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Targeting the chemokine network in atherosclerosis

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ABSTRACT

Chemokines and their receptors represent a potential target for immunotherapy in chronic inflammation. They comprise a large family of cytokines with chemotactic activity, and their cognate receptors are expressed on all cells of the body. This network dictates leukocyte recruitment and activation, angiogenesis, cell proliferation and maturation. Dysregulation of chemokine and chemokine receptor expression as well as function participates in many pathologies including cancer, autoimmune diseases and chronic inflammation. In atherosclerosis, a lipid-driven chronic inflammation of middle-sized and large arteries, chemokines and their receptors participates in almost all stages of the disease from initiation of fatty streaks to mature atherosclerotic plaque formation. Atherosclerosis and its complications are the main driver of mortality and morbidity in cardiovascular diseases (CVD). Hence, exploring new fields of therapeutic targeting of atherosclerosis is of key importance. This review gives an overview of the recent advances on the role of key chemokines and chemokine receptors in atherosclerosis, addresses chemokine-based biomarkers at biochemical, imaging and genetic level in human studies, and highlights the clinial trials targeting atherosclerosis.

1. Introduction

Atherosclerosis is the primary cause of cardiovascular diseases (CVD) and is initiated by endothelial cell (EC) activation through modified lipids such as oxidized low-density lipoprotein (ox-LDL) [1,2]. Activated ECs release chemokines, which allow for the recruitment of immune cells into the subendothelial space called the intima. The recruited leukocytes produce inflammatory mediators, thus augmenting the ongoing chronic inflammatory process at arterial sites, orchestrating monocyte to macrophage differentiation, foam cell formation, migration, and proliferation of smooth muscle cells (SMCs). As the disease progresses, the complex inflammatory milieu leads to rupture of unstable lesions and subsequent thrombosis, which might cause severe clinical complications [1]. Chemokines participate in almost all the stages from initiation of a fatty streak to mature lesion formation, and better understanding of their pathophysiological role would be of great value to clinical management. Chemokines, are a family of small, secreted, and structurally related cytokines with chemotactic properties and play a crucial role in inflammation and immunity [3].

This review recapitulates the role of key chemokines and chemokine receptors in atherosclerotic animal models, discusses advances in using chemokines or chemokine receptors as biomarkers or imaging tracers for diagnosis and risk stratification in human CVD, overviews genetic alterations in genes coding for chemokine and chemokine receptor and explores their association with CVD, and summarizes (ongoing) clinical trials.

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2. Chemokines and their receptors in experimental atherosclerosis (animal models) - recent insights

Chemokines and their receptors are multifunctional orchestrators of acute and chronic immune responses facilitating for example leukocyte mobilization, adhesion, migration, differentiation and homing to different tissues. Given the aforementioned immune regulatory properties, the chemokine (-receptor) network extensively contributes to various stages of atherosclerosis, such as leukocyte trafficking and infiltration into atherosclerotic lesions, foam cell formation and cell maturation within the plaque. In this section, novel findings on the role of a list of chemokines and their receptors (Table 1) in atherosclerosis will be revealed, highlighting potential new mechanisms which could be novel targets for future therapeutic approaches.

2.1. Chemokines in atherosclerosis

2.1.1. CCL chemokine ligands

CCL2 (also known as monocyte chemotactic protein 1 [MCP]-1) is a proatherosclerotic chemokine regulating the trafficking of inflammatory cells and thereby leukocyte migration into the artery wall [4]. Surprisingly, CCL2 release from myeloid cells was shown to be affected in a circadian fashion at different times of the day. Winter et al. showed that CCL2 dependent myeloid cell recruitment to atherosclerotic lesions oscillates in a diurnal fashion with a peak during the night phase. CCR2 antagonism at two different times (noon and midnight) in Apolipoprotein E deficient (*Apoe*$^{-/-}$) mice fed with western diet (WD) for 4 weeks, revealed a significant reduction in plaque size only at midnight compared to the vehicle control [5]. Hence, chrono-pharmacological targeting of CCL2 mediated signaling could be of therapeutic interest.

CCL3 (also known as macrophage inflammatory protein 1α) is an inflammatory chemokine inducing chemotaxis of different leukocyte subsets, including monocytes/macrophages and T-lymphocytes (T cells) via CC chemokine receptors (CCR)1, CCR4, or CCR5 [6]. Hematopoietic deficiency of CCL3 was shown to significantly reduce aortic sinus lesion formation in LDL receptor (LDLR) deficient (*Ldlr*$^{-/-}$) mice after 12 weeks of WD [7]. In addition, atorvastatin treatment alleviates atherosclerotic plaque burden in Apolipoprotein E deficient (*Apoe*$^{-/-}$) mice fed a WD for 16 weeks through downregulation of CCL3 expression [8]. Taken together, CCL3 seems to be pro-atherogenic.

CCL5 (also known as RANTES: regulated upon activation, normal T cell expressed and secreted) is another important chemokine mediating immune cell recruitment and infiltration to the atherosclerotic plaque [9]. For example, platelet derived CCL5 and human neutrophil peptide 1 (HNP1) liaise together to form stable heteromers to facilitate classical monocyte recruitment to sites of inflammation. Blocking of this HNP1-CCL5 interaction by dose dependent treatment of CX3CR1 reporter mice (*Apoe*$^{-/-}$ *Cx3cr1*$^{egfp/WT}$; WD for 4 weeks) with the peptidic inhibitor SKY leads to inhibition of HNP1-CCL5 induced adhesion of classical monocytes [10]. Antagonizing CCL5-CXCL4 heteromers with MKEY, a synthetic cyclic peptide inhibitor of CXCL4-CCL5 heterodimer formation, significantly reduced neutrophil recruitment as well as monocyte infiltration in the infarcted areas of C57BL/6 mice [11]. Concluding, blocking CCL5 heteromerization with its various interaction partners could be a promising way to diminish leukocyte recruitment to sites of inflammation.

CCL17 is mainly expressed by a subset of myeloid dendritic cells (DCs) and mediates chemotactic activity of T cells through its receptor CCR4. CCL17 deficiency in *Apoe*$^{-/-}$ mice limited atherosclerotic lesion growth, which was ascribed to an enhanced number of regulatory T cells (Treg) in the absence of CCL17 [12]. Moreover, nuclear magnetic resonance spectroscopy revealed heteromeric interaction of CCL17 and CCL5. Inhibition of these heterodimers by a specific peptide (CAN) in *Apoe*$^{-/-}$ mice fed WD for 6 weeks leads to reduction in lesion size in the aortic root underlining the proatherogenic role of CCL17 [13].

In-vivo studies in BALB/C mice suggest selective expression of CCL19 by mature DCs [14]. Higher expression of CCL19/CCL21 was observed within the atherosclerotic plaques in aortae of *Apoe*$^{-/-}$ mice, also in regions with predominantly CD68^{+} macrophages and in areas rich in CD3^{+} T cells. In the same study, *in-vitro* experiments suggest involvement of CCL19 and especially CCL21 in increasing the release of inflammatory cytokines and enhance matrix metalloproteinase (MMP) activity in THP-1 derived macrophages [15]. In conclusion, this study emphasized the increased CCL19 and CCL21 levels in atherosclerosis and how the dysfunction in their regulation could lead to altered responses in T cells and macrophages. This leads to reduced plaque stability and helps atheroprogression. Contrary to this study, in an *in-vivo* study including transplantation of bone marrow from *plt/plt* mice, which lack the expression of CCL19 and CCL21-Ser, into irradiated *Ldlr*$^{-/-}$ mice lead to significant upregulation of leukocyte recruitment into the atherosclerotic plaques. This indicates that reduced expression of CCL19 and CCL21 in *plt/plt/Ldlr*$^{-/-}$ leads to reduced pro-inflammatory activation and higher plaque stability. Additionally, in the same study downregulation of CCL19 reduced the uptake of oxLDL in the lesion macrophages of *plt/plt/Ldlr*$^{-/-}$ mice thereby resulting in smaller lesion macrophages and it was accompanied by increased plaque stability [16]. These findings suggest the contradictory role of CCL19 and CCL21 in atherosclerosis and further *in-vivo* studies are needed to unravel their exact immune-regulatory role in atherogenesis and plaque stability.

