Chemokines as Therapeutic Targets in Cardiovascular Disease The Road Behind, The Road Ahead

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Chemokines as Therapeutic Targets in Cardiovascular Disease

The Road Behind, The Road Ahead

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Abstract—With the incidence and impact of atherosclerotic cardiovascular disease and its clinical manifestations still rising, therapeutic options that target the causal mechanisms of this disorder are highly desired. Since the CANTOS trial (Canakinumab Antiinflammatory Thrombosis Outcome Study) has demonstrated that lowering inflammation can be beneficial, focusing on mechanisms underlying inflammation, for example, leukocyte recruitment, is feasible. Being key orchestrators of leukocyte trafficking, chemokines have not lost their attractiveness as therapeutic targets, despite the difficult road to drug approval thus far. Still, innovative therapeutic approaches are being developed, paving the road towards the first chemokine-based therapeutic against inflammation. In this overview, recent developments for chemokines and for the chemokine-like factor MIF (macrophage migration inhibitory factor) will be discussed.

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Key Words: atherosclerosis ■ bone marrow ■ cardiovascular disease ■ chemokines ■ myocardial infarction

Cardiovascular disease (CVD) is the major cause of death worldwide, causing 17.7 millions of deaths and 45% of all noncommunicable diseases in 2015 according to the World Health Organization.[1] Inflammation crucially contributes to the development and progression of atherosclerosis[2] and also shapes remodeling processes in the heart following myocardial infarction (MI).[3] Over the last decades, studies in preclinical models have uncovered a multitude of inflammatory mediators impacting on the pathology of CVD,[2–4] offering interesting leads for novel therapeutic targets. However, clinical translation into effective strategies for patient treatment is lagging behind. Nonetheless, the CANTOS trial (Canakinumab Antiinflammatory Thrombosis Outcome Study) recently demonstrated that blocking the proinflammatory IL (interleukin)-1β in patients with previous MI reduced systemic inflammation, recurrent cardiovascular events and cardiovascular death,[5] and thereby was the first to show that anti-inflammatory treatment strategies can be beneficial in patients with atherosclerotic CVD.

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This review focuses on the role of chemokines as potential therapeutic targets in CVD as they are the key regulators of recruitment and adhesion of leukocytes to inflamed arteries in the setting of atherosclerosis and into the myocardium of the ischemic heart.[3] After we highlight a selection of key chemokines and the chemokine-like cytokine MIF (macrophage migration inhibitory factor) in CVD, with main focus on atherosclerosis and ischemic heart disease, we will discuss clinical studies targeting chemokines in CVD, present novel translational approaches based on these molecules for diagnosis and therapy in CVD, as well as discuss current hurdles in the road to clinical translation.

Chemokines in CVD

Chemokines are small (8–12 kDa) chemotactic cytokines, which have an important role in directing the migration of blood cells to target tissues. Chemokines are classified into 4 groups, with the CC- and CXC-types being the most common. Besides their importance in governing leukocytes to sites of inflammation, chemokines and their receptors also have homeostatic functions (eg, homing of lymphocytes to lymphoid organs, regulating egress of stem cells, and leukocytes from the bone marrow). Chemokines might work together in a sense that some trigger integrin activation to induce firm arrest of leukocytes on activated endothelium, whereas others guide the leukocytes to subendothelial locations by chemotaxis. In this way and by regulating circulating leukocyte counts, chemokines may contribute

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Nonstandard Abbreviations and Acronyms

ACKR	atypical chemokine receptor
CVD	Cardiovascular disease
C(X)CL	C(X)C chemokine ligand
C(X)CR	C(X)C chemokine receptor
HNP1	human neutrophil peptide 1
IL-1β	interleukin 1β
MI	myocardial infarction
MIF	macrophage migration inhibitory factor
miRNA	micro RNA
siRNA	short interfering RNA
SMC	smooth muscle cell

to the development of atherosclerosis. Further, chemokines can affect leukocyte activation, monocyte survival, foam cell formation, smooth muscle cell (SMC) proliferation, cell egress from lesions, (lymph-)angiogenesis as well as thrombus formation,[4] all with important impact on CVD (Figure). Among the various chemokines identified to alter MI[3] and atherosclerosis,[4] we will focus here on novel findings as well as promising therapeutic characteristics of the chemokines CCL5 (CC-chemokine ligand 5), CX_3CL1 (CXXXC-chemokine ligand 1), CCL2, CXCR2 (CXC-chemokine receptor 2), and CXCR3 as well as the CXCL12/CXCR4 axis, as these have been shown to have distinct roles in atherosclerosis.

