CXCR2 chemokine receptor -a master regulator in cancer and physiology

Gwendal Lazennec, Krishna Rajarathnam, Ann Richmond

CXCR2 chemokine receptor – a master regulator in cancer and physiology

Gwendal Lazennec [1, 2*], Krishna Rajarathnam[3] and Ann Richmond [4, 5, 6]

[1] CNRS, SYS2DIAG-ALCEDIAG, Cap delta, 1682 rue de la Valsière, Montpellier, France.

[2] CNRS, GDR 3697 "Microenvironment of tumor niches", Micronit, France.

[3] Department of Biochemistry and Molecular Biology, Department of Microbiology and Immunology, Sealy Center for Structural Biology and Molecular Biophysics, University of Texas Medical Branch, Galveston, TX, USA

[4] Department of Veterans Affairs, Tennessee Valley Healthcare System, Nashville, TN, USA

[5] Vanderbilt University School of Medicine, Department of Pharmacology, Nashville, TN, USA.

[6] Vanderbilt Ingram Cancer Center, Vanderbilt University Medical Center, Nashville, TN, USA.

Running title: Update on CXCR2 function

[*] **Correspondence:** gwendal.lazennec@sys2diag.cnrs.fr (G. Lazennec)

ABSTRACT

The understanding of the roles of chemokine receptor CXCR2 and its ligands in cancer, inflammation and immunity has been strongly modified by recent findings. The contribution of tissue-specific knockout of this receptor shows that it is involved in, among other things, cancer, central nervous system function, metabolism, reproduction, Covid-19, and response to circadian cycles. Moreover, the involvement of CXCR2 in neutrophil function has been revisited not only in physiology, but also for its major contribution to cancers. The recent unfolding of a role for CXCR2 in numerous cancers has led to an extensive evaluation of multiple CXCR2 antagonists in preclinical and clinical studies. In this review, we discuss the potential of targeting CXCR2 for cancer treatment.

KEYWORDS

CXCR2, chemokine receptor, neutrophil, inflammation, cancer, glycosaminoglycan

Recent developments about CXCR2 function

Chemokines (see glossary), a large family of more than 40 members, are classified into four subfamilies according to the presence of cysteine residues in their N-terminal region (CCL, CXCL, XCL and CX3CL), which are acting through seven transmembrane G-coupled chemokine receptors (CXCR, CCR, XCR, CX3CR). Chemokines are involved in the communication between cells, both in paracrine and distant ways as they can travel through the circulation and cross tissue barriers. Chemokines can have both homeostatic and inflammatory functions [1, 2].

Among them, the chemokine receptor CXCR2 is expressed in diverse cell types - including mainly neutrophils, and to a lesser extent, endothelial cells, macrophages, oligodendrocytes, neurons, neural crest-derived cells, and some cancer cells. It binds several ligands, which allows some redundancy, but recent studies indicate that the phenotype of each chemokine is distinct and finetunes cellular responses that are also coupled to CXCR1 (see box 1 and Fig. 1 for basics about CXCR2). CXCR2 plays diverse roles in pathophysiology from combating bacterial and viral infections to development, blood vessel narrowing, diseases of several organs including the heart, lung, liver and the brain, and in several cancer etiologies [2-5]. The main functions described earlier focused on its pro-angiogenic role and its critical function of chemotaxis of neutrophils. CXCR2 field has attracted recently a considerable attention, leading to a major breakthrough in the understanding of the function of this receptor, far beyond the initial expectations. In this review, we will summarize the recent advances concerning CXCR2 function, including its novel physiological roles based on whole or tissue-specific knockout of *Cxcr2*, its involvement in neutrophil and hematopoietic stem cell (HSC) action, in Covid-19 and in cancer, the latter being the most active field concerning CXCR2. Finally, we discuss the latest advances in structure and function of CXCR2, and finish with the main achievements in targeting CXCR2.

Recent findings with KO of CXCR2 in physiology

In this section, we will summarize the major advances in CXCR2 function in physiology (Fig. 2, Key figure), based on whole and tissue specific *Cxcr2* KO animals, which are mainly related to central nervous system (CNS), pituitary, reproductive function and metabolism. The involvement of CXCR2 in cancer will be covered later. In mice, in addition to high expression in neutrophils, CXCR2 is differentially expressed in other cell types. Therefore, determining the exact role of CXCR2 in any specific tissue or cell compartment from *Cxcr2* knock-out mouse

or CXCR2 inhibitor experiments remains a challenge. It is also important to point out that Cre-lines intended to target specific cells are frequently less specific than expected and lead to unwanted deletion in other cell types, which requires caution with the interpretations in some models. A breakthrough came when Ransohoff's laboratory developed the *Cxcr2*$^{fl/fl}$ mouse crossed with Mx-Cre mice, allowing induction of Cre-recombinase with polyinosinic-polycytidylic acid (poly I:C), and targeted disruption of Cxcr2 in neutrophils, leading to a defective migration of neutrophils in sterile peritonitis [6].

In the CNS, earlier work had shown that CXCR2 was mainly expressed in oligodendrocytes and neutrophils. Cell-specific knock-out of *Cxcr2* in oligodendrocytes (PLP-CreER - *Cxcr2*$^{fl/fl}$ mice) enabled a better remyelination after induced **experimental autoimmune encephalomyelitis (EAE)** [7]. Interestingly, with the same model, there was no effect on the clinical severity of multiple sclerosis (MS) symptoms after viral infection with the JHM strain of mouse hepatitis virus [8]**.** However, mice with *Cxcr2* loss in oligodendroglia exhibited increased remyelination. Khaw et al. also reported that the targeted deletion of *Cxcr2* in neutrophils (*MRP8-CRE-Cxcr2*$^{fl/fl}$ orCxcr2 cKO mice) could protect against CNS neurodegeneration from heat-killed *Mycobacterium tuberculosis* and MOG35-55 peptide induced EAE [9]. Targeted deletion of *Cxcr2* in CD4+ T cells leads to reduced effects of type-B EAE, due to diminished CD4+ T- cell migration into the spinal cord gray matter [10].

The pituitary has been recently shown to be a site of CXCR2 action. This was discovered following the observation that challenging *Cxcr2*-KO animals to chronic infections leads to major reproductive defects in females including, alterations of mammary gland development, reduced uterus size and altered functionality of the ovary with the absence of corpus luteum [11]. *Cxcr2*-KO females are unable to cycle in these conditions due to altered levels of circulating steroid hormones as well as pituitary hormones such as LH, FSH and PRL, resulting from a complete modification of the transcriptome of the pituitary gland that mimics autoimmune hypophysitis, an inflammatory pituitary disease.

As with other types of infections, there are emerging studies concerning the link between Covid-19 and CXCR2, which are described in Box 2.

The role of CXCR2 in regulating metabolism has been revisited using cell specific *Cxcr2 KO* mice. Initial reports had shown that *Cxcr2*$^{-/-}$ mice are protected against obesity-induced insulin resistance and that antibodies against CXCL5, a CXCR2 ligand had the same effect [12].

Baragetti et al. showed that loss of *Cxcr2* in neutrophils resulted in improved insulin response, less weight gain, and reduced homing of neutrophils to the liver [13]. Mice with neutrophil-specific loss of Cxcr2 were protected from high-fat diet mediated aging. *Cxcr2* is also acting directly on adipose tissue, as *Cxcr2* KO leads to a thinner skin in females but not males, due to a reduced subcutaneous adipose layer and smaller adipocytes [14]. Adipocytes themselves express CXCR2 and this expression is higher than in pre-adipocytes. Moreover, in contrast to the down regulation of CXCL5 observed during adipocyte differentiation [12], Kusuyama et al. observed an up-regulation and a promoting effect of CXCL3 during the differentiation [15]. CXCL3 promotes differentiation through an ERK (Extracellular signal-regulated kinase) and JNK (c-Jun N-terminal kinase) activation of C/EBPβ and δ.

The involvement of CXCR2 and its ligands in Cancer

CXCR2 has been demonstrated to play a key role in tumorigenesis through several routes: 1) recruitment of **myeloid-derived suppressor cells (MDSC)** and **tumor associated neutrophils (TANs)** into the pre-metastatic niche or into the tissues undergoing tumor initiation and progression; 2) direct autocrine effects on tumor growth; 3) effects on angiogenesis.

Here, we will summarize the main recent findings in each type of cancer. For reference to earlier work on CXCR2 and its ligands in cancer, refer to the following reviews [2, 5].

Among all cell types, expression of CXCR2 is highest in neutrophils in both mice and human. As neutrophils have been shown to be essential in many of the recent studies involving CXCR2 in cancer, we have also covered the latest discoveries concerning the function of CXCR2 in neutrophils (Box 3) and hematopoietic stem cells (Box 4) in a non-cancer context, which are crucial to understand how this receptor can also act in cancer.

Breast cancer (BCa)

Early work had shown that aggressive **triple negative breast cancers (TNBC)** were expressing higher levels of CXCR2 ligands compared to luminal tumors and that breast epithelial tumor cells themselves produced high levels of CXCR2 ligands, including in particular CXCL1, CXCL2, CXCL5 and CXCL8. This overexpression of CXCR2 ligands in TNBC was due to the location of their genes in a narrow genomic cluster in 4q21 and leads to increased proliferation, metastasis and chemoresistance [16-18]. Interestingly, CXCR2 itself is mainly expressed by

neutrophils found in the tumor and is associated with more aggressive breast tumors, in particular TNBC [19]. It is worth mentioning that to date, this is the only study that tested in parallel several anti-CXCR2 antibodies and correlated these results with *in situ* hybridization (ISH) experiments, confirming a staining of granulocytes and not of tumor cells in BCa. There is a general issue on the development of specific antibodies against chemokine receptors, and unfortunately, the number of correctly validated antibodies, with adequate controls such as knock out or knock in cells and combination with RNA data, remains low. Similarly, Rot et al. have recently highlighted this issue concerning the decoy chemokine receptor ACKR1 and additional controls were essential to confirm the lack of specificity of some antibodies [20].

The prognostic value of CXCR2 in BCa remains controversial. Boissière-Michot et al. showed that low CXCR2 levels correlate with reduced immune infiltrate and poorer overall survival. In contrast, TNBCs with higher CXCR2 levels are characterized by an increased immune infiltrate and a better prognosis [21]. Other studies have shown that CXCR2 correlates with high grade BCa and worst prognosis, but the anti-CXCR2 antibody used was different and stained mostly tumor cells [22], whereas other studies show that tumor cells express only low levels of CXCR2 RNA and protein [19]. However, the expression and activation of CXCR2 on breast cancer tumor cells has been associated with increased stemness of the breast cancer cells [23].

Hadadi et al. showed in mixed model background FVB/C57BL/6 of **Polyoma middle tumor antigen** (***PyMT)*** mice, that exposing mice to jetlag increases cancer cell dissemination and lung metastasis [24], reminding the potential of circadian cycle on neutrophils function. Though the lung neutrophil levels in jetlagged compared to control mice were similar, lung metastasis could be reduced by treating mice with the CXCR2 inhibitor SB265610.

Using a targeted *Cxcr2* deletion in myeloid cells in a C57BL/6 background (*Cxcr2 Mye*$^{\Delta/\Delta}$ mice or *LysM-Cre –Cxcr2*$^{fl/fl}$), Yang et al. observed a significant reduction in orthotopic tumor growth and outgrowth of BCa cells in a lung metastasis model compared to WT mice [25]. Tumors growing in *Cxcr2Mye*$^{\Delta/\Delta}$ mice exhibited significantly reduced MDSCs and increased intratumoral CXCL11, which was shown to come from an increase in tumor infiltrating B1b cells. The increased CXCL11 expression enhanced infiltration and activation of effector CD8+ T cells. Interestingly, treatment of tumor-bearing mice with a CXCR1/CXCR2 antagonist (SX-682) also inhibited tumor growth, reduced intratumoral MDSCs, increased B1b and CD8+T cells in the TME, and in addition, enhanced the response to anti-PD-1 to reduce tumor growth.