2.1.2. CXC chemokine ligands

CXC chemokine ligands like CXCL1, CXCL2, CXCL4, CXCL12 and CXCL16 play various roles in the progression of atherosclerosis. ECs release CXCL1 upon accumulation of oxLDL-induced lysophosphatidic acid and high levels of CXCL1 lead to recruitment and migration of leukocytes to the carotid bifurcation, while depletion of CXCL1 reduces macrophage accumulation and reduces atherosclerotic progression [17]. Evasin-3, a pharmacological inhibitor of CXCL1 and CXCL2, fostered reduction of MMP-9 and intraplaque neutrophil recruitment in *Apoe*$^{-/-}$ mice (fed WD for on 11 weeks and cast placement at week 2) [18]. Inhibition of CXCL1 in *Apoe*$^{-/-}$ mice (fed WD for 4 weeks) with infusion of an antibody to mCXCL1 led to reduced lesion area, and decline of monocyte and macrophage expansion [19]. In addition,

Table 1
Key chemokine ligands and chemokine receptors in atherosclerosis in this review.

Chemokines ligands	
CCL chemokine ligands	Chemokine receptors
CCL2	CCR2
CCL3	CCR1, CCR4, CCR5
CCL5	CCR1, CCR3, CCR5
CCL17	CCR4
CCL19	CCR7
CCL21	CCR7
CXCL1	CXCR2
CXCL2	CXCR2
CXCL4	CCR1, CXCR3
CXCL12	CXCR4, CXCR7
CXCL16	CXCR6
Chemokine receptors	
CC chemokine receptors	Chemokine ligands
CCR1	CCL7, CCL13, CCL3, CCL5, CCL23, CCL14, CCL15, CXCL4, CCL16
CCR2	CCL2, CCL7, CCL8, CCL12 (mouse only), CCL13, CCL16 (human only)
CCR5	CCL3, CCL4, CCL5, CCL8
CCR7	CCL19, CCL21
CXCR2	CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, CXCL8
CXCR4	CXCL12, CCR5
CXCR6	CXCL16
CX3CR1	CX3CL1

CXCL4, a platelet-derived chemokine, interacts with CCL5 to make stable heterodimers, amplifying monocyte and neutrophil recruitment, as well as neutrophil extracellular trap formation. Inhibition of this interaction was shown to inhibit atherosclerosis in mice [11,20].

CXCL12, also known as stromal cell derived factor 1 (SDF-1) alpha, and its receptor CXCR4 are involved in the healing of injured or ischemic vascular tissue. One study suggested an atheroprotective role of CXCL12 in *Apoe*$^{-/-}$ mice by recruitment of SMC progenitor cells [21]. Here, CXCL12 contributes to stabilization of atherosclerotic plaques by inducing fibrous cap thickening and SMC progenitor cell recruitment to the plaque [22]. However, another recent study evaluated the role of cell specific CXCL12 depletion using ubiquitous knockout, SMC knockout, hematopoietic knockout, non-hematopoietic knockout and endothelial cell specific knockout mice on *Apoe*$^{-/-}$ background. In this study, EC-specific CXCL12 deficiency in *Apoe*$^{-/-}$ mice implies an atheroprogressive role of CXCL12 when of endothelial/resident cellular origin [23]. Therefore, source and context of CXCL12 release have a crucial impact on its role in vascular inflammation.

Unlike other chemokines, CXCL16 contains mucin stalk, transmembrane and cytoplasmic domains. It can be present as membrane bound or as a soluble form and is expressed on activated T cells and natural killer T cells [24]. The soluble form is linked to the migration of T cells to atherosclerotic plaques. *Cxcl16*$^{-/-}$*Ldlr*$^{-/-}$ mice showed an increased number of macrophages in aortic arches and increased atherosclerosis lesion size compared to control animals [25]. In an *in vivo* study, overexpression of circulatory CXCL16 induced by intravenous injection of a lentiviral vector carrying CXCL16 transgene into *Apoe*$^{-/-}$ mice promoted a vulnerable plaque phenotype (59% higher plaque vulnerability) compared to control mice [26]. Together these data suggest a pro-atherosclerotic role of soluble CXCL16.

Extensive studies on key chemokine ligands like CCL2, CCL3, CCL5, CCL17, CXCL1, CXCL2, CXCL4, CXCL12 and CXCL16 suggest their role in atherosclerosis and pave the way for the therapeutic targeting of chemokines in atherosclerosis. The studies involving targeting of chemokine ligands in a circadian fashion were missing until Winter et al. explored the chrono-pharmacological targeting of CCL2 in early stages of atherosclerosis. The authors detected the substantial localization of immobilized CCL2 on carotid endothelium covering atherosclerotic lesions and at sites, with rhythmic myeloid cell adhesion in hypercholesteremic mice. On the transcriptional level, high Ccl2 expression in circulating monocytes and neutrophils lead to high levels of CCL2 in plasma especially at early morning hours. Additionally, time and site dependent *in-vivo* analyses of myeloid cell adhesion to the carotid endothelium and to the cremasteric microcirculation showed high leukocyte adhesion at morning in carotid endothelium as compared to microcirculation. Additionally, at early morning, disruption of CCL2 signaling lead to reduced adhesion of myeloid cells to carotid endothelium of hypercholesteremic *Cx3cr1*$^{GFP/WT}$*Apoe*$^{-/-}$ mice. Contrary to this, at the same time, CCR2 inhibition had no effect on cell adhesion in the cremasteric microcirculation. This study provides a novel targeting approach of a time- and site-specific inhibition of CCL2 at early stages of atherosclerosis. However, it would be interesting to perform such a targeting strategy during later stages of atherosclerosis and to investigate if the other organs also have the similar differences in micro and macro-circulation.

2.2. Roles of chemokine receptors in atherosclerosis

Functional effects of chemokines are mediated by their binding to their (corresponding) cell surface receptors, and the ligand repertoire of chemokine receptors is typically class restricted. Therefore, we would summarize the advances of three important classes of the receptors: CC, CXC and CX3C chemokine receptors. In addition, the majority of receptors are able to interact with many different ligands of the same class and ligands as well as receptors may also form heteromers, rendering the chemokine ligand-receptor network multifaceted and promiscuous. Thus, it is not surprising that ligand and receptor responses may differ depending on ligand or receptor availability, which are also reliant on the type of inflammation present. Hence, this network promiscuity in the context of a chronic inflammation like atherosclerosis increases the complexity of identifying therapeutic targets.

2.2.1. CC chemokine receptors

CCR1 has a detrimental role in atherogenesis by mediating monocyte recruitment, as evident by a reduced number of classical monocytes in aortas in *Ccr1*$^{-/-}$*Apoe*$^{-/-}$ mice both after 4 weeks and 8 weeks of WD [19]. However, transplantation of *Ccr1*$^{-/-}$ bone marrow into *Ldlr*$^{-/-}$ mice unveiled an increased lesion size and higher macrophage and T cell accumulation at sites of inflammation after exposure to a high cholesterol diet for 12 weeks compared to *Ccr1*$^{+/+}$ bone marrow recipients [27]. The complexity of hematopoietic cells and different mouse strains might be responsible for the different roles of CCR1 in atherosclerosis. Cell specific deletion of *CCR1* would be necessary to uncover this discrepancy.

