Cell-based therapies for rheumatoid arthritis: opportunities and challenges

Yu-Jing Li and Zhu Chen

Abstract: Rheumatoid arthritis (RA) is the most common immune-mediated inflammatory disease characterized by chronic synovitis that hardly resolves spontaneously. The current treatment of RA consists of nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, conventional disease-modifying antirheumatic drugs (cDMARDs), biologic and targeted synthetic DMARDs. Although the treat-to-target strategy has been intensively applied in the past decade, clinical unmet needs still exist since a substantial proportion of patients are refractory or even develop severe adverse effects to current therapies. In recent years, with the deeper understanding of immunopathogenesis of the disease, cell-based therapies have exhibited effective and promising interventions to RA. Several cell-based therapies, such as mesenchymal stem cells (MSC), adoptive transfer of regulatory T cells (Treg), and chimeric antigen receptor (CAR)-T cell therapy as well as their beneficial effects have been documented and verified so far. In this review, we summarize the current evidence and discuss the prospect as well as challenges for these three types of cellular therapies in RA.

Keywords: chimeric antigen receptor T cell, mesenchymal stem cells, regulatory T cells, rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is one of the most common autoimmune diseases characterized by chronic synovitis which mainly involved in small joints, affecting up to 1% of the general population worldwide. The repertoire of therapeutic drugs of RA has been growing in the past decades. The current treatment of RA consists of nonsteroidal anti-inflammatory drugs (NSAIDs), glucocorticoids, conventional disease-modifying antirheumatic drugs (cDMARDs), and biologic agents, among which cDMARDs has long been the major therapy. Actually, the past decades have witnessed tremendous innovations in RA therapy. On one hand, more and more potential druggable targets have been identified and developed, such as cells [e.g. B cells and fibroblast-like synoviocytes (FLS)], cytokines [e.g. tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF)], signaling pathways [e.g. Janus kinase family (JAK), IL-1-receptor-associated kinase 4 (IRAK-4)], and epigenetic regulators (e.g. DNA methylation, histone modifications, and noncoding RNAs). On the other hand, the treat-to-target (T2T) strategy has been intensively advocated in clinical practice, leading to the improved outcome of RA patients. However, despite the advent of novel biologic agents, increasing adverse events such as opportunistic infections preclude their broad application in clinic. Furthermore, a substantial proportion of patients remain resistant (or have limited efficiency) to current treatments. Hence, more effective and safer therapies for RA are an unmet clinical need.

With the advancement of the research on the immunopathogenesis of RA and the progress of immunological techniques, cellular immunotherapy represented by mesenchymal stem cells (MSCs) has attracted increasing attention. Several preclinical and clinical studies have verified the efficacy and safety of MSC in RA, which
has been reviewed elsewhere. In addition, adoptive regulatory T cells (Treg) and chimeric antigen receptor (CAR)-T cells therapy also exhibit their application potential in a variety of autoimmune diseases including RA. This review summarizes current evidence in cell-based therapies for RA. The underlying mechanisms as well as the challenges in clinical application are also discussed.

**Dysregulated immune homeostasis in RA**

Dysregulation of various innate and adaptive immune cells, cytokines, and other regulatory mediators are involved in the progression of RA. Knowledge of the imbalance between the pro-inflammatory mediators and anti-inflammatory mediators is vital for understanding the pathophysiology of RA and designing new therapeutic strategies. It is well established that in active RA patients, the amount of peripheral T helper 17 (Th17) cell and mRNA expression of its transcription factor RORγt are significantly increased, compared with inactive RA patients and healthy people. The quantity of serum Th17 cells of RA patients is proved to be positively correlated with the disease activity score in 28 joints (DAS28), C-reactive protein (CRP), and anticitrullinated protein antibodies (ACPAs), indicating the principal role of Th17 cells in the inflammatory progression in RA. In comparison, peripheral Tregs and forkhead-box protein P3 (Foxp3), an indispensable transcription factor driving the differentiation of Treg cells, decrease in RA patients, causing Th17/Treg imbalance as well as an increase in disease severity. In a collagen-induced arthritis (CIA) mouse model, adoptive transfusion with Tregs reduced Th17 cells and alleviated both arthritis and bone destruction. Results from above studies validated that RA is a Th17-driven disease and Th17/Treg imbalance acts as a key factor in RA. Meanwhile, indirect evidence from previous studies reported that M1/M2 macrophage ratio greater than 1 in RA is associated with higher erythrocyte sedimentation rate (ESR), CRP, and more osteoclasts which exert a critical role in bone resorption and joint destruction. A more recent study using new nanoparticles has demonstrated highly effective in damping inflammation via inducing M1 macrophage apoptosis and M2 macrophage polarization, suggesting the M1/M2 imbalance might be another crucial pro-inflammatory mediator in the progression of RA.

Both Th17/Treg imbalance and M1/M2 imbalance give rise to dysregulation of numerous cytokines, Th17 and M1 macrophages in inflamed synovial tissue are the primary source of pro-inflammatory cytokines such as IL-1β, IL-17, IL-23, and TNF-α, which exacerbate the inflammation as well as the joint damage. Among them, IL-17 and TNF-α display a critical impact on the pathogenesis of RA. IL-17 and TNF-α initiate the secretion of degradative enzymes such as prostaglandins E2 (PGE2), matrix metalloproteinase (MMP)-1, MMP-9, and cathepsin K, leading to cartilage destruction. Both cytokines induce the production of many other pro-inflammatory cytokines such as IL-1β, IL-6, and IL-8, which not only aggregate the degradation of extracellular matrix protein but also stimulate the formation of inflammatory microenvironment in the synovium via CXCL8/IL-8. In RA, bone absorption is closely associated with the imbalance between excessive bone damage caused by osteoclasts and suppression of bone formation by osteoblasts. Accumulating evidence indicates that receptor activator for nuclear factor kappa-B ligand (RANKL) accelerates the formation of osteoclasts, resulting in severe bone erosion and cartilage degradation. IL-17 and TNF-α in synovial fibroblasts greatly enhance RANKL expression. In a RANKL transgenic mice model, a distinct reduction in bone mass was exhibited, caused by a rise in the osteoclast number. Osteoclasts activated by IL-17 and TNF-α secrete SEMA4D to further suppress bone formation. On osteoblasts, the Plexin-B1 binds SEMA4D and indirectly attenuates insulin-like growth factor-I (IGF-I) signaling that enhances osteoblast differentiation. To sum up, IL-17 and TNF-α promote bone erosion and inhibit bone formation. Notably, IL-17 also has the property of enhancing endothelial cell migration and the production of vascular endothelial growth factor (VEGF), prompting angiogenesis and pannus growth.

On the contrary, studies have illustrated that serum anti-inflammatory cytokines such as IL-4 and IL-5 in RA patients are remarkably lower than healthy people. IL-4 possesses both immunosuppressive and anti-osteoclastogenic role in the modulation of RA. In a murine CIA model treated with IL-4, a significant decrease in serum levels of rheumatoid factor (RF), CRP, antinuclear antibodies (ANA), and TNF-α was demonstrated. In addition, IL-9, IL-10, IL-13, IL-27, and IL-33 are all profound anti-arthritis
cytokines promoting resolution of inflammation via various manners, which were summarized in our previous work.39

MSC transplantation

Immunoregulatory effects of MSC

MSCs are stem cells that have the potential to regulate inflammatory processes and differentiate into mesenchymal lineages including osteoblasts and chondrocytes.42 MSCs are reported to generate a tolerogenic microenvironment by suppressing various innate and adaptive immune cells.43,44 Exerting strong immunomodulatory effects, MSC transplantation is currently viewed as a promising therapy for multiple diseases such as graft-versus-host disease (GVHD),45 inflammatory bowel disease (IBD),46 and kidney injury.47 The underlying mechanisms may attribute to soluble factors produced by the MSCs and responses carried out by both innate and adaptive immune cells (Figure 1).

Activated MSCs promote IL-10 and indoleamine 2,3-dioxygenase (IDO) expression, attenuating M1 macrophage function and supporting M2 macrophage activation.48,49 This is further confirmed that exosomes isolated from MSCs significantly decrease the level of M1 macrophage marker inducible nitric oxide synthase (iNOS) and promote M2 polarization.50,51 CD1c+ dendritic cells (DCs) are tolerogenic DCs which produce high levels of IL-10 and suppress T-cell proliferation in an IL-10-dependent manner.52 Umbilical cord-derived MSCs (UC-MSCs) are found to express FMS-related tyrosine kinase 3-ligand (FLT3L), which promotes the production and prevents the apoptosis of tolerogenic CD1c+ DCs; whereas knockdown of FLT3L in MSCs eliminates such effect, suggesting that FLT3L is indispensable for MSCs to exert immunosuppressive function.53

In adaptive immune systems, MSCs inhibit effector T cells (Teff) and B cells while prompt Tregs and Bregs, restoring the balanced inflammatory setting.54 In a fulminant hepatic failure model, MSCs were found to increase the ratio of Tregs/Th17 and downregulate TNF-α, interferon (IFN)-γ, IL-1β, and IL-6, restoring the anti-inflammatory state.55 MSCs mediate immunoregulatory responses partly by regulating DCs. Periapical lesions (PL)-MSC-developed DCs possess pro-tolerogenic properties and induce Tregs via IDO-1, immunoglobulin-like transcript (ILT)-3, and ILT-4.56 Apart from DCs, various soluble regulators contribute to the immunosuppressive effect of MSCs. TNF-α is a
priming factor to trigger the immunomodulatory effect of MSCs. TNF-α in inflammatory setting stimulates the expression of intercellular adhesion molecule (ICAM)-1, a pivotal regulator in MSCs. In models of IBD and GVHD, administration of bone marrow (BM)-MSCs that overexpressed ICAM-1 showed decreasing Th17 and IL-17, and an increase in Tregs and transcription of Foxp3.57,58 TNF-α-induced protein 3 (TNFAIP3), an ubiquitin-modifying enzyme, participates in the maintaining of immune homeostasis and prevents autoimmune diseases.59 TNFAIP3 in MSCs inhibits TNF-α generation and promotes IL-10 proliferation; whereas knockdown of TNFAIP3 weakens the immunosuppressive capacity of MSCs both in vitro and in vivo as indicated that inhibition of T-cell proliferation is reversed.60 In RA, MSC-derived exosomes are found to inhibit the activation of FLS, cells that exert a key role on the progression of RA, through miR-143-3p/TNFAIP3/NF-κB pathway, emphasizing the vital role of TNFAIP3.61 Moreover, hypoxia-inducible factor 1α (HIF1α) is a metabolic regulator required for MSC. Silencing of HIF1α in MSC is found to increase the ratio of Th17/Tregs, associated with the metabolic alteration and reduced immunosuppressive mediators like ICAM, IL-6, and nitric oxide (NO).62 In adipose tissue-derived MSCs (AD-MSC), the level of MMP9 is increased. Interestingly, inhibition of MMP9 significantly diminishes the suppressive effect on T<sub>eff</sub> cells of AD-MSC, suggesting that MMP9 plays a critical role in the immunomodulatory activity of MSCs.63 Astonishingly, IL-17 promotes immunosuppression rather than inflammation in the presence of MSCs. In a concanavalin A-induced hepatitis mouse model, physiologically pro-inflammatory cytokine IL-17 dramatically enhanced the immunosuppression of MSCs on T<sub>eff</sub> cells in an iNOS-dependent manner.64 Programmed cell death 1 ligand 1 (PDL1) is a costimulatory molecule which exerts strong negative effects on immune responses.65 In a CIA model, the immunomodulatory function of MSCs was demonstrated to be highly related to PDL1 as blockage of PDL1 was associated with noticeable mitigation of MSCs-based immunosuppression. On the contrary, the immunosuppressive effect was enhanced when PDL1 was overexpressed.66 MSCs also restrict inflammatory responses via mitochondria. Adoptive transfer of MSC-derived mitochondria was proved to increase the expression of Foxp3, cytotoxic T lymphocyte associated antigen-4 (CTLA-4), and transforming growth factor-β (TGF-β), which are involved in T<sub>eff</sub> cells suppression. In a GVHD mouse model, MSC-derived mitochondria indirectly led to significant reduction in tissue damage by alleviating CD4<sup>+</sup> and CD8<sup>+</sup> T-cell infiltration.67 In addition, MSCs exert suppressive function on B cells. In a study during which BM-MSCs and B cells were cocultured, proliferation of B cells was found to be inhibited by MSCs through a cessation in the G0/G1 phase of the cell cycle.68 MSCs also downregulate the function of B cells and induce the generation of regulatory B cells (Breg) which secrete IL-10.69 Besides, MSC-derived extracellular vesicles (MSC-EVs) are indispensable for MSC-based immunosuppression, which convert immune cells to immunosuppressive phenotypes via the delivery of immunoregulatory miRNAs and proteins.70

