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IM - REVIEW

Therapeutic inhibition of CXCR1/2: where do we stand?

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Abstract

Mounting experimental evidence from in vitro and in vivo animal studies points to an essential role of the CXCL8-CXCR1/2 axis in neutrophils in the pathophysiology of inflammatory and autoimmune diseases. In addition, the pathogenetic involvement of neutrophils and the CXCL8-CXCR1/2 axis in cancer progression and metastasis is increasingly recognized. Consequently, therapeutic targeting of CXCR1/2 or CXCL8 has been intensively investigated in recent years using a wide array of in vitro and animal disease models. While a significant benefit for patients with unwanted neutrophil-mediated inflammatory conditions may be expected from a potential clinical use of inhibitors, their use in severe infections or sepsis might be problematic and should be carefully and thoroughly evaluated in animal models and clinical trials. Translating the approaches using inhibitors of the CXCL8-CXCR1/2 axis to cancer therapy is definitively a new and promising research avenue, which parallels the ongoing efforts to clearly define the involvement of neutrophils and the CXCL8-CXCR1/2 axis in neoplastic diseases. Our narrative review summarizes the current literature on the activation and inhibition of these receptors in neutrophils, key inhibitor classes for CXCR2 and the therapeutic relevance of CXCR2 inhibition focusing here on gastrointestinal diseases.

Keywords Inflammation · Neutrophils · CXCR2 · Chemokine receptor inhibition · Cancer · Gut

Abbreviations

ALI	Acute lung injury
BAL	Bronchoalveolar lavage fluid
C5a	Complement 5a
cAMP	Cyclic adenosine monophosphate
CD11b	Cluster of differentiation 11b
COPD	Chronic obstructive pulmonary disease
COVID-19	Coronavirus disease 2019
CRAC	Calcium release activated channel
CXCR	C-X-C motif chemokine receptor
CXCL	C-X-C motif chemokine
ESL1	E-selectin ligand 1
fMLP	*N*-Formylmethionine-leucyl-phenylalanine
G31P	CXCL8 (3–72) K11R/G31P, a competitive inhibitor of CXCR1/2
GPCR	G-protein coupled receptor
GRK	G-protein coupled receptor kinases
IL	Interleukin
IP_3	Inositol triphosphate
I/R	Ischemia/reperfusion
JAK	Janus kinase
KC	Keratinocyte-derived chemokine (synonym for CXCL1)
LFA-1	Leukocyte function-associated antigen 1
LPS	Lipopolysaccharide
MAPK	Map kinase
MDSC	Myeloid-derived suppressor cells
MPO	Myeloperoxidase
NE	Neutrophil elastase
NET	Neutrophil extracellular trap
NOD	Non-obese diabetic
TNF-α	Tumor necrosis factor alpha
PI3K	Phosphoinositide 3-kinase
PD1	Programmed death ligand 1
PTx	Pertussis toxin
ROS	Reactive oxygen species
PLC	Phospholipase C

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PSGL-1	P-selectin glycoprotein ligand 1
SOCE	Store-operated calcium entry
STIM	Stromal interaction molecule
TAN	Tumor-associated neutrophils
TLR	Toll-like receptor

Introduction/background

CXCR1 and 2 are two chemokine receptors which are expressed mainly on leukocytes but also on endothelial cells and cancer cells. CXCR1/2 play a major role in the pathophysiology of a wide spectrum of inflammatory conditions. Consequently, their inhibition presents a great therapeutic potential [1]. This review focuses on the activation and inhibition of these receptors in neutrophils, key inhibitor classes, and the therapeutic relevance of inhibition.

Neutrophilic granulocytes are key cells of the innate immune system, as well as important for modulating processes of adaptive immunity [2]. Neutrophils are also referred to as polymorphonuclear cells and possess a range of enzymes and inflammatory mediators in their granules as well as a wide receptor repertoire, including the expression of CXCR1/2 (Fig. 1). With the help of this defense weaponry, they unfold a spectrum of functions including shaping immune responses, mediating tissue injury and repair, and killing microorganisms. The mechanisms used for killing pathogens, including phagocytosis, degranulation, and neutrophil extracellular trap (NET) formation, are furthermore involved in tissue injury during inflammatory and autoimmune diseases. In addition, the complex role of neutrophils in cancer pathophysiology is beginning to be unraveled [3, 4].

In a healthy state, neutrophils circulate in the peripheral blood. Upon signals from the inflammation site, e.g., through the local production/release of chemokines, neutrophils are recruited into the affected tissue. Recruitment of neutrophils from the intravascular compartment into tissue is a tightly regulated process following a cascade of activation and adhesion events consisting in tethering, rolling, and adhesion to the inflamed vessel wall with subsequent postarrest modifications and eventually transmigration into inflamed tissue (Fig. 2) [5, 6]. Among the receptors and ligands involved, the CXCR1/2-CXCL8 axis plays an important role, particularly in the induction of firm neutrophil arrest, but also in the subsequent steps including postarrest modifications and transmigration [7].

The CXCR1/2 receptors

CXCR1/2 (formerly termed IL-8 receptor alpha and beta) are class A (rhodopsin-like) G-protein-coupled receptors with 7