CCL5, its Receptor CCR5, and its Heterophilic Interactions

The chemokine CCL5 and its primary receptor CCR5 (CC-chemokine receptor 5) have widely established roles in the development of atherosclerosis and MI. Unlike CCR1, manipulation or knockdown of CCR5 and its ligands (notably CCL5) was shown to have beneficial effects on the disease outcome in respective animal models.[4,6] A possible use of CCL5 levels as a biomarker for CVD has been indicated in several studies.[7–10] However, a case-cohort study from samples of the MONICA/KORA (Monitoring Trends and Determinants in Cardiovascular Disease/Kooperative Gesundheitsforschung in der Region Augsburg) Augsburg studies questioned the utility of CCL5 levels as biomarker for cardiovascular risk.[11]

As opposed to binding to its (membrane-bound) receptors CCR1 and CCR5, CCL5 also binds to other soluble inflammatory factors such as chemokines (eg, CXCL4, CCL17, and CXCL12) and defensins (eg, HNP1 [human neutrophil

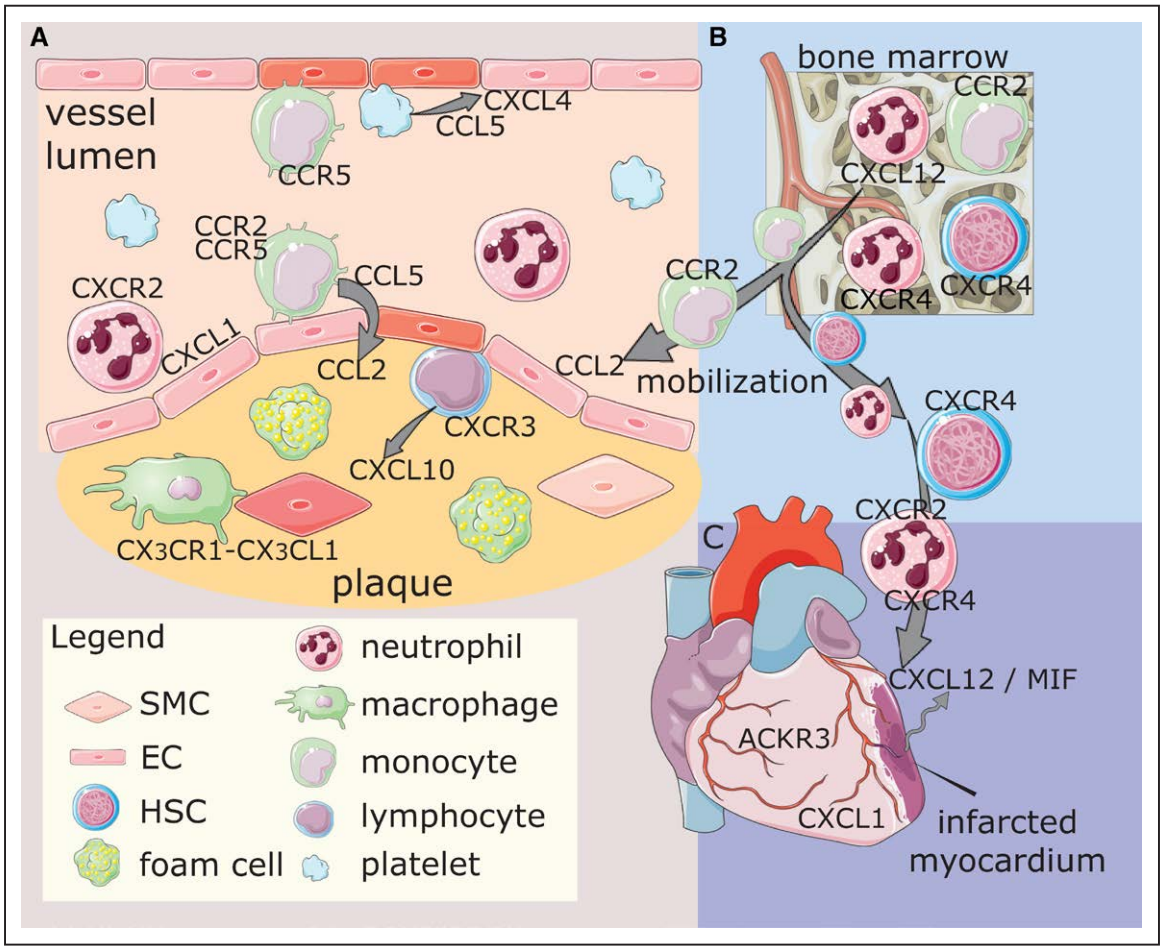

Figure. Involvement of the chemokine system in cardiovascular disease. The chemokine system can influence the progression of atherosclerosis and cardiac disease by regulating the recruitment of leukocytes (eg, classical monocytes and lymphocytes) to developing plaques (**A**), by transmitting survival signals between smooth muscle cells (SMC) and macrophages (**A**), by regulating egress of monocytes, neutrophils or hematopoietic stem cells (HSC) from the bone marrow (**B**) and their homing to plaques (**A**) or to infarcted myocardium (**C**). Platelets can prime monocyte recruitment by the deposition of chemokines (**A**). ACKR3 indicates atypical chemokine receptor 3; EC, endothelial cell; MIF, macrophage migration inhibitory factor; and SMC, smooth muscle cell.

peptide 1]). In this way, CCL5 and its binding partners can mutually influence their activities. For example, binding of CCL5 to CXCL4, both deposited by platelets on endothelium, increases the recruitment of monocytes to activated endothelium shown in both in vitro experiments and in mouse models.[4] Similar applies for an interaction of CCL5 with CCL17, which facilitates heterodimer formation of CCR5 and CCR4 on the surface of dendritic cells.[12] Heterodimer formation between chemokine receptors is well established to modulate their activity.[13] Also here, the interaction between CCR5 and CCR4 prolonged receptor surface retention and chemotaxis of dendritic cells.[12] Not only chemokines interact with CCL5, but also the defensin HNP1 (human neutrophil peptide 1) was recently found to bind to CCL5, with synergistic effects on the adhesion of classical monocytes.[14]

The CCL2/CCR2 Axis