In a different model of BCa, the transgenic mice *PyMT*, which spontaneously develop mammary tumors, Timaxian et al. showed an increase in CXCR2 ligand in the mammary gland, and an increase of neutrophils in the tumor, the spleen and the circulation [26]. When *PyMT* mice on FVB background were crossed with Pan-*Cxcr2*-KO mice (*PyMT/ Cxcr2*$^{-/-}$ mice), mammary tumor weight increased, as well as of lung metastasis [26]. Interestingly, they observed a strong increase in the number of TANs in the mammary gland of *PyMT/Cxcr2*$^{-/-}$ mice compared to *PyMT/Cxcr2 WT* mice. RNAseq data revealed that mammary *Cxcr2*$^{-/-}$ TAN had a more pronounced TAN2 profile compared to WT TANs. In addition, *Cxcr2*$^{-/-}$ TANs had lost the ability to kill tumor cells, suggesting that *Cxcr2*$^{-/-}$ TANs are favoring tumor growth. Acharyya et al. did not observe an effective inhibition of lung metastasis when using CXCR2 inhibitor SB265610 with human BCa xenografts in athymic mice [27]. However, studies by Sharma showed that silencing CXCR2 in mouse breast cancer cell lines in a xenograft model enhances tumor growth and metastasis [28]. The study by Romero-Moreno using the *PyMT*/FVB model, in an *ex vivo* setting [18], demonstrated that injection of the *PyMT* cells into *ex vivo* bone cultures resulted in an increase of CXCL5 production that stimulates cancer cell production and this could be reduced by treatment with the CXCR2 inhibitor SB225002 [18]. An additional link between CXCR2 and neutrophils in cancer has been proposed by Teijeira et al. who showed that the treatment of neutrophils from healthy donors or of granulocytic-MDSCs isolated from the blood of cancer patients, with CXCR2 ligands induces **NETosis** [29]. Mice xenografted with murine 4T1 breast cells show a strong splenomegaly with an increased presence of $CD11b^+$ $Ly6G^+$ neutrophils in the tumor and in the circulation. There was also an increased number of NETs in $Ly6G^+$ stained areas of the tumor, but this was less evident in the spleen. These data suggest that NETosis could reduce cytotoxic immunity mediated by CD8 T lymphocytes and natural killer cells (NK), by protecting tumor cells. These authors also show that dual CXCR1/CXCR2 inhibitor repertaxin reduces NETs in the tumor.

The differences in terms of CXCR2 action between various BCa studies might be related to models themselves: targeted *Cxcr2* KO vs pan *Cxcr2* KO, mouse strain used (C57Bl/6 versus FVB) and differences in *in vivo* model recapitulating the complete tumor onset in the mammary gland vs injection of various types of tumor cells in the tail vein leading to lung metastasis. Compared to other cancers, BCa might represent a unique case in which neutrophil CXCR2 is rather protective against primary tumor progression, but not lung metastasis, but the pro or anti-tumoral action of CXCR2 remains a matter of debate.

Prostate Cancer (PCa)

The major issue of PCa remains its hormone escape from anti-androgen-based therapies, even with recently developed novel drugs and thus the appearance of castration-resistant (CR) prostate cancers. Early work had shown that more aggressive prostate tumors produce higher levels of CXCR2 ligands such as CXCL5 and CXCL8 [30]. Lopez-Bujanda et al. showed that CXCL8 RNA levels decreased upon treating the androgen responsive LNCaP cell line with dihydrotestosterone, whereas it increased by the anti-androgen enzalutamide [31]. Moreover, when comparing castration sensitive (CS) and resistant (CR) human prostate cell lines, they observed a higher production of CXCL8 by CR cell lines. In a setting of castration resistance, they observed an increased recruitment of PMN-MDSCs that could be blocked with an anti-CXCR2 antibody. However, CXCR2 blockade alone was ineffective for tumor growth, but it could synergistically increase the potential of the immune checkpoint inhibitor anti-CTLA4 to delay the onset of castration resistance.

PCa patients with high CXCR2 or CXCR1 in tumors exhibit accelerated biochemical recurrence (BCR). Ionizing radiations (IR) increase CXCL8 levels only in PTEN deficient PCa cells *in vitro*, whereas CXCR1 and CXCR2 were induced by IR, independently of PTEN status [32]. Silencing of *CXCR2* or *CXCR1* alone had minimal effect of cell growth *in vitro*, whereas double silencing of *CXCR1* and *CXCR2* decrease the viability of PTEN-deficient tumor cells exposed to IR. In a PCa xenograft model with the PTEN-deficient castration resistant C4-2 cell line, the CXCR2 inhibitor AZD5069 could reduce tumor growth, both in the absence and the presence of IR treatment.

Li et al. have reported that the subunit of the SWI/SNF chromatin remodeling complex Arid1A levels were decreased in tumors of $Pten^{pc-/-}$ compared to WT prostate [33]. The KO of *Arid1a* gene in $Pten^{pc-/-}$ was sufficient to decrease survival and increase tumor growth in mice. PCa patients with low levels of ARID1A in the tumor displayed shorter recurrence free survival and higher levels of intra-tumoral CXCL2 and CXCL3. Blocking CXCR2 with SB225002 antagonist could abolish the enhanced growth of $Pten^{pc-/-}$ $Arid1a^{pc-/-}$ tumors as well as the elevation of PMN-MDSCs in tumors.

Several studies have also highlighted the potential role of CXCR2 in macrophage function of PCa. Di Mitri et al. observed that the tumors of murine prostate cancer model $Pten^{pc-/-}$ $Trp53^{pc-/-}$ mice were infiltrated by $CD11b^{+}$ $Ly6G^{-}$ $F4/80^{+}$ TAMs that expressed CXCR2 at similar levels

as CD11b$^+$ Ly6G$^+$ F4/80$^-$ TANs and their presence increased during tumor progression [34]. In addition, there was an increase of CXCL1, CXCL2 and CXCL5 levels in the tumor. The treatment of BM derived macrophages with CXCL2 polarized them towards anti-inflammatory macrophages characterized by high expression of arginase and CD206 and the ability to suppress CD8$^+$ T lymphocytes proliferation. In addition, CXCR2 levels were increased in BM derived macrophages activated by IL4/IL13. The anti-inflammatory state driven by IL4/IL13 treatment could be reverted by the CXCR2 antagonists SB2205002 and SB265610. Treatment of *Pten$^{pc-/-}$ Trp53$^{pc-/-}$* animals with the CXCR2 antagonist AZD5069 reduced tumor growth and increased the expression of senescence markers, without affecting epithelial cell viability. AZD5069 reduced the pro-angiogenic F4/80$^+$ CD11c$^{+/-}$ CD206$^+$ macrophages levels in favor of pro-inflammatory F4/80$^+$ CD11c$^+$ CD206$^-$ macrophages that secrete TNFα, leading to a re-education of TAMs. Surprisingly, CXCR2 blockade did not affect the recruitment of CD11b$^+$ Ly6G$^+$ F4/80$^-$ TANs in *Pten$^{pc-/-}$ Trp53$^{pc-/-}$* tumors. Injection of *Cxcr2*-KO BM derived macrophages into *Pten$^{pc-/-}$ Trp53$^{pc-/-}$* animals led to the recruitment of infiltrating TAMs polarized towards a pro-inflammatory phenotype, which secreted TNFα, leading to tumor growth inhibition, and induction of senescence and DNA damage.

Another study has shown the importance and CAFs in the EMT of PCa cells through a CXCR2-dependent mechanism. Using a spontaneous mouse model of PCa (*dCKO: PB-CRE-Pten$^{fl/fl}$ Hic1 $^{fl//fl}$* mice), Wu et al. reported a strong infiltration of M2 TAMs upon secretion of TGFβ by PCa epithelial tumor cells deprived of Hic1 [35]. TGFβ promoted the polarization of M2 TAMs and expression of CXCR4. TGFβ also induced the production of CXCL12 by CAFs, which in turn stimulated the production of CXCL5 by M2 macrophages. Finally, CXCL5 produced by TAMs was able to promote the EMT of PCa cancer cells through a CXCR2 dependent mechanism.

Most of luminal PCa cells express the androgen receptor (AR) and the prostate specific antigen (KLK3) but not CXCR2. However, about 1% of tumor cells are quiescent neuroendocrine (NE) cells devoid of AR and KLK3, but expressing CXCL8 and CXCR2 [36]. As PCa tumors progress to high grade tumors, there is an increase in the number of CXCR2$^+$ NE cells. Purified CXCR2$^+$ NE cells from PCA tumors, compared to CXCR2$^-$ luminal PCa cells, have enriched signatures for stem cell, EMT, extracellular organization tumorigenesis and angiogenesis. Moreover, CXCR2$^+$ NE had a higher capability to form spheroids compared to CXCR2$^-$ luminal cells. In addition, CR cell line C4-2B cultured in the presence of the anti-androgen enzalutamide showed an increase in CXCR2$^+$ cell numbers. Similarly, forced expression of CXCR2 in LNCaP luminal PCa cells, led

to resistance to enzalutamide and transformation to a more basal, EMT and neural phenotype. Inversely, knockout of *CXCR2* in enzalutamide-resistant C4-2B/MDVR cells, which display a NE feature, increased their expression of luminal and epithelial markers. Delivery of the CXCR1/CXCR2 inhibitor navarixin (SCH 527123) or knockout of *CXCR2* in C4-2B/MDVR reduced tumor growth. $CXCR2^+$ NE cells create a niche for AR^+ $CXCR2^-$ luminal leading to hormone resistance.

Pancreatic ductal adenocarcinoma (PDAC)

The role of CXCR2 in PDAC depends on the studies and the models used and the results are currently subject to discussion.

Chen et al. showed that PDAC tumor progression and suppression of anti-tumor immunity was enhanced when collagen 1 (Col1) is deleted in myofibroblast [37]. Reduced Col1 expression in myofibroblasts led to Sox9-mediated up-regulation of CXCL5 in cancer cells, which in turn increased the recruitment of $CD206^+$ $F4/80^+$ $Arg1^+$ MDSCs. Co-inhibition of CXCR2 and CCR2 reversed the tumor progression events arising when Col1 is deleted in myofibroblasts.

Bianchi et al. reported that CXCL1 levels were increased in Kras-Tp53 tumors [38]. The source of CXCL1 was tumor cells themselves, whereas CXCR2 was nearly exclusively present on PMN-MDSCs. Clusters of CXCL1 Pan-CK^+ tumor cells also contained $CD11b^+$ $CD15^+$ $CXCR2^+$ PMN-MDSCs and $CD68^+$ $CD163^+$ M2-like macrophages but lacked $CD8^+$ T cells [38]. Knocking down CXCL1 in cancer cells strongly reduced tumor growth. Tumor Necrosis Factor-α (TNFα) production by PMN-MDSCs up-regulated CXCL1 in tumor cells through CREB induction. In addition, PMN-MDSCs polarized CAFs into pro-inflammatory CAFs (iCAFs) with an activated IL-6/STAT3 signaling pathway.

Knock down of Lysine (K)-specific demethylase 6A (KDM6A) in the pancreas led to an increase in the number of TANs in the tumor of *LSL-Kras$^{G12D/+}$ LSL-p53$^{R712H/+}$/ Kdm6A$^{fl/fl}$* mice [39]. Loss of KDM6A in pancreatic tumor cells results in increased CXCL1 transcription, neutrophil recruitment, NET formation and enhanced tumor growth [39].

In the KPC orthotopic model of PDAC, Evrard et al. observed an increase in the number of both immature ($Ly6G^{lo/+}$ $CXCR2^-$ $CD101^-$) and mature ($Ly6G^+$ $CXCR2^+$ $CD101^+$) neutrophils in the **bone marrow (BM)** and blood of KPC mice compared to naïve mice [40]. However, an increase in immature neutrophils ($Ly6G^{lo/+}$ $CXCR2^-$ $CD101^-$) in the circulation and tumor was linked to tumor burden, suggesting that immature neutrophils favor tumor growth.

Using the inducible *Kras/p53* transgenic model, Gulhati et al. were able to identify five myeloid cell clusters (myeloid_c1-5), with myeloid_c2 and myeloid_c3 displaying high levels of CXCR2 and Ly6G [41]. Interestingly, while the combined PD1+ CTLA4 therapy targeting immune checkpoint proteins had no effect on the survival of tumor-bearing mice, targeting neutrophils with a Gr-1 antibody or with the CXCR1/CXCR2 inhibitor, SX-682, was sufficient to greatly enhance survival. In addition, the combination of PD1+ CTLA4 with SX682 treatment further increased the survival of the animals compared to SX682 alone -treated animals.

Hepatocellular cancer

In patients with primary sclerosing cholangitis or colitis, Gram-negative bacteria can activate TLR4-mediated induction of CXCL1 expression in the liver, which recruits $CXCR2^{+}$ polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) and promotes tumor growth [42]. Antibiotic treatment blocked bacterial-induced CXCL1 expression and accumulation of PMN-MDSCs. Antibody neutralization of CXCL1 as well as CXCR2 inhibition by treatment with SB225002 blocked also MDSC recruitment and reduced tumor burden and progression in a DSS-colitis mouse model.

