Chemokines and chemokine receptors have been implicated in inflammatory cell recruitment and angiogenesis underlying the pathogenesis of rheumatoid arthritis (RA) and other inflammatory rheumatic diseases. Numerous CXC, CC, C and CX3C chemokines and their receptors have been detected in the arthritic synovium and numerous strategies, including biologics, peptide and other small molecule inhibitors of chemokines and their receptors have given promising results in preclinical studies performed in animal models of arthritis. However, most recent human RA trials using antibodies and synthetic compounds have failed. Reasons for negative results of these RA trials include overlapping actions of multiple chemokines, dose-dependency, both antagonistic and agonistic effects of chemokines, chemokine degradation by proteases, as well as effects of anti-inflammatory, regulatory cells. Recent studies have suggested that CCRI may still be a good target and previous trials may have failed because of the need of sustained CCRI occupancy throughout the treatment. Therefore, modulation of receptor occupancy may be a feasible option to increase the efficacy of chemokine receptor targeting.

**KEYWORDS**

Rheumatoid arthritis, chemokines, chemokine receptors, targeting

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Nevertheless, these classifications are not fully justified as some primarily homeostatic chemokines involved in lymphoid development have also been implicated in inflammatory states, such as arthritis.1,4

CXC chemokines in arthritis
In CXC chemokines, two conserved C residues are separated by one unconserved amino acid. These mediators chemoattract neutrophils, lymphocytes and monocytes into the synovium. The underlying molecular mechanisms include leukocyte integrin expression and L-selectin shedding, cytoskeletal reorganisation, neutrophil degranulation and phagocytosis, as well as the production of proteases and other inflammatory mediators.1,4-6,9

The most relevant CXC chemokines involved in the pathogenesis of arthritis are CXCL1 (grot), CXCL4 (PF4), CXCL5 (ENA-78), CXCL6 (GCP-2), CXCL7 (CTAP-III), CXCL8 (IL-8), CXCL9 (Mig), CXCL10 (IP-10), CXCL12 (SDF-1), CXCL13 (BCA-1) and CXCL16. All these chemokines are abundantly expressed in the sera, synovial fluids and tissues of arthritis patients.3-5,13-33 Synovial macrophages are the major source of most CXC chemokines and they are sensitive to change after effective treatment.1,16,19,24,34-36 The pro-angiogenic or angiostatic function of CXC chemokines has been linked to the presence or absence of the ELR amino acid motif in their protein sequence, respectively.7,9,20,37 Indeed, the ELR-containing CXCL1, CXCL5, CXCL7, CXCL8 and CXCL16 promote 7,9,16,20,29,30-40 while the ELR-lacking CXCL4, CXCL9 and CXCL10 inhibit synovial neovascularisation.8,9,41 In addition, all these CXC chemokines exert mostly inflammatory rather than homeostatic function (table 1).

CXCL12, CXCL13 and CXCL16 are more peculiar CXC chemokines in many ways. First, while the other CXC chemokines described above have common receptors, CXCL12, CXCL13 and CXCL16 are specific ligands for CXCR4, CXCR5 and CXCR6, respectively.1,14,24,26,27,29-31,42-45 Second, these three chemokines are primarily homeostatic chemokines involved in lymphoid organisation, but they have also been implicated in synovial inflammation, as well as synovial lymphoid neogenesis.1,14,29,31,42,44 Regarding angiogenesis, CXCL12 promotes neovascularisation despite lacking the ELR motif.8,39,46 CXCL12 induces CXCR4-dependent integrin-mediated lymphocyte and monocyte adhesion and migration, as well as osteoclastogenesis, bone resorption and thus radiographic progression in RA.24,26,27,44,49 CXCL13 is also expressed by synovial fibroblasts, T cells and endothelial cells and follicular dendritic cells within the RA synovium.30,45 CXCL16 is secreted by synovial macrophages and fibroblasts and is involved in mononuclear cell recruitment into the RA synovium.33,44-49

