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REVIEW

Emerging Role of Fractalkine in the Treatment of Rheumatic Diseases

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Abstract: Rheumatoid arthritis (RA) is an autoimmune disorder that affects joints and is characterized by synovial hyperplasia and bone erosion associated with neovascularization and infiltration of proinflammatory cells. The introduction of biological disease-modifying anti-rheumatic drugs has dramatically changed the treatment of RA over the last 20 years. However, fewer than 50% of RA patients enter remission, and 10–15% are treatment refractory. There is currently no cure for RA. Fractalkine (FKN, also known as CX3CL1) is a cell membrane-bound chemokine that can be induced on activated vascular endothelial cells. FKN has dual functions as a cell adhesion molecule and a chemoattractant. FKN binds specifically to the chemokine receptor CX3CR1, which is selectively expressed on subsets of immune cells such as patrolling monocytes and killer lymphocytes. The FKN–CX3CR1 axis is thought to play important roles in the initiation of the inflammatory cascade and can contribute to exacerbation of tissue injury in inflammatory diseases. Accordingly, studies in animal models have shown that inhibition of the FKN–CX3CR1 axis not only improves rheumatic diseases but also reduces associated complications, such as pulmonary fibrosis and cardiovascular disease. Recently, a humanized anti-FKN monoclonal antibody, E6011, showed promising efficacy with a dose-dependent clinical response and favorable safety profile in a Phase 2 clinical trial in patients with RA (NCT02960438). Taken together, the preclinical and clinical results suggest that E6011 may represent a new therapeutic approach for rheumatic diseases by suppressing a major contributor to inflammation and mitigating concomitant cardiovascular and fibrotic diseases. In this review, we describe the role of the FKN–CX3CR1 axis in rheumatic diseases and the therapeutic potential of anti-FKN monoclonal antibodies to fulfill unmet clinical needs.

Keywords: fractalkine, CX3CR1, humanized anti-fractalkine monoclonal antibody (E6011), $CD16^{+}$ monocyte, rheumatic diseases

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disorder that primarily affects joints. The disease is characterized by synovial hyperplasia and bone erosion associated with neovascularization, infiltration of proinflammatory cells, and increased cytokine production. These pathological inflammatory features are generated locally by the selective invasion and accumulation of immune cells in the lesion.[1] The step-wise process by which immune cells are recruited from the blood, extravasate through interactions with vascular endothelial cells, and migrate into tissue is a tightly regulated process involving a number of chemotactic factors and cell adhesion molecules.[2]

Chemokines are a family of molecules that play important roles in the migration of leukocytes through binding to specific cell-surface receptors.[3] The

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approximately 50 members of the chemokine family are classified into CC, CXC, CX3C, and C subfamilies,[3] whereas the 19 known chemokine receptors are all members of the G protein-coupled 7-transmembrane superfamily of receptors.[3,4] The first step of leukocyte migration to sites of inflammation involves transient and weak selectin-mediated interactions between rolling leukocytes and vascular endothelial cells (Figure 1A). Next, integrins expressed by leukocytes are activated by chemokines presented on glycosaminoglycans. This is followed by firm adhesion of the leukocytes to the endothelium, extravasation, and transmigration into the tissue, where the cells move along a chemoattractant gradient towards the site of inflammation (Figure 1A).[5–8] The soluble form of macrophage inflammatory protein-1β (MIP-1β) is one example of a chemokine that induces firm adhesion of T cells to endothelial cells. We reported that MIP-1β immobilized by binding to cell-surface proteoglycans induces integrin-mediated adhesion of T cells much more efficiently than does soluble MIP-1β. At one time, chemokines were thought to be exclusively secreted as soluble molecules that were indispensable to forming