Melanoma

Yang et al. recently addressed how CXCR2 regulates melanoma tumor growth by co-targeting *Cxcr2* at the same time that expression of mutant $Braf^{V600E}$ and loss of *Pten* were initiated in melanocytes [43]. In a second model*, Cxcr2* was deleted in melanocytes coincident with the induction of $Nras^{Q61R}$ expression and *Ink4a* deletion. Loss of *Cxcr2* expression during tumor induction reduced tumor growth and/or incidence, reprogrammed the transcriptome of tumor cells and the tumor microenvironment, and increased anti-tumor immunity. Induction of the transcription factor TFCP2L1 coincided with reduced expression of growth-related genes and increased expression of tumor suppressors. Moreover, in a second model where CXCR2 is deleted in myeloid cells, there is a consequential inhibition of recruitment of MDSCs into the TME and an increase in $CD8^{+}$ T effector cell activity, resulting in a more anti-tumor immune microenvironment and inhibition of melanoma tumor growth [25]. Inhibitors of CXCR2 also inhibit melanoma tumor growth [44] and can enhance response to immune checkpoint inhibitors [25, 43]. Both a small molecule antagonist of both CXCR1 and CXCR2, (SX682), and a humanized antibody to CXCL8 (BMS-986253) shown to inhibit solid tumor

growth [45] are currently in clinical trials in combination with anti-PD1 for treatment of anti-PD1 resistant metastatic melanoma.

Lung Cancer

In a study of patients with lung adenocarcinoma (LUAD) and patients with lung squamous cell carcinoma (NSCLC), CXCR2 was shown to be upregulated in lung tumor cells as well as in stromal cells and high CXCR2 expression correlated with poorer survival [46]. Mouse models of lung cancer cells showed that these tumors express elevated CXCL1, CXCL2, CXCL5 levels in comparison to normal lung tissue. Inhibition of CXCR2 with SB225002 *in vitro* stimulates apoptosis, senescence, EMT and impair tumor cell proliferation. In mouse models, blockade of CXCR2 reduces tumor growth and the infiltration of neutrophils into the tumor, enhanced $CD8^+$ T cell activation and increased the sensitivity to cisplatin.

Overactivation of RAS/ERK pathway in lung is frequent and involves SHP2. Using the SHP2 inhibitor SHP099, Tang et al showed that it was effective on mice intravenously injected with $Kras^{G12D}$ $Trp53^{-/-}$ NSCLC [47]. Moreover, they observed that SHP099 induced the expression of CXCL1 and CXCL5 mainly in tumor cells, two potent chemoattractant of PMN-MDSCs. Treatment of animals with SHP099 or the CXCR1/CXCR2 inhibitor SX682 partially reduced tumor growth, whereas the combination of SHP099 and SX682 suppressed tumor growth and prolonged survival.

Using mouse models of lung metastasis with subcutaneous injection of LLC lung cancer cells, Lu et al. observed that early after primary tumor resection, LLC tumor cells are not yet present in the lung, while $CD11b^+$ $GR1^+$ MDSCs are already there [48]. Moreover, the transfer of monocytic MDSCs ($CD11b^+$ $Ly6C^{hi}$ $Ly6G^-$) from the BM of mice xenografted with LLC lung cancer cells, led to an increase of lung metastasis, whereas the transfer of PMN-MDSCs ($CD11b^+$ $Ly6C^{low}$ $Ly6G^+$) had no effect, suggesting that monocytes MDSCs are the major drivers of lung metastasis. The treatment of mice with low doses of histone deacetylase inhibitors, which do not affect primary tumor growth, could decrease the number of MDSCs in the lung, as well as metastasis. Histone deacetylase inhibitors reduced the expression of the chemokine receptor CCR2 in lung and BM monocytic MDSCs, while inhibiting CXCR2 expression in the lung and BM PMN-MDSCs.

In a urethane-induced model of inflammation driven lung cancer, TNFα induces the expression of both MHCII and CXCR2 on alveolar type-II (AT-II) lung cells [49]. Consequently, there is an

associated expansion of Treg cells in an MHC-II-dependent manner. Blocking TNFα reversed the urethane mediated lung inflammation and down-regulated MHCII as well as blocked expansion of the Treg population of CD4+ T cells. Ablation of CXCR2 activity also eliminates the induction of MHC-II and the expansion of Treg cells. In summary, TNFα induces CXCR2 which when activated by its ligands induces NF-κB and the MAPK-ERK/JNK pathways which in turn induces MHC-II expression and Tregs.

Glioblastoma

Two thirds of glioblastoma (GBM) tumors show an amplification of EGFR and many of them express the constitutively active in frame deletion mutant EGFRvIII. GBM expressing EGFRvIII secrete CXCL1/2/3 and accumulate more PMN-MDSCs than GBM expressing wild type EGFR [50]. As a result, there is resistance to anti-PD-1 and CTLA-4 therapy. Inhibition of CXCR2 with AZD5069 resulted in reduction in PNM-MDSCs, increased intratumoral cytotoxic CD8+ T cells, and improved response to immune checkpoint inhibitors in mice with EGFRvIII expressing GBM.

Thyroid cancer

A common genetic mutation of *BRAF* (*BRAF*^{V600E}) is found in 40-80% of papillary thyroid cancers (PTC), leading to MAPK signaling activation and to a higher cancer recurrence. Moreover, T-box transcription factor 3 (TBX3) expression is up-regulated by BRAF^{V600E} induction of the MAPK pathway. Zhang et al. used the PTC *TPO-Cre; LSL-BRAF*^{V600E} (*mPTC* mice) mouse model to show that targeted deletion of Tbx3 in PTC cells decreased incidence of tumors [51]. There was a strong decrease of CXCL1, CXCL2 and CXCL3 in *mPTC-Tbx3*$^{-/-}$ compared to *mPTC* tumors, and inhibition of IKKβ/NF-κB signaling reversed TBX3-mediated induction of CXCL1-3. In addition, the knock-down of TLR2, an upstream regulator factor of IKKβ/NF-κB pathway, prevented the induction of CXCR2 ligands by TBX3. Ectopic expression of CXCL1 or CXCL2 could restore tumor growth of xenografted human PTC cells silenced with TBX3. In mPTC-Tbx3$^{-/-}$ tumors, there was a decrease of PMN-MDSCs, but not of monocytic MDSCs. Finally, treatment of mPTC-Tbx3$^{-/-}$ mice with the CXCR2 inhibitor SB265610 was sufficient to reduce tumor growth.

Bone Cancer

Single cell sequencing of $CD34^+$ HSPC from patients with myeloproliferative neoplasm (MPN) revealed an hyperactivation TNFα/NFκB pathways, along with higher levels of CXCL1, CXCL3 and CXCL8 in patients with high fibrosis [52]. In the *hMPL*^{W515L} adoptive mouse transfer model of fibrosis, when cKit+ murine BM cells were stimulated with CXCL8, there was enhanced pERK and pSTAT3 signaling that was lost in *Cxcr2*$^{-/-}$ bone marrow cells. Mice transplanted with hMPL^{W515L} *Cxcr2*$^{-/-}$ expressing cells displayed reductions in white blood cells and reduced numbers of neutrophils, megakaryocytes, and reduced bone marrow and spleen fibrosis.

A summary of the different factors contributing to the regulation of the expression of CXCR2 and its ligands in the different types of cancers is presented in Fig. 3.

Effectiveness of drugs targeting CXCR2

Targeting chemokine receptors remains a challenge and so far only two drugs have been approved by FDA: AMD3100 (CXCR4 antagonist) used for peripheral blood stem cell transplantation and Maraviroc (CCR5 antagonist) for preventing HIV infection [53]. To understand the action of CXCR2 and its ligands, it is also essential to have a better knowledge of signaling activities of each ligand that bind to the receptor and how their function is regulated by binding to **glycosaminoglycans** (see box 5).

We already mentioned some CXCR2 drugs used in preclinical models, and we will restrict this part to those tested clinically recently. A large number of high-affinity small molecule CXCR2 inhibitors with a nanomolar (nM) affinity are now available [4, 5, 54]. Two main strategies have been used: 1) targeting the receptor itself with non-competitive allosteric or reversible antagonists 2) targeting the ligands of CXCR2 and in particular CXCL8 with antibodies. Of particular note, some of CXCR2 inhibitors also antagonize CXCR1 (dual antagonists), which can make the interpretation of the results sometimes difficult. Current CXCR2 antagonists tested in clinical trials are summarized in Table 1.

Over the years, several CXCR2 inhibitors, discovered through the pharma drug pipeline, have undergone and/or are currently undergoing phase-I and phase-II clinical trials for diseases from COPD, asthma, cystic fibrosis, pancreatic islet transplantation, reperfusion injury, coronary heart disease, influenza, pancreatitis, and several cancers [45, 55-66]. The only trial that advanced to phase-III for pancreatic islet transplantation in type I diabetes failed to show efficacy compared to the control group [60]. A few clinical studies that also used antibodies

to target CXCL8 and treat COPD, COVID-19 or metastatic tumors have shown partial efficacy or are currently undergoing [45, 67].

CXCR2 inhibitors have either shown limited efficacy in clinical trials despite showing promise in clinical models, or are still under current evaluation. It is thus too early to draw conclusions. Among the issues that could delay drug development, is the fact that CXCR2 is activated by several chemokines, and at least two of them can also act on CXCR1. Moreover, we are lacking mechanistic insights on how inhibition of CXCR2 signaling effects multiple cell types in the clinical context. One specific cell target is certainly the neutrophil, which represents the cell expressing the highest level of CXCR2 and for which many novel roles have been discovered recently (Box 3). In response to infection and injury, CXCR2 comes into play for rapidly mobilizing neutrophils to the insult site. However, CXCR2 is rapidly internalized on exposure to chemokine, and while the receptor can rapidly recycle, high levels of chemokine can lead to receptor trafficking to the lysosomes for degradation [68]. Considering that the phenotype of blood and recruited neutrophils in terms of their ability to inflict tissue damage varies, it is important to know whether the drug should inhibit neutrophil or MDSC trafficking to the tissue or dampen the activity of primed and activated neutrophils or MDSCs that are already in the tissue. Therefore, the mode of administration could impact the outcome, and for instance, targeted local administration such as using a nebulizer rather than systemic administration for lung pathologies could be more effective.

Most clinical studies measure only blood neutrophil levels and a few studies have characterized CXCR2 levels and/or report neutrophil activity such as chemotaxis as to define its phenotype compared to a healthy cohort. These data could be limiting and not sufficient to fully understand CXCR2 function in the disease context, and a more comprehensive characterization including those of tissue neutrophil CXCR2 levels and of neutrophil phenotype (such as pro-inflammatory, primed, or exhausted; mature or immature) similar to animal model studies are necessary for better design of clinical trials and dosage regimen. Furthermore, most clinical trials have focused on the efficacy of the CXCR2 inhibitor for chronic diseases, and more trials that test the efficacy for acute inflammatory diseases as well as cancers are needed.

Concluding remarks

The knowledge of CXCR2 and its ligands has widely increased in the last years far beyond its initial roles in angiogenesis and inflammation. Unsuspected functions of CXCR2 have been discovered in physiopathology including its action in CNS, pituitary, metabolism, circadian rhythm or viral pathologies such as Covid-19. Moreover, the cancer field has been the major area of investigation of the potential of CXCR2 and its ligands, revealing a crucial involvement of tumor microenvironment affecting neutrophils, macrophages, MDSCs, CAFs as well as tumor cells. The pro- or anti-tumoral role of CXCR2 may be dependent on the type of cancer and its stage of progression. However, preclinical studies have raised a very strong interest in using antagonists to target CXCR2, and a number of clinical trials with CXCR2 antagonists or antibodies against CXCL8 in cancers are ongoing (see Table 1 and clinician's corner). Future questions to be addressed concerning the roles of CXCR2 and its ligands are summarized in the Outstanding questions box. This challenging area, like what is observed when targeting other chemokine receptors, will definitely require a better knowledge of CXCR2 expression in various cell populations and its action, which will inform clinical trial design. The possibility of targeting CXCR2 in combination with immune checkpoint inhibitors and other disease driving pathways, will be interesting to achieve efficient control of CXCR2 action and maximize the response to therapy. The recent discoveries on CXCR2 function have widened the spectrum of its actions in many diseases and it remains many questions to answer, to achieve a better targeting of this receptor and its ligands in clinics.

Box 1: CXCR1 and CXCR2 basics

In humans, seven chemokines characterized by the conserved N-terminal 'ELR' motif – CXCL1, CXCL2, CXCL3, CXCL5, CXCL6, CXCL7, and CXCL8 – are agonists for the CXCR2 receptor (Fig. 1). For human CXCR1, CXCL8 alone is a high affinity agonist and all other CXCR2 ligands, including CXCL6, are low-affinity agonists. At a cellular level, CXCR2 activation triggers trimeric G-protein and β-arrestin-mediated signaling cascades including in particular PI3K/Akt and MAPK/ERK pathways, which play dual roles in directed cell migration and molecular processes required for eliminating invading pathogens, initiating tissue repair, modulating angiogenesis, growth, and other functions at the target site [69]. ELR-chemokines are selectively and differentially expressed by different cell types and tissues for normal homeostatic functions and in different disease conditions [70-72], indicating *in vivo* CXCR2 signaling activity and phenotype cannot be simply correlated to *in vitro* CXCR2 activity determined from cellular assays, and that the context is critical. As CXCR1 and CXCR2 share some common ligands, there could be an overlap in their functions, but several studies have shown that they trigger different responses, in particular the ability of CXCR1 to stimulate phospholipase D activation [73]. Moreover, they display distinct patterns of expression in humans and mice [74]. For instance, in mouse neutrophils, the main receptor expressed is CXCR2, whereas in humans, both CXCR1 and CXCR2 are playing major roles. *Cxcr1* and *Cxcr2* KO mouse models are also revealing distinct phenotypes [75].