**Table 1. Chemokines and chemokine receptors relevant for RA**

<table>
<thead>
<tr>
<th>Chemokine receptor</th>
<th>Chemokine ligand</th>
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<tbody>
<tr>
<td><strong>CXC chemokine receptors</strong></td>
<td></td>
</tr>
<tr>
<td>CXCR1 (A)</td>
<td>IL-8/CXCL8 (A,H), GCP-2/CXCL6</td>
</tr>
<tr>
<td>CXCR2 (A)</td>
<td>IL-8/CXCL8 (A,H), ENA-78/CXCL13 (A), groα/CXCL4 (A), CTAP-III/CXCL7, GCP-2/CXCL6</td>
</tr>
<tr>
<td>CXCR3 (A)</td>
<td>IP-10/CXCL10, PF4/CXCL4, Mig/CXCL9</td>
</tr>
<tr>
<td>CXCR4 (A)</td>
<td>SDF-1/CXCL12</td>
</tr>
<tr>
<td>CXCR5</td>
<td>BCA-1/CXCL13</td>
</tr>
<tr>
<td>CXCR6</td>
<td>CXCL16 (A)</td>
</tr>
<tr>
<td>CXCR7</td>
<td>ITAC/CXCL11, SDF-1/CXCL12</td>
</tr>
<tr>
<td><strong>C-C chemokine receptors</strong></td>
<td></td>
</tr>
<tr>
<td>CCR1 (A,H)</td>
<td>MIP-1α/CCL3 (A), RANTES/CCL5 (A), MCP-3/CCL7, HCC-1/CCL14, HCC-2/CCL15, HCC-4/CCL16</td>
</tr>
<tr>
<td>CCR2 (A)</td>
<td>MCP-1/CCL2 (A,H), MCP-3/CCL17, HCC-4/CCL16</td>
</tr>
<tr>
<td>CCR3</td>
<td>RANTES/CCL5, MCP-2/CCL8, MCP-3/CCL17, HCC-2/CCL15</td>
</tr>
<tr>
<td>CCR4</td>
<td>TARC/CCL17, KCKT</td>
</tr>
<tr>
<td>CCR5 (A,H)</td>
<td>MIP-1α/CCL3 (A), RANTES/CCL5 (A), MCP-2/CCL8, HCC-1/CCL14</td>
</tr>
<tr>
<td>CCR6</td>
<td>MIP-3α/CCL20</td>
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<tr>
<td>CCR7</td>
<td>SLC/CCL21</td>
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<tr>
<td><strong>C chemokine receptors</strong></td>
<td></td>
</tr>
<tr>
<td>XCR1</td>
<td>Lymphotactin/XCL1</td>
</tr>
<tr>
<td>C-X-C chemokine receptors</td>
<td></td>
</tr>
<tr>
<td>CXCR1</td>
<td>Fractalkine/CXCL1</td>
</tr>
</tbody>
</table>

*Already targeted in animal models (A) or human (H) trials. See text for abbreviations.

**CC chemokines**
CCL2 (MCP-1), CCL3 (MIP-1α), CCL5 (RANTES), CCL7 (MCP-3), CCL8 (MCP-2), CCL13 (MCP-4), CCL14 (HCC-1), CCL15 (HCC-2), CCL16 (HCC-3), CCL17 (TARC), CCL18 (PARC), CCL19 (ELC), CCL20 (MIP-3α) and CCL21 (SLC) have all been detected in arthritic sera and synovia (table 1).25,30,50-68 These CC chemokines are chemotactic for monocytes and lymphocytes. Among these chemokines, CCL20 preferentially recruits Th17 cells,64 induces both osteoblast proliferation and osteoclast differentiation and collaborates with the RANK ligand system in the uncoupling between new bone formation and bone resorption in RA.59-70 CCL13 has been associated with the cartilage of the RA joint and is released by articular chondrocytes.71,72 Regarding chemokine function, CCL2, CCL3, CCL5 and CCL13 exert mainly inflammatory functions. Among primarily homeostatic CC chemokines, CCL17, CCL18, CCL19 and CCL21 have been implicated in synovial lymphoid neogenesis in arthritis, as well as physiological lymphoid organisation.30,50,51,57,58

