Dissecting the role of the CXCL12/CXCR4 axis in acute myeloid leukaemia

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Dissecting the role of the CXCL12/CXCR4 axis in Acute Myeloid Leukaemia

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Introduction

Acute Myeloid Leukaemia (AML) is the most common acute leukaemia in adults with the lowest survival rate[1] and an incidence of 602 cases per 100,000 person-years in the United Kingdom[23]. Despite recent advances in its treatment, it remains a therapeutic challenge due to its clinical and biological heterogeneity. AML is a clonal disorder of haemopoietic stem cells. It is characterised by the abnormal proliferation, differentiation and accumulation of immature myeloid cells, which reduces the production of healthy haemopoietic cells[4]. It can arise as a "de novo" malignancy in previously healthy individuals, secondary to other haematological disorders or following previous treatment[3]. Patients usually presents with fatigue, weight loss and signs of bone marrow failure, such as anaemia, thrombocytopenia and neutropenia, and a history of bruising and recurrent infections. Diagnosis involves the presence of more than 20% of blasts in the bone marrow and lineage is usually confirmed by immunophenotyping. It is an aggressive disease and left untreated can lead to death within months.

Prognosis can be affected by both patient related and disease related factors. Old age and a low performance status are associated with a high risk profile and poor prognosis[5, 6]. Cytogenetic analysis at diagnosis is also recognised as an important prognostic factor. In current clinical practice, patients are routinely risk stratified according to their FMS-like tyrosine kinase 3 (FLT3) and Nucleophosmin 1 (NMP1) mutational status to determine the most effective treatment[7]. Two FLT3 mutations are found in AML, the tyrosine kinase domain mutations (TKD, about 5-10% of patients) and in-frame, internal tandem duplication (ITD, about 23% of patients)[8]. FLT3 ITDs mutations in patients with AML are more prevalent with increasing age and are associated with an overall poor clinical outcome[9]. NPM1 mutations are most prevalent in patients with normal karyotype and are associated with a favourable outcome[10]. Conventional treatment includes induction chemotherapy to induce complete remission and consolidation to eliminate any residual leukaemic cells from the bone marrow. This often involves bone marrow transplantation, for patients who are deemed fit enough.

One of the biggest challenges in AML is the high relapse rate following an initial response to chemotherapy. New therapeutic strategies are needed, focusing on the elimination of the remnant chemo-resistant leukaemic cells which 'hide' within the protective niches of the bone marrow (BM). Here, they are surrounded by other cell types, including stromal cells, that promote their survival by enabling them to evade destruction by both their own immune system[11-15] and intra-vascular therapies. This ultimately leads to progression of chemotherapy resistant AML[16]. The mechanism of stromal -mediated protection of leukaemic cells, and their anchorage in the BM, is complex and involves many cytokines, chemokines and adhesion molecules. As shown in Figure 1, the stromal cell-derived factor-1 (SDF-1, also known as CXCL12) and it's receptor, CXCR4 have been implicated as critical mediators. Targeting this axis is an exciting therapeutic strategy and is therefore the focus of this review.