CCR2, receptor for CCL2, mediates monocyte chemotaxis. CCR2-targeted siRNA treatment in *Apoe*$^{-/-}$ mice reduced recruitment of Ly-6C (high) monocytes, attenuated infarct inflammation, and curbed post-myocardial infarction left ventricular remodeling [28]. *Apoe*$^{-/-}$ mice treated with the CCR2 antagonist (15a) exhibited a reduced number of circulating CCR2+ monocytes and atherosclerotic burden in aortic roots after 6 weeks WD [29]. As aforementioned, blockade of CCR2-CCL2 signaling abolished oscillatory leukocyte adhesion and timed pharmacological CCR2 neutralization during the activity phase resulting in reduced atherogenesis without disturbing microvascular recruitment [5]. Hence, CCR2-based therapy would be of great potential to treat atherosclerosis.

CCR5 exerts an atherogenic phenotype as apparent from mice lacking CCR5. CCR5 deficiency resulted in less lesional macrophages in diet-induced atherosclerosis, and neointima formation was reduced in an IL10-dependent manner [30,31]. Recently, CCR5 blockade by maraviroc, a negative allosteric modulator of CCR5, was also demonstrated to hinder enhanced recruitment in MT4-MMP (membrane type-4 matrix metalloproteinase, also named MMP17)-deficient Ly6C^{low} monocytes to early atherosclerotic lesions in MT4-MMP$^{-/-}$ bone marrow transplanted *Ldlr*$^{-/-}$ recipients fed on WD for 8 weeks [32].

CCR7 is identified to regulate T cell homeostasis and stimulate DC maturation. Deletion of *CCR7* in *Apoe*$^{-/-}$ mice led to increased plaque burden by increasing T cell content in atherosclerotic lesions after WD for 8 weeks, and transplantation of *Ccr7*$^{-/-}$*Apoe*$^{-/-}$ bone marrow into *Ccr7*$^{+/+}$*Apoe*$^{-/-}$ mice followed by a WD for 8 weeks resulted in increased lesion size [33]. In contrast, CCR7 deficiency was reported to attenuate plaque burden in *Ldlr*$^{-/-}$ mice after 12 weeks of WD. Moreover, transfer of CCR7$^{+/+}$ T cells primed with oxLDL-pulsed DCs into *Ccr7*$^{-/-}$*Ldlr*$^{-/-}$ recipient mice fed a WD for 12 weeks revealed an increased atherosclerosis plaque burden to an extent comparable to *Ldlr*$^{-/-}$ mice [34]. This discrepancy might be attributable to the complex role of CCR7 signaling in different animal strains in the context of atherosclerosis. Cell-specific CCR7 knockout mice would be important to fully address the role of this receptor in T cells/macrophages/DC homing and T-cell development/activation under atherogenic conditions.

2.2.2. CXC and CX3C chemokine receptors

CXCR2 is known for its function in neutrophil mobilization and participates in early atherosclerotic lesion formation [35,36]. More recently, a macrophage migration inhibitory factor (MIF) -derived cyclopeptide was described to inhibit key inflammatory and atherogenic MIF activities through interaction with CXCR2 [37]. All the above findings are indicative of a pro-atherogenic role of CXCR2. On the other hand, CXCR2 activation by its ligand CXCL5 has been described to increase cholesterol efflux in macrophages via upregulation of ABCA1, thus playing a protective role in atherogenesis by limiting foam cell

formation [38]. Hence, the selective blockade of CXCR2/MIF interaction while keeping the CXCR2/CXCL5 interaction intact would be of importance in CXCR2-targeted therapy against atherosclerosis.

The chemokine receptor CXCR4 is a key regulator of cell homeostasis and seems to have atheroprotective functions. Recent studies showed that B-cell-specific knockout of CXCR4 in mice reduces bone marrow IgM production and plasma IgM levels, an effect which could be restored by the overexpression of CXCR4 [39,40]. Moreover, vascular CXCR4 suppresses atherosclerosis by sustaining endothelial barrier function through WNT/β-catenin signaling, and supporting a contractile SMC phenotype [41]. The longevity-associated variant (LAV) of the bactericidal/permeability-increasing fold-containing family B member 4 (BPIFB4) has been found significantly enriched in long-living individual and its transfer was shown to limit atherosclerosis and skewed macrophages towards an M2-resolving phenotype through modulation of CXCR4 [42]. However, CXCR4/MIF interactions were described to mediate atherogenesis. The dual effect of CXCR4 has hampered the therapeutic application of CXCR4 agonist/antagonist in the treatment of atherosclerosis. Inspiringly, a soluble CXCR4 ectodomain mimics (msR4M-L1) was designed and has been proved to exhibite high affinity to MIF, with no binding to CXCL12. Fluorescence polarization and microscale thermophoresis in a binding-competition approach also confirmed an intact cardioprotective axis of MIF/CD74 in the presence of msR4M-L1. Next, the MIF/msR4M-L1 core binding region was mapped and the complex formation was verified using alanine mutants of both msR4M-L1 and MIF. The selective msR4M-L1/MIF binding was then specially blocked MIF/CXCR4-driven cell signaling response without any interference of CXCL12/CXCR4-elicited signaling in a yeast system that expresses human CXCR4. Moreover, HEK293-CD74 transfectant data showed that msR4M-L1did not affect the MIF binding to cell-surface CD74. In this study, msR4M-L1 dose-dependently inhibited MIF-triggered uptake of fluorescently labeled oxidized low-density lipoprotein and motility of human monocytes. Monocyte adhesion on human aortic endothelial was also inhibited by msR4M-L1 both under static condition and shear stress. In addition, msR4M-L1 localized to atherosclerotic plaques in a MIF-specific manner and inhibited MIF-mediated atherogenic leukocyte recruitment *ex vivo*. *Apoe*$^{-/-}$ mice receiving msR4M-L1 in parallel to HFD for 4.5 weeks exhibited reduced plaque burden, lesional macrophage accumulation and reduction of multiple inflammatory cytokines in the circulation [43]. Given the fact that chemokine ligand-receptor network promiscuity has been obstacle for chemokine ligands (receptors) as therapeutic targets in clinical application, the engineered chemokine-selective- GPCR-ectodomain-based mimicry principle that distinguishes disease-exacerbating from -protective signaling in this study would raise more attention to the chemokine-based strategy and could be of great potential for the treatment or prevention of other inflammatory diseases.

CXCR6 is reported to promote T cell homing, interferon-gamma production, and macrophage accumulation into the aortic wall and mice lacking CXCR6 on *Apoe*$^{-/-}$ background had reduced atherosclerosis compared to *Apoe*$^{-/-}$ controls after WD for 17 weeks or chow diet for 56 weeks [44]. CXCR6 was also shown to accelerate foam cell formation in atherosclerosis and regulates the recruitment of pro-inflammatory IL-17 A-producing T cells into atherosclerotic aortas [45,46]. Together these data suggest a pro-atherosclerotic role of CXCR6.