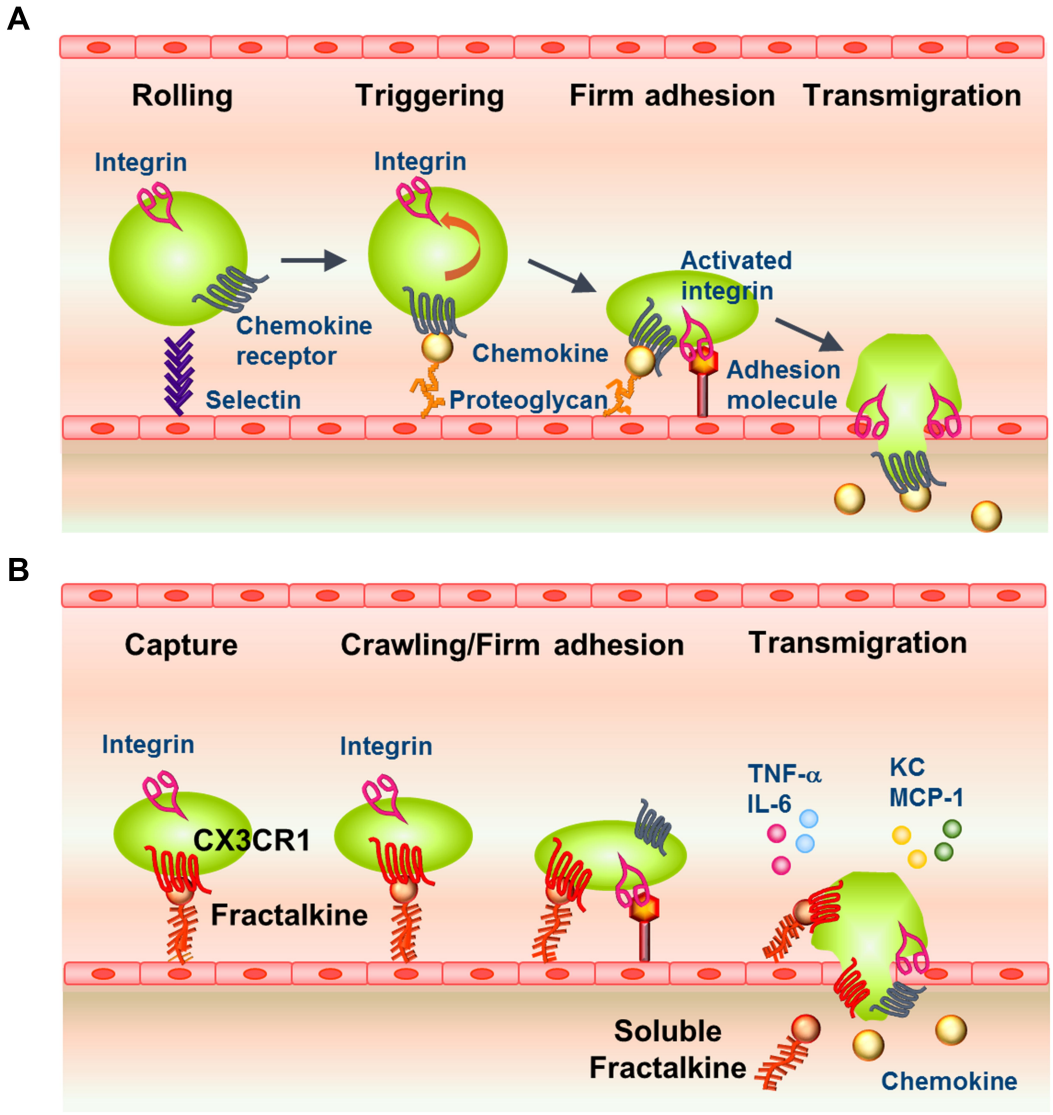

Figure 1 Classical and Fractalkine–CX3CR1-Mediated Pathways of Leukocyte Recruitment to Inflamed Tissue. (**A**) Model of the classical pathway for leukocyte extravasation into sites of inflammation *via* an adhesion and transmigration cascade. Leukocytes adhere to the endothelial layer through selectins (tethering and rolling), which is followed by engagement of chemokine receptors and integrin activation (firm adhesion), and transmigration into the underlying tissue. (**B**) Model of the involvement of fractalkine-mediated pathways in the adhesion and transmigration of CX3CR1^{high} leukocytes from the circulation into inflamed tissue. Fractalkine–CX3CR1 engagement enhances the transient capture and attachment of leukocytes to endothelial cells, which is followed by crawling/firm adhesion (activation of integrins by chemokines), production of inflammatory cytokines, and transmigration through the endothelial layer to the sites of inflammation.

Abbreviations: TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; KC, keratinocyte chemoattractant; MCP-1, macrophage chemoattractant protein-1.

a local chemokine gradient via binding to proteoglycans;[9] however, it is now known that chemokines are also synthesized as membrane-bound molecules that do not interact with proteoglycans.[4]

Fractalkine (FKN, also known as CX3CL1) is the only known member of the CX3C chemokine family and is a functionally unique membrane-bound chemokine possessing multiple biological functions.[2] Auffray et al showed that FKN–CX3CR1 interactions enable a subset of monocytes, known as patrolling cells, to crawl along the resting endothelium in a manner dependent on both FKN and the integrin LFA-1.[10] Under inflammatory conditions, binding of FKN to CX3CR1 plays multiple roles in maintaining immune homeostasis by supporting the movement of patrolling monocytes on vascular endothelial cells, facilitating the rapid migration of circulating leukocytes into inflamed tissues (Figure 1B), and contributing to the survival of leukocyte subsets. Considering these roles, it is not surprising that the FKN–CX3CR1 axis is involved in the pathogenesis of many inflammatory diseases.

In this review, we focus on the physiological and pathological roles of FKN in various rheumatic diseases. We also discuss the therapeutic potential of the anti-FKN monoclonal antibody (mAb) E6011 (Eisai Co. Ltd.),[11] which has a distinct mode of action compared with cytokine inhibitors and holds promise as a strategy to meet the unmet medical needs of patients with rheumatic diseases.