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C and CXC chemokines in RA
The C chemokine family contains two members, XCL1 (lymphotactin) and XCL2 (SCM-1). Among them, XCL1 is involved in T cell accumulation in the RA joint.74,75 The single member of the CX_C family is CXCL1 (fractalkine). This chemokine is chemotactic for mononuclear cells, mediates T cell adhesion and cytokine production, and regulates the cytoskeletal structure, proliferation and migration of synovial fibroblasts.76-79 CXCL1 is also an angiogenic mediator80 and has been associated with rheumatoid vasculitis81 and accelerated atherosclerosis leading to increased cardiovascular morbidity in RA.82-84

CHEMOKINE RECEPTORS IN ARTHRITIS
Chemokine receptors are 7-transmembrane domain receptors expressed on the target cells. Some chemokine receptors, such as CXCR2, CCR1, CCR2, CCR3 or CCR5, have multiple ligands, while others including CXCR4, CCR5, CXCR6, CCR8 or CCR9 are specific receptors for one single ligand (table 1).4,5
In general, all CXCRs have been implicated in the pathogenesis of arthritis. CXCR1 and CXCR2 recognise the most relevant inflammatory and angiogenic CXC chemokines described above.4,5,81 CXCR3 may be the most important receptor in leukocyte homing into Th1 type inflammatory sites, such as the RA synovium.82-85 As described above, CXCR4 is involved in CXCL12-dependent ingress of lymphocytes into the RA synovium.86 CXCR4, CCR5 and CXCR6 bind their respective homeostatic chemokine ligands, CXCL12, CXCL13 and CXCL16. Thus, as mentioned above, these CXCRs are involved in both physiological lymphoid organisation and synovial lymphoid neogenesis.86-88,90,91
Among CCRs, CCR1, CCR2, CCR3, CCR4, CCR5, CCR6 and CCR7 are abundantly expressed in the RA synovium and on synovial cells.4,5,10-12,83,84-86,88 CCR2 and CCR3 are also present on articular chondrocytes.89 CCR5 may be the most prominent CCR characterising Th1 inflammatory infiltrates.85,87 In some studies, a single nucleotide polymorphism leading to the production of the truncated ∆32-CCR5 non-functional receptor allele was found to be protective against RA including extra-articular manifestations and joint erosions,94-99 but results have been variable.96-97 The protective role of this polymorphism was also suggested in juvenile98 and psoriatic arthritis.99 CCR6 is involved in the ingress of Th17 lymphocytes into the rheumatoid joint.64,100 CCR7 has been associated with synovial lymphoid neogenesis in murine arthritis.86 In a comparative study on CCRs, peripheral blood monocytes mainly expressed CCR1 and CCR2, suggesting that these receptors were involved in monocyte recruitment from the circulation. In contrast, CCR3 and CCR5 expression was upregulated in RA synovial fluids indicating that these CCRs were important in monocyte retention in the joint.88 Regarding the C and CX_C chemokine receptors, XCR1 is expressed on RA synovial lymphocytes, macrophages and fibroblasts, while XCR1 has been detected on macrophages and dendritic cells.4,76-100 CXCR1 has been implicated in the recruitment of Th1 type lymphocytes into the joint.101

