of different biologics on cardiovascular biomarkers (Front Pharmacol. 2021 Nov 4;12:774808).

In conclusion, this research topic explored and summarised many different aspects of therapies with biological treatments in inflammatory and immune mediated diseases. The effects of monoclonal antibodies (belimumab, ADA, TCZ, etanercept, and WBP216) as well as chemical compounds (arsenic trioxide) and medicinal herbs have been evaluated in addition to the investigation of different aspects influencing autoimmune and inflammatory diseases ranging from non-coding RNA in EXOs to gut microbial communities' activity.

## AUTHOR CONTRIBUTIONS

AG and FO wrote the editorial and AB and ME revised it.

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 24 November 2020
doi: 10.3389/fphar.2020.587534

# Regional Differences in the Gut Microbiota and Gut-Associated Immunologic Factors in the Ileum and Cecum of Rats With Collagen-Induced Arthritis

*Huihui Xu[1†], Jinfeng Cao[1†], Xiaoya Li[2,3†], Xiangchen Lu[2,4], Ya Xia[2,4], Danping Fan[2,3], Hongyan Zhao[1]*, Dahong Ju[1]* and Cheng Xiao[2,5]**

[1] Beijing Key Laboratory of Research of Chinese Medicine on Prevention and Treatment for Major Diseases, Experimental Research Center, China Academy of Chinese Medical Science, Beijing, China, [2] Institute of Clinical Medicine, China-Japan Friendship Hospital, Beijing, China, [3] The Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences/Peking Union Medical College, Beijing, China, [4] School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China, [5] Department of Emergency, China-Japan Friendship Hospital, Beijing, China

OPEN ACCESS

**Edited by:**
Alessandra Bortoluzzi,
University of Ferrara, Italy

**Reviewed by:**
Mario M. Zaiss,
University Hospital Erlangen, Germany
Muxin Gong,
Capital Medical University, China

***Correspondence:**
Hongyan Zhao
zhaohongyan1997@163.com
Dahong Ju
judahong@126.com
Cheng Xiao
xc2002812@126.com

†These authors have contributed equally to this work

**Specialty section:**
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

**Received:** 26 July 2020
**Accepted:** 15 October 2020
**Published:** 24 November 2020

**Citation:**
Xu H, Cao J, Li X, Lu X, Xia Y, Fan D, Zhao H, Ju D and Xiao C (2020) Regional Differences in the Gut Microbiota and Gut-Associated Immunologic Factors in the Ileum and Cecum of Rats With Collagen-Induced Arthritis. Front. Pharmacol. 11:587534. doi: 10.3389/fphar.2020.587534

Rheumatoid arthritis (RA) is a common autoimmune disease characterized by chronic inflammation and a multifactorial etiology. We previously showed that gut microbiota dysbiosis in the rat ileum is involved in the development of collagen-induced arthritis (CIA). The gut microbiota in the distinct gastrointestinal tract (GIT) plays region-specific roles, but information on the different roles of the microbiota in distinct GIT compartments of CIA rats is limited. This study aimed to evaluate the region-specific differences in the gut microbial communities and certain gut-associated immunologic factors in the ileum and cecum of CIA rats. Ileal and cecal digesta were collected from CIA and control rats for microbiome analysis. We determined the microbial richness, diversity and taxa as well as the expression of interleukin (IL)-1β and IL-17A in the epithelium and lamina propria of the ileum and cecum mucosal layers. The CIA-induced microbiota alterations in the ileum differed from those in the cecum. The ileal microbiota were more markedly influenced in CIA, as revealed by sharp reductions in the abundances of the families Enterococcaceae, Lactobacillaceae and Streptococcaceae and the genera *Lactobacillus* and *Lactococcus.* Moreover, significant increases in IL-1β, and IL-17A mRNA expression were detected in only the ileal epithelium and lamina propria of the mucosal layer. Therefore, the microbial characteristics in the ileum were consistent with the immune-mediated inflammatory features of CIA, suggesting that the ileal microbiota might better represent the CIA-induced inflammatory responses than the cecal microbiota and that these responses might partially impact the progression of RA by regulating intestinal mucosal immunity.

**Keywords: gut microbiota, intestinal mucosal immunity, rheumatoid arthritis, ileum, cecum, collagen-induced arthritis**

## INTRODUCTION

Rheumatoid arthritis (RA), one of the most frequently occurring autoimmune diseases, is characterized by synovial inflammation, joint cartilage and bone destruction. The onset and progression of RA is believed to require both genetic and environmental factors (Forbes et al., 2016). The role of the gut microbiota in the occurrence and development of RA is increasingly being appreciated. Recently, numerous studies have shown that dysbiosis of the gut microbiota community might function as a crucial environmental factor that triggers the pathogenesis of RA (Liu et al., 2013). The gut microbiota plays a pivotal role in the maintenance of immune system homeostasis, particularly the barrier function of the intestinal mucosa, and an imbalanced interaction between the gut microbiota and the host intestinal mucosal immune system can increase the risk of immune-mediated inflammatory disease (Maynard et al., 2012; Honda and Littman, 2016). We previously found that the intestinal immune response is actively involved in the pathogenesis of collagen-induced arthritis (CIA) in rats, and Peyer's patch (PP) cells, which are an important component of the intestinal mucosal immune system, could induce immune tolerance to enhance CIA treatment (Xiao et al., 2009). The balance between the immune response and immunologic tolerance in the intestinal tract is important for the maintenance of homeostasis. Bagchi et al. showed that the intestinal mucosal immune response is enhanced in CIA rats, showing increased ratios of $CD4^+/CD8^+$ cells in the epithelium and lamina propria of the small intestine (Wang et al., 2015). Gut-associated lymphoid tissue, which consists of PPs, intestinal intraepithelial lymphocytes (IELs), lamina propria lymphocytes (LPLs) and mesenteric lymph nodes, is the largest lymphoid organ of the human body and is crucial for human health (Chandran et al., 2003), and PPs and other immunocytes of considerable proportions are mainly distributed along the ileum (Ishii et al., 2010).

The gastrointestinal tract (GIT) of vertebrates is in contact with numerous commensal microbiota and exogenous antigens, and the microbial communities are integral to the maintenance of intestinal morphology and nutrient digestion and metabolism and are critical modulators of host immune homeostasis (Donaldson et al., 2018; Mao et al., 2018). The distinct components of the intestine should be regarded as separate entities due to their different regulatory properties in mucosal immunity (Mann et al., 2016). We previously found obvious differences in $CD4^+$ and $CD8^+$ T cell alterations in PPs, IELs and LPLs between normal and CIA mice, which suggests that the correlations between enteric immune responses and CIA in diverse compartments might differ (Xiao et al., 2006). Different locations in the GIT provide different nutrient and physicochemical conditions for the gut microbiota community. A previous study suggested that the functional heterogeneity of each GIT niche gives rise to regional-specific differences in the gut microbiota (Martinez-Guryn et al., 2019). Our previous study showed that the ileal microbial community of rats with CIA differed from that of the control group (Li et al., 2020). The ileum mainly functions to digest food, absorb nutrients, and develop intestinal mucosal immunity to protect against pathogens (Williams et al., 2015). The cecum is an important site of water and electrolyte absorption, digesta retention and microbial fermentation. The ileum and cecum, which are part of the small and large intestines, respectively, have distinct structures, physiological functions and bacterial loads. Therefore, these areas might exhibit striking differences in their bacterial colonization and related immunologic factor modulation in RA. Because the CIA model shares many clinical, histopathological and immunological features with RA patients, it is always used to investigate novel approaches for the prevention of RA (Zhao et al., 2018). However, only a few previous CIA-related experimental studies have focused on distinguishing gut microbiota dysbiosis in the ileum from that in other intestinal compartments, and information regarding the correlation of the gut microbiota in distinct compartments with mucosal immune responses is scarce. Interleukin (IL)-1β and IL-17A are important inflammatory cytokines implicated in the pathogenesis of RA, and blockage of these molecules alleviates the severity of CIA in mice (Wu et al., 2016). Moreover, some anti-IL-17A monoclonal antibodies could improve RA signs and symptoms in RA patients, and no strong adverse safety signals were noted (Genovese et al., 2010; Genovese et al., 2014). In this study, we evaluated alterations in the gut microbial communities in the ileum and cecum of CIA rats via the high-throughput sequencing of bacterial 16S rRNA and compared the mRNA protein expression of IL-1β and IL-17A in the ileum and cecum of CIA rats.

## MATERIALS AND METHODS

### Animals

Eighteen adult male Sprague-Dawley rats (190 ± 10 g) were all obtained from the Research Institute of Experimental Animals, Chinese Academy of Medical Science (animal license number: SCXK (Beijing) 2014-0013). The rats were maintained under specific pathogen-free conditions in a conventional animal housing facility at the experimental animal center of China-Japan Friendship Hospital (experimental animal room license number: SCXK (Beijing) 2016-0043) under a 12-h light/12-h dark cycle with 45–65% humidity and a temperature of 20–22°C. Rats were housed in 545 × 395 × 200 mm cages with a maximum of five animals per cage and given free access to the same standard diet and sterile water. Rats were acclimated and cohoused for a 1 week period before the experiments started.

### Induction of Collagen-Induced Arthritis and Animal Grouping

The rats were randomly divided into two groups, the control group (n = 8) and the CIA group (n = 10). Arthritis was induced through an intradermal tail vein injection of 100 μg of bovine type II collagen (Chondrex) in a 1:1 emulsion mixed with an equal amount of incomplete Freund's adjuvant (Chondrex). Seven days later, a booster injection of the same preparation was administered at the base of the tail. Every 3 days after the booster immunization, the rats were assessed for arthritis

severity according to the arthritis index (AI). The AI scores, which range from 0 to 4, are defined as follows: 0) normal; 1) detectable arthritis with slight erythema; 2) significant erythema and swelling; 3) severe erythema plus edema from joint to digit; and 4) maximal edema with arthrolysis. The total arthritis score was then calculated as the sum of the scores of each hind paw. On the 10th day after the primary immunization, the ankle joints were not swollen in one rat, which was excluded. Animals in both the normal and CIA groups were given the same deionized water and standard diet for another 4 weeks.

## Bacterial DNA Extraction, Amplification and Sequencing

At the end of the experiment (38 days after the primary immunization), the rats were sacrificed via anesthesia, and the intestinal contents of the ileum and cecum were harvested, immediately shock-frozen in liquid nitrogen and stored at −80°C for further analysis. Microbial genomic DNA from the ileal and cecal digesta samples was extracted using a QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quantity and quality of extracted DNAs were measured using a NanoDrop instrument and FastQC software. The PCR products were purified by silane magnetic beads of an appropriate size, and the purified amplicons were sequenced using a Qubit fluorometer (version 2.0, Invitrogen) according to standard protocols. The V3-V4 region of the bacterial 16S rRNA gene was amplified by PCR using the primers 341F and 805R in 25-μL reaction volumes, which included 0.15 μl of 2G Robust DNA polymerase (5 U/μl; KAPA). The PCR thermal cycle was 95°C for 5 min followed by 25 cycles of 95°C for 30 s, annealing at 55°C for 30 s, 72°C for 60 s and a final extension at 72°C for 10 min.

## High-Throughput Sequencing and Microbiome Analysis

We mainly used Quantitative Insights Into Microbial Ecology (QIIME) software for the quality-based filtering of the raw sequences. The universal primer pair was designed to target highly conserved regions of bacterial DNA, and high-throughput sequencing of bacterial 16S rRNA gene marker amplicons encoding the V3 and V4 regions was performed on the Illumina HiSeq 2500 PE250 platform by BioMiao Biological Technology (Beijing) Co., Ltd. The paired-end reads were detected by HTQC (version 1.92.3) to remove ambiguous base sequences and merged into a complete read; the chimeric sequences were identified and removed using Mothur (version 1.38.1).

After the detection of chimeras, the remaining high-quality sequences were analyzed and clustered into operational taxonomic units (OTUs) based on a nucleic acid similarity cutoff of 97% using the QIIME software package and the Usearch612 pipeline. The OTUs were then annotated using the Ribosomal Database Project classifier to obtain the taxonomic assignments, and the Python Nearest Alignment Space Termination method was adopted to align the representative sequences against the Greengenes 16S rDNA database. The relative abundances (%) were estimated at the phylum, class, order, family and genus levels. The data were then processed to calculate the $\alpha$- and $\beta$-diversity and thus assess the differences between the groups. The $\alpha$-diversity analysis (including the Shannon, Simpson, Chao1, and observed species indices) and the ß-diversity analysis (UniFrac distance) were performed using QIIME.

## Real-Time PCR

Partial gut tissue samples of the ileum and cecum were collected from rats of each group and processed on ice immediately after dissection. The ileum and cecum were opened longitudinally, washed three times with saline and stored at −80°C. The relative mRNA expression levels of IL-1β and IL-17A in the tissue homogenates obtained from the ileum and cecum were determined by real-time quantitative PCR, and GAPDH was used as an endogenous control. Briefly, total RNA was extracted using TRIzol reagent (Invitrogen), and the obtained RNA was used to generate cDNA using a Reverse Transcription System (Promega) according to the manufacturer's directions. Real-time quantitative PCR was performed using 2 × SYBR Green qPCR Mix (Aidlab Biotechnologies Co., Ltd.). Denaturation was performed at 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. The following primers were used: IL-1β, forward ACAGCAGCATCTCGACAAGAGC and reverse CCACGGGCAAGACATAGGTAGC, and IL-17A forward TGTGTCAATGCGGAGGGAAAGC and reverse CACACCCACCAGCATCTTCTCG. The relative gene expression levels were calculated by the $2^{-\triangle\triangle Ct}$ method.

## Immunohistochemistry

Partial tissue samples of the ileum and cecum were harvested after the contents were collected, flushed with physiological saline, dissected longitudinally and fixed in formaldehyde. The tissues were subsequently embedded in paraffin, and sections of 4 μm thickness were cut from the paraffin-embedded tissues. IL-1β, and IL-17A were immunolocalized in tissues according to the manufacturer's instructions. The paraffin sections were dewaxed using routine methods, incubated with 3% $H_2O_2$ for 10 min and treated with primary antibodies against rat IL-1β (Santa Cruz Biotechnology) and IL-17A (Abcam, Cambridge, MA, United States) at 37°C for 1 h. The sections were then incubated with poly-HRP anti-rabbit IgG for 10 min at room temperature, stained with 3,3-diaminobenzidine (Fuzhou Maixin Biotech. Co., Ltd.) and counterstained with hematoxylin.

## Statistical Analysis

The statistical analyses were performed using SPSS 20.0 software. The normality of variable distribution was analyzed using the Shapiro-Wilk test. The microbial diversity data that exhibited a normal distribution were assessed using the independent-sample t-test procedure. The Mann-Whitney test was used to assess the variables with a non-normal distribution. Data were expressed as means ± SEMs. Differences were considered significant if $p \leq 0.05$. The ß-diversity was visualized using principal coordinate analysis (PCoA) and nonmetric multidimensional scaling (NMDS). The OTU relative abundance values were

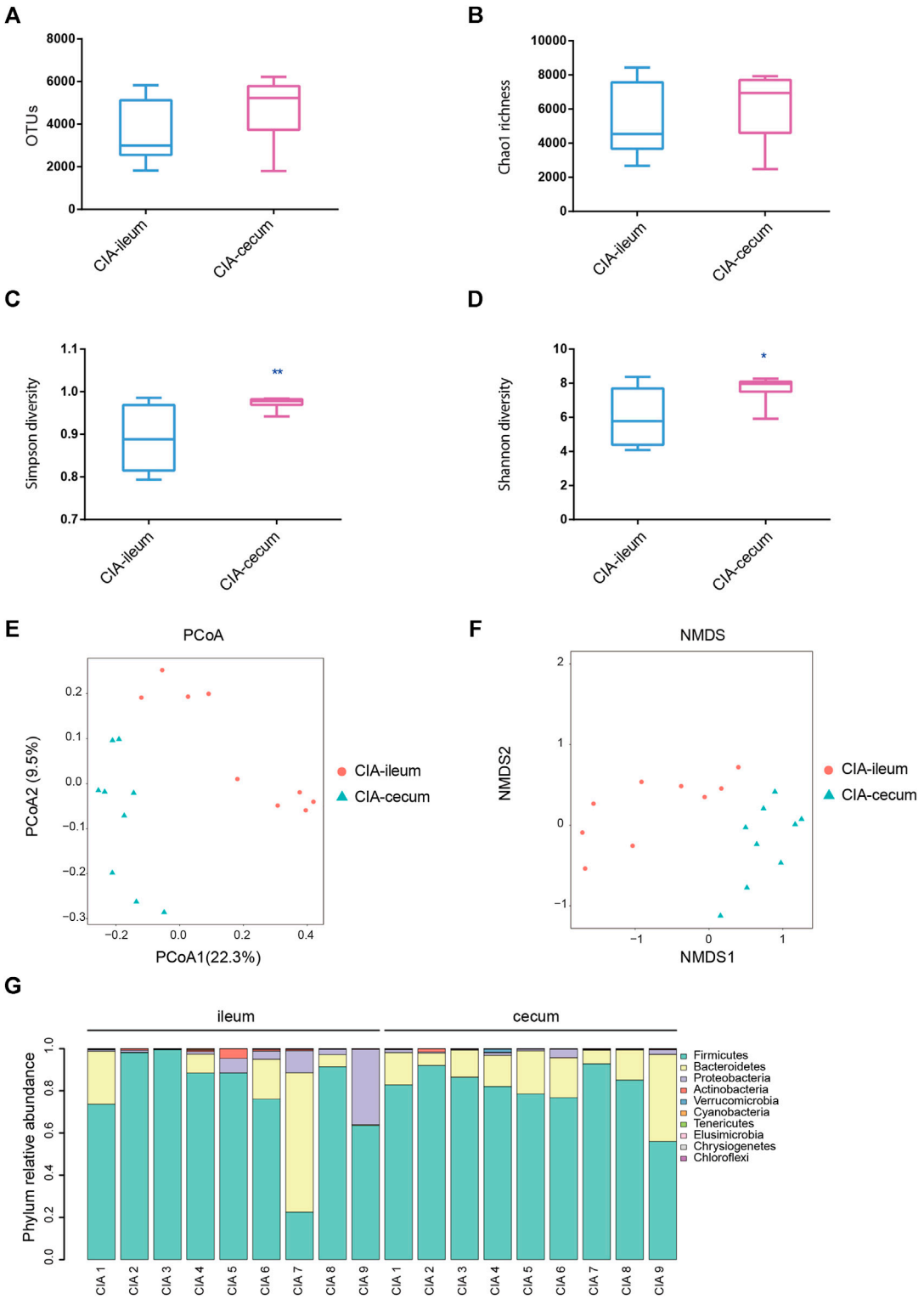


**FIGURE 1 |** The microbial communities in the ileum and cecum of the collagen-induced arthritis (CIA) group were obtained by 16S sequencing. The rats were induced with bovine collagen II on days 0 and 7 and sacrificed via anesthesia 6 weeks after the primary immunization. **(A)** Analysis of the observed species in the ileal and cecal microbial communities. **(B)** Analysis of the Chao richness estimators of the ileal and cecal microbial communities. **(C)** Analysis of the Simpson diversity indices of the ileal and cecal microbial communities. **(D)** Analysis of the Shannon diversity indices of the ileal and cecal microbial communities. **(E)** Community structure as determined by principal coordinate analysis. **(F)** Nonmetric multidimensional scaling ordination of the microbiome. **(G)** Phylum relative abundances of the microbiota in each sample of CIA rats. The values are presented as the means ± SDs. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the ileum.

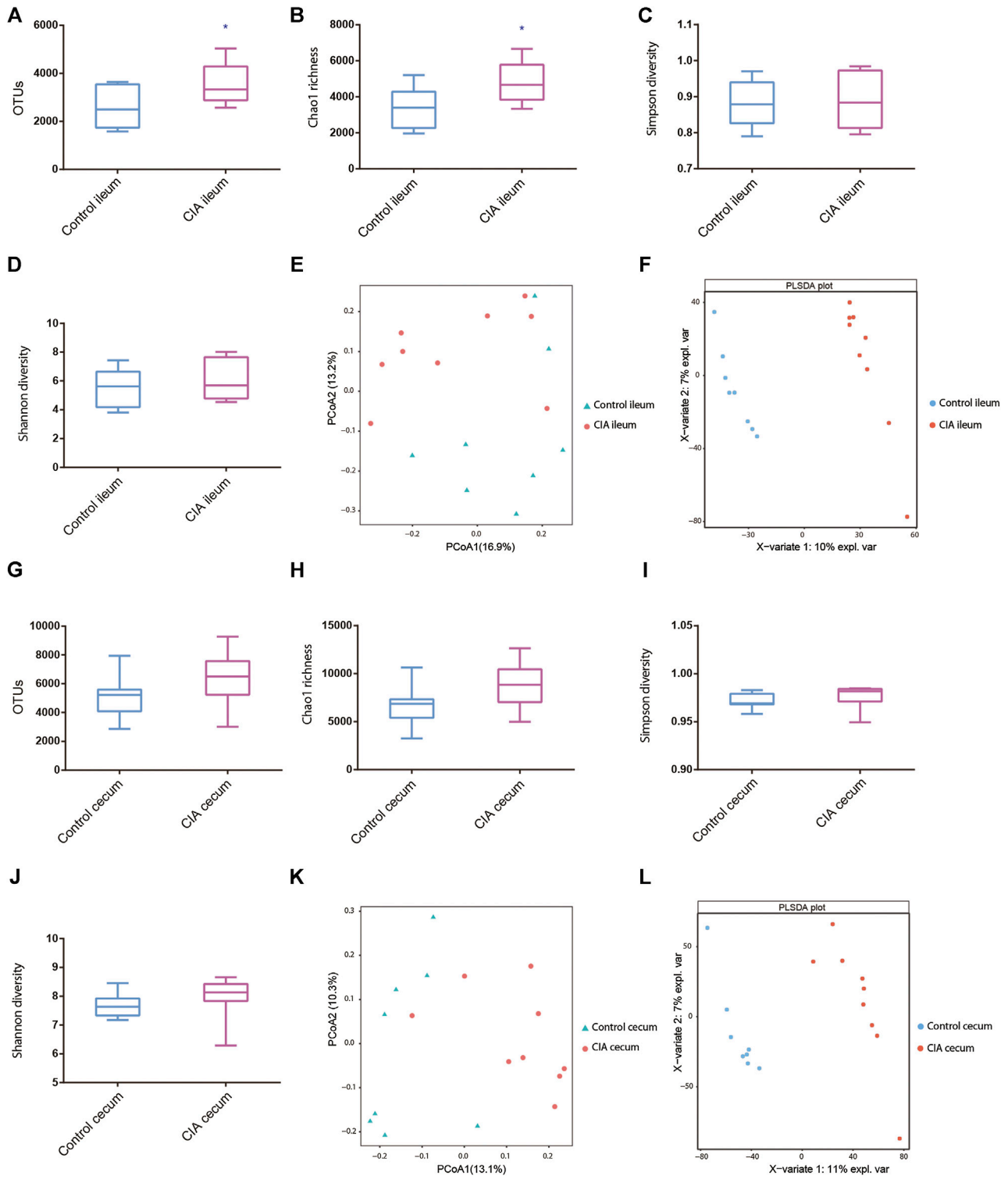


**FIGURE 2 |** Ileal and cecal microbiota alterations in collagen-induced arthritis (CIA) rats compared with control rats based on the $\alpha$- and $\beta$-diversity. **(A–D)** Alterations in the observed species, Chao richness estimator, Simpson diversity and Shannon diversity indices of the ileal microbial community. **(E,F)** Principal coordinate analysis (PCoA) and partial least squares discriminant analysis (PLSDA) of the ileum as determined by the unweighted UniFrac distance in CIA and control rats. **(G–J)** Alterations in the observed species, Chao richness estimator, Simpson diversity and Shannon diversity indices of the cecal microbial community. **(K,L)** PCoA and PLSDA of the cecum as determined by the unweighted UniFrac distance in CIA and control rats. The values are presented as the means ± SDs. *$p < 0.05$ compared with the control group.

analyzed using the linear discriminant analysis effect size (LEfSe) algorithm to identify taxa, and the effect size of each differentially abundant feature was estimated by linear discriminant analysis.

# RESULTS

## Collagen-Induced Arthritis Rats Exhibit Different Diversities and Community Structures in the Ileum and Cecum

Significant differences in the severity of ankle joint swelling were found between the control and CIA groups (**Supplementary Figure S1**). A total of 3,346,939 V3-V4 16S rRNA sequence reads were obtained from 17 samples (control group n = 8; CIA group n = 9). Although no significant differences in the OTUs or Chao1 richness estimators were found between the ileum and cecum in the control groups, the ileum and cecum of healthy rats showed obvious differences in bacterial diversity (**Supplementary Figure S2**). Following CIA treatment, the rats displayed significant differences in the microbial richness values in the ileum and cecum, as demonstrated by the Shannon and Simpson indices (**Figures 1C,D**). No significant differences in the OTUs or Chao richness estimators were found between the ileum and cecum in the CIA groups (**Supplementary Figures S1A,B**). As shown in **Figure 1E**, the PCoA results based on ß-diversity revealed separation of the microbial community in the ileum from that in the cecum, and the divergence in the distribution of the microbiota was also significant (ANOSIM, R = 0.578, $p < 0.01$). Additionally, similar visual separation was achieved by NMDS ordination (**Figure 1F**). In summary, the ileal and cecal communities of the CIA group showed obvious differences in bacterial diversity and community. After CIA treatment, Firmicutes (77.99%), Bacteroidetes (13.93%) and Proteobacteria (6.99%) were the three predominant phyla in the ileum, and based on their relative abundances, Firmicutes (81.41%) and Bacteroidetes (16.67%) remained the two predominant phyla in the cecum (**Figure 1G**).

## Collagen-Induced Arthritis Rats Exhibit Alterations in Ileal and Cecal Microbial Richness and Composition

In the ileum, the CIA rats exhibited significant increases in the observed species and Chao1 indices compared with those of the healthy rats (**Figures 2A,B**). However, in the cecum, although CIA rats also tended to exhibit increased OTUs and Chao1 values compared with those of the control rats, no significant difference was found between the two groups (**Figures 2G,H**). Additionally, no significant differences in the microbial diversities in the ileum and cecum were observed between the CIA and healthy rats, as demonstrated by the Shannon and Simpson indices (**Figures 2C, D, I, J**).

The PCoA results provided a visual separation of the microbial communities in the ileum between the CIA and control groups (**Figure 2E**), which indicated that CIA tended to affect the microbial community in the ileum of rats. Moreover, the PCoA results also revealed a visual separation of the microbial community in the cecum between the CIA and control groups (**Figure 2K**). However, the results were not significantly different between the ileum and cecum. Similar visual separation results were also obtained with partial least squares discriminant analysis (PLSDA) (**Figures 2F,L**).

## Collagen-Induced Arthritis Alters the Relative Abundances of the Microbiota at the Family Levels

To account for the differences in microbial diversity, we further identified the bacteria at the family and genus levels. We selected families with a relative abundance >1% in at least one group, as shown in the heat maps presented in **Figures 3A,C**. The family-level analysis showed that the abundances of 16 and 19 families were significantly changed in the ileum (**Supplementary Figure S3A**) and cecum (**Supplementary Figure S3B**) of the CIA group, respectively. Among the families with a relative abundance greater than 0.5% in at least one group, Enterococcaceae ($p < 0.01$), Lactobacillaceae ($p < 0.05$) and Streptococcaceae ($p < 0.05$) presented significantly lower abundances in the ileum of the CIA group than in that of the control group (**Figure 3B**). However, in the cecum, the abundance of the bacterial family Peptostreptococcaceae ($p < 0.05$) was significantly increased after CIA induction (**Figure 3D**).

## Collagen-Induced Arthritis Alters the Relative Abundances of the Microbiota at the Genus Levels

The genus-level changes in genera with a relative abundance greater than 0.5% in at least one group are shown as a heat map in **Figures 4A,C**. At the genus level, the abundances of 28 and 30 genera were changed significantly in the ileum and cecum of the CIA group, respectively. CIA induction significantly reduced the abundances of *Lactobacillus*, *Lactococcus*, unclassified Enterococcaceae *Enterococcus* ($p < 0.05$) and other genera of Enterococcaceae ($p < 0.01$) in the ileum but increased the abundance of *Clostridium* in the ileum ($p < 0.05$, **Figure 4B**). *Lactobacillus*, *Lactococcus*, and *Clostridium* were the three most abundant genera in the ileum that exhibited significant changes in abundance after CIA induction (>1.5% in at least one group). Among these genera, *Lactobacillus* was the most abundant (21.15% in the control group and 10.48% in the CIA group). In the cecum, unclassified *Clostridiales*, unclassified Lachnospiraceae and *Oscillospira* were the three most abundant genera in both the control and CIA groups, but no significant differences in the abundances of these genera were found. Interestingly, all the genera that exhibited changes in the cecum presented an extremely low relative abundance (<0.5%). In summary, these results indicate that CIA induction significantly changed the gut microbiota communities, particularly in the ileum digesta. Thus, the LEfSe algorithm was then used to further identify specific bacterial taxa that are characteristic of the ileum of the CIA group but not in that of the control group. Consequently, the abundances of 23 taxa were found different between the CIA and control groups. The abundances of 15 taxa

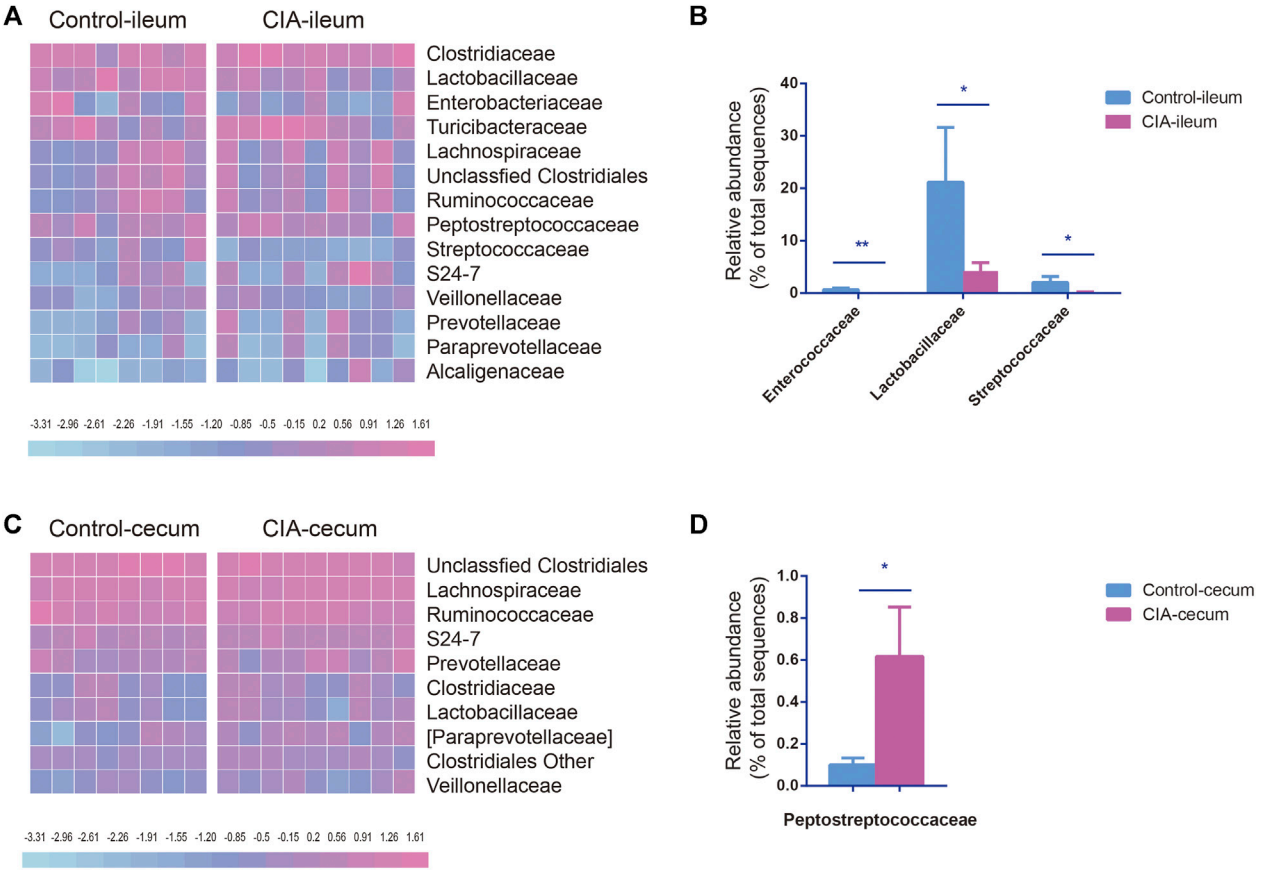


**FIGURE 3 |** Comparison of the family-level compositions of the ileal and cecal microbiota communities in the control and collagen-induced arthritis (CIA) groups. **(A)** Heat map of the relative abundances at the family level. **(B)** Significantly changed microbial families found in the ileum. **(C)** Heat map of the relative abundances at the family level. **(D)** Significantly changed microbial families found in the cecum. The values are presented as the means ± SDs. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control group.

were increased in the control group, and those of eight taxa were increased in the CIA group (**Figure 4D**).

## The Relative mRNA and Protein Expression of Interleukin-1β, and Interleukin-17A Is Increased in the Ileum of the Collagen-Induced Arthritis Group

Proinflammatory cytokines and autoantibodies are involved in the upregulation of inflammatory responses. IL-1β and IL-17A are markers of inflammatory responses in the intestine. To evaluate whether mucosa-mediated inflammatory cytokine production differs between the ileum and cecum, we assessed the mRNA expression of IL-1β, and IL-17A in the ileum and cecum of the CIA and control groups by real-time PCR. Interestingly, significantly increased mRNA levels of IL-1β, and IL-17A were found in only the ileum after CIA induction (**Figures 5A,E**). No significant difference in IL-1β, and IL-17A mRNA expression in the cecum was found between CIA and control rats (**Figures 5C,G**). The protein expression in the epithelium and lamina propria of the mucosal layer was detected by immunohistochemistry as shown in **Figure 5** (right panel).

## DISCUSSION

RA is a systemic autoimmune disease that results in joint inflammation, cartilage damage and bone destruction, and researchers have become increasingly interested in studying the interplay of gut microbiota dysbiosis and the pathogenesis of RA (Maeda and Takeda, 2017). Microbial analyses of fecal samples have been used in a variety of studies to explore this relationship because these samples can be conveniently obtained and are thought to represent all gut microbial alterations of the host. However, the GIT is a multiorgan system, and each of its regions exhibits functional heterogeneity to regulate digestion, nutrient absorption, immunity and metabolism. These properties give rise to regional differences among the extensive gut microbial compositions in the diverse GIT segments of the host (Martinez-Guryn et al., 2019). A previous study revealed that in healthy conditions, the lower GIT is composed of distinct microbial populations along the small intestine, cecum and colon, and the small intestine exhibits lower microbial diversity than the cecum and colon (Donaldson et al., 2016). Differences in the gut microbiota between the ileum and cecum have been found in studies of diverse species. Through terminal restriction fragment

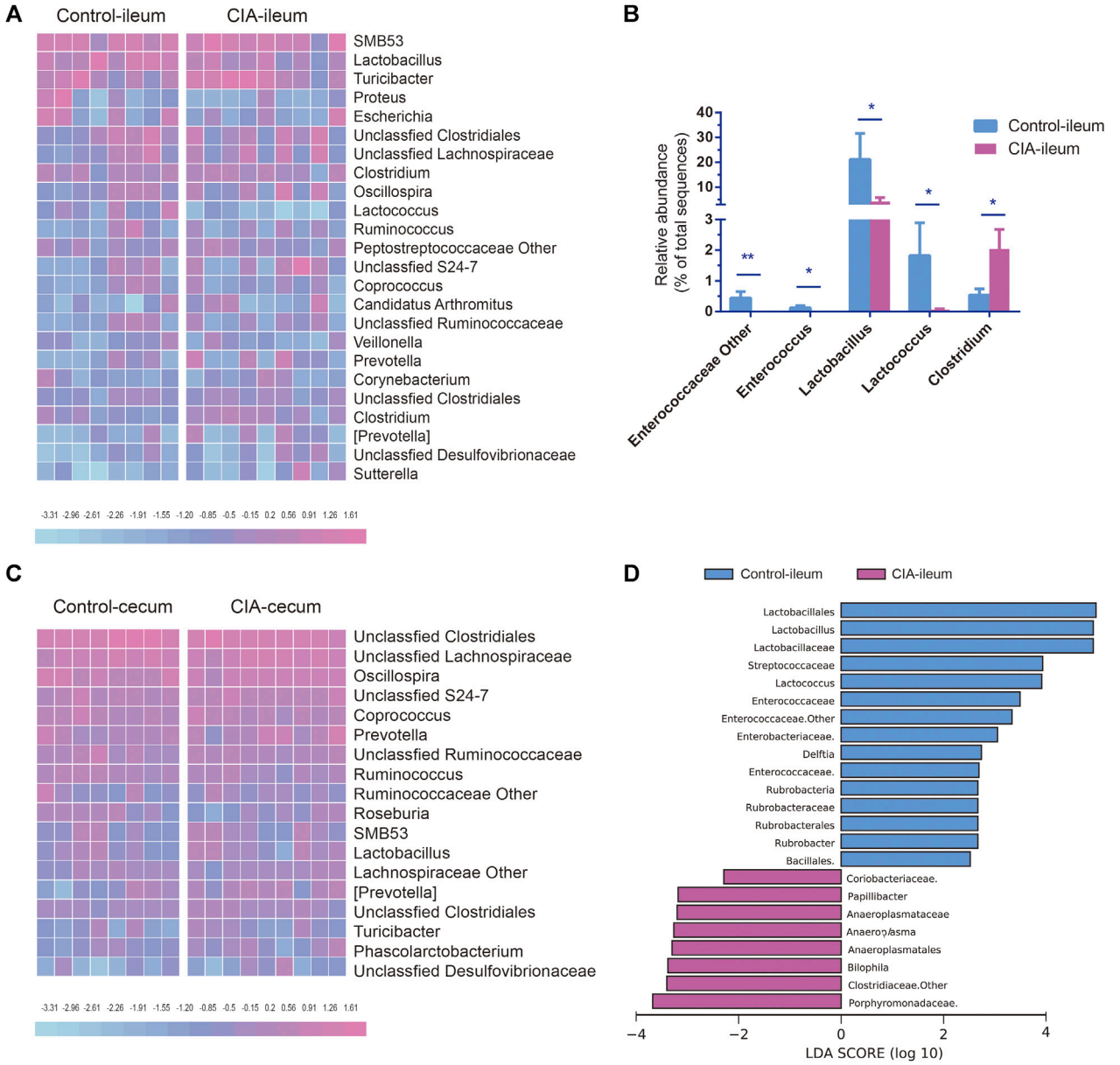


**FIGURE 4 |** Comparison of the genus-level microbial communities in the ileum and cecum of the control and collagen-induced arthritis (CIA) groups and taxa-level changes in the ileal microbiota. **(A)** Heat map of the relative abundances at the genus level. **(B)** Significantly changed microbial genera found in ileal digesta. **(C)** Heat map of the relative abundances at the genus level. **(D)** The linear discriminant analysis effect size algorithm revealed taxonomic changes in the ileal microbiota. The bar plot shows the significantly altered taxa ($p < 0.05$) based on the effect size [linear discriminant analysis score ($\log_{10}$) > ±2). The values are presented as the means ± SDs. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control group.

length polymorphism (T-RFLP) and 16S rRNA sequence analyses, Gong et al. found that the microbial community in the ileum was less diverse than that in the cecum of broiler chickens (Gong et al., 2002). Yu et al. demonstrated that early antibiotic exposure exerts varying effects on the microbial communities in the ileum and cecum, and this treatment has more substantial effects in the ileum than in the cecum by altering the diversity, richness and structure of the genera (Yu et al., 2018). In a study of aged rats, Lee et al. revealed that the ß-diversity of the microbiota composition in the ileum was higher than that in the cecum, but the microbiota a-diversity was lower in the ileum than in the cecum. Lactobacillaceae were found at higher abundances in the ileum than in the cecum, whereas Ruminococcaceae and Lachnospiraceae were more abundant in the cecum (Lee et al., 2018). The microbial properties are affected by CIA induction, and the gut microbiota in different locations of the GIT exist in different physicochemical, nutrient and immune-related conditions. A previous study demonstrated

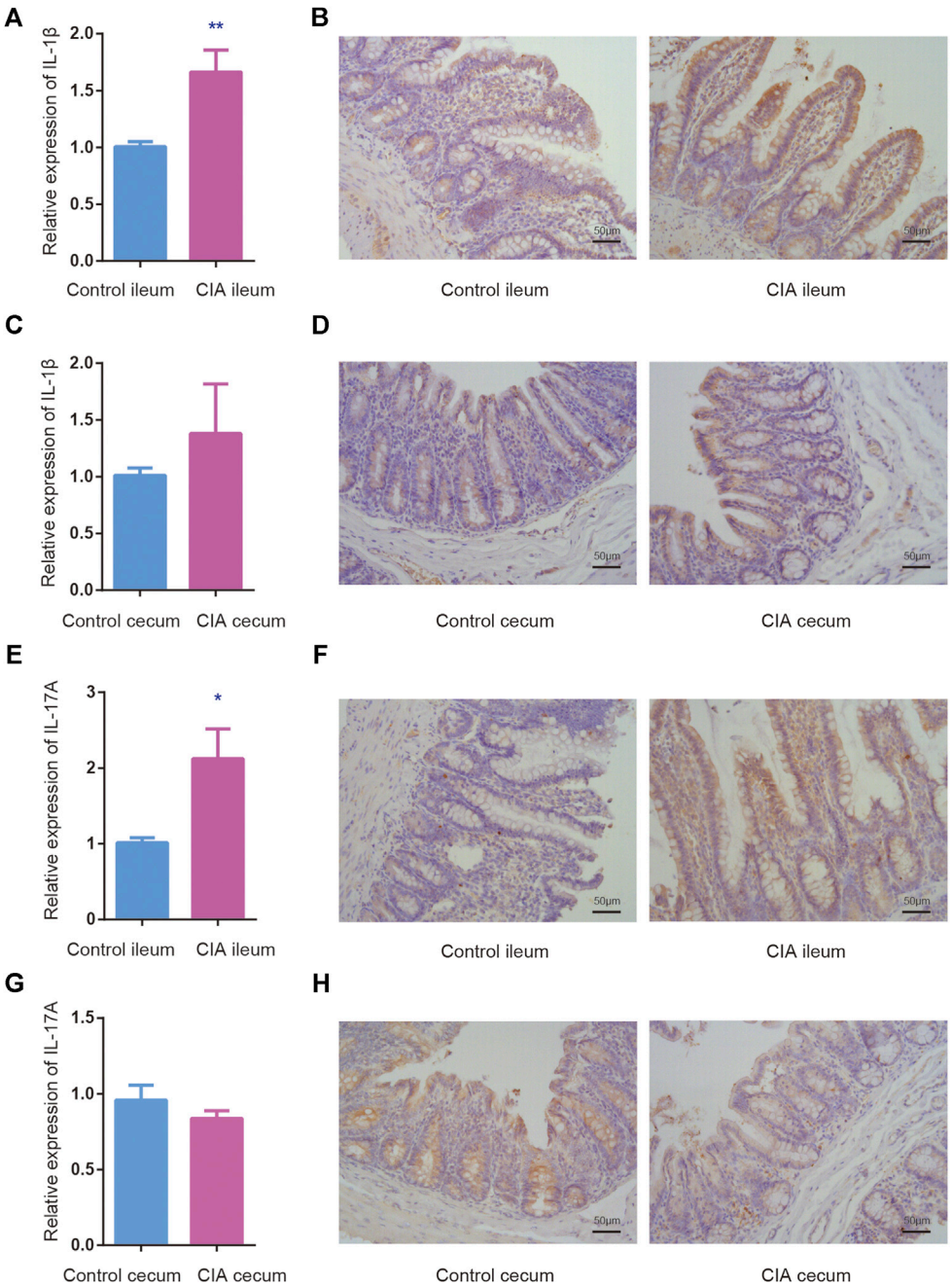


**FIGURE 5 |** The relative mRNA and protein expression of interleukin (IL)-1β and IL-17A was upregulated in the mucosal layer of ileum of the collagen-induced arthritis (CIA) group. The mRNA expression was determined relative to the mean value of the control group. **(A,B)** The relative mRNA and protein expression of IL-1β in the mucosal layer of the ileum. **(C,D)** The relative mRNA and protein expression of IL-1β in the mucosal layer of the cecum. **(E,F)** The relative mRNA and protein expression of IL-17A in the mucosal layer of the ileum. **(G,H)** The relative mRNA and protein expression of IL-17A in the mucosal layer of the cecum. The data are presented as the means ± SEMs. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared with the control group.

that the changes induced by CIA in the gut microbial communities of mice differ between ileal and colonic microbes (Doonan et al., 2019). Interestingly, when transferred into germ-free mice, fecal ileal samples from only arthritic mice immunized with "arthritogenic" collagen type II + complete Freund's adjuvant increased gut permeability, while those from the other regimen did not (Tajik et al., 2020). Therefore, the measurement of only fecal samples might not be sufficient for exploring the onset and development of CIA, which are mediated by the numerous gut microbiota in the host. Characterizing the microbial alterations in distinct intestinal compartments of CIA rats might provide more representative samples for exploring the

pathogenesis of arthritis. In the present study, we compared the specific differences in the microbiota that colonize the ileum and cecum of CIA rats and detected the regulation of certain gut-associated immunologic factors.

The main functions of the large intestine are to extract water and salt from solid wastes, whereas the small intestine mainly functions to absorb nutrients and minerals from the diet. The ileum and cecum, which are part of the small and large intestines, respectively, contain microbial populations with different structures. Herein, 16S rRNA gene sequencing showed that the microbiota in the ileal digesta of healthy control rats was significantly less diverse than that in the cecum, as demonstrated by the Shannon and Simpson indices, and this finding appears to be consistent with that obtained in a previous study (Donaldson et al., 2016). In CIA rats, less bacterial diversity was also found in the ileum than in the cecum based on the Shannon and Simpson indices. A previous study indicated that the $\alpha$-diversity of the gut microbiota in fecal samples of CIA rats did not differ from that of control rats (Shan et al., 2018). In this study, a comparison of the ileal microbiota of CIA rats with control rats revealed that CIA induction significantly increased the richness indices, but the diversity indices did not show significant differences. Furthermore, both the richness and diversity indices of the cecum samples of the CIA group were not significantly different from those in the control group. Consequently, the alterations in the ileal and cecal bacteria induced by CIA exhibited *a*-diversity-based differences. In addition, the structure of the gut microbiota might periodical change in the CIA rat model, as demonstrated by long-term experiments performed by Peng et al., (2019). Jeong Y et al. found that the phylum Bacteroidetes is enriched in early RA patients (Jeong et al., 2019), and Rogier et al. found that the abundance of the family Lactobacillaceae, which belongs to the Firmicutes family, is reduced during the preclinical phase of arthritis (Rogier et al., 2017). At the phylum level, three predominant bacteria in the ileum were herein altered in CIA rats compared with the control group. Firmicutes (81.49%), Proteobacteria (15.20%) and Bacteroidetes (2.41%) were the three major groups in the ileum of the healthy control group. After CIA induction, the three predominant bacterial phyla in the ileum changed to Firmicutes (77.99%), Bacteroidetes (13.93%), and Proteobacteria (6.99%). However, the three dominant bacterial phyla in the cecum of the CIA group did not differ from those in the control group. At the family level, the abundances of the families Enterococcaceae, Lactobacillaceae and Streptococcaceae were significantly reduced in the ileum of the CIA group compared with the control group ileum. In the cecum, the abundance of the family Peptostreptococcaceae was significantly increased after CIA induction.

At the genus level, CIA induction influenced the ileal microbiota by substantially decreasing the abundances of *Lactobacillus* and *Lactococcus.* Lactic acid bacteria constitute a representative intervention for reducing autoimmunity and thus recovering immune homeostasis. The genus *Lactobacillus*, which belongs to the family Lactobacteriaceae, is the predominant genus in the small intestine. Some strains of *Lactobacillus* might exert beneficial effects on the immunomodulation of the immune response during CIA progression. Esvaran et al. showed that the oral administration of *Lactobacillus* species, such as *L. fermentum* PC1, to CIA mice could significantly decrease joint inflammation by increasing the production of the cytokines IL-4 and IL-10 and decreasing IL-12 production (Esvaran and Conway, 2019). A previous study suggested that *L. acidophilus* can protect rats from arthritis symptoms (Amdekar and Singh, 2016). Hosoya et al. showed that *L. helveticus* SBT2171 suppresses the excessive proliferation of LPS-stimulated mouse T lymphocytes and B lymphocytes and exerts an immunosuppressive effect *in vivo*, which indicates a possible mechanism for the alleviation of CIA (Hosoya et al., 2014). Yamashita et al. demonstrated that both the oral administration and intraperitoneal inoculation of *L. helveticus* SBT2171 attenuated CIA-related inflammatory symptoms, and intraperitoneal inoculation even decreased the numbers of CIA-exacerbating immune cells, including B cells and $CD4^+$ T cells, and downregulated the production of IL-6 and bovine type II collagen-specific antibodies in CIA mice (Yamashita et al., 2017). Liu et al. found that pretreatment of CIA mice with *L. salivarius* UCC118 or *L. plantarum* WCFS1 isolated from RA patients markedly reduced the Th17 cell fraction, increased the Treg fraction, and enhanced the antiarthritic and anti-inflammatory effects; the former even notably increased the serum levels of the anti-inflammatory cytokine IL-10 (Liu et al., 2016). The abundance of the genus *Lactococcus* in the ileum of the CIA group was also markedly lower than that in the control group. The oral administration of an adapted recombinant strain, *L. lactis*, which was engineered to express murine IL-35, inhibits IL-17 and IFN-$\gamma$ and increases IL-10 production derived from $CCR6^+$ and $CCR6^-$ $Foxp3^+$ $^{or}$ $^-$ $CD39^+$ $CD4^+$ T cells to suppress the incidence and progression of CIA (Maddaloni et al., 2018). The feeding of arthritic mice with food-based *Lactococcus* engineered to express CFA/I fimbriae prevents arthritis by inducing $CD39^+$ Tregs to secrete TGF-β and IL-10 and thus inhibit TNF-α production and neutrophil influx into the joints (Maddaloni et al., 2015). Although the relative abundances of ileal *Lactobacillus* and *Lactococcus* were significantly reduced in the CIA group, a significantly increased abundance of *Clostridium* was observed. Some species of Clostridia are commensal, whereas others are pathogenic. The abundance of *Clostridium* III is higher in RA cohorts than in healthy controls, as revealed in a comparative study (Forbes et al., 2018). Different intestinal *Clostridium* species (*C. perfringens*, *C. histolyticum*, *C. clostridioforme*, *C. leptum*, *C. sporosphaeroides* and *Blautia coccoides*) evoke distinct responses regarding the production of cytokines, including TNF-α, IL-8 and IL-10, by human mononuclear cells and might subsequently influence immune responses (Tuovinen et al., 2013).

Considerable evidence indicates that the gut microbiota regulates the mucosal immune system, including multiple types of immunocytes that contribute to the development of RA (Lucchino et al., 2019). The results from experimental animal studies indicate that gut microbiota dysbiosis serves as a potential trigger of enteric mucosal immune responses and can thus lead to the development of CIA (Jubair et al., 2018). Given the different changes in the bacterial load between the ileum and cecum of CIA

rats, we investigated whether the mRNA expression of immunological factors, specifically IL-1β and IL-17A, in these two compartments was altered by CIA induction to broaden our understanding of the interplay between gut microbiota and immunity in CIA rats. Interestingly, the mRNA expression of IL-1β and IL-17A in the epithelium and lamina propria of the mucosal layer of the ileum and cecum also showed differential regulation of the enteric mucosal immune responses between the CIA and control groups. The published data have shown that CIA induction increases the levels of the proinflammatory cytokines IL-1β and IL-17A (Chen et al., 2019; Hui et al., 2019). IL-17A is the primary cytokine of Th17 cells (Maddur et al., 2012), and IL-1β can induce IL-17A secretion from $CD4^+$ T cells, which have been implicated in the pathogenesis of RA (Yang et al., 2008). A recent study showed that partial elimination of the gut microbiota by broad-spectrum antibiotics during the establishment of CIA regulates the mucosal T helper cell balance and inhibits IL-17A mRNA in the terminal ileum, which is a main site for microbiota-induced T cell regulation, whereas the expression of IL-17 in colon tissue is not affected by CIA. These researchers also analyzed the percentage of local Th17 cells in joint-draining lymph nodes and found that it was markedly higher in CIA mice at the preclinical phase than in the control mice (Rogier et al., 2017). Dendritic cells (DCs) have the unique ability to polarize specific types of T cell responses due to the production of polarizing cytokines. Mann et al. suggested that ileal DCs play a more inflammatory role than colonic DCs because more ileal DCs produce the proinflammatory cytokine IL-1β than colonic DCs (Mann et al., 2016). Thus, we detected the expression of IL-1β and IL-17A in the ileal and cecal epithelium and lamina propria of the mucosal layer. Higher levels of IL-1β and IL-17A expression were found in the ileal epithelium and lamina propria of the mucosal layer, but no significant differences in their expression in the cecal epithelium and lamina propria of the mucosal layer were found between the CIA and healthy control groups. Because all the changed bacterial families and genera in the cecum presented extremely low relative abundances, differences in bacteria with an extremely low abundance in the cecum might not be responsible for the progression of CIA. However, the ileal microbiota was more markedly influenced, as demonstrated by sharp reductions in the abundances of the family Lactobacillaceae and the genera *Lactobacillus* after CIA. In addition, because the microbial profile of the cecal digesta sample was not closely correlated with the gut-associated immunologic factors IL-1β and IL-17A, the changes in the ileal bacteria might be more reflective of the impact on CIA development than the alterations in the cecal bacteria. However, further studies need to explore the different species in these genera that exhibit significant changes in the ileal digesta and the effects of their diverse metabolites. In addition, the neighboring intestinal immunomodulatory cells that located in distinct intestinal mucosa may be the most likely intermediary by which the gut microbiota can influence the occurrence and development of RA (Xu et al., 2020), so the mechanisms by which the dysbiosis of microbiota and their metabolites in distinct intestinal positions affect intestinal Th17 cells and other related immunocyte responses should be investigated.

## CONCLUSION

In summary, this work demonstrates that the different alterations in bacterial taxa in the ileum and cecum and the expression of the *Lactobacillus* 16S rRNA gene in the ileum exhibit the most marked changes after CIA. The features of the ileal microbial community identified by differential abundance analysis are consistent with the immune-mediated inflammatory features of CIA, which suggests that the ileal microbiota might better represent the CIA-induced inflammatory responses than the cecal microbiota. These findings might be partially related to the altered induction of excessive mucosal IL-1β and IL-17A expression in the ileum, which highlights the interactions between the microbiota and CIA. The alterations in the abundances of specific bacteria in the ileum of the host further emphasize the potential effects on CIA development. These taxa might serve as biomarkers for the detection and diagnosis of RA in the clinic and might also be common components of RA etiology in different tissues. The potential effects of these observed changes on the intestinal mucosal system in RA need to be further investigated in the future.

## DATA AVAILABILITY STATEMENT

The datasets generated for this study can be found in NCBI accession PRJNA668272.

## ETHICS STATEMENT

The animal experimental protocols were approved by the Research Ethics Committee of the Institute of Clinical Medical Sciences, China-Japan Friendship Hospital (No. 180207).

## AUTHOR CONTRIBUTIONS

HX, JC, and XYL contributed equally to this paper. HX drafted the manuscript. HX, JC, and XYL conducted the experiments. XCL analyzed the data. YX and DF participated in discussions related to the paper. CX formulated the concept and designed the paper. HZ and DJ revised this paper. All the authors read and approved the final manuscript.

## FUNDING

This study was supported by the National Natural Science Foundation of China (grant numbers 81673844 and 81573845) and the Fundamental Research Funds for the Central Public Welfare Research Institutes (grant number ZZ2018001).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.587534/full#supplementary-material

## REFERENCES


Amdekar, S., and Singh, V. (2016). Lactobacillus acidophilus maintained oxidative stress from reproductive organs in collagen-induced arthritic rats. *J. Hum. Reprod. Sci.* 9 (1), 41–46. doi:10.4103/0974-1208.178638

Chandran, P., Satthaporn, S., Robins, A., and Eremin, O. (2003). Inflammatory bowel disease: dysfunction of GALT and gut bacterial flora (I). *Surgeon* 1 (2), 63–75. doi:10.1016/s1479-666x(03)80118-x

Chen, G., Zhang, Z., Liu, Y., and Lu, J., Qi, X., Fang, C., et al. (2019). Efficacy and safety of Zuogui Pill in treating osteoporosis: study protocol of a systematic review. *Medicine* 98 (8), e13936. doi:10.1097/md.0000000000013936

Donaldson, G. P., Ladinsky, M. S., Yu, K. B., Sanders, J. G., Yoo, B. B., Chou, W. C., et al. (2018). Gut microbiota utilize immunoglobulin A for mucosal colonization. *Science* 360 (6390), 795–800. doi:10.1126/science.aaq0926

Donaldson, G. P., Lee, S. M., and Mazmanian, S. K. (2016). Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* 14 (1), 20–32. doi:10.1038/nrmicro3552

Doonan, J., Tarafdar, A., Pineda, M. A., Lumb, F. E., Crowe, J., Khan, A. M., et al. (2019). The parasitic worm product ES-62 normalises the gut microbiota bone marrow axis in inflammatory arthritis. *Nat. Commun.* 10 (1), 1554. doi:10.1038/s41467-019-09361-0

Esvaran, M., and Conway, P. L. (2019). Lactobacillus fermentum PC1 has the capacity to attenuate joint inflammation in collagen-induced arthritis in DBA/1 mice. *Nutrients* 11 (4), 785. doi:10.3390/nu11040785.

Forbes, J. D., Chen, C. Y., Knox, N. C., Marrie, R.-A., El-Gabalawy, H., Kievit, T. d., et al. (2018). A comparative study of the gut microbiota in immune-mediated inflammatory diseases-does a common dysbiosis exist? *Microbiome* 6 (1), 221. doi:10.1186/s40168-018-0603-4

Forbes, J. D., Domselaar, G. V., and Bernstein, C. N. (2016). The gut microbiota in immune-mediated inflammatory diseases. *Front. Microbiol.* 7, 1081. doi:10.3389/fmicb.2016.01081

Genovese, M. C., Greenwald, M., Cho, C. S., Berman, A., Jin, L., Cameron, G. S., et al. (2014). A phase II randomized study of subcutaneous ixekizumab, an anti-interleukin-17 monoclonal antibody, in rheumatoid arthritis patients who were naive to biologic agents or had an inadequate response to tumor necrosis factor inhibitors. *Arthritis Rheum.* 66 (7), 1693–1704. doi:10.1002/art.38617

Genovese, M. C., Van den Bosch, F., Roberson, S. A., Bojin, S., Biagini, I. M., Ryan, P., et al. (2010). LY2439821, a humanized anti-interleukin-17 monoclonal antibody, in the treatment of patients with rheumatoid arthritis: a phase I randomized, double-blind, placebo-controlled, proof-of-concept study. *Arthritis Rheum.* 62 (4), 929–939. doi:10.1002/art.27334

Gong, J., Forster, R. J., Yu, H., Chambers, J. R., Wheatcroft, R., Sabour, P. M., et al. (2002). Molecular analysis of bacterial populations in the ileum of broiler chickens and comparison with bacteria in the cecum. *FEMS Microbiol. Ecol.* 41 (3), 171–179. doi:10.1111/j.1574-6941.2002.tb00978.x

Honda, K., and Littman, D. R. (2016). The microbiota in adaptive immune homeostasis and disease. *Nature* 535 (7610), 75–84. doi:10.1038/nature18848

Hosoya, T., Sakai, F., Yamashita, M., Shiozaki, T., Endo, T., Ukibe, K., et al. (2014). Lactobacillus helveticus SBT2171 inhibits lymphocyte proliferation by regulation of the JNK signaling pathway. *PloS One* 9 (9), e108360. doi:10.1371/journal.pone.0108360

Hui, W., Yu, D., Cao, Z., and Zhao, X. (2019). Butyrate inhibit collagen-induced arthritis via Treg/IL-10/Th17 axis. *Int. Immunopharm.* 68, 226–233. doi:10.1016/j.intimp.2019.01.018

Ishii, H., Isomoto, H., Shikuwa, S., Hayashi, T., Inoue, N., Yamaguchi, N., et al. (2010). Peyer's patches in the terminal ileum in ulcerative colitis: magnifying endoscopic findings. *J. Clin. Biochem. Nutr.* 46 (2), 111–118. doi:10.3164/jcbn.09-77

Jeong, Y., Kim, J. W., You, H. J., Park, S.-J., Lee, J., Ju, J. H., et al. (2019). Gut microbial composition and function are altered in patients with early rheumatoid arthritis. *J. Clin. Med.* 8 (5), 693. doi:10.3390/jcm8050693

Jubair, W. K., Hendrickson, J. D., Severs, E. L., Schulz, H. M., Adhikari, S., Ir, D., et al. (2018). Modulation of inflammatory arthritis in mice by gut microbiota through mucosal inflammation and autoantibody generation. *Arthritis Rheum.* 70 (8), 1220–1233. doi:10.1002/art.40490

Lee, S. M., Kim, N., Park, J. H., Nam, R. H., Yoon, K., and Lee, D. H. (2018). Comparative analysis of ileal and cecal microbiota in aged rats. *J. Cancer Prev.* 23 (2), 70–76. doi:10.15430/jcp.2018.23.2.70

Li, X., Lu, C., Fan, D., Lu, X., Xia, Y., Zhao, H., et al. (2020). Human umbilical mesenchymal stem cells display therapeutic potential in rheumatoid arthritis by regulating interactions between immunity and gut microbiota via the aryl hydrocarbon receptor. *Front Cell Dev. Biol.* 8, 131. doi:10.3389/fcell.2020.00131

Liu, X., Zhang, J., Zou, Q., Zhong, B., Wang, H., Mou, F., et al. (2016). Lactobacillus salivarius isolated from patients with rheumatoid arthritis suppresses collagen-induced arthritis and increases Treg frequency in mice. *J. Interferon Cytokine Res.* 36 (12), 706–712. doi:10.1089/jir.2016.0057

Liu, X., Zou, Q., Zeng, B., Fang, Y., and Wei, H. (2013). Analysis of fecal Lactobacillus community structure in patients with early rheumatoid arthritis. *Curr. Microbiol.* 67 (2), 170–176. doi:10.1007/s00284-013-0338-1

Lucchino, B., Spinelli, F. R., Iannuccelli, C., Guzzo, M. P., Conti, F., and Franco, M. D. (2019). Mucosa-environment interactions in the pathogenesis of rheumatoid arthritis. *Cells* 8 (7), 700. doi:10.3390/cells8070700

Maddaloni, M., Kochetkova, I., Hoffman, C., and Pascual, D. W. (2018). Delivery of IL-35 by Lactococcus lactis ameliorates collagen-induced arthritis in mice. *Front. Immunol.* 9. 2691. doi:10.3389/fimmu.2018.02691

Maddaloni, M., Kochetkova, I., Jun, S., Callis, G., Thornburg, T., and Pascual, D. W. (2015). Milk-based nutraceutical for treating autoimmune arthritis via the stimulation of IL-10- and TGF-beta-producing CD39+ regulatory T cells. *PLoS One* 10 (1), e0117825. doi:10.1371/journal.pone.0117825

Maddur, M. S., Miossec, P., Kaveri, S. V., and Bayry, J. (2012). Th17 cells: biology, pathogenesis of autoimmune and inflammatory diseases, and therapeutic strategies. *Am. J. Pathol.* 181 (1), 8–18. doi:10.1016/j.ajpath.2012.03.044

Maeda, Y., and Takeda, K. (2017). Role of gut microbiota in rheumatoid arthritis. *J. Clin. Med.* 6 (6), 60. doi:10.3390/jcm6060060

Mann, E. R., Bernardo, D., English, N. R., Landy, J., Al-Hassi, H. O., Peake, S. T. C., et al. (2016). Compartment-specific immunity in the human gut: properties and functions of dendritic cells in the colon versus the ileum. *Gut* 65 (2), 256–270. doi:10.1136/gutjnl-2014-307916

Mao, K., Baptista, A. P., Tamoutounour, S., Zhuang, L., Bouladoux, N., Martins, A. J., et al. (2018). Innate and adaptive lymphocytes sequentially shape the gut microbiota and lipid metabolism. *Nature* 554 (7691), 255–259. doi:10.1038/nature25437

Martinez-Guryn, K., Leone, V., and Chang, E. B. (2019). Regional diversity of the gastrointestinal microbiome. *Cell Host Microbe* 26 (3), 314–324. doi:10.1016/j.chom.2019.08.011

Maynard, C. L., Elson, C. O., Hatton, R. D., and Weaver, C. T. (2012). Reciprocal interactions of the intestinal microbiota and immune system. *Nature* 489 (7415), 231–241. doi:10.1038/nature11551

Peng, J., Lu, X., Xie, K., Xu, Y., He, R., Guo, L., et al. (2019). Dynamic alterations in the gut microbiota of collagen-induced arthritis rats following the prolonged administration of total glucosides of paeony. *Front. Cell. Infect. Microbiol.* 9, 204. doi:10.3389/fcimb.2019.00204

Rogier, R., Evans-Marin, H., Manasson, J., van der Kraan, P. M., Walgreen, B., et al. (2017). Alteration of the intestinal microbiome characterizes preclinical inflammatory arthritis in mice and its modulation attenuates established arthritis. *Sci. Rep.* 7 (1), 15613. doi:10.1038/s41598-017-15802-x

Shan, J., Peng, L., Qian, W., Xie, T., Kang, A., Gao, B., et al. (2018). Integrated serum and fecal metabolomics study of collagen-induced arthritis rats and the therapeutic effects of the zushima tablet. *Front. Pharmacol.* 9, 891. doi:10.3389/fphar.2018.00891

Tajik, N., Frech, M., Schulz, O., Schett, G., and Zaiss, M. M. (2020). Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. *Nat. Commun.* 11 (1), 1995. doi:10.1038/s41467-020-15831-7

Tuovinen, E., Keto, J., Nikkila, J., Mättö, J., and Lähteenmäki, K. (2013). Cytokine response of human mononuclear cells induced by intestinal Clostridium species. *Anaerobe* 19, 70–76. doi:10.1016/j.anaerobe.2012.11.002

Wang, T., Wei, Z. F., Dou, Y. N., Xia, Y.-f., and Dai, Y. (2015). [Effect of madecassoside on intestinal mucosal immunity in collagen-induced arthritis rats]. *Zhong Yao Cai* 38 (2), 333–338

Williams, K., Milner, J., Boudreau, M. D., Gokulan, K., Cerniglia, C. E., and Khare, S. (2015). Effects of subchronic exposure of silver nanoparticles on intestinal microbiota and gut-associated immune responses in the ileum of sprague-dawley rats. *Nanotoxicology* 9 (3), 279–289. doi:10.3109/17435390.2014.921346

Wu, Q., Wang, Y., Wang, Q., Yu, D., Wang, Y., Song, L., et al. (2016). The bispecific antibody aimed at the vicious circle of IL-1β and IL-17A, is beneficial for the

collagen-induced rheumatoid arthritis of mice through NF-κB signaling pathway. *Immunol. Lett.* 179, 68–79. doi:10.1016/j.imlet.2016.09.001

Xiao, C., Lu, C., Zhao, L., Liu, Z., Zhang, W., He, Y., et al. (2006). The effects of triptolide on enteric mucosal immune responses of DBA/1 mice with collagen-induced arthritis. *Planta Med.* 72 (14), 1268–1272. doi:10.1055/s-2006-947240

Xiao, C., Zhao, L., Liu, Z., Lu, C., Zhao, N., Yang, D., et al. (2009). The effect of triptolide on CD4+ and CD8+ cells in the Peyer's patch of DA rats with collagen induced arthritis. *Nat. Prod. Res.* 23 (18), 1699–1706. doi:10.1080/14786410802187783

Xu, H., Zhao, H., Fan, D., Liu, M., Cao, J., Xia, Y., et al. (2020). Interactions between gut microbiota and immunomodulatory cells in rheumatoid arthritis. *Mediat. Inflamm.* 2020, 1430605. doi:10.1155/2020/1430605

Yamashita, M., Matsumoto, K., Endo, T., Ukibe, K., Hosoya, T., Matsubara, Y., et al. (2017). Preventive effect of Lactobacillus helveticus SBT2171 on collagen-induced arthritis in mice. *Front. Microbiol.* 8, 1159. doi:10.3389/fmicb.2017.01159

Yang, L., Anderson, D. E., Baecher-Allan, C., Hastings, W. D., Bettelli, E., Oukka, M., et al. (2008). IL-21 and TGF-beta are required for differentiation of human T(H)17 cells. *Nature* 454 (7202), 350–352. doi:10.1038/nature07021

Yu, M., Mu, C., Zhang, C., Yang, Y., Su, Y., and Zhu, W. (2018). Marked response in microbial community and metabolism in the ileum and cecum of suckling piglets after early antibiotics exposure. *Front. Microbiol.* 9, 1166. doi:10.3389/fmicb.2018.01166

Zhao, H., Xu, H., Zuo, Z., Wang, G., Liu, M., Guo, M., et al. (2018). Yi shen juan Bi pill ameliorates bone loss and destruction induced by arthritis through modulating the balance of cytokines released by different subpopulations of T cells. *Front. Pharmacol.* 9, 262. doi:10.3389/fphar.2018.00262

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 02 February 2021
doi: 10.3389/fphar.2020.635823

# Biomarkers of Response to Biologic Therapy in Juvenile Idiopathic Arthritis

*Varvara Choida[1,2], Margaret Hall-Craggs[3], Bethany R. Jebson[1,4], Corinne Fisher[1,2], Maria Leandro[1,2], Lucy R. Wedderburn[1,4,5] and Coziana Ciurtin[1,2]**

[1]Centre for Adolescent Rheumatology Versus Arthritis at UCL UCLH and GOSH, Division of Medicine, University College London, London, United Kingdom, [2]Department of Adolescent Rheumatology, University College London Hospital, London, United Kingdom, [3]Centre for Medical Imaging, University College London, London, United Kingdom, [4]University College London Great Ormond Street Institute for Child Health, London, United Kingdom, [5]NIHR Biomedical Research Centre at Great Ormond Street Hospital, London, United Kingdom

**Background:** Juvenile idiopathic arthritis (JIA) is the most common chronic inflammatory arthritis of childhood, characterized by various clinical phenotypes associated with variable prognosis. Significant progress has been achieved with the use of biologic treatments, which specifically block pro-inflammatory molecules involved in the disease pathogenesis. The most commonly used biologics in JIA are monoclonal antibodies and recombinant proteins targeting interleukins 1 (IL-1) and 6 (IL-6), and tumor necrosis factor α (TNF-α). Several biomarkers have been investigated in JIA.

**Aims:** To assess the level of evidence available regarding the role of biomarkers in JIA related to guiding clinical and therapeutic decisions, providing disease prognostic information, facilitating disease activity monitoring and assessing biologic treatment response in JIA, as well as propose new strategies for biologic therapy-related biomarker use in JIA.

**Methods:** We searched PubMed for relevant literature using predefined key words corresponding to several categories of biomarkers to assess their role in predicting and assessing biologic treatment response and clinical remission in JIA.

**Results:** We reviewed serological, cellular, genetic, transcriptomic and imaging biomarkers, to identify candidates that are both well-established and widely used, as well as newly investigated in JIA on biologic therapy. We evaluated their role in management of JIA as well as identified the unmet needs for new biomarker discovery and better clinical applications.

**Conclusion:** Although there are no ideal biomarkers in JIA, we identified serological biomarkers with potential clinical utility. We propose strategies of combining biomarkers of response to biologics in JIA, as well as routine implementation of clinically acceptable imaging biomarkers for improved disease assessment performance.

**Keywords: juvenile idiopathic arthritis, serological biomarkers, imaging biomarkers, cellular biomarkers, biologics**

OPEN ACCESS

***Edited by:***
*Francesca Oliviero, University of Padua, Italy*

***Reviewed by:***
*Claudio Ferrante, University of Studies G. d'Annunzio Chieti and Pescara, Italy*
*Haroon Khan, Abdul Wali Khan University Mardan, Pakistan*

****Correspondence:***
*Coziana Ciurtin*
*c.ciurtin@ucl.ac.uk*

***Specialty section:***
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

***Received:*** *30 November 2020*
***Accepted:*** *31 December 2020*
***Published:*** *02 February 2021*

***Citation:***
*Choida V, Hall-Craggs M, Jebson BR, Fisher C, Leandro M, Wedderburn LR and Ciurtin C (2021) Biomarkers of Response to Biologic Therapy in Juvenile Idiopathic Arthritis. Front. Pharmacol. 11:635823. doi: 10.3389/fphar.2020.635823*

## INTRODUCTION

Juvenile idiopathic arthritis (JIA) is a heterogeneous group of diseases, with onset before the age of 16. JIA has been divided into seven subtypes with distinct clinical presentations, according to the International League of Associations for Rheumatology (ILAR) classification criteria (Petty et al., 1998). More specifically, the categories are systemic-onset JIA (SJIA), persistent or extended oligoarticular JIA, polyarticular rheumatoid factor (RF) positive and polyarticular RF negative JIA, enthesitis-related arthritis (ERA), psoriatic arthritis (PsA) and undifferentiated arthritis. There is a variety of composite scores and outcomes to quantify and monitor the disease activity in JIA (McErlane et al., 2013). The Juvenile Arthritis Disease Activity Score (JADAS) is a composite score consisted of the physician and patient/guardian global assessment (visual analogue scale 0–10 cm), the number of active joints and the normalized values of C-reactive protein (CRP) or erythrocyte sediment ratio (ESR) out of 10 (Consolaro et al., 2016). The American College of Rheumatology (ACR) Pediatric response criteria (ACR Pedi 30/50/70 and 90) evaluate 30, 50, 70, and 90% improvement in response to treatment, respectively (Giannini et al., 1997; Ruperto et al., 1998). They include two additional core outcome variables to JADAS: the number of limited joints and functional ability, measured by the Childhood Health Assessment Questionnaire (CHAQ). There are established definitions for inactive disease, clinical remission on treatment (inactive disease for ≥6 months) and off treatment (inactive disease for ≥12 months) (Wallace et al., 2004), as well as for flares (Brunner et al., 2002). However, these definitions do not work equally well for all JIA subtypes because of the heterogeneity of patients' clinical presentation, and alternative definitions have surfaced and used as outcomes in research studies (Heiligenhaus et al., 2012; Consolaro et al., 2016; Zanwar et al., 2018).

The emergence of biologic treatments has changed the prognosis for many JIA patients, whose condition did not improve adequately on conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs), mainly methotrexate, or experienced side effects because of them. TNF-α inhibitors, such as etanercept (human dimeric fusion protein which functions as a decoy receptor and binds to soluble TNF-α), adalimumab (human monoclonal antibody -mAb- which binds with high affinity both soluble and membrane-bound TNF-α) and infliximab (chimeric mAb which blocks both soluble and trans-membrane TNF-α) are widely used in JIA. In fact, etanercept is one of the most frequently prescribed biologics for JIA in many countries, including the United Kingdom (Geikowski et al., 2014; Kearsley-Fleet et al., 2020). Other biologics include tocilizumab (humanized mAb which blocks both soluble and trans-membrane IL-6), anakinra (human IL-1 receptor agonist which blocks IL-1 type 1 receptor) and canakinumab (human mAb against IL-1β), abatacept (human cytotoxic T-lymphocyte-associated protein 4 immunoglobulin fusion protein, acting as T-cell co-stimulatory blockade) and rituximab, a chimeric anti-CD20 mAb causing B-cell depletion. The efficacy of biologics varies depending on the disease subtype, although there is lack of head-to-head clinical trials between different biologics (Davies et al., 2017). Despite the positive short-term outcomes in numerous studies (Ungar et al., 2013), many patients switch biologics due to primary inefficacy, loss of response or adverse effects. Data from biologic registries in the United Kingdom, suggest that 23% of patients receive at least two biologic drugs, 5% at least three and 1% four or more biologic drugs within a median observational period of 2.2 years (Kearsley-Fleet et al., 2020). The retention rate of biologics declines with time, from 92.9% in the first year of treatment to 68.1% at 4 years, according to a Portuguese registry (Mourao et al., 2016). About one third of JIA patients retained their first anti-TNF treatment in 10 years, according to a local registry (Favalli et al., 2017). In addition, tapering or discontinuation of biologic treatment is a reasonable option in the context of clinical remission. Unfortunately, in many cases treatment requires to be resumed, due to worsening disease control. Therefore, there is a great need for biomarkers to guide clinical decisions, such as commencing, switching or tapering biologic DMARDs (bDMARDs).

Biomarkers are characteristics that are objectively measured and indicate the presence or severity of a disease state. Therapeutic biomarkers reflect biological, pathogenic or pharmacologic processes as indicators of a therapeutic effect, whilst surrogate markers are biomarkers that serve as a substitute for a clinically meaningful endpoint and can provide evidence to help predict the effect of a therapeutic intervention (Hunter et al., 2010). In this review, we present the results of a comprehensive search of the literature via PubMed in order to identify clinical, serological, genetic, cellular and imaging biomarkers which can assist clinicians in their efforts to personalize bDMARDs prescription and adjust treatment strategies for JIA patients in a judicious manner. As the largest body of evidence regarding potential biomarker utility is related to treatment with etanercept in JIA, and SJIA represents the most severe JIA phenotype, we will be dedicating particular attention to studies investigating this specific treatment and disease type.

## BASELINE CLINICAL CHARACTERISTICS OF JIA AS PREDICTORS OF RESPONSE TO BIOLOGIC TREATMENTS

There have been multiple studies, comprising large number of patients, which assessed the baseline characteristics as predictors of response to etanercept, which has been one of the best studied biologic treatments in JIA (**Table 1**). Various patient characteristics, such as lower CHAQ scores reflecting better functional levels (Otten et al., 2011a; Geikowski et al., 2014; Kearsley-Fleet et al., 2016), lack of concurrent steroid treatment (Geikowski et al., 2014; Kearsley-Fleet et al., 2016) and younger age (Solari et al., 2013; Geikowski et al., 2014; Kearsley-Fleet et al., 2016) appeared to be favourable characteristics for successful treatment with etanercept. Patients with SJIA were less likely to have a positive response to etanercept, compared to other JIA types (Otten et al., 2011a; Geikowski et al., 2014), whereas the persistent oligoarticular type was associated with the highest response rate to etanercept (Alexeeva et al., 2017).

**TABLE 1 |** Baseline clinical, serological and therapeutic characteristics as predictors of response to etanercept in JIA.

| Ref. | Study design | N patients | Results |
|---|---|---|---|
| Geikowski et al. (2014) | Prospective observational multi-centre | 863 | Baseline predictors of ACR Pedi 70 after 6 months of treatment were:<br>• High ESR (OR 1.02; 95% CI 1.01, 1.03)<br>• Lower CHAQ-DI (OR 0.70; 95% CI 0.56, 0.88)<br>• Lower age at start of treatment (OR 0.94; 95% CI 0.91, 0.98)<br>• Treatment without corticosteroids (OR 0.68; 95% CI 0.49, 0.94)<br>• Any JIA type other than SJIA (OR 0.28; 95% CI 0.16, 0.52), model AUC 0.646 |
| Kearsley-Fleet et al. (2016) | Prospective observational multi-centre | 496 | Baseline predictors of ACR Pedi 90 after 1 year of treatment were:<br>• Shorter disease duration (OR 0.91; 95% CI 0.85, 0.97)<br>• Lack of concurrent steroid treatment (OR 0.57; 95% CI 0.35, 0.93)<br>• History of chronic anterior uveitis (OR 2.26; 95% CI 1.08, 4.71) |
| Otten et al. (2011a) | Prospective observational multi-centre | 262 | Baseline predictors of excellent response, compared to intermediate or poor response* after 15 months of treatment were:<br>• Lower CHAQ score (OR 0.49; 95% CI, 0.33–0.74),<br>• Low number of DMARDs (including methotrexate) used before introduction of ETN (OR 0.64; 95% CI, 0.43–0.95),<br>• Younger age of disease onset (OR 0.92; 95% CI, 0.84–0.99). |
| Mo et al. (2020) | Retrospective single-centre | 87 | A machine learning model to predict response (AUC 79.17%) included:<br>• Tender joint count<br>• Time interval (disease onset to treatment initiation),<br>• Lymphocyte count<br>• Weight |
| Solari et al. (2013) | Retrospective single-centre | 173 | Predictors of inactive disease were:<br>• Age at disease onset<3.6 years [HR 1.61 (1.04–2.49)]<br>• Absence of wrist involvement [HR 2.19 (1.38–3.48)] |
| Alexeeva et al. (2017) | Prospective open-label | 197 | Clinical phenotype predicted response:<br>• More patients with persistent oligoarticular (65.5%) vs. RF negative polyarticular (23.4%) or ERA (38.5%) achieved an excellent response to treatment at 1 year. |
| Su et al. (2017) | Retrospective single-centre | 58 | CID at 6 months post-treatment was not predicted by:<br>• Age of disease onset<br>• Gender<br>• JIA subtypes (only extended oligoarticular, polyarticular, SJIA included)<br>• Number of active joints at disease onset<br>• Duration from disease onset to starting treatment<br>• ESR, CRP, and CHAQ scores.<br>No difference in IL- 12p70, TNF-α, IL-10, IL-6, and IL-1β levels before and 6 months post ETN treatment, between the patients who achieved or not CID at 6 months |

*ACR, American college of rheumatology; AUC, area under the curve; CHAQ-DI, childhood health assessment questionnaire disability index; CI, confidence interval; CID, clinically inactive disease; CRP, c-reactive protein; DAS, disease activity score; DMARDs, disease modifying anti-rheumatic drugs; ESR, erythrocyte sediment ratio; ETN, etanercept; ERA, enthesitis-related arthritis; HR, hazard ratio; (95% confidence interval); IL, interleukin; ILAR, International League of Associations for Rheumatology; JADAS, juvenile arthritis disease activity score; JIA, juvenile idiopathic arthritis; OR, odds ratio; Pedi, pediatric; RF, rheumatoid factor; TNF, tumor necrosis factor.*

Interestingly, shorter disease duration was a positive predictor of therapeutic benefit (Kearsley-Fleet et al., 2016; Mo et al., 2020) in contrast to the number of DMARDs used before the initiation of etanercept treatment, which was associated negatively with treatment response (Otten et al., 2011a). Taken together, these findings support the use of etanercept early in the disease course for non-systemic JIA.

Data from 62 polyarticular JIA patients who completed a long extension clinical trial of adalimumab, suggested that the achievement of JADAS-27 (assessing 27 joints) clinical remission was more likely in early responders, who met either the ACR Pedi 50 or above response criteria or JADAS-27 threshold for inactive or low disease activity at 4, 8, 12 and 16 weeks (Lovell et al., 2020). Patients with ERA who had raised body mass index (BMI) were less likely to achieve inactive disease after 1 year irrespective of treatments, including biological agents; 19/72 of ERA patients were on anti-TNF treatment (Makay et al., 2016).

## THERAPEUTIC DRUG MONITORING AND ANTI-DRUG ANTIBODIES AS BIOMARKERS OF EFFICACY AND TOXICITY OF BIOLOGIC TREATMENTS IN JIA

The clinical utility of TDM and measurement of ADA has been investigated intensively in patients with inflammatory bowel disease (IBD), predominantly in relation to infliximab and adalimumab (Papamichael et al., 2019). Monitoring of trough concentrations and ADA can be 1) proactive, in order to titrate dosing, with a view to improving clinical outcomes and drug survival, or 2) reactive, to guide decisions upon the emergence of secondary loss of response (SLR). For example, a retrospective study in ulcerative colitis showed that patients who developed SLR on adalimumab or infliximab, despite adequate trough levels, had longer duration of response when switched to a different class

of biologics compared to receiving a different anti-TNF-α agent (Yanai et al., 2015).

The formation of ADA is documented with all the licensed biologic treatments in JIA. However, the relation between ADA and treatment failure or adverse effects, the persistent or transient nature of ADA, as well as their prevalence in relation to treatment duration, vary across the different biologics (Doeleman et al., 2019). For instance, antibodies against etanercept, abatacept and canakinumab are non-neutralizing and are not linked with loss of efficacy (Mori et al., 2012; Sun et al., 2016; Doeleman et al., 2019; Verstegen et al., 2020). Similarly, despite the increased prevalence of ADA in patients treated with anakinra (82% at 12 months), the majority of patients develop non-neutralizing antibodies and do not lose treatment response (Lovell et al., 2000; Ilowite et al., 2009). In comparison, adalimumab and infliximab ADA are associated with reduced trough levels and loss of efficacy (Ruperto et al., 2010; Skrabl-Baumgartner et al., 2015; Marino et al., 2018; Brunelli et al., 2020). Although the prevalence of tocilizumab ADA is low in JIA, 43% of patients with neutralizing ADA experienced treatment failure compared with 6% of JIA patients with no detectable ADA (Brunner et al., 2015). Concomitant use of methotrexate has a protective role against the development of adalimumab ADA [risk ratio 0.33; 95% Confidence interval (95% CI) 0.21, 0.52] (Doeleman et al., 2019). The above findings regarding adalimumab were also reported in relation to patients receiving this drug for JIA-associated uveitis (Skrabl-Baumgartner et al., 2019). In addition, the risk of infusion reactions in patients treated with tocilizumab, infliximab or rilonacept increased in the presence of ADA (Ruperto et al., 2007; Lovell et al., 2013; Yokota et al., 2014).

In conclusion, there is a potential clinical role of monitoring ADA and trough concentrations, especially in patients receiving adalimumab and infliximab monotherapy (Doeleman et al., 2019). However, detecting biologic drug trough levels is not always practical, especially for patients who self-administer their medication subcutaneously as their blood tests should be coordinated prior to their next dose administration. Moreover, establishing concentration thresholds for therapeutic benefit is challenging, because results are likely to vary depending on the selected assays, clinical endpoints, or even type of JIA.

## POTENTIAL ROLE OF MEASURING PROINFLAMMATORY PROTEINS IN SERUM AS BIOMARKERS OF THERAPEUTIC RESPONSE TO BIOLOGIC TREATMENTS IN JIA

The myeloid-related S100 proteins (low molecule proteins named S100 as they are soluble in 100%, i.e., saturated, ammonium sulfate at neutral pH): S100A12 and the S100A8/S100A9 complex (also known as myeloid-related protein 8/14–MRP8/14 or calprotectin) are proinflammatory proteins secreted by myeloid cells. This family of proteins have been widely investigated in the rheumatological field and have shown significant utility as biomarkers to predict disease severity, response to treatment and disease flare in conditions including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE) and JIA (Soyfoo et al., 2009; Nordal et al., 2016). Their efficacy as potential biomarkers for response to treatment in JIA was initially investigated in patients treated with methotrexate monotherapy. A prospective study of 87 patients, with all types of JIA represented, demonstrated that patients with higher MRP8/14 levels before initiating methotrexate were more likely to have a better response from treatment at 6-months follow-up (Moncrieffe et al., 2013). Similarly, a multi-centre study, including 88 patients from three national biologic registries who received etanercept or adalimumab as their first biologic treatment, showed that baseline MRP8/14 levels were significantly higher in responders compared to non-responders (Anink et al., 2015). Treatment response was defined as achieving at least ACRPedi50 response within 6 months of treatment. Levels above 1,193 ng/ml predicted treatment efficacy of anti-TNF biologic with 66% sensitivity, 81% specificity and an area under the curve (AUC) of 0.76. Furthermore, there was a greater reduction in the levels of MRP8/14 in patients who achieved inactive disease vs. those who did not. In the same patient cohort, S100A12 baseline levels were also higher in patients who met the treatment response criteria (Gohar et al., 2018). A concentration above 213 ng/ml predicted a minimum ACRPedi50 response with 58.6% sensitivity and 80.6% specificity (AUC = 0.734). Moreover, the mean S100A12 levels decreased significantly after 4 weeks of etanercept treatment in 21 patients with polyarticular and oligoarticular JIA (Foell et al., 2004). When tested alone or incorporated into multivariate models, S100A8/S100A9 proteins have shown higher predictive power when determining treatment response than clinical variables such as ESR or CRP (42, 44). However recently, a Dutch study involving 123 patients with early non-systemic JIA, mostly RF negative polyarticular subtype, reported no difference in baseline MRP8/14 levels between responders (patients who achieved at least a ACRPedi50 response) and non-responders, though patients in this study received different DMARDs (Barendregt et al., 2020). Another observational study which measured the baseline levels of MRP8/14 in 152 non-systemic JIA patients before starting anti-TNF treatment, demonstrated that patients who reached inactive disease at 12 months had higher levels compared to patients who did not (Alberdi-Saugstrup et al., 2017). However, a cut-off concentration of 500 ng/ml was associated with very low sensitivity of predicting inactive disease and discontinuation due to lack of efficacy (22 and 39% respectively), with specificities of 80 and 83% respectively. At the same time, the selected cut-off level could not predict treatment response based on any of the ACR Pedi criteria.

Long-lasting efficacy is a desirable outcome of biologic treatment in JIA. A potentially promising biomarker for long-term retention of treatment with etanercept is the change in TNF-α levels, as documented in a cohort of 41 non-systemic JIA patients with a median follow-up of 90 months (Kahn et al., 2016). Patients who experienced benefit and remained on treatment had more increase in their TNF-α levels at 6 weeks post-treatment onset than those who did not. TNF-α was detected in serum as complexes between etanercept (acting as decoy TNF-α receptor) and soluble

TNF-α. This might not apply to other anti-TNF agents, as another study found that TNF-α and interleukin-17 (IL-17) levels during the first 6 months of treatment were significantly higher amongst JIA patients treated successfully with etanercept (n = 6) compared to adalimumab (n = 7) (Walters et al., 2016).

## CELL BIOMARKERS AS PREDICTORS OF RESPONSE TO BIOLOGIC TREATMENT

In RA, there is evidence of an increased percentage of regulatory T-cells (Treg) in responders to adalimumab compared to non-responders, therefore the Treg subpopulation has been suggested as a potential biomarker of response (McGovern et al., 2012; Nguyen et al., 2018). A study in polyarticular JIA, including 30 patients treated with etanercept, methotrexate and prednisolone, explored the different Treg subsets in patients with active vs. inactive disease status and found that patients with active disease had a higher percentage of human leukocyte antigen-D related (HLA-DR) + Treg cells compared to patients with inactive disease (Rossetti et al., 2017). Interestingly, these Treg clonotypes were more closely related to synovial rather than circulating Treg cells and remained suppressive. Moreover, polyarticular and oligoarticular JIA patients on remission were found to have a significantly lower increase in the percentage of switched memory B-cells compared to active patients, during treatment with TNF inhibitors and methotrexate. On the other hand, patients on methotrexate alone had a similar rise in the frequency of this cell subset, irrespective of disease activity (Marasco et al., 2018), suggesting that switched memory B-cells could be a potential biomarker of response to biologic treatment in JIA.

## GENETIC AND TRANSCRIPTOMIC BIOMARKERS OF RESPONSE TO BIOLOGIC TREATMENT IN JIA

Various genetic biomarkers have been investigated to assess their potential as predictor biomarkers of clinical response in JIA, with the majority of studies focused on response to methotrexate treatment as first line therapy in JIA (Hinks et al., 2011; Ramsey et al., 2019). Human leukocyte antigen B27 (HLA-B27) positivity in JIA patients was associated with double the odds of not being in clinical remission of treatment at the end of 8 years follow-up irrespective of treatment (Berntson et al., 2013). Despite previous studies identifying numerous single nucleotide polymorphisms (SNPs) at distinct loci associated with systemic JIA, only the high expression of IL1RN (the gene encoding IL1 receptor antagonist) alleles correlated strongly with lack of response to anakinra therapy (Arthur et al., 2018).

Analysis of gene expression profiles from SJIA achieving the adapted ACR JIA response criteria following initiation of treatment with canakinumab (including IL-1β, IL-1 receptors (IL1-R1 and IL1-R2), IL-1 receptor accessory protein (IL1-RAP), and IL-6) found the strongest clinical response was observed in patients with higher baseline expression of dysregulated genes and a strong early transcriptional response (Brachat et al., 2017). This suggests that successful treatment with canakinumab led to downregulation of innate immune response genes.

However, gene transcriptional profiling of peripheral blood mononuclear cells (PBMCs) of patients with polyarticular JIA with active disease vs. remission (on methotrexate monotherapy or methotrexate combined with biologic treatment) revealed underlying biologic differences which seem to represent a disease signature, as even JIA patients with well controlled disease had persistent transcriptomic differences compared to healthy children (Knowlton et al., 2009; Moncrieffe et al., 2010). The hepatocyte nuclear factor 4 alpha (HNF4α), which is expressed by T cells and granulocytes, emerged in another study as a key factor in controlling genes associated with JIA remission on treatment (including biologic therapies) (Jiang et al., 2013).

## IMAGING BIOMARKERS OF RESPONSE TO BIOLOGIC TREATMENTS

Imaging is most commonly used to confirm diagnosis, evaluate disease activity and response to therapy (Magni-Manzoni et al., 2009; Muller et al., 2009; Rebollo-Polo et al., 2011; Brown et al., 2012; Bugni Miotto e Silva et al., 2014). There is very little published on the use of imaging biomarkers to predict response and outcome of therapy. Ultrasound and magnetic resonance imaging (MRI) are the most commonly used imaging modalities/biomarkers to assess disease as they are sensitive to identifying inflammation and use non-ionizing radiation.

As far as ultrasound is concerned, one study with 42 JIA patients used a comprehensive (44-joint) power Doppler ultrasound (PDUS) assessment at 0, 3 and 6 months of starting additional DMARD or biologic treatment, in order to measure treatment response. A reduced 10-joint PDUS was deducted and found to have good sensitivity to change at 6 months of treatment (Collado et al., 2013). Another prospective study reported that the number of ultrasound positive joints (out of 28) decreased significantly after 24 weeks treatment with etanercept. The same study concluded that a higher number of ultrasound positive joints at baseline was seen in patients who achieved ACRPedi50 response compared to patients who did not, and that it was an independent predictive factor of response (odds ratio – OR = 1.438, 95% CI: 1.091–1.897) (Zhou and Gu, 2019). On the other hand, there are conflicting results as to whether positive ultrasound findings in JIA patients with inactive disease can predict flares, although it should be noted that a minority of patients were on biologic treatment in these studies (Magni-Manzoni et al., 2013; Miotto et al., 2017; De Lucia et al., 2018; Zhao et al., 2018).

Conventional MRI has been used in clinical trials to assess treatment response in RA (Woodworth et al., 2017), psoriatic arthritis (NCT03783026) and axial spondyloarthritis (van der Heijde et al., 2018). More specifically, the Outcome Measures in Rheumatology Clinical Trial (OMERACT) RA MRI score (RAMRIS), which evaluates the wrist and second to fifth

metacarpophalangeal joints for osteitis, synovitis, erosions and joint space narrowing, is a valid biomarker in RA. It has demonstrated responsiveness, as early as 2 weeks post treatment (Conaghan et al., 2014) and is predictive of radiographic progression (Baker et al., 2014; Conaghan et al., 2014; Conaghan et al., 2019).

In a similar way, the Juvenile Arthritis Magnetic Resonance Imaging Score (JAMRIS) derives from MRI knee examination. Synovial hypertrophy, a component of the score, changed significantly in 15 consecutive JIA patients who were treated for 12 months with DMARDs and/or TNF-α blockers (Hemke et al., 2013). The Spondyloarthritis Research Consortium of Canada (SPARCC) scoring system for the assessment of sacroiliac joints has also been evaluated in juvenile spondyloarthritis; the standardized response mean calculated from paired MRI examinations before and after treatment (18/35 on biologic treatment) was moderate (Panwar et al., 2019). Moreover, a retrospective analysis of serial MRI scans of the sacroiliac joints in ERA patients, before and after initiation of TNF inhibitors, using again the SPARCC score, showed reduction of inflammation after treatment, but progression of structural damage (Bray et al., 2019). In addition to the aforementioned semi-quantitative scores, apparent diffusion coefficient (ADC) is a potential quantitative MRI biomarker for sacroiliitis (Vendhan et al., 2016). A study in patients with ERA treated with biologics showed that the reduction in ADC values after biologic treatment was greater in responders vs. non-responders (Bray et al., 2017).

There are inherent limitations in the usefulness of semi-quantitative scores as biomarkers of treatment response. The main drawback of ultrasound and MRI derived inflammation scores is that they are based on subjective interpretation of images by radiologists, which introduces bias and measurement error. This is more complicated when children are assessed, as the distinction between true inflammation and skeletal immaturity is challenging. On the other hand, quantitative imaging biomarkers are less operator-dependent and therefore have better reproducibility. Importantly, they offer a numerical value to facilitate comparison between serial scans. Although further work is needed for the technical and clinical validation of such biomarkers (Barendregt et al., 2019; Hall-Craggs et al., 2019; European Society of Radiology, 2020), they provide an opportunity for more robust measurement of treatment response and the ability to establish thresholds that guide clinical treatment.

## VARIOUS PREDICTOR BIOMARKERS FOR SUCCESSFUL WITHDRAWAL OF BIOLOGICS TREATMENT IN JIA

The ultimate goal for patients with JIA, as with other chronic diseases, is to achieve remission off medications, with obvious benefits for the patient as well as society, through improved productivity and reduced costs of health care. A systematic review of treatment withdrawal in JIA patients in remission described that the frequency of flares ranged from 30 to 100% in different studies (Halyabar et al., 2019). Data from a Canadian inception cohort showed that the probability of flare (defined as no longer fulfilling the criteria of inactive disease) within 12 months of attaining inactive disease was 42.5% and that of requiring treatment intensification was 26.6% (Guzman et al., 2016). After treatment withdrawal the corresponding numbers were 31.7 and 25%, although specifically for SJIA the risks were significantly lower, 6.2 and 3%, respectively. The identified risk factors for flares were RF positive polyarthritis, positive antinuclear antibodies (ANA) and features of severe disease before achieving inactive disease status, such as joint count over four or use of biologic treatment. In terms of long-term prognosis, results from the Nordic JIA study, an inception cohort study, suggested that only 32.8% (108/329) of participants achieved clinical remission (CR) defined as inactive disease without medications for 12 months, after 18 years of follow-up (Glerup et al., 2020). Patients with persistent oligoarticular and systemic-onset JIA achieved CR at the highest rate (54.2 and 53.8% respectively), in contrast to ERA, where only 8.1% of patients were successful. The systemic-onset category demonstrated also the highest probability of maintaining remission off biologic treatment, in comparison with other categories, in a multi-centre retrospective analysis (Simonini et al., 2018). In terms of the polyarticular JIA phenotype, a prospective study revealed that a significantly higher proportion of patients with RF positive polyarticular disease (7/17 or 40%) on anti-TNF therapy failed to maintain clinically inactive disease (CID) at 6 months, compared to patients with extended oligoarticular (1/18 or 6%) and RF negative polyarticular JIA (19/102 or 18%) (Lovell et al., 2018). Out of 107 patients who remained inactive for 6 months, 67 (63%) flared within 8 months of discontinuation of the biologic. Older age at disease onset, (hazard ratio–HR 0.92; 95%-CI 0.85–0.99), shorter disease duration (HR 1.12; 1.04–1.21), shorter duration from disease onset to achieving CID (HR 1.1; 95% CI 1.01–1.20) and shorter CID duration prior to discontinuation of biologic therapy (HR 1.16; 95% 1.01, 1.33) were associated with a reduced likelihood of flaring. In a retrospective analysis which included only RF negative polyarticular and oligoarticular JIA types, positive ANA, male sex and raised CRP were identified as risk factors for flaring after discontinuation of etanercept, but could account only for 14% of the variability of the prediction (Aquilani et al., 2018). Shorter duration of etanercept treatment (6.1 vs. 15.8 months) before discontinuation and faster attainment of CID were recorded in patients who did not relapse after discontinuation of etanercept, compared to relapsers (Su et al., 2017). However, data from the Dutch Arthritis and Biologicals in Children (ABC) registry depicted the opposite association, which is that shorter duration of treatment (28.6 vs. 45 months) was recorded in the 15/39 patients who flared after stopping etanercept treatment as in remission (Otten et al., 2011a). In addition, data from a German biologic registry, showed that 11.7% of patients achieved drug-free remission at a mean follow-up of 9.1 years (Minden et al., 2019). In this study, they discovered that patients who initiated biologic treatment (etanercept in 91% cases) within 2 years of disease onset had higher chance of achieving remission off drugs (defined as clinical JADAS-10

score, assessing up to 10 active joints ≤1) at last follow-up, compared to others who started treatment between 2 and 5 years (OR: 0.28; 95% CI 0.12–0.64) or after 5 years (OR: 0.12; 95% CI 0.05–0.27) of disease onset. The researchers also demonstrated that earlier biologic treatment (<2 years) was associated with a higher proportion of patients with no functional limitations and optimal well-being in young adulthood compared to late treatment (>5 years). Furthermore, shorter disease duration (0.5 vs. 1.1 years) was associated with a successful gradual discontinuation of adalimumab in 29/35 patients with ERA, who had attained inactive disease and been on treatment for at least 2 years (Papailiou S et al., 2020). In contrast to the hopeful results of this retrospective study, data from the ABC registry revealed that despite the high rates of good response to etanercept in psoriatic JIA patients, 5/6 patients who ceased treatment at 22 months flared at a median of 2 months (Otten et al., 2011b). Finally, longer retention of the first biologic, as well as increased frequency of treatment suspension due to remission was observed in patients aged less than 16 years at the initiation of biologic therapy, as per data from a Spanish biologic registry (Bethencourt Baute et al., 2018).