ELR-chemokines share similar structures, exist as monomers and dimers and interact with tissue glycosaminoglycans (GAGs) [76, 77]. Structural, biophysical, and animal model studies indicate that these properties are intimately coupled, and that they act in concert to regulate CXCR2 activation that is uniquely tailored for each chemokine [78, 79]. Therefore, understanding CXCR2 function and phenotype in the context of *in vivo* milieu is dependent on understanding of the shared and distinct properties of its ligands and how they are shaped by the local environment that is highly context dependent.

Box 2: CXCR2 and Covid-19

SARS-CoV-2 pandemic has highlighted the major adverse effect of massive uncontrolled inflammation in the outcome of infected patients, with a "cytokine storm" leading to tissue damage [80, 81]. Elevated counts of circulating neutrophils have been reported for patients in

critical condition [82]. The role of CXCR2 and its ligands in the severity of the disease is emerging and could explain the differences between individuals, in ability to overcome the infection. In particular, the mortality of male patients is higher than female patients [81] and this is correlated with higher CXCL8 and IL-18 levels in male subjects [83]. By using transcriptomic data from nasopharyngeal swabs of SARS-CoV-2 patients, Freire et al. found a network of dysregulated genes. This includes neutrophil related genes such as *CXCR2*, *CXCR1*, *IL-1β, S100A9*, *ITGAM* and *DNBL*, which were down-regulated in females compared to males and could account for the better outcome for females [84]. In the same line, Black/African American (AA) SARS-CoV-2 patients showed a higher mortality compared to other ethnic groups and this involves the infiltration of the lung by $CXCR2^{+}$ mature neutrophils from the blood, which once in the lung, were reprogrammed to produce higher levels of inflammatory molecules, including CXCL8 and IL-1β [85].

Recent work based on cytometry and proteomic approaches, has shown that SARS-CoV-2 patients with severe disease display a subpopulation of immature neutrophils with a hyperactive state [86]. Circulating neutrophils of patients with severe symptoms displayed low levels of CD62L (a marker of primed neutrophils) as well as high expression of CD66b and CD63 (secondary and primary granule markers, respectively) and high expression of CXCR2, confirming the activated state of such neutrophils.

Box 3: Role of CXCR2 in tissue-specific heterogeneity of neutrophils

Until recently, the precise role of CXCR2 in neutrophils had been relatively poorly addressed and novel findings are summarized in Fig. I. In zebrafish, both *cxcr1* and *cxcr2* promote the motility of neutrophils, *cxcr1* stimulated the clustering of neutrophils, whereas *cxcr2* favored the dispersion [87].

Cxcr2-KO mice displayed diminished maturation of splenic neutrophils, based on CD101 and Ly6G expression and RNAseq profiling of neutrophils [88], while there was an increase of aged $CD62L^{lo}$ $CXCR4^{hi}$ neutrophils in the spleen. *Cxcr2*-KO splenic neutrophils were less phagocytic compared to WT neutrophils, with decreased production of **reactive oxygen species (ROS)** and F-actin, but this was in contrast to bone marrow (BM) *Cxcr2*-KO neutrophils [88]. Similarly, single cell RNA sequencing (scRNAseq) showed that in contrast to blood neutrophils, which are Ly6G+ $CXCR2^{+}$, BM neutrophils were heterogeneous, with immature

neutrophils characterized by a Ly6G$^{lo/+}$ CXCR2^{-} CD101^{-} phenotype, whereas mature neutrophils were Ly6G^{+} CXCR2^{+} CD101^{+} [40]. Moreover, BM proliferating CXCR4^{+} ckit^{+} CD62L^{lo} Ly6G^{lo} CXCR2^{-} neutrophils were distinct from CXCR4^{lo} ckit^{lo} CD62^{hi} Ly6G^{+} CXCR2^{+} non-proliferating neutrophils. Another scRNAseq study of BM, peripheral and spleen mouse neutrophils enabled the identification of 8 neutrophil clusters with varying maturation [89]. Among these populations, CXCR2^{hi} neutrophils defined by Evrard et al. corresponded to G4, G5a, G5b and G5c clusters, the most mature found in peripheral tissues [89].

A major recent breakthrough has been the discovery that neutrophil function is closely correlated to the circadian clock. To characterize circadian rhythm, the notion of **Zeitgeber time** (ZT) has been introduced (see glossary for more details). It was shown an active release of neutrophils from the BM between ZT17 and 5 and a clearance between ZT5 and 13 [90]. Aged neutrophils (CXCR4^{hi} CD62L^{lo}) display a peak of accumulation at ZT5 (dark) and these neutrophils display higher levels of circadian genes Bmal1 and Clock and a decrease of CD62l and CXCR2. The situation is the opposite at ZT13 (light), with a decreased expression of Bmal1. Knock-out of *Cxcr2* or *Cxcr4* in neutrophils prevents the decreased expression of Bmal1 observed at ZT13. Bmal1 can bind to *Cxcl2* promoter, which is required for aging and up-regulation of CD62l.

In humans, CXCL8 can trigger both chemotaxis, NETosis (see glossary) and ROS production [91]. However, only high concentrations of CXCL8 enhanced NETosis, whereas motility stimulation was achieved at lower concentrations. Pharmacological inhibition of CXCR2 reduced NETosis and chemotaxis and inhibition of CXCR1 decreased NETosis.

Box 4: CXCR2 and hematopoietic stem cells

Recent work provide compelling evidence that CXCR2 plays an important role in mobilizing hematopoietic stem cell (HSC) from the BM. Karpova et al. showed that the cotreatment of mice with the very late antigen 4 (VLA4) inhibitor CWHM-823 and CXCR2 agonist CXCL2 synergistically enhance HSC mobilization from the BM, and that endothelial CXCR2 plays a role in this process by using targeted deletion of *Cxcr2* in endothelial cells [92]. In the same line, Capitano et al. showed that the secreted nuclear protein DEK increases cytokine-induced HSC expansion to regulate hematopoiesis *in vivo* [93]. Interestingly DEK displays an ELR (Glu-Leu-Arg) motif, similar to other CXCR2 ligands and acts extracellularly presumably through CXCR2 binding, as *Cxcr2* KO prevents DEK action [93].

Box 5: CXCR2 and glycosaminoglycans interactions and ligand binding

CXCR2-driven cell trafficking is mediated by chemokine gradients achieved by GAG interactions [94], with an essential role of endothelial GAGs [95, 96]. Chemokines bind to GAGs heparan sulfate, chondroitin sulfate, and dermatan sulfate. ELR-chemokines are basic proteins, and lysine residues dominate at the GAG-binding interface. Lysine sidechains are highly dynamic, suggesting structural plasticity plays an important role in chemokine-GAG interactions [69, 97]. Recent studies show that the chemokine dimer binds GAGs with higher affinity, conserved basic residues and those that are unique to a given chemokine mediate binding, and GAG binding surfaces among chemokines and binding interfaces for a given chemokine for different GAGs can vary significantly [69, 78, 98]. Animal studies show that i) the recruitment activity of the chemokine monomer and dimer are distinctly different, (ii) monomer-dimer equilibrium regulates recruitment, (iii) recruitment profiles vary between chemokines and tissues, (iv) despite lower receptor activity, the dimer can recruit more neutrophils than the monomer, indicating GAG interactions promote high local concentrations of chemokine that are needed for activating signaling pathways for migration, and (v) GAG interactions also indirectly regulate surface receptor density and thereby trafficking, as the chemokine, in addition to signaling, also induces receptor endocytosis [76]. ELR-chemokines also form heterodimers and their GAG interactions are distinctly different from those of homodimers [99-101]. Considering ELR-chemokines dimerize at micromolar concentrations but activate CXCR2 at nanomolar concentrations, this makes a compelling case that the *in vivo* chemokine concentration, and by extension monomer to dimer ratio, can vary significantly. This can be attributed to different GAG interactions and to small local volumes in the proximity of the cell surface compared to the center of the vessel along the migration path. GAG-bound chemokine can directly bind the receptor or GAG interactions can indirectly determine the chemokine levels available for CXCR2 signaling. The CXCR2 N-terminal domain (N-domain) is a critical ligand binding site, and structural studies show that several ELR-chemokine residues are involved in binding to both the CXCR2 N-domain and GAGs and that the GAG-bound monomers are unable to bind the N-domain [68, 70]. Structures of a ternary complex of CXCR2 bound to CXCL8 dimer and CXCL8 monomer reveal that only one monomer of the dimer is involved in receptor interactions with the second monomer pointed away,

indicating that it is available for GAG interactions [75]. Whether receptor-bound dimer can bind GAGs especially in the context of *in vivo* milieu needs to be determined.

Clinician's corner

- So far, the use of specific drugs, inhibitors and antibodies targeting chemokine receptors has shown very little success in clinic. This also applies to CXCR2 inhibitors as treatment for chronic inflammatory diseases. There are several potential issues in correlating preclinical studies with clinical trials: 1) the fact that CXCR1 and CXCR2 level of expression by varying cell types and functions may be different in mouse versus human; 2) an inhibitor that targets both CXCR2 and CXCR1 could provide different results in mouse versus human and an inhibitor specific for CXCR2 may show promise in mice but be less effective in humans where more CXCR1 is expressed; 3) accessibility of the receptor to the inhibitors that directly bind the receptor in part due to ligand induced receptor internalization and recycling and also to GAG interactions with receptors.
- To achieve a significant response in clinics, it will be important to know what cell types are expressing CXCR2, the level of expression, the activation of the receptor and the expression of other ligand competing receptors. Moreover, the recent advent of scRNAseq and CITE-seq analyses have provided new tools to dissect the expression of CXCR2 in a tissue environment at the single cell level [102].
- Many studies have focused on inhibiting CXCR2 in neutrophils, but neutrophils do not constitute a homogeneous population of cells, but rather a continuum of adapting cells with different degrees of differentiation, activation, and chemokine receptor expression. Some neutrophils do not express CXCR2. This heterogeneity also differs between tissues. Thus, targeting such cells as a whole might be illusory and explain some of the issues observed in clinical trials.
- Clinical trials combining CXCR2 antagonists with other drugs as well as checkpoint inhibitors are ongoing, and the results will be interesting. In cancers, it may be necessary to target CXCR2 and at the same time target genetic tumor drivers and inhibitors of CD8 T cell function. In inflammatory diseases, it may be necessary to target more than one chemokine or G protein- coupled receptor, as CXCR2 can

crosstalk with other G-coupled receptors. We will learn from each trial how to better design these approaches.

Figure Legends

Fig. I (embedded in box 3): A central action of CXCR2 in neutrophils in a non-cancer situation

Neutrophils are subjected to circadian rhythm with an increased number of neutrophils during daytime compared to nighttime. This goes along with increased aging, increased expression of CXCR4 and decreased expression of CD62L. Neutrophil CXCR2 levels are lower in daytime and are less able to inhibit aging. Mechanistically, Bmal1 binds to *Cxcl2* promoter, increases its production, which in turn activates CXCR2. CXCR2 has multiple actions including enhancing maturation of neutrophils with elevated levels of CD101 and Ly6G. CXCR2 will also inhibit the proliferation of neutrophils, while increasing ROS production, which in turn increases NETosis and phagocytosis. The figure was made manually in Adobe illustrator.

Fig. 1: CXCR1 and CXCR2 ligands

Upper panel: In humans, CXCR2 ligands are CXCL1 (MGSA/GROα), CXCL2 (GROβ), CXCL3 (GROγ), CXCL5 (ENA-78), CXCL6 (GCP-2), CXCL7 (NAP-2) and CXCL8 (IL-8). For human CXCR1, CXCL8 alone is a high affinity agonist and all others are low-affinity agonists. In mice, there is a drop in redundancy, with only five ligands: CXCL1 (KC), CXCL2 (MIP-2), CXCL3 (DCIP), CXCL5/6 (LIX) and CXCL7 (NAP-2). The former names of these ligands given before the unified nomenclature [103] are also indicated in parenthesis. CXCR2 ligands exhibit a N-terminal motif 'glu-leu-arg (ELR)' which is absolutely critical for CXCR2 activation. **Lower panel**: The main CXCR2 and dual (CXCR1/2) inhibitors are indicated here. The figure was made manually in Adobe illustrator.