The CCL2/CCR2 axis regulates the inflammatory recruitment of classical monocytes in atherosclerosis as well as in the infarcted heart, and also the egress of monocytes from the bone marrow.[3,4] Lipid nanoparticles carrying a short interfering RNA against CCR2 reduced inflammatory monocyte recruitment and infarction size after cardiac ischemia-reperfusion injury, as well as atherosclerosis.[15,16] In acute coronary syndrome CCL2 plasma levels associated with an increased risk for death or MI,[17] and a recent study applying a proteomics approach in patients with heart failure with preserved ejection fraction revealed CCL2 as an inflammatory biomarker associated with heart failure severity and outcome.[18] Further, recent insights revealed a circadian rhythmicity in CCL2/CCR2-mediated chemotaxis, with chronopharmacological targeting of CCR2 reducing atherosclerosis without disturbing microvascular leukocyte recruitment.[19] Although the effect on advanced stages of atherosclerosis was not yet investigated in this study, these findings reveal interesting new perspectives taking into account timed therapeutic treatment.

CXCR2, 3 and Their Ligands

The CXC-chemokine group can be classified by ELR$^+$ (glutamic acid-leucine-arginine) and ELR$^-$ chemokines, depending on the presence of an N-terminal 3-amino acid stretch. The ELR$^+$ chemokines (eg, CXCL1, 2, 5, and 8) can bind to and activate CXCR1 and 2, which are important receptors for neutrophil recruitment. Those without an ELR-motive generally activate CXCR3 (eg, CXCL9, 10, and 11) and CXCR4 (CXCL12), attracting lymphocytes (CXCR3), or keeping neutrophils and hematopoietic stem cells in the bone marrow (CXCR4). Animal studies have revealed roles for CXCR2 and CXCR3 and their ligands in atherosclerosis and MI. For example, CXCL1 is involved in the recruitment of monocytes and of macrophage accumulation during atherosclerosis.[20,21] The role of CXCR2 in MI is not as clearly defined, since it exerts both detrimental and protective effects,[22] for example, a detrimental circadian rhythm-dependent recruitment of CXCR2-positive neutrophils.[23] The role of CXCR3 in atherosclerosis and MI is well established and involves the recruitment and phenotype of T cells.[24] This is exemplified by deletion of CXCL10 in mice, which resulted in a decrease of CD4+ T-cell counts in plaques, whereas FoxP3-positive regulatory T cells were increased. This might explain the overall reduction in atherosclerosis observed in these mice.[25]

The Special Ones

CX_3CL1 and CXCL16

Among the various chemokines, some are somewhat peculiar, such as CX_3CL1 and CXCL16. Both are membrane-bound, have a glycosylated mucin-like stalk, and are sensitive for (limited) proteolysis by ADAM (a disintegrin and metalloprotease) metalloproteases.[26] The membrane-bound forms of CX_3CL1 and CXCL16 bind to CX_3CR1 and CXCR6, respectively, and can support adhesive interactions of leukocytes under shear flow. The proteolytically released forms containing the chemokine domains can chemoattract immune cells to sites of inflammation. Interestingly, CXCL16 also has a function as scavenger receptor for oxidized lipoproteins, besides its activity as chemoattractant. This might also explain its protective role in models of atherosclerosis.[27] In contrast, its receptor CXCR6 appears to promote atherosclerosis.[28] Currently, CXCL16 is mainly in focus as a mediator of cancer progression.

