Targeting GM-CSF in rheumatoid arthritis

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ABSTRACT
Granulocyte-macrophage colony-stimulating factor (GM-CSF) is well-known as a haemopoietic growth factor. However, it is also essential in regulating functions of mature myeloid cells such as macrophages. Preclinical studies and observations of flares of arthritis in patients following GM-CSF treatment supported its important contribution to the pathogenesis of rheumatoid arthritis (RA). As the most advanced compound, mavrilimumab, a monoclonal antibody against GM-CSF receptor, has already completed phase II trials with a long term of follow-up period of 74 weeks. During this exposure period, an acceptable sustained safety and tolerability profile has been observed addressing the concerns of development of cytopenias or pulmonary alveolar proteinosis. Of note, a rapid and sustained efficacy and normalisation of acute phase reactants were consistently shown in studies both targeting GM-CSF and its receptor. Its tumour necrosis factor (TNF) independent mode of action with concurrent blockade of GM-CSF as well as IL-17 signalling reported from preclinical studies supports the assumption that it can be a useful biologic and an alternative agent in TNF inhibitor resistant patients with RA. Therefore, subsequent studies are warranted to investigate the safety and efficacy of GM-CSF blocking agents in different subgroups of RA.

Introduction
Although treatment of rheumatoid arthritis (RA) has evolved tremendously in recent years, still an important number of patients fail to reach the target, disease remission or low disease activity (LDA) (1). Blockade of any single cytokine or cellular subset cannot control the disease in all patients, which necessitates investigations of other cytokines or cellular mechanisms responsible for RA pathogenesis. To most clinicians, granulocyte-macrophage colony-stimulating factor (GM-CSF) is well-known as a haemopoietic growth factor used to treat neutropenia following chemotherapy. It was a long-time concern that targeted therapies against this cytokine could cause severe side effects such as neutropenia or pulmonary alveolar proteinosis. Therefore, during the early development phase of compounds targeting GM-CSF or its receptor special attention was paid to this potential adverse event revealing no evidence for such an associated risk profile. On the other hand the so far available results clearly showed rapid and sustained effects on disease activity and patient reported outcomes in RA (2).

GM-CSF
GM-CSF, a small secreted cytokine is a haemopoietic growth factor responsible for proliferation and differentiation of myeloid cells from bone marrow progenitors. Besides myeloid cells, T and B cells and tissue resident cells including chondrocytes, fibroblasts, osteoblasts, microglia, endothelial and epithelial cells can secrete GM-CSF (3-5). GM-CSF binds to a heterodimeric GM-CSF receptor (GM-CSFR) composed of a specific-ligand binding α-chain (GM-CSFRα) and a signal-transducing β-chain (GM-CSFRβ), which is shared with IL-3 and IL-5 receptors (6, 7). GM-CSFR activation leads to downstream signalling pathways of Janus kinase-signal transducer and activator of transcription-suppressor of cytokine signalling (JAK-STAT-SOCS) as well as other pathways including mitogen-activated protein kinases (MAPK), phosphatidylinositol 3 kinase (PI3K), and NFκB (7-11). The cytoplasmic tail of GM-CSFRα also has the capacity to interact with signalling pathways (12). GM-CSF is not solely a growth factor responsible for proliferation of myeloid cells, but also essential in regulating functions of mature myeloid cells such as chemotaxis and cell adhesion, dendritic cell function, expression of...
pro-inflammatory cytokines, phagocytosis, and microbial killing (6, 13, 14). It has a central role in regulating innate immunity. Regarding in vitro studies, GM-CSF can polarise macrophages into M1-like macrophages producing inflammatory cytokines instead of M2-like macrophages which in contrast produce an anti-inflammatory medium (15, 16). However, in vivo studies suggested also association with M2 polarisation in the lung and collaboration of other immune factors such as interferon-gamma (IFNγ), interferon regulatory factor 5 (IRF-5) or transforming growth factor-β (TGFβ) family member activin A (17-20). In the pulmonary mucosa, the epithelium is a major producer of GM-CSF. This GM-CSF plays a critical role locally in regulating microbial defense and surfactant clearance by alveolar macrophage population. Defects in GM-CSF or its receptor led to death of mice causing pulmonary disease characterised by accumulation of surfactant-like proteins and increased susceptibility to microbial infection (21). However, a surprising finding in this study was a relatively normal myelopoiesis.

