At the Bedside: Profiling and treating patients with CXCR4-expressing cancers

Miguel Martin[1] | **Ingrid A. Mayer**[2] | **Annemiek M.E. Walenkamp**[3] | **Constantin Lapa**[4] | **Michael Andreeff**[5] | **Alexandra Bobirca**[6]

[1]Oncology Department, Instituto de Investigación Sanitaria Gregorio Marañón, Universidad Complutense de Madrid, Madrid, Spain

[2]Division of Hematology/Oncology, Vanderbilt University Medical Center, Nashville, Tennessee, USA

[3]University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[4]Nuclear Medicine, Medical Faculty, University of Augsburg, Augsburg, Germany

[5]Section of Molecular Hematology and Therapy, Department of Leukemia, The University of Texas, Maryland Anderson Cancer Center, Houston, Texas, USA

[6]Polyphor Ltd, Allschwil, Switzerland

Correspondence
Miguel Martín, MD, PhD, Oncology Department, Instituto de Investigación Sanitaria Gregorio Marañón, Universidad Complutense de Madrid, C/Dr. Esquerdo 46, Madrid 28009, Spain;
Email: mmartin@geicam.org
Ingrid A. Mayer, MD, MSCI, Ingram Professor of Cancer Research, Professor of Medicine, Division of Hematology/ Oncology, Vanderbilt University Medical Center, 2220 Pierce Ave. 777 PRB, Nashville, TN 37232, USA.
Email: ingrid.mayer@vumc.org

Abstract

The chemokine receptor, C-X-C chemokine receptor type 4 (CXCR4) and its ligand, C-X-C motif chemokine 12, are key mediators of hematopoietic cell trafficking. Their roles in the proliferation and metastasis of tumor cells, induction of angiogenesis, and invasive tumor growth have been recognized for over 2 decades. CXCR4 is a promising target for imaging and therapy of both hematologic and solid tumors. To date, Sanofi Genzyme's plerixafor is the only marketed CXCR4 inhibitor (i.e., Food and Drug Administration-approved in 2008 for stem cell mobilization). However, several new CXCR4 inhibitors are now being investigated as potential therapies for a variety of fluid and solid tumors. These small molecules, peptides, and Abs include balixafortide (POL6326, Polyphor), mavorixafor (X4P-001, X4 Pharmaceuticals), motixafortide (BL-8040, BioLineRx), LY2510924 (Eli Lilly), and ulocuplumab (Bristol-Myers Squibb). Early clinical evidence has been encouraging, for example, with motixafortide and balixafortide, and the CXCR4 inhibitors appear to be generally safe and well tolerated. Molecular imaging is increasingly being used for effective patient selection before, or early during CXCR4 inhibitor treatment. The use of radiolabeled theranostics that combine diagnostics and therapeutics is an additional intriguing approach. The current status and future directions for radioimaging and treating patients with CXCR4-expressing hematologic and solid malignancies are reviewed. See related review - At the Bench: Pre-Clinical Evidence for Multiple Functions of CXCR4 in Cancer. *J. Leukoc. Biol.* xx: xx–xx; 2020.

KEYWORDS

chemokine, CXCL12, CXCR4 antagonist, CXCR4 inhibitor, radioimaging, tumor

ABBREVIATIONS: ADCC, antibody-dependent cell mediated cytotoxicity; ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; BC, breast cancer; BM, bone marrow; BMS, Bristol-Myers Squibb; Bor, bortezomib; BTK, Bruton's tyrosine kinase; CLL, chronic lymphocytic leukemia; CR, complete remission; CRC, colorectal cancer; CRi, complete remission with incomplete blood count recovery; CT, computed tomography; CXCL12, C-X-C motif chemokine 12; CXCR4, C-X-C chemokine receptor type 4; Dex, dexamethasone; DLBCL, diffuse large B cell lymphoma; DOTATATE, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-(Tyr^3)-octreotate; ER, estrogen receptor; ERK, extracellular signal-regulated kinase; ERT, endoradiotherapy; FDA, Food and Drug Administration; FL, follicular lymphoma; FLAG-Ida, fludarabine, idarubicin, cytarabine, and G-CSF; FLT3, FMS-like tyrosine kinase 3; FS, frameshift; GAG, glycosaminoglycan; GBM, glioblastoma multiforme; GEJ, gastro-esophageal junction;; HCC, hepatocellular carcinoma; HER2, human epidermal growth factor receptor 2; HiDAC, high-dose cytarabine; HSC, hematopoietic stem cells; HV, healthy volunteer; I, iodine; ITD, internal tandem duplication; Len, lenalidomide; MBC, metastatic breast cancer; MDS, myelodysplastic syndrome; MEC, mitoxantrone, etoposide, and cytarabine; mrd, minimal residual disease; NFB, N-terminal 4-fluoro-benzoyl; NHL, non-Hodgkin lymphoma; NOTA, 1,4,7-triazacyclononane-N,N′,N″-triacetic acid; NS, nonsense; ORR, objective response rate; OS, overall survival; PCa, prostate cancer; PD-L1, programmed death-ligand 1; PDX, patient-derived xenograft; PET, positron emission tomography; PETHEMA, Programa Español de Tratamientos en Hematología; PFS, progression-free survival; POETIC, pediatric Oncology Experimental Therapeutics Investigators' Consortium; PSA, prostate-specific antigen; QD, quaque die (Once daily); RCC, renal cell carcinoma; rrAML, relapsed/refractory acute myeloid leukemia; SCLC, small cell lung cancer; SDF-1α, stromal cell-derived factor 1α; SOC, standard of care; SPECT, single photon emission computed tomography; TN, triple negative; UK, United Kingdom; US, United States; VEGF, vascular endothelial growth factor; WHIM, warts, hypogammaglobulinemia, infections, and myelokathexis; WM, Waldenstrom macroglobulinemia.