CX3CR1 activation plays a crucial role in recruitment, stimulation and the survival of monocytes/macrophages in the pathogenesis of atherosclerosis. DC-restricted antigen-uptake receptor (DEC205) targeted DNA vaccine against CX3CR1 protects against atherogenesis in 34 week old *Apoe*$^{-/-}$ mice fed on chow diet [47]. Further, designed VHH (variable domains of camelid heavy chain-only) antibodies to overcome the failure in antagonistic monoclonal antibodies to CX3CR1, have recently been shown to block the CX3CR1 receptor *in vivo* and concomitantly reduced the progression of atherosclerosis in *Apoe*$^{-/-}$ mice fed WD for 16 weeks [48]. Since the presence of its unique ligand CX3CL1 (fractalkine) was confirmed in aorta of *Ldlr*$^{-/-}$ mice, engineered Treg overexpressing CX3CR1 were generated to guide Treg selectively to the plaque. In line, adoptive transfer of CX3CR1^{+}Tregs in *Ldlr*$^{-/-}$ mice resulted in reduced plaque progression and lipid deposition and ameliorated plaque stability [49]. Adoptive cell therapy to enhance immuno-suppressive functions specifically at sites of vascular inflammation are a promising therapeutic approach to limit atherosclerosis progression.

3. Chemokines in diagnosis and disease severity in human atherosclerosis

3.1. Chemokine and chemokine receptor expression in human atherosclerosis

There is a growing body of evidence describing genes altered at the onset or at advanced stages of atherogenesis. Analytical tools that are available today (e.g. gene expression microarrays or RNA sequencing) allow for the detection of the alteration in gene expression of every single known gene in the atherogenic process. Taking advantage of publicly available datasets, we searched the atherosclerosis related datasets with a sample size of more than ten in each group to diminish the false positive rate from limited sample sizes (except for one dataset of five patients with familial hypercholesterolemia) in the Gene Expression Omnibus repository. Thereafter we compared the gene expression of chemokines and chemokine receptors described above in human plaque specimens or blood samples *versus* corresponding controls (results are summarized in Fig. 1 and Table 2), thus providing information on how the chemokine network differs in atherosclerotic tissues from control samples.

It should be noted that different studies have not always produced a consistent outcome. For example, CCL2 and CCR2 were higher expressed in atheroma plaques compared to distant macroscopically intact tissue from the same patient (Accession number: GSE43292 [50]), but the expression of CCL2 or CCR2 did not differ between atherosclerotic arterial wall and distal part of mammary artery as non-atherosclerotic arterial wall (GSE40231 [51]). The latter is likely to be attributed to temporal expression differences of some chemokines during different stages of atherogenesis. For instance, multiple chemokines (CCL3, CCL5, CCL19, CCL21, CXCL12, CXCL16) and chemokine receptors (CCR1, CCR2, CCR5, CCR7, CXCR4, CX3CR1) were enhanced in advanced atherosclerotic plaques *versus* early-stage lesions (GSE28829 [52]). However, we did not observe any differences in peripheral blood mononuclear cell (PBMC) samples from obstructive coronary artery disease compared to non-obstructive coronary artery disease in terms of chemokine or receptors expression in GSE90074 [53]. In addition, chemokines and receptors did not differ in peripheral blood samples from atherosclerosis and non-atherosclerosis subjects with larger sample size in GSE20129 [54]. These findings might suggest a more plaque-relevant chemokine or chemokine receptors alterations during atherogenesis. Familial hypercholesterolemia (FH) patients exhibited higher CCR7 in blood compared to control subjects in GSE13985 (no publication available). Overall, CCL5, CCL19 and CXCR4 were higher in plaque specimens compared to control tissue or higher in advanced atherosclerotic plaques *versus* early-stage plaque. Taken together these gene expression analysis reveals interesting and significant changes of chemokine and chemokine receptor expression in plaque and blood samples of human origin. However, association does not necessarily mirror causality and hence has still to be accompanied by mechanistic studies linking cause and consequence.

3.2. Biomarkers and imaging tools for diagnosis and prognosis

Identification of biomarkers correlating with the disease severity of coronary artery disease (CAD) has led to important advances in prevention and treatment. As chemokines participate in the initiation and development of atherosclerosis, their titers are frequently used as