GM-CSF and RA
GM-CSF is expressed in the synovial membrane and levels of GM-CSF are elevated in synovial fluid of RA patients (22, 23). The GM-CSFRα is also up-regulated in synovial tissue and on circulating mononuclear cells from RA subjects (24, 25). Synovial tissue macrophage populations are associated with articular damage and decrease in numbers of macrophages is a sensitive biomarker for response to treatment in patients with RA (26-28). Since GM-CSF plays a central role in macrophage differentiation, survival and activation, inhibiting GM-CSF activity can affect macrophage function and may provide clinical benefit in RA. TH17 cells have an important place also in RA pathogenesis and GM-CSF contributes to the differentiation and pathogenicity of these cells (29, 30). Figure 1 provides a schematic representation summarising the association of GM-CSF with cells and cytokines important in RA pathogenesis.

In animal models of arthritis, while deficiency and blockade of GM-CSF is protective, overexpression or injection of GM-CSF is associated with flares of arthritis (31-35). Other in vitro studies suggest a role for GM-CSF pathway blockade in tumour necrosis factor (TNF) inhibitor resistant situations and as a part of combinations strategies e.g. with IL-17 or p38 inhibitors (32, 36-39). Finally, the observation of flares of arthritis following administration of recombinant GM-CSF to treat neutropenia in patients with Felty’s syndrome or after chemotherapy suggested a potential role for targeting GM-CSF and its receptors in RA (40-42).

Clinical studies
GM-CSFR inhibition
Phase I. Mavrilimumab (CAM-3001) is a high-affinity human monoclonal antibody to the GM-CSFRα chain with competitive antagonistic effect on GM-CSF signalling. The first in-human study targeting the innate arm of the immune system via the GM-CSF pathway was performed with mavrilimumab in adult onset RA patients with a Disease Activity Score 28-joint assessment (DAS28) ≥4.8 (mild to moderate RA) under a stable dosage of methotrexate (MTX) for ≥3 months (43). This was a double-blind placebo-controlled study with a 5:1 randomisation (mavrilimumab:placebo) where patients received single escalating intravenous (IV) doses of mavrilimumab (0.01, 0.03, 0.1, 0.3, 1.0, 3.0 and 10.0 mg/kg) or placebo on study Day 0 and were followed up for 24 weeks. Overall, 32 patients were enrolled and completed the planned evaluations including safety analysis. The primary objective was safety of the compound, while secondary objectives included pharmacokinetics and effect on disease activity. As a surrogate marker for biologic activity, inhibition of the induction of SOCS3 mRNA by GM-CSF was shown with doses of 0.3 mg/kg just after 4 hours of dosing and this effect was sustained over 2 weeks. Of note, at base-line and 63% of patients had normal acute phase reactants at the study entry.
Of the n=10 out of 27 subjects treated with mavrilimumab who had elevated erythrocyte sedimentation rate (ESR) (≥ 20 mm/hr) at baseline, normalised values were measured in n=9 of them after treatment. None of the patients had significant haematological changes or deterioration of lung functions. Of note, adverse events (AEs) were similar between placebo and mavrilimumab-treated groups, mostly mild and not related to the drug dosage.

**Phase II trials.** The phase 2A multicentre, randomised, double-blind, placebo-controlled study (EARTH) of mavrilimumab included 233 adult European subjects with moderate-to-severely active seropositive and MTX-resistant RA (DAS28 ≥3.2) on a stable MTX dose over at least 4 weeks prior to screening (44). Patients with active or latent tuberculosis (TB), clinically significant chronic or recurrent infections including hepatitis C or chronic active hepatitis B were excluded. Furthermore, a history of previous treatment with more than one biologic therapy for RA that was discontinued for lack of efficacy was also an exclusion criteria. Subjects received 10, 30, 50 or 100mg of mavrilimumab subcutaneous (SC) or placebo every other week (eow) for 12 weeks and patients were followed-up for additional 12 weeks. The primary endpoint defined as an improvement in DAS28-CRP of at least 1.2 at Week 12 was significantly more frequently achieved in the mavrilimumab-treated group compared to placebo (55.7% vs. 34.7%; p=0.003). Of note, differences in response rates were observed already by Week 2, increased throughout the study period and were dose related with best improvement in the 100 mg group. Additionally, all categories of the American College of Rheumatology (ACR) criteria (ACR20: 69.2% vs. 40.0%, p=0.005; ACR50: 30.8% vs. 12.0%, p=0.021; ACR70: 17.9% vs. 4.0%, p=0.030), as well as the Health Assessment Questionnaire Disability Index (HAQ-DI) (-0.48 vs. -0.25, p=0.005) were significantly improved. The proportion of patients achieving a moderate or good European League Against Rheumatism (EULAR) response was significantly higher compared to placebo (67.7 vs. 50.7%; p=0.025) with the highest proportion of moderate or good EULAR responders in the 100 mg group (46.2% and 30.8%, respectively). AEs were generally mild or moderate and no relevant hypersensitivity reactions, serious infections or clinically significant deterioration of lung functions were noted. As add-on to the EARTH study, the same protocol was performed in 51 Japanese patients (45). With the exception of ACR70, the 100mg dose showed a high degree of consistency in almost all efficacy endpoints with comparable safety profile to the European patients.