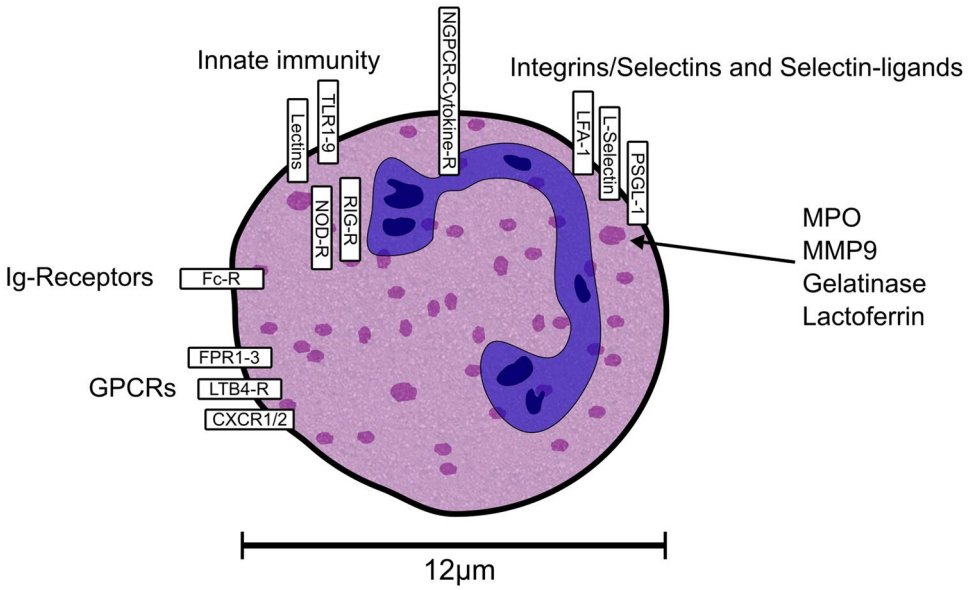

Fig. 1 The Neutrophil. Neutrophils are terminally differentiated leukocytes, around 12 µm in diameter. They are histologically characterized by a "neutral" color (as opposed to basophilic or eosinophilic granulocytes), segmented nucleus, and granules. Granules contain anti-microbial and anti-tissue proteins including myeloperoxidase (MPO), neutrophil elastase (NE), matrix metalloproteases (MMPs), gelatinase and lactoferrin. Their receptor profiles include G-protein-coupled receptors (GPCRs) like CXC-receptors 1 and 2 (CXCR1/2), Formyl-peptide receptors 1 and 2 (FPRs), and Leukotriene B4 receptors (LTB4-R), as well as Fc-receptors, innate immunity receptors like toll-like-receptors 1–9 (TLR1-9), lectins, RIG- and NOD-like receptors, and non-G-protein-coupled cytokine receptors (NGPCR-Cytokine-R) like interleukin 4 receptor or interleukin 1 receptor 1; as well as adhesion receptors like L-selectin, P-selectin glycoprotein ligand 1 (PSGL-1), leukocyte function-associated antigen 1 (LFA-1), Macrophage antigen-1 (Mac-1) [18, 181]

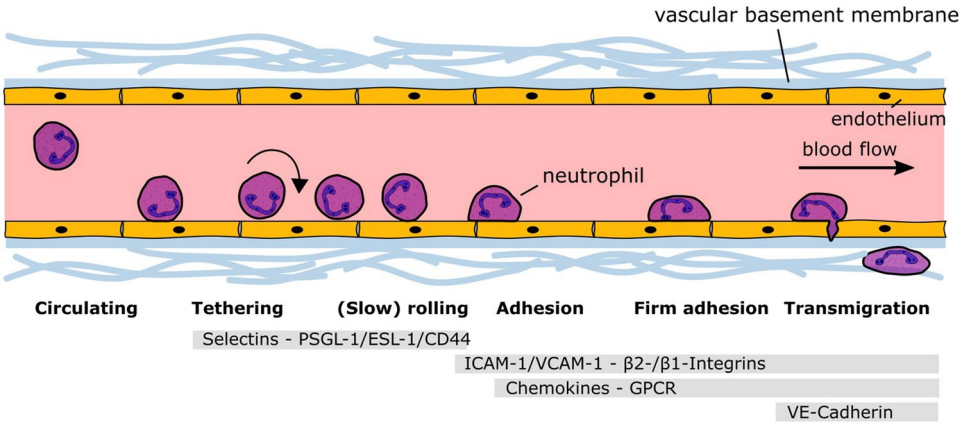

Fig. 2 Role of CXCR1/2 in the Neutrophil Recruitment Cascade. The recruitment of neutrophils is proceeding along a cascade of adhesion and activation steps. Free flowing neutrophils are initially tethered to the endothelial lining via selectins (e.g., E- and P-selectin) and corresponding selectin ligands (e.g., PSGL1 or ESL1) mediating rolling along the endothelium. During rolling, neutrophils receive activation signals, e.g., through selectin/selectin ligand interactions and through binding of chemokines (e.g. CXCL8) to their receptors (e.g. CXCL2). These signals lead to a conformational change of neutrophil-expressed β_2 integrins which further slows down rolling velocity and eventually mediates firm neutrophil arrest. Relevant chemokine receptors on neutrophils are CXCR1 and 2 interacting with chemokine CXCL8 (Il-8, in humans) or CXCL1 (KC, in mice). After firm adhesion, neutrophils undergo postarrest modifications including β2-integrin clustering, spreading, adhesion strengthening, and crawling. Latter is needed for finding a suitable transmigration spot. Finally, the cell exits the vessel through or between the endothelial cells and penetrates the vascular basement membrane to reach the inflamed tissue. Molecules here include VE-cadherin and others [5, 182]. Figure adapted from [183]

transmembrane domains. They are expressed on neutrophils, macrophages and mast cells and other leukocytes as well as on non-immune cells such as endothelial cells and cancer cells [8]. CXCR1 and 2 bind C-X-C motif chemokines carrying the glutamic acid-leucine-arginine (ELR) motif, mainly the chemokines CXCL1 through 8. In humans, high affinity ligands for CXCR1 are CXCL6 and CXCL8, and for CXCR2 CXCL1-3 and 5–8 [9]. In mice and rats, no homologue of human CXCL8 has been described. However, CXCL1 (also called keratinocyte-derived chemokine/KC) is considered its functional homologue [10].

Recently, the 3D structure of CXCR1 was resolved by Park and colleagues using nuclear magnetic resonance (NMR) spectroscopy (PDB: 2LNL) showing three extracellular loops and three intracellular loops. Of the intracellular loops particularly the third one is proposed to play a crucial role for the signal transduction to G proteins [11]. The structure of CXCR2 has been resolved so far only in complex with the guanine nucleotide exchange factor PDZ-RhoGEF (PDB: 5TYT) [12].

The resolution of CXCR1's 3D structure paved the way for further in silico analyses, including its ligand binding sites and modes. These models propose that the N loop of CXCL8 (see also below) and the N terminal domain of CXCR1 interact electrostatically, which enables the N terminal ELR motif of CXCL8 to move closer to the extracellular loops of the receptor (mediated through hydrophobic interactions).Finally, firm binding of the two molecules is mediated through electrostatic interactions [13].

The chemokine CXCL8/IL-8

Most research on CXCR1/2 inhibitors focused on the role of CXCL8. The chemokine CXCL8, also known as interleukin 8 (IL-8), is a variable-length protein that many cells can secrete, including monocytes, macrophages, fibroblasts, hepatocytes, epithelial and endothelial cells [14]. It belongs to the C-X-C family of chemokines, meaning the first two cysteine amino acids are separated by another amino acid. CXCL8 is synthesized as a 99 amino acid long precursor protein and then cleaved depending on cell type and stimulus [15]. CXCL8 secretion is often induced by stimulation with interleukin 1β (IL-1β) or tumor necrosis factor α (TNF-α). Its 3D structure was resolved by NMR spectroscopy in 1990 (PDB: 1IL8). The main features are two antiparallel alpha helices on top of a six-stranded platform of beta sheets [16].