Physiological Functions of the FKN–CX3CR1 Axis

FKN is the sole member of the CX3C-type chemokine family and consists of a chemokine domain, a mucin domain, and a transmembrane domain with a short cytoplasmic tail.[12] FKN is also unique compared with other classical secreted chemokines in that its membrane-bound form is fully functional as an adhesion molecule,[4] thereby obviating the need for an association with proteoglycans. Indeed, cells expressing CX3CR1, the FKN receptor, bind rapidly and with high affinity to plate-immobilized FKN as well as to FKN-expressing cells.[4,13,14] The soluble form of FKN is generated by proteolytic cleavage mediated by ADAM 10 (a disintegrin-like metalloproteinase 10) or ADAM17 (also known as tumor necrosis factor-α converting enzyme).[15,16]

Membrane-bound FKN is expressed on endothelial cells, fibroblast-like synoviocytes (FLSs), intestinal epithelial cells, osteoblasts, neurons, and astrocytes.[17] FKN is upregulated on several of these cell types, especially endothelial cells, FLSs, and intestinal epithelium, upon exposure to inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1α (IL-1α), and interferon-γ (IFN-γ).[18]

The FKN receptor CX3CR1 is expressed on subsets of cytotoxic lymphocytes, including natural killer cells, effector memory T cells, and $\gamma\delta^+$ T cells, all of which express the lytic molecules perforin and granzyme B and exhibit marked cytotoxicity.[4] CX3CR1 is also expressed on monocytes/macrophages, dendritic cells, and osteoclast precursors (OCPs).[19,20] Circulating peripheral blood monocytes can be classified into three subsets: $CD14^{high}CD16^{-}$ (classical), $CD14^{high}CD16^{int}$ (intermediate), and $CD14^{int}CD16^{high}$ (non-classical monocytes).[20–22] Among these three subsets, intermediate and non-classical monocytes are known to express high levels of CX3CR1.[23,24] Consequently, FKN preferentially mediates the migration of the two $CX3CR1^{high}$ subsets (intermediate and non-classical monocytes; hereafter referred to as $CD16^{+}$ monocytes).[24]

In mice, $CX3CR1^{high}$ monocytes have been shown to play a role in monitoring vascular abnormalities by acting as patrolling cells, as described above. Haskell et al reported that CX3CR1-expressing cells adhere more rapidly to immobilized FKN than they do to vascular cell adhesion molecule-1 on endothelial cells,[25] suggesting that the FKN–CX3CR1 interaction may be dominant in vivo.

FKN–CX3CR1 Signaling Cascades

FKN–CX3CR1 binding also triggers G protein-mediated signaling that enhances the avidity of integrin–ligand binding.[13,26,27] Activation of CX3CR1 by FKN initiates a signaling cascade through Gαi/o that involves activation of extracellular signal-regulated kinase (a mitogen-activated protein kinase), phosphoinositide 3-kinase, Akt/protein kinase B, and mobilization of intracellular Ca^{2+}. These signals promote the survival of $CX3CR1^{high}$ macrophages.[28–31] These events result in firm attachment of $CX3CR1^{high}$ monocytes to the vascular endothelium, and the cells then produce chemokines and cytokines that initiate local inflammation and recruit neutrophils and $CX3CR1^{low}$ monocytes to the lesion (Figure 1B).[32] Through these mechanisms, FKN facilitates the rapid recruitment and extravasation of circulating leukocytes,[26] thereby amplifying the inflammatory reaction.

White et al reported that FKN contributes to the survival of macrophages in both mice and humans.[29] In CX3CR1-deficient mice, macrophage survival is impaired under normal physiological conditions as well as during liver inflammation and atherosclerosis.

FKN–CX3CR1 Axis Involvement in the Pathogenesis of Rheumatic Diseases

RA

The synovium is the primary target of immune cells in RA, and infiltrated activated macrophages and lymphocytes are abundant in the affected synovial tissue. These cells secrete a variety of inflammatory cytokines that further activate joint-resident cells such as FLSs, chondrocytes, and osteoclasts. In turn, the locally amplified tissue inflammation results in hyperproliferation of FLSs and joint destruction through activation of osteoclasts and copious production of matrix metalloproteinases.[33]

Joint-infiltrated CX3CR1^{high} T cells strongly adhere to FLSs in the synovium in an FKN-dependent manner, and they also produce IFN-γ and exhibit cytotoxic activity.[34] The number of circulating CX3CR1^{high} T cell is also elevated in the circulation of RA patients.[35] A recent global transcriptomics study suggested that peripheral T helper (Tph) cells express CX3CR1. Tph cells can activate B cells and induce antibody production, which may contribute to tissue inflammation in RA.[36]

In mice with type II collagen-induced arthritis, a model of RA, administration of an anti-FKN mAb efficiently suppresses arthritis, as reflected by a decrease in arthritis score and a reduction in cartilage oligomeric matrix protein and matrix metalloproteinase-3 levels in plasma.[37,38] In contrast, anti-FKN treatment did not affect plasma levels of serum amyloid A, anti-type II collagen antibody, TNF-α, or IL-6, but it significantly suppressed TNF-α and IL-6 mRNA expression in the affected joints.[38] Moreover, an increase in cell death in the inflamed synovium could be detected immediately after the administration of an anti-FKN mAb to mice with type II collagen-induced arthritis.[39] In these studies, histological analysis showed suppression of cartilage and bone destruction accompanied by a marked decrease in the number of osteoclasts.[37,38] These results establish that an anti-FKN mAb can suppress local joint destruction in models of RA.

