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1 **CXCR2 chemokine receptor – a master regulator in cancer and physiology**

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17 **Running title: Update on CXCR2 function**

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22 **ABSTRACT**

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

32

33 **KEYWORDS**

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

35 **Recent developments about CXCR2 function**

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

42 Among them, the chemokine receptor CXCR2 is expressed in diverse cell types - including
43 mainly neutrophils, and to a lesser extent, endothelial cells, macrophages, oligodendrocytes,
44 neurons, neural crest-derived cells, and some cancer cells. It binds several ligands, which
45 allows some redundancy, but recent studies indicate that the phenotype of each chemokine
46 is distinct and finetunes cellular responses that are also coupled to CXCR1 (see box 1 and Fig.
47 1 for basics about CXCR2). CXCR2 plays diverse roles in pathophysiology from combating
48 bacterial and viral infections to development, blood vessel narrowing, diseases of several
49 organs including the heart, lung, liver and the brain, and in several cancer etiologies [2-5].
50 The main functions described earlier focused on its pro-angiogenic role and its critical
51 function of chemotaxis of neutrophils. CXCR2 field has attracted recently a considerable
52 attention, leading to a major breakthrough in the understanding of the function of this
53 receptor, far beyond the initial expectations. In this review, we will summarize the recent
54 advances concerning CXCR2 function, including its novel physiological roles based on whole
55 or tissue-specific knockout of *Cxcr2*, its involvement in neutrophil and hematopoietic stem
56 cell (HSC) action, in Covid-19 and in cancer, the latter being the most active field concerning
57 CXCR2. Finally, we discuss the latest advances in structure and function of CXCR2, and finish
58 with the main achievements in targeting CXCR2.

59

60 **Recent findings with KO of CXCR2 in physiology**

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

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

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

85 The pituitary has been recently shown to be a site of CXCR2 action. This was discovered
86 following the observation that challenging *Cxcr2*-KO animals to chronic infections leads to
87 major reproductive defects in females including, alterations of mammary gland development,
88 reduced uterus size and altered functionality of the ovary with the absence of corpus luteum
89 [11]. *Cxcr2*-KO females are unable to cycle in these conditions due to altered levels of
90 circulating steroid hormones as well as pituitary hormones such as LH, FSH and PRL, resulting
91 from a complete modification of the transcriptome of the pituitary gland that mimics
92 autoimmune hypophysitis, an inflammatory pituitary disease.

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

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

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

108

109 **The involvement of CXCR2 and its ligands in Cancer**

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

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

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

121

122 *Breast cancer (BCa)*

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

129 neutrophils found in the tumor and is associated with more aggressive breast tumors, in
130 particular TNBC [19]. It is worth mentioning that to date, this is the only study that tested in
131 parallel several anti-CXCR2 antibodies and correlated these results with *in situ* hybridization
132 (ISH) experiments, confirming a staining of granulocytes and not of tumor cells in BCa. There
133 is a general issue on the development of specific antibodies against chemokine receptors, and
134 unfortunately, the number of correctly validated antibodies, with adequate controls such as
135 knock out or knock in cells and combination with RNA data, remains low. Similarly, Rot et al.
136 have recently highlighted this issue concerning the decoy chemokine receptor ACKR1 and
137 additional controls were essential to confirm the lack of specificity of some antibodies [20].
138 The prognostic value of CXCR2 in BCa remains controversial. Boissière-Michot et al. showed
139 that low CXCR2 levels correlate with reduced immune infiltrate and poorer overall survival. In
140 contrast, TNBCs with higher CXCR2 levels are characterized by an increased immune infiltrate
141 and a better prognosis [21]. Other studies have shown that CXCR2 correlates with high grade
142 BCa and worst prognosis, but the anti-CXCR2 antibody used was different and stained mostly
143 tumor cells [22], whereas other studies show that tumor cells express only low levels of CXCR2
144 RNA and protein [19]. However, the expression and activation of CXCR2 on breast cancer
145 tumor cells has been associated with increased stemness of the breast cancer cells [23].
146 Hadadi et al. showed in mixed model background FVB/C57BL/6 of **Polyoma middle tumor**
147 **antigen (PyMT)** mice, that exposing mice to jetlag increases cancer cell dissemination and lung
148 metastasis [24], reminding the potential of circadian cycle on neutrophils function. Though
149 the lung neutrophil levels in jetlagged compared to control mice were similar, lung metastasis
150 could be reduced by treating mice with the CXCR2 inhibitor SB265610.
151 Using a targeted *Cxcr2* deletion in myeloid cells in a C57BL/6 background (*Cxcr2 Mye^{Δ/Δ}* mice
152 or *LysM-Cre –Cxcr2^{fl/fl}*), Yang et al. observed a significant reduction in orthotopic tumor growth
153 and outgrowth of BCa cells in a lung metastasis model compared to WT mice [25]. Tumors
154 growing in *Cxcr2Mye^{Δ/Δ}* mice exhibited significantly reduced MDSCs and increased
155 intratumoral CXCL11, which was shown to come from an increase in tumor infiltrating B1b
156 cells. The increased CXCL11 expression enhanced infiltration and activation of effector CD8+
157 T cells. Interestingly, treatment of tumor-bearing mice with a CXCR1/CXCR2 antagonist (SX-
158 682) also inhibited tumor growth, reduced intratumoral MDSCs, increased B1b and CD8+T
159 cells in the TME, and in addition, enhanced the response to anti-PD-1 to reduce tumor growth.