CXCL8 primarily functions as a chemoattractant for neutrophils, i.e. CXCL8 triggers the recruitment of neutrophils to the site of inflammation through interaction with neutrophil-expressed CXCR1/2 [17]. CXCR1 and 2 both have similar affinities for CXCL8 (Kd 0.7–3.6 nM; [20, 21]) and intradermal application of CXCL8 results in edema and neutrophil accumulation at the site of injection, in most studies with no signs of additional inflammatory symptoms such as pain or itching. Interestingly, neutrophil recruitment is significantly enhanced by transendothelial transport of extravascular chemokines to the luminal site of the inflamed endothelium mediated by endothelium expressed Duffy antigen receptor [18]. In vivo, chemokines including

CXCL8 are predominantly bound to heparan sulfates on the glycocalyx of endothelial cells and presented to intravascular neutrophils [18]. Intravascular application of CXCL8 leads to severe systemic granulocytopenia, followed by granulocytosis [19].

Of note, CXCL8 exists both in a monomeric and a dimeric form, which may have different effects on CXCR1/2 regarding desensitization and receptor internalization [22].

In rats and mice, it has been demonstrated that application of recombinant human CXCL8, which is not found physiologically in those animals, also reliably leads to firm arrest of rolling neutrophils [23, 24].

Physiological functions and signal transduction of CXCR1/2

Binding of CXCL8 to its receptors causes activation of neutrophils, which can be seen by chemotaxis toward the gradient, increased adhesion and transmigration, increased reactive oxygen species (ROS) production and activation of Ca^{2+} signaling [1]. On a molecular level, G protein, phosphoinositol 3-kinase (PI3K) and Ras/MAP kinase (MAPK) signaling pathways are involved to induce the above-mentioned effector functions (Fig. 3). Interestingly, receptor function might be dependent on the specific bound chemokine (*biased agonism*) [25].

CXCR1/2 are coupled to heterotrimeric G proteins. In vitro experiments using COS7 cells revealed that G_q alpha proteins, $G_{\alpha i2}$ and $G_{\alpha i3}$ are involved in signal transduction [26]. Upon ligand binding, the beta and gamma subunits dissociate from the α subunit and activate subsequent pathways, most notably Phospholipase C (PLC) or PI3K (Fig. 3). The activation (dissociation) of the βγ subunit alone is sufficient for critical neutrophil functions such as chemotaxis [27].

CXCL8 binding to CXCR1, but not to CXCR2, results in respiratory burst through the activation of Phospholipase D [28, 29].

The activation of PLC-β2 and PLC-β3 via the formation of inositol triphosphate (IP_3) and subsequent activation of IP_3 sensitive receptors on the endoplasmatic reticulum (ER) leads to ER Ca^{2+} depletion and concomitant increase in cytosolic Ca^{2+} levels, a process which is pertussis-toxin sensitive [26]. After depletion of ER stores, Ca^{2+} channels on the plasma membrane open and more Ca^{2+} flows

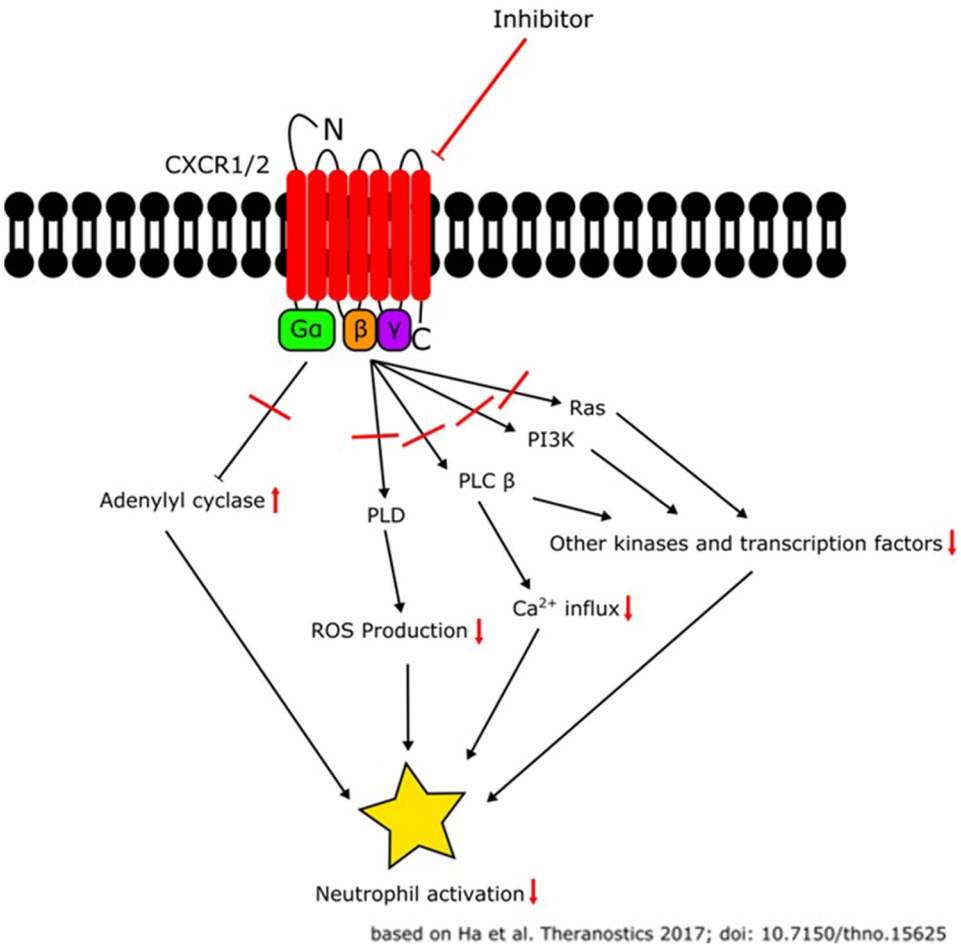

Fig. 3 Inhibition of intracellular CXCR1/2 mediated signaling in neutrophils. Binding of CXCL8 to CXCR1/2 leads to inhibition of adenylyl cyclase, and activation of different enzymes including phospholipase D (PLD), phospholipase Cβ (PLCβ), PI3K and Ras. Inhibition of CXCR1/2 activates adenylyl cyclase, and attenuates the activation of the other enzymes, leading to decreased neutrophil activation. For details and references, see text

in from the extracellular space, which is also referred to as store-operated calcium entry (SOCE) [30]. SOCE is induced by the interaction of the ER calcium sensor protein stromal interaction molecule 1 (STIM1) with calcium release activated Ca^{2+} (CRAC) channels on the plasma membrane including Orai1 and Orai2 [29]. Ca^{2+} signaling is essential for a wide range of neutrophil functions including β_2-integrin outside in signaling, cytoskeletal rearrangement, migration, phagocytosis, ROS production and degranulation [29].