A study of human *TNF* transgenic mice, which express human *TNF-α* but are mouse *Ccr2*-deficient and lack classical monocytes, showed that joint destruction is predominantly mediated by osteoclasts differentiated from OCPs, which are themselves derived from CD115^{+}CX3CR1^{high}Ly6c^{low}CCR2^{low} non-classical monocytes.[40] The interaction between FKN and CX3CR1 is important for normal osteoclast differentiation and efficient bone resorption in normal mice.[41–43] FKN is highly expressed on osteoblasts located on the bone surface in conditions associated with inflamed joints, such as RA. In addition, immobilized FKN is involved in firm adhesion of CX3CR1-expressing OCPs to the plate.[41,43,44]

FKN blockade has been shown to inhibit migration of macrophages and OCPs into the inflamed synovium.[37,38] Interestingly, drugs currently used for RA treatment, such as etanercept (TNF-α inhibitor) and tofacitinib (Janus kinase [JAK] inhibitor), do not directly inhibit OCP migration.[45] These results suggest that anti-FKN mAbs may act through multiple modes of action; namely, an anti-inflammatory effect via inhibition of the accumulation of inflammatory cells; induction of FLS cell death; and a bone-preserving effect via a reduction of osteoclasts in the affected joints. These observations further support the possible utility of anti-FKN mAbs as an alternative therapy for RA.

Interstitial lung disease (ILD) is a common extraarticular complication of RA that worsens the prognosis and increases mortality in refractory cases.[46] The role of FKN in the pathogenesis of ILD is currently unknown; however, the FKN–CX3CR1 axis has been implicated in lung involvement in patients with systemic sclerosis (SSc)[47,48] and amyopathic dermatomyositis (ADM),[49,50] suggesting that this mechanism might also be involved in RA-associated ILD. In a bleomycin-induced model of pulmonary fibrosis, mice lacking *Cx3cr1* had reduced levels of pulmonary fibrosis, suggesting that FKN contributes to an inflammatory state that exacerbates ILD.[51] Thus, further studies should investigate anti-FKN therapy as a potential treatment for RA-associated ILD.

SSc

SSc is characterized by fibrosis and vascular alterations that affect various organs, including the skin, lungs, esophagus, intestines, and heart. Hasegawa et al reported that patients with SSc have large numbers of CX3CR1-expressing macrophages in the lung and skin tissues compared with healthy subjects. SSc patients show strong FKN expression on endothelial cells in the skin and on endothelial cells, type II pneumocytes, and airway epithelial cells in the lungs.[47,48]

Several studies, including a large cohort study of 292 SSc patients, have shown that serum FKN concentrations are higher in SSc patients than in healthy subjects, and high FKN is associated with a higher titer of anti-topoisomerase -I-antibody, the presence of digital ischemia, and more severe pulmonary fibrosis.[47,48,52] Serum FKN is decreased by glucocorticoid treatment with or without cyclophosphamide.[47] Polymorphisms at positions 249I and 280M of the *CX3CR1* sequence are thought to be associated with pulmonary arterial hypertension, which is a life-threatening complication in SSc patients.[53] These findings suggest that FKN is a biomarker of SSc-related ILD and may contribute to the pathogenesis of SSc. Cultured normal human lung fibroblasts produce FKN after incubation with IL-1β and IFN-γ,[54] which are stimulators of pulmonary fibrosis and inflammation.[55] Thus, pulmonary fibrosis might be exacerbated by FKN induced in inflammatory conditions.

Studies in mice have demonstrated that the FKN–CX3CR1 axis is an important contributor to the accumulation of macrophages and fibroblasts at wound sites.[56] In the bleomycin-induced skin fibrosis model of SSc, serum FKN and lesional FKN expression are increased. Administration of an anti-FKN mAb or *Cx3cr1* deficiency significantly suppresses the dermal thickness, collagen content, and capillary loss caused by bleomycin.[57] In a murine model of SSc induced by transforming growth factor-β and connective tissue growth factor, skin fibrosis and macrophage infiltration are attenuated by anti-FKN mAb treatment or *Cx3cr1* deficiency.[58] Anti-FKN mAb administration to bleomycin-treated mice suppresses skin fibrosis and skin infiltration of $CX3CR1^{high}$ cells, monocytes/macrophages, and $CD3^+$ T cells.[57] In addition, FKN is reportedly involved in liver and kidney fibrosis in mice, both of which are attenuated by *Cx3cr1* deficiency.[59–62] Although the role of FKN in the pathogenesis of pulmonary fibrosis is not fully understood, increased expression of CX3CR1 on fibroblasts and M2 type macrophages, which play a pivotal role in fibrosis, is observed in the bleomycin-induced pulmonary fibrosis mouse model. Notably, this effect is attenuated in *Cx3cr1*-deficient mice via a reduction in fibrocyte and M2 macrophage infiltration.[51]

Polymyositis and Dermatomyositis (PM and DM)