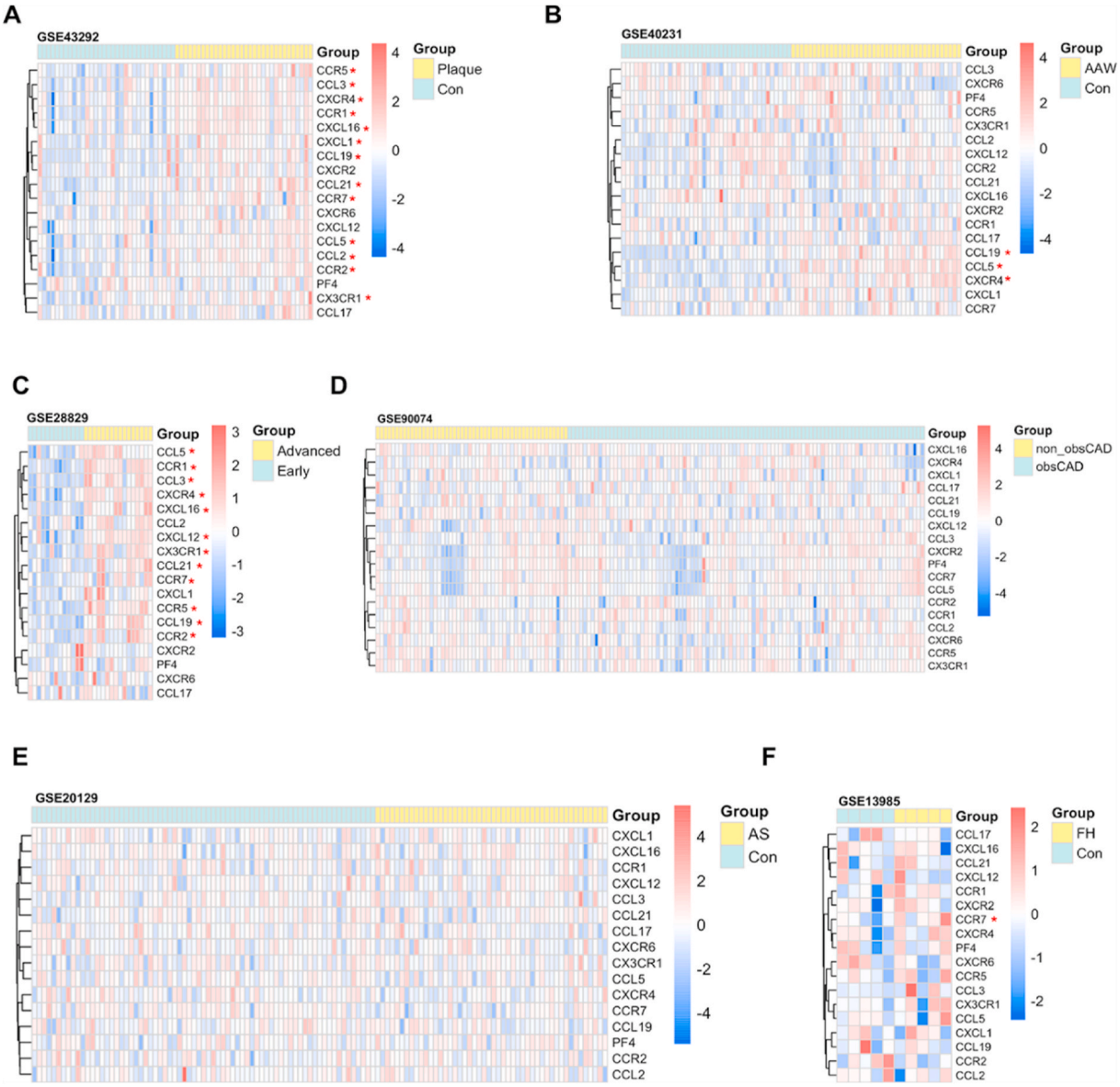

Fig. 1. Expressions of chemokines and chemokine receptors in plaque tissues or blood from human atherosclerosis related GEO datasets.
Heatmap generated from (A) 32 atheroma plaques (Plaque) and 32 distant macroscopically intact tissue (Con) from the same patient in GSE43292; (B) 40 atherosclerotic arterial walls (AAW) and 40 non-atherosclerotic arterial walls (Con, distal part of mammary artery) in GSE40231; (C) 16 advanced atherosclerotic plaques (Advanced) and 13 early-stage (Early) plaques in GSE28829; (D) 93 obstructive coronary artery disease (obsCAD) and 50 non-obstructive coronary artery disease (non_obsCAD) PBMC samples in GSE90074; (E) 48 atherosclerosis (AS) and 71 non-atherosclerosis (Con) peripheral blood samples from the Multi-Ethnic Study of Atherosclerosis cohort in GSE20129. (F) Blood collected from 5 patients diagnosed with familial hypercholesterolemia (FH) and 5 controls subjects in GSE13985. Differentially expressed genes were obtained by limma package in R. Genes with a fold change >1.2 or <0.8 and adjusted p value < 0.05 *versus* control group in heatmap are denoted with a red asterisk.

biomarkers for diagnosis and prognosis in CAD patients (Table 3).

Hypercholesterolemia is one of the risk factors for CVD. Notably, for the population with hypercholesterolemia in the absence of CAD, Rallidis et al. revealed that simvastatin significantly decreases CCL2 levels in these patients, suggesting that CCL2 is a sensitive marker to detect the anti-inflammatory effects of simvastatin in blood, especially for early stages of atherosclerosis [55]. In addition, there was a postprandial decrease in chemokines (CCL2, CCL3, CCL4) related to early atherosclerotic processes after an oral unsaturated fat load tested in familial hypercholesterolemia patients, while no alteration in chemokine levels was observed in normolipidemic controls [56]. In addition, elevated levels of chemokine CCL5 in patients with stable angina pectoris may also identify patients prone to plaque formation and atherosclerosis [57].

Chemokines are also used to assess the disease severity in ongoing coronary heart disease. Elevated baseline levels of CCL2 were associated with both traditional risk factors for atherosclerosis and an increased risk for clinical adverse events (death or myocardial infarction) in a large cohort of patients with acute coronary syndromes [58]. In addition, circulating CCL2 levels with a cut-off of 61.95 pg/mL had 91% sensitivity and 91% specificity for predicting CAD patients. Combined analysis of CCL2 with IL-6 levels predicts higher mortality in CAD patients with high sensitivity and specificity and could provide more information for clinical management [59]. Plasma CCL5 levels have also been reported to correlate with cardio-cerebral atherosclerosis burden in patients with ischemic cerebrovascular disease [60]. Serum CCL17 levels were shown to be positively associated with the type and severity of CAD independently of traditional cardiovascular risk factors [61]

Table 2
Alteration of genes encoding chemokine ligands (receptors) in human atherosclerosis.

Data set	Sample description	Changes
GSE43292	32 atheroma plaques *vs.* 32 distant macroscopically intact tissues	Increased: CCL2, CCL3, CCL5, CCL19, CCL21, CXCL1, CXCL16, CCR1, CCR2, CCR5, CCR7, CXCR4, CX3CR1
GSE40231	40 atherosclerotic arterial walls *vs.* 40 non-atherosclerotic arterial walls	Increased: CCL5, CCL19, CXCR4
GSE28829	16 advanced atherosclerotic plaques *vs.* 13 early-stage plaques	Increased: CCL3, CCL5, CCL19, CCL21, CXCL12, CXCL16, CCR1, CCR2, CCR5, CCR7, CXCR4, CX3CR1
GSE90074	PBMCs from 93 obstructive coronary artery disease *vs.* 50 non-obstructive coronary artery disease	No significance changes
GSE20129	PBMCs from 48 atherosclerosis *vs.* 71 non-atherosclerosis	No significance changes
GSE13985	Blood from 5 patients with familial hypercholesterolemia *vs.* 5 controls	Increased: CCR7

Table 3
An overview of current biomarkers in cardiovascular disease.

Biomarker	Clinical condition	Clinical significance
CCL2	Early stage of AS	Simvastatin significantly decreases CCL2 levels in hypercholesterolemia
		Postprandial decrease in CCL2 after an oral unsaturated fat load tested in FH
	CAD	Increased risk for clinical adverse events predicting CAD
		Higher in AMI and unstable angina pectoris
		Predictor of restenosis after percutaneous transluminal coronary angioplasty
CCL3	Early stage of AS	Postprandial decrease in CCL3 after an oral unsaturated fat load tested in FH
CCL4	Early stage of AS	Postprandial decrease in CCL4 after an oral unsaturated fat load tested in FH
CCL5	Stable angina pectoris	Patients with elevated CCL5 prone to AS increase of CCL5 might represent a sign of restenosis
	Refractory unstable angina pectoris	Predictor of clinical adverse events
		Higher in AMI and unstable angina pectoris
CCL17	CAD	positively associated with the type and severity of CAD
CXCL12	AMI	Predictor of clinical adverse events
CXCL16	ACS	Predictor of clinical adverse events
CCL18	Refractory unstable angina pectoris	Predictor of clinical adverse events
CX3CL1	CAD	Higher in AMI and unstable angina pectoris elevated early after PCI

AS: atherosclerosis; FH: familial hypercholesterolemia; CAD: coronary artery disease; AMI: acute myocardial infarction; ACS: acute coronary syndrome; PCI: percutaneous coronary intervention.

while CXCL12 could serve as an independent predictor of death or repetitive acute myocardial infarction and new-onset heart failure [62]. In patients with acute coronary syndrome, CXCL16 levels at admission are independently related to adverse clinical outcomes, especially cardiovascular death, indicating that CXCL16 levels are beneficial for risk stratification under these conditions [63].

As the plaque continues to develop it may become unstable and rupture. At the advanced or unstable stage, biomarkers to assess the disease severity or predict clinical outcome are of essential importance for disease monitoring. Kraaijeveld et al. revealed CCL5 and CCL18 titers to be significantly elevated in patients with refractory unstable angina pectoris compared to stabilized patients, hence combined measurement of CCL5 and CCL18 may predict future cardiovascular adverse events [64]. Similarly, CCL18 serum levels correlate with coronary calcification and segment involvement score, and CCL5 demonstrates an independent association with the presence of obstructive CAD, as well as the occurrence of primary cardiac events [65]. Higher levels of CCL2, CCL5 and CX3CL1 are found in acute myocardial infarction and unstable angina pectoris patients [66]. In addition, CX3CL1 levels are elevated the first 12 h after percutaneous coronary intervention in patients with acute myocardial infarction [67]. Enhanced CCL19 titers were also considered to be a biomarker for identifying high-risk patients in which more urgent intervention may be indicated [68].

Restenosis after percutaneous coronary intervention is still a major clinical problem. Platelet, leukocytes and EC activation due to the procedure are recognized as possible inflammatory triggers fostering restenosis [69,70]. The increase of CCL5 might represent a sign of restenosis after percutaneous coronary intervention in patients with stable angina pectoris [71]. CCL2 plasma levels measured 15 days after percutaneous transluminal coronary angioplasty were also described as an independent predictor of restenosis [70].