**Capacity of MSC in bone and cartilage regeneration**

MSCs possess regenerative property to repair parenchymal tissue and organs through differentiating into lineages of mesenchymal tissues, including MSC-osteoblast-osteocyte and MSC-chondroblast-chondrocyte lineages.71 Increasing evidence have demonstrated that MSCs prompt bone regeneration, mineralization, and enhance bone healing.72 It has been proved that MSCs promote osteoblast differentiation and are able to repair both preclinical calvarial bone defects and clinical diseases like severe mandibular ridge resorption.73-75 MSCs foster a regenerative microenvironment by secreting a broad spectrum of paracrine factors including MSC-EVs.76 MSC-EVs have been verified to enhance bone formation as shown in significantly increased osteogenic gene expression like type I collagen (COL I), alkaline phosphatase (ALP), osteocalcin (OCN), and osteopontin (OPN).77 In MSC-EVs, the pro-osteogenic microRNAs such as miR-10b and miR-21 are increased, while the anti-osteogenic microRNAs like miR-31, miR-144, and miR-221 are decreased.71 miR-135 is seen as another osteogenesis-related microRNA which is increased during the osteogenesis of AD-MSCs. miR-135 enhances the expression of bone markers and extracellular matrix calcium deposition through miR-135/Homeobox A2 (Hoxa2)/Runx2 pathway, whereas the knockdown of miR-135 blocks such processes. Exosomes isolated from BM-MSCs (BMSC-Exos) also significantly enhance bone growth by promoting angiogenesis.78 Exosome mimetics (EMs) possess an intrinsic homing effect with
high stability. It has been demonstrated that EMs in MSCs could enhance osteogenesis via inhibition of miR-29a.\textsuperscript{79} Indian hedgehog (Ihh) signaling pathway is a major member of endochondral bone development, which participates in the regenerative capacity of MSCs.\textsuperscript{80} MSCs that overexpressing Ihh possess a strong effect in promoting bone repair and the chondrogenesis.\textsuperscript{81} Pro-inflammatory cytokines in the inflammatory setting also promote osteogenesis. Activator protein 2a (AP2a) is found to promote differentiation in MSCs by enhancing Runx2 activity. AP2a also targets and directly represses transcription of BARX1, a downstream gene of AP2a inhibiting osteogenic differentiation potential in MSCs, indirectly prompting bone regeneration.\textsuperscript{82} Special AT-rich sequence-binding protein 2 (SATB2) is a downstream effector of bone morphogenetic protein (BMP) signaling involved in osteoblast differentiation and bone regeneration.\textsuperscript{83} SATB2 is found to enhance the expression of bone matrix proteins and osteogenic transcription factors in MSCs.\textsuperscript{84} Besides, MSCs effectively differentiate into osteoblasts and mineralize through IL-1/\textit{Wnt}-5a/Ror2 pathway, confirmed by enhanced ALP activity and expression of Runx2.\textsuperscript{85} In addition, MSCs indirectly promote bone regeneration by inhibiting the function of osteoclasts. MSC-EVs suppress the formation of osteoclasts via osteoprotegerin (OPG)-RANKL-RANK signaling pathway, thus indirectly promote the formation of bone tissues.\textsuperscript{86}

\textbf{MSC transplantation in RA}

Many preclinical studies indicated that in mouse models of CIA, treatment with MSCs obtained from various sources significantly alleviated synovitis and bone erosion.\textsuperscript{87,88} It has been demonstrated that human MSCs significantly attenuated inflammatory arthritis in CIA mice, with significantly lower inflammation and erosion score.\textsuperscript{89} In CIA rats treated with MSCs, joint injury and inflammation were significantly improved and pathological changes were ameliorated, including lymphocyte infiltration, synovial hyperplasia, cartilage damage, joint destruction, and excessive formation of pannus in the ankles.\textsuperscript{90} MSC transplantation also significantly downregulated the number of osteoclasts, IL-17 and TNF-\textit{\alpha} while enhanced the mRNA expression of IL-10 and TGF-\textit{\beta1} in the ankle joints.\textsuperscript{91} In another RA rat model, improvement of paw edema, RA score, cytokine assay, and antioxidant state was exhibited in rats that received MSCs.\textsuperscript{92} Results from other experiments showed that MSC treatment alleviated signs of synovitis in CIA mice, reverting to the values of healthy rats. This was evident by the decrease in the levels of RF, CRP and ANA.\textsuperscript{41,92} Moreover, TNF-\textit{\alpha} and monocyte chemoattractant protein-1 (MCP-1) levels decreased with MSC treatment. Similarly, gene expression data showed amelioration in mice receiving MSC therapy where cartilage oligomeric matrix protein (Comp), tissue inhibitor metalloproteinase-1 (Timp1), MMP-1, and IL-1 receptor (Il-1r) gene expression levels decreased.\textsuperscript{41,93} MSCs were also shown to promote Treg differentiation and reduce Th17 differentiation.\textsuperscript{89} In CIA mice after MSC transplantation, studies also found that both the number and function of follicular helper T (Tfh) cells were downregulated, which are capable of assisting B cells for the production of high-affinity autoantibodies.\textsuperscript{94} MSCs can mediate T cell apoptosis via FasL/Fas pathway, resulting in immune tolerance and ameliorating the severity of CIA in mice.\textsuperscript{95} Micro-CT imaging confirmed that osteoporosis and bone destruction in rats were significantly improved, and pathological analysis of the joint revealed that MSCs potentially inhibit synovial hyperplasia and cartilage destruction in CIA rats.\textsuperscript{96}

Also, several clinical studies have confirmed the safety and potential of MSCs on RA patients.\textsuperscript{97,98} In clinical trials, MSCs transplantation has been proved to be safe and effective (Table 1). The administration of many sources of MSCs was shown to be well tolerated, without evidence of dose-related toxicity.\textsuperscript{99,100} After MSCs transplantation, health assessment questionnaire (HAQ) and DAS28 of 1 and 3 years after treatment significantly decreased.\textsuperscript{97,101} Levels of ESR, CRP, RF, and anti-cyclic citrullinated peptide (anti-CCP) of 1 and 3 years after MSCs treatment were also lower in RA patients in various clinical trials.\textsuperscript{97,102} Further study showed that the Treg/Th17 ratio was increased in the MSCs therapy group, supporting that MSCs play important roles in regulating immune homeostasis in RA.\textsuperscript{103,104} MSCs can significantly inhibit T cells in RA patients and regulate the expression of cytokines in the pathophysiologic process of RA.\textsuperscript{105} After MSCs transplantation, patients showed improved immune balance as manifested by decreased IL-1\textit{\beta}, IL-6, IL-8, and TNF-\textit{\alpha} and increased TGF-\textit{\beta1} and IL-10 levels.\textsuperscript{98,104,106} In a phase I clinical trial, plasma concentration of B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) in refractory RA
| Clinical trial identifier | Status | Clinical phase | Country | Methods | Number of patients enrolled | Outcome measures | References |
|--------------------------|--------|----------------|---------|---------|----------------------------|-----------------|------------|
| NCT01873625              | Completed | II/III | Iran | MSC | 60 | Pain, physical activity, walking distance and imaging | Shadmanfar et al. | |
| NCT01643116              | Completed | I/II | Spain | AD-MSC | 53 | AE, serious AE and proportion of ACR20/ACR50/ACR70 | Ahara-Garcia et al. | |
| NCT01547091              | Completed | I/II | China | hUC-MSC + DMARDs | 200 | Safety, RA serology, DAS28 and assessment of pain | Qi et al., 2011; Wang et al., 2013; Wang et al., 2014 | |
| NCT01851070              | Completed | II | United States | MPCs | 48 | Safety and efficacy | He et al. | |
| NCT01985464              | Unknown | I/II | Panama | UC-MSC | 20 | AE, CRP, ESR, anticitrulline antibody, RF, HAQ, DAS28, EULAR | https://clinicaltrials.gov/ct2/show/NCT01985464?term=NCT01985464&draw=2&rank=1 | |
| NCT02221258              | Completed | I | Korea | UC-MSC | 9 | Safety | Park et al. | |
| NCT02643823              | Unknown | I | China | hUC-MSC + DMARDs | 40 | Severity of AE, RA serology and DAS28 |https://clinicaltrials.gov/ct2/show/NCT02643823?term=NCT02643823&draw=2&rank=1 | |
| NCT03333981              | Completed | I | China | UC-MSC | 9 | Safety | Ghoryani et al., 2016; Ghoryani et al., 2017; Gowari et al., 2018 | |
| NCT02634233              | Completed | I | United States | UC-MSC | 20 | AE, CRP, ESR, anticitrulline antibody, RF, HAQ, DAS28, EULAR | Ghoryani et al., 2016; Shiroyani et al., 2017 | |
| NCT03067870              | Not recruiting | I | Jordan | BM-MSC | 100 | VAS, physical activity and resurfacing of articular cartilage | https://clinicaltrials.gov/ct2/show/NCT03067870?term=NCT03067870&draw=2&rank=1 | |
| NCT03798028              | Unknown | II | China | UC-MSC | 250 | HBG, FVC, or DLCO, or all three, ACR 20/50/70, white blood cell and platelet count, rate of blood routine hemoglobin, high resolution CT of lung and 6-min walking distance | https://clinicaltrials.gov/ct2/show/NCT03798028?term=NCT03798028&draw=2&rank=1 | |
| NCT03186417              | Recruiting | I | United States | hMSC | 20 | DLT, spirometry, AE, and DAS28-CRP | He et al., 2018 | |