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

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

192

193 *Prostate Cancer (PCa)*

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

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

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

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

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

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

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

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

262

263 *Pancreatic ductal adenocarcinoma (PDAC)*

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

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

271 Bianchi et al. reported that CXCL1 levels were increased in Kras-Tp53 tumors [38]. The source
272 of CXCL1 was tumor cells themselves, whereas *CXCR2* was nearly exclusively present on PMN-
273 MDSCs. Clusters of CXCL1 Pan-CK⁺ tumor cells also contained CD11b⁺ CD15⁺ *CXCR2*⁺ PMN-
274 MDSCs and CD68⁺ CD163⁺ M2-like macrophages but lacked CD8⁺ T cells [38]. Knocking down
275 CXCL1 in cancer cells strongly reduced tumor growth. Tumor Necrosis Factor- α (TNF α)
276 production by PMN-MDSCs up-regulated CXCL1 in tumor cells through CREB induction. In
277 addition, PMN-MDSCs polarized CAFs into pro-inflammatory CAFs (iCAF) with an activated IL-
278 6/STAT3 signaling pathway.

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

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

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

295

296 *Hepatocellular cancer*

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

304

305 *Melanoma*

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

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

322

323 *Lung Cancer*

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

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

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

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

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

358

359 *Glioblastoma*

360 Two thirds of glioblastoma (GBM) tumors show an amplification of EGFR and many of them
361 express the constitutively active in frame deletion mutant EGFRvIII. GBM expressing EGFRvIII
362 secrete CXCL1/2/3 and accumulate more PMN-MDSCs than GBM expressing wild type EGFR
363 [50]. As a result, there is resistance to anti-PD-1 and CTLA-4 therapy. Inhibition of CXCR2 with
364 AZD5069 resulted in reduction in PNM-MDSCs, increased intratumoral cytotoxic CD8+ T cells,
365 and improved response to immune checkpoint inhibitors in mice with EGFRvIII expressing
366 GBM.

367

368 *Thyroid cancer*

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

382

383 *Bone Cancer*

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

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

393

394 **Effectiveness of drugs targeting CXCR2**

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

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

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

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

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

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

444

445

446

447 **Concluding remarks**

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

467

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471

472 **Box 1: CXCR1 and CXCR2 basics**

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

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

498

499 **Box 2: CXCR2 and Covid-19**

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

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

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

521

522

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

524 Until recently, the precise role of CXCR2 in neutrophils had been relatively poorly addressed
525 and novel findings are summarized in Fig. 1. In zebrafish, both *cxcr1* and *cxcr2* promote the
526 motility of neutrophils, *cxcr1* stimulated the clustering of neutrophils, whereas *cxcr2* favored
527 the dispersion [87].