As far as laboratory markers are concerned, the S100 proteins have been reported to not only predict response to biologic treatment, but also the risk of flaring post methotrexate and biologic treatment withdrawal (Foell et al., 2010; Anink et al., 2015). MRP8/14 above 720 ng/ml predicted flares within 6 months of discontinuation of etanercept in 26 patients with non-systemic JIA with an AUC of 0.75 (Anink et al., 2015). Moreover, higher levels of vascular endothelial growth factor (VEGF) and S100A12 were found in 9/22 of patients who relapsed after achieving remission, defined as absence of arthritic findings, disease activity score assessing 28 joints in RA (DAS-28) < 2.6, low CRP and matrix metalloproteinase-3 (MMP-3), on methotrexate and/or biologic treatment (Yamasaki et al., 2016). More specifically, S100A12 > 177 ng/ml and VEGF >158 pg/ml predicted relapse with 92.3 and 76.9% sensitivity, respectively and 77.8% specificity for both markers. Furthermore, raised levels of S100A12 during inactive disease was found to predict relapse with an AUC of 0.77 (Foell et al., 2004). On the other hand, two subsequent studies did not replicate these findings. In the first study, MRP8/14 or S100A12 were not significantly different between 39 patients with extended oligoarticular of polyarticular disease who flared within 8 months of anti-TNF treatment withdrawal and 67 patients who remained clinically inactive off biologic treatment (Hinze et al., 2019). In the other study, MRP8/14 was tested in two cohorts of non-systemic JIA patients, including 88 patients (27 on anti-TNF treatment) with inactive disease after 12 months of treatment (Barendregt et al., 2020). Levels of MRP8/14 did not predict the development of joint inflammation defined as an active joint count ≥1 at 6 or 12 months post treatment cessation on either cohort. A summary of evidence regarding MRP8/14 in non-systemic JIA created by the authors of the last study uncovered the discrepancies that exist between the published predictive models, which might be explained by the inconsistent definition of outcomes, dissimilar representation of JIA subtypes and treatments, as well as different assays used to measure serological biomarkers (Barendregt et al., 2020). With regards to systemic-onset JIA subtype, a study with 15 patients who stopped anakinra treatment after achieving an adapted ACRPedi90 response at 3 months, demonstrated that S100A12 levels were significantly raised in eight patients who relapsed later (Vastert et al., 2014). There is also limited evidence that higher MRP8/14 levels can predict relapse after anakinra withdrawal, based on two patients flaring and two who remaining inactive (Holzinger et al., 2012). Levels of the autoantibody targeting the oncoprotein DEK (anti-DEK) were found to be significantly elevated in 30 patients with polyarticular JIA who flared within 8 months after ceasing anti-TNF treatment, compared to 59 patients who did not flare. The difference in the anti-DEK levels between the groups was significant based on samples taken after patients flared, whilst anti-DEK levels at the time of discontinuation could not predict the outcome (Mor-Vaknin et al., 2018). Finally, an increased population frequency of an inflammatory CD4 memory subset ($CD3^+CD4^+CD45RA^-TNF\alpha^+$) predicted relapse at 8 months after discontinuation of biologic therapy (AUC = 0.939) in polyarticular JIA patients with inactive disease prior to treatment cessation (Leong et al., 2019).

## POTENTIAL CLINICAL USE OF BIOLOGIC TREATMENT-RELATED BIOMARKERS IN SJIA

Biologic treatments have improved significantly the outcomes in SJIA. Controlling disease activity in SJIA is especially important, as active disease is associated with a higher risk for development of macrophage activation syndrome (MAS), which is a life-threatening complication. The treatment choices include the use of non-steroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, methotrexate, anakinra, canakinumab, tocilizumab and TNFα blockers. There is no consensus on the treatment strategy, although anakinra is the biologic of choice when there are features of MAS (Ringold et al., 2013; Specialised Commissioning Team, 2015). Therefore, biomarkers predictive of response are needed to inform treatment decisions, in order to reduce the risk of complications related to the disease, but also diminish drug-related toxicity, particularly from steroids.

Several studies have reported clinical and laboratory findings that are associated with the achievement of inactive disease, the majority of them concerning therapy with anakinra (**Table 2**). Several biomarkers have been identified as useful in predicting the response to treatment with anakinra: early initiation of treatment increased the odds of achieving inactive disease and high neutrophil count at baseline was associated with good clinical response, whereas increased number of active joints at baseline was a negative prognostic factor for clinical improvement on treatment. Early treatment response appears to predict long-term response to both IL-1 and IL-6 blockers.

The proinflammatory proteins MRP8/14 and S100A12 can be useful as diagnostic and therapeutic prognostic: both markers rise in active disease. The diagnostic accuracy of MRP8/14 exceeded the accuracy of established inflammatory markers such as CRP and ESR (Holzinger et al., 2012), whereas S100A12 can help differentiate between SJIA and other causes of systemic

**TABLE 2 |** Predictors for biologic treatment response in SJIA.

| Ref | Medication | Study design | N patients | Results |
|---|---|---|---|---|
| Gattorno et al. (2008) | ANA | Prospective | 22 (10 responders) | Complete responders had a lower number of active joints vs. non responders (median 3.5 vs. 7) and a higher number of neutrophils (median 19.3 vs. 9.1 × $10^3$/$mm^3$), |
| Nigrovic et al. (2011) | ANA | Retrospective, multi-centre | 46 | Incomplete responders were younger at onset vs. complete responders (median age 5.2 vs. 10.2 years), (OR 1.5 per year; 95% CI 1.1–2.0) |
| Holzinger et al. (2012) | ANA, ETN | Prospective | 52 12 on biologics | • MRP8/14 decreased markedly in responders to biologic treatment (12/12) and in responders (6/12) to methotrexate<br>• MRP8/14 detects flares vs. inactive disease with outstanding diagnostic accuracy (AUC: 0.957 ± 0.019)<br>•MRP8/14 > 740 ng/ml can predict relapse in next 6 months (AUC: 0.91), 13/26 inactive patients relapsed |
| Vastert et al. (2014) | ANA | Prospective single-centre | 20 (15 responders) | • S100A8/9 (MRP8/14), S100A12 and IL-8 decreased markedly in responders (ACR Pedi 90) at 3 months<br>• Lower levels of IL-8, S100A12, S100A8/9 at 3 months in 7/15 patients with ID who succeeded to discontinue treatment within a year (significant only for S100A12) |
| Pardeo et al. (2015) | ANA | Retrospective, single-centre | 25 (14 responders) | Earlier treatment from disease onset associated with ID at 6 months (median 1.9 vs. 24.5 months). |
| Saccomanno et. al. (2019) | ANA | Retrospective single-centre | 62 (24 responders) | Predictors of complete clinical response at 1 year included:<br>•Disease duration ≤3.9 years (OR 6.78; 95% CI 1.30–35.27),<br>•Active joint count ≤10 (OR 8.25; 95% CI 1.26–53.91),<br>•Ferritin >444 ng/ml (OR 4.75; 95% CI 1.16–19.50),<br>•Systemic manifestation score >3 (OR 6.44; 95% CI 1.38–24.62), AUC: 0.83 |
| Kearsley-Fleet et al. (2019) | ANA, TCZ | Prospective, multi-centre | 76 | Baseline characteristics not associated with response (ACR Pedi 90, MDA or ID) |
| Ter Haar et al. (2019) | ANA | Prospective, single-centre | 42 (32 ID at 1 year) | • ID at 1 month after ANA treatment predicted ID at 1 year (OR 27; 95% CI 4.17–539.74), AUC: 0.84<br>• Neutrophils>9 × $10^9$/L at baseline predict ID at 1 year (OR 38.67; 95% CI 6.53–362.73), AUC: 0.85 |
| Ruperto et al. (2018) | CAN | Open-label, long-term extension study | 144 (96 early responders) | Early responders (completed glucocorticoid tapering in part I of trial 2) achieved greater decrease in JADAS during the study as compared with late responders (mixed model; $p < 0.01$) |
| Bielak et al. (2018) | TCZ | Prospective, multi-centre | 46 | • 7/17 (41%) patients showing inactive disease at the last visit had a response to TCZ within 5 weeks<br>• Polycyclic course was associated with greater odds of clinical response (OR 7.0; 95% CI 1.8–27.2) compared to monocyclic or polyarticular course of SJIA |

*ANA, Anakinra; ACR, American college of Rheumatology; AUC, area under the curve; CAN, canakinumab; CI, confidence interval; ETN, etanercept; ID, clinically inactive disease; MDA, minimal disease activity; MRP, myeloid-related protein; OR, odds ratio; Pedi, pediatric; SJIA, systemic juvenile idiopathic arthritis; TCZ, tocilizumab.*

inflammation (Wittkowski et al., 2008). Moreover, their values decreased sharply in patients who displayed significant clinical improvement with treatment, such as fulfilling the ACRPedi90 criteria of response or the Wallace criteria of inactive disease (Holzinger et al., 2012; Vastert et al., 2014). Importantly, low levels during inactive disease were associated with successful tapering of anakinra, whilst levels of MRP8/14 above a cut-off were predictive of relapse (based on limited number of patients) (Holzinger et al., 2012; Vastert et al., 2014).

## DISCUSSION

There have been previous reviews exploring the broad subject of biomarker identification in JIA (Consolaro et al., 2015; Gohar et al., 2016; Shoop-Worrall et al., 2019). This review exposed a diverse group of potential biomarkers, including inherent patient characteristics, clinical, laboratory, genetic, transcriptomic and imaging features, which are associated with short-term and long-term therapeutic goals, such as the attainment of inactive disease on biologic treatment and the sustainment of clinical remission after treatment withdrawal.

The JIA clinical phenotype, as defined by the ILAR classification is an important prognostic factor for long-term disease outcome as patients with persistent oligoarticular and systemic JIA subtypes are more likely to achieve remission without medications. However, JIA phenotype also influenced the response to biologic treatment as patients with persistent oligoarticular JIA had a higher chance to respond to etanercept than patients with polyarticular subtypes, whereas RF positive polyarticular category was associated with a higher risk of flares on anti-TNF treatment. As discussed above, longer disease duration at the onset of biologic treatment, higher CHAQ scores, concurrent steroid administration and previous use of multiple DMARDs are negative predictive factors of response to anti-TNF agents, suggesting that the timing of initiation of biologic treatment is crucial. Biologic treatment

initiation early in the disease course was associated not only with better clinical response to etanercept and anakinra (the latter for patients with SJIA), but also with a higher chance of treatment discontinuation due to remission and better functional outcomes in young adulthood. This is an important observation as this is a factor that can be influenced by clinicians, whereas the same does not apply for the age of disease onset and JIA subtype. Moreover, clinical improvement within weeks from biologic initiation in patients with SJIA, but also in polyarticular JIA patients on adalimumab, is predictive of a future well-controlled disease.

All things considered, it should be noted that there is limited information about clinical predictors of response to biologics other than etanercept and anakinra, as there is longer experience with these biologic treatments in JIA, which is reflected in the available information from national JIA registries. This is also the reason for focusing our review on detailing biomarkers of response to etanercept across various JIA phenotypes and to anakinra in SJIA.

Nonetheless, there is no doubt that data from national registries have deepened our understanding about long-term outcomes of patients with JIA and have allowed us to assess the efficacy and safety of various biologic treatments and discover predictors of treatment success. There is immense potential from the development of national registries. Their growth will ensure that more extensive data can be collected, as efficiently as possible, while also expanding the collaboration and data sharing between nations as treatment recommendations and access to biologic treatment differ world-wide. One of the major challenges is ensuring that data collection is continued without interruptions during transition of JIA patients to adult care. The COVID-19 Global Rheumatology Alliance is a recent example of successful international collaboration resulting in the accumulation of important knowledge related to the risk of COVID-19 infection in immunosuppressed patients, which has informed the management of rheumatology patients during the pandemic (Robinson and Yazdany, 2020).

In terms of laboratory tests, MRP8/14 and S100A12 have emerged as the most promising biomarkers for predicting treatment response to methotrexate and bDMARDs, as well as indicating whether there is an increased risk of flare during inactive disease, which might deter clinicians from tapering treatment. However, not all studies have confirmed their ability to predict flares for non-systemic JIA patients and there was a small number of SJIA patients included. Moreover, the added value of MRP8/14 to the prediction model for treatment response based on clinical features alone was small; $R^2$ increased from 0.50 to 0.54 (Anink et al., 2015), raising further questions about their clinical utility. Further prospective studies with larger number of patients are needed to ratify these encouraging results. The findings from the interventional study PREDICT-JIA, which used S100A12 and high sensitivity CRP for treatment withdrawal stratification are expected in the near future (ISRCTN69963079).

More studies are also required to delineate the pharmacokinetics of biologics and examine whether the proactive measurement of trough levels, along with dose titration can improve patient outcomes and drug retention or support a safer tapering strategy. The presence of neutralizing ADA appears to be linked with potential loss of efficacy or infusion reactions, in the cases of adalimumab, infliximab and tocilizumab. However, many questions remain unanswered, such as if proactive monitoring of ADA and trough levels can reduce the risk of loss of response due to dose titration, or if in the light of secondary inefficacy, drug level and immunogenicity to biologic agents can aid the choice of the subsequent biologic treatment.

As far as imaging is concerned, there is paucity of validated imaging biomarkers in JIA, compared to RA. This might be explained by the different distribution of joint inflammation in JIA, often involving multiple large joints, which are more difficult to image, compared to the small joints of hands or feet alone often affected in RA. In addition, it is less feasible to organize scans for younger children with JIA. An imaging technique which offers whole-body coverage could be a logical option for assessment of JIA patients with different clinical presentation for the detection of subclinical synovitis. Whole-body MRI (WBMRI) with contrast has been used to assess for musculoskeletal inflammation in studies for RA, PsA and ankylosing spondylitis (AS) (Axelsen et al., 2014; Poggenborg et al., 2015), therefore we propose that this imaging technique could potentially have wide imaging biomarker utility across all JIA phenotypes. The value of MR imaging has been better appreciated in ERA. The presence of sacroiliitis on MRI is not only diagnostic, but helps to shape therapeutic decisions, as axial inflammation responds better to bDMARDs than conventional therapy. Moreover, improvement of sacroiliitis with treatment can be detected by MRI, suggesting that MRI is a sensitive to change imaging biomarker for response to biologic treatment. More recently, the use of quantitative imaging MRI techniques to objectify change in inflammation offers additional benefits (Hall-Craggs et al., 2018). More specifically, these measures are objective and reproducible as they are less dependent on the radiologist experience.

Ultrasound examination of multiple (eight) large joints using Power Doppler (PD) was feasible for assessment of patients with JIA, taking on average 30 min to complete (Zhao et al., 2018). Ultrasound should be able to provide a clear distinction between chronic synovitis defined as hypoechoic synovial hypertrophy commonly found in patients with longstanding disease and joint damage, and active synovitis, diagnosed by the presence of PD signal within the joint. However, in the above studies (Magni-Manzoni et al., 2013; De Lucia et al., 2018; Zhao et al., 2018), researchers compared clinical findings in JIA with positive ultrasound findings, which included gray scale and/or PD signal abnormalities. Interestingly, Magni-Manzoni et al. reported that PD signal was seen more frequently in the patients who stayed inactive than in patients who flared during follow-up (Magni-Manzoni et al., 2013). In comparison with adults, physiological intra-articular vascularity is a common finding in young people who are still to complete

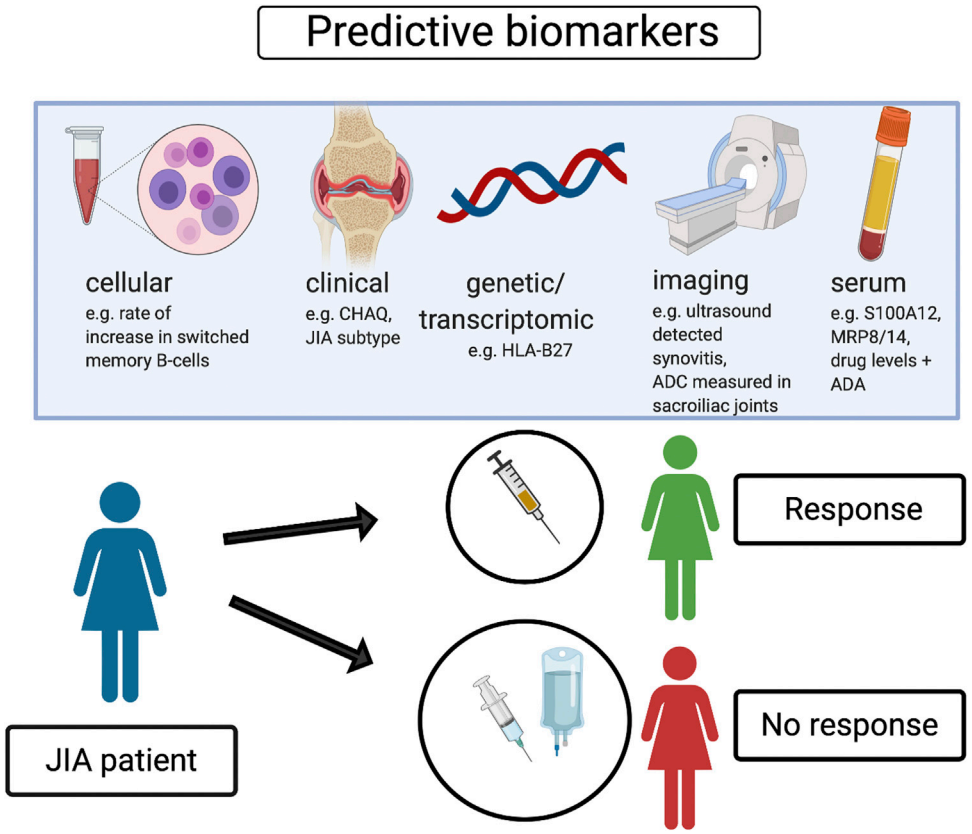


**FIGURE 1 |** Potential predictive biomarkers of response to biologic treatment.

their growth, which makes the interpretation of joint inflammation in the context of JIA more challenging. In order to minimize over-reporting of active synovitis, the ultrasound OMERACT initiative amended the definitions of ultrasound-detected joint pathology in children (Collado et al., 2018). Although there is some evidence that ultrasound can detect reduction in joint inflammation after treatment, further research is needed to validate ultrasound as a tool to guide clinical management in patients with clinically inactive disease.

In conclusion, specific clinical features, serum proinflammatory proteins, selected cellular subsets and newly emerging transcriptomic signatures, in addition to imaging outcomes have been identified as potential positive or negative prediction markers of response to biologic treatment, as well as achievement of remission without treatment. Further research studies are needed to develop and validate individual or composite biomarkers with clinical applicability that could improve biologic treatment management in patients with JIA, as well as personalized treatment strategies. We propose some potential predictive biomarkers related to biologic treatment response in JIA which could be associated with patient benefit and optimization of treatment strategies (**Figure 1**).

## AUTHOR CONTRIBUTIONS

Conception and design CC and VC. Literature review VC, BJ, MH-C, and CC. Drafting manuscript VC. Revising manuscript MH-C, BJ, CF, ML, LW, and CC. All authors approved the final version of the manuscript.

## FUNDING

This work is funded by an Action Medical grant to MHC and CC (1063), a Center of Excellence (Center for Adolescent Rheumatology vs. Arthritis) grant to LW and CC (21593) as well as grants from NIHR Biomedical Research Center at University College London Hospital to CC (BRC/III 525, BRC/III 773). LW and BJ are supported by grants from vs. Arthritis (20164 and 21593), MRC (MR/R013926/1), GOSH Children's Charity and the NIHR Biomedical Research Center at GOSH. VC is supported by a grant from British Society of Rheumatology (4190033). CF is supported by a grant from vs. Arthritis (20762) and NIHR Biomedical Research Center at University College London Hospital. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

## REFERENCES


Alberdi-Saugstrup, M., Nielsen, S., Mathiessen, P., Nielsen, C. H., and Müller, K. (2017). Low pretreatment levels of myeloid-related protein-8/14 and C-reactive protein predict poor adherence to treatment with tumor necrosis factor inhibitors in juvenile idiopathic arthritis. *Clin. Rheumatol.* 36, 67–75. doi:10.1007/s10067-016-3375-x

Alexeeva, E. I., Namazova-Baranova, L. S., Bzarova, T. M., Valieva, S. I., Denisova, R. V., Sleptsova, T. V., et al. (2017). Predictors of the response to etanercept in patients with juvenile idiopathic arthritis without systemic manifestations within 12 months: results of an open-label, prospective study conducted at the National Scientific and Practical Center of Children's Health, Russia. *Pediatr. Rheumatol. Online J.* 15, 51. doi:10.1186/s12969-017-0178-9

Anink, J., Van Suijlekom-Smit, L. W. A., Otten, M. H., Prince, F. H. M., Van Rossum, M. A. J., Dolman, K. M., et al. (2015). MRP8/14 serum levels as a predictor of response to starting and stopping anti-TNF treatment in juvenile idiopathic arthritis. *Arthritis Res. Ther.* 17, 200. doi:10.1186/s13075-015-0723-1

Aquilani, A., Marafon, D. P., Marasco, E., Nicolai, R., Messia, V., Perfetti, F., et al. (2018). Predictors of flare following etanercept withdrawal in patients with rheumatoid factor-negative juvenile idiopathic arthritis who reached remission while taking medication. *J. Rheumatol.* 45, 956–961. doi:10.3899/jrheum.170794

Arthur, V. L., Shuldiner, E., Remmers, E. F., Hinks, A., Grom, A. A., Foell, D., et al. (2018). IL1RN variation influences both disease susceptibility and response to recombinant human interleukin-1 receptor antagonist therapy in systemic juvenile idiopathic arthritis. *Arthritis Rheumatol.* 70, 1319–1330. doi:10.1002/art.40498

Axelsen, M. B., Eshed, I., Duer-Jensen, A., Møller, J. M., Pedersen, S. J., and Østergaard, M. (2014). Whole-body MRI assessment of disease activity and structural damage in rheumatoid arthritis: first step towards an MRI joint count. *Rheumatology (Oxford)* 53, 845–853. doi:10.1093/rheumatology/ket425

Baker, J. F., Ostergaard, M., Emery, P., Hsia, E. C., Lu, J., Baker, D. G., et al. (2014). Early MRI measures independently predict 1-year and 2-year radiographic progression in rheumatoid arthritis: secondary analysis from a large clinical trial. *Ann. Rheum. Dis.* 73, 1968–1974. doi:10.1136/annrheumdis-2013-203444

Barendregt, A. M., Bray, T. J. P., Hall-Craggs, M. A., and Maas, M. (2019). Emerging quantitative MR imaging biomarkers in inflammatory arthritides. *Eur. J. Radiol.* 121, 108707. doi:10.1016/j.ejrad.2019.108707

Barendregt, A. M., Veldkamp, S. R., Hissink Muller, P. C. E., van de Geer, A., Aarts, C., van Gulik, E. C., et al. (2020). MRP8/14 and neutrophil elastase for predicting treatment response and occurrence of flare in patients with juvenile idiopathic arthritis. *Rheumatology (Oxford)* 59, 2392–2401. doi:10.1093/rheumatology/kez590

Berntson, L., Nordal, E., Aalto, K., Peltoniemi, S., Herlin, T., Zak, M., et al. (2013). HLA-B27 predicts a more chronic disease course in an 8-year followup cohort of patients with juvenile idiopathic arthritis. *J. Rheumatol.* 40, 725–731. doi:10.3899/jrheum.121257

Bethencourt Baute, J. J., Sanchez-Piedra, C., Ruiz-Montesinos, D., Medrano San Ildefonso, M., Rodriguez-Lozano, C., Perez-Pampin, E., et al. (2018). Persistence and adverse events of biological treatment in adult patients with juvenile idiopathic arthritis: results from BIOBADASER. *Arthritis Res. Ther.* 20, 227. doi:10.1186/s13075-018-1728-3

Bielak, M., Husmann, E., Weyandt, N., Haas, J.-P., Hügle, B., Horneff, G., et al. (2018). IL-6 blockade in systemic juvenile idiopathic arthritis–achievement of inactive disease and remission (data from the German AID-registry). *Pediatr. Rheumatol. Online J.* 16, 22. doi:10.1186/s12969-018-0236-y

Brachat, A. H., Grom, A. A., Wulffraat, N., Brunner, H. I., Quartier, P., Brik, R., et al. (2017). Early changes in gene expression and inflammatory proteins in systemic juvenile idiopathic arthritis patients on canakinumab therapy. *Arthritis Res. Ther.* 19, 13. doi:10.1186/s13075-016-1212-x

Bray, T. J. P., Vendhan, K., Ambrose, N., Atkinson, D., Punwani, S., Fisher, C., et al. (2017). Diffusion-weighted imaging is a sensitive biomarker of response to biologic therapy in enthesitis-related arthritis. *Rheumatology (Oxford)* 56, 399–407. doi:10.1093/rheumatology/kew429

Bray, T. J. P., Lopes, A., Fisher, C., Ciurtin, C., Sen, D., and Hall-Craggs, M. A. (2019). Sacroiliac joint ankylosis in young spondyloarthritis patients receiving biologic therapy: observation of serial magnetic resonance imaging scans. *Arthritis Rheumatol.* 71, 594–598. doi:10.1002/art.40750

Brown, A., Hirsch, R., Laor, T., Hannon, M. J., Levesque, M. C., Starz, T., et al. (2012). Do patients with juvenile idiopathic arthritis in clinical remission have evidence of persistent inflammation on 3T magnetic resonance imaging? *Arthritis Care Res. (Hoboken)* 64, 1846–1854. doi:10.1002/acr.21774

Brunelli, J. B., Silva, C. A., Pasoto, S. G., Saa, C. G. S., Kozu, K. T., Goldenstein-Schainberg, C., et al. (2020). Anti-adalimumab antibodies kinetics: an early guide for juvenile idiopathic arthritis (JIA) switching. *Clin. Rheumatol.* 39, 515–521. doi:10.1007/s10067-019-04798-6

Brunner, H. I., Lovell, D. J., Finck, B. K., and Giannini, E. H. (2002). Preliminary definition of disease flare in juvenile rheumatoid arthritis. *J. Rheumatol.* 29, 1058–1064.

Brunner, H. I., Ruperto, N., Zuber, Z., Keane, C., Harari, O., Kenwright, A., et al. (2015). Efficacy and safety of tocilizumab in patients with polyarticular-course juvenile idiopathic arthritis: results from a phase 3, randomised, double-blind withdrawal trial. *Ann. Rheum. Dis.* 74, 1110–1117. doi:10.1136/annrheumdis-2014-205351

Bugni Miotto e Silva, V., de Freitas Tavares da Silva, C., de Aguiar Vilela Mitraud, S., Nely Vilar Furtado, R., Esteves Hilário, M. O., Natour, J., et al. (2014). Do patients with juvenile idiopathic arthritis in remission exhibit active synovitis on joint ultrasound? *Rheumatol. Int.* 34, 937–945. doi:10.1007/s00296-013-2909-7

Collado, P., Naredo, E., Calvo, C., Gamir, M. L., Calvo, I., García, M. L., et al. (2013). Reduced joint assessment vs. comprehensive assessment for ultrasound detection of synovitis in juvenile idiopathic arthritis. *Rheumatology (Oxford)* 52, 1477–1484. doi:10.1093/rheumatology/ket148

Collado, P., Windschall, D., Vojinovic, J., Magni-Manzoni, S., Balint, P., Bruyn, G. A. W., et al. (2018). Amendment of the OMERACT ultrasound definitions of joints' features in healthy children when using the Doppler technique. *Pediatr. Rheumatol. Online J.* 16, 23. doi:10.1186/s12969-018-0240-2

Conaghan, P. G., Østergaard, M., Troum, O., Bowes, M. A., Guillard, G., Wilkinson, B., et al. (2019). Very early MRI responses to therapy as a predictor of later radiographic progression in early rheumatoid arthritis. *Arthritis Res. Ther.* 21, 214. doi:10.1186/s13075-019-2000-1

Conaghan, P. G., Peterfy, C., Olech, E., Kaine, J., Ridley, D., Dicarlo, J., et al. (2014). The effects of tocilizumab on osteitis, synovitis and erosion progression in rheumatoid arthritis: results from the ACT-RAY MRI substudy. *Ann. Rheum. Dis.* 73, 810–816. doi:10.1136/annrheumdis-2013-204762

Consolaro, A., Giancane, G., Schiappapietra, B., Davì, S., Calandra, S., Lanni, S., et al. (2016). Clinical outcome measures in juvenile idiopathic arthritis. *Pediatr. Rheumatol. Online J.* 14, 23. doi:10.1186/s12969-016-0085-5

Consolaro, A., Varnier, G. C., Martini, A., and Ravelli, A. (2015). Advances in biomarkers for paediatric rheumatic diseases. *Nat. Rev. Rheumatol.* 11, 265–275. doi:10.1038/nrrheum.2014.208

Davies, R., Gaynor, D., Hyrich, K. L., and Pain, C. E. (2017). Efficacy of biologic therapy across individual juvenile idiopathic arthritis subtypes: a systematic review. *Semin. Arthritis Rheum.* 46, 584–593. doi:10.1016/j.semarthrit.2016.10.008

De Lucia, O., Ravagnani, V., Pregnolato, F., Hila, A., Pontikaki, I., Gattinara, M., et al. (2018). Baseline ultrasound examination as possible predictor of relapse in patients affected by juvenile idiopathic arthritis (JIA). *Ann. Rheum. Dis.* 77, 1426–1431. doi:10.1136/annrheumdis-2017-211696

Doeleman, M. J. H., Van Maarseveen, E., and Swart, J. F. (2019). Immunogenicity of biologic agents in juvenile idiopathic arthritis: a systematic review and meta-analysis. *Rheumatology (Oxford)* 58, 1839–1849. doi:10.1093/rheumatology/kez030

European Society of Radiology (2020). ESR statement on the validation of imaging biomarkers. *Insights Imaging* 11, 76. doi:10.1186/s13244-020-00872-9

Favalli, E. G., Pontikaki, I., Becciolini, A., Biggioggero, M., Ughi, N., Romano, M., et al. (2017). Real-life 10-year retention rate of first-line anti-TNF drugs for inflammatory arthritides in adult- and juvenile-onset populations: similarities and differences. *Clin. Rheumatol.* 36, 1747–1755. doi:10.1007/s10067-017-3712-8

Foell, D., Wittkowski, H., Hammerschmidt, I., Wulffraat, N., Schmeling, H., Frosch, M., et al. (2004). Monitoring neutrophil activation in juvenile rheumatoid arthritis by S100A12 serum concentrations. *Arthritis Rheum.* 50, 1286–1295. doi:10.1002/art.20125

Foell, D., Wulffraat, N., Wedderburn, L. R., Wittkowski, H., Frosch, M., Gerss, J., et al. (2010). Methotrexate withdrawal at 6 vs. 12 months in juvenile idiopathic

arthritis in remission: a randomized clinical trial. *J. Am. Med. Assoc.* 303, 1266–1273. doi:10.1001/jama.2010.375

Gattorno, M., Piccini, A., Lasiglié, D., Tassi, S., Brisca, G., Carta, S., et al. (2008). The pattern of response to anti-interleukin-1 treatment distinguishes two subsets of patients with systemic-onset juvenile idiopathic arthritis. *Arthritis Rheum.* 58, 1505–1515. doi:10.1002/art.23437

Geikowski, T., Becker, I., Horneff, G., and German BIKER Registry Collaborative Study Group (2014). Predictors of response to etanercept in polyarticular-course juvenile idiopathic arthritis. *Rheumatology (Oxford)* 53, 1245–1249. doi:10.1093/rheumatology/ket490

Giannini, E. H., Ruperto, N., Ravelli, A., Lovell, D. J., Felson, D. T., and Martini, A. (1997). Preliminary definition of improvement in juvenile arthritis. *Arthritis Rheum.* 40, 1202–1209. doi:10.1002/1529-0131(199707)40:7<1202::AID-ART3>3.0.CO;2-R

Glerup, M., Rypdal, V., Arnstad, E. D., Ekelund, M., Peltoniemi, S., Aalto, K., et al. (2020). Long-term outcomes in juvenile idiopathic arthritis: eighteen years of follow-up in the population-based nordic juvenile idiopathic arthritis cohort. *Arthritis Care Res.* 72, 507–516. doi:10.1002/acr.23853

Gohar, F., Anink, J., Moncrieffe, H., Van Suijlekom-Smit, L. W. A., Prince, F. H. M., van Rossum, M. A. J., et al. (2018). S100A12 is associated with response to therapy in juvenile idiopathic arthritis. *J. Rheumatol.* 45, 547–554. doi:10.3899/jrheum.170438

Gohar, F., Kessel, C., Lavric, M., Holzinger, D., and Foell, D. (2016). Review of biomarkers in systemic juvenile idiopathic arthritis: helpful tools or just playing tricks? *Arthritis Res. Ther.* 18, 163. doi:10.1186/s13075-016-1069-z

Guzman, J., Oen, K., Huber, A. M., Watanabe Duffy, K., Boire, G., Shiff, N., et al. (2016). The risk and nature of flares in juvenile idiopathic arthritis: results from the ReACCh-Out cohort. *Ann. Rheum. Dis.* 75, 1092–1098. doi:10.1136/annrheumdis-2014-207164

Hall-Craggs, M. A., Bray, T. J. P., Ciurtin, C., and Bainbridge, A. (2019). Quantitative magnetic resonance imaging has potential for assessment of spondyloarthritis: arguments for its study and use. *J. Rheumatol.* 46, 541–542. doi:10.3899/jrheum.181049

Hall-Craggs, M. A., Bray, T. P. J., and Bainbridge, A. P. (2018). Quantitative imaging of inflammatory disease: are we missing a trick? *Ann. Rheum. Dis.* 77, 1689–1691. doi:10.1136/annrheumdis-2018-213614

Halyabar, O., Mehta, J., Ringold, S., Rumsey, D. G., and Horton, D. B. (2019). Treatment withdrawal following remission in juvenile idiopathic arthritis: a systematic review of the literature. *Paediatr. Drugs* 21, 469–492. doi:10.1007/s40272-019-00362-6

Heiligenhaus, A., Foeldvari, I., Edelsten, C., Smith, J. R., Saurenmann, R. K., Bodaghi, B., et al. (2012). Proposed outcome measures for prospective clinical trials in juvenile idiopathic arthritis-associated uveitis: a consensus effort from the multinational interdisciplinary working group for uveitis in childhood. *Arthritis Care Res. (Hoboken)* 64, 1365–1372. doi:10.1002/acr.21674

Hemke, R., van Rossum, M. A., van Veenendaal, M., Terra, M. P., Deurloo, E. E., de Jonge, M. C., et al. (2013). Reliability and responsiveness of the juvenile arthritis MRI scoring (JAMRIS) system for the knee. *Eur. Radiol.* 23, 1075–1083. doi:10.1007/s00330-012-2684-y

Hinks, A., Moncrieffe, H., Martin, P., Ursu, S., Lal, S., Kassoumeri, L., et al. (2011). Association of the 5-aminoimidazole-4-carboxamide ribonucleotide transformylase gene with response to methotrexate in juvenile idiopathic arthritis. *Ann. Rheum. Dis.* 70, 1395–1400. doi:10.1136/ard.2010.146191

Hinze, C. H., Foell, D., Johnson, A. L., Spalding, S. J., Gottlieb, B. S., Morris, P. W., et al. (2019). Serum S100a8/A9 and S100A12 levels in children with polyarticular forms of juvenile idiopathic arthritis: relationship to maintenance of clinically inactive disease during anti-tumor necrosis factor therapy and occurrence of disease flare after discontinuation of therapy. *Arthritis Rheumatol.* 71, 451–459. doi:10.1002/art.40727

Holzinger, D., Frosch, M., Kastrup, A., Prince, F. H., Otten, M. H., Van Suijlekom-Smit, L. W. A., et al. (2012). The Toll-like receptor 4 agonist MRP8/14 protein complex is a sensitive indicator for disease activity and predicts relapses in systemic-onset juvenile idiopathic arthritis. *Ann. Rheum. Dis.* 71, 974–980. doi:10.1136/annrheumdis-2011-200598

Hunter, D. J., Losina, E., Guermazi, A., Burstein, D., Lassere, M. N., and Kraus, V. (2010). A pathway and approach to biomarker validation and qualification for osteoarthritis clinical trials. *Curr. Drug Targets* 11, 536–545. doi:10.2174/138945010791011947

Ilowite, N., Porras, O., Reiff, A., Rudge, S., Punaro, M., Martin, A., et al. (2009). Anakinra in the treatment of polyarticular-course juvenile rheumatoid arthritis: safety and preliminary efficacy results of a randomized multicenter study. *Clin. Rheumatol.* 28, 129–137. doi:10.1007/s10067-008-0995-9

Jiang, K., Frank, M., Chen, Y., Osban, J., and Jarvis, J. N. (2013). Genomic characterization of remission in juvenile idiopathic arthritis. *Arthritis Res. Ther.* 15, R100. doi:10.1186/ar4280

Kahn, R., Berthold, E., Gullstrand, B., Schmidt, T., Kahn, F., Geborek, P., et al. (2016). Circulating complexes between tumour necrosis factor-alpha and etanercept predict long-term efficacy of etanercept in juvenile idiopathic arthritis. *Acta Paediatr.* 105, 427–432. doi:10.1111/apa.13319

Kearsley-Fleet, L., Beresford, M. W., Davies, R., de Cock, D., Baildam, E., Foster, H. E., et al. (2019). Short-term outcomes in patients with systemic juvenile idiopathic arthritis treated with either tocilizumab or anakinra. *Rheumatology (Oxford)* 58, 94–102. doi:10.1093/rheumatology/key262

Kearsley-Fleet, L., Davies, R., Lunt, M., Southwood, T. R., and Hyrich, K. L. (2016). Factors associated with improvement in disease activity following initiation of etanercept in children and young people with Juvenile Idiopathic Arthritis: results from the British Society for Paediatric and Adolescent Rheumatology Etanercept Cohort Study. *Rheumatology (Oxford)* 55, 840–847. doi:10.1093/rheumatology/kev434

Kearsley-Fleet, L., Heaf, E., Davies, R., Baildam, E., Beresford, M. W., Foster, H. E., et al. (2020). Frequency of biologic switching and the outcomes of switching in children and young people with juvenile idiopathic arthritis: a national cohort study. *Lancet Rheumatol.* 2, e217–e226. doi:10.1016/S2665-9913(20)30025-4

Knowlton, N., Jiang, K., Frank, M. B., Aggarwal, A., Wallace, C., Mckee, R., et al. (2009). The meaning of clinical remission in polyarticular juvenile idiopathic arthritis: gene expression profiling in peripheral blood mononuclear cells identifies distinct disease states. *Arthritis Rheum.* 60, 892–900. doi:10.1002/art.24298

Leong, J. Y., Chen, P., Yeo, J. G., Ally, F., Chua, C., Nur Hazirah, S., et al. (2019). Immunome perturbation is present in patients with juvenile idiopathic arthritis who are in remission and will relapse upon anti-TNFα withdrawal. *Ann. Rheum. Dis.* 78, 1712–1721. doi:10.1136/annrheumdis-2019-216059

Lovell, D. J., Brunner, H. I., Reiff, A. O., Jung, L., Jarosova, K., Němcová, D., et al. (2020). Long-term outcomes in patients with polyarticular juvenile idiopathic arthritis receiving adalimumab with or without methotrexate. *RMD Open* 6, e001208. doi:10.1136/rmdopen-2020-001208

Lovell, D. J., Giannini, E. H., Reiff, A., Cawkwell, G. D., Silverman, E. D., Nocton, J. J., et al. (2000). Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. *N. Engl. J. Med.* 342, 763–769. doi:10.1056/NEJM200003163421103

Lovell, D. J., Giannini, E. H., Reiff, A. O., Kimura, Y., Li, S., Hashkes, P. J., et al. (2013). Long-term safety and efficacy of rilonacept in patients with systemic juvenile idiopathic arthritis. *Arthritis Rheum.* 65, 2486–2496. doi:10.1002/art.38042

Lovell, D. J., Johnson, A. L., Huang, B., Gottlieb, B. S., Morris, P. W., Kimura, Y., et al. (2018). Risk, timing, and predictors of disease flare after discontinuation of anti-tumor necrosis factor therapy in children with polyarticular forms of juvenile idiopathic arthritis with clinically inactive disease. *Arthritis Rheumatol.* 70, 1508–1518. doi:10.1002/art.40509

Magni-Manzoni, S., Epis, O., Ravelli, A., Klersy, C., Veisconti, C., Lanni, S., et al. (2009). Comparison of clinical versus ultrasound-determined synovitis in juvenile idiopathic arthritis. *Arthritis Rheum.* 61, 1497–1504. doi:10.1002/art.24823

Magni-Manzoni, S., Scirè, C. A., Ravelli, A., Klersy, C., Rossi, S., Muratore, V., et al. (2013). Ultrasound-detected synovial abnormalities are frequent in clinically inactive juvenile idiopathic arthritis, but do not predict a flare of synovitis. *Ann. Rheum. Dis.* 72, 223–228. doi:10.1136/annrheumdis-2011-201264

Makay, B., Gücenmez, Ö. A., and Ünsal, E. (2016). Inactive disease in enthesitis-related arthritis: association of increased body mass index. *J. Rheumatol.* 43, 937–943. doi:10.3899/jrheum.151208

Marasco, E., Aquilani, A., Cascioli, S., Moneta, G. M., Caiello, I., Farroni, C., et al. (2018). Switched memory B cells are increased in oligoarticular and polyarticular juvenile idiopathic arthritis and their change over time is related to response to tumor necrosis factor inhibitors. *Arthritis Rheumatol.* 70, 606–615. doi:10.1002/art.40410

Marino, A., Real-Fernández, F., Rovero, P., Giani, T., Pagnini, I., Cimaz, R., et al. (2018). Anti-adalimumab antibodies in a cohort of patients with juvenile idiopathic arthritis: incidence and clinical correlations. *Clin. Rheumatol.* 37, 1407–1411. doi:10.1007/s10067-018-4057-7

McErlane, F., Beresford, M. W., Baildam, E. M., Thomson, W., and Hyrich, K. L. (2013). Recent developments in disease activity indices and outcome measures for juvenile idiopathic arthritis. *Rheumatology (Oxford)* 52, 1941–1951. doi:10.1093/rheumatology/ket150

McGovern, J. L., Nguyen, D. X., Notley, C. A., Mauri, C., Isenberg, D. A., and Ehrenstein, M. R. (2012). Th17 cells are restrained by Treg cells via the inhibition of interleukin-6 in patients with rheumatoid arthritis responding to anti-tumor necrosis factor antibody therapy. *Arthritis Rheum.* 64, 3129–3138. doi:10.1002/art.34565

Minden, K., Horneff, G., Niewerth, M., Seipelt, E., Aringer, M., Aries, P., et al. (2019). Time of disease-modifying antirheumatic drug start in juvenile idiopathic arthritis and the likelihood of a drug-free remission in young adulthood. *Arthritis Care Res. (Hoboken)* 71, 471–481. doi:10.1002/acr.23709

Miotto E Silva, V. B., Mitraud, S. A. V., Furtado, R. N. V., Natour, J., Len, C. A., and Terreri, M. T. S. E. L. R. A. (2017). Patients with juvenile idiopathic arthritis in clinical remission with positive power Doppler signal in joint ultrasonography have an increased rate of clinical flare: a prospective study. *Pediatr. Rheumatol. Online J.* 15, 80. doi:10.1186/s12969-017-0208-7

Mo, X., Chen, X., Ieong, C., Zhang, S., Li, H., Li, J., et al. (2020). Early prediction of clinical response to etanercept treatment in juvenile idiopathic arthritis using machine learning. *Front. Pharmacol.* 11, 1164. doi:10.3389/fphar.2020.01164

Moncrieffe, H., Hinks, A., Ursu, S., Kassoumeri, L., Etheridge, A., Hubank, M., et al. (2010). Generation of novel pharmacogenomic candidates in response to methotrexate in juvenile idiopathic arthritis: correlation between gene expression and genotype. *Pharmacogenet. Genomics.* 20, 665–676. doi:10.1097/FPC.0b013e32833f2cd0

Moncrieffe, H., Ursu, S., Holzinger, D., Patrick, F., Kassoumeri, L., Wade, A., et al. (2013). A subgroup of juvenile idiopathic arthritis patients who respond well to methotrexate are identified by the serum biomarker MRP8/14 protein. *Rheumatology (Oxford)* 52, 1467–1476. doi:10.1093/rheumatology/ket152

Mor-Vaknin, N., Rivas, M., Legendre, M., Mohan, S., Yuanfan, Y., Mau, T., et al. (2018). High levels of DEK autoantibodies in sera of patients with polyarticular juvenile idiopathic arthritis and with early disease flares following cessation of anti-tumor necrosis factor therapy. *Arthritis Rheumatol.* 70, 594–605. doi:10.1002/art.40404

Mori, M., Takei, S., Imagawa, T., Imanaka, H., Nerome, Y., Higuchi, R., et al. (2012). Safety and efficacy of long-term etanercept in the treatment of methotrexate-refractory polyarticular-course juvenile idiopathic arthritis in Japan. *Mod. Rheumatol.* 22, 720–726. doi:10.1007/s10165-011-0578-5

Mourão, A. F., Santos, M. J., Melo Gomes, J. A., Martins, F. M., Mendonça, S. C., Oliveira Ramos, F., et al. (2016). Effectiveness and long-term retention of anti-tumour necrosis factor treatment in juvenile and adult patients with juvenile idiopathic arthritis: data from Reuma.pt. *Rheumatology (Oxford)* 55, 697–703. doi:10.1093/rheumatology/kev398

Müller, L., Kellenberger, C. J., Cannizzaro, E., Ettlin, D., Schraner, T., Bolt, I. B., et al. (2009). Early diagnosis of temporomandibular joint involvement in juvenile idiopathic arthritis: a pilot study comparing clinical examination and ultrasound to magnetic resonance imaging. *Rheumatology (Oxford)* 48, 680–685. doi:10.1093/rheumatology/kep068

Nguyen, D. X., Cotton, A., Attipoe, L., Ciurtin, C., Doré, C. J., and Ehrenstein, M. R. (2018). Regulatory T cells as a biomarker for response to adalimumab in rheumatoid arthritis. *J. Allergy Clin. Immunol.* 142, 978–980. doi:10.1016/j.jaci.2018.04.026

Nigrovic, P. A., Mannion, M., Prince, F. H. M., Zeft, A., Rabinovich, C. E., van Rossum, M. A. J., et al. (2011). Anakinra as first-line disease-modifying therapy in systemic juvenile idiopathic arthritis: report of forty-six patients from an international multicenter series. *Arthritis Rheum.* 63, 545–555. doi:10.1002/art.30128

Nordal, H. H., Brun, J. G., Hordvik, M., Eidsheim, M., Jonsson, R., and Halse, A. K. (2016). Calprotectin (S100A8/A9) and S100A12 are associated with measures of disease activity in a longitudinal study of patients with rheumatoid arthritis treated with infliximab. *Scand. J. Rheumatol.* 45, 274–281. doi:10.3109/03009742.2015.1107128

Otten, M. H., Prince, F. H., Armbrust, W., Ten Cate, R., Hoppenreijs, E. P., Twilt, M., et al. (2011a). Factors associated with treatment response to etanercept in juvenile idiopathic arthritis. *J. Am. Med. Assoc.* 306, 2340–2347. doi:10.1001/jama.2011.1671

Otten, M. H., Prince, F. H., Ten Cate, R., van Rossum, M. A., Twilt, M., Hoppenreijs, E. P., et al. (2011b). Tumour necrosis factor (TNF)-blocking agents in juvenile psoriatic arthritis: are they effective? *Ann. Rheum. Dis.* 70, 337–340. doi:10.1136/ard.2010.135731

Panwar, J., Tse, S. M. L., Lim, L., Tolend, M. A., Radhakrishnan, S., Salman, M., et al. (2019). Spondyloarthritis research Consortium of Canada scoring system for sacroiliitis in juvenile spondyloarthritis/enthesitis-related arthritis: a reliability, validity, and responsiveness study. *J. Rheumatol.* 46, 636–644. doi:10.3899/jrheum.180222

Papailiou S, D. F., Tsolia, M. N., and Maritsi, D. N. 2020. Attainment of inactive disease following discontinuation of adalimumab monotherapy in patients with era. 26th European Paediatric Rheumatology Congress, Virtual. *Pediatric Rheumatology* 11. doi:10.1186/s12969-020-00469-y

Papamichael, K., Vogelzang, E. H., Lambert, J., Wolbink, G., and Cheifetz, A. S. (2019). Therapeutic drug monitoring with biologic agents in immune mediated inflammatory diseases. *Expert Rev. Clin. Immunol.* 15, 837–848. doi:10.1080/1744666X.2019.1630273

Pardeo, M., Pires Marafon, D., Insalaco, A., Bracaglia, C., Nicolai, R., Messia, V., et al. (2015). Anakinra in systemic juvenile idiopathic arthritis: a single-center experience. *J. Rheumatol.* 42, 1523–1527. doi:10.3899/jrheum.141567

Petty, R. E., Southwood, T. R., Baum, J., Bhettay, E., Glass, D. N., Manners, P., et al. (1998). Revision of the proposed classification criteria for juvenile idiopathic arthritis: durban, 1997. *J. Rheumatol.* 25, 1991–1994.

Poggenborg, R. P., Eshed, I., Østergaard, M., Sørensen, I. J., Møller, J. M., Madsen, O. R., et al. (2015). Enthesitis in patients with psoriatic arthritis, axial spondyloarthritis and healthy subjects assessed by 'head-to-toe' whole-body MRI and clinical examination. *Ann. Rheum. Dis.* 74, 823–829. doi:10.1136/annrheumdis-2013-204239

Ramsey, L. B., Moncrieffe, H., Smith, C. N., Sudman, M., Marion, M. C., Langefeld, C. D., et al. (2019). Association of SLCO1B1 *14 allele with poor response to methotrexate in juvenile idiopathic arthritis patients. *ACR Open Rheumatol.* 1, 58–62. doi:10.1002/acr2.1008

Rebollo-Polo, M., Koujok, K., Weisser, C., Jurencak, R., Bruns, A., and Roth, J. (2011). Ultrasound findings on patients with juvenile idiopathic arthritis in clinical remission. *Arthritis Care Res. (Hoboken)* 63, 1013–1019. doi:10.1002/acr.20478

Ringold, S., Weiss, P. F., Beukelman, T., DeWitt, E. M., Ilowite, N. T., Kimura, Y., et al. (2013). 2013 update of the 2011 American College of Rheumatology recommendations for the treatment of juvenile idiopathic arthritis: recommendations for the medical therapy of children with systemic juvenile idiopathic arthritis and tuberculosis screening among children receiving biologic medications. *Arthritis Rheum.* 65, 2499–2512. doi:10.1002/art.38092

Robinson, P. C., and Yazdany, J. (2020). The COVID-19 Global Rheumatology Alliance: collecting data in a pandemic. *Nat. Rev. Rheumatol.* 16, 293–294. doi:10.1038/s41584-020-0418-0

Rossetti, M., Spreafico, R., Consolaro, A., Leong, J. Y., Chua, C., Massa, M., et al. (2017). TCR repertoire sequencing identifies synovial Treg cell clonotypes in the bloodstream during active inflammation in human arthritis. *Ann. Rheum. Dis.* 76, 435–441. doi:10.1136/annrheumdis-2015-208992

Ruperto, N., Brunner, H. I., Quartier, P., Constantin, T., Wulffraat, N. M., Horneff, G., et al. (2018). Canakinumab in patients with systemic juvenile idiopathic arthritis and active systemic features: results from the 5-year long-term extension of the phase III pivotal trials. *Ann. Rheum. Dis.* 77, 1710–1719. doi:10.1136/annrheumdis-2018-213150

Ruperto, N., Lovell, D. J., Cuttica, R., Wilkinson, N., Woo, P., Espada, G., et al. (2007). A randomized, placebo-controlled trial of infliximab plus methotrexate for the treatment of polyarticular-course juvenile rheumatoid arthritis. *Arthritis Rheum.* 56, 3096–3106. doi:10.1002/art.22838

Ruperto, N., Lovell, D. J., Cuttica, R., Woo, P., Meiorin, S., Wouters, C., et al. (2010). Long-term efficacy and safety of infliximab plus methotrexate for the treatment of polyarticular-course juvenile rheumatoid arthritis: findings from an open-label treatment extension. *Ann. Rheum. Dis.* 69, 718–722. doi:10.1136/ard.2009.100354

Ruperto, N., Ravelli, A., Falcini, F., Lepore, L., de Sanctis, R., Zulian, F., et al. (1998). Performance of the preliminary definition of improvement in juvenile chronic arthritis patients treated with methotrexate. Italian Pediatric Rheumatology Study Group. *Ann. Rheum. Dis.* 57, 38–41. doi:10.1136/ard.57.1.38

Saccomanno, B., Tibaldi, J., Minoia, F., Bagnasco, F., Pistorio, A., Guariento, A., et al. (2019). Predictors of effectiveness of anakinra in systemic juvenile idiopathic arthritis. *J. Rheumatol.* 46, 416–421. doi:10.3899/jrheum.180331

Shoop-Worrall, S. J. W., Wu, Q., Davies, R., Hyrich, K. L., and Wedderburn, L. R. (2019). Predicting disease outcomes in juvenile idiopathic arthritis: challenges, evidence, and new directions. *Lancet Child Adolesc. Health* 3, 725–733. doi:10.1016/S2352-4642(19)30188-9

Simonini, G., Ferrara, G., Pontikaki, I., Scoccimarro, E., Giani, T., Taddio, A., et al. (2018). Flares after withdrawal of biologic therapies in juvenile idiopathic arthritis: clinical and laboratory correlates of remission duration. *Arthritis Care Res. (Hoboken)* 70, 1046–1051. doi:10.1002/acr.23435

Skrabl-Baumgartner, A., Erwa, W., Muntean, W., and Jahnel, J. (2015). Anti-adalimumab antibodies in juvenile idiopathic arthritis: frequent association with loss of response. *Scand. J. Rheumatol.* 44, 359–362. doi:10.3109/03009742.2015.1022213

Skrabl-Baumgartner, A., Seidel, G., Langner-Wegscheider, B., Schlagenhauf, A., and Jahnel, J. (2019). Drug monitoring in long-term treatment with adalimumab for juvenile idiopathic arthritis-associated uveitis. *Arch. Dis. Child.* 104, 246–250. doi:10.1136/archdischild-2018-315060

Solari, N., Palmisani, E., Consolaro, A., Pistorio, A., Viola, S., Buoncompagni, A., et al. (2013). Factors associated with achievement of inactive disease in children with juvenile idiopathic arthritis treated with etanercept. *J. Rheumatol.* 40, 192–200. doi:10.3899/jrheum.120842

Soyfoo, M. S., Roth, J., Vogl, T., Pochet, R., and Decaux, G. (2009). Phagocyte-specific S100A8/A9 protein levels during disease exacerbations and infections in systemic lupus erythematosus. *J. Rheumatol.* 36, 2190–2194. doi:10.3899/jrheum.081302

Specialised Commissioning Team, N. E. (2015). Appendix A: suggested treatment flow-chart for JIA. Available: https://www.england.nhs.uk/commissioning/wp-content/uploads/sites/12/2015/07/appx-a-jia-e03Pd.pdf (Accessed November 13, 2020).Online

Su, Y., Yang, Y.-H., and Chiang, B. L. (2017). Treatment response to etanercept in methotrexate refractory juvenile idiopathic arthritis: an analysis of predictors and long-term outcomes. *Clin. Rheumatol.* 36, 1997–2004. doi:10.1007/s10067-017-3682-x

Sun, H., Van, L. M., Floch, D., Jiang, X., Klein, U. R., Abrams, K., et al. (2016). Pharmacokinetics and pharmacodynamics of canakinumab in patients with systemic juvenile idiopathic arthritis. *J. Clin. Pharmacol.* 56, 1516–1527. doi:10.1002/jcph.754

Ter Haar, N. M., van Dijkhuizen, E. H. P., Swart, J. F., van Royen-Kerkhof, A., El Idrissi, A., Leek, A. P., et al. (2019). Treatment to target using recombinant interleukin-1 receptor antagonist as first-line monotherapy in new-onset systemic juvenile idiopathic arthritis: results from a five-year follow-up study. *Arthritis Rheumatol.* 71, 1163–1173. doi:10.1002/art.40865

Ungar, W. J., Costa, V., Burnett, H. F., Feldman, B. M., and Laxer, R. M. (2013). The use of biologic response modifiers in polyarticular-course juvenile idiopathic arthritis: a systematic review. *Semin. Arthritis Rheum.* 42, 597–618. doi:10.1016/j.semarthrit.2012.10.006

van der Heijde, D., Sieper, J., Maksymowych, W. P., Lambert, R. G., Chen, S., Hojnik, M., et al. (2018). Clinical and MRI remission in patients with nonradiographic axial spondyloarthritis who received long-term open-label adalimumab treatment: 3-year results of the ABILITY-1 trial. *Arthritis Res. Ther.* 20, 61. doi:10.1186/s13075-018-1556-5

Vastert, S. J., de Jager, W., Noordman, B. J., Holzinger, D., Kuis, W., Prakken, B. J., et al. (2014). Effectiveness of first-line treatment with recombinant interleukin-1 receptor antagonist in steroid-naive patients with new-onset systemic juvenile idiopathic arthritis: results of a prospective cohort study. *Arthritis Rheumatol.* 66, 1034–1043. doi:10.1002/art.38296

Vendhan, K., Bray, T. J. P., Atkinson, D., Punwani, S., Fisher, C., Sen, D., et al. (2016). A diffusion-based quantification technique for assessment of sacroiliitis in adolescents with enthesitis-related arthritis. *Br. J. Radiol.* 89, 20150775. doi:10.1259/bjr.20150775

Verstegen, R. H. J., McMillan, R., Feldman, B. M., Ito, S., and Laxer, R. M. (2020). Towards therapeutic drug monitoring of TNF inhibitors for children with juvenile idiopathic arthritis: a scoping review. *Rheumatology (Oxford)* 59, 386–397. doi:10.1093/rheumatology/kez285

Wallace, C. A., Ruperto, N., and Giannini, E.Childhood Arthritis and Rheumatology Research Alliance, Pediatric.Rheumatology International Trials Organization, Pediatric Rheumatology Collaborative Study Group (2004). Preliminary criteria for clinical remission for select categories of juvenile idiopathic arthritis. *J. Rheumatol.* 31, 2290–2294.

Walters, H. M., Pan, N., Lehman, T. J. A., Adams, A., Kalliolias, G. D., Zhu, Y. S., et al. (2016). The impact of disease activity and tumour necrosis factor-α inhibitor therapy on cytokine levels in juvenile idiopathic arthritis. *Clin. Exp. Immunol.* 184, 308–317. doi:10.1111/cei.12782

Wittkowski, H., Frosch, M., Wulffraat, N., Goldbach-Mansky, R., Kallinich, T., Kuemmerle-Deschner, J., et al. (2008). S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever of unknown origin. *Arthritis Rheum.* 58, 3924–3931. doi:10.1002/art.24137

Woodworth, T. G., Morgacheva, O., Pimienta, O. L., Troum, O. M., Ranganath, V. K., and Furst, D. E. (2017). Examining the validity of the rheumatoid arthritis magnetic resonance imaging score according to the OMERACT filter-a systematic literature review. *Rheumatology (Oxford)* 56, 1177–1188. doi:10.1093/rheumatology/kew445

Yamasaki, Y., Takei, S., Imanaka, H., Nerome, Y., Kubota, T., Nonaka, Y., et al. 2016). Prediction of long-term remission of oligo/polyarticular juvenile idiopathic arthritis with S100A12 and vascular endothelial growth factor. *Mod. Rheumatol.* 26, 551–556. doi:10.3109/14397595.2015.1109784

Yanai, H., Lichtenstein, L., Assa, A., Mazor, Y., Weiss, B., Levine, A., et al. (2015). Levels of drug and antidrug antibodies are associated with outcome of interventions after loss of response to infliximab or adalimumab. *Clin. Gastroenterol. Hepatol.* 13, 522–530. doi:10.1016/j.cgh.2014.07.029

Yokota, S., Imagawa, T., Mori, M., Miyamae, T., Takei, S., Iwata, N., et al. (2014). Longterm safety and effectiveness of the anti-interleukin 6 receptor monoclonal antibody tocilizumab in patients with systemic juvenile idiopathic arthritis in Japan. *J. Rheumatol.* 41, 759–767. doi:10.3899/jrheum.130690

Zanwar, A., Phatak, S., and Aggarwal, A. (2018). Prospective validation of the juvenile spondyloarthritis disease activity index in children with enthesitis-related arthritis. *Rheumatology (Oxford)* 57, 2167–2171. doi:10.1093/rheumatology/key246

Zhao, Y., Rascoff, N. E., Iyer, R. S., Thapa, M., Reichley, L., Oron, A. P., et al. (2018). Flares of disease in children with clinically inactive juvenile idiopathic arthritis were not correlated with ultrasound findings. *J. Rheumatol.* 45, 851–857. doi:10.3899/jrheum.170681

Zhou, L., and Gu, X. (2019). Correlation of ultrasonography synovitis with disease activity and clinical response to etanercept treatment in juvenile idiopathic arthritis patients. *Braz. J. Med. Biol. Res.* 52, e8565. doi:10.1590/1414-431X20198565

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

CLINICAL TRIAL
published: 18 February 2021
doi: 10.3389/fphar.2021.617265

# Modeling and Simulation to Support Phase Ib/IIa Dose Selection for WBP216, A Long Half-Life Fully Human Monoclonal Antibody Against Interleukin-6

*Xiange Tang*[1,2], *Xiaofeng Zeng*[3], *Xiaoduo Guan*[1,2], *Rui Chen*[1,2]* *and Pei Hu*[1,2]*

[1]Clinical Pharmacology Research Center, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China, [2]Key Laboratory of Clinical PK and PD Investigation for Innovative Drugs, Beijing, China, [3]Department of Rheumatology, Peking Union Medical College Hospital, Chinese Academy of Medical Science and Peking Union Medical College, Beijing, China

OPEN ACCESS

**Edited by:**
*Anna Lisa Giuliani, University of Ferrara, Italy*

**Reviewed by:**
*Tianyan Zhou, Peking University, China*
*Xiaodong Liu, China Pharmaceutical University, China*

***Correspondence:**
*Rui Chen*
*chenrui04@126.com*
*Pei Hu*
*hubei01_pumch@163.com*

**Specialty section:**
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

**Received:** *14 October 2020*
**Accepted:** *14 January 2021*
**Published:** *18 February 2021*

**Citation:**
*Tang X, Zeng X, Guan X, Chen R and Hu P (2021) Modeling and Simulation to Support Phase Ib/IIa Dose Selection for WBP216, A Long Half-Life Fully Human Monoclonal Antibody Against Interleukin-6. Front. Pharmacol. 12:617265. doi: 10.3389/fphar.2021.617265*

WBP216 is an innovative IL-6 antibody, presenting high affinity to IL-6 and a long half-life (40–60 days). To optimize the dosage regimen for future clinical trials, pharmacokinetics (PK) and pharmacodynamics (PD) of WBP216 would be firstly characterized in Chinese rheumatoid arthritis (RA) patients. PK, CRP and DAS28 data of WBP216 were collected from 26 RA patients in a single ascending dose study. Non-linear mixed effects modeling was used for a population PK/PD analysis. A two-compartment model with a sequential zero-first order absorption and a first order elimination best described PK behavior of WBP216. Apparent systemic clearance was 0.015 L/h, central volume was 8.04 L. CRP as the fast-decreasing endpoint and DAS28 as the slow-reacting endpoint were both fitted well through an indirect response model. The baseline of ALT and free IL-6 were found associated with PK/PD parameters during covariates exploration. Simulation results confirmed that a loading dose regimen either of administration at weeks 0, 2, and 6 or doubling the maintenance dose level, followed by maintenance dosing of 75–150 mg every 8 weeks, was expected to provide a best risk/benefit ratio in future clinical studies. We hope this first PK/PD study of WBP216 in Chinese RA patients will help in the clinical development of WBP216 in future and provide a reference to the dosage optimization of similar antibodies with long half-life.

**Clinical Trial Registration:** CTR20170306

**Keywords: population pharmacokinetic, population pharmacodynamic, antibody, rheumatoid arthritis, dose optimization, model**

## INTRODUCTION

WBP216 is an IgG1 antibody that binds and neutralizes IL-6 and is designed as a biologic anti-rheumatic drug. Rheumatoid arthritis (RA) is featured by progressive articular disability, systemic inflammation, and high morbidity, which stem from a complex interaction between various inflammatory cells and cytokines (McInnes and Schett, 2011; Smolen et al., 2016). Biological therapies are recommended for treating RA refractory to synthetic chemical drugs (Newsome, 2002).

IL-6 is one of major pro-inflammatory cytokines involved in RA pathogenesis. Inhibition of the IL-6 signaling pathway helps to reduce inflammation and pain in patients with RA (Ohsugi and Kishimoto, 2008; Raimondo et al., 2017). Tocilizumab, as an anti interleukin 6 receptor (IL-6R) monoclonal antibody, is launched in 2009 for the indication of RA, whose successful clinical use has proved the key role of IL-6 in RA pathogenesis (Choy et al., 2002).

WBP216 is a fully humanized monoclonal antibody that has showed its significant efficacy in treatment of RA in phase I trial. WBP216 has a strong affinity for interleukin 6 (IL-6), with an affinity constant in the picomolar range. WBP216 prevents the interaction of IL-6 and its receptor, thus reducing pro-inflammatory activity. Unlike regular IgG1 antibodies, WBP216 includes YTE mutations in the fragment crystallization (Fc). These mutations increase the ability of the Fc fragment to bind the neonatal Fc receptor (FcRn) (Oganesyan et al., 2009), which protect WBP216 from intracellular degradation, and thereby extending its half-life to >40 days and bringing some different pharmacokinetic (PK) characters. Thus, WBP216 has the potential to relieve RA effectively and for a sustained period.

Due to the blockade of IL-6 signaling, WBP216 can directly inhibit the hepatic production of C-reactive protein (CRP) directly (Bastida et al., 2019) and the erythrocyte sedimentation rate (ESR). Thus, the levels of inflammatory markers (CRP and ESR) rapidly decrease after the initiation of IL-6 blocker treatment, even prior to any improvement in swollen or tender joint counts. Therefore CRP alone is not sufficient to assess efficacy. According to the American College of Rheumatology (ACR) response criteria, the disease activity score based on 28 joint (DAS28) in its two versions employing ESR or CRP are most frequently used in clinical trials and also in clinical practice. DAS28 includes assessment of swollen/tender counts for 28 joints and patient/evaluator physician global assessment, and usually decreases slowly after RA treatments compared to CRP and ESR (Smolen et al., 2003). Hence, rapidly and slowly decreasing pharmacodynamic (PD) biomarkers should be assessed together to comprehensively evaluate RA medications.

Population PK/PD modeling and simulation has proven to be a useful approach in facilitating drug development (Budha et al., 2015). We report here the population PK/PD analysis of WBP216 PK and serum CRP and DAS28-ESR data in RA patients from phase Ia, and model-based simulation results. The goal of this modeling and simulation is to optimize dose levels, dosing intervals and loading dose regimens for such a long half-life antibody in Phase Ib/IIa, a multiple ascending dose (MAD) study, which will help to improve the drug development efficiency. Moreover, a population PK/PD analysis in this phase can lead to a better understanding of PK/PD characteristics of WBP216. PK/PD data obtained directly from RA patients instead of healthy volunteers allows to recommend more accurate dosing regimens that can provide significant CRP and DAS28-ESR reduction while minimizing the frequency of subcutaneous injections.

Therefore, we aimed to establish a population PK/PD model of WBP216 using phase Ia PK/PD data in RA patients and then use the model to make phase Ib/IIa dose regimen decision in this study. To our knowledge, this is the first report of the PK/PD of WBP216 in Chinese RA population. Our study will provide valuable information for the development and application of WBP216 in clinic.

# METHODS

## Study Population and Data

Briefly, phase Ia (CTR20170306) was a multi-center, randomized, double-blinded, single ascending dose study of WBP216 in RA patients. The study protocol was designed in full compliance with Good Clinical Practice and the Declaration of Helsinki and approved by the independent ethics committee of Peking Union Medical College Hospital and Beijing hospital (Beijing, China). Subjects eligible to take part in this study were RA patients diagnosed over 6 months, treated with basic RA medications (e.g., methotrexate and leflunomide) stably for at least 28 days, aged from 18 to 70 years and with a body mass index (BMI) of 19–30 kg/m$^2$. All subjects signed the Informed Consent Form before their participation.

A total of 36 subjects were enrolled into 5 dose cohorts (10, 30, 75, 150 or 300 mg) respectively and randomized to receive WBP216 or placebo subcutaneously, with 3 subjects receiving active WBP216 and 1 subject receiving placebo in the lowest dose cohort (i.e., 10 mg) and with 6 subjects receiving WBP216 and 2 subjects receiving placebo in other each dose group. Serum samples were collected at pre-dose and 0.083, 0.5, 1, 3, 7, 14, 21, 28, 42, 56, 84, 112, 168 days post-dose to obtain total WBP216 concentration and CRP data. Total WBP216 was assayed by validated methods whose linear calibration ranges were 39.1–10,000 ng/ml for WBP216. The CRP and ESR measurements were conducted by the clinical laboratory center of the hospitals. The LLOQ for CRP and ESR are 0.01 mg/L and 1 mm/h, respectively. DAS28-ESR and ACR scores were estimated by doctors at baseline and on days 7, 28, 56, 84, 112, 140 and 168, with 112/140 days measurements only for the 75–300 mg groups. If any anti-drug antibody (ADA) measurement was positive in any subject during the trial period, this subject was described as ADA positive.

## Characterization of WBP216 PK Properties

The YTE mutations in the Fc fragment of WBP216 may result in non-standard extended PK properties, which is one of our study focus. In order to explore the PK characteristics, PK parameters were firstly calculated based on individual plasma concentration-time-profiles using non-compartment analysis (NCA) by validated Phoenix WinNonlin version 8.1 software (Pharsight Corporation, Mountain View, CA, USA). PK parameters through NCA were then analyzed and explored using WinNonlin or PRISM (GraphPad 8.0.1, San Diego, CA, USA). A power model (**Eq. 1**) proposed by Gough et al. was applied to assess the dose proportionality (Kevin Gough, 1995).

$$\log(Y) = \mu + \beta \times \log(\text{dose}) \quad (1)$$

in which $Y$ denotes PK parameters such as area under curve (AUC) or maximum concentration ($C_{max}$). This approach

assumes that the underlying relationship between log($Y$) and log(dose) is linear. $\beta = 1$ indicates total dose proportionality. In our study, a less stringent criterion was used (Hummel et al., 2009), given the small sample size in phase I across multiple dose groups. The estimate of $\beta$ together with CI falling completely within the range of [0.5–2] was quantified as dose proportionality.

## Population PK and PD Model Development

The relationship between drug exposure and response was evaluated using nonlinear mixed effects models (Phoenix NLME, version 8.1, Certara). First-order conditional estimation, extended least squares method (FOCE-ELS) was used to estimate pop PK/PD model parameters. The final structural model was determined by the objective function value (OFV) and Akaike information criterion (AIC). A sequential modeling strategy was used for fitting the models to the phase Ia data. The population PK model was first developed and then the individual post hoc parameters from the final PK model were used to predict the individual WBP216 concentrations to drive the drug effect on CRP or DAS28 time profiles using appropriate PD models.

We tried one, two or three-compartment PK model with a first order, saturate or sequential zero-first order absorption compartment. The elimination phase was also analyzed by fitting to first order, saturate elimination or target-mediated-drug-disposition (TMDD) models. Based on the mechanism of action, an indirect-response model was chosen as a starting point for PD model development for CRP or DAS28. A linear model, an $E_{max}$ model or a sigmoidal $E_{max}$ model (Hill equation) were applied to characterize the relationship between WBP216 serum concentrations and those disease activity measures.

Inter-individual variability (IIV) was assumed to follow a log-normal distribution and was described using exponential model (**Eq. 2**).

$$P_{ij} = \theta_i \times e^{\eta ij} \tag{2}$$

where $P_{ij}$ represents the individual value of the parameter for the $i$th parameter in the $j$th individual, $\theta_i$ depicts the population typical value for the $i$th parameter, and $\eta ij$ represents random effect in $j$th individual sampled from a normal distribution with a mean of zero and variance of $\omega^2$.

The residual unexplained variability of WBP216 concentration and PD observation was described by a proportional or additive error model, respectively (**Eqs 3**, **4**).

$$Y_{obs} = Y_{pred} \times (1 + \varepsilon_1) \tag{3}$$

$$Y_{obs} = Y_{pred} + \varepsilon_2 \tag{4}$$

where $Y_{obs}$ and $Y_{pred}$ are the observed and predicted serum concentration in plasma or disease activity measures. And $\varepsilon_1$ is the proportional and $\varepsilon_2$ is the additive component of the residual error model, respectively. Both of $\varepsilon_1$ and $\varepsilon_2$ are assumed to be normally distributed in the range of (0, $\sigma^2$).

After collinearity diagnostics and correlation analysis, possible covariates including weight, age, sex, baseline serum albumin (ALB), alanine amiotransferase (ALT), creatinine clearance (CLcr), total bilirubin (TBIL), free IL-6, ADA (negative or positive) etc. were tested on both PK and PD parameters. Continuous covariates were described using the power function and categorical covariate were modeled by exponential function, see **Eqs 5** and **6**.

$$\text{Effect}_i = \left(\text{Cov}_{ij}/\text{Cov}_{\text{median}}\right)^{\theta\text{covi}} \tag{5}$$

$$\text{Effect}_i = e^{\text{Covij}\cdot\theta\text{covi}} \tag{6}$$

where $Effect_i$ is the multiplicative factor for covariate $i$, $Cov_{ij}$ is the covariate value for individual $j$, $Cov_{median}$ is the median covariate value, and $\theta cov_i$ is the exponent or parameter for covariate $i$ model.

Potential covariates were incorporated into the base model one by one using stepwise forward inclusion. When OFV decreased by 6.63 (at $p < 0.01$), the covariate was selected for inclusion to develop a full model, followed by the backward elimination. The covariates were subtracted one at a time in a stepwise manner as well once OFV increased above 10.83 (at $p < 0.001$, df = 1), until all remaining covariates in model were statistically significant.

## Model Evaluation

During the process of models building, the goodness of fit (GOF) of different models was compared on the basis of OFV and AIC. GOF was graphically evaluated by inspecting plots of the individual or population predicted vs. observed values, and plots of the conditionally weighted residuals (CWRES) vs. population predictions or time.

Models were also validated internally using prediction-corrected visual predictive checks (pcVPC) as well (Bergstrand et al., 2011). On the basis of 1,000 times pcVPC simulation, the 90% prediction interval (PI) was compared with the 90% interval of the prediction-corrected observations. Bootstrap analysis was also performed for the final model along with a total of 500 data sets resampling randomly from the original data set (Ette, 1997). We reported the calculated 90% confidence interval (CI) of model parameters from successfully minimized runs.

## Simulations for Phase Ib Dose Selection

Simulation was conducted in Phoenix NLME (version 8.1, Certara, USA) based on a Monte-Carlo simulation approach. Up to 100 Phase Ib trials were simulated using the uncertainty distribution in parameter estimates. 27 patients in each simulated trial were simulated using the IIV log-normal distribution in both PK and PD parameters. The distribution of covariates still leveraged those of phase Ia data set. Serum CRP levels and DAS28 were simulated for a range of maintenance doses (30–300 mg) under three different dosing frequencies: every 4 weeks (Q4W), every 8 weeks (Q8W), and every 12 weeks (Q12W). The duration of drug effect was simulated up to week 72 with weekly virtual PD sampling. It was assumed that the PK/PD relationship based on the Phase I study lasting 24weeks could be extrapolated to longer term studies.

Tocilizumab has proven its successful clinical efficacy, so it was used as the reference for comparison of clinical endpoints. Since mean CRP is decreased by around 90% and mean DAS28 could be reduced by over 56.5% using the recommended dosage of tocilizumab (ACTEMRA® HIGHLIGHTS OF PRESCRIBING

**TABLE 1 |** Descriptive statistics of the demographic characteristics, laboratory data and disease activity (baseline values) of the RA patients included in the population PK/PD model ($n$ = 26).

| Covariates | Value (Mean ± SD) |
|---|---|
| Demographic | |
| Sex-female, *n* (%) | 23 (88.5%) |
| Age (years) | 49.7 ± 10.1 |
| Weight (kg) | 61.5 ± 7.9 |
| Height (cm) | 161.0 ± 7.0 |
| Laboratory data | |
| Albumin (ALB, g/L) | 40.2 ± 3.4 |
| Alanine transaminase (ALT, U/L) | 14.1 ± 7.0 |
| Total bilirubin (TBIL, μmol/L) | 10.8± 3.9 |
| Creatinine clearance (CLcr, ml/min) | 104.6 ± 31.8 |
| Positive ADA, n (%) | 3 (11.5%) |
| Free IL-6 (baseline, pg/mL) | 54.8 ± 87.9 |
| CRP (baseline, mg/L) | 14.7± 19.9 |
| Disease activity | |
| DAS28 (baseline) | 5.3 ± 0.9 |

INFORMATION; U.S. Food and Drug Administration), ΔCRP≥90% and ΔDAS28 ≥ 56.5% were set as our target efficacy to optimize WBP216 phase Ib maintenance dose levels and dosing frequencies.

WBP216 would take a long time to achieve steady state exposure and efficacy because of its slow elimination rate constants. Because RA patients would require a rapid pain relief, a loading regimen of WBP216 would be necessary. Hence, we simulated two categories of loading regimen to achieve steady state exposure: Firstly, WBP216 was given in a more intensive frequency at an initial three-administrations, including 0–4–8th week, 0–2–4th week or 0–2–6th week; The second simulation used a loading dose, that doubled the confirmed maintenance dose level.

# RESULTS

## Clinical Data Summary

Up to 36 RA patients took active medicine and placebo in a ratio of 3:1, respectively, wherein 27 patients received WBP216. One of the subjects in the 75 mg group showed a huge fluctuation of CRP level after 21 days from administration, very different from other subjects. The CWRES of there CRP samples were also greater than six during CRP model exploration. This subject was considered as an outlier and excluded from this PK/PD model analysis. Descriptive statistics (baseline values) of potential covariates of 26 patients tested in the PK/PD analysis were summarized in **Table 1**. A total of 391 PK samples were obtained during the phase Ia and PD data consisted of 384 CRP samples and 241 DAS28 samples. Since fewer than 10% of PK samples were below the lower limit of quantification (LLOQ), they were handled by M1 method (Keizer et al., 2015). PK concentrations whose corresponding ADA was positive were all above the LLOQ, so they were not discarded.

## Inspection of WBP216 PK Properties

We performed an NCA analysis for different dose groups before population PK model development to understand the PK properties fully because of the unique YTE mutations in WBP216. **Figure 1** showed that mean apparent clearance (CL/*F*) tended to increase with increasing dose, contrary to the clearance change pattern of TMDD, which generally has a high clearance in lower dose groups (Mager, 2006). Large variability of CL/*F* among individuals was observed in both 75 and 300 mg dose groups. High individual variability of apparent volume (V/*F*) was also observed in higher dose levels (75–300 mg). Like CL/*F*, V/*F* presented an increasing trend over dose levels. The phenomenon of CL/*F* and V/*F* changing with dose levels was speculated to result from the changes of either the actual increased CL and V or decreased bioavailability (*F*). To clarify the real reason, the half-life of WBP216 was analyzed. Half-life ($t_{1/2}$ = 0.693*V/CL) is considered not to be impacted by *F*. In this analysis of half-life, the value of $t_{1/2}$ distributed evenly in five dose groups, and the mean $t_{1/2}$ remained almost the same in different groups, except for the 150 mg group, likely caused by lower CL/*F* and higher V/*F* value in this group. Those changing trends of CL/*F*, V/*F* and $t_{1/2}$ suggested that the decrease of *F* with doses was probably the cause of the increase of CL/*F* and V/*F*. Mean $t_{1/2}$ is 40–60 days, indicating the potential for a long dosing interval in therapeutic use. The power model fitted ln (AUC) over ln (dose) data well (**Figure 1D**). Parameter $\beta$ was calculated as 0.652 with 90% confidence interval (CI), [0.43, 0.87], part of which fell outside the lower limit of prespecified range [0.5, 2] (Hummel et al., 2009). This dose-dependent study indicated little lower than proportional increases in exposure (AUC).

## Population PK/PD Model Development

According to AIC value and goodness of fit (GOF) plots, the selected final proposed PK/PD model structure is shown in **Figure 2**. The WBP216 serum concentrations were best described by a two-compartment PK model with sequential zero-first order absorption and first order elimination (see **Eqs 7–9**). An indirect-response model with an drug $E_{max}$ inhibition of the CRP or DAS28 zero order rate production constant ($K_{in}$) best described the disease measures (see **Eqs 10**, **11**). Residual variability was characterized by a proportional error model for serum concentration, CRP and DAS28. All PK/PD model parameters are summarized in **Table 2**.

$$\frac{dA_{depot}}{dt} = \frac{Dose}{Td} - ka \times A_{depot} \quad \left(t = 0,\ A_{depot} = 0\right) \tag{7}$$

$$\frac{dA1}{dt} = ka \times A_{depot} - CL\Big/F \times C1 - Q\Big/F \times (C1 - C2) \quad (t = 0, A1 = 0) \tag{8}$$

$$\frac{dA2}{dt} = Q\Big/F \times (C1 - C2) \quad (t = 0,\ A2 = 0) \tag{9}$$

$$\frac{dCRP}{dt} = K_{in,CRP} \times \left(1 - \frac{E_{max,CRP} \times C1}{EC_{50,CRP} + C1}\right) - K_{out,CRP} \times CRP \tag{10}$$

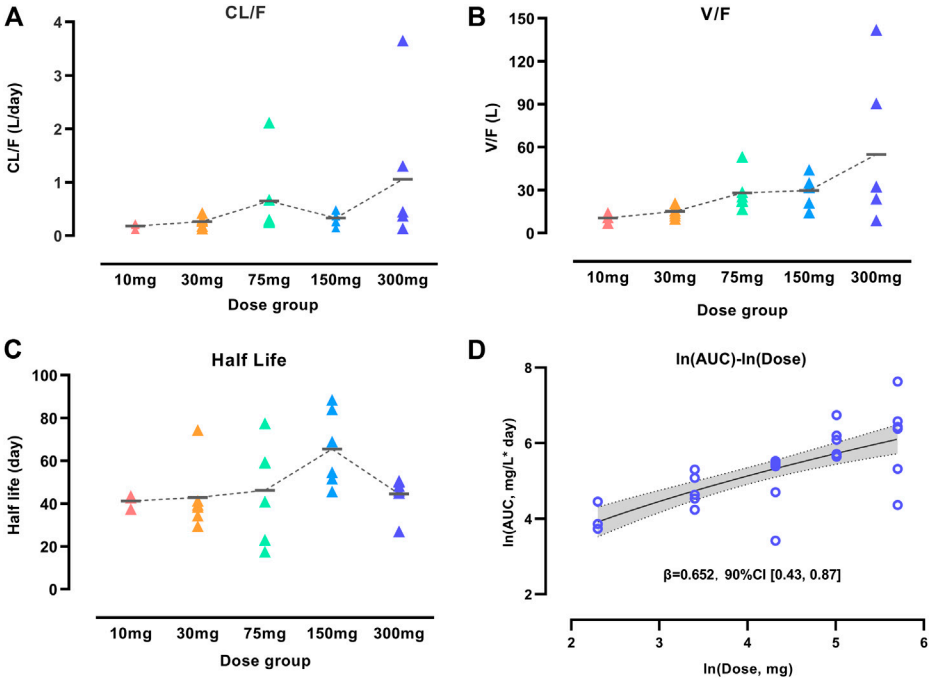


**FIGURE 1 |** Characterization of WBP216 pharmacokinetic properties. Apparent clearance vs. dose groups **(A)**, Apparent volume vs. dose groups **(B)**, Comparison of half-life in different dose groups **(C)**. The short horizontal line represents the mean value of CL/F, V/F and t1/2, respectively, in five dose groups. Dose proportionality assessment using power model **(D)**. The dashed lines are the connection of those mean value; The solid line denotes the expected mean value and the shaded region denotes the 90% confidence interval.

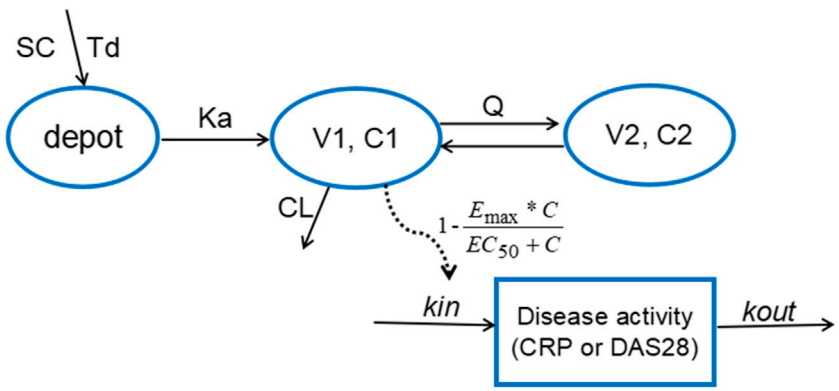


**FIGURE 2 |** The proposed PK/PD model structure. Parameters are abbreviated as follows: SC = subcutaneous injection; Td = time of zero order release; Ka = first order absorption rate; C1 = concentration in central compartment; V1 = central volume; CL = clearance from central compartment; C2 = concentration in peripheral compartment; V2 = volume of peripheral compartment; Q = clearance between central and peripheral compartments; $E_{max}$ = maximum drug efficacy; $EC_{50}$ = concentration needed for 50% of $E_{max}$; $K_{in}$ = zero order rate constant for response production; $K_{out}$ = first order rate for response loss.

$$\frac{\mathrm{dDAS28}}{\mathrm{d}t} = K_{\mathrm{in,DAS28}} \times \left(1 - \frac{E_{\mathrm{max},DAS28} \times C1}{EC_{50,DAS28} + C1}\right) - K_{\mathrm{out,DAS28}} \times \mathrm{DAS28} \quad (11)$$

Wherein $A_{\mathrm{depot}}$, A1, A2 representing WBP216 amounts in absorption depot, systemic central and peripheral compartments respectively, were equal to zero when $t$ = 0. C1 and C2 were the concentration in central and peripheral compartment, respectively and equal to zero before dosing. Response was CRP or DAS28, and was equal to its baseline value before drug administration.

The final PK model fitted the observed concentration data better by assuming that after WBP216 was subcutaneously administered, drug was released from the injection site at a

**TABLE 2 |** Summary of final population pharmacokinetic and pharmcodynamic model parameters in rheumatoid arthritis patients.

| Parameters | Definition | Estimated value (RSE%) | IIV(RSE%) | Bootstrap of estimates median (90%CI) | Bootstrap of IIV median (90%CI) | Shrinkage |
|---|---|---|---|---|---|---|
| PK model | | | | | | |
| Ka (1/hr) | Subcutaneous absorption rate constant | 0.007 (20.8) | 54.1% (13.3) | 0.007 (0.005, 0.01) | 50.7% (24.6%, 60.9%) | 14.3% |
| Td (hr) | Release time of zero order rate | 2.183 (29.0) | 128.4% (21) | 2.173 (1.221, 3.246) | 124.5% (73.5%, 194.8%) | 16.9% |
| CL/F ( L/h) | Clearance from central compartment | 0.015 (12.4) | 52.7% (13.2) | 0.014 (0.011, 0.018) | 50.3% (37.5%, 61.0%) | –0.4% |
| V1/F (L) | Volume of central compartment | 8.039 (30.9) | 117.1% (11.1) | 8.268 (5.024, 14.24) | 112.4% 90.5%, 132.4%) | 0.4% |
| V2/F (L) | Volume of peripheral compartment | 10.298 (11.6) | 48.2% (27.0) | 10.141 (7.27, 12.124) | 45.8% (30.9%, 69.9%) | 18.1% |
| Q/F (L/h) | Clearance between central and peripheral compartment | 0.062 (20.9) | NE | 0.060 (0.048, 0.120) | NA | NA |
| $\theta_{ALT\sim CL}$ | Covariate about ALT on CL | –0.833 (30.9) | NA | –0.830 (–1.113, –0.196) | NA | NA |
| $\sigma_{PK}$ | Proportional error for serum concentration | 0.119 (8.9) | NA | 0.118 (0.102, 0.137) | NA | NA |
| CRP model | | | | | | |
| $K_{in,CRP}$ (mg/(L*hr)) | Zero-order constant for response production | 0.185 (19.7) | 93.2% (14.3) | 0.187 (0.071, 0.323) | 86.9% (66.0%, 107.5%) | 4.5% |
| $K_{out,CRP}$ (1/hr) | First-order rate constant for response loss | 0.026 (4.6) | NE | 0.026 (0.024, 0.029) | NA | NA |
| $EC_{50,CRP}$ (ug/L) | The concentration to achieve 50% $E_{max,CRP}$ | 194.37 (24.7) | 113.0% (16.6) | 202.887 (124.151, 578.5) | 107.9% (79.8%, 138.8%) | 8.6% |
| $E_{max,CRP}$ | The maximum effect of drug | 1 (fixed) | NA | NA | NA | NA |
| $\Theta_{BaseFreeIL-6\sim kin}$ | Covariate about baseline of free IL-6 on $K_{in,CRP}$ | 0.695 (14.9) | NA | 0.710 (0.519, 0.855) | NA | NA |
| $\Theta_{BaseFreeIL-6\sim EC50}$ | Covariate about baseline of free IL-6 on $EC_{50,CRP}$ | –0.772 (17.6) | NA | –0.773 (–1.021, –0.557) | NA | NA |
| $\sigma_{CRP}$ | Proportional error for CRP | 0.523 (13.1) | NA | 0.524 (0.422, 0.640) | NA | NA |
| DAS28 model | | | | | | |
| $K_{in,DAS28}$ (1/hr) | Zero-order constant for response production | 0.003 (11.1) | NE | 0.003 (0.002, 0.004) | NA | NA |
| $K_{out,DAS28}$ (1/hr) | First-order rate constant for response loss | 0.0006 (11.5) | 17.0% (12.8) | 0.0006 (0.0004, 0.0009) | 16.5% (11.9%, 21.5%) | 8.1% |
| $EC_{50,DAS28}$ (ug/L) | The concentration to achieve 50% $E_{max,DAS28}$ | 1,576.3 (15.6) | 95.5% (13.5) | 1,602.1 (1,014.4, 2,513.9) | 92.2% (55.9%, 120%) | 15.4% |
| $E_{max,DAS28}$ | The maximum effect of drug | 1 (fixed) | NA | NA | NA | NA |
| $\sigma_{DAS28}$ | Proportional error for DAS28 | 0.18 (12.0) | NA | 0.177 (0.143, 0.217) | NA | NA |

*RSE%: relative standard errors; IIV: Inter-individual variability; 90% CI: 90% confidence interval; NE: not estimate; NA: not applicable.*

zero order rate to a depot compartment, and was then absorbed to a central compartment. Td is the period of zero order release and Ka denotes the first order absorption rate. The reason of selecting such a more complex absorption model will be discussed later. Based on the mechanism of action, WBP216 neutralizes IL-6 and inhibits CRP production, and further slow down disease progression, so it is plausible to set the drug inhibition on response production rate, $K_{in}$ (Sharma and Jusko, 1998). As shown in **Table 2**, the relative standard errors (RSE%) for almost all fixed-effect parameters were ≤30.9%. The uncertainties for random-effect parameters were <27%. Overall, the precision of parameters estimates was acceptable. Parameters showed various inter-individual variability, ranging from 17 to 128.4%. $E_{max}$ for CRP was set to 1 since its value was always very close to 1 in all tested runs. $E_{max}$ for DAS28 was also fixed to 1, thus leading to a straightforward convergence of the model. All applicable shrinkage was below 18.1%, which was smaller than reported cut-off value 30% and could assure accurate IIV estimates and avoidance of misleading diagnostic plots (Savic and Karlsson, 2009).

The correlation diagnosis chart of various covariates was presented in **Supplementary Figure S1**. The potential impacts of demographics and laboratory data baseline on PK/PD parameters of WBP216 were tested using a stepwise covariate modeling procedure. Those statistical significant covariate effects were identified and retained in the final model: ALT on CL, baseline free IL-6 on $EC_{50,CRP}$ and $K_{in,CRP}$ (see **Eqs 12–14** and **Supplementary Table S2**). Continuous covariates were described using the power function, centered by the median value. Apparent clearance decreased with increasing ALT with exponent –0.833, which explained around 18% of CL/F inter-individual variability (IIV). Higher baseline free IL-6 levels could result in increased $K_{in,CRP}$ (exponent 0.695), while lead to its

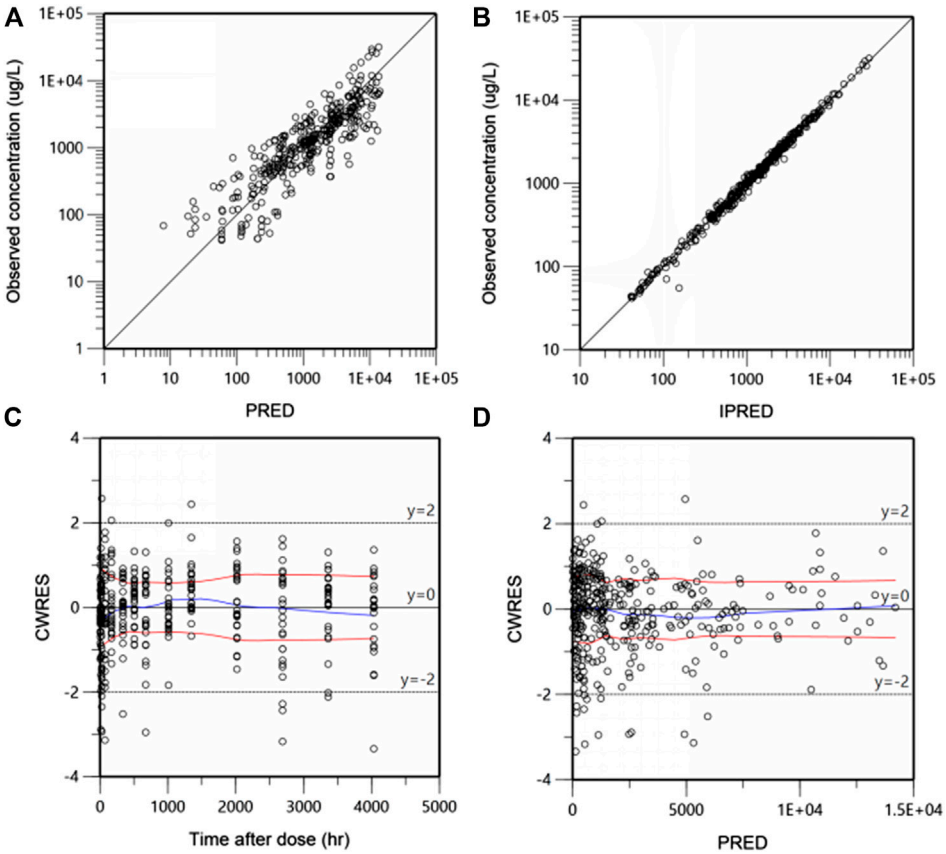


**FIGURE 3 |** Goodness-of-fit plots for the final population pharmacokinetic (PK) model. Population-predicted concentration vs. observed concentrations (ug/L) **(A)**, Individual-predicted concentration vs. observed concentrations **(B)**, The conditional weighted residuals (CWRES) over time after dose (h) **(C)**, CWRES against population predicted concentration **(D)**. The blue lines are the smoothed LOESS regression lines and the red lines represent LOESS regression to the absolute values of the dependent variable with its negative reflection.

$EC_{50,CRP}$ reduction (exponent −0.772). Baseline free IL-6 accounted for 59.2% IIV of $K_{in,CRP}$ and 55.6% IIV of $EC_{50,CRP}$. No covariates were found statistically significant in parameters for DAS28. Covariate associated parameters are also presented in **Table 2**.

$$CL/F = \theta_{CL/F} \times \left(\frac{ALT}{11}\right)^{-0.833} \times e^{\eta CL/F} \quad (12)$$

$$K_{in,CRP} = \theta_{Kin,CRP} \times \left(\frac{BaseFreeIL\text{-}6}{24.9}\right)^{0.695} \times e^{\eta_{K_{in,CRP}}} \quad (13)$$

$$EC_{50,CRP} = \theta_{EC_{50,CRP}} \times \left(\frac{BaseFreeIL\text{-}6}{24.9}\right)^{-0.772} \times e^{\eta EC50,CRP} \quad (14)$$

## Model Diagnosis and Evaluation

Goodness-of-fit (GOF) plots for the final PK model in serum are shown in **Figure 3**. Plots of the population- and individual-predicted concentration vs. observed concentrations demonstrate no major bias. The conditionally weighted residuals (CWRES) were symmetrically distributed about zero axis and most points laid within the acceptable range (−2 to 2), suggesting that little to no bias accompanied with concentration or time. The GOF plots for CRP and DAS28 model were presented in **Supplementary Figures S2 and S3**, respectively, which also performed well in visual diagnostic.

The predictive performance was evaluated internally by pcVPC. Plots of pcVPC were presented in **Figure 4**. We can see that the 5th, 50th and 95th percentiles of prediction-corrected observations and predicted data were fairly consistent, especially a better match for DAS28. The 95th percentile of predicted PK data through VPC is a slight under-prediction and the CRP model over-predicted drug inhibition at 5th percentile slightly, which will be discussed in the part of discussion. Despite these small deviations, the 90% prediction interval of simulated data covered most of the observations. The bootstrap results are also shown in **Table 2**. The typical values of parameters and IIV estimates in the final model were pretty close to the median values from bootstrap validation, and fell within 90%CI of bootstrap parameters completely, which indicated high stability and precision of the final model.

Overall, the good performance of GOF, pcVPC plots and bootstrap estimations reconfirmed that the final PK/PD model was adequately developed and the predictive performance was sufficient to capture PK/PD observations.

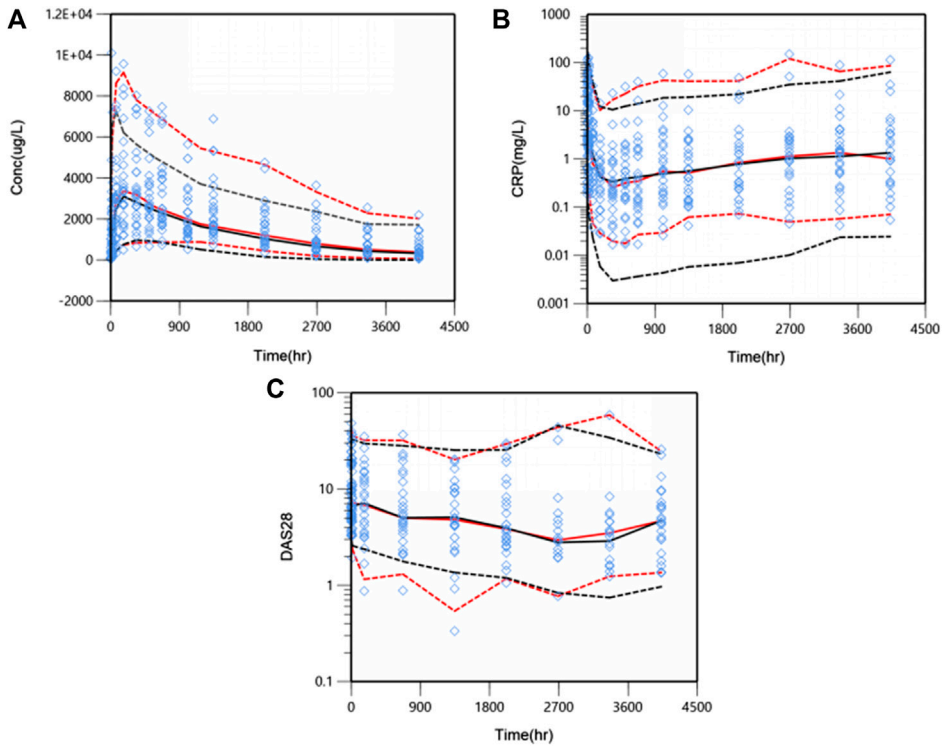


**FIGURE 4 |** Prediction-corrected visual predictive check (pcVPC) results for PK/PD model. WBP216 serum concentrations vs. time within 24 weeks **(A)**, CRP against time within 24 weeks **(B)**, DAS28 over time within 24 weeks **(C)**. Open diamonds represent observation points. Red dash/solid lines denotes 5th, 50th, and 95th percentiles for prediction-corrected observations; black dash/solid lines are 5th, 50th, and 95th percentiles for prediction, respectively.

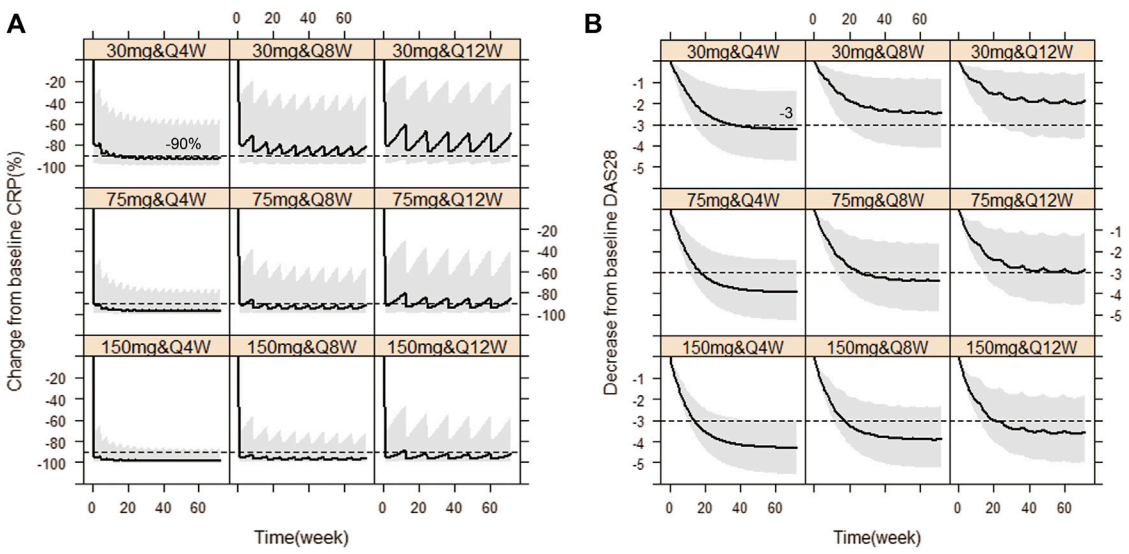


**FIGURE 5 |** Simulated CRP and DAS28 changes over time under nine dose regimens using final population PK/PD model. The percent change from baseline CRP against time **(A)**, The decrease from baseline DAS28 over time **(B)**. The solid line denotes the 50th percentile of model simulation results and the shaded region presents the 80% prediction interval. Dashed lines show −90% and −3 therapeutic targets for CRP and DAS28, respectively.

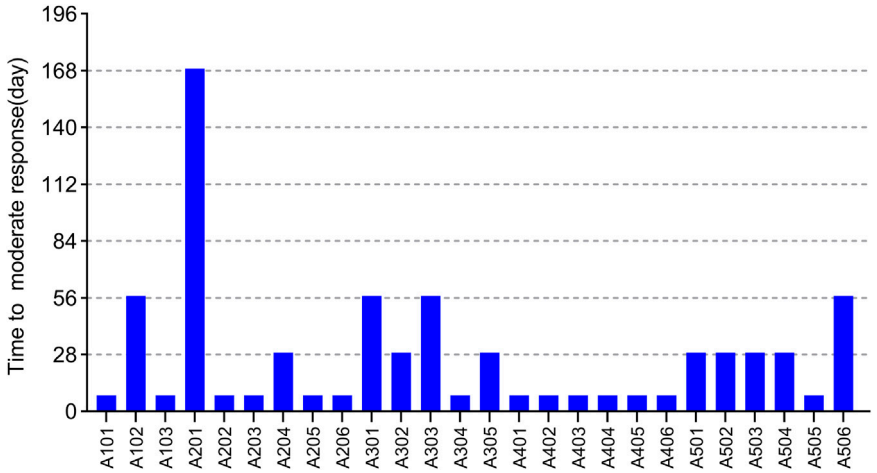


**FIGURE 6 |** The time required to reach moderate response of 26 subjects in phase Ia trial. Each bar represents one subject.