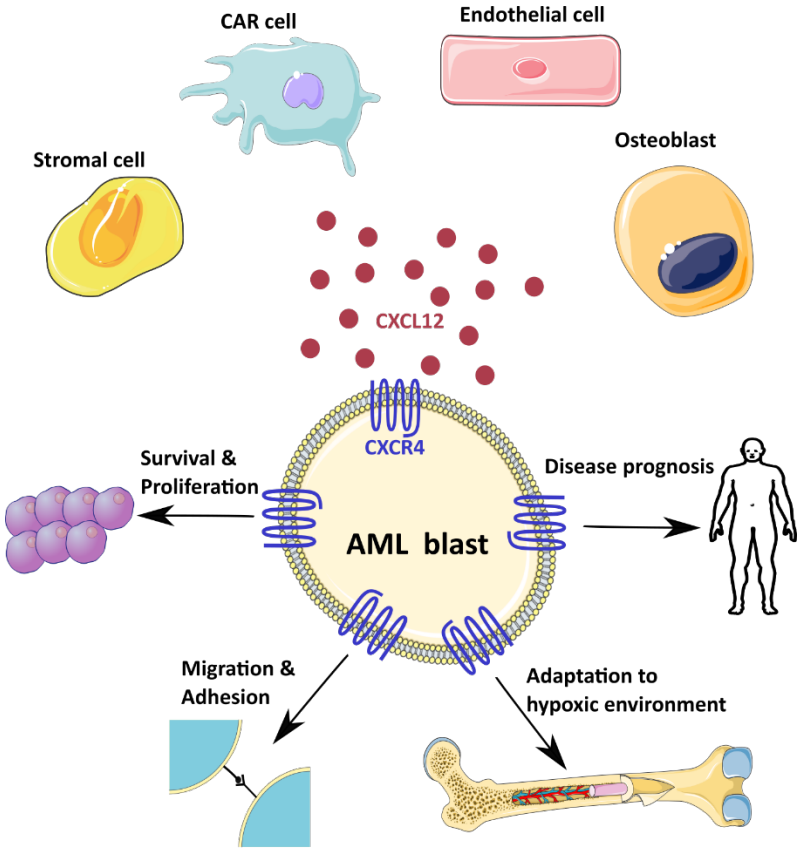

Figure 1: Summary of the main effects of CXCL12/CXCR4 axis in the AML microenvironment

The CXCL12/CXCR4 axis

CXCR4 expression

CXCL12 binds to CXCR4, a G-protein coupled chemokine receptor, which is functionally expressed in several cell types, including the haematopoietic stem cells (HSC). Similar to normal HSC, the majority of AML blast cells, predominately primitive subsets[17], also express CXCR4[18]. Interestingly, undifferentiated AML shows a very limited pattern of chemokine production and chemokine receptor expression, where only CXCR4 is expressed. AML cells with monocytic differentiation or myeloid maturation produce higher levels of chemokines and show a more diverse receptor repertoire which includes CCR1, CCR2, CCR5, CCR6, CXCR1 alongside CXCR4[19]. Although AML cells express variable levels of external CXCR4 on their cell surface, high levels of internal CXCR4 is also found. In particular, Tavor et al showed that internal CXCR4 expression was elevated in all AML cases, including cells that do not express surface CXCR4[20]. Mohle et all also concluded that AML differentiation-related expression of CXCR4 results in preferential activity of CXCL12 in myelomonocytic blasts[21]. Interestingly, Sison et al showed that treatment of AML cell lines (MOLM-14 and MV4-11) with chemotherapy resulted in surface CXCR4 upregulation[22]. This indicates that chemotherapy can dynamically affect CXCR4 expression in order to contribute to stromal protection of leukaemic cells from further chemotherapy-induced apoptosis, thus contributing to leukaemic cell chemoresistance. In the same study, treatment with a CXCR4 inhibitor preferentially decreased stromal protection from higher chemotherapy-induced upregulation of surface CXCR4.

CXCL12 expression

CXCL12 is secreted by several hematopoietic cells, including the more mature $CD34^+CD38^+$ progenitor cells[23]. In vitro experiments have shown that CXCL12 can be detected intracellularly (cytoplasm), on the cell membrane, and in the culture supernatant[24]. Tavor et al investigated the CXCL12 expression on AML cells and confirmed that it is expressed both intracellularly as well as on the cell surface. These results suggest that the malignant cells also secrete this chemokine[20]. However, primary human AML cells have been shown to release detectable CXCL12 in only 10 out of 68 of patients[18] so autocrine CXCL12 /CXCR4 loops are not common in AML. The major sources of CXCL12 in AML BM are stromal cells and CXCL12-abundant reticular (CAR) cells, which are progenitors of mesenchymal stem cells surrounding the sinusoids or located near the endosteum[25]. Endothelial cells and osteoblasts also secrete lower levels[26]. Mice lacking CXCL12 have reduced myeloid progenitors in the bone marrow but not in the foetal liver, indicating that this chemokine is mainly responsible for bone marrow retention and myelopoiesis[27].