Typically expressed in the brain and by activated endothelial cells and vascular SMC, CX_3CL1 mediates cross-talk of SMC with CX_3CR1-expressing macrophages in the plaque interior, leading to increased expression of inflammatory molecules.[29] In addition, arterial-resident macrophages originate from CX_3CR1^+ embryonic precursors and their survival and maintenance is upheld by CX_3CR1–CX_3CL1 interactions.[30,31] Also on platelets, the CX_3CL1–CX_3CR1 axis is involved in the activation and adhesion of platelets to vascular cells.[32–34] Interestingly in mice, CX_3CR1 on platelets is upregulated by hyperlipidemia and might mediate the adhesion of platelets to the denuded vessel wall.[34] In humans, plasma levels of soluble CX_3CL1 may serve as an indicator for vascular dysfunction and of all-cause mortality in patients with heart failure.[35,36]

The CXCL12/CXCR4 Axis

The CXCL12/CXCR4 chemokine ligand/receptor axis plays a complex and double-edged role in CVD.[37] CXCL12 is classified as a homeostatic chemokine, regulating the homing of CXCR4-positive progenitor cells and leukocytes in the bone marrow as well as their release to the periphery on injury or stress. This function in directing progenitor cell mobilization has been linked to a protective role of the CXCL12/CXCR4 axis in myocardial ischemia.[38] Further, a cardioprotective role of the CXCL12/CXCR4 axis in myocardial ischemia has been ascribed to increased neoangiogenesis,[39–41] cardiomyocyte protection and cardioprotective signaling,[39] and a phase II trial (STOP-HF [St Vincent's Screening To Prevent Heart Failure]) examining single endocardial CXCL12 overexpression by gene therapy revealed the potential to improve cardiac function in patients with ischemic heart failure after MI.[42] In injury-induced restenosis the CXCL12/CXCR4 axis improves reendothelialization of denudated arteries through the mobilization of endothelial progenitor cells[37,43,44] and an enhanced endothelial proliferation.[45] Also, in diet-induced atherosclerosis, vascular protection by endothelial apoptotic bodies was mechanistically linked to the induction of protective CXCL12/

CXCR4 signaling in the endothelium as well as to progenitor cell recruitment.[46] Further, both endothelial as well as SMC CXCR4 were recently revealed to be atheroprotective by preserving endothelial barrier function and sustaining contractile SMC phenotype, respectively.[47] Notably, recent Mendelian randomization studies and conditional analysis revealed CXCL12 as a causal mediator of CAD, identifying a SNP (single nucleotide polymorphism) near *CXCL12* to be independently associated with CXCL12 plasma levels and increased risk for CAD.[48,49] Such Mendelian randomization studies represent a novel epidemiological study approach by incorporating genetic variant analyses to investigate a potential causal relationship between a risk factor and clinical outcome. Experimental data further indicate that effects of CXCL12 driving atherosclerosis rely on CXCL12 production in arterial endothelial cells.[49] Indeed, findings that somatic deficiency in *CXCR4* did not reveal equivalent protective effects as deletion in vascular cell types,[47] imply proatherogenic functions of CXCR4 in a different, for example, bone marrow–derived compartment.

On the contrary, CXCR4 was also demonstrated to negatively impact on MI potentially associated with the recruitment of proinflammatory cells to the ischemic heart,[50,51] whereas in injury-induced restenosis, the mobilization of smooth muscle progenitor cells by the CXCL12/CXCR4 axis was linked to increased neointima formation.[37,52] In cardiac fibroblasts, CXCL12/CXCR4 signaling induced increased fibroblast proliferation and collagen production.[53] Altogether, these findings support the concept of a double-edged role of the CXCL12/CXCR4 axis in CVD, likely reflecting disease subtype- as well as cell type-specific effects. Therefore, cell type-specific and regional targeting have to be considered in future clinical translation strategies targeting the CXCL12/CXCR4 axis.