Moreover, chemokines are also gaining attention as novel imaging tracers to elucidate biological mechanisms. For example, ^{68}Ga-Pentixafor, a specific CXCR4 ligand, has been used in motion-corrected targeted positron emission tomography (PET)/computed tomography (CT) to enable *in vivo* characterization of CXCR4 expression in small culprit and non-culprit coronary atherosclerotic lesions after acute myocardial infarction [72]. Further, arterial wall ^{68}Ga-pentixafor uptake was described to be associated with cardiovascular risk factors and calcified plaque burden [73], which may hold promise for identification of vulnerable plaques. Recently, a pilot study revealed that CXCR4-directed ^{68}Ga-Pentixa for PET/CT identified more lesions than ^{18}F-FDG PET/CT [74]. In addition, ^{111}In-DOTA-DAPTA (CCR5 antagonist) has been shown to detect atherosclerotic lesions in *Apoe*$^{-/-}$ mice, suggesting that ^{111}In-DOTA-DAPTA can specifically target CCR5 in atherosclerotic lesions [75].

All these findings suggest that chemokines and chemokine like mimetics may be useful to assess individual atherosclerotic burden, as a surrogate marker for CAD screening or a predictor of clinical cardiovascular events and may function as imaging tracers for a better understanding of characteristics of atherosclerotic lesions.

3.3. Association of single-nucleotide polymorphisms (SNPs) in chemokine genes with atherosclerosis

SNPs occur within a gene or in a regulatory region near a gene and may play a direct role in disease pathology by directly affecting gene function. Table 4 gives a brief overview of SNPs associated with chemokine function in atherosclerosis, which we will describe in more detail in the following paragraphs.

Vascular CXCR4 has been shown to preserve endothelial barrier function, and maintains a contractile SMC phenotype in a mouse model of diet induced atherosclerosis while the C-allele of SNP rs2322864 in the human *CXCR4* gene results in decreased CXCR4 expression and is

Table 4
Association of SNPs in chemokines or chemokine receptors with atherosclerosis.

SNP	Gene	Role in atherosclerosis	PMID
rs2322864	*CXCR4*	C allele is associated with increased risk for coronary heart disease	28450349
rs2228014	*CXCR4*	A allele is associated with increased risk for coronary heart disease	29581828
rs3732379	*CX3CR1*	249I allele is associated with decreased risk for atherosclerosis; an elevated risk of restenosis after stenting.	22731642, 16411402
rs3732378	*CX3CR1*	280 M allele is associated with decreased risk for atherosclerosis. 280 M allele might counteract the elevated risk of restenosis by 249I after stenting.	22731642, 16411402
rs1024611	*CCL2*	G allele results in greater production of MCP-1; is associated with increased risk of coronary atherosclerosis in an asymptomatic population and CAD; is associated with greater reductions in high-sensitivity C-reactive protein levels after CCR2 blockade	16934270, 23930970, 21247529
rs2107538	*CCL5*	T allele may increase genetic susceptibility of CAD	26688689
rs1799987	*CCL5*	Higher frequency of GG or GA genotype in acute coronary syndrome individuals than AA genotype	26688689
rs333	*CCL5*	Δ32 is associated with decreased risk for cardiovascular disease	18436884
rs4508917	*CXCL10*	Higher frequency of the GG genotype in ischemic heart disease patients	27152707
rs4359426	*CCL22*	Lower frequency of the AA genotype in ischemic heart disease patients	27152707
rs223828	*CCL17*	T allele is associated with increased CCL17 promoter activity and increased risk for CAD	28794385
rs223899	*CCL17*	T allele is associated with increased risk for CAD	28794385
rs880175	*CXCL12*	T allele is associated with increased risk for CAD	30012324
rs1482478	*CXCL12*	A allele is associated with increased risk for CAD	30012324

associated with increased risk for CAD [41]. Another study showed similar results that C-allele of SNP rs2322864 and A-allele of SNP rs2228014 were associated with increased risk of CAD [76].

Monocytes play a critical role in atherosclerosis and express CX3CR1, which acts as either a monocyte chemokine receptor or an adhesion molecule and is highly expressed also in foam cells and coronary artery SMCs in human atherosclerotic arteries, but not in normal human arterial tissue [77]. Evidence shows that two SNPs, in the *CXCR1* gene, namely rs3732379 (V249I) and rs3732378 (T280 M), were associated with a decreased risk of atherosclerosis [78]. In retrospective studies, these *CX3CR1* polymorphisms have consistently been associated with reduced prevalence of atherosclerotic disease endpoints, including cerebrovascular disease [79] and restenosis after coronary stenting [80], which is in line with a meta-analysis with pooled data retrieved from seven case-control studies unveiling the association of *CX3CR1* polymorphisms with individual's susceptibility to CAD [81]. The protective role of a methionine at position 280 (280 M) might contribute to a reduced and delayed binding of CX3CL1 to the CX3CR1 receptor and decreased CX3CL1-induced chemotaxis [82]. As another monocyte biology related genetic polymorphism rs1024611 in the promoter region of the *CCL2* gene (−2578 A > G) results in greater production of CCL2 protein and is associated with an excess risk of coronary atherosclerosis in an asymptomatic population [83]. Similarly, G-allele of *CCL2* (−2518 A > G) is also associated with increased risk of CAD in the population of patients from the Indian state Punjab [84]. Furthermore, a randomized, double-blind, placebo-controlled study demonstrates that patients with G allele of rs1024611 (−2518 A > G) in CCL2 had significantly greater reductions in high-sensitivity C-reactive protein levels after CCR2 blockade, suggesting CCR2 blockade might be tailored and beneficial for subsets of CAD patients with G allele at this SNP locus against atherosclerosis [85].

As the CCL5-CCR5 axis is pivotal to leukocyte recruitment several studies also worked on the association of CCL5 or CCR5 polymorphisms with atherosclerosis. The SNP rs2107538 (−403C > T) in the *CCL5* gene with T allele may increase genetic susceptibility of CAD. The frequencies of SNP rs1799987 in CCR5 (CCR5-59029) with GG or GA genotype were higher than AA genotype in acute coronary syndrome individuals [86]. *CCR5* Δ32 (rs333) allele has been linked with higher high-density lipoprotein, lower triglycerides, lower C-reactive protein levels, decreased intima-media thickness, and cardiovascular disease risk [87,88]. These SNPs may be incorporated to genotype score and provide a prognostic value to cardiovascular clinical events.

Evidence has demonstrated that the up-regulation of Th1- and Th17 cell-associated inflammatory responses and down-regulation of anti-inflammatory responses of Treg-/Th2 cell-related chemokines was associated with CVD [89]. For example, CXCL10 binds to its receptor CXCR3 and mainly acts as a chemoattractant for Th1 lymphocytes [90], while CCL20 attracts CCR6-expressing Th17 cells [91] and CCL22 orchestrates Th2- and Treg migration via its receptor CCR4 [92]. Evidence shows that ischemic heart disease patients exhibited a higher frequency of the GG genotype at SNP rs4508917 in the *CXCL10* gene, whereas the same patients have a lower frequency of the AA genotype at SNP rs4359426 in the *CCL22* gene compared to healthy subjects. However, no differences were observed between subjects with different genotypes at these two SNP loci with respect to the related serum levels of each chemokine, which does not exclude functional differences of the gene product [93].