A phase IIb study of mavrilimumab (EARTH EXPLORER 1) enrolled 326 patients with moderate to severe adult onset RA (DAS28-CRP ≥3.2; ≥4 swollen joint; mean DAS28-CRP at baseline: 5.77) from Europe and South America with an inadequate response to at least one of disease-modifying anti-rheumatic drugs (DMARDs) (46-52). Patients were randomised to receive 1 of 3 SC mavrilimumab dosages (150, 100, 30 mg eow) or placebo plus MTX (7.5–25.0 mg/week). At Week 12, a statistically significant difference (p<0.001) in DAS28-CRP as the primary endpoint was seen for all dosages of mavrilimumab vs. placebo and this effect was sustained until Week 24 (p<0.001). Furthermore, a significantly greater percentage of mavrilimumab-treated patients met the ACR20 core primary endpoint vs. placebo for all dosages at Week 24 (p<0.001). In summary, mavrilimumab (particularly 150 mg eow) showed rapid and clinically meaningful effects across a number of disease activity parameters with an acceptable safety and tolerability profile over the 24-week study period.

In another phase II study of mavrilimumab (EARTH EXPLORER 2), moderate-to-severe active RA patients who have had an inadequate response to one or two anti-TNF agents were randomised to SC mavrilimumab 100 mg (eow) or golimumab 50 mg (monthly) for 24 weeks (53). The study has been completed, however, results have not been published so far.

An open label extension study was performed in adult RA patients who had completed EARTH Explorer 1 and 2 Phase IIb studies or were rescued as inadequate responders at Week 12 (54). The aim of the study was to evaluate the long-term safety and efficacy of mavrilimumab 100 mg eow through 74 weeks of treatment in patients with moderate to severe RA. Primary endpoint was to assess the long-term risk benefit ratio of mavrilimumab 100 mg eow via evaluation of treatment-emergent adverse events (TEAEs), treatment-emergent serious adverse events (TSAEs), laboratory parameters and pulmonary function tests. Exploratory endpoints included DAS28–CRP LDA (<3.2), DAS28–CRP remission rate (<2.6), ACR20/50/70 response rates and change in modified Total Sharp Score (mTSS) erosion/narrowing scores. Among the 440 patients receiving mavrilimumab in this study, the most common TSAEs (n/rate/100 patient years) were arthralgia symptoms (coded as “osteoarthritis”) (4[0.7]) and bronchitis (3[0.5]). The most common TEAEs of special interest were bronchitis (29[4.8]) and respiratory tract infection(12[2.0]). Within the clinical programme, no monocytopenia was reported and there were no cases of pulmonary alveolar proteinosis. Overall, also no other pulmonary safety concerns were identified, and pulmonary function tests demonstrated a generally transient 20% reduction to <80% of predicted values in a few patients. With respect to efficacy, mavrilimumab demonstrated sustained efficacy with DAS28-CRP <3.2 and <2.6 rates of 57.3% and 38.5%, respectively. After 74 weeks of treatment, 68% of patients showed no radiographic progression (<0.5 change in mTSS vs. baseline). In conclusion, all performed clinical trials reached their primary outcome and demonstrated the benefit of inhibiting the GM-CSFR-α pathway on RA disease activity. Importantly, mavrilimumab showed an acceptable sustained safety and tolerability profile, with no significant pulmonary findings over the 74-week treatment duration. Since mavrilimumab produced sustained and clinically meaningful effects across a number of disease activity parameters
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An alternative approach to interfere with GM-CSF signalling would be the direct neutralisation of the cytokine. The first in patient Study of an anti-GM-CSF monoclonal antibody (MOR103) in RA included 96 patients with active RA (DAS28≤5.1) (55). The primary outcome measure was the adverse event rate and safety profile, while secondary endpoints included DAS28, ACR core set measures, EULAR response criteria and magnetic resonance imaging of synovitis (MRI synovitis). By using three different dosages of MOR103 (0.3, 1.0 and 1.5 mg/kg) compared to placebo, a rapid and significant clinical improvement was demonstrated compared to placebo, most pronounced at the 1.0 mg/kg dosage in this study. Short-term safety and tolerability remained in the range of placebo. AEs were generally mild or moderate and the most common AE was nasopharyngitis.