TARGETING OF CHEMOKINES AND CHEMOKINE RECEPTORS
Chemokines and chemokine receptors may be targeted by indirect, non-specific as well as by direct, chemokine-specific approaches. These strategies have been tested in animal models of arthritis, in vitro cultures of human RA synovial cells and tissues, as well as in a limited number of human RA clinical trials (table 1).102-104
Inhibition of chemokine and chemokine receptor expression by immunosuppressive therapy
Some non-steroidal anti-inflammatory drugs (NSAID), corticosteroids, traditional disease-modifying antirheumatic drugs (DMARD) and biologics exert multiple anti-inflammatory actions including chemokine and chemokine receptor inhibition. In early studies, NSAIDs and corticosteroids attenuated CXCL8 and CCL2 production in vitro, as well as in arthritis models.103,106 A recently developed dual cyclooxygenase-lipoxygenase inhibitor, ML3000, downregulated CXCL9, CXCL10 and CXCL11 expression in RA synovial fibroblasts.107 Among traditional DMARDs, sulfasalazine, sulfapyridine, methotrexate (MTX) and leflunomide inhibited the production of various CXC and CC chemokines in synovial cell and explant cultures in vitro, as well as in animal models of arthritis and RA in vivo.108-114 There have been an increasing number of studies with biologics, primarily anti-TNF agents. Infliximab and etanercept may suppress the release of CXCL1, CXCL8, CXCL10, CXCL16, CCL2, CCL3, CCL20, CXCL1 and possibly other chemokines in RA.112-115 The IL-6 receptor inhibitor tocilizumab also suppressed CCL20 production in RA.115 As IL-6 signalling plays a crucial role in the stimulation of chemokine production in RA,116 tocilizumab may inhibit the release of other chemokines as well. B cell inhibition by rituximab alters the CXCL8 network117 and decreases CCL5 production.118 Efficacy of rituximab has been associated with surface CCR5 density.119 With respect to chemokine receptors, TNF-α blockade also reduced CCR3, CCR5, CCR6 and CXCR1 expression on T cells120-122 and resulted in the clearance of CXCR3; T
cells from the synovium.\textsuperscript{17} The efficacy of rituximab was correlated with increased CCR5 density on peripheral blood T cells in RA.\textsuperscript{17} Chemokine inhibition by biologics may have relevance for safety of anti-TNF therapy as infliximab reduced the secretion of CCL5, CCL2 and CCL3 in response to Mycobacteria.\textsuperscript{178} Some other synthetic compounds, as well as natural products, may also influence chemokine secretion. For example, antioxidants, such as N-acetyl-L-cysteine and 2-oxothiazolidine-4-carboxylate, the bioflavonoid quercetin, as well as the lipid-lowering simvastatin, inhibited the expression of CXCL8 and CCL2 by activated, cultured human synovial fibroblasts.\textsuperscript{158-160} Epigallocatechin-3-gallate (EGCG), a compound derived from green tea, as well as green tea extracts suppressed the production of multiple chemokines including CXCL1, CXCL5, CCL2 and CCL5 by cultured RA synovial fibroblasts, bone cells and in a rat model of arthritis.\textsuperscript{161-163} Activation of peroxisome proliferator-activated receptor γ (PPARγ) suppresses CCL2 expression in monocytes,\textsuperscript{161} thus PPARγ agonists, such as glitazones, may inhibit chemokine production. Some traditional Oriental medicines, such as triptolide, lingzhi, curcumin, tongbiling, honokiol, cool-cold and others exert antiarthritic effects, which may, in part, be explained by chemokine and chemokine receptor inhibition.\textsuperscript{164-166}

### Specific chemokine targeting

In various animal models of arthritis, neutralising antibodies to CXCL1, CXCL5, CXCL8, CXCL16, CCL2, CCL3, CCL5, CCL24 and CXCL1 blocked arthritis both therapeutically and preventatively.\textsuperscript{167-169} A novel inhibitor of endogenous CCL2, p8A-MCP-1, also improved rat adjuvant-induced arthritis (AIA).\textsuperscript{17} Peptide inhibitors of CXCL4, CCL2 and CCL5 also attenuated murine and rat arthritis.\textsuperscript{158-160}

The efficacy of chemokine targeting may be increased by combining various specific strategies. For example, in murine AIA, a combination of CXCL1 and CCL2 resulted in more pronounced effects than did CCL2 blockade alone.\textsuperscript{171} In a rabbit arthritis model, the combination of anti-CXCL1 and anti-CXCL8 antibodies inhibited arthritis better than did any of the two antibodies alone.\textsuperscript{172} The downside of more effective chemokine blockade could be an increased risk of side effects such as infections. There has only been a very limited number of published human anti-chemokine trials. In the only available, published trial on inhibition of a chemokine ligand in RA, an anti-CCL2 antibody, ABN912, was evaluated in a randomised, placebo-controlled clinical trial. In this study, 33 patients received the active compound, while 12 received placebo. Serial arthroscopic biopsies were performed. ABN912 treatment was well tolerated, but there was no detectable clinical benefit or significant changes in synovial biomarkers.\textsuperscript{173}

In summary, after promising preclinical studies in arthritis models using antibodies and peptide inhibitors to chemokines, only one human RA trial yielding negative results has been published. This trial was completed in 2006 and not followed by others, suggesting that targeting of a single chemokine may not be effective in arthritis. Preclinical studies using combined chemokine blockade already suggested that simultaneous targeting of multiple chemokines may be the future strategy. As chemokine receptors may recognise multiple inflammatory chemokine ligands, more trials have been conducted using CCR antagonists.