## Simulations for Phase Ib/IIa Dose Selection

Based on the observations, we chose dose levels of 30, 75 and 150 mg as potential maintenance doses so as to rapidly reach the target effect (mean ΔCRP≥90%; ΔDAS28 ≥ 56.5%, i.e. ΔDAS28 ≥ 3 when average baseline DAS28 is 5.3) at steady state, equivalent to tocilizumab (ACTEMRA® HIGHLIGHTS OF PRESCRIBING INFORMATION; U.S. Food and Drug Administration). Then, simulations of those different maintenance dose levels and three varying dose frequencies Q4W, Q8W and Q12W were performed, respectively. **Figure 5** showed the Monte-Carlo simulations of CRP and DAS28 changing from baseline over time under different dose regimens using the final PK/PD model. Consistent with observations, the simulation results showed that CRP would decrease very rapidly to the nadir within the first week, while DAS28 changed slowly and reached a plateau after 24 weeks. Multiple dose regimens could decrease the mean CRP levels more than 90% from baseline and reduce DAS28 by more than 3 units, except for 30 mg Q8W, 30 mg Q12W, 75 mg Q12W scenario. The optimal regimens, in terms of achieving mean ΔCRP≥90% and ΔDAS28 ≥ 3, seemed to be 75 and 150 mg as maintenance doses administered every 8 weeks. Approximately 81% and 92% of virtual patients achieved DAS28-ESR <2.6 (the cutoff of RA remission defined by EULAR), when dosed at 75 mg Q8W and 150 mg Q8W, respectively. The 30 mg dose level did not achieve target efficacy when dosed every 8 or 12 weeks. Dosing every 4 weeks was a bit over-dosing and inconvenient to use in clinical practice, however, Q12W dosing frequency resulted in larger fluctuation in efficacy, especially in CRP, and led to a lower proportion of patients nearby target reference line no matter what dose level.

EULAR (European league against rheumatism) response criteria state (Broeder et al., 2002; Wells et al., 2009) (see **Supplementary Table S1**): moderate responders were patients with an improvement of >0.6 and a present DAS28 score of <5.1, or an improvement of >1.2 and a present DAS28 score of >5.1. We predefined that once patients start having a moderate response to WBP216, they would begin to feel pain relief intuitively. Patients' time needed to reach at least moderate response are summarized in **Figure 6**. The results showed that over half of patients got pain relief within around 10 days, while 12/26 patients did not reach moderate response until at least 28 days from drug administration. WBP216 caused a gradual increase in clinical efficacy during the phase Ia period, which was consistent with the patients' reports of slow onset of drug action.

Therefore, in order to allow patients to benefit from WBP216 therapy more quickly, loading dose regimens were designed. On

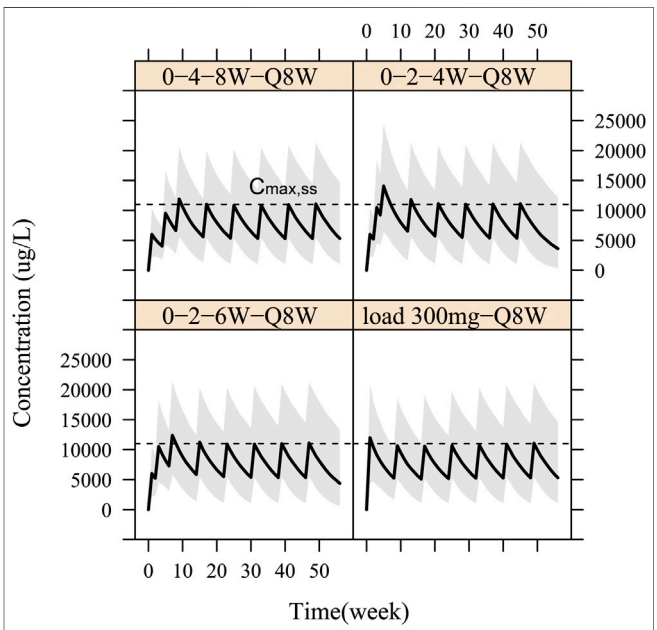


**FIGURE 7 |** Simulation for pharmacokinetic profiles according to four different loading schedules with 150 mg Q8W as maintenance regimen. The solid line denotes the 50th percentile of model simulation results and the shaded region represents the 80% prediction interval. Dashed lines are maximum concentration at steady state ($C_{max,ss}$).

the basis that the optimal dosing frequency was Q8W during maintenance therapy, we simulated four kinds of "loading dose schedules", **Figure 7** showed examples of varying loading schedules followed by a maintenance dose level of 150 mg Q8W: (1) Loading dose at weeks 0, 4 and 8 followed by Q8W still took more than 8 weeks to reach steady state exposure. (2) Loading dose at weeks 0, 2 and 4 followed by Q8W caused a steep WBP216 concentration increase, exceeding the peak concentration achieved by the maximum dose level (300 mg) in phase Ia, which may raise potential safety concerns. (3) Dosing at weeks 0, 2 and 6 followed by Q8W would reach plateau exposure at the second drug administration. (4) A loading dose of 300 mg, doubling the maintenance dose level, resulted in steady state instantly at the first dosing. Hence, the last two loading dose schedules are highly recommended for future clinical trials of WBP216. The simulated DAS28 profiles under the optimized loading dose regimen (3) and (4) were presented in **Supplementary Figure S4**, showing that the target efficacy was reached three weeks in advance.

## DISCUSSION

We developed a population PK/PD model that characterized the relationship between WBP216 serum concentrations and the changes in CRP and DAS28. This is the first report of the PK/PD of WBP216 in Chinese RA patients.

Rheumatoid arthritis is a complex autoimmune-mediated inflammatory disease involving both genetic and environmental factors (Smolen et al., 2016). Many factors in RA patients such as the disease process, complications, and concomitant medications could cause significant PK and PD differences between patients and healthy volunteers. Different from some IL-6 (R) antibodies that have been developed PK/PD models based on first-in-human studies with data from healthy volunteers (Xu et al., 2011), our modeling and simulation study in RA patients directly could avoid the aforementioned confounding factors and recommend more accurate dose regimens for phase Ib/IIa trials.

A two-compartment PK model with sequential zero-first order absorption and first order elimination fitted the PK data well. During the process of characterizing PK properties, we found the bioavailability of subcutaneous administration decreased gently with increasing dose levels. This atypical absorption may because large antibodies from the SC site are transported through the tissue interstitium and into the lymphatic system slowly accompanied by tissue metabolism and hydration etc (Richter et al., 2012; Jung et al., 2018). Adopting a base PK model structure with sequential zero-first order absorption (OFV = 4,935) performed significantly better than a traditional two-compartment model with first order absorption rate (OFV = 5,088) or a saturated absorption model of adding the Michaelis–Menten equation to bioavailability (OFV = 5,026). Although the NCA analysis suggested a slight trend of absorption saturation, it seemed not obvious enough to fit a typical M–M equation best in absorption phase. Linear clearance was sufficient to describe WBP216 elimination rather than a linear plus Michaelis–-Menten elimination of tocilizumab (Abdallah et al., 2017), probably because WBP216 binds to free IL-6 while tocilizumab binds the IL-6R involving complex internalization. The typical CL/*F* (0.015 L/h) from model estimates was slightly smaller than CL/*F* of sirukumab (0.019 L/h, corrected by *F*) (Xu et al., 2011), a IL-6 antibody, but the estimated V1/*F* (8.1 L) of WBP216 seemed to be larger than 4.1 L in sirukumab and 5.6 L in tocilizumab. Therefore WBP216 has a longer half-life. PK parameters showed high inter-individual variability, however, only ALT was identified as a covariate accounting for 18% of the variability of CL/F. CL/F was negatively correlated with ALT with exponent −0.833. Chunze Li etc. also reported that clearance of trastuzumab emtansine correlated significantly with baseline albumin and AST (Li et al., 2017). And the clearance of another IL-6 antibody, siltuximab, was found to be impacted negatively by ALT as well in its poplulation PK analyis containing 460 participants (Nikanjam et al., 2019). It is generally accepted that a therapeutic antibody is unlikely to be impacted by functional hepatic impairment (Dirks and Meibohm, 2010). We did not have sufficient data to explain this phenomenon. Dose adjustment for hepatic dysfunction was undetermined, and this would require a large target population to validate this point further. Some studies reported that weight was an important factor affecting CL/F (Abdallah et al., 2017) or V1/F (Li et al., 2018). Our study did not find this covariate, perhaps because of limited sample size with narrow weight range (61.5 ± 7.9 kg) in this phase Ia trial and the stringent covariate entry/elimination criteria set. In addition, only 3/27 patients exhibited ADA positive (**Table 1**), so ADA was correspondingly identified as a non-significant covariate influencing pharmacokinetic behaviors. All ADA samples of the outlier subject were detected negative, which could not impact ADA conclusion after exclusion.

We did not adopt the strategy of modeling the PK and PD data concurrently because the high variability of CRP or DAS28 would affect the estimate precision of PK parameters. An indirect response model with inhibition on response production was applied for both CRP and DAS28 endpoints based on WBP216's mechanism of action. Fast decreasing CRP is able to reflect the binding IL-6 ability of WBP216 directly since hepatic production of CRP is mainly mediated by IL-6 (Vermeire et al., 2004), while slow changing DAS28 tracks RA-related clinical efficacy closely. Unlike some early clinical studies that only focused on a fast-decreasing biomarker (i.e., CRP) (Xu et al., 2011; Mayer et al., 2015; Li et al., 2018), we evaluated both fast- and slow-decreasing endpoints simultaneously to understand the efficacy of WBP216 comprehensively. As **Table 2** shown, whatever $K_{in,CRP}$ or $K_{out,CRP}$ had greater value than those of DAS28, hinting high turnover rate of CRP. $K_{in,DAS28}$ 0.003 $h^{-1}$ and $K_{out,DAS28}$ $6*10^{-4}$ $h^{-1}$ were very closed to reported values of tocilizumab ($K_{in,DAS28}$ 0.0037 $h^{-1}$; $K_{out,DAS28}$ $7.2 \times 10^{-4}$ $h^{-1}$) (Bastida et al., 2019). While Levi et al. reported $K_{in,DAS28}$ 0.011 $h^{-1}$ and $K_{out,DAS28}$ $15.8 \times 10^{-4}$ $h^{-1}$ using data from 4 phase III studies of tocilizumab, which was almost 2 fold of our estimated DAS28 parameters (Levi et al., 2013). The differences in above reports may because the DAS28 baseline of subjects was about 5.3 in our and Bastida's

studies instead of 6.8 in Levi's research. The $EC_{50,CRP}$ (194.37 ug/L) for CRP was fairly smaller than that of DAS28 (1,576.3 ug/L), which indicated a higher concentration was needed for half-maximally decreasing DAS28. Covariates analysis showed that baseline free IL-6 was expected to be positively correlated with $K_{in,CRP}$, since CRP production is mainly stimulated by IL-6 in body. However, no covariates were discovered affecting $K_{in}$ and $K_{out}$ of DAS28. Baseline of free IL-6 was negatively associated with the $EC_{50}$ for CRP. The addition of baseline free IL-6 in model was able to explain 59.2% and 55.6% of IIV for $K_{in,CRP}$ and $EC_{50,\ CRP}$ respectively. Those limited sample size and narrow demographic or laboratory data may not provide fairly accurate covariate impacts on PK/PD parameters but they provided a reference for future covariate analysis in larger population.

The final population PK/PD model was evaluated by GOF plots, pcVPC and bootstrap. Slight deviations were observed in the 95th percentile of serum concentration and 5th percentile of CRP between respective predicted and prediction-corrected observations (**Figure 4**). It was noteworthy that one of patients in 300 mg group had extremely high PK exposure, over three fold of other subjects, which raised the 95th percentile of the observed concentration significantly. WBP216 was able to inhibit CRP to a very low level, almost close to zero, while the lower limit of quantitation of CRP can only reach 0.01 mg/L. Our model is expected to have a higher uncertainty near zero because of high IIV and residual errors, so the model predicted a lower CRP 5th percentile value than that of observed data. However, this slight deviation did not affect overall predictive ability since the 90% prediction interval of simulated data covered the majority of the observations.

WBP216 is the IL-6 monoclonal antibody with the longest half-life (40–60 days) by far. By comparison, the half-life of tocilizumab is reported as 11–13days and siltuximab as around 21day, leading to a dosing regimen of once every three or four weeks in clinical practice (ACTEMRA® and SYLVANT®, HIGHLIGHTS OF PRESCRIBING INFORMATION; FDA). According to our simulation results (**Figure 5**), the long half-life of WBP216 would allow it to be optimally administered once every 8 weeks. Dosing every 4 weeks did not offer any advantage since excess drug exposure would occur. Although the simulation results showed 150 mg Q12W seemed also acceptable, the Q8W dosing frequency was able to maintain a more stable change in CRP and DAS28. A score of DAS28-ESR<2.6 defines RA remissions (Anderson et al., 2012; Smolen et al., 2016). In our simulations with the baseline DAS28 around 5.3, when dosing 75 mg Q8W and 150 mg Q8W, approximately 81% and 92% of virtual patients were able to achieve DAS28-ESR <2.6 after 24 weeks therapy, respectively. So both of dose levels (75 and 150 mg) will deserve to be tested in future clinical studies.

A few subjects in phase Ia complained of getting limited relief until one or two months after drug administration (**Figure 6**). This prompted us to design four loading schedules used for simulation. An initial loading regimen of dosing at weeks 0, 2 and 6, followed by a maintenance regimen of Q8W, achieved steady state at the second administration, which was consist with dosage regimen of infliximab (RENFLEXIS, HIGHLIGHTS OF PRESCRIBING INFORMATION; FDA). Another good loading dose option is to double the maintenance dose level. The simulated DAS28 profiles under the two optimized loading dose regimen did show that three weeks were saved to reach the target efficacy with the maintenance dose of 150 mg Q8W (**Supplementary Figure S4**).

Although ACR20/50/70 endpoints were also measured in this early clinical study, there was no obvious dose-dependent relationship in the probability of achieving ACR20/50/70 efficacy in this phase Ia study. The combination of both early biomarkers (CRP and DAS28) helped to build the PK/PD relationship. A population PK/PD approach proved again useful in integrating all available PK/PD data during early clinical phases. Our study provided a relatively complete paradigm to accelerate clinical development for similar drugs as well.

The limitations of the model are as follows: (1) Baseline ALT and free IL-6 considered to be statistically significant were observed as covariates. Nevertheless, the limited number of patients in our study and the strict entry criteria set for the clinical trial may result in ambiguous covariate effects. Therefore, the confirmation of covariate effects should be kept in mind in future studies, which will have more data of target population added in. (2) In order to design dose regimen for a MAD study, it was assumed that the PK/PD relationship based on 36 patients in the Phase Ia study lasting 24weeks can be extrapolated to a larger target population and a longer term study. Attention should be paid to this model hypothesis when drawing conclusions from the simulation results. Patients with different disease states, disease progression, drug resistance and combination may invalidate this hypothesis.

In summary, a population PK/PD model was first successfully established for WBP216. Fast-decreasing (CRP) and slow-decreasing (DAS28) biomarkers were modeled concurrently to assess efficacy of WBP216 fully. For WBP216 with an exceptionally long half-life (40–60 days), two kinds of loading dose regimens are recommended for the next clinical studies. We expect that the modeling and simulation will be valuable for dose selection during future clinical trials, and provide a reference for the PK/PD studies of similar antibodies.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by correspondence authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by peking union medical college hospital; Beijing

hospital. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

RC and PH designed and organized the clinical trial. XZ collected clinical datas and involved in discussion of results. RC and XT conceived the model research. XT and XG performed the model research. XT analyzed the data and wrote the manuscript.

## FUNDING

This work was supported by the Clinical Research Fund from Wujieping Medical Foundation (No. 320.6750.19090–12); the Drug Development and Application Fund from Chinese Pharmacological Society (No. 2018DL001); the Beijing Key Laboratory (No. Z151100001615061); Chinese National Major Project for New Drug Innovation (No. 2019ZX09734001) and CAMS Innovation Fund for Medical Sciences (CIFMS) (No. 2016-I2M-1-010). Those funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

## ACKNOWLEDGMENTS

We are grateful to WuXi AppTec Co., Ltd and AstraZaneca PLC. for supporting the clinical study financially and proofreading. We also thank Cibo Huang from Beijing Hospital for performing part of trials.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.617265/full#supplementary-material.

## REFERENCES

Abdallah, H., Hsu, J. C., Lu, P., Fettner, S., Zhang, X., Douglass, W., et al. (2017). Pharmacokinetic and pharmacodynamic analysis of subcutaneous tocilizumab in patients with rheumatoid arthritis from 2 randomized, controlled trials: SUMMACTA and BREVACTA. *J. Clin. Pharmacol.* 57 (4), 459–468. doi:10.1002/jcph.826

Anderson, J., Caplan, L., Yazdany, J., Robbins, M. L., Neogi, T., Michaud, K., et al. (2012). Rheumatoid arthritis disease activity measures: American College of Rheumatology recommendations for use in clinical practice. *Arthritis Care Res.* 64 (5), 640–647. doi:10.1002/acr.21649

Bastida, C., Soy, D., Ruiz-Esquide, V., Sanmarti, R., and Huitema, A. (2019). Exposure-response modeling of tocilizumab in rheumatoid arthritis using continuous composite measures and their individual components. *Br. J. Clin. Pharmacol.* 85 (8), 1710–1718. doi:10.1111/bcp.13954

Bergstrand, M., Hooker, A. C., Wallin, J. E., and Karlsson, M. O. (2011). Prediction-corrected visual predictive checks for diagnosing nonlinear mixed-effects models. *AAPS J.* 13 (2), 143–151. doi:10.1208/s12248-011-9255-z

Broeder, A. D., Creemers, M. C. W., Gestel, A. M. V., and Riel, P. L. C. M. (2002). Dose titration using the Disease Activity Score (DAS28) in rheumatoid arthritis patients treated with anti-TNF-alpha. *Rheumatology* 41 (6), 638–642. doi:10.1093/rheumatology/41.6.638

Budha, N. R., Leabman, M., Jin, J. Y., Wada, D. R., Baruch, A., Peng, K., et al. (2015). Modeling and simulation to support phase 2 dose selection for RG7652, a fully human monoclonal antibody against proprotein convertase subtilisin/Kexin Type 9. *AAPS J.* 17 (4), 881–890. doi:10.1208/s12248-015-9750-8

Choy, E. H., Isenberg, D. A., Garrood, T., Farrow, S., Ioannou, Y., Bird, H., et al. (2002). Therapeutic benefit of blocking interleukin-6 activity with an anti-interleukin-6 receptor monoclonal antibody in rheumatoid arthritis: a randomized, double-blind, placebo-controlled, dose-escalation trial. *Arthritis Rheum.* 46 (12), 3143–3150. doi:10.1002/art.10623

Dirks, N. L., and Meibohm, B. (2010). Population pharmacokinetics of therapeutic monoclonal antibodies. *Clin. Pharmacokinet.* 49 (10), 633–659. doi:10.2165/11535960-000000000-00000

Ette, E. I. (1997). Stability and performance of a population pharmacokinetic model. *J. Clin. Pharmacol.* 37 (6), 486–495. doi:10.1002/j.1552-4604.1997.tb04326.x

Hummel, J., McKendrick, S., Brindley, C., and French, R. (2009). Exploratory assessment of dose proportionality: review of current approaches and proposal for a practical criterion. *Pharmaceut. Stat.* 8 (1), 38–49. doi:10.1002/pst.326

Jung, Y. S., Chae, D., and Park, K. (2018). Population PK-PD model of pegylated Interferon Alfa-2a in healthy Korean men. *J. Pharmaceut. Sci.* 107 (12), 3171–3178. doi:10.1016/j.xphs.2018.08.017

Keizer, R. J., Jansen, R. S., Rosing, H., Thijssen, B., Beijnen, J. H., Schellens, J. H., et al. (2015). Incorporation of concentration data below the limit of quantification in population pharmacokinetic analyses. *Pharmacol Res Perspect* 3 (2), e131. doi:10.1002/prp2.131

Kevin Gough, M. H. (1995). Assessment of dose proportionality: report from the statisticians in the pharmaceutical Industry/pharmacokinetics UK joint working party. *Drug Inform. J.* 29, 1039–1048. doi:10.1177/009286159502900324

Levi, M., Grange, S., and Frey, N. (2013). Exposure-response relationship of tocilizumab, an anti-IL-6 receptor monoclonal antibody, in a large population of patients with rheumatoid arthritis. *J. Clin. Pharmacol.* 53 (2), 151–159. doi:10.1177/0091270012437585

Li, C., Agarwal, P., Gibiansky, E., Jin, J. Y., Dent, S., Goncalves, A., et al. (2017). A phase I pharmacokinetic study of trastuzumab emtansine (T-DM1) in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer and normal or reduced hepatic function. *Clin. Pharmacokinet.* 56 (9), 1069–1080. doi:10.1007/s40262-016-0496-y

Li, C., Shoji, S., and Beebe, J. (2018). Pharmacokinetics and C-reactive protein modelling of anti-interleukin-6 antibody (PF-04236921) in healthy volunteers and patients with autoimmune disease. *Br. J. Clin. Pharmacol.* 84 (9), 2059–2074. doi:10.1111/bcp.13641

Mager, D. E. (2006). Target-mediated drug disposition and dynamics. *Biochem. Pharmacol.* 72 (1), 1–10. doi:10.1016/j.bcp.2005.12.041

Mayer, C. L., Xie, L., Bandekar, R., Qi, M., van de Velde, H., Reddy, M., et al. (2015). Dose selection of siltuximab for multicentric Castleman's disease. *Canc. Chemother. Pharmacol.* 75 (5), 1037–1045. doi:10.1007/s00280-015-2720-0

McInnes, I. B., and Schett, G. (2011). The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* 365 (23), 2205–2219. doi:10.1056/NEJMra1004965

Newsome, G. (2002). Guidelines for the management of rheumatoid arthritis: 2002 update. *J. Am. Acad. Nurse Pract.* 14 (10), 432–437. doi:10.1111/j.1745-7599.2002.tb00072.x

Nikanjam, M., Yang, J., and Capparelli, E. V. (2019). Population pharmacokinetics of siltuximab: impact of disease state. *Canc. Chemother. Pharmacol.* 84 (5), 993–1001. doi:10.1007/s00280-019-03939-7

Oganesyan, V., Damschroder, M. M., Woods, R. M., Cook, K. E., Wu, H., and Dall'Acqua, W. F. (2009). Structural characterization of a human Fc fragment engineered for extended serum half-life. *Mol. Immunol.* 46 (8–9), 1750–1755. doi:10.1016/j.molimm.2009.01.026

Ohsugi, Y., and Kishimoto, T. (2008). The recombinant humanized anti-IL-6 receptor antibody tocilizumab, an innovative drug for the treatment of rheumatoid arthritis. *Expet Opin. Biol. Ther.* 8 (5), 669–681. doi:10.1517/14712598.8.5.669

Raimondo, M. G., Biggioggero, M., Crotti, C., Becciolini, A., and Favalli, E. G. (2017). Profile of sarilumab and its potential in the treatment of rheumatoid arthritis. *Drug Des. Dev. Ther.* 11, 1593–1603. doi:10.2147/DDDT.S100302

Richter, W. F., Bhansali, S. G., and Morris, M. E. (2012). Mechanistic determinants of biotherapeutics absorption following SC administration. *AAPS J.* 14 (3), 559–570. doi:10.1208/s12248-012-9367-0

Savic, R. M., and Karlsson, M. O. (2009). Importance of shrinkage in empirical bayes estimates for diagnostics: problems and solutions. *AAPS J.* 11 (3), 558–569. doi:10.1208/s12248-009-9133-0

Sharma, A., and Jusko, W. J. (1998). Characteristics of indirect pharmacodynamic models and applications to clinical drug responses. *Br. J. Clin. Pharmacol.* 45 (3), 229–239. doi:10.1046/j.1365-2125.1998.00676.x

Smolen, J. S., Aletaha, D., and McInnes, I. B. (2016). Rheumatoid arthritis. *Lancet* 388 (10055), 2023–2038. doi:10.1016/S0140-6736(16)30173-8

Smolen, J. S., Breedveld, F. C., Schiff, M. H., Kalden, J. R., Emery, P., Eberl, G., et al. (2003). A simplified disease activity index for rheumatoid arthritis for use in clinical practice. *Rheumatology* 42 (2), 244–257. doi:10.1093/rheumatology/keg072

Vermeire, S., Van Assche, G., and Rutgeerts, P. (2004). C-reactive protein as a marker for inflammatory bowel disease. *Inflamm. Bowel Dis.* 10 (5), 661–665. doi:10.1097/00054725-200409000-00026

Wells, G., Becker, J., Teng, J., Dougados, M., Schiff, M., Smolen, J., et al. (2009). Validation of the 28-joint Disease Activity Score (DAS28) and European League against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Ann. Rheum. Dis.* 68 (6), 954–960. doi:10.1136/ard.2007.084459

Xu, Z., Bouman-Thio, E., Comisar, C., Frederick, B., Van Hartingsveldt, B., Marini, J. C., et al. (2011). Pharmacokinetics, pharmacodynamics and safety of a human anti-IL-6 monoclonal antibody (sirukumab) in healthy subjects in a first-in-human study. *Br. J. Clin. Pharmacol.* 72 (2), 270–281. doi:10.1111/j.1365-2125.2011.03964.x

**Conflict of Interest:** The authors declare that this clinical trial study received funding from WuXi AppTec Co., Ltd. and AstraZaneca PLC, and PK/PD modeling study received funding from Clinical Research Fund from Wujieping Medical Foundation, the Drug Development and Application Fund from Chinese Pharmacological Society, the Beijing Key Laboratory, Chinese National Major Project for New Drug Innovation and CAMS Innovation Fund for Medical Sciences (CIFMS). Those funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 16 March 2021
doi: 10.3389/fphar.2021.635654

# The Efficacy of Tocilizumab in the Treatment of Patients with Refractory Immune-Mediated Necrotizing Myopathies: An Open-Label Pilot Study

*Sizhao Li, Wenli Li, Wei Jiang, Linrong He, Qinglin Peng, Guochun Wang and Xin Lu**

*Department of Rheumatology, China-Japan Friendship Hospital, Beijing, China*

OPEN ACCESS

**Edited by:**
*Francesca Oliviero, University of Padua, Padua, Italy*

**Reviewed by:**
*Francoise Degoul, Institut National de la Santé et de la Recherche Médicale (INSERM), Paris, France*
*Jiansheng Huang, Vanderbilt University Medical Center, Nashville, United States*

***Correspondence:**
*Xin Lu*
*luxin_n@163.com*

**Specialty section:**
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

**Received:** *30 November 2020*
**Accepted:** *26 January 2021*
**Published:** *16 March 2021*

**Citation:**
*Li S, Li W, Jiang W, He L, Peng Q, Wang G and Lu X (2021) The Efficacy of Tocilizumab in the Treatment of Patients with Refractory Immune-Mediated Necrotizing Myopathies: An Open-Label Pilot Study. Front. Pharmacol. 12:635654. doi: 10.3389/fphar.2021.635654*

**Objective:** To evaluate the efficacy of tocilizumab (TCZ) in adult patients with refractory immune-mediated necrotizing myopathies (IMNMs) and investigate possible predictive biomarkers of the response to treatment with TCZ.

**Methods:** Patients with refractory IMNM were enrolled in this open-label pilot observational study and received intravenous TCZ treatment. The clinical response was assessed after 6 months of TCZ treatment according to the 2016 American College of Rheumatology–European League Against Rheumatism (ACR–EULAR) response criteria for adult dermatomyositis and polymyositis. Muscle biopsies were performed to investigate muscle fiber regeneration by immunohistochemical staining of CD56. Serum levels of interleukin (IL)-6 were measured using a multiplex bead-based flow fluorescent immunoassay. The levels of muscle IL-6 mRNA were detected by real-time polymerase chain reaction.

**Results:** A total of 11 patients with refractory IMNM were enrolled in the study, including 3 anti-3-hydroxy-3-methylglutaryl-CoA reductase- and 8 anti-signal recognition particle-positive patients. Seven (63.6%) of these patients achieved clinically significant responses according to the 2016 ACR–EULAR myositis response criteria. Responders had higher baseline serum IL-6 and muscle IL-6 mRNA levels and percentage of CD56-positive muscle fibers than non-responders. Baseline serum IL-6 levels and the percentage of CD56-positive muscle fibers were positively correlated with total improvement score after 6 months of TCZ treatment. Furthermore, muscle fiber necrosis and muscle fiber size variation decreased in repeated muscle biopsies in five responders.

**Conclusion:** Patients with refractory IMNM may respond to TCZ. Baseline serum IL-6 and muscle IL-6 mRNA levels and the percentage of CD56-positive muscle fibers may predict the response to TCZ treatment in these patients.

**Keywords: immune-mediated necrotizing myopathy, tocilizumab, treatment, biomarker, interleukin-6**

# INTRODUCTION

Immune-mediated necrotizing myopathies (IMNMs) are a novel subgroup of idiopathic inflammatory myopathies (IIMs) characterized by significantly elevated serum creatine kinase (CK) levels, severe proximal muscle weakness, and resistance to conventional therapy (Dalakas, 2015; Allenbach et al., 2018b). Anti-3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and anti-signal recognition particle (SRP) antibodies have been recognized as serum biomarkers for IMNM (Allenbach et al., 2018b). Despite intense immunosuppressant treatment, the outcomes of IMNM are worse than those of other IIM subtypes, such as dermatomyositis (DM) and polymyositis (PM). Recent studies have indicated that approximately half of anti-HMGCR- and anti-SRP-positive patients with IMNM still have marked muscle weakness 2 years after aggressive treatment (Pinal-Fernandez et al., 2017; Tiniakou et al., 2017). Therefore, there is a need to discover novel and targeted therapeutics, such as biologics, to improve the prognosis of these patients and investigate the potential targeting mechanism involved in the pathogenesis of the disease.

Interleukin (IL)-6, a pleiotropic cytokine that regulates muscle function, is produced and released locally by skeletal muscle fiber in different pathological conditions. Under physiological conditions, IL-6 maintains muscle homeostasis and positively regulates muscle function (Baeza-Raja and Muñoz-Cánoves, 2004; Serrano et al., 2008). In contrast, it negatively regulates the muscle phenotype under some pathological stimuli (Munoz-Canoves et al., 2013). Pelosi et al. have reported that the treatment of C2C12 myoblasts with recombinant IL-6 significantly inhibits the differentiation of C2C12 myogenic cells (Pelosi et al., 2014). Interestingly, the inhibition of IL-6 activity by an anti-IL-6 receptor (IL-6R) antibody promotes muscle fiber regeneration in both cardiotoxin-induced muscle injury and dystrophin/utrophin double-knockout mice (Fujita et al., 2014; Wada et al., 2017). Allenbach et al. recently demonstrated that upregulated IL-6 expression and impaired muscle regeneration are detected in the muscles of patients with anti-HMGCR and anti-SRP myopathies, which suggests that the IL-6 pathway may be involved in the pathogenesis of IMNM (Allenbach et al., 2018a).

In this pilot study, we evaluated the efficacy of tocilizumab (TCZ), a recombinant anti-IL-6R monoclonal antibody, on both clinical and histological parameters in patients with refractory IMNM and investigate the potential role of IL-6 in the pathogenesis of IMNM.

# METHODS

## Patient Selection

Patients with refractory IMNM were enrolled in this study. The inclusion criteria were as follows: 1) anti-SRP- or anti-HMGCR-positive and 2) disease worsening after treatment with high-dose glucocorticoids (equivalent of prednisone 1.0 mg/kg/d for at least 1 month) and at least one immunosuppressant, including azathioprine (AZA), methotrexate (MTX), cyclosporine (CSA), tacrolimus (TAC), or intravenous immunoglobulin (IVIG) at a known effective dose for at least 3 months. Disease worsening was defined as 1) manual muscle testing (MMT) decreasing by ≥ 20% and worsening of physician global activity by ≥ 2 cm on a 10 cm visual analog scale (VAS), 2) worsening of global extramuscular activity by ≥ 2 cm on a 10 cm VAS, or 3) worsening of any three of six International Myositis Assessment and Clinical Studies (IMACS) core set measures (CSMs) by ≥30% (Tjarnlund et al., 2018).

The exclusion criteria were cancer-associated myositis, other connective tissue disease overlap myositis, infection-, drug-, or toxin-induced myopathies, and muscle histopathological findings suggestive of muscular dystrophy, metabolic myopathy, or congenital myopathy.

## Study Design

This study was a 6-month one-arm open-label pilot study. The patients were administered 8 mg/kg intravenous TCZ infused every 4 weeks for six rounds, following a standard dosing protocol. The dose of prednisolone was reduced following a standardized reduction schedule. Stable doses of other immunosuppressive or immunomodulatory agents, including AZA, MTX, CSA, TAC, and IVIG, were administered after TCZ administration.

## Outcome Assessment and Response Criteria

The 2016 American College of Rheumatology–European League Against Rheumatism IIM response criteria for adult DM/PM were used to evaluate the outcomes of patients (Aggarwal et al., 2017). Total improvement score (TIS), which is the sum of the improvement scores in each of the IMACS CSMs and provides a quantitative assessment of improvement for each patient, were calculated 3 and 6 months after TCZ treatment. A TIS of ≥20, 40 and 60 represents minimal, moderate, or major improvement, respectively. A response was defined as a TIS ≥20 at 3 or 6 months.

Overall safety and tolerability of TCZ during the entire treatment period were assessed by adverse events (AEs) and severe AEs (SAEs).

## Detection of anti-HMGCR and anti-SRP Antibodies and Serum IL-6 Levels

The levels of anti-HMGCR antibodies were measured using an enzyme-linked immunosorbent assay (Raybiotech, China) according to a previously described method (Ge et al., 2015). Levels of antibodies against SRP were determined using immunoblotting (EUROLINE, Lubeck, Germany) in accordance with the standard methods (Euroline Myositis Profile 3 immuno line-blot; Euroimmun). The levels of IL-6 in patients' serum were measured using the multiplex bead-based flow fluorescent immunoassay (Raisecare, Qingdao, China) according to the manufacturer's instructions.

**TABLE 1 |** Baseline demographics and clinical characteristics of patients with IMNM.

| Variables[a] | All patients (n = 11) |
|---|---|
| Sex ratio, (F/M) | 7/4 |
| Anti-SRP positive, n (%) | 8 (72.7%) |
| Anti-HMGCR positive, n (%) | 3 (27.3%) |
| Age at symptom onset, years | 42 (34–53) |
| Muscle weakness | 11 (100%) |
| Max CK before any treatment, IU/L | 7,411 (4,375–10,891) |
| Dysphagia, n (%) | 6 (54.5%) |
| Interstitial lung disease, n (%) | 2 (18.2%) |
| Skin rash, n (%) | 1 (9.1%) |
| Duration of previous therapy, months | 6 (5–8) |
| Previous medication | |
| Initial treatment of high-dose glucocorticoid, n (%) | 11 (100%) |
| Methotrexate, n (%) | 6 (54.5%) |
| Azathioprine, n (%) | 3 (27.3%) |
| Cyclosporine, n (%) | 1 (9.1%) |
| Methotrexate and tacrolimus, n (%) | 1 (9.1%) |
| Additional IVIG besides immunosuppressant, n (%) | 4 (36.4%) |

[a]*Presented as median (interquartile range).*

*IMNM, immune-mediated necrotizing myopathy; SRP, signal recognition particle; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; CK, creatine kinase (normal range: 26–200 IU/L); IVIG, intravenous immunoglobulin.*

## Muscle Biopsies and Immunohistochemical Analysis

Muscle biopsies were performed at the baseline and after 6 months. The specimens were frozen in isopentane prechilled with liquid nitrogen and stored at -80°C until processing. Hematoxylin and eosin staining, combined eosin and dystrophin immunostaining (NCL-DYS1, Leica, United Kingdom), and immunohistochemical staining of NCAM/CD56 (ab6123, Abcam, United Kingdom) were performed on 8-μm frozen sections as previously described (Arouche-Delaperche et al., 2017; Allenbach et al., 2018a). The half area of each section was quantitative analyzed. For each patient, the total number of muscle fibers and necrotic and regenerating muscle fibers was manually determined. The Feret diameter of each muscle fiber outlined by the immunostaining of dystrophin was automatically measured using ImageJ v1.51u. Myofiber necrosis, defined as pale and/or hyalinized muscle fibers combined with the loss of sarcolemmal integrity/coarse appearance, was evaluated on combined eosin and dystrophin immunostaining (Aggarwal et al., 2017). NCAM/CD56-positive fibers represent regenerating muscle fibers.

## RNA Extraction and Quantitative Real Time-Polymerase Chain Reaction (qPCR)

Total muscle RNA was extracted using TRIzol reagent (Thermo, Waltham, United States). RNA was reverse-transcribed into cDNA using a PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Dalian, China). qPCR was performed using the TB Green Fast qPCR mix in a 7,500 Real-Time PCR system (Applied Biosystems, Singapore) according to the manufacturer's instructions. The expression levels of IL-6 were normalized to those of GAPDH.

The primers used were as follows: IL-6: forward 5′-GAAAGC AGCAAAGAGGCACT-3′ and reverse 5′-AGCTCTGGCTTG TTCCTCAC-3′; GAPDH: forward 5′-CCTCCTGCACCACCA ACTGCTT-3′ and reverse 5′-GAGGGGCCATCCACAGTCTTC T-3′.

## Statistical Analysis

Statistical analysis was performed using SPSS 22.0. Differences between responders and non-responders were compared using the Mann-Whitney U test. To compare the parameters before and after TCZ treatment, a paired-samples *t*-test was used. The correlation between clinical outcome and biomarkers was analyzed using Spearman's rank correlation. $p < 0.05$ was considered significant.

# RESULTS

## Baseline Characteristics of Patients

A total of 11 patients were enrolled in this study. The mean age of disease onset was 42 years (range, 34–81 years) and seven females and four males were included. Eight were anti-SRP-positive and three were anti-HMGCR-positive. All patients had muscle weakness and significant CK level elevation. Among these patients, 54.5% had complications with dysphagia, 18.2% had interstitial lung disease, and 9.1% had a skin rash. All patients were initially treated with high-dose glucocorticoids and/or immunosuppressants for more than 3 months before enrollment (**Table 1**).

## Clinical Response to Tocilizumab Treatment

Seven (63.6%) of these patients achieved the threshold of a minimal clinically significant improvement after 3 months of treatment with TCZ and were classified as responders. Four (36.4%) were non-responders and none worsened 6 months after TCZ treatment. Four (36.4%) and three (27.3%) patients attained a moderate and major improvement after 3 months of treatment with TCZ, respectively. At 6 months, seven (63.6%) patients achieved a major improvement. The median TIS was 50 and 75 at 3 and 6 months in all patients, respectively. All six IMACS CSMs except extramuscular global activity improved at 3 and 6 months compared with the baseline for the whole patient population (**Table 2**).

## Biomarkers for Predicting the Outcomes

The serum IL-6 levels of responders at the baseline were higher than those of non-responders ($p = 0.008$; **Figure 1A**). In all patients, serum IL-6 levels were higher after 6 months of treatment than at the baseline (median 14.5 pg/ml, range 9.2–25.4; **Figure 1B**) but there was no difference between responders and non-responders. There was a positive correlation between the baseline serum IL-6 level and TIS after 6 months (**Figure 1C**).

**TABLE 2 |** Six IMACS core set measures in patients with IMNM at the baseline and after 3 and 6 months of treatment with tocilizumab.

| Variable[a] | Baseline | Month 3 | Month 6 |
|---|---|---|---|
| Physician global activity, VAS (10 cm) | 6.0 (5.7–6.5) | 5.0 (3.8–5.5) | 3.0 (1.5–5.5) |
| Patient global activity, VAS (10 cm) | 7.0 (6.0–7.0) | 4.2 (4.0–6.0) | 2.5 (2.3–6.0) |
| MMT-8 (0–80) | 49 (42–52) | 51 (49–58) | 60 (53–65) |
| HAQ (0–3) | 1.6 (1.1–1.85) | 0.9 (0.6–1.3) | 0.55 (0.3–0.85) |
| CK, IU/L (26–200) | 975 (730–1751) | 491 (185–702) | 240 (86–416) |
| Extramuscular activity, VAS (10 cm) | 2.0 (1.5–2.2) | 2.0 (1.5–2.2) | 2.0 (1.5–2.2) |

[a]*Presented as median (interquartile range).*

*IMNM, immune-mediated necrotizing myopathy; IMACS, International Myositis Assessment and Clinical Studies; VAS, visual analog scale; MMT-8, Manual Muscle Test-8; HAQ, Health Assessment Questionnaire; CK, creatine kinase (reference: 26–200 IU/L).*

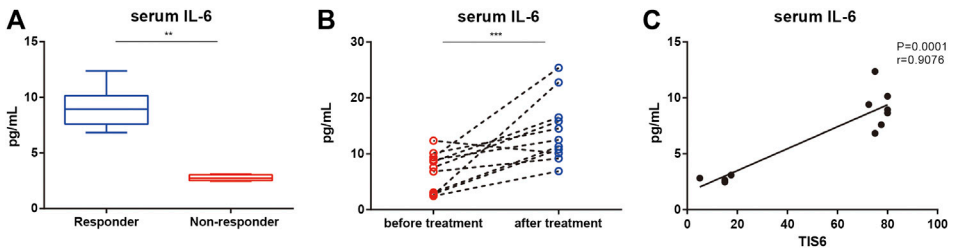


**FIGURE 1 |** Serum levels of interleukin (IL)-6 and its association with the clinical response of patients with immune-mediated necrotizing myopathy before and after treatment with tocilizumab (TCZ). **(A)** Serum levels of IL-6 between responders and non-responders at the baseline. **(B)** Serum levels of IL-6 of patents before and after 6 months of treatment with TCZ. **(C)** Correlation of serum IL-6 levels of patients at the baseline and the total improvement scores (TIS) after 6 months.

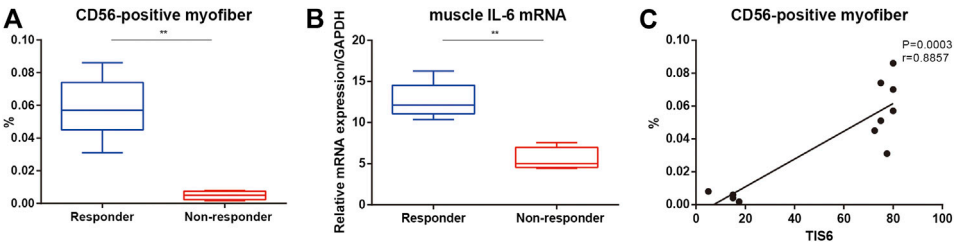


**FIGURE 2 |** CD56-positive muscle fibers and muscle interleukin (IL)-6 mRNA levels in patients with immune-mediated necrotizing myopathy. **(A)** Percentage of CD56-positive muscle fibers between responders and non-responders at the baseline. **(B)** Muscle IL-6 mRNA levels between responders and non-responders at the baseline. **(C)** Correlation of the percentage of CD56-positive myofibers at the baseline and the total improvement scores (TIS) after 6 months.

Responders had a higher baseline muscle IL-6 mRNA level and percentage of CD56-positive muscle fibers than non-responders (**Figures 2A,B**). There was a positive correlation between TIS after 6 months of TCZ treatment and the baseline percentage of CD56 positive fibers in all patients (**Figure 2B**).

Repeated muscle biopsies were performed in five patients who responded to treatment with TCZ. After 6 months of treatment, the percentage of necrotic muscle fibers decreased from 2.36 ± 0.76 to 0.6 ± 0.39% ($p$ = 0.0028; **Figures 3A,B**). The percentage of regenerating muscle fibers in post-treatment muscle biopsies was significantly lower than that in pre-treatment muscles (5.98 ± 2.12% vs. 1.16 ± 0.7%, $p$ = 0.0007; **Figures 3C,D**). The percentage of myofibers with a Feret diameter of less than 40 μm significantly decreased after treatment with TCZ, leading to a redistribution of muscle fibers with a Feret diameters between 40 and 100 μm (**Figures 3E,F**).

## Safety Data

There were 10 transient AEs in five patients, namely, mild hypofibrinogenemia without bleeding (n = 2), antifungal cream-treated tinea corporis (n = 1), oral antibiotic-treated respiratory tract infection (n = 2), allergic rash (n = 2), and leukocytopenia (n = 3). Only hypofibrinogenemia was considered to be related to TCZ. All these AEs were mild and none resulted in TCZ dose reduction or withdrawal. There were no SAEs.

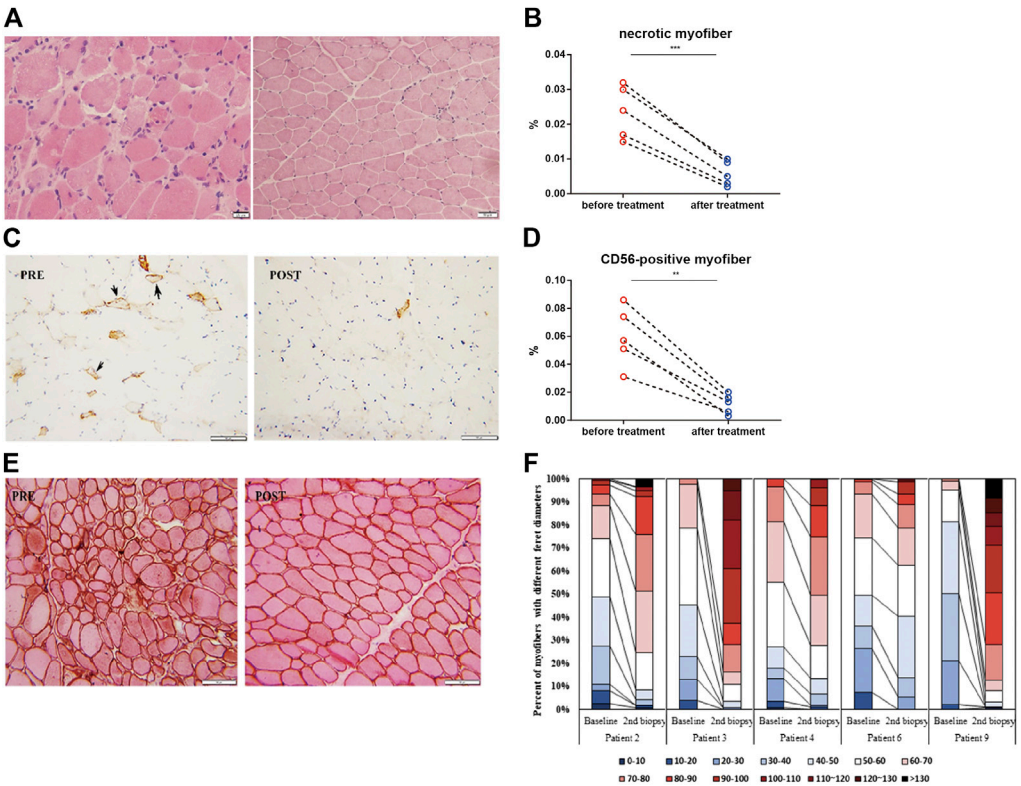


**FIGURE 3 |** Histopathological changes in muscle biopsies in the responder group (n = 5; 4 anti-SRP-positive, 1 anti-HMGCR-positive) before and after 6 months of treatment with tocilizumab. **(A, C)** Hematoxylin and eosin staining **(A)** and immunohistochemical staining of CD56 **(brown) (C)** in muscle biopsy of one anti-SRP-positive patient before (PRE) and after (POST) treatment with tocilizumab. **(B,D)** Changes in the percentage of necrotic myofibers **(B)** and CD56-positive muscle fibers **(D)** before and after treatment with tocilizumab among five responders. **(E)** Combined eosin and dystrophin immunohistochemical staining in muscle biopsy of one anti-SRP-positive patient before (PRE) and after (POST) treatment with tocilizumab. **(F)** Quantification of the percentage of muscle fibers with different Feret diameter and their distribution before and after treatment with tocilizumab (Scale bar, 20 μm).

## DISCUSSION

In this preliminary study, we demonstrated that 7 out of 11 patients with refractory IMNM responded well to treatment with TCZ, and 63.6% achieved a major improvement according to the new response criteria for myositis improvement after 6 months of TCZ treatment. Furthermore, baseline serum IL-6 levels, muscle IL-6 mRNA levels, and the percentage of regenerating muscle fibers (CD56-positive myofibers) may be effective markers to predict the response to TCZ treatment. Additionally, muscle biopsies indicated that the redistribution of muscle fibers with a Feret diameter between 40 and 100 μm after TCZ treatment suggest a positive effect of TCZ treatment on the regeneration of muscle fibers.

TCZ has been widely used in the treatment of various refractory autoimmune disorders, such as giant cell arteritis and rheumatoid arthritis (Smolen et al., 2008; Stone et al., 2017). Three previous case reports have suggested that IL-6 blockade is effective in IIM (Narazaki et al., 2011; Kondo et al., 2014; Beaumel et al., 2016). In this prospective study, we demonstrated that an IL-6R antagonist was effective in the clinical improvement of patients with refractory IMNM. In the muscle biopsy, we compared baseline IL-6 mRNA levels between responders and non-responders. Interestingly, the baseline muscle IL-6 mRNA levels in responders were higher than those in non-responders, which suggests that muscle IL-6 mRNA may be a useful marker for the prediction of therapeutic response. However, there was no correlation between the change in muscle IL-6 mRNA levels before and after TCZ treatment, which may be related to the small number of muscle biopsies after treatment. In the future, we will further investigate the relationship between muscle IL-6 mRNA and TCZ action in paired and large samples to estimate the role of IL-6 in IMNM.

In this study, we focused on the characteristics of repeated muscle biopsies in five patients who responded well to TCZ treatment. Before TCZ treatment, all patients received aggressive therapy. Although previous immunomodulatory therapy resulted in a significant decrease in CK levels in these patients, the patients continued to clinically deteriorate with muscle weakness and a very low MMT-8 score. Previous studies have established that there is a discrepancy between decreased CK levels and worsened muscle strength in patients with muscular disorders. A common explanation for this phenomenon is that muscle strength improvement could be delayed by muscle fiber regeneration. A previous study demonstrated that muscle remodeling occurs up

to 30 days after a single electrical stimulation (Mackey et al., 2011). In these five patients, the average duration of routine immunotherapy before the first muscle biopsy was 5 months, which was sufficient to complete the process of muscle regeneration. However, we still observed the prominent and excessive regeneration of muscle fibers with a significant decrease in muscle fiber diameter. In previous basic research, these pathological features were reported when myoblast fusion was disturbed during muscle regeneration (Hindi et al., 2017). In this study, we detected a higher level of muscle IL-6 mRNA and percentage of regenerating muscle fibers in responders than in non-responders. Moreover, we observed a positive correlation between the percentage of CD56-positive myofibers and clinical improvement score, and muscle fibers with a Feret diameter between 40 and 100 μm were redistributed and regenerated after TCZ treatment. Taken together, these results suggest that long-lasting elevated IL-6 levels may lead to severe muscle weakness via the reduction of myoblast fusion during muscle regeneration. Blocking the IL-6 signal using TCZ could recover muscle regeneration and improve muscle performance in patients with IMNM.

However, there are limitations to this study. First, the open-label design of this study may have affected the objective evaluation of the disease by physicians and patients. Second, owing to the small number of patients included in this study and lack of a control arm, caution is required in the interpretation of the results.

In conclusion, TCZ treatment may be beneficial for patients with refractory IMNM. The response to TCZ can be predicted by baseline serum IL-6 and muscle IL-6 mRNA levels and the percentage of CD56-positive muscle fibers in these patients. Additionally, this study provides new insights into the role of IL-6 in the pathogenesis of IMNM. In the future, a randomized placebo-controlled trial of IL-6 blockade involving more patients with IMNM is needed to confirm our results.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Research Review Committee and Ethical Review Committee of the China–Japan Friendship Hospital. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

LS contributed to the enrollment and follow-up of patients, data analysis, and drafting of the manuscript. LW helped with immunohistochemical staining. PQ helped with flow fluorescent immunoassays and performed PCR experiments. JW and HL helped with data collection and the assessment of disease activity. WG helped revise the manuscript. LX supervised all aspects of the study, including study design, data interpretation, and manuscript revision. All authors have read and approved the final manuscript.

## FUNDING

This work was supported by the Beijing Municipal Science and Technology Commission (Z191100006619012) and National Natural Science Foundation of China (81571603, 81701615, 81601833).

## REFERENCES

Aggarwal, R., Rider, L. G., Ruperto, N., Bayat, N., Erman, B., Feldman, B. M., et al. (2017). 2016 American College of Rheumatology/European league against rheumatism criteria for minimal, moderate, and major clinical response in adult dermatomyositis and polymyositis: an international myositis assessment and clinical studies group/paediatric rheumatology international trials organisation collaborative initiative. *Ann. Rheum. Dis.* 76 (5), 792–801. doi:10.1136/annrheumdis-2017-211400

Allenbach, Y., Arouche-Delaperche, L., Preusse, C., Radbruch, H., Butler-Browne, G., Champtiaux, N., et al. (2018a). Necrosis in anti-SRP$^+$ and anti-HMGCR$^+$myopathies: role of autoantibodies and complement. *Neurology* 90 (6), e507–e517. doi:10.1212/WNL.0000000000004923

Allenbach, Y., Mammen, A. L., Benveniste, O., and Stenzel, W. (2018b). 224th ENMC international workshop: clinico-sero-pathological classification of immune-mediated necrotizing myopathies Zandvoort, The Netherlands, 14-16 october 2016. *Neuromuscul. Disord.* 28 (1), 87–99. doi:10.1016/j.nmd.2017.09.016

Arouche-Delaperche, L., Allenbach, Y., Amelin, D., Preusse, C., Mouly, V., Mauhin, W., et al. (2017). Pathogenic role of anti-signal recognition protein and anti-3-Hydroxy-3-methylglutaryl-CoA reductase antibodies in necrotizing myopathies: myofiber atrophy and impairment of muscle regeneration in necrotizing autoimmune myopathies. *Ann. Neurol.* 81 (4), 538–548. doi:10.1002/ana.24902

Baeza-Raja, B., and Muñoz-Cánoves, P. (2004). p38 MAPK-induced nuclear factor-kappaB activity is required for skeletal muscle differentiation: role of interleukin-6. *Mol. Biol. Cell* 15 (4), 2013–2026. doi:10.1091/mbc.e03-08-0585

Beaumel, A., Muis-Pistor, O., Tebib, J. G., and Coury, F. (2016). Antisynthetase syndrome treated with tocilizumab. *Jt. Bone Spine* 83 (3), 361–362. doi:10.1016/j.jbspin.2015.03.016

Dalakas, M. C. (2015). Inflammatory muscle diseases. *N. Engl. J. Med.* 373 (4), 393–394. doi:10.1056/NEJMc1506827

Fujita, R., Kawano, F., Ohira, T., Nakai, N., Shibaguchi, T., Nishimoto, N., et al. (2014). Anti-interleukin-6 receptor antibody (MR16-1) promotes muscle regeneration via modulation of gene expressions in infiltrated macrophages. *Biochim. Biophys. Acta (BBA)-Gen. Subjects* 1840 (10), 3170–3180. doi:10.1016/j.bbagen.2014.01.014

Ge, Y., Lu, X., Peng, Q., Shu, X., and Wang, G. (2015). Clinical characteristics of anti-3-hydroxy-3-methylglutaryl coenzyme A reductase antibodies in Chinese patients with idiopathic inflammatory myopathies. *PLoS One* 10 (10), e0141616. doi:10.1371/journal.pone.0141616

Hindi, S. M., Shin, J., Gallot, Y. S., Straughn, A. R., Simionescu-Bankston, A., Hindi, L., et al. (2017). MyD88 promotes myoblast fusion in a cell-autonomous manner. *Nat. Commun.* 8 (1), 1624. doi:10.1038/s41467-017-01866-w

Kondo, M., Murakawa, Y., Matsumura, T., Matsumoto, O., Taira, M., Moriyama, M., et al. (2014). A case of overlap syndrome successfully treated with

tocilizumab: a hopeful treatment strategy for refractory dermatomyositis? *Rheumatology* 53 (10), 1907–1908. doi:10.1093/rheumatology/keu234

Mackey, A. L., Brandstetter, S., Schjerling, P., Bojsen-Moller, J., Qvortrup, K., Pedersen, M. M., et al. (2011). Sequenced response of extracellular matrix deadhesion and fibrotic regulators after muscle damage is involved in protection against future injury in human skeletal muscle. *Faseb J.* 25 (6), 1943–1959. doi:10.1096/fj.10-176487

Munoz-Canoves, P., Scheele, C., Pedersen, B. K., and Serrano, A. L. (2013). Interleukin-6 myokine signaling in skeletal muscle: a double-edged sword? *FEBS J.* 280 (17), 4131–4148. doi:10.1111/febs.1233810.1111/febs.12454

Narazaki, M., Hagihara, K., Shima, Y., Ogata, A., Kishimoto, T., and Tanaka, T. (2011). Therapeutic effect of tocilizumab on two patients with polymyositis. *Rheumatology* 50 (7), 1344–1346. doi:10.1093/rheumatology/ker152

Pelosi, M., De Rossi, M., Barberi, L., and Musarò, A. (2014). IL-6 impairs myogenic differentiation by downmodulation of p90RSK/eEF2 and mTOR/p70S6K axes, without affecting AKT activity. *Biomed. Res. Int.* 2014, 206026. doi:10.1155/2014/206026

Pinal-Fernandez, I., Parks, C., Werner, J. L., Albayda, J., Paik, J., Danoff, S. K., et al. (2017). Longitudinal course of disease in a large cohort of myositis patients with autoantibodies recognizing the signal recognition particle. *Arthritis Care Res.* 69 (2), 263–270. doi:10.1002/acr.22920

Serrano, A. L., Baeza-Raja, B., Perdiguero, E., Jardí, M., and Muñoz-Cánoves, P. (2008). Interleukin-6 is an essential regulator of satellite cell-mediated skeletal muscle hypertrophy. *Cell Metab.* 7 (1), 33–44. doi:10.1016/j.cmet.2007.11.011

Smolen, J. S., Beaulieu, A., Rubbert-Roth, A., Ramos-Remus, C., Rovensky, J., Alecock, E., et al. (2008). Effect of interleukin-6 receptor inhibition with tocilizumab in patients with rheumatoid arthritis (OPTION study): a double-blind, placebo-controlled, randomised trial. *Lancet* 371 (9617), 987–997. doi:10.1016/s0140-6736(08)60453-5

Stone, J. H., Tuckwell, K., Dimonaco, S., Klearman, M., Aringer, M., Blockmans, D., et al. (2017). Trial of tocilizumab in giant-cell arteritis. *N. Engl. J. Med.* 377 (4), 317–328. doi:10.1056/NEJMoa1613849

Tiniakou, E., Pinal-Fernandez, I., Lloyd, T. E., Albayda, J., Paik, J., Werner, J. L., et al. (2017). More severe disease and slower recovery in younger patients with anti-3-hydroxy-3-methylglutaryl-coenzyme A reductase-associated autoimmune myopathy. *Rheumatology* 56 (5), 787–794. doi:10.1093/rheumatology/kew470

Tjarnlund, A., Tang, Q., Wick, C., Dastmalchi, M., Mann, H., Tomasova Studynkova, J., et al. (2018). Abatacept in the treatment of adult dermatomyositis and polymyositis: a randomised, phase IIb treatment delayed-start trial. *Ann. Rheum. Dis.* 77 (1), 55–62. doi:10.1136/annrheumdis-2017-211751

Wada, E., Tanihata, J., Iwamura, A., Takeda, S., Hayashi, Y. K., and Matsuda, R. (2017). Treatment with the anti-IL-6 receptor antibody attenuates muscular dystrophy via promoting skeletal muscle regeneration in dystrophin-/utrophin-deficient mice. *Skeletal Muscle* 7 (1), 23. doi:10.1186/s13395-017-0140-z

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 16 March 2021
doi: 10.3389/fphar.2021.626342

# Research Progress of Therapeutic Enzymes and Their Derivatives: Based on Herbal Medicinal Products in Rheumatoid Arthritis

*Ming Cai*[1,2,3], *Wei-Jian Ni*[4,5], *Lan Han*[2,3], *Wei-Dong Chen*[2,3] *and Dai-Yin Peng*[2,3]*

[1]Department of Pharmacy, The Second Affiliated Hospital of Anhui University of Chinese Medicine, Hefei, China, [2]Anhui University of Chinese Medicine, Hefei, China, [3]Key Laboratory of Chinese Medicinal Formula Research, Anhui University of Chinese Medicine, Hefei, China, [4]School of Pharmacy, Anhui Medical University, The Key Laboratory of Anti-inflammatory and Immune Medicines, Ministry of Education, Hefei, China, [5]Department of Pharmacy, Anhui Provincial Hospital, The First Affiliated Hospital of USTC, University of Science and Technology of China, Hefei, China

Rheumatoid arthritis (RA) acts as one of the most common, agnogenic and chronic inflammatory-autoimmune disorder which is characterized by persistent synovitis, cartilage destruction, and joint deformities, leads to a wide range of disabilities, and increased mortality, thus imposing enormous burdens. Several drugs with anti-inflammatory and immunomodulatory properties such as celecoxib, diclofenac and methotrexate are being selected as conventional drugs in the allopathic system of medicine for the treatment of RA in clinic. However, there are some serious side effects more or less when using these drugs because of their short poor bioavailability and biological half-life for a long time. These shortcomings greatly promote the exploration and application of new low- or no-toxicity drugs for treating the RA. Meanwhile, a growing number of studies demonstrate that several herbs present certain anti-inflammatory and anti-arthritic activities through different enzymes and their derivatives, which indicate that they are promising therapeutic strategies when targeting these mediators based on herbal medicinal products in RA research. This review article summarizes the roles of the main enzymes and their derivatives during the pathogenesis of RA, and clearly clarifies the explicit and potential targeted actions of herbal medicinal products that have anti-RA activity. Our review provides timely and critical reference for the scientific rationale use of herbal medicinal products, with the increasing basic research and clinical application of herbal medicinal products by patients with RA.

**Keywords: rheumatoid arthritis, herbal medicinal products, inflammatory, immune, enzyme, enzyme derivatives**

OPEN ACCESS

***Edited by:***
*Anna Lisa Giuliani,*
*University of Ferrara, Italy*

***Reviewed by:***
*Marina Korotkova,*
*Karolinska Institutet (KI), Sweden*
*Rafael Scaf De Molon,*
*São Paulo State University, Brazil*
*Federica Furini,*
*University of Ferrara, Italy*

***Correspondence:**
*Dai-Yin Peng*
*pengdaiyin@163.com*

***Specialty section:***
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

***Received:*** *05 November 2020*
***Accepted:*** *07 January 2021*
***Published:*** *16 March 2021*

***Citation:***
*Cai M, Ni W-J, Han L, Chen W-D and Peng D-Y (2021) Research Progress of Therapeutic Enzymes and Their Derivatives: Based on Herbal Medicinal Products in Rheumatoid Arthritis. Front. Pharmacol. 12:626342. doi: 10.3389/fphar.2021.626342*

**Abbreviations:** COX-2, cyclooxygenase-2; cPLA2, cytosolic phospholipase A2; PG201, a multi-component phytopharmaceutical derived from 12 Oriental herbal medicines, Chaenomelis Fructus, Achyranthis Radix, Acanthopanax Cortex, Cinnamomi Cortex, Gentianae Macrophyllae Radix, Clematidis Radix, Angelica Gigantis Radix, Cnidii Rhizoma, Gastrodiae Rhizoma, Carthami Flos, Saposhnikoviae Radix, and Dipsaci Radix; HMBA, 2-hydroxy-4-methoxy benzoic acid; HOEC, (+)-2-(1-hydroxyl-4-oxocyclohexyl) ethyl caffeate; IDO, indoleamine 2, 3-dioxygenase; iNOS, inducible macrophage type NOS; LOX, lipoxygenase; MMP, matrix metalloproteases; NOS, nitric oxide synthase; OA, osteoarthritis; POEa/POEe, ethyl acetate and ethyl ether extract of P. orientale; RA, rheumatoid arthritis; SOG, sec-O-glucosylhamaudol; sPLA2, secreted phospholipase A2; TGP, total glucosides of paeony; TWHF, tripterygium wilfordii Hook. F.

## INTRODUCTION

Rheumatoid arthritis (RA) is one of the most common agnogenic and chronic inflammatory-autoimmune disorder that major targets the synovium, joints, and cartilage, which causes irreversible joint damage, and causes severe extra-articular manifestations and complications (Fert-Bober et al., 2020). During the occurrence and development of rheumatoid arthritis, both environmental and genetic are important factors involved (Scherer et al., 2020). In the initiation phase of rheumatoid arthritis, there is the autoreactive T cells activation, the T cell mobilization and recruitment along with other leukocytes into the disease area, including joints, synovium, and cartilage (Fang et al., 2020). For the moment, these leukocytes produce multiple enzymes, various inflammatory cytokines and mediators such as phospholipase $A_2$ ($PLA_2$), prostaglandins (PG), and diverse cytokines (interleukins, tumor necrosis factor, etc), which induce the synovial and joint inflammation and finally cause the damage in joint tissue through triggering different signaling cascades intracellular and extracellular (McInnes and Schett, 2011; Hong et al., 2020). Therefore, targeting these enzymes and their derivatives is a potential therapeutic strategy for the treatment of rheumatoid arthritis. In recent years, using the well-defined biochemical and pharmacological inhibitors to suppress rheumatoid arthritis in experimental animal models have been reported many times (Croia et al., 2019). Interestingly, studies find that many of these enzymes, derivatives and related signaling pathways can be targeted and intervened by several medicinal products, for instance, herbal medicinal products which belong to the traditional medicine or complementary and alternative medicine (Mateen et al., 2016; Wang et al., 2020). Therefore, this review article summarizes the roles of main enzymes and their derivatives of inflammation during the occurrence and pathogenesis of RA, to provide insight into how the herbal medicinal products that target these enzymes and their derivatives may lead to the prevention of RA.

In this review, the herbal medicinal products we discuss below are examined through many experiments for their anti-inflammatory, anti-arthritic and immunoregulatory activities. Reviewing the related literature, we find that the *in vitro* researches are performed by those cultured defined cell types, including chondrocytes (Feng and Qiu, 2018), macrophages (McHugh, 2017; 2019), and fibroblasts (Croft et al., 2019), while the *in vivo* studies are based on multiple well-established experimental RA models, such as collagen-induced arthritis (CIA) (Kim et al., 2015; Li et al., 2017), adjuvant-induced arthritis (AIA) (Pan et al., 2017; Wang et al., 2017), as well as streptococcal cell wall-induced arthritis. In these studies, specific purified compounds, extractives and monomer derived from the herbal medicinal product are appended to the cultured cells in the case of inflammatory stimulants such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α) and lipopolysaccharide (LPS) for the *in vitro* experiments. The cells used for studies are taken from mice, rats or from the authoritative cell lines (Cheng et al., 2020; Yang et al., 2020). For the *in vivo* tests, the herbal medicinal products are investigated as an extract or a purified bioactive compound (Xiong et al., 2019). During the *in vivo* research, the intraperitoneal injection and oral administration are considered as the two-principal means of intervention (Bao et al., 2019). The parameters for assessing the RA model situation and the herbal medicinal products therapeutic efficacy are consisted of clinical criteria for grading such as phenotypic (weight change, paw volume), biochemical changes, histopathological analysis and RA biomarkers, et al.

## THERAPEUTIC ENZYMES AND THEIR DERIVATIVES IN RA

As we know, RA is a common and agnogenic chronic inflammatory disorder. And meanwhile, inflammation is a kind of physiological stress reaction of the organism response to different external stimulus and internal anomaly such as infection, trauma, and immune reactions (Cronstein et al., 2020). During the initial period and perpetuation of inflammatory reaction, there are a variety of enzymes and their derivatives act in concert (Spel and Martinon, 2020). In this article, we discuss the detailed characteristics of these major enzymes and their derivatives including phospholipase $A_2$ ($PLA_2$), cyclooxygenase (COX) and prostaglandins (PGs), lipoxygenase (LOX), matrix metalloproteases (MMPs)/tissue inhibitors of metalloproteases (TIMPs), nitric oxide synthase (NOS) and nitric oxide (NO), as well as indoleamine 2, 3-dioxygenase (IDO), and demonstrate the targeting of these spots by natural herbal and synthetic products resulting in the prevention of RA (**Table 1**). Also, we try to clarify the mechanisms of these enzymes and their derivatives involved in the RA progression.

### Phospholipase $A_2$ ($PLA_2$)

$PLA_2$ is a kind of intensively studied hydrolase which can catalyze-hydrolyze the membrane phospholipids at the position of sn-2 thereby producing the lysophospholipid and fatty acid products (Martin et al., 2020). Then, the produced free fatty acid (FFA) can be broken down into various importance biological lipid mediators, and the lysophospholipid products also play vital roles in the corresponding biological processes and physiological activities (Duchez et al., 2019). $PLA_2$ is of high pharmaceutical value protein because it can be responsible for the arachidonic acid release from membranes, and subsequent transformation of fatty acid to leukotrienes and prostaglandins, which plays important roles in the subsequent inflammatory response (Magrioti and Kokotos, 2013). In organisms, there are more than 14 different species of $PLA_2$ enzymes, which may play different or similar roles in many biological processes. Among these 14 isoforms, four main subtypes of $PLA_2$ include the cytosolic phospholipase $A_2$ ($cPLA_2$), calcium-independent phospholipase $A_2$ ($iPLA_2$), secreted phospholipase $A_2$ ($sPLA_2$), and the platelet activating factor acetyl hydrolase/oxidized lipid lipoprotein-associated phospholipase $A_2$ ($LpPLA_2$) are widely studied and identified by researchers (Kozaki et al., 2015; Sodergren et al., 2015). $cPLA_2$ is one of the main subtypes produced in the inflammation area and also the only one $PLA_2$ with a catalyzed-hydrolysis

**TABLE 1 |** List of herbal medicinal products involved in targeting the enzymes in rheumatoid arthritis.

| Herbal products | Botanical source | Principle extracted | Study Phase | Subject | Target enzyme | Outcomes | References |
|---|---|---|---|---|---|---|---|
| PG201 | 12 herbs compound | Ethanol extract | Clinical study | Raw 264.7 macrophage cell; DBA/1 mice; Rabbit and OA patients | cPLA2 | Relieve RA | Park et al. (2005), Yoo et al. (2014), and Kim et al. (2016) |
| Boerhaavia diffusa | Nyctaginaceae tuberous root | Ethanol extract | Preclinical study | Swiss albino mice; Swiss albino rat | sPLA2 | Relieve RA | Giresha et al. (2017) |
| HMBA | Hemidesmus indicus R. BR | Ethanol extract | Preclinical study | New Zealand strain rabbit BALBc mice | sPLA2 | Relieve RA | Gomes et al. (2012) |
| Danshen extract | Root of salvia miltiorrhiza bunge | 80% ethanol extract | Clinical study | Human | sPLA2 | Relieve RA | Chen et al. (2017) |
| TWHF extract | Tripterygium wilfordii hook F | Methanol extract; Chloroform extract | Preclinical study | Synovial fibroblast | COX-2 | Relieve RA | Tao et al. (1998) and Lin et al. (2007) |
| Myricetin | Euphorbia dracunculoides | Ethanol + water extract | Preclinical study | BV2 cell Mice | COX-2 | Relieve RA | Pan et al. (2019) |
| Xanthones extract | Swertia chirayita | Petroleum ether extract; Ethyl acetate extract | Preclinical study | Raw 264.7 macrophage cell | COX-2 | Relieve RA | Hu et al. (2019) |
| Bacopa monniera extract | Bacopa monniera linn | Methanolic extract | Preclinical study | Wistar rat, Rat mononuclear cells | 5-LOX/ 15-LOX | Relieve RA | Viji and Helen (2008) |
| HOEC | Incarvillea mairei var | 80% ethanol extract | Preclinical study | Wistar rats | 5-LOX/ 15-LOX | Relieve RA | Yang et al. (2018) |
| SOG | Saposhnikovia divaricata (turcz.) schischk | Ethyl acetate/n-butanol/ ethanol/water (1:1:0.1:2, v/v/v/v) | Preclinical study | Raw 264.7 macrophage cell | 5-LOX | Relieve RA | Zhao et al. (2016) and Liu et al. (2020) |
| TGP | Root of paeonia lactiflora pallas | Ethanol extract | Preclinical study | DBA/1 mice | MMP-1/ MMP-3 | Relieve RA | Li et al. (2019) and Can et al. (2020) |
| Celastrol | Root bark of tripterygium wilfordii hook. f | Ethylacetate extract | Preclinical study | Sprague-dawley rat | MMP-9 | Relieve RA | Xiao et al. (2018) |
| Ikarisoside | Epimedium koreanum | Methanol extract | Preclinical study | Raw 264.7 macrophage cell | MMP-9 | Relieve RA | Choi et al. (2010) |
| Ursolic acid | Ocimum sanctum | Methanol crude extract | Preclinical study | Albino wistar rat | MMP-2/ MMP-9 | Relieve RA | Radhiga et al. (2019) |
| Catechins | Green tea derived from the leaves of camellia sinensis | Acetone extract, Aqueous extract | Preclinical study | Human synovial fibroblast | MMP-2 | Relieve RA | Fechtner et al. (2017) |
| POEa/POEe | Polygonum orientale L | Ethyl acetate extract; Ethyl ether extract | Preclinical study | Sprague-dawley rat | iNOS | Relieve RA | Gou et al. (2018) |
| Celastrus ethylacetate extract | Celastrus aculeatus merr | Ethylacetate extract | Preclinical study | Sprague-dawley rat | NOS | Relieve RA | Bai et al. (2014) |
| Celastrol | Root bark of *tripterygium wilfordii hook. f* | Ethylacetate extract | Preclinical study | Wistar rat | iNOS | Anti-inflammation | Kannaiyan et al. (2011) |
| Hemerocallis citrine, Ethanol extract | Hemerocallis citrine | Ethanol extract | Preclinical study | Wistar rat | IDO | Anti-inflammation | Liu et al. (2014) |
| Feiji recipe | 12 herbs compound | Herbal mixture | Clinical study | C57BL/6 mice | IDO | Regulate immune | Luo et al. (2018) |

*Note: Preclinical: Research involving animals and cells; Clinical study: Research involving human specimens.*

peculiarity for arachidonic acid (AA) at the position of sn-2 in phospholipids (Hartz et al., 2019). As AA is the precursor of eicosanoids, so, $cPLA_2$ can be considered as the pivotal enzyme involved in the production of eicosanoids and therefore, is an important enzyme in some inflammation diseases, e.g., RA (Sommerfelt et al., 2015). In addition to the above functions, $cPLA_2$ can boost the enzymatic activity of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase in monocytes and neutrophils to produce the superoxides during the process of inflammation (Raichel et al., 2008). Therefore, inhibiting the $cPLA_2$ could simultaneously decrease the activities of multiple lipid materials, which facilitate the neutrophils recruitment to the inflammation area, and accelerate the superoxides production and release. Several studies show that $cPLA_2$ is expressed in RA synovium (Malaviya et al., 2006), and has been performing significant roles during the progression of inflammatory in several models of arthritis (Courties et al., 2011). In the rheumatoid arthritis synovial fibroblasts (RASFs) of human, the expression level of $cPLA_2$ is increased by proinflammatory cytokines such as TNF-α and IL-1β (Chi et al., 2011). Then, the elevated $cPLA_2$ acts as an important regulator of those key players including interleukin-8 (IL-8), prostaglandin E2 ($PGE_2$), stromelysin-1 (matrix metalloproteinase 3, MMP3) and COX2 in the pathology of RA, which results in the destruction,

angiogenesis of bone and cartilage and the neutrophil recruitment (Sommerfelt et al., 2013). Common research has long held that the cartilage and bone degradation are the two major hallmarks of RA, therefore reduction or prevention of these destructive processes should be a central therapeutic objective. Also, in murine collagen induced arthritis, $cPLA_2$ is also recognized to be one of the vital regulators of the neutrophil recruitment and inflammatory reaction, which highlights the promising biological relevance of $cPLA_2$ in synovitis and arthritis (Raichel et al., 2008). Given all the evidence above, regulating and intervening the enzymatic activity of $cPLA_2$ by specific $cPLA_2$ inhibitors and then promoting the normalization of downstream signals probably be considered as a promising alternative or supplement strategy to the current therapeutic methods for the treatment of RA. Recent research finds that herbal extracts and their purified compounds can selectively suppress the production of $cPLA_2$ and eventually prevent the inflammatory process. Therein, a study demonstrates that *PG201* is an extract from a mixture contained 12 different herbs by ethanol. While *PG201* reduces the protein expression of $cPLA_2$, it does not affect the mRNA expression level of $cPLA_2$, which leads to the decreased production of $PGE_2$, thus declining the concentrations of IL-1β, IL-6 and CC chemokine ligand-2 (CCL2) in supernatant and synovial tissues, eventually plays important anti-inflammation and anti-arthritic activity in LPS induced inflammatory cells (Raw264.7 cell) and RA rat model (Shin et al., 2003; Choi et al., 2012). All these effects mainly dependent on that *PG201* can substantially reduce the activator protein-1 (AP-1) and cyclic adenosine monophosphate-responsive element-binding (CREB) protein transcription factors' DNA-binding activities, rather than nuclear factor-κB. Similarly, several fatty acids also can be hydrolyzed by $sPLA_2$ at the position of sn-2 in the substrate phospholipid. However, the detailed mechanism of $sPLA_2$ in the generation of eicosanoid is not clear in mammalian cells (Takada and Fujita, 2017). According to a study, the mice expressing $sPLA_2$ enzyme ($sPLA_2$ transgene) developed a deteriorated arthritis, which seemed more severe of arthritis than that of in mice lacking $sPLA_2$. Since lipidomic analyses underlined the important roles of $sPLA_2$ during the production of diverse PGs, especially $PGI_2$, one possibility is that $sPLA_2$ may aggravate the arthritis through increasing the levels of those eicosanoids. Meantime, the results of a primary study indicated that $sPLA_2$ is induced production and activation in the process of inflammation and has been in a high level in the synovial fluid of RA patients (Dore and Boilard, 2019). So far, a number of studies focusing on this subject have no more accurate conclusions, and clinical trials of $sPLA_2$ against RA cannot reach a satisfactory effect (Krizaj, 2014). But one study shows that the ethanol extract of *Boerhaavia diffusa*, a popular medicinal herb, concentration-dependently inhibits the $sPLA_2$ enzymes to different degrees. Then, the inhibition of $sPLA_2$ enzymes will neutralize the indirect hemolytic activity, mouse paw edema, and other RA symptoms induced by $sPLA_2$ (Giresha et al., 2017). Also, both the Chinese herbal drug *Salvia miltiorrhiza* extract and the Indian synthetic herbal compounds 2-hydroxy-4-methoxy benzoic acid (HMBA) show a good inhibitory effect on $sPLA_2$ induced toxicities (Gomes et al., 2012; Chen et al., 2017). Although results show good effects, some critical issues still exist and further investigations should to be done to verify the exact active ingredients. In view of the above findings, $cPLA_2$ and $sPLA_2$ are two valuable therapeutic targets, hence targeting the $PLA_2$ enzyme mediator herbal products (such as *PG201*, *Boerhaavia diffusa*, *Salvia miltiorrhiza* extract and HMBA) development is likely to be a potential therapeutic strategy during the process of RA treatment.

## Cyclooxygenase (COX) and Prostaglandins (PGs)

Cyclooxygenase is an important enzyme that produces eicosanoids which regulate multiple pathological and physiological process. It converts AA into prostaglandin H2 (PGH2). Then, the $PGH_2$ can be further catalyzed by different synthases to produce 5 main bioactive prostaglandins including PGD2, PGE2, PGF2, PGI2, and thromboxane A2 (TXA2) (Qureshi and Dua, 2020). Early studies have confirmed that there are two main subtypes of cyclooxygenase that are found in the human body (Nokhbehsaim et al., 2020). The first one is known as cyclooxygenase-1 (COX-1), which is considered as a beneficial enzyme and constitutively expressed in many kinds of tissues and cells, and acts as part of normal cellular housekeeping, such as maintaining the stomach's lining, whereas the inducible enzyme named cyclooxygenase-2 (COX-2), in contrast, is induced by some particular conditions such as mitogenic and inflammatory stimuli, etc (Leng et al., 2018). Both types of COX make a class of compounds including prostaglandins, which produce signals that are short-lived and only affect nearby cells, or the same cells produce them. Long ago before regulation of COX-2 gene expression had been recorded in synovial tissues of both human and rodent. In the experimental arthritis, the expression level of COX-2 has been confirmed to increase in consistent with the clinical disease development and closely correlated with the infiltration of synovial mononuclear cell (Masferrer et al., 1994). In the human synovial tissues from RA, osteoarthritis (OA) and non-arthritic traumatic injury patients, the significantly express signal of COX was captured in a distinct structure called the synovial lining layer and multiple cell types including subsynovial synoviocytes, mononuclear inflammatory cells and vascular endothelial cells (Crofford, 1997). And the level of COX-2 was proved to be closely correlated with the infiltration degree of mononuclear cell, which provided a measurement manner for the synovial inflammation (Sano et al., 1992). During the occurrence and development of arthritis, the inflammatory cytokines such as IL-1β and TNF-α can induce the expression of COX-2 in synovial fibroblasts via stimulating the NF-κB and mitogen-activated protein kinase (MAPK) signals. Subsequently, COX-2 promoted the generation and accumulation of prostanoids in the synovium tissue (Nakano et al., 2020). Among the variety of prostaglandins, PGE2 and thromboxane A2 (TXA2) are the two powerful inflammation bioactive substances that contribute to the development of RA. In the course of RA, PGE2 causes the vasodilatation and subsequently brings about the neutrophils recruitment to the affected progression of joints (Peng et al.,

2019). The neutrophils recruitment in joints is attributed to the generation of IL-17 induced by IL-23, as well as the damaged production of interferon-$\gamma$ (IFN-$\gamma$) and IL-12. Furthermore, PGE2 regulates the degradation of matrix to influence the destruction of cartilage (Jiang L. et al., 2020). Under the inflammation condition, PGE2 can also induce the angiogenesis through accelerating the vascular endothelial growth factor (VEGF) production. Besides, research finds that PGE2 can not only promote the intension of inflammatory pain by also enhancing the sensitization of bradykinin and histamine-induced nociceptive stimuli, but also accelerate the plasma extravasation induced edema during the RA disease. In the meantime, studies have been shown that the effect of IL-1, IL-6 and TNF-α on bone resorption is mainly by a PGE2 dependent way (Jia et al., 2019). Extracts from a famous Chinese herbal called *Tripterygium wilfordii Hook. F* (TWHF) have been demonstrated effectively when treating the patients with inflaming and autoimmune diseases, for example, RA. In the subsequent experiments, researchers found that the chloroform/methanol extract (T2), ethyl acetate extract (EA) extract and the triptolide component could suppress the production of PGE2 via blocking the COX-2 upregulation in RASF of RA in a dose-dependent pattern ($p < 0.05$) in joint tissues of CIA mice (Tao et al., 1998; Lin et al., 2007). These alterations indicated that TWHF possesses promising immunosuppressive and anti-inflammatory activities on RA. In LPS stimulated neuroinflammation, treatment with *Myricetin*, a natural flavanol, can attenuate the expression of COX-2, which remarkably inhibits the generation of PGE2, IL-1β, and TNF-α, and eventually ameliorate the neuroinflammation (Jang et al., 2020). In human chondrocytes stimulated by IL-1β, *Myricetin* could increase the ration of p-Akt/Akt to activate the PI3K/Akt signaling pathway. Then, the activated pathway enhances the Nrf2/HO-1 pathway activation to abolish the NF-κB mediated inflammation, eventually suppresses the expression of COX-2 and inhibits the generation of inflammation mediators such as PEG2, IL-6 and TNF-α to ameliorate the development of OA (Pan et al., 2019). The above results supporting *Myricetin* can be regarded as a potential anti-RA herbal product by targeting the COX-2 and related PGE2 in the further research. Also, *Xanthones* extracts derived from *Swertia chirayita*, a famous Chinese herb, show a good anti-inflammatory property by inhibiting the expression of COX-2 and PGE2 in murine macrophage cells, which can be considered as a potential anti-RA herbal product for further study (Hu et al., 2019). Therefore, it is a beginning point for finding more products to decrease the level of PGE2 through suppressing the activity or expression of COX-2 during RA treatment. TXA2, the other product derived from COX, acts as a paracrine/autocrine hormone to induce the human platelets with as fast irreversible aggregation. It is also a potent smooth muscle contraction inducer by binding to its specific receptor, TXA2 receptor (Kashiwagi et al., 2019). Research shows that the biosynthesis of TXA2 at the molecular level in RA patients is obviously stronger than that of in healthy control subjects, suggesting its role in RA development. Further studies indicate that TXA2 binds to its receptor to active several intracellular signaling, which causes the transcription factor NF-κB activation, and subsequently increases the levels of IL-1 and TNF-α, leading to the synovial cell pathology in RA (Wang et al., 2015). Therefore, targeting TXA2 provides a potential therapeutic strategy during the RA treatment by herbal medicinal products.

## Lipoxygenase (LOX) and Leukotrienes (LT)

Lipoxygenase (LOX) is a kind of non-heme iron-containing dioxygenases that can catalyze the oxidation of fatty acid. Until now, the main subtypes of LOX, such as 5-LOX, 12-LOX, and 15-LOX, have been validated by several studies, which can stereospecifically combine with atom of oxygen at carbon atom 5, 12, or 15, respectively (Mackel et al., 2020). That much had been shown many times before: LOX transduction system is one major signaling pathways during inflammatory process of RA and that the synovial fluid of RA patients has a multitude of leukotrienes (Gheorghe et al., 2009). Studies show that 5-LOX and 15-LOX are primarily existing in the synovium of RA and OA, and therein 15-LOX is a lipid-per oxidizing enzyme that predominantly expressed in eosinophils, macrophages, fibroblasts and most articular tissues, including cartilage, synovium, and bone and its products are found in human synovial fluids, while 5-LOX is mainly located in the lining/sublining macrophages, neutrophils, and mast cells (Colamorea et al., 1999; Klein et al., 2004). In the human body, IL-13 induces the expression of 15-LOX in blood monocytes, while IL-4 stimulates the 15-LOX in RA synovial cells, monocytes, mast cells and dendritic cells. Then, 15-LOX converts AA to 15-hydroperoxy-eicosatetraenoic acid (15-HETE), which undergoes further conversion to form the15-hydroxyeicosatetraenoic acid (Wan et al., 2020). In the RA joint, the intermediate 15-HETE suppresses the generation of leukotriene B4 (LTB4) to regulate the leukocytes infiltration. Also, 15-HETE inhibits the mitogenesis of T-lymphocyte and the secretion of eosinophil leukotrienes C4 (LTC4), to prevent the neutrophil migration, and inhibit superoxide anion production and degranulation from activated neutrophil, which leads to the down-regulation of the inflammatory process in RA joints, indicating that 15-LOX has a protective effect on RA through forming the anti-inflammatory lipoxins (Wan et al., 2019). Some research shows that the expression of 15-LOX is elevated by IL-1β and TNF-α in RASF, while knockout of 15-LOX significantly reduces the cartilage destruction and inflammatory arthritis in C57/B6 mice induced by Freund's Complete Adjuvant H37Ra (FCA; containing the 1 mg/ml *Mycobacterium tuberculosis* H37Ra) (Wu et al., 2012). Besides, treating with 15-(S)-HETE can also promote the osteoclasts differentiation (Kronke et al., 2009). The above studies indicate the indispensable role of 15-LOX during the arthritis pathogenesis, thus providing a valuable target for drug discovery and development when treating inflammatory arthritis. In RA, 12-LOX represents a similar anti-inflammatory enzyme operative, while the 5-LOX subtype catalyzes the synthesis of leukotriene B4 (LTB4) from arachidonic acid, and it is known to accelerate the pathogenesis of RA in contrast with 15-HETE. To confirm the role of 5-LOX in RA, RASF are pretreated with two kinds of 5-LOX inhibitors MK-886 (5 mM) and NDGA (5 and 10 mM) for 1 h. For the next 6 h, cells are treated with TNF-α (10 ng/ml). Researchers find that

pretreatment of 5-LOX inhibitors can significantly decrease the TNF-α-induced IL-6 protein level and the expression of monocyte chemotactic protein-1 (MCP-1)/CCL-2, and the results are similar to those of 5-LOX knockdown (Lin et al., 2014). Furthermore, *in vivo* study demonstrates that 5-LOX inhibitor can alleviate the TNF-α induced phenotypic changes and even systemic inflammation. The alterations induced by 5-LOX inhibitors and 5-LOX knockdown suggest a valuable therapeutic strategy targeting 5-LOX for treating the RA (Chen et al., 2006).

Several studies show that many agents can inhibit the activity of LOX, which displays a significant inhibitory effect against joint inflammation. Among them, some herbal products have attracted people's attention. *Bacopa monniera Linn* is described in the ayurvedic materia medica, as a therapeutically useful herb for the treatment of inflammation (Viji and Helen, 2008). In the carrageenan-induced rat paw edema model, *Bacopa monniera Linn* and its methanolic extract can significantly inhibit the activity of 5-LOX, while increasing the 15-LOX activity. Then, the decline in 5-LOX and elevation of 15-LOX activity leads to the decreased $LTB_4$ production, which shows a significant effect in decreasing edema and the inflammation process. The result indicates that *Bacopa mon*niera *Linn* and its methanolic extracts may be a potential herbal product for treating joint inflammation and even RA when targeting LOX (+)-2-(1-hydroxyl-4-oxocyclohexyl) ethyl caffeate (HOEC) is an important herbal ingredient product isolated from *Incarvillea mairei var,* which has long been used as folk medicine for the treatment of inflammatory related diseases in China. Research found HOEC as an inhibitor of 5-LOX and 15-LOX *in vitro* to significantly inhibit both the two LOXs, thus suppressing the LOX related pathway in the beginning of arthritis. The inhibition of LOX pathway reduced the production of LTC4 and 15-HETE, but had little effect on LTB4 expression, eventually alleviating the clinical symptoms of arthritis, such as synovial hypeplasia, multiple cartilage destruction and pannus (Yang et al., 2018). These alterations suggest that treatment of herbal product HOEC exhibit a significant anti-RA effect by targeting the LOXs, which should be paid attention to and further studied. Also, a major herbal compound named *sec-O-glucosylhamaudol* (SOG), which is derived from *Saposhnikovia divaricata* (Turcz.) Schischk, has been reported to have anti-5-LOX activity, suggesting that SOG might have therapeutic effects on inflammatory disease, such as acute lung injury and RA (Zhao et al., 2016; Liu et al., 2020). Based on the above findings, targeting the lipoxygenase may be a great therapeutic strategy in treating inflammatory disease including RA and these herbal medicinal products will develop into effective medicines for RA treatment with the deepening and development of research.

## Matrix Metalloproteases (MMPs)/Tissue Inhibitors of Metalloproteinases (TIMPs)

Under the environment of RA, mononuclear/macrophages are activated to produce various cell factors and inflammatory mediators (Siouti and Andreakos, 2019). Among these mediators, the inflammatory factors such as IL-1β and TNF-α activate and accelerate the generation of matrix metalloproteases (MMPs), which will significantly increase the total activities of MMPs indirectly. The enzyme family can irreversibly promote the degradation of extracellular matrix (ECM) ingredients, including the collagen and fibronectin in the place of articular cartilage and bone (Viana et al., 2020). The major components of cartilage are type II collagen and proteoglycans, while type I collagen primarily constitutes the bone. Studies indicate that the process of MMPs mediated collagen degradation is the rate-limiting step during the damage of cartilage and bone. In the joints area, synovial cells produce the MMP-1, while MMP-13 synthesized by chondrocytes acid reside in cartilage (Li and Li, 2019; Nishi et al., 2019). Under the circumstances, MMP-13 degrades many substances including collagen, proteoglycan molecule and aggrecan. In a clinical study, researchers found the expression levels of MMP-1 and MMP-13 are increased in RA patients, and the baseline levels of serum MMP-1 and MMP-13 are correlated with disease progression, which can be used for predicting the radiographic and functional outcome in the early RA (Green et al., 2003). Regulated upon activation, normal T cell expressed and secreted (RANTES)/CCL5 is a chemokine produced by the majority of cell types, such as synovial fibroblasts, chondrocytes and activated T cells, etc. that participate in the pathogenesis of RA. In human RASFs, a study demonstrated that RANTES/CCL5 can induce the expression of MMP-1 and MMP-13, thus destroying the native collagen structure (Agere et al., 2017). Besides, RASFs produced MMP-1 and MMP-13, and the increased levels of these two MMPs in synovial fluid and tissue biopsies of RA patients offer some solid evidence for their function in tissue destruction (Yoshihara et al., 2000; Miller et al., 2009). In the inflammatory arthritis mice model and SFs, Firestein et al. proposed that JNK signaling pathway can mediate the production of multiple cytokines such as IL-1β and TNF-α, and these cytokines subsequently induced the MMP-1 and MMP-13 expression (Han et al., 2001). In recent studies, researchers used JAK inhibitor or anti-TNF-α as intervention means and the results showed the obvious efficacy in ameliorating the tissue destruction, which was partly relation with the downregulation of MMP-1 and MMP-13 (Catrina et al., 2002; Boyle et al., 2015). The findings above indicated that MMP-1 and MMP-13 play a certain role in the RA progression, which should be considered as promising therapeutic target when treated with anti-RA agents, including herbal medicinal products. In the meantime, research also showed that the expression of other MMPs, such as MMP-2, MMP-3, MMP-9, MMP-12, and MMP-14, is obviously elevated in RA. And these enzymes degraded the components of non-collagenous protein of matrix, which results in the complete joint damage (Wang X. et al., 2019). Besides, MMPs also play a pivotal role in the angiogenesis progress, which is one of the critical components of the inflammatory arthritis pathogenic process (Withrow et al., 2016). Taken together, suppressing the activities of pathogenic MMPs in the section of joint, bone, and synovial cells can prevent or significantly decrease the joint and bone destruction, thereby alleviating the pain of arthritis patients and benefiting with an improved quality of life. Several studies report that TIMPs 1-4 are the natural inhibitors of MMPs, and they can

diminish the pro-inflammatory cytokines and tissue damage in the joint (Hyc et al., 2016). Therefore, plenty of effort has been invested in finding and designing the effective suppressant and herbal products of MMPs' activity and/or synthesis that display great anti-RA activity in several arthritis animal models. So far, there have been some herbal medicinal products that show good treatment results in treating the arthritis, especially RA. *Total glucosides of paeony* (TGP), a Chinese herbal product with extensive popularity, shows the obvious inflammation and pain inhibiting effects in a rat model of RA. Then, an in-depth study finds that part of its anti-RA effect is attributed to the suppression of the TNF-α and IL-1β production via macrophage-like synoviocyte (MLS), and that of MMP-1 and MMP-3 by FLS (Li et al., 2019). Moreover, this interdependent inhibition effect on different inflammatory enzymes and mediators can be explained by the fact that IL-1β and TNF-α regulates the expression and activity of MMP-1 and MMP-3 (Can et al., 2020). The anti-RA clinical application of *Pavlin* is well validated by the results acquired by above studies. Likewise, in a separate study, researchers find that *Triphala guggul*, an ayurvedic herbal medicinal product, shows a certain inhibition of key enzymes including hyaluronidase, collagenase and MMPs, which are involved in the tissue damage in RA (Sumantran et al., 2007). *Tanshinone IIA* (Tan IIA), the primary phytochemical extracted from a famous Chinese herbal Salvia Miltiorrhiza Bunge, is reported to be capable of promoting the RA-FLS apoptosis and inhibit arthritis progression in an AIA mouse model (Wang Z. et al., 2019). Moreover, RA patients treated at clinic with Tan IIA showed significant improvements in their clinical symptoms. After exploring, a researcher found that Tan *IIA* could effectively inhibit the increased mRNA expression of multiple matrix metalloproteinases (including MMP2, MMP3, MMP8, and MMP9) and proinflammatory factors in RA-FLSs stimulated by induced by TNF-α, resulting in the inhibition of inflammatory reactivity and end of knee joint destruction, which indicates the promising therapeutic role in the treatment of RA and shows potential to improve the life quality of RA patients (Du et al., 2020). Besides, Celastrol, Ikarisoside, and AKBA (mastic acid active ingredient) suppress the activity of MMP9, one of the transcription factor NF-κB-mediated genes (Choi et al., 2010; Xiao et al., 2018). Ursolic acid inhibits the expression of MMP-9, while the catechins extracted from *green tea* shows a good inhibitory effect on the expression and activity of MMP1 and MMP13 (Fechtner et al., 2017; Radhiga et al., 2019). *PG201*, the aforementioned new herbal drug, was reported to amplify the expression of TIMP-2, thus elevating the ration of TIMP2/MMP2 in synovial tissue and fluid in CIA rabbits and mice. For the arthritis progression, down-regulation of MMPs and up-regulation of TIMPs could prevent the collagen and proteoglycan release from cartilage to relieve the cartilage destruction and degradation (Park et al., 2005). Encouragingly, *PG201* (Layla, PMG Pharmaceutical) for treating OA had received the new drug application (NDA) approval in March 2012 (Yoo et al., 2014; Kim et al., 2016). Therefore, targeting the MMPs/TIMPs system may be a brilliant therapeutic strategy in treating the RA and these herbal medicinal products will develop into great medicines for RA treatment to a large degree.

## Nitric Oxide Synthase (NOS) and Nitric Oxide (NO)

Nitric oxide (NO) is a kind of radical gas with molecules synthesized from the guanidino group of L-arginine by nitric oxide synthase (NOS), which plays important roles in human body, including anti-tumor, anti-bacterial, wound healing, and vasodilation effects (Gartside et al., 2020). However, things always have two sides. When the level of endogenous NO is excess, severe inflammatory diseases such as inflammatory periodontal disease and joint disease including RA set in (Zaichko et al., 2020). NOS contain different isoforms: inducible macrophage type NOS (iNOS), endothelial cell NOS (ecNOS) and brain NOS (bNOS) (Gartside et al., 2020). Under the stimulation of a variety of immunological factors, pro-inflammatory cytokines can induce the non-hematopoietic cells, like fibroblasts, to produce the iNOS. Similar to that of NO, the production of iNOS may have either a toxic effect or a protective effect. Studies using non-selective NOS inhibitors showed the suppression of arthritis in rats, thus suggesting positive inhibitory effects of iNOS in acute and chronic joint inflammation, which indicate the possibility of direct NO toxic effects in RA (Stefanovic-Racic et al., 1994). In antigen-induced arthritis (AiA) mouse, Andreas et al. found that the development of AIA is related to the overexpression of iNOS in synovial microcirculation of knee joint, the elevated NO production and the increased leukocyte adhesion/infiltration in synovium (Veihelmann et al., 2001). Meantime, in the C57/Bl6 mice with AIA, the iNOS expresses, after stimulation, up to ten times more NO than the two constitutive forms of NOS (Schmitt-Sody et al., 2007). When detailed mechanisms are explored, researchers found that the activation of NF-κB in multiple arthritis models including RA, CIA and AIA is necessary for both the initiation and development of inflammation (Makarov, 2001). Overexpression of NF-κB has been reported to target the transcriptional process of iNOS mRNA, thus increasing the levels of iNOS gradually, which will catalyze and produce NO in arthritis models (Tak and Firestein, 2001). Under the inflammatory condition of RA patients, overexpression of iNOS is essential for the increased NO production (Aktan, 2004). At this point, researchers find that NO can stimulate the generation of pathogenic cytokines such as IL-1β, TNF-α, IFN-$\gamma$, and collagenase (Zaichko et al., 2020). Also, NO induces certain chemokines that contribute to the disease progression in RA. After decreasing the production of NO by inhibiting NOS, the arthritic symptoms were reduced. The above results demonstrate that suppressing the activity of iNOS could decrease the level of NO, eventually revealing an anti-arthritis effect, especially in RA, CIA and AIA. Based on these findings, the study of herbals shows that anti-oxidants extracted from a number of herbals can scavenge the NO and other free radicals. Besides, some herbals-derived compounds can suppress the activity of iNOS. *Daphne genkwa* is a herb with important anti-inflammatory effects. Some research showed that flavonoid is the main active ingredient. In LPS-induced RAW264.7 macrophages, T lymphocytes and fibroblast-like synoviocytes, *Daphne genkwa* can obviously inhibit the NF-κB pathway and down-regulate the expression of iNOS mRNA,

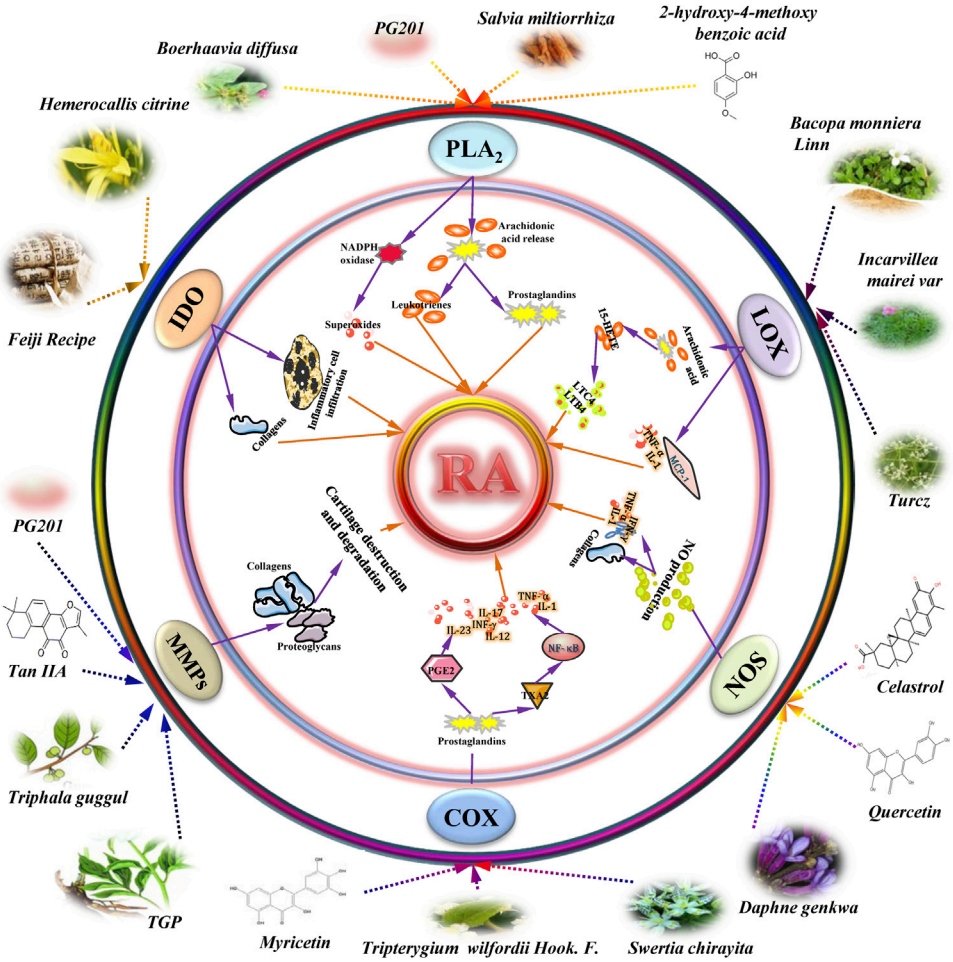


**FIGURE 1 |** Schematic model representing the anti-rheumatoid arthritis (RA) effects of herbal medicinal products on therapeutic enzymes and their derivatives targets in RA. Additional abbreviations: 15-HETE: 15-hydroperoxy-eicosatetraenoic acid; COX-2: cyclooxygenase-2; $PLA_2$: phospholipase $A_2$; IDO: indoleamine 2, 3-dioxygenase; IFN-γ: interferon gamma; IL-1: interleukin-1; LTB4: leukotriene B4; LTC4: leukotrienes C4; LOX: lipoxygenase; MMP: matrix metalloproteinase; NOS: Nitric oxide synthase; NO: nitric oxide; PGE2: prostaglandin E2; Tan IIA: tanshinone IIA; TGP: total glycosides of paeony; TNF-α: tumor necrosis factor α; TXA2: thromboxane A2.

which leads to the decreased expression of iNOS protein. After that, the decreased iNOS will reduce the secretion levels of NO and IL-6 in inflammatory RA-FLSs (Sun et al., 2020). According to one study, oral administration of POEa and POEe (ethyl acetate and ethyl ether extract of P. orientale, respectively) to rats can ameliorate the adjuvant arthritis (AA), and this is associated with decreased generation of various inflammatory mediators, including NO by macrophage (Gou et al., 2018). In another study, *Celastrus aculeatus* exerts its anti-inflammatory and anti-arthritis activity as tested in the AA model. After treating with *Celastrus* ethylacetate extract, rats show a significant decrease of the NOS expression and the NO levels both in serum and culture supernate of antigen-stimulated draining lymph node cells (Bai et al., 2014). Besides, *Celastrol*, as an ingredient of *Celastrus* and other celastraceae family of herbals, has been shown to modulate the expression of iNOS (Kannaiyan et al., 2011). Therefore, targeting the iNOS may be a potential therapeutic strategy in treating the RA and these herbal medicinal products will develop into great medicines for RA treatment.