PM and DM are inflammatory diseases involving infiltration of T cells and macrophages into the muscles, and both diseases are often complicated by pulmonary fibrosis. The affected muscle tissue of patients with PM or DM and the lungs of those with ILD express FKN on infiltrated mononuclear cells and endothelial cells, and infiltrated T cells and macrophages in these organs express CX3CR1.[63] Levels of ADAM17, which cleaves FKN to generate a soluble form, is significantly higher in the serum of patients with inflammatory myositis than in healthy subjects,[64] suggesting that FKN is secreted into the lung tissue. Similarly, serum FKN is higher in PM or DM patients than in healthy subjects[63,65] and its level correlates with disease activity, as reflected by serum creatine kinase, manual muscle tests, and alveolar–arterial oxygen pressure difference.[63] Notably, in ADM patients who have antibodies against CADM-140/MDA5 (clinically amyopathic dermatomyositis-140/melanoma differentiation-associated gene 5), serum FKN levels and the anti-CADM-1/MDA5 antibody titer not only correlated with each other but also both correlated with disease activity.[49,50] Takada et al also reported that the anti-CADM-140/MDA5 antibody titer can predict the course of rapidly progressing ADM-related ILD.[50]

Anti-FKN mAb administration ameliorates myositis in mice with experimental autoimmune myositis, a model of human PM.[66] In these mice, FKN is expressed on infiltrated mononuclear cells and endothelial cells in the affected muscle, and CX3CR1 is expressed on $CD4^+$ and $CD8^+$ T cells and macrophages.[66]

Systemic Lupus Erythematosus (SLE)

SLE is an autoimmune disease that can affect many organs, including the skin, joints, central nervous system, and kidneys.[67] The serum concentration of FKN is higher in patients with SLE than in healthy subjects or patients with RA or primary Sjogren's syndrome.[68,69] In addition, serum FKN correlates significantly with the disease activity index of SLE patients, as well as with biomarkers such as anti-double stranded DNA antibodies, anti-Sm antibodies, immune complexes, and complement hemolytic activity (CH50).[69]

SLE-related lupus nephritis (LN) is a significant cause of morbidity and mortality.[70] The LN classification proposed by the International Society of Nephrology/Renal Pathological Society (ISN/RPS) is used to provide information on disease activity and/or chronicity and to guide treatment.[71] Glomerular expression of FKN and kidney infiltration by $CX3CR1^{high}CD16^+$ monocytes are both elevated in patients with ISN/RPS class III or IV LN who present with proliferative glomerulonephritis.[72] Reflecting

the local pathology, FKN levels in serum and urine are also higher in patients with class III or IV LN than in patients with other classes.[73] Similarly, in MRL/lpr mice, which spontaneously develop a form of glomerulonephritis that resembles class IV LN, FKN expression and $CD16^{+}$ monocyte infiltration in glomeruli both increase in parallel with LN progression.[74] Moreover, the mononuclear cell infiltration and glomerular damage in these mice are reduced by administration of an FKN antagonist.[74,75] These findings therefore support the involvement of FKN in the pathogenesis of LN via recruitment of monocytes into the kidney.

FKN levels are significantly higher in cerebrospinal fluid samples from patients with SLE-associated involvement of the central nervous system (termed neuropsychiatric SLE) than from healthy subjects, and the FKN levels correlate with treatment effects.[76] Similarly, both cerebrospinal fluid and serum levels of FKN are significantly higher in patients with diffuse neuropsychiatric SLE than in patients with other SLE subtypes or in healthy subjects.[76] These results further support a role for FKN in the pathogenesis of SLE, at least neuropsychiatric SLE, and suggest that FKN could serve as a therapeutic target and/or a biomarker for SLE disease activity.

IgG4-Related Disease (IgG4-RD)

IgG4-RD is a relatively recently recognized immunological disease characterized by an elevated serum IgG4 concentration and immune-mediated fibroinflammatory processes. Infiltration of IgG4-positive plasma cells is observed in various organs, including the lacrimal glands, salivary glands, pancreas, kidneys, lungs, and retroperitoneum.[77] The main histopathological findings of the involved organs are storiform fibrosis formed by spindle cells that resemble fibroblasts, obliterative phlebitis, and ectopic lymphoid structures.

A recent study indicated that CX3CR1-expressing Tph-like cells, which can recruit B cells and T follicular helper cells, contribute to the typical pathological findings of tissue injury and ectopic lymphoid structure formation in IgG4-RD. Patients with this disorder have elevated levels of $CX3CR1^{high}$ Tph-like cells in the blood, and the percentage of these cells correlates positively with the number of involved organs and the IgG4-RD Responder Index score. $CX3CR1^{high}$ Tph-like cells express abundant levels of cytotoxic mediators such as granzyme A and perforin, leading to pathological tissue damage in IgG4-RD lesions. Thus, $CX3CR1^{high}$ Tph-like cells could be a potential clinical biomarker and/or a therapeutic target for inhibiting the progression of IgG4-RD.[78]

Cardiovascular Disease (CVD)