528 *Cxcr2*-KO mice displayed diminished maturation of splenic neutrophils, based on CD101 and
529 Ly6G expression and RNAseq profiling of neutrophils [88], while there was an increase of
530 aged CD62L^{lo} CXCR4^{hi} neutrophils in the spleen. *Cxcr2*-KO splenic neutrophils were less
531 phagocytic compared to WT neutrophils, with decreased production of **reactive oxygen**
532 **species (ROS)** and F-actin, but this was in contrast to bone marrow (BM) *Cxcr2*-KO neutrophils
533 [88]. Similarly, single cell RNA sequencing (scRNAseq) showed that in contrast to blood
534 neutrophils, which are Ly6G⁺ CXCR2⁺, BM neutrophils were heterogeneous, with immature

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

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

552 In humans, CXCL8 can trigger both chemotaxis, NETosis (see glossary) and ROS production
553 [91]. However, only high concentrations of CXCL8 enhanced NETosis, whereas motility
554 stimulation was achieved at lower concentrations. Pharmacological inhibition of CXCR2
555 reduced NETosis and chemotaxis and inhibition of CXCR1 decreased NETosis.

556

557 **Box 4: CXCR2 and hematopoietic stem cells**

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

567

568 **Box 5: CXCR2 and glycosaminoglycans interactions and ligand binding**

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

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

600

601 **Clinician's corner**

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

629 crosstalk with other G-coupled receptors. We will learn from each trial how to better
630 design these approaches.
631

632 **Figure Legends**

633

634 **Fig. 1 (embedded in box 3): A central action of CXCR2 in neutrophils in a non-cancer**
635 **situation**

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

644

645 **Fig. 1: CXCR1 and CXCR2 ligands**

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

655

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

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

663

664

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

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

674

675

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681

682 **Declaration of interests**

683 The authors declare no competing interests.

684

685 **References**

686

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

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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 head 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	<i>PipelinReview.com</i> NCT04628481 ^{XXI} Maria De Pizzol. [107] NCT02814838 ^{XXII}

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

			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 ^{xxxIII} NCT04599140 ^{xxxIV} NCT04574583 ^{xxxV}

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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>
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- ^{XVI}<https://classic.clinicaltrials.gov/ct2/show/NCT03034967>
- ^{XVII}<https://clinicaltrials.gov/study/NCT03136380>
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- ^{XXXII}<https://classic.clinicaltrials.gov/ct2/show/NCT04245397>
- ^{XXXIII}<https://classic.clinicaltrials.gov/ct2/show/NCT03161431>

952 ^{XXXIV} <https://classic.clinicaltrials.gov/ct2/show/NCT04599140>
953 ^{XXXV} <https://classic.clinicaltrials.gov/ct2/show/NCT04574583>

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955 All trials are registered to clinicaltrials.gov

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958 **Glossary**

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

962 **Chemokines:** Chemokines (Chemotactic cytokines) are small basic proteins (molecular weight ~ 8 to
963 10 kDa) that share the following characteristics – reversibly exist as monomers and dimers, activate
964 receptors that belong to the G protein-coupled receptor (GPCR) class, and bind glycosaminoglycans
965 that regulate receptor signaling and function.

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

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

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

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

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

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

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

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

990 **Zeitgeber time (ZT):** This notion defines the external signals that define the physiological periodicity of
991 24h and the endogenous clock that will condition the circadian rhythm. It is divided into a light phase
992 and dark phase. In mice, dark phase correspond to Zeitgeber times (ZT) 12 to 24, whereas the light
993 phase is between ZT0 and 12. The circadian clock is shifted by 12h in humans compared to mice as
994 mice are more active in the dark compared to the light period.