## Indoleamine 2, 3-Dioxygenase (IDO)

Tryptophan is an essential amino acid that is critical for normal cell survival and proliferation, and obviously important for the development and functioning of many organs in the human body (Hu et al., 2020). Some studies found that tryptophan can be catabolized by indoleamine 2, 3-dioxygenase (IDO) to form the kynurenine, which can induce the apoptosis of T cells (Jiang X. et al., 2020). IDO, as the only one rate-limiting enzyme outside the liver that catalyzes tryptophan to kynurenine, is expressed in dendritic cells (DC) and activated macrophages, but not in the T cells. IDO positive DCs exert a significant role in the induction and maintenance of peripheral tolerance through the generation/activation of regulatory T cells (Treg) and consumption of self-reactive T cells. A study from Ozkan reports that the serum concentration of tryptophan markedly decreases, while the levels

of kynurenine significantly increase in RA patients, indicating the integral role of IDO in RA disease (Merlo et al., 2017). Meanwhile, the concentration of kynurenine is higher in synovial fluid of RA than that of in OA patients and correlates with proinflammatory cytokines such as IL-1β, IL-6, and IL-8 expression (Bertazzo et al., 1999). Subsequently, IDO overexpression has been detected in the RA synovial, as well as in OA synovial. Under the inflamed synovium microenvironment, the expression of IDO was evaluated at a mRNA level in human FLS isolated from RA patients (Massalska et al., 2019). Some studies have found that IDO expression showed a direct effect on RA-related chondrocytes proliferation and collagen II in the matrix that suggests a possible effect on the MMPs (Chang et al., 2018). In the RA model, IDO could markedly diminish the accumulation of pathogenic Th1 and Th17 cells in the arthritic joints, thereby alleviating the severity of this disease. But with the deepening of the research, some results also show that inhibiting the activity of IDO may recede instead of aggravate RA. In-depth mechanism study finds that the IDO activity can be regulated by $CD4^+$ $CD25^+$ Treg and interferon-$\gamma$ (IFN-$\gamma$). Moreover, the over-expression of enzyme tryptophanyl-tRNA-synthetase (TTS) in cytoplasmic can load the tryptophan into its specific tRNA, to form the chromoyl-tRNA complex, and this complex can antagonize the IDO-mediated deprivation of tryptophan, which will diminish the accumulation of T cells in arthritic joints and eventually recede the RA (Kim et al., 2018). Therefore, targeting the IDO could be a promising therapeutic strategy. So far, there has been no direct evidence that herbal medicine can alleviate the RA progression through targeting the IDO. However, in the depression-like model of rat, ethanol extracts from *Hemerocallis citrine* can attenuate the upregulation of proinflammatory cytokines and IDO (Liu et al., 2014). Meantime, *Feiji Recipe*, a compound of Chinese herbal medicine product, can significantly reduce the expression of IDO in C57BL/6 orthotopic mouse model (Luo et al., 2018). As of now, herbal products have not been studied much for their ability to modulate RA via altering IDO activity, but fortunately it also provides us with the great opportunity to apply the herbal products in the basic research and clinical treatment of RA in the future.

## CONCLUSION

Targeting the enzymes and their derivatives is likely to become a potential therapeutic strategy in the treatment of RA and these targeted herbal medicinal products would develop into great medicines for RA treatment. The major benefit of using these herbal products and their active ingredients due to their limited or almost no undesirable side effects. Hence, the interdisciplinary efforts of researchers are intended to explore the detailed biology functions of existing herbal products, to screen and identify novel herbal products, and to define the exact molecular mechanisms should be reinforced. These actions would facilitate and boost the discovery, screening, and development of safe and effective herbal products for treating RA and other inflammation, and autoimmune-mediated disorders.

## AUTHOR CONTRIBUTIONS

MC and W-JN designed the "ideas"; W-JN helped MC to deal with the information efficiently; MC wrote the manuscript; LH, W-DC and D-YP revised the manuscript. All authors agree to be accountable for the content of the work.

## FUNDING

This work was supported by the National Key Research and Development Plan (Nos. 2017YFC1701600, 2017YFC1701601); the National Natural Science Foundation of China (Nos. 81803602, 81473387, 81503291); the Key Project of the National Science Fund of Anhui Province (No. KJ2017A277); and the Natural Science Foundation of Anhui Province (No. 1708085QH207).

## REFERENCES

Agere, S. A., Akhtar, N., Watson, J. M., and Ahmed, S. (2017). RANTES/CCL5 induces collagen degradation by activating MMP-1 and MMP-13 expression in human rheumatoid arthritis synovial fibroblasts. *Front. Immunol.* 8, 1341. doi:10.3389/fimmu.2017.01341

Aktan, F. (2004). iNOS-mediated nitric oxide production and its regulation. *Life Sci.* 75 (6), 639–653. doi:10.1016/j.lfs.2003.10.042

Bai, S.-T., Chen, P.-H., Chen, Y.-Y., Lin, X.-C., Liu, J.-S., and Tong, L. (2014). Ethyl acetate extract fromCelastrus aculeatusMerr. Suppresses synovial inflammation in adjuvant arthritis rats through apoptosis induction of CD4+CD25+FOXP3+T cells. *Evid.-Based Complement. Altern. Med.* 2014, 1. doi:10.1155/2014/460136

Bao, Y., Sun, Y.-W., Ji, J., Gan, L., Zhang, C.-F., Wang, C.-Z., et al. (2019). Genkwanin ameliorates adjuvant-induced arthritis in rats through inhibiting JAK/STAT and NF-κB signaling pathways. *Phytomedicine* 63, 153036. doi:10.1016/j.phymed.2019.153036

Bertazzo, A., Punzi, L., Bertazzolo, N., Pianon, M., Pozzuoli, A., Costa, C. V. L., et al. (1999). Tryptophan catabolism in synovial fluid of various arthropathies and its relationship with inflammatory cytokines. *Adv. Exp. Med. Biol.* 467, 565–570. doi:10.1007/978-1-4615-4709-9_70

Boyle, D. L., Soma, K., Hodge, J., Kavanaugh, A., Mandel, D., Mease, P., et al. (2015). The JAK inhibitor tofacitinib suppresses synovial JAK1-STAT signalling in rheumatoid arthritis. *Ann. Rheum. Dis.* 74 (6), 1311–1316. doi:10.1136/annrheumdis-2014-206028

Can, V. C., Locke, I. C., Kaneva, M. K., Kerrigan, M. J. P., Merlino, F., De Pascale, C., et al. (2020). Novel anti-inflammatory and chondroprotective effects of the human melanocortin MC1 receptor agonist BMS-470539 dihydrochloride and human melanocortin MC3 receptor agonist PG-990 on lipopolysaccharide activated chondrocytes. *Eur. J. Pharmacol.* 872, 172971. doi:10.1016/j.ejphar.2020.172971

Catrina, A. I., Lampa, J., Ernestam, S., Af, K. E., Bratt, J., Klareskog, L., et al. (2002). Anti-tumour necrosis factor (TNF)-alpha therapy (etanercept) down-regulates serum matrix metalloproteinase (MMP)-3 and MMP-1 in rheumatoid arthritis. *Rheumatology* 41 (5), 484–489. doi:10.1093/rheumatology/41.5.484

Chang, R.-Q., Li, D.-J., and Li, M.-Q. (2018). The role of indoleamine-2,3-dioxygenase in normal and pathological pregnancies. *Am. J. Reprod. Immunol.* 79 (4), e12786. doi:10.1111/aji.12786

Chen, A.-D., Wang, C. L., Wang, C.-L., Qin, Y., Tian, L., Chen, L.-B., et al. (2017). The effect of Danshen extract on lipoprotein-associated phospholipase A2 levels in

patients with stable angina pectoris: study protocol for a randomized controlled trial - the DOLPHIN study. *Trials* 18 (1), 606. doi:10.1186/s13063-017-2336-2

Chen, M., Lam, B. K., Kanaoka, Y., Nigrovic, P. A., Audoly, L. P., Austen, K. F., et al. (2006). Neutrophil-derived leukotriene B4 is required for inflammatory arthritis. *J. Exp. Med.* 203 (4), 837–842. doi:10.1084/jem.20052371

Cheng, W.-X., Zhong, S., Meng, X.-B., Zheng, N.-Y., Zhang, P., Wang, Y., et al. (2020). Cinnamaldehyde inhibits inflammation of human synoviocyte cells through regulation of jak/stat pathway and ameliorates collagen-induced arthritis in rats. *J. Pharmacol. Exp. Ther.* 373 (2), 302–310. doi:10.1124/jpet.119.262907

Chi, P.-L., Luo, S.-F., Hsieh, H.-L., Lee, I.-T., Hsiao, L.-D., Chen, Y.-L., et al. (2011). Cytosolic phospholipase A2induction and prostaglandin E2release by interleukin-1β via the myeloid differentiation factor 88-dependent pathway and cooperation of p300, Akt, and NF-κB activity in human rheumatoid arthritis synovial fibroblasts. *Arthritis Rheum.* 63 (10), 2905–2917. doi:10.1002/art.30504

Choi, H. J., Park, Y. R., Nepal, M., Choi, B.-Y., Cho, N.-P., Choi, S. H., et al. (2010). Inhibition of osteoclastogenic differentiation by Ikarisoside A in RAW 264.7 cells via JNK and NF-κB signaling pathways. *Eur. J. Pharmacol.* 636 (1-3), 28–35. doi:10.1016/j.ejphar.2010.03.023

Choi, J., Kim, S.-H., and Kim, S. (2012). Suppressive effects of PG201, an antiarthritic botanical formulation, on lipopolysaccharide-induced inflammatory mediators in Raw264.7 cells. *Exp. Biol. Med.* 237 (5), 499–508. doi:10.1258/ebm.2011.011203

Colamorea, T., Di Paola, R., Macchia, F., Guerrese, M.-C., Tursi, A., Butterfield, J. H., et al. (1999). 5-Lipoxygenase upregulation by dexamethasone in human mast cells. *Biochem. Biophysical Res. Commun.* 265 (3), 617–624. doi:10.1006/bbrc.1999.1732

Courties, G., Baron, M., Presumey, J., Escriou, V., van Lent, P., Scherman, D., et al. (2011). Cytosolic phospholipase A2α gene silencing in the myeloid lineage alters development of Th1 responses and reduces disease severity in collagen-induced arthritis. *Arthritis Rheum.* 63 (3), 681–690. doi:10.1002/art.30174

Crofford, L. J. (1997). COX-1 and COX-2 tissue expression: implications and predictions. *J. Rheumatol. Suppl.* 49, 15–19.

Croft, A. P., Campos, J., Jansen, K., Turner, J. D., Marshall, J., Attar, M., et al. (2019). Distinct fibroblast subsets drive inflammation and damage in arthritis. *Nature* 570 (7760), 246–251. doi:10.1038/s41586-019-1263-7

Croia, C., Bursi, R., Sutera, D., Petrelli, F., Alunno, A., and Puxeddu, I. (2019). One year in review 2019: pathogenesis of rheumatoid arthritis. *Clin. Exp. Rheumatol.* 37 (3), 347–357.

Cronstein, B. N., Buyon, J. P., and Abramson, S. B. (2020). Gerald weissmann: inflammation in rheumatic disease. *Ann. Rheum. Dis.* 79 (4), 435–436. doi:10.1136/annrheumdis-2019-216770

Dore, E., and Boilard, E. (2019). Roles of secreted phospholipase A2 group IIA in inflammation and host defense. *Biochim. Biophys. Acta (Bba) - Mol. Cell Biol. Lipids* 1864 (6), 789–802. doi:10.1016/j.bbalip.2018.08.017

Du, H., Wang, Y., Zeng, Y., Huang, X., Liu, D., Ye, L., et al. (2020). Tanshinone IIA suppresses proliferation and inflammatory cytokine production of synovial fibroblasts from rheumatoid arthritis patients induced by TNF-α and attenuates the inflammatory response in AIA mice. *Front. Pharmacol.* 11, 568. doi:10.3389/fphar.2020.00568

Duchez, A.-C., Boudreau, L. H., Naika, G. S., Rousseau, M., Cloutier, N., Levesque, T., et al. (2019). Respective contribution of cytosolic phospholipase A2α and secreted phospholipase A2 IIA to inflammation and eicosanoid production in arthritis. *Prostaglandins Other Lipid Mediators* 143, 106340. doi:10.1016/j.prostaglandins.2019.106340

Fang, W., Zhang, Y., and Chen, Z. (2020). Innate lymphoid cells in inflammatory arthritis. *Arthritis Res. Ther.* 22 (1), 25. doi:10.1186/s13075-020-2115-4

Fechtner, S., Singh, A., Chourasia, M., and Ahmed, S. (2017). Molecular insights into the differences in anti-inflammatory activities of green tea catechins on IL-1β signaling in rheumatoid arthritis synovial fibroblasts. *Toxicol. Appl. Pharmacol.* 329, 112–120. doi:10.1016/j.taap.2017.05.016

Feng, F.-B., and Qiu, H.-Y. (2018). Effects of Artesunate on chondrocyte proliferation, apoptosis and autophagy through the PI3K/AKT/mTOR signaling pathway in rat models with rheumatoid arthritis. *Biomed. Pharmacother.* 102, 1209–1220. doi:10.1016/j.biopha.2018.03.142

Fert-Bober, J., Darrah, E., and Andrade, F. (2020). Insights into the study and origin of the citrullinome in rheumatoid arthritis. *Immunol. Rev.* 294 (1), 133–147. doi:10.1111/imr.12834

Gartside, S. E., Yurttaser, A. E., Burns, A. L., Jovanović, N., Smith, K. J., Amegashiti, N. S., et al. (2020). A role for nitric oxide in serotonin neurons of the midbrain raphe nuclei. *Eur. J. Neurosci.* 51 (9), 1881–1899. doi:10.1111/ejn.14713

Gheorghe, K. R., Korotkova, M., Catrina, A. I., Backman, L., af Klint, E., Claesson, H.-E., et al. (2009). Expression of 5-lipoxygenase and 15-lipoxygenase in rheumatoid arthritis synovium and effects of intraarticular glucocorticoids. *Arth. Res. Ther.* 11 (3), R83. doi:10.1186/ar2717

Giresha, A. S., Pramod, S. N., Sathisha, A. D., and Dharmappa, K. K. (2017). Neutralization of inflammation by inhibiting in vitro and in vivo secretory phospholipase A2 by ethanol extract of Boerhaavia diffusa L. *Pharmacognosy Res.* 9 (2), 174–181. doi:10.4103/0974-8490.204650

Gomes, A., Bhattacharya, S., Mukherjee, S., Gomes, A., and Gomes, A. (2012). Inhibition of toxic actions of phospholipase A2 isolated & characterized from the Indian Banded Krait (Bungarus fasciatus) venom by synthetic herbal compounds. *Indian J. Med. Res.* 136 (1), 40–45.

Gou, K.-J., Zeng, R., Ren, X.-D., Dou, Q.-L., Yang, Q.-B., Dong, Y., et al. (2018). Anti-rheumatoid arthritis effects in adjuvant-induced arthritis in rats and molecular docking studies of Polygonum orientale L. extracts. *Immunol. Lett.* 201, 59–69. doi:10.1016/j.imlet.2018.11.009

Green, M. J., Gough, A. K., Devlin, J., Smith, J., Astin, P., Taylor, D., et al. (2003). Serum MMP-3 and MMP-1 and progression of joint damage in early rheumatoid arthritis. *Rheumatology* 42 (1), 83–88. doi:10.1093/rheumatology/keg037

Han, Z., Boyle, D. L., Chang, L., Bennett, B., Karin, M., Yang, L., et al. (2001). c-Jun N-terminal kinase is required for metalloproteinase expression and joint destruction in inflammatory arthritis. *J. Clin. Invest.* 108 (1), 73–81. doi:10.1172/JCI12466

Hartz, A. M. S., Rempe, R. G., Soldner, E. L. B., Pekcec, A., Schlichtiger, J., Kryscio, R., et al. (2019). Cytosolic phospholipase A2 is a key regulator of blood-brain barrier function in epilepsy. *FASEB J.* 33 (12), 14281–14295. doi:10.1096/fj.201901369RR

Hong, J.-I., Park, I. Y., and Kim, H. A. (2020). Understanding the molecular mechanisms underlying the pathogenesis of arthritis pain using animal models. *Ijms* 21 (2), 533. doi:10.3390/ijms21020533

Hu, Q., Jin, L., Zeng, J., Wang, J., Zhong, S., Fan, W., et al. (2020). Tryptophan metabolite-regulated Treg responses contribute to attenuation of airway inflammation during specific immunotherapy in a mouse asthma model. *Hum. Vaccin. Immunother.* 16 (8), 1891–1899. doi:10.1080/21645515.2019.1698900

Hu, T.-Y., Ju, J.-M., Mo, L.-H., Ma, L., Hu, W.-H., You, R.-R., et al. (2019). Anti-inflammation action of xanthones from Swertia chirayita by regulating COX-2/NF-κB/MAPKs/Akt signaling pathways in RAW 264.7 macrophage cells. *Phytomedicine* 55, 214–221. doi:10.1016/j.phymed.2018.08.001

Hyc, A., Moskalewski, S., and Osiecka-Iwan, A. (2016). Influence of cartilage interstitial fluid on the mRNA levels of matrix proteins, cytokines, metalloproteases and their inhibitors in synovial membrane. *Int. J. Mol. Med.* 38 (3), 937–942. doi:10.3892/ijmm.2016.2684

Jang, J.-H., Lee, S. H., Jung, K., Yoo, H., and Park, G. (2020). Inhibitory effects of Myricetin on lipopolysaccharide-induced neuroinflammation. *Brain Sci.* 10 (1), 32. doi:10.3390/brainsci10010032

Jia, X.-Y., Chang, Y., Sun, X.-J., Wei, F., Wu, Y.-J., Dai, X., et al. (2019). Regulatory effects of paeoniflorin-6′-O-benzene sulfonate (CP-25) on dendritic cells maturation and activation via PGE2-EP4 signaling in adjuvant-induced arthritic rats. *Inflammopharmacology* 27 (5), 997–1010. doi:10.1007/s10787-019-00575-8

Jiang, L., Xu, K., Li, J., Zhou, X., Xu, L., Wu, Z., et al. (2020). Nesfatin-1 suppresses interleukin-1β-induced inflammation, apoptosis, and cartilage matrix destruction in chondrocytes and ameliorates osteoarthritis in rats. *Aging* 12 (2), 1760–1777. doi:10.18632/aging.102711

Jiang, X., Lin, Q., Xu, L., Chen, Z., Yan, Q., Chen, L., et al. (2020). Indoleamine-2,3-Dioxygenase mediates emotional deficits by the kynurenine/tryptophan pathway in the ethanol addiction/withdrawal mouse model. *Front. Cel. Neurosci.* 14, 11. doi:10.3389/fncel.2020.00011

Kannaiyan, R., Shanmugam, M. K., and Sethi, G. (2011). Molecular targets of celastrol derived from Thunder of God Vine: potential role in the treatment of inflammatory disorders and cancer. *Cancer Lett.* 303 (1), 9–20. doi:10.1016/j.canlet.2010.10.025

Kashiwagi, H., Yuhki, K.-i., Imamichi, Y., Kojima, F., Kumei, S., Tasaki, Y., et al. (2019). Prostaglandin F2α facilitates platelet activation by acting on prostaglandin E2 receptor subtype EP3 and thromboxane A2 receptor TP in mice. *Thromb. Haemost.* 119 (8), 1311–1320. doi:10.1055/s-0039-1688906

Kim, H. J., Kim, H. M., Ryu, B., Lee, W.-S., Shin, J.-S., Lee, K.-T., et al. (2016). Constituents of PG201 (Layla), a multi-component phytopharmaceutical, with

inhibitory activity on LPS-induced nitric oxide and prostaglandin E2 productions in macrophages. *Arch. Pharm. Res.* 39 (2), 231–239. doi:10.1007/s12272-015-0654-z

Kim, J., Kim, Y., Yi, H., Jung, H., Rim, Y. A., Park, N., et al. (2015). Eupatilin ameliorates collagen induced arthritis. *J. Korean Med. Sci.* 30 (3), 233–239. doi:10.3346/jkms.2015.30.3.233

Kim, K.-W., Kim, H. J., Kim, B.-M., Kwon, Y.-R., Kim, H.-R., and Kim, Y.-J. (2018). Epigenetic modification of mesenchymal stromal cells enhances their suppressive effects on the Th17 responses of cells from rheumatoid arthritis patients. *Stem Cel Res Ther* 9 (1), 208. doi:10.1186/s13287-018-0948-4

Klein, R. F., Allard, J., Avnur, Z., Nikolcheva, T., Rotstein, D., Carlos, A. S., et al. (2004). Regulation of bone mass in mice by the lipoxygenase gene Alox15. *Science* 303 (5655), 229–232. doi:10.1126/science.1090985

Kozaki, T., Tagashira, M., Yamanishi, K., Ellis, B., Kayanoki, T., Ooishi, R., et al. (2015). Evaluation of drug-drug interaction between the novel cPLA2inhibitor AK106-001616 and methotrexate in rheumatoid arthritis patients. *Xenobiotica* 45 (7), 615–624. doi:10.3109/00498254.2014.1000430

Krizaj, I. (2014). Roles of secreted phospholipases $A_2$ in the mammalian immune system. *Protein Pept. Lett.* 21 (12), 1201–1208.

Krönke, G., Uderhardt, S., Katzenbeisser, J., and Schett, G. (2009). The 12/15-lipoxygenase pathway promotes osteoclast development and differentiation. *Autoimmunity* 42 (4), 383–385. doi:10.1080/08916930902832488

Leng, P., Li, D., Sun, Y., Wang, Y., and Zhang, H. (2018). Effects of human cyclooxygenase-2 gene silencing on synovial cells of rheumatoid arthritis mediated by lentivirus. *Artif. Cell Nanomed. Biotechnol.* 46 (Suppl. 3), S274–S280. doi:10.1080/21691401.2018.1491479

Li, H., Cao, X.-Y., Dang, W.-Z., Jiang, B., Zou, J., and Shen, X.-Y. (2019). Total Glucosides of Paeony protects against collagen-induced mouse arthritis via inhibiting follicular helper T cell differentiation. *Phytomedicine* 65, 153091. doi:10.1016/j.phymed.2019.153091

Li, J., Wei, Y., Li, X., Zhu, D., Nie, B., Zhou, J., et al. (2017). Herbal formula Xian-Fang-Huo-Ming-Yin regulates differentiation of lymphocytes and production of pro-inflammatory cytokines in collagen-induced arthritis mice. *BMC Complement. Altern. Med.* 17 (1), 12. doi:10.1186/s12906-016-1526-x

Li, Z., and Li, Y. (2019). Effect of growth hormone releasing hormone on chondrocytes of osteoarthritis. *Korean J. Intern. Med.* 2019, 399. doi:10.3904/kjim.2018.399

Lin, H.-C., Lin, T.-H., Wu, M.-Y., Chiu, Y.-C., Tang, C.-H., Hour, M.-J., et al. (2014). 5-Lipoxygenase inhibitors attenuate TNF-α-induced inflammation in human synovial fibroblasts. *PLoS One* 9 (9), e107890. doi:10.1371/journal.pone.0107890

Lin, N., Liu, C., Xiao, C., Jia, H., Imada, K., Wu, H., et al. (2007). Triptolide, a diterpenoid triepoxide, suppresses inflammation and cartilage destruction in collagen-induced arthritis mice. *Biochem. Pharmacol.* 73 (1), 136–146. doi:10.1016/j.bcp.2006.08.027

Liu, G., Xie, J., Shi, Y., Chen, R., Li, L., Wang, M., et al. (2020). Sec-O-glucosylhamaudol suppressed inflammatory reaction induced by LPS in RAW264.7 cells through inhibition of NF-κB and MAPKs signaling. *Biosci. Rep.* 40 (2), 230. doi:10.1042/BSR20194230

Liu, X.-L., Luo, L., Liu, B.-B., Li, J., Geng, D., Liu, Q., et al. (2014). Ethanol extracts from Hemerocallis citrina attenuate the upregulation of proinflammatory cytokines and indoleamine 2,3-dioxygenase in rats. *J. Ethnopharm.* 153 (2), 484–490. doi:10.1016/j.jep.2014.03.001

Luo, B., Que, Z.-J., Zhou, Z.-Y., Wang, Q., Dong, C.-S., Jiang, Y., et al. (2018). Feiji Recipe inhibits the growth of lung cancer by modulating T-cell immunity through indoleamine-2,3-dioxygenase pathway in an orthotopic implantation model. *J. Integr. Med.* 16 (4), 283–289. doi:10.1016/j.joim.2018.04.008

Mackel, J. J., Garth, J. M., Blackburn, J. P., Jones, M., and Steele, C. (2020). 12/15-Lipoxygenase deficiency impairs neutrophil granulopoiesis and lung proinflammatory responses to Aspergillus fumigatus. *J. Immunol.* 204 (7), 1849–1858. doi:10.4049/jimmunol.1900808

Magrioti, V., and Kokotos, G. (2013). Phospholipase A2inhibitors for the treatment of inflammatory diseases: a patent review (2010 - present). *Expert Opin. Ther. Patents* 23 (3), 333–344. doi:10.1517/13543776.2013.754425

Makarov, S. S. (2001). NF-kappa B in rheumatoid arthritis: a pivotal regulator of inflammation, hyperplasia, and tissue destruction. *Arthritis Res.* 3 (4), 200–206. doi:10.1186/ar300

Malaviya, R., Ansell, J., Hall, L., Fahmy, M., Argentieri, R. L., Olini, G. C., et al. (2006). Targeting cytosolic phospholipase A2 by arachidonyl trifluoromethyl ketone prevents chronic inflammation in mice. *Eur. J. Pharmacol.* 539 (3), 195–204. doi:10.1016/j.ejphar.2006.03.018

Martin, R., Gutierrez, B., Cordova, C., San Roman, A., Alvarez, Y., Hernandez, M., et al. (2020). Secreted phospholipase A2-IIA modulates transdifferentiation of cardiac fibroblast through EGFR transactivation: an inflammation-fibrosis link. *Cells* 9 (2), 396. doi:10.3390/cells9020396

Masferrer, J. L., Zweifel, B. S., Manning, P. T., Hauser, S. D., Leahy, K. M., Smith, W. G., et al. (1994). Selective inhibition of inducible cyclooxygenase 2 *in vivo* is antiinflammatory and nonulcerogenic. *Proc. Natl. Acad. Sci.* 91 (8), 3228–3232. doi:10.1073/pnas.91.8.3228

Massalska, M., Kuca-Warnawin, E., Janicka, I., Plebanczyk, M., Pawlak, D., Dallos, T., et al. (2019). Survival of lymphocytes is not restricted by IDO-expressing fibroblast from rheumatoid arthritis patients. *Immunopharm. Immunotoxicol.* 41 (2), 214–223. doi:10.1080/08923973.2019.1569048

Mateen, S., Zafar, A., Moin, S., Khan, A. Q., and Zubair, S. (2016). Understanding the role of cytokines in the pathogenesis of rheumatoid arthritis. *Clinica Chim. Acta* 455, 161–171. doi:10.1016/j.cca.2016.02.010

McHugh, J. (2017). FLIPping the switch on macrophages. *Nat. Rev. Rheumatol.* 13 (7), 390. doi:10.1038/nrrheum.2017.90

McHugh, J. (2019). FRA1 in macrophages promotes arthritis. *Nat. Rev. Rheumatol.* 15 (6), 320. doi:10.1038/s41584-019-0233-7

McInnes, I. B., and Schett, G. (2011). The pathogenesis of rheumatoid arthritis. *N. Engl. J. Med.* 365 (23), 2205–2219. doi:10.1056/NEJMra1004965

Merlo, L. M. F., Grabler, S., DuHadaway, J. B., Pigott, E., Manley, K., Prendergast, G. C., et al. (2017). Therapeutic antibody targeting of indoleamine-2,3-dioxygenase (Ido2) inhibits autoimmune arthritis. *Clin. Immunol.* 179, 8–16. doi:10.1016/j.clim.2017.01.016

Miller, M.-C., Manning, H. B., Jain, A., Troeberg, L., Dudhia, J., Essex, D., et al. (2009). Membrane type 1 matrix metalloproteinase is a crucial promoter of synovial invasion in human rheumatoid arthritis. *Arthritis Rheum.* 60 (3), 686–697. doi:10.1002/art.24331

Nakano, R., Kitanaka, T., Namba, S., Kitanaka, N., Suwabe, Y., Konno, T., et al. (2020). Non-transcriptional and translational function of canonical NF-κB signaling in activating ERK1/2 in IL-1β-induced COX-2 expression in synovial fibroblasts. *Front. Immunol.* 11, 579266. doi:10.3389/fimmu.2020.579266

Nishi, K., Gondaira, S., Okamoto, M., Nebu, T., Koiwa, M., Ohtsuka, H., et al. (2019). Effect of Mycoplasma bovis on expression of inflammatory cytokines and matrix metalloproteinases mRNA in bovine synovial cells. *Vet. Immunol. Immunopathol.* 216, 109920. doi:10.1016/j.vetimm.2019.109920

Nokhbehsaim, M., Nogueira, A. V. B., Nietzsche, S., Eick, S., and Deschner, J. (2020). Regulation of cyclooxygenase 2 by filifactor alocis in fibroblastic and monocytic cells. *Mediators Inflamm.* 2020, 1. doi:10.1155/2020/4185273

Pan, T., Cheng, T.-f., Jia, Y.-r., Li, P., and Li, F. (2017). Anti-rheumatoid arthritis effects of traditional Chinese herb couple in adjuvant-induced arthritis in rats. *J. Ethnopharmacology* 205, 1–7. doi:10.1016/j.jep.2017.04.020

Pan, X., Chen, T., Zhang, Z., Chen, X., Chen, C., Chen, L., et al. (2019). Activation of Nrf2/HO-1 signal with Myricetin for attenuating ECM degradation in human chondrocytes and ameliorating the murine osteoarthritis. *Int. Immunopharmacol.* 75, 105742. doi:10.1016/j.intimp.2019.105742

Park, K. C., Park, E. J., Kim, E. R., Kim, Y., Chung, S. H., Cho, B. W., et al. (2005). Therapeutic effects of PG201, an ethanol extract from herbs, through cartilage protection on collagenase-induced arthritis in rabbits. *Biochem. Biophys. Res. Commun.* 331 (4), 1469–1477. doi:10.1016/j.bbrc.2005.04.030

Peng, A., Lu, X., Huang, J., He, M., Xu, J., Huang, H., et al. (2019). Rheumatoid arthritis synovial fibroblasts promote TREM-1 expression in monocytes via COX-2/PGE2 pathway. *Arthritis Res. Ther.* 21 (1), 169. doi:10.1186/s13075-019-1954-3

Qureshi, O., and Dua, A. (2020). *COX inhibitors*, Treasure Island, FL: StatPearls.

Radhiga, T., Senthil, S., Sundaresan, A., and Pugalendi, K. (2019). Ursolic acid modulates MMPs, collagen-I, α-SMA, and TGF-β expression in isoproterenol-induced myocardial infarction in rats. *Hum. Exp. Toxicol.* 38 (7), 785–793. doi:10.1177/0960327119842620

Raichel, L., Berger, S., Hadad, N., Kachko, L., Karter, M., Szaingurten-Solodkin, I., et al. (2008). Reduction of cPLA2αoverexpression: an efficient anti-inflammatory therapy for collagen-induced arthritis. *Eur. J. Immunol.* 38 (10), 2905–2915. doi:10.1002/eji.200838545

Sano, H., Hla, T., Maier, J. A., Crofford, L. J., Case, J. P., Maciag, T., et al. (1992). *In vivo* cyclooxygenase expression in synovial tissues of patients with rheumatoid

arthritis and osteoarthritis and rats with adjuvant and streptococcal cell wall arthritis. *J. Clin. Invest.* 89 (1), 97–108. doi:10.1172/JCI115591

Scherer, H. U., Häupl, T., and Burmester, G. R. (2020). The etiology of rheumatoid arthritis. *J. Autoimmun.* 110, 102400. doi:10.1016/j.jaut.2019.102400

Schmitt-Sody, M., Gottschalk, O., Metz, P., Zysk, S., Hausdorf, J., von Schulze Pellengahr, C., et al. (2007). Endothelial iNOS versus platelet iNOS: responsibility for the platelet/leukocyte endothelial cell interaction in murine antigen induced arthritis *in vivo*. *Inflamm. Res.* 56 (6), 262–268. doi:10.1007/s00011-007-6171-x

Shin, S. S., Jin, M., Jung, H. J., Kim, B., Jeon, H., Choi, J. J., et al. (2003). Suppressive effects of PG201, an ethanol extract from herbs, on collagen-induced arthritis in mice. *Rheumatology* 42 (5), 665–672. doi:10.1093/rheumatology/keg209

Siouti, E., and Andreakos, E. (2019). The many facets of macrophages in rheumatoid arthritis. *Biochem. Pharmacol.* 165, 152–169. doi:10.1016/j.bcp.2019.03.029

Södergren, A., Karp, K., Bengtsson, C., Möller, B., Rantapää-Dahlqvist, S., and Wållberg-Jonsson, S. (2015). Is lipoprotein-associated phospholipase A2 a link between inflammation and subclinical atherosclerosis in rheumatoid arthritis?. *Biomed. Res. Int.* 2015, 1. doi:10.1155/2015/673018

Sommerfelt, R. M., Feuerherm, A. J., Jones, K., and Johansen, B. (2013). Cytosolic phospholipase A2 regulates TNF-induced production of joint destructive effectors in synoviocytes. *PLoS One* 8 (12), e83555. doi:10.1371/journal.pone.0083555

Sommerfelt, R. M., Feuerherm, A. J., Skuland, T., and Johansen, B. (2015). Cytosolic phospholipase A2 modulates TLR2 signaling in synoviocytes. *PLoS One* 10 (4), e0119088. doi:10.1371/journal.pone.0119088

Spel, L., and Martinon, F. (2020). Inflammasomes contributing to inflammation in arthritis. *Immunol. Rev.* 294 (1), 48–62. doi:10.1111/imr.12839

Stefanovic-Racic, M., Meyers, K., Meschter, C., Coffey, J. W., Hoffman, R. A., and Evans, C. H. (1994). N-monomethyl arginine, an inhibitor of nitric oxide synthase, suppresses the development of adjuvant arthritis in rats. *Arthritis Rheum.* 37 (7), 1062–1069. doi:10.1002/art.1780370712

Sumantran, V. N., Kulkarni, A. A., Harsulkar, A., Wele, A., Koppikar, S. J., Chandwaskar, R., et al. (2007). Hyaluronidase and collagenase inhibitory activities of the herbal formulation Triphala guggulu. *J. Biosci.* 32 (4), 755–761. doi:10.1007/s12038-007-0075-3

Sun, Y.-w., Bao, Y., Yu, H., Chen, Q.-j., Lu, F., Zhai, S., et al. (2020). Anti-rheumatoid arthritis effects of flavonoids from Daphne genkwa. *Int. Immunopharmacol.* 83, 106384. doi:10.1016/j.intimp.2020.106384

Tak, P. P., and Firestein, G. S. (2001). NF-κB: a key role in inflammatory diseases. *J. Clin. Invest.* 107 (1), 7–11. doi:10.1172/JCI11830

Takada, Y., and Fujita, M. (2016). Secreted phospholipase A2 type IIA (sPLA2-IIA) activates integrins in an allosteric manner. *Adv. Exp. Med. Biol.* 925, 103–115. doi:10.1007/5584_2016_95

Tao, X., Schulze-Koops, H., Ma, L., Cai, J., Mao, Y., and Lipsky, P. E. (1998).Effects of Tripterygium wilfordii hook F extracts on induction of cyclooxygenase 2 activity and prostaglandin E2 production. *Arthritis Rheum.* 41, 130–138. doi:10.1002/1529-0131(199801)41:1<130::aid-art16>3.0.co;2-4

Veihelmann, A., Landes, J., Hofbauer, A., Dorger, M., Refior, H. J., Messmer, K., et al. (2001). Exacerbation of antigen-induced arthritis in inducible nitric oxide synthase-deficient mice. *Arthritis Rheum.* 44.(6), 1420–1427. doi:10.1002/1529-0131(200106)44:6<1420::aid-art237>3.0.co;2-k

Viana, M. N., Leiguez, E., Gutiérrez, J. M., Rucavado, A., Markus, R. P., Marçola, M., et al. (2020). A representative metalloprotease induces PGE2 synthesis in fibroblast-like synoviocytes via the NF-κB/COX-2 pathway with amplification by IL-1β and the EP4 receptor. *Sci. Rep.* 10 (1), 3269. doi:10.1038/s41598-020-59095-z

Viji, V., and Helen, A. (2008). Inhibition of lipoxygenases and cyclooxygenase-2 enzymes by extracts isolated from Bacopa monniera (L.) Wettst. *J. Ethnopharmacol.* 118 (2), 305–311. doi:10.1016/j.jep.2008.04.017

Wan, Y., Li, D., Lv, Y., Wu, M., Li, L., and Yin, Z. (2019). Elevated levels of 15-lipoxygenase-1 contribute to the abnormal phenotypes of osteoblasts in human osteoarthritis. *Life Sci.* 239, 116980. doi:10.1016/j.lfs.2019.116980

Wan, Y., Lv, Y., Li, L., and Yin, Z. (2020). 15-Lipoxygenase-1 in osteoblasts promotes TGF-β1 expression via inhibiting autophagy in human osteoarthritis. *Biomed. Pharmacother.* 121, 109548. doi:10.1016/j.biopha.2019.109548

Wang, M. J., Huang, Y., Huang, R. Y., Chen, X. M., Zhou, Y. Y., Yu, W. L., et al. (2015). Determination of role of thromboxane A2 in rheumatoid arthritis. *Discov. Med.* 19 (102), 23–32.

Wang, W., Zhou, H., and Liu, L. (2020). The role of Chinese herbal medicine in the management of adverse drug reactions of leflunomide in treating rheumatoid arthritis. *Phytomedicine* 68, 153136. doi:10.1016/j.phymed.2019.153136

Wang, X., He, X., Zhang, C.-F., Guo, C.-R., Wang, C.-Z., and Yuan, C.-S. (2017). Anti-arthritic effect of berberine on adjuvant-induced rheumatoid arthritis in rats. *Biomed. Pharmacother.* 89, 887–893. doi:10.1016/j.biopha.2017.02.099

Wang, X., Tang, K., Wang, Y., Chen, Y., Yang, M., Gu, C., et al. (2019). Elevated microRNA-145-5p increases matrix metalloproteinase-9 by activating the nuclear factor-κB pathway in rheumatoid arthritis. *Mol. Med. Rep.* 20 (3), 2703–2711. doi:10.3892/mmr.2019.10499

Wang, Z., Li, J., Zhang, J., and Xie, X. (2019). Sodium tanshinone IIA sulfonate inhibits proliferation, migration, invasion and inflammation in rheumatoid arthritis fibroblast-like synoviocytes. *Int. Immunopharmacol.* 73, 370–378. doi:10.1016/j.intimp.2019.05.023

Withrow, J., Murphy, C., Liu, Y., Hunter, M., Fulzele, S., and Hamrick, M. W. (2016). Extracellular vesicles in the pathogenesis of rheumatoid arthritis and osteoarthritis. *Arthritis Res. Ther.* 18 (1), 286. doi:10.1186/s13075-016-1178-8

Wu, M.-Y., Lin, T.-H., Chiu, Y.-C., Liou, H.-C., Yang, R.-S., and Fu, W.-M. (2012). Involvement of 15-lipoxygenase in the inflammatory arthritis. *J. Cell Biochem.* 113 (7), 2279–2289. doi:10.1002/jcb.24098

Xiao, S., Zhang, M., He, Z., and Wang, D. (2018). Celastrol attenuates symptoms of preeclampsia in rats by inhibiting matrix metalloproteinase-9. *Eur. J. Pharmacol.* 830, 33–38. doi:10.1016/j.ejphar.2018.04.025

Xiong, Y., Ma, Y., Kodithuwakku, N. D., Fang, W., Liu, L., Li, F., et al. (2019). Protective effects of Clematichinenoside AR against inflammation and cytotoxicity induced by human tumor necrosis factor-α. *Int. Immunopharmacol.* 75, 105563. doi:10.1016/j.intimp.2019.04.010

Yang, P., Qian, F., Zhang, M., Xu, A.-L., Wang, X., Jiang, B., et al. (2020). Zishen Tongluo formula ameliorates collagen-induced arthritis in mice by modulation of Th17/Treg balance. *J. Ethnopharmacol.* 250, 112428. doi:10.1016/j.jep.2019.112428

Yang, W., Wang, X., Xu, L., Li, H., and Wang, R. (2018). LOX inhibitor HOEC interfered arachidonic acid metabolic flux in collagen-induced arthritis rats. *Am. J. Transl Res.* 10 (8), 2542–2554.

Yoo, W.-H., Yoo, H.-G., Park, S.-H., Baek, H.-j., Lee, Y. J., Shim, S. C., et al. (2014). Efficacy and safety of PG201 (Layla) and celecoxib in the treatment of symptomatic knee osteoarthritis: a double-blinded, randomized, multi-center, active drug comparative, parallel-group, non-inferiority, phase III study. *Rheumatol. Int.* 34 (10), 1369–1378. doi:10.1007/s00296-014-2964-8

Yoshihara, Y., Nakamura, H., Obata, K., Yamada, H., Hayakawa, T., Fujikawa, K., et al. (2000). Matrix metalloproteinases and tissue inhibitors of metalloproteinases in synovial fluids from patients with rheumatoid arthritis or osteoarthritis. *Ann. Rheum. Dis.* 59 (6), 455–461. doi:10.1136/ard.59.6.455

Zaichko, K., Stanislavchuk, M., and Zaichko, N. (2020). Circadian fluctuations of endothelial nitric oxide synthase activity in females with rheumatoid arthritis: a pilot study. *Rheumatol. Int.* 40 (4), 549–554. doi:10.1007/s00296-020-04525-4

Zhao, A., Li, L., Li, B., Zheng, M., and Tsao, R. (2016). Ultrafiltration LC-ESI-MSn screening of 5-lipoxygenase inhibitors from selected Chinese medicinal herbs Saposhnikovia divaricata, Smilax glabra, Pueraria lobata and Carthamus tinctorius. *J. Funct. Foods* 24, 244–253. doi:10.1016/j.jff.2016.04.011

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

SYSTEMATIC REVIEW
published: 26 April 2021
doi: 10.3389/fphar.2021.673984

# Efficacy and Safety of Adalimumab in Noninfectious Uveitis: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

*Biao Li, Haoran Li, Li Zhang and Yanlin Zheng**

*Department of Ophthalmology, Affiliated Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, China*

**Background:** Patients with noninfectious uveitis (NIU) are at risk of systemic side effects of long-term glucocorticoid therapy and uncontrolled inflammatory complications. In urgent need to identify more aggressive therapies, adalimumab (ADA) may be the right choice.

**Objectives:** To summarize the current evidence from randomized controlled trials (RCTs) regarding the efficacy and safety of ADA in the treatment of NIU.

**Methods:** We searched Pubmed, Embase, Web of Science, Cochrane Library databases, and Clinical Trials Registry for qualifying articles from their inception to November 19, 2020, with no language restriction. Randomized controlled trials comparing ADA with conventional routine treatment in noninfectious uveitis patients of any age, gender, or ethnicity were included. The primary outcome was the time to treatment failure (TF). The secondary outcomes were the change in best-corrected visual acuity (BCVA), change in the anterior chamber (AC) cell grade, change in vitreous haze (VH) grade, and adverse events (AEs).

**Main results:** The six studies comprised 605 participants in all, and the sample size of each study ranged from 16 to 225. The overall pooled results of the primary outcome (HR = 0.51; 95% CI, 0.41 to –0.63) showed that ADA nearly halved the risk of treatment failure compared to placebo for NIU patients. The pooled mean difference of change in BCVA was -0.05 (95% CI, –0.07 to –0.02). The pooled mean difference of change in AC cell grade and VH grade was –0.29 (95% CI, –0.62 to –0.05) and –0.21 (95% CI, –0.32 to –0.11), respectively. The incidence of AEs in the ADA group was numerically higher than that of AEs in the placebo group (2,237 events and 9.40 events per patient-year, equivalent to 1,257 events and 7.79 events per patient-year).

**Conclusion:** This meta-analysis of six RCTs further confirmed that ADA considerably lowered the risk of treatment failure or visual loss, and moderately reduced AC cell grades and VH grades with slightly more AEs, as compared to placebo. ADA is both effective and safe in treating NIU.

**Systematic Review Registration:** [https://clinicaltrials.gov], identifier [CRD42020217909].

**Keywords: adalimumab, noninfectious uveitis, anti-TNF-α, treatment, meta-analysis**

OPEN ACCESS

***Edited by:***
*Maria Efthymiou,*
*University College London,*
*United Kingdom*

***Reviewed by:***
*Inês Cardoso Leal,*
*Santa Maria Hospital, Portugal*
*Victor Llorens,*
*Hospital Clínic de Barcelona, Spain*

****Correspondence:***
*Yanlin Zheng*
*zhengyanlin@cdutcm.edu.cn*

***Specialty section:***
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

***Received:*** *28 February 2021*
***Accepted:*** *07 April 2021*
***Published:*** *26 April 2021*

***Citation:***
*Li B, Li H, Zhang L and Zheng Y (2021) Efficacy and Safety of Adalimumab in Noninfectious Uveitis: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Front. Pharmacol. 12:673984. doi: 10.3389/fphar.2021.673984*

## INTRODUCTION

Noninfectious uveitis (NIU) encompasses a heterogeneous collection of ocular disorders related to different etiologies, characterized by intraocular inflammation in the absence of infection (Airody et al., 2016; Dick et al., 2016). It is generally believed that noninfectious uveitis is an immune-mediated ocular inflammation frequently accompanied by systemic autoimmune diseases such as juvenile idiopathic arthritis, Behcet syndrome, or ankylosing spondylitis (Cordero-Coma and Sobrin, 2015; Schwartzman and Schwartzman, 2015). The mean prevalence of uveitis in Europe is 144.85 in 100,000 people, while NIU approximately accounts for 70% of uveitis (Llorenç et al., 2015). Simultaneously, the gross prevalence of NIU in American adults is roughly calculated to be 121/100,000 (Dick et al., 2016). It is estimated that the risk of blindness or low vision in patients with NIU is ten times higher than that in people without NIU (Durrani et al., 2004). NIU accounted for approximately 20% of legal blindness in developed countries, causing a massive burden to society (Nussenblatt, 2005; Wakefield and Chang, 2005; Jabs et al., 2013).

The treatment principle of NIU is to control intraocular inflammation, prevent relapses of inflammation, and reduce drug-related side effects. Currently, corticosteroids and immunosuppressants remain the mainstay of treatment drugs, which sometimes fail to control inflammation and frequently cause well-known ocular and systemic adverse effects (Sen et al., 2014; Miloslavsky et al., 2017; Niederer et al., 2017; Suhler et al., 2017). Therefore, it is urgent to identify more effective and safer therapies that target specific immune response mediators to achieve and maintain inflammation remission. Moreover, adalimumab (ADA) may be the one (Suhler et al., 2013; Levy-Clarke et al., 2014).

Adalimumab (Humira®; AbbVie Inc.) is a full-length human monoclonal antibody that uniquely targets TNF-α and counteracts its biological activity (Burmester et al., 2013; Airody et al., 2016; Balevic and Rabinovich 2016). Currently, ADA is the only biologic that has been proven effective to NIU by randomized-control, double-blind phase III studies (Jaffe et al., 2016; Nguyen et al., 2016). Consequently, the United States, European countries, Japan, and China have successively approved ADA to treat NIU. Although it received approval in these countries, ADA has not yet been widely used worldwide.

The most recent systematic review on the ADA for NIU dates back to 2018 (Ming et al., 2018), while several relevant articles have been published after that. Moreover, all the existing systematic reviews on this topic are mainly based on observational studies and rarely include randomized controlled trials (RCTs) which lead to their low quality of evidence. So we herein conducted a systematic review with meta-analysis to synthesize the currently accessible evidence from RCTs to assess ADA's efficacy and safety in NIU.

## ARTICLE TYPES

A Systematic Review and Meta-Analysis of RCTs.

## MANUSCRIPT FORMATTING

## Methods

This meta-analysis was conducted following the Preferred Reporting Items for Systematic Review and Meta-analysis statements (Shamseer et al., 2015), with the protocol registered in the Prospero database (CRD42020217909).

### Study Design and Interventions

The main inclusion criteria are as follows: 1) randomized controlled trials comparing ADA with conventional routine treatment (such as local and systemic corticosteroids, immunosuppressants) in patients of any age, gender, or ethnicity with a diagnosis of NIU; 2) the mean follow-up duration was more than three months; 3) sample size greater than 10; 4) AC cell grade and VH grade were evaluated by Standardization of Uveitis Nomenclature (SUN) criteria (Jabs, Nussenblatt, and Rosenbaum, 2005). We excluded studies that met any of the following conditions: 1) duplicate reports on the same study; 2) inadequate data or information; 3) control group was not placebo.

### Data Sources and Search Strategy

Pubmed, Embase, Web of Science, Cochrane Library, and Clinical Trials Registry were searched for relevant literature from their inception to November 19, 2020, regardless of language. If necessary, the researchers were contacted for more data. The search was limited to abstract/keyword/title fields. The search terms included uveitis, iridocyclitis, retinitis, retinal vasculitis, panuveitis, uveit*, adalimumab, ADA, Humira, TNF, TNF-a, anti-tumor necrosis factor-alpha, randomized controlled trial, and clinical trial. The Boolean operators appropriately connected these keywords. Study design types were restricted to randomized controlled trials (RCTs).

### Study Selection and Exclusion Processes

According to the inclusion criteria, two reviewers (Biao Li and Haoran Li) independently assessed the relevant studies for eligibility. Any disagreements were resolved through discussion among ourselves.

### Outcomes Assessment

The primary outcome was the time to treatment failure (TTF), a rigorous composite outcome composed of four components (new ocular inflammatory lesions, BCVA, AC cell grade, and VH grade). "Treatment failure" was defined by the presence of one or more of the following factors: 1. new active, inflammatory lesions relative to baseline; 2. a two-step increase in anterior chamber cell or vitreous haze grade; 3. a worsening of best-corrected visual acuity by 15 or more letters on the Early Treatment Diabetic Retinopathy Study chart, relative to the best state previously achieved, in at least one eye; 4. sustained non-improvement with entry grade of ≥3; 5. use of concomitant medications not allowed; and 6. intermittent or continuous suspension of study treatment (adalimumab or placebo) for a cumulative period of longer than four weeks. The secondary efficacy outcomes included change in BCVA (logMAR), change in AC cell grade, and VH grade (according to SUN). The

safety outcome was the number and the rate of adverse events (per patient-years).

### Data Extraction

Two authors (Biao Li and Haoran Li) extracted the data from the included publications into standard forms independently and cross-checked them to ensure accuracy. Differences were settled by discussion and transferred to a third author if needed. The information captured included: first author's last name, published date, number of patients in each group, demographic data, follow-up time, and definitions of endpoints. If the same registered trial data appeared in multiple articles, the article with the latest or most comprehensive data was included.

### Data Analysis

Two reviewers (Biao Li and Li Zhang) independently evaluated the quality of the included studies using the recommended Cochrane Collaboration tool for assessing the risk of bias, which consists of seven types of risks of bias: random sequence generation; incomplete outcome data; allocation concealment; selective reporting; blinding of participants and personnel; blinding of outcome assessment; for-profit bias. Disagreements were settled by discussion and transferred to a third author if needed. We conduct our statistical analysis using review manager version 5.3 software according to the intention to treat analysis method. The evaluation of outcomes was done per eye, except TF and AEs, mainly pooled per patient. For continuous endpoints such as BCVA, AC cell grade, and VH grade, we preferentially retrieved mean differences with 95% CI. When meta-analysis was not proper for certain types of data we narratively summarize the relevant results.

The heterogeneity of the pooled results was assessed by Cochrane's Q test and Higgins' $I^2$. If apparent heterogeneity existed ($p < 0.1$ or $I^2 > 50\%$), the pooled results were estimated using the random-effects model. Alternatively, the fixed-effects model was adopted. Besides, we deleted each study to assess each study's impact on the overall risk estimate to examine the results' robustness. Subgroup analysis was initially planned according to the type of uveitis, study location, follow-up time duration, and participants' age. Unfortunately, we do not have enough sample size to perform these analyses.

We originally planned to examine the publication bias of included studies by funnel plots and Egger's test. Unfortunately, we lack enough studies to conduct these analyses.

### Confidence in Cumulative Evidence

The GRADE system was used to access the evidence's quality of every efficacy outcomes. GRADE system scored the evidence of each outcome in five aspects: limitations of the study design and execution, inconsistency, indirectness, imprecision of results, and publication bias. Accordingly, we classify ADA treatment's recommendation level as very low, low, medium, or high.

## Results

### Study Selection

Our literature search yielded 918 articles (Pubmed: 335; Embase: 292; Web of Science: 161; Cochrane Library: 113; ClinicalTtrials.gov: 17). After removing duplicates, 616 articles remain. Of these, 568 were excluded after screening for the titles and abstracts. After the full-text examination of the remaining 48 articles, five articles (6 trials, there was one article containing the data of two RCTs) met inclusion criteria for our meta-analysis (**Figure 1**).

### Study Characteristic

All six studies used intention-to-treat analysis to evaluate efficacy and safety outcomes. Six hundred thirty-six patients were enrolled in six studies, including 318 in the ADA group and 287 in the control group. The sample size ranged from 16 to 225. Overall, 61.23% (368/601) of patients were female, with the sex distribution favored females in each of the six studies. The mean age varied from 8.90 to 50.90 years old, and the mean uveitis duration was between 43.75 and 94.56 months. **Table 1** summarises the characteristics of the six selected RCTs.

### Quality Assessment

We judged for-profit bias in all six studies as high risk since five of the included studies were sponsored by pharmaceutical companies (AbbVie), while the remaining one studies had participants who received remuneration such as speaker's fees from AbbVie.

Since only outcome assessors were blinded in Mackensen's study, the risk of performance bias was considered high, and the detection bias was assessed as low risk. In the other five studies, the risk regarding blinding were all considered low risk since the methods of blinding patients, doctors, and outcome assessors were described in detail. The remaining four types of risk of bias were all considered low risk in six studies because the corresponding evidence can be found in the article. As prospective trial registrations were accomplished in all studies and their prespecified outcomes were reported, selective reporting was considered low risk. **Figure 2** demonstrates the risk of bias in each study.

### Synthesis of Results

The pooled results included the time to treatment failure, change in AC cell grade, change in VH grade, and change in BCVA (logMAR). **Table 2** demonstrates the relevant results of the including studies.

#### *Time to Treatment Failure*

The hazard ratio (HR) to treatment failure was reported in five studies. All studies reported the HRs ranging from 0.25 to 0.57, except for the VISUAL I Japan (HR = 1.2, 95% CI, 0.41–3.51). The pooled results of all the five studies (HR = 0.51; 95% CI, 0.41–0.63) showed that ADA nearly halved the risk of treatment failure compared to placebo. Heterogeneity was not significant by the Q statistic (6.49 on 4 df, $p = 0.17$) and by $I^2$ (38%). (**Figure 3**)

#### *Change in BCVA (logMAR)*

A total of five studies involving 856 patients presented the change in BCVA (logMAR) after the intervention. The mean difference of the change in BCVA ranged between −0.08 and 0.04 ($p < 0.05$). The pooled estimate mean difference favored

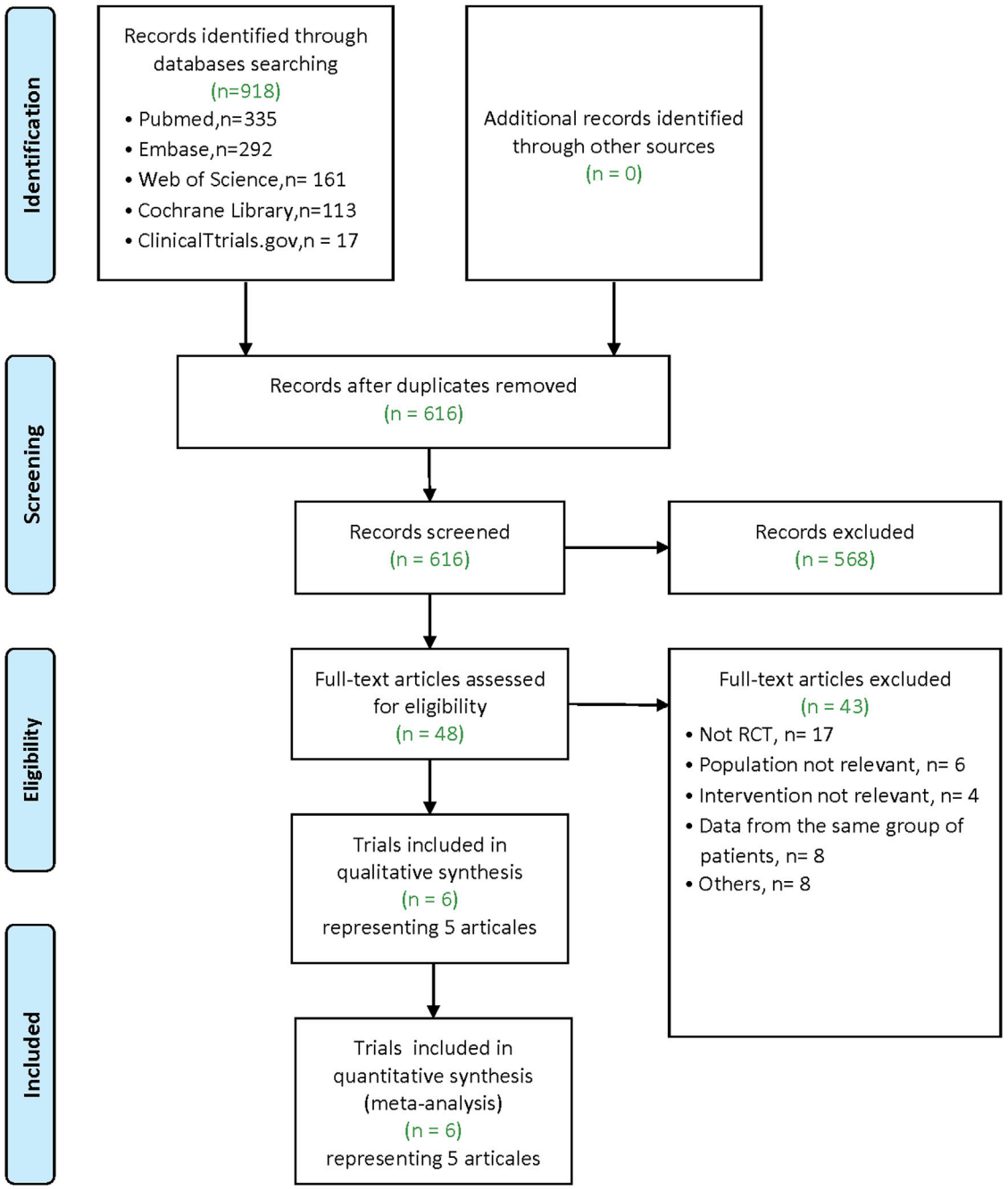


**FIGURE 1 |** Flow diagram.

patients who received ADA (MD = −0.05, 95% CI, −0.07 to −0.02, $p = 0.0004$). The Q statistic (2.91 on 5 df, $p = 0.71$) and $I^2$ (0%) indicated low heterogeneity in the pooled studies. (**Figure 4**)

### *Change in AC Cell Grade*

Of the six studies evaluating change in AC cell grade, five studies found that the ADA group's AC cell grade improved significantly more than the placebo group, while one trial reported the opposite result. The mean difference was ranging from −0.79 to 0.22 ($p < 0.05$). The pooled mean difference was −0.29 (95% CI, −0.62 to 0.05), not showing a significant difference between ADA and placebo groups. Both the Q statistic (38.58 on 5 df, $p < 0.01$) and by $I^2$ (87%) demonstrated significant heterogeneity, which indicated that a random-effects model was preferable.

Additionally, we excluded each study's estimates to examine each study's influence on the overall results. After excluding

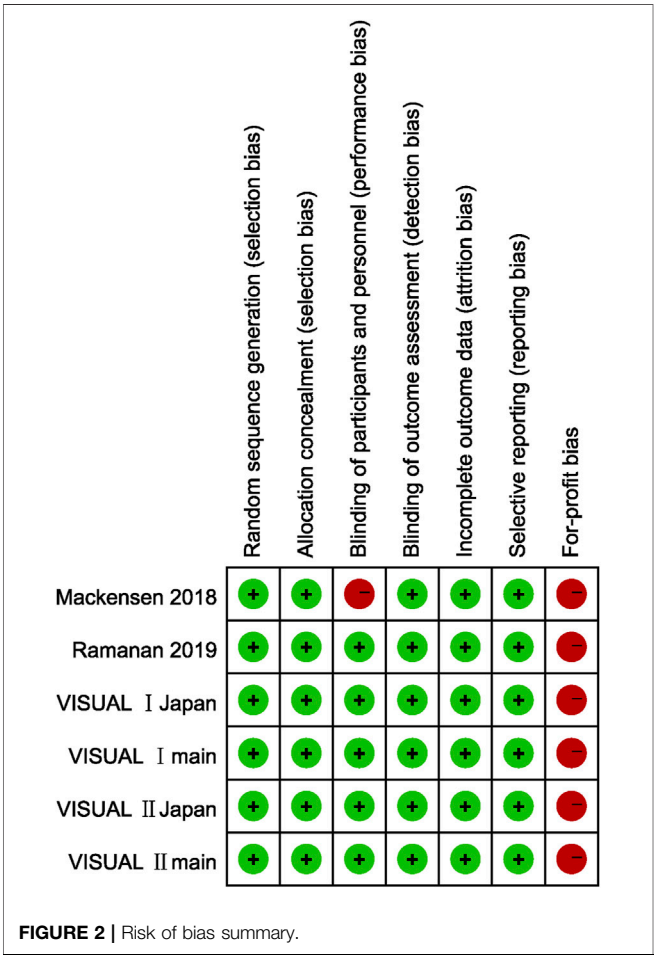


**FIGURE 2 |** Risk of bias summary.

VISUAL Ⅰ Japan, the overall results have changed a lot that the diamond marker does not intersect with 0, showing that the AC cell grade was significantly better in the ADA group than the placebo group (MD = −0.39, 95% CI: −0.72, −0.06).

In VISUAL I Japan, AC cell grade was numerically higher in the ADA group (MD = 0.22; 95% CI, −0.17, −0.61) since one patient in the placebo group did not experience treatment failure from the beginning to end. Given the small sample size, this patient strongly impacted all the outcomes including AC cell grade. (**Figure 5**)

### *Change in VH Grade*

Five studies reported Change in VH grade, and the mean difference was ranging from −0.54 to −0.13 ($p < 0.05$). The pooled mean difference in VH grade change was −0.21, with a 95% CI (−0.32, −0.11), suggesting that the VH grade improved significantly more in the ADA group than the control group. Heterogeneity was low by the Q statistic (3.70 on 4 df, $p = 0.45$) and by $I^2$ (0%). After removing any one study, the pooled results did not change significantly, and the estimates in each case were well within the confidence range of the overall estimate. (**Figure 6**)

### *Safety*

All six RCTs have safety information with 601 patients. A total of 3,494 AEs occurred during 406.5 patient-years, and the overall incidence of AEs was 8.60 events per patient-year. There were 2,237 AEs in the ADA group, and the overall incidence was 9.40 events per patient-year, numerically higher than those of the 1,257 total events and 7.79 events per patient-year in the placebo group. **Table 3** shows the results of the safety. Six studies reported a total of 59 serious adverse events, of which 66% (39) occurred in the ADA group, indicating that the risk of serious adverse events in the ADA group was twice that of the placebo group.

The most common AEs were injection-site reactions and allergic reactions. The AEs reported in six studies were similar to those reported in previous studies and no new AEs occurred.

## Risk of Bias Across Studies

It cannot be performed due to the small sample size.

# Discussion

## Summary of Main Findings

This meta-analysis of six RCTs including 605 patients systematically reviewed ADA's efficacy and safety in NIU. The results show that ADA almost halved the risk of NIU patients' treatment failure by significantly improving BCVA and reducing the AC cell grade and VH grade. The incidence of ADA-related AEs was generally low, and the safety profile was

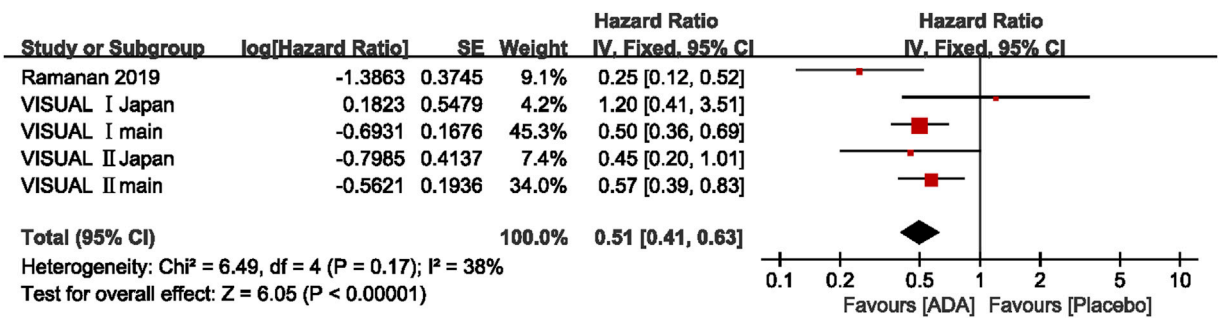


**FIGURE 3 |** Forest plot of time to treatment failure.

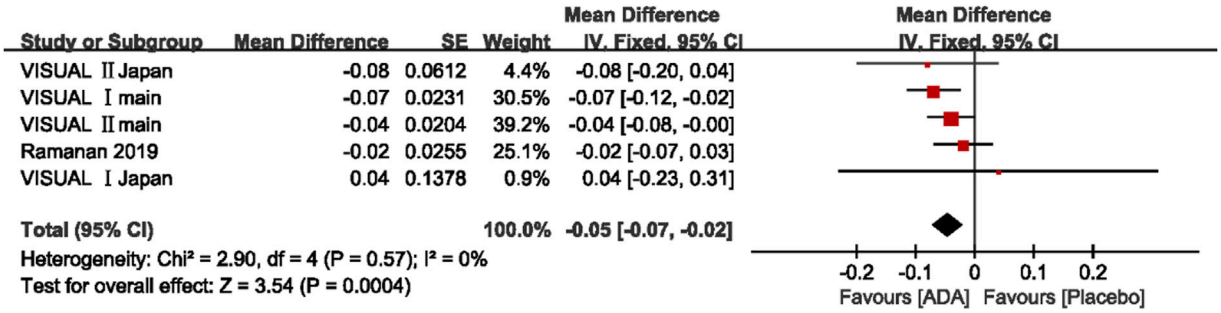


**FIGURE 4 |** Forest plot of BCVA.

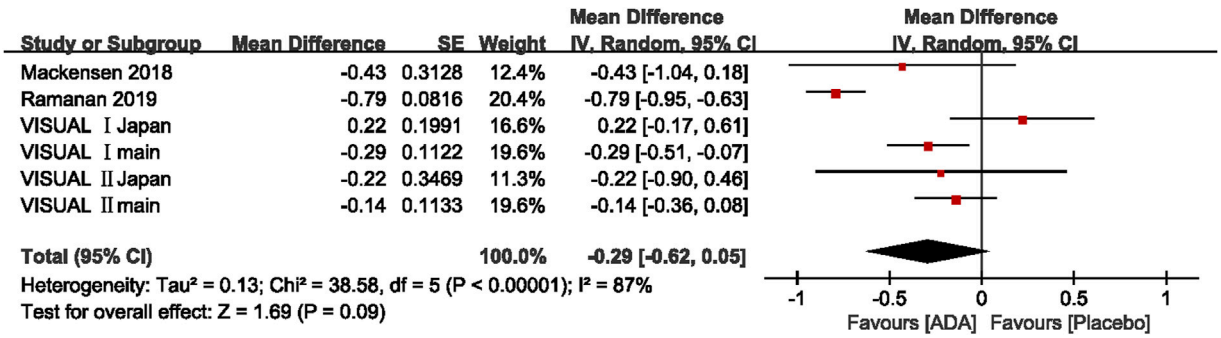


**FIGURE 5 |** Forest plot of AC cell grade.

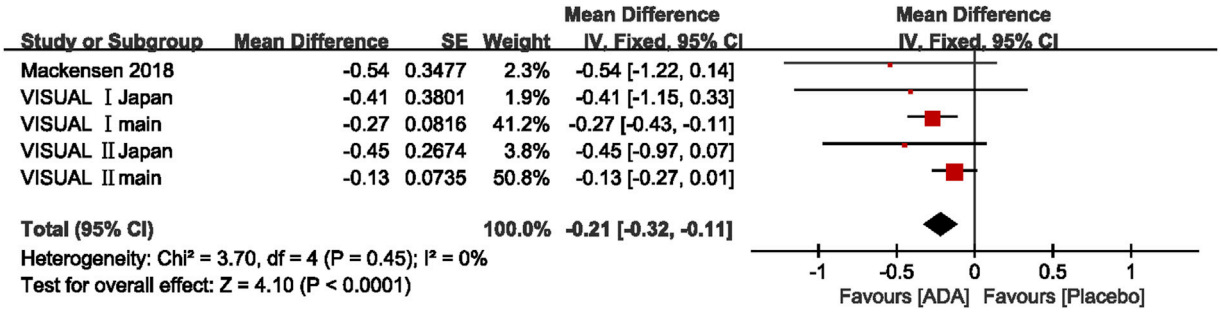


**FIGURE 6 |** Forest plot of VH grade.

similar to other reports in previous studies. Significant differences favoring ADA over placebo was seen for two secondary endpoints (change in BCVA and VH grade). Outcomes regarding AC cell grade in the ADA group were numerically superior to that in the placebo group.

## GRADE

According to the GRADE, the certainty of the evidence concerning four efficacy outcomes were all judged as moderate. **Table 4** shows the summary of GRADE's. Moderate-quality evidence shows that ADA considerably lowered the risk of treatment failure or visual impairment, moderately reduced AC cell grades and VH grades in NIU.

## Comparison to Prior Reviews

The efficacy and safety results mentioned above were consistent with those of a meta-analysis by Shuai Ming et al. Their review included 20 non-RCTs and three RCTs. In contrast, our study included six RCTs and excluded non-RCTs, leading to a higher quality of evidence.

A similar article entitled "Anti-TNF Drugs for Chronic Uveitis in Adults—A Systematic Review and Meta-Analysis of Randomized Controlled Trials" has been published by Leal

**TABLE 1 |** Characteristics of the included RCTs.

| | Study, study dates, setting, type of study, registration number | Sample size (ADA/placebo), mean age, % of females, type of uveitis, uveitis duration | Population: diagnosis | Intervention and comparator | Outcomes |
|---|---|---|---|---|---|
| VISUAL I main | • August 2010–August 2014<br>• 67 sites, 18 countries<br>• Multicenter, double-masked, randomized Placebo-controlled Phase 3 trial<br>• NCT 01138657 | • *n* = 217 (110/107), age 42.65 (14.89) years 57% female<br>• Active uveitis<br>• Intermediate 22%, posterior 33%, pan 45%<br>• Bilateral 91%, unilateral 9%<br>• 45.53 (62.53) months | • Idiopathic 37%<br>• Sarcoidosis 8%<br>• Behçet's disease 7%<br>• VKH 12%<br>• Birdshot chorioretinopathy 20%<br>• Multifocal choroiditis and panuveitis 5%<br>• Other 10% | • Intervention: subcutaneous ADA, 80 mg loading dose followed by 40 mg dose eow<br>• comparator: placebo<br>• prednisone burst for all at week 0, tapering to 0 by week < 15 | • Primary outcome: TTF (worsening of one or more of AC grade, VH grade, BCVA, or new inflammatory lesions) at/after week 6, one or more eyes<br>• Secondary outcomes: BCVA, change in VH or AC grade, % change in CRT, time to MO, change in VFQ-25 score, AEs |
| VISUAL II main | • August 2010–May 2015<br>• 72 sites, 22 countries<br>• Multicenter, double-masked, randomized placebo-controlled, phase 3 trial | • *n* = 225 (114/111), age 42.56 (13.43) years 57% female<br>• Inactive uveitis<br>• Intermediate 21%, posterior 33%, pan 46%<br>• Bilateral 96%, unilateral 4%<br>• 61.17 (65.97) months | • Idiopathic 31% I<br>• Sarcoidosis 14%<br>• Behçet's disease 7%<br>• VKH 23%<br>• birdshot chorioretinopathy 13%<br>• multifocal choroiditis and Panuveitis 3%<br>• other 9% | • Intervention: subcutaneous ADA, 80 mg loading dose followed by 40 mg dose eow<br>• Comparator: placebo<br>• Prednisone burst for all at week 2, tapering to 0 by week 15 | • Primary outcome: TTF (worsening of one or more of AC grade, VH grade, BCVA, or new inflammatory lesions) at after week 2, one or more eyes<br>• Secondary outcomes: BCVA, change in VH or AC grade, % change in CRT, time to MO, change in VFQ-25 score, AEs |
| VISUAL I Japan | • August 2010–August 2014<br>• 7 sites in Japan<br>• Multicenter, double-masked, randomized Placebo-controlled Phase 3 trial<br>• NCT 01138657 | • *n* = 16 (8/8), age 50.9 (14.72) years, 59% female<br>• Active uveitis<br>• Intermediate 6%, posterior 13%, pan 81%<br>• Bilateral 87.5% Unilateral 12.5%<br>• 57.15 (75.70) months | • Idiopathic 44%<br>• Sarcoidosis 38%<br>• Behçet's disease 12%<br>• VKH 6% | • Intervention: subcutaneous ADA, 80 mg loading dose followed by 40 mg dose eow<br>• Comparator: Placebo<br>• Prednisone burst for all at week 0, tapering to 0 by week 15 | • Primary outcome: TTF (worsening of one or more of AC grade, VH grade BCVA, or new inflammatory lesions) at/after week 6, one or more eyes<br>• Secondary outcomes: BCVA, change in VH or AC grade, % changein CRT, time to MO, change in VFQ-25 score, AEs |
| VISUAL II Japan | • August 2010–May 2015<br>• 7 sites in Japan<br>• Multicenter, double-masked, randomized Placebo-controlled Phase 3 trial<br>• NCT 01124838 | • *n* = 32 (16/16), age 46.8 (12.49) years, 59% female<br>• Active uveitis<br>• Intermediate 0%, posterior 9%, pan 91%<br>• Bilateral 91%, unilateral 9%<br>• 43.75 (38.13) months | • Idiopathic 25%<br>• Sarcoidosis 31%<br>• Behçet's disease 3%<br>• VKH 38%<br>• Other 3% | • Intervention: subcutaneous ADA, 80 mg loading dose followed by 40 mg dose eow<br>• Comparator: placebo<br>• Prednisone burst for all at week 2, tapering to 0 by week 15 | • Primary outcome: TTF (worsening of one or more of AC grade, VH grade, BCVA, or new inflammatory lesions) at after week 2, one or more eyes<br>• Secondary outcomes: BCVA, change in VH or AC grade, % change in CRT, time to MO, change in VFQ-25 score, AEs |
| Mackensen 2018 | • May 2007–August 2012<br>• 2 centers<br>• Randomized, prospective, controlled Two-center clinical trial<br>• NCT 00348153 | • *n* = 25 (10/15), age 36 years, 60% female<br>• Active uveitis<br>• Anterior 60%, posterior + pan 40%-<br>• 94.56 months | • JIA: 8%<br>• Spondyloarthritis: 16%<br>• GPA/Behçet's/ sarcoidosis: 20%<br>• HLA-B27B: 24% | • Intervention: ADA (40 mg subcutaneous injection eow)<br>• Comparator: blank<br>• Both arms continued previous immunosuppressive therapy and received a corticosteroid bolus of 1 mg/kg bw, with a fixed standardized tapering scheme | • Primary outcome: change in visual acuity<br>• Secondary outcomes: extent of macular edema, intraocular inflammatory Activity (SUN), the number of treatment arm switchers, the cumulative systemic corticosteroid dose, AEs |

(Continued on following page)

**TABLE 1 |** (*Continued*) Characteristics of the included RCTs.

| | Study, study dates, setting, type of study, registration number | Sample size (ADA/placebo), mean age, % of females, type of uveitis, uveitis duration | Population: diagnosis | Intervention and comparator | Outcomes |
|---|---|---|---|---|---|
| Ramanan 2019 | • October 2011–June 2015<br>• 14 centers in the United Kingdom<br>• Randomized, parallel-group, double-blind Placebo-controlled Multicenter clinical trial<br>• ISRCTN 10065623 | • *n* = 90 (60/30), age 8.90 (3.88) years, 78% female<br>• Active uveitis<br>• Bilateral 28%, unilateral 72%<br>• 63.96 (42.36) months | JIA-associated uveitis 100% | • Intervention: ADA (20 mg/0.8 ml for patients weighing <30 kg or 40 mg/0.8 ml for patients weighing ≥30 kg by subcutaneous injection eow)<br>• Comparator: placebo<br>• All participants received a stable dose of MTX | • Primary outcome: TTF (multicomponent score as defined by set criteria based on the SUN criteria)<br>• Secondary outcomes: number of participants failing treatment, BCVA use of corticosteroids, safety, tolerability, compliance |

*ADA, adalimumab; eow, every other week; AC, anterior chamber; AE, adverse event; CRT, central retinal thickness; JIA, juvenile idiopathic arthritis; LFP laser flare photometry; MTX, methotrexate; SL; SUN, standardization of uveitis nomenclature; TTF, time to treatment failure; VH, vitreous haze.*

**TABLE 2 |** Summary of the results of individual studies.

| | Time to treatment failure, HR | BCVA (change) (logMAR), MD | AC cell grade (change), MD | VH grade (change), MD |
|---|---|---|---|---|
| VISUAL I main | 0.50 (0.36, 0.70) | −0.07 (−0.11, −0.02) | −0.29 (−0.51, −0.07) | −0.27 (−0.43, −0.11) |
| VISUAL II main | 0.57 (0.39, 0.84) | −0.04 (−0.08, 0.01) | −0.14 (−0.37, 0.08) | −0.13 (−0.28, 0.01) |
| VISUAL I Japan | 1.20 (0.41, 3.54) | 0.04 (−0.22, 0.31) | 0.22 (−0.17, 0.61) | −0.41 (−1.15, 0.34) |
| VISUAL II Japan | 0.45 (0.20, 1.03) | −0.08 (−0.20, 0.04) | −0.22 (−0.90, 0.46) | −0.45 (−0.98, 0.07) |
| Mackensen 2018 | NA | NA | −0.43 (−1.05, 0.18) | −0.54 (−1.22, 0.14) |
| Ramanan 2019 | 0.25 (0.12, 0.51) | −0.02 (−0.07, 0.02) | −0.79 (−0.96, −0.63) | NA |

*NA, not available; MD, mean difference; SD, standard deviation; HR, hazard ratio; BCVA, best-corrected visual acuity; AC, anterior chamber; VH, vitreous haze; logMAR, logarithm of the minimum angle of resolution.*

**TABLE 3 |** summary of safety result.

| AE summery | Sample size (ADA/placebo) | ADA | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | AEs, no. of events | AEs, events/ patient-years | SAEs, no. of events | SAEs, events/ patient-years | AEs, no. of events | AEs, events/ patient-years | SAEs, no. of events | SAEs, events/ patient-years |
| VISUAL 1 main | 217 (110/107) | 657 | 10.524 | 18 | 0.288 | 430 | 9.717 | 6 | 0.136 |
| VISUAL 2 main | 225 (114/111) | 831 | 8.790 | 13 | 0.138 | 642 | 9.050 | 10 | 0.141 |
| VISUAL 1 Japan | 16 (8/8) | 28 | 12.101 | 1 | 0.431 | 25 | 7.962 | 0 | 0 |
| VISUAL 2 Japan | 32 (16/16) | 48 | 6.743 | 1 | 0.140 | 16 | 7.344 | 1 | 0.459 |
| Mackensen 2018 | 25 (10/15) | 54 | 7.665 | 1 | 0.142 | 30 | 5.475 | 0 | 0 |
| Ramanan 2019 | 90 (60/30) | 619 | 10.600 | 5 | 0.086 | 114 | 7.210 | 3 | 0.190 |

*ADA, adalimumab; AE, adverse event; SAE, serious adverse event.*

et al. on May 24, 2019. Leal's study included three RCTs concerning two drugs: adalimumab and etanercept, with a sample size of 458. In comparison, our meta-analysis included six studies related to one single drug (ADA) with a sample size of 605. Moreover, the efficacy analysis was not conducted in Leal's study because of the significant heterogeneity between interventions. Our study pooled the six studies for efficacy analysis using uniform outcome measures such as BCVA, change in AC cell grade, and VH grade (SUN). This review is the first meta-analysis including only RCTs to summarize the current evidence regarding ADA's efficacy and safety in NIU, with strengths in the relatively higher quality of evidence and multicomponent primary endpoint.

**TABLE 4 |** GRADE's summary of finding.

**Adalimumab compared to Placebo for non-infectious uveitis**

Patient or population: non-infectious uveitis
Setting:
Intervention: Adalimumab
Comparison: Placebo

| Outcomes No of participants (studies) | Relative effect (95% CI) | Anticipated absolute effects (95% CI) Difference | Certainty | What happens |
|---|---|---|---|---|
| Time to treatment failure<br>No of participants: 667 (5 RCTs) | HR 0.51 (0.41 to 0.63) [Time to treatment failure] | | ⊕⊕⊕◯<br>MODERATE | — |
| Change in BCVA (logMAR)<br>№ of participants: 580 (5 RCTs) | – | – MD 0.05 lower (0.07 lower to 0.02 lower) | ⊕⊕⊕◯<br>MODERATE | — |
| Change in AC cell grade<br>№ of participants: 592 (6 RCTs) | – | – MD 0.29 lower (0.62 lower to 0.05 higher) | ⊕⊕⊕◯<br>MODERATE | — |
| Change in VH grade<br>№ of participants: 502 (5 RCTs) | – | – MD 0.21 lower (0.32 lower to 0.11 lower) | ⊕⊕⊕◯<br>MODERATE | — |

**The risk in the intervention group (and its 95% confidence interval) is based on the assumed risk in the comparison group and the relative effect of the intervention (and its 95% CI). CI: Confidence interval; HR: Hazard Ratio; MD: Mean difference*

*GRADE Working Group grades of evidence*

*High certainty: We are very confident that the true effect lies close to that of the estimate of the effect*

*Moderate certainty: We are moderately confident in the effect estimate: The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different*

*Low certainty: Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimate of the effect*

*Very low certainty: We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimate of effect*

### Limitations

The principal limitation is that high heterogeneity was observed in our analysis. Differences in types of uveitis, patient's age, concomitant medications, and outcome measures may contribute to the heterogeneity. It is important to note that we did not restrict participants' age, so we recruited both adult and pediatric patients in this meta-analysis. In adults, ADA's main indications in NIU are intermediate, posterior forms of uveitis and panuveitis. In children with uveitis, ADA's main indication is JIA-associated uveitis and idiopathic uveitis, which is mainly anterior uveitis. We originally planned to conduct subgroup analysis in this article based on the location and type of uveitis, but due to the small sample size and the inability to extract relevant data from some studies, we gave up the subgroup analysis. In the future, more studies on the treatment of single uveitic disease with ADA are needed.

Besides, it is well known that RCT is not an ideal type of study to identify safety results because the relatively small sample size and short follow-up time make it challenging to identify rare adverse events. Therefore, the RCTs included in this review are not sufficient to study AEs thoroughly.

A further limitation was that the four VISUAL trials were sponsored by one pharmaceutical company (AbbVie) and the remaining two studies had participants who received remuneration such as the speaker's fees from AbbVie, which may seriously affect the results.

### Implication

In the future, independent non-company sponsored RCTs are needed to further provide more objective and robust evidence. Besides, it is necessary to further compare ADA with conventional immunosuppressors regarding efficacy and safety in NIU.

## CONCLUSION

This meta-analysis of six RCTs comparing ADA with placebo for NIU further confirmed that ADA considerably lowered the risk of treatment failure or visual impairment, and moderately reduced AC cell grades and VH grades with slightly more AEs.

## DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding author/s.

## FUNDING

Supported by Sichuan Provincial Department of Science and Technology, China (2019YFH0117).

## AUTHOR CONTRIBUTIONS

Conceptualization: BL. Data curation: BL and HL. Methodology: BL and ZL. Drafting of the article: BL and HL. Final approval: BL and YZ.

## REFERENCES


Airody, A., Heath, G., Lightman, S., and Gale, R. (2016). Non-infectious uveitis: optimising the therapeutic response. *Drugs* 76 (1), 27–39. doi:10.1007/s40265-015-0502-y

Balevic, S. J., and Rabinovich, C. E. (2016). Profile of adalimumab and its potential in the treatment of uveitis. *Drug Des. Devel Ther.* 10, 2997–3003. doi:10.2147/dddt.S94188

Burmester, G. R., Panaccione, R., Gordon, K. B., McIlraith, M. J., and Lacerda, A. P. M. (2013). Adalimumab: long-term safety in 23 458 patients from global clinical trials in rheumatoid arthritis, juvenile idiopathic arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis and crohn's disease. *Ann. Rheum. Dis.* 72 (4), 517–524. doi:10.1136/annrheumdis-2011-201244

Cordero-Coma, M., and Sobrin, L. (2015). Anti-tumor necrosis factor-α therapy in uveitis. *Surv. Ophthalmol.* 60 (6), 575–589. doi:10.1016/j.survophthal.2015.06.004

Dick, A. D., Tundia, N., Sorg, R., Zhao, C., Chao, J., Joshi, A., et al. (2016). Risk of Ocular Complications in patients with noninfectious intermediate uveitis, posterior uveitis, or panuveitis. *Ophthalmology* 123 (3), 655–662. doi:10.1016/j.ophtha.2015.10.028

Durrani, O. M., Tehrani, N. N., Marr, J. E., Moradi, P., Stavrou, P., and Murray, P. I. (2004). Degree, duration, and causes of visual loss in uveitis. *Br. J. Ophthalmol.* 88 (9), 1159–1162. doi:10.1136/bjo.2003.037226

Jabs, D. A., Nussenblatt, R. B., and Rosenbaum, J. T. (2005). Standardization of uveitis nomenclature for reporting clinical data. results of the first international workshop. *Am. J. Ophthalmol.* 140 (3), 509–516. doi:10.1016/j.ajo.2005.03.057

Jabs, D. A., Busingye, J., and Busingye, Jacqueline. (2013). Approach to the diagnosis of the uveitides. *Am. J. Ophthalmol.* 156 (2), 228–236. doi:10.1016/j.ajo.2013.03.027

Jaffe, G. J., Dick, A. D., Brézin, A. P., Nguyen, Q. D., Thorne, J. E., Kestelyn, P., et al. (2016). Adalimumab in patients with active noninfectious uveitis. *N. Engl. J. Med.* 375 (10), 932–943. doi:10.1056/NEJMoa1509852

Levy-Clarke, G., Jabs, D. A., Read, R. W., Rosenbaum, J. T., Vitale, A., and Van GelderVan Gelder, R. N. (2014). Expert panel recommendations for the use of anti-tumor necrosis factor biologic agents in patients with ocular inflammatory disorders. *Ophthalmology* 121 (3), 785–796. doi:10.1016/j.ophtha.2013.09.048

Llorenç, V., Mesquida, M., Sainz de la Maza, M., Keller, J., Molins, B., Espinosa, G., et al. (2015). Epidemiology of uveitis in a western urban multiethnic population. the challenge of globalization. *Acta Ophthalmol.* 93 (6), 561–567. doi:10.1111/aos.12675

Miloslavsky, E. M., NadenBijlsma, R. P., Bijlsma, J. W. J., Brogan, P. A., Brown, E. S., Brunetta, P., et al. (2017). Development of a glucocorticoid toxicity index (gti) using multicriteria decision analysis. *Ann. Rheum. Dis.* 76 (3), 543–546. doi:10.1136/annrheumdis-2016-210002

Ming, S., Xie, K. H. He., He, H., Li, Y., and Lei, B. (2018). Efficacy and safety of adalimumab in the treatment of non-infectious uveitis: a meta-analysis and systematic review. *Drug Des. Devel. Ther.* 12, 2005–2016. doi:10.2147/dddt.S160431

Nguyen, Q. D., Merrill, P. T., Jaffe, G. J., Dick, A. D., Kurup, S. K., Sheppard, J., et al. (2016). Adalimumab for prevention of uveitic flare in patients with inactive non-infectious uveitis controlled by corticosteroids (VISUAL II): a multicentre, double-masked, randomised, placebo-controlled phase 3 trial. *The Lancet* 388 (10050), 1183–1192. doi:10.1016/s0140-6736(16)31339-3

Niederer, R. L., Sharief, L., Bar, A., Lightman, S. L., and Tomkins-Netzer, O. (2017). Predictors of long-term visual outcome in intermediate uveitis. *Ophthalmology* 124 (3), 393–398. doi:10.1016/j.ophtha.2016.11.013

Nussenblatt, R. (2005). Treating intraocular inflammatory disease in the 21st century. *Arch. Ophthalmol.* 123 (7), 1000–1001. doi:10.1001/archopht.123.7.1000

Schwartzman, S., and Schwartzman, M. (2015). The use of biologic therapies in uveitis. *Clinic Rev. Allerg. Immunol.* 49 (3), 307–316. doi:10.1007/s12016-014-8455-6

Sen, H. N., Vitale, S., Gangaputra, S. S., Nussenblatt, R. B., Liesegang, T. L., Levy-Clarke, G. A., et al. (2014). Periocular corticosteroid injections in uveitis. *Ophthalmology* 121 (11), 2275–2286. doi:10.1016/j.ophtha.2014.05.021

Shamseer, L., Moher, D. D. G., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al. (2015). Preferred reporting items for systematic review and meta-analysis protocols (prisma-p) 2015: elaboration and explanation. *BMJ* 349, g7647. doi:10.1136/bmj.g7647

Suhler, E. B., Lowder, C. Y., Goldstein, D. A., Giles, T., Lauer, A. K., Kurz, P. A., et al. (2013). Adalimumab therapy for refractory uveitis: results of a multicentre, open-label, prospective trial. *Br. J. Ophthalmol.* 97 (4), 481–486. doi:10.1136/bjophthalmol-2012-302292

Suhler, E. B., Thorne, J. E., Mittal, M., Betts, K. A., Tari, S., Camez, A., et al. (2017). Corticosteroid-related adverse events systematically increase with corticosteroid dose in noninfectious intermediate, posterior, or panuveitis. *Ophthalmology* 124 (12), 1799–1807. doi:10.1016/j.ophtha.2017.06.017

Wakefield, D., and Chang, J. H. (2005). Epidemiology of uveitis. *Int. Ophthalmol. Clin.* 45 (2), 1–13. doi:10.1097/01.iio.0000155938.83083.94


**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 21 May 2021
doi: 10.3389/fphar.2021.666971

# Characterization of B- and T-Cell Compartment and B-Cell Related Factors Belonging to the TNF/TNFR Superfamily in Patients With Clinically Active Systemic Lupus Erythematosus: Baseline BAFF Serum Levels Are the Strongest Predictor of Response to Belimumab after Twelve Months of Therapy

*Silvia Piantoni[1*†], Francesca Regola[1†], Stefania Masneri[1†], Michele Merletti[1], Torsten Lowin[2], Paolo Airò[1], Angela Tincani[1], Franco Franceschini[1], Laura Andreoli[1†‡] and Georg Pongratz[2†‡]*

[1]Rheumatology and Clinical Immunology Unit, ASST Spedali Civili of Brescia, Department of Clinical and Experimental Sciences, University of Brescia, Brescia, Italy, [2]Department of Rheumatology and Hiller Research Center for Rheumatology, University Hospital Düsseldorf, Düsseldorf, Germany

**Background:** Patients with systemic lupus erythematosus (SLE) show increased serum levels of tumor necrosis factor (TNF)/TNF receptor (R) superfamily member, e.g. BAFF (B lymphocyte stimulator). Belimumab, a monoclonal antibody against soluble BAFF, is used for treatment of SLE. Although B cells are the main target, a BAFF-dependent T-cell activation pathway also plays a role. High levels of anti-DNA antibodies and low complement at baseline are known predictors of response to Belimumab.

**Objectives:** To explore the association of circulating lymphocytes and serum levels of B-cell related TNF/TNFR superfamily members with response to Belimumab in SLE patients.

**Methods:** Twenty-one SLE patients received Belimumab. Clinical evaluation and laboratory tests were performed at baseline, at 6 and 12 months. TNF super-family members (BAFF, APRIL, sBCMA, sCD40L, sTACI, TWEAK) were tested by high-sensitivity ELISA in all patients, and lymphocyte immunophenotyping was performed by flow cytometry in ten subjects. SLE-disease activity was assessed by SLEDAI-2K score. Linear regression modeling was used to investigate parameters influencing SLEDAI-2K and anti-dsDNA antibody titers over time and for predictive models.

**Results:** Clinical improvement was observed in all patients. A global reduction of circulating B cells, especially naïve, was detected, without variation in the T-cell compartment. All TNF family members decreased, whereas APRIL remained constant.

OPEN ACCESS

**Edited by:**
Francesca Oliviero,
University of Padua, Italy

**Reviewed by:**
Ioannis Parodis,
Karolinska Institutet (KI), Sweden
Alvaro Gomez,
Karolinska Institutet (KI), Sweden

***Correspondence:**
Silvia Piantoni
slv.piantoni@gmail.com

**†ORCID:**
Silvia Piantoni
orcid.org/0000-0003-0913-0197
Francesca Regola
orcid.org/0000-0001-6560-1112
Stefania Masneri
orcid.org/0000-00001-5327-0758
Laura Andreoli
orcid.org/0000-0002-9107-3218
Georg Pongratz
orcid.org/0000-0002-2185-8458
‡These authors share senior authorship

**Specialty section:**
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

**Received:** 11 February 2021
**Accepted:** 30 April 2021
**Published:** 21 May 2021

**Citation:**
Piantoni S, Regola F, Masneri S, Merletti M, Lowin T, Airò P, Tincani A, Franceschini F, Andreoli L and Pongratz G (2021) Characterization of B- and T-Cell Compartment and B-Cell Related Factors Belonging to the TNF/TNFR Superfamily in Patients With Clinically Active Systemic Lupus Erythematosus: Baseline BAFF Serum Levels Are the Strongest Predictor of Response to Belimumab after Twelve Months of Therapy. Front. Pharmacol. 12:666971. doi: 10.3389/fphar.2021.666971

The increase in serum levels of C3 ($p$ = 0.0004) and sTACI ($p$ = 0.0285) was associated with a decrease of SLEDAI-2K. The increase of C4 ($p$ = 0.027) and sBCMA ($p$ = 0.0015) and the increase of $CD8^+$ T cells ($p$ = 0.0160) were associated with a decrease, whereas an increase of sCD40L in serum ($p$ = 0.0018) and increased number of $CD4^+$ T cells ($p$ = 0.0029) were associated with an increase, in anti-dsDNA antibody titers, respectively. Using stepwise forward inclusion, the minimal model to predict SLEDAI-2K response at 12 months included BAFF ($p$ = 3.0$e$ − 07) and SLEDAI-2K ($p$ = 7.0$e$ − 04) at baseline. Baseline APRIL levels also showed an association, although the overall model fit was weaker.

**Conclusion:** In our real-life cohort, baseline serum levels of BAFF were the best predictor of response to Belimumab, confirming post-hoc results of the BLISS study and suggesting the utility of this particular biomarker for the identification of patients who are more likely to respond.

**Keywords: systemic lupus erythematosus, belimumab, biomarkers, TNF/TNFR superfamily-related factors, adaptive immunity**

## INTRODUCTION

An imbalance of B- and T-cell activity and differentiation was described as a crucial pathogenetic event in systemic lupus erythematosus (SLE) (Nagy et al., 2005). The pivotal role of autoantibodies was accepted as one of the main events in SLE and all factors which are involved in their development were studied as potential triggers of the disease (Nagy et al., 2005). BAFF (B-cell activating factor), also known as BLyS (B lymphocyte stimulator), is a member of Tumor necrosis factor/Tumor necrosis factor receptor (TNFS/TNFR) superfamily, also including APRIL (a proliferation-inducing ligand), their common receptors TACI (transmembrane activator and calcium-modulator and cyclophilin ligand interactor) and BCMA (B cell maturation antigen), CD40 ligand (CD40L) and TWEAK (TNF-related weak inducer of apoptosis). These factors, which show a high degree of structural homology with TNF, are described to be widely involved in the pathogenesis of SLE and in other systemic autoimmune diseases and they are newly identified as possible target of therapies (Ware, 2013).

Experimental data on SLE mouse models showed that BAFF and APRIL act in a concert to support humoral memory (Samy et al., 2017). BAFF is crucial for the development of self-reactive B cells from the transitional stage, which are more dependent on BAFF for their survival than memory B cells (Lesley et al., 2004; Samy et al., 2017). APRIL seems to act at a later stage, promoting the establishment of long-lived plasma cells (Belnoue et al., 2008). Both are involved in B-cell activation and class-switch recombination. BAFF and APRIL bind to TACI and BCMA, while BAFF additionally binds to a third receptor, BAFF-R. These receptors are expressed on the membrane and shed by B lineage cells during their differentiation (Darce et al., 2007) and BAFF-R and BCMA are described to be expressed also by T cells (Ng et al., 2004). CD40L ligand is the molecule which binds CD40, a stimulatory receptor expressed on dendritic cells, macrophages and B cells. It is crucial in IgG immunoglobulin class switching (Peters et al., 2009). CD40L is also fundamental as co-stimulatory molecule displayed on the membrane of T cells during the early phase of activation (Grewal and Flavell, 1996). CD40/CD40L blockade has been successful in preventing or stabilizing SLE nephritis in murine models (Kalled et al., 1998). TWEAK, produced by a large amount of myeloid and immune cells, is a factor acting primarily on tissue cells. In fact, its receptor, the fibroblast growth factor-inducible 14, is highly expressed on non-hematopoietic cells and up-regulated by injury-associated factors (Burkly, 2014). Dysfunction of TWEAK or its receptor has been described in the pathogenesis of lupus nephritis (Zhao et al., 2007).

Several drugs blocking the above factors were tested in clinical trials for their use in selected SLE patients. Only Belimumab, a fully humanized monoclonal IgG1λ antibody neutralizing soluble BAFF, has been approved for treatment of clinically active SLE (Navarra et al., 2011). A pooled subgroup analyses of Belimumab trials over 52 weeks of treatment (BLISS-52) demonstrated a greater therapeutic benefit in patients with increased disease activity at baseline, as measured by Safety of Estrogens in Lupus Erythematosus National assessment-Systemic Lupus Erythematosus Disease Activity Index (SELENA-SLEDAI) (Furie et al., 2011), anti-double strand (ds) DNA positivity, low complement or corticosteroid treatment (Navarra et al., 2011). As suggested by the post-hoc analyses of the Belimumab trials (BLISS-52 and BLISS-76) (Zhao et al., 2007; Burkly, 2014), baseline BAFF levels were proposed to be potentially useful in identifying SLE patients in which Belimumab might be expected to be more successful (Roth et al., 2016).