Survival and proliferation

Suzuki et al investigated the effect of CXCL12 on $CD4^+$ T cells and showed that cell survival was promoted through this pathway by two main mechanisms: increased transcription of survival genes and posttranslational inactivation of apoptotic genes[28]. The CXCL12 /CXCR4 interaction phosphorylates CXCR4, which then promotes calcium flux and activates signalling pathways, including MEK/ERK, JAK/STAT, and PI3K/AKT axes, thus promoting cell survival[29]. CXCL12 promotes the survival of AML cells and the addition of blocking CXCL12 antibodies or CXCR4 inhibitors significantly decreases it[20]. Schelker et al showed that *in vitro* co-culturing of AML cells on BM-derived human mesenchymal stem cells resulted in greater proliferation, which was significantly reduced when CXCR4 was blocked[30]. Sugiyama et al developed an *in vivo* model using CXCR4 deficient mice and demonstrated that the absence of CXCR4 reduced the HSC pool in the adult bone marrow[25]. Furthermore, in another study the use of Plerixafor, a known CXCR4 antagonist, resulted in decreased proliferation of FLT3-ITD positive blast cells[31].

Migration

The CXCL12 /CXCR4 axis is fundamental for haematopoietic stem cell migration. CXCL12 was the first chemoattractant described for human $CD34^+$ progenitor cells, in which a transient elevation of cytoplasmic calcium was seen with a subsequent chemotactic response[32]. Mohle et al. used conditioned medium from a stromal cell line to show that $CD34^+$ progenitor cells migrate across endothelium in response to CXCL12. They also used recombinant CXCL12 to confirm efficient migration of $CXCR4^+$ leukaemic blasts and a CXCR4 antagonist to effectively inhibit the migratory effect of both[17]. Kalinkovich et al investigated the relationship between the CXCL12/CXCR4 axis and microparticles, which are vesicles shed from the plasma membrane of cells[33]. AML patients had elevated $CXCR4^+$ microparticles and total CXCL12 levels in their peripheral blood and bone marrow when compared to healthy volunteers. Interestingly, the majority of $CXCR4^+$ microparticles were $CD45^+$ in AML patients and $CD41^+$ in normal individuals. The microparticles enhanced the migration of the AML cell line HL-60 to CXCL12 *in vitro* and increased their homing to the bone marrow of irradiated mice; effects reduced by a CXCR4 inhibitor. Burger et al showed that CXCR4 activation and Very Late Antigen-4 (VLA-4) binding are fundamental for AML cell migration beneath stromal cells, a phenomenon called pseudoemperipolesis[34]. Some studies have investigated the phenotype of the migrated cells and showed that CXCL12 preferentially induces transmigration of primitive

$CD34^+CD38^-CXCR4^+$ cells[35]. Interestingly, Voermans et al showed that primary AML cells migrate towards CXCL12, independently of AML subtype[36]. Preferential or diminished migration was observed by leukaemic cells expressing CD34/CD38/HLA-DR and CD14/CD36 respectively. Finally, AML $CD34^+$ cells derived from the bone marrow showed significantly higher CXCL12-induced migration compared to $CD34^+$ cells derived from peripheral blood. Liesveld et al used the CXCR4 inhibitor AMD3100 to confirm inhibition of trans-endothelial transmigration by AML blasts[37].