MIF and ACKR3 Further Complicate the CXCL12/CXCR4 Axis

The understanding of (patho-)physiological effects of the CXCL12/CXCR4 axis is further complicated by macrophage MIF as alternative ligand of CXCR4, and by ACKR3 (atypical chemokine receptor 3; previously CXCR7) as alternative receptor for CXCL12 and MIF.[54] In vitro analyses, animal studies as well as clinical epidemiological studies support a proatherogenic role of MIF.[55] The role of MIF in MI seems more complex, with studies revealing both MIF-induced cardioprotective signaling in the context of cardiac ischemia[56] versus MIF-dependent inflammatory processes aggravating ischemia-induced myocardial damage,[57] as summarized in detail recently by Tilstam et al[58] Subsequently, cell type-specific effects were identified, with leukocyte-derived MIF being proinflammatory versus cardiac cell-derived MIF being cardioprotective.[59,60] Although this complicates the concept of therapeutically targeting MIF in CVD, it was recently suggested that common *MIF* promoter variants might guide the therapeutic optimization of MIF levels: one may strive to decrease *MIF* levels in high *MIF* expressors at high cardiovascular risk to reduce atherosclerotic burden, whereas increase *MIF* levels in low *MIF* expressors when aiming for increased cardiac protection after ischemia.[58] Although no clinical studies are currently registered to investigate MIF increase, anti-MIF strategies investigated in clinical trials for cancer and autoimmunity[58] may support clinical translation in the CVD field also.

A role for ACKR3 in cholesterol uptake in adipose tissue and thereby in regulating blood cholesterol levels was previously linked to a regulatory effect of ACKR3 on atherosclerosis.[61] Further, a role in endothelial proliferation and angiogenesis may underlie a protective role of ACKR3 in injury-induced neointima formation as well as in cardiac remodeling after MI,[62] which may offer interesting future therapeutic strategies in CVD. In general, ACKRs present a further aspect in leukocyte trafficking and in modulating immune responses.[63] For example, ACKR2 controls lymphangiogenesis by capturing CCL2, thereby regulating the proximity of prolymphangiogenic macrophages to the developing vessels.[64] Since the presence of lymphatic vessels also influences the progression of atherosclerosis,[65] a role for ACKR2 could be envisioned.

Towards Drugs Targeting Chemokines: How Far Are We?

Despite the multitude of preclinical studies revealing the importance of chemokines in CVD, clinical translation has not yet been successful. In fact, only few clinical applications are currently on the market. The CCR5 antagonist Maraviroc (Celsentri/Selzentry; Pfizer) is approved for US and European markets for the treatment of HIV. The CXCR4 antagonist AMD3465 (Plerixafor; Genzyme Corporation) is in use to mobilize hematopoietic stem and progenitor cells for autologous transplantation in patients with non-Hodgkin lymphoma. Recently, also a CCR4-directed humanized monoclonal antibody was approved by the Food and Drug Administration for the treatment of T-cell lymphoma and T-cell leukemia. However, chemokine-based drug applications to treat CVD are not yet available in the clinic. The reasons are manifold and may include poor target and dosage selection, timing of administration, insufficiently defined (patho)physiological role of a therapeutic target and the vastness of the chemokine system.[66]

Preclinical results are summarized in Table 1 and recent and ongoing clinical studies that examine targeting the previously discussed chemokines/chemokine receptors in CVD, are summarized in Table 2.

Since the mid-1990s, CCL5 and CCR5 have been in sharp focus as a drug target for the treatment of HIV, as CCR5 serves as a cofactor for cellular entry of the so-called R5 type of HIV-1 strains. Extension of the N-terminus of CCL5 resulted in CCR1 and 5 inhibitors, for example, Met-RANTES and AOP-RANTES.[81] Although these modified CCL5 variants also showed potential as HIV-1 blockers, it was a small molecular CCR5-antagonist that eventually entered the market (Maraviroc). The trafficking of T cells, monocytes and neutrophils being a major function of CCR5, antagonists for CCR5 were also shown to be effective in preventing atherosclerosis and myocardial ischemia-reperfusion injury in mice.[67,82] Since atherosclerosis is a common complication in patients with HIV, particularly during treatment with antiviral protease inhibitors, the use of CCR5-antagonists is actually considered for its prevention. In fact, Maraviroc counteracted the increase in inflammation caused by protease inhibitors and reduced atherosclerotic plaque development in mice.[68] Results for