(Continued)
| Clinical trial identifier | Status | Clinical phase | Date (start-completion) | Country | Methods | Number of patients enrolled | Outcome measures | References |
|--------------------------|--------|----------------|-------------------------|---------|---------|-----------------------------|----------------|------------|
| NCT03691909              | Completed | I/II          | 2018–2020               | United States | AD-MSC  | 15                           | AE, TNF-α, IL-6, CRP, ESR, and Joint Count 66/68 | https://clinicaltrials.gov/ct2/show/NCT03691909?term=NCT03691909&draw=2&rank=1 |
| NCT03618784              | Recruiting | I/II        | 2018–2021               | Korea | UC-MSC  | 33                           | AE, ACR20/50/70, EULAR, DAS 28-ESR, KHAQ, CDAI, Pain VAS, and change in cytokine | https://clinicaltrials.gov/ct2/show/NCT03618784?term=NCT03618784&draw=2&rank=1 |
| NCT03828344              | Active, not recruiting | I  | 2020–2022               | United States | UC-MSC  | 16                           | AE and serious AE, ACR20/50/70, DAS28-CRP, HAQ-DI, SDAI, RF, and anti-CCP | https://clinicaltrials.gov/ct2/show/NCT03828344?term=NCT03828344&draw=2&rank=1 |
| NCT04971980              | Recruiting | I/II        | 2021–2022               | China | UC-MSC  | 9                            | AE, changes of vital signs, complete blood count, blood biochemical, coagulation function, routine urine analysis, urine pregnancy test, cardiac rate, ACR20/50/70, TNF-α, IL-6, DAS28, HAQ, SDAI, and CDAI | https://clinicaltrials.gov/ct2/show/NCT04971980?term=NCT04971980&draw=2&rank=1 |
| NCT04170426              | Active, not recruiting | I/II      | 2021–2024               | United States | AD-MSC  | 54                           | AE, serious AE, and proportion of ACR20 patients | https://clinicaltrials.gov/ct2/show/NCT04170426?term=NCT04170426&draw=1&rank=1 |
| NCT05003934              | Recruiting | I  | 2021–2025               | Antigua and Barbuda | AlloRx  | 20                           | AE, DAS28, and VAS | https://clinicaltrials.gov/ct2/show/NCT05003934?term=NCT05003934&draw=2&rank=1 |

ACR, American College of Rheumatology; AD, adipose tissue; AE, adverse events; anti-CCP, anti-cyclic citrullinated peptide; BM, bone marrow; CDAI, clinical disease activity index; CRP, C-reactive protein; DAS28, Disease Activity Score using 28 joint counts; DLCO, carbon monoxide diffusing capacity; DLT, Dose limiting toxicity; DMARD, disease-modifying anti-rheumatic drugs; ESR, erythrocyte sedimentation rate; EULAR, European League against Rheumatism; FVC, forced vital capacity; HAQ, Quality of Life Questionnaire; HBG, blood routine hemoglobin; hUC, human umbilical cord; IFN, interferon; IL, interleukin; KHAQ, Korean Health assessment questionnaire; MPC, Mesenchymal Precursor Cells; MSC, mesenchymal stem cells; RA, rheumatoid arthritis; RF, rheumatoid factor; SDAI, Simplified Disease Activity Index; TNF, tumor necrosis factor; Treg, regulatory T cell; VAS, visual analogue scale.
patients, cytokines from a large family of TNF promoting differentiation and survival of B cells, significantly decreased after the MSCs transplantation. Consistently, the gene expression of BAFF-receptor significantly decreased on month 6 after the MSC transplantation. Surprisingly, IFN-γ strongly enhance the clinical effects of MSCs. In a phase I/II randomized controlled trial, the clinical symptoms, disease activity indexes, and antibodies levels were more promptly ameliorated in patients receiving MSCs together with IFN-γ compared with single MSCs treatment. Moreover, the ratio of Tregs/Th17 was also enhanced more rapidly. In addition, researchers found that IFN-γ may play a vital role in predicting clinical responses of RA patients to MSCs as high level of IFN-γ was related to decreasing DAS28 and high Tregs/Th17 ratio. Evidence from above clinical trials supports the notion that MSCs transplantation may be a promising, safe and effective therapy strategy to treat RA.

**Adoptive regulatory T cells immunotherapy**

Tregs are a subset of specialized CD4+ helper T cells defined by the expression of the IL-2 receptor α-chain (CD25) and the transcription factor Foxp3. Having a pivotal function in maintaining immune tolerance by down-regulating aberrant immune stimulations, Tregs are reported to be important in the prevention of autoimmune diseases such as autoimmune gastritis, thyroiditis, graft rejection and multiple sclerosis. Depletion of Tregs results in an exacerbation of arthritis and development of gastritis and thyroiditis. Adoptive Tregs immunotherapy has been proved critically vital in the prevention of autoimmune disease, tissue repair, and regeneration, suggesting that manipulation of these cells is a promising alternative treatment to treat or even cure autoimmune diseases. Currently, researchers have successfully used Tregs in the treatment of established colitis. Prophylactic treatment with CD4+ CD25+ Tregs has also been reported to diminish the severity of other autoimmune diseases such as GVHD, experimental autoimmune encephalomyelitis (EAE), and diabetes. In RA, dysregulation of self-tolerance leads to generation of autoreactive lymphocytes which target tissue-specific auto-antigens in joints. The ultimate treatment strategy should be to re-induce self-tolerance before severe tissue damage. Therefore, Tregs are ideal options. However, compared with the healthy people, apparent deficiencies are found in numbers and functions of Tregs in RA patients. The link between defects of Tregs in RA patients and mechanisms of autoimmunity establishes the foundation for using adoptive Tregs therapy to treat RA.

**Effects of Tregs on immune modulation**

Tregs downregulate immune response by modulating proliferation, activation, and cytokine production of APC, CD4+, CD8+ T cells and B cells. Tregs exert immunosuppressive effects mainly by two mechanisms: contact-dependent or contact-independent suppression. Lymphocyte activation gene 3 (LAG-3) and CTLA-4 are surface markers expressed on Tregs acting in contact-dependent modulation. By binding to MHC-II, LAG-3 mitigates the costimulatory capacity of DCs and the immune response of T cells. CTLA-4 downregulates costimulatory markers CD80/CD86 on APCs, inhibiting antigen presentation of APCs and inhibition of T-cell activation. In contact-independent manners, Tregs secrete anti-inflammatory cytokines like TGF-β, IL-10, and IL-3 and cytolytic factors such as granzyme A, granzyme B, and perforin. Apart from the above well-known mechanisms, Tregs also efficiently induce mitochondrion-dependent cell death of DC through Bax and Bak (members of pro-apoptotic Bcl-2 family)-dependent cell death mechanisms, thus regulating antigen-specific immune responses and restoring immune tolerance. Induced CD4+ Foxp3+ Tregs (iTregs) stimulate splenic CD11c+ DCs and generate DCiTregs which is a tolerogenic phenotype and secretes high levels of anti-inflammatory cytokines in CIA model like IL-10, TGF-β, and IDO, exhibiting immunosuppressive activity. In Tregs, V-domain Ig suppressor of T cell activation (VISTA), a novel immunoglobulin superfamily ligand, is highly expressed. VISTA is able to suppress T-cell function, whereas anti-VISTA mono-antibody significantly converts resting CD8+ T cell into active cells. SUMO-specific proteases (SENPs) are enzymes mediating deSUMOylation. SENPs are found to be important regulators for the stability and function of Treg by enhancing deSUMOylation and inhibiting the transcription of T eff while increased SUMOylation prompts T eff activation. Tregs-derived TGF-β1 also participates in the maintaining of autoimmune suppression and peripheral tolerance, and block of expression of TGF-β1 is demonstrated to result in allergy and autoimmunity.

Progranulin (PGRN) is an...
anti-inflammatory growth factor that is broadly expressed in numerous cells including immune cells. In PGRN-deficient mice, severe inflammatory arthritis is developed and addition of PGRN could reverse such arthritis. In one CIA model, it was demonstrated that PGRN exerted immunosuppressive capacity by enhancing the secretion of IL-10 by Tregs, which is related to Foxo4 and Stat3.133

Adoptive Treg cells immunotherapy in arthritis

In adoptive Tregs immunotherapy, autologous or allogeneic Tregs are isolated, ex vivo activated, expanded, and then infused to the patient.134 Adoptive Tregs immunotherapy have been proved effective in animal models of autoimmune diseases such as CIA, colitis,135 autoimmune cholangitis,136 and glomerulonephritis.137 Of note, adoptive Tregs immunotherapy has been used in clinical trials to prevent systemic lupus erythematosus (SLE),138 type 1 diabetes mellitus (T1DM),138 and GVHD19 for its successful outcomes.

In CIA models, studies have verified that adoptively transferred Tregs quickly appeared in synovial tissue after injection and blocked T-cell proliferation as well as type II collagen (CII)–specific proliferation, distinctly decreasing the severity and decelerating disease progression.110 Nonetheless, one biologic characteristic of Tregs is functional instability and conversion phenotypically to detrimental T eff.119 The foremost bottleneck of adoptive Tregs therapy is maintaining the stability and plasticity of Tregs. Moreover, Tregs in peripheral blood constitute only 1% to 3% of the mature CD4 + T cell subpopulation in mice and humans, which is another challenge for clinical application.140 The prerequisites for successful adoptive Tregs immunotherapy are identification of specific auto-antigen, effective methods to expand Tregs, and proper epigenetic regulation of Tregs stability.

Obtaining antigen-specific Tregs. It has been reported that polyclonal Tregs prevented and almost ceased arthritis progression.23 However, polyclonal Tregs transfer may cause temporary suppression of the whole immune system.141 Antigen-specific Tregs, developing antigen-specific tolerance by inhibiting Ag-specific T eff cells while also permitting an intact immune response toward other antigens, can be a suitable treatment strategy with low systemic side effects.142 T-cell receptor (TCR), responsible for recognizing fragments of antigens, is essentially required for maturation and function of Tregs.143 To generate self-Ag specific Tregs, it is a good choice to use TCR transduction. In one study, OTI1-TCR gene was transferred into purified CD4 + CD25 + T cells to redirect the specificity of naturally occurring Tregs. Adoptive transfer of gene modified Tregs into recipient mice has been proved to achieve targeted immune suppression, improving inflammatory knee swelling and reducing the numbers of Th17 cells.144 Although TCR transduction in T cells has been proved to be safe, feasible, and applicable, there are still safety concerns caused by cross-reactivity with healthy tissues.145 Another approach to generate antigen-specific Tregs is to culture CD4 + T cells from CIA animals with TGF-β1, IL-2, retinoic acid, APCs, and CII then expand such CII-specific Tregs with anti-CD3 antibody, anti-CD28 antibody, TGF-β1, IL-2, and retinoic acid. Adoptive transfer of these antigen-specific Tregs has been shown to be stable in vivo and reverse the CIA progression by suppressing the proliferation of CD4 + T cells and the key inflammatory cytokine TNF-α.146

Type I Tregs (Tr1) are unique Tregs producing high levels of IL-10. In CII-specific TCR transgenic mice, CII-specific Tr1 (Col-Treg) cells were isolated and expanded. Infusion of Col-Treg cells significantly dampened the proliferation of antigen-specific T eff and reduced the incidence and severity of arthritis symptoms as measured by reductions in paw swelling, arthritic scores, and CII-specific antibody titers, providing a great perspective for the development of novel therapeutic approaches to RA.147 Although specific disease-inducing antigens are important, it is unknown which antigens are critical for RA. Bystander antigens such as Peptide19 has been demonstrated to be similarly effective in inducing T-cell regulation and suppressing arthritis.148,149 Peptide19 is a predominant epitope and a target molecule driving epitope spreading and bystander suppression in autoimmune diseases.150 Adoptively transferred peptide 19-specific naïve CD4 + CD25 + CD45RA + Tregs (45RA-Tregs) into mice had a strong power to suppress T-cell function and alleviate CIA, as indicated by the complete elimination of footpad swelling, prolonged survival, little histopathologic changes, and preferential localization of CD4 + CD25 + Tregs at the articular joints.148
Expansion of antigen-specific Tregs. To reach a successful adoptive Tregs therapy for RA patients, sufficient number of infused cells and effective expansion of Ag-specific Tregs without losing their specificity or capacities are required. There are two major strategies to augment the number of Tregs: expanding antigen-specific Tregs or converting antigen-specific conventional T cells into Tregs ex vivo.