The objective of the present study is to characterize circulating peripheral B and T lymphocytes together with the evaluation of soluble B-cell related factors belonging to the TNF/TNFR

**TABLE 1 |** Demographic, clinical and laboratory features of SLE patients at baseline.

| | Total patients (*n* = 21) | Patients with lymphocyte immuno-phenotyping (*n* = 10) | *p* |
|---|---|---|---|
| Demographic features | | | |
| Women (*n*, %) | 19 (90) | 8 (80) | 0.5773 |
| Age, years | 41 (31–58) | 39 (33–45) | 0.5671 |
| Disease duration, years | 10 (2–23) | 12 (7–20) | 0.4443 |
| SLE Manifestations | | | |
| Cutaneous manifestations (malar rash and/or discoid rash, oral ulcers) | 18 (86) | 9 (90) | 1.0000 |
| Articular involvement (arthritis/Jaccoud's arthropathy) | 19 (90) | 9 (90) | 1.0000 |
| Renal involvement | 11 (52) | 5 (50) | 1.0000 |
| Hematological involvement | 11 (52) | 4 (40) | 0.7043 |
| NPSLE | 5 (24) | 3 (30) | 1.0000 |
| Serositis (pulmonary/pericardic effusion) | 4 (19) | 3 (30) | 0.6518 |
| Antiphospholipid syndrome | 3 (14) | 2 (20) | 1.0000 |
| SLEDAI 2K-score | 6 (4–10) | 6 (4–9) | 0.5054 |
| Laboratory Parameters | | | |
| Blood count of leukocytes ($X10^9$/L) (*nv* = 4–9) | 6 (2–8) | 6 (5–8) | 0.3859 |
| Serum levels of C3 (mg/dl) (*nv* = 80–160) | 72 (52–97) | 68 (53–90) | 0.6266 |
| Serum levels of C4 (mg/dl) (*nv* = 10–40) | 9 (6–18) | 10 (7–17) | 0.5965 |
| Serum levels of anti-dsDNA (UI/ml) (*nv* < 7) | 26 (7–128) | 37 (8–289) | 0.6051 |
| aCL positivity (IgM and/or IgG) (*n*, %) | 6 (29) | 2 (20) | 1.0000 |
| Anti-b2GPI positivity (IgM and/or IgG) (*n*, %) | 6 (29) | 2 (20) | 1.0000 |
| LA positivity (*n*, %) | 6 (29) | 3 (30) | 1.0000 |
| Treatment[a] | | | |
| Dosage of prednisone (mg/day) | 9 (5–23) | 8 (5–23) | 0.6113 |
| Use of hydroxychloroquine at 5 mg/kg/day (*n*, %) | 15 (71) | 7 (70) | 1.0000 |
| Use of immunosuppressant drugs (*n*, %) | 16 (76) | 7 (70) | 0.4648 |

*Data are expressed as median (10th–90th percentile), if not otherwise specified. NPSLE, neuropsychiatric systemic lupus erythematosus; SLEDAI-2K score, systemic lupus erythematosus disease activity index 2000; C3 and C4: complement factor 3 and 4; Anti-dsDNA, anti-double-stranded DNA autoantibody; aCL, anti-cardiolipin antibodies; Ig, immunoglobulin; Anti-b2GPI, antibeta2-glycoprotein I antibodies; LA, lupus anticoagulant; nv, normal values.*

[a]*Seven patients were on treatment with mycophenolate mofetil, four patients with methotrexate, four with azathioprine, one with cyclosporine.*

superfamily in a real-life cohort of clinically active SLE patients treated with Belimumab, in order to explore the potential role of these pathogenetic factors as predictors of response to therapy.

# PATIENTS AND METHODS

## Patients

Twenty-one consecutive patients with SLE, classified according to the revised American College of Rheumatology (ACR) criteria (Hochberg, 1997), and treated with Belimumab according to common clinical practice, were enrolled in this study. Written informed consent was obtained from all patients. Their main clinical, laboratory and demographic features, obtained from clinical records, are presented in **Table 1**. 76% of patients took immunosuppressants: seven were on treatment with mycophenolate mofetil at the median dose (10th–90th percentile) of 2 (1.6–2) g/die, four with methotrexate at 15 (12–15) mg/week, four with azathioprine at 100 (75–100) mg/die, one with cyclosporine at 250 mg/die. SLE Disease Activity Index 2000 (SLEDAI-2K) score was used to determine disease activity (Romero-Diaz et al., 2011).

The study was approved by the local institutional ethics committee (approval number 2793) and conducted in accordance with the Declaration of Helsinki.

## Laboratory Parameters

Peripheral blood samples of 21 patients were obtained at the start of the study (T0) and every six months of treatment (T6 and T12). Only one dosage of the TNF/TNFR superfamily members at T12 of one patient was missing (**Figure 1**).

Anti-dsDNA autoantibodies were determined by FARR assay (Kodak Clinical Diagnostics, Amersham, United Kingdom) and C3 and C4 levels by nephelometry (Siemens Healthcare, Deerfield, IL, United States).

BAFF, APRIL, sTACI, sBCMA, sCD40L, and TWEAK levels were measured by respective commercially available ELISAs (human Duo Set; R&D Systems, Inc., Minneapolis, MN, United States), according to manufacturer's guidelines.

## Flow Cytometry

In the first ten enrolled subjects, lymphocyte immunophenotyping was performed by flow cytometry.

One hundred microliters of whole blood were stained for 30 min at 4°C using monoclonal antibodies conjugated with fluorochromes (Beckman Coulter Inc., Fullerton, CA, United States) to identify B and T-cell surface markers by flow cytometry (Cytomics NAVIOS, Beckman Coulter), as previously described (Piantoni et al., 2018; Regola et al., 2019). Absolute cell count was determined by single platform analysis using Flow-Count beads (Beckman Coulter), according to manufacturer's guidelines.

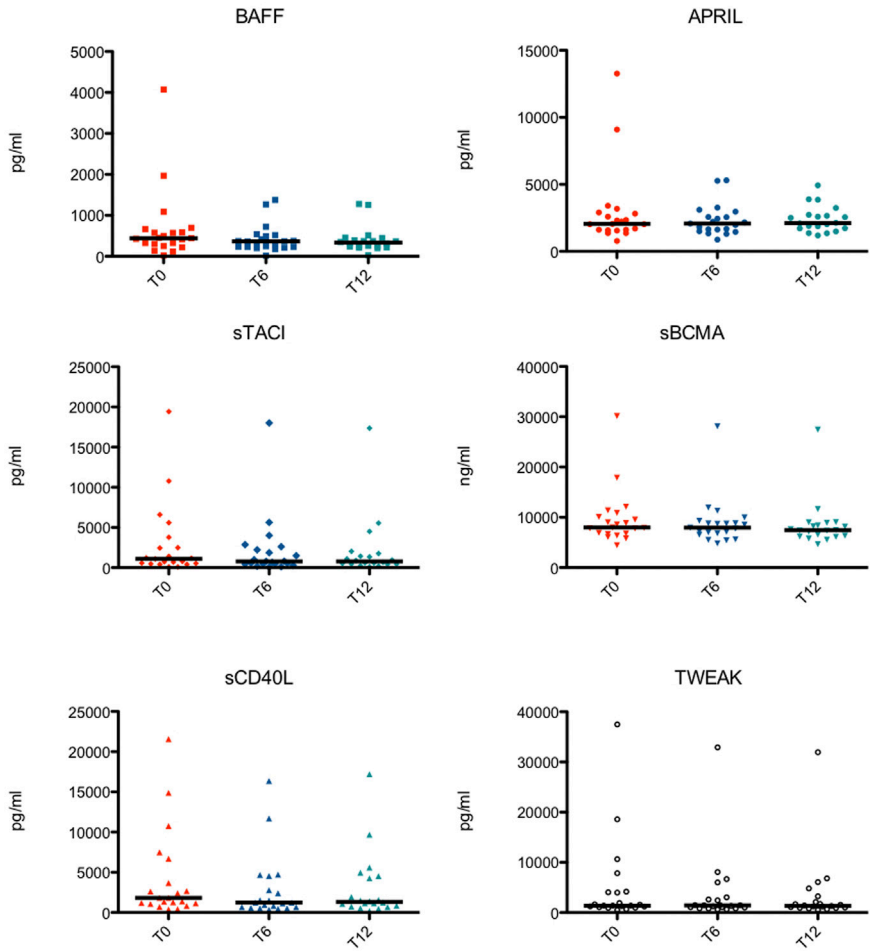


**FIGURE 1 |** Serological levels of the TNF Superfamily members at different time points in each subject. BAFF, B cell activating factor; APRIL, a proliferation-inducing ligand; sTACI, soluble transmembrane activator and calcium-modulator and cyclophilin ligand interactor; sBCMA, soluble B cell maturation antigen; sCD40L, soluble CD40 ligand; TWEAK, TNF-related weak inducer of apoptosis.

## Statistical Analysis

Data are expressed as median (10th–90th percentile). Comparisons between groups were made with Mann-Whitney test or Wilcoxon signed-rank test, when appropriated. Spearman rank test was used to evaluate the correlations between quantitative variables. Chi-square test or Fisher's exact test were applied for comparison between qualitative variables. Robust Mixed linear regression modeling (R package: robustlmm Koller (2016)) was used to investigate parameters influencing SLEDAI-2K and anti-ds DNA antibody titers over time. Adjustment for intra-individual effects was done by including patient ID as a random intercept in the linear regression model. Absolute numbers of $CD19^+$, $CD4^+$, $CD8^+$, $CD4^+CD28^-$ and T regulatory cells as well as leukocytes and serum levels of C3, C4, BAFF, APRIL, sTACI, sBCMA, sCD40L, TWEAK and anti-dsDNA antibodies were included in the initial model. Model selection was done by stepwise backwards exclusion. The time variable was kept in all models. Predictive linear models were built by stepwise forward inclusion using the same basic model structure. Statistical analysis was performed by using the software package GraphPad Prism six software and R software package version 4.0.3. [R Core Team (2020), R Foundation for Statistical Computing, Vienna, Austria]. $p$-values ($p$) $\leq 0.050$ were considered as statistically significant.

# RESULTS

## Clinical and Laboratory Features of Systemic Lupus Erythematosus Patients Treated With Belimumab

Twenty-one patients received Belimumab intravenously at standard regimen (10 mg/kg at 0–15–30 days and then every 4 weeks).

Enrolled patients were 2 males and 19 females with a median age of 41 (31–58) years. The disease duration at time of Belimumab start was 10 (2–23) years. The baseline SLEDAI-2K score was 6 (4–10), the anti-dsDNA level was 26 (7–128) UI/ml, and their C3 and C4 level was 72 (52–97) and 9 (6–18) mg/dl,

**TABLE 2 |** Comparisons of the clinical and laboratory features of 21 SLE patients at different time points.

| | T0 (*n* = 21) | T6 (*n* = 21) | T12 (*n* = 21) | p T0 vs. T6 | p T0 vs. T12 | p T6 vs. T12 |
|---|---|---|---|---|---|---|
| Disease activity | | | | | | |
| SLEDAI 2K-score | 6 (4–10) | 4 (2–6) | 4 (2–5) | **0.0007** | **0.0025** | 0.9045 |
| Laboratory Parameters | | | | | | |
| Serum levels of C3 (mg/dl) (*nv* = 80–160) | 72 (52–97) | 74 (50–103) | 73 (50–101) | 0.9404 | 0.6789 | 0.6600 |
| Serum levels of C4 (mg/dl) (*nv* = 10–40) | 9 (6–18) | 11 (3–18) | 11 (6–20) | 0.3480 | 0.1394 | 0.3486 |
| Serum levels of anti-dsDNA (UI/ml) (*nv* < 7) | 26 (7–128) | 28 (5–228) | 28 (5–110) | 0.5699 | 0.2114 | 0.3396 |
| Treatment | | | | | | |
| Dosage of prednisone (mg/day) | 9 (5–23) | 6 (3.6–12.5) | 4 (3–10.5) | **0.0030** | **0.0010** | **0.0468** |

*Data are expressed as median (10th–90th percentile). SLEDAI-2K score, Systemic Lupus Erythematosus disease Activity Index 2000; C3 and C4: complement factor 3 and 4; Anti-dsDNA, anti-double-stranded DNA autoantibody; nv, normal values. In bold* p ≤ *0.050.*

**TABLE 3 |** Serum Levels Changes of TNF related Biomarkers during belimumab treatment.

| | T0 (*n* = 21) | T6 (*n* = 21) | T12 (*n* = 21) | p T0 vs. T6 | p T0 vs. T12 | p T6 vs T12 |
|---|---|---|---|---|---|---|
| BAFF (pg/ml) | 444.8 (134.4–1,091.2) | 366.5 (201.5–722.7) | 334.7 (209.2–589.9) | **0.0547** | **0.0215** | 0.8124 |
| APRIL (pg/ml) | 2053.3 (1,369.2–3,407.9) | 2,085.3 (1,346.8–3,268.5) | 2,117.6 (1,368.1–3,862.2) | 0.1678 | 0.4304 | 0.9273 |
| sTACI (pg/ml) | 1,096.5 (397.3–6,599.9) | 782.3 (181.7–4,011.3) | 777.6 (243.0–4,610.9) | **0.0003** | **0.0006** | **0.0215** |
| sBCMA (ng/ml) | 7,982.7 (6,099.2–12,114.7) | 7,954.4 (5,523.5–11,303.0) | 7,437.8 (5,771.4–9,319.0) | **0.0319** | **0.0010** | 0.1054 |
| sCD40L (pg/ml) | 1,817.3 (703.2–10,754.0) | 1,243.1 (522.2–4,721.5) | 1,326.5 (522.2–5,999.5) | **0.0005** | **0.0136** | 0.0759 |
| TWEAK (pg/ml) | 1,381.3 (938.9–10,656.5) | 1,446.8 (815.5–6,671.6) | 1,365.2 (812.0–6,151.8) | **0.0010** | **0.0020** | 0.1327 |

*Data are expressed as median (10th–90th percentile). In bold* p ≤ *0.050. BAFF: B cell activating factor; APRIL: a proliferation-inducing ligand; sTACI: soluble transmembrane activator and calcium-modulator and cyclophilin ligand interactor; sBCMA, soluble B cell maturation antigen; sCD40L: soluble CD40 ligand; TWEAK: TNF-related weak inducer of apoptosis.*

respectively. No significant differences were found between the whole group of patients and the subgroup of patients tested for lymphocyte immunophenotyping (**Table 1**).

During Belimumab treatment there was a significant improvement in SLEDAI-2K activity index, while no significant change was observed in anti-dsDNA, C3 and C4 levels. Prednisone dosage was progressively reduced, while concomitant immunosuppressive therapies remained unchanged (**Table 2**).

## Analysis of Changes in B- and T-Cell Compartment During Belimumab Treatment

After treatment with Belimumab, B lymphocytes decreased in patients with SLE, both in percentages (T0, T6 and T12 = 8.1, 3.8 and 3.1 % of CD19 on total lymphocytes) and absolute numbers (T0, T6 and T12 = 82.3, 17.3 and 21.1 cell/μl). In particular, there was a decrease of naïve B cells (T0, T6 and T12 = 45.5, 25.1 and 19.1% of CD19 on total lymphocytes; T0, T6 and T12 = 20.8, 1.5 and 1.4 cell/ul) while percentage switched memory B cells increased (T0, T6 and T12 = 18.4, 41.4 and 48.9% of CD19 on total lymphocytes; T0, T6 and T12 = 13.3, 5.8 and 6.0 cell/ul).

The percentage and the absolute number of unswitched memory and transitional B cells did not change significantly.

Comparing distributions of $CD4^+$, $CD8^+$, regulatory T cells, and naïve, central memory, effector memory, terminal differentiated effector memory, CD28 negative subsets among $CD4^+$ and $CD8^+$ T cells, before and after therapy with Belimumab, we did not observe any significant changes over time (**Supplementary Table S1**). These results confirmed our previous observation in a larger cohort (Regola et al., 2019).

## Analysis of Serum Level Changes of Tumor Necrosis Factor Superfamily Biomarkers During Belimumab Treatment

The following biomarkers belonging to the TNF/TNFR superfamily were tested in serum of the 21 enrolled patients: BAFF, APRIL, sTACI, sBCMA, sCD40L, and TWEAK (**Table 3**).

Serum levels of BAFF (T0, T6 and T12 = 444.8, 366.5 and 334.7 pg/ml), as well as sTACI (T0, T6 and T12 = 1,096.5, 782.3 and 777.6 pg/ml) significantly decreased over time.

Serum levels of APRIL remained stable over time (T0, T6 and T12 = 2,053.3, 2,085.5 and 2,117.6 pg/ml).

sBCMA serum levels decreased, but significantly only between 6 and 12 months following start of Belimumab treatment (T0, T6 and T12 = 7,982.7, 7,954.4 and 7437.8 ng/ml). On the other hand, changes in sCD40L were observed mostly in the first 6 months of therapy (T0, T6 and T12 = 1,817.3, 1243.1 and 1,326.5 pg/ml).

Serum levels of TWEAK, after an initial increase, significantly decreased one year after initiation of Belimumab treatment (T0, T6 and T12 = 1,381.3, 1,446.8 and 1,365.2 pg/ml).

Serological levels of the TNF/TNFR Superfamily members at different time points in each subject are shown in **Figure 1**.

**TABLE 4 |** Results of robust mixed linear regression model for predictors of SLEDAI-2K. Individual Patient ID was included as random effect in the model. Predictor values were measured at all timepoints.

| Dependent: SLEDAI-2K | | | | |
|---|---|---|---|---|
| **Predictors** | **Estimate** | **Std. error** | ***t*. value** | ***p*** |
| (Intercept) | 11.4386 | 1.1960 | 9.5644 | <0.001 |
| Month 6 | **−2.9902** | **0.5108** | **−5.8544** | **<0.001** |
| Month 12 | **−3.3201** | **0.5197** | **−6.3886** | **<0.001** |
| C3 | **−0.0574** | **0.0140** | **−4.1083** | **0.0004** |
| sTACI | **−0.0002** | **0.0001** | **−2.3424** | **0.0285** |

*MODEL FIT: AIC = 300.13, BIC = 315.02 Pseudo-$R^2$ (fixed effects) = 0.47 Pseudo-$R^2$ (total) = 0.63. In bold* p ≤ *0.050. SLEDAI-2K score, systemic lupus erythematosus disease activity index 2,000; sTACI: soluble transmembrane activator and calcium-modulator and cyclophilin ligand interactor; C3: complement factor 3.*

**TABLE 5 |** Results of robust mixed linear regression model for predictors of anti-dsDNA antibody titer. Individual Patient ID was included as random effect in the model. Predictor values were measured at all timepoints.

| Dependent: anti-dsDNA antibody titer | | | | |
|---|---|---|---|---|
| **Predictors** | **Estimate** | **Std. error** | ***t* value** | ***P*value** |
| (Intercept) | 335.7107 | 76.5060 | 4.3880 | 0.0007 |
| Month 6 | 37.1296 | 30.4093 | 1.2210 | 0.2369 |
| Month 12 | −29.3079 | 30.7385 | −0.9535 | 0.3528 |
| C4 | **−8.0294** | **2.1743** | **−3.6928** | **0.0027** |
| sBCMA | **−0.0285** | **0.0076** | **−3.7340** | **0.0015** |
| sCD40L | **0.0308** | **0.0086** | **3.5974** | **0.0018** |
| #CD4 T cells | **0.4038** | **0.1212** | **3.3317** | **0.0029** |
| #CD8 T cells | **−0.4150** | **0.1558** | **−2.6639** | **0.0160** |

*MODEL FIT: AIC = 384.81, BIC = 400.93 Pseudo-$R^2$ (fixed effects) = 0.76 Pseudo-$R^2$ (total) = 0.87. In bold* p ≤ *0.050. sBCMA, soluble B cell maturation antigen; sCD40L, soluble CD40 Ligand; C4: complement factor 4; # absolute number of cells.*

## Correlation Between Baseline Number of B- and T-Cell Subsets and Serum Levels of Tumor Necrosis Factor Superfamily Members, and Their Change Over Time

The percentage of CD19 $^+$ cells at baseline showed a correlation with baseline levels of sBCMA ($r = 0.7, p = 0.02$) and TWEAK ($r = 0.86$, $p = 0.002$) (**Supplementary Table S2**).

A significant correlation between the percentage of variation of $CD19^+$ and the decrease of sTACI ($r = 0.7$, $p = 0.02$) and TWEAK ($r = 0.8, p = 0.002$), respectively, was found at 12 months of follow-up.

No correlation was found between TNF/TNFR superfamily members and the number of T-cell subsets (and their respective variations) (data not shown).

## Determination of Parameters Associated With SLEDAI and Anti-dsDNA Antibody Changes During Belimumab Treatment

Regression analysis confirmed that SLEDAI-2K decreases with duration of Belimumab treatment, with an average reduction of 2.9 (+/− 0.51) at T6 and 3.3 (+/− 0.52) points at T12 (**Table 4**). In addition, an increase in serum levels of C3 and sTACI was associated with SLEDAI-2K. On average SLEDAI-2K was 0.5 (+/- 0.1) points lower with every 10 mg/dl increase of C3. For sTACI, this association was weaker with SLEDAI-2K reduced by 0.02 (+/− 0.01) points for every 100 pg/ml increase in sTACI (**Table 4**). When including time after treatment initiation as an interacting factor into the model, the association with C3 remained, however, the association with TACI was not significant anymore (**Supplementary Table S3**).

**TABLE 6 |** Linear regression model to predict percent improvement of SLEDAI-2K after one year, dependent on SLEDAI-2K at baseline and BAFF levels measured at baseline.

| Dependent: Percent improvement of SLEDAI2K after one year | | | | | |
|---|---|---|---|---|---|
| **Baseline predictors** | **Estimate** | **5%** | **95%** | ***t* val.** | ***p*** |
| (Intercept) | −0.438 | −20.491 | 19.614 | −0.038 | 9.7*e* − 01 |
| BAFF | **0.018** | **0.014** | **0.022** | **8.107** | **3.0*e* − 07** |
| SLEDAI-2K | **4.574** | **2.646** | **6.501** | **4.128** | **7.0*e* − 04** |

*MODEL FIT: F (2,17) = 13.27,* p = *<0.01* $R^2$ *= 0.61 Adj.* $R^2$ *= 0.56. In bold* p ≤ *0.050. SLEDAI-2K score, systemic Lupus Erythematosus disease activity index 2,000; BAFF: B cell activating factor.*

Further mixed linear regression analysis revealed that month into treatment did not significantly change anti-dsDNA serum titers (**Table 5**). However, an increase of serum C4, sBCMA and/or absolute cell number of $CD8^+$ T cells was associated with a decrease, whereas an increase of CD40L and/or number of $CD4^+$ T cells was associated with an increase in anti-dsDNA antibody titers (**Table 5**).

In a last step, we explored possible predictive models using baseline parameters to estimate percent improvement of SLEDAI-2K after 12 months of therapy. Using stepwise forward inclusion, the minimal model to best describe response to Belimumab after 12 months included BAFF serum level and SLEDAI-2K at baseline. The model predicted more than half of the change in SLEDAI-2K at T12 ($R^2 = 0.61$; Adj. $R^2 = 0.56$, $p < 0.01$, **Table 6**). Every increase in serum BAFF of 100 pg/ml at baseline results in a reduction of SLEDAI-2K after one year of treatment of 1.8% (95% CI: 1.4–2.2%) on average. In addition, for every SLEDAI-2K increase of one point at baseline, SLEDAI-2K at T12 into Belimumab treatment decreases about 4.6% (95% CI: 2.6–6.5) (**Table 6**). Since correlation analysis showed high interdependence of all measured members of the TNF superfamily members, we also determined if BAFF as a predictor of response can be substituted by any of the other determined TNF family members. As shown in **Supplementary Table S3** most of the TNF family members will not suffice as predictors for SLEDAI response after 12 months, however, baseline APRIL serum level also showed a significant association, although the overall model fit was slightly weaker than using serum baseline BAFF as predictor (**Supplementary Table S4**).

# DISCUSSION

Belimumab, a monoclonal antibody targeting BAFF, has been approved since 2011 as an add-on therapy in adult SLE patients

who have an active disease despite standard treatment. Its efficacy and safety were demonstrated in four randomized controlled trials for prolonged use of the drug (Ruiz-Irastorza and Bertsias, 2020). In our cohort, the drug showed its beneficial effects in reducing disease activity over a 12-month-period, as demonstrated by the reduction of the SLEDAI-2K index. However, the variations of anti-dsDNA titer and complement levels were not significant, which was in contrast to previous reports (Furie et al., 2011; Navarra et al., 2011). This difference may be related to the selection of patients who had a mild serological activity at baseline. In general, the main indication for adding Belimumab in our patients was actually to reduce steroid dose. In fact, the well-known effect in reducing cumulative exposure to glucocorticoids was evident as early as 6 months into therapy.

Despite the fact that the post-hoc analysis of trial data showed a greater response in patients with high clinical or serological disease activity (van Vollenhoven et al., 2012), no biomarker has been validated yet for the routine management of patients who are candidate for Belimumab. To address this issue, we considered TNFSF/TNFRSF related factors which had the potential to serve as biomarkers for SLE disease assessment and monitoring of immunomodulatory therapy. In fact, the detection of these factors, which play a role in the pathogenesis of SLE regulating crosstalk between immune cells, could be easily standardized, being measured in peripheral blood in a reproducible way. Among others, circulating levels of BAFF and APRIL, which have an important role in selection, maturation and survival of B cells, are a matter of interest for SLE because their production is enhanced in response to B cell activation through Toll-like receptor (TLR)-9, interferons (IFNs), interleukin (IL)-10 and granulocyte colony-stimulating factor (G-CSF), all involved in SLE pathogenesis (Koyama et al., 2005; Petri et al., 2008; Salazar-Camarena et al., 2016). In addition, an increase in BAFF levels has been described in association with increased disease activity and anti-dsDNA antibodies (Petri et al., 2008). In our study, BAFF progressively reduced over one year of therapy with Belimumab associated with clinical improvement in patients, and a weak correlation with reduction of naïve and transitional B cells, as suggested by the mechanism of action of Belimumab and as reported by us before (Regola et al., 2019). Such correlation was not confirmed in the present study, reinforcing the concept that the main effect of Belimumab on subpopulations could act through the blocking of membrane BAFF, and not of the soluble form (Regola et al., 2019). The post-hoc analysis of phase III randomized clinical trials showed that BAFF levels ≥ 2 ng/ml at enrollment were an independent prognostic factor for an increased risk of moderate and severe lupus flares in patients randomized to receive standard therapy only (Petri et al., 2013). Another analysis from the same study found that patients with BAFF serum levels ≥ 2 ng/ml at baseline had higher response parameters than those with lower BAFF levels, in the Belimumab arm (Petri et al., 2013). In the same way, it was demonstrated that serum BAFF levels ≥ 1.2 ng/ml predicted an increased probability and shorter time to reach response in a cohort of Swedish SLE patients (Parodis et al., 2017). According to this, we demonstrated an association between higher baseline BAFF serum levels and a greater reduction in SLEDAI-2K score after 12 months of therapy, reinforcing the evidence that determination of BAFF levels at the beginning of therapy, together with evaluation of clinical disease activity, could be useful in predicting response to the drug. In our study, BAFF levels decreased during Belimumab therapy, in contrast with what was demonstrated in another report in which BAFF levels increased with time (Parodis et al., 2017). A possible explanation of this observed dissimilarity between studies could be related to the possible different pre-analytic processing of samples which may have caused a modification in the structure of the BAFF molecule, influencing its detection. To better address this aspect, further investigations are necessary to identify if the detected serum BAFF represents only the active form of the molecule or even the inactive form which is complexed with the drug. It has also to be clarified if the proportion of circulating BAFF is representative with the amount that is compartmented in the tissues or expressed on membranes, in order to better identify possible clinical associations.

As an alternative biomarker, although weaker associated with response than BAFF, we showed that baseline serum level of APRIL could also be useful. In the APRIL-SLE clinical trial, BAFF levels above the median at baseline were correlated with an increased risk of British Isles Lupus Assessment Group (BILAG) A or B flare (Gordon et al., 2003) in the placebo group (Isenberg et al., 2015) and patients with high baseline serum values of both BAFF and APRIL showed the greatest effect size. While there are some studies that demonstrated a direct correlation between BAFF and anti-dsDNA serum levels (Stohl et al., 2003; Petri et al., 2008), conflicting results were reported about the possible correlation between APRIL levels and SLE disease activity in terms of activity indices or autoantibody levels (Stohl et al., 2004; Koyama et al., 2005). Furthermore, the reverse trend displayed by APRIL as compared to BAFF confirms the possibility that these two factors could play an opposite role in SLE (Morel et al., 2009).

TACI and BCMA, the common receptor of BAFF and APRIL, were described to be involved in immunoglobulin class switching (He et al., 2010) and in promoting plasma cells survival (O'Connor et al., 2004), respectively. Recent studies demonstrated that the soluble form of these receptors, sTACI and sBCMA, act as decoy receptors with a role in immunomodulatory pathways, being the result of a proteolytic shedding partially dependent on ligand binding and receptor interactions (Meinl et al., 2018). Circulating sTACI, identified as a potential biomarker in autoimmune diseases, is shed from the membrane of activated B cells and plasma cells (Hoffmann et al., 2015). It functions as an immunoregulator, because its decoy function reduces BAFF- and APRIL-mediated survival of different B cell subpopulations (Salazar-Camarena et al., 2020). The reduction of B-cell hyperactivation after therapy with Belimumab could explain the parallel decrease of sTACI, that was demonstrated to be increased in SLE patients in correlation with disease activity (Hoffmann et al., 2015). According with its potential clinical value, we showed that variation in sTACI was related to the improvement of the clinical condition of our

patients, as measured by SLEDAI-2K. However, its independent contribution is weak, as demonstrated by our model. Recently, the expression of BCMA on B cells was shown to decrease in active SLE (Salazar-Camarena et al., 2016), however the soluble form, sBCMA, was increased in serum and correlated with disease activity and anti-dsDNA levels (Salazar-Camarena et al., 2020). BCMA was found to be expressed also on the surface of T cells regulating their expansion within the lymph node germinal centers (GC) (Coquery et al., 2015). Recently, it was also demonstrated that the decoy function of circulating sBCMA is only relevant for APRIL and not for BAFF, especially in conditions of over-production, such as in SLE (Coquery et al., 2015). Confirming the relevance of sBCMA as marker of B-cell activation (Laurent et al., 2015; Vincent et al., 2019), we showed an association with autoantibody titers.

The interaction between CD40 on B cells and its ligand (CD40L) which is transiently expressed by T cells and released in soluble form by activated $CD4^+$ T cells, is another crucial event that takes place in GC with a role in enhancing humoral response (Basso et al., 2004). It plays a central role in SLE, considering the importance of T-cell dependent humoral immune responses in its pathogenesis (Nagy et al., 2005). Soluble CD40L (sCD40L) in serum or its expression in tissues is upregulated in SLE patients, and often associated with disease severity (Yazdany and Davis, 2004). As shown for sBCMA, its circulating levels revealed an association with anti-dsDNA titers in our cohort, confirming previous findings (Stohl et al., 2003; Petri et al., 2008).

TWEAK is a circulating trimeric molecule which exerts its effect at tissue level. Its relevance in SLE is linked to the constitutive presence in kidneys, with an upregulation during injuries (Schwartz et al., 2006). The lack of association between serum TWEAK levels or their change over time, and clinical or serological parameters of our patients, could be explained by the fact that patients were not enrolled during an acute phase of a nephritis. Its reduction over time in this cohort is unclear and, apparently, without a biological significance. Further evaluations of this tissue-specific parameter could be performed in the future on a specific subset of patients, considering the potential antiproteinuric effects of Belimumab that have emerged from the latest studies (Dooley et al., 2013; Kang et al., 2017).

Some correlations were found between circulating number of B cells and the TNFSF/TNFRSF related factors at baseline. It suggested their inter-relation, but the lack of correlation with specific B-cell subsets may be explained with the presence of other factors which are involved in the maturation and function of B-cells during the disease course. Indeed, the partial evaluation of potentially involved circulating factors, the lack of complementary functional studies, along with the limited number of enrolled patients and the lack of a control group, are limitations of this real-life observational study.

However, our analysis of B- and T-cell compartment modifications during Belimumab therapy sheds a light on the potential usefulness of peripheral B-cell immunophenotyping in SLE patients, confirming our previous results (Regola et al., 2019) and the evidence showed by other researchers in that field (Ramsköld et al., 2019). In particular, it was showed that the long-term longitudinal evaluation of B cells could have important implications in the evaluation of a cellular response to the treatment, that could lead to clinical improvement (Ramsköld et al., 2019). Despite these suggestions, the routinely monitoring of circulating cells could be difficult to be introduced into clinical practice, also on the basis of a lack of evidence in predicting response. In conclusion, only the determination of baseline BAFF serum levels might be useful and feasible to predict the response to therapy, as an add-on biomarker to those already tested in clinical practice.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Brescia Ethics Committee, Asst Spedali Civili (approval number 2793). The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

SP, TL, LA, and GP contributed to conception and design of the study. SP, FR, PA, AT, FF, and LA enrolled the patients and obtained the biological samples. FR, TL, MM, and SM performed the experiments and organized the database. SP, FR, and GP performed the statistical analysis. SP, FR, LA, and GP wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.666971/full#supplementary-material

## REFERENCES

Basso, K., Klein, U., Niu, H., Stolovitzky, G. A., Tu, Y., Califano, A., et al. (2004). Tracking CD40 Signaling during Germinal Center Development. *Blood*. 104 (13), 4088–4096. doi:10.1182/blood-2003-12-4291

Belnoue, E., Pihlgren, M., McGaha, T. L., Tougne, C., Rochat, A.-F., Bossen, C., et al. (2008). APRIL Is Critical for Plasmablast Survival in the Bone Marrow and Poorly Expressed by Early-Life Bone Marrow Stromal Cells. *Blood* 111 (5), 2755–2764. doi:10.1182/blood-2007-09-110858

Burkly, L. C. (2014). TWEAK/Fn14 axis: the Current Paradigm of Tissue Injury-Inducible Function in the Midst of Complexities. *Semin. Immunol.* 26 (3), 229–236. doi:10.1016/j.smim.2014.02.006

Coquery, C. M., Loo, W. M., Wade, N. S., Bederman, A. G., Tung, K. S., Lewis, J. E., et al. (2015). BAFF Regulates Follicular Helper T Cells and Affects Their

Accumulation and Interferon-γ Production in Autoimmunity. *Arthritis Rheumatol.* 67 (3), 773–784. doi:10.1002/art.38950

Darce, J. R., Arendt, B. K., Wu, X., and Jelinek, D. F. (2007). Regulated Expression of BAFF-Binding Receptors during Human B Cell Differentiation. *J. Immunol.* 179 (11), 7276–7286. doi:10.4049/jimmunol.179.11.7276

Dooley, M. A., Houssiau, F., Aranow, C., D'Cruz, D. P., Askanase, A., Roth, D. A., et al. (2013). Effect of Belimumab Treatment on Renal Outcomes: Results from the Phase 3 Belimumab Clinical Trials in Patients with SLE. *Lupus*. 22, 63–72. doi:10.1177/0961203312465781

Furie, R., Petri, M., Zamani, O., Cervera, R., Wallace, D. J., Tegzová, D., et al. (2011). A Phase III, Randomized, Placebo-Controlled Study of Belimumab, a Monoclonal Antibody that Inhibits B Lymphocyte Stimulator, in Patients with Systemic Lupus Erythematosus. *Arthritis Rheum.* 63, 3918–3930. doi:10.1002/art.30613

Gordon, C., Sutcliffe, N., Skan, J., Stoll, T., and Isenberg, D. A. (2003). Definition and Treatment of Lupus Flares Measured by the BILAG Index. *Rheumatology*. 42 (11), 1372–1379. doi:10.1093/rheumatology/keg382

Grewal, I. S., and Flavell, R. A. (1996). The Role of CD40 Ligand in Costimulation and T-Cell Activation. *Immunol. Rev.* 153, 85–106. doi:10.1111/j.1600-065x.1996.tb00921.x

He, B., Santamaria, R., Xu, W., Cols, M., Chen, K., Puga, I., et al. (2010). The Transmembrane Activator TACI Triggers Immunoglobulin Class Switching by Activating B Cells through the Adaptor MyD88. *Nat. Immunol.* 11 (9), 836–845. doi:10.1038/ni.1914

Hochberg, M. C. (1997). Updating the American College of Rheumatology Revised Criteria for the Classification of Systemic Lupus Erythematosus. *Arthritis Rheum.* 40 (9), 1725. doi:10.1002/art.1780400928

Hoffmann, F. S., Kuhn, P. H., Laurent, S. A., Hauck, S. M., Berer, K., Wendlinger, S. A., et al. (2015). The Immunoregulator Soluble TACI Is Released by ADAM10 and Reflects B Cell Activation in Autoimmunity. *J. Immunol.* 194 (2), 542–552. doi:10.4049/jimmunol.1402070

Isenberg, D., Gordon, C., Licu, D., Copt, S., Rossi, C. P., and Wofsy, D. (2015). Efficacy and Safety of Atacicept for Prevention of Flares in Patients with Moderate-To-Severe Systemic Lupus Erythematosus (SLE): 52-week Data (APRIL-SLE Randomised Trial). *Ann. Rheum. Dis.* 74 (11), 2006–2015. doi:10.1136/annrheumdis-2013-205067

Kalled, S. L., Cutler, A. H., Datta, S. K., and Thomas, D. W. (1998). Anti-CD40 Ligand Antibody Treatment of SNF1 Mice with Established Nephritis: preservation of Kidney Function. *J. Immunol.* 160, 2158–2165.

Kang, S., Fedoriw, Y., Brenneman, E. K., Truong, Y. K., Kikly, K., and Vilen, B. J. (2017). BAFF Induces Tertiary Lymphoid Structures and Positions T Cells within the Glomeruli during Lupus Nephritis. *J. Immunol.* 198, 2602–2611. doi:10.4049/jimmunol.1600281

Koller, M. (2016). Robustlmm: An R Package for Robust Estimation of Linear Mixed-Effects Models. *J. Stat. Softw.* 75 (6), 1–24. doi:10.18637/jss.v075.i06

Koyama, T., Tsukamoto, H., Miyagi, Y., Himeji, D., Otsuka, J., Miyagawa, H., et al. (2005). Raised Serum APRIL Levels in Patients with Systemic Lupus Erythematosus. *Ann. Rheum. Dis.* 64 (7), 1065–1067. doi:10.1136/ard.2004.022491

Laurent, S. A., Hoffmann, F. S., Kuhn, P. H., Cheng, Q., Chu, Y., Schmidt-Supprian, M., et al. (2015). γ-Secretase Directly Sheds the Survival Receptor BCMA from Plasma Cells. *Nat. Commun.* 11 (6), 7333. doi:10.1038/ncomms8333

Lesley, R., Xu, Y., Kalled, S. L., Hess, D. M., Schwab, S. R., Shu, H. B., et al. (2004). Reduced Competitiveness of Autoantigen-Engaged B Cells Due to Increased Dependence on BAFF. *Immunity*. 20 (4), 441–453. doi:10.1016/s1074-7613(04)00079-2

Meinl, E., Thaler, F. S., and Lichtenthaler, S. F. (2018). Shedding of BAFF/APRIL Receptors Controls B Cells. *Trends Immunol.* 39 (9), 673–676. doi:10.1016/j.it.2018.07.002

Morel, J., Roubille, C., Planelles, L., Rocha, C., Fernandez, L., Lukas, C., et al. (2009). Serum Levels of Tumour Necrosis Factor Family Members a Proliferation-Inducing Ligand (APRIL) and B Lymphocyte Stimulator (BLyS) Are Inversely Correlated in Systemic Lupus Erythematosus. *Ann. Rheum. Dis.* 68 (6), 997–1002. doi:10.1136/ard.2008.090928

Nagy, G., Koncz, A., and Perl, A. (2005). T- and B-Cell Abnormalities in Systemic Lupus Erythematosus. *Crit. Rev. Immunol.* 25 (2), 123–140. doi:10.1615/critrevimmunol.v25.i2.30

Navarra, S. V., Guzmán, R. M., Gallacher, A. E., Hall, S., Levy, R. A., Jimenez, R. E., et al. (2011). Efficacy and Safety of Belimumab in Patients with Active Systemic Lupus Erythematosus: a Randomised, Placebo-Controlled, Phase 3 Trial. *Lancet*. 377, 721–723. doi:10.1016/S0140-6736(10)61354-2

Ng, L. G., Sutherland, A. P. R., Newton, R., Qian, F., Cachero, T. G., Scott, M. L., et al. (2004). B Cell-Activating Factor Belonging to the TNF Family (BAFF)-R Is the Principal BAFF Receptor Facilitating BAFF Costimulation of Circulating T and B Cells. *J. Immunol.* 173 (2), 807–817. doi:10.4049/jimmunol.173.2.807

O'Connor, B. P., Raman, V. S., Erickson, L. D., Cook, W. J., Weaver, L. K., Ahonen, C., et al. (2004). BCMA Is Essential for the Survival of Long-Lived Bone Marrow Plasma Cells. *J. Exp. Med.* 199 (1), 91–98. doi:10.1084/jem.20031330

Parodis, I., Sjöwall, C., Jönsen, A., Ramsköld, D., Zickert, A., Frodlund, M., et al. (2017). Smoking and Pre-existing Organ Damage Reduce the Efficacy of Belimumab in Systemic Lupus Erythematosus. *Autoimmun. Rev.* 16 (4), 343–351. doi:10.1016/j.autrev.2017.02.005

Peters, A. L., Stunz, L. L., and Bishop, G. A. (2009). CD40 and Autoimmunity: the Dark Side of a Great Activator. *Semin. Immunol.* 21 (5), 293–300. doi:10.1016/j.smim.2009.05.012

Petri, M., Stohl, W., Chatham, W., McCune, W. J., Chevrier, M., Ryel, J., et al. (2008). Association of Plasma B Lymphocyte Stimulator Levels and Disease Activity in Systemic Lupus Erythematosus. *Arthritis Rheum.* 58 (8), 2453–2459. doi:10.1002/art.23678

Petri, M. A., van Vollenhoven, R. F., Buyon, J., Levy, R. A., Navarra, S. V., Cervera, R., et al. (2013). Baseline Predictors of Systemic Lupus Erythematosus Flares: Data from the Combined Placebo Groups in the Phase III Belimumab Trials. *Arthritis Rheum.* 65 (8), 2143–2153. doi:10.1002/art.37995

Piantoni, S., Regola, F., Zanola, A., Andreoli, L., Dall'Ara, F., Tincani, A., et al. (2018). Effector T-Cells Are Expanded in Systemic Lupus Erythematosus Patients with High Disease Activity and Damage Indexes. *Lupus*. 27, 143–149. doi:10.1177/0961203317722848

Ramsköld, D., Parodis, I., Lakshmikanth, T., Sippl, N., Khademi, M., Chen, Y., et al. (2019). B Cell Alterations during BAFF Inhibition with Belimumab in SLE. *EBioMedicine*. 40, 517–527. doi:10.1016/j.ebiom.2018.12.035

Regola, F., Piantoni, S., Lowin, T., Archetti, S., Reggia, R., Kumar, R., et al. (2019). Association between Changes in BLyS Levels and the Composition of B and T Cell Compartments in Patients with Refractory Systemic Lupus Erythematosus Treated with Belimumab. *Front. Pharmacol.* 10, 433. doi:10.3389/fphar.2019.00433

Romero-Diaz, J., Isenberg, D., and Ramsey-Goldman, R. (2011). Measures of Adult Systemic Lupus Erythematosus: Updated Version of British Isles Lupus Assessment Group (BILAG 2004), European Consensus Lupus Activity Measurements (ECLAM), Systemic Lupus Activity Measure, Revised (SLAM-R), Systemic Lupus Activity Questionnaire for Population Studies (SLAQ), Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K), and Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI). *Arthritis Care Res.* 11 (63 Suppl. 0 11), S37–S46. doi:10.1002/acr.20572

Roth, D. A., Thompson, A., Tang, Y., Hammer, A. E., Molta, C. T., and Gordon, D. (2016). Elevated BLyS Levels in Patients with Systemic Lupus Erythematosus: Associated Factors and Responses to Belimumab. *Lupus*. 25 (4), 346–354. doi:10.1177/0961203315604909

Ruiz-Irastorza, G., and Bertsias, G. (2020). Treating Systemic Lupus Erythematosus in the 21st Century: New Drugs and New Perspectives on Old Drugs. *Rheumatology*. 59 (Suppl. l), v69–v81. doi:10.1093/rheumatology/keaa403

Salazar-Camarena, D. C., Ortiz-Lazareno, P. C., Cruz, A., Oregon-Romero, E., Machado-Contreras, J. R., Muñoz-Valle, J. F., et al. (2016). Association of BAFF, APRIL Serum Levels, BAFF-R, TACI and BCMA Expression on Peripheral B-Cell Subsets with Clinical Manifestations in Systemic Lupus Erythematosus. *Lupus*. 25 (6), 582–592. doi:10.1177/0961203315608254

Salazar-Camarena, D. C., Palafox-Sánchez, C. A., Cruz, A., Marín-Rosales, M., and Muñoz-Valle, J. F. (2020). Analysis of the Receptor BCMA as a Biomarker in Systemic Lupus Erythematosus Patients. *Sci. Rep.* 10 (1), 6236. doi:10.1038/s41598-020-63390-0

Samy, E., Wax, S., Huard, B., Hess, H., and Schneider, P. (2017). Targeting BAFF and APRIL in Systemic Lupus Erythematosus and Other Antibody-Associated Diseases. *Int. Rev. Immunol.* 36 (1), 3–19. doi:10.1080/08830185.2016.1276903

Schwartz, N., Su, L., Burkly, L. C., Mackay, M., Aranow, C., Kollaros, M., et al. (2006). Urinary TWEAK and the Activity of Lupus Nephritis. *J. Autoimmun.* 27 (4), 242–250. doi:10.1016/j.jaut.2006.12.003

Stohl, W., Metyas, S., Tan, S. M., Cheema, G. S., Oamar, B., Roschke, V., et al. (2004). Inverse Association between Circulating APRIL Levels and Serological and Clinical Disease Activity in Patients with Systemic Lupus Erythematosus. *Ann. Rheum. Dis.* 63 (9), 1096–1103. doi:10.1136/ard.2003.018663

Stohl, W., Metyas, S., Tan, S. M., Cheema, G. S., Oamar, B., Xu, D., et al. (2003). B Lymphocyte Stimulator Overexpression in Patients with Systemic Lupus Erythematosus: Longitudinal Observations. *Arthritis Rheum.* 48 (12), 3475–3486. doi:10.1002/art.11354

van Vollenhoven, R. F., Petri, M. A., Cervera, R., Roth, D. A., Ji, B. N., Kleoudis, C. S., et al. (2012). Belimumab in the Treatment of Systemic Lupus Erythematosus: High Disease Activity Predictors of Response. *Ann. Rheum. Dis.* 71 (8), 1343–1349. doi:10.1136/annrheumdis-2011-200937

Vincent, F. B., Kandane-Rathnayake, R., Koelmeyer, R., Hoi, A. Y., Harris, J., Mackay, F., et al. (2019). Analysis of Serum B Cell-Activating Factor from the Tumor Necrosis Factor Family (BAFF) and its Soluble Receptors in Systemic Lupus Erythematosus. *Clin. Transl Immunol.* 8 (4), e01047. doi:10.1002/cti2.1047

Ware, C. F. (2013). Protein Therapeutics Targeted at the TNF Superfamily. *Adv. Pharmacol.* 66, 51–80. doi:10.1016/B978-0-12-404717-4.00002-0

Yazdany, J., and Davis, J. (2004). The Role of CD40 Ligand in Systemic Lupus Erythematosus. *Lupus.* 13 (5), 377–380. doi:10.1191/0961203304lu1030oa

Zhao, Z., Burkly, L. C., Campbell, S., Schwartz, N., Molano, A., Choudhury, A., et al. (2007). TWEAK/Fn14 Interactions Are Instrumental in the Pathogenesis of Nephritis in the Chronic Graft-Versus-Host Model of Systemic Lupus Erythematosus. *J. Immunol.* 179 (11), 7949–7958. doi:10.4049/jimmunol.179.11.7949

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 25 May 2021
doi: 10.3389/fphar.2021.656124

# Single Cell RNA-Seq Analysis Identifies Differentially Expressed Genes of Treg Cell in Early Treatment-Naive Rheumatoid Arthritis By Arsenic Trioxide

*Chunling Li*[1,2], *Tianshu Chu*[1], *Zhiyi Zhang*[2]* *and Yue Zhang*[2,3,4]*

[1]Department of Rheumatology and Immunology, Henan Provincial People's Hospital, People's Hospital of Zhengzhou University, Zhengzhou, China, [2]Department of Rheumatology and Immunology, First Affiliated Hospital of Harbin Medical University, Harbin, China, [3]Department of Rheumatology, Immunology and Gerontology, South China Hospital, Health Science Center, Shenzhen University, Shenzhen, China, [4]Shenzhen Futian Hospital of Rheumatic Diseases, Shenzhen, China

OPEN ACCESS

**Edited by:**
Francesca Oliviero,
University of Padua, Italy

**Reviewed by:**
Agnieszka Paradowska-Gorycka,
National Institute of Geriatrics, Rheumatology and Rehabilitation, Poland
Barbara Rossi,
University of Verona, Italy

***Correspondence:**
Zhiyi Zhang
zhangzhiyi2014@163.com
Yue Zhang
toronto101@163.com

**Specialty section:**
This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology

**Received:** 20 January 2021
**Accepted:** 28 April 2021
**Published:** 25 May 2021

**Citation:**
Li C, Chu T, Zhang Z and Zhang Y (2021) Single Cell RNA-Seq Analysis Identifies Differentially Expressed Genes of Treg Cell in Early Treatment-Naive Rheumatoid Arthritis By Arsenic Trioxide. Front. Pharmacol. 12:656124. doi: 10.3389/fphar.2021.656124

**Objective:** Early treatment-naïve rheumatoid arthritis (RA) has defective regulatory T (Treg) cells and increased inflammation response. In this study, we aim to illustrate the regulation of Treg cells in pathogenesis of early rheumatoid arthritis by arsenic trioxide ($As_2O_3$).

**Methods:** We studied the effects of $As_2O_3$ on gene expression in early treatment-naïve RA Treg cells with single cell RNA-seq (scRNA-seq). Treg cells were sorted from peripheral blood mononuclear cells (PBMCs) and purified by fluorescence-activated cell sorting (FACS) and cultured with or without $As_2O_3$ (at 0.1 μM) for 24 h. Total RNA was isolated and sequenced, and functional analysis was performed against the Gene Ontology (GO) database. Results for selected genes were confirmed with RT-qPCR.

**Results:** $As_2O_3$ exerts no significant effect on $CD4^+$ T-cell apoptosis under physical condition, and selectively modulate $^{CD4+}$ T cells toward Treg cells not Th17 cells under special polarizing stimulators. $As_2O_3$ increased the expression of 200 and reduced that of 272 genes with fold change (FC) 2.0 or greater. Several genes associated with inflammation, Treg-cell activation and differentiation as well as glucose and amino acids metabolism were among the most strongly affected genes. GO function analysis identified top ten ranked significant biological process (BPs), molecular functions (MFs), and cell components (CCs) in treatment and nontreatment Treg cells. In GO analysis, genes involved in the immunoregulation, cell apoptosis and cycle, inflammation, and cellular metabolism were enriched among the significantly affected genes. The KEGG pathway enrichment analysis identified the forkhead box O (FoxO) signal pathway, apoptosis, cytokine–cytokine receptor interaction, cell cycle, nuclear factor-kappa B (NF-κB) signaling pathway, tumor necrosis factor α (TNF-α), p53 signaling pathway, and phosphatidylinositol 3′-kinase (PI3K)-Akt signaling pathway were involved in the pathogenesis of early treatment-naïve RA.

**Conclusion:** This is the first study investigating the genome-wide effects of $As_2O_3$ on the gene expression of treatment-naïve Treg cells. In addition to clear anti-inflammatory and

immunoregulation effects, $As_2O_3$ affect amino acids and glucose metabolism in Treg cells, an observation that might be particularly important in the metabolic phenotype of treatment-naïve RA.

**Keywords: rheumatoid arthritis, arsenic trioxide, regulatory T cell, T helper 17 cell, single cell RNA-seq**

## INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease characterized by inflammatory synovitis and progressive destruction of joint cartilage and bone, leading to swelling, pain, stiffness, and loss of function (Han et al., 2008; Smolen et al., 2016). T cells are a critical regulator in the pathogenesis of RA as they accumulate in the lesions of joints, resulting in tissue-destruction and sustaining chronic inflammation (Goronzy and Weyand, 2017).

The pathological mechanism of RA remains unclear; however, it has been reported that the imbalance between T helper 17 (Th17) and regulatory T cells (Tregs) underlines the pathogenesis of RA, Th17 cells have proinflammatory effects, and the proportion of Th17 cells is higher in RA patients, and the content is positively correlated with the activity of RA disease (Niu et al., 2012; Jin et al., 2018; Li et al., 2019). Tregs, however, have immunosuppressive function and play an important role in the induction and maintenance of self-tolerance. The reduced content and dysfunction of Tregs are closely related to the occurrence and development of RA. Moreover, the connection of the Treg/Th17 cells imbalance is relevant for the development and/or progression of RA, which is in turn associated with the autoimmunity, chronic inflammation, and articular destruction in joints of RA patients (McInnes and Schett, 2007; Su et al., 2019). Previous literatures demonstrated that Treg cells mainly mediate the anti-inflammatory responses by producing IL-10 and transforming growth factor (TGF-β) suppression cytokines and maintain the state of autoimmune tolerance (Wang et al., 2017). IL-17 can induce proinflammatory cytokines production, chemokines (such as CCR6), and matrix metalloproteinases (such as MMP13), which result in tissue invasion and destruction as well as damage of articular cartilage and bone (Koenders et al., 2006; Miossec and Kolls, 2012). Tregs injected into collagen induced arthritis (CIA) mice can control the inflammatory responses and alleviate pathological damage (Safari et al., 2018). Thus, improving the Treg/Th17 cells balance shows some promise for the treatment of RA.

A couple of genome-wide association studies (GWAS) have previously been performed comparing RA either to osteoarthritis or to healthy donors (Stahl et al., 2010; Eyre et al., 2011). Two larger studies have utilized microarray and RNA-seq, respectively, to compare RA and healthy individuals $CD4^+$ T cells, identifying a number of differentially expressed genes involved in differentiation, immune response, apoptosis, cell cycle regulation, and cellular metabolism (Sumitomo et al., 2018). Recent research has yielded biological therapies and small molecules to target signaling pathways and pathogenic components involved in inflammation and immunity, but in spite of these reasonably successful treatments, very few RA patients are able to achieve and stay in a state of drug-free remission. Innovative strategies are needed to obtain new insights into mechanisms which underline disease pathogenesis and to identify new treatments.

In current studies, $As_2O_3$ has shown substantial efficacy in treatment of patients with newly diagnosed or relapsed acute promyelocytic leukemia (APL) and other type of cancer and those characterized by the proliferation of immature immune cells, due to its substantial ability to induce apoptosis and mitogen activated protein kinase (MAPK) expression (Zhang et al., 2001). Furthermore, $As_2O_3$ exerts therapeutic effects on lymphoproliferative and severe autoimmune disease manifested in MRL/lpr mice (Bobé et al., 2006). Additionally, our previous accumulating data revealed that $As_2O_3$ contributes the balance of Treg/Th17 cells and affects both of their related signal cytokines pathways in treatment-naïve RA and CIA (Li et al., 2019a; Li et al., 2019b). Furthermore, we have discovered that $As_2O_3$ significantly suppress angiogenesis and induced fibroblast like synoviocytes (FLS) apoptosis in CIA and RA (Mei et al., 2011; Zhang et al., 2017). Moreover, our previous experiments found that $A_2O_3$ with vitamin D rescues the defective VDR-PPAR-$\gamma$ functional module of autophagy synergistically in RA (Wang et al., 2019).

RA patients have an increased susceptibility to metabolic syndrome during the progression of their disease, associated with disruption of lipid and glucose metabolism (Cojocaru et al., 2012). There is also evidence on metabolic derangements in RA, and impairments in, for example, glycolysis, amino acids metabolism, oxidative stress, and mitochondrial respiration have been reported (Weyand and Goronzy, 2017; Falconer et al., 2018). Glycolysis deprivation was found to impair Th17 differentiation dramatically, while defective glycolysis supported the development of Treg cells. Replacement of glucose with galactose, treatment with 2-DG (an inhibitor of hexokinase, the first rate-limiting enzyme of glycolysis), and lack of crucial regulators of T-cell glycolytic metabolism, all resulted in diminished Th17 development but enhanced Treg-cell differentiation (Shi et al., 2011; Kalim et al., 2018; Kono et al., 2018; Cluxton et al., 2019). Conversely, inhibition of fatty acid oxidation results in diminished differentiation to Th17 cells, but increased development of Tregs (Gualdoni et al., 2016). Due to their established effects on these metabolic pathways in other cell types, $As_2O_3$ could plausibly affect RA pathogenesis via affecting Treg metabolism.

In the present study, we set out to study the effects of the $As_2O_3$ on gene expression in treatment-naïve RA Treg cells. The aim was to identify significantly modulated pathways and/or functional categories of genes that might be important in the pathogenesis of RA.

**TABLE 1 |** General information of early treatment-naïve RA patients used for scRNA-seq and qRT-PCR analysis.

| Clinical and laboratory characteristics of treatment-naïve RA* for scRNA-seq and qRT-PCR | | |
|---|---|---|
| | **scRNA-seq** | **qRT-PCR** |
| Age, y | 43 ± 4.52 | 44 ± 7.81 |
| Sex (male/female) | 1/2 | 1/5 |
| Morning stiffness | 2 (67) | 5 (83) |
| Serum rheumatoid factor | 3 (100) | 4 (67) |
| Serum anti-CCP | 3 (100) | 5 (83) |
| Bone erosions | 1 (33) | 2 (33) |
| DAS28, mean ± S.E.M (range) | 5.5 ± 0.4 (4.2-8.5) | 5.7 ± 0.5 (4.0-8.9) |
| Swollen joint count, mean ± S.E.M (range) | 5.2 ± 1.8 (2-8) | 5.5 ± 1.9 (2-16) |
| Tender joint count, mean ± S.E.M (range) | 4.9 ± 1.7 (2-10) | 12.6 ± 2.5 (2-24) |
| Disease duration (month), mean ± S.E.M (range) | 5.2 ± 1.6 (1-12) | 7.6 ± 2.3 (2-24) |

**Patients were diagnosed as having rheumatoid arthritis (RA) if they met ≥4 of criteria listed. Except where indicated otherwise, values are the number (%) of patients. Anti-CCP = anti-cyclic citrullinated peptide; DAS28 = Disease Activity Score in 28 joints.*

# MATERIALS AND METHODS

## Early Treatment-Naïve RA Patient Collection

Treatment-naïve RA patients were obtained from the first affiliated hospital of Harbin Medical University, Department of Rheumatology. Recruited RA patients adhered to the Helsinki Declaration. All the three early treatment-naïve RA patients were seropositive for rheumatoid factor (RF) and or/anti-citrullinated peptide antibodies (ACPA), fulfilled 2010 ACR/EULAR criteria for RA and had active disease with a DAS28 > 3.2. The three early treatment-naïve RA patients were collected for RNA-seq and six treatment-naïve RA patients were collected for validation by qRT-PCR (**Table 1**). All the RA patients fulfilled the ACR criteria, according to clinical and radiological imaging (Aletaha et al., 2010). All participants gave written informed consent according to the Declaration of Helsinki. Ethics approval for the study was obtained from Harbin Medical University Research and Ethics Committee, Henan Provincial People's Hospital Research and Ethics Committee, Zhengzhou University Research and Ethics Committee.

## Apoptosis Assay

Naïve $CD4^+$ T cells were isolated from early treatment-naïve RA patients and cultured in the presence or absence of $As_2O_3$ with anti-CD3/CD28 activation and IL-2 exists. The detailed procedures as described in our previous study (Li et al., 2019).

## Flow Cytometry

For intracellular cytokine detection, cells were stimulated with the corresponding Cell Activation Cocktail (with Brefeldin A) (Biolegend, San Diego, CA) for 6 h. The detailed procedures described in our previous study (Li et al., 2019).

## Cytokine Quantification

The cytokines IL-17A and IL-10 (D1700 and D1000, respectively, purchased from R&D systems, Minneapolis, United States) and MMP13 (E-EL-H0134c, Elabscience Biotechnology, Wuhan, China) were determined using enzyme-linked immunosorbent assay (ELISA). The ELISA was performed according to the manufacturer's instructions.

## Immunofluorescence Staining

Cultured cells were fixed in 4% paraformaldehyde, followed by penetrating and blocking serum for 1 h, Rabbit anti-STAT3 (ab68153, Abcam, Cambridge, MA, United States) and Rabbit anti-Foxp3 (BA 2032–1, Boster, Wuhan, China) were used as the primary antibody. Samples were washed three times and incubated with FITC secondary antibodies (PV6001, ZSGB-BIO, Beijing, China), and DAPI (Sigma) was used for staining nuclei. Images were captured using a microscope (Leica, Mannheim, Germany).

## Single Treg Cell Sorting

Treg cells were purified from PBMCs by high-speed cell sorter system (Moflo XDP, Beckman coulter, United States). The detailed procedures are described in our previous literature (Li et al., 2019). The working schematic model is shown in **Figure 1A**.

## RNA Quantification and Qualification

RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer spectrophotometer (IMPLEN, CA, United States). RNA concentration was measured using Qubit RNA Assay Kit in Qubit 2.0 Flurometer (Life Technologies, CA, United States). RNA concentration and integrity were assessed using the RNA Nano 6000 Assay Kit of the Bioanalyzer 2100 system (Agilent Technologies, CA, United States).

## GO and KEGG Enrichment Analysis of Differentially Expressed Genes

To assess the function of identified DEGs, the functional analysis and clustering tool from Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7 was used to perform Gene Ontology (GO) function enriched analysis based on DEGs obtained from RA Treg cells. GO enrichment analysis of DEGs was implemented by the GOseq

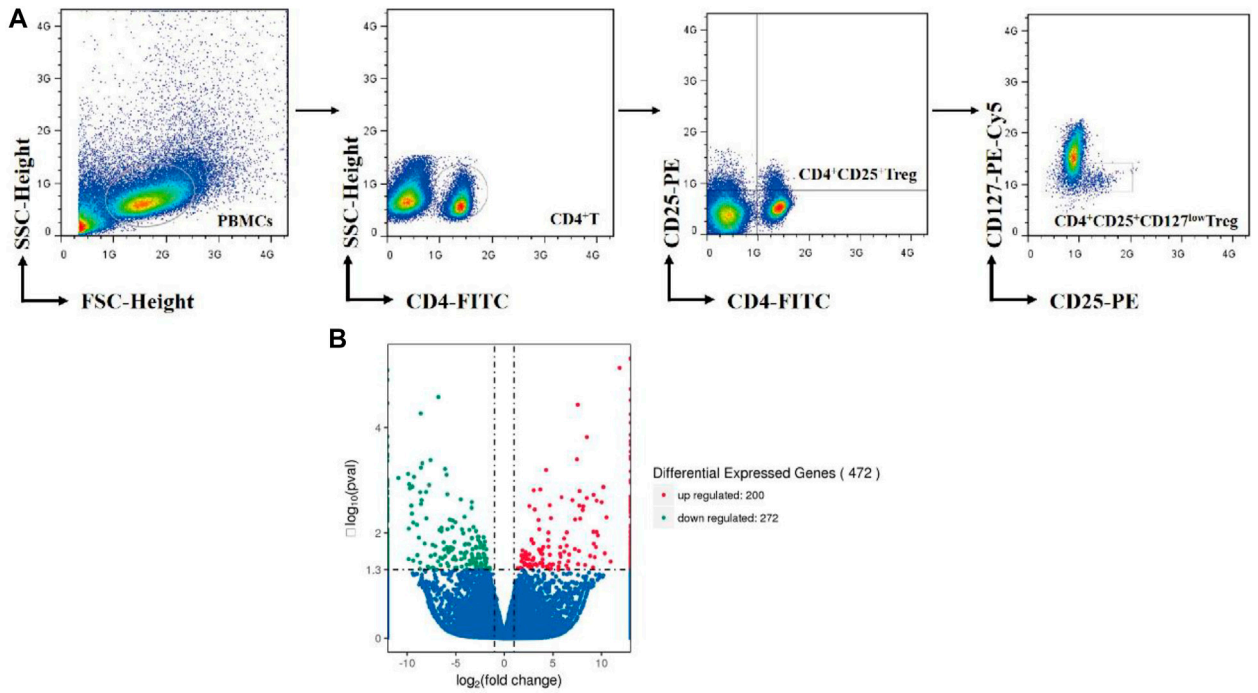


**FIGURE 1 | (A)** Overview of Treg-cell isolation by cell sorter. **(B)** Volcano Plots include all differential expressed genes absolute value log2-fold change >0.5. Red nodes represent upregulation and blue nodes represent downregulation in treatment Treg cells compared to nontreatment Treg cells.

R package, in which gene length bias was corrected. DAVID provides a comprehensive set of functional annotation tools for investigators to understand the biological meaning based on a large list of genes (http://david.ncifcrf.gov/). For any given gene list, DAVID tools can identify enriched biological themes (GO terms), discover enriched functional-related genes cluster, and visualize genes on BioCarta and KEGG pathway maps (Huang et al., 2009). Therefore, DAVID was carried out to identify the enriched GO functions including the biological processes (BPs), molecular functions (MFs), and cell components (CCs). KEGG Orthology Based Annotation System (KOBAS 2.0) (http://kobas.cbi.edu.cn) was employed to identify biological pathways from the identified DEGs involved in the diseases. It performs statistical tests to identify statistically significantly enriched pathways and diseases using biological knowledge from five well-known pathway databases and GO (Xie et al., 2011). Thus, the KOBAS 2.0 was used to identify the enriched KEGG pathway based on adjusted $p$ values. KEGG pathways including five or more DEGs genes were considered as the biologically meaningful analysis.

## Validating RNA-Seq Data Using Real-Time PCR

Total RNA was extracted from Treg cells according to the instructions of RNA extraction kit (Trizol Reagent, Invitrogen, Carlsbad, CA, United States). cDNA obtained from the reverse transcriptase reaction and subjected to quantitative RT-qPCR using SYBR Green PCR Master Mix (Bio-Rad, California, United States) and using the ABI Prism 7500 Sequence detection system (Applied Biosystems). Primers for human glyceraldehyde-3-phosphate dehydrogenase (GAPDH), cyclin-dependent kinase inhibitor 3(CDKN3), sushi domain containing 4 (SUSD4), histone cluster 4 H4 (HIST4H4), ubiquitin-specific peptidase 7 (USP7), histidine ammonia lyase (HAL), protein tyrosine phosphatase, non-receptor type 13 (PTPN13), DNA fragmentation factor subunit beta (DFFB), receptor interacting serine/threonine kinase 1 (RIPK1), and methyltransferase like 3 (METTL3) were purchased from Takara. The primer and concentrations were optimized according to the manufacturer's instructions in SYBR Green PCR Master Mix Protocol. Relative expression levels of the nine selected genes were calculated by using the $2^{-\Delta\Delta Ct}$ method. The detailed following methods were performed according to our previous literature (Zhang et al., 2017).

## Statistical Analysis

Statistical significance was determined using GraphPad Prism Software (Version 6 for Windows; GraphPad Prism, San Diego, CA, United States). Simple comparisons were made using unpaired, two-tailed Student's $t$-test for parametric data or Mann–Whitney test for nonparametric data, as indicated. Multigroup comparisons of the means were carried out by one-way analysis of variance test with post hoc contrasts by Tukey test. $p$ values of 0.05 or less was considered statistically significant. All data are presented as mean ± S.E.M. Transcripts with significantly differential expressions of Treg cells by $As_2O_3$ were identified using hypergeometric test. The resulting

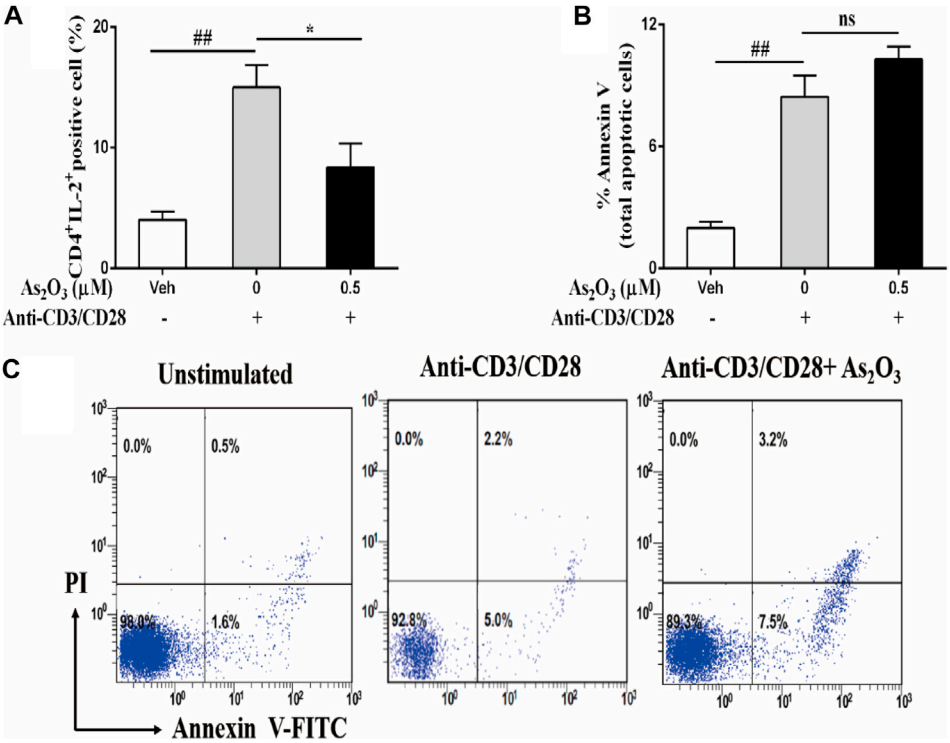


**FIGURE 2 |** $As_2O_3$ suppress IL-2 expression from activated $CD4^+$ T cells without affecting their viability. PBMCs ($1 \times 10^6$ cells per well) were left untreated or treated with $As_2O_3$ (0.5 μM) for 30 min. Cells were then stimulated with anti-CD3/CD28 for 8 h. Subsequently, cells were stained on the surface and intracellularly, and analyzed by flow cytometry. **(A)** Percentage of intracellular production of IL-2 from $CD4^+$ T cell is shown as means ± SEM of six independent experiments. **(B,C)** Cell apoptosis of $CD4^+$ T cells after activated with anti-CD3/CD28 through staining for annexin V and PI was detected by flow cytometry analysis. Data are means ± SEM of six independent experiments. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs* nonactivated PBMCs cells; $^{*}p < 0.05$, $^{**}p < 0.01$ *vs* PBMCs nontreated with $As_2O_3$ (one-way ANOVA).

*p*-values were adjusted using the Benjamini and Hochberg's method for controlling the false discovery rate (FDR). DEGs were identified by applying the Benjamini and Hochberg method with adjusted *p* values of <0.05. DAVID v6.7 was used to carry out GO function enriched analysis based on the DEGs. KOBAS 2.0 was used to identify the enriched KEGG pathway based on the adjusted *p* values using Benjamini and Hochberg method.

# RESULTS

## $As_2O_3$ Modulates IL-2 Production From PBMCs Without Affecting Viability

The immunomodulatory activity of $As_2O_3$ on PBMCs cells responses was also determined by the reduction of the growth factor IL-2 compared with IL-2-producing anti-CD3/CD28 activated PBMCs (**Figure 2A**). This effect was not due to an induction of the cell death, as assessed by annexin V staining that was used as a marker for apoptosis in combination with propidium iodide (PI), to distinguish between apoptotic and necrotic cells. After treatment 24 h, $As_2O_3$ treatment of PBMCs did not induce cell apoptosis, ruling out the potential cytotoxic role of $As_2O_3$ (**Figures 2B,C**). The decrease of IL-2 did not result in a significant decrease in PBMCs proliferation.

## $As_2O_3$ Critically Affect Th17 and Treg Differentiation and Related Signal Cytokines

Th17 and Treg subsets are both derived from naïve $CD4^+$ T cells in peripheral blood upon antigen stimulation and specific polarizing cytokines. Because $As_2O_3$ dampened the inflammatory response of IL-17–producing cell from PBMCs, we next investigated whether $As_2O_3$ could directly affect their differentiation from naïve $CD4^+$ T cells into Th17 cells lineages. To obtain this aim, a standard naïve $CD4^+$ T cells differentiation assay was performed by polyclonal stimulation with anti-CD3/CD28 and specific polarizing cytokines in the presence of $As_2O_3$ (**Figure 3A**).

Under specific polarizing conditions, highly purified naïve $CD4^+$ T cells displayed significantly higher amounts of intracellularly produced and extracellularly released IL-17 and MMP13, as compared to nonpolarized (Th0) cells (**Figure 3C**), in particular, in non-skewed Th0 cells, which produce low level IL-17 and MMP13. However, $As_2O_3$ significantly reduced Th17 generation, acting both on intracellular production and extracellular release

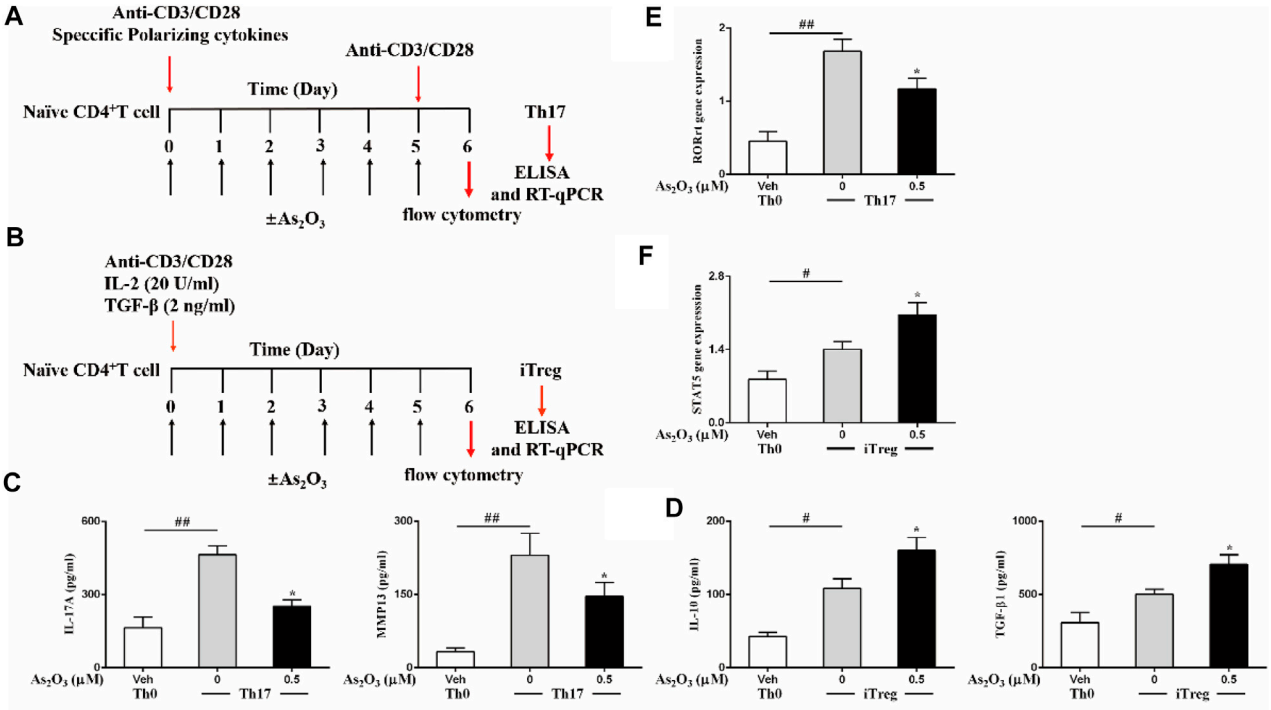


**FIGURE 3 |** $As_2O_3$ affects Treg and Th17 cells differentiation. **(A,B)** Schematic representative of Th17 and Treg-cell differentiation. **(C,D)** ELISA of IL-17A and MMP13 in supernatants of Th17 cells polarized, IL-10 and TGF-β release in supernatants of Treg-cell production in the presence or absence of $As_2O_3$ (0.5 μM), measured after 24 h of restimulation with anti-CD3/CD28. **(E,F)** RT-qPCR analysis of the expression RORγt for Th17 cells, of STAT5 in Treg cells. Cycling threshold (CT) values are normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Data are represented as means ± SEM of six independent experiments. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. nonpolarized $CD4^+$ T cells; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. Th17 cells nontreated with $As_2O_3$ (one-way ANOVA).

from Th17 cells, suggesting that $As_2O_3$ affects not only Th17 cells induction but also specific functional properties. To address whether Th17 polarization was associated with the acquisition of their typical features, we also measured the mRNA encoding for the transcription factor RORγt known to be critical for their differentiation. As we expected, Th17 condition induced the highest expression of their specific transcription factors. The presence of $As_2O_3$ during Th17 polarization led to decreased RORγt in Th17 cells (**Figure 3E**). These findings support a pivotal role of $As_2O_3$ in hindering *de novo* Th17 differentiation.

In light of the role of $As_2O_3$ in resolving inflammation and because Treg cells is an important cell subset involved in modulating and maintaining self-regulation of the immune system, we also investigated whether $As_2O_3$ could affect the generation of induced Treg cells. This cell subset develops from naïve $CD4^+$ T cells upon antigen stimulation and transforming growth factor-β (TGF-β) exposure. To obtain this aim, highly purified naïve $CD4^+$ T cells were cultured under Treg-inducing conditions in the presence of $As_2O_3$ (**Figure 3B**). We found that $As_2O_3$ potentiated Treg differentiation, with $As_2O_3$ enhancing STAT5 expression compared to control Treg cells (**Figure 3F**). $As_2O_3$-induced *de novo* generation of Treg cells was also paralleled by their capacity to increase their suppressive cytokines IL-10 and TGF-β1 (**Figure 3D**), suggesting that $As_2O_3$ affect not only Treg induction but also specific functional properties.

## $As_2O_3$ Promotes $CD4^+CD25^+$ Treg Cells Proliferation

To investigate the effect of $As_2O_3$ on $CD4^+CD25^+$ Treg cells proliferation, $CD4^+CD25^-$ T and $CD4^+CD25^+$ Treg cells were isolated from PBMCs in treatment-naïve RA patients (**Figure 4A**). $CD4^+CD25^+$ Treg cells generally represent anti-inflammatory subtype. To examine whether $As_2O_3$ directly affect $CD4^+CD25^+$ Treg-cell proliferation, $CD4^+CD25^+$ Treg cells were cultured with physical condition anti-CD3/CD28 stimulation, as well as $As_2O_3$ was added in the well for 3 days. Interestingly, we noticed that $As_2O_3$ dramatically increased the proportion of $CD4^+CD25^+$ Treg cells compared with without $As_2O_3$ treatment of $CD4^+CD25^+$ Treg cells (**Figure 4B**). To address whether $As_2O_3$ affects $CD4^+CD25^+$ Treg-cell polarization was associated with the acquisition of their typical features, we also measured the mRNA encoding for the transcription factor known to be critical for their differentiation Foxp3. As expected, $As_2O_3$ induced higher expression of Foxp3 compared with $CD4^+CD25^+$ Treg cells alone (**Figure 4C**).

## $As_2O_3$ Affects the Phenotype of Treg and Th17 Cells

To examine the effect of $As_2O_3$ in the phenotypic characteristics of Treg cells, we used flow cytometry to assess the expression of Foxp3 and surface markers known to be associated with either Th17 or

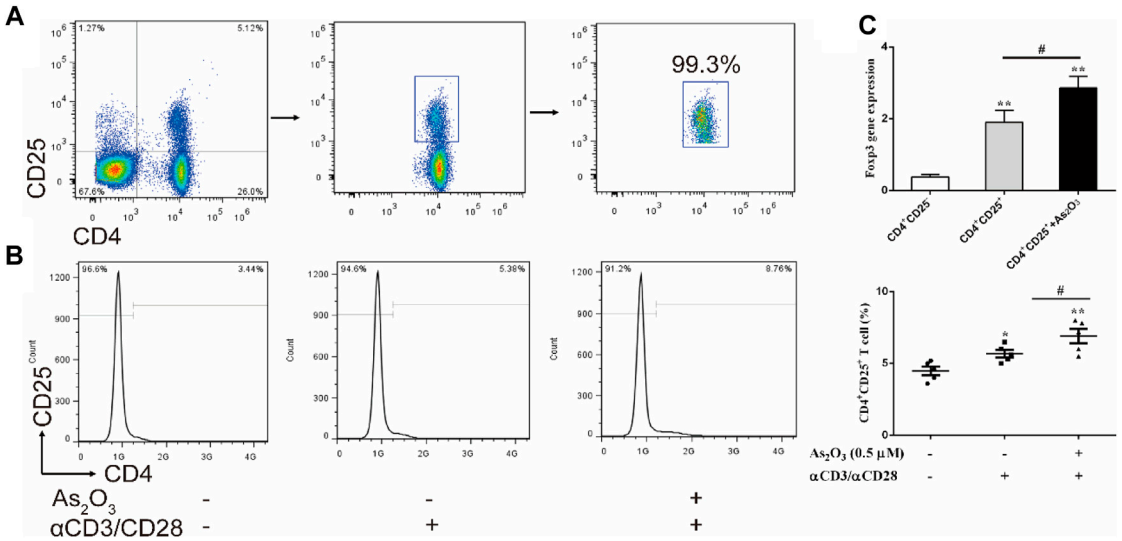


**FIGURE 4 |** $As_2O_3$ increased $CD4^+CD25^+$ Treg-cell proportion by regulating Foxp3 expression. **(A)** $CD4^+CD25^+$ Treg cells and $CD4^+CD25^-$ T cells were isolated from PBMCs in treatment-naïve RA patients. The purity of obtained cells was ≥99% confirmed by flow cytometry. **(B)** Histograms represent the proportion of $CD4^+CD25^+$ Treg cells from treatment-naïve RA patients in absence or presence of $As_2O_3$ (0.5 μM) and with or without anti-CD3/CD28 activation *in vitro*. **(C)** qRT-PCR for detection of Foxp3 from purified $CD4^+CD25^+$ Treg-cell subsets. The statistical analysis was performed using one-way ANOVA. Data are represented as the mean ± SEM in six independent experiments. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs* nontreatment cells; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. $CD4^+CD25^-$ T cells from treatment-naïve RA patients.

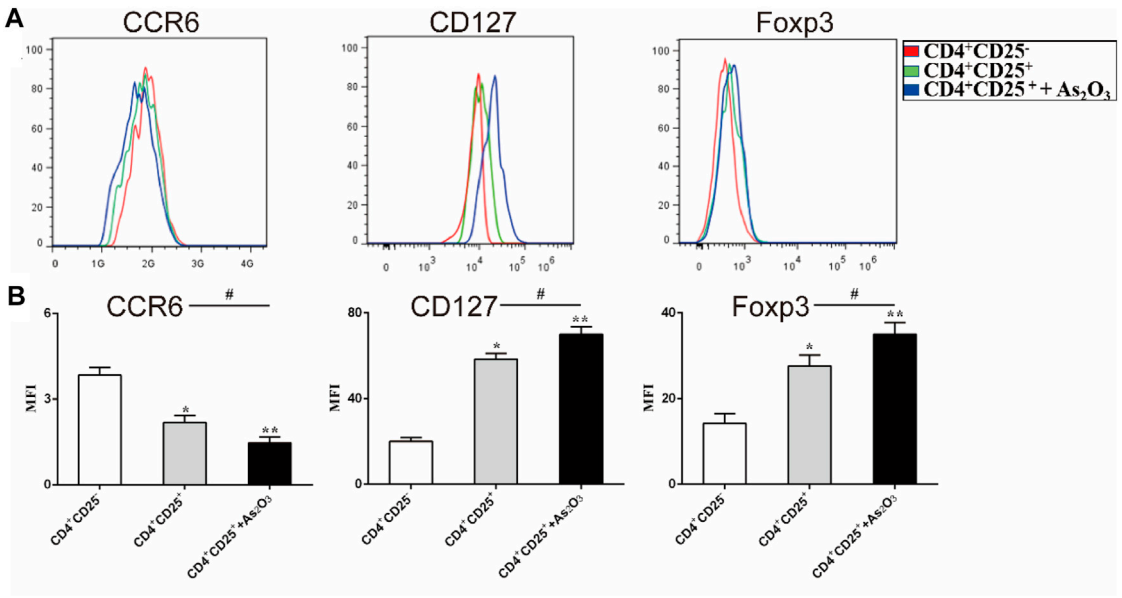


**FIGURE 5 |** $As_2O_3$ affect phenotype of $CD4^+CD25^+$ Treg cells. **(A)** Histogram represent that surface expression of CCR6, CD127, and intracellular expression of Foxp3 were measured in $CD4^+CD25^-$ T cells, $CD4^+CD25^+$ Treg cells and coculture in the presence or absence of $As_2O_3$. **(C)** The expression levels of the surface and intracellular markers were quantified as mean fluorescence intensity (MFI). The statistical analysis was performed using one-way ANOVA. Data are represented as the mean ± SEM in six independent experiments. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ vs. nontreatment cells; $^{*}p < 0.05$, $^{**}p < 0.01$ vs. $CD4^+CD25^-$ T cells from treatment-naïve RA patients.

Treg cells. CCR6 is human Th17 cell marker. The expression of CCR6 was slightly but significantly lower in $CD4^+CD25^+$ Treg cells than in $CD4^+CD25^-$ Treg cells (mean fluorescence intensity (MFI): 2.18 ± 0.24 vs 3.84 ± 0.27, $p < 0.05$), whereas the expression levels of the Treg-associated regulatory molecule CD127 and Foxp3 were significantly higher in $CD4^+CD25^+$ Treg cells than in $CD4^+CD25^-$

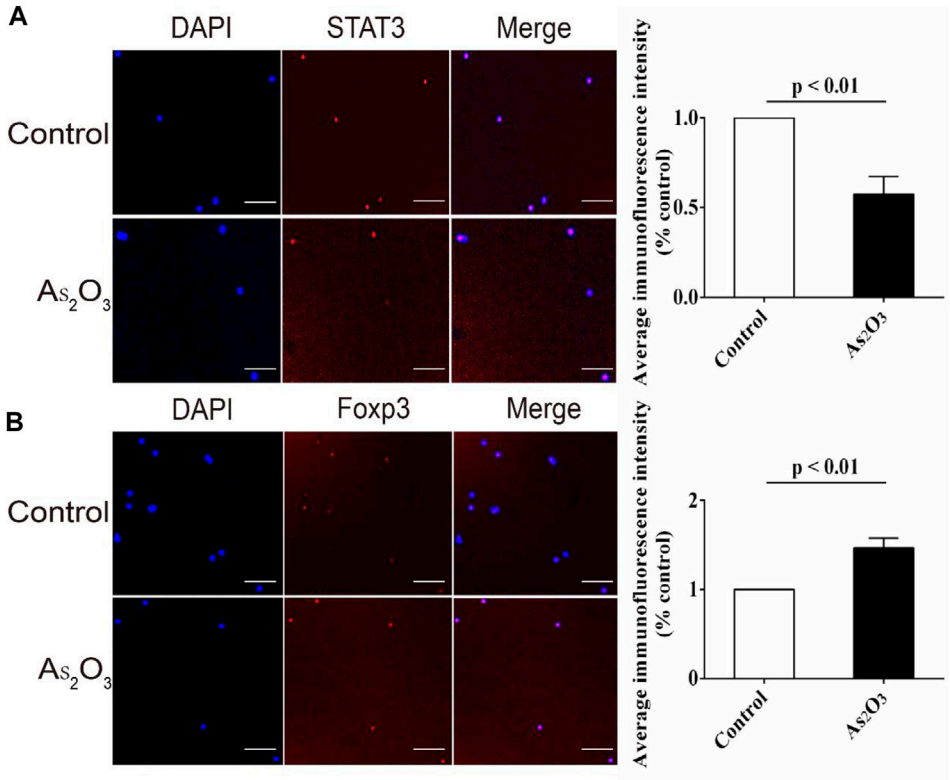


**FIGURE 6 |** $As_2O_3$ increased gene expression of transcription factor Foxp3 for Treg cell while decreased STAT3 for Th17 cell from treatment-naïve RA patients. **(A,B)** PBMCs were extracted and cultured with anti-CD3/CD28 in presence or absence of $As_2O_3$ for 24 h. Representative images of anti-STAT3 and anti-Foxp3 fluorescence (red), nuclear DAPI (blue), and merged with the bright field (Merge) in vehicle and $As_2O_3$ treatment. Scale bars: 100 μm $As_2O_3$ decreased the apparent STAT3 fluorescence intensity while increased that of Foxp3 compared with vehicle group. STAT3 and Foxp3 levels were detected and quantified by the fluorescence microscopy. The statistical analysis was performed using one-way ANOVA. Data are represented as the mean ± SEM in six independent experiments.

Treg cells (MFI: 58.4 ± 2.65 vs 20.2 ± 1.7 and 22.2 ± 1.02 17.6 ± 2.5, respectively). Interestingly, we noticed that $As_2O_3$ downregulated CCR6 expression, while upregulated CD127 and Foxp3 expression in $CD4^+CD25^+$ Treg cells (**Figures 5A,B**). These results show that $As_2O_3$ enhances Treg-cell immunosuppression by regulating phenotype of Treg and Th17 cells.

## $As_2O_3$ Inhibited Nuclear Translocation of STAT3 in Early Treatment-Naïve RA Patients

We studied the effects of $As_2O_3$ on STAT3 in Th17 and Foxp3 in Treg cells by immunofluorescent staining. We determined the effects of $As_2O_3$ on STAT3 and Foxp3 function by observing nuclear translocation. As shown, STAT3 in nucleus was reduced by $As_2O_3$ treatment (**Figure 6A**), while the Foxp3 was increased (**Figure 6B**).

## Pathway Enrichment Analysis

The pathway enrichment analysis identified 22 differentially expressed pathways. Considering that the biological functions identified and previous study results for Treg cells, eight potential critical pathways participate in regulation of Treg-cell function by $As_2O_3$ from early treatment-naïve RA patients (**Table 2**). One upregulated enriched pathway found to be involved in FoxO acetylation which affects Foxp3 transcription and Treg-cell development. Seven downregulated enriched pathways were also determined, including apoptosis, cytokine–cytokine receptor interaction, cell cycle, PI3K-Akt signaling pathway, nuclear factor kappa-B signaling pathway, calcium signaling pathway, and p53 signaling pathway (**Table 2**).

## Differentially Expressed Genes

After normalization and correction for multiple testing, 100 genes were upregulated more than 2.0-fold in $As_2O_3$-treated cells compared to control cells, and 136 downregulated by the same factor (FC<-2.0). In total, 472 genes were found to be differentially expressed in $As_2O_3$-treated vs. control Treg cells in a statistically manner (FDR-corrected $p$ value <0.05). We have identified 472 DEGs including 200 upregulated genes and 272 downregulated genes in treatment Treg cells compared to nontreatment Treg cells with $As_2O_3$ (**Figure 1B**). Twelve most strongly up- and downregulated genes are listed in **Table 3**.

The list of the most strongly upregulated genes include genes involved in the regulation of cell proliferation, immunoregulation, apoptosis, and amino acids and glycolysis metabolism. Among the most strongly downregulated genes are those linked to cell proliferation and differentiation, extracellular signal activation, and inflammation.

**TABLE 2 |** KEGG pathways enriched in treatment Treg cells of early treatment-naïve RA patients.

| KEGG pathway | Class | Number of genes (%) | Nominal *p* value[a] | Adjusted *p* value[b] |
|---|---|---|---|---|
| Upregulated in treatment Treg group | — | — | — | — |
| FoxO signaling pathway | Signal transduction | 26 (20.5) | $9.5 \times 10^{-5}$ | $1.4 \times 10^{-3}$ |
| Downregulated in treatment Treg group | — | — | — | — |
| Apoptosis | Development | 46 (23.1) | $3.4 \times 10^{-5}$ | $4.4 \times 10^{-3}$ |
| Cell cycle | Development | 35 (22.4) | $5.3 \times 10^{-6}$ | $5.9 \times 10^{-4}$ |
| Rheumatoid arthritis | Immune disease | 28 (20.6) | $5.4 \times 10^{-5}$ | $7.3 \times 10^{-3}$ |
| Nuclear factor-kappa B signaling pathway | Signal transduction | 24 (26.5) | $1.8 \times 10^{-4}$ | $3.5 \times 10^{-2}$ |
| Cytokine–cytokine receptor interaction | Immune system | 27 (23.2) | $1.6 \times 10^{-5}$ | $7.7 \times 10^{-3}$ |
| T cell receptor signaling pathway | Signaling molecules and interaction | 22(21.5) | $5.7 \times 10^{-5}$ | $6.7 \times 10^{-3}$ |

[a]*Nominal* p *value was calculated by hypergeometric test.*
[b]*Adjusted* p *values was corrected from nominal* p *values by Benjamini and Hochberg multiple testing correction.*

**TABLE 3 |** Eight most strongly up- and downregulated genes in $As_2O_3$-treated RA Treg cells relative to control.

**Genes most strongly upregulated by $As_2O_3$**

| Genes | Name | Function | $Log_2$-fold change | Nominal *p* value[a] |
|---|---|---|---|---|
| CDKN3 | Cyclin-dependent kinase inhibitor 3 | Cell cycle | 11.8 | $49 \times 10^{-2}$ |
| SUSD4 | Sushi domain containing 4 | Immunity | 7.6 | $4.8 \times 10^{-2}$ |
| HIST4H4 | Histone cluster 4 H4 | Histone regulation | 5.5 | $3.2 \times 10^{-2}$ |
| PSAT1 | Phosphoserine aminotransferase 1 | Amino acid synthesis | 4.8 | $4.3 \times 10^{-2}$ |
| PDK-3 | Pyruvate dehydrogenase kinase | Glucose and fatty acid metabolism | 4.2 | $4.6 \times 10^{-2}$ |
| CRAT | Carnitine O-acetyltransferase | Fatty acid metabolism | 3.7 | $3.8 \times 10^{-2}$ |
| USP7 | Ubiquitin-specific peptidase 7 | Ubiquitination metabolism | 3.1 | $4.1 \times 10^{-2}$ |
| HAL | Histidine ammonia lyase | Histidine metabolism | 2.5 | $1.8 \times 10^{-2}$ |

**Genes most strongly downregulated by $As_2O_3$**

| Genes | Name | Function | $Log_2$-fold change | Nominal *p* value[a] |
|---|---|---|---|---|
| DFFB | DNA fragmentation factor subunit beta | Regulate Apoptosis | −8.4 | $4.9 \times 10^{-2}$ |
| PTPN13 | Protein tyrosine phosphatase, nonreceptor type 13 | Regulate apoptosis | −7.6 | $2.5 \times 10^{-2}$ |
| RIPK1 | Receptor interacting serine/threonine kinase 1 | Regulate Apoptosis | −6.4 | $4.6 \times 10^{-2}$ |
| UBR5 | Ubiquitin protein ligase E3 component n-recognin 5 | DNA damage | −5.5 | $4.9 \times 10^{-2}$ |
| PBRM1 | Polybromo 1 | Negative cell proliferation | −5.2 | $3.3 \times 10^{-2}$ |
| METTL3 | Methyltransferase like 3 | RNA methylation | −4.1 | $6.1 \times 10^{-2}$ |
| SLC45A4 | Solute carrier family 45 member 4 | Anion transport | −3.3 | $4.9 \times 10^{-2}$ |
| FLCN | Folliculinum | GTPase activation | −2.4 | $4.7 \times 10^{-4}$ |

[a]*Nominal* p *value was calculated by Fisher's exact test.*

## GO Function Enrichment Analysis

We selected nine significantly overrepresented BPs, including regulation of mitotic cell cycle, regulation of DNA recombination, and protein ubiquitination. There were nine significantly overrepresented MFs, including peptide transporter activity, RNA binding, glycogen synthase activity, kinase activator activity, and phosphatase activity. We also detected five significantly overrepresented CCs, including mitochondrial chromosome, cyclin E1–CDK2 complex, and DNA helicase complex (**Figure 7**). In summary, the significantly overrepresented BPs, MFs, and CCs were dramatically different between nontreatment Treg and treatment Treg cell with $As_2O_3$.

## Genes Involved in Inflammation and Immunoregulation

As chronic inflammatory autoimmune disease and changes in Treg cell frequency and dysfunction are central features in the pathogenesis, we set out to separately study genes linked to these processes (**Table 4**). Several proinflammatory factors such as hypoxia inducible factor 1 alpha subunit (HIF1α, fold change −8.4), matrix metallopeptidase 3 (MMP3, fold change −3.2), C-C motif chemokine receptor 6 (CCR6, fold change −7.6), and SMAD family member 3 (SMAD3, fold change −4.8) were significantly downregulated by $As_2O_3$, while the anti-inflammatory peroxisome proliferator activated receptor gamma (PPARγ, fold change 6.4) and interleukin 10 (IL-10, fold change 2.6) were upregulated. Genes affecting immune response, such as forkhead box O1 (Foxo1, fold change 11.8) and cytotoxic T-lymphocyte associated protein 4 (CTLA-4, fold change 4.6), were similarly upregulated.

## Amino Acid and Carbohydrate Metabolism

RA is also known to be associated with metabolic syndrome. We separately studied genes for proteins participating in the main pathways of amino acid and carbohydrate metabolism (amino acid biosynthesis, glycolysis) (Weyand and Goronzy, 2017; Falconer et al., 2018). $As_2O_3$ did not have a marked (fold change>2.0) effect

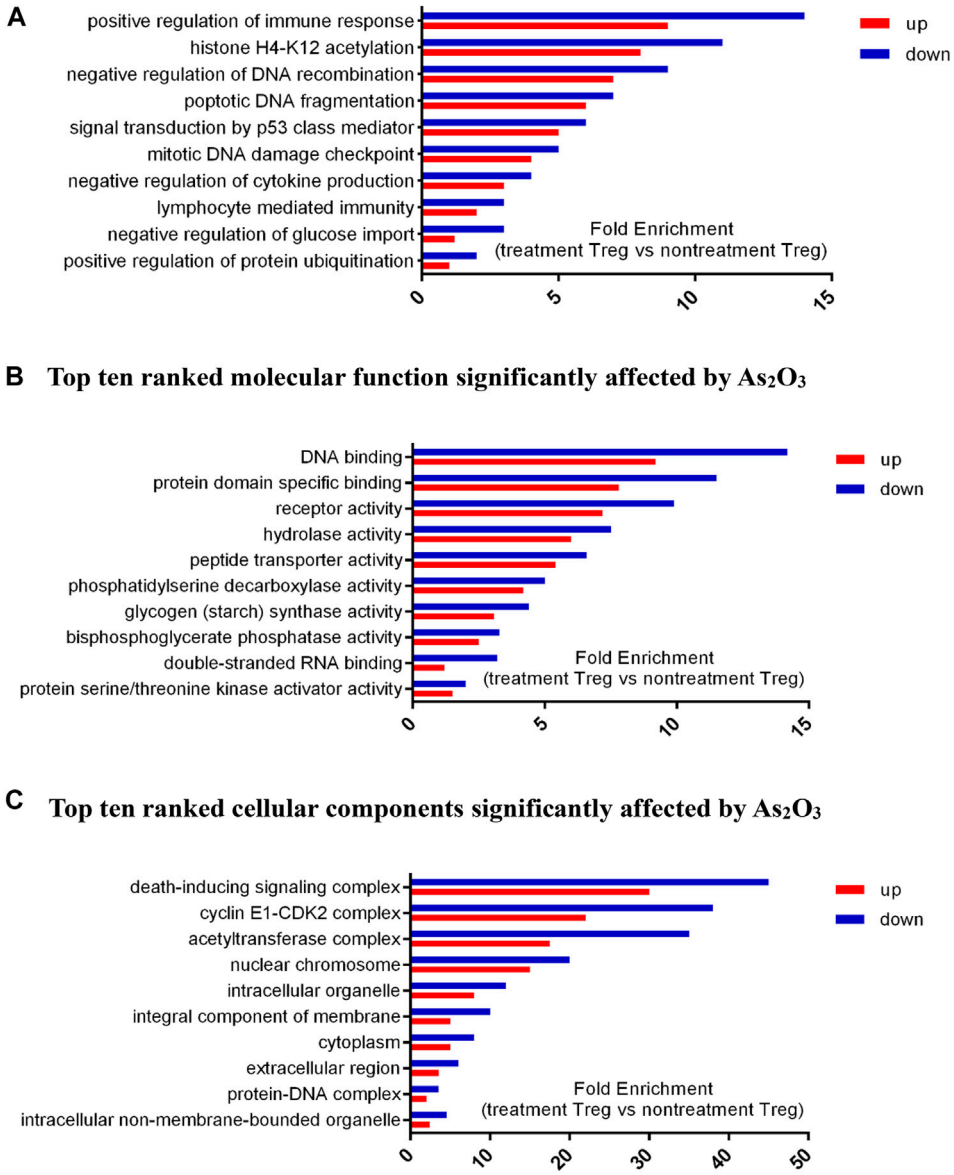


**FIGURE 7 |** Differential expressed genes (DEGs) profiles of Gene Ontology (GO) terms (biological process, molecular function, and cellular component) in treatment Treg cells and nontreatment Treg cells from early treatment-naïve RA patients. **(A)** Top ten ranked biological process significantly affected by $As_2O_3$.. **(B)** Top ten ranked molecular function significantly affected by $As_2O_3$.. **(C)** Top ten ranked cellular components significantly affected by $As_2O_3$.

on any of these genes, with the single exception upregulation of acyl-CoA thioesterase 4 (ACOT4) (fold change 2.9).

## qRT-PCR Validates Differential Expressed Genes

Five upregulated genes (CDKN3, SUSD4, USP7, HAL, and HIST4H4) and four downregulated gene (DFFB, PTPN13, RIPK1, and METTL3) in early treatment-naïve RA patients were selected for RT-qPCR. The nine selected genes expression were consistent between RNA-seq and RT-qPCR analysis, confirming the accuracy of our data (**Figure 8**).

# DISCUSSION

In this study, we have presented a comprehensive analysis of the frequency, phenotype, cytokine profile, and gene expression profile of $CD4^+CD25^+CD127^{low}$ Treg and Th17 cells from $CD4^+$ T cells of PB from patients with early treatment-naïve RA patients.

Our findings have indicated that $As_2O_3$ increased $CD4^+CD25^+$ Treg-cell expression significantly in PBMCs of early treatment-naïve RA patients.