As mentioned, CCL17 was shown to be an independent risk factor for CAD. The same research group further identified the SNPs rs223899 and rs223828 in the *CCL17* gene to be significantly associated with CAD after adjustment of traditional risk factors of CAD in a Chinese cohort. The T allele of rs223828 was shown to be associated with serum CCL17 concentrations and functional assays further displayed an enhanced *CCL17* promoter activity in individuals with the T allele of rs223828 compared to the major allele C in at the *CCL17* locus [94]. These findings indicate that rs223828 is a functional SNP and the corresponding increase of serum CCL17 enables its atherogenic effects by restraint of Treg maintenance and enhancement of platelet aggregation in coagulation [95].

Taken together, epidemiological analyses have identified many biomarker-CAD relationships, however, whether they are truly indicative of a causal association is unknown. Mendelian randomization (MR) is a method of using measured variation in genes of known function to examine the causal effect of a modifiable exposure (eg. biomarker) on disease direction in observational studies. Recently, 237 CAD-associated biomarkers and suggested deleterious effects of the SNPs rs880175 and rs1482478 in the *CXCL12* gene on CAD (odds ratio = 1.69). These two SNPs were positively correlated with CXCL12 serum levels in the CARDIoGRAM consortium data and MR results were corroborated using genetically predicted biomarker levels in the validation cohort of UK Biobank. Hence, genetically elevated CXCL12 were associated with an increased risk of CAD in the target and validation cohort [96]. Understanding the mechanism by which these markers mediate CAD will provide novel insights into CAD and could lead to new approaches to prevention.

Although the increment of genome-wide association studies provided the chance to examine the association of numerous SNPs with CAD. However, the studies on the characteristics of the variants on the basis of their nature and more in-depth associative studies are quite sparse and required at the current era.

Computational methods would be of great aid in identification of change in structure, capacity and strength of proteins as a result of some

variation to segregate between the neutral (harmless) SNPs and SNPs of functional importance [97]. Protein-ligand docking would be good choice to observe the structural variance in interacting behaviors of native and mutants [98]. Therefore, the effect of SNPs on protein structure behavior in CAD would provide more information to identify and predict pathogenic SNPs and warrant more investigation in future studies.

4. Treatment targeting chemokines in atherosclerosis

4.1. Pre-clinical studies targeting chemokines and their receptors

Various preclinical (mouse) studies suggest that targeting chemokines e.g. by small peptide-based constructs could interfere with atherosclerosis progression. Targeting of CCL2, CCL5, CCL8 and CXCL9 via miR-146a/-181b was shown to reduce monocyte adhesion to ECs thereby reducing atherosclerosis [99] and disrupting chemokine heteromers in diet-induced atherosclerosis mouse models interferes with monocyte recruitment. For example, inhibition of CCL5/CXCL4 heteromer formation with MKEY leads to a reduction of lesion area and macrophage accumulation in aortic arch, abdominal aorta and aortic root [20]. CAN, another peptide-based inhibitor blocked CCL5-CCL17 heterodimerization thereby abolishing lesion growth in the aortic root [13]. Blocking of HNP1-CCL5 interaction by SKY peptide also reduced adhesion of monocytes and thus showing atheroprotective effects [10].

Preclinical evaluation on interference with chemokine receptors has also gained attention. For instance, siRNA treatment targeting CCR2 in *Apoe*$^{-/-}$ mice leads to reduced monocyte accumulation in atherosclerotic plaques [100]. Chrono-pharmacological inhibition of CCL2 by using CCR2 antagonist (RS102895) also reduced atherosclerotic lesion size [5]. CCR5 inhibitor maraviroc showed protective effects reducing by reducing lesional macrophage numbers as well as by diminishing the expression of endothelial adhesion molecules and CCL5 in the lesions [101].

TAK-779 is a non-peptide CCR5/CXCR3 antagonist supplemented to *Ldlr*$^{-/-}$ mice, to antagonize CCR5 and CXCR3 showed inhibition of T cell migration to lesions [102] while both, TAK-779 and NBI-74330, another CXCR3 antagonist, reduced atherosclerotic plaque formation [103]. Preclinical studies on CX3CR1 inhibition involving its antagonist F1 (an amino terminus–modified CX3CR1 ligand) and M3 (44-kDa protein encoded by the murine gamma herpesvirus 68) shows their negative effect on atherosclerotic progression in both *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice [104,105]. ACKR3 activation by CCX771 leads to inhibition of the binding of CXCL12 to ACKR3 in *Apoe*$^{-/-}$ mice and resulted in reduced macrophage accumulation and decreased lesion size [106]. Fig. 2 has depicted all the chemokine targets used in the preclinical studies to ameliorate atherosclerosis.

Pre-clinical studies so far have focused on novel inhibition methods like miRNA-based therapy, heterodimer inhibition via peptides and non-peptide antagonists. Chemokine ligands like CCL2, CCL5, CCL8 and CXCL9 mediate monocyte adhesion to endothelium and thus help in atheroprogression. Inhibition of these chemokine ligands by using novel miRNA-based therapy ameliorates atherosclerosis. Studies targeting chemokine receptors via siRNA treatments and their inhibitors like maraviroc also show similar effects. This as a whole shows the relevance of chemokine ligands and their receptors targeting in atherosclerosis. Various targeting approaches like miRNA, siRNA, chrono-pharmacological targeting, peptide and non-peptide antagonist show promising atheroprotective effects in mice. With differences in metabolism, immune responses and physical activity between species,