In neutrophils, PI3Kγ is activated by the βγ subunits of G-protein coupled receptors (GPCRs) [31] and is required for neutrophil adhesion (specifically postarrest adhesion strengthening) under flow [32]. It is also involved in mediating chemotaxis [33].

One well characterized downstream function of CXCL8 in neutrophils is the activation of β_2-integrins including leukocyte function-associated antigen 1 (LFA-1, CD11a/CD18, $\alpha_L\beta_2$) [34]. On neutrophils, β_2-integrins play a major role in mediating slow rolling and adhesion of neutrophils to the inflamed endothelium (Fig. 2).

Similar to other GPCRs, CXCR1/2 show receptor internalization after ligand binding. Following agonist binding to CXCR1/2, receptor phosphorylation by a GPCR kinase (GRK) occurs, which in turn facilitates binding of β-arrestin to the receptors leading to receptor desensitization and finally clathrin-mediated endocytosis via the AP-2 adaptor protein [35–38]. The receptor is cleared of the agonist and can then be recycled back to the cell membrane. CXCR1 and 2 undergo internalization to a similar degree, however, differ in their recycling characteristics. There is evidence that receptor internalization occurs only at rather high concentrations of chemokines suggesting an involvement particularly in the later stages of chemotaxis [38]. It is noteworthy to mention here that these in vitro experiments were done with soluble chemokines, whereas chemokines under in vivo conditions are also immobilized e.g., on endothelial cells, which might influence receptor internalization.

Besides internalization, CXCR1/2 can also be desensitized by the activation of other receptors, including formyl-methionyl-leucyl-phenylalanine (fMLP) or Complement 5a (C5a) receptors. CXCR1 activation in turn can desensitize fMLP and C5a receptors [39]. A similar desensitization can also be observed between CXCR1/2 and C–C chemokine receptor 5 (CCR5) [40]. This phenomenon is known as class desensitization and usually leads to attenuated cell signaling upon ligand binding [39, 41].

Chemotaxis is one of the best studied effects following activation of the neutrophil CXCL8-CXCR1/2 axis. Neutrophils sense and follow a gradient of chemokine, under physiological conditions to the site of inflammation. The process is dependent on intracellular Ca^{2+} [42], PI3K, Janus kinase 3 (JAK3) [43] and tyrosine kinases Cbl and Akt [44].

Different chemokines/ligands trigger different functions upon binding to the same receptor. This phenomenon, well known for GPCRs in general [45], was recently also demonstrated specifically for CXCR1/2: CXCL1 and CXCL6 lead to attenuated intracellular cAMP and Ca^{2+} signaling compared to equimolar CXCL8 stimulation [25].

On an organism level, CXCL8 and CXCR2 also play a critical role in angiogenesis, as demonstrated by many in vitro and in vivo studies [46]. This is especially important for tumor proliferation, as inhibiting CXCR1/2 has also been shown to be beneficial in certain entities of cancer (see section "Therapeutic targeting of CXCR1/2 in disease").

Therapeutic inhibition of CXCR1/2

A variety of inhibitors of CXCR1 and/or 2 have been described to date and are summarized in Table 1. One of the first to be used was the toxin of *Bordetella pertussis* (Pertussis toxin; PTx), which was discovered to inhibit neutrophil activation with impaired granule enzyme secretion [47] and reduced CXCL1-mediated adhesion in mice in vivo [24]. On a molecular level, PTx catalyzes the ADP-ribosylation of the Gα subunits $G_{\alpha i1\text{-}3}$ and $G_{\alpha o1\text{-}2}$ which prevents downstream G-protein signaling. Because of its inherent wide range of (side-)effects in humans, it is currently not therapeutically used. However, it remains a valuable tool in the research of chemokine-mediated neutrophil activation [48, 49].

The diaryl urea class of chemokine receptor inhibitors includes the compounds SKF83589, SB225002, SB332235, SB265610, SB656933 (Elubrixin) and GSK1325756 (Danirixin). All compounds share two phenyl groups connected by a urea group and are CXCR2-selective. Limited data is available on SKF83589, the first of its class [50]. SB225002 was developed from SKF83589 and first described in 1998, where its binding to CXCR2 was characterized using radioligand binding assays and its effects on the receptors investigated using calcium signaling, chemotaxis and neutrophil recruitment assays. The compound attenuated Ca^{2+} mobilization in response to CXCL1, and CXCL8 (only in HL60 cells). It reliably inhibited neutrophil chemotaxis in response to both CXCL1 and CXCL8, and lowered systemic neutrophil counts following i.v. administration of CXCL8 [51]. SB332235 was first described in a publication from 2002, where its binding was characterized using a radioligand binding assay, and its inhibitory action was demonstrated by Ca^{2+} mobilization assays, chemotaxis assays and an experimental arthritis model in rabbits [52]. SB332235 inhibited neutrophil chemotaxis in vitro and attenuated arthritis, as measured by lower leukocyte counts and chemokine concentrations in synovial fluid [52]. SB265610 was described in 2009 as an allosteric inverse agonist of the CXCR2 receptor, but apparently not pursued further to clinical research [53]. SB656933 (Elubrixin) was

Table 1 Inhibitors of CXCR1/2

Name	Class	References
Allosteric inhibition		
SKF83589	Diarylurea	[50]
SB225002		[51]
SB656933		[54]
SB332235		[52]
GSK1325756 (Danirixin)		[56]
SB468477	Cyanoguanidine	[184]
Reparixin (Repertaxin)	R-ibuprofen derivate	[185]
Ladarixin (DF2156A)	Trifluoromethanesulfonate phenyl propanamide	[77]
DF2755A		[80]
DF2162		[74]
SCH-527123 (Navarixin)		[82, 83]
SX-576	Boronic acid	[60]
SX-517		[59]
AZD8309	Bicyclic thiazolopyrimidine	[186]
AZD5069		[94]
AZ10397767		[88]
PD0220245	Quinoxaline	[109]
Competition or binding of CXCL8		
CXCL8 K11R G31P	Mutated peptide	[159]
DD-NAc-PGP isomer	Small peptide	[100]
TNF-stimulated gene 6 protein (TSG6)	Protein	[187]
Inhibition of associated G protein		
Pepducin × 1/2pal-i1	Lipid-conjugated peptide	[106]
Pertussis toxin	Peptide	[24]
Unknown		
SB455821	Unknown	[55]
Antileukinate	Protein	[97]

first characterized in 2011 using a CXCL1-induced CD11b expression assay and experimental ozone-induced airway inflammation model in humans [54]. It inhibited CD11b expression, as well as neutrophil recruitment and activation (as measured by myeloperoxidase [MPO] release) in a dose-dependent manner. It was tested in clinical phase 1 and 2 studies for cystic fibrosis, chronic obstructive pulmonary disease (COPD) and ulcerative colitis (Table 2).