Tregs-specific targeted gene proliferation stimulators and immune complexes are broadly used to promote the expansion of Tregs. One basic adoptive cell therapy is to expand Tregs with anti-CD3, anti-CD28, IL-2, and rapamycin, increasing the number of Tregs up to thousands of times without losing the inhibitory activity. In a prospective, open-label, phase I to IIa study treating patients with RA, low-dose IL-2 was demonstrated to be well tolerated and effective, which activated and expanded specific Treg without Teff cells activation, thus increasing the Treg/Teff. There are other strategies for expanding Tregs. For instance, CD28 superagonist stimulation in vitro is more efficient in promoting Tregs proliferation compared with anti-CD3/antiCD28 method. Studies have also verified that employing superagonistic anti-CD28 antibodies and IL-2 was a promising protocol for large-scale in vitro generation of Tregs. Tregs can be expanded by APCs. Vitamin D3, glucocorticoids, IL-10, TGF-β, and vasoactive intestinal peptide induce tolerogenic DCs, expanding Treg cells indirectly. Induced Tregs expanded by mature tolerogenic DCs (iTregmtDC), suppressed CD4+ T-cell proliferation and Th17-cell differentiation to a greater extent and reduced the severity and progression of CIA more significantly than iTregs. iTregmtDC also has the capacity to increase the production of IFN-γ, TGF-β, and IL-10, and reduce the level of TNF, IL-17, IL-6 and anti-CII IgG. In addition, immature BM-derived DCs (iDCs) could induce the expansion of CD4+ CD49b+ Tregs, which reverse clinical symptoms of arthritis and provide continuing protection. In mice adoptively transferred with CD4+ CD49b+ Tregs, histologic examination of arthritic joints revealed a decreased inflammatory synovitis, pannus formation, and inflammatory cell infiltration. In one adjuvant arthritis model, BM cells were cultured in the presence of GM-CSF, giving rise to a population of CD11c+ MHCII+ CD45R+ CD8- DCs (BMDCs). CD4+ T cells cocultured with the BMDCs were expanded to 90% of Foxp3+ Tregs. Such ex vivo–generated Tregs were proved to suppress the arthritis upon their reintroduction to the host.

Retroviral Foxp3 gene transfer converts naive CD4+ T cells into Tregs, whose phenotype is similar to the naturally occurring CD4+ CD25+ Foxp3+ Tregs. Study has proved that CII primed Foxp3-transduced T cells in CIA rats significantly reduced arthritis severity, T-cell infiltration, level of CII-specific IgG, and pro-inflammatory cytokine including IL-6, TNF-α, and IL-17. In another murine model of RA, FoxP3 with anti-apoptotic Bcl-2 family molecule Bcl-xL were linked and transduced into CD4+ T cells, which differentiated into functional Tregs. Adoptive transfer of Tregs expressing both Foxp3 and Bcl-xL was demonstrated effective in suppressing RA than CD4+ T cells expressing Foxp3 alone and sustained survival in vitro.

Apart from CD4+ Tregs, CD8+ Tregs also exert immunosuppressive effect. In a CIA model, human CD8+ T cells from peripheral blood mononuclear cells were induced and expanded with TGF-β1, rapamycin along with anti-CD3/28 and IL-2 in vitro, which turned into hCD8+ Tregs. After adoptive transfusion, hCD8+ Tregs exhibited vigorous suppression ability in CIA mice as indicated by the reduced clinical scores, levels of anti-collagen IgG antibody, cartilage erosion, CD4+ Treg proliferation, number of Th17, mRNA expression of IL-17, RANKL, and MMP1 and CD80/86 expression on DCs. B cells can also induce Tregs, known as Treg-of-B cells, which express LAG3 and IL-10. In a CIA model, Treg-of-B cells alleviated the disease severity as well as local and systemic inflammation by inhibiting IL-6, TNF-α, and TRAP, a common biomarker of functional osteoclasts. Ag-specific Tregs can be developed from induced pluripotent stem cells (iPSCs) as well. Adoptive transfer of Ag-specific iPSC-Tregs into Ag-induced arthritis (AIA) mice was shown to reduce joint inflammation and swelling and to prevent bone loss.

Maintaining the stability of expanded Tregs is another bottleneck in applications of adoptive Tregs immunotherapy. As an essential factor in the differentiation and function of Tregs, Foxp3 as well as its stable expression is required. In a recent experiment, adoptive transfer of TNF
receptor type II (TNFRII)$^+$ Tregs increased Foxp3 gene methylation in Tregs, ameliorating the inflammation and preventing aggravation of arthritis.$^{162}$ In contrast, the deletion of TNFRII resulted in increased severity of experimental arthritis and reduced both numbers and capacity of Tregs.$^{163}$ It is reported that phosphorylation of Foxp3 is regulated by a TGF-β activated kinase 1 (TAK1)-Nemo-like kinase (NLK) signaling pathway. TAK1-NLK pathway promotes the phosphorylation and thus enhances the stability of Foxp3 by inhibiting the combination with STUB1 E3-ubiquitin protein ligase. The lack of NLK in Tregs is found to increase the incidence of autoimmunity.$^{164}$ Moreover, as mentioned before, numerous genes and signal pathways are involved in the function of Tregs. Therefore, it is reasonable to infer that upregulating the expression of involved genes such as CTLA-4, PD-1 may increase the stability of Tregs.$^{165}$

Notably, one recent study has verified the concept of using Treg activation to treat RA. Tregalizumab is a monoclonal anti-CD4 IgG antibody selectively activating Tregs but not Teff cells.$^{166}$ In a phase IIb, randomized, placebo-controlled trial, the efficacy and safety of tregalizumab were measured in patients with active RA.$^{167}$ Although tregalizumab was shown to exert biological effects on CD4 modulation, it was not clinically effective compared with placebo. This was probably because that the expected effect would have been achieved after a longer treatment period due to some special mechanisms of Tregs activation. Therefore, it is reasonable to speculate that tregalizumab might be used in early phase or preclinical phase of RA in the future.

**Emerging CAR-T cells in the treatment of RA**

Malfunction of T-cell tolerance plays a principal role in the pathogenesis of many autoimmune diseases including RA. Therefore, restoring immune tolerance is of great significance. Nevertheless, current treatments have little effect on immune tolerance inverse. CAR-T cells are bioengineered redirected T lymphocytes expressing specific receptor in the membrane that can recognize a specific antigen of a target cell without MHC restriction, thus interacting with target cells.$^{168}$ To create CAR-T cells, T cells from peripheral blood of a patient are separated and then CAR genes are inserted into T cell genome, followed by expansion of manufactured CAR-T cells and infusion back into the patient.$^{169}$ Nowadays, the major use of CAR-T cells is in the treatment of various cancers. Recently, the CAR-T cells are also utilized in preclinical experiments of RA, colitis, SLE, pemphigus vulgaris (PV), EAE and type 1 diabetes, bringing new hope of treatment choice for autoimmune diseases.$^{170,171}$ In the preclinical models above, three different approaches have been used, including identifying a specific antigen in a target cell and initiating cytotoxic activity by CAR-T cells, generating a cytotoxic effect on autoantibody-releasing B cell by chimeric autoantibody receptor T cells (CAAR-T), and binding to a specific antigen in a target cell to exert regulatory function of Tregs by chimeric antigen receptor in regulatory T lymphocytes (CAR-Treg).$^{172}$ CAAR-T has a variation of modified CAR and consists of a specific antigen, a transmembrane domain, and an intracellular signaling domain.$^{173}$ CAAR-T targets autoreactive B cells and exerts selective cytotoxicity function only against immune cells that carry receptors to specific auto-antigen without universal immunosuppression. When the specific antigen of the CAAR-T cells encounters and binds to the specific auto-antibodies expressed on B cells, the B cells will be destroyed, thus reducing the generation of autoantibodies.$^{171}$ CAR-Tregs regulate autoimmune T cells by inducing anergy and immunological ignorance.$^{174}$ CAR-Tregs generate immunosuppressive cytokines such as IL-10, IL-35, and TGF-β and induce apoptosis of Teff cells via Fas-ligand, granzyme B/A, and perforin, thus suppressing T eff cells.$^{175}$

**CAR-T and autoimmune diseases**

Five-module receptor complexes consist of a TCR module, three CD3 signaling modules, and a coreceptor module, playing an indispensable role in T cells.$^{176}$ Biomimetic five-module chimeric antigen receptor (5MCAR), which consists of a surrogate coreceptor module and a chimeric receptor module composed of specific antigen and elements of the TCR, has been found to eliminate pathogenic T cells and mediate autoimmune disease in mice.$^{176}$ In a nonobese diabetic (NOD) mice model, adoptively transferred 5MCAR–T cells could rapidly home to inflamed pancreases and attenuate T1DM by targeting autoantibody CD4$^+$ T cells.$^{176}$ CD4$^+$ insulin-reactive T cells target the insulin β-chain 9–23 peptide (B:9–23) in NOD. IA$^d$ (a single MHC class II molecule)–insulin B:9–23 complex is crucial for the initiation of autoimmune responses in
the NOD mouse.\textsuperscript{177} mAb287 can selectively bind to IA\textsuperscript{g7}-B9–23 complexes and prevent T1DM.\textsuperscript{178} One study has verified that 287-CAR T cells selectively migrated to pancreatic lymph nodes after adoptive transfer and delayed disease onset, inhibiting islet autoimmunity.\textsuperscript{179} CD19 is a B-cell surface marker that is intimately involved in B-cell signaling, proliferation, and differentiation.\textsuperscript{180} In the (NZB × NZW) F1 and MRL\textsuperscript{fas/fas} mouse models of lupus, CD8\textsuperscript{+} T cells expressing CD19-targeted CARs persistently depleted CD19\textsuperscript{+} B cells and effectively eliminated autoantibody production, reversing disease manifestations and extending life spans of mice.\textsuperscript{181} A more recent study proved that in MRL-lpr mice, a spontaneous murine SLE disease model, adoptive transfer of anti-CD19 CAR T cells not only exerted durable therapeutic efficacy as sustained B-cell depletion but also prevented disease progression before the onset of symptoms.\textsuperscript{180}

In the antibody-mediated autoimmune disease PV, CAAR comprising the major PV auto-antigen desmoglein (Dsg) 3 was engineered to T cells. Dsg3 CAAR-T cells were shown to exhibit specific cytotoxicity against cells expressing anti-Dsg3 BCRs both in vitro and in vivo, dramatically decreasing Dsg3 serum autoantibody titers and ameliorating the symptoms.\textsuperscript{171}