We report in this article for the first time, to our knowledge, that $As_2O_3$ can modulate $CD4^+CD25^+$ Treg-cell differentiation

**TABLE 4 |** Selected genes linked to inflammation and immunoregulation in $As_2O_3$-treated RA Treg cells relative to control.

| Inflammation and immunoregulation | | | |
|---|---|---|---|
| **Genes** | **Name** | **$Log_2$-fold change** | **Nominal *p* value[a]** |
| Foxo1 | Forkhead box O1 | 11.8 | $4.9 \times 10^{-2}$ |
| Foxp3 | Forkhead box P3 | 8.6 | $1.3 \times 10^{-2}$ |
| CD28 | CD28 molecule | 6.5 | $4.3 \times 10^{-2}$ |
| PPARγ | Peroxisome proliferator activated receptor gamma | 6.4 | $2.3 \times 10^{-2}$ |
| CD25 | Interleukin 2 receptor subunit alpha | 5.8 | $2.8 \times 10^{-2}$ |
| CTLA4 | Cytotoxic T-lymphocyte associated protein 4 | 4.6 | $4.8 \times 10^{-2}$ |
| RUNX3 | Runt related transcription factor 3 | 4.3 | $6.3 \times 10^{-2}$ |
| STAT5 | Signal transducer and activator of transcription 5 | 3.7 | $4.6 \times 10^{-2}$ |
| TLR8 | Toll like receptor 8 | 2.8 | $2.5 \times 10^{-2}$ |
| IL-10 | Interleukin 10 | 2.6 | $2.5 \times 10^{-2}$ |
| TGF-β | Transforming growth factor beta 1 | 2.5 | $2.2 \times 10^{-2}$ |
| IL-15 | Interleukin 15 | 2.1 | $4.1 \times 10^{-2}$ |
| STAT3 | Signal transducer and activator of transcription 3 | −9.4 | $6.1 \times 10^{-2}$ |
| RORc | RAR-related orphan receptor C | −8.8 | $4.9 \times 10^{-2}$ |
| HIF1α | Hypoxia inducible factor 1 alpha subunit | −8.4 | $4.6 \times 10^{-2}$ |
| CCR6 | C–C motif chemokine receptor 6 | −7.6 | $3.1 \times 10^{-2}$ |
| CXCR3 | C-X-C motif chemokine receptor 3 | −6.5 | $4.9 \times 10^{-2}$ |
| SOCS1 | Suppressor of cytokine signaling 1 | −6.0 | $3.3 \times 10^{-2}$ |
| IFN-γ | Interferon gamma | −5.3 | $4.9 \times 10^{-2}$ |
| SMAD3 | SMAD family member 3 | −4.8 | $4.7 \times 10^{-2}$ |
| IL-6 | Interleukin 6 | −4.4 | $4.7 \times 10^{-4}$ |
| MMP3 | Matrix metallopeptidase 3 | −3.2 | $2.6 \times 10^{-3}$ |
| IL-17A | Interleukin 17A | −2.5 | $3.4 \times 10^{-2}$ |

[a]*Nominal* p *value was calculated by Fisher's exact test.*

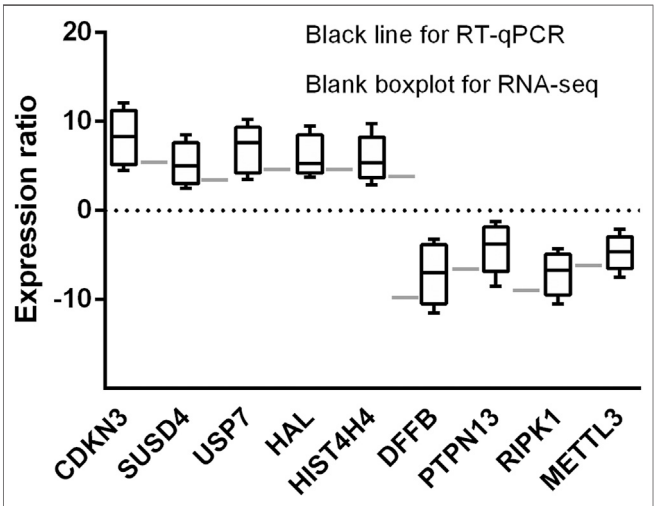


**FIGURE 8 |** Histogram indicates the expression change of the nine selected genes detected by RT-qPCR (black bars, $n = 6$) and scRNA-seq (white bars, $n = 3$). The results showing the scRNA-seq analysis and RT-qPCR were consistent with expression change of the nine identified genes.

and affect its function, thus improving immune dysfunction. Audrey et al. reported that $As_2O_3$ increased the antitumor immune response through the depletion of Treg numbers mediated by oxidative stress in tumor-bearing mice (Thomas-Schoemann et al., 2012). Furthermore, our previous studies revealed that $As_2O_3$ could induce RA FLS apoptosis through NF-κB signaling pathway (Mei et al., 2011), inhibiting angiogenesis via modulating TSP-1-TGF-β-CTGF-VEGF functional module (Zhang et al., 2017) and rescuing the defective VDR-PPARγ functional module of autophagy (Wang et al., 2019). We also systematically reviewed that $As_2O_3$ is a feasible treatment option based on its ability to protect against inflammation (Zhang et al., 2018). Therefore, it is becoming increasingly clear that $As_2O_3$ might take part in the control of immune response by promoting the differentiation of $CD4^+CD25^+$ Treg cells and related phenotype expression.

Based on our previous findings, we conducted scRNA-seq analysis elicited the exact therapeutic mechanism of Treg cells by $As_2O_3$ in early treatment-naïve RA patients. $As_2O_3$ was found to affect the expression of a large number of genes in early treatment-naïve RA Treg cells. Among the most strongly affected genes were several involved in inflammation, immunoregulation, and amino acid and carbohydrate metabolism.

Immune imbalance along with autoimmune dysfunction is a central feature of RA. In our data, $As_2O_3$ reduced the expression of well-known Th17 transcription factor such as signal transducer and activator of transcription 3 (STAT3). In addition, $As_2O_3$ downregulated the expression of hypoxia-inducible factor 1 alpha subunit (HIF1α). As the relative expression of Treg/Th17–related cytokines during the course of RA, the effects of $As_2O_3$ on immune balance are likely to depend on the phase of the disease process.

RA has been reported to be associated with Treg/Th17 cell imbalance (Li et al., 2019a; Li et al., 2019b). Activation of the Foxo signaling pathway appears to counteract these effects and particularly, forkhead box O1 (Foxo1) has been shown to enhance Treg-cell differentiation and stability (Kerdiles et al.,

2010). Interestingly, the expression of Foxo1 was strongly upregulated by $As_2O_3$ in the present data. Furthermore, $As_2O_3$ strongly enhanced the expression of Runx3, which has been shown to regulate Foxp3 expression (Loo et al., 2020).

These genes were predicted to target Foxo signaling pathway that play a major role in Treg-cell formation and function. Foxo induces the transcription factor Foxp3 expression. Single-cell RAN-seq revealed that the upregulated pathway mainly involves the Foxo signaling pathway–related protein acetylation and deubiquitination in Treg cell from early treatment-naïve RA patients by $As_2O_3$. The downregulated pathway mainly involves apoptosis activated by DNA fragmentation factor, cyclin E1, ATPase plasma membrane $Ca2^+$ transporting 4, mitochondrial ribosomal protein, ubiquitin protein ligase in intracellular membrane, and extracellular region. Considering above of the findings, metabolic disorder and epigenetic modification may involve in the regulation of Treg cell by $As_2O_3$ from early treatment-naïve RA patients.

The highly conserved role of Foxo transcription factor in cell cycle inhibition and apoptosis has been extensively studied in the past decade. Previous literature has uncovered that Foxo transcription factors in T-cell fate specification, especially with regard to Treg-cell differentiation play a critical role by integrating PI3K-Akt and TGF-β-SMAD signaling pathways (Kerdiles et al., 2010). Preclinical studies demonstrated that dysfunctional differentiation of naïve T cell in RA patients is critically induced by insufficient of the histone acetyltransferase, which leads to the deficiency of Foxp3 acetylation and subsequently Foxp3 degradation (Su et al., 2019). Other researchers have previously shown that Foxp3 deubiquitination partially but significantly increase Treg cells and that partial rescue of Treg-cell development (Zhao et al., 2015). Therefore, we gain a hypothesis that $As_2O_3$ may affect Treg-cell function by enhancing Foxo acetylation and attenuating ubiquitination subsequently orchestrate a program of Foxp3 gene expression and Treg-cell differentiation. Thirteen identified MFs imply that $As_2O_3$ promotes Treg-cell differentiation and enhanced function may partially affect its epidemic modification. Foxo transcription factor genetic program controls aspects of Treg-cell differentiation and that are dispensable for the maintenance for Foxp3 expression and $Foxp3^+$ cells expansion in response to homeostatic or inflammatory cues. Substrate-binding F-box protein SKP2 binds to ubiquitin ligase induces Foxo ubiquitination and subsequent proteasome degradation of Foxo (Huang and Tindall, 2011). This study revealed that $As_2O_3$ perhaps increase Treg differentiation and development by enhance Foxo acetylation and inhibit Foxo ubiquitination.

Our previous studies revealed that $As_2O_3$ at low concentration (0.1–0.5 μM) primarily promote Treg-cell differentiation and does not induce its apoptosis. RNA-seq analysis showed that $As_2O_3$ inhibits apoptosis which manifested tightly to the inactivation of cytokine–cytokine receptor interaction, cell cycle, PI3K-Akt signaling pathway, NK-κB signaling pathway, calcium signaling pathway, and p53 signaling pathway. Our data suggested that $As_2O_3$ perhaps attenuates DNA fragmentation factor subunit beta (DFFB) activity subsequently inhibit apoptosis (Han et al., 2020). Furthermore, $As_2O_3$ interaction may prevent apoptosis-inducing factor oxidation in mitochondria exposed to stress and undergoing apoptosis (Bano and Prehn, 2018). $As_2O_3$ tampered released NF-κB dimers activated through various posttranslational modifications and translocate to the nucleus where they bind to specific DNA sequences and inhibit transcription of target genes (Hayden and Ghosh, 2008).

An interesting example is pyruvate dehydrogenase kinase 1 (PDK1), which was one of the genes most strongly downregulated by $As_2O_3$. As this gene inactivates pyruvate dehydrogenase and prevents pyruvate convert into lactate or acetyl-CoA, inhibition of PDK1 during Th17-cell polarization by dichloroacetate (DCA) is sufficient to block Th17-cell differentiation (Bantug et al., 2018). Several genes promoting amino acid synthesis and transport, such as solute carrier family 1 member 5 (SLC1A5), which encodes a glutamine transporter and limits Th17-cell generation while promote Treg-cell generation (Nakaya et al., 2014), were also downregulated. However, genes coding for the enzymes participating in the major pathways of carbohydrate and amino acid metabolism (glycolysis and amino biosynthesis) were not significantly affected. Of the RA-associated glycolysis metabolism genes, glucose transporter (GLUT1) is a central mediator of carbohydrate metabolism. Its expression has been shown to be increased in RA (Falconer et al., 2018), and GLUT1 was found to be downregulated by $As_2O_3$ in the present study.

Cell cycle kinase, serine–protein kinase (ATM), is involved in the amino acid metabolism. T cells from RA that have low levels of ATM commit to the Th1 and Th17, rather than Treg (Weyand and Goronzy, 2017). These are examples of $As_2O_3$-induced normalization of the expression of carbohydrate and amino acid metabolism–related genes in RA Treg cells.

## CONCLUSION

In conclusion, $As_2O_3$ was found to cause a major phenotype switch in RA Treg. This is the first study investigating the genome-wide effects of $As_2O_3$ on the gene expression of treatment naïve Treg cells. In addition to promoting apoptosis, inhibiting angiogenesis, restoring immune imbalance, suppressing inflammation. $As_2O_3$ was also found to affect glucose-amino acid metabolism related genes. Since, targeting metabolism may be the potential therapeutic for treatment-naïve RA patients.

## DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Harbin Medical University and the Ethics Committee of Zhengzhou University.

## AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

## FUNDING

This work is supported by National Natural Science Foundation of China to ZZ (No. 81771749) and YZ (No. 81771748).

## ACKNOWLEDGMENTS

We would like to thank Juan Zhang and Weiyan Wang for their insightful suggestions, Yanli Wang for technical assistance with cell sorting. The study partially originated from previous thesis (Li, 2019).

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.656124/full#supplementary-material

## REFERENCES

Aletaha, D., Neogi, T., Silman, A. J., Funovits, J., Felson, D. T., Bingham, C. O., 3rd, et al. (2010). 2010 Rheumatoid Arthritis Classification Criteria: An American College of Rheumatology/European League against Rheumatism Collaborative Initiative. *Arthritis Rheum.* 62 (9), 2569–2581. doi:10.1002/art.27584

Bano, D., and Prehn, J. H. M. (2018). Apoptosis-Inducing Factor (AIF) in Physiology and Disease: The Tale of a Repented Natural Born Killer. *EBioMedicine* 30, 29–37. doi:10.1016/j.ebiom.2018.03.016

Bantug, G. R., Galluzzi, L., Kroemer, G., and Hess, C. (2018). The Spectrum of T Cell Metabolism in Health and Disease. *Nat. Rev. Immunol.* 18 (1), 19–34. doi:10.1038/nri.2017.99

Bobé, P., Bonardelle, D., Benihoud, K., Opolon, P., and Chelbi-Alix, M. K. (2006). Arsenic Trioxide: A Promising Novel Therapeutic Agent for Lymphoproliferative and Autoimmune Syndromes in MRL/lpr Mice. *Blood* 108 (13), 3967–3975. doi:10.1182/blood-2006-04-020610

Cluxton, D., Petrasca, A., Moran, B., and Fletcher, J. M. (2019). Differential Regulation of Human Treg and Th17 Cells by Fatty Acid Synthesis and Glycolysis. *Front. Immunol.* 10, 115. doi:10.3389/fimmu.2019.00115

Cojocaru, M., Cojocaru, I. M., Silosi, I., and Vrabie, C. D. (2012). Metabolic Syndrome in Rheumatoid Arthritis. *Maedica (Bucur)* 7 (2), 148–152.

Eyre, S., Ke, X., Lawrence, R., Bowes, J., Panoutsopoulou, K., Barton, A., et al. (2011). Examining the Overlap between Genome-wide Rare Variant Association Signals and Linkage Peaks in Rheumatoid Arthritis. *Arthritis Rheum.* 63 (6), 1522–1526. doi:10.1002/art.30315

Falconer, J., Murphy, A. N., Young, S. P., Clark, A. R., Tiziani, S., Guma, M., et al. (2018). Review: Synovial Cell Metabolism and Chronic Inflammation in Rheumatoid Arthritis. *Arthritis Rheumatol.* 70 (7), 984–999. doi:10.1002/art.40504

Goronzy, J. J., and Weyand, C. M. (2017). Successful and Maladaptive T Cell Aging. *Immunity* 46 (3), 364–378. doi:10.1016/j.immuni.2017.03.010

Gualdoni, G. A., Mayer, K. A., Göschl, L., Boucheron, N., Ellmeier, W., and Zlabinger, G. J. (2016). The AMP Analog AICAR Modulates the T Reg/T H 17 axis through Enhancement of Fatty Acid Oxidation. *FASEB J.* 30 (11), 3800–3809. doi:10.1096/fj.201600522r

Han, D. S. C., Ni, M., Chan, R. W. Y., Chan, V. W. H., Lui, K. O., Chiu, R. W. K., et al. (2020). The Biology of Cell-free DNA Fragmentation and the Roles of DNASE1, DNASE1L3, and DFFB. *Am. J. Hum. Genet.* 106 (2), 202–214. doi:10.1016/j.ajhg.2020.01.008

Han, G. M., O'Neil-Andersen, N. J., Zurier, R. B., and Lawrence, D. A. (2008). CD4+CD25high T Cell Numbers Are Enriched in the Peripheral Blood of Patients with Rheumatoid Arthritis. *Cell Immunol* 253 (1-2), 92–101. doi:10.1016/j.cellimm.2008.05.007

Hayden, M. S., and Ghosh, S. (2008). Shared Principles in NF-Kb Signaling. *Cell* 132 (3), 344–362. doi:10.1016/j.cell.2008.01.020

Huang, D. W., Sherman, B. T., and Lempicki, R. A. (2009). Systematic and Integrative Analysis of Large Gene Lists Using DAVID Bioinformatics Resources. *Nat. Protoc.* 4 (1), 44–57. doi:10.1038/nprot.2008.211

Huang, H., and Tindall, D. J. (2011). Regulation of FOXO Protein Stability via Ubiquitination and Proteasome Degradation. *Biochim. Biophys. Acta (Bba) - Mol. Cel Res.* 1813 (11), 1961–1964. doi:10.1016/j.bbamcr.2011.01.007

Jin, S., Chen, H., Li, Y., Zhong, H., Sun, W., Wang, J., et al. (2018). Maresin 1 Improves the Treg/Th17 Imbalance in Rheumatoid Arthritis through miR-21. *Ann. Rheum. Dis.* 77, 1644–1652. doi:10.1136/annrheumdis-2018-213511

Kalim, K. W., Yang, J.-Q., Li, Y., Meng, Y., Zheng, Y., and Guo, F. (2018). Reciprocal Regulation of Glycolysis-Driven Th17 Pathogenicity and Regulatory T Cell Stability by Cdc42. *J.I.* 200 (7), 2313–2326. doi:10.4049/jimmunol.1601765

Kerdiles, Y. M., Stone, E. L., Beisner, D. L., McGargill, M. A., Ch'en, I. L., Stockmann, C., et al. (2010). Foxo Transcription Factors Control Regulatory T Cell Development and Function. *Immunity* 33 (6), 890–904. doi:10.1016/j.immuni.2010.12.002

Koenders, M. I., Joosten, L. A. B., and van den Berg, W. B. (2006). Potential New Targets in Arthritis Therapy: Interleukin (IL)-17 and its Relation to Tumour Necrosis Factor and IL-1 in Experimental Arthritis. *Ann. Rheum. Dis.* 65 (Suppl. 3), iii29–iii33. doi:10.1136/ard.2006.058529

Kono, M., Yoshida, N., Maeda, K., Skinner, N. E., Pan, W., Kyttaris, V. C., et al. (2018). Pyruvate Dehydrogenase Phosphatase Catalytic Subunit 2 Limits Th17 Differentiation. *Proc. Natl. Acad. Sci. USA* 115 (37), 9288–9293. doi:10.1073/pnas.1805717115

Li, C. (2019). *Study on the Balance Mechanism of Treg/Th17 cells in rheumatoid arthritis by arsenic trioxide by regulating STAT3*. Harbin: Harbin Medical University. doi:10.1136/annrheumdis-2019-eular.4762

Li, C., Zhang, J., Wang, W., Wang, H., Zhang, Y., and Zhang, Z. (2019a). Arsenic Trioxide Improves Treg and Th17 Balance by Modulating STAT3 in Treatment-Naïve Rheumatoid Arthritis Patients. *Int. Immunopharmacology* 73, 539–551. doi:10.1016/j.intimp.2019.05.001

Li, C., Zhang, J., Wang, W., Wang, H., Zhang, Y., and Zhang, Z. (2019b). Data on Arsenic Trioxide Modulates Treg/Th17/Th1/Th2 Cells in Treatment-Naïve Rheumatoid Arthritis Patients and Collagen-Induced Arthritis Model Mice. *Data in Brief* 27, 104615. doi:10.1016/j.dib.2019.104615

Loo, C.-S., Gatchalian, J., Liang, Y., Leblanc, M., Xie, M., Ho, J., et al. (2020). A Genome-wide CRISPR Screen Reveals a Role for the Non-canonical Nucleosome-Remodeling BAF Complex in Foxp3 Expression and Regulatory T Cell Function. *Immunity* 53 (1), 143–157. doi:10.1016/j.immuni.2020.06.011

McInnes, I. B., and Schett, G. (2007). Cytokines in the Pathogenesis of Rheumatoid Arthritis. *Nat. Rev. Immunol.* 7 (6), 429–442. doi:10.1038/nri2094

Mei, Y., Zheng, Y., Wang, H., Gao, J., Liu, D., Zhao, Y., et al. (2011). Arsenic Trioxide Induces Apoptosis of Fibroblast-like Synoviocytes and Represents Antiarthritis Effect in Experimental Model of Rheumatoid Arthritis. *J. Rheumatol.* 38 (1), 36–43. doi:10.3899/jrheum.100299

Miossec, P., and Kolls, J. K. (2012). Targeting IL-17 and TH17 Cells in Chronic Inflammation. *Nat. Rev. Drug Discov.* 11 (10), 763–776. doi:10.1038/nrd3794

Nakaya, M., Xiao, Y., Zhou, X., Chang, J.-H., Chang, M., Cheng, X., et al. (2014). Inflammatory T Cell Responses Rely on Amino Acid Transporter ASCT2 Facilitation of Glutamine Uptake and mTORC1 Kinase Activation. *Immunity* 40 (5), 692–705. doi:10.1016/j.immuni.2014.04.007

Niu, Q., Cai, B., Huang, Z.-C., Shi, Y.-Y., and Wang, L.-L. (2012). Disturbed Th17/Treg Balance in Patients with Rheumatoid Arthritis. *Rheumatol. Int.* 32 (9), 2731–2736. doi:10.1007/s00296-011-1984-x

Safari, F., Farajnia, S., Arya, M., Zarredar, H., and Nasrolahi, A. (2018). CRISPR and Personalized Treg Therapy: New Insights into the Treatment of

Rheumatoid Arthritis. *Immunopharmacology and Immunotoxicology* 40 (3), 201–211. doi:10.1080/08923973.2018.1437625

Shi, L. Z., Wang, R., Huang, G., Vogel, P., Neale, G., Green, D. R., et al. (2011). HIF1α-dependent Glycolytic Pathway Orchestrates a Metabolic Checkpoint for the Differentiation of TH17 and Treg Cells. *J. Exp. Med.* 208 (7), 1367–1376. doi:10.1084/jem.20110278

Smolen, J. S., Aletaha, D., and McInnes, I. B. (2016). Rheumatoid Arthritis. *The Lancet* 388 (10055), 2023–2038. doi:10.1016/s0140-6736(16)30173-8

Stahl, E. A., Raychaudhuri, S., Raychaudhuri, S., Remmers, E. F., Xie, G., Eyre, S., et al. (2010). Genome-wide Association Study Meta-Analysis Identifies Seven New Rheumatoid Arthritis Risk Loci. *Nat. Genet.* 42 (6), 508–514. doi:10.1038/ng.582

Su, Q., Jing, J., Li, W., Ma, J., Zhang, X., Wang, Z., et al. (2019). Impaired Tip60-Mediated Foxp3 Acetylation Attenuates Regulatory T Cell Development in Rheumatoid Arthritis. *J. Autoimmun.* 100, 27-39. doi:10.1016/j.jaut.2019.02.007

Sumitomo, S., Nagafuchi, Y., Tsuchida, Y., Tsuchiya, H., Ota, M., Ishigaki, K., et al. (2018). A Gene Module Associated with Dysregulated TCR Signaling Pathways in CD4 + T Cell Subsets in Rheumatoid Arthritis. *J. Autoimmun.* 89, 21–29. doi:10.1016/j.jaut.2017.11.001

Thomas-Schoemann, A., Batteux, F., Mongaret, C., Nicco, C., Chéreau, C., Annereau, M., et al. (2012). Arsenic Trioxide Exerts Antitumor Activity through Regulatory T Cell Depletion Mediated by Oxidative Stress in a Murine Model of Colon Cancer. *J.Immunol.* 189 (11), 5171–5177. doi:10.4049/jimmunol.1103094

Wang, W., Li, C., Zhang, Z., and Zhang, Y. (2019). Arsenic Trioxide in Synergy with Vitamin D Rescues the Defective VDR-PPAR-Gamma Functional Module of Autophagy in Rheumatoid Arthritis. *PPAR Res.* 2019, 6403504. doi:10.1155/2019/6403504

Wang, Y., Zhou, C., Gao, H., Li, C., Li, D., Liu, P., et al. (2017). Therapeutic Effect of Cryptotanshinone on Experimental Rheumatoid Arthritis through Downregulating P300 Mediated-STAT3 Acetylation. *Biochem. Pharmacol.* 138, 119–129. doi:10.1016/j.bcp.2017.05.006

Weyand, C. M., and Goronzy, J. J. (2017). Immunometabolism in Early and Late Stages of Rheumatoid Arthritis. *Nat. Rev. Rheumatol.* 13 (5), 291–301. doi:10.1038/nrrheum.2017.49

Xie, C., Mao, X., Huang, J., Ding, Y., Wu, J., Dong, S., et al. (2011). KOBAS 2.0: a Web Server for Annotation and Identification of Enriched Pathways and Diseases. *Nucleic Acids Res.* 39, W316–W322. doi:10.1093/nar/gkr483

Zhang, J., Li, C., Zheng, Y., Lin, Z., Zhang, Y., and Zhang, Z. (2017). Inhibition of Angiogenesis by Arsenic Trioxide via TSP-1-TGF-B1-CTGF-VEGF Functional Module in Rheumatoid Arthritis. *Oncotarget* 8 (43), 73529–73546. doi:10.18632/oncotarget.19867

Zhang, J., Zhang, Y., Wang, W., Li, C., and Zhang, Z. (2018). Double-Sided Personality: Effects of Arsenic Trioxide on Inflammation. *Inflammation* 41 (4), 1128–1134. doi:10.1007/s10753-018-0775-x

Zhang, T.-D., Chen, G.-Q., Wang, Z.-G., Wang, Z.-Y., Chen, S.-J., and Chen, Z. (2001). Arsenic Trioxide, a Therapeutic Agent for APL. *Oncogene* 20 (49), 7146–7153. doi:10.1038/sj.onc.1204762

Zhao, Y., Guo, H., Qiao, G., Zucker, M., Langdon, W. Y., and Zhang, J. (2015). E3 Ubiquitin Ligase Cbl-B Regulates Thymic-Derived CD4+CD25+ Regulatory T Cell Development by Targeting Foxp3 for Ubiquitination. *J.I.* 194 (4), 1639–1645. doi:10.4049/jimmunol.1402434

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

REVIEW
published: 15 June 2021
doi: 10.3389/fphar.2021.672515

# From Bed to Bench and Back: TNF-α, IL-23/IL-17A, and JAK-Dependent Inflammation in the Pathogenesis of Psoriatic Synovitis

*Ettore Silvagni[1], Sonia Missiroli[2], Mariasole Perrone[2], Simone Patergnani[2], Caterina Boncompagni[2], Alessandra Bortoluzzi[1], Marcello Govoni[1], Carlotta Giorgi[2], Stefano Alivernini[3], Paolo Pinton[2†] and Carlo Alberto Scirè[1,4*†]*

[1]Rheumatology Unit, Department of Medical Sciences, Università degli Studi di Ferrara and Azienda Ospedaliero-Universitaria S. Anna, Cona, Italy, [2]Department of Medical Sciences, Section of Experimental Medicine, Laboratory for Technologies of Advanced Therapies, University of Ferrara, Ferrara, Italy, [3]Rheumatology Unit, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy, [4]Epidemiology Research Unit, Italian Society for Rheumatology, Milan, Italy

OPEN ACCESS

**Edited by:**
*Pallavi R. Devchand, University of Calgary, Canada*

**Reviewed by:**
*Kyle L. Flannigan, University of Calgary, Canada*
*Francesca Bianchini, University of Florence, Italy*

***Correspondence:**
*Carlo Alberto Scirè*
*carloalberto.scire@unife.it*

*[†]These authors have contributed equally to this paper*

**Specialty section:**
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

**Received:** *25 February 2021*
**Accepted:** *18 May 2021*
**Published:** *15 June 2021*

**Citation:**
*Silvagni E, Missiroli S, Perrone M, Patergnani S, Boncompagni C, Bortoluzzi A, Govoni M, Giorgi C, Alivernini S, Pinton P and Scirè CA (2021) From Bed to Bench and Back: TNF-α, IL-23/IL-17A, and JAK-Dependent Inflammation in the Pathogenesis of Psoriatic Synovitis. Front. Pharmacol. 12:672515. doi: 10.3389/fphar.2021.672515*

Psoriatic arthritis (PsA) is a chronic inflammatory immune-mediated disease with a burdensome impact on quality of life and substantial healthcare costs. To date, pharmacological interventions with different mechanisms of action, including conventional synthetic (cs), biological (b), and targeted synthetic (ts) disease-modifying antirheumatic drugs (DMARDs), have been proven efficacious, despite a relevant proportion of failures. The current approach in clinical practice and research is typically "predictive": the expected response is based on stratification according to clinical, imaging, and laboratory data, with a "heuristic" approach based on "trial and error". Several available therapeutic options target the TNF-α pathway, while others are directed against the IL-23/IL-17A axis. Janus kinase inhibitors (JAKis), instead, simultaneously block different pathways, endowing these drugs with a potentially "broad-spectrum" mechanism of action. It is not clear, however, whether targeting a specific pathway (e.g., TNF-α or the IL-23/IL-17 axis) could result in discordant effects over other approaches. In particular, in the case of "refractory to a treatment" patients, other pathways might be hyperactivated, with opposing, synergistic, or redundant biological significance. On the contrary, refractory states could be purely resistant to treatment as a whole. Since chronic synovitis is one of the primary targets of inflammation in PsA, synovial biomarkers could be useful in depicting specific biological characteristics of the inflammatory burden at the single-patient level, and despite not yet being implemented in clinical practice, these biomarkers might help in selecting the proper treatment. In this narrative review, we will provide an up-to-date overview of the knowledge in the field of psoriatic synovitis regarding studies investigating the relationships among different activated proinflammatory processes suitable for targeting by different available drugs. The final objective is to clarify the state of the art in the field of personalized medicine for psoriatic disease, aiming at moving beyond the current treatment schedules toward a patient-centered approach.

**Keywords: psoriatic arthritis, TNF-alfa, IL-23/IL-17 axis, JAK/STAT pathway, synovial histopathology, synovial biopsy, targeted therapies, personalized medicine**

## INTRODUCTION

Psoriatic arthritis (PsA) is a chronic systemic immune-mediated inflammatory disease belonging to the spondyloarthritis (SpA) spectrum. PsA occurs at frequencies ranging from 6% to 42% of patients with skin psoriasis, according to different studies (Scher et al., 2019; Zabotti et al., 2020), or affects family members of psoriatic patients (Gladman et al., 2005). Skin disease is considered the main risk factor for PsA development, and although the occurrence of joint disease is not predictable, an incidence risk of approximately 20% is approximated after more than 30 years of skin psoriasis, with higher rates in the context of nail, scalp, or inverse psoriasis; this risk is also affected by the severity of the cutaneous manifestations. In the context of PsA, involvement of the joints, entheses, and skin is challenging for clinicians, and dactylitis, nail dystrophy, uveitis, and spine manifestations represent clinical endotypes susceptible to different management approaches. Progressive damage accrual, along with inflammatory manifestations of the disease, is highly disabling for patients, with impacts on quality of life and healthcare costs (Singh and Strand, 2009). Comorbidities associated with repercussions related to cardiovascular risk, such as obesity and metabolic syndrome, are intrinsic parts of psoriatic "disease".

This clinical heterogeneity is reflected in complex pathophysiology, knowledge of which is crucial to hypothesizing a therapeutic approach targeting the ongoing pathological process (Veale and Fearon, 2018; Scher et al., 2019; Russell et al., 2021). Infiltration of both innate and adaptive immune cells in different target organs and tissues results in significant production of different proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, IL-22, IL-23, IL-17A, and IL-18, inducing further inflammatory mediators release and damage progression. New evidence in PsA pathogenesis has provided further insights into the molecular pathways involved in either cutaneous or articular manifestations of the disease, and genetic, epigenetic, environmental, cellular, and molecular aspects have been clarified, driving the development of different targeted therapies (Jadon et al., 2020). From a clinical point of view, drugs targeting different molecules primarily involved in chronic inflammation, such as TNF-α, the IL-23/IL-17A axis, or Janus kinases/signal transducers and activators of transcription (JAK/STATs), are now available (Gossec et al., 2016, 2020; Singh et al., 2018). This multiplicity of treatment options poses relevant questions about how to best interfere with different proinflammatory processes in individual PsA patients because clinicians lack reliable tools to select the best therapeutic pathway to target to optimize clinical response. A priori, tissue-specific biomarkers are the most promising candidates to stratify patients based on the actual ongoing pathogenic process and are suitable for targeting by pharmacological treatments, as demonstrated by some attempts in other chronic inflammatory joint diseases, such as rheumatoid arthritis (RA) (Humby et al., 2021). Despite important discoveries in this field, there are still great obstacles to the goal of "personalized arthritis medicine' in the context of PsA (Jadon et al., 2020).

The main aim of this narrative review is to provide an up-to-date overview of the knowledge in the field of psoriatic synovitis, focusing specifically on studies investigating the relationships among activated proinflammatory immune pathways, following hyperactivation of TNF-α, the IL-23/IL-17A axis, or JAK/STAT-dependent inflammation. Moreover, we will summarize the most robust and innovative evidence on the synovial membrane as a biomarker of response to treatment in PsA patients.

## Targeted Therapies for Psoriatic arthritis

Bedside data on the efficacy of treatments for PsA come from randomized controlled trials (RCTs) and observational studies, as well as systematic literature reviews (SLRs) (Kerschbaumer et al., 2020) informing current clinical practice guidelines (Coates et al., 2016; Gossec et al., 2016, Gossec et al., 2020; Singh et al., 2018). First-line pharmacological treatment strategies include nonsteroidal anti-inflammatory drugs (NSAIDs) and/or local injection of glucocorticoids (GCs). Conventional synthetic (cs) disease-modifying antirheumatic drugs (DMARDs) (e.g., methotrexate (MTX)) are selected in the case of elective peripheral joint involvement, while PsA patients refractory to csDMARDs should be treated with biological (b) DMARDs or oral targeted synthetic (ts) DMARDs. Current treatment guidelines suggest the use of JAK inhibitors (JAKis) in the case of bDMARD treatment failure or when other biologics are contraindicated. Since the amount of data on JAKis adoption in PsA will increase in the coming years, the positioning of these drugs might be revised when treatment recommendations are updated. This stepwise approach is generally accepted worldwide. Currently available bDMARDs for the management of PsA, which were designed based on the growing knowledge of disease pathogenesis, include 5 different TNF inhibitors (TNFis) (infliximab (IFX), etanercept (ETA), adalimumab (ADA), certolizumab pegol (CTZ), golimumab (GOL)), and their available biosimilars, the anti-IL-12/IL-23 p40 common subunit antibody ustekinumab, the anti-IL-17A antibodies secukinumab (SEC) and ixekizumab (IXE), and the selective T-cell co-stimulation modulator abatacept. Additionally, apremilast, tofacitinib, and upadacitinib are the only oral tsDMARDs available for PsA approved by the Food and Drug Administration (FDA) and European Medicine Association (EMA). The first inhibits phosphodiesterase-4 (PDE4). Tofacitinib blocks JAK1 and JAK3, with a functional effect on JAK2 (Mease et al., 2017a; Gladman et al., 2017), while upadacitinib is a selective JAK1 inhibitor (Mease et al., 2021b). Moreover, other drugs for the systemic management of PsA are in different phases of development. The anti-IL-23 biologics risankizumab (Clinical trial registration, KEEPsAKE 1, 2021; Clinical trial registration, KEEPsAKE 2, 2021), tildrakizumab (Clinical trial registration, INSPIRE 1, 2021; Clinical trial registration, INSPIRE 2, 2021), and guselkumab (Mease et al., 2020a; Deodhar et al., 2020) and the anti-IL17 receptor (IL-17R) antibody brodalumab (Mease et al., 2021a), which is already approved for psoriasis management, are under investigation in PsA, and the bispecific immunoglobulin bimekizumab, which targets IL-17A and IL-17F, is also being studied (Ritchlin et al., 2020a). Among

**TABLE 1 |** b/tsDMARDs that have been approved or are in different phases of development for the systemic management of PsA.

| bDMARDs | Mechanism of action | EMA/FDA approval or phase of development |
|---|---|---|
| Etanercept | TNFi | RA; PsA; PsO; JIA; AS/nrAS |
| Adalimumab | TNFi | RA; PsA; PsO; JIA; AS/nrAS; SH; CD/UC; chronic uveitis |
| Infliximab | TNFi | RA; PsA; PsO; AS/nrAS; CD/UC |
| Golimumab | TNFi | RA; PsA; JIA; AS/nrAS; UC |
| Certolizumab pegol | TNFi | RA; PsA; PsO; AS/nrAS |
| Ustekinumab | p40 common subunit (IL-12 and IL-23) inhibitor | PsA; PsO; CD/UC |
| Secukinumab | IL-17A inhibitor | PsA; PsO; AS/nrAS |
| Ixekizumab | IL-17A inhibitor | PsA; PsO; AS/nrAS |
| Abatacept | CD80/CD86-mediated Co-stimulation inhibitor | RA; PsA |
| Risankizumab | p19 subunit (IL-23) inhibitor | PsO<br>*PsA (phase II trial completed and phase III trials ongoing)* |
| Tildrakizumab | p19 subunit (IL-23) inhibitor | PsO<br>*PsA (phase II trial completed and phase III trials ongoing)* |
| Guselkumab | p19 subunit (IL-23) inhibitor | PsO<br>*PsA (phase II–III trials completed)* |
| Brodalumab | IL-17RA inhibitor | PsO<br>*PsA (phase III trials completed)* |
| Bimekizumab | IL-17A and IL-17F bispecific antibody | *PsO (phase IIb trial completed and phase III trials ongoing)*<br>*PsA (phase IIb trial completed and phase III trials ongoing)* |
| tsDMARDs | Mechanism of action | EMA/FDA approval or phase of development |
| Apremilast | PDE4 inhibitor | PsA; PsO; BD |
| Tofacitinib | JAK1/3 inhibitor | RA; PsA; UC |
| Upadacitinib | JAK1 inhibitor | RA; PsA |
| Filgotinib | JAK1 inhibitor | RA; *PsA (phase II completed and phase III trials active—not recruiting)* |

*b/tsDMARDs, biological/targeted synthetic disease-modifying antirheumatic drugs; PsA, psoriatic arthritis; EMA, European Medicines Agency; FDA, U.S. Food and Drug Administration; TNFi, tumor necrosis factor-alpha inhibitor; RA, rheumatoid arthritis; PsO, psoriasis; JIA, juvenile idiopathic arthritis; AS/nrAS, ankylosing spondylitis/nonradiographic axial spondylarthritis; SH, suppurative hidradenitis; CD, Crohn's disease; UC, ulcerative colitis; IL, interleukin; IL-17RA, IL-17 receptor A; PDE4, phosphodiesterase-4; BD, Behcet's disease; JAK, Janus kinase.*

tsDMARDs, filgotinib is promising (Clinical trial registration, PENGUIN 1, 2021; Clinical trial registration, PENGUIN 2, 2021). **Table 1** summarizes the drugs for the management of PsA that are currently approved or in different phases of development.

Among the high number of drugs registered for the management of PsA in recent years, the majority of available therapeutic options act against the TNF-α pathway, while others are directed against the IL-23/IL-17A axis, and JAKis virtually encompass the intersections of a number of pathways based on their potential "broad-spectrum" mechanism of action, blocking different type I and II cytokines (e.g., IL-6, IL-23, IL-22, and interferons (IFNs)). This wide availability of drugs enables interference with the most important cytokines and nodes involved in disease pathogenesis, with the clinical aim of reducing signs and symptoms of the disease and preventing joint/bone damage and disability accrual. From a pathogenetic point of view, instead, the possibility of interfering with single or multiple crossroads directly involved in disease susceptibility and synergism could reduce inflammation in its entirety at the site of the disease (i.e., the skin, entheses, and synovium), decelerating the progression to more advanced stages of illness.

In regard to bedside application, the choice of the preferred bDMARD as a first-line biological treatment in patients with peripheral arthritis and selection of the correct strategy after failure of the first bDMARD (historically a TNFi) are aspects of interest, despite available evidence supporting clinical decisions being scant (Silvagni et al., 2019; Chimenti et al., 2020). Indeed, after almost 20 years of TNFi availability in the field of PsA, the capability to treat PsA with this class of drugs in the clinic has been reinforced by long-term efficacy and safety data (Fagerli et al., 2018; Haugeberg et al., 2018). TNFis are usually considered among first-line biological treatment strategies in different clinical settings, while experience with recently developed anti-IL-17A agents is obviously lower. However, similar efficacy rates have been shown between TNFis and non-TNFis in RCTs, with higher responses in first-line treatment strategies (McInnes et al., 2013; Mease et al., 2017b) than in second-line options (Ritchlin et al., 2014; McInnes et al., 2015; Mease et al., 2015; Nash et al., 2017). Moreover, the recent European League Against Rheumatisms (EULAR) recommendations suggested preferring an anti-IL-17A or anti-IL-12/23 agent in cases with relevant skin involvement (Gossec et al., 2020), and this remains, at present, the only acknowledgment of personalized systemic treatment in this context. However, in the 2018 American College of Rheumatology (ACR) guidelines, TNFis are conditionally suggested as the first-line treatment strategy over anti-IL-17A and anti-IL-12/23 antibodies (Singh et al., 2018) on the basis of the more robust amount of clinical data.

Information regarding the comparative effectiveness of drugs with different modes of action in PsA is steadily increasing. Indirect evidence from RCTs and observational studies cautiously suggests a higher efficacy for IL-17A inhibition at the cutaneous level, with respect to joint involvement, while the use of TNFis has produced more comparable rates of response between the skin and joints (Boutet et al., 2018). This was confirmed by network meta-analyses. TNFis demonstrated

**TABLE 2 |** Main differences in clinical response between TNFis and non-TNF b/tsDMARDs in PsA phase III RCTs directly comparing the treatment arms.

| Study | Treatment arms | Population | Primary objective | Results |
|---|---|---|---|---|
| EXCEED trial, McInnes et al. (2020) | SEC 300 mg or ADA 40 mg | Active bDMARDs-naïve PsA | Superiority of SEC vs ADA for ACR20 at 52 weeks | Primary objective not met (SEC was noninferior to ADA, SEC 67%; ADA 59%, $p = 0.0239$). |
| SPIRIT-H2H trial, Mease et al. (2020b); Smolen et al. (2020) | IXE 160/80 mg or ADA 40 mg | Active bDMARDs-naïve PsA | Superiority of IXE vs ADA for simultaneous achievement of ACR50 and PASI100 at 24 weeks | IXE was superior to ADA (IXE 36%; ADA 28%, $p = 0.036$); results were maintained at 52 weeks (IXE 39%; ADA 26%, $p < 0.001$). |
| Enthesial CLearance In PSoriatic Arthritis (ECLIPSA), Araujo et al. (2019) | UST 45/90 mg or TNFi (open label) | Active bDMARDs-naïve PsA with active enthesitis | SPARCC = 0 at 24 weeks | UST was superior to TNFis (UST 74%; TNFis 42%, $p = 0.018$) but not at the joint levels (SJC + TJC = 0: UST 41%; TNFis 34%). |
| SPIRIT-P1 study, Mease et al. (2017b); Coates et al. (2017) | IXE 80 mg every 4 weeks, IXE 80 mg every 2 weeks, ADA 40 mg, or placebo | Active csDMARDs-IR PsA | Superiority of IXE vs placebo for ACR20 at 24 weeks | IXE was superior to placebo, similar effect compared to ADA (IXE 4 W 58%; IXE 2 W 62%; placebo 30%, $p < 0.001$; ADA 57%). |
| Oral Psoriatic Arthritis Trial (OPAL) Broaden, Mease et al. (2017a) | Tofacitinib 5 mg BID or 10 mg BID, ADA 40 mg, or placebo | Active csDMARDs-IR PsA | Superiority of tofacitinib vs placebo for ACR20 at 12 weeks | Tofacitinib was superior to placebo, similar effect compared to ADA (Tofa 5 mg BID 50%; Tofa 10 mg BID 61%; placebo 33%, $p < 0.001$; ADA 52%). |

*TNFis, TNF inhibitors; b/tsDMARDs, biological/targeted synthetic disease-modifying antirheumatic drugs; PsA, psoriatic arthritis; RCTs, randomized controlled trials; SEC, secukinumab; ADA, adalimumab; ACR, American College of Rheumatology; ACR20, ACR response 20%; IXE, ixekizumab; PASI, Psoriasis Area Severity Index; UST, ustekinumab; W, week; SPARCC, Spondyloarthritis Research Consortium of Canada; csDMARDs-IR, conventional synthetic DMARDs insufficient responders; BID, bis in die.*

substantially higher ACR responses (i.e., articular symptoms) than other biologics and tsDMARDs, although the differences were numerically low (Gladman et al., 2020; Ruyssen-Witrand et al., 2020), and the effect of prior exposure to bDMARDs did not result in higher efficacy for other drugs with different mechanisms of action. On the contrary, both TNFis (except for etanercept) and anti-IL17A agents produced consistent cutaneous responses (i.e., Psoriasis Area Severity Index (PASI) response) compared to placebo. Recently, head-to-head RCTs directly comparing different active treatment strategies have provided relevant practical information. In the EXCEED trial (McInnes et al., 2020), 853 active bDMARD-naïve PsA patients were randomly assigned to the anti-IL-17A SEC (300 mg monthly) or the TNFi ADA, with the primary objective of demonstrating the superiority of SEC over ADA at 52 weeks (ACR20 response). The primary endpoint was not reached; however, SEC demonstrated an efficacy profile similar to that of ADA (odds ratio [OR]: 1.30, 95% confidence interval [95% CI]: 0.98–1.72) with no new safety signals with respect to registration RCTs. In the SPIRIT-H2H trial (Mease et al., 2020b; Smolen et al., 2020), the primary objective was the simultaneous achievement of joint and skin responses (ACR50 and PASI100) with IXE compared to ADA. IXE was superior to ADA in terms of the combined skin and joint primary endpoint ($p = 0.036$ at 24 weeks, $p < 0.001$ at 52 weeks) and non-inferior to ADA in ACR responses. Additional RCTs compared TNFis with non-TNFis, and although these trials were not designed to primarily evaluate the comparison, numerical differences in terms of efficacy endpoints, at least for the peripheral joints involvement, were not clinically meaningful (Mease et al., 2017b; Coates et al., 2017). **Table 2** highlights the differences in clinical responses between TNFis and non-TNFis in phase III RCTs in PsA comparing different treatment arms.

In line with these results, the decision on the biologic to adopt in each PsA patient is substantially empirical, and guidelines are not restrictive in this sense, with even more elusive data on the sequencing of therapies. EULAR recommendations (Gossec et al., 2020) suggest a preferred option for an anti-IL-17A or anti-IL-12/23 agent over TNFis and JAKis in cases with severe skin involvement, while the 2015 Group for Research and Assessment of Psoriasis and Psoriatic Arthritis (GRAPPA) guidelines tried to enlist specific treatments for individual clinical domains (e.g., axial, enthesis, dactylitis, and peripheral joint) and in cases with selected comorbidities (Ogdie et al., 2020). However, individual treatment decisions remain based on "heuristic" approaches, being not tailored to the biological features of the disease. Based on these issues, this approach exposes patients to possible primary inefficacy, unexpected side effects or several failed biological treatments before achieving clinical amelioration. As almost 40% of patients do not appropriately respond to their first-line biological treatment (Ritchlin et al., 2020b), the search for predictive biomarkers able to depict treatment response a priori is one the major unmet needs in the field, and addressing this need includes a global reconsideration of RCT development, aiming to tailor treatment decisions at the "single-patient" level (Miyagawa et al., 2018; Leijten et al., 2019; Pitzalis et al., 2020).

## Treatment Response Biomarkers in Psoriatic Arthritis

As underlined above, determining biomarkers related to early diagnosis, damage, prognosis, and treatment response is one of the major unmet needs in the field of PsA; as such, it is included in the research agendas of the most relevant international treatment guidelines (Ogdie et al., 2020). Biomarkers are currently defined

as measurable indicators of disease status. Despite the growing number of studies aimed at identifying diagnostic, prognostic, and treatment selection biomarkers (Generali et al., 2016; Mahendran and Chandran, 2018; Mahmood et al., 2018), no validated biomarkers are yet available for clinical use in PsA (Scher et al., 2019). Special interest lies in identifying peripheral blood, synovial fluid (SF), and synovial membrane biomarkers of response to drugs with different mechanisms of action (Jadon et al., 2020).

## Genetic Biomarkers

Genetic biomarkers to predict clinical response, mostly to TNFis, have been investigated in both psoriasis and PsA (O'Rielly et al., 2019). Early reports require confirmation in defined clinical subsets, with homogenization of inclusion criteria in clinical presentation, course of the disease, and the genotyping and molecular expression of specific cells and tissues. Polymorphisms in the TNFAIP3 (Ovejero-Benito et al., 2019), TNF-α 308A, IL-6 174 (Fabris et al., 2016), and TNF489A (Murdaca et al., 2014) alleles were related to the clinical efficacy of different TNFis in observational studies. Genetic and epigenetic modifications have also been exploited to highlight the treatment response to other bDMARDs, such as ustekinumab, but the evidence is mostly available in psoriasis rather than PsA (Ovejero-Benito et al., 2018; Dand et al., 2019). Since the amount of research data will increase as the availability of new b/tsDMARDs increases, the amount of genetic biomarker data will rise accordingly. However, PsA is a multifactorial disease, and genetic predisposition accounts for only a portion of the pathogenic process, with environmental factors significantly influencing the course of this disease (Veale and Fearon, 2018). Thus, it is not surprising that genetic biomarkers have not yet entered clinical practice.

## Serum Biomarkers

Since serum biomarkers are the most easily accessible measures, a relatively high number of studies have focused on serum levels of different proinflammatory molecules, clearly demonstrating increased levels of IL-17A, IL-23, IL-6, IL-1β, IL-21, transforming growth factor (TGF)-β, TNF-α, and interferon-gamma (IFN-$\gamma$) in the serum and SF of patients with SpA, including PsA, compared to those of controls (Londono et al., 2012; Raychaudhuri and Raychaudhuri, 2017). Among biomarkers of response to treatment, baseline C-reactive protein (CRP), IL-6 (Muramatsu et al., 2017), matrix metalloproteinase 3 (MMP-3) (Chandran et al., 2013), low-molecular-mass hyaluronan (Hellman et al., 2019), and C3 levels (Chimenti et al., 2012) were found to be predictive of TNFi therapy response in prospective studies (Gratacós et al., 2007; Kristensen et al., 2008; Scrivo et al., 2020). Lower IL-6 levels were associated with clinical response to ustekinumab (Muramatsu et al., 2017). Longitudinal decreases in the plasma concentrations of IL-6, vascular endothelial growth factor (VEGF), MMP3, and chitinase-3 like-1 (YKL-40) (Pedersen et al., 2010) and increases in serum cartilage oligomeric matrix protein (COMP) (Chandran et al., 2013) levels were linked to clinical response to TNFis. Moreover, from a panel of 92 serum proteins, pyridinoline, adiponectin, prostatic acid phosphate (PAP), and factor VII were identified as predictors of response to golimumab in a prospective observational study (Wagner et al., 2013). However, the serum concentrations of metabolites are influenced by several factors, and although PsA is a systemic condition, none of these biomarkers have been validated in clinical trials. Therefore, their roles are mostly mechanistic rather than decisional.

## Peripheral Blood Cellular Biomarkers

Within the cellular compartment, several studies have demonstrated elevated frequencies of IL-17-positive T-cells in patients with PsA (Leipe et al., 2010; Dolcino et al., 2015), with even higher numbers in the SF (Raychaudhuri et al., 2012; Menon et al., 2014). Peripheral T-cell phenotyping was exploited in one of the first attempts to apply a precision medicine approach in PsA. Miyagawa et al. (2018) directly compared, across a proof-of-concepts open-label study, two different treatment strategies in a population of patients with active PsA and an insufficient response to MTX (26 patients in a strategic treatment group versus 38 in a standard administration group following EULAR recommendations). Before starting therapy, FACS analysis of peripheral blood lymphocytes was performed to phenotypically characterize circulating T cells. Patients with a higher T helper 1 (Th1) cell status received the anti-IL-12/IL-23 antibody ustekinumab, while those with a higher Th17 cell level were treated with the IL-17A blocker SEC. A TNFi or SEC was given if the peripheral blood T-cell population was enriched in both the Th1 and Th17 clusters, while only the TNFi was administered when both were downregulated. This tailored approach with specific interventions based on distinct T-cell phenotypes and presumed activated proinflammatory pathways resulted in better clinical outcomes at 6 months. Specifically, low disease activity measured by the Simplified Disease Activity Index (SDAI) and Disease Activity Score on 28 joints (DAS28) and ACR20 responses were achieved more often in the group of PsA patients receiving a tailored approach than in the conventional treatment approach group, in which no relevant biologic-dependent treatment decision was made. Similar outcomes were not obtained for cutaneous manifestations, for which the proportions of patients achieving PASI75 and PASI90 were not significantly different between the groups. According to the authors, it was the strategy itself that contributed to the achievement of a favorable response to treatment, instead of the type of bDMARD selected. This study, even if preliminary, is a forerunner in the application of a biomarker-driven approach to address conditions such as PsA.

## Synovial Biomarkers

The analysis of cells and pathways in synovial tissue reveals findings that are not always exhibited by peripheral blood sampling. The anatomical proximity of the synovial membrane to the hypothesized inflammatory source emphasizes the putative roles of synovial biomarkers and their early modifications after treatment initiation. When chronic inflammatory arthritis occurs in the context of psoriatic disease, histological features include marked hyperplasia of the intimal lining layer containing

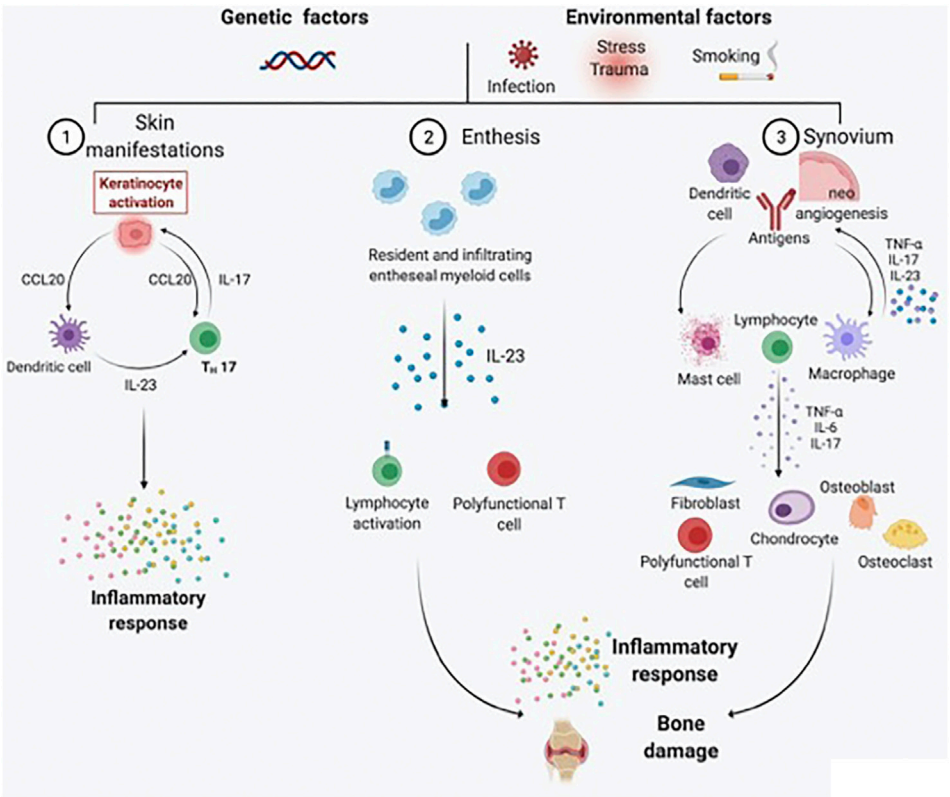


**FIGURE 1 |** Schematic representation of the main pathogenic processes driving PsA development and chronicity. PsA is a heterogeneous chronic disease, with skin, entheseal, and synovial tissue involvement occurring in different proportions across patients under genetic and environmental triggers. For skin manifestations, activated dendritic cells secrete predominantly IL-23, which in turn induces the differentiation of naïve T cells into Th17 cells. IL-17 is responsible for keratinocyte activation and subsequent perpetuation of skin inflammation. At the entheseal level, resident and infiltrating entheseal myeloid cells produce IL-23, which is responsible for lymphocyte activation and the inflammatory response, as well as bone damage. Psoriatic synovitis, on the contrary, is characterized by tortuous and immature neoangiogenesis, with antigen presentation by dendritic cells and macrophages leading to lymphocyte activation. As a result, the synovial inflammatory infiltrate is rich in activated lymphocytes, mast cells, and macrophages. Polyfunctional T cells are responsible for the production of several types of proinflammatory cytokines (e.g., TNF-α, IL-17A, GM-CSF, and IFN-γ) in the synovial membrane and synovial fluid. The proinflammatory cytokine milieu further activates fibroblast-like synoviocytes, chondrocytes, osteoblasts, and osteoclasts, resulting in bone damage.

fibroblast-like synoviocytes (FLSs) and macrophages and infiltration of the synovial sublining by both innate and adaptive immune cells, which are responsible for inflammatory mediator release, neoangiogenesis induction and cartilage and bone destruction. Inflammatory infiltrates in PsA consist of different immune cells, including macrophages, mast cells, polymorphonuclear (PMN) cells, and lymphocytes (B cells, T cells, and plasma cells), responsible for the significant production of different proinflammatory cytokines, including TNF, IL-1β, IL-6, IL-22, IL-23, IL-17A, and IL-18 (van Kuijk and Tak, 2011; Veale and Fearon, 2018) (**Figure 1**). Based on these observations, tracing inflammatory cells driving synovial inflammation in patients with undifferentiated inflammatory arthritis helped in the identification of tissue-dependent markers for predicting the development of defined chronic arthritis, such as PsA, within 1-year of follow-up (Alivernini et al., 2018). PsA synovitis partially differs from RA, as it is characterized by prominent neoangiogenesis with tortuous, immature, and elongated vessels (Kruithof et al., 2005a; Fromm et al., 2019); numerous macrophages in the lining (but not the sublining) layer (Rycke et al., 2005; Ambarus et al., 2012); and an increase in IL-17-positive infiltrating mast cells (Noordenbos et al., 2012). The presence of clonally expanded populations of CD8 T cells resistant to effective treatment (Curran et al., 2004; Penkava et al., 2020) suggests an antigen-driven T-cell response promoting inflammation. Although lymphoid aggregates and plasma cells are generally less represented in PsA than in RA (Alivernini et al., 2019; Nerviani et al., 2019), follicle-like structures found in the synovial tissue of treatment-naïve PsA patients are active, as shown by the presence of $CD21^{pos}$ or $CD23^{pos}$ follicular dendritic cells, together with the expression of activation/proliferation markers such as Ki67 and Bcl6, and are associated with the presence of autoantibodies in PsA patients at disease onset (Frasca et al., 2018). Moreover, consistent remodeling of bone metabolism is found in PsA, with bone neoformation markers interconnected with catabolic markers (allowing the presence of erosions along with new-bone growth) (Rahimi and Ritchlin, 2012; van Tok et al., 2018). The research utility of synovial biomarker discovery relies on the development of short-term

clinical trials testing new drugs in early stages of pharmacological development (Gerlag and Tak, 2008; Codullo and McInnes, 2011), limiting the full period of the study to the time course of small proof-of-principle trials ("to-go-or-not-to-go") (Sande et al., 2011). With this design in mind, studies investigating predictive synovial biomarkers of response to treatments have identified, in PsA, a reduction in sublining macrophages after effective TNFi treatment (Goedkoop et al., 2004a; Cañete et al., 2004; Rycke et al., 2005; Kruithof et al., 2006; van Kuijk et al., 2008; Pontifex et al., 2011). However, $CD3^{pos}$ T-cell and MMP (MMP-3 and MMP-13) reductions appear to be the most sensitive biomarker variations associated with an effective treatment response to TNFis (Goedkoop et al., 2004a; van Kuijk et al., 2008; Pontifex et al., 2011; van Kuijk and Tak, 2011). Recent studies have used protein profiles generated from proteomic analysis of powdered synovial tissues to compare patients with a response to ETA and ADA therapy with nonresponders (Ademowo et al., 2016; Collins et al., 2016). Different sets of biomarkers have been proposed, involving acute-phase proteins, annexins, cytoskeletal proteins, the hypoxia response, angiogenesis, and apoptotic signaling. Again, validation of baseline synovial predictive biomarkers to demonstrate superiority for a biomarker-driven approach with respect to recommended treatment algorithms has not been undertaken to date.

On the contrary, studies investigating the synovial impact of drugs, mostly TNFis, have helped elucidate the effects of these drugs at the synovial level. TNFis, as an example, do not enhance apoptotic markers in either RA (Smeets et al., 2003) or PsA (Goedkoop et al., 2004a), but they are able to decrease inflammatory cytokine levels, interfere with inflammatory cell homing from the peripheral circulation via a reduction in chemokine and adhesion molecule production, and reduce neovascularization of the tissue (Baeten et al., 2001; Kruithof et al., 2005b; Gerlag and Tak, 2008). Researchers have found decreases in VEGF (Cañete et al., 2004), von Willebrand's factor, αVβ integrin, and the adhesion molecules ICAM-1 and VCAM-1 (Baeten et al., 2001; Cañete et al., 2004; Goedkoop et al., 2004a, Goedkoop et al., 2004b) after TNFi treatment. Conversely, synovial mechanisms of the response to IL-17A blockers are not as widely understood. Van Mens et al. (2018) focused on longitudinal synovial modifications following SEC administration. After 12 weeks, there was a significant decrease in $CD15^{pos}$ neutrophils and in $CD68^{pos}$ macrophages in the sublining layer, with an increase in IL-17A-positive mast cells and reductions in IL-6, MMP-8, CCL-20, and IL-17A mRNA expression (Mens et al., 2018; Chen et al., 2019). The in vitro administration of an anti-IL-17A agent to FLS cultures was effective in reducing IL-17A-induced IL-6 production, with no differences between PsA and RA FLS (Frommer et al., 2019). SEC was also tested in vitro in SF mononuclear cell (SFMC) cultures and cocultures of FLS and peripheral blood mononuclear cells (PBMCs), producing a reduction in the release of monocyte chemoattractant protein 1 (MCP-1) after 48 hours (Nielsen et al., 2020). Finally, the adoption of a blocker of IL-17-receptor A (IL-17RA) was tested in PsA FLS cultures, highlighting reductions in IL-6 and IL-8 release into supernatants after stimulation with IL-17A (Raychaudhuri et al., 2012). In addition, the cellular effects of abatacept at the articular level (Szentpetery et al., 2017) have been longitudinally investigated in 14 patients starting abatacept or placebo treatment. Global synovitis and vascularity scores were significantly reduced after abatacept treatment compared with placebo treatment. The authors did not find significant changes in synovial CD3, CD8, or CD31 expression during the study period, while there was a significant reduction in FOXP3-positive $CD4^{pos}$ regulatory T cells (Tregs).

Despite all these promising findings, these is not enough evidence to allow genetic, serum, cellular, or synovial biomarkers to be included in treatment decision-making in clinical practice, mainly because most studies were performed to assess only one class of drugs, without stratifying patient groups based on different treatment responses. Moreover, explanations for the effect of a single drug might be more complex than simply targeting a soluble molecule or receptor, given the heterogeneity of drugs in terms of structures and pharmacokinetics (Humby et al., 2017). Therefore, the main treatment selection rules remain mostly empirical, and treatment outcomes, both in RCTs and in clinical practice, remain essentially based on clinical measures.

## TNF-α-, IL-23/IL-17-, and JAK/STAT-Dependent Signal Transduction Axes

Moving toward the bench side, the availability of efficacious drugs that directly target specific proinflammatory cytokines means, from a biological point of view, that interfering with TNF-α, IL-23/IL-17A axis, or JAK/STAT-dependent inflammation could disrupt several downstream signal transduction axes, with subsequent positive effects at the systemic and local levels. Knowledge of these signal transduction axes is, therefore, important to understand how each cytokine/node is dependent on or independent from others (**Figure 2**).

### TNF-α

TNF-α is a key cytokine in the pathogenesis of SpA, and skin manifestations, as well as enthesis, joint, and spine involvement, represent the epiphenomenon of hyperactivated TNF-α-dependent inflammation as a result of innate and adaptive immune response activation (Palladino et al., 2003; Tracey et al., 2008; Croft and Siegel, 2017). TNF-α is part of the TNF superfamily, and its activities in health and pathology are pleiotropic. Many different immune and nonimmune cell types can produce this cytokine, including fibroblasts and keratinocytes. TNF-α can be found in either a soluble form or a transmembrane (tm) form bound to cells. The soluble form (sTNF-α) is released after enzymatic cleavage of the cell surface–bound precursor (tmTNF-α) by TNF-alpha-converting enzyme (TACE) (Higuchi and Aggarwal, 1994). Both sTNF-α and tmTNF-α are biologically active. TNF-α binds two distinct receptors: type I (TNFR1, also known as p55 and CD120a) and type II (TNFR2, also known as p75 and CD120b). Both receptors are transmembrane glycoproteins with multiple cysteine-rich repeats in the extracellular N-terminal domain. Signal

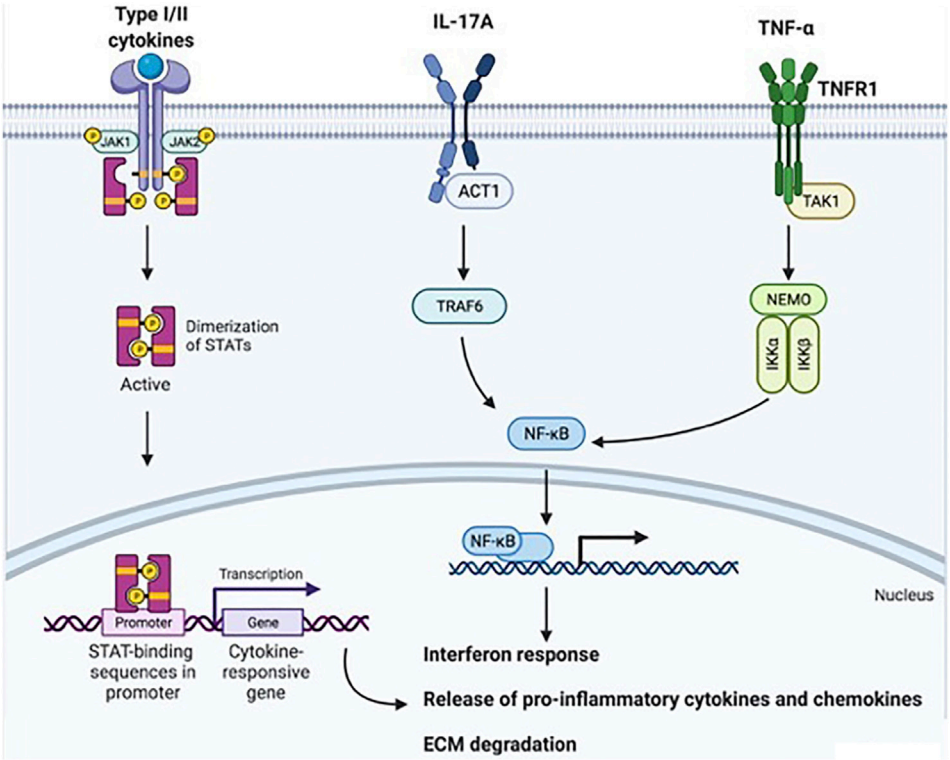


**FIGURE 2 |** Downstream signal transduction mechanisms following TNF-α, IL-17A, or JAK/STAT-coupled receptor activation. Type I and II cytokines (e.g., IL-23, IL-22, IL-6, and type I, II, and III IFNs) bind JAK/STAT-associated receptors. JAK proteins are associated with the cytoplasmic domain of these receptors, and when cytokines bind to the receptor, JAKs undergo autophosphorylation and phosphorylate other JAKs. STATs recognize JAKs, bind their cognate receptors and become phosphorylated by JAKs. STATs then translocate to the nucleus, bind DNA and activate the transcription of target genes for the interferon response, proinflammatory mediator production, and ECM degradation. IL-17A binds IL-17R, a transmembrane heterodimer of IL17RA and IL-17RC. This binding induces Act-1 activation, which in turn activates TRAF6 and, accordingly, NF-kB. NF-kB migrates to the nucleus and induces target gene transcription. TNF-α, either in its soluble or transmembrane form, binds TNFR1 or TNFR2. After binding, in the classical proinflammatory axis induced by TNF-α-dependent cellular activation, TAK1 engages NEMO. NEMO activation results in the phosphorylation of specific serine residues in inhibitory proteins of NF-κB (IκBs) by IKK-1 and IKK-2, leading to IκB polyubiquitination and proteasome-dependent degradation. This process releases NF-κB proteins, which translocate to the nucleus and induce target gene transcription. Taken together, these mechanisms result in an interferon response, proinflammatory chemokine and cytokine release, and extracellular matrix degradation. Abbreviations: TNF-α, tumor necrosis factor-alpha; IL, interleukin; JAK/STAT, Janus Kinase/signal transducer and activator of transcription; ECM, extracellular matrix; IL-17R, IL-17 Receptor; Act1, NF-kappa-B activator 1; TRAF6, TNFR-associated factor 6; NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells; TNFR, tumor necrosis factor-alpha receptor; TAK1, transforming growth factor-alpha-activated kinase 1; NEMO, NF-κB essential modulator; IKK, inhibitor of IκB kinase; IκBs, inhibitory proteins of NF-κB. This image was created with © BioRender 2021.

transduction mediated by TNF-α receptor activation can alternatively lead to activation of nuclear factor kappa-B (NF-κB) or to apoptosis, depending on the metabolic state of the cell (Kuwano and Hara, 2000; Hayden and Ghosh, 2014). It is relevant to note that tmTNF-α can also act as a signal transducer and not only as a ligand. In this case, binding to tmTNF-α by TNFRs, or even TNFis, can induce reverse signaling and trigger cell activation or cytokine suppression and apoptosis in tmTNF-α-expressing cells. TNFR1 is constitutively expressed on virtually all nucleated cell types, whereas TNFR2 is inducible and preferentially expressed on endothelial and hematopoietic cells (Sedger and McDermott, 2014). The cytoplasmic region of TNFR1 contains a death domain that couples TNFR1 to either of 2 distinct signaling pathways via binding of the adapter protein TNFR-associated death domain. The first pathway leads to the activation of nuclear factor kappa-B1 (NF-κB1), a family of transcription factors that controls many inflammatory genes, while a distinct signaling pathway leads to caspase-8- and caspase-3-dependent apoptosis. In a "network concept" of the role of TNF-α in inflammation, TNF-α is considered an early and important trigger and mediator of downstream mechanisms, with a variety of feedback loops managing chronicity. However, it is not the only key cytokine involved in inflammatory pathways at the basis of chronic inflammatory arthritis development and perpetuation.

## IL-17

IL-17-dependent signaling has been identified as a key modulator of synovial inflammation and joint destruction in various arthropathies, and its role in PsA pathogenesis, not only in skin manifestations (Robert and Miossec, 2019), is crucial (Doyle et al., 2012; Raychaudhuri et al., 2012; Raychaudhuri and Raychaudhuri, 2017). In particular, IL-17 is essential for increased expansion of Th17 cells, amplification and perpetuation

of enthesitis, promotion of bone resorption via stimulation of receptor activator of nuclear factor-kB ligand (RANKL) expression, and modulation of inflammatory pain. The IL-17 family is composed of 6 different forms. IL-17A is the most active form, with 30-fold higher activity than IL-17F. IL-17A can also be part of an active heterodimer with IL-17F, which is thought to have intermediate activity between IL-17A and IL-17F. Cellular production of IL-17 is complex, and different cells are involved in its production. Naïve $CD4^{pos}$ T cells that differentiate into Th17 cells in response to stimulation by IL-23 are considered the main producers of this cytokine, but other cell types (e.g., $CD8^{pos}$ T cells, γδ T cells, NK cells, mast cells, polymorphonuclear cells, and group 3 innate lymphoid cells) consistently contribute to its production (Blijdorp et al., 2018; Chen et al., 2019). IL-17 receptor is a receptor complex formed by IL-17RA and IL-17RC (a heterodimeric transmembrane IL-17RA and IL-17RC complex). The binding of IL-17 to IL-17R leads to Act1 engagement, activation of the TNFR-associated factor (TRAF) 6 protein and subsequent NF-κB-mediated transcription of proinflammatory cytokines, among which IL-22 increases IL-17 function and activates osteoclasts and IL-21 promotes the differentiation of follicular Th cells. Conversely, IL-17E, also called IL-25, can bind another receptor formed by IL-17RA and IL-17RB, blocking downstream Th1/Th17 activation and, in contrast, increasing Th2 activity.

### JAK/STAT-Dependent Signaling

In contrast, JAK/STAT-dependent signal transduction mediates the responses to a variety of different type I and II cytokines (Schwartz et al., 2016). It is relevant to note that neither TNF-α nor IL-17A signals via JAK/STAT-coupled receptors. However, IL-23, one of the key cytokines involved in Th17 polarization, and the IL-17-dependent downstream cytokines IL-22 and IL-21 bind to JAK/STAT-associated receptors. Type I cytokines include common gamma-chain cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21), common beta-chain cytokines (IL-3, IL-5, and granulocyte–macrophage colony-stimulating factor [GM-CSF]), IL-6, IL-23, and IL-12. Among type II cytokines, type I, II, and III IFNs and IL-10-related cytokines (IL-10, IL-22, and IL-20) are involved. JAK/STAT-coupled receptors have an extracellular cytokine-binding domain and a cytoplasmic domain that associates with JAKs. JAKs comprise four different proteins (JAK1, JAK2, JAK3, and tyrosine kinase 2 [TYK2]); when cytokines bind to the extracellular portion of their receptors, JAKs start working as phosphotransferases, transferring a phosphate group from ATP to tyrosine residues in their substrates. JAKs can transfer a phosphate group to themselves (autophosphorylation) or to other JAKs (transphosphorylation). Once JAKS are phosphorylated, they are recognized by STATs. The primary function of STATs relies on transmitting signals from type I and II cytokine receptors to the nucleus. There are currently seven known STATs: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. Prior to activation, STATs reside in the cytosol, but after cytokines bind to receptors, STATs bind to their cognate receptors and are phosphorylated by JAKs. After this modification, STATs translocate to the nucleus, where they bind DNA and activate the transcription of target genes, resulting in activation of key interferon response genes, production of proinflammatory cytokines and chemokines that perpetuate synovial inflammation, and activation of products that destroy the extracellular matrix (ECM) (e.g., MMPs), mediating cartilage and bone damage. Different JAK and STAT proteins can transmit signals from specific cytokines; however, a certain degree of functional promiscuity exists.

Among cytokines crucial in driving Th17 polarization, IL-23 is composed of two subunits, p19 and p40, which are linked by a disulfide bond. While the p19 subunit is an element unique to IL-23, the p40 subunit is shared with IL-12. IL-23 binds to its receptor IL-23R, which is coupled with JAK2 and Tyk2, members of the JAK family, which in turn mediate the downstream phosphorylation of STAT3 and its subsequent migration to the nucleus.

Given the broad range of cytokines that bind to JAK/STAT-associated receptors, targeting cytokines involved in JAK/STAT signal transduction could mean interfering with wider immune pathways than targeting only TNF-α or IL-17A per se, even if neither TNF-α nor IL-17A signaling occurs via JAK/STAT-coupled receptors. However, it is not clear how deep and complex the connections among these immune pathways might be.

## Relationships Among Different Proinflammatory "Immune Pathways" in PsA Synovitis

The clinical identification of patients refractory to specific targeting of TNF-α or the IL-23/IL-17 axis raises questions about the possibility of using a different available targeted therapy in refractory patients, with the aim of inhibiting the supposed "opposing" pathway. Few studies, however, have tried to investigate the specific clue regarding the connections between TNF-α and IL-23/IL-17 inflammatory effects in the context of PsA synovitis to clarify how targeting different nodes could modify their close interactions (**Table 3**).

### First Historical Highlights

In 2012, Noordenbos et al. (2012) investigated the role of mast cells in the pathogenesis of PsA synovitis. In their work, an analysis of paired synovial biopsy tissue samples obtained before and after 12 weeks of effective treatment with ETA demonstrated a significant decrease in the overall number of IL-17-positive cells, with no overall decrease in the number of IL-17-positive mast cells. The authors suggested that IL-17-positive mast cells could be resistant to clinically effective TNF-α inhibition. Similarly, Fiocco et al. (2010) demonstrated that PsA patients receiving intra-articular treatment with ETA showed reductions in the SF levels of different cytokines. As the main result of this study, post-treatment IL-1b, IL-6, and IL-22 levels were significantly reduced when compared with the corresponding baseline values, while IL-17A levels remained unchanged. In the same period, other researchers (Mitra et al., 2012; Raychaudhuri et al., 2012) investigated the effect of inhibiting the IL-17A axis in the PsA synovium. In a preliminary work, Raychaudhuri et al. (2012) cultured PsA FLS with IL-17A or TNF-α and found significant

**TABLE 3 |** Main studies investigating the relationships between TNF-α- and IL-23/IL-17A-driven proinflammatory pathways in psoriatic synovitis.

| Study | Type of study | Laboratory technique | Main results |
|---|---|---|---|
| Fiocco et al. (2010) | Longitudinal SF analysis before and at the last time SF sample was available for aspiration after IA-ETA treatment. | Luminex analysis | Longitudinal decreases in IL-1b, IL-6, and IL-22 levels in the SF AND no variation in IL-17A. |
| Noordenbos et al. (2012) | Longitudinal ST analysis before and after 12 weeks of systemic ETA treatment. | Double immunofluorescence | Longitudinal decrease in IL-positive cells AND no variation in IL-17-positive mast cells. |
| Mens et al. (2018) | Longitudinal ST analysis before and after 12 weeks of systemic SEC treatment. | Quantitative RT-PCR | Longitudinal reductions in IL-17A, IL-6, CCL-20, and MMP-3 mRNA levels AND no variations in IL-8 or TNF-α levels. |
| Chen et al. (2019) | Longitudinal ST analysis before and after 12 weeks of systemic SEC treatment. | IHC | Longitudinal reduction in IL-17A-positive non-mast cells AND increase in IL-17A-positive mast cells. |
| Raychaudhuri et al. (2012) | In vitro study, FLSs treated with IL-17A, TNF-α, and an IL-17RA blocker. | ELISA | TNF-α and IL-17A in vitro similarly increase IL-6, IL-8, and MMP-3 production in FLS cultures, while the IL-17RA blocker reduces the production. |
| Mitra et al. (2012) | In vitro study, FLSs treated with IL-22 and TNF-α. | Proliferation assays (MTT and CFSE dilution assays). | IL-22 and TNF-α-induced FLS proliferation AND IL-22 + TNF-α had a synergistic effect on FLS proliferation. |
| Gao et al. (2016) | In vitro study, synovial explant cultures treated with tofacitinib or DMSO. | ELISA | Ample effect of tofacitinib on in vitro cytokine production, reducing IL-8, IL-6, MCP-1, and MMP-3. IL-17A not detectable; TNF-α not evaluated. |
| Raychaudhuri et al. (2017) | In vitro study, SFMCs treated with tofacitinib. | FACS | Tofacitinib reduced IL-23-induced CD4 + CD11a + CD45RO + IL-17 + T cells. |
| Frommer et al. (2019) | In vitro study, FLSs treated with IL-17A, TNF-α, SEC, and ADA. | ELISA | IL-17A increases IL-6 release into FLS culture supernatants; TNF-α had a synergistic effect on increasing IL-6 release AND SEC and ADA had similar effects on IL-6 release inhibition. |
| Xu et al. (2020) | In vitro study, SFMC, and FLS coculture with SEC or ADA. | ELISA | SEC reduced IL-17A, IL-8 and IL-6 release; ADA reduced IL-8, TNF-α, and MMP-3/13. |
| Wade et al. (2019) | In vitro study, synovial cell suspensions treated in vitro with the PDE4 inhibitor rolipram. | ST single-cell suspension analysis + FACS. | Cells triple positive for GM-CSF, TNF-α, and IL-17 or IFN-γ were enriched in the PsA synovium compared to the peripheral blood and correlated with disease activity AND they were reduced by in vitro administration of rolipram. |
| Steel et al. (2020) | Cross-sectional study. | FACS | IL-17-positive CD8 T cells triple positive for GM-CSF, TNF-α, and IFN-γ, were enriched in the SF compared with the peripheral blood. |

*TNF-α, tumor necrosis factor-alpha; IL, interleukin; SF, synovial fluid; IA, intra-articular; ETA, etanercept; ST, synovial tissue; SEC, secukinumab; RT-PCR, real-time polymerase chain reaction; mRNA, messenger RNA; IHC, immunohistochemistry; FLS, fibroblast-like synoviocyte; IL-17RA, interleukin 17 receptor A; ELISA, enzyme-linked immunosorbent assay; MMP, matrix metalloproteinase; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; CFSE, carboxy fluorescein succinimidyl ester; DMSO, dimethylsulfoxide; MCP-1, monocyte chemoattractant protein-1; SFMCs, synovial fluid mononuclear cells; FACS, Hi-D fluorescence-activated cell sorting; ADA, adalimumab; PDE4, phosphodiesterase-4; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon.*

increases in IL-6 and IL-8 levels in both experiments. Blocking IL-17A activity with an IL-17RA blocker resulted in reductions in IL-6, IL-8, and MMP-3 production. In the work of Mitra et al. (2012), PsA-derived FLS cultured in the presence or absence of recombinant human IL-22, a proinflammatory cytokine that is produced by activated Th17 and Th22 cell subsets, induced FLS proliferation, an effect that was further enhanced when TNF-α was added to the culture. These studies have raised a relevant issue in the field of PsA concerning possible interference among multiple and different IL-mediated immune pathways using a "same targeted mechanism" drug. Conversely, they have cast doubts on the existence of different activated synovial proinflammatory immune processes in PsA patients, since a synergistic effect of IL-17A and TNF-α might be the most conceivable explanation.

## Longitudinal Synovial Biopsy Studies

In fact, the recent availability of anti-IL17A agents for the clinical management of PsA provides an opportunity to explore the synovial effects of SEC in terms of interactions with TNF-dependent inflammation. In a recent report by Van Mens et al. (2018), the authors performed synovial biopsies before administering the anti-IL17A SEC to peripheral SpA patients, including PsA subjects, and found significant reductions in IL-17A, IL-6, and MMP-3 mRNA levels but not in TNF-α levels 12 weeks after the start of the new drug. Furthermore, Chen et al. (2019) demonstrated that the number of IL-17A-positive mast cells was increased after 12 weeks of SEC treatment in peripheral SpA patients (particularly when a clinical response was achieved), while that of IL-17A-positive non-mast cells was significantly reduced, suggesting different types of IL-17 metabolism in different types of immune cells.

## In Vitro Culture Systems

Exploiting in vitro culture systems, other researchers have tried to solve the problem from another perspective. Frommer et al. (2019) demonstrated a synergistic effect for IL-17A and TNF-α in promoting IL-6 release into culture supernatants of PsA-FLS, while ADA and SEC were similarly effective in inhibiting IL-6 release. In 2016, another group of researchers (Gao et al., 2016; McGarry et al., 2017) performed studies investigating the synovial effect of the tsDMARD tofacitinib in PsA. In their works, the

authors cultured PsA-derived FLS and whole-tissue synovial explants in the presence or absence of 1 μM tofacitinib citrate. Tofacitinib, deemed to have a wider synovial effect than TNF-α inhibitors and IL17A blockers, inhibited PsA-derived FLS invasion and migration and reduced IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1) release into supernatants compared with a vehicle control. In this work, however, the IL-17A levels in PsA synovial explant cultures were undetectable, while TNF-α levels were not investigated. Raychaudhuri et al. (2017) demonstrated that tofacitinib was able to reduce the number of IL-23-induced IL-17-positive memory T cells in PBMCs and SFMCs derived from PsA patients. As it is known that neither IL-17A nor TNF-α signals via the JAK/STAT pathway, one of the explanations for the link between tofacitinib treatment and IL-17-positive T-cell reduction is the inhibition of IL-23 activity (which signals via JAK/STAT-coupled receptors) by tofacitinib and the associated further reduction in IL-17A production by different downstream effectors. This interconnection among several cytokines remains crucial when considering the complex relationships among different inflammatory pathways. In fact, it has been demonstrated in RA that TNF-α and the IL-23/IL-17A axis produce synergistic effects on bone damage (Kirkham et al., 2006) and chemotactic activity toward T cells and dendritic cells in the synovium (Chabaud et al., 2001). One of the mechanisms thought to mediate IL-17A and TNF-α synergism involves inflammatory protein mRNA transcript stabilization, independent of TRAF-6-dependent signal transduction (Hartupee et al., 2009). The synergistic effect of IL-17 and TNF-α might be dependent on the cell type, as demonstrated in several cell cultures, including RA-FLS (Noack et al., 2019) and CD14pos myeloid cell populations in normal enthesis soft tissue and perientheseal bone (Bridgewood et al., 2019). Moreover, since several cellular elements are key characteristics of psoriatic synovitis, the adoption of more sophisticated in vitro models of chronic synovitis might be of value. Xu et al. (2020) co-cultured CD4 T cells isolated from PsA SF with allogeneic FLS and treated the in vitro system with ADA or SEC. SEC significantly reduced IL-17A and IL-6 release into the cellular supernatant, while ADA reduced TNF-α and MMP-3/13. Both drugs reduced IL-8 levels, while IFN-$\gamma$ was not reduced by either of the treatments. In summary, although SEC and ADA showed overlapping activity affecting mediator release, a differential effect was demonstrated, with the anti-IL-17A agent mostly inhibiting inflammatory mediator release and TNF-α antagonism impacting structural mediators (e.g., MMPs).

## Lessons from Genetics, Animal Models, Skin, and Systemic Counterparts

As it is not completely accepted that the synovium is the appropriate tissue to evaluate the etiopathogenesis of psoriatic disease, in which skin and entheseal manifestations account for a large part (**Figure 1**), and given the relative difficulty of obtaining samples of entheseal or bone tissue from live patients, researchers have tried to investigate the specific clue about the presence of different/opposing pathways in PsA using animal models of arthritis, as well as cutaneous biopsies, circulating cells, and genomic profiling studies. Belasco et al. (2015) analyzed paired PsA synovial tissue and skin samples. They demonstrated higher IL-23/IL-17-related gene expression in cutaneous biopsies, while TNF and IFN-$\gamma$-signatures were quite homogenously expressed in both sites. These findings point toward the existence of distinct phenotypic inflammatory activities that govern pathology in the skin compared with that in the joints but do not explain why a significant proportion of patients with polyarthritis become refractory to TNFis. Recently, these data were confirmed by Nerviani et al. (2020), who evaluated 14 matched synovial tissue and skin biopsies from PsA patients. The relative gene expression of TNF-α was homogeneous in both the skin and the synovium, while IL-23A, IL-12B, and IL-23R showed higher expression in lesional skin than in the synovium.

Studies of murine models of arthritis are helpful in understanding links between TNF- and IL-17-dependent inflammation and have shown that treating collagen-induced arthritis (CIA) mice with TNF-blocking agents results in a rebound increase in lymph node Th17 cells, with a converse reduction in synovial Th17 cells (Notley et al., 2008). Similar rebounds in splenic and lymph node Th17 cells were also demonstrated in genetic TNFR2 loss-of-function mice (Miller et al., 2015). The addition of IL-17A was able to induce an experimental model of arthritis independent of TNF (Koenders et al., 2005b; Koenders et al., 2005a, Koenders et al., 2006). Studies of murine models of psoriasis have similarly proven increases in cutaneous and serum IL-17A and IL-22 levels after TNF-α blockade (Ma et al., 2010). Whether these findings apply to patients with exacerbated skin psoriasis under TNFi treatment (Brown et al., 2017) is not known. The results obtained in mouse models of arthritis show promise for clarifying the relationships among different immune pathways but—at the same time—are challenging the assumption that these two cytokine subsets are mutually antagonistic. Again, these mouse models are not fully generalizable to humans, indicating the need for further research.

In fact, studies of human patients with skin psoriasis have failed to demonstrate rebounds in IL-17A and IL-22 at the cutaneous level after TNFi treatment (Zaba et al., 2007, Zaba et al., 2009; Johnston et al., 2014), despite an increase in circulating Th17 cells (Hull et al., 2015). Tissue resistance to IL-17A, mediated by downregulation of IL-17RC, was found to be an early modification after ETA treatment in psoriatic patients. Cutaneous expression of IL-17A-related genes was unchanged in cutaneous biopsies from refractory patients (Zaba et al., 2009). Similar results were obtained following ineffective tofacitinib treatment (Krueger et al., 2016). With particular regard to blood biomarkers, Kim et al. (2018) investigated the effect of systemic treatment with etanercept or tofacitinib in psoriatic patients on relevant proinflammatory and cardiovascular protein biomarkers. The main result of their work was that after 4 weeks of treatment, both tofacitinib and etanercept reduced IL-6, CCL20, and CXCL10 levels, but the IL-17A level was significantly reduced only in responders for either treatment. Recently, in a study by Miyagawa et al. (2018), systemic treatment with SEC resulted in a reduction in circulating Th17 cells and an increase in Th1 cells. The authors' explanation of this rebound relied on the inhibition of the expression of IL-12 receptors on naïve T cells modulated by IL-17. The reduction in IL-17 levels after SEC treatment could revert this inhibitory effect, thus

facilitating the expression of IL-12 receptors and inducing differentiation into Th1 cells. Nonetheless, this rebound was not associated with clinical worsening of disease.

### Future Perspectives for Precision Medicine Approaches

Considering the synovial environment, Wade and coworkers (Wade et al., 2019; Canavan et al., 2020) investigated the presence of polyfunctional T cells in synovial tissues from PsA patients, hypothesizing that an effective treatment could interfere with this specific cluster of cells, which are able to produce a wide variety of different cytokines. The authors demonstrated that a significant proportion of synovial T-cell subsets were triple-positive for GM-CSF, TNF, and IL-17 or IFN-$\gamma$ compared with matched blood subsets and that these polyfunctional T cells were positively correlated with disease activity (measured with the Disease Activity in PSoriatic Arthritis (DAPSA) score), while single cytokine-producing T cells were not. Moreover, in vitro administration of a phosphodiesterase 4 (PDE4) inhibitor (rolipram) to synovial cell cultures significantly reduced the number of polyfunctional triple-positive T cells. Additionally, CD8 T cells were found to be polyfunctional in PsA SF, producing IL-17A, IFN$\gamma$, TNF-α, GM-CSF, and IL-22 (Steel et al., 2020). These studies suggest that a single T-cell population might be able to orchestrate diverse inflammatory pathways, specifically at the synovial level, and treatments able to interfere with the activity of all of these pathways, or strategies based on the recognition of patterns of cytokine expression at the single-patient level, might be more effective than others targeting a single mechanism or determined empirically.

The possibility of translating this seminal evidence into clinical practice is fascinating. In a similar condition such as RA, a recent RCT, for the first time, exploited information derived from synovial histology and mRNA expression analysis to inform selected treatment decisions (Humby et al., 2021). The combination of peripheral T-cell phenotyping (Miyagawa et al., 2018) with single-cell analysis at target tissue levels might also be used to inform treatment schedules in the context of PsA. In particular, not only cutaneous samples but also the synovial membrane should be evaluated to identify, at a particular time point in the disease history, the most active pathway at the single-patient level. In addition, it is still not known how "big data" analytics (e.g., machine learning and artificial intelligence) might impact personalized medicine approaches, providing individual molecular and clinical data to be compared with population-based data (Ritchlin et al., 2020b). Since rapid evolution is expected in the near future, the possibility of obtaining data from international multicenter consortia involving different types of target tissues, along with a refined definition of clinical endotypes linked with drug response or sequential treatment history, might impact management schedules and timelines, definitely changing the "heuristic" strategy to a personalized "precision medicine" approach.

## DISCUSSION AND CONCLUSION

The rules driving the treatment paradigm of "the right drug for the right patient at the right moment" in PsA have not been determined yet. At the bedside, many treatments with different mechanisms of action have been proven to be effective, and from a clinical point of view, it seems reasonable to think that TNF-α and IL-23/IL-17A inhibitors block different pathogenic processes, with JAKis inhibiting a wider number of cytokine nodes. However, it is unclear whether the clinical response to TNFis implicitly indicates a TNF-α-driven disease not amenable to IL-23/IL-17 targeting. Since biomarkers have not entered clinical practice to drive treatment decisions in PsA, the paradigms guiding therapy selection, and strategy adoption remain almost empirical. In contrast, at the bench, the analysis of cytokine downstream signaling pathways has revealed several axes that only appeared to be disconnected, and studies analyzing longitudinal synovial tissue modifications after the start of TNFis or IL-17A blockers, as well as in vitro models of PsA synovitis, genomic profiling studies, animal models, and skin or peripheral blood cell population analyses, have revealed much more complex interconnections. TNF-α and IL-17A demonstrate overlapping and synergistic activities, with differential variations depending on the type of cells analyzed, creating the possibility of "adaptation" to alternative key nodes at the tissue level. Again, the presence of polyfunctional cells pertaining contemporarily to alternative nodes might be central in driving disease aggressiveness and treatment resistance. Therefore, the analysis of tissue-derived samples might help in unveiling the interconnections among different nodes and selecting the most active node (or the driving pathway) suitable for targeting by available drugs to optimize an even more ambitious approach based on "precision medicine" in this highly challenging chronic disease, in accordance with a "patient-centered" perspective.

## AUTHOR CONTRIBUTIONS

All authors contributed to (1) the conception of the study, (2) drafting the article or revising it critically for important intellectual content, and (3) the final approval of the version to be submitted.

## ACKNOWLEDGMENTS

The authors would like to thank the clinicians and nurses of the Rheumatology Unit, Ferrara University, for their help in the clinical evaluation of patients with chronic inflammatory arthropathies. PP is grateful to Camilla degli Scrovegni for continuous support. PP was supported by the Progetti di Rilevante Interesse Nazionale Grant, PRIN2017E5L5P3. CG was supported by the European Research Council Grant, 853057-InflaPML.

## REFERENCES


Ademowo, O. S., Hernandez, B., Collins, E., Rooney, C., Fearon, U., van Kuijk, A. W., et al. (2016). Discovery and Confirmation of a Protein Biomarker Panel with Potential to Predict Response to Biological Therapy in Psoriatic Arthritis. *Ann. Rheum. Dis.* 75, 234–241. doi:10.1136/annrheumdis-2014-205417

Alivernini, S., Bruno, D., Tolusso, B., Bui, L., Petricca, L., Gigante, M. R., et al. (2019). Differential Synovial Tissue Biomarkers Among Psoriatic Arthritis and Rheumatoid Factor/anti-Citrulline Antibody-Negative Rheumatoid Arthritis. *Arthritis Res. Ther.* 21, 116. doi:10.1186/s13075-019-1898-7

Alivernini, S., Tolusso, B., Petricca, L., Bui, L., Di Mario, C., Gigante, M. R., et al. (2018). Synovial Predictors of Differentiation to Definite Arthritis in Patients with Seronegative Undifferentiated Peripheral Inflammatory Arthritis: microRNA Signature, Histological, and Ultrasound Features. *Front. Med.* 5. doi:10.3389/fmed.2018.00186

Ambarus, C. A., Noordenbos, T., de Hair, M. J., Tak, P. P., and Baeten, D. L. (2012). Intimal Lining Layer Macrophages but Not Synovial Sublining Macrophages Display an IL-10 Polarized-like Phenotype in Chronic Synovitis. *Arthritis Res. Ther.* 14, R74. doi:10.1186/ar3796

Araujo, E. G., Englbrecht, M., Hoepken, S., Finzel, S., Kampylafka, E., Kleyer, A., et al. (2019). Effects of Ustekinumab versus Tumor Necrosis Factor Inhibition on Enthesitis: Results from the Enthesial Clearance in Psoriatic Arthritis (ECLIPSA) Study. *Semin. Arthritis Rheum.* 48, 632–637. doi:10.1016/j.semarthrit.2018.05.011

Baeten, D., Kruithof, E., Van den Bosch, F., Demetter, P., Van Damme, N., Cuvelier, C., et al. (2001). Immunomodulatory Effects of Anti-tumor Necrosis Factor ? Therapy on Synovium in Spondylarthropathy: Histologic Findings in Eight Patients from an Open-Label Pilot Study. *Arthritis Rheum.* 44, 186–195. doi:10.1002/1529-0131(200101)44:1<186::AID-ANR25>3.0.CO;2-B

Belasco, J., Louie, J. S., Gulati, N., Wei, N., Nograles, K., Fuentes-Duculan, J., et al. (2015). Comparative Genomic Profiling of Synovium versus Skin Lesions in Psoriatic Arthritis. *Arthritis Rheumatol.* 67, 934–944. doi:10.1002/art.38995

Blijdorp, I. C., Menegatti, S., Van Mens, L. J., van de Sand, M. G., Chen, S., Hreggvidsdottir, H. S., et al. (2018). IL-22-and GM-CSF-Expressing but Not IL-17A-expressing Group 3 Innate Lymphoid Cells Are Expanded in the Inflamed Spondyloarthritis Joint. *Arthritis Rheumatol.*

Boutet, M.-A., Nerviani, A., Gallo Afflitto, G., and Pitzalis, C. (2018). Role of the IL-23/IL-17 Axis in Psoriasis and Psoriatic Arthritis: The Clinical Importance of its Divergence in Skin and Joints. *Ijms* 19, 530. doi:10.3390/ijms19020530

Bridgewood, C., Watad, A., Russell, T., Palmer, T. M., Marzo-Ortega, H., Khan, A., et al. (2019). Identification of Myeloid Cells in the Human Enthesis as the Main Source of Local IL-23 Production. *Ann. Rheum. Dis.* 78, 929–933. doi:10.1136/annrheumdis-2018-214944

Brown, G., Wang, E., Leon, A., Huynh, M., Wehner, M., Matro, R., et al. (2017). Tumor Necrosis Factor-α Inhibitor-Induced Psoriasis: Systematic Review of Clinical Features, Histopathological Findings, and Management Experience. *J. Am. Acad. Dermatol.* 76, 334–341. doi:10.1016/j.jaad.2016.08.012

Canavan, M., Wade, S. M., Veale, D. J., and Fearon, U. (2020). Enrichment of polyfunctional T cells in PsA synovial tissue. Response to: 'Polyfunctional TEM cells in psoriatic arthritis synovium skewed towards Th17 cells' by Raychaudhuri et al. *Ann. Rheum. Dis.*, annrheumdis–2019. doi:10.1136/annrheumdis-2019-216814

Cañete, J. D., Pablos, J. L., Sanmartí, R., Mallofré, C., Marsal, S., Maymó´, J., et al. (2004). Antiangiogenic Effects of Anti-tumor Necrosis Factor α Therapy with Infliximab in Psoriatic Arthritis. *Arthritis Rheum.* 50, 1636–1641. doi:10.1002/art.20181

Chabaud, M., Page, G., and Miossec, P. (2001). Enhancing Effect of IL-1, IL-17, and TNF-α on Macrophage Inflammatory Protein-3α Production in Rheumatoid Arthritis: Regulation by Soluble Receptors and Th2 Cytokines. *J. Immunol.* 167, 6015–6020. doi:10.4049/jimmunol.167.10.6015

Chandran, V., Shen, H., Pollock, R. A., Pellett, F. J., Carty, A., Cook, R. J., et al. (2013). Soluble Biomarkers Associated with Response to Treatment with Tumor Necrosis Factor Inhibitors in Psoriatic Arthritis. *J. Rheumatol.* 40, 866–871. doi:10.3899/jrheum.121162

Chen, S., Noordenbos, T., Blijdorp, I., van Mens, L., Ambarus, C. A., Vogels, E., et al. (2019). Histologic Evidence that Mast Cells Contribute to Local Tissue Inflammation in Peripheral Spondyloarthritis by Regulating interleukin-17A Content. *Rheumatology* 58, 617–627. doi:10.1093/rheumatology/key331

Chimenti, M. S., Perricone, C., Graceffa, D., Di Muzio, G., Ballanti, E., Guarino, M. D., et al. (2012). Complement System in Psoriatic Arthritis: a Useful Marker in Response Prediction and Monitoring of Anti-TNF Treatment. *Clin. Exp. Rheumatol.* 30, 23–30.

Chimenti, M. S., D'Antonio, A., Conigliaro, P., Ferrigno, S., Vendola, A., Ferraioli, M., et al. (2020). An Update for the Clinician on Biologics for the Treatment of Psoriatic Arthritis. *Btt* 14, 53–75. doi:10.2147/BTT.S260754

Clinical trial registration, INSPIRE 1 (2021a). A Phase III, Randomized, Double-Blind, Single-Dose, Placebo-Controlled Study to Demonstrate the Efficacy and Safety of Tildrakizumab in Subjects with Active Psoriatic Arthritis I (INSPIRE 1). clinicaltrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT04314544 (Accessed February 18, 2021).

Clinical trial registration, INSPIRE 2 (2021b). A Phase III, Randomized, Double-Blind, Placebo-Controlled Study to Demonstrate the Efficacy and Safety of Tildrakizumab in Anti-TNF Naïve Subjects with Active Psoriatic Arthritis II (INSPIRE 2). clinicaltrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT04314531 (Accessed February 18, 2021).

Clinical trial registration, KEEPsAKE 1 (2021c). A Phase 3, Randomized, Double-Blind, Study Comparing Risankizumab to Placebo in Subjects with Active Psoriatic Arthritis (PsA) Who Have a History of Inadequate Response to or Intolerance to at Least One Disease Modifying Anti-rheumatic Drug (DMARD) Therapy (KEEPsAKE 1). clinicaltrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03675308 (Accessed February 18, 2021).

Clinical trial registration, KEEPsAKE 2 (2021d). A Phase 3, Randomized, Double-Blind Study Comparing Risankizumab to Placebo in Subjects with Active Psoriatic Arthritis Including Those Who Have a History of Inadequate Response or Intolerance to Biologic Therapy(Ies) (KEEPsAKE 2). clinicaltrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT03671148 (Accessed February 18, 2021).

Clinical trial registration, PENGUIN 1 (2021e). A Phase 3, Randomized, Double-Blind, Placebo and Adalimumab-Controlled Study to Evaluate the Efficacy and Safety of Filgotinib in Subjects with Active Psoriatic Arthritis Who Are Naive to Biologic DMARD. Therapy.clinicaltrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT04115748 (Accessed February 18, 2021).

Clinical trial registration, PENGUIN 2 (2021f). A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of Filgotinib in Subjects with Active Psoriatic Arthritis Who Have an Inadequate Response or Are Intolerant to Biologic DMARD Therapy. clinicaltrials.Gov. Available at: https://clinicaltrials.gov/ct2/show/NCT04115839 (Accessed February 18, 2021).