Epidemiological studies have shown that RA is associated with a significantly increased risk of CVD-related morbidity and mortality.[79] Numerous reports have documented the involvement of the FKN–CX3CR1 axis in atherosclerosis and cardiovascular events.[80,81] The FKN–CX3CR1 axis participates in the atherosclerotic pathological process by mediating the recruitment of leukocytes and their interaction with vascular cells.[82] Interestingly, polymorphisms in the human *CX3CR1* gene are genetic risk factors for coronary artery disease and atherosclerosis. These polymorphisms are associated with a significant decrease in the number of FKN-binding sites per cell.[83]

Clinical research has shown that elevated $CD16^{+}$ monocyte counts are associated with an increased risk of cardiovascular events.[84] $CD14^{low}CD16^{high}$ monocytes are associated with more advanced vascular dysfunction, as measured by nitric oxide bioavailability and vascular production of reactive oxygen species.[85] Plasma FKN levels are significantly increased in patients with unstable angina pectoris and plaque rupture compared with healthy subjects.[86] These studies, supported by a growing body of evidence demonstrating the significant role of $CD16^{+}$ monocytes in atherosclerosis development, suggest that $CD16^{+}$ monocytes are a potential target for the development of new therapeutic strategies in atherosclerosis.[84]

Cx3cr1 deficiency has been shown to prevent the development of arteriosclerosis in *apolipoprotein E*-deficient ($Apoe^{-/-}$) mice.[87,88] CX3CR1 is expressed on intimal dendritic cells, and these cells are less abundant in the aortas of $Cx3cr1^{-/-}/Apoe^{-/-}$ mice compared with $Apoe^{-/-}$ mice.[89] In this study, *Cx3cr1* deficiency was found to impair the accumulation of dendritic cells in the aortic wall and markedly reduce the atherosclerotic burden.[89]

Clinical Development of E6011, a Humanized Anti-FKN mAb, in RA

Insufficiently treated RA leads to severe joint damage, disability, decreased quality of life, and other comorbidities. At present, the predominant treatments are disease-modifying anti-rheumatic drugs (DMARDs). They include conventional synthetic DMARDs, of which methotrexate is the anchor drug, as well as biological and synthetic DMARDs that target TNF-α, the IL-6 receptor, T cell costimulatory

molecules, CD20 on B cells, and intracellular signaling molecules such as JAKs. Recent guidelines for the management of RA recommended that sustained remission or low disease activity should be rapidly attained in every patient.[90–92] However, about 50–70% of patients fail to achieve remission or to maintain low disease activity, even if they initially respond well to current therapies.[93,94]

We recently conducted a phase 2, multicenter, randomized, double-blind, placebo-controlled study of the humanized anti-FKN mAb E6011 to evaluate its safety and efficacy in Japanese RA patients inadequately responding to methotrexate (NCT02960438).[95] Patients were randomly assigned to receive placebo or E6011 at 100 mg, 200 mg, or 400/200 mg in a 2:1:2:2 ratio. Subjects in the 100 mg, 200 mg, and placebo groups were dosed at Weeks 0, 1, and 2, and every 2 weeks subsequently; subjects in the 400/200 mg group received 400 mg at Weeks 0, 1, 2, 4, 6, 8, and 10 and then received 200 mg every 2 weeks subsequently. During the 24-week double-blind period, patients received the study drug subcutaneously at Weeks 0, 1, and 2, and then every 2 weeks thereafter. The primary endpoint was the American College of Rheumatology 20 (ACR20) response rate at Week 12. Using a non-responder imputation (NRI) method, the response rates were 37.0%, 39.3%, 48.1%, and 46.3% in the placebo, 100 mg, 200 mg, and 400/200 mg groups, respectively (not statistically significant). However, the secondary endpoint (ACR20 response rate at Week 24) was significantly different for the 200 mg group (53.7%, $P < 0.025$) and 400/200 mg group (57.4%, $P < 0.025$) compared with the placebo group (35.2%). In the biomarker analysis, we focused on $CD16^+$ monocytes due to their importance in RA pathophysiology and their high expression of the FKN receptor CX3CR1. The full patient population was dichotomized into "high" and "low" $CD16^+$ monocyte subgroups using a cutoff value of 10.35%, the median percentage of $CD16^+$ monocytes at baseline. Subjects in the high group (>10.35% $CD16^+$ monocytes at baseline) showed a greater dose-dependent ACR20 response compared with subjects in the low group at Week 24: the response rates were 30.0% vs 43.3%, 46.7% vs 20.0%, 57.7% vs 54.5%, and 69.6% vs 45.5% for the placebo, 100, 200, and 400/20 mg groups, respectively [NRI method]; Figure 2). These results indicated that the baseline percentage of $CD16^+$ monocytes could predict the response to E6011, and additionally suggest the possibility of a precision medicine approach to E6011 therapy, although further research in this area will be needed.

In this clinical study, adverse events that occurred in ≥5% of subjects in any E6011 group were nasopharyngitis, upper respiratory tract infection, stomatitis, bronchitis, back pain, pharyngitis, and dental caries. Thus, E6011 was well tolerated with no notable safety concerns at doses of 100, 200, and 400/200 mg when administered subcutaneously for 24 weeks.