Adhesion

AML and normal HSC cells adhere to the BM through three main receptors: CXCR4, VLA-4 and CD44[38]. The adhesion of HSC to the BM niche via the CXCL12 /CXCR4 axis is a cooperative process, and is stronger than that by N-cadherin binding, which is also important in cell adhesion[39]. In addition to the effect on survival and migration of HSC and AML blasts, it has been shown that CXCL12 can enhance the activity of integrins thus improving adhesion and retention of $CD34^+$ cells in the BM. During stem cell transplantation, CXCL12 was shown to mediate the homing of human progenitor cells to the BM. The CXCL12 /CXCR4–mediated bone marrow cell anchorage is crucial and it's disruption leads to the release of hematopoietic cells into the circulation, a process regulated by various proteolytic enzymes[40]. Liesveld et al used a static functional co-culture assay, where he cultured stromal or endothelial cells with AML blasts. This study found that the addition of a CXCR4 inhibitor in the co-culture system, did not affect the adhesion of AML cells on the endothelial monolayers. However, treatment with the same inhibitor decreased the expression of other receptors known to be involved in cell adhesion on endothelial cells[37]. Wagner et al demonstrated that primitive human progenitor haematopoietic cell subsets have higher affinity for human mesenchymal stem cells and adherent $CD34^+$ cells express higher levels of genes coding for adhesion proteins and extracellular matrix including fibronectin, cadherin, and vascular cell adhesion molecule-1 (VCAM-1)[41]. Finally, CXCL12 signalling has been shown to induce VLA-4 and Lymphocyte function-associated antigen 1 (LFA-1) on human cord blood cells[42] which in turn induces $CD34^+$ cell adhesion to structures that carry VCAM-1 and intracellular adhesion molecule-1 (ICAM-1)[43].

VLA-4 expression has been associated with chemoresistance in AML. It is an integrin that can bind to VCAM-1, which is expressed by endothelial and stromal cells as well as fibronectin. In contrast to endothelial cells, only low levels of ICAM-1 or VCAM-1 are expressed on stromal cells[35]. *In vitro* studies have confirmed that VCAM-1/VLA-4 pathway is critical for the NF-kB activation in stromal cells and leukaemia cells interaction, which can promote chemoresistance[44]. Matsunaga et al investigated the interactions between VLA-4 on leukaemic cells and fibronectin on stromal cells. This interaction was found to be crucial in mediating adhesion of AML cells on stromal cells and in the presence of fibronectin, VLA-4 positive AML cells were resistant to cytotoxic drugs through the PI-3K/AKT/Bcl-2 signalling pathway[45]. In the same study, the combination of blocking VLA-4 and administering cytosine arabinoside (AraC) achieved 100% survival in a mouse model compared to a very low survival in mice treated with AraC alone[45]. Petty et al investigated the cross talk between CXCL12/CXCR4 and VCAM-1/VLA-4 pathways in neutrophil retention in the BM. They showed that CXCL12 signalling increases VLA-4 adhesion *in vitro*. Blocking both receptors caused a synergistic effect, releasing neutrophils from the BM *in vivo*[46].

Hyaluronic acid (HA) is the main ligand for the adhesion receptor CD44 and their interaction is essential for $CD34^+$ stem/progenitor cell homing into the BM, a process blocked when anti-CD44 antibody is used. Jin et al used an *in vivo* model to show that the administration of a monoclonal

antibody against CD44 resulted in significant reduction in leukaemic repopulation by interfering with the leukaemic stem cell- BM microenvironment[47]. Interestingly, CXCL12 was shown to stimulate progenitor cell adhesion to immobilised HA, which was found to be highly expressed on the endosteum and sinusoidal endothelium where CXCL12 is also abundant. These findings are evidence in support of a potentially significant crosstalk between the two pathways in HSC trans-endothelial migration and anchorage to the BM niche[48].

E-selectin is another important adhesion molecule that plays a fundamental role in AML cell migration and homing. Using an *in vitro* assay, it was shown to contribute to the adhesion of AML blasts on human endothelium[49]. Chien et al used a human AML *in vivo* model to show that an E-selectin inhibitor successfully mobilised AML blasts by blocking their adhesion[50]. In another study, Noguchi et al showed that a minor E-selectin ligand, CD65, was an independent risk factor for extravascular infiltration of AML[51].