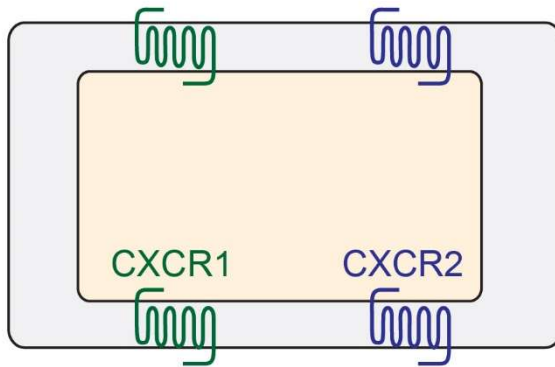
995

- 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?**

Human cells

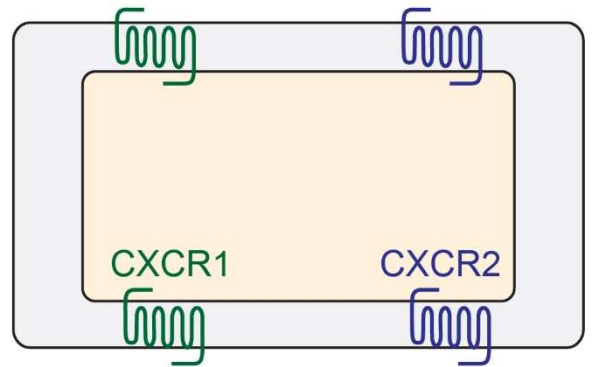


Ligands

CXCL6
CXCL8

CXCL1
CXCL2
CXCL3
CXCL5
CXCL6
CXCL7
CXCL8

Mouse Cells



CXCL5/6

CXCL1
CXCL2
CXCL3
CXCL5/6
CXCL7

Drugs

CXCR2-specific

AZD5059
Danirixin (GSK1325756)
DF2156A
SB225002
SB265610
SC656933

Dual (CXCR1/2)