Fig. 2 (Key Figure): Novel roles of CXCR2 in physiology in a non-cancer situation

CXCR2 has been recently shown to worsen the severity of Sars-CoV-2 infections through the recruitment of neutrophils. CXCR2 promotes EAE and preventing remyelination in CNS. CXCR2 is also acting in the control of the hypothalamus-pituitary-gonad axis through pituitary, ovary, uterus and mammary gland, in the context of chronic infections. Finally, CXCR2 promotes obesity-induced insulin resistance by acting in particular in neutrophils and macrophages in adipose tissue and liver. The figure was made manually in Adobe illustrator.

Fig.3: A diversity of signals regulating CXCR2 and its ligands in cancer cells

This scheme summarizes some of the factors regulating the expression of CXCR2 and its ligands in different types of cancer cells. These factors can be specific of a particular type of cancer and should not be taken as a valid view for all cases. Black signs represent inhibition signals, whereas red arrows represent stimulation signals. ARID1a: AT-rich interaction domain 1A, HDAC: Histone deacetylase, KDM6a: lysine demethylase 6a, PTEN: phosphatase and tensin homolog, SHP2: Src homology 2-containing protein tyrosine phosphatase 2, SOX9: SRY-box transcription factor 9, TBX3: T-box transcription factor 3, TLR4: Toll Like Receptor 4, TNFα: Tumor necrosis factor-alpha. The figure was made manually in Adobe illustrator.

Acknowledgments

This work was supported by la Ligue contre le Cancer to GL. Krishna Rajarathnam was supported by NIH grant R21AI160613. Ann Richmond was supported by NIH grants CA116021 and CA243326 and grants from the Department of Veterans Affairs 101BX002301 and 51K6BX005225.

Declaration of interests

The authors declare no competing interests.

References

1. Lopez-Cotarelo, P. et al. (2017) Beyond Chemoattraction: Multifunctionality of Chemokine Receptors in Leukocytes. Trends Immunol 38 (12), 927-941.
2. Lazennec, G. and Richmond, A. (2010) Chemokines and chemokine receptors: new insights into cancer-related inflammation. Trends Mol Med 16 (3), 133-44.
3. SenGupta, S. et al. (2019) Getting TANned: How the tumor microenvironment drives neutrophil recruitment. J Leukoc Biol 105 (3), 449-462.
4. Cheng, Y. et al. (2019) Potential roles and targeted therapy of the CXCLs/CXCR2 axis in cancer and inflammatory diseases. Biochim Biophys Acta Rev Cancer 1871 (2), 289-312.
5. Ha, H. et al. (2017) Role of the CXCL8-CXCR1/2 Axis in Cancer and Inflammatory Diseases. Theranostics 7 (6), 1543-1588.
6. Liu, L. et al. (2013) Functional defect of peripheral neutrophils in mice with induced deletion of CXCR2. Genesis 51 (8), 587-95.
7. Liu, L. et al. (2015) Spatiotemporal ablation of CXCR2 on oligodendrocyte lineage cells: Role in myelin repair. Neurol Neuroimmunol Neuroinflamm 2 (6), e174.
8. Marro, B.S. et al. (2019) Disrupted CXCR2 Signaling in Oligodendroglia Lineage Cells Enhances Myelin Repair in a Viral Model of Multiple Sclerosis. J Virol 93 (18).
9. Khaw, Y.M. et al. (2020) Neutrophil-selective deletion of Cxcr2 protects against CNS neurodegeneration in a mouse model of multiple sclerosis. J Neuroinflammation 17 (1), 49.
10. Khaw, Y.M. et al. (2021) Astrocytes lure CXCR2-expressing CD4(+) T cells to gray matter via TAK1-mediated chemokine production in a mouse model of multiple sclerosis. Proc Natl Acad Sci U S A 118 (8).
11. Timaxian, C. et al. (2020) The health status alters the pituitary function and reproduction of mice in a Cxcr2-dependent manner. Life Sci Alliance 3 (3), e201900599.
12. Chavey, C. et al. (2009) CXC ligand 5 is an adipose-tissue derived factor that links obesity to insulin resistance. Cell Metab 9 (4), 339-49.
13. Baragetti, A. et al. (2023) Neutrophil aging exacerbates high fat diet induced metabolic alterations. Metabolism 144, 155576.
14. Dyer, D.P. et al. (2019) The chemokine receptor CXCR2 contributes to murine adipocyte development. J Leukoc Biol 105 (3), 497-506.
15. Kusuyama, J. et al. (2016) CXCL3 positively regulates adipogenic differentiation. J Lipid Res 57 (10), 1806-1820.
16. Bieche, I. et al. (2007) CXC chemokines located in the 4q21 region are up-regulated in breast cancer. Endocr Relat Cancer 14 (4), 1039-1052.
17. Freund, A. et al. (2003) IL-8 expression and its possible relationship with estrogen-receptor-negative status of breast cancer cells. Oncogene 22 (2), 256-65.
18. Romero-Moreno, R. et al. (2019) The CXCL5/CXCR2 axis is sufficient to promote breast cancer colonization during bone metastasis. Nat Commun 10 (1), 4404.
19. Boissiere-Michot, F. et al. (2020) Prognostic Value of CXCR2 in Breast Cancer. Cancers (Basel) 12 (8).
20. Rot, A. et al. (2022) Murine bone marrow macrophages and human monocytes do not express atypical chemokine receptor 1. Cell Stem Cell 29 (7), 1013-1015.

21. Boissiere-Michot, F. et al. (2021) CXCR2 Levels Correlate with Immune Infiltration and a Better Prognosis of Triple-Negative Breast Cancers. Cancers (Basel) 13 (10).
22. Chu, H. et al. (2020) C-X-C motif chemokine receptor type 2 correlates with higher disease stages and predicts worse prognosis, and its downregulation enhances chemotherapy sensitivity in triple-negative breast cancer. Transl Cancer Res 9 (2), 840-848.
23. Zhang, R. et al. (2023) PMN-MDSCs modulated by CCL20 from cancer cells promoted breast cancer cell stemness through CXCL2-CXCR2 pathway. Signal Transduct Target Ther 8 (1), 97.
24. Hadadi, E. et al. (2020) Chronic circadian disruption modulates breast cancer stemness and immune microenvironment to drive metastasis in mice. Nat Commun 11 (1), 3193.
25. Yang, J. et al. (2021) Targeted Deletion of CXCR2 in Myeloid Cells Alters the Tumor Immune Environment to Improve Antitumor Immunity. Cancer Immunol Res 9 (2), 200-213.
26. Timaxian, C. et al. (2021) Pivotal Role for Cxcr2 in Regulating Tumor-Associated Neutrophil in Breast Cancer. Cancers (Basel) 13 (11).
27. Acharyya, S. et al. (2012) A CXCL1 paracrine network links cancer chemoresistance and metastasis. Cell 150 (1), 165-78.
28. Sharma, B. et al. (2013) Targeting CXCR2 enhances chemotherapeutic response, inhibits mammary tumor growth, angiogenesis, and lung metastasis. Mol Cancer Ther 12 (5), 799-808.
29. Teijeira, A. et al. (2020) CXCR1 and CXCR2 Chemokine Receptor Agonists Produced by Tumors Induce Neutrophil Extracellular Traps that Interfere with Immune Cytotoxicity. Immunity 52 (5), 856-871 e8.
30. Vindrieux, D. et al. (2009) Emerging roles of chemokines in prostate cancer. Endocr Relat Cancer 16, 663-673.
31. Lopez-Bujanda, Z.A. et al. (2021) Castration-mediated IL-8 promotes myeloid infiltration and prostate cancer progression. Nat Cancer 2 (8), 803-818.
32. Armstrong, C.W.D. et al. (2020) Clinical and functional characterization of CXCR1/CXCR2 biology in the relapse and radiotherapy resistance of primary PTEN-deficient prostate carcinoma. NAR Cancer 2 (3), zcaa012.
33. Li, N. et al. (2022) ARID1A loss induces polymorphonuclear myeloid-derived suppressor cell chemotaxis and promotes prostate cancer progression. Nat Commun 13 (1), 7281.
34. Di Mitri, D. et al. (2019) Re-education of Tumor-Associated Macrophages by CXCR2 Blockade Drives Senescence and Tumor Inhibition in Advanced Prostate Cancer. Cell Rep 28 (8), 2156-2168 e5.
35. Wu, T. et al. (2022) Targeting HIC1/TGF-beta axis-shaped prostate cancer microenvironment restrains its progression. Cell Death Dis 13 (7), 624.
36. Li, Y. et al. (2019) Targeting cellular heterogeneity with CXCR2 blockade for the treatment of therapy-resistant prostate cancer. Sci Transl Med 11 (521).
37. Chen, Y. et al. (2021) Type I collagen deletion in alphaSMA(+) myofibroblasts augments immune suppression and accelerates progression of pancreatic cancer. Cancer Cell 39 (4), 548-565 e6.
38. Bianchi, A. et al. (2023) Cell-Autonomous Cxcl1 Sustains Tolerogenic Circuitries and Stromal Inflammation via Neutrophil-Derived TNF in Pancreatic Cancer. Cancer Discov 13 (6), 1428-1453.
39. Yang, J. et al. (2022) KDM6A Loss Recruits Tumor-Associated Neutrophils and Promotes Neutrophil Extracellular Trap Formation in Pancreatic Cancer. Cancer Res 82 (22), 4247-4260.
40. Evrard, M. et al. (2018) Developmental Analysis of Bone Marrow Neutrophils Reveals Populations Specialized in Expansion, Trafficking, and Effector Functions. Immunity 48 (2), 364-379 e8.
41. Gulhati, P. et al. (2023) Targeting T cell checkpoints 41BB and LAG3 and myeloid cell CXCR1/CXCR2 results in antitumor immunity and durable response in pancreatic cancer. Nat Cancer 4 (1), 62-80.
42. Zhang, Q. et al. (2021) Gut Microbiome Directs Hepatocytes to Recruit MDSCs and Promote Cholangiocarcinoma. Cancer Discov 11 (5), 1248-1267.
43. Yang, J. et al. (2023) CXCR2 expression during melanoma tumorigenesis controls transcriptional programs that facilitate tumor growth. Mol Cancer 22 (1), 92.
44. Groth, C. et al. (2021) Blocking Migration of Polymorphonuclear Myeloid-Derived Suppressor Cells Inhibits Mouse Melanoma Progression. Cancers (Basel) 13 (4).
45. Bilusic, M. et al. (2019) Phase I trial of HuMax-IL8 (BMS-986253), an anti-IL-8 monoclonal antibody, in patients with metastatic or unresectable solid tumors. J Immunother Cancer 7 (1), 240.
46. Cheng, Y. et al. (2021) Targeting CXCR2 inhibits the progression of lung cancer and promotes therapeutic effect of cisplatin. Mol Cancer 20 (1), 62.
47. Tang, K.H. et al. (2022) Combined Inhibition of SHP2 and CXCR1/2 Promotes Antitumor T-cell Response in NSCLC. Cancer Discov 12 (1), 47-61.
48. Lu, Z. et al. (2020) Epigenetic therapy inhibits metastases by disrupting premetastatic niches. Nature 579 (7798), 284-290.
49. Guo, N. et al. (2020) Lung adenocarcinoma-related TNF-alpha-dependent inflammation upregulates MHC-II on alveolar type II cells through CXCR-2 to contribute to Treg expansion. FASEB J 34 (9), 12197-12213.