Compared with CAR-T and CAAR-T, CAR-Tregs are more commonly investigated in autoimmune diseases. CAR redirects Tregs to the site where autoimmune activity happens, increasing their suppressive efficiency without systemic immunosuppression. In an acute experimental colitis model, tripartite chimeric receptor (TPCR) was developed, which composed of an antibody variable region specific for 2,4,6-Trinitrophenol (TNP) that was attached to the extracellular and transmembrane domain of the CD28 costimulatory molecule and intracellular domain of the stimulatory Fc-\gamma receptor chain. Adoptive transfer of Tregs that transgenically expressing TPCR resulted in antigen-specific Tregs accumulation and activation at inflamed colonic sites, leading to antigen-specific suppression and significant alleviation of acute experimental colitis induced by 2,4,6-trinitrobenzenesulphonic acid (TNBS).\textsuperscript{182} In T-cell transfer colitis model, carcinoembryonic antigen (CEA) transgenic CAR was redirected and introduced into Tregs to generate CEA-specific CAR-Treg. CEA CAR-Tregs were able to accumulate in the colons and suppress the severity of colitis of diseased mice.\textsuperscript{183} In the murine EAE model, murine Foxp3 gene along with CAR targeting myelin oligodendrocyte glycoprotein (MOG) were transduced to CD4\textsuperscript{+} T cells, generating MOG CAR-Tregs. Localizing to the central nervous system (CNS) and binding to MOG\textsuperscript{+} oligodendrocytes, CNS-targeting CAR-Tregs exhibited powerful protective effect and efficiently suppressed ongoing inflammation and diminished symptoms.\textsuperscript{184} In another T1D model, CAR was used to redirect T-cell specificity toward insulin and T_{eff} cells were transformed to Tregs by Foxp3 transduction. Such insulin-targeted CAR-Tregs exerted stable suppressive function in the pancreas of diabetic mice.\textsuperscript{185} Vitiligo is an autoimmune disease in which ganglioside D3 (GD3) antigen is found in stressed melanocytes and contributes to melanogenesis.\textsuperscript{186} In a mouse model of progressive vitiligo, GD3-responsive CAR was introduced to Tregs. GD3 CAR-Treg protected melanocytes from T-cell-mediated destruction and significantly delayed the progress of depigmentation without obvious side effects.\textsuperscript{187}

**CAR-T in RA**

Since a CAR or CAAR targets only a single cell type, application of such novel treatment strategy to RA is limited, in which various types of autoreactive responses present. One study first designed antigenic peptides for specific recognition by pathogenic B cells and provided a direction for the customized treatment of RA according to patient’s specific auto-antigen profiles.\textsuperscript{17} In RA, ACPAs are one of the most specific serological markers which is associated with disease development.\textsuperscript{188} In this experiment, four citrullinated peptide epitopes including citrullinated vimentin, citrullinated type II collagen, citrullinated fibrinogen and tenasin C, and a cyclocitrulline peptide-1 were selected as ligands for targeting autoreactive B cells. Then, engineered T cells expressing a fixed anti-fluorescein isothiocyanate (FITC) CAR were constructed and tested to eliminate specific autoreactive B cells via recognition of the above FITC-labeled auto-antigenic peptide epitopes. It has been proved that specifically redirected anti-FITC CAR-T cells had the potential of recognizing corresponding FITC-labeled citrullinated peptide epitope and lysis autoreactive B-cell subsets from RA patients in vitro successfully.\textsuperscript{17}
Perspective and challenges
With accumulating evidence and progressing technologies, cell-based therapy has shown preliminary therapeutic potential for RA. However, thorough preclinical and clinical investigations are required to apply cell-based therapy in clinical practice, since such novel practical therapeutic strategy still faces multiple challenges. Most investigations of MSC-based therapeutic approaches are focused on evaluating the safety and efficacy, while the formulation, route of administration, dose, and frequency of MSC remain to be defined. In addition, whether MSC transplantation is effective only for a certain number of disease phenotypes is not known since MSC transplantation has not shown significant benefits in refractory RA. Moreover, although MSCs are reported to exert the significant immunosuppression on T cells in presence of strong inflammation, less inflammation milieu may weaken the suppression of MSCs on T cells, even promote T-cell responses. Likewise, adoptive Tregs therapy has similar shortcomings such as the probability of conversion into pathogenic cells. Lack of specific antigen on the surface of Tregs also makes it difficult to purify Tregs and thus increases the risk of contamination with T eff. As for CAR-based therapy, a major barrier in the CAR design for autoimmune therapy is the relative scarcity of cell-surface tissue-specific antigens since it is difficult to find proper specific antigens that target interested cells in autoimmune diseases. Stability, durability, safety, effectiveness, manufacturing, and persistence must be confirmed for CAR-T therapy to apply in clinic.

Author contribution(s)
Yu-jing Li: Investigation; Methodology; Writing – original draft.
Zhu Chen: Conceptualization; Investigation; Supervision; Writing – review & editing.

ORCID iD
Zhu Chen https://orcid.org/0000-0003-0828-1583

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Natural Science Foundation of China (grant numbers 81871227 and 81501344).

Conflict of interest statement
The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

References
1. Okamoto K, Nakashima T, Shinohara M, et al. Osteoimmunology: the conceptual framework unifying the immune and skeletal systems. Physiol Rev 2017; 97: 1295–1349.
2. Aletaha D and Smolen JS. Diagnosis and management of rheumatoid arthritis: a review. JAMA 2018; 320: 1360–1372.
3. Huang J, Fu X, Chen X, et al. Promising therapeutic targets for treatment of rheumatoid arthritis. Front Immunol 2021; 12: 686155.
4. Barnas JL, Looney RJ and Anolik JH. B cell targeted therapies in autoimmune disease. Curr Opin Immunol 2019; 61: 92–99.
5. Lin YJ, Anzaghe M and Schulke S. Update on the pathomechanism, diagnosis, and treatment options for rheumatoid arthritis. Cells 2020; 9: 880.
6. Nygaard G and Firestein GS. Restoring synovial homeostasis in rheumatoid arthritis by targeting fibroblast-like synoviocytes. Nat Rev Rheumatol 2020; 16: 316–333.
7. van Vollenhoven R. Treat-to-target in rheumatoid arthritis – are we there yet? Nat Rev Rheumatol 2019; 15: 180–186.
8. Becede M, Alasti F, Gessl I, et al. Risk profiling for a refractory course of rheumatoid arthritis. Semin Arthritis Rheum 2019; 49: 211–217.
9. Lopez-Santalla M, Bueren JA and Garin MI. Mesenchymal stem/stromal cell-based therapy for the treatment of rheumatoid arthritis: an update on preclinical studies. EBioMedicine 2021; 69: 103427.
10. Cheung TT and McInnes IB. Future therapeutic targets in rheumatoid arthritis? Semin Immunopathol 2017; 39: 487–500.
11. Luo Y, Wu W, Gu J, et al. Human gingival tissue-derived MSC suppress osteoclastogenesis and bone erosion via CD39-adenosine signal pathway in autoimmune arthritis. EBioMedicine 2019; 43: 620–631.
12. Liu R, Zhao P, Tan W, et al. Cell therapies for refractory rheumatoid arthritis. Clin Exp Rheumatol 2018; 36: 911–919.
13. Sarsenova M, Issabekova A, Abisheva S, et al. Mesenchymal stem cell-based therapy for...
rheumatoid arthritis. *Int J Mol Sci* 2021; 22: 11592.

14. Lopez-Santalla M, Fernandez-Perez R and Garin MI. Mesenchymal stem/stromal cells for rheumatoid arthritis treatment: an update on clinical applications. *Cells* 2020; 9: 1852.

15. Hwang JJ, Rim YA, Nam Y, et al. Recent developments in clinical applications of mesenchymal stem cells in the treatment of rheumatoid arthritis and osteoarthritis. *Front Immunol* 2021; 12: 631291.

16. Lv X, Wang L, Zou X, et al. Umbilical cord mesenchymal stem cell therapy for regenerative treatment of rheumatoid arthritis: opportunities and challenges. *Drug Des Devel Ther* 2021; 15: 3927–3936.

17. Zhang B, Wang Y, Yuan Y, et al. In vitro elimination of autoreactive B cells from rheumatoid arthritis patients by universal chimeric antigen receptor T cells. *Ann Rheum Dis* 2021; 80: 176–184.

18. Dall’Era M, Pauli ML, Remedios K, et al. Adoptive Treg cell therapy in a patient with systemic lupus erythematosus. *Arthritis Rheumatol* 2019; 71: 431–440.

19. Brunstein CG, Miller JS, McKenna DH, et al. Umbilical cord blood-derived T regulatory cells to prevent GVHD: kinetics, toxicity profile, and clinical effect. *Blood* 2016; 127: 1044–1051.

20. Wang W, Shao S, Jiao Z, et al. The Th17/Treg imbalance and cytokine environment in peripheral blood of patients with rheumatoid arthritis. *Rheumatol Int* 2012; 32: 887–893.

21. Lucas C, Perdriger A and Ame P. Definition of B cell helper T cells in rheumatoid arthritis and their behavior during treatment. *Semin Arthritis Rheum* 2020; 50: 867–872.

22. Zhong W, Zhao L, Liu T, et al. IL-22-producing CD4+T cells in the treatment response of rheumatoid arthritis to combination therapy with methotrexate and leflunomide. *Sci Rep* 2017; 7: 41143.

23. Sun J, Yang Y, Huo X, et al. Efficient therapeutic function and mechanisms of human polyclonal CD8(+)CD103(+)Foxp3(+) regulatory T cells on collagen-induced arthritis in mice. *J Immunol Res* 2019; 2019: 8575407.

24. Park H, Li Z, Yang XO, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; 6: 1133–1141.

25. Koenders MI and van den Berg WB. Translational mini-review series on Th17 cells: are T helper 17 cells really pathogenic in autoimmunity? *Clin Exp Immunol* 2010; 159: 131–136.

26. Fukui S, Iwamoto N, Takatani A, et al. M1 and M2 monocytes in rheumatoid arthritis: a contribution of imbalance of M1/M2 monocytes to osteoclastogenesis. *Front Immunol* 2017; 8: 1958.

27. Yang Y, Guo L, Wang Z, et al. Targeted silver nanoparticles for rheumatoid arthritis therapy via macrophage apoptosis and re-polarization. *Biomaterials* 2021; 264: 120390.

28. Hui W, Yu D, Cao Z, et al. Butyrate inhibit collagen-induced arthritis via Treg/IL-10/Th17 axis. *Int Immunopharmacol* 2019; 68: 226–233.

29. Tardito S, Martinelli G, Soldano S, et al. Macrophage M1/M2 polarization and rheumatoid arthritis: a systematic review. *Autoimmun Rev* 2019; 18: 102397.

30. Van Raemdonck K, Umar S, Palasiewicz K, et al. CCL21/CCR7 signaling in macrophages promotes joint inflammation and Th17-mediated osteoclast formation in rheumatoid arthritis. *Cell Mol Life Sci* 2020; 77: 1387–1399.

31. Laan M, Cui ZH, Hoshino H, et al. Neutrophil recruitment by human IL-17 via C-X-C chemokine release in the airways. *J Immunol* 1999; 162: 2347–2352.