Navarixin (SCH527123)
Reparixin (Repertaxin)
SX-682

Fig. 1

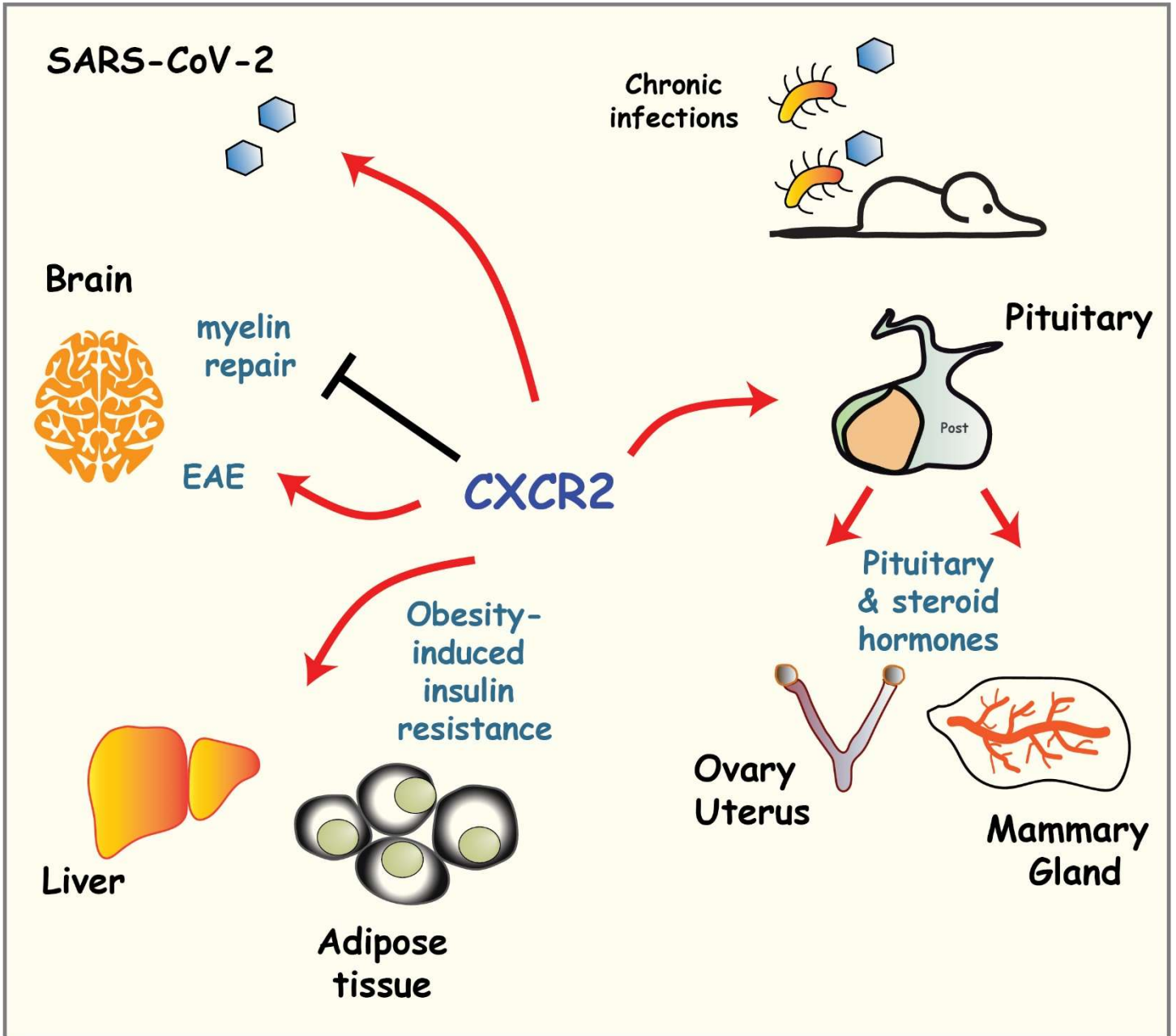