50. Yeo, A.T. et al. (2023) Driver Mutations Dictate the Immunologic Landscape and Response to Checkpoint Immunotherapy of Glioblastoma. Cancer Immunol Res 11 (5), 629-645.
51. Zhang, P. et al. (2022) Targeting myeloid derived suppressor cells reverts immune suppression and sensitizes BRAF-mutant papillary thyroid cancer to MAPK inhibitors. Nat Commun 13 (1), 1588.
52. Dunbar, A.J. et al. (2023) CXCL8/CXCR2 signaling mediates bone marrow fibrosis and is a therapeutic target in myelofibrosis. Blood 141 (20), 2508-2519.
53. Lai, W.Y. and Mueller, A. (2021) Latest update on chemokine receptors as therapeutic targets. Biochem Soc Trans 49 (3), 1385-1395.
54. Zhang, X. et al. (2019) The role of CXCR2 in acute inflammatory responses and its antagonists as anti-inflammatory therapeutics. Curr Opin Hematol 26 (1), 28-33.
55. Rennard, S.I. et al. (2015) CXCR2 Antagonist MK-7123. A Phase 2 Proof-of-Concept Trial for Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med 191 (9), 1001-11.
56. O'Byrne, P.M. et al. (2016) Efficacy and safety of a CXCR2 antagonist, AZD5069, in patients with uncontrolled persistent asthma: a randomised, double-blind, placebo-controlled trial. Lancet Respir Med 4 (10), 797-806.
57. Madan, A. et al. (2019) Efficacy and Safety of Danirixin (GSK1325756) Co-administered With Standard-of-Care Antiviral (Oseltamivir): A Phase 2b, Global, Randomized Study of Adults Hospitalized With Influenza. Open Forum Infect Dis 6 (4), ofz163.
58. Lazaar, A.L. et al. (2018) Effect of the CXCR2 antagonist danirixin on symptoms and health status in COPD. Eur Respir J 52 (4).
59. Todd, C.M. et al. (2016) The effects of a CXCR1/CXCR2 antagonist on neutrophil migration in mild atopic asthmatic subjects. Pulm Pharmacol Ther 41, 34-39.
60. Maffi, P. et al. (2020) Targeting CXCR1/2 Does Not Improve Insulin Secretion After Pancreatic Islet Transplantation: A Phase 3, Double-Blind, Randomized, Placebo-Controlled Trial in Type 1 Diabetes. Diabetes Care 43 (4), 710-718.
61. Opfermann, P. et al. (2015) A pilot study on reparixin, a CXCR1/2 antagonist, to assess safety and efficacy in attenuating ischaemia-reperfusion injury and inflammation after on-pump coronary artery bypass graft surgery. Clin Exp Immunol 180 (1), 131-42.
62. Joseph, J.P. et al. (2017) CXCR2 Inhibition - a novel approach to treating CoronAry heart DiseAse (CICADA): study protocol for a randomised controlled trial. Trials 18 (1), 473.
63. Schott, A.F. et al. (2017) Phase Ib Pilot Study to Evaluate Reparixin in Combination with Weekly Paclitaxel in Patients with HER-2-Negative Metastatic Breast Cancer. Clin Cancer Res 23 (18), 5358-5365.
64. Goldstein, L.J. et al. (2020) A window-of-opportunity trial of the CXCR1/2 inhibitor reparixin in operable HER-2-negative breast cancer. Breast Cancer Res 22 (1), 4.
65. Hastrup, N. et al. (2015) The effects of the CXCR2 antagonist, MK-7123, on bone marrow functions in healthy subjects. Cytokine 72 (2), 197-203.
66. Cullberg, M. et al. (2018) Pharmacokinetics of the Oral Selective CXCR2 Antagonist AZD5069: A Summary of Eight Phase I Studies in Healthy Volunteers. Drugs R D 18 (2), 149-159.
67. Mahler, D.A. et al. (2004) Efficacy and safety of a monoclonal antibody recognizing interleukin-8 in COPD: a pilot study. Chest 126 (3), 926-34.
68. Fan, G.H. et al. (2004) Rab11-family interacting protein 2 and myosin Vb are required for CXCR2 recycling and receptor-mediated chemotaxis. Mol Biol Cell 15 (5), 2456-69.
69. Rajarathnam, K. et al. (2019) How do chemokines navigate neutrophils to the target site: Dissecting the structural mechanisms and signaling pathways. Cell Signal 54, 69-80.
70. Dhayni, K. et al. (2023) Aortic valve calcification is promoted by interleukin-8 and restricted through antagonizing CXCR2. Cardiovasc Res.
71. Caxaria, S. et al. (2023) Disease modification and symptom relief in osteoarthritis using a mutated GCP-2/CXCL6 chemokine. EMBO Mol Med 15 (1), e16218.
72. Graca, F.A. et al. (2023) Platelet-derived chemokines promote skeletal muscle regeneration by guiding neutrophil recruitment to injured muscles. Nat Commun 14 (1), 2900.
73. Jones, S.A. et al. (1996) Different functions for the interleukin 8 receptors (IL-8R) of human neutrophil leukocytes: NADPH oxidase and phospholipase D are activated through IL-8R1 but not IL-8R2. Proc Natl Acad Sci U S A 93 (13), 6682-6.
74. Moepps, B. et al. (2006) A homolog of the human chemokine receptor CXCR1 is expressed in the mouse. Mol Immunol 43 (7), 897-914.
75. Swamydas, M. et al. (2016) CXCR1-mediated neutrophil degranulation and fungal killing promote Candida clearance and host survival. Sci Transl Med 8 (322), 322ra10.
76. Sawant, K.V. et al. (2021) Neutrophil recruitment by chemokines Cxcl1/KC and Cxcl2/MIP2: Role of Cxcr2 activation and glycosaminoglycan interactions. J Leukoc Biol 109 (4), 777-791.

77. Sepuru, K.M. and Rajarathnam, K. (2019) Structural basis of chemokine interactions with heparan sulfate, chondroitin sulfate, and dermatan sulfate. J Biol Chem 294 (43), 15650-15661.
78. Rajarathnam, K. et al. (2018) Glycosaminoglycan Interactions Fine-Tune Chemokine-Mediated Neutrophil Trafficking: Structural Insights and Molecular Mechanisms. J Histochem Cytochem 66 (4), 229-239.
79. Rajarathnam, K. and Desai, U.R. (2020) Structural Insights Into How Proteoglycans Determine Chemokine-CXCR1/CXCR2 Interactions: Progress and Challenges. Front Immunol 11, 660.
80. Merad, M. and Martin, J.C. (2020) Pathological inflammation in patients with COVID-19: a key role for monocytes and macrophages. Nat Rev Immunol 20 (6), 355-362.
81. Song, J.W. et al. (2020) Immunological and inflammatory profiles in mild and severe cases of COVID-19. Nat Commun 11 (1), 3410.
82. Liang, W. et al. (2020) Development and Validation of a Clinical Risk Score to Predict the Occurrence of Critical Illness in Hospitalized Patients With COVID-19. JAMA Intern Med 180 (8), 1081-1089.
83. Takahashi, T. et al. (2020) Sex differences in immune responses that underlie COVID-19 disease outcomes. Nature 588 (7837), 315-320.
84. Freire, P.P. et al. (2021) The relationship between cytokine and neutrophil gene network distinguishes SARS-CoV-2-infected patients by sex and age. JCI Insight 6 (10).
85. Eddins, D.J. et al. (2023) Transcriptional reprogramming of infiltrating neutrophils drives lung pathology in severe COVID-19 despite low viral load. Blood Adv 7 (5), 778-799.
86. Rice, C.M. et al. (2023) Hyperactive immature state and differential CXCR2 expression of neutrophils in severe COVID-19. Life Sci Alliance 6 (2).
87. Coombs, C. et al. (2019) Chemokine receptor trafficking coordinates neutrophil clustering and dispersal at wounds in zebrafish. Nat Commun 10 (1), 5166.
88. Delobel, P. et al. (2022) CXCR2 intrinsically drives the maturation and function of neutrophils in mice. Front Immunol 13, 1005551.
89. Xie, X. et al. (2020) Single-cell transcriptome profiling reveals neutrophil heterogeneity in homeostasis and infection. Nat Immunol 21 (9), 1119-1133.
90. Adrover, J.M. et al. (2019) A Neutrophil Timer Coordinates Immune Defense and Vascular Protection. Immunity 50 (2), 390-402 e10.
91. Teijeira, A. et al. (2021) Differential Interleukin-8 thresholds for chemotaxis and netosis in human neutrophils. Eur J Immunol 51 (9), 2274-2280.
92. Karpova, D. et al. (2019) Targeting VLA4 integrin and CXCR2 mobilizes serially repopulating hematopoietic stem cells. J Clin Invest 129 (7), 2745-2759.
93. Capitano, M.L. et al. (2019) Secreted nuclear protein DEK regulates hematopoiesis through CXCR2 signaling. J Clin Invest 129 (6), 2555-2570.
94. Crijns, H. et al. (2020) Targeting Chemokine-Glycosaminoglycan Interactions to Inhibit Inflammation. Front Immunol 11, 483.
95. Derler, R. et al. (2017) Glycosaminoglycan-Mediated Downstream Signaling of CXCL8 Binding to Endothelial Cells. Int J Mol Sci 18 (12).
96. Gschwandtner, M. et al. (2017) Glycosaminoglycans are important mediators of neutrophilic inflammation in vivo. Cytokine 91, 65-73.
97. Joseph, P.R.B. et al. (2017) Heparin-bound chemokine CXCL8 monomer and dimer are impaired for CXCR1 and CXCR2 activation: implications for gradients and neutrophil trafficking. Open Biol 7 (11).
98. Sepuru, K.M. et al. (2018) Structural basis, stoichiometry, and thermodynamics of binding of the chemokines KC and MIP2 to the glycosaminoglycan heparin. J Biol Chem 293 (46), 17817-17828.
99. Brown, A.J. et al. (2017) Chemokine CXCL7 Heterodimers: Structural Insights, CXCR2 Receptor Function, and Glycosaminoglycan Interactions. Int J Mol Sci 18 (4).
100. Sepuru, K.M. and Rajarathnam, K. (2021) Structural basis of a chemokine heterodimer binding to glycosaminoglycans. Biochem J 478 (5), 1009-1021.
101. Sawant, K.V. et al. (2023) Chemokine Cxcl1-Cxcl2 heterodimer is a potent neutrophil chemoattractant. J. Leuko. Biol (in press).
102. Hao, Y. et al. (2021) Integrated analysis of multimodal single-cell data. Cell 184 (13), 3573-3587 e29.
103. Zlotnik, A. and Yoshie, O. (2000) Chemokines: a new classification system and their role in immunity. Immunity 12 (2), 121-7.
104. Evans, T.R.J. et al. (2023) A phase I/II study of the CXCR2 inhibitor, AZD5069, in combination with durvalumab, in patients (pts) with advanced hepatocellular carcinoma (HCC). Journal of Clinical Oncology 41 (4_suppl), TPS631-TPS631.
105. Lazaar, A.L. et al. (2020) CXCR2 antagonist for patients with chronic obstructive pulmonary disease with chronic mucus hypersecretion: a phase 2b trial. Respir Res 21 (1), 149.

902 106. Roberts, G. et al. (2019) Randomized, Double-Blind, Placebo-Controlled Study of the Safety, Tolerability,
903 and Clinical Effect of Danirixin in Adults With Acute, Uncomplicated Influenza. Open Forum Infect Dis 6 (4),
904 ofz072.
905 107. Piemonti, L. et al. (2022) Ladarixin, an inhibitor of the interleukin-8 receptors CXCR1 and CXCR2, in
906 new-onset type 1 diabetes: A multicentre, randomized, double-blind, placebo-controlled trial. Diabetes Obes
907 Metab 24 (9), 1840-1849.
908 108. Sordi, V. et al. (2023) Post hoc analysis of a randomized, double-blind, prospective trial evaluating a
909 CXCR1/2 inhibitor in new-onset type 1 diabetes: endo-metabolic features at baseline identify a subgroup of
910 responders. Front Endocrinol (Lausanne) 14, 1175640.
911 109. Xie, Y. et al. (2023) Expanding role of CXCR2 and therapeutic potential of CXCR2 antagonists in
912 inflammatory diseases and cancers. Eur J Med Chem 250, 115175.
913

914

915 **Table 1 : Inhibitors of CXCR2 tested in clinical studies.**

916

Compound	Specificity	Supplier	Activity	Reference
Monoclonal antibody to CXCL8	CXCL8 800mg IV infusion at month 0 followed by 400mg monthly	Abgenix, Inc.	Improves dyspnoea, but not lung function or health status	[67]
AZD-5069	CXCR2 selective reversible antagonist IC50= 0.79 nM 5, 15, 45 mg Twice daily	Astrazeneca	Uncontrolled persistent asthma—without positive outcome Suppresses hepatic neutrophil recruitment and in concert with anti-PD-1 suppresses non-alcoholic steatohepatitis-HCC tumor growth Phase I/II for PDAC, metastatic nead and neck cancer Phase I/II in combination with enzalutamide, in patients with metastatic castration resistant prostate cancer	[56] [104] NCT02499328[I] NCT03177187[II]
AZD-8039	CXCR2	Astrazeneca	Control of airway inflammation	NCT00860821[III]
BMS-986253	CXCL8 monoclonal Ab to CXCL8 Doses: 4,8,16 or 32 mg/kg IV every 2 weeks for 52 weeks	BMS	Phase I/II ongoing clinical trials in metastatic or unresectable solid tumors	[45] NCT03400332[IV] NCT02451982[V] NCT03026140[VI] NCT04572451[VII] NCT04123379[VIII] NCT03400332[IX] NCT04848116[X] NCT03689699[XI]