SB455821, a CXCR2 inhibitor, inhibited neutrophil transmigration in vitro, as well as in vivo in a murine peritonitis assay. Interestingly, in response to zymosan, it did not inhibit neutrophil recruitment into the peritoneum of mice [55].

GSK1325756 (Danirixin), also a diarylurea compound, was investigated in Ca^{2+} mobilization and CD11b upregulation assays. In addition, it was tested in a lipopolysaccharide (LPS)- or ozone-induced acute lung injury (ALI) model in rats, where it showed lower disease scores when pretreated with GSK1325756 [56, 57]. Multiple clinical phase 1 and 2 trials for COPD and viral respiratory infections were subsequently performed (please refer to Table 2 for a detailed list of trials). However, due to insufficient efficacy in disease improvement in these trials, GlaxoSmithKline recently stopped Danirixin development for COPD [58].

Another class of inhibitors are boronic acid containing molecules. The first of its class is SX-517, which was first characterized in 2014 [59]. Its pharmacodynamics was analyzed in a radioligand binding assay, and its effects were studied functionally in human neutrophils in vitro and in a murine in vivo inflammation model involving a dorsal air pouch [59]. Another compound, SX-576, led to reduced neutrophil influx in an ozone-induced ALI rat model [60]. Further studies were conducted by the team to improve their solubility and oral bioavailability [61]. The successor SX-682 then proved to attenuate myeloid-derived suppressor cells (MDSC) influx in head and neck cancer as well as in castration-resistant prostate cancer, thereby increasing the efficacy of immunotherapy [62–64]. SX-682 has recently entered phase 1 trial for melanoma treatment [65].

Another group of dual CXCR1/2 small molecule antagonists include Ladarixin, Reparixin (Repertaxin), DF2162 and DF2755A. Reparixin, a R-ibuprofen derivative, was described in 2004 and tested in a GPCR signaling and

Table 2 Clinical trials for CXCR1/2 inhibitors

Compound	Clinical indication	Selectivity	Company	Phase	References
SX-682	Advanced melanoma	CXCR2	Syntrix Biosystems	Phase 1	NCT03161431
Ladarixin (DF2156A)	Insulin-dependent Diabetes mellitus type 1	CXCR1/2 dual	Dompè SpA	Phase 2 Phase 3	NCT02814838 NCT04628481
Reparixin (Repertaxin)	Lung transplant/ischemia–reperfusion injury	CXCR1		Phase 2	NCT00224406
	Post surgical I/R following coronary artery bypass graft	CXCR1		Phase 1	EudraCT 2004-001,138-18 [194]
	T1D islet cell transplantation Metastatic breast cancer			Phase 2 Phase 2	NCT01220856 NCT05212701
AZD8309	Airway inflammation	CXCR2	AstraZeneca	(Basic science)	ISRCTN46666382 NCT00860821
AZD5069	Airway inflammation	CXCR2	AstraZeneca	Phase 1	NCT01735240 NCT01332903 NCT01480739 NCT01083238 NCT00953888 NCT01100047 NCT01051505 NCT01989520 NCT01890148 NCT01962935 NCT02583477
	Metastatic castration-resistant prostate cancer	CXCR2	AstraZeneca	Phase 1	NCT03177187
	Severe asthma	CXCR2	AstraZeneca	Phase 1	NCT01704495
	Bronchiectasis	CXCR2	AstraZeneca	Phase 1	NCT01255592
	COPD	CXCR2	AstraZeneca	Phase 2	NCT01233232
	Solid tumors	CXCR2	AstraZeneca	Phase 2	NCT02499328
SB-656933	Ulcerative colitis	CXCR2	GlaxoSmithKline	Phase 2	NCT00748410
SCH 527,123 MK-7123	COPD	CXCR2	Merck Sharp & Dohme Corp	Phase 2	NCT01006616
	Asthma	CXCR2	Merck Sharp & Dohme Corp	Phase 2	NCT00688467 NCT00632502
	Advanced solid tumors	CXCR2	Merck Sharp & Dohme Corp	Phase 2	NCT03473925
	Psoriasis	CXCR2	Merck Sharp & Dohme Corp	Phase 2	NCT00684593
Monoclonal Anti-CXCL8	COPD	CXCL8	Abgenix Inc	Phase 2	[195]
GSK1325756 (Danirixin)	COPD and viral respiratory infections	CXCR2	GlaxoSmithKline	Phase 1	NCT01209052 NCT01209104 NCT02201303 NCT03457727 NCT02453022 NCT01453478 NCT03136380 NCT01267006 NCT02169583
				Phase 2	NCT02130193 NCT03034967 NCT02469298 NCT02927431 NCT03250689 NCT03170232

chemotaxis assay, and in a rat liver I/R injury model, where it showed attenuated Ca^{2+} signaling, reduced migration and reduced neutrophil infiltration into the liver [66]. The efficacy of Reparixin was also evaluated in a type 1 diabetes mouse model and spinal cord injury rat model, where it led to reduced neutrophil infiltration, improved glycemia and improved neurological scores, respectively [67–69]. In a human trial for type 1 diabetes islet transplantation however, no further benefits compared to placebo could be shown [70]. Similarly, it was investigated for advanced triple-negative breast cancer in a human trial, where it did not show a prolonged progression-free survival compared to placebo [71]. On the other hand, in a human trial in patients with SARS-CoV-2 infection (COVID-19) pneumonia, Reparixin led to an improvement in clinical outcomes compared to the standard of care [72]. DF2162's activity was evaluated using radioligand binding and chemotaxis assays [73]. Here, it inhibited chemotaxis while it did not affect CXCL8 binding. Further evaluation was performed in a rat arthritis, mouse nociception and lung fibrosis model, where it attenuated inflammation/fibrosis as measured by neutrophil influx, local chemokine production and histological scores [74, 75]. Ladarixin, also known as DF2156A, was developed in 2012 and characterized using radioligand binding and chemotaxis assays, where it showed similar inhibitory activity for CXCR1 and 2, an optimized pharmacokinetic profile and overall inhibited neutrophil chemotaxis [76]. Ladarixin was first tested in a mouse sponge-induced angiogenesis model of chronic inflammation, where it reduced neutrophil migration, TNF-α production and new vessel formation [76]. Apart from that, it was also tested in in vitro adipocyte models, mouse type 1 diabetes and rat cerebral ischemia/reperfusion (I/R) models. In these tested animal models, Ladarixin showed improved outcomes as measured by increased insulin sensitivity, delayed diabetes development/lower glycemia, improved neurological scores following cerebral I/R and reduced neutrophil infiltration [67, 76–78]. In a human trial, Ladarixin short-term treatment did not show any appreciable effects on preserving residual beta cell function in new-onset type 1 diabetes patients [79]. DF2755A was recently described using radioligand binding and chemotaxis assays [80]. It inhibited chemotaxis without affecting binding of CXCL8. In a mouse mechanical nociception and post-surgical pain model, it was able to lower inflammatory hyperalgesia as measured by higher paw redrawal thresholds [80]. Moreover, it had been reported recently that oral treatment with DF2755A can prevent and reverse peripheral neuropathy associated to non-Hunner interstitial cystitis/bladder pain syndrome by directly inhibiting chemokine-induced excitation of sensory neurons in a cyclophosphamide-induced non-ulcerative interstitial cystitis rat model [81].