Namilumab. Namilumab (MT203) is another monoclonal antibody targeting GM-CSF and has been evaluated in patients with mild to moderate RA in a phase Ib study, PRIORA (56). Patients were on stable MTX doses and received three single injections of namilumab 150 or 300mg or placebo on days 0, 15 and 29 with a follow-up period of 12 weeks. Namilumab was generally well tolerated with a similar safety outcome across the treatment groups. By Day 29, greater improvements in DAS28 (ESR and CRP) and joint counts could be observed in namilumab-treated patients compared to placebo. Additionally, DAS28-ESR response rates were higher in namilumab group than placebo at Day 56 (71.4% vs. 28.6%). Currently, another phase I and two phase II trials of namilumab

Table I. Clinical trials of agents targeting GM-CSF pathway in rheumatoid arthritis.

<table>
<thead>
<tr>
<th>Drug/ ClinicalTrials.gov Identifier</th>
<th>Target</th>
<th>Drug regime</th>
<th>Primary outcome measures</th>
<th>Trial status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mavrilimumab NCT00771420 (43)</td>
<td>GM-CSFR</td>
<td>a single, escalating IV doses of mavrilimumab (0.01, 0.03, 0.1, 0.3, 1.0, 3.0 and 10.0mg/kg) or placebo, plus MTX</td>
<td>Incidence and severity of AEs</td>
<td>Phase I published</td>
</tr>
<tr>
<td>Mavrilimumab NCT01050998 (44)</td>
<td>GM-CSFR</td>
<td>10mg, 30mg, 50mg, or 100mg SC mavrilimumab eow or placebo, plus MTX</td>
<td>Improvement in DAS28-CRP</td>
<td>Phase II published</td>
</tr>
<tr>
<td>Mavrilimumab NCT01050998 (Japanese patients) (45)</td>
<td>GM-CSFR</td>
<td>10mg, 30mg, 50mg, or 100mg SC mavrilimumab eow or placebo, plus MTX</td>
<td>Improvement in DAS28-CRP</td>
<td>Phase II published</td>
</tr>
<tr>
<td>Mavrilimumab NCT01706926 (46-52)</td>
<td>GM-CSFR</td>
<td>150, 100, 30mg SC mavrilimumab eow or placebo, plus MTX</td>
<td>Improvement in DAS28-CRP and ACR20</td>
<td>Phase III abstracts presented</td>
</tr>
<tr>
<td>Mavrilimumab NCT01715896 (53)</td>
<td>GM-CSFR</td>
<td>Mavrilimumab 100mg SC eow or golimumab 50mg SC monthly alternating with placebo, plus MTX</td>
<td>ACR20, 50, 70 responses, DAS-28&lt;2.6, HAQ-DI improvement&gt;0.25</td>
<td>Phase II completed, no results posted</td>
</tr>
<tr>
<td>Mavrilimumab NCT01712399 (54)</td>
<td>GM-CSFR</td>
<td>Mavrilimumab 100 mg SC eow plus MTX</td>
<td>Incidence and severity of AEs</td>
<td>Phase II abstract presented</td>
</tr>
<tr>
<td>MOR103 NCT01023256 (55)</td>
<td>GM-CSF</td>
<td>Weekly escalating IV doses of MOR103 (0.3, 1.0 and 1.5mg/kg) or placebo</td>
<td>Incidence and severity of AEs</td>
<td>Phase I/IIa published</td>
</tr>
<tr>
<td>Namilumab NCT01317797 (56)</td>
<td>GM-CSF</td>
<td>Weekly escalating SC doses of namilumab (150mg and 300mg) or placebo, plus MTX</td>
<td>Incidence and severity of AEs</td>
<td>Phase I abstract presented</td>
</tr>
<tr>
<td>Namilumab NCT02254599 (57)</td>
<td>GM-CSF</td>
<td>Single SC doses of namilumab (80mg, 150mg and 300mg) or placebo</td>
<td>Incidence and severity of AEs</td>
<td>Phase I recruiting</td>
</tr>
<tr>
<td>Namilumab NCT02379091 (58)</td>
<td>GM-CSF</td>
<td>SC doses of namilumab (20mg, 80mg and 150mg) or placebo, plus MTX</td>
<td>DAS28-CRP response</td>
<td>Phase II recruiting</td>
</tr>
<tr>
<td>Namilumab NCT02393378 (59)</td>
<td>GM-CSF</td>
<td>Namilumab 300mg SC at Week 0 followed by 150mg eow or adalimumab 40mg eow, plus MTX</td>
<td>Change in synovitis, erosion and bone marrow edema (ostitis) based on OMERACT RAMRIS</td>
<td>Phase II recruiting</td>
</tr>
<tr>
<td>MORAb-002 NCT01357759 (60)</td>
<td>GM-CSF</td>
<td>A single, escalating IV doses of MORAb-002 or placebo</td>
<td>Incidence and severity of AEs</td>
<td>Phase I completed, no results posted</td>
</tr>
<tr>
<td>KB003 NCT00995449 (61)</td>
<td>GM-CSF</td>
<td>70mg, 200mg, or 600mg KB003 IVx5doses or placebo IVx5doses</td>
<td>Incidence and severity of AEs</td>
<td>Phase II terminated (program refocus)</td>
</tr>
</tbody>
</table>