Table 1. Preclinical Studies of Chemokines or Chemokine Receptors

Target	Compound	Model (Mouse)	Reference
CCR5/CXCR3	TAK779*	Atherosclerosis	van Wanrooij et al[67]
CCR5	Maraviroc*	Atherosclerosis	Cipriani et al[68]
CCR5	Met-RANTES†	Myocardial infarction	Projahn et al[40]
CX_3CR1	F1†	Atherosclerosis	Poupel et al[69]
CX_3CR1	M3‡	Atherosclerosis	Ravindran et al[70]
CX_3CR1	AZ12201182*	Stent restenosis	Ali et al[71]
CCL2/5/8	siRNA/liposomes	Atherosclerosis	Ma et al[72]
CCR2	siRNA/liposomes	Atherosclerosis	Leuschner et al[15]
CCR2	RS102982*	Atherosclerosis/ myocardial infarction	Winter et al[19]
CCR2	siRNA/liposomes	Myocardial infarction	Majmudar et al[16]
CCL5/CXCL4	MKEY peptide§	Atherosclerosis	von Hundelshausen et al[12] and Koenen et al[73]
CCL5/CCL17	CAN peptide§	Atherosclerosis	von Hundelshausen et al[12]
CCL5/HNP1	SKY peptide§	Myocardial infarction	Winter et al[19]
MIF	Antibody and COR100140*	Myocardial infarction	White et al[57]
ACKR3	CCX771*	Atherosclerosis	Li et al[61]
ACKR3	TC14012*	Myocardial infarction	Hao et al[62]
CXCL1, 2	Evasin-3‡	Myocardial ischemia/ reperfusion	Montecucco et al[74]
CCL5, 11	Evasin-4‡	Myocardial infarction	Braunersreuther et al[75]
CXCR3	NBI-74330*	Atherosclerosis	van Wanrooij et al[76]
CXCR3	AMG487*	Cardiac remodeling	Koren et al[77]

Compounds that have shown beneficial effects in myocardial infarction/remodeling or atherosclerosis studies in the mouse. ACKR3 indicates atypical chemokine receptor 3; CCL5, CC-chemokine ligand 5; CCR5, CC-chemokine receptor 5; CXCL, CXXXC-chemokine ligand; and MIF, macrophage migration inhibitory factor.

*Small molecule chemokine receptor antagonist.

†Modified chemokine.

‡Chemokine-neutralizing protein.

§Chemokine heteromer formation-antagonists.

humans are pending, as a clinical trial directed at the effects of Maraviroc on atherosclerosis in HIV patients was recently completed (URL: http://www.clinicaltrials.gov. Unique identifier: NCT03402815).

Also for CX_3CL1 and CX_3CR1, preclinical testing of antagonists is ongoing. The use of an N-terminally modified antagonistic CX_3CL1 variant (termed F1) reduced platelet adhesion to TNF-α (tumor necrosis factor-α) activated SMC.[34] When administered to mice, F1 reduced atherosclerosis through the inhibition of monocyte adhesion.[69] A viral CX_3CL1-binding protein M3 reduced atherosclerosis through a similar mechanism.[70] Further, monocyte adhesion was counteracted in a study implementing a novel small-molecular CX_3CR1 antagonist AZ12201182 in drug-eluting stents, resulting in a 60% reduction of stenosis in a porcine model of coronary artery stenting.[71] A viral broad-spectrum chemokine inhibitor M3 from murine gamma herpesvirus 68 was found to inhibit atherosclerosis by the inhibition of CCL2, CCL5, and CX_3CL1.[70] Likewise, a tick-derived group of small chemokine binding proteins, termed the Evasins, have shown therapeutic potential in mouse models of myocardial ischemia or infarction.[74,75]

Bindarit, which inhibits the expression of the chemokines CCL2, CCL7, CCL8, and IL12, reduced in-stent late loss (of lumen) after percutaneous coronary intervention in patients with ischemic heart disease, however, without effect on major adverse cardiovascular events.[78] Further, the humanized monoclonal anti-CCR2 antibody MLN1202 was shown to reduce high-sensitivity C-reactive protein levels as biomarker of inflammation in patients at high risk for atherosclerotic CVD,[79] but a phase II clinical trial evaluating the effect of this antibody on arterial inflammation in patients with stable atherosclerosis (URL: http://www.clinicaltrials.gov. Unique identifier: NCT02388971) was withdrawn.