Adaptation to hypoxic environment

CXCR4 has also been shown to have a central role in cell adaption to the hypoxic environment. One of the key features of the HSC in the BM microenvironment is their hypoxic profile. They have been shown to exhibit increased pimonidazole (Pimo)[52], a known marker of hypoxia, and express Hypoxia-inducible transcription factor 1 alpha (HIF-1α)[53]. HIF-1α has been detected in many cell culture systems under 5% oxygen (40mmHg) and usually gets degraded by proteasomes under normoxic conditions (21% oxygen)[54]. Interestingly Ceradini et al showed that HIF-1α regulates CXCL12 gene expression in endothelial cells, which increases the migration and adhesion of CXCR4 positive circulatory cells to ischaemic tissue[55]. Using osteosarcoma cells, Guan et al confirmed that hypoxia promotes the expression of HIF-1a and that CXCR4 expression can be upregulated by HIF-1a in hypoxia, a phenotype which persists after the cells are transferred to normoxic conditions[56]. CXCR4 is known to play an important role in the pathology of Chronic Lymphocytic Leukaemia (CLL)[57] and HIF-1a mRNA expression was shown to correlate with that of CXCR4 in CLL patients. In further support, Valsecchi et al demonstrated that co-culturing CLL cells with HS5 stromal cells induces an increase in HIF-1a mRNA which also increases the expression of HIF-1a target genes including CXCR4[58].

Prognosis

The prognostic impact of the CXCL12/CXCR4 axis in leukaemia has been studied in detail. High and intermediate CXCR4 expression on AML cells is associated with decreased overall and relapse-free survival[59]. Raised CXCR4 expression on $CD34^+$ cells in particular is associated with even poorer prognosis and a higher relapse rate. In a study by Rombouts et al it was shown to be significantly higher in FLT3-ITD AML, one of the most frequent mutations in AML, conferring poor response to chemotherapy[60]. However, in another study, CXCR4 expression was shown to be associated with poor prognosis in AML patients irrespective of FLT3 gene mutation status[61].

CXCR4 inhibitors

Several CXCR4 inhibitors have been developed and used in pre-clinical and clinical models for mobilisation of haemopoietic cells. They can be small molecules (e.g. AMD3100), peptides (e.g. BL-8040), or monoclonal antibodies (e.g. Ulocuplumab)[62]. A few of the most important inhibitors that have been tested throughout the years are described below.

Plerixafor (AMD3100)

Plerixafor, previously known as AMD3100, is a CXCR4 receptor antagonist, which was developed to block HIV but found to cause leukocytosis in a phase 1 study in normal volunteers[63]. This finding led to further development of the drug, and it was shown to increase circulating $CD34^+$cells in healthy volunteers and cancer patients when administered alone or in combination with granulocyte colony stimulating factor (G-CSF). G-CSF is known to decrease CXCL12 and upregulate CXCR4, thus also induces stem cell mobilisation[64, 65]. Liles et al reported administration of Plerixafor in 26 healthy volunteers resulted in leukocytosis with only mild, transient toxicity[66]. Plerixafor is now approved for the mobilisation of autologous hematopoietic stem and progenitor cells in patients with multiple myeloma and non-Hodgkin lymphoma[67].

To investigate the effect of blocking and reducing the CXCL12/CXCR4 axis on AML cells, Shen at al devised a biomimetic polystyrene scaffold model propagated with osteoblasts, stromal cells and AML cells. Their results confirmed that combining G-CSF and Plerixafor blocked the protective effect of the other cells on the AML and enabled greater levels of chemotherapy induced apoptosis [68]. Nervi et al used a murine acute promyelocytic leukaemia (APL) model and Plerixafor to examine the interaction of the leukaemia cells with the BM niche. Administration of Plerixafor to mice mobilised leukaemic cells into the peripheral circulation and the spleen, inducing a 1.6-fold increase in total leukocytes and a 9-fold increase in circulating blasts. Plerixafor treatment of the leukaemic mice significantly enhanced chemotherapy efficacy. They also showed that co-culturing of the murine APL cells with M2-10B4 stromal cell line protected them against chemotherapy-induced apoptosis, mainly through soluble factors released from stromal cells (non-contact dependent)[69]. Zhang et al provided insight into the mechanism of action of Plerixafor and another small peptide CXCR4 inhibitor, ALX40-4C. Both drugs were shown to induce CXCL12-like G-protein activation in CXCR4-expressing cells[70]. A number of early phase clinical trials involving Plerixafor in AML have been initiated, investigating its effect in combination with chemotherapy. The most important are summarized below:

Author	Uy GL[71]	Deol A[64]	Cooper TM[72]	Martínez-Cuadrón D[74]	Roboz et al[75]	Michelis et al[73]
Year	2012	2013	2017	2018	2018	2019
Aim	Assess toxicities, remission rate and blast mobilisation	Mobilize stem cells prior to transplantation	Determine a tolerable & biologically active dose	Establish a safe and efficient dose	Investigate the safety and efficacy	Determine the safety and tolerability of Plerixafor
Patients	52 patients with relapsed/ refractory AML	49 patients who failed at least 1 mobilisation attempt	19 patients (13 AML, 5 ALL, 1 MDS)	41 patients with AML	69 patients with newly diagnosed AML	12 patients with AML
Treatment	Plerixafor plus mitoxantrone, etoposide, and cytarabine	Plerixafor and G-CSF	Plerixafor plus cytarabine and etoposide	Plerixafor plus FLAG-IDA	Plerixafor plus Decitabine	Plerixafor plus fludarabine and busulfan
Results	Dose escalated without toxicities. Overall complete remission rate was 46%. There was a 2-fold mobilisation in leukaemic blasts into the peripheral circulation.	2.5×10^6 $CD34^+$ cells/kg were collected in 67% of patients. The cumulative incidence of MDS/AML at 42 months was 17%	Plerixafor mobilised blasts into the peripheral blood (3.4-fold). Surface CXCR4 expression correlated with degree of mobilisation	49% achieved complete remission, 7% died during induction. Median overall and disease-free survivals were 9.9 and 13 months, respectively	Overall response rate was 43%. Most common toxicities were myelosuppression and infection. Plerixafor induced mobilisation of leukaemia stem cells	17% relapsed post-transplant and 50% were alive at the last follow-up. The median follow-up of survivors was 67 months.
Conclusion	The addition of Plerixafor to cytotoxic chemotherapy is feasible and encouraging in AML	Secondary MDS/AML in transplanted patients after Plerixafor mobilisation needs further studying	Plerixafor, in combination with high-dose cytarabine and etoposide, was well tolerated	The combination treatment resulted in a relatively high complete remission with an acceptable toxicity profile	Plerixafor can be safely added to decitabine in poor-prognosis, elderly AML patients	Plerixafor administration is safe and well tolerated. Further study in a larger cohort is warranted

AMD3465

AMD3465 is a selective small molecule CXCR4 antagonist that was initially shown to have a therapeutic potential by mobilizing leucocytes in mice and dogs[76]. It has been described as more potent than Plerixafor and inhibits surface CXCR4 expression on AML cell membrane in a dose dependent manner[77]. Zeng et al showed that AMD3465 induced cell mobilisation in AML cell lines and enhanced the chemotherapy induced apoptosis of primary AML blasts in stromal co-culture systems. AMD3465 in combination with chemotherapy resulted in complete leukaemia eradication using an *in vivo* model. FLT3 mutated AML have been shown to be resistant to both chemotherapy and FLT3 inhibition, and it has been suggested that this is due to the pro-survival pathways (PI3K/Akt and MEK/ERK), which are activated by the stroma[67]. Using transfected murine cell lines, Zeng et al showed that AMD3465 enhanced the apoptotic effects of an FLT3 inhibitor on FLT3-mutated cells, an effect which was greater in hypoxic conditions. Finally, AMD3465 was shown to mobilise FLT3 mutated leukaemic cells (7.5 fold increase in peripheral blood) and enhance apoptosis *in vivo*[29].