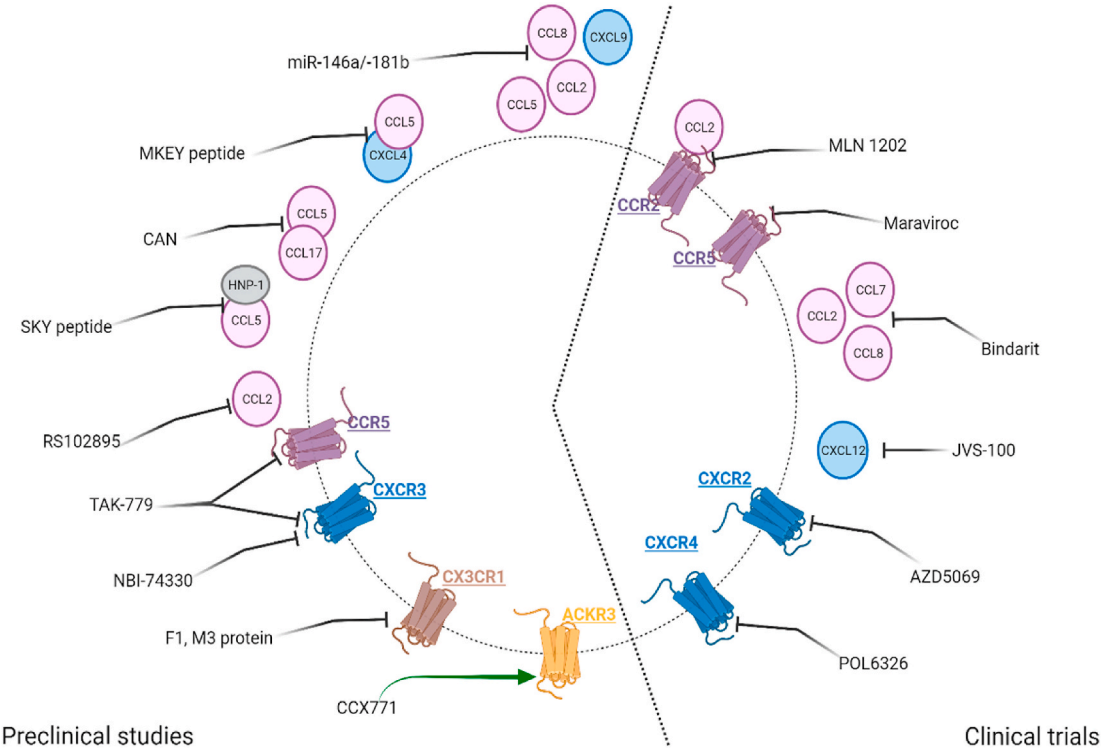

Fig. 2. Overview of preclinical studies and clinical trials involving targeting of chemokines and their receptors in atherosclerosis.
Left side of the circle represents preclinical studies. Inhibition of CCL2, CCL5, CCL8 and CXCL9 was performed using miR-146a/-181b. For inhibition of CCL5/CXCL4 and CCL5/CCL17 heteromers, MKEY peptide and CAN peptide were used, respectively. SKY peptide antagonized the interaction of HNP-1 and CCL5. RS102895 was used to inhibit CCL2. Chemokine receptors CCR5, CXCR3 and CX3CR1 were inhibited/antagonized by TAK-779, NBI-74330 and F1/M3 protein, respectively. CCX771 acts as an agonist to stimulate ACKR3 function. Right side of the circle represents clinical trials. Targeting of chemokine receptors CCR2, CCR5, CXCR2 and CXCR4 was performed using MLN1202, Maraviroc, AZD5069 and POL6326, respectively. Bindarit was used to antagonize CCL2, CCL8 and CCL7. A non-viral, naked DNA plasmid JVS-100 was used as human CXCL12 inhibitor.

additional studies are important to analyze if these targets show similar effects to target atheroprogression in humans.

4.2. Clinical studies/trials

Preclinical studies have demonstrated profound effects of chemokines and their receptors on inflammatory responses related to development and progression of atherosclerosis. Some of the above-summarized findings have already found their way into clinical studies. To delineate ongoing clinical trials in the field of chemokines and chemokine receptors in CVD we performed a literature research using https://www.clinicaltrialsregister.eu/and https://www.clinicaltrials.gov/websites and could extract the following findings:

MLN1202 is a monoclonal antibody used in a phase II study to analyze the effect of CCR2 inhibition on serum C-reactive protein levels. CCR2 antagonism by MLN1202 led to reduction in serum C-reactive protein levels in subjects at higher risk of atherosclerosis [85]. However, a phase III trial will be required to ascertain the efficacy of MLN1202 in atherosclerosis. Another proof of mechanism phase II clinical trial with MLN1202 was conducted in patients with stable atherosclerosis and examined its effect on arterial inflammation (ClinicalTrials.gov identifier (NCT number): NCT02388971). However, this study was withdrawn.

Maraviroc, a CCR5 antagonist, induced anti-atherosclerotic effect in atherosclerosis prone mice and in preliminary data in humans [101]. One phase IV clinical study examines effects of maraviroc as a treatment of atherosclerotic progression in HIV patients. However, the human phase IV study results are pending (ClinicalTrials.gov Identifier: NCT03402815).

Chemokines like CCL2, CCL7 and CCL8 are discussed to regulate restenosis processes. Therefore, in a phase II trial, Bindarit was used to selectively inhibit CCL2, CCL7 and CCL8 and was evaluated for its therapeutic potential to prevent restenosis in patients after undergoing percutaneous coronary intervention. This trial showed a trend towards Bindarit induced reduction in in-segment late loss, but was not statistically significant [107].

Phase II trial included the safety and efficacy of endocardial dosing of JVS-100 (a non-viral, naked DNA plasmid encoding human CXCL12 inhibitor) to improve cardiac performance in patients with lowest left ventricular ejection fraction during high-risk ischemic cardiomyopathy [108]. This study failed to demonstrate statistical significance on its primary endpoint of improved composite score at 4 months after treatment.

In a randomized controlled trial, CXCR2 antagonist AZD5069 showed potent inhibition of calcium flux and CD11b expression on neutrophils which lead to reversible reduction of neutrophils in the circulation [109]. Another ongoing randomized controlled Phase II trial (EudraCT number: 2016-000775-24) evaluates if AZD5069 improves endothelial function in patients undergoing cutaneous coronary intervention for atherosclerotic coronary disease [110]. The secondary endpoint of this trial is to correlate inhibition of CXCR2 to the alteration of plaque towards more stable plaques and reduced restenosis.

Further, a phase II clinical trial (ClinicalTrials.gov Identifier: NCT01905475, EudraCT Number: 2012-003229-91) examining the effect of POL6326, a CXCR4 antagonist on heart function, and healing with regard to infarct size in patients with acute myocardial infarction has been completed, but with results not yet available. Clinical trials with therapeutic agents/molecules targeting chemokines in atherosclerosis are summarized in Fig. 2.

Considering the amount of pre-clinical studies, relatively few chemokine targets are under evaluation in humans. Most of the clinical studies are in Phase II, from which, some of them like Bindarit and JVS-100 do not show any statistically significant results. Others based on antagonists like AZD5069 and POL6326 are either ongoing or completed but results are still not available. Only maraviroc which is CCR5 antagonist, reached in phase IV trails in HIV patients with atherosclerosis progression. However, the results from this study is still pending. Failure of most of the phase II studies to reach statistical significance and their possible withdrawal could be because of the complexity of human physiology, underlying comorbidities. Additional studies with different approaches to target chemokines without major side effects are needed.

5. Conclusion and future perspectives

Expression and function of chemokines and their receptors are altered in patients with CVD, affecting their conventional role in mobilization of immune cells to the sites of inflammation, but also have an impact on cellular processes such as cell proliferation, migration, and cytokine expression. Moreover, hypoxia, shear stress, and inflammatory cytokines influence expression of chemokines and their receptors in CVD. The picture is further complicated by age, sex, ethnicity, and genetic variation, as well as differential CVD pathology of these patients, not to forget comorbidities like diabetes mellitus or chronic kidney which present with altered inflammatory processes different from CVD patients without these issues [111].

Understanding all these mechanisms that foster and influence development of CVD and related comorbidities are of great importance and central to be able to take targeted therapeutic approaches to the next level. Animal experiments have proven indispensable in uncovering mechanisms driving chemokine (receptor) biology in the pathophysiology of atherosclerotic CVD, however, translation of these findings into humans is not trivial and demands further effort. Gene expression analysis for example reveals interesting and significant changes of chemokine and chemokine receptor expression in plaque and blood samples of human origin. However, association does not necessarily mirror causality therefore mechanistic studies linking cause and consequence are needed, a limitation which could be overcome by Mendelian randomization analyses. Further, chemokines and chemokine-like mimetics may be useful as biomarkers to assess individual atherosclerotic burden functioning as imaging tracers (e.g. CXCR4) or as predictors of clinical cardiovascular events as (e.g. CCL5 or CCL18). In addition (ongoing) clinical trials point at a beneficial role of drugs e.g. inhibiting CCR5 or CXCR2 in CVD. In addition, optimization of therapy to address circadian rhythmicity may also improve drug efficacy [5]. In parallel, nanomedicine-based approaches targeting e.g. vascular adhesion molecules to deliver therapeutic cargo [112] or downregulation of e.g. CCR2 expression [100] have gained attention in CVD therapeutic approaches. Down this road also a direct, local targeting of chemokines by microRNAs may be promising in reducing atherosclerosis [113]. However, spatial as well as temporal aspects of therapeutic application need to be considered and careful future evaluation has to show at which costs systemic blocking of chemokines or their receptors in CVD *versus* tissue or cell specific targeting approaches may come. Unraveling the chemokine network, its diversity, effector functions, and regulation of the immune response is essential to develop successful future therapeutic strategies.

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Author contributions

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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