### Chemokine receptor blockade: how to proceed after disappointing clinical trials?

Some CXCR antagonists have been used in animal models, but they were not tested in human RA. For example, an anti-CXCR3 antibody inhibited AIA.\textsuperscript{174} Synthetic oral antagonists of CXCR1, CXCR2 and CXCR4 inhibited arthritis in various rodent models.\textsuperscript{175,176} To our knowledge, no clinical trial results obtained with any CXCR antagonists in arthritis have been published. CCR1, CCR2 and CCR5 bind multiple CC chemokine ligands including CCL3, CCL5, CCL7, CCL8, CCL14, CCL15 and CCL16 that have been implicated in the pathogenesis of RA,\textsuperscript{177-179} Therefore, numerous synthetic or biological CCR1, CCR2 and CCR5 antagonists have been developed in recent years.\textsuperscript{180,181,182} Dual targeting of CCR2 and CCR5 is underway.\textsuperscript{177}

CCR1 is a receptor for CCL3, CCL5, CCL7, CCL14, CCL15 and CCL16 (table 1). Among CCR1 antagonists, in early preclinical studies, J-113863 diminished synovitis and joint destruction in murine collagen-induced arthritis (CIA).\textsuperscript{183} Met-RANTES, a dual CCR1/CCR5 antagonist, inhibited both murine CIA and rat AIA.\textsuperscript{184-186} This was followed by the development and introduction of two oral CCR1 antagonists, CP-481,715 and MLN3897, to human RA trials.\textsuperscript{187,188} CP-481,715 was evaluated in a phase I clinical trial to assess pharmacokinetics and safety. It was administered to 78 healthy individuals in escalating doses up to 3000 mg. This drug was well-tolerated.\textsuperscript{189} CP-481,715 has also been evaluated in a two-week phase Ib, proof-of-concept study in RA patients using a dose of 300 mg per eight hours. Altogether 16 RA patients were randomised 3:1 with active/placebo treatment for 14 days, and it decreased the number of total and intimal macrophages, as well as CCR1+ cells in the synovium. About one-third of the patients also fulfilled the ACR20 criteria for clinical improvement.\textsuperscript{190} In a subsequent phase IIa study, RA patients with active disease despite MTX treatment received either 10 mg of MLN3897 or placebo orally once daily with concomitant MTX therapy. Although MLN3897 was well-tolerated, no difference in ACR20 was found between the active and placebo group. Interestingly,
MLN3897 was associated with a relatively high degree (≥ 90%) of CCR1 occupancy throughout the trial as determined by CCL3 internalisation assay. CCR2 recognises CCL2, CCL7 and CCL16 (table 1). Some CCR2 inhibitors have also entered animal studies and then clinical trials. While low doses of the MC-21 anti-CCR2 monoclonal antibody markedly improved murine CIA, high doses of this antibody rather had pro-inflammatory effects. MKo812, another small molecule CCR2 inhibitor, had no effect on the severity of CIA. In a phase IIa clinical trial with a CCR2 blocking antibody MLN1202, 32 patients with active RA received three infusions of either placebo or anti-CCR2 antibody at 0.5 mg/kg or 1.5 mg/kg over a period of six weeks. The antibody reduced the levels of free CCR2 on CD14+ monocytes; however, no clinical benefit could be demonstrated.

CCR5 binds CCL3, CCL5, CCL8 and CCL14 (table 1). As discussed above, in earlier studies, Met-RANTES and other small molecule antagonists, such as SCH-X of CCR5, showed some efficacy in preclinical arthritis studies. Recently, a small molecule CCR5 antagonist, AZD5962, was tried in preclinical, phase I and IIb studies. Ligand-binding and chemotaxis studies supported the biological activity of this compound. In the phase IIb trial, 371 patients with active RA received 20 mg, 50 mg, 100 mg or 150 mg oral AZD5962 once daily, placebo or open-label etanercept 50 mg subcutaneously once weekly. There was no significant difference in the number of patients receiving the active compound or placebo. Furthermore, etanercept was more effective than AZD5962 or placebo. A phase Ib trial was conducted with SCH351125, a small molecule oral CCR5 inhibitor. Among 32 patients with active RA, 20 received the active compound and 12 received placebo. No synovial, MRI or clinical efficacy could be proven. Another CCR5 inhibitor, maraviroc, has been tried in phase II-III trials in HIV infection and AIDS, as well as to a phase II trial in RA. Thus, CCR2 and CCR5 blockade yielded disappointing results in RA, and results for CCR1 blockade have been variable. Do we now have to bury the idea of blocking chemokines and their receptors in arthritis and other immune-mediated inflammatory disorders? Maybe we should think again. There have been several issues that may interfere with the efficacy of chemokine and chemokine receptor blockade in RA (table 2).