SCH527123, also known as MK-7132 or Navarixin, is another small molecule allosteric inhibitor of CXCR1 and 2 [82, 83]. In addition to inhibiting neutrophil activation, recruitment and chemotaxis, it was tested in multiple pulmonary inflammation models, where it could attenuate or block local neutrophil influx, goblet cell hyperplasia and excessive mucus production [82]. In a trial in healthy humans, a reduction of ozone-induced airway neutrophilia could be demonstrated for the compound [84]. Navarixin was also tested in a murine and piglet Influenza model, where it improved survival and attenuated lung injury [85]. In an colorectal cancer model, Navarixin was able to inhibit tumor growth, spreading and angiogenesis in vivo [86]. Those findings were translated into different phase 2 clinical trials in COPD patients (see Table 2). Here, Navarixin showed improvement in pulmonary function (increase in forced expiratory volume in 1 s, FEV1) and decreased sputum neutrophil numbers, while it also led to dose-dependent neutropenia [87]. Other phase 2 clinical trials were focusing on asthma and psoriasis. Here, no disease improvement could be observed (Table 2). The most recent trial is investigating a beneficial effect of Navarixin in advanced or metastatic solid tumors. Results are still pending.

Another CXCR2-antagonist, AZD8309, is a pyrimidine-based compound, which selectively blocks CXCR2 [88]. Its oral application was examined in a clinical trial of inhaled LPS in healthy volunteers as a model for neutrophilic airway inflammation. AZD8309 led to reduced sputum neutrophil counts, reduced neutrophil elastase (NE) activity and reduced generation of CXCL1 [89]. A recent study used the compound in a murine pancreatitis model, where it successfully attenuated neutrophil influx, intrapancreatic activation of proteases and thereby reduced disease severity [90]. A subsequently developed compound, AZD5069 [91] was assessed in multiple phase 1 trials (see Table 2) and showed predictive linear pharmacokinetics with no relevant disturbances by food uptake, patient age or ethnicity and an optimal dosing at twice a day [92]. Other phase 1 studies investigated the safety of the compound in asthma and metastatic ductal adenocarcinoma (Table 2). Currently, a phase 1 and a phase 2 trial on the combination with Enzalutamide in metastatic castration-resistant prostate cancer is ongoing. In a phase 2 trial for uncontrolled persistent and severe asthma, no significant reduction in the amount of exacerbations could be demonstrated [93]. Another phase 2 study in patients with COPD showed AZD5069 to be well tolerated overall, but found it caused systemic neutropenia in some cases [94, 95]. The results for an application of the compound to treat relapsed metastatic squamous cell carcinomas of the head and neck have not been published yet (NCT02499328). Another similar compound called AZ10397767 is thiazolopyrimidine-based and an inhibitor for both CXCR2 and

CCR2 [88]. In an in vivo lung adenocarcinoma model, AZ10397767 could attenuate neutrophil influx and tumor growth, but not CXCR2 dependent angiogenesis in mice [96].

Besides small molecule inhibitors, peptide-based CXCR1/2 inhibitors have been developed. Those include Antileukinate, Nac-PGP, CXCL8 K11R G31P or pepducins. Antileukinate was first described in 1995 and successfully characterized and tested in radioligand binding, enzyme release, chemotaxis assays, as well as in a rabbit skin edema model and a murine bleomycin-induced acute lung injury model. The peptide attenuated neutrophil activation, chemotaxis in response to CXCL8 in vitro, and inflammation/neutrophil recruitment in vivo [97, 98]. N-Acetyl-Proline-Guanine-Proline (Nac-PGP) is a peptide which was found in the degraded extracellular matrix following airway inflammation and neutrophil influx [99] and described as a competitive CXCR1/2 antagonist [100]. CXCL8 (3–73) K11R G31P (short G31P) is a CXCL8 analog with two mutations (at positions 11 and 31, respectively), and was reported to have a higher affinity for CXCR1 and 2 than native CXCL8, while suppressing neutrophil activation and chemotaxis [101, 102]. It was also effective in attenuating pulmonary inflammation in an experimental *K. pneumoniae* pneumonia guinea pig model, as measured by neutrophil counts in bronchioalveolar lavage (BAL) fluid, MPO release and lung histological analysis [103].

Pepducins are lipid-conjugated proteins which target intracellular loops of G proteins. Lipids, such as palmitate, are appended N-terminally to intracellular loops, e.g., i3 or i1, of G-protein coupled receptors. The lipid allows these molecules to float in the cell membrane and disrupt the activation of G proteins via interfering with the intracellular loops of these receptor [104]. They are named after the receptor they target, then the conjugated lipid, and finally the intracellular loop, for example × 1/2-pal-i1 is a pepducin targeting CXCR1/2 (× 1/2), has palmitate conjugated (pal), and interacts with the first intracellular loop (i1). In a 2005 study [105], it was shown that pepducin × 1/2-pal-i3 and pepducin × 1/2-LCA-i1 inhibit neutrophil function in vitro as well as in vivo. This was evidenced by absent Ca^{2+} influx upon CXCL8 binding, reduced leukocyte recruitment in vitro, as well as in a murine peritonitis assay. The administration of the pepducins protected the mice from death due to septic peritonitis, even if the administration occurred delayed [105].