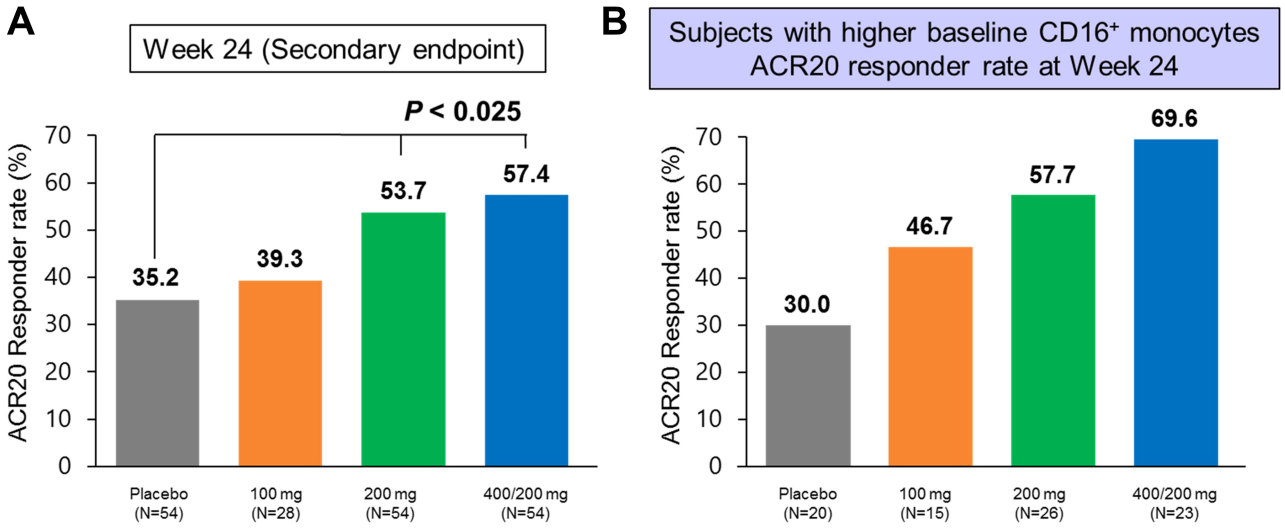

Figure 2 Results of a Phase 2 Clinical Trial of E6011, a Humanized Anti- FKN mAb, in Subjects with RA (NCT02960438). (**A**) ACR20 response rate of the full cohort at Week 24 (NRI). (**B**) ACR20 response rate at Week 24 in the patient subset with a high percentage of $CD16^+$ monocytes at baseline. Subjects were divided into high and low groups using the median percentage of $CD16^+$ monocytes at baseline (10.35%). Reproduced from *ACR/ARP Annu Meet*, A Phase 2 Study of E6011, an Anti-Fractalkine Monoclonal Antibody, in Patients with Rheumatoid Arthritis Inadequately Responding to Biologics, Tanaka T, Takeuchi T, Yamanaka H, et al. 9(Supplement 70):1-3553, copyright 2018, with permission from BMJ Publishing Group Ltd.[45]

Abbreviations: ACR20, American College of Rheumatology 20% response criteria; FKN, fractalkine; mAb, monoclonal antibody; NRI, non-responder imputation; RA, rheumatoid arthritis.

In a second study of E6011 in RA patients with an inadequate response to biologics, subcutaneous administration of E6011 at 400 mg was well tolerated but did not show significant efficacy compared with placebo at Week 12 (NCT02960490).[96] However, an exploratory pharmacokinetic exposure analysis indicated that subjects with higher serum trough concentrations of E6011 showed a trend towards efficacy, albeit not significant. Based on these results, further investigation of E6011 is warranted to determine the optimal clinical dose and evaluation period in RA.

Future Perspectives for the Treatment of RA with E6011

Despite considerable advances in RA treatments and strategies, some patients fail to achieve and/or sustain remission. Those patients require new treatment options with novel mechanisms of action. Several therapeutic antibodies are in clinical development for RA, including modulators of inflammatory cytokines (IL-6, IL-10), inflammatory growth factors (granulocyte-macrophage colony-stimulating factor), and adhesion molecules (cadherin-11) (Table 1).[97] Among these investigational drugs, inhibition of cadherin-11 is a particularly novel approach that targets synovial fibroblasts in RA.[98] Although it will be interesting to evaluate the efficacy of adding anti-cadherin-11 mAb (RG6125) on top of anti-TNF therapy in RA patients with an inadequate response to anti-TNF alone, unfortunately, no discernable therapeutic effect of RG6125 in combination with TNF blockers has been demonstrated to date.[98]

In addition to monotherapy with new drugs, there is a pressing need to investigate novel combinations or sequential treatments with targeted therapies for patients refractory to currently available therapies,[97,99] who are arguably the patient population with the most urgent unmet medical needs.[100] It should be emphasized that patients with a history of treatment with multiple biologics and/or small molecules should not be excluded from clinical trials. Of particular interest is the testing of combination therapies in refractory patients, which should also be studied alongside novel targeted therapies. E6011 is a biologic classified as a cell trafficking inhibitor but it is not a direct cytokine inhibitor. Theoretically, adding E6011 on top of anti-cytokine therapy may be a feasible option for treatment-refractory RA patients.