1 | INTRODUCTION

The chemokine receptor, C-X-C chemokine receptor type 4 (CXCR4) and its ligand, C-X-C motif ligand 12 (CXCL12), also known as stromal cell-derived factor 1α (SDF-1α), are key mediators of hematopoietic cell trafficking, and play an important role in solid tumor growth, survival, angiogenesis, and the metastatic process.[1] In addition to facilitating the growth of primary tumors through processes including modulation of angiogenesis and cell proliferation signaling pathways and recruitment of immune cells to tumor sites, CXCR4 allows tumor cells to migrate to sites where CXCL12 is highly expressed, for example, the bone marrow (BM). Inhibition of CXCR4 has the potential to disrupt multiple processes that allow tumors to grow and spread. Given the multiple functions of the CXCR4/CXCL12 axis in several tumor types, this chemokine receptor represents a promising target for imaging and therapy.

To date, the only marketed inhibitor of CXCR4 is plerixafor (AMD3100), which was approved by the Food and Drug Administration (FDA) in 2008.[2] However, a number of companies are investigating CXCR4 inhibitors in the clinical space, including Sanofi Genzyme's plerixafor, BioLineRx's motixafortide (BL-8040), Eli Lilly's LY2510924, Bristol-Myers Squibb's (BMS's) ulocuplumab, X4 Pharmaceuticals' mavorixafor (X4P-001), and Polyphor's balixafortide. This review summarizes the clinical use of CXCR4 inhibitors for patient profiling and the treatment of hematological and solid tumor indications and builds on the preclinical evidence described in the matching Bench review by Luker et al. in this issue.

2 | PATIENT PROFILING AND TREATMENT USING RADIOIMAGING

There are several obstacles known to be associated with current biopsy-based target assessment, such as poor accessibility of lesions, tumor heterogeneity within and between lesions, and sampling errors. Therefore, molecular imaging is increasingly used for effective patient selection before, or early during anticancer treatment. Molecular imaging can also add knowledge on pharmacokinetic parameters, drug-target engagement, patient stratification, and responses to treatment.[3] It includes a wide range of techniques including radiolabeling a compound of interest followed by visualization with single photon emission computed tomography (SPECT) or positron emission tomography (PET). Radiolabeling can be performed using a variety of radionuclides, which are preferably matched to the compound on the basis of size and half-life. Imaging can provide information on drug behavior in vivo, whole-body drug target visualization, and heterogeneity in drug target expression.

The role of CXCR4/CXCL12 in the proliferation and metastasis of tumor cells, induction of angiogenesis, and invasive tumor growth, has been recognized for >2 decades, and described in detail in the matching "Bench" review in this edition. Given the multiple functions of the CXCR4/CXCL12 axis in several tumor types, this chemokine receptor represents a promising target for imaging and therapy. Noninvasive molecular imaging of CXCR4 expression became feasible through the introduction of radiolabeled receptor ligands that allow for whole-body SPECT or PET.[4] For example, labeling of AMD3100 with ^{99m}Tc resulted in specific binding in organs with high levels of CXCR4 expression and CXCR4-positive tumors. The development of [^{68}Ga]-pentixafor can be regarded as a milestone for clinical PET imaging of CXCR4 expression.[4] Proof-of-concept visualization with this tracer could be demonstrated for hematological malignancies such as leukemia, lymphoma, multiple myeloma (MM), and certain solid cancers like adrenocortical carcinoma and small cell lung cancer (SCLC), and also for other pathological conditions, such as splenosis, stroke, atherosclerosis, and myocardial infarction.[4]