Later, another pepducin, × 1/2-pal-i1, was synthesized and tested for its clinical use, as well as for histological effects on neutrophils, cytokines and lipids in experimental murine alcoholic steatohepatitis (mASH). Although neutrophils and CXCR1/2 have not been addressed systematically in this disease (model) before, the inhibitor reduced the incidence and mortality of mASH. It reversed mASH, downregulated CXCL1/TNF-α/IL1b expression, and reduced neutrophil and lipid accumulation in the liver [106].

CXCR2 inhibitors that have been disclosed, but so far not extensively tested in vivo, include a pyrimidine-5-carbonitrile- [107] and triazolopyrimidine- [108], and a 2-amino-3-heteroaryl-quinoxaline-based compound [109]. In addition, a nicotinanilid [110], and a nicotinamide [111] based compound were synthesized, which are both potent CXCR2 inhibitors.

Finally, new methods for the discovery of CXCR1/2 inhibitor compounds based on in silico modeling are emerging as recently described [112].

Therapeutic targeting of CXCR1/2 focusing on gastrointestinal and metabolic diseases

As already mentioned above, blocking CXCR1, but more so CXCR2, have been shown to significantly reduce neutrophil recruitment, associated tissue damage and disease severity in many clinical disease models. In this section, the therapeutic effects of pharmacological CXCR1/2 blockade are summarized with an emphasis on gastrointestinal and metabolic diseases (Table 3).

Inflammatory bowel disease

Ulcerative colitis (UC) is a chronic autoimmune disease of the intestinal mucosa, mainly the rectum and colon. The inflammation of the intestine generates symptoms such as bloody diarrhea, malabsorption and pain, usually in a biphasic manner (flare-up and remission). Discovered pathophysiological features include the relevance of innate lymphoid cells, T helper cells, IL-13 and IL-4 as drivers of the disease [113]. Also, dysbiosis and TLR2 and 4 upregulation is seen, though it is unclear whether these are causes or consequences. Current treatment options include 5-aminosalicylates, corticosteroids, 5-mercaptopurine, anti-TNF antibodies, and–as ultima ratio–proctocolectomy [113]. Neutrophils have been reported to be involved in the pathogenesis of UC: depletion of neutrophils and inhibition of leukocyte adhesion in a rat model attenuated experimental UC, and in humans, CXCL8 and CXCR1/2 expression is increased in UC and can be correlated with different disease phases and severity [114, 115]. Neutrophil infiltration is also one of the major criteria in two histological grading systems for UC [116]. Similar to rheumatoid arthritis, elevated S100A8/A9 (calprotectin) levels can be seen in UC, and correlate with disease severity, so that fecal S100A8/A9 levels are an established biomarker to monitor disease activity [117]. In murine studies, mice lacking CXCR2 were protected to a certain degree from experimental colitis [118, 119]. The small molecule CXCR2 inhibitor SB225002 also improved acute colitis in mice in vivo [120]. Further

Table 3 Potential therapeutic use of CXCR1/2 inhibitors

Experimental disease model	Inhibitor(s)	Effects	References
Alcoholic steatohepatitis (mouse)	Pepducin × 1/2pal-i1	Reduced neutrophil infiltration; normalization of histology	[106]
Coecal ligation and puncture (mouse)	Different pepducins	Reduced mortality	[105]
	Anti-CXCR2 Ab	Delayed neutrophil infiltration, reduced mortality	[188]
Type 1 diabetes (mouse)	Reparixin, Ladarixin	Delayed diabetes development, lower glycemia after T1D development	[67]
Transient cerebral ischemia (rat)	Ladarixin CXCL8 G31P	Reduced cerebral MPO, ischemic volume; improved neurological outcome	[77, 189]
Intestinal ischemia (rat)	CXCL8 G31P	Reduced neutrophil infiltration, improved histology	[190]
Dust-induced lung inflammation (mouse)	CXCL8 G31P	Reduced neutrophil infiltration, chemokine levels, improved histology	[191]
Acute lung injury (mouse)	Reparixin	Reduced neutrophil infiltration, improved vascular leakage, improved gas exchange	[185]
Bleomycin-induced lung fibrosis (mouse)	DF2162	Reduced neutrophil infiltration, reduced fibrosis	[75]
Spinal cord injury (rat)	Reparixin	Reduced chemokines, lesion area; increased neurons, clinical scores	[68]
Hepatic reperfusion injury (rat)	Reparixin	Reduced neutrophil infiltration, liver enzymes, necrosis	[66]
	Ladarixin		[76]
Sponge-induced angiogenesis (mouse)	Ladarixin	Reduced neutrophil infiltration, hemoglobin levels	
Rheumatoid arthritis (mouse)	SCH-563705	Reduced chemokines in synovial fluid, decreased disease severity including histology	[192]
Cigarette smoke-induced lung inflammation (mouse)	SCH-N	Reduced neutrophil infiltration in BAL, improved histology, increase of MIP-2 and KC in BAL	[193]
Ozone-induced airway inflammation (human)	SB-656933	Dose-dependent reduction of neutrophils and activation in sputum	[54]

inhibitory and clinical studies have not yet been reported, despite the evidence of neutrophil contribution to UC [115].

For the other major inflammatory bowel disease, Crohn's disease (CD), positive correlation of CXCL8 with disease activity has been reported in around half of studies in a meta-analysis, so CXCL8 as biomarker still remains controversial [121]. Neutrophils, and specifically neutrophil extracellular traps, seem to contribute to inflammation and tissue destruction in both CD and UC [122].

Alcoholic steatohepatitis (ASH) is a sterile inflammation of the liver resulting from excessive long-term alcohol consumption. ASH usually manifests itself through liver failure, and if left untreated leads to cirrhosis and eventually end-stage liver disease and death. Pathophysiologically, chronic alcohol consumption severely disturbs liver fatty acid, ROS and enzyme metabolism. Histologically, liver tissue from ASH patients shows accumulation of fat (steatosis), infiltration of immune cells including neutrophils termed Mallory-Denk bodies, and perivenular fibrosis [123]. Treatment is usually symptomatic, and abstinence does not guarantee remission of the disease. Immunomodulators like corticosteroids and anti-TNFα drugs have been tested with mixed results [124]. Chemokines which seem to be involved in the recruitment of neutrophils to liver tissue include CXCL2 and to a lesser extent CXCL1 [125]. In a murine ASH model, CXCR1/2 pepducins were able to stop the progression, as well as reverse the condition as evidenced by reduced neutrophils liver infiltration and normalization of liver histology [106].

Cancer and inhibition of the of the CXCL8-CXCR1/2 axis

The role of neutrophils and chemokines has been well established in various malignancies. Tumors interact in many ways with various immune cell populations aiming to be tolerated by both adaptive and innate immunity to guarantee tumor growth including induction of angiogenesis in solid tumors [8]. A meta-analysis on various cancer entities concluded that accumulation of neutrophils in tumor tissue is a negative prognostic factor in cancer overall [126].