Presentations at the 2019 Advances in Targeted Therapies meeting emphasized the need to better define "refractory" states both phenotypically and molecularly. Recently, Tasaki et al reported a longitudinal study that monitored the drug response of RA patients using multi-omics analysis of peripheral blood constituents.[101] Even RA patients who achieved clinical remission by treatment with tocilizumab or infliximab may not reach molecular remission, which is defined as a molecular profile similar to that of healthy individuals. Interestingly, that study found that the transcriptional residual molecular signature (RMS) of $CD16^{+}$ monocytes is upregulated in RA patients

Table 1 Cytokine-Targeting Therapies in Development for RA

Target	Drug Name/Code	Company	Clinical Trial Phase
IL-6	Sirukumab (CNTO-136, Plivensia)	GSK, Janssen	III
GM-CSF	Otilimab (GSK 3,196,165)	GSK	III
TNF-α	Ozoralizumab (ATN-103, TS-152)	Ablynx, Taisho	III
IL-6	Olokizumab (OKZ)	R-Pharm	III
IL-6	Clazakizumab (BMS-945,429, ALD518)	CSL Behring	IIb
IL-6 receptor	Vobarilizumab (ALX0061)	Ablynx	IIb
IL-10	Dekavil (F8IL10)	Philogen	II
GM-CSF	Namilumab (MT203)	Takeda	II
GM-CSF receptor-α	Mavrilimumab (CAM-3001)	MedImmune	II
TNF-α, IL-17A	Remtolumab (ABT-122)	AbbVie	II
GM-CSF	Gimsilumab (MORAb-022)	Morphotek	I
GM-CSF	Lenzilumab (KB003)	Humanigen	Terminated
Cadherin-11	RG6125	Roche	Terminated

Notes: Compiled from information in reference[97] and ClinicalTrials.gov (https://clinicaltrials.gov).
Abbreviations: IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; TNF-α, tumor necrosis factor-α; mAb, monoclonal antibody; GSK, GlaxoSmithKline.

compared with healthy individuals,[101] suggesting the possibility that the $CD16^+$ monocyte RMS may be indicative of incomplete response to treatment. Moreover, the $CD16^+$ monocyte RMS observed in RA patients was also found in patients with inflammatory bowel disease and obesity.[101] Thus, these results suggest that the clinical importance of the transcriptional RMS may not be limited to RA, and that $CD16^+$ monocytes could be a biomarker to identify molecular remission in patients with diseases other than RA. Based on these observations, we speculate that E6011 monotherapy or combination therapy with other biologics or JAK inhibitors could be an option to achieve molecular remission in refractory RA patients.

Conclusion

The preceding discussion highlighted the need to study novel targeted therapies, novel combinations, and new sequential treatment strategies with existing therapies for the treatment of refractory RA.[100] By blocking cell adhesion and signaling, the anti-FKN mAb E6011 has a distinct mode of action from other drugs currently under investigation for RA, which include cytokine/cytokine receptor inhibitors (eg, infliximab, tocilizumab), modulators of T cell costimulation (eg, abatacept), and JAK inhibitors (eg, tofacitinib, baricitinib). This unique feature may allow E6011 to be used not only for monotherapy but also for combination therapy with other drugs for patients with refractory RA. E6011 may also reduce the risk of CVD in RA patients by decreasing the abundance of $CD16^+$ monocytes, thereby acting as a cardioprotective drug. As noted above, recent studies have shown that the $CD16^+$ monocytes transcriptional RMS may be useful as a hallmark for molecular remission in RA. The results of clinical and preclinical studies indicate that E6011 also has the potential to promote "total health care" in patients with rheumatic and other diseases involving $CD16^+$ monocytes, and could present a new therapeutic strategy for the treatment of patients with refractory RA.

Abbreviations

ACR20, American College of Rheumatology 20; ADAM 10, a disintegrin-like metalloproteinase 10; ADM, amyopathic dermatomyositis; *Apoe*$^{-/-}$, *apolipoprotein E*-deficient; CADM-140/MDA5, clinically amyopathic dermatomyositis-140/melanoma differentiation-associated gene 5; CH50, complement hemolytic activity; CVD, cardiovascular disease; DM, dermatomyositis; DMARDs, disease-modifying antirheumatic drugs; FKN, fractalkine; FLS, fibroblast-like synoviocyte; IFN-γ, interferon-γ; IL-1α, interleukin-1α; ILD, interstitial lung disease; ISN/RPS, International Society of Nephrology/Renal Pathological Society; JAK, Janus kinase; LN, lupus nephritis; mAb, monoclonal antibody; MIP-1β, macrophage inflammatory protein-1β; NRI, non-responder imputation; OCP, osteoclast precursor; PM, polymyositis; RA, rheumatoid arthritis; RMS, residual molecular signature; SLE, systemic lupus erythematosus; SSc, systemic sclerosis; TNF-α, tumor necrosis factor-α; Tph, peripheral T helper cell.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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