BL-8040 (formerly known as BTK140)

BL-8040 (BKT140) is a new generation peptide CXCR4 inhibitor with higher affinity than Plerixafor and causes significant and preferential apoptosis in leukaemia cells[78]. In addition, it has been shown to induce disease regression in primary AML xenograft models[79]. BL-8040 has been used in combination with chemotherapy in a safety and efficacy clinical trial and given to patients with relapsed/refractory AML. The study showed promising initial results, demonstrating that it has potent anti-leukaemic activity, can mobilise leukaemic blasts from the BM to the periphery and may improve the clinical response in combination with chemotherapy[80]. More recent clinical trials demonstrated that BL-8040 induced rapid mobilisation of human $CD34^+$ cells and can be a safe and effective monotherapy prior to transplantation[81]. The combination of BL-8040 and cytarabine in relapsed/refractory AML improves response rate and when used as a single agent BL-8040 can induce mobilisation, differentiation and apoptosis of AML blasts[82]. Abraham et al investigated the effect of BL-8040 on AML cell survival and mobilisation. They showed that BL-8040 induced AML cell apoptosis both *in vitro* and *in vivo* via upregulation of miR-15a/miR-16-1, which subsequently caused downregulation of anti-apoptotic genes including BCL-2, MCL-1 and cyclin-D1. Survival signals via the AKT/ERK pathways were also inhibited, which contributed to the apoptotic effect. Finally, Abraham et al shoed that co-targeting CXCR4 with BCL-2 or FLT3 inhibitors enhanced the apoptotic effect, which provides a rationale for further research in combination therapies to achieve synergistic effects[83].

LY2510924

Cho et al reported that another potent peptide CXCR4 inhibitor, LY2510924, inhibited CXCL12-induced chemotaxis and pro-survival signals of AML cells more effectively than Plerixafor. *In vitro*, LY2510924 inhibited AML cell proliferation and reduced stromal induced protection against chemotherapy. *In vivo*, LY2510924 induced mobilisation of leukaemic cells into the circulation. They concluded that LY2510924 effectively disrupts CXCL12/CXCR4, thus providing an effective anti-leukaemia agent both as a monotherapy as well as in combination with chemotherapy[84].

TN140

TN140 is a small peptide inhibitor that is not yet approved for clinical use. The *in vivo* effects of TN140 were investigated using a murine model of AML. TN140 was shown to functionally block CXCR4 and lead to a reduction in leukaemic cells in the BM following their mobilisation to the blood. After treatment, the leukaemic cells recovered from the BM had a marked decrease in ERK phosphorylation

which suggests that TN140 affects survival signalling pathways. Most importantly, TN140 reduced relapse after secondary transplantation and treated mice had a prolonged survival. Using an *in vitro* assay the authors confirmed that treatment with TN140 inhibits the migration response of CXCL12 and disrupts the adhesion of AML cells on stroma[79].

Ulocuplumab (BMD-936564/MDX-1338)

Kuhne et al. investigated the effect of potent fully human IgG4 monoclonal antibody, Ulocuplumab, that recognises human CXCR4 in AML, non–Hodgkin lymphoma (NHL), chronic lymphocytic leukaemia (CLL) and multiple myeloma and has a longer half-life than Plerixafor. *In vitro* models showed that Ulocuplumab inhibits calcium flux and CXCL12-induced migration. It also has antitumor activity when given as a monotherapy[85]. The first in man phase I clinical trial assessing the clinical benefit of Ulocuplumab in relapsed/refractory AML resulted in improved response rate when used in combination with mitoxantrone, etoposide and cytarabine (MEC). The overall complete remission (CR) and complete remission with incomplete blood count (CRi) recovery rate was 51% versus 24-28%, which has been historically achieved using MEC alone[86].

Conclusion

AML is the most common adult acute leukaemia with the lowest survival rate. It remains a therapeutic challenge due to its heterogeneity and high relapse rate. The CXCL12/CXCR4 axis is central to its pathogenesis and affects leukaemic blast migration, survival and adhesion to the protective BM niche. Here it is sheltered from the toxic effects of chemotherapy. Blocking the CXCL12/CXCR4 axis is an attractive therapeutic strategy and several new CXCR4 inhibitors have been developed with promising initial results. Further investigation into the development of a 'multi-hit' therapy that targets several signalling pathways related to AML cell adhesion and maintenance in the BM is essential. The successful release of the AML cells from the BM into the circulation would enable them to be targeted by conventional chemotherapeutic drugs.

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