It Is thought that neutrophils are recruited to the tumor tissue and by releasing their enzymes and destroying the ECM, they pave the way for tumor cells to grow [127]. Besides recruiting tumor-associated neutrophils (TANs) and other immune cells to the tumor [128, 129], the activation of CXCR2 can directly and indirectly lead to tumor cell proliferation [130, 131], inhibit physiological cell proliferation [132–134], cause tumor cell migration enabling metastasis [135, 136] and induce angiogenesis [137, 138]. Studies on

CXCR2 provide evidence that at least for gastric [139–142], cholangiocellular [143], colorectal [144, 145], pancreatic [146, 147], breast [148], prostate [149], and lung cancer [150], absence or inhibition of the receptor leads to a better outcome of the malignancies in terms of tumor volume, and angiogenesis. By contrast, in studies investigating gastric cancer [151], triple-negative breast cancer [152, 153], and renal cancer [154], a protective effect of CXCR2 was shown.

Studies using small molecule CXCR1/2 inhibitors are starting to emerge, for example in vitro and in vivo inhibition of malignant melanoma cell growth using Ladarixin [155, 156]. In addition, the combination of Reparixin with antineoplastic agent docetaxel reduced the tumor size in a model of human breast cancer cell lines and breast cancer patient-derived xenografts [157] demonstrating that Reparixin is able to reduce in vivo the tumor-initiating ability of breast cancer cells by affecting the cancer stem cell population. In a colon cancer cell model, treatment of mice with SCH-527123 or SCH-479833 attenuated liver metastasis formation [158]. G31P application in a mouse prostate cancer cell model resulted in reduced tumor growth and reduced tumor vascularization [159]. In Ras-driven cancers, inhibition of CXCL8 using an antibody attenuated their growth due to increased tumor cell death [160]. Recently, it was shown that CXCR1/2 inhibitors can reduce tumor volume in vivo in renal cell carcinoma and squamous cell carcinoma resistant to standard treatment [161]. One study specifically confirmed that inhibiting CXCR1/2 on neutrophils, but not on endothelial cells or tumor cells, significantly reduced neutrophil accumulation, tumor growth and metastasis formation in pancreatic cancer [147, 162]. CXCR2 inhibition and subsequent reduced accumulation of neutrophil precursors can also potentiate anti-programmed death ligand 1 (PD1) immunotherapy in some cancer models, especially melanoma [64, 65, 163]. However, despite the growing evidence of a potentially critical role of the CXCL8-CXCR1/2 axis in cancer progression [164], interfering with neutrophil recruitment into tumor tissue as a therapeutic approach only begins to emerge and more evidence linking the CXCL8-CXCR1/2 axis to neutrophils in cancer is warranted.

Metabolic syndrome and atherosclerosis

The metabolic syndrome describes risk factors, and their eventually occurring metabolic diseases such as type 2 diabetes and atherosclerosis [165]. The link between obesity, diabetes and inflammation originates in dysfunctional adipocytes and pro-inflammatory macrophages releasing inflammatory mediators, which are responsible for a chronic low-level inflammation, which again has been shown to associate with diabetes development [78, 166]. Here, CXCL8 and CXCL1, but also other ELR-CXC chemokines like CXCL5 have been identified as key adipocytokines, their levels correlating with obesity [167–169]. Atherosclerosis is one of the most important chronic inflammatory disorders in the circle of metabolic dysregulation. Here, hypercholesterinemia and other factors lead to atherosclerotic plaque formation, which progressively occludes the vessel over time. This also bears the risk of rupture and mobilization of plaque content into the vessel with subsequent downstream occlusion and ultimately organ damage [170]. In atherosclerotic plaques various CXCR2 ligands were found in humans as well as in mice demonstrating together with CXCR2 to be a driving force in atheroprogression [171–173]. Furthermore, stimulation of CXCR1/2 by a CXCL8-homologue induced development and progression of atherosclerotic plaques in LDL-receptor deficient mice illustrating an important role of CXCR2 in atherosclerosis [174, 175]. This seems to be particularly true for the early phase of the disease where neutrophils are recruited to atherosclerotic lesions in a CXCR2 dependent manner [173].

Diabetes mellitus

The role of neutrophils and the CXCL8-CXCR1/2 axis including its inhibition have been intensively investigated for a variety of autoimmune diseases [176]. Increased CXCL1 was identified as a possible marker for β-cell destructive autoimmune activity in the pancreas during onset of type 1 diabetes [177], leading to a build-up of blood glucose and all its (potential) consequences. Pathophysiologically, immune cells including neutrophils and T cells attack and destroy β-cells [178]. Mounting evidence shows the CXCL8-CXCR1/2 axis plays an important role in the recruitment of neutrophils to the pancreatic tissue [179]. Traditionally, T1D is treated with lifelong parenteral insulin substitution. However, new immunomodulatory drugs, including CXCR1/2 inhibitors, are starting to emerge: treatment of mice with SB225002 in an experimental T1D model attenuated neutrophil recruitment to the pancreas almost completely [180]. Reparixin was tested in mice and humans regarding the outcome of islet cell transplantations and was found to consistently attenuate disease progression as evidenced by elevated C-peptide levels and lower insulin requirement [69]. Ladarixin has been shown to block and reverse T1D development in non-obese diabetic (NOD) mice. This was associated with inhibition of insulitis and modification of leukocytes distribution in blood, spleen, bone marrow and lymph nodes [68].

Conclusion

The chemokine receptors CXCR1/2 on neutrophils have been identified as key players in many inflammatory disorders. Therefore, therapeutic inhibition of CXCR1/2 (or its ligands such as CXCL8) might be beneficial and help

to reduce neutrophil recruitment in those disorders with unwanted neutrophil recruitment including inflammatory bowel disease, atherosclerosis, rheumatoid arthritis, and others.

Recent evidence also suggests a critical role of neutrophils in cancer development and progression and studies highlighting the potential therapeutic uses of inhibitors of this axis are beginning to emerge, especially with malignancies in the gastrointestinal tract including pancreas, and colon, but also in other organs such as skin (melanoma), and kidney.

Taken together, for many neutrophil-driven disease entities, preclinical evidence of the efficacy of CXCR1/2 inhibitors is accumulating. Further studies and clinical trials are now warranted to clarify and potentially solidify the therapeutic use of CXCR1/2 inhibitors.

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Declarations

Conflict of interest MS received support by a project grant from Dompè Farmaceutici S.p.A. RB is a preclinical pharmacology consultant of Dompé Farmaceutici S.p.a.

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