			Trials for COVID patients	NCT04050462[XII] NCT05148234[XIII] NCT04347226[XIV]
Danirixin (GK1325756)	CXCR2 Selective reversible antagonist IC 50=12.5nM 15 mg BID 5mg/kg in mice 75mg/ BID	GSK	Breast cancer metastasis suppression and renewal of breast cancer stem cell suppression Unfavorable for COPD (NCT03250689[XV]: terminated) (NCT03034967[XVI]: completed) (NCT03136380[XVII]: completed) (NCT02130193[XVIII]: completed) (NCT01006616[XIX]: terminated) Uncomplicated influenza-well tolerated but no difference in viral load in Phase IIa study (NCT02927431[XX]: terminated)	[105] [106]
Ladarixin (DF2156A)	CXCR1/CXCR2 non-competitive dual allosteric antagonist IC50= 0.7nM 400mg bid for patients-3 cycles of 14 days on/14 days off	DOMPE	Treatment of new-onset Type I Diabetes- in Phase III Clinical Trial Newly diagnosed type I diabetes patients showed no improvement in	*PipelinReview.com* NCT04628481[XXI] Maria De Pizzol. [107] NCT02814838[XXII]

			preserving beta cell function (Phase 2 trial) Post-hoc analysis of a Phase 2 multicenter trial show it may be useful for HIGH-daily insulin requirement baseline patients	[108]
Navarixin (SCH 527123 or MK-7123)	CXCR2/CXCR1 Allosteric antagonist IC 50 =2.6 nM and 36nM, respectively 70mg/kg In patients, 50mg	Merck and Ligand Pharmaceuticals	Phase II for NSCLC, castration resistant prostate cancer and colorectal cancer and also for asthma, psoriasis Anti-inflammatory for COPD (NCT 01006616[XXVII])- results showed improved FEV Evaluated as anti-inflammatory for adults hospitalized with influenza combined with oseltamivir-inconclusive outcome-but safe	NCT00688467[XXIII] NCT00632502[XXIV] NCT03473925[XXV] NCT00684593[XXVI] [55] [57]
QBM076	CXCR2 25 – 150 mg bid/8 weeks	Novartis	COPD	NCT 01972776[XXVIII](terminated)
Reparixin (Repertaxin)	CXCR1/2 noncompetitive allosteric inhibitor IC 50 =1 nM and 100nM, respectively	DOMPE	Phase II for TNBC tested for improved transplant outcome following intra-hepatic infusion of pancreatic islets in	[64] NCT01817959[XXIX] [60]

			patients with Type 1 diabetes Phase III: no efficacy	
RIST4721	CXCR2	Aristea Therapeutics	Inflammatory Response in Healthy Male Subjects Using a Standardized Blister Model Discontinued for safety reasons	NCT04105959[XXX]
SX-682	CXCR1/ CXCR2 allosteric dual inhibitor IC50= 42 nm and 20 nM respectively	Syntrix	Ongoing Phase I and Phase II clinical trials for melanoma, PDAC, MDS, CRC, NSCLC, metastatic cancer, Myelodysplastic Syndrome	[109] NCT04477343[XXXI] NCT04245397[XXXII] NCT03161431[XXIII] NCT04599140[XXXIV] NCT04574583[XXXV]

Footnotes

[I] https://classic.clinicaltrials.gov/ct2/show/NCT02499328
[II] https://classic.clinicaltrials.gov/ct2/show/NCT03177187
[III] https://classic.clinicaltrials.gov/ct2/show/NCT00860821
[IV] https://clinicaltrials.gov/study/NCT03400332?a=20
[V] https://classic.clinicaltrials.gov/ct2/show/NCT02451982
[VI] https://classic.clinicaltrials.gov/ct2/show/NCT03026140
[VII] https://classic.clinicaltrials.gov/ct2/show/NCT04572451
[VIII] https://classic.clinicaltrials.gov/ct2/show/NCT04123379
[IX] https://clinicaltrials.gov/study/NCT03400332?a=20
[X] https://classic.clinicaltrials.gov/ct2/show/NCT04848116
[XI] https://classic.clinicaltrials.gov/ct2/show/NCT03689699
[XII] https://classic.clinicaltrials.gov/ct2/show/NCT04050462
[XIII] https://classic.clinicaltrials.gov/ct2/show/NCT05148234
[XIV] https://classic.clinicaltrials.gov/ct2/show/NCT04347226
[XV] https://classic.clinicaltrials.gov/ct2/show/NCT03250689
[XVI] https://classic.clinicaltrials.gov/ct2/show/NCT03034967
[XVII] https://clinicaltrials.gov/study/NCT03136380
[XVIII] https://classic.clinicaltrials.gov/ct2/show/NCT02130193
[XIX] https://classic.clinicaltrials.gov/ct2/show/NCT01006616
[XX] https://classic.clinicaltrials.gov/ct2/show/NCT02927431
[XXI] https://classic.clinicaltrials.gov/ct2/show/NCT04628481
[XXII] https://classic.clinicaltrials.gov/ct2/show/NCT02814838
[XXIII] https://classic.clinicaltrials.gov/ct2/show/NCT00688467
[XXIV] https://classic.clinicaltrials.gov/ct2/show/NCT00632502
[XXV] https://classic.clinicaltrials.gov/ct2/show/NCT03473925
[XXVI] https://clinicaltrials.gov/study/NCT00684593
[XXVII] https://classic.clinicaltrials.gov/ct2/show/NCT01006616
[XXVIII] https://classic.clinicaltrials.gov/ct2/show/NCT01972776
[XXIX] https://classic.clinicaltrials.gov/ct2/show/NCT01817959
[XXX] https://classic.clinicaltrials.gov/ct2/show/NCT04105959
[XXXI] https://classic.clinicaltrials.gov/ct2/show/NCT04477343
[XXXII] https://classic.clinicaltrials.gov/ct2/show/NCT04245397
[XXXIII] https://classic.clinicaltrials.gov/ct2/show/NCT03161431

[xxxiv] https://classic.clinicaltrials.gov/ct2/show/NCT04599140
[xxxv] https://classic.clinicaltrials.gov/ct2/show/NCT04574583

All trials are registered to clinicaltrials.gov

Glossary

Bone marrow (BM): Spongy tissue of some bones comprising hematopoietic cells, adipose tissue and stromal cells and involved in the production of blood cells.

Chemokines: Chemokines (Chemotactic cytokines) are small basic proteins (molecular weight ~ 8 to 10 kDa) that share the following characteristics – reversibly exist as monomers and dimers, activate receptors that belong to the G protein-coupled receptor (GPCR) class, and bind glycosaminoglycans that regulate receptor signaling and function.

Experimental autoimmune encephalomyelitis (EAE): EAE is an experimental model for the inflammatory demyelinating disease, multiple sclerosis. EAE is mediated by myelin-specific T cells, which are initially activated at the peripheral lymphoid organs and then reach the CNS.

Glycosaminoglycan (GAG): GAGs are linear polysaccharides and their chemical complexity arises due to differences in backbone structure and sulfation pattern. GAGs are selectively expressed by most cell types including the endothelium and epithelium, and are also present in the extracellular matrix (ECM) and the glycocalyx that forms a formidable barrier between the vasculature and the tissue.

Myeloid-derived suppressor cells (MDSCs): These are myeloid cells with the ability to inhibit immune response. They are divided in PMN-MDSC or Granulocitic-MDSCs (G-MDSCs) with a phenotype related to neutrophils or M-MDSC with a phenotype related to monocytes.

NETosis: Formation of neutrophil extracellular traps, which corresponds to the release in the microenvironment of modified chromatin decorated with bactericidal proteins from granules and cytoplasm by neutrophils. These structures will trap and kill microbes.

Polyoma middle tumor-antigen (PyMT): PyMT refers in this article to a particular transgenic mouse in which the PyMT gene is under the control of the mammary-specific promoter (mouse mammary tumor virus) leading to the development of tumors in the mammary gland.

Reactive oxygen species (ROS): Derivatives of the molecular oxygen that are generated by NADPH pathway in neutrophil-mediated killing of microbes in the phagosomes and are also released to the extracellular milieu. ROS are also produced in the mitochondria due to oxidative phosphorylation.

Tumor associated neutrophils (TANs): TANs are neutrophils that can be found in tumors and can have either pro- (TAN2) or anti-tumoral (TAN1) properties.

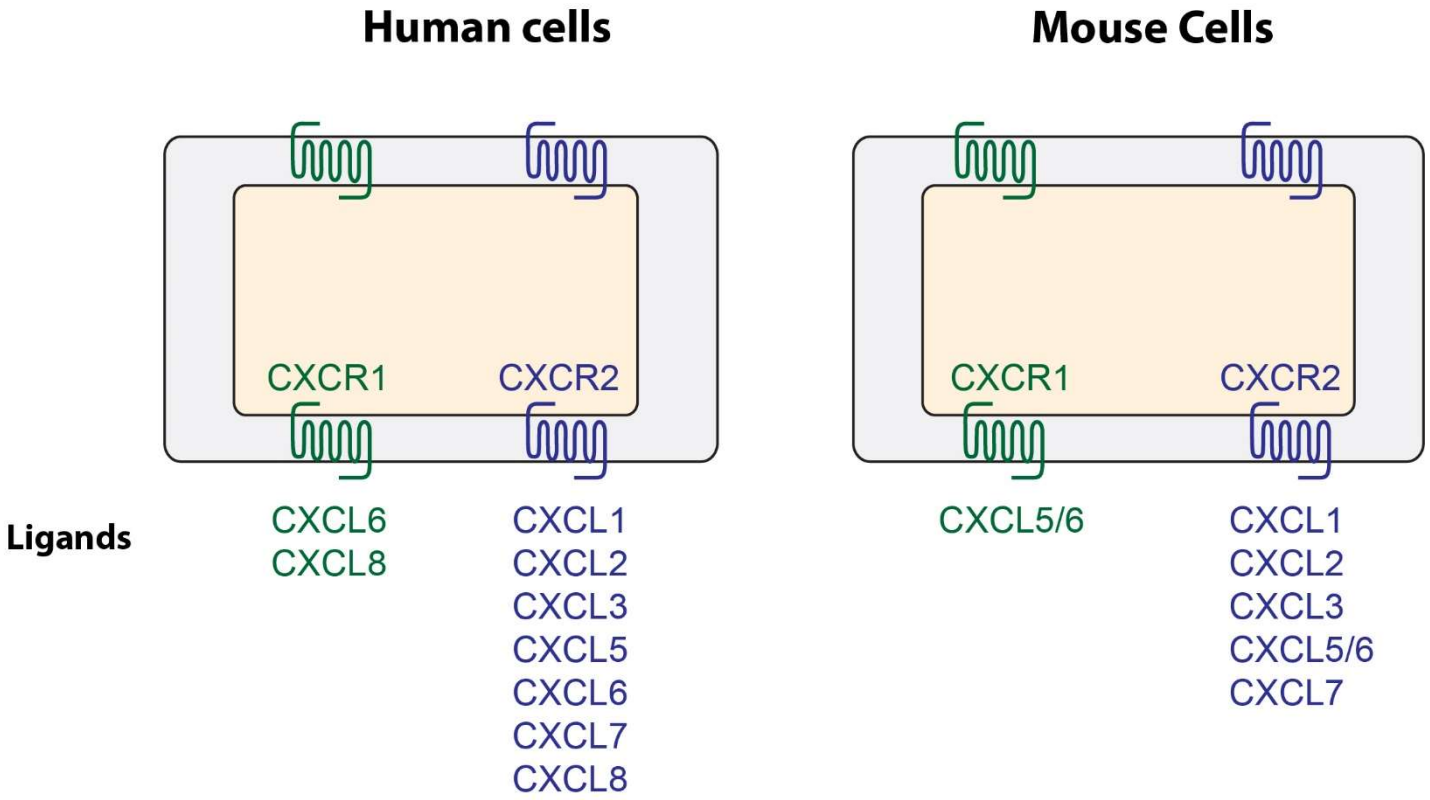
Triple negative breast cancer (TNBC): This refers to a particular type of breast cancer that does not express estrogen receptor (ER), progesterone receptor (PR) and exhibit amplification of the oncogene Her-2 in contrast to luminal (ER^+ PR^+) breast cancers and Her-2^+ breast cancers.

Zeitgeber time (ZT): This notion defines the external signals that define the physiological periodicity of 24h and the endogenous clock that will condition the circadian rhythm. It is divided into a light phase and dark phase. In mice, dark phase correspond to Zeitgeber times (ZT) 12 to 24, whereas the light phase is between ZT0 and 12. The circadian clock is shifted by 12h in humans compared to mice as mice are more active in the dark compared to the light period.

- Recent studies indicate CXCR2 plays diverse and novel roles in several cancers and basic physiology, viral infection such as Covid-19 that goes beyond its widely accepted role in bacterial infections. The pro or anti-tumoral action of CXCR2 may vary depending on which cells are expressing the receptor and this remains a subject of debate.
- CXCR2 is expressed by multiple cell types including neutrophils, endothelial, immune, stromal and some epithelial cells but recently developed single cell technologies are needed to validate this expression at the protein and RNA levels. The tissue-specific heterogeneity in CXCR2 expression and function in neutrophils has been clarified with respect to maturation, aging and circadian rhythm. Such studies in other cell types may reveal underlying complexity for CXCR2 function in tissues.
- CXCR2 ligands show diverse glycosaminoglycan interactions, suggesting that the phenotype of each chemokine is distinct and plays non-overlapping roles in the physiological context.
- Recent availability of structures of CXCR2 bound to ligands or antagonists and the analysis of downstream effectors of CXCR2 should facilitate designing more potent drugs and better understanding of CXCR2 signaling function in pathophysiology.