A single endocardial CXCL12 dosing using a CXCL12-coding plasmid (JVS-100) in the phase II STOP-HF trial revealed the potential to improve cardiac function 12 months after treatment in patients with left ventricular ejection fraction below 26% following MI.[42] A second study examining single endocardial overexpression of CXCL12 in patients with ischemic heart disease using a nonviral DNA plasmid (ACRX-100) has recently been evaluated for safety and preliminary efficacy on cardiac improvement (Phase I), but results are not yet available. Further, a clinical trial examining the effect of CXCR4 antagonism on cell mobilization, heart function, and infarct size in patients with acute MI (CATCH-AMI [CXCR4 Antagonism for Cell Mobilisation and Healing in Acute Myocardial Infarction], Phase II) has been completed, but with results not yet available. Another clinical application being investigated is the use of CXCR4-directed imaging as diagnostic tool for atherosclerotic burden,[83] with CXCR4-directed endoradiotherapy revealed to have an anti-inflammatory effect on atherosclerotic plaques.[84] For MIF and ACKR3, no clinical studies examining a potential therapeutic application in CVD are currently registered.

With also CXCR2 mediating (CXCL8- and MIF-mediated) inflammatory monocyte and neutrophil recruitment in atherosclerosis,[85,86] the CICADA trial (CXCR2 Inhibition – A Novel Approach to Treating Coronary Heart Disease) is currently examining the effect of CXCR2 inhibition on cardiovascular surrogate parameters as coronary flow reserve and coronary plaque structure in patients with CAD undergoing percutaneous coronary intervention.[80]

Table 2. Clinical Trials Evaluating Chemokine s or Chemokine Receptors Registered at http://www.clinicaltrials.gov or EudraCTR

Target	Compound (Trial Name)	Condition	Aim of Study	Phase	Study Outcome and Status	Identifier
CCR5	Maraviroc	HIV patients with atherosclerosis	Efficacy of Maraviroc in Modulating Atherosclerosis in HIV Patients	Phase IV	Completed, results pending	NCT03402815
CXCL12	JVS-100 (CXCL12-coding plasmid) for single, endomyocardial injection	Ischemic heart failure	Safety and efficacy (primary end point: improved heart failure composite score at 4 months)	Phase II	Primary end point not met, but improved cardiac function 12 mo after treatment in patients with LVEF <26% following MI[42]	NCT01643590
CXCL12	ACRX-100 (nonviral DNA plasmid for transient expression of CXCL12) for single, endomyocardial injection	Ischemic heart failure	Safety, tolerability and preliminary efficacy (cardiac function, cardiac perfusion, improvement in NYHA classification)	Phase I	Completed, results not yet available	NCT01082094
CXCR4	POL6326 (CXCR4 antagonist) [CXCR4 Antagonism for Cell Mobilization and Healing in Acute Myocardial Infarction (CATCH-AMI)]	Large reperfused st-elevation myocardial infarction	As a stem cell mobilizer, effect on heart function and infarct size, safety and tolerability	Phase II	Completed, results not yet available	NCT01905475; EudraCT 2012-003229-91
CCL2	Bindarit (inhibits the expression of CCL2, CCL7, CCL8, and IL-12)	Coronary restenosis	Efficacy and safety after coronary stenting	Phase II	No significant reduction in in-segment late loss or MACE, but significant reduction in in-stent late loss[78]	NCT01269242
CCR2	MLN1202 (Anti-CCR2 humanized monoclonal antibody)	Atherosclerosis	Effect on C-reactive protein	Phase II	Significant reduction in circulating levels of C-reactive protein.[79]	NCT00715169
CXCR2	AZD5069 (CXCR2 inhibitor)	PCI in coronary artery disease	Effect on heart function (coronary flow reserve; diastolic function) and coronary plaque inflammation	Phase II	Ongoing, no results available yet[80]	EudraCT 2016-000775-24

A specific search for interventional studies related to CVD and heart disease (excluding peripheral artery disease) was performed for CCR5, CXCL12/CXCR4, CCL2/CCR2, and CXCL2. Comparable searches for CX3CL/R1, CXCR3, MIF, and ACKR3 did not return any result. ACKR3 indicates atypical chemokine receptor 3; CATCH-AMI, CXCR4 Antagonism for Cell Mobilisation and Healing in Acute Myocardial Infarction; CCR5, CC-chemokine receptor 5; CVD, cardiovascular disease; CXCL, CXXXC-chemokine ligand; CXCR2, CXC-chemokine receptor 2; and MIF, macrophage migration inhibitory factor.