Fig. 2

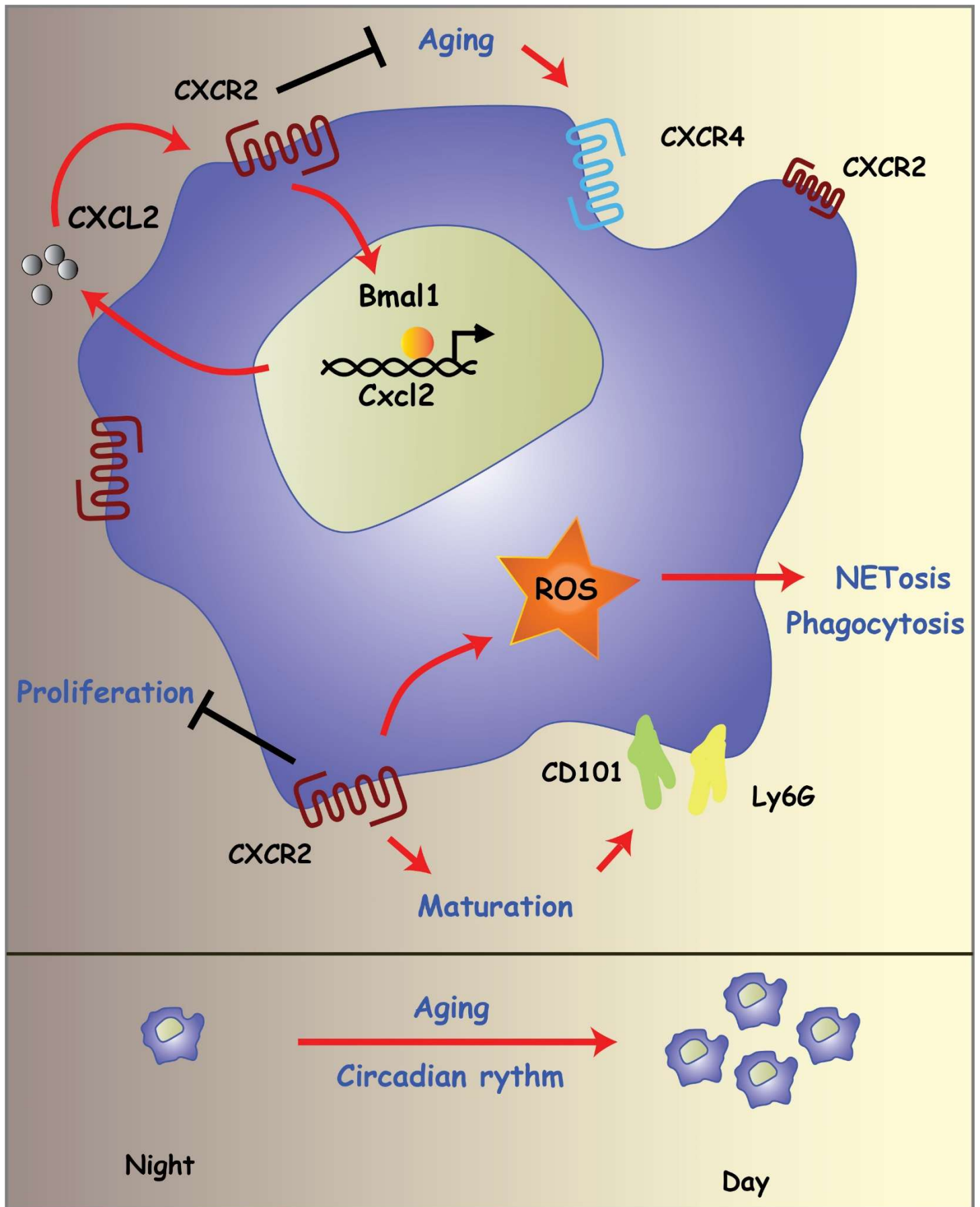


Fig. 3

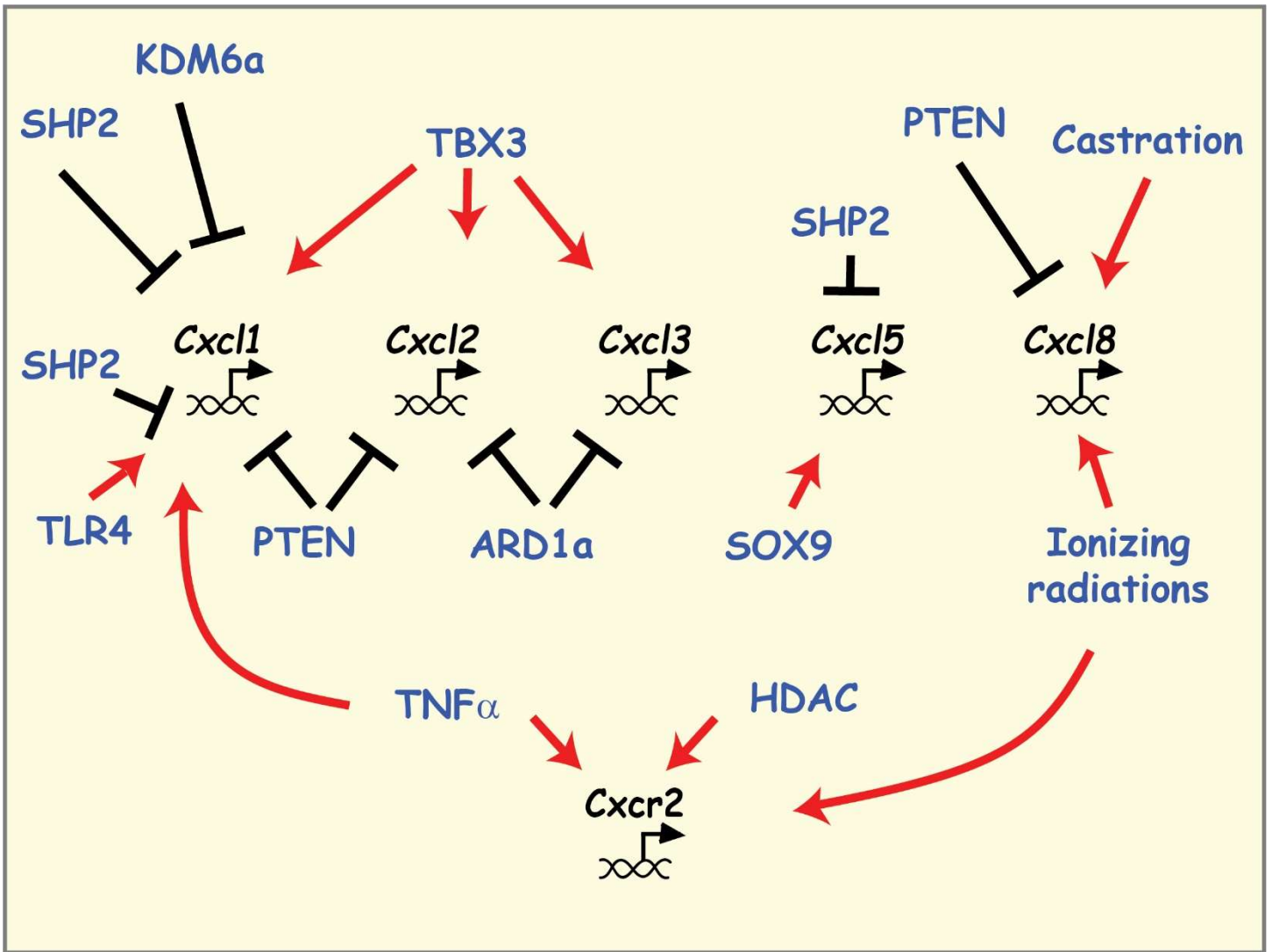


Fig. 4