ACR: American College of Rheumatology; AE: adverse event; CRP: C-reactive protein; DAS28: Disease Activity Score-28; eow: every other week; G-CSF: granulocyte colony-stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; HAQ-DI: Health Assessment Questionnaire Disability Index; IV: intravenous; MTX: methotrexate; OMERACT: Outcome measures in Rheumatology; RAMRIS: rheumatoid arthritis magnetic resonance imaging studies; SC: subcutaneous.

(DAS28-CRP <3.2, DAS28–CRP <2.6, ACR20/50/70), further studies are warranted to confirm the promising results in larger cohorts and to find an optimal positioning for mavrilimumab in the treatment algorithm in patients with RA.

GM-CSF inhibition

MOR103. An alternative approach to interfere with GM-CSF signalling would be the direct neutralisation of the cytokine. The first in patient Study of an anti-GM-CSF monoclonal antibody (MOR103) in RA included 96 patients with active RA (DAS28≤5.1) (55). The primary outcome measure was the adverse event rate and safety profile, while secondary endpoints included DAS28, ACR core set measures, EULAR response criteria and magnetic resonance imaging of synovitis (MRI synovitis). By using three different dosages of MOR103 (0.3, 1.0 and 1.5 mg/kg) compared to placebo, a rapid and significant clinical improvement was demonstrated compared to placebo, most pronounced at the 1.0 mg/kg dosage in this study. Short-term safety and tolerability remained in the range of placebo. AEs were generally mild or moderate and the most common AE was nasopharyngitis.

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are recruiting patients (57-59). Current status and characteristics of the studies targeting GM-CSF pathway are summarised in Table I.

Discussion and conclusion

Data from both preclinical and clinical studies suggest that targeting GM-CSF and its receptor is a reasonable treatment option in RA. The favourable safety together with early and sustained efficacy and consistency of results in patients from different continents support the initiation of worldwide phase III studies. Although the obtained ACR20 and ACR50 responses are comparable to marketed biologic agents, the TNF-independent mode of action of GM-CSF blockade may provide an alternative treatment option in patients resistant to other biologics. Moreover, data from preclinical studies support the hypothesis that targeting GM-CSF can be a part of possible future combination therapies especially with concurrent blockade of IL-17. Finally, the introduction of new therapies with novel targets has the potential to decrease the proportion of refractory patients in RA. The most important issue in the near future will be the selection of right biological DMARD on an individual basis.

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