Novel Translational Approaches for Diagnosis and Therapy in CVD

Despite the low clinical translation in regard to mainly systemic targeting chemokines but also adhesion molecules and integrins to reduce CVD burden, insights into the function of these molecules in inflammation and CVD have triggered the development of early stage imaging approaches as well as local targeting methods, primarily based on recent advances in nanotechnology. Indeed, nanomedicine seems highly promising for imaging and local drug delivery in CVD in general and atherosclerosis particularly.[87] Different types of nanocarriers targeting adhesion molecules (ICAM1 [intercellular adhesion molecule-1] and VCAM1 [vascular cell adhesion molecule-1]), selectins (E-selectin), and integrins ($\alpha v\beta 3$ integrin) using antibodies or protein-binding peptides have been examined for imaging of inflamed endothelium and atherosclerotic lesions as well as for targeted delivery, as reviewed in detail recently.[87,88] For example, systemic application of nanoparticles carrying CCR2-silencing short interfering RNA reduced CCR2 expression in monocytes, monocyte accumulation in plaques as well as infarct size and inflammation after MI.[15,16] Also, VCAM-1-targeted liposomes carrying CCR2 antagonists were shown to bind activated endothelium and reduce monocyte adhesion and transmigration,[89] and VCAM1-targeted nanoparticles containing anti-miR-712 were more efficient in inhibiting plaque formation in mice compared with naked anti-miR-172.[90] Packaging of miR-146a/181b, which reduces TRAF6/NF-κB (tumor necrosis factor receptor-associated factor 6/nuclear factor kappa B) signaling, into an E-selectin-targeting vector reduced expression of chemokines CCL2, CCL5, CCL8, and CXCL9, endothelial monocyte adhesion as well as atherosclerosis in mice,[72] suggesting that also a direct, local targeting of chemokines by microRNAs may be promising in reducing atherosclerosis.[91] A comparable approach is used by nature itself, with endothelial apoptotic bodies being atheroprotective by transporting miRNA-126 to neighboring endothelial cells to increase CXCL12/CXCR4-mediated vascular protection.[46]

In addition to the above approaches, the interactions of CCL5 with CXCL4, CCL17 and HNP1 present interesting

therapeutic targets. Rationally designed synthetic peptides, termed MKEY, CAN, and SKY were implemented to block the interactions of CCL5 with CXCL4, CCL17, and HNP1, respectively. This resulted in a reduction of atherosclerotic plaque size and in a reduced ischemia-reperfusion injury in mice treated with the respective peptides.[6,12,14,92] Although none of the above peptides has entered clinical trials, these examples illustrate that interactions of CCL5 with other proteins might be an interesting approach for the treatment of CVD, with less risk for immunologic side-effects as a total functional blockade of CCL5.

Conclusions and Future Perspectives

Despite promising findings in preclinical studies, clinical translation targeting chemokines into therapeutic strategies for CVD currently lags behind. Failure to translate many promising chemokine-based animal studies to the treatment of patients with MI has raised skepticism to our ability to develop clinical applications based on promising preclinical findings.[3] An important hurdle is the high heterogeneity of patients, with factors as age, sex, ethnicity, genetic variation, and differential CVD pathology highly increasing complexity of pathological processes and thereby clinical translation potential. Also, comorbidities may highly affect disease pathology and thereby the efficacy of therapeutic strategies. For example, patients with diabetes mellitus or chronic kidney disease have a highly increased risk of CVD at least partly caused by increased and altered inflammatory processes in these patients,[93] and unraveling comorbidity-associated alterations is essential for successful clinical translation in defined patient groups, supporting more personalized treatment approaches. Another hurdle is the double-edged role that inflammatory mechanisms and specific molecules may play in atherosclerosis[37] as well as in cardiac repair after MI,[3] indicating that both spatial as well as temporal aspects of therapeutic application need to be considered. Temporal optimization of therapy to address circadian rhythmicity, as shown for early atherosclerotic processes, may also improve drug efficacy and reduce side effects.[19] Another approach might be to take the above hurdles into account when developing preclinical (animal) models, to have a more robust outcome before entering clinical evaluation.

Despite these hurdles that still need to be overcome, recent developments in nanomedicine have triggered nanoparticle-based local targeting as preferential approach over systemic interference, for example, by specifically targeting activated endothelium over adhesion molecules and selectins to target proinflammatory chemokines locally in atherosclerotic lesions. And although studies investigating the usefulness of nanoparticle targeting were mainly performed in a preclinical setting and nanoparticle-based approaches still require additional evaluation of side effects,[87] these approaches may hold great promise for the implementation of novel diagnostic and therapeutic applications.

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Highlights

- Atherosclerosis is an inflammatory disease that precedes myocardial infarction or stroke. No true causative treatment for atherosclerosis or its complications is available yet.
- Chemokines constitute a large family and are important regulators of leukocyte migration and other cellular responses. Several chemokines have key roles in inflammation and thus present interesting drug targets.
- This review highlights recent developments for chemokines and for the chemokine-like factor MIF (macrophage migration inhibitory factor).