Coates, L. C., Kavanaugh, A., Mease, P. J., Soriano, E. R., Laura Acosta-Felquer, M., Armstrong, A. W., et al. (2016). Group for Research and Assessment of Psoriasis and Psoriatic Arthritis 2015 Treatment Recommendations for Psoriatic Arthritis. *Arthritis Rheumatol.* 68, a–n. doi:10.1002/art.39573

Coates, L. C., Kishimoto, M., Gottlieb, A., Shuler, C. L., Lin, C.-Y., Lee, C. H., et al. (2017). Ixekizumab Efficacy and Safety with and without Concomitant Conventional Disease-Modifying Antirheumatic Drugs (cDMARDs) in Biologic DMARD (bDMARD)-Naïve Patients with Active Psoriatic Arthritis (PsA): Results from SPIRIT-P1. *RMD Open* 3, e000567. doi:10.1136/rmdopen-2017-000567

Codullo, V., and McInnes, I. B. (2011). Synovial Tissue Response to Treatment in Psoriatic Arthritis. *Torj* 5, 133–137. doi:10.2174/1874312901105010133

Collins, E. S., Butt, A. Q., Gibson, D. S., Dunn, M. J., Fearon, U., van Kuijk, A. W., et al. (2016). A Clinically Based Protein Discovery Strategy to Identify Potential Biomarkers of Response to Anti-TNF-α Treatment of Psoriatic Arthritis. *Prot. Clin. Appl.* 10, 645–662. doi:10.1002/prca.201500051

Croft, M., and Siegel, R. M. (2017). Beyond TNF: TNF Superfamily Cytokines as Targets for the Treatment of Rheumatic Diseases. *Nat. Rev. Rheumatol.* 13, 217–233. doi:10.1038/nrrheum.2017.22

Curran, S. A., FitzGerald, O. M., Costello, P. J., Selby, J. M., Kane, D. J., Bresnihan, B., et al. (2004). Nucleotide Sequencing of Psoriatic Arthritis Tissue before and during Methotrexate Administration Reveals a Complex Inflammatory T Cell Infiltrate with Very Few Clones Exhibiting Features that Suggest They Drive the Inflammatory Process by Recognizing Autoantigens. *J. Immunol.* 172, 1935–1944. doi:10.4049/jimmunol.172.3.1935

Dand, N., Duckworth, M., Baudry, D., Russell, A., Curtis, C. J., Lee, S. H., et al. (2019). HLA-C*06:02 Genotype Is a Predictive Biomarker of Biologic Treatment Response in Psoriasis. *J. Allergy Clin. Immunol.* 143, 2120–2130. doi:10.1016/j.jaci.2018.11.038

De Rycke, L., Baeten, D., Foell, D., Kruithof, E., Veys, E. M., Roth, J., et al. (2005). Differential Expression and Response to Anti-TNFalpha Treatment of Infiltrating versus Resident Tissue Macrophage Subsets in Autoimmune Arthritis. *J. Pathol.* 206, 17–27. doi:10.1002/path.1758

Deodhar, A., Helliwell, P. S., Boehncke, W.-H., Kollmeier, A. P., Hsia, E. C., Subramanian, R. A., et al. (2020). Guselkumab in Patients with Active Psoriatic Arthritis Who Were Biologic-Naive or Had Previously Received TNFα Inhibitor Treatment (DISCOVER-1): a Double-Blind, Randomised, Placebo-Controlled Phase 3 Trial. *The Lancet* 395, 1115–1125. doi:10.1016/S0140-6736(20)30265-8

Dolcino, M., Ottria, A., Barbieri, A., Patuzzo, G., Tinazzi, E., Argentino, G., et al. (2015). Gene Expression Profiling in Peripheral Blood Cells and Synovial Membranes of Patients with Psoriatic Arthritis. *PLoS ONE* 10, e0128262. doi:10.1371/journal.pone.0128262

Doyle, M. S., Collins, E. S., FitzGerald, O. M., and Pennington, S. R. (2012). New Insight into the Functions of the Interleukin-17 Receptor Adaptor Protein Act1 in Psoriatic Arthritis. *Arthritis Res. Ther.* 14, 226. doi:10.1186/ar4071

Fabris, M., Quartuccio, L., Fabro, C., Sacco, S., Lombardi, S., Ramonda, R., et al. (2016). The -308 TNFα and the -174 IL-6 Promoter Polymorphisms Associate with Effective Anti-tnfα Treatment in Seronegative Spondyloarthritis. *Pharmacogenomics J.* 16, 238–242. doi:10.1038/tpj.2015.49

Fagerli, K. M., Kearsley-Fleet, L., Watson, K. D., Packham, J., Contributors Group, B.-R., Symmons, D. P. M., et al. (2018). Long-term Persistence of TNF-Inhibitor Treatment in Patients with Psoriatic Arthritis. Data from the British Society for Rheumatology Biologics Register. *RMD Open* 4, e000596. doi:10.1136/rmdopen-2017-000596

Fiocco, U., Sfriso, P., Oliviero, F., Roux-Lombard, P., Scagliori, E., Cozzi, L., et al. (2010). Synovial Effusion and Synovial Fluid Biomarkers in Psoriatic Arthritis to Assess Intraarticular Tumor Necrosis Factor-α Blockade in the Knee Joint. *Arthritis Res. Ther.* 12, R148. doi:10.1186/ar3090

Frasca, L., Palazzo, R., Chimenti, M. S., Alivernini, S., Tolusso, B., Bui, L., et al. (2018). Anti-LL37 Antibodies Are Present in Psoriatic Arthritis (PsA) Patients: New Biomarkers in PsA. *Front. Immunol.* 9. doi:10.3389/fimmu.2018.01936

Fromm, S., Cunningham, C. C., Dunne, M. R., Veale, D. J., Fearon, U., and Wade, S. M. (2019). Enhanced Angiogenic Function in Response to Fibroblasts from Psoriatic Arthritis Synovium Compared to Rheumatoid Arthritis. *Arthritis Res. Ther.* 21, 297. doi:10.1186/s13075-019-2088-3

Frommer, K., Rehart, S., Müller-Ladner, U., and Neumann, E. (2019). Thu0028 Comparison of Il-17a and Tnf Induced Cytokine Secretion by Rheumatoid and Psoriatic Arthritis Synovial Fibroblasts and Their Inhibition by Biologics. *Ann. Rheum. Dis.* 78, 281–282. doi:10.1136/annrheumdis-2019-eular.3321

Gao, W., McGarry, T., Orr, C., McCormick, J., Veale, D. J., and Fearon, U. (2016). Tofacitinib Regulates Synovial Inflammation in Psoriatic Arthritis, Inhibiting STAT Activation and Induction of Negative Feedback Inhibitors. *Ann. Rheum. Dis.* 75, 311–315. doi:10.1136/annrheumdis-2014-207201

Generali, E., Scirè, C. A., Favalli, E. G., and Selmi, C. (2016). Biomarkers in Psoriatic Arthritis: a Systematic Literature Review. *Expert Rev. Clin. Immunol.* 12, 651–660. doi:10.1586/1744666X.2016.1147954

Gerlag, D. M., and Tak, P. P. (2008). Novel Approaches for the Treatment of Rheumatoid Arthritis: Lessons from the Evaluation of Synovial Biomarkers in Clinical Trials. *Best Pract. Res. Clin. Rheumatol.* 22, 311–323. doi:10.1016/j.berh.2008.02.002

Gladman, D. D., Antoni, C., Mease, P., Clegg, D., and Nash, P. (2005). Psoriatic Arthritis: Epidemiology, Clinical Features, Course, and Outcome. *Ann. Rheum. Dis.* 64, ii14–ii17. doi:10.1136/ard.2004.032482

Gladman, D. D., Orbai, A.-M., Gomez-Reino, J., Chang-Douglass, S., Leoncini, E., Burton, H. E., et al. (2020). Network Meta-Analysis of Tofacitinib, Biologic Disease-Modifying Antirheumatic Drugs, and Apremilast for the Treatment of Psoriatic Arthritis. *Curr. Ther. Res.* 93, 100601. doi:10.1016/j.curtheres.2020.100601

Gladman, D., Rigby, W., Azevedo, V. F., Behrens, F., Blanco, R., Kaszuba, A., et al. (2017). Tofacitinib for Psoriatic Arthritis in Patients with an Inadequate Response to TNF Inhibitors. *N. Engl. J. Med.* 377, 1525–1536. doi:10.1056/NEJMoa1615977

Goedkoop, A. Y., Kraan, M. C., Picavet, D. I., de Rie, M. A., Teunissen, M. B., Bos, J. D., et al. (2004b). Deactivation of Endothelium and Reduction in Angiogenesis in Psoriatic Skin and Synovium by Low Dose Infliximab Therapy in Combination with Stable Methotrexate Therapy: a Prospective Single-centre Study. *Arthritis Res. Ther.* 6, R326–R334. doi:10.1186/ar1182

Goedkoop, A. Y., Kraan, M., Teunissen, M., Picavet, D., de Rie, M. A., Bos, J., et al. (2004a). Early Effects of Tumour Necrosis Factor Blockade on Skin and Synovial Tissue in Patients with Active Psoriasis and Psoriatic Arthritis. *Ann. Rheum. Dis.* 63, 769–773. doi:10.1136/ard.2003.018085

Gossec, L., Baraliakos, X., McInnes, I., Kerschbaumer, A., de Wit, M., Dougados, M., et al. (2020). Response to: "Comment on: "EULAR recommendations for the management of psoriatic arthritis with pharmacological therapies: 2019 update" by Gossec et al" by Wei et al. *Ann. Rheum. Dis.* 79, 700–712. doi:10.1136/annrheumdis-2020-218456

Gossec, L., Smolen, J. S., Ramiro, S., de Wit, M., Cutolo, M., Dougados, M., et al. (2016). European League against Rheumatism (EULAR) Recommendations for the Management of Psoriatic Arthritis with Pharmacological Therapies: 2015 Update. *Ann. Rheum. Dis.* 75, 499–510. doi:10.1136/annrheumdis-2015-208337

Gratacós, J., Casado, E., Real, J., and Torre-Alonso, J. C. (2007). Prediction of Major Clinical Response (ACR50) to Infliximab in Psoriatic Arthritis Refractory to Methotrexate. *Ann. Rheum. Dis.* 66, 493–497. doi:10.1136/ard.2006.060079

Hartupee, J., Liu, C., Novotny, M., Sun, D., Li, X., and Hamilton, T. A. (2009). IL-17 Signaling for mRNA Stabilization Does Not Require TNF Receptor-Associated Factor 6. *J. Immunol.* 182, 1660–1666. doi:10.4049/jimmunol.182.3.1660

Haugeberg, G., Michelsen, B., Tengesdal, S., Hansen, I. J. W., Diamantopoulos, A., and Kavanaugh, A. (2018). Ten Years of Follow-Up Data in Psoriatic Arthritis: Results Based on Standardized Monitoring of Patients in an Ordinary Outpatient Clinic in Southern Norway. *Arthritis Res. Ther.* 20. doi:10.1186/s13075-018-1659-z

Hayden, M. S., and Ghosh, S. (2014). Regulation of NF-Kb by TNF Family Cytokines. *Semin. Immunol.* 26, 253–266. doi:10.1016/j.smim.2014.05.004

Hellman, U., Engström-Laurent, A., Larsson, A., and Lindqvist, U. (2019). Hyaluronan Concentration and Molecular Mass in Psoriatic Arthritis: Biomarkers of Disease Severity, Resistance to Treatment, and Outcome. *Scand. J. Rheumatol.* 48, 284–293. doi:10.1080/03009742.2019.1577490

Higuchi, M., and Aggarwal, B. B. (1994). TNF Induces Internalization of the P60 Receptor and Shedding of the P80 Receptor. *J. Immunol.* 152, 3550–3558.

Hull, D. N., Williams, R. O., Pathan, E., Alzabin, S., Abraham, S., and Taylor, P. C. (2015). Anti-tumour Necrosis Factor Treatment Increases Circulating T Helper Type 17 Cells Similarly in Different Types of Inflammatory Arthritis. *Clin. Exp. Immunol.* 181, 401–406. doi:10.1111/cei.12626

Humby, F. C., Al Balushi, F., Lliso, G., Cauli, A., and Pitzalis, C. (2017). Can Synovial Pathobiology Integrate with Current Clinical and Imaging Prediction Models to Achieve Personalized Health Care in Rheumatoid Arthritis?. *Front. Med.* 4. doi:10.3389/fmed.2017.00041

Humby, F., Durez, P., Buch, M. H., Lewis, M. J., Rizvi, H., Rivellese, F., et al. (2021). Rituximab versus Tocilizumab in Anti-TNF Inadequate Responder Patients with Rheumatoid Arthritis (R4RA): 16-week Outcomes of a Stratified, Biopsy-Driven, Multicentre, Open-Label, Phase 4 Randomised Controlled Trial. *The Lancet* 397, 305–317. doi:10.1016/S0140-6736(20)32341-2

Jadon, D. R., Stober, C., Pennington, S. R., and FitzGerald, O. (2020). Applying Precision Medicine to Unmet Clinical Needs in Psoriatic Disease. *Nat. Rev. Rheumatol.* 16, 609–627. doi:10.1038/s41584-020-00507-9

Johnston, A., Guzman, A. M., Swindell, W. R., Wang, F., Kang, S., and Gudjonsson, J. E. (2014). Early Tissue Responses in Psoriasis to the Antitumour Necrosis Factor-α Biologic Etanercept Suggest Reduced Interleukin-17 Receptor Expression and Signalling. *Br. J. Dermatol.* 171, 97–107. doi:10.1111/bjd.12937

Kerschbaumer, A., Smolen, J. S., Dougados, M., de Wit, M., Primdahl, J., McInnes, I., et al. (2020). Pharmacological Treatment of Psoriatic Arthritis: a Systematic Literature Research for the 2019 Update of the EULAR Recommendations for the Management of Psoriatic Arthritis. *Ann. Rheum. Dis.* 79, 778–786. doi:10.1136/annrheumdis-2020-217163

Kim, J., Tomalin, L., Lee, J., Fitz, L. J., Berstein, G., Correa-da Rosa, J., et al. (2018). Reduction of Inflammatory and Cardiovascular Proteins in the Blood of Patients with Psoriasis: Differential Responses between Tofacitinib and Etanercept after 4 Weeks of Treatment. *J. Invest. Dermatol.* 138, 273–281. doi:10.1016/j.jid.2017.08.040

Kirkham, B. W., Lassere, M. N., Edmonds, J. P., Juhasz, K. M., Bird, P. A., Lee, C. S., et al. (2006). Synovial Membrane Cytokine Expression Is Predictive of Joint Damage Progression in Rheumatoid Arthritis: A Two-Year Prospective Study (The DAMAGE Study Cohort). *Arthritis Rheum.* 54, 1122–1131. doi:10.1002/art.21749

Koenders, M. I., Joosten, L. A. B., and van den Berg, W. B. (2006). Potential New Targets in Arthritis Therapy: Interleukin (IL)-17 and its Relation to Tumour Necrosis Factor and IL-1 in Experimental Arthritis. *Ann. Rheum. Dis.* 65, iii29–iii33. doi:10.1136/ard.2006.058529

Koenders, M. I., Kolls, J. K., Oppers-Walgreen, B., Van Den Bersselaar, L., Joosten, L. A. B., Schurr, J. R., et al. (2005a). Interleukin-17 Receptor Deficiency Results in Impaired Synovial Expression of Interleukin-1 and Matrix Metalloproteinases 3, 9, and 13 and Prevents Cartilage Destruction during Chronic Reactivated Streptococcal Cell wall-induced Arthritis. *Arthritis Rheum.* 52, 3239–3247. doi:10.1002/art.21342

Koenders, M. I., Lubberts, E., Oppers-Walgreen, B., van den Bersselaar, L., Helsen, M. M., Kolls, J. K., et al. (2005b). Induction of Cartilage Damage by Overexpression of T Cell interleukin-17A in Experimental Arthritis in Mice Deficient in Interleukin-1. *Arthritis Rheum.* 52, 975–983. doi:10.1002/art.20885

Kristensen, L. E., Gülfe, A., Saxne, T., and Geborek, P. (2008). Efficacy and Tolerability of Anti-tumour Necrosis Factor Therapy in Psoriatic Arthritis Patients: Results from the South Swedish Arthritis Treatment Group Register. *Ann. Rheum. Dis.* 67, 364–369. doi:10.1136/ard.2007.073544

Krueger, J., Clark, J. D., Suárez-Fariñas, M., Fuentes-Duculan, J., Cueto, I., Wang, C. Q., et al. (2016). Tofacitinib Attenuates Pathologic Immune Pathways in Patients with Psoriasis: A Randomized Phase 2 Study. *J. Allergy Clin. Immunol.* 137, 1079–1090. doi:10.1016/j.jaci.2015.12.1318

Kruithof, E., Baeten, D., De Rycke, L., Vandooren, B., Foell, D., Roth, J., et al. (2005a). Synovial Histopathology of Psoriatic Arthritis, Both Oligo- and Polyarticular, Resembles Spondyloarthropathy More Than it Does Rheumatoid Arthritis. *Arthritis Res. Ther.* 7, R569–R580. doi:10.1186/ar1698

Kruithof, E., Baeten, D., Van den Bosch, F., Mielants, H., Veys, E., and De Keyser, F. (2005b). Histological Evidence that Infliximab Treatment Leads to Downregulation of Inflammation and Tissue Remodelling of the Synovial Membrane in Spondyloarthropathy. *Ann. Rheum. Dis.* 64, 529–536. doi:10.1136/ard.2003.018549

Kruithof, E., De Rycke, L., Vandooren, B., De Keyser, F., FitzGerald, O., McInnes, I., et al. (2006). Identification of Synovial Biomarkers of Response to Experimental Treatment in Early-phase Clinical Trials in Spondylarthritis. *Arthritis Rheum.* 54, 1795–1804. doi:10.1002/art.21914

Kuwano, K., and Hara, N. (2000). Signal Transduction Pathways of Apoptosis and Inflammation Induced by the Tumor Necrosis Factor Receptor Family. *Am. J. Respir. Cel Mol. Biol.* 22, 147–149. doi:10.1165/ajrcmb.22.2.f178

Leijten, E. F., Radstake, T. R., McInnes, I. B., and Jacobs, J. W. (2019). Limits of Traditional Evidence-Based Medicine Methodologies Exemplified by the Novel Era in Psoriatic Arthritis Drug Development. *Expert Rev. Clin. Immunol.* 15, 441–444. doi:10.1080/1744666X.2019.1580144

Leipe, J., Grunke, M., Dechant, C., Reindl, C., Kerzendorf, U., Schulze-Koops, H., et al. (2010). Role of Th17 Cells in Human Autoimmune Arthritis. *Arthritis Rheum.* 62, 2876–2885. doi:10.1002/art.27622

Londono, J., Romero-Sanchez, M. C., Torres, V. G., Bautista, W. A., Fernandez, D. J., Quiroga, J. d. A., et al. (2012). Associação entre os níveis séricos de potenciais biomarcadores com a presença de fatores relacionados à atividade clínica e ao mau prognóstico em espondiloartrites. *Rev. Bras. Reumatol.* 52, 536–544. doi:10.1590/s0482-50042012000400006

Ma, H.-L., Napierata, L., Stedman, N., Benoit, S., Collins, M., Nickerson-Nutter, C., et al. (2010). Tumor Necrosis Factor α Blockade Exacerbates Murine Psoriasis-like Disease by Enhancing Th17 Function and Decreasing Expansion of Treg Cells. *Arthritis Rheum.* 62, 430–440. doi:10.1002/art.27203

Mahendran, S., and Chandran, V. (2018). Exploring the Psoriatic Arthritis Proteome in Search of Novel Biomarkers. *Proteomes* 6, 5. doi:10.3390/proteomes6010005

Mahmood, F., Coates, L. C., and Helliwell, P. S. (2018). Current Concepts and Unmet Needs in Psoriatic Arthritis. *Clin. Rheumatol.* 37, 297–305. doi:10.1007/s10067-017-3908-y

McGarry, T., Gao, W., Veale, D. J., and Fearon, U. (2017). Response to: 'Regulatory Role of the JAK STAT Kinase Signalling System on the IL-23/IL-17 Cytokine axis in Psoriatic Arthritis' by Raychaudhuriet Al. *Ann. Rheum. Dis.* 76, e37. doi:10.1136/annrheumdis-2017-211081

McInnes, I. B., Behrens, F., Mease, P. J., Kavanaugh, A., Ritchlin, C., Nash, P., et al. (2020). Secukinumab versus Adalimumab for Treatment of Active Psoriatic Arthritis (EXCEED): a Double-Blind, Parallel-Group, Randomised, Active-Controlled, Phase 3b Trial. *The Lancet* 395, 1496–1505. doi:10.1016/S0140-6736(20)30564-X

McInnes, I. B., Kavanaugh, A., Gottlieb, A. B., Puig, L., Rahman, P., Ritchlin, C., et al. (2013). Efficacy and Safety of Ustekinumab in Patients with Active Psoriatic Arthritis: 1 Year Results of the Phase 3, Multicentre, Double-Blind, Placebo-Controlled PSUMMIT 1 Trial. *The Lancet* 382, 780–789. doi:10.1016/s0140-6736(13)60594-2

McInnes, I. B., Mease, P. J., Kirkham, B., Kavanaugh, A., Ritchlin, C. T., Rahman, P., et al. (2015). Secukinumab, a Human Anti-interleukin-17a Monoclonal Antibody, in Patients with Psoriatic Arthritis (FUTURE 2): a Randomised, Double-Blind, Placebo-Controlled, Phase 3 Trial. *The Lancet* 386, 1137–1146. doi:10.1016/S0140-6736(15)61134-5

Mease, P., Hall, S., FitzGerald, O., van der Heijde, D., Merola, J. F., Avila-Zapata, F., et al. (2017a). Tofacitinib or Adalimumab versus Placebo for Psoriatic Arthritis. *N. Engl. J. Med.* 377, 1537–1550. doi:10.1056/NEJMoa1615975

Mease, P. J., Helliwell, P. S., Hjuler, K. F., Raymond, K., and McInnes, I. (2021a). Brodalumab in Psoriatic Arthritis: Results from the Randomised Phase III AMVISION-1 and AMVISION-2 Trials. *Ann. Rheum. Dis.* 80, 185–193. doi:10.1136/annrheumdis-2019-216835

Mease, P. J., Lertratanakul, A., Anderson, J. K., Papp, K., Van den Bosch, F., Tsuji, S., et al. (2021b). Upadacitinib for Psoriatic Arthritis Refractory to Biologics: SELECT-PsA 2. *Ann. Rheum. Dis.* 80, 312–320. doi:10.1136/annrheumdis-2020-218870

Mease, P. J., McInnes, I. B., Kirkham, B., Kavanaugh, A., Rahman, P., van der Heijde, D., et al. (2015). Secukinumab Inhibition of Interleukin-17A in Patients with Psoriatic Arthritis. *N. Engl. J. Med.* 373, 1329–1339. doi:10.1056/NEJMoa1412679

Mease, P. J., Rahman, P., Gottlieb, A. B., Kollmeier, A. P., Hsia, E. C., Xu, X. L., et al. (2020a). Guselkumab in Biologic-Naive Patients with Active Psoriatic Arthritis (DISCOVER-2): a Double-Blind, Randomised, Placebo-Controlled Phase 3 Trial. *The Lancet* 395, 1126–1136. doi:10.1016/S0140-6736(20)30263-4

Mease, P. J., Smolen, J. S., Behrens, F., Nash, P., Liu Leage, S., Li, L., et al. (2020b). A Head-To-Head Comparison of the Efficacy and Safety of Ixekizumab and Adalimumab in Biological-Naïve Patients with Active Psoriatic Arthritis: 24-week Results of a Randomised, Open-Label, Blinded-Assessor Trial. *Ann. Rheum. Dis.* 79, 123–131. doi:10.1136/annrheumdis-2019-215386

Mease, P. J., van der Heijde, D., Ritchlin, C. T., Okada, M., Cuchacovich, R. S., Shuler, C. L., et al. (2017b). Ixekizumab, an interleukin-17A Specific Monoclonal Antibody, for the Treatment of Biologic-Naive Patients with Active Psoriatic Arthritis: Results from the 24-week Randomised, Double-Blind, Placebo-Controlled and Active (Adalimumab)-controlled Period of the Phase III Trial SPIRIT-P1. *Ann. Rheum. Dis.* 76, 79–87. doi:10.1136/annrheumdis-2016-209709

Menon, B., Gullick, N. J., Walter, G. J., Rajasekhar, M., Garrood, T., Evans, H. G., et al. (2014). Interleukin-17 + CD8 + T Cells Are Enriched in the Joints of Patients with Psoriatic Arthritis and Correlate with Disease Activity and Joint Damage Progression. *Arthritis Rheumatol.* 66, 1272–1281. doi:10.1002/art.38376

Miller, P. G., Bonn, M. B., and McKarns, S. C. (2015). Transmembrane TNF-TNFR2 Impairs Th17 Differentiation by Promoting Il2 Expression. *J.I.* 195, 2633–2647. doi:10.4049/jimmunol.1500286

Mitra, A., Raychaudhuri, S. K., and Raychaudhuri, S. P. (2012). Functional Role of IL-22 in Psoriatic Arthritis. *Arthritis Res. Ther.* 14, R65. doi:10.1186/ar3781

Miyagawa, I., Nakayamada, S., Nakano, K., Kubo, S., Iwata, S., Miyazaki, Y., et al. (2018). Precision Medicine Using Different Biological DMARDs Based on Characteristic Phenotypes of Peripheral T Helper Cells in Psoriatic Arthritis. *Rheumatology* 58, 336–344. doi:10.1093/rheumatology/key069

Muramatsu, S., Kubo, R., Nishida, E., and Morita, A. (2017). Serum Interleukin-6 Levels in Response to Biologic Treatment in Patients with Psoriasis. *Mod. Rheumatol.* 27, 137–141. doi:10.3109/14397595.2016.1174328

Murdaca, G., Gulli, R., Spanò, F., Lantieri, F., Burlando, M., Parodi, A., et al. (2014). TNF-α Gene Polymorphisms: Association with Disease Susceptibility and

Response to Anti-TNF-α Treatment in Psoriatic Arthritis. *J. Invest. Dermatol.* 134, 2503–2509. doi:10.1038/jid.2014.123

Nash, P., Kirkham, B., Okada, M., Rahman, P., Combe, B., Burmester, G. R., et al. (2017). Ixekizumab for the Treatment of Patients with Active Psoriatic Arthritis and an Inadequate Response to Tumour Necrosis Factor Inhibitors: Results from the 24-week Randomised, Double-Blind, Placebo-Controlled Period of the SPIRIT-P2 Phase 3 Trial. *Lancet* 389, 2317–2327. doi:10.1016/S0140-6736(17)31429-0

Nerviani, A., Boutet, M.-A., Tan, W. S. G., Goldmann, K., Purkayastha, N., Lajtos, T. A., et al. (2020). IL-23 Skin and Joint Profiling in Psoriatic Arthritis: Novel Perspectives in Understanding Clinical Responses to IL-23 Inhibitors. *Ann. Rheum. Dis.* 80, 591–597. doi:10.1136/annrheumdis-2020-218186

Nerviani, A., Ribera, G. L., Boutet, M. A., Goldmann, K., Kelly, S., Bombardieri, M., et al. (2019). Op0113 Histological and Molecular Portrait of the Synovial Tissue in Early Treatment-Naïve Psoriatic Arthritis in Comparison with Rheumatoid Arthritis. *Ann. Rheum. Dis.* 78, 130–131. doi:10.1136/annrheumdis-2019-eular.3634

Nielsen, M. A., Lomholt, S., Mellemkjær, A., Andersen, M. N., Buckley, C. D., and Kragstrup, T. W. (2020). Responses to Cytokine Inhibitors Associated with Cellular Composition in Models of Immune-Mediated Inflammatory Arthritis. *ACR Open Rheuma* 2, 3–10. doi:10.1002/acr2.11094

Noack, M., Beringer, A., and Miossec, P. (2019). Additive or Synergistic Interactions between IL-17A or IL-17F and TNF or IL-1β Depend on the Cell Type. *Front. Immunol.* 10. doi:10.3389/fimmu.2019.01726

Noordenbos, T., Yeremenko, N., Gofita, I., van de Sande, M., Tak, P. P., Cañete, J. D., et al. (2012). Interleukin-17-positive Mast Cells Contribute to Synovial Inflammation in Spondylarthritis. *Arthritis Rheum.* 64, 99–109. doi:10.1002/art.33396

Notley, C. A., Inglis, J. J., Alzabin, S., McCann, F. E., McNamee, K. E., and Williams, R. O. (2008). Blockade of Tumor Necrosis Factor in Collagen-Induced Arthritis Reveals a Novel Immunoregulatory Pathway for Th1 and Th17 Cells. *J. Exp. Med.* 205, 2491–2497. doi:10.1084/jem.20072707

O'Rielly, D. D., Jani, M., Rahman, P., and Elder, J. T. (2019). The Genetics of Psoriasis and Psoriatic Arthritis. *J. Rheumatol. Suppl.* 95, 46–50. doi:10.3899/jrheum.190119

Ogdie, A., Coates, L. C., and Gladman, D. D. (2020). Treatment Guidelines in Psoriatic Arthritis. *Rheumatology* 59, i37–i46. doi:10.1093/rheumatology/kez383

Ovejero-Benito, M. C., Reolid, A., Sánchez-Jiménez, P., Saiz-Rodríguez, M., Muñoz-Aceituno, E., Llamas-Velasco, M., et al. (2018). Histone Modifications Associated with Biological Drug Response in Moderate-To-Severe Psoriasis. *Exp. Dermatol.* 27, 1361–1371. doi:10.1111/exd.13790

Ovejero-Benito, M. C., Muñoz-Aceituno, E., Reolid, A., Fisas, L. H., Llamas-Velasco, M., Prieto-Pérez, R., et al. (2019). Polymorphisms Associated with Anti-TNF Drugs Response in Patients with Psoriasis and Psoriatic Arthritis. *J. Eur. Acad. Dermatol. Venereol.* 33, e175–e177. doi:10.1111/jdv.15431

Palladino, M. A., Bahjat, F. R., Theodorakis, E. A., and Moldawer, L. L. (2003). Anti-TNF-α Therapies: the Next Generation. *Nat. Rev. Drug Discov.* 2, 736–746. doi:10.1038/nrd1175

Pedersen, S. J., Hetland, M. L., Sørensen, I. J., Østergaard, M., Nielsen, H. J., and Johansen, J. S. (2010). Circulating Levels of Interleukin-6, Vascular Endothelial Growth Factor, YKL-40, Matrix Metalloproteinase-3, and Total Aggrecan in Spondyloarthritis Patients during 3 Years of Treatment with TNFα Inhibitors. *Clin. Rheumatol.* 29, 1301–1309. doi:10.1007/s10067-010-1528-x

Penkava, F., Velasco-Herrera, M. D. C., Young, M. D., Yager, N., Nwosu, L. N., Pratt, A. G., et al. (2020). Single-cell Sequencing Reveals Clonal Expansions of Pro-inflammatory Synovial CD8 T Cells Expressing Tissue-Homing Receptors in Psoriatic Arthritis. *Nat. Commun.* 11, 4767. doi:10.1038/s41467-020-18513-6

Pitzalis, C., Choy, E. H. S., and Buch, M. H. (2020). Transforming Clinical Trials in Rheumatology: towards Patient-Centric Precision Medicine. *Nat. Rev. Rheumatol.* 16, 590–599. doi:10.1038/s41584-020-0491-4

Pontifex, E. K., Gerlag, D. M., Gogarty, M., Vinkenoog, M., Gibbs, A., Burgman, I., et al. (2011). Change in CD3 Positive T-Cell Expression in Psoriatic Arthritis Synovium Correlates with Change in DAS28 and Magnetic Resonance Imaging Synovitis Scores Following Initiation of Biologic Therapy - a Single centre, Open-Label Study. *Arthritis Res. Ther.* 13, R7. doi:10.1186/ar3228

Rahimi, H., and Ritchlin, C. T. (2012). Altered Bone Biology in Psoriatic Arthritis. *Curr. Rheumatol. Rep.* 14, 349–357. doi:10.1007/s11926-012-0259-1

Raychaudhuri, S. K., Abria, C., and Raychaudhuri, S. P. (2017). Regulatory Role of the JAK STAT Kinase Signalling System on the IL-23/IL-17 Cytokine axis in Psoriatic Arthritis. *Ann. Rheum. Dis.* 76, e36. doi:10.1136/annrheumdis-2016-211046

Raychaudhuri, S. P., Raychaudhuri, S. K., and Genovese, M. C. (2012). IL-17 Receptor and its Functional Significance in Psoriatic Arthritis. *Mol. Cel. Biochem.* 359, 419–429. doi:10.1007/s11010-011-1036-6

Raychaudhuri, S. P., and Raychaudhuri, S. K. (2017). Mechanistic Rationales for Targeting interleukin-17A in Spondyloarthritis. *Arthritis Res. Ther.* 19. doi:10.1186/s13075-017-1249-5

Ritchlin, C., Rahman, P., Kavanaugh, A., McInnes, I. B., Puig, L., Li, S., et al. (2014). Efficacy and Safety of the anti-IL-12/23 P40 Monoclonal Antibody, Ustekinumab, in Patients with Active Psoriatic Arthritis Despite Conventional Non-biological and Biological Anti-tumour Necrosis Factor Therapy: 6-month and 1-year Results of the Phase 3, Multicentre, Double-Blind, Placebo-Controlled, Randomised PSUMMIT 2 Trial. *Ann. Rheum. Dis.* 73, 990–999. doi:10.1136/annrheumdis-2013-204655

Ritchlin, C. T., Pennington, S. R., Reynolds, N. J., and FitzGerald, O. (2020b). Moving toward Precision Medicine in Psoriasis and Psoriatic Arthritis. *J. Rheumatol. Suppl.* 96, 19–24. doi:10.3899/jrheum.200122

Ritchlin, C. T., Kavanaugh, A., Merola, J. F., Schett, G., Scher, J. U., Warren, R. B., et al. (2020a). Bimekizumab in Patients with Active Psoriatic Arthritis: Results from a 48-week, Randomised, Double-Blind, Placebo-Controlled, Dose-Ranging Phase 2b Trial. *The Lancet* 395, 427–440. doi:10.1016/S0140-6736(19)33161-7

Robert, M., and Miossec, P. (2019). IL-17 in Rheumatoid Arthritis and Precision Medicine: From Synovitis Expression to Circulating Bioactive Levels. *Front. Med.* 5. doi:10.3389/fmed.2018.00364

Russell, T., Bridgewood, C., Rowe, H., Altaie, A., Jones, E., and McGonagle, D. (2021). Cytokine "fine Tuning" of Enthesis Tissue Homeostasis as a Pointer to Spondyloarthritis Pathogenesis with a Focus on Relevant TNF and IL-17 Targeted Therapies. *Semin. Immunopathol.* 43, 193–206. doi:10.1007/s00281-021-00836-1

Ruyssen-Witrand, A., Perry, R., Watkins, C., Braileanu, G., Kumar, G., Kiri, S., et al. (2020). Efficacy and Safety of Biologics in Psoriatic Arthritis: a Systematic Literature Review and Network Meta-Analysis. *RMD Open* 6, e001117. doi:10.1136/rmdopen-2019-001117

Sande, M. G. H. van. de., Gerlag, D. M., Lodde, B. M., Baarsen, L. G. M. van., Alivernini, S., Codullo, V., et al. (2011). Evaluating Antirheumatic Treatments Using Synovial Biopsy: a Recommendation for Standardisation to Be Used in Clinical Trials. *Ann. Rheum. Dis.* 70, 423–427. doi:10.1136/ard.2010.139550

Scher, J. U., Ogdie, A., Merola, J. F., and Ritchlin, C. (2019). Preventing Psoriatic Arthritis: Focusing on Patients with Psoriasis at Increased Risk of Transition. *Nat. Rev. Rheumatol.* 15, 153–166. doi:10.1038/s41584-019-0175-0

Schwartz, D. M., Bonelli, M., Gadina, M., and O'Shea, J. J. (2016). Type I/II Cytokines, JAKs, and New Strategies for Treating Autoimmune Diseases. *Nat. Rev. Rheumatol.* 12, 25–36. doi:10.1038/nrrheum.2015.167

Scrivo, R., Giardino, A. M., Salvarani, C., Foti, R., Afeltra, A., Viapiana, O., et al. (2020). An Observational Prospective Study on Predictors of Clinical Response at Six Months in Patients with Active Psoriatic Arthritis Treated with Golimumab. *Clin. Exp. Rheumatol.* 38, 107–114.

Sedger, L. M., and McDermott, M. F. (2014). TNF and TNF-Receptors: From Mediators of Cell Death and Inflammation to Therapeutic Giants - Past, Present and Future. *Cytokine Growth Factor. Rev.* 25, 453–472. doi:10.1016/j.cytogfr.2014.07.016

Silvagni, E., Bortoluzzi, A., Ciancio, G., and Govoni, M. (2019). Biological and Synthetic Target DMARDs in Psoriatic Arthritis. *Pharmacol. Res.* 149, 104473. doi:10.1016/j.phrs.2019.104473

Singh, J. A., Guyatt, G., Ogdie, A., Gladman, D. D., Deal, C., Deodhar, A., et al. (2018). 2018 American College of Rheumatology/National Psoriasis Foundation Guideline for the Treatment of Psoriatic Arthritis. *Arthritis Care Res.* 71, 2–29. doi:10.1002/acr.23789

Singh, J. A., and Strand, V. (2009). Health Care Utilization in Patients with Spondyloarthropathies. *Rheumatology* 48, 272–276. doi:10.1093/rheumatology/ken472

Smeets, T. J. M., Kraan, M. C., van Loon, M. E., and Tak, P.-P. (2003). Tumor Necrosis Factor ? Blockade Reduces the Synovial Cell Infiltrate Early after

Initiation of Treatment, but Apparently Not by Induction of Apoptosis in Synovial Tissue. *Arthritis Rheum.* 48, 2155–2162. doi:10.1002/art.11098

Smolen, J. S., Mease, P., Tahir, H., Schulze-Koops, H., de la Torre, I., Li, L., et al. (2020). Multicentre, Randomised, Open-Label, Parallel-Group Study Evaluating the Efficacy and Safety of Ixekizumab versus Adalimumab in Patients with Psoriatic Arthritis Naïve to Biological Disease-Modifying Antirheumatic Drug: Final Results by Week 52. *Ann. Rheum. Dis.* 79, 1310–1319. doi:10.1136/annrheumdis-2020-217372

Steel, K. J. A., Srenathan, U., Ridley, M., Durham, L. E., Wu, S. Y., Ryan, S. E., et al. (2020). Polyfunctional, Proinflammatory, Tissue-Resident Memory Phenotype and Function of Synovial Interleukin-17A + CD 8 + T Cells in Psoriatic Arthritis. *Arthritis Rheumatol.* 72, 435–447. doi:10.1002/art.41156

Szentpetery, A., Heffernan, E., Gogarty, M., Mellerick, L., McCormack, J., Haroon, M., et al. (2017). Abatacept Reduces Synovial Regulatory T-Cell Expression in Patients with Psoriatic Arthritis. *Arthritis Res. Ther.* 19. doi:10.1186/s13075-017-1364-3

Tok, M. N., Duivenvoorde, L. M., Kramer, I., Ingold, P., Pfister, S., Roth, L., et al. (2019). Interleukin-17A Inhibition Diminishes Inflammation and New Bone Formation in Experimental Spondyloarthritis. *Arthritis Rheumatol.* 71, 612–625. doi:10.1002/art.40770

Tracey, D., Klareskog, L., Sasso, E. H., Salfeld, J. G., and Tak, P. P. (2008). Tumor Necrosis Factor Antagonist Mechanisms of Action: A Comprehensive Review. *Pharmacol. Ther.* 117, 244–279. doi:10.1016/j.pharmthera.2007.10.001

van Kuijk, A. W. R., Gerlag, D. M., Vos, K., Wolbink, G., de Groot, M., de Rie, M. A., et al. (2008). A Prospective, Randomised, Placebo-Controlled Study to Identify Biomarkers Associated with Active Treatment in Psoriatic Arthritis: Effects of Adalimumab Treatment on Synovial Tissue. *Ann. Rheum. Dis.* 68, 1303–1309. doi:10.1136/ard.2008.091389

van Kuijk, A. W. R., and Tak, P. P. (2011). Synovitis in Psoriatic Arthritis: Immunohistochemistry, Comparisons with Rheumatoid Arthritis, and Effects of Therapy. *Curr. Rheumatol. Rep.* 13, 353–359. doi:10.1007/s11926-011-0181-y

Van Mens, L. J. J., Sande, M. G. H., Menegatti, S., Chen, S., Blijdorp, I. C. J., Jong, H. M., et al. (2018). Brief Report: Interleukin-17 Blockade with Secukinumab in Peripheral Spondyloarthritis Impacts Synovial Immunopathology without Compromising Systemic Immune Responses. *Arthritis Rheumatol.* 70, 1994–2002. doi:10.1002/art.40581

Veale, D. J., and Fearon, U. (2018). The Pathogenesis of Psoriatic Arthritis. *The Lancet* 391, 2273–2284. doi:10.1016/S0140-6736(18)30830-4

Wade, S. M., Canavan, M., McGarry, T., Low, C., Wade, S. C., Mullan, R. H., et al. (2019). Association of Synovial Tissue Polyfunctional T-Cells with DAPSA in Psoriatic Arthritis. *Ann. Rheum. Dis.* 78, 350–354. doi:10.1136/annrheumdis-2018-214138

Wagner, C. L., Visvanathan, S., Elashoff, M., McInnes, I. B., Mease, P. J., Krueger, G. G., et al. (2013). Markers of Inflammation and Bone Remodelling Associated with Improvement in Clinical Response Measures in Psoriatic Arthritis Patients Treated with Golimumab. *Ann. Rheum. Dis.* 72, 83–88. doi:10.1136/annrheumdis-2012-201697

Xu, X., Davelaar, N., Mus, A. M., Asmawidjaja, P. S., Hazes, J. M. W., Baeten, D. L. P., et al. (2020). Interleukin-17A Is Produced by CD4 + but Not CD8 + T Cells in Synovial Fluid Following T Cell Receptor Activation and Regulates Different Inflammatory Mediators Compared to Tumor Necrosis Factor in a Model of Psoriatic Arthritis Synovitis. *Arthritis Rheumatol.* 72, 1303–1313. doi:10.1002/art.41271

Zaba, L. C., Cardinale, I., Gilleaudeau, P., Sullivan-Whalen, M., Suárez-Fariñas, M., Fuentes-Duculan, J., et al. (2007). Amelioration of Epidermal Hyperplasia by TNF Inhibition Is Associated with Reduced Th17 Responses. *J. Exp. Med.* 204, 3183–3194. doi:10.1084/jem.20071094

Zaba, L. C., Suárez-Fariñas, M., Fuentes-Duculan, J., Nograles, K. E., Guttman-Yassky, E., Cardinale, I., et al. (2009). Effective Treatment of Psoriasis with Etanercept Is Linked to Suppression of IL-17 Signaling, Not Immediate Response TNF Genes. *J. Allergy Clin. Immunol.* 124, 1022–1030. doi:10.1016/j.jaci.2009.08.046

Zabotti, A., Tinazzi, I., Aydin, S. Z., and McGonagle, D. (2020). From Psoriasis to Psoriatic Arthritis: Insights from Imaging on the Transition to Psoriatic Arthritis and Implications for Arthritis Prevention. *Curr. Rheumatol. Rep.* 22. doi:10.1007/s11926-020-00891-x

**Conflict of Interest:** ES and CS have received research support from Abbvie. SA has received research grants from Pfizer and Abbvie. CS has received consultancy fees from Abbvie and BMS.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Pharmacology

ORIGINAL RESEARCH
published: 17 September 2021
doi: 10.3389/fphar.2021.727901

# The Functional Roles of RNAs Cargoes Released by Neutrophil-Derived Exosomes in Dermatomyositis

*Liya Li*[1,2], *Xiaoxia Zuo*[1,3,4], *Di Liu*[1], *Hui Luo*[1,3,4] *and Honglin Zhu*[1,3,4]*

[1]The Department of Rheumatology and Immunology, Xiangya Hospital of Central South University, Changsha, China, [2]The Department of Rheumatology and Immunology, The Third Xiangya Hospital, Central South University, Changsha, China, [3]Provincial Clinical Research Center for Rheumatic and Immunologic Diseases, Xiangya Hospital, Changsha, China, [4]National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Changsha, China

Dermatomyositis (DM) is an idiopathic inflammatory myopathy characterized by cutaneous manifestations. We first identified the profiles of noncoding RNAs (lncRNAs and miRNAs) in peripheral neutrophil exosomes (EXOs) of DM patients and explored their potential functional roles. Bioinformatics analyses were performed with R packages. Real-time quantitative PCR was used to validate the altered RNAs in DM neutrophil EXO-stimulated human dermal microvascular endothelial cells (HDMECs) and human skeletal muscle myoblasts (HSkMCs). In DM neutrophil EXOs, 124 upregulated lncRNAs (with 1,392 target genes), 255 downregulated lncRNAs (with 1867 target genes), 17 upregulated miRNAs (with 2,908 target genes), and 15 downregulated miRNAs (with 2,176 target genes) were identified. GO analysis showed that the differentially expressed (DE) lncRNAs and DE miRNAs participated in interleukin-6 and interferon-beta production, skeletal muscle cell proliferation and development, and endothelial cell development and differentiation. KEGG analysis suggested that DE lncRNAs and DE miRNAs were enriched in the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways. Many novel and valuable DE lncRNAs and DE miRNAs interacted and cotargeted in the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways. Our study suggests that neutrophil EXOs participate in DM pathogenesis through lncRNAs and miRNAs in the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways.

**Keywords: dermatomyositis, neutrophil-derived exosome, lncRNA, miRNA, PI3K-Akt, MAPK, AMPK, FoxO**

OPEN ACCESS

***Edited by:***
*Maria Efthymiou,*
*University College London,*
*United Kingdom*

***Reviewed by:***
*Juan Du,*
*Capital Medical University, China*
*Hui Li,*
*Guangxi University, China*

***Correspondence:**
*Honglin Zhu*
*honglinzhu@csu.edu.cn*

***Specialty section:***
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

***Received:*** *20 June 2021*
***Accepted:*** *20 August 2021*
***Published:*** *17 September 2021*

***Citation:***
*Li L, Zuo X, Liu D, Luo H and Zhu H (2021) The Functional Roles of RNAs Cargoes Released by Neutrophil-Derived Exosomes in Dermatomyositis. Front. Pharmacol. 12:727901. doi: 10.3389/fphar.2021.727901*

**Abbreviations:** DM, dermatomyositis; IFN, interferon; PI3K, phosphoinositol-3-kinase; Akt, v-akt murine thymoma viral oncogene homologue; MAPK, mitogen-activated protein kinase; AMPK, adenosine monophosphate-activated protein kinase; FoxO, Forkhead box O; EXOs, exosomes; EVs, extracellular vesicles; HDMECs, human dermal microvascular endothelial cells; miRNAs, microRNAs; lncRNAs, long noncoding RNAs; JDM, juvenile DM; NCs, normal controls; DE, differentially expressed; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; HSkMCs, human skeletal muscle myoblasts; IGF, insulin-like growth factor.

## INTRODUCTION

Dermatomyositis (DM) is an idiopathic inflammatory myopathy with characteristic cutaneous manifestations and symmetrical progressive proximal weakness (Bohan and Peter, 1975a; Bohan and Peter, 1975b; Callen, 2000). The hallmark histopathological features of DM include perifascicular atrophy and inflammatory infiltration (by macrophages/neutrophils, $CD20^+$ B cells, $CD4^+$ T cells and plasmacytoid dendritic cells) in muscle (Dalakas, 2015; DeWane et al., 2019). Although the mechanism of DM is largely unknown, some factors are thought to contribute to DM pathogenesis, such as MHC polymorphisms, epigenetic modifications, type I interferon (IFN) signalling, myositis-specific antibodies and many other pathways, such as the phosphoinositol-3-kinase (PI3K)–v-akt murine thymoma viral oncogene homologue (Akt), mitogen-activated protein kinase (MAPK), adenosine monophosphate-activated protein kinase (AMPK) and Forkhead box O (FoxO) signalling pathways (Lahoria et al., 2016; Gao et al., 2017; Tartar et al., 2018; Gao et al., 2019).

In recent years, neutrophils have been recognized as participants in imbalances among autoimmune responses directed at tissue-specific antigens with the ability to inhibit and control these responses (Wahren-Herlenius and Dörner, 2013) and as intermediates between effectors and regulatory mechanisms (Navegantes et al., 2017). Neutrophils play pathogenic roles by releasing various molecules, such as proteases, cytokines, reactive oxygen species and exosomes (EXOs), extracellularly after triggering the immune complex (Glennon-Alty et al., 2018). Neutrophils also interact with macrophages, dendritic cells, B and T cells, and natural killer cells that produce IFN-$\gamma$, regulating the immune response (Jaillon et al., 2013). In DM patients, an increased baseline peripheral blood neutrophil-to-lymphocyte ratio is associated with pulmonary involvement, disease activity and worse overall survival (Yang et al., 2017; Gao et al., 2018a; Ha et al., 2018). In our previous study, proteinases and proteins in the neutrophil cytoplasm were found to play important roles in muscle inflammatory cell infiltration and vascular damage in DM patients (Gao et al., 2018b; Xiao et al., 2019). All of the above results suggest that neutrophils play important roles in the pathogenesis of DM; however, the detailed mechanisms need further research.

EXOs are extracellular vesicles (EVs) that are 30–150 nm in diameter and are secreted by most cells. EXOs express specific surface markers, including tumour susceptibility gene 101, integrins and tetraspanins (CD63, CD9, and CD81/82) (Yáñez-Mó et al., 2015). EXOs contain selective cargoes of RNAs, DNAs and proteins, which are valuable sources of intercellular signalling molecules, biomarkers and treatment targets (Li et al., 2018; Li et al., 2019). For example, serum EXOs can restore cellular function *in vitro* and can be employed for the treatment and noninvasive diagnosis of dysferlinopathy (Dong et al., 2018). In addition, EXOs released from inflamed myotubes induce myoblast inflammation and inhibit myogenic mechanisms while stimulating atrophic signals (Kim et al., 2018). Neutrophil-derived EXOs (neutrophil EXOs) also play important roles in immune responses (Rossaint et al., 2016). In generalized pustular psoriasis, proteomic analysis of neutrophil EXO contents has identified olfactomedin 4 as the critical differentially expressed (DE) protein that mediates autoimmune inflammatory responses (Shao et al., 2019). In the progression of asthma, neutrophil EXOs can modulate immune responses, enhance the proliferation of airway smooth muscle cells, and promote airway remodelling (Vargas et al., 2016). In chronic obstructive pulmonary disease (COPD), activated neutrophil EXOs predominate in lung fluids. These EXOs can degrade extracellular matrix by protecting surface neutrophil elastase from alpha-1 antitrypsin inhibition. Transferring activated neutrophil EXOs causes a COPD-like phenotype in murine lungs in which alveoli are destroyed via neutrophil elastase (Genschmer et al., 2019). In our previous study, neutrophil EXOs from systemic sclerosis patients were found to inhibit the proliferation and migration of human dermal microvascular endothelial cells (HDMECs) (Li et al., 2020a; Li et al., 2020b). Therefore, we hypothesized that neutrophil EXOs might lead to muscle damage in DM patients.

Recently, noncoding RNA regulatory mechanisms involving microRNAs (miRNAs, <50 nucleotides) and long noncoding RNAs (lncRNAs, >200 nucleotides) have been widely studied in the context of DM (Peng et al., 2016; Gao et al., 2019; Mazzone et al., 2019). Many miRNAs and lncRNAs are associated with disease activity and participate in the pathogenesis of DM (Satoh et al., 2005; Misunova et al., 2016; Peng et al., 2016). In juvenile DM (JDM) patients, the miRNAs of plasma EXOs are capable of altering transcriptional programmes within endothelial cells (Jiang et al., 2019). Here, we analysed the noncoding RNAs (lncRNAs and miRNAs) profiles of neutrophil EXOs from DM patients and sought insights into their predicted functional roles, such as their roles in pathogenetic mechanisms, and their potential utility as new biomarkers and therapeutic targets.

## MATERIALS AND METHODS

### Patients and Controls

We collected blood from 20 DM patients and 22 normal controls (NCs) who were all Han Chinese. Among these participants, 5 patients with DM as primary diagnosis and 5 age- and sex-matched NCs were selected for lncRNA and miRNA sequencing analyses. The clinical characteristics of 5 DM patients for neutrophil EXOs RNAseq are shown in **Supplementary Table S1**. All patients fulfilled the 1975 Bohan and Peter diagnostic criteria for DM and were enrolled at the Department of Rheumatology and Immunology at the Xiangya Hospital of Central South University (Bohan and Peter, 1975a; Bohan and Peter, 1975b). The clinical characteristics of the participants, including their demographic characteristics, serological characteristics, organ involvement and medications, are shown in **Supplementary Table S2**.

### Isolation and Culture of Neutrophils

Peripheral whole blood samples (10 ml) were collected in EDTA anticoagulant-coated tubes and processed within 2 h. Neutrophils were isolated by density gradient equilibrium centrifugation using Histopaque-1077 and Histopaque-1119 (Sigma-Aldrich,

St. Louis, United States) according to the manufacturer's instructions. The neutrophil pellets were used for culture or stored at −80°C until analysis. For neutrophil culture, the cell pellets (cell viability>90%) were resuspended in the appropriate volume ($2–5\times10^6$ cell/ml) of 1,640 medium (Gibco, Carlsbad, United States) supplemented with 10% EXO-free foetal bovine serum (FBS, Gibco), which was prepared by collecting the supernatant after ultracentrifugation of FBS at 150,000 × *g* for 3 h at 4°C (the same procedure was used for the EXO-free FBS described below). The neutrophils were incubated for 2 h at 37°C in a humidified atmosphere containing 5% $CO_2$. The cell pellets and supernatants were collected separately for further analysis.

## Isolation and Identification of Exosomes and EXO RNAs

We isolated EXOs from cultured neutrophil supernatants using an exoEasy Maxi Kit (Qiagen, Frederick, United States) according to the manufacturer's instructions. The neutrophil EXOs were dissolved in 600 μl of eluting buffer and stored at −80°C until analysis. Neutrophil EXOs were identified as cup-shaped double-membrane structures by transmission electron microscopy (FEI Tecnai G2 Spirit Twin, Hillsboro, United States) via negative staining (**Supplementary Figure S1A**), were confirmed to express CD63 with a FACSCalibur flow cytometer (BD Biosciences, San Jose, United States) using EXOome-Human CD63 Isolation/Detection Dynabeads (Invitrogen, Thermo Fisher Scientific, Lithuania) and anti-human CD63-PE antibodies (12–0,639, eBioscience, United States) according to the manufacturer's protocol (**Supplementary Figure S1B**) and were verified to meet the expected EXO size by dynamic light scattering with a Zetasizer Nano ZS instrument (Malvern Instrument, Marvin, United Kingdom) (**Supplementary Figure S1C**).

Total RNA was extracted from the neutrophil EXOs with TRIzol (Invitrogen Life Technologies, California, United States) according to the manufacturer's protocol. An Agilent 2,200 TapeStation (Agilent Technologies, California, United States) and a Qubit® 2.0 Fluorometer (Life Technologies) were used to assess the quantity and integrity of the total RNA and the purified library products described below.

## RNA Library Construction, Sequencing and Data Analysis

RNAs libraries were created with an NEBNext® Multiplex Small RNA Library Prep Set for Illumina (NEB, Ipswich, United States) for small RNA and an NEBNext® Ultra™ RNA Library Prep Kit for Illumina for lncRNA, according to the manufacturer's instructions. Paired-end sequencing (PE150) with the Illumina 3,000 platform was performed (RiboBio Co.,Guangzhou, China). The number of lncRNAs and miRNAs detected in neutrophil EXOs are shown in **Supplementary Table S3**. The sequencing reads were preprocessed with fastQC software and Trimmomatic tools (v 0.36) to assess the read quality and to filter out poor-quantity reads, HISAT2 was used to map the reads to the human reference genome hg19; uniquely mapped reads were assigned to annotated genes with HTSeq (for lncRNAs) and the Burrows-Wheeler Aligner (for miRNAs). The miRDeep2 database was used to identify known mature miRNAs based on miRBase (v21) (www.miRBase.org) and to predict novel miRNAs. DESeq2 was used to identify the significantly DE lncRNAs (adjusted $p < 0.05$ and $|\log2(\text{fold change})| > 1.5$), while the R package edgeR was used to identify the significantly DE miRNAs (adjusted $p < 0.05$ and $|\log2(\text{fold change})| > 1$).

## Bioinformatics Analysis for Differentially Expressed lncRNAs and Differentially Expressed miRNAs

LncRNAs regulate potential target genes through two major mechanisms: cis regulation and trans regulation. Target genes in cis were obtained by integrating the DE lncRNAs and their adjacent (within 10 kb) DE mRNAs. After extracting the sequences of the DE lncRNAs and DE mRNAs, both BLAST software (for initial screening) and RNAplex software (for re-screening) were used to identify potential target genes of the lncRNAs in trans. The target genes for selected miRNAs were predicted with the targetScan, miRDB, miRTarBase and miRWalk software programs. Moreover, the miRanda, PITA and RNAhybrid software programs were used to identify common elements between the lncRNAs and miRNAs and to predict the miRNAs associated with lncRNAs of interest.

The target genes of the DE lncRNAs and DE miRNAs were subjected to pathway analysis with the Gene Ontology (GO) (http://geneontology.org/) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg) databases. GO and KEGG enrichment analyses were performed with the R package clusterProfiler (Yu et al., 2012). Interaction and coexpression networks for lncRNAs, miRNAs and mRNAs were constructed with Cytoscape software (http://apps.cytoscape.org/).

## Cell Culture

HDMECs were obtained from Cell Biolabs (# CBR130858, San Diego, United States) and cultured in DMEM (Gibco) with 10% EXO-free FBS. Human skeletal muscle myoblasts (HSkMCs) were purchased from ScienCell and cultured in Skeletal Muscle Cell Medium (ScienCell, San Diego, United States) with 5% EXO-free FBS. All cells were cultured at 37°C in a 5% $CO_2$ humidified incubator. Both HDMECs and HSkMCs were used when they reached 60–70% confluence and were then stimulated with 20% neutrophil EXOs (volume concentration, 160 μl of neutrophil EXO eluting buffer and 640 μl of medium) per well for 48 h in 24-well plates. Each experiment was repeated three times with three samples per group.

## Validation Using Real-Time Quantitative PCR

Total RNA from neutrophils, HDMECs and HSkMCs was extracted using TRIzol according to the manufacturer's instructions. For lncRNAs, cDNA was prepared using a PrimeScript™ RT Reagent Kit (Perfect Real Time, Takara, Japan). Real-time quantitative PCR was performed using TB Green PCR Master Mix (Takara, Japan) on a 7,500 Real-Time PCR System (Applied Biosystems, California, United States).

**TABLE 1 |** DE lncRNAs in the neutrophil exosomes of DM patients (Top 30).

| lncRNAs | $log_2$(FC) | *p* value | lncRNAs | $log_2$(FC) | *p* value |
|---|---|---|---|---|---|
| ENST00000600489.1 | 5.432 | 0.000 | NR_123718.1 | −2.136 | 0.000 |
| NR_136569.1 | 5.423 | 0.000 | NR_135530.1 | −2.337 | 0.000 |
| ENST00000594492.1 | 3.713 | 0.000 | NR_040001.2 | −2.368 | 0.000 |
| NR_003013.1 | 3.216 | 0.000 | ENST00000444796.1 | −2.434 | 0.001 |
| ENST00000592523.1 | 3.207 | 0.000 | ENST00000453561.2 | −2.609 | 0.000 |
| ENST00000607284.1 | 2.839 | 0.000 | ENST00000593824.1 | −3.242 | 0.000 |
| NR_024393.1 | 2.633 | 0.001 | ENST00000503403.1 | −3.375 | 0.000 |
| ENST00000526936.1 | 2.163 | 0.001 | ENST00000572850.1 | −3.755 | 0.000 |
| ENST00000428280.1 | 1.970 | 0.001 | NR_110815.1 | −4.137 | 0.001 |
| ENST00000419196.1 | 1.737 | 0.001 | ENST00000519840.1 | −4.254 | 0.000 |
| ENST00000503469.2 | 1.577 | 0.001 | ENST00000587907.1 | −4.275 | 0.000 |
| NR_135626.1 | −1.764 | 0.001 | NR_104232.1 | −4.649 | 0.000 |
| ENST00000438753.1 | −1.899 | 0.000 | ENST00000581910.1 | −4.667 | 0.001 |
| ENST00000450365.1 | −1.929 | 0.001 | ENST00000592296.1 | −5.274 | 0.000 |
| NR_038194.1 | −2.013 | 0.001 | ENST00000577199.1 | −5.982 | 0.000 |

*DE: differentially expressed; DM: dermatomyositis; FC: fold change.*

*GAPDH* expression was used as the endogenous control. The gene-specific primers are shown in **Supplementary Table S4**. The relative expression of miRNAs was measured with a miDETECT A Track™ miRNA qRT-PCR Starter Kit (RiboBio.Co., Guangzhou, China) on the 7,500 Real-Time PCR System. The miRNA-specific primers and the Uni-Reverse Primer were purchased from RiboBio Co. (Guangzhou, China). The miRNA primers product information is shown in **Supplementary Table S5**. U6B small nuclear RNA was used as the endogenous control to normalize the sample data. Each group had more than six samples.

## Statistical Analysis

All data except for the RNA sequencing data were analysed with GraphPad Prism 5 software, and statistical significance was set as a two-sided $p < 0.05$. Numerical variables with a normal distribution are presented as the mean ± SEM and were analysed by unpaired t-test. Data with a nonnormal distribution are shown as the median and were assessed with the Wilcoxon rank sum test.

# RESULTS

## Differentially Expressed lncRNAs in Dermatomyositis Neutrophil Exosomes

We identified 379 DE lncRNAs in neutrophil EXOs in DM patients compared with NCs, including 124 upregulated and 255 downregulated lncRNAs [**Supplementary Data S1**]. The top 30 DE lncRNAs are shown in **Table 1** and **Supplementary Table S6**. Bioinformatics analysis identified 1,392 target genes for the upregulated lncRNAs and 1867 target genes for the downregulated lncRNAs [**Supplementary Data S2**]. GO analysis of these target genes revealed that the regulation of interleukin-6 production, IFN-β production and skeletal muscle cell proliferation terms were enriched; KEGG analysis indicated that the FoxO signalling pathway, endocytosis and JAK-STAT signalling pathway were involved (**Table 3** and **Supplementary Tables S7, S8**).

## Differentially Expressed miRNAs in Dermatomyositis Neutrophil Exosomes

We identified a total of 32 DE miRNAs between DM patients and NCs from the miRNA profiles of neutrophil EXOs, including 17 upregulated and 15 downregulated miRNAs (**Table 2** and **Supplementary Table S9**). Bioinformatics analysis predicted 2,908 target genes for the upregulated miRNAs and 2,176 target genes for the downregulated miRNAs [**Supplementary Data S3**]. GO enrichment analysis suggested that both of these miRNA target genes were involved in actin filament organization, muscle tissue development, endothelial cell development and differentiation and activation of MAPK activity; KEGG analysis indicated that the PI3K–Akt, MAPK, AMPK, and FoxO signalling pathways, among others, were enriched (**Table 3** and **Supplementary Tables S10, S11**).

## Interaction Relationships of Differentially Expressed lncRNAs and Differentially Expressed miRNAs in the Phosphoinositol-3-Kinase–Akt, Mitogen-Activated Protein Kinase, Adenosine Monophosphate-Activated Protein Kinase and FoxO Signalling Pathways

LncRNAs can regulate miRNAs in some cellular processes. Here, we analysed the relationships between the DE lncRNAs and DE miRNAs in DM neutrophil EXOs and found that 91 lncRNAs (58 downregulated and 33 upregulated lncRNAs) and 23 miRNAs (11 downregulated and 12 upregulated miRNAs) might interact with each other [**Supplementary Figure S2**]. From the GO and KEGG analyses above, we found that the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways were enriched for the predicted target genes of both DE lncRNAs and DE miRNAs. Thus, we analysed the lncRNAs, miRNAs and target genes involved in these four pathways. We found

**TABLE 2 |** DE miRNAs in the neutrophil exosomes of DM patients ($n$ = 32).

| miRNAs | $log_2$(FC) | $p$ value | miRNAs | $log_2$(FC) | $p$ value |
|---|---|---|---|---|---|
| hsa-miR-3614-5p | 4.262 | 0.000 | hsa-miR-1273h-3p | 3.138 | 0.049 |
| hsa-miR-1180-3p | 4.250 | 0.007 | hsa-miR-4792 | −2.993 | 0.002 |
| hsa-miR-451a | 1.705 | 0.014 | hsa-miR-1323 | −2.938 | 0.005 |
| hsa-miR-23a-5p | 2.905 | 0.014 | hsa-miR-516a-5p | −3.424 | 0.005 |
| hsa-miR-183-5p | 1.296 | 0.015 | hsa-miR-512-3p | −3.163 | 0.008 |
| hsa-miR-486-3p | 2.303 | 0.016 | hsa-miR-372-3p | −12.485 | 0.010 |
| hsa-miR-223-5p | 1.286 | 0.020 | hsa-miR-4488 | −3.933 | 0.022 |
| hsa-miR-1268a | 12.006 | 0.022 | hsa-let-7f-1-3p | −12.101 | 0.027 |
| hsa-miR-424-3p | 1.291 | 0.030 | hsa-miR-520a-3p | −2.520 | 0.030 |
| hsa-miR-16-2-3p | 1.031 | 0.031 | hsa-miR-424-5p | −1.967 | 0.033 |
| hsa-miR-363-3p | 1.394 | 0.033 | hsa-miR-542-3p | −2.116 | 0.039 |
| hsa-miR-122-5p | 1.251 | 0.036 | hsa-miR-4433b-5p | −12.189 | 0.039 |
| hsa-miR-1278 | 11.949 | 0.039 | hsa-miR-1307-5p | −3.503 | 0.043 |
| hsa-miR-548ad-5p | 3.300 | 0.044 | hsa-miR-195-5p | −1.511 | 0.046 |
| hsa-miR-548ae-5p | 3.300 | 0.044 | hsa-miR-27b-3p | −1.632 | 0.047 |
| hsa-miR-182-5p | 1.099 | 0.045 | hsa-miR-518e-3p | −3.719 | 0.047 |

*DE: differentially expressed; DM: dermatomyositis; FC: fold change.*

**TABLE 3 |** GO and KEGG enrichment analysis for target genes of DE lncRNAs and DE miRNAs in DM neutrophil exosomes.

| **GO enrichment for target genes of DE lncRNAs** | **$p$ value (up/down[a])** |
|---|---|
| regulation of interleukin-6 production | 0.013/0.015 |
| regulation of interferon-beta production | 0.011/0.002 |
| regulation of skeletal muscle satellite cell proliferation | 0.045/0.019 |
| **KEGG enrichment for target genes of DE lncRNAs** | **$p$ value (up/down[a])** |
| FoxO signaling pathways | 0.037/0.247 |
| Endocytosis | 0.049/0.214 |
| JAK-STAT signaling pathway | 0.153/0.013 |
| **GO enrichment for target genes of DE miRNAs** | **$p$ value (up/down[a])** |
| actin filament organization | 0.000/0.001 |
| muscle tissue development | 0.000/0.000 |
| endothelial cell development and differentiation | 0.000/0.000 |
| activation of MAPK activity | 0.000/0.001 |
| **KEGG enrichment for target genes of DE miRNAs** | **$p$ value (up/down[a])** |
| PI3K-Akt signaling pathways | 0.000/0.000 |
| MAPK signaling pathways | 0.000/0.000 |
| AMPK signaling pathways | 0.000/0.000 |
| FoxO signaling pathways | 0.000/0.000 |
| Endocytosis | 0.002/0.017 |
| Regulation of actin cytoskeleton | 0.001/0.000 |
| Wnt signaling pathway | 0.002/0.002 |
| mTOR signaling pathway | 0.009/0.002 |
| TNF signaling pathway | 0.012/0.001 |
| Th17 cell differentiation | 0.013/0.009 |
| VEGF signaling pathway | 0.048/0.032 |

[a]*The* p *value of predicted target genes for upregulated/downregulated lncRNAs/miRNAs.*
*GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.*
*DE: differentially expressed; DM: dermatomyositis.*

that 43 predicted target genes were able to be regulated by both lncRNAs and miRNAs. Finally, we obtained an interaction network that included 50 lncRNAs, 18 miRNAs and their 43 cotarget mRNAs (**Table 4**). Among the relationships, 3 of the lncRNA-miRNA pairs (in which the miRNAs were regulated by lncRNAs) were NR_046101.1–hsa-miR-3614-5p, ENST00000444868.2–hsa-miR-195-5p and ENST00000433036–hsa-miR-512-3p.

## Analysis of Differentially Expressed lncRNAs and Differentially Expressed miRNAs in the Phosphoinositol-3-Kinase–Akt, Mitogen-Activated Protein Kinase, Adenosine Monophosphate-Activated Protein Kinase and FoxO Signalling Pathways in Neutrophils and Neutrophil EXO–Stimulated Human Dermal Microvascular Endothelial Cells and Human Skeletal Muscle Myoblasts

Skeletal muscle cells and endothelial cells are target cells in the pathogenesis of DM (Yáñez-Mó et al., 2015; Gao et al., 2018b; Gao et al., 2019). To further analyze the functions of neutrophil EXOs, we validated the expression of DE lncRNAs and DE miRNAs in neutrophils and in neutrophil EXO-stimulated HDMECs and HSkMCs. As shown in **Figure 1** and **Supplementary Figure S3**, 10 DE lncRNAs (6 upregulated and 4 downregulated lncRNAs) and 10 DE miRNAs (5 upregulated and 5 downregulated miRNAs) involved in the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways were chosen for validation analysis.

Nine of the 10 DE lncRNAs (ENST00000510274.1, ENST00000609726.1, NR_110761.1, NR_125423.1, ENST00000592296.1, ENST00000584643.1, ENST00000428280.1, ENST00000591854.1 and NR_039978.1) were significantly DE in HDMECs-stimulated by neutrophil EXOs, ENST00000433036.1 was not. Eight of the lncRNAs were significantly DE in HSkMCs-stimulated by neutrophil EXOs, and five of them were DE in neutrophils. The results for most of them were consistent with the results of the lncRNA profile analysis (**Figure 1A**).

**TABLE 4 |** Co-target genes for DE lncRNAs and DE miRNAs in the PI3K-Akt, MAPK, AMPK, FoxO signaling pathways.

| Co-target genes (*n* = 43) | DE lncRNAs (*n* = 50) | DE miRNAs (*n* = 18) |
|---|---|---|
| *ANGPT2* | ENST00000581633.1, NR_125914.1, NR_125915.1 | hsa-miR-542-3p |
| *ATG12* | ENST00000584643.1 | hsa-miR-183-5p |
| *BCL2* | ENST00000588307.1 | hsa-miR-486-3p, hsa-miR-182-5p |
| *CACNG3* | ENST00000567624.1 | hsa-miR-486-3p |
| *CACNG8* | **NR_046101.1**[a], ENST00000565495.1, ENST00000539313.1, ENST00000600508.1, ENST00000584643.1 | **hsa-miR-3614-5p**[a] |
| *CPT1A* | ENST00000582536.1, NR_038219.1, ENST00000591918.2, ENST00000504658.1, ENST00000592296.1, ENST00000508878.1, ENST00000526936.1, ENST00000511517.1 | hsa-miR-512-3p, hsa-miR-372-3p, hsa-miR-520a-3p |
| *CREB1* | ENST00000448256.1, ENST00000609726.1 | hsa-let-7f-1-3p, hsa-miR-512-3p, hsa-miR-3614-5p, hsa-miR-182-5p, hsa-miR-122-5p, hsa-miR-27b-3p |
| *CREB3L1* | ENST00000527239.1 | hsa-miR-182-5p |
| *CRK* | NR_104232.1, NR_039978.1, ENST00000519451.1, ENST00000609726.1, ENST00000544086.1, ENST00000539313.1 | hsa-miR-372-3p, hsa-miR-195-5p, hsa-miR-424-5p |
| *EEF2K* | ENST00000510274.1, ENST00000504658.1, ENST00000428280.1, ENST00000584643.1, ENST00000591918.2 | hsa-miR-1323 |
| *ELK4* | ENST00000582269.1 | hsa-miR-3614-5p, hsa-miR-520a-3p, hsa-miR-195-5p, hsa-miR-424-5p, hsa-miR-372-3p |
| *ERBB3* | NR_125423.1, ENST00000581633.1 | hsa-miR-512-3p |
| *FGF5* | ENST00000582269.1 | hsa-miR-542-3p |
| *FGFR1* | NR_125423.1, ENST00000544086.1 | hsa-miR-424-5p, hsa-miR-486-3p |
| *FGFR2* | ENST00000444868.2, ENST00000585761.1 | hsa-miR-1323 |
| *FLT1* | ENST00000609726.1, ENST00000539313.1, ENST00000581633.1 | hsa-miR-372-3p |
| *FOXO3* | ENST00000444868.2 | hsa-miR-182-5p |
| *GHR* | ENST00000606096.1 | hsa-miR-195-5p |
| *GRB2* | NR_134920.1, ENST00000585921.1, ENST00000609726.1 | hsa-miR-1323 |
| *IL1R1* | ENST00000544086.1 | hsa-miR-1323 |
| *IL2RA* | NR_038219.1 | hsa-miR-122-5p |
| *IL6R* | ENST00000592296.1, ENST00000584643.1 | hsa-miR-3614-5p, hsa-miR-451a |
| *IRAK4* | NR_039978.1, NR_125915.1, NR_125914.1 | hsa-miR-372-3p, hsa-miR-520a-3p, hsa-miR-512-3p |
| *ITGA2* | ENST00000565495.1 | hsa-miR-424-5p, hsa-miR-372-3p, hsa-miR-195-5p |
| *ITGB8* | NR_110119.1, ENST00000584643.1 | hsa-miR-183-5p, hsa-miR-182-5p, hsa-miR-372-3p |
| *MAP3K13* | NR_110761.1, ENST00000399866.3, ENST00000584643.1, ENST00000436249.3 | hsa-miR-183-5p, hsa-miR-542-3p |
| *MDM2* | ENST00000366360.2, ENST00000533008.1 | hsa-miR-542-3p, hsa-miR-3614-5p, hsa-miR-1278 |
| *MEF2C* | NR_109940.1, ENST00000510274.1 | hsa-miR-182-5p, hsa-miR-183-5p |
| *MRAS* | NR_023922.2 | hsa-miR-424-5p |
| *NFATC3* | ENST00000569088.1 | hsa-miR-512-3p |
| *NR4A1* | NR_037177.1, ENST00000462717.1, ENST00000568362.1, ENST00000433036.1 | hsa-miR-542-3p |
| *OSMR* | NR_110,761.1, ENST00000425192.1, ENST00000428280.1, ENST00000558478.1, ENST00000584643.1 | hsa-miR-1323 |
| *PIK3CD* | ENST00000591918.2 | hsa-miR-4792 |
| *PIK3R2* | ENST00000585921.1 | hsa-miR-486-3p |
| *PPM1B* | ENST00000585761.1 | hsa-miR-1323, hsa-miR-182-5p |
| *RAP1A* | NR_023922.2 | hsa-miR-512-3p |
| *RPS6KA3* | NR_125423.1, NR_134920.1, NR_039978.1, NR_125914.1, NR_125915.1, ENST00000591854.1, ENST00000581633.1, ENST00000582536.1, **ENST00000444868.2**[b] | hsa-miR-183-5p, hsa-miR-372-3p, **hsa-miR-195-5p**[b] |
| *SLC2A4* | **ENST00000433036.1**[c] | hsa-miR-520a-3p,**hsa-miR-512-3p**[c] |
| *SMAD2* | ENST00000582269.1, ENST00000606096.1, ENST00000462717.1, ENST00000366360.2 | hsa-miR-1278 |
| *SMAD4* | NR_023922.2, ENST00000510274.1, ENST00000568362.1 | hsa-miR-27b-3p, hsa-miR-183-5p, hsa-miR-182-5p |
| *STAT3* | ENST00000526936.1, ENST00000444868.2 | hsa-miR-486-3p |
| *STMN1* | ENST00000582269.1, ENST00000366360.2, ENST00000462717.1 | hsa-miR-1268a |
| *THEM4* | NR_046101.1, NR_038219.1 | hsa-miR-512-3p, hsa-miR-183-5p, hsa-miR-372-3p |

[a, b, c]*The lncRNA-miRNA pairs of interaction relationship.*

*DE: differentially expressed.*

Among the 10 DE miRNAs, all of them (hsa-miR-3614-5p, hsa-miR-451a, hsa-miR-1268a, hsa-miR-486-3p, hsa-miR-424-5p, hsa-miR-122-5p, hsa-let-7f-1-3p, hsa-miR-1323, hsa-miR-195-5p, and hsa-miR-27b-3p) were expressed at substantially higher levels in the DM group than in the NC group for both neutrophils and HDMECs-stimulated by neutrophil EXOs. Among them, hsa-miR-

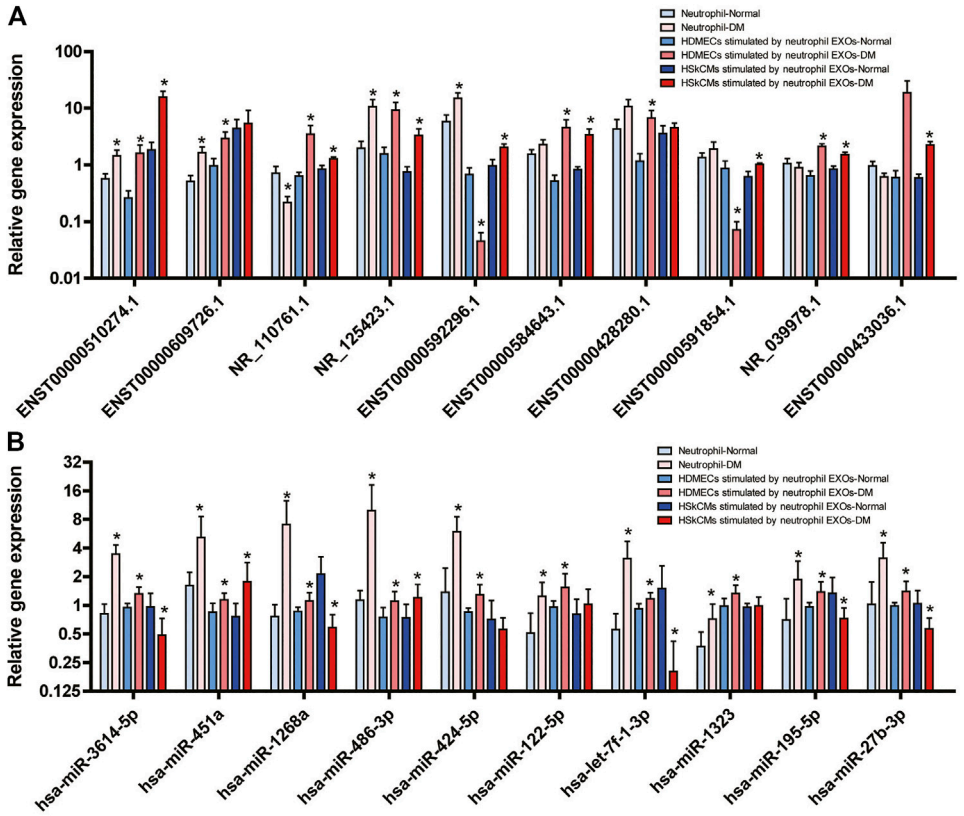


**FIGURE 1 |** Expression levels of DE lncRNAs **(A)** and DE miRNAs **(B)** in the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways validated by real-time quantitative PCR in neutrophils of DM patients and in HDMECs and HSkMCs after stimulation with DM neutrophil EXOs (*DM vs. NC).

3614-5p, hsa-miR-451a, hsa-miR-1268a, hsa-miR-486-3p, and hsa-miR-122-5p were significantly DE consistent with the results of the miRNA profile analysis. Among HSkMCs-stimulated by neutrophil EXOs, the expression levels of hsa-miR-451a and hsa-miR-486-3p were higher in the DM group than in the NC group, while those of hsa-miR-3614-5p, hsa-miR-1268a, hsa-let-7f-1-3p, hsa-miR-195-5p and hsa-miR-27b-3p, were lower (**Figure 1B**).

## DISCUSSION

Increasing amounts of data have recently highlighted the potential contributions of EXOs to vascular injury and muscle damage in DM pathogenesis. In the current study, 10 DE miRNAs in plasma EXOs were identified JDM patients compared with NCs. JDM-derived plasma EXOs can be taken up by human aortic endothelial cells, and induce alteration in multiple genes (59 genes) in these cells, which suggests potential mechanisms by which plasmas EXOs can ultimately target vascular tissue in JDM (Jiang et al., 2019). In addition, DM plasma-derived EVs have been found to trigger proinflammatory cytokine (IFNβ, TNFα and IL-6) release and STING signalling pathway activation in circulating immune cells. The activated STING pathway is preferentially mediated by EV-dsDNA (Li et al., 2021). DE proteins have also been found in DM serum EVs. The number of serum Plexin D1+ EVs is positively associated with muscle pain or weakness in DM patients, and correlates with the levels of aldolase, white blood cells, neutrophils and platelets. In DM patients after treatment (in clinical remission), the serum levels of Plexin D1+ EVs are significantly decreased (Uto et al., 2021). As EXOs contain multiple RNAs, DNAs, lipids, and proteins, EXOs can be selectively taken up by any neighbouring or distant cells, and reprogram the recipient cells. Thus, they have extremely strong potential to be diagnostic biomarkers and therapeutic nanocarriers, and might also participate in multiple processes of DM pathogenesis.

In this study, the noncoding RNAs profiles were also changed in DM neutrophil EXOs. Functional analysis suggested that the DE lncRNAs and DE miRNAs might participate in interleukin-6 and IFN-β production, skeletal muscle cell proliferation and development, and endothelial cell development and differentiation, which are associated with DM histopathology. Our validation results and interaction analyses revealed that, many novel and valuable DE lncRNAs and DE miRNAs were cotargeted and altered in the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways in the neutrophil EXO–stimulated HDMECs and HSkMCs. In the pathogenesis of DM, immune activation resulted in capillary destruction and leaded to ischemia and microinfarction, hypoperfusion, and perifascicular atrophy. Vascular endothelial cells injury was the initiating factor of DM pathogenesis, and then caused skeletal muscle damage. The functions and transcriptome levels of endothelial cells and skeletal muscle cells are different. So the

reactions to neutrophil EXOs stimulation were not the same, leading to the expression of DE lncRNAs and miRNAs were not accordant. Several studies have demonstrated that many miRNAs of the PI3K–Akt–FoxO pathway participate in the posttranscriptional regulation of skeletal muscle genes, including miR-19b-3p, miR-99a-5p, miR-100-5p, miR-222-3p, miR-324-3p, and miR-486-5p (Urbánek and Klotz, 2017; Brown and Webb, 2018).

PI3K–Akt, MAPK and AMPK are major effectors of insulin metabolic action and are essential for glucose homeostasis (Schultze et al., 2012). Once the insulin/insulin-like growth factor (IGF) signalling pathway is stimulated, insulin and IGF receptors are autophosphorylated and recruit downstream PI3K–Akt, MAPK and AMPK signalling pathway members. PI3K is a lipid kinase that generates phosphatidylinositol triphosphate and subsequently activates Akt, a serine/threonine kinase that converts extracellular stimuli into a wide range of cellular responses. The MAPK family is also a protein serine/threonine kinase family that includes c-Jun N-terminal kinase (JNK), p38 MAPK and extracellular-regulated protein kinases. AMPK is mainly known as a conserved and ubiquitously expressed energy sensor (that senses increases in AMP:ATP and ADP:ATP ratios) in eukaryotic cells. In addition to their diverse functions in cellular metabolism, the PI3K–Akt, MAPK and AMPK pathways have also been widely involved throughout evolution in regulating physiological processes such as gene expression, mitosis, motility, proliferation, survival, apoptosis and differentiation, and they might be attractive therapeutic targets for diabetes, cancers and autoimmune diseases (Cargnello and Roux, 2011; Mayer and Arteaga, 2016; Olivier et al., 2018).

FoxO transcription factors are conserved regulators of a variety of cellular processes, including cell cycle regulation, redox balance, proteostasis, apoptosis, metabolism and DNA damage repair. In all species, FoxO transcription factors are subject to extensive posttranslational modification, including phosphorylation, acetylation, ubiquitination and methylation (Link, 2019). Such modification of FoxO activity can be regulated by the PI3K–Akt, MAPK and AMPK signalling pathways upon stimulation by growth factor signalling, oxidative and genotoxic stress, and nutrient deprivation (Brown and Webb, 2018). The complex PI3K–Akt, MAPK, AMPK and FoxO signalling networks play decisive roles in the maintenance of skeletal muscle homeostasis and regulate the differentiation, proliferation and regeneration of muscle cells (Wu et al., 2017; Tia et al., 2018), either together or separately.

According to studies on DM pathogenesis, skeletal muscle inflammation induces muscle atrophy by inhibiting PI3K–Akt–mediated myogenic signals, which are activated by AMPK, p38 MAPK, JNK, mTOR, and IGF-1 (Stitt et al., 2004; Li et al., 2005; Kim et al., 2017; Kim et al., 2018). In a mouse model of autoimmune myositis and in differentiated C2C12 myotubes, cellular immune stimulation and intracellular β-amyloid (Aβi) have been found to potentially independently drive muscle atrophy through the PI3K–Akt–FoxO pathways (Lee et al., 2012). Furthermore, EXOs released from inflammatory C2C12 myotubes likely contribute to inflammation-induced muscle atrophy (Kim et al., 2018). These findings suggest that the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways play remarkable roles in muscle inflammation and damage in DM, possibly even through EXOs.

At present, inhibitors of PI3K–Akt (mTOR and ETP-45658), AMPK (doxorubicin hydrochloride and dorsomorphin dihydrochloride) and MAPK (PD98059, PD184352 and PD0325901) are already being successfully used in the clinic for the treatment of diverse cancer types and inflammatory diseases; in contrast, the AMPK activator metformin is the only drug targeting protein kinase activity that is widely used today, demonstrating the dual roles of AMPK activation (Arana-Argáez et al., 2010; Farhan et al., 2017). The findings of the current study might provide important insights to aid in the search for new DM therapeutics.

## CONCLUSION

This study identified the noncoding RNAs profiles of neutrophil EXOs. Bioinformatics analysis showed that the predicted target genes of DE lncRNAs and DE miRNAs were enriched in the PI3K–Akt, MAPK, AMPK and FoxO signalling pathways, which suggests their roles in the pathogenesis of DM. These molecules may be useful diagnostic and prognostic biomarkers in the future.

## DATA AVAILABILITY STATEMENT

The bioinformatics analysis datasets presented in this study can be found in the article/**Supplementary Material**. The raw data for RNAs sequencing are provided in the repository, the accession number is GSE155281.

## ETHICS STATEMENT

All blood samples were approved by the Ethical Committee of Xiangya Hospital of Central South University (No. 201303293, 201404360, 201212074) in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The patients/participants provided their written informed consent to participate in this study.

## AUTHOR CONTRIBUTIONS

LL and HZ performed the research and wrote the manuscript; DL and HL collected and managed the clinical samples and data; HZ and XZ devised the research study and revised the manuscript. All authors read and approved the final manuscript.

## FUNDING

This study was funded by grants from the National Key Research and Development Program of China (2016YFC0903900), National Natural Science Foundation of China (81671622, 81771765, 81701621), and Hunan Provincial Natural Science Foundation (2018JJ3823, 2019JJ40503).

## ACKNOWLEDGMENTS

The authors thank all the members of the Department of Rheumatology and Immunology, Xiangya Hospital, Central South University for their clinical support.

## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.727901/full#supplementary-material

## REFERENCES

Arana-Argáez, V. E., Delgado-Rizo, V., Pizano-Martínez, O. E., Martínez-Garcia, E. A., Martín-Márquez, B. T., Muñoz-Gómez, A., et al. (2010). Inhibitors of MAPK Pathway ERK1/2 or P38 Prevent the IL-1{beta}-induced Up-Regulation of SRP72 Autoantigen in Jurkat Cells. *J. Biol. Chem.* 285 (43), 32824–32833. doi:10.1074/jbc.M110.121087

Bohan, A., and Peter, J. B. (1975). Polymyositis and Dermatomyositis (First of Two Parts). *N. Engl. J. Med.* 292 (7), 344–347. doi:10.1056/NEJM197502132920706

Bohan, A., and Peter, J. B. (1975). Polymyositis and Dermatomyositis (Second of Two Parts). *N. Engl. J. Med.* 292 (8), 403–407. doi:10.1056/NEJM197502202920807

Brown, A. K., and Webb, A. E. (2018). Regulation of FOXO Factors in Mammalian Cells. *Curr. Top. Dev. Biol.* 127, 165–192. doi:10.1016/bs.ctdb.2017.10.006

Callen, J. P. (2000). Dermatomyositis. *The Lancet* 355 (9197), 53–57. doi:10.1016/s0140-6736(99)05157-0

Cargnello, M., and Roux, P. P. (2011). Activation and Function of the MAPKs and Their Substrates, the MAPK-Activated Protein Kinases. *Microbiol. Mol. Biol. Rev.* 75 (1), 50–83. doi:10.1128/MMBR.00031-10

Dalakas, M. C. (2015). Inflammatory Muscle Diseases. *N. Engl. J. Med.* 372 (18), 1734–1747. doi:10.1056/NEJMra1402225

DeWane, M. E., Waldman, R., and Lu, J. (2019). Dermatomyositis Part I: Clinical Features and Pathogenesis. *J. Am. Acad. Dermatol.* 82, 267. doi:10.1016/j.jaad.2019.06.1309

Dong, X., Gao, X., Dai, Y., Ran, N., and Yin, H. (2018). Serum Exosomes Can Restore Cellular Function *In Vitro* and Be Used for Diagnosis in Dysferlinopathy. *Theranostics* 8 (5), 1243–1255. doi:10.7150/thno.22856

Farhan, M., Wang, H., Gaur, U., Little, P. J., Xu, J., and Zheng, W. (2017). FOXO Signaling Pathways as Therapeutic Targets in Cancer. *Int. J. Biol. Sci.* 13 (7), 815–827. doi:10.7150/ijbs.20052

Gao, M. Z., Huang, Y. L., Wu, X. D., Xu, Q. W., Ji, R., Gu, B., et al. (2018). Red Blood Cell Distribution Width and Neutrophil to Lymphocyte Ratio Are Correlated with Disease Activity of Dermatomyositis and Polymyositis. *J. Clin. Lab. Anal.* 32 (1), e22209. doi:10.1002/jcla.22209

Gao, S., Luo, H., Zhang, H., Zuo, X., Wang, L., and Zhu, H. (2017). Using Multi-Omics Methods to Understand Dermatomyositis/polymyositis. *Autoimmun. Rev.* 16 (10), 1044–1048. doi:10.1016/j.autrev.2017.07.021

Gao, S., Zhang, H., Zuo, X., Xiao, Y., Liu, D., Zhu, H., et al. (2019). Integrated Comparison of the miRNAome and mRNAome in Muscles of Dermatomyositis and Polymyositis Reveals Common and Specific miRNA-mRNAs. *Epigenomics* 11 (1), 23–33. doi:10.2217/epi-2018-0064

Gao, S., Zuo, X., Liu, D., Xiao, Y., Zhu, H., Zhang, H., et al. (2018). The Roles of Neutrophil Serine Proteinases in Idiopathic Inflammatory Myopathies. *Arthritis Res. Ther.* 20 (1), 134. doi:10.1186/s13075-018-1632-x

Genschmer, K. R., Russell, D. W., Lal, C., Szul, T., Bratcher, P. E., Noerager, B. D., et al. (2019). Activated PMN Exosomes: Pathogenic Entities Causing Matrix Destruction and Disease in the Lung. *Cell* 176 (1-2), 113–e15. doi:10.1016/j.cell.2018.12.002

Glennon-Alty, L., Hackett, A. P., Chapman, E. A., and Wright, H. L. (2018). Neutrophils and Redox Stress in the Pathogenesis of Autoimmune Disease. *Free Radic. Biol. Med.* 125, 25–35. doi:10.1016/j.freeradbiomed.2018.03.049

Ha, Y. J., Hur, J., Go, D. J., Kang, E. H., Park, J. K., Lee, E. Y., et al. (2018). Baseline Peripheral Blood Neutrophil-To-Lymphocyte Ratio Could Predict Survival in Patients with Adult Polymyositis and Dermatomyositis: A Retrospective Observational Study. *PLoS One* 13 (1), e0190411. doi:10.1371/journal.pone.0190411

Jaillon, S., Galdiero, M. R., Del Prete, D., Cassatella, M. A., Garlanda, C., and Mantovani, A. (2013). Neutrophils in Innate and Adaptive Immunity. *Semin. Immunopathol* 35 (4), 377–394. doi:10.1007/s00281-013-0374-8

Jiang, K., Karasawa, R., Hu, Z., Chen, Y., Holmes, L., O'Neil, K. M., et al. (2019). Plasma Exosomes from Children with Juvenile Dermatomyositis Are Taken up by Human Aortic Endothelial Cells and Are Associated with Altered Gene Expression in Those Cells. *Pediatr. Rheumatol. Online J.* 17 (1), 41. doi:10.1186/s12969-019-0347-0

Kim, J. S., Lee, Y. H., Chang, Y. U., and Yi, H. K. (2017). PPARγ Regulates Inflammatory Reaction by Inhibiting the MAPK/NF-κB Pathway in C2C12 Skeletal Muscle Cells. *J. Physiol. Biochem.* 73 (1), 49–57. doi:10.1007/s13105-016-0523-3

Kim, S., Lee, M. J., Choi, J. Y., Park, D. H., Kwak, H. B., Moon, S., et al. (2018). Roles of Exosome-like Vesicles Released from Inflammatory C2C12 Myotubes: Regulation of Myocyte Differentiation and Myokine Expression. *Cell Physiol Biochem* 48 (5), 1829–1842. doi:10.1159/000492505

Lahoria, R., Selcen, D., and Engel, A. G. (2016). Microvascular Alterations and the Role of Complement in Dermatomyositis. *Brain* 139 (Pt 7), 1891–1903. doi:10.1093/brain/aww122

Lee, H. K., Rocnik, E., Fu, Q., Kwon, B., Zeng, L., Walsh, K., et al. (2012). Foxo/atrogin Induction in Human and Experimental Myositis. *Neurobiol. Dis.* 46 (2), 463–475. doi:10.1016/j.nbd.2012.02.011

Li, B. G., Hasselgren, P. O., and Fang, C. H. (2005). Insulin-like Growth Factor-I Inhibits Dexamethasone-Induced Proteolysis in Cultured L6 Myotubes through PI3K/Akt/GSK-3beta and PI3K/Akt/mTOR-dependent Mechanisms. *Int. J. Biochem. Cel Biol* 37 (10), 2207–2216. doi:10.1016/j.biocel.2005.04.008

Li, L., Zuo, X., Liu, D., Luo, H., and Zhu, H. (2020). The Profiles of miRNAs and lncRNAs in Peripheral Blood Neutrophils Exosomes of Diffuse Cutaneous Systemic Sclerosis. *J. Dermatol. Sci.* 98 (2), 88–97. doi:10.1016/j.jdermsci.2020.02.009

Li, L., Zuo, X., Xiao, Y., Liu, D., Luo, H., and Zhu, H. (2020). Neutrophil-derived Exosome from Systemic Sclerosis Inhibits the Proliferation and Migration of Endothelial Cells. *Biochem. Biophys. Res. Commun.* 526 (2), 334–340. doi:10.1016/j.bbrc.2020.03.088

Li, S., Li, Y., Chen, B., Zhao, J., Yu, S., Tang, Y., et al. (2018). exoRBase: a Database of circRNA, lncRNA and mRNA in Human Blood Exosomes. *Nucleic Acids Res.* 46 (D1), D106–D12. doi:10.1093/nar/gkx891

Li, Y., Bax, C., Patel, J., Vazquez, T., Ravishankar, A., Bashir, M. M., et al. (2021). Plasma-derived DNA Containing-Extracellular Vesicles Induce STING-Mediated Proinflammatory Responses in Dermatomyositis. *Theranostics* 11 (15), 7144–7158. doi:10.7150/thno.59152

Li, Y., Zhao, J., Yu, S., Wang, Z., He, X., Su, Y., et al. (2019). Extracellular Vesicles Long RNA Sequencing Reveals Abundant mRNA, circRNA, and lncRNA in Human Blood as Potential Biomarkers for Cancer Diagnosis. *Clin. Chem.* 65 (6), 798–808. doi:10.1373/clinchem.2018.301291

Link, W. (2019). Introduction to FOXO Biology. *Methods Mol. Biol.* 1890, 1–9. doi:10.1007/978-1-4939-8900-3_1

Mayer, I. A., and Arteaga, C. L. (2016). The PI3K/AKT Pathway as a Target for Cancer Treatment. *Annu. Rev. Med.* 67, 11–28. doi:10.1146/annurev-med-062913-051343

Mazzone, R., Zwergel, C., Artico, M., Taurone, S., Ralli, M., Greco, A., et al. (2019). The Emerging Role of Epigenetics in Human Autoimmune Disorders. *Clin. Epigenetics* 11 (1), 34. doi:10.1186/s13148-019-0632-2

Misunova, M., Salinas-Riester, G., Luthin, S., Pommerenke, C., Husakova, M., Zavada, J., et al. (2016). Microarray Analysis of Circulating Micro RNAs in the Serum of Patients with Polymyositis and Dermatomyositis Reveals a Distinct Disease Expression Profile and Is Associated with Disease Activity. *Clin. Exp. Rheumatol.* 34 (1), 17–24.

Navegantes, K. C., de Souza Gomes, R., Pereira, P. A. T., Czaikoski, P. G., Azevedo, C. H. M., and Monteiro, M. C. (2017). Immune Modulation of Some Autoimmune Diseases: the Critical Role of Macrophages and Neutrophils in the Innate and Adaptive Immunity. *J. Transl Med.* 15 (1), 36. doi:10.1186/s12967-017-1141-8

Olivier, S., Foretz, M., and Viollet, B. (2018). Promise and Challenges for Direct Small Molecule AMPK Activators. *Biochem. Pharmacol.* 153, 147–158. doi:10.1016/j.bcp.2018.01.049

Peng, Q. L., Zhang, Y. M., Yang, H. B., Shu, X. M., Lu, X., and Wang, G. C. (2016). Transcriptomic Profiling of Long Non-coding RNAs in Dermatomyositis by Microarray Analysis. *Sci. Rep.* 6, 32818. doi:10.1038/srep32818

Rossaint, J., Kühne, K., Skupski, J., Van Aken, H., Looney, M. R., Hidalgo, A., et al. (2016). Directed Transport of Neutrophil-Derived Extracellular Vesicles Enables Platelet-Mediated Innate Immune Response. *Nat. Commun.* 7, 13464. doi:10.1038/ncomms13464

Satoh, T., Okano, T., Matsui, T., Watabe, H., Ogasawara, T., Kubo, K., et al. (2005). Novel Autoantibodies against 7SL RNA in Patients with Polymyositis/dermatomyositis. *J. Rheumatol.* 32 (9), 1727–1733.

Schultze, S. M., Hemmings, B. A., Niessen, M., and Tschopp, O. (2012). PI3K/AKT, MAPK and AMPK Signalling: Protein Kinases in Glucose Homeostasis. *Expert Rev. Mol. Med.* 14, e1. doi:10.1017/S1462399411002109

Shao, S., Fang, H., Zhang, J., Jiang, M., Xue, K., Ma, J., et al. (2019). Neutrophil Exosomes Enhance the Skin Autoinflammation in Generalized Pustular Psoriasis via Activating Keratinocytes. *FASEB J.* 33 (6), 6813–6828. doi:10.1096/fj.201802090RR

Stitt, T. N., Drujan, D., Clarke, B. A., Panaro, F., Timofeyva, Y., Kline, W. O., et al. (2004). The IGF-1/PI3K/Akt Pathway Prevents Expression of Muscle Atrophy-Induced Ubiquitin Ligases by Inhibiting FOXO Transcription Factors. *Mol. Cel* 14 (3), 395–403. doi:10.1016/s1097-2765(04)00211-4

Tartar, D. M., Chung, L., and Fiorentino, D. F. (2018). Clinical Significance of Autoantibodies in Dermatomyositis and Systemic Sclerosis. *Clin. Dermatol.* 36 (4), 508–524. doi:10.1016/j.clindermatol.2018.04.008

Tia, N., Singh, A. K., Pandey, P., Azad, C. S., Chaudhary, P., and Gambhir, I. S. (2018). Role of Forkhead Box O (FOXO) Transcription Factor in Aging and Diseases. *Gene* 648, 97–105. doi:10.1016/j.gene.2018.01.051

Urbánek, P., and Klotz, L. O. (2017). Posttranscriptional Regulation of FOXO Expression: microRNAs and beyond. *Br. J. Pharmacol.* 174 (12), 1514–1532. doi:10.1111/bph.13471

Uto, K., Ueda, K., Okano, T., Akashi, K., Takahashi, S., Nakamachi, Y., et al. (2021). Identification of Plexin D1 on Circulating Extracellular Vesicles as a Potential Biomarker of Polymyositis and Dermatomyositis. *Rheumatology (Oxford)*, keab588. doi:10.1093/rheumatology/keab588

Vargas, A., Roux-Dalvai, F., Droit, A., and Lavoie, J. P. (2016). Neutrophil-Derived Exosomes: A New Mechanism Contributing to Airway Smooth Muscle Remodeling. *Am. J. Respir. Cel Mol Biol* 55 (3), 450–461. doi:10.1165/rcmb.2016-0033OC

Wahren-Herlenius, M., and Dörner, T. (2013). Immunopathogenic Mechanisms of Systemic Autoimmune Disease. *Lancet* 382 (9894), 819–831. doi:10.1016/S0140-6736(13)60954-X

Wu, C. L., Satomi, Y., and Walsh, K. (2017). RNA-seq and Metabolomic Analyses of Akt1-Mediated Muscle Growth Reveals Regulation of Regenerative Pathways and Changes in the Muscle Secretome. *BMC Genomics* 18 (1), 181. doi:10.1186/s12864-017-3548-2

Xiao, Y., Zhu, H., Li, L., Gao, S., Liu, D., Dai, B., et al. (2019). Global Analysis of Protein Expression in Muscle Tissues of Dermatomyositis/polymyosisits Patients Demonstrated an Association between Dysferlin and Human Leucocyte Antigen A. *Rheumatology* 58, 1474. doi:10.1093/rheumatology/kez085

Yáñez-Mó, M., Siljander, P. R., Andreu, Z., Zavec, A. B., Borràs, F. E., Buzas, E. I., et al. (2015). Biological Properties of Extracellular Vesicles and Their Physiological Functions. *J. Extracell Vesicles* 4, 27066. doi:10.3402/jev.v4.27066

Yang, W., Wang, X., Zhang, W., Ying, H., Xu, Y., Zhang, J., et al. (2017). Neutrophil-lymphocyte Ratio and Platelet-Lymphocyte Ratio Are 2 New Inflammatory Markers Associated with Pulmonary Involvement and Disease Activity in Patients with Dermatomyositis. *Clin. Chim. Acta* 465, 11–16. doi:10.1016/j.cca.2016.12.007

Yu, G., Wang, L. G., Han, Y., and He, Q. Y. (2012). clusterProfiler: an R Package for Comparing Biological Themes Among Gene Clusters. *OMICS* 16 (5), 284–287. doi:10.1089/omi.2011.0118

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers in Pharmacology

REVIEW
published: 04 November 2021
doi: 10.3389/fphar.2021.774808

# Cardiometabolic Comorbidities in Patients With Psoriasis: Focusing on Risk, Biological Therapy, and Pathogenesis

*Jiangluyi Cai*[1,2†], *Lian Cui*[2,3†], *Yu Wang*[1,2†], *Ying Li*[1,2], *Xilin Zhang*[1,2*] *and Yuling Shi*[1,2*]

[1]Department of Dermatology, Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai, China, [2]Institute of Psoriasis, Tongji University School of Medicine, Shanghai, China, [3]Department of Dermatology, Shanghai Tenth People's Hospital, Tongji University School of Medicine, Shanghai, China

Psoriasis is a chronic inflammatory disease characterized by erythematous scaly plaques, accompanied by systemic damage that leads to the development of multiple comorbidities. In particular, the association between psoriasis and cardiometabolic comorbidities, including cardiovascular diseases (CVDs), obesity, diabetes mellitus, and metabolic syndrome, has been verified in a considerable number of clinical trials. Moreover, the increased risk of cardiometabolic comorbidities positively correlates with psoriasis severity. Biologic therapy targeting inflammatory pathways or cytokines substantially improves the life quality of psoriasis patients and may affect cardiometabolic comorbidities by reducing their incidences. In this review, we focus on exploring the association between cardiometabolic comorbidities and psoriasis, and emphasize the benefits and precautions of biologic therapy in the management of psoriasis with cardiometabolic comorbidities. The pathogenic mechanisms of cardiometabolic comorbidities in psoriasis patients involve common genetic factors, lipid metabolism, insulin resistance, and shared inflammatory pathways such as tumor necrosis factor-$\alpha$ and interleukin-23/Th-17 pathways.