32. Takayanagi H. Osteoimmunology: shared mechanisms and crosstalk between the immune and bone systems. *Nat Rev Immunol* 2007; 7: 292–304.

33. Cho E, Chen Z, Ding M, et al. PMSA prevents osteoclastogenesis and estrogen-dependent bone loss in mice. *Bone* 2021; 142: 115707.

34. Mizuno A, Kanno T, Hoshi M, et al. Transgenic mice overexpressing soluble osteoclast differentiation factor (sODF) exhibit severe arthritis. *Nat Rev Immunol* 1999; 162: 2347–2352.

35. Negishi-Koga T, Shinohara M, Komatsu N, et al. Suppression of bone formation by osteoclastic expression of semaphorin 4D. *Nat Med* 2011; 17: 1473–1480.

36. Pickens SR, Volin MV, Mandelin AM, et al. IL-17 contributes to angiogenesis in rheumatoid arthritis. *J Immunol* 2010; 184: 3233–3241.

37. Yang P, Qian FY, Zhang MF, et al. Targeted silver nanoparticles for rheumatoid arthritis therapy via macrophage apoptosis and re-polarization. *Biomaterials* 2021; 264: 120390.

38. Qu CH, Hou Y, Bi YF, et al. Diagnostic values of serum IL-10 and IL-17 in rheumatoid arthritis and their correlation with serum...
14–3-3eta protein. *Eur Rev Med Pharmacol Sci* 2019; 23: 1899–1906.

39. Chen Z, Bozec A, Ramming A, et al. Anti-inflammatory and immune-regulatory cytokines in rheumatoid arthritis. *Nat Rev Rheumatol* 2019; 15: 9–17.

40. Lubberts E, Joosten LA, Chabaud M, et al. IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. *J Clin Invest* 2000; 105: 1697–1710.

41. Haikal SM, Abdeltawab NF, Rashed LA. Mesenchymal stem cells increase skin graft survival time and up-regulate PD-L1 expression in splenocytes of mice. *Immunol Lett* 2017; 182: 39–49.

42. Dey D, Fischer NG, Dragon AH, et al. Culture and characterization of various porcine integumentary-connective tissue-derived mesenchymal stromal cells to facilitate tissue adhesion to percutaneous metal implants. *Stem Cell Res Ther* 2021; 12: 604.

43. Hu C and Li L. The immunoregulation of mesenchymal stem cells plays a critical role in improving the prognosis of liver transplantation. *J Transl Med* 2019; 17: 412.

44. Meisel R, Zibert A, Laryea M, et al. Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation. *Blood* 2004; 103: 4619–4621.

45. Boberg E, von Bahr L, Afram G, et al. Treatment of chronic GVHD with mesenchymal stromal cells induces durable responses: a phase II study. *Stem Cells Transl Med* 2020; 9: 1190–1202.

46. Soontararak S, Chow L, Johnson V, et al. Mesenchymal stem cells (MSC) derived from induced pluripotent stem cells (iPSC) equivalent to adipose-derived MSC in promoting intestinal healing and microbiome normalization in mouse inflammatory bowel disease model. *Stem Cells Transl Med* 2018; 7: 456–467.

47. Saad A, Dietz AB, Herrmann SMS, et al. Autologous mesenchymal stem cells increase cortical perfusion in renovascular disease. *J Am Soc Nephrol* 2017; 28: 2777–2785.

48. Saldana L, Bensiamar F, Valles G, et al. Immunoregulatory potential of mesenchymal stem cells following activation by macrophage-derived soluble factors. *Stem Cell Res Ther* 2019; 10: 58.

49. Moravej A, Geramizadeh B, Azarpira N, et al. Mesenchymal stem cells increase skin graft survival time and up-regulate PD-L1 expression in splenocytes of mice. *Immunol Lett* 2017; 182: 39–49.

50. Wei F, Li Z, Crawford R, et al. Immunoregulatory role of exosomes derived from differentiating mesenchymal stromal cells on inflammation and osteogenesis. *J Tissue Eng Regen Med* 2019; 13: 1978–1991.

51. Ren W, Hou J, Yang C, et al. Extracellular vesicles secreted by hypoxia pre-challenged mesenchymal stem cells promote non-small cell lung cancer cell growth and mobility as well as macrophage M2 polarization via miR-21-5p delivery. *J Exp Clin Cancer Res* 2019; 38: 62.

52. Qian C and Cao X. Naturally occurring CD1c+ human regulatory dendritic cells: immunoregulators that are expanded in response to E. *Eur J Immunol* 2012; 42: 1388–1392.

53. Yuan X, Qin X, Wang D, et al. Mesenchymal stem cell therapy induces FLT3L and CD1c(+) dendritic cells in systemic lupus erythematosus patients. *Nat Commun* 2019; 10: 2498.

54. Huang B, Cheng X, Wang H, et al. Mesenchymal stem cells and their secreted molecules predominantly ameliorate fulminant hepatic failure and chronic liver fibrosis in mice respectively. *J Transl Med* 2016; 14: 45.

55. Yoshizumi Y, Yukawa H, Iwaki R, et al. Immunomodulatory effects of adipose tissue-derived stem cells on concanavalin A-induced acute liver injury in mice. *Cell Med* 2017; 9: 21–33.

56. Dokic J, Tomic S, Markovic M, et al. Mesenchymal stem cells from periapical lesions modulate differentiation and functional properties of monocyte-derived dendritic cells. *Eur J Immunol* 2013; 43: 1862–1872.

57. Tang B, Li X, Liu Y, et al. The therapeutic effect of ICAM-1-overexpressing mesenchymal stem cells on acute graft-versus-host disease. *Cell Physiol Biochem* 2018; 46: 2624–2635.

58. Li X, Wang Q, Ding L, et al. Inter cellular adhesion molecule-1 enhances the therapeutic effects of MSCs in a dextran sulfate sodium-induced colitis model by promoting MSCs homing to murine colons and spleens. *Stem Cell Res Ther* 2019; 10: 267.

59. Das T, Bergen IM, Koudstaal T, et al. DNGR1-mediated deletion of A20/Tnfaip3 in dendritic cells alters T and B-cell homeostasis and promotes autoimmune liver pathology. *J Autoimmun* 2019; 102: 167–178.

60. Dang RJ, Yang YM, Zhang L, et al. A20 plays a critical role in the immunoregulatory function of...
mesenchymal stem cells. *J Cell Mol Med* 2016; 20: 1550–1560.

61. Su Y, Liu Y, Ma C, et al. Mesenchymal stem cell-originated exosomal IncRNA HAND2-AS1 impairs rheumatoid arthritis fibroblast-like synoviocyte activation through miR-143-3p/ TNFAIP3/NF-kappaB pathway. *J Orthop Surg Res* 2021; 16: 116.

62. Montesinos JJ, Lopez-Garcia L, Cortes-Morales VA, et al. Human bone marrow mesenchymal stem/stromal cells exposed to an inflammatory environment increase the expression of ICAM-1 and release microvesicles enriched in this adhesive molecule: analysis of the participation of TNF-alpha and IFN-gamma. *J Immunol Res* 2020; 2020: 8839625.

63. Lavini-Ramos C, Silva HM, Soares-Schanoski A, et al. MMP9 integrates multiple immunoregulatory pathways that discriminate high suppressive activity of human mesenchymal stem cells. *Sci Rep* 2017; 7: 874.

64. Han X, Yang Q, Lin L, et al. Interleukin-17 enhances immunosuppression by mesenchymal stem cells. *Cell Death Differ* 2014; 21: 1758–1768.

65. Dong H, Zhu G, Tamada K, et al. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med* 1999; 5: 1365–1369.

66. Wu W, Xiao ZX, Zeng D, et al. B7-H1 promotes the functional effect of human gingiva-derived mesenchymal stem cells on collagen-induced arthritis murine model. *Mol Ther* 2020; 28: 2417–2429.

67. Court AC, Le-Gatt A, Luz-Crawford P, et al. Mitochondrial transfer from MSCs to T cells induces Treg differentiation and restricts inflammatory response. *EMBO Rep* 2020; 21: e48052.

68. Corcione A, Benvenuto F, Ferretti E, et al. Human mesenchymal stem cells modulate B-cell functions. *Blood* 2006; 107: 367–372.

69. Cho KA, Lee JK, Kim YH, et al. Mesenchymal stem cells ameliorate B-cell-mediated immune responses and increase IL-10-expressing regulatory B cells in an EBI3-dependent manner. *Cell Mol Immunol* 2017; 14: 895–908.

70. Harrell CR, Jovicic N, Djonov V, et al. Mesenchymal stem cell-derived exosomes and other extracellular vesicles as new remedies in the therapy of inflammatory diseases. *Cells* 2019; 8: 1605.

71. Wang X and Thomsen P. Mesenchymal stem cell-derived small extracellular vesicles and bone regeneration. *Basic Clin Pharmacol Toxicol* 2021; 128: 18–36.

72. Li W, Liu Y, Zhang P, et al. Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration. *ACS Appl Mater Interfaces* 2018; 10: 5240–5254.

73. Kuhn LT, Liu Y, Boyd NL, et al. Developmental-like bone regeneration by human embryonic stem cell-derived mesenchymal cells. *Tissue Eng Part A* 2014; 20: 365–377.

74. Wang Z, Han L, Sun T, et al. Osteogenic and angiogenic lineage differentiated adipose-derived stem cells for bone regeneration of calvarial defects in rabbits. *J Biomed Mater Res A* 2021; 109: 538–550.

75. Gjerde C, Mustafa K, Hellem S, et al. Cell therapy induced regeneration of severely atrophied mandibular bone in a clinical trial. *Stem Cell Res Ther* 2018; 9: 213.

76. Yuan P, Li Z, Shao B, et al. Extracellular vesicles derived from starving BMSCs enhance survival of chondrocyte aggregates in grafts by attenuating chondrocyte apoptosis and enabling stable cartilage regeneration for craniofacial reconstruction. *Acta Biomater* 2022; 140: 659–673.

77. Takeuchi R, Katagiri W, Endo S, et al. Exosomes from conditioned media of bone marrow-derived mesenchymal stem cells promote bone regeneration by enhancing angiogenesis. *PLoS ONE* 2019; 14: e0225472.

78. Huang Y, He B, Wang L, et al. Bone marrow mesenchymal stem cell-derived exosomes promote rotator cuff tendon-bone healing by promoting angiogenesis and regulating M1 macrophages in rats. *Stem Cell Res Ther* 2020; 11: 496.

79. Fan J, Lee CS, Kim S, et al. Generation of small RNA-modulated exosome mimetics for bone regeneration. *ACS Nano* 2020; 14: 11973–11984.

80. Joeng KS and Long F. The Gli2 transcriptional activator is a crucial effector for Ihh signaling in osteoblast development and cartilage vascularization. *Development* 2009; 136: 4177–4185.

81. Zou S, Chen T, Wang Y, et al. Mesenchymal stem cells overexpressing Ihh promote bone repair. *J Orthop Surg Res* 2014; 9: 102.

82. Lin X, Yang H, Wang L, et al. AP2a enhanced the osteogenic differentiation of mesenchymal stem cells by inhibiting the formation of YAP/
RUNX2 complex and BARX1 transcription. Cell Prolif 2019; 52: e12522.