"Outstanding questions"

- **What is the role of CXCR2 in acute vs. chronic diseases?**
- **How does CXCR2 signaling vary in response to different ligands?**
- **What novel strategies could be used to design drugs and antibodies against CXCR2 in cancer?**
- **What are the roles of CXCR2 in changes in endothelial GAGs in cancer microvessels?**



Drugs	CXCR2-specific	Dual (CXCR1/2)
	AZD5059	Navarixin (SCH527123)
	Danirixin (GSK1325756)	Reparixin (Repertaxin)
	DF2156A	SX-682
	SB225002	
	SB265610	
	SC656933	

Fig. 1

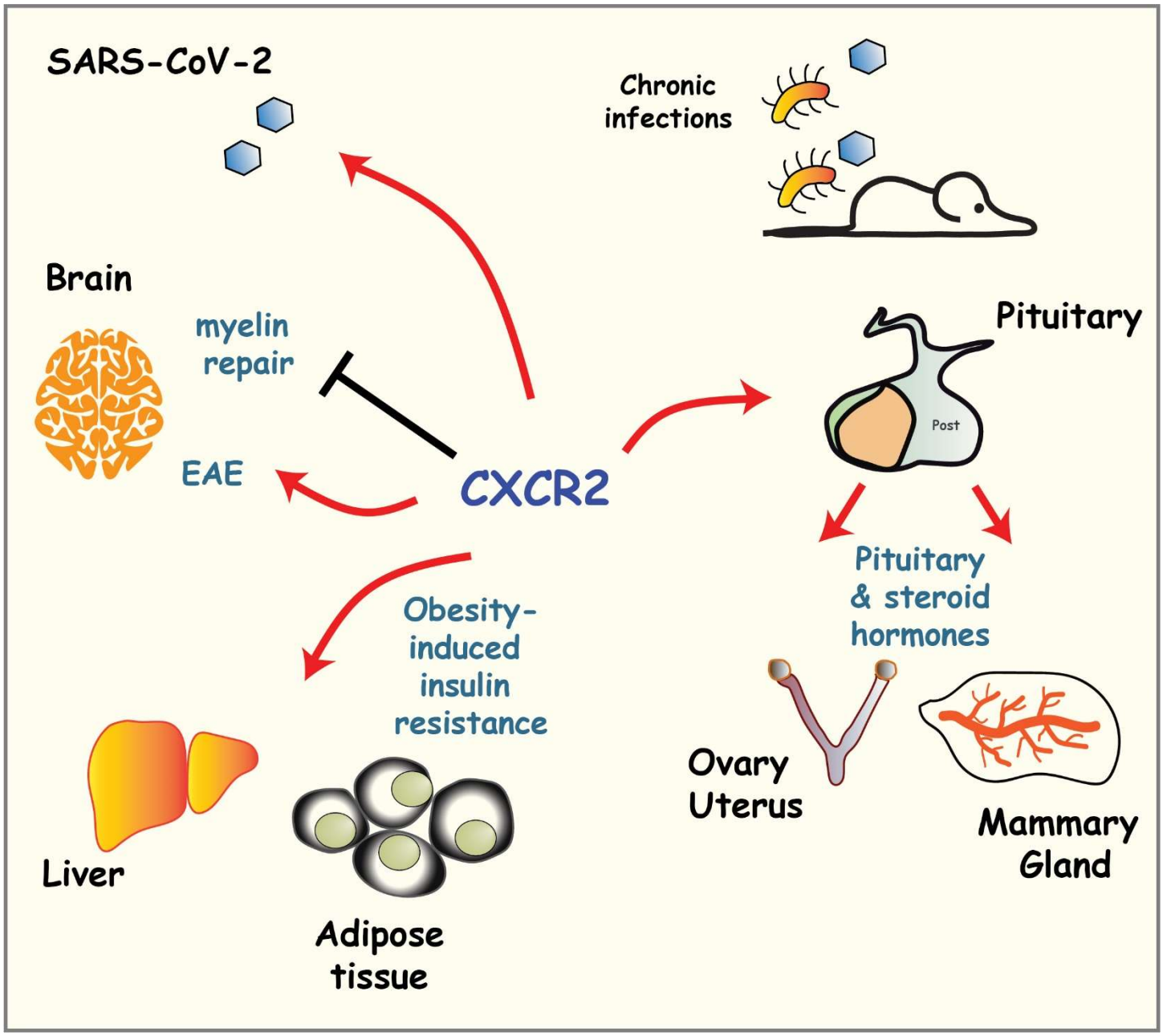

SARS-CoV-2
Chronic infections
Brain
myelin repair
EAE
CXCR2
Pituitary
Post
Pituitary & steroid hormones
Obesity-induced insulin resistance
Ovary
Uterus
Mammary Gland
Liver
Adipose tissue

Fig. 2

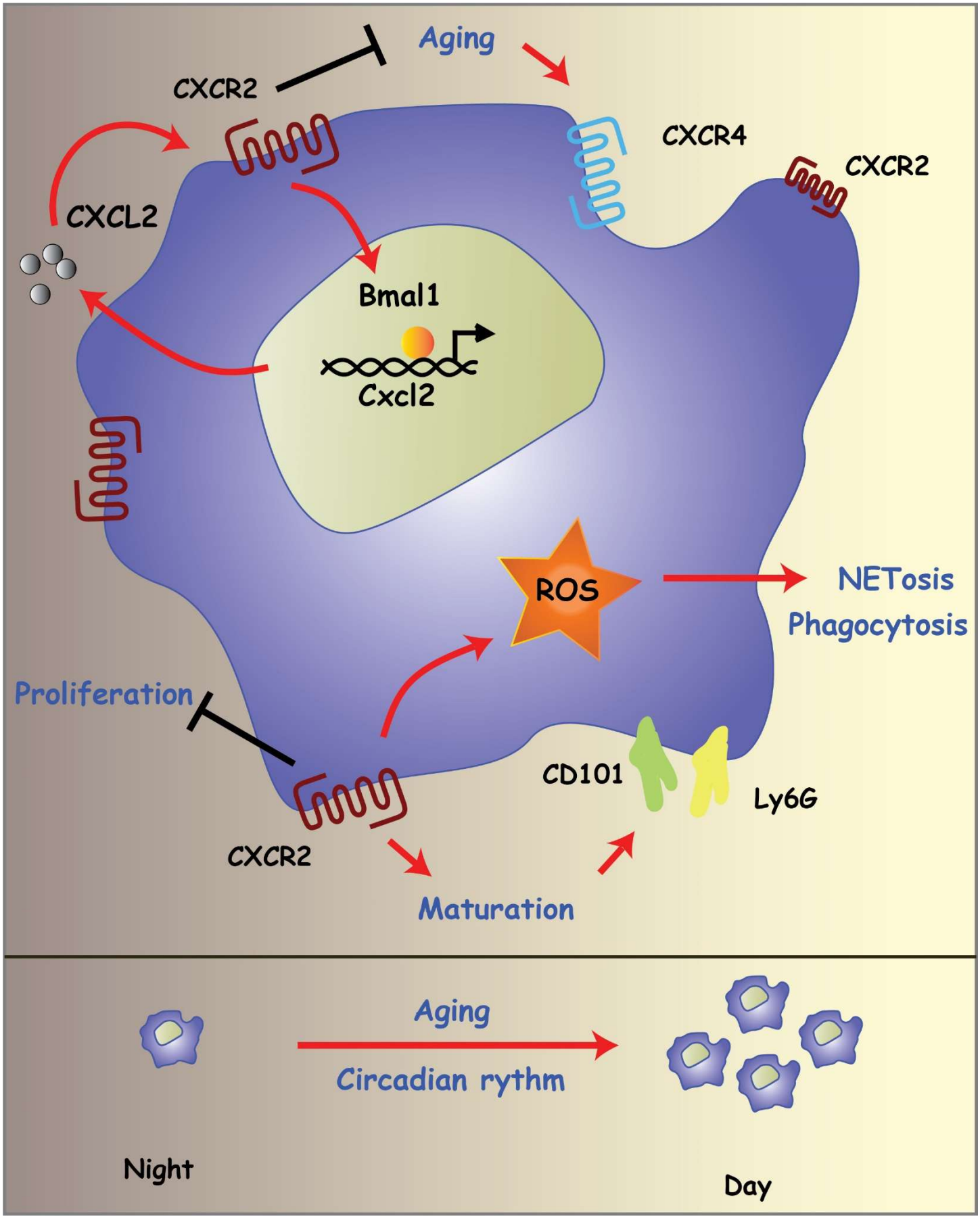

Aging
CXCR2
CXCR4
CXCR2
CXCL2
Bmal1
Cxcl2
ROS
NETosis
Phagocytosis
Proliferation
CD101
Ly6G
CXCR2
Maturation
Aging
Circadian rythm
Night
Day

Fig. 3

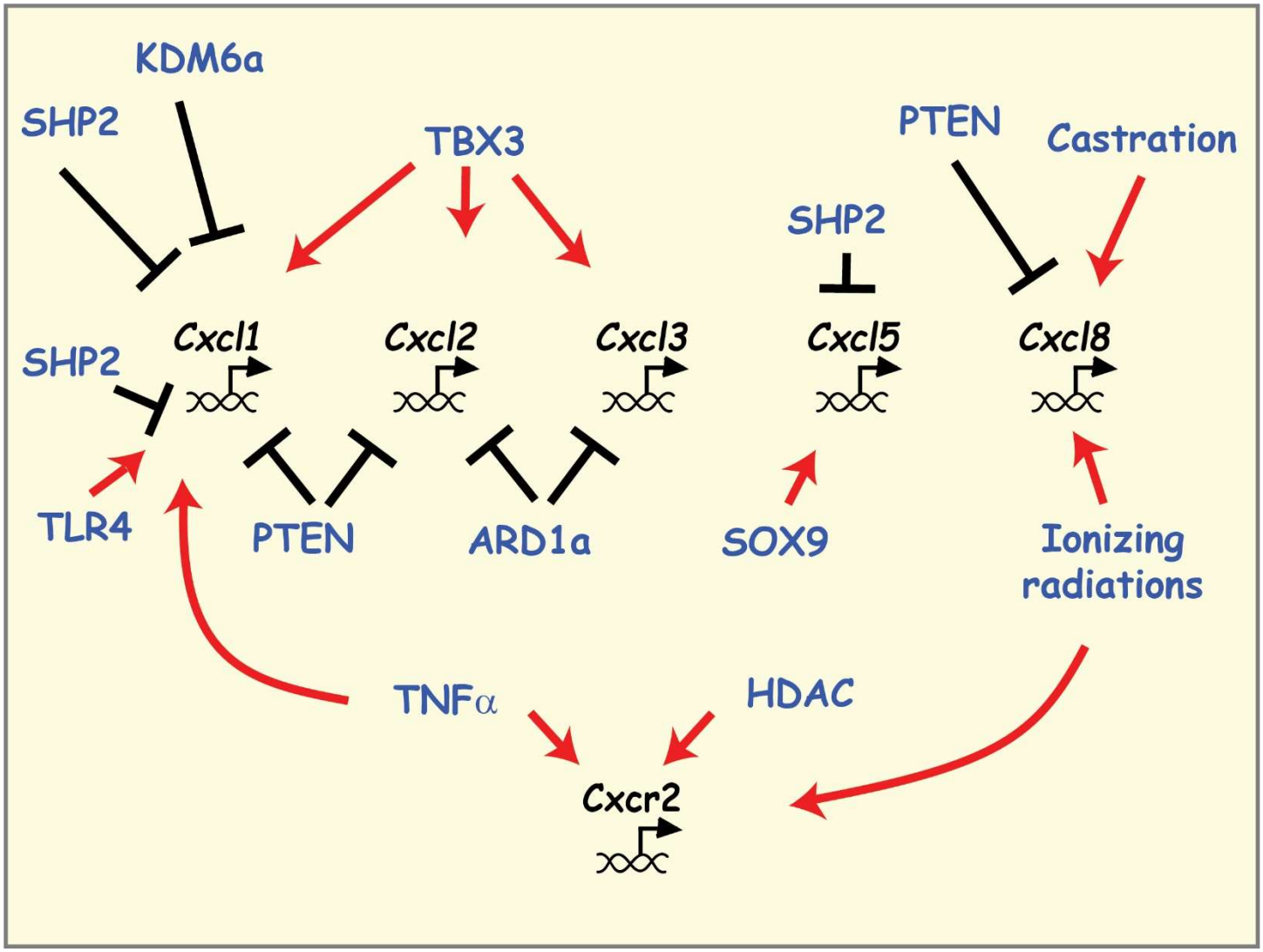

KDM6a
SHP2
TBX3
PTEN
Castration
SHP2
SHP2
Cxcl1
Cxcl2
Cxcl3
Cxcl5
Cxcl8
TLR4
PTEN
ARD1a
SOX9
Ionizing radiations
TNFα
HDAC
Cxcr2

Fig. 4