**Keywords: psoriasis, biologics, cardiovascular disease, diabetes mellitus, obesity, metabolic syndrome**

OPEN ACCESS

***Edited by:***
*Anna Lisa Giuliani,*
*University of Ferrara, Italy*

***Reviewed by:***
*Mark Lebwohl,*
*Icahn School of Medicine at Mount Sinai, United States*
*Eva Reali,*
*University of Milano-Bicocca, Italy*

***Correspondence:**
*Yuling Shi*
*shiyuling1973@tongji.edu.cn*
*Xilin Zhang*
*xilin206@163.com*

*†These authors have contributed equally to this work*

***Specialty section:***
*This article was submitted to Inflammation Pharmacology, a section of the journal Frontiers in Pharmacology*

***Received:*** *13 September 2021*
***Accepted:*** *14 October 2021*
***Published:*** *04 November 2021*

***Citation:***
*Cai J, Cui L, Wang Y, Li Y, Zhang X and Shi Y (2021) Cardiometabolic Comorbidities in Patients With Psoriasis: Focusing on Risk, Biological Therapy, and Pathogenesis. Front. Pharmacol. 12:774808. doi: 10.3389/fphar.2021.774808*

## INTRODUCTION

Psoriasis is a chronic immune-mediated inflammatory disease characterized by erythematous scaly plaques that commonly develop on extensor surfaces. Its histopathological features are hyperkeratosis, parakeratosis, epidermal acanthosis, and the infiltration of immune cells. Psoriasis has been estimated to affect 2–3% of the world population and has deleterious effects on the quality of life of patients (Griffiths and Barker, 2007).

With recent advances in the understanding of psoriasis, the disease is increasingly being considered as a systemic inflammatory disorder rather than only involving the skin and joints. An increased risk of inflammatory comorbidities such as cardiovascular diseases (CVDs) and metabolic diseases, collectively called cardiometabolic comorbidities, has been reported in psoriasis patients. There is increasing awareness that cardiovascular risk factors would enhance the potential risks of cardiovascular morbidity and mortality in affected psoriasis patients (Neimann et al., 2006). Psoriasis is also typically related to metabolic diseases including obesity, diabetes mellitus, and metabolic syndrome, which manifests as a combination of central obesity, hypertension, insulin

resistance, and dyslipidemia (Johnsson et al., 2012). The incidences of major adverse cardiovascular events (MACEs), a composite endpoint comprised of myocardial infarction, cerebrovascular accident, and cardiovascular death, have been reported to be higher in patients with severe psoriasis (Abuabara et al., 2010; Mehta et al., 2010; Ahlehoff et al., 2012). Indeed, with an increase in the severity of psoriasis, patients are prone to a higher risk of CVDs, suggesting that the prevalence of cardiovascular events positively correlates with psoriasis severity. Analogous positive trends have also been identified between metabolic disorders and psoriasis independent of obesity and other risk factors (Yeung et al., 2013).

There has been a continuous innovation in the management of psoriasis along with the advances in understanding of its pathogenic mechanisms and essential inflammatory pathways (Boehncke and Schön, 2015). Targeted immunotherapy, including the agonists of tumor necrosis factor-$\alpha$ (TNF-$\alpha$), interleukin-(IL)-17 and IL-23, has achieved great success (Mahil et al., 2016). First-generation biologics involves a group of TNF-$\alpha$ inhibitors, including monoclonal antibodies adalimumab, infliximab, certolizumab-pegol, and golimumab as well as its fusion protein etanercept. Second-generation biologics are composed of IL-12/23 and IL-17 inhibitor family: anti–IL-12/23p40 antibody ustekinumab; anti–IL-23p19 antibodies guselkumab, risankizumab, and tildrakizumab; anti–IL-17A antibodies secukinumab and ixekizumab; and anti–IL-17 receptor a antibody brodalumab (Rønholt and Iversen, 2017). They have now become prevalent regimens for the induction and maintenance of curative effect in patients with severe psoriasis that a higher proportion of patients have achieved Psoriasis Area Severity Index 75 (PASI 75) and PASI 90 after long-time treatment (Papp et al., 2005; Reich et al., 2005; Saurat et al., 2008). Their rapid efficacy has greatly improved the life quality of psoriasis patients (Rønholt and Iversen, 2017).

Because of the frequent association between psoriasis and cardiometabolic disorders, the impact of psoriasis therapy on the risk of cardiometabolic comorbidities have aroused our attention. In the clinical trials aimed at investigating the efficacy and safety profile of biologic therapies for psoriasis, the number of MACE cases was generally higher in the biologic groups than in the placebo groups. However, the intergroup difference was not statistically significant. This prompted us to recognize the cardiovascular risk during the medication of biologics in psoriasis patients (D'Adamio et al., 2019). TNF-$\alpha$ inhibitors reduce the risk of cardiovascular events and improve the cardiovascular outcome in patients with psoriasis, (Lee et al., 2019a). IL-17A, another important biologic target of psoriasis, plays a vital role in the pathogenesis of both psoriasis, and atherosclerotic plaques that accumulating evidence supports a beneficial influence of its agonists on related cardiometabolic comorbidities (Roubille et al., 2015; Lockshin et al., 2018). On the other hand, cardiometabolic comorbidities also affect the therapeutic effects of biologics (Boyd and Kavanaugh, 2015). Therefore, the impact of biologic therapies on cardiometabolic comorbidities should not be ignored during the treatment of psoriasis.

In this review, we explored the association between biologics and cardiometabolic comorbidities in patients with psoriasis to determine optimal systemic management of psoriasis by early screening and intervention for cardiometabolic comorbidities. We discuss the overlapping mechanisms between psoriasis and associated cardiometabolic comorbidities, especially the shared inflammatory pathways between psoriasis and cardiometabolic diseases linked to a sequence of inflammatory cascade reactions driven by increased T helper 1 (Th1), Th17 lymphocytes and associated proinflammatory cytokines, including TNF-$\alpha$, IL-1$\beta$, IL-17 and IL-23 (Davidovici et al., 2010). Other overlapping pathogenic mechanisms between psoriasis and comorbid cardiometabolic disorders have been proposed to be caused by common genetic factors (Lu et al., 2013), secretion of adipokines (Deng and Scherer, 2010; Robati et al., 2014), lipoprotein particles (Salahuddin et al., 2015), insulin resistance (Gyldenløve et al., 2015), angiogenesis (Malecic and Young, 2017), endothelial dysfunction (Karbach et al., 2014), and oxidative stress (Armstrong et al., 2011; Lockshin et al., 2018).

# MANUSCRIPT FORMATTING

## Risk of Cardiometabolic Diseases in Psoriasis

### Psoriasis and Cardiovascular Diseases

The increased risk of MACEs in patients with psoriasis has been discussed for decades. Previous studies showed a markedly increased incidence of cardiovascular diseases among psoriasis patients (Miller et al., 2013). A prominent role of chronic inflammation in CVDs was firstly noted (Ridker et al., 1997). Subsequently, a prospective population-based cohort study in the United Kingdom discovered that there existed a higher cardiovascular risk in patients with systemic chronic inflammatory conditions including psoriasis (Gelfand et al., 2006; Ridker, 2010). The study prompted the process of defining the association between psoriasis and cardiovascular events. Gelfand et al reported that psoriasis was an independent risk factor for myocardial infarction (MI), especially in young individuals with severe psoriasis (Gelfand et al., 2006). Furthermore, a cohort study using the General Practice Research Database demonstrated that patients with severe psoriasis are at a higher risk of cardiovascular mortality after controlling for major cardiovascular risk factors, providing stronger evidence that severe psoriasis may be an independent risk factor for cardiovascular death (Mehta et al., 2010). However, the higher prevalence of conventional cardiovascular risk factors, which are comprised of smoking, diabetes, lipid abnormalities, and hypertension, in psoriasis patients has been confirmed in many observational studies (Stern, 2010). It is well-established that psoriasis increases the risk of MI and ischemic stroke, and more recent studies have linked psoriasis with an increased risk of other CVDs such as heart failure and atrial fibrillation (Ahlehoff et al., 2012; Khalid et al., 2014). The risk of cardiovascular events is also linked to a positive dose-response relationship with

objectively-measured psoriasis severity and cumulative duration of psoriasis (Yeung et al., 2013; Egeberg et al., 2017).

## Psoriasis and Obesity

For a long time, obesity has been considered as a cardiovascular risk factor along with other factors including smoking, hypertension, and hyperlipidemia. It has been shown to be more prevalent in psoriasis patients than in patients without psoriasis (Shapiro et al., 2012). The relationship between obesity and psoriasis is estimated to be interrelated that obesity correlates with an increased risk of psoriasis and psoriasis might conversely lead to the occurrence of obesity (Budu-Aggrey et al., 2019). A recent meta-analysis of prospective studies showed that the relative risk of the relevance between psoriasis risk and per 5-unit increment in body mass index (BMI), per 10-cm increment in waist circumference, per 0.1-unit increment in the waist-to-hip ratio, per 5 kg of weight gain was 1.19 (95% CI: 1.10–1.28), 1.24 (95% CI: 1.17–1.31), 1.37 (95% CI: 1.23–1.53), and 1.11 (95% CI: 1.07–1.16), respectively, concluding that risk of psoriasis increases with the degree of obesity measured by the four abovementioned aspects (Aune et al., 2018). As the severity of psoriasis has a positive correlation with weight gain, several studies investigated the impact of weight loss on psoriasis severity through low-energy diet or bariatric surgery (Jensen et al., 2013; Shelling and Kirsner, 2013; Debbaneh et al., 2014). They found that weight loss in overweight patients with psoriasis showed a declined trend of psoriasis severity manifested as a reduction of PASI and improved quality of life (Jensen et al., 2013; Shelling and Kirsner, 2013; Debbaneh et al., 2014). It is possible that psoriasis might develop first and bring about obesity. This view is supported by the substantial lipid abnormalities in psoriasis patients and the increased incidence of adiposity, especially central adiposity, following the development of psoriasis in children (Mallbris et al., 2006; Paller et al., 2013).

## Psoriasis and Diabetes Mellitus

Diabetes mellitus, considered as one of the traditional cardiovascular-related risk factors that can contribute to cardiovascular morbidity and mortality, has been linked to psoriasis in many clinical trials and meta-analyses (Miller et al., 2013). In a Danish nationwide cohort study, the incidence rate of new-onset diabetes mellitus increased among patients with psoriasis compared with the population without psoriasis and was positively correlated with psoriasis severity after correcting for confounding factors such as age, sex, concomitant medication, comorbidity, and socioeconomic status (Khalid et al., 2013). This phenomenon was also identified in a meta-analysis of 44 observational studies that showed a higher risk of type 2 diabetes mellitus (T2DM) [odds ratio (OR): 2.10, 95% CI: 1.73–2.55] in patients with severe psoriasis (Wu et al., 2015). In addition to diabetes mellitus itself, its related systemic complications also show a positive relationship with moderate-to-severe psoriasis, the severity of which was objectively measured by the body surface area (BSA) affected, independently of other risk factors including obesity and smoking (Yeung et al., 2013). The risk of psoriasis development is higher in diabetes patients, which is associated with drug exposure in those treated with anti-diabetic therapies. An increasing risk of psoriasis is linked to the frequent use of insulin (adjusted OR: 1.29, 95% CI: 1.18–1.42, $p < 0.001$). On the other hand, a reduction in the risk of psoriasis is related to the frequent use of thiazolidinedione (TZD) (adjusted OR: 0.89, 95% CI: 0.81–0.98) compared with low-frequency TZD users (Wu et al., 2015). Conversely, concomitant medication during the treatment of psoriasis could also modulate the risk of T2DM incidence (Lee et al., 2014).

A Danish population-based twin study revealed that genetic and environmental factors play a role in the comorbidity of psoriasis and T2DM (Lønnberg et al., 2016). Pleiotropic susceptibility loci CDKAL1 contributes to the occurrence of psoriasis as well as diabetes mellitus (Wolf et al., 2008), which may upregulate inflammatory cytokines in psoriasis and thus promote insulin resistance, an independent risk factor of T2DM (Gelfand, 2016). A study indicated that insulin resistance or impaired insulin sensitivity exists in psoriasis patients with normal glucose tolerance, which may further result in the development of diabetes mellitus (Gyldenløve et al., 2015). Systemic inflammation may be a potential shared pathophysiologic pathway between psoriasis and diabetes mellitus. Inflammatory mediators such as TNF-$\alpha$, IL-6, leptin, and adiponectin affect the regulation of insulin sensitivity by various interactions *via* signaling pathways between insulin receptors and cytokines or adipocytokines (Davidovici et al., 2010; Donath, 2014).

## Psoriasis and Metabolic Syndrome

Metabolic syndrome is a comprehensive term for a cluster of interrelated metabolic disorders such as abdominal obesity, hypertension, insulin resistance, dysglycemia, and dyslipidemia (Eckel et al., 2010). It is associated with higher risks of cardiometabolic diseases, including coronary artery diseases and type 2 diabetes mellitus, and all-cause mortality (Gami et al., 2007). An increased prevalence of metabolic syndrome was confirmed in patients with psoriasis compared with general population in a meta-analysis of 12 observational studies (pooled OR: 2.26, 95% CI: 1.70–3.01) (Armstrong et al., 2013). A subsequent meta-analysis reached a similar conclusion after adjusting for confounders (pooled OR: 1.42, 95% CI: 1.28–1.65) (Rodríguez-Zúñiga and García-Perdomo, 2017), and showed that the risk of metabolic syndrome was closely related to psoriasis severity (Parodi et al., 2014). Dyslipidemia and hypertension are considered essential components of metabolic syndrome and had a higher prevalence among patients with psoriasis than in control groups in previous research (Kim et al., 2019; Snekvik et al., 2019). The potential risk of triggering psoriasis among patients with metabolic syndrome was found to increase in a prospective study; the association between individual components of metabolic syndrome and the incidence of psoriasis was also explored in this study (Kim et al., 2019). Some individual components such as a low level of high-density lipoprotein (HDL) cholesterol, a high level of triglycerides, and abdominal obesity promote psoriasis

development (Snekvik et al., 2019). However, the conclusion about the impact of elevated blood pressure and fasting plasma glucose levels on the incidence of psoriasis remains controversial (Wu et al., 2014; Kim et al., 2017; Kim et al., 2019). Hence, more research is needed to determine the association between these factors.

## Effect of Tumor Necrosis Factor-α Inhibitors on Cardiometabolic Outcomes in Psoriasis

### Effect on Cardiovascular Diseases

Psoriasis accompanied by the occurrence of high-risk cardiovascular events is associated with the spread of inflammation through blood vessels by the interactions of cytokines. Various studies have demonstrated that the risk of cardiovascular comorbidities in psoriasis patients reduced after treatment with TNF-α inhibitors. TNF-α has been identified as a pivotal cytokine in the pathogenesis of both atherosclerosis and autoimmune diseases such as rheumatoid arthritis (RA) and psoriasis (McKellar et al., 2009). Treatment with TNF-α inhibitors in psoriasis patients could reverse early atherosclerosis at the initial stage presented as significantly reduced arterial intima-media thickness (IMT) values without irreversible atherosclerotic plaque (which indicates the development of subclinical atherosclerosis) and decreased signal intensity on β-2-(18F)-fluoro-2-deoxy-D-glucose-Positron emission tomography/computed tomography (FDG-PET/CT), implying less vascular inflammation (Jókai et al., 2013; Dey et al., 2017). However, a randomized controlled trial (RCT) showed that the target-to-background ratio (TBR) of carotid vessel walls, an indicator of vascular inflammation, had a modest increase after adalimumab treatment for 52 weeks (Bissonnette et al., 2017). Anti-TNF-α therapy also has a favorable effect on the improvement of arterial stiffness, measured by the gold standard aortic pulse wave velocity (aPWV), in patients with psoriatic arthritis (Angel et al., 2010). Despite that a recent systematic review showed no significant effect of TNF-α inhibitors on the subclinical indicators of atherosclerosis in inflammatory diseases including psoriasis, a positive effect of TNF-α biologics on the clinical outcomes of CVDs *via* alternate pathways, such as primary disease remission or reduced prothrombotic tendency, cannot be ruled out (Knowles et al., 2020).

The cardioprotective effect of TNF-α inhibitors that reduce the risk of MI compared with topical agents has been demonstrated in a retrospective cohort study (Wu and Poon, 2013a; Wu and Poon, 2013b). Coronary microvascular dysfunction as a result of systematic inflammation in psoriasis patients was ameliorated manifested as an increase in the coronary flow reserve (CFR) from 2.2 ± 0.7 to 3.02 ± 0.8 ($p < 0.0001$) after anti-TNF-α therapy (Piaserico et al., 2016). Psoriasis patients receiving biologic agents including TNF-α inhibitors showed almost no difference in the progression of asymptomatic coronary artery diseases (CAD) in a follow up, while CT imaging data suggested a significant increase in the procession of CAD in the control group (Herédi et al., 2016). Another study confirmed a decreased burden of non-calcified coronary plaques after anti-TNF-α therapy compared with the patients who did not receive biologic treatment ($p < 0.01$) (Elnabawi et al., 2019a). Clinical or subclinical cardiac dysfunction such as left ventricular diastolic dysfunction and right ventricular systolic dysfunction is slightly more prevalent in patients with psoriasis, which has been uncovered to be ameliorated upon TNF-α inhibitor therapy (Ahlehoff et al., 2016; Herédi et al., 2016).

Whether the risk of heart failure reduces or increases in psoriasis patients treated with TNF-α inhibitors has been discussed for a long time. It has been reported that TNF-α has detrimental effects on chronic heart failure (CHF). However, there is no conclusive evidence to prove the specific therapeutic effect of TNF-α inhibitors on CHF in psoriasis patients (Hori and Yamaguchi, 2013). Multiple clinical trials have demonstrated that etanercept does not affect hospitalization or mortality due to CHF (Mann et al., 2004; Campanati et al., 2020). A high dose of infliximab exacerbates the CHF condition in psoriasis patients with CHF (Chung et al., 2003). The dose-dependent association between the deterioration of CHF and the application of TNF-α inhibitors has prompted the cautious usage of this agent in patients with CHF (Campanati et al., 2020). The New York Heart Association recommends that TNF-α inhibitors are contraindicated in patients with class 3 or 4 CHF as well as those with class 1 or 2 CHF whose ejection fraction is lower than 50% (Menter et al., 2008).

Psoriasis patients with cumulative exposure to TNF-α inhibitors for 6 months had more than 11.2% reduction of cardiovascular event risk compared with those who received phototherapy [hazard ratio (HR): 0.89, 95% confidence interval (CI): 0.79–0.99, $p = 0.048$] in a large-scale observational cohort study (Wu et al., 2018). A similar conclusion was reached in another study that the risk of cardiovascular events in patients receiving TNF-α inhibitors was lower than those receiving methotrexate (Wu J. J. et al., 2017). A meta-analysis of five studies including 49,795 patients with plaque psoriasis or psoriatic arthritis also verified the efficacy of anti–TNF-α therapy in decreasing the incidence of cardiovascular events (Yang et al., 2016). Another meta-analysis of 38 RCTs involving 18,024 patients treated with biologic therapy including TNF-α inhibitors reported 10 cases of MACEs in nine RCTs. However, a pooled analysis showed no significant statistical difference in the incidence of MACEs in patients treated with biologic therapy compared with those treated with conventional therapy or placebo (OR: 1.45, 95% CI: 0.34–6.24) (Rungapiromnan et al., 2017). Therefore, more RCTs are needed to provide the basis for the selection and rational usage of biologic agents in order to minimize the cardiovascular risk in patients with psoriasis (Gelfand, 2018).

### Effects on Cardiovascular Biomarkers

The risk of CVDs, including coronary heart diseases and peripheral arterial diseases, can be predicted and characterized by cardiovascular biomarkers such as C-reactive protein (CRP) and vascular endothelial growth factor, which are related to systemic inflammation and endothelial dysfunction (Heeschen et al., 2003; Melander et al., 2009). The serum levels of these factors decreased after TNF-α inhibitor therapy for over 24 weeks

in a prospective study (Boehncke et al., 2011). Another study investigated six additional cardiovascular risk markers, including vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1, E-selectin, matrix metalloproteinase-9, myeloperoxidase, and total plasminogen activator inhibitor-1. They are linked to BMI and waist-hip ratio, and participate in the onset and development of cardiometabolic diseases, especially metabolic syndrome, in psoriasis patients (Sigurdardottir et al., 2014).

### Effects on Metabolic Disorders

An association between weight gain and TNF-$\alpha$ inhibitor has been reported. And, different types of TNF-$\alpha$ inhibitors were associated with distinct characteristics of weight gain among the patients (Saraceno et al., 2008). The efficacy and response of TNF-$\alpha$ inhibitors with fixed-dose medications such as etanercept are impaired in obese individuals (Clark and Lebwohl, 2008). Furthermore, the results from a prospective study suggested that successful weight loss (≥5% from baseline values) by concomitant dietary intervention in obese patients resulted in a higher rate of disease improvement defined as minimal disease activity in patients with psoriatic arthritis (Di Minno et al., 2014). Therefore, in order to treat psoriasis and associated obesity, appropriate diet, physical exercise as well as weight loss are necessary to improve the therapeutic effect of biologic agents (Dalamaga and Papadavid, 2019). The clinical response to infliximab or ustekinumab is not affected by body weight in the treatment of psoriasis patients, because they are dosed in a weight-based manner (Clark and Lebwohl, 2008; Dalamaga and Papadavid, 2019).

A retrospective cohort study among RA and psoriasis patients revealed a decreased risk of diabetes mellitus among patients treated with TNF-$\alpha$ inhibitors compared with those treated with other nonbiologic agents (Solomon et al., 2011). Studies concerning the effects of etanercept on insulin sensitivity, which is a pivotal factor during the onset of metabolic syndrome and diabetes mellitus, show that etanercept has a positive effect on improving fasting glucose levels by attenuating insulin resistance (Marra et al., 2007; Stanley et al., 2011). Contradictory results have been reported in a RCT that the application of etanercept failed to change insulin secretion and sensitivity in psoriatic patients (Martínez-Abundis et al., 2007). Insulin sensitivity has been improved by another TNF-$\alpha$ inhibitor adalimumab in psoriasis patients without diabetes (Pina et al., 2015).

There is evidence that TNF-$\alpha$ inhibitors may have favorable effects on certain conditions of metabolic syndrome in the management of psoriasis. However, further exploration is needed to more precisely determine the influence (Channual et al., 2009). The impact of anti–TNF-$\alpha$ treatment on the lipid profile of psoriasis patients has not been concluded so far. Recently, a prospective cohort study reported that TNF-$\alpha$ inhibitors are beneficial for regulating the metabolic state by decreasing the levels of total cholesterol and low-density lipoprotein cholesterol (Botelho et al., 2020), but no significant difference occurred after adalimumab treatment in another study (Bissonnette et al., 2013).

## Effect of IL-12/23 Inhibitors on Cardiometabolic Outcomes in Psoriasis

Biologics targeting IL-23 include two types of monoclonal antibodies, namely anti–IL-12/23p40, including ustekinumab, briakinumab, and anti–IL-23p19, including guselkumab, tildrakizumab, and risankizumab. Multiple RCTs and pooled analyses have proven the safety of IL12/23p40 inhibitors; however, one of the anti–IL-12/23 compounds, briakinumab, was withdrawn from the market due to an increased cardiovascular risk since it caused frequent MACEs in the early phase (Gordon et al., 2012; Langley et al., 2013).

### Clinical Evidence for the IL-12/23p40 Inhibitor Ustekinumab

The safety profile of another IL-12/23 inhibitor ustekinumab aroused concern after the withdrawal of briakinumab due to the high incidence of MACEs, and further studies aimed to evaluate the cardiovascular risk of this class of compounds. During a 3-years follow-up study, the combined MACE rate per 100 patient-years was 0.44 (95% CI: 0.27–0.67) in a pooled analysis of phase II/III clinical studies of ustekinumab on moderate-to-severe psoriasis, and the comparison of the standardized incidence ratios of psoriasis patients treated with ustekinumab with general population suggests that the effect of ustekinumab on MACEs is neither detrimental nor beneficial (Reich et al., 2011). A meta-analysis of 22 RCTs involving 10,183 psoriasis patients also showed no significant difference in the MACE rate between patients receiving anti–IL-12/IL-23 and placebo, with a Mantel-Haenszel risk difference of 0.012 events/person-year (95% CI: −0.001 to 0.026; $p = 0.12$) (Ryan et al., 2011), but a higher risk of MACEs in patients receiving IL-12/23 antibodies in comparison with placebo was found after the evaluation of the same trials using another statistical technique named the Peto OR method (Tzellos et al., 2013). The increased rate of MACEs in patients treated with anti–IL-23 therapy was confirmed in a meta-analysis of randomized clinical trials that mainly covered individuals with high cardiovascular risk (Ait-Oufella et al., 2019). A recent case-time-control study also identified a significant association between the initiation of treatment with ustekinumab and the early occurrence of severe cardiovascular events (OR: 4.17; 95% CI: 1.19–14.59) (Choi et al., 2020). Data from the PHOENIX 1 study of long-term ustekinumab treatment in patients with an extended duration of exposure showed a favorable safety profile and stable clinical response (Kimball et al., 2013). The influence of IL-12/23 inhibitors on cardiovascular safety was also compared with those of other biologics approved for the treatment of psoriasis in some trials, which reported a comparable risk of MACEs among ustekinumab, TNF-$\alpha$ inhibitors, and IL-17 inhibitors (Kimball et al., 2013; Ikonomidis et al., 2017; Gelfand, 2018). The risk of atrial fibrillation and MACEs did not substantially differ between treatment initiated with ustekinumab and TNF-$\alpha$ inhibitors in a cohort study (Lee et al., 2019a). However, in another analysis, ustekinumab showed a greater improvement in vascular, coronary and myocardial function, which was reflected in improved global longitudinal strain, left ventricular twisting, percent difference

between peak twisting and untwisting at mitral valve opening (% untwMVO), and CFR as well as reduced circulating N-terminal pro-B-type natriuretic peptide (NT-proBNP) levels (Ikonomidis et al., 2017). To investigate the effect of biological therapy on the characteristics of coronary plaque phenotypes that partly determine the risk of MI, 290 participants treated with biologics were included in a 1-year follow-up study performed with serial coronary computed tomography angiography and the collection of clinical and laboratory data, and an improvement in the high-sensitivity-c-reactive protein (hs-CRP) level and a reduction in the non-calcified plaque burden were observed in the anti–IL-12/23 treatment group, which was inferior to the effects of anti-TNF-$\alpha$ and anti–IL-17 in the prospective, observational study (Hjuler et al., 2016). The incidence of a lipid-rich necrotic core, which is a high-risk coronary plaque feature, was also found to decrease after 1 year of biological therapy, including anti–TNF-$\alpha$, anti–IL-12/23, and anti–IL-17 therapy, compared with the nonbiologic therapy group (Poizeau et al., 2020). Similarly, anti–IL-12/23 and anti–IL-17 therapy was associated with a significant reduction in coronary inflammation measured by the perivascular fat attenuation index in a prospective cohort study (Hjuler et al., 2016).

Although anti–TNF-$\alpha$ therapy appears to increase the weight and BMI of patients, this effect does not appear in patients treated with anti–IL-12/23 therapy with weight-adjusted doses, suggesting that ustekinumab could be considered to treat overweight and obese patients with psoriasis (Wu et al., 2020). However, the significance of weight loss during the biological treatment of obese patients cannot be ignored because multiple studies have proven that a reduction in weight improves the clinical response to ustekinumab (Zhu et al., 2009; Gisondi et al., 2016). No significant change in the mean lipid levels after the biological therapy, including ustekinumab or placebo, for 1 year at follow-up was observed in another study (Hjuler et al., 2016). Similar results were also observed for BMI, lipid and glucose levels, which remained unchanged at the 1-year follow-up assessment of psoriasis patients treated with biologics (Elnabawi et al., 2019b).

### Ongoing Research About IL-23p19 Inhibitors

Agents targeting the p19 subunit of the IL-23 cytokine pathway have been approved for treatment only for 3 years, so the data for the incidence of MACEs in the clinical trials for the safety profile of IL-23p19 inhibitors is not sufficient to determine its influence on the cardiovascular risk of psoriasis patients (Blauvelt et al., 2017; Papp et al., 2017). Three completed phase 3 trials of guselkumab in the treatment of psoriasis, including VOYAGE 1 (NCT02207231), VOYAGE 2 (NCT02207244), and NAVIGATE (NCT02203032) reported low rates (<1.5%) of MACE occurrence over the study period in all groups (Blauvelt et al., 2017; Reich et al., 2017; Langley et al., 2018). A pooled analysis of three RCTs on tildrakizumab, including a phase 2b study (NCT01225731) and reSURFACE 1 (NCT01722331) and 2 (NCT01729754), also reported low rates of MACEs (Blauvelt et al., 2018). A low incidence of MACE was also reported in two phase 3 trials of risankizumab vs ustekinumab: UltIMMa-1 (NCT02684370) and UltIMMa-2 (NCT02684357) (Gordon et al., 2018). A total of 109 studies on the efficacy and safety of the therapy for psoriasis involving biologic agents were included in a network meta-analysis suggesting that no significant difference exists in the risk of MACE occurrence among guselkumab, tildrakizumab, ustekinumab, certolizumab, infliximab, adalimumab, and etanercept, versus placebo (Sbidian et al., 2017). More data are needed to confirm the effect of the anti–IL-23p19 agents on cardiovascular and metabolic risk.

## Relationship Between IL-17 Inhibitors and Cardiovascular Risk in Psoriasis

### IL-17 and Atherosclerosis

The existing data evaluating the effects of IL-17 inhibitors as newly-approved clinical biologic agents targeting IL-17 signaling on the risk of CVDs in psoriasis patients are insufficient. However, experimental data have shown that overexpression of IL-17A in keratinocytes of the murine model [K14-IL-17A (ind/+)] induced systemic vascular inflammation, arterial hypertension, and endothelial dysfunction, all of which can lead to an increased risk of CVDs (Karbach et al., 2014). IL-17A, which is involved in the development of psoriasis, is also implicated in the pathogenesis of CVDs, which suggests that IL-17A-mediated inflammation may become a potential overlapping pathological mechanism between psoriasis and its cardiovascular comorbidities (Lockshin et al., 2018). Research on atherosclerosis yielded conflicting results, suggesting that the IL-17 mainly produced by Th17 cells may promote or prevent atherosclerotic plaque development and stability, which are determined by specific inflammatory condition (Taleb et al., 2015). Atherosclerotic plaque stability is enhanced by the cytokine profile of increased IL-17 levels and decreased level of interferon-$\gamma$ (IFN-$\gamma$) in the local microenvironment, whereas the increased production of IL-17 and IFN-$\gamma$ has a synergistic pro-inflammatory effect in promoting disease progression (Taleb et al., 2015). Other studies also suggested that IL-17 maintained the stability of atherosclerotic plaques by promoting the generation of collagen and smooth muscle cells and downregulating the level of VCAM-1 (Taleb et al., 2009; Gisterå et al., 2013). However, the findings regarding the percentage of circulating Th17 cells and related cytokine IL-17A in patients with acute coronary syndromes are discordant that some reported increasing levels and others showed no significant difference in comparison with patients without coronary artery diseases (Cheng et al., 2008; Eid et al., 2009). The association between IL-17-mediated inflammation and atherosclerotic plaque conformation as well as the instability has yielded evidence for the hypothesis that psoriasis patients have a high risk of MI (Chen et al., 2010). Given the association between IL-17 and atherosclerosis in psoriasis, the effects of IL-17 inhibitors on cardiovascular comorbidities in the treatment of psoriasis should be evaluated carefully.

### IL-17 Inhibitors and Cardiovascular Events

In phase III clinical trial programs of IL-17 inhibitors, a small section of patients experienced a few cases of MACEs (Langley

et al., 2014; Griffiths et al., 2015; Lebwohl et al., 2015). Low levels of IL-17 in patients has been shown to be associated with a higher risk of recurrent MI and death in the related research (Simon et al., 2013). In a meta-analysis including 5,951 patients from 9 RCTs that compared the efficacy and safety of IL-17 inhibitors with placebo, six cases of MACEs were reported in 2,143 patients treated with IL-17 inhibitors whereas 700 patients treated with placebo reported only one MACE across four studies; however, no significant difference was observed between IL-17 inhibitors and placebo (Wu D. et al., 2017). Another meta-analysis of the effect of biologic therapy on the risk of MACEs showed 2 MACEs in 514 patients receiving IL-17 inhibitors among 8 MACEs in patients treated with various biologics ($n$ = 12,596) and 2 MACEs in the placebo group ($n$ = 5,092), and the pooled analysis of biologics and placebo, as well as various separate agents including TNF-$\alpha$ inhibitors, anti–IL-17A agents, or anti–IL-12/23 agents, did not show a significant difference in the risk of MACEs (Rungapiromnan et al., 2017). In a prospective, observational study of 215 psoriasis patients receiving biologic therapy recruited over a 1-year follow-up, an improvement in hs-CRP and HDL cholesterol level was observed in the anti–IL17-treated groups, and the most significant reduction (up to 12%) in coronary plaque burden appeared in the anti–IL-17 therapy group among all biologic-treated groups (Elnabawi et al., 2019a). Exposure-adjusted incidence rates of cardiovascular events were comparable between the secukinumab (300 mg/150 mg) group and etanercept group in the pooled analysis of 10 clinical trials over 52 weeks in 3,993 psoriasis patients (van de Kerkhof et al., 2016). In phase III clinical trials of brodalumab AMAGINE-1, five MACEs occurred, including two in patients treated with placebo and three in patients receiving constant 210 mg of brodalumab (Papp et al., 2016). Moreover, in the AMAGINE-2 and AMAGINE-3 studies, deaths occurred due to cardiovascular problems such as stroke and cardiac arrest (Lebwohl et al., 2015). A study evaluating the long-term safety of ixekizumab from 13 clinical trials reported 84 cases of MACEs among 5,697 psoriasis patients, with no significant increase in the cardiovascular risk or the incidence (Armstrong et al., 2020). Overall, data obtained primarily from pivotal clinical trials of short-term studies on anti–IL-17 therapy in patients with moderate-to-severe psoriasis plaque did not demonstrate an increased risk of CVDs. However, considering the limitations of these data, more long-term studies are necessary to determine the cardiovascular risk.

Prospective CARIMA trials (NCT02559622) that incorporated patients with moderate-to-severe psoriasis with or without severe CVD aimed to evaluate the influence of secukinumab on endothelial dysfunction over 52 weeks, and indicated a protective effect of secukinumab on CVD by improving the endothelial function and flow-mediated dilation in psoriasis patients (von Stebut et al., 2019). Recently prospective studies on the impact of IL-17 inhibitors on cardiovascular risk are ongoing such as Vascular Inflammation in Psoriasis (VIP) trials of secukinumab (VIP-S; NCT02690701), which is evaluating the cardiovascular risk in psoriasis patients from many aspects, including aortic inflammation and cardiometabolic biomarkers.

### IL-17 Inhibitors and Obesity

Inflammation propagation in obese adipose tissues is facilitated by IL-17A without impairing the adipogenesis and insulin response induced by the inflammatory environment (Pestel et al., 2020). In real-life cohorts including a majority of psoriasis patients with cardiometabolic comorbidities, real-world data of the baseline characteristics and clinical response to secukinumab suggested that patients with high BMI and obesity appeared to show a lower persistence of the curative effect and an adverse effect of PASI ≤3 response at week 78 (Rompoti et al., 2020). In contrast to the weight-enhancing effects of TNF-$\alpha$ inhibitor treatment, anti–IL-17 therapy as well as anti–IL-12/23 therapy appears to show no increase in body weight and BMI (Wu et al., 2020). Risk-benefit analyses of four RCTs that enrolled 2,403 patients with plaque psoriasis showed that patients with body weight less than 90 kg treated with 150 mg secukinumab and patients with body weight of 90 kg or more treated with 300 mg secukinumab had comparable efficacy and safety, suggesting that weight is a pivotal factor to be considered in dosing regimen recommendation (Lee et al., 2019b). However, another IL-17 inhibitor brodalumab showed no significant difference in efficacy and safety between nonobese and obese patients in post-hoc analysis of the AMAGINE-2 and AMAGINE-3 trials (Hsu et al., 2020). The impact of the IL-17A inhibitor secukinumab on adipose tissue and cutaneous inflammation in patients with moderate-to-severe psoriasis is being explored prospectively in the ongoing ObePso-S trial (NCT03055494) (Korman, 2020).

The findings from studies exploring the effect of biologic therapy on cardiometabolic syndrome in the treatment of psoriasis are summarized in **Table 1**.

## Overlapping Mechanisms Between Cardiometabolic Diseases and Psoriasis

Laboratory research has explored mechanistic links between psoriasis and CVDs related to the inflammatory cascades integrated by activated immune cells and upregulation of proinflammatory cytokines and mediators that influence both psoriasis and atherosclerosis (Flammer and Ruschitzka, 2012). Activation of the innate immune system is considered to be indispensable to initiate the inflammatory cascade in psoriasis, and components of the innate immune system, including neutrophils, dendritic cells, macrophages, and pro-inflammatory cytokines such as TNF-$\alpha$ and IL-18 have been shown to exist in psoriatic adipose tissue. Deranged lipid distribution and impaired adipose function have been confirmed to accelerate the build-up of atherosclerotic plaques in cardiovascular diseases (Sajja et al., 2018). The critical T-cell differentiation into Th1 and Th17 cells, which is stimulated by IL-12 and IL-23 and leads to the release of cytokines such as TNF-$\alpha$, IFN-$\gamma$, IL-17, and IL-22 in the process of adaptive immunity, overlaps between psoriasis and cardiometabolic diseases leading to augmented keratinocyte proliferation and angiogenesis

**TABLE 1 |** Effect of biologics on cardiometabolic comorbidities.

| Target therapy | Biologics | Effect of biologics on | | | |
|---|---|---|---|---|---|
| | | Cardiovascular diseases | Obesity | Metabolic syndrome | Diabetes mellitus |
| TNF-$\alpha$ inhibitors | Adalimumab infliximab certolizumab-pegol etanercept | Various studies have demonstrated a reduced cardiovascular risk Wu and Poon (2013a), Wu and Poon (2013b), decreased level of cardiovascular bio-markers Boehncke et al. (2011), Sigurdardottir et al. (2014), and improved arterial stiffness after treatment Angel et al. (2010). Controversy exists in the effect of vascular inflammation Bissonnette et al. (2013), Bissonnette et al. (2017), Dey et al. (2017). | Observational data indicated an increase in weight gain after treatment Saraceno et al. (2008). A prospective study indicated a better efficacy and response of biologics after weight loss Di Minno et al. (2014). Infliximab (dosed on weight) is not affected by weight Clark and Lebwohl (2008), Dalamaga and Papadavid (2019). | A prospective cohort study reported TNF-$\alpha$ inhibitors are beneficial to regulate metabolic state by decreasing levels of total cholesterol and low-density lipoprotein cholesterol Botelho et al. (2020), but a more precise impact needs further exploration Channual et al. (2009). | Contradictory results have been reported that the effect of etanercept on insulin sensitivity Marra et al. (2007), Martínez-Abundis et al. (2007), Stanley et al. (2011). Adalimumab improved insulin sensitivity in non-diabetic patients affected by psoriasis in a prospective study Pina et al. (2015). |
| IL-12/ 23p40 inhibitors | Ustekinumab briakinumab | Briakinumab was withdrawn from the market due to the increased cardiovascular risk Gordon et al. (2012), Langley et al. (2013). The effect of ustekinumab on MACEs demonstrated in several studies is neither detrimental nor beneficial Reich et al. (2011), Ryan et al. (2011). Another analysis reported a greater improvement of vascular, coronary, and myocardial function after ustekinumab treatment Ikonomidis et al. (2017). | Anti-IL-12/23 therapy appears no increase in body weight and BMI Wu et al. (2020). | No significant change of mean lipid levels after ustekinumab treatment for 1 year at follow-up was observed in a study Hjuler et al. (2016). And similar results were also found in another study that body mass index, lipids, or glucose remained unchanged at 1-year follow-up of psoriasis treated with biologics Elnabawi et al. (2019b). | |
| IL-17 inhibitors | Ecukinumab ixekizumab brodalumab | IL-17 has the double effect of promoting or preventing atherosclerotic plaques Taleb et al. (2015). IL-17 therapy didn't demonstrate an increased risk of cardiovascular diseases in a few short-term studies Wu et al. (2017b), Rungapiromnan et al. (2017), Armstrong et al. (2020). More long-term prospective studies are ongoing. | IL-17 inhibitors appear no increase in body weight and BMI Wu et al. (2020). Weight is a pivotal factor to influence the efficacy and response of anti-IL17 therapy Lee et al. (2019b), Rompoti et al. (2020). | — | — |
| IL-23p19 inhibitors | Guselkumab tildrakizumab risankizumab | Current clinical trials are not sufficient to draw conclusion about its impact on cardiometabolic risk of psoriasis patients, only can reflect the cardiovascular safety in a short term. | | | |

(Lockshin et al., 2018). Another potential mechanism of insulin resistance can be induced by the chronic inflammatory state in psoriatic diseases, and both of them may contribute to the early stage of the formation of atherosclerotic plaques leading to cardiometabolic diseases by causing endothelial dysfunction and increased intima-media thickness in patients with psoriasis (Siegel et al., 2013). Adipokines, a group of proteins secreted by adipocytes, including leptin, visfatin, and resistin, promote the inflammatory condition in psoriasis patients by the correlation with immune cells and pro-inflammatory factors, which in turn results in the appearance of an abnormal serum adipokine profile in patients with psoriasis (Toussirot et al., 2014). Genetic and environmental factors play a role in the comorbidity of psoriasis and metabolic disorders (Lønnberg et al., 2016). For example, the pleiotropic susceptibility loci CDKAL1 contributes to the occurrence of psoriasis as well as diabetes mellitus (Wolf et al., 2008), which may upregulate the inflammatory cytokines in psoriasis promoting insulin resistance, an independent risk factor of T2DM (Gelfand, 2016). One study indicated that psoriatic patients with normal glucose tolerance show insulin resistance or impaired insulin sensitivity, which may further develop into diabetes (Gyldenløve et al., 2015). The inflammatory condition affecting the systemic circulation in psoriasis is regarded as a promoter of components of metabolic syndrome such as insulin resistance, vascular dysfunction, dyslipidemia, and the systemic inflammation also becomes an inducer of endothelial dysfunction, oxidative stress, and increased angiogenesis (Gisondi et al., 2018). Therefore,

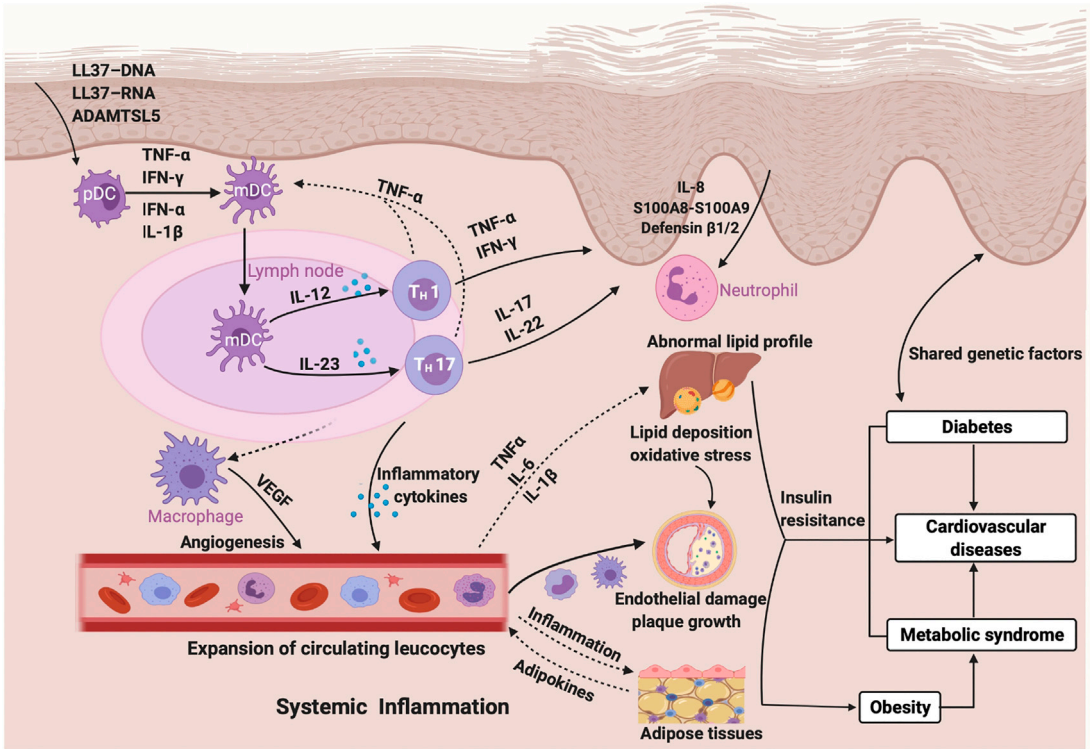


**FIGURE 1 |** Psoriasis, cardiometabolic comorbidities, and their common inflammatory pathway.

systemic inflammation may be the foremost shared pathophysiology pathways between psoriasis and cardiometabolic diseases, which awaits future exploration (**Figure 1**).

## Suggestions for the Management of Cardiometabolic Diseases in Psoriasis

Cardiovascular risks in psoriatic patients should be addressed with vigilance, and screening for the traditional cardiovascular risk factors and inflammatory markers such as CRP should be performed to predict the incidence of cardiovascular events (Kimball et al., 2008). Appropriate treatment options should be implemented considering the systemic condition of patients to reduce the risk and severity of cardiovascular disorders (Kristensen et al., 2015). Early determination of the cardiovascular risk in patients with psoriasis is recommended to facilitate effective management and prevent the occurrence of cardiovascular events in psoriatic patients, and precautions against cardiovascular events may also reduce the severity of psoriasis (Hu and Lan, 2017). The significance of weight control and monitoring should be emphasized because weight loss can improve the severity of psoriasis in obese patients and decrease the risk of cardiovascular events (Debbaneh et al., 2014). In the majority of studies, weight or BMI was a critical factor that negatively affected the therapeutic response to biologics and disease-modifying anti-rheumatic drugs; therefore, more attention should be paid to weight or BMI control during the treatment (Batalla et al., 2015). Screening the risk of diabetes mellitus in patients with psoriasis and implementation of preventive measures against the occurrence of diabetes mellitus should also receive importance, especially in patients with high affected BSA, since data shows that every 10% increase in BSA affected by psoriasis is accompanied by a 20% increase in diabetes risk (Wan et al., 2018). During the treatment of psoriasis, attention should be paid to the relationship between concomitant medications and the hazard of diabetes mellitus to select appropriate drugs for treatment, and blood glucose monitoring of patients should be strengthened (Lee et al., 2014). Given the mutual interaction of psoriasis and metabolic syndrome, preventive management of individual components of metabolic syndrome can also prevent the inflammation infiltrating into the skin to cause psoriasis. On the other hand, the significance of monitoring blood pressure, fasting plasma glucose level, HDL cholesterol, triglyceride level, and waist circumference to determine the development of metabolic syndrome after the diagnosis of psoriasis cannot be ignored. It is worth mentioning that there are certain other comorbidities and concomitant conditions, such as pediatric age group, pregnancy, and concomitant chronic infections, in addition to cardiometabolic comorbidities discussed in this review. All the comorbidities and concomitant conditions should also be considered when selecting optimal treatment

for psoriasis patients to guarantee its safety and efficacy (Kaushik and Lebwohl, 2019a; Kaushik and Lebwohl, 2019b).

## AUTHOR CONTRIBUTIONS

All authors contributed to the literature search, analysis of the articles and preparation of the manuscript.

## FUNDING

This work was sponsored by grants from National Natural Science Foundation of China (No.487 81673050, 81872522, 82073429), Innovation Program of Shanghai Municipal Education Commission (No. 2019-01-07-00-07-E00046), the Program of Science and Technology Commission of Shanghai Municipality (No. 18140901800), Excellent Subject Leader Program of Shanghai Municipal Commission of Health and Family Planning (No. 2018BR30), Clinical Research Program of Shanghai Hospital Development Center (No. SHDC2020CR1014B, SHDC12018X06) and Program of Shanghai Academic Research Leader (No. 20XD1403300). This work was supported by grants from Department of Dermatology, Shanghai Skin Disease Hospital, Tongji University School of Medicine, Shanghai, China; Institute of Psoriasis, Tongji University School of Medicine, Shanghai, China.

## REFERENCES

Abuabara, K., Azfar, R. S., Shin, D. B., Neimann, A. L., Troxel, A. B., and Gelfand, J. M. (2010). Cause-specific Mortality in Patients with Severe Psoriasis: a Population-Based Cohort Study in the U.K. *Br. J. Dermatol.* 163 (3), 586–592. doi:10.1111/j.1365-2133.2010.09941.x

Ahlehoff, O., Gislason, G. H., Jørgensen, C. H., Lindhardsen, J., Charlot, M., Olesen, J. B., et al. (2012). Psoriasis and Risk of Atrial Fibrillation and Ischaemic Stroke: a Danish Nationwide Cohort Study. *Eur. Heart J.* 33 (16), 2054–2064. doi:10.1093/eurheartj/ehr285

Ahlehoff, O., Hansen, P. R., Gislason, G. H., Frydland, M., Bryld, L. E., Elming, H., et al. (2016). Myocardial Function and Effects of Biologic Therapy in Patients with Severe Psoriasis: a Prospective Echocardiographic Study. *J. Eur. Acad. Dermatol. Venereol.* 30 (5), 819–823. doi:10.1111/jdv.13152

Ait-Oufella, H., Libby, P., and Tedgui, A. (2019). Anticytokine Immune Therapy and Atherothrombotic Cardiovascular Risk. *Arterioscler Thromb. Vasc. Biol.* 39 (8), 1510–1519. doi:10.1161/ATVBAHA.119.311998

Angel, K., Provan, S. A., Gulseth, H. L., Mowinckel, P., Kvien, T. K., and Atar, D. (2010). Tumor Necrosis Factor-Alpha Antagonists Improve Aortic Stiffness in Patients with Inflammatory Arthropathies: a Controlled Study. *Hypertension* 55 (2), 333–338. doi:10.1161/HYPERTENSIONAHA.109.143982

Armstrong, A., Paul, C., Puig, L., Boehncke, W. H., Freeman, M., Torii, H., et al. (2020). Safety of Ixekizumab Treatment for up to 5 Years in Adult Patients with Moderate-To-Severe Psoriasis: Results from Greater Than 17,000 Patient-Years of Exposure. *Dermatol. Ther. (Heidelb)* 10 (1), 133–150. doi:10.1007/s13555-019-00340-3

Armstrong, A. W., Harskamp, C. T., and Armstrong, E. J. (2013). Psoriasis and Metabolic Syndrome: a Systematic Review and Meta-Analysis of Observational Studies. *J. Am. Acad. Dermatol.* 68 (4), 654–662. doi:10.1016/j.jaad.2012.08.015

Armstrong, A. W., Voyles, S. V., Armstrong, E. J., Fuller, E. N., and Rutledge, J. C. (2011). Angiogenesis and Oxidative Stress: Common Mechanisms Linking Psoriasis with Atherosclerosis. *J. Dermatol. Sci.* 63 (1), 1–9. doi:10.1016/j.jdermsci.2011.04.007

Aune, D., Snekvik, I., Schlesinger, S., Norat, T., Riboli, E., and Vatten, L. J. (2018). Body Mass index, Abdominal Fatness, Weight Gain and the Risk of Psoriasis: a Systematic Review and Dose-Response Meta-Analysis of Prospective Studies. *Eur. J. Epidemiol.* 33 (12), 1163–1178. doi:10.1007/s10654-018-0366-z

Batalla, A., González-Fernández, D., González-Lara, L., Abalde, T., Salgado-Boquete, L., Queiro-Silva, R., et al. (2015). Cardiovascular Risk Factors Influence Response to Biological Therapies in Psoriasis. *J. Am. Acad. Dermatol.* 73 (2), 327–329. doi:10.1016/j.jaad.2015.04.041

Bissonnette, R., Harel, F., Krueger, J. G., Guertin, M. C., Chabot-Blanchet, M., Gonzalez, J., et al. (2017). TNF-α Antagonist and Vascular Inflammation in Patients with Psoriasis Vulgaris: A Randomized Placebo-Controlled Study. *J. Invest. Dermatol.* 137 (8), 1638–1645. doi:10.1016/j.jid.2017.02.977

Bissonnette, R., Tardif, J. C., Harel, F., Pressacco, J., Bolduc, C., and Guertin, M. C. (2013). Effects of the Tumor Necrosis Factor-α Antagonist Adalimumab on Arterial Inflammation Assessed by Positron Emission Tomography in Patients with Psoriasis: Results of a Randomized Controlled Trial. *Circ. Cardiovasc. Imaging* 6 (1), 83–90. doi:10.1161/CIRCIMAGING.112.975730

Blauvelt, A., Papp, K. A., Griffiths, C. E., Randazzo, B., Wasfi, Y., Shen, Y. K., et al. (2017). Efficacy and Safety of Guselkumab, an Anti-interleukin-23 Monoclonal Antibody, Compared with Adalimumab for the Continuous Treatment of Patients with Moderate to Severe Psoriasis: Results from the Phase III, Double-Blinded, Placebo- and Active Comparator-Controlled VOYAGE 1 Trial. *J. Am. Acad. Dermatol.* 76 (3), 405–417. doi:10.1016/j.jaad.2016.11.041

Blauvelt, A., Reich, K., Papp, K. A., Kimball, A. B., Gooderham, M., Tyring, S. K., et al. (2018). Safety of Tildrakizumab for Moderate-To-Severe Plaque Psoriasis: Pooled Analysis of Three Randomized Controlled Trials. *Br. J. Dermatol.* 179 (3), 615–622. doi:10.1111/bjd.16724

Boehncke, S., Salgo, R., Garbaraviciene, J., Beschmann, H., Hardt, K., Diehl, S., et al. (2011). Effective Continuous Systemic Therapy of Severe Plaque-type Psoriasis Is Accompanied by Amelioration of Biomarkers of Cardiovascular Risk: Results of a Prospective Longitudinal Observational Study. *J. Eur. Acad. Dermatol. Venereol.* 25 (10), 1187–1193. doi:10.1111/j.1468-3083.2010.03947.x

Boehncke, W.-H., and Schön, M. P. (2015). Psoriasis. *The Lancet* 386 (9997), 983–994. doi:10.1016/s0140-6736(14)61909-7

Botelho, K. P., Pontes, M. A. A., Rodrigues, C. E. M., and Freitas, M. V. C. (2020). Prevalence of Metabolic Syndrome Among Patients with Psoriasis Treated with TNF Inhibitors and the Effects of Anti-TNF Therapy on Their Lipid Profile: A Prospective Cohort Study. *Metab. Syndr. Relat. Disord.* 18 (3), 154–160. doi:10.1089/met.2019.0092

Boyd, T., and Kavanaugh, A. (2015). Clinical Guidelines: Addressing Comorbidities in Systemic Inflammatory Disorders. *Nat. Rev. Rheumatol.* 11 (12), 689–691. doi:10.1038/nrrheum.2015.129

Budu-Aggrey, A., Brumpton, B., Tyrrell, J., Watkins, S., Modalsli, E. H., Celis-Morales, C., et al. (2019). Evidence of a Causal Relationship between Body Mass index and Psoriasis: A Mendelian Randomization Study. *Plos Med.* 16 (1), e1002739. doi:10.1371/journal.pmed.1002739

Campanati, A., Diotallevi, F., Martina, E., Paolinelli, M., Radi, G., and Offidani, A. (2020). Safety Update of Etanercept Treatment for Moderate to Severe Plaque Psoriasis. *Expert Opin. Drug Saf.* 19 (4), 439–448. doi:10.1080/14740338.2020.1740204

Channual, J., Wu, J. J., and Dann, F. J. (2009). Effects of Tumor Necrosis Factor-Alpha Blockade on Metabolic Syndrome Components in Psoriasis and Psoriatic Arthritis and Additional Lessons Learned from Rheumatoid Arthritis. *Dermatol. Ther.* 22 (1), 61–73. doi:10.1111/j.1529-8019.2008.01217.x

Chen, S., Crother, T. R., and Arditi, M. (2010). Emerging Role of IL-17 in Atherosclerosis. *J. Innate Immun.* 2 (4), 325–333. doi:10.1159/000314626

Cheng, X., Yu, X., Ding, Y. J., Fu, Q. Q., Xie, J. J., Tang, T. T., et al. (2008). The Th17/Treg Imbalance in Patients with Acute Coronary Syndrome. *Clin. Immunol.* 127 (1), 89–97. doi:10.1016/j.clim.2008.01.009

Choi, H., Uceda, D. E., Dey, A. K., Abdelrahman, K. M., Aksentijevich, M., Rodante, J. A., et al. (2020). Treatment of Psoriasis with Biologic Therapy Is Associated with Improvement of Coronary Artery Plaque Lipid-Rich Necrotic Core: Results from a Prospective, Observational Study. *Circ. Cardiovasc. Imaging* 13 (9), e011199. doi:10.1161/CIRCIMAGING.120.011199

Chung, E. S., Packer, M., Lo, K. H., Fasanmade, A. A., and Willerson, J. T. (2003). Randomized, Double-Blind, Placebo-Controlled, Pilot Trial of Infliximab, a Chimeric Monoclonal Antibody to Tumor Necrosis Factor-Alpha, in Patients with Moderate-To-Severe Heart Failure: Results of the Anti-TNF Therapy

against Congestive Heart Failure (ATTACH) Trial. *Circulation* 107 (25), 3133–3140. doi:10.1161/01.CIR.0000077913.60364.D2

Clark, L., and Lebwohl, M. (2008). The Effect of Weight on the Efficacy of Biologic Therapy in Patients with Psoriasis. *J. Am. Acad. Dermatol.* 58 (3), 443–446. doi:10.1016/j.jaad.2007.11.011

D'Adamio, S., Silvaggio, D., Lombardo, P., Bianchi, L., Talamonti, M., and Galluzzo, M. (2019). The Safety of Anti-interleukins Monoclonal Antibodies for the Treatment of Psoriasis. *Expert Opin. Drug Saf.* 18 (11), 1031–1041. doi:10.1080/14740338.2019.1663168

Dalamaga, M., and Papadavid, E. (2019). Can We Better Strategize Our Choice of Pharmacotherapy for Patients with Co-morbid Psoriasis and Obesity. *Expert Opin. Pharmacother.* 20 (11), 1303–1308. doi:10.1080/14656566.2019.1603294

Davidovici, B. B., Sattar, N., Prinz, J., Jörg, P. C., Puig, L., Emery, P., et al. (2010). Psoriasis and Systemic Inflammatory Diseases: Potential Mechanistic Links between Skin Disease and Co-morbid Conditions. *J. Invest. Dermatol.* 130 (7), 1785–1796. doi:10.1038/jid.2010.103

Debbaneh, M., Millsop, J. W., Bhatia, B. K., Koo, J., and Liao, W. (2014). Diet and Psoriasis, Part I: Impact of Weight Loss Interventions. *J. Am. Acad. Dermatol.* 71 (1), 133–140. doi:10.1016/j.jaad.2014.02.012

Deng, Y., and Scherer, P. E. (2010). Adipokines as Novel Biomarkers and Regulators of the Metabolic Syndrome. *Ann. N. Y Acad. Sci.* 1212, E1–E19. doi:10.1111/j.1749-6632.2010.05875.x

Dey, A. K., Joshi, A. A., Chaturvedi, A., Lerman, J. B., Aberra, T. M., Rodante, J. A., et al. (2017). Association between Skin and Aortic Vascular Inflammation in Patients with Psoriasis: A Case-Cohort Study Using Positron Emission Tomography/Computed Tomography. *JAMA Cardiol.* 2 (9), 1013–1018. doi:10.1001/jamacardio.2017.1213

Di Minno, M. N., Peluso, R., Iervolino, S., Russolillo, A., Lupoli, R., and Scarpa, R. (2014). Weight Loss and Achievement of Minimal Disease Activity in Patients with Psoriatic Arthritis Starting Treatment with Tumour Necrosis Factor α Blockers. *Ann. Rheum. Dis.* 73 (6), 1157–1162. doi:10.1136/annrheumdis-2012-202812

Donath, M. Y. (2014). Targeting Inflammation in the Treatment of Type 2 Diabetes: Time to Start. *Nat. Rev. Drug Discov.* 13 (6), 465–476. doi:10.1038/nrd4275

Eckel, R. H., Alberti, K. G., Grundy, S. M., and Zimmet, P. Z. (2010). The Metabolic Syndrome. *Lancet* 375 (9710), 181–183. doi:10.1016/S0140-6736(09)61794-3

Egeberg, A., Skov, L., Joshi, A. A., Mallbris, L., Gislason, G. H., Wu, J. J., et al. (2017). The Relationship between Duration of Psoriasis, Vascular Inflammation, and Cardiovascular Events. *J. Am. Acad. Dermatol.* 77 (4), 650–e3. doi:10.1016/j.jaad.2017.06.028

Eid, R. E., Rao, D. A., Zhou, J., Lo, S. F., Ranjbaran, H., Gallo, A., et al. (2009). Interleukin-17 and Interferon-Gamma Are Produced Concomitantly by Human Coronary Artery-Infiltrating T Cells and Act Synergistically on Vascular Smooth Muscle Cells. *Circulation* 119 (10), 1424–1432. doi:10.1161/CIRCULATIONAHA.108.827618

Elnabawi, Y. A., Dey, A. K., Goyal, A., Groenendyk, J. W., Chung, J. H., Belur, A. D., et al. (2019). Coronary Artery Plaque Characteristics and Treatment with Biologic Therapy in Severe Psoriasis: Results from a Prospective Observational Study. *Cardiovasc. Res.* 115 (4), 721–728. doi:10.1093/cvr/cvz009

Elnabawi, Y. A., Oikonomou, E. K., Dey, A. K., Mancio, J., Rodante, J. A., Aksentijevich, M., et al. (2019). Association of Biologic Therapy with Coronary Inflammation in Patients with Psoriasis as Assessed by Perivascular Fat Attenuation Index. *JAMA Cardiol.* 4 (9), 885–891. doi:10.1001/jamacardio.2019.2589

Flammer, A. J., and Ruschitzka, F. (2012). Psoriasis and Atherosclerosis: Two Plaques, One Syndrome. *Eur. Heart J.* 33 (16), 1989–1991. doi:10.1093/eurheartj/ehr425

Gami, A. S., Witt, B. J., Howard, D. E., Erwin, P. J., Gami, L. A., Somers, V. K., et al. (2007). Metabolic Syndrome and Risk of Incident Cardiovascular Events and Death: a Systematic Review and Meta-Analysis of Longitudinal Studies. *J. Am. Coll. Cardiol.* 49 (4), 403–414. doi:10.1016/j.jacc.2006.09.032

Gelfand, J. M. (2018). Commentary: Does Biologic Treatment of Psoriasis Lower the Risk of Cardiovascular Events and Mortality?: A Critical Question that We Are Only Just Beginning to Answer. *J. Am. Acad. Dermatol.* 79 (1), 69–70. doi:10.1016/j.jaad.2018.03.046

Gelfand, J. M., Neimann, A. L., Shin, D. B., Wang, X., Margolis, D. J., and Troxel, A. B. (2006). Risk of Myocardial Infarction in Patients with Psoriasis. *JAMA* 296 (14), 1735–1741. doi:10.1001/jama.296.14.1735

Gelfand, J. M. (2016). Psoriasis, Type 2 Diabetes Mellitus, and Obesity: Weighing the Evidence. *JAMA Dermatol.* 152 (7), 753–754. doi:10.1001/jamadermatol.2016.0670

Gisondi, P., Del Giglio, M., and Girolomoni, G. (2016). Considerations for Systemic Treatment of Psoriasis in Obese Patients. *Am. J. Clin. Dermatol.* 17 (6), 609–615. doi:10.1007/s40257-016-0211-7

Gisondi, P., Fostini, A. C., Fossà, I., Girolomoni, G., and Targher, G. (2018). Psoriasis and the Metabolic Syndrome. *Clin. Dermatol.* 36 (1), 21–28. doi:10.1016/j.clindermatol.2017.09.005

Gisterå, A., Robertson, A. K., Andersson, J., Ketelhuth, D. F., Ovchinnikova, O., Nilsson, S. K., et al. (2013). Transforming Growth Factor-β Signaling in T Cells Promotes Stabilization of Atherosclerotic Plaques through an Interleukin-17-dependent Pathway. *Sci. Transl Med.* 5 (196), 196ra100. doi:10.1126/scitranslmed.3006133

Gordon, K. B., Langley, R. G., Gottlieb, A. B., Papp, K. A., Krueger, G. G., Strober, B. E., et al. (2012). A Phase III, Randomized, Controlled Trial of the Fully Human IL-12/23 mAb Briakinumab in Moderate-To-Severe Psoriasis. *J. Invest. Dermatol.* 132 (2), 304–314. doi:10.1038/jid.2011.304

Gordon, K. B., Strober, B., Lebwohl, M., Augustin, M., Blauvelt, A., Poulin, Y., et al. (2018). Efficacy and Safety of Risankizumab in Moderate-To-Severe Plaque Psoriasis (UltIMMa-1 and UltIMMa-2): Results from Two Double-Blind, Randomised, Placebo-Controlled and Ustekinumab-Controlled Phase 3 Trials. *Lancet* 392 (10148), 650–661. doi:10.1016/S0140-6736(18)31713-6

Griffiths, C. E., and Barker, J. N. (2007). Pathogenesis and Clinical Features of Psoriasis. *Lancet* 370 (9583), 263–271. doi:10.1016/S0140-6736(07)61128-3

Griffiths, C. E., Reich, K., Lebwohl, M., van de Kerkhof, P., Paul, C., Menter, A., et al. (2015). Comparison of Ixekizumab with Etanercept or Placebo in Moderate-To-Severe Psoriasis (UNCOVER-2 and UNCOVER-3): Results from Two Phase 3 Randomised Trials. *Lancet* 386 (9993), 541–551. doi:10.1016/S0140-6736(15)60125-8

Gyldenløve, M., Storgaard, H., Holst, J. J., Vilsbøll, T., Knop, F. K., and Skov, L. (2015). Patients with Psoriasis Are Insulin Resistant. *J. Am. Acad. Dermatol.* 72 (4), 599–605. doi:10.1016/j.jaad.2015.01.004

Heeschen, C., Dimmeler, S., Hamm, C. W., Boersma, E., Zeiher, A. M., and Simoons, M. L. (2003). Prognostic Significance of Angiogenic Growth Factor Serum Levels in Patients with Acute Coronary Syndromes. *Circulation* 107 (4), 524–530. doi:10.1161/01.cir.0000048183.37648.1a

Herédi, E., Végh, J., Pogácsás, L., Gáspár, K., Varga, J., Kincse, G., et al. (2016). Subclinical Cardiovascular Disease and It's Improvement after Long-Term TNF-α Inhibitor Therapy in Severe Psoriatic Patients. *J. Eur. Acad. Dermatol. Venereol.* 30 (9), 1531–1536. doi:10.1111/jdv.13649

Hjuler, K. F., Bøttcher, M., Vestergaard, C., Bøtker, H. E., Iversen, L., and Kragballe, K. (2016). Association between Changes in Coronary Artery Disease Progression and Treatment with Biologic Agents for Severe Psoriasis. *JAMA Dermatol.* 152 (10), 1114–1121. doi:10.1001/jamadermatol.2016.1984

Hori, M., and Yamaguchi, O. (2013). Is Tumor Necrosis Factor-α Friend or Foe for Chronic Heart Failure. *Circ. Res.* 113 (5), 492–494. doi:10.1161/CIRCRESAHA.113.302024

Hsu, S., Green, L. J., Lebwohl, M. G., Wu, J. J., Blauvelt, A., and Jacobson, A. A. (2020). Comparable Efficacy and Safety of Brodalumab in Obese and Nonobese Patients with Psoriasis: Analysis of Two Randomized Controlled Trials. *Br. J. Dermatol.* 182 (4), 880–888. doi:10.1111/bjd.18327

Hu, S. C., and Lan, C. E. (2017). Psoriasis and Cardiovascular Comorbidities: Focusing on Severe Vascular Events, Cardiovascular Risk Factors and Implications for Treatment. *Int. J. Mol. Sci.* 18 (10), 2211. doi:10.3390/ijms18102211

Ikonomidis, I., Papadavid, E., Makavos, G., Andreadou, I., Varoudi, M., Gravanis, K., et al. (2017). Lowering Interleukin-12 Activity Improves Myocardial and Vascular Function Compared with Tumor Necrosis Factor-A Antagonism or Cyclosporine in Psoriasis. *Circ. Cardiovasc. Imaging* 10 (9), e006283. doi:10.1161/CIRCIMAGING.117.006283

Jensen, P., Zachariae, C., Christensen, R., Geiker, N. R., Schaadt, B. K., Stender, S., et al. (2013). Effect of Weight Loss on the Severity of Psoriasis: a Randomized Clinical Study. *JAMA Dermatol.* 149 (7), 795–801. doi:10.1001/jamadermatol.2013.722

Johnsson, H., McInnes, I. B., and Sattar, N. (2012). Cardiovascular and Metabolic Risks in Psoriasis and Psoriatic Arthritis: Pragmatic Clinical Management Based on Available Evidence. *Ann. Rheum. Dis.* 71 (4), 480–483. doi:10.1136/annrheumdis-2011-200567

Jókai, H., Szakonyi, J., Kontár, O., Marschalkó, M., Szalai, K., Kárpáti, S., et al. (2013). Impact of Effective Tumor Necrosis Factor-Alfa Inhibitor Treatment on Arterial Intima-media Thickness in Psoriasis: Results of a Pilot Study. *J. Am. Acad. Dermatol.* 69 (4), 523–529. doi:10.1016/j.jaad.2013.06.019

Karbach, S., Croxford, A. L., Oelze, M., Schüler, R., Minwegen, D., Wegner, J., et al. (2014). Interleukin 17 Drives Vascular Inflammation, Endothelial Dysfunction, and Arterial Hypertension in Psoriasis-like Skin Disease. *Arterioscler Thromb. Vasc. Biol.* 34 (12), 2658–2668. doi:10.1161/ATVBAHA.114.304108

Kaushik, S. B., and Lebwohl, M. G. (2019). Psoriasis: Which Therapy for Which Patient: Psoriasis Comorbidities and Preferred Systemic Agents. *J. Am. Acad. Dermatol.* 80 (1), 27–40. doi:10.1016/j.jaad.2018.06.057

Kaushik, S. B., and Lebwohl, M. G. (2019). Psoriasis: Which Therapy for Which Patient: Focus on Special Populations and Chronic Infections. *J. Am. Acad. Dermatol.* 80 (1), 43–53. doi:10.1016/j.jaad.2018.06.056

Khalid, U., Ahlehoff, O., Gislason, G. H., Kristensen, S. L., Skov, L., Torp-Pedersen, C., et al. (2014). Psoriasis and Risk of Heart Failure: a Nationwide Cohort Study. *Eur. J. Heart Fail.* 16 (7), 743–748. doi:10.1002/ejhf.113

Khalid, U., Hansen, P. R., Gislason, G. H., Lindhardsen, J., Kristensen, S. L., Winther, S. A., et al. (2013). Psoriasis and New-Onset Diabetes: a Danish Nationwide Cohort Study. *Diabetes care* 36 (8), 2402–2407. doi:10.2337/dc12-2330

Kim, E. S., Han, K., Kim, M. K., Park, Y. M., Baek, K. H., Moon, S. D., et al. (2017). Impact of Metabolic Status on the Incidence of Psoriasis: a Korean Nationwide Cohort Study. *Sci. Rep.* 7 (1), 1989. doi:10.1038/s41598-017-01983-y

Kim, H. N., Han, K., Park, Y. G., and Lee, J. H. (2019). Metabolic Syndrome Is Associated with an Increased Risk of Psoriasis: A Nationwide Population-Based Study. *Metabolism* 99, 19–24. doi:10.1016/j.metabol.2019.07.001

Kimball, A. B., Gladman, D., Gelfand, J. M., Gordon, K., Horn, E. J., Korman, N. J., et al. (2008). National Psoriasis Foundation Clinical Consensus on Psoriasis Comorbidities and Recommendations for Screening. *J. Am. Acad. Dermatol.* 58 (6), 1031–1042. doi:10.1016/j.jaad.2008.01.006

Kimball, A. B., Papp, K. A., Wasfi, Y., Chan, D., Bissonnette, R., Sofen, H., et al. (2013). Long-term Efficacy of Ustekinumab in Patients with Moderate-To-Severe Psoriasis Treated for up to 5 Years in the PHOENIX 1 Study. *J. Eur. Acad. Dermatol. Venereol.* 27 (12), 1535–1545. doi:10.1111/jdv.12046

Knowles, L., Nadeem, N., and Chowienczyk, P. J. (2020). Do anti-tumour Necrosis Factor-α Biologics Affect Subclinical Measures of Atherosclerosis and Arteriosclerosis? A Systematic Review. *Br. J. Clin. Pharmacol.* 86 (5), 837–851. doi:10.1111/bcp.14215

Korman, N. J. (2020). Management of Psoriasis as a Systemic Disease: what Is the Evidence. *Br. J. Dermatol.* 182 (4), 840–848. doi:10.1111/bjd.18245

Kristensen, S. L., McInnes, I. B., and Sattar, N. (2015). Psoriasis, Psoriatic Arthritis and Cardiovascular Risk: Are We Closer to a Clinical Recommendation. *Ann. Rheum. Dis.* 74 (2), 321–322. doi:10.1136/annrheumdis-2014-206617

Langley, R. G., Elewski, B. E., Lebwohl, M., Reich, K., Griffiths, C. E., Papp, K., et al. (2014). Secukinumab in Plaque Psoriasis-Rresults of Two Phase 3 Trials. *N. Engl. J. Med.* 371 (4), 326–338. doi:10.1056/NEJMoa1314258

Langley, R. G., Papp, K., Gottlieb, A. B., Krueger, G. G., Gordon, K. B., Williams, D., et al. (2013). Safety Results from a Pooled Analysis of Randomized, Controlled Phase II and III Clinical Trials and Interim Data from an Open-Label Extension Trial of the Interleukin-12/23 Monoclonal Antibody, Briakinumab, in Moderate to Severe Psoriasis. *J. Eur. Acad. Dermatol. Venereol.* 27 (10), 1252–1261. doi:10.1111/j.1468-3083.2012.04705.x

Langley, R. G., Tsai, T. F., Flavin, S., Song, M., Randazzo, B., Wasfi, Y., et al. (2018). Efficacy and Safety of Guselkumab in Patients with Psoriasis Who Have an Inadequate Response to Ustekinumab: Results of the Randomized, Double-Blind, Phase III Navigate Trial. *Br. J. Dermatol.* 178 (1), 114–123. doi:10.1111/bjd.15750

Lebwohl, M., Strober, B., Menter, A., Gordon, K., Weglowska, J., Puig, L., et al. (2015). Phase 3 Studies Comparing Brodalumab with Ustekinumab in Psoriasis. *N. Engl. J. Med.* 373 (14), 1318–1328. doi:10.1056/NEJMoa1503824

Lee, J. E., Wang, J., Florian, J., Wang, Y. M., Kettl, D., Marcus, K., et al. (2019). Effect of Body Weight on Risk-Benefit and Dosing Regimen Recommendation of Secukinumab for the Treatment of Moderate to Severe Plaque Psoriasis. *Clin. Pharmacol. Ther.* 106 (1), 78–80. doi:10.1002/cpt.1478

Lee, M. P., Desai, R. J., Jin, Y., Brill, G., Ogdie, A., and Kim, S. C. (2019). Association of Ustekinumab vs TNF Inhibitor Therapy with Risk of Atrial Fibrillation and Cardiovascular Events in Patients with Psoriasis or Psoriatic Arthritis. *JAMA Dermatol.* 155 (6), 700–707. doi:10.1001/jamadermatol.2019.0001

Lee, M. S., Lin, R. Y., and Lai, M. S. (2014). Increased Risk of Diabetes Mellitus in Relation to the Severity of Psoriasis, Concomitant Medication, and Comorbidity: a Nationwide Population-Based Cohort Study. *J. Am. Acad. Dermatol.* 70 (4), 691–698. doi:10.1016/j.jaad.2013.11.023

Lockshin, B., Balagula, Y., and Merola, J. F. (2018). Interleukin 17, Inflammation, and Cardiovascular Risk in Patients with Psoriasis. *J. Am. Acad. Dermatol.* 79 (2), 345–352. doi:10.1016/j.jaad.2018.02.040

Lønnberg, A. S., Skov, L., Skytthe, A., Kyvik, K. O., Pedersen, O. B., and Thomsen, S. F. (2016). Association of Psoriasis with the Risk for Type 2 Diabetes Mellitus and Obesity. *JAMA Dermatol.* 152 (7), 761–767. doi:10.1001/jamadermatol.2015.6262

Lu, Y., Chen, H., Nikamo, P., Qi Low, H., Helms, C., Seielstad, M., et al. (2013). Association of Cardiovascular and Metabolic Disease Genes with Psoriasis. *J. Invest. Dermatol.* 133 (3), 836–839. doi:10.1038/jid.2012.366

Mahil, S. K., Capon, F., and Barker, J. N. (2016). Update on Psoriasis Immunopathogenesis and Targeted Immunotherapy. *Semin. Immunopathol* 38 (1), 11–27. doi:10.1007/s00281-015-0539-8

Malecic, N., and Young, H. S. (2017). Excessive Angiogenesis Associated with Psoriasis as a Cause for Cardiovascular Ischaemia. *Exp. Dermatol.* 26 (4), 299–304. doi:10.1111/exd.13310

Mallbris, L., Granath, F., Hamsten, A., and Ståhle, M. (2006). Psoriasis Is Associated with Lipid Abnormalities at the Onset of Skin Disease. *J. Am. Acad. Dermatol.* 54 (4), 614–621. doi:10.1016/j.jaad.2005.11.1079

Mann, D. L., McMurray, J. J., Packer, M., Swedberg, K., Borer, J. S., Colucci, W. S., et al. (2004). Targeted Anticytokine Therapy in Patients with Chronic Heart Failure: Results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). *Circulation* 109 (13), 1594–1602. doi:10.1161/01.CIR.0000124490.27666.B2

Marra, M., Campanati, A., Testa, R., Sirolla, C., Bonfigli, A. R., Franceschi, C., et al. (2007). Effect of Etanercept on Insulin Sensitivity in Nine Patients with Psoriasis. *Int. J. Immunopathol Pharmacol.* 20 (4), 731–736. doi:10.1177/039463200702000408

Martínez-Abundis, E., Reynoso-von Drateln, C., Hernández-Salazar, E., and González-Ortiz, M. (2007). Effect of Etanercept on Insulin Secretion and Insulin Sensitivity in a Randomized Trial with Psoriatic Patients at Risk for Developing Type 2 Diabetes Mellitus. *Arch. Dermatol. Res.* 299 (9), 461–465. doi:10.1007/s00403-007-0784-3

McKellar, G. E., McCarey, D. W., Sattar, N., and McInnes, I. B. (2009). Role for TNF in Atherosclerosis? Lessons from Autoimmune Disease. *Nat. Rev. Cardiol.* 6 (6), 410–417. doi:10.1038/nrcardio.2009.57

Mehta, N. N., Azfar, R. S., Shin, D. B., Neimann, A. L., Troxel, A. B., and Gelfand, J. M. (2010). Patients with Severe Psoriasis Are at Increased Risk of Cardiovascular Mortality: Cohort Study Using the General Practice Research Database. *Eur. Heart J.* 31 (8), 1000–1006. doi:10.1093/eurheartj/ehp567

Melander, O., Newton-Cheh, C., Almgren, P., Hedblad, B., Berglund, G., Engström, G., et al. (2009). Novel and Conventional Biomarkers for Prediction of Incident Cardiovascular Events in the Community. *JAMA* 302 (1), 49–57. doi:10.1001/jama.2009.943

Menter, A., Gottlieb, A., Feldman, S. R., Van Voorhees, A. S., Leonardi, C. L., Gordon, K. B., et al. (2008). Guidelines of Care for the Management of Psoriasis and Psoriatic Arthritis: Section 1. Overview of Psoriasis and Guidelines of Care for the Treatment of Psoriasis with Biologics. *J. Am. Acad. Dermatol.* 58 (5), 826–850. doi:10.1016/j.jaad.2008.02.039

Miller, I. M., Ellervik, C., Yazdanyar, S., and Jemec, G. B. (2013). Meta-analysis of Psoriasis, Cardiovascular Disease, and Associated Risk Factors. *J. Am. Acad. Dermatol.* 69 (6), 1014–1024. doi:10.1016/j.jaad.2013.06.053

Neimann, A. L., Shin, D. B., Wang, X., Margolis, D. J., Troxel, A. B., and Gelfand, J. M. (2006). Prevalence of Cardiovascular Risk Factors in Patients with Psoriasis. *J. Am. Acad. Dermatol.* 55 (5), 829–835. doi:10.1016/j.jaad.2006.08.040

Paller, A. S., Mercy, K., Kwasny, M. J., Choon, S. E., Cordoro, K. M., Girolomoni, G., et al. (2013). Association of Pediatric Psoriasis Severity with Excess and

central Adiposity: an International Cross-Sectional Study. *JAMA Dermatol.* 149 (2), 166–176. doi:10.1001/jamadermatol.2013.1078

Papp, K. A., Blauvelt, A., Bukhalo, M., Gooderham, M., Krueger, J. G., Lacour, J. P., et al. (2017). Risankizumab versus Ustekinumab for Moderate-To-Severe Plaque Psoriasis. *N. Engl. J. Med.* 376 (16), 1551–1560. doi:10.1056/NEJMoa1607017

Papp, K. A., Reich, K., Paul, C., Blauvelt, A., Baran, W., Bolduc, C., et al. (2016). A Prospective Phase III, Randomized, Double-Blind, Placebo-Controlled Study of Brodalumab in Patients with Moderate-To-Severe Plaque Psoriasis. *Br. J. Dermatol.* 175 (2), 273–286. doi:10.1111/bjd.14493

Papp, K. A., Tyring, S., Lahfa, M., Prinz, J., Griffiths, C. E., Nakanishi, A. M., et al. (2005). A Global Phase III Randomized Controlled Trial of Etanercept in Psoriasis: Safety, Efficacy, and Effect of Dose Reduction. *Br. J. Dermatol.* 152 (6), 1304–1312. doi:10.1111/j.1365-2133.2005.06688.x

Parodi, A., Aste, N., Calvieri, C., Cantoresi, F., Carlesimo, M., Fabbri, P., et al. (2014). Metabolic Syndrome Prevalence in Psoriasis: a Cross-Sectional Study in the Italian Population. *Am. J. Clin. Dermatol.* 15 (4), 371–377. doi:10.1007/s40257-014-0074-8

Pestel, J., Chehimi, M., Bonhomme, M., Robert, M., Vidal, H., and Eljaafari, A. (2020). IL-17A Contributes to Propagation of Inflammation but Does Not Impair Adipogenesis And/or Insulin Response, in Adipose Tissue of Obese Individuals. *Cytokine* 126, 154865. doi:10.1016/j.cyto.2019.154865

Piaserico, S., Osto, E., Famoso, G., Zanetti, I., Gregori, D., Poretto, A., et al. (2016). Treatment with Tumor Necrosis Factor Inhibitors Restores Coronary Microvascular Function in Young Patients with Severe Psoriasis. *Atherosclerosis* 251, 25–30. doi:10.1016/j.atherosclerosis.2016.05.036

Pina, T., Armesto, S., Lopez-Mejias, R., Genre, F., Ubilla, B., Gonzalez-Lopez, M. A., et al. (2015). Anti-TNF-α Therapy Improves Insulin Sensitivity in Non-diabetic Patients with Psoriasis: a 6-month Prospective Study. *J. Eur. Acad. Dermatol. Venereol.* 29 (7), 1325–1330. doi:10.1111/jdv.12814

Poizeau, F., Nowak, E., Kerbrat, S., Le Nautout, B., Droitcourt, C., Drici, M. D., et al. (2020). Association between Early Severe Cardiovascular Events and the Initiation of Treatment with the Anti-interleukin 12/23p40 Antibody Ustekinumab. *JAMA Dermatol.* 156 (11), 1208–1215. doi:10.1001/jamadermatol.2020.2977

Reich, K., Armstrong, A. W., Foley, P., Song, M., Wasfi, Y., Randazzo, B., et al. (2017). Efficacy and Safety of Guselkumab, an Anti-interleukin-23 Monoclonal Antibody, Compared with Adalimumab for the Treatment of Patients with Moderate to Severe Psoriasis with Randomized Withdrawal and Retreatment: Results from the Phase III, Double-Blind, Placebo- and Active Comparator-Controlled VOYAGE 2 Trial. *J. Am. Acad. Dermatol.* 76 (3), 418–431. doi:10.1016/j.jaad.2016.11.042

Reich, K., Langley, R. G., Lebwohl, M., Szapary, P., Guzzo, C., Yeilding, N., et al. (2011). Cardiovascular Safety of Ustekinumab in Patients with Moderate to Severe Psoriasis: Results of Integrated Analyses of Data from Phase II and III Clinical Studies. *Br. J. Dermatol.* 164 (4), 862–872. doi:10.1111/j.1365-2133.2011.10257.x

Reich, K., Nestle, F. O., Papp, K., Ortonne, J. P., Evans, R., Guzzo, C., et al. (2005). Infliximab Induction and Maintenance Therapy for Moderate-To-Severe Psoriasis: a Phase III, Multicentre, Double-Blind Trial. *Lancet* 366 (9494), 1367–1374. doi:10.1016/S0140-6736(05)67566-6

Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P., and Hennekens, C. H. (1997). Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men. *N. Engl. J. Med.* 336 (14), 973–979. doi:10.1056/NEJM199704033361401

Ridker, P. M. (2010). Psoriasis, Inflammation, and Vascular Risk: a Problem More Than Skin Deep. *Eur. Heart J.* 31 (8), 902–904. doi:10.1093/eurheartj/ehq042

Robati, R. M., Partovi-Kia, M., Haghighatkhah, H. R., Younespour, S., and Abdollahimajd, F. (2014). Increased Serum Leptin and Resistin Levels and Increased Carotid Intima-media wall Thickness in Patients with Psoriasis: Is Psoriasis Associated with Atherosclerosis. *J. Am. Acad. Dermatol.* 71 (4), 642–648. doi:10.1016/j.jaad.2014.06.006

Rodríguez-Zúñiga, M. J. M., and García-Perdomo, H. A. (2017). Systematic Review and Meta-Analysis of the Association between Psoriasis and Metabolic Syndrome. *J. Am. Acad. Dermatol.* 77 (4), 657–e8. doi:10.1016/j.jaad.2017.04.1133

Rompoti, N., Sidiropoulou, P., Panagakis, P., Stratigos, A., Papoutsaki, M., Stefanaki, E., et al. (2020). Real-world Data from a Single Greek centre on the Use of Secukinumab in Plaque Psoriasis: Effectiveness, Safety, Drug Survival, and Identification of Patients that Sustain Optimal Response. *J. Eur. Acad. Dermatol. Venereol.* 34 (6), 1240–1247. doi:10.1111/jdv.16202

Rønholt, K., and Iversen, L. (2017). Old and New Biological Therapies for Psoriasis. *Int. J. Mol. Sci.* 18 (11), 2297. doi:10.3390/ijms18112297

Roubille, C., Richer, V., Starnino, T., McCourt, C., McFarlane, A., Fleming, P., et al. (2015). The Effects of Tumour Necrosis Factor Inhibitors, Methotrexate, Non-steroidal Anti-inflammatory Drugs and Corticosteroids on Cardiovascular Events in Rheumatoid Arthritis, Psoriasis and Psoriatic Arthritis: a Systematic Review and Meta-Analysis. *Ann. Rheum. Dis.* 74 (3), 480–489. doi:10.1136/annrheumdis-2014-206624

Rungapiromnan, W., Yiu, Z. Z. N., Warren, R. B., Griffiths, C. E. M., and Ashcroft, D. M. (2017). Impact of Biologic Therapies on Risk of Major Adverse Cardiovascular Events in Patients with Psoriasis: Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Br. J. Dermatol.* 176 (4), 890–901. doi:10.1111/bjd.14964

Ryan, C., Leonardi, C. L., Krueger, J. G., Kimball, A. B., Strober, B. E., Gordon, K. B., et al. (2011). Association between Biologic Therapies for Chronic Plaque Psoriasis and Cardiovascular Events: a Meta-Analysis of Randomized Controlled Trials. *JAMA* 306 (8), 864–871. doi:10.1001/jama.2011.1211

Sajja, A. P., Joshi, A. A., Teague, H. L., Dey, A. K., and Mehta, N. N. (2018). Potential Immunological Links between Psoriasis and Cardiovascular Disease. *Front. Immunol.* 9, 1234. doi:10.3389/fimmu.2018.01234

Salahuddin, T., Natarajan, B., Playford, M. P., Joshi, A. A., Teague, H., Masmoudi, Y., et al. (2015). Cholesterol Efflux Capacity in Humans with Psoriasis Is Inversely Related to Non-calcified burden of Coronary Atherosclerosis. *Eur. Heart J.* 36 (39), 2662–2665. doi:10.1093/eurheartj/ehv339

Saraceno, R., Schipani, C., Mazzotta, A., Esposito, M., Di Renzo, L., De Lorenzo, A., et al. (2008). Effect of Anti-tumor Necrosis Factor-Alpha Therapies on Body Mass index in Patients with Psoriasis. *Pharmacol. Res.* 57 (4), 290–295. doi:10.1016/j.phrs.2008.02.006

Saurat, J. H., Stingl, G., Dubertret, L., Papp, K., Langley, R. G., Ortonne, J. P., et al. (2008). Efficacy and Safety Results from the Randomized Controlled Comparative Study of Adalimumab vs. Methotrexate vs. Placebo in Patients with Psoriasis (CHAMPION). *Br. J. Dermatol.* 158 (3), 558–566. doi:10.1111/j.1365-2133.2007.08315.x

Sbidian, E., Chaimani, A., Garcia-Doval, I., Do, G., Hua, C., Mazaud, C., et al. (2017). Systemic Pharmacological Treatments for Chronic Plaque Psoriasis: a Network Meta-Analysis. *Cochrane Database Syst. Rev.* 12, CD011535. doi:10.1002/14651858.CD011535.pub2

Shapiro, J., Cohen, A. D., Weitzman, D., Tal, R., and David, M. (2012). Psoriasis and Cardiovascular Risk Factors: a Case-Control Study on Inpatients Comparing Psoriasis to Dermatitis. *J. Am. Acad. Dermatol.* 66 (2), 252–258. doi:10.1016/j.jaad.2010.11.046

Shelling, M. L., and Kirsner, R. S. (2013). Practice Gaps. Gaining Insights into the Relationship of Obesity, Weight Loss, and Psoriasis. *JAMA Dermatol.* 149 (7), 801–802. doi:10.1001/jamadermatol.2013.3383

Siegel, D., Devaraj, S., Mitra, A., Raychaudhuri, S. P., Raychaudhuri, S. K., and Jialal, I. (2013). Inflammation, Atherosclerosis, and Psoriasis. *Clin. Rev. Allergy Immunol.* 44 (2), 194–204. doi:10.1007/s12016-012-8308-0

Sigurdardottir, G., Ekman, A. K., Ståhle, M., Bivik, C., and Enerbäck, C. (2014). Systemic Treatment and Narrowband Ultraviolet B Differentially Affect Cardiovascular Risk Markers in Psoriasis. *J. Am. Acad. Dermatol.* 70 (6), 1067–1075. doi:10.1016/j.jaad.2013.12.044

Simon, T., Taleb, S., Danchin, N., Laurans, L., Rousseau, B., Cattan, S., et al. (2013). Circulating Levels of Interleukin-17 and Cardiovascular Outcomes in Patients with Acute Myocardial Infarction. *Eur. Heart J.* 34 (8), 570–577. doi:10.1093/eurheartj/ehs263

Snekvik, I., Nilsen, T. I. L., Romundstad, P. R., and Saunes, M. (2019). Metabolic Syndrome and Risk of Incident Psoriasis: Prospective Data from the HUNT Study, Norway. *Br. J. Dermatol.* 180 (1), 94–99. doi:10.1111/bjd.16885

Solomon, D. H., Massarotti, E., Garg, R., Liu, J., Canning, C., and Schneeweiss, S. (2011). Association between Disease-Modifying Antirheumatic Drugs and Diabetes Risk in Patients with Rheumatoid Arthritis and Psoriasis. *JAMA* 305 (24), 2525–2531. doi:10.1001/jama.2011.878

Stanley, T. L., Zanni, M. V., Johnsen, S., Rasheed, S., Makimura, H., Lee, H., et al. (2011). TNF-alpha Antagonism with Etanercept Decreases Glucose and Increases the Proportion of High Molecular Weight Adiponectin in Obese

Subjects with Features of the Metabolic Syndrome. *J. Clin. Endocrinol. Metab.* 96 (1), E146–E150. doi:10.1210/jc.2010-1170

Stern, R. S. (2010). Psoriasis Is Not a Useful Independent Risk Factor for Cardiovascular Disease. *J. Invest. Dermatol.* 130 (4), 917–919. doi:10.1038/jid.2009.446

Taleb, S., Romain, M., Ramkhelawon, B., Uyttenhove, C., Pasterkamp, G., Herbin, O., et al. (2009). Loss of SOCS3 Expression in T Cells Reveals a Regulatory Role for Interleukin-17 in Atherosclerosis. *J. Exp. Med.* 206 (10), 2067–2077. doi:10.1084/jem.20090545

Taleb, S., Tedgui, A., and Mallat, Z. (2015). IL-17 and Th17 Cells in Atherosclerosis: Subtle and Contextual Roles. *Arterioscler Thromb. Vasc. Biol.* 35 (2), 258–264. doi:10.1161/ATVBAHA.114.303567

Toussirot, Ã. r., Aubin, F. o., and Dumoulin, G. (2014). Relationships between Adipose Tissue and Psoriasis, with or without Arthritis. *Front. Immunol.* 5, 368. doi:10.3389/fimmu.2014.00368

Tzellos, T., Kyrgidis, A., and Zouboulis, C. C. (2013). Re-evaluation of the Risk for Major Adverse Cardiovascular Events in Patients Treated with anti-IL-12/23 Biological Agents for Chronic Plaque Psoriasis: a Meta-Analysis of Randomized Controlled Trials. *J. Eur. Acad. Dermatol. Venereol.* 27 (5), 622–627. doi:10.1111/j.1468-3083.2012.04500.x

van de Kerkhof, P. C., Griffiths, C. E., Reich, K., Leonardi, C. L., Blauvelt, A., Tsai, T. F., et al. (2016). Secukinumab Long-Term Safety Experience: A Pooled Analysis of 10 Phase II and III Clinical Studies in Patients with Moderate to Severe Plaque Psoriasis. *J. Am. Acad. Dermatol.* 75 (1), 83–e4. doi:10.1016/j.jaad.2016.03.024

von Stebut, E., Reich, K., Thaçi, D., Koenig, W., Pinter, A., Körber, A., et al. (2019). Impact of Secukinumab on Endothelial Dysfunction and Other Cardiovascular Disease Parameters in Psoriasis Patients over 52 Weeks. *J. Invest. Dermatol.* 139 (5), 1054–1062. doi:10.1016/j.jid.2018.10.042

Wan, M. T., Shin, D. B., Hubbard, R. A., Noe, M. H., Mehta, N. N., and Gelfand, J. M. (2018). Psoriasis and the Risk of Diabetes: A Prospective Population-Based Cohort Study. *J. Am. Acad. Dermatol.* 78 (2), 315–e1. doi:10.1016/j.jaad.2017.10.050

Wolf, N., Quaranta, M., Prescott, N. J., Allen, M., Smith, R., Burden, A. D., et al. (2008). Psoriasis Is Associated with Pleiotropic Susceptibility Loci Identified in Type II Diabetes and Crohn Disease. *J. Med. Genet.* 45 (2), 114–116. doi:10.1136/jmg.2007.053595

Wu, C. Y., Shieh, J. J., Shen, J. L., Liu, Y. Y., Chang, Y. T., and Chen, Y. J. (2015). Association between Antidiabetic Drugs and Psoriasis Risk in Diabetic Patients: Results from a Nationwide Nested Case-Control Study in Taiwan. *J. Am. Acad. Dermatol.* 72 (1), 123–130. doi:10.1016/j.jaad.2014.08.042

Wu, D., Hou, S. Y., Zhao, S., Hou, L. X., Jiao, T., Xu, N. N., et al. (2017). Efficacy and Safety of Interleukin-17 Antagonists in Patients with Plaque Psoriasis: a Meta-Analysis from Phase 3 Randomized Controlled Trials. *J. Eur. Acad. Dermatol. Venereol.* 31 (6), 992–1003. doi:10.1111/jdv.14125

Wu, J. J., Guérin, A., Sundaram, M., Dea, K., Cloutier, M., and Mulani, P. (2017). Cardiovascular Event Risk Assessment in Psoriasis Patients Treated with Tumor Necrosis Factor-α Inhibitors versus Methotrexate. *J. Am. Acad. Dermatol.* 76 (1), 81–90. doi:10.1016/j.jaad.2016.07.042

Wu, J. J., and Poon, K. Y. (2013). Association of Ethnicity, Tumor Necrosis Factor Inhibitor Therapy, and Myocardial Infarction Risk in Patients with Psoriasis. *J. Am. Acad. Dermatol.* 69 (1), 167–168. doi:10.1016/j.jaad.2013.02.019

Wu, J. J., and Poon, K. Y. (2013). Association of Gender, Tumor Necrosis Factor Inhibitor Therapy, and Myocardial Infarction Risk in Patients with Psoriasis. *J. Am. Acad. Dermatol.* 69 (4), 650–651. doi:10.1016/j.jaad.2013.04.035

Wu, J. J., Sundaram, M., Cloutier, M., Gauthier-Loiselle, M., Guérin, A., Singh, R., et al. (2018). The Risk of Cardiovascular Events in Psoriasis Patients Treated with Tumor Necrosis Factor-α Inhibitors versus Phototherapy: An Observational Cohort Study. *J. Am. Acad. Dermatol.* 79 (1), 60–68. doi:10.1016/j.jaad.2018.02.050

Wu, M. Y., Yu, C. L., Yang, S. J., and Chi, C. C. (2020). Change in Body Weight and Body Mass index in Psoriasis Patients Receiving Biologics: A Systematic Review and Network Meta-Analysis. *J. Am. Acad. Dermatol.* 82 (1), 101–109. doi:10.1016/j.jaad.2019.07.103

Wu, S., Han, J., Li, W. Q., and Qureshi, A. A. (2014). Hypertension, Antihypertensive Medication Use, and Risk of Psoriasis. *JAMA Dermatol.* 150 (9), 957–963. doi:10.1001/jamadermatol.2013.9957

Yang, Z. S., Lin, N. N., Li, L., and Li, Y. (2016). The Effect of TNF Inhibitors on Cardiovascular Events in Psoriasis and Psoriatic Arthritis: an Updated Meta-Analysis. *Clin. Rev. Allergy Immunol.* 51 (2), 240–247. doi:10.1007/s12016-016-8560-9

Yeung, H., Takeshita, J., Mehta, N. N., Kimmel, S. E., Ogdie, A., Margolis, D. J., et al. (2013). Psoriasis Severity and the Prevalence of Major Medical Comorbidity: a Population-Based Study. *JAMA Dermatol.* 149 (10), 1173–1179. doi:10.1001/jamadermatol.2013.5015

Zhu, Y., Hu, C., Lu, M., Liao, S., Marini, J. C., Yohrling, J., et al. (2009). Population Pharmacokinetic Modeling of Ustekinumab, a Human Monoclonal Antibody Targeting IL-12/23p40, in Patients with Moderate to Severe Plaque Psoriasis. *J. Clin. Pharmacol.* 49 (2), 162–175. doi:10.1177/0091270008329556

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

# Advantages of publishing in Frontiers

**OPEN ACCESS**
Articles are free to read for greatest visibility and readership

**FAST PUBLICATION**
Around 90 days from submission to decision

**HIGH QUALITY PEER-REVIEW**
Rigorous, collaborative, and constructive peer-review

**TRANSPARENT PEER-REVIEW**
Editors and reviewers acknowledged by name on published articles

**Frontiers**
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

**Visit us:** www.frontiersin.org
**Contact us:** frontiersin.org/about/contact

**REPRODUCIBILITY OF RESEARCH**
Support open data and methods to enhance research reproducibility

**DIGITAL PUBLISHING**
Articles designed for optimal readership across devices

**FOLLOW US**
@frontiersin

**IMPACT METRICS**
Advanced article metrics track visibility across digital media

**EXTENSIVE PROMOTION**
Marketing and promotion of impactful research

**LOOP RESEARCH NETWORK**
Our network increases your article's readership