2.1 | CXCR4-directed imaging in hematologic and solid malignancies

2.1.1 | Hematologic malignancies

Beyond hematopoietic cells, CXCR4 expression is high among several hematologic malignancies, including non-Hodgkin lymphoma (NHL), MM, chronic lymphocytic leukemia (CLL), and acute myeloid leukemia (AML). To date, most experience with [^{68}Ga]-pentixafor PET imaging has been gained in patients with MM. Following general proof-of-concept in a xenograft mouse model and a few patients with NHL and MM,[5] first disease-specific studies in MM were performed, providing superior or complimentary results (as compared to ^{18}F-fluorodeoxyglucose PET) in 9 of 14 and 23 of 24 patients, respectively.[6,7] Of note, marked inter- as well as intraindividual differences of receptor expression as a sign of myeloma clonal heterogeneity were recorded. In MM patients, CXCR4-targeted PET imaging could be further explored for prognostic stratification and patient selection for CXCR4-directed therapies.

Further proof-of-concept studies have shown the clinical applicability of [^{68}Ga]-pentixafor in both AML and CLL patients: In AML, where the CXCR4/CXCL12 axis is crucially involved in attraction and retention of leukemic cells into the protective BM niche, CXCR4-directed imaging with [^{68}Ga]-pentixafor was able to identify patients with CXCR4-positive AML.[8] Another study revealed that the BM involvement of CLL is associated with significant tracer uptake compared with healthy BM in CXCR4-directed PET imaging using [^{68}Ga]-pentixafor.[9]

These proof-of-concept studies depict the potential of CXCR4-directed PET imaging as a diagnostic tool, and aid in response evaluation in hematologic malignancies. Besides, a promising application might be the selection of patients for personalized therapeutic strategies like CXCR4-directed endoradiotherapy (ERT). Apart from inherent heterogeneity of receptor expression by various tumor clones, this is also partly due to highly dynamic CXCR4 expression levels that are particularly volatile after administration of chemotherapy.[10] Interestingly, up-regulation of surface CXCR4 by chemotherapy could potentially be induced to foster the effects of subsequent ERT.[11]

2.1.2 | Solid malignancies

CXCR4 overexpression has been described in various solid cancers, including breast cancer (BC), prostate cancer (PCa), colorectal cancer

(CRC), and lung,[12] and there is evidence that inhibitors like AMD3465 can act as antitumor agents in BC for example.[13] As with hematologic malignancies, CXCR4 expression in solid tumors denotes more aggressive disease and is associated with an unfavorable prognosis.[14] In line with this, a study by Werner et al. found an inverse correlation between tumor differentiation and CXCR4 surface expression as assessed by immunohistochemistry of surgical samples in neuroendocrine neoplasms.[15]

However, in contrast to the CXCR4 expression profile expected from in vitro studies, the first in vivo, non-invasive whole-body PET/computed tomography (CT) studies revealed a more heterogeneous, modest, and in some cases absent receptor expression of solid cancers[16] with only a few solid tumor types such as SCLC and adrenocortical cancer demonstrating pronounced overexpression of CXCR4 as assessed by PET/CT.[17,18]

In a recently published study, Fang et al. demonstrated increased CXCR4 expression in esophageal malignancies, with immune cells (neutrophils and T cells) rather than esophageal fibroblasts or endothelial cells being the major source of the PET signal.[19] Finally, a first pilot studies demonstrated the general feasibility of CXCR4-directed imaging for detection of glioma.[20,21]

2.1.3 | Theranostics

Radiolabeled compounds are used to determine a treatment strategy by combining therapeutics and diagnostics in the same agent.[22] Theranostic approaches in oncology are particularly interesting because specific Abs are designed against targets on the tumor cell membrane and immune cells as well as targets in the tumor microenvironment. Noninvasive molecular imaging techniques, such as SPECT and PET, provide information on the whole-body distribution of radiolabeled mAbs and Ab-related therapeutics. Examples of successful theranostic agents include the use of radio-iodine for both diagnosis and therapy of benign and malignant thyroid disease: a diagnostic scan with ^{123}I-, ^{124}I-, or a low activity of ^{131}I-iodide is followed by therapy with high activity ^{131}I-iodide.[23] Similar strategies are used for adrenergic tumors such as pheochromocytoma and neuroblastoma. More recently established theranostics include somatostatin receptor-targeting peptides for diagnosis and treatment of neuroendocrine tumors with agents such as [^{68}Ga]-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid-(Tyr^3)-octreotate (DOTATATE) and [^{177}Lu]-DOTATATE,[24] respectively. Finally, theranostic targeting of the prostate-specific membrane Ag has attracted considerable attention given its very promising diagnostic and therapeutic potential.[25]

Although many underlying mechanisms and their implications for disease progression are still unknown, most tumors have a worsening prognosis with increasing CXCR4 expression.[14] For