83. Zhao X, Qu Z, Tickner J, et al. The role of SATB2 in skeletogenesis and human disease. Cytokine Growth Factor Rev 2014; 25: 35–44.

84. Chen Q, Zheng L, Zhang Y, et al. Special AT-rich sequence-binding protein 2 (Satb2) synergizes with Bmp9 and is essential for osteo/odontogenic differentiation of mouse incisor mesenchymal stem cells. Cell Prolif 2021; 54: e13016.

85. Sonomoto K, Yamaoka K, Oshita K, et al. Interleukin-1beta induces differentiation of human mesenchymal stem cells into osteoblasts via the Wnt-5a/receptor tyrosine kinase-like orphan receptor 2 pathway. Arthritis Rheum 2012; 64: 3355–3363.

86. Liu L, Guo S, Shi W, et al. Bone marrow mesenchymal stem cell-derived small extracellular vesicles promote periodontal regeneration. Tissue Eng Part A 2021; 27: 962–976.

87. An L, Chu T, Wang L, et al. Frequent injections of high-dose human umbilical cord mesenchymal stem cells slightly aggravate arthritis and skeletal muscle cachexia in collagen-induced arthritic mice. Exp Ther Med 2020; 22: 1272.

88. Zhai Q, Dong J, Zhang X, et al. Mesenchymal stem cells enhance therapeutic effect and prevent adverse gastrointestinal reaction of methotrexate treatment in collagen-induced arthritis. Stem Cells Int 2021; 2021: 8850820.

89. Nam Y, Jung SM, Rim YA, et al. Intraperitoneal infusion of mesenchymal stem cell attenuates severity of collagen antibody induced arthritis. PLoS ONE 2018; 13: e0198740.

90. Shu J, Pan L, Huang X, et al. Transplantation of human amnion mesenchymal cells attenuates the disease development in rats with collagen-induced arthritis. Clin Exp Rheumatol 2015; 33: 484–490.

91. Li X, Lu C, Fan D, et al. 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 2020; 8: 131.

92. Abdelmawgoud H and Saleh A. Anti-inflammatory and antioxidant effects of mesenchymal and hematopoietic stem cells in a rheumatoid arthritis rat model. Adv Clin Exp Med 2018; 27: 873–880.

93. Wang YH, Yang ZQ, Zhu SF, et al. Comparative study of methotrexate and human umbilical cord mesenchymal stem cell transplantation in the treatment of rheumatoid arthritis. J Biol Regul Homeost Agents 2018; 32: 599–605.

94. Liu R, Li X, Zhang Z, et al. Allogeneic mesenchymal stem cells inhibited T follicular helper cell generation in rheumatoid arthritis. Sci Rep 2015; 5: 12777.

95. Roudsari PP, Alavi-Moghadam S, Rezaei-Tavirani M, et al. The outcome of stem cell-based therapies on the immune responses in rheumatoid arthritis. Adv Exp Med Biol 2021; 1326: 159–186.

96. Gao J, Zhang G, Xu K, et al. Bone marrow mesenchymal stem cells improve bone erosion in collagen-induced arthritis by inhibiting osteoclasia-related factors and differentiating into chondrocytes. Stem Cell Res Ther 2020; 11: 171.

97. Qi T, Gao H, Dong Y, et al. Cervus and cucumis peptides combined umbilical cord mesenchymal stem cells therapy for rheumatoid arthritis. Medicine 2020; 99: e21222.

98. Ghoryani M, Shariati-Sarabi Z, Tavakkol-Afshari J, et al. The sufficient immunoregulatory effect of autologous bone marrow-derived mesenchymal stem cell transplantation on regulatory T cells in patients with refractory rheumatoid arthritis. J Immunol Res 2020; 2020: 3562753.

99. Alvaro-Gracia JM, Jover JA, Garcia-Vicuna R, et al. Intravenous administration of expanded allogeneic adipose-derived mesenchymal stem cells in refractory rheumatoid arthritis (Cx611): results of a multicentre, dose escalation, randomised, single-blind, placebo-controlled phase Iib/Iia clinical trial. Ann Rheum Dis 2017; 76: 196–202.

100. Shadmanfar S, Labibzadeh N, Emadedin M, et al. Intra-articular knee implantation of autologous bone marrow-derived mesenchymal stromal cells in rheumatoid arthritis patients with knee involvement: results of a randomized, triple-blind, placebo-controlled phase 1/2 clinical trial. Cytotherapy 2018; 20: 499–506.

101. Wang L, Huang S, Li S, et al. Efficacy and safety of umbilical cord mesenchymal stem cell therapy for rheumatoid arthritis patients: a prospective phase I/II study. Drug Des Devel Ther 2019; 13: 4331–4340.

102. Ghoryani M, Shariati-Sarabi Z, Tavakkol-Afshari J, et al. Amelioration of clinical symptoms of patients with refractory rheumatoid
arthritis following treatment with autologous bone marrow-derived mesenchymal stem cells: a successful clinical trial in Iran. Biomed Pharmacother 2019; 109: 1834–1840.

103. Wang L, Wang L, Cong X, et al. Human umbilical cord mesenchymal stem cell therapy for patients with active rheumatoid arthritis: safety and efficacy. Stem Cells Dev 2013; 22: 3192–3202.

104. Yang Y, He X, Zhao R, et al. Serum IFN-gamma levels predict the therapeutic effect of mesenchymal stem cell transplantation in active rheumatoid arthritis. J Transl Med 2018; 16: 165.

105. Pedrosa M, Gornes J, Laranjeira P, et al. Immunomodulatory effect of human bone marrow-derived mesenchymal stromal/stem cells on peripheral blood T cells from rheumatoid arthritis patients. J Tissue Eng Regen Med 2020; 14: 16–28.

106. Park EH, Lim HS, Lee S, et al. Intravenous infusion of umbilical cord blood-derived mesenchymal stem cells in rheumatoid arthritis: a phase Ia clinical trial. Stem Cells Transf Med 2018; 7: 636–642.

107. Gowhari Shabgah A, Shariati-Sarabi Z, Tavakkol-Afshari J, et al. A significant decrease of BAFF, APRIL, and BAFF receptors following mesenchymal stem cell transplantation in patients with refractory rheumatoid arthritis. Gene 2020; 732: 144336.

108. He X, Yang Y, Yao M, et al. Combination of human umbilical cord mesenchymal stem (stromal) cell transplantation with IFN-gamma treatment synergistically improves the clinical outcomes of patients with rheumatoid arthritis. Ann Rheum Dis 2020; 79: 1298–1304.

109. Fontenot JD, Gavin MA and Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol 2003; 4: 330–336.

110. Morgan ME, Flierman R, van Duivenvoorde LM, et al. Effective treatment of collagen-induced arthritis by adoptive transfer of CD25+ regulatory T cells. Arthritis Rheum 2005; 52: 2212–2221.

111. Sakaguchi S, Sakaguchi N, Asano M, et al. Immunologic self-tolerance maintained by CD25+ regulatory T cells suppress antigen-specific autoreactive immune responses and central nervous system inflammation during active experimental autoimmune encephalomyelitis. J Immunol 2002; 169: 4712–4716.

112. Tarbell KV, Petit LS, Zuo X, et al. Cutting edge: CD4+CD25+ regulatory T cells eliminate autoreactive T cells. J Exp Med 2007; 204: 191–201.

113. Miao J and Zhu P. Functional defects of Treg cells: new targets in rheumatic diseases, including ankylosing spondylitis. Curr Rheumatol Rep 2018; 20: 30.

114. Kim HR, Park HJ, Son J, et al. Tumor microenvironment dictates regulatory T cell phenotype: upregulated immune checkpoints reinforce suppressive function. J Immunother Cancer 2019; 7: 339.

115. Toor SM, Murshed K, Al-Dhaheri M, et al. Immune checkpoints in circulating and tumor-infiltrating CD4(+) T cell subsets in colorectal cancer patients. Front Immunol 2019; 10: 2936.

116. Jafari S, Molavi O, Kahroba H, et al. Clinical application of immune checkpoints in targeted immunotherapy of prostate cancer. Cell Med Life Sci 2020; 77: 3693–3710.

117. Maruhashi T, Okazaki IM, Sugiuira D, et al. LAG-3 inhibits the activation of CD4(+) T cells that recognize stable pMHCII through its conformation-dependent recognition of pMHCII. Nat Immunol 2018; 19: 1415–1426.
124. Tekguc M, Wing JB, Osaki M, et al. Treg-expressed CTLA-4 depletes CD80/CD86 by trogocytosis, releasing free PD-L1 on antigen-presenting cells. *Proc Natl Acad Sci USA* 2021; 118: e2023791118.

125. Frank MJ, Reagan PM, Bartlett NL, et al. In situ vaccination with a TLR9 agonist and local low-dose radiation induces systemic responses in untreated indolent lymphoma. *Cancer Discov* 2018; 8: 1258–1269.

126. Chen M, Felix K and Wang J. Immune regulation through mitochondrion-dependent dendritic cell death induced by T regulatory cells. *J Immunol* 2011; 187: 5684–5692.

127. Yang J, Yang Y, Fan H, et al. Tolerogenic splenic IDO (+) dendritic cells from the mice treated with induced-Treg cells suppress collagen-induced arthritis. *J Immunol Res* 2014; 2014: 831054.

128. Le Mercier I, Chen W, Lines JL, et al. VISTA regulates the development of protective antitumor immunity. *Cancer Res* 2014; 74: 1933–1944.

129. Kondo Y, Ohno T, Nishii N, et al. Differential contribution of three immune checkpoint (VISTA, CTLA-4, PD-1) pathways to antitumor responses against squamous cell carcinoma. *Oral Oncol* 2016; 57: 54–60.

130. Yeh ET, Gong L and Kamitani T. Ubiquitin-like proteins: new wines in new bottles. *Gene* 2000; 248: 1–14.

131. Yu X, Lao Y, Teng XL, et al. SENP3 maintains the stability and function of regulatory T cells via BACH2 deSUMOylation. *Nat Commun* 2018; 9: 3157.

132. Turner JA, Stephen-Victor E, Wang S, et al. Regulatory T cell-derived TGF-beta1 controls multiple checkpoints governing allergy and autoimmunity. *Immunity* 2020; 53: 1202–1214.e1206.

133. Fu W, Hu W, Shi L, et al. Foxo4- and Stat3-dependent IL-10 production by progranulin in regulatory T cells restrains inflammatory arthritis. *FASEB J* 2017; 31: 1354–1367.

134. Miyara M, Ito Y and Sakaguchi S. TREG-cell therapies for autoimmune rheumatic diseases. *Nat Rev Rheumatol* 2014; 10: 543–551.

135. Voskens CJ, Stoica D, Roessner S, et al. Safety and tolerability of a single infusion of autologous ex vivo expanded regulatory T cells in adults with ulcerative colitis (ER-TREG 01): protocol of a phase 1, open-label, fast-track dose-escalation clinical trial. *BMJ Open* 2021; 11: e049208.

136. Tanaka H, Zhang W, Yang GX, et al. Successful immunotherapy of autoimmune cholangitis by adoptive transfer of forkhead box protein 3 (+) regulatory T cells. *Clin Exp Immunol* 2014; 178: 253–261.

137. Scalapino KJ and Daikh DI. Suppression of glomerulonephritis in NZB/NZW lupus prone mice by adoptive transfer of ex vivo expanded regulatory T cells. *PLoS ONE* 2009; 4: e6031.