<table>
<thead>
<tr>
<th>Table 2. Examples of potential difficulties and caveats in human chemokine and chemokine receptor blockade trials</th>
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</thead>
<tbody>
<tr>
<td>• Redundancy of the chemokine system</td>
</tr>
<tr>
<td>• Agonist action of certain ligands on one receptor, and antagonist action on others</td>
</tr>
<tr>
<td>• Cleavage of chemokines by proteases</td>
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<tr>
<td>• Unwanted inhibition of anti-inflammatory cells</td>
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<tr>
<td>• Interference with homeostatic function</td>
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<tr>
<td>• Levels of receptor occupancy were not high enough at all times</td>
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</table>

Chemokines are key producers of pro-inflammatory cytokines and chemokines and they represent biomarkers sensitive to change after effective treatment. Therefore, an in vitro monocyte migration assay was used to determine the effects of CCR1, CCR2 and CCR5 inhibition. Monocyte chemotaxis was induced by CCL2, CCL5 or by an RA synovial fluid pool and the effects of anti-CCR1, anti-CCR2 and anti-CCR5 blocking antibodies, as well as those of BX147, a small molecule CCR1 inhibitor, were tested. As expected, anti-CCR2 and anti-CCR5 antibodies inhibited CCL2- and CCL5-induced chemotaxis, respectively. However, the anti-CCR2 and anti-CCR5 antibodies did not influence RA synovial fluid-mediated monocyte migration, not even when used in combination. In contrast, both the anti-CCR1 antibody and the CCR1 synthetic inhibitor blocked RA synovial fluid-induced monocyte chemotaxis. These results suggest that while CCR2 and CCR5 may not be critical for monocyte migration into synovial compartments, CCR1 seems to mediate this process. Indeed, CCR2 and CCR5 antibody blockade be dependent on the dose of the CCR antagonists.
failed to reduce synovial cell infiltration in clinical trials.\textsuperscript{46,68,73} Although preclinical studies indicated that dual targeting of CCR2 and CCR5 may be beneficial in animal models of arthritis,\textsuperscript{77} these data raise concerns about potential efficacy of CCR2/CCR5 blockade in RA patients.\textsuperscript{82} In contrast, CCR1 blockade inhibited synovial macrophage infiltration in a proof-of-principle study in RA patients\textsuperscript{79,73,80} and also in the recent study showing the effects of in vitro migration towards synovial fluid.\textsuperscript{81} Therefore, the fact that CCR1 blockade failed in some clinical trials does not necessarily mean that CCR1 is not a good target.\textsuperscript{66} It appears critical to achieve very high levels of receptor occupancy at all times during the in order to inhibit monocyte migration into the synovial compartment in vivo.\textsuperscript{69} Indeed, sustained CCR1 occupancy has been associated with effective anti-inflammatory response in other models of inflammation.\textsuperscript{195}

**SUMMARY**

In this review, we have discussed the potential role of chemokines and chemokine receptors in the pathogenesis of arthritis. Numerous CXC, as well as some CC and CX\(C\) chemokines and their respective receptors, have been implicated in leukocyte ingress into the inflamed synovium. Despite promising results in preclinical studies using anti-chemokine and anti-chemokine receptor biologics and small molecule compounds, nearly all human RA trials failed. Recent studies suggest that, at least with CCR1 inhibitors, chemokine receptor occupancy during the whole day is critical. This insight may open new opportunities for future clinical trials in RA as well as other inflammatory conditions.

**REFERENCES**


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