138. Bluestone JA, Buckner JH, Fitch M, et al. Type 1 diabetes immunotherapy using polyclonal regulatory T cells. *Sci Transl Med* 2015; 7: 315ra189.

139. Wright GP, Stauss HJ and Ehrenstein MR. Therapeutic potential of Tregs and Ehrenstein MR. Therapeutic potential of Tregs and Ehrenstein MR. *Semin Immunol* 2011; 23: 195–201.

140. Safinia N, Leech J, Hernandez-Fuentes M, et al. Promoting transplantation tolerance; adoptive regulatory T cell therapy. *Clin Exp Immunol* 2013; 172: 158–168.

141. Shitara K and Nishikawa H. Regulatory T cells: a potential target in cancer immunotherapy. *Ann N Y Acad Sci* 2018; 1417: 104–115.

142. McGovern JL, Wright GP and Stauss HJ. Engineering specificity and function of therapeutic regulatory T cells. *Front Immunol* 2017; 8: 1517.

143. Li MO and Rudensky AY. T cell receptor signalling in the control of regulatory T cell differentiation and function. *Nat Rev Immunol* 2016; 16: 220–233.

144. Wright GP, Notley CA, Xue SA, et al. Adoptive therapy with redirected primary regulatory T cells results in antigen-specific suppression of arthritis. *Proc Natl Acad Sci USA* 2009; 106: 19078–19083.

145. van Loenen MM, de Boer R, Amir AL, et al. Mixed T cell receptor dimers harbor potentially harmful neoreactivity. *Proc Natl Acad Sci USA* 2009; 106: 10972–10977.

146. Sun G, Hou Y, Gong W, et al. Adoptive induced antigen-specific Treg cells reverse inflammation in collagen-induced arthritis mouse model. *Inflammation* 2018; 41: 485–495.

147. Asnagli H, Martire D, Belmonte N, et al. Type II as an efficient cell-based therapy in arthritis. *Arthritis Res Ther* 2014; 16: R115.

148. Kim HJ, Cha GS, Joo JY, et al. Targeting the epitope spreader Pep19 by naive human CD45RA(+) regulatory T cells dictates a distinct suppressive T cell fate in a novel form of
immunotherapy. J Periodontal Implant Sci 2017; 47: 292–311.

149. Zonneveld-Huijssoon E, Roord ST, de Jager W, et al. Bystander suppression of experimental arthritis by nasal administration of a heat shock protein peptide. Ann Rheum Dis 2011; 70: 2199–2206.

150. Kwon EY, Cha GS, Jeong E, et al. Pep19 drives epitope spreading in periodontitis and periodontitis-associated autoimmune diseases. J Periodontal Res 2016; 51: 381–394.

151. Mosanya CH and Isaacs JD. Tolerising cellular therapies: what is their promise for autoimmune disease? Ann Rheum Dis 2019; 78: 297–310.

152. Zavvar M, Abdolmaleki M, Farajifard H, et al. Collagen II-primed Foxp3 transduced T cells ameliorate collagen-induced arthritis in rats: the effect of antigenic priming on T regulatory cell function. Iran J Allergy Asthma Immunol 2018; 17: 361–371.

153. He X, Smeets RL, van Rijssen E, et al. Single CD28 stimulation induces stable and polyclonal expansion of human regulatory T cells. Sci Rep 2017; 7: 43003.

154. Beyersdorf N, Hanke T, Kerkau T, et al. CD28 superagonists put a break on autoimmunity by preferentially activating CD4+CD25+ regulatory T cells. Autoimmun Rev 2006; 5: 40–45.

155. Yang J, Liu L, Yang Y, et al. Adoptive cell therapy of induced regulatory T cells expanded by tolerogenic dendritic cells on murine autoimmune arthritis. J Immunol Res 2017; 2017: 7573154.

156. Charbonnier LM, Han G, Quentin J, et al. Adoptive transfer of IL-10-secreting CD4+CD49b+ regulatory T cells suppresses ongoing arthritis. J Autoimmun 2010; 34: 390–399.

157. Aricha R, Reuveni D, Fuchs S, et al. Suppression of experimental autoimmune myasthenia gravis by autologous T regulatory cells. J Autoimmun 2016; 67: 57–64.

158. Haque R, Lei F, Xiong X, et al. Foxp3 and Bcl-xL cooperatively promote regulatory T cell persistence and prevention of arthritis development. Arthritis Res Ther 2010; 12: R66.

159. Chen SY, Hsu WT, Chen YL, et al. Lymphocyte-activation gene 3(+) (LAG3(+) ) forkhead box protein 3(+) (FOXP3(–)) regulatory T cells induced by B cells alleviates joint inflammation in collagen-induced arthritis. J Autoimmun 2016; 68: 75–85.

160. Hsu LH, Li KP, Chu KH, et al. A B-1a cell subset induces Foxp3(-) T cells with regulatory activity through an IL-10-independent pathway. Cell Mol Immunol 2015; 12: 354–365.

161. Haque M, Fino K, Sandhu P, et al. Development of stem cell-derived antigen-specific regulatory T cells against autoimmunity. J Vis Exp 2016; 117: 54720.

162. Santinon F, Batignes M, Mebrek ML, et al. Involvement of tumor necrosis factor receptor type II in Foxp3 stability and as a marker of Treg cells specifically expanded by anti-tumor necrosis factor treatments in rheumatoid arthritis. Arthritis Rheumatol 2020; 72: 576–587.

163. Tseng WY, Huang YS, Clanchy F, et al. TNF receptor 2 signaling prevents DNA methylation at the Foxp3 promoter and prevents pathogenic conversion of regulatory T cells. Proc Natl Acad Sci USA 2019; 116: 21666–21672.

164. Fleskens V, Minotti CM, Wua X, et al. Nemo-like kinase drives Foxp3 stability and is critical for maintenance of immune tolerance by regulatory T cells. Cell Rep 2019; 26: 3600–3612.e3606.

165. Safari F, Farajinia S, Arya M, et al. CRISPR and personalized Treg therapy: new insights into the treatment of rheumatoid arthritis. Immunopharmacol Immunotoxicol 2018; 40: 201–211.

166. Becker C, Kubach J, Wijdenes J, et al. CD4-mediated functional activation of human CD4+CD25+ regulatory T cells. Eur J Immunol 2007; 37: 1217–1223.

167. van Vollenhoven RF, Keystone EC, Strand V, et al. Efficacy and safety of tregalizumab in patients with rheumatoid arthritis and an inadequate response to methotrexate: results of a phase IIb, randomised, placebo-controlled trial. Ann Rheum Dis 2018; 77: 495–499.

168. June CH and Sadelain M. Chimeric antigen receptor therapy. N Engl J Med 2018; 379: 64–73.

169. Rosenberg SA and Restifo NP. Adoptive cell transfer as personalized immunotherapy for human cancer. Science 2015; 348: 62–68.

170. Jin X, Xu Q, Pu C, et al. Therapeutic efficacy of anti-CD19 CAR-T cells in a mouse model of systemic lupus erythematosus. Cell Mol Immunol 2021; 18: 1896–1903.

171. Ellebrecht CT, Bhoj VG, Nace A, et al. Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease. Science 2016; 353: 179–184.
172. Santamaria-Alza Y and Vasquez G. Are chimeric antigen receptor T cells (CAR-T cells) the future in immunotherapy for autoimmune diseases? *Inflamm Res* 2021; 70: 651–663.

173. Chen Y, Sun J, Liu H, *et al.* Immunotherapy deriving from CAR-T cell treatment in autoimmune diseases. *J Immunol Res* 2019; 2019: 5727516.

174. Zmievskaya E, Valiullina A, Ganeeva I, *et al.* Application of CAR-T cell therapy beyond oncology: autoimmune diseases and viral infections. *Biomedicines* 2021; 9: 59.

175. Boroughs AC, Larson RC, Choi BD, *et al.* Chimeric antigen receptor costimulation domains modulate human regulatory T cell function. *JCI Insight* 2019; 5: e126194.

176. Kobayashi S, Thelin MA, Parrish HL, *et al.* A biomimetic five-module chimeric antigen receptor ((5M)CAR) designed to target and eliminate antigen-specific T cells. *Proc Natl Acad Sci USA* 2020; 117: 28950–28959.

177. Simone E, Daniel D, Schloot N, *et al.* T cell receptor restriction of diabetogenic autoimmune NOD T cells. *Proc Natl Acad Sci USA* 1997; 94: 2518–2521.

178. Zhang L, Crawford F, Yu L, *et al.* Monoclonal antibody blocking the recognition of an insulin peptide-MHC complex modulates type 1 diabetes. *Proc Natl Acad Sci USA* 2014; 111: 2656–2661.

179. Zhang L, Sosinowski T, Cox AR, *et al.* Chimeric antigen receptor (CAR) T cells targeting a pathogenic MHC class II:peptide complex modulate the progression of autoimmune diabetes. *J Autoimmun* 2019; 96: 50–58.

180. Mei HE, Schmidt S and Dorner T. Rationale of anti-CD19 immunotherapy: an option to target autoreactive plasma cells in autoimmunity. *Arthritis Res Ther* 2012; 14(Suppl. 5): S1.

181. Kansal R, Richardson N, Neeli I, *et al.* Sustained B cell depletion by CD19-targeted CAR T cells is a highly effective treatment for murine lupus. *Sci Transl Med* 2019; 11: eaav1648.

182. Eliav E, Adam N, Waks T, *et al.* Amelioration of colitis by genetically engineered murine regulatory T cellsredirected by antigen-specific chimeric receptor. *Gastroenterology* 2009; 136: 1721–1731.

183. Blat D, Zigmond E, Alteber Z, *et al.* Suppression of murine colitis and its associated cancer by carcinoembryonic antigen-specific regulatory T cells. *Mol Ther* 2014; 22: 1018–1028.

184. Fransson M, Piras E, Burman J, *et al.* CAR/FoxP3-engineered T regulatory cells target the CNS and suppress EAE upon intranasal delivery. *J Neuroinflammation* 2012; 9: 112.

185. Tenspolde M, Zimmermann K, Weber LC, *et al.* Regulatory T cells engineered with a novel insulin-specific chimeric antigen receptor as a candidate immunotherapy for type 1 diabetes. *J Autoimmun* 2019; 103: 102289.

186. Birke S, Gao L, Zeng G, *et al.* Down-regulation of GD3 ganglioside and its O-acetylated derivative by stable transfection with antisense vector against GD3-synthase gene expression in hamster melanoma cells: effects on cellular growth, melanogenesis, and dendricity. *J Neurochem* 2000; 74: 547–554.

187. Mukhatayev Z, Dellacecca ER, Cosgrove C, *et al.* Antigen specificity enhances disease control by Tregs in vitiligo. *Front Immunol* 2020; 11: 581433.

188. Schwenzer A, Jiang X, Mikuls TR, *et al.* Identification of an immunodominant peptide from citrullinated tenascin-C as a major target for autoantibodies in rheumatoid arthritis. *Ann Rheum Dis* 2016; 75: 1876–1883.

189. Zhou X, Bailey-Bucktrout SL, Jeker LT, *et al.* Instability of the transcription factor Foxp3 leads to the generation of pathogenic memory T cells in vivo. *Nat Immunol* 2009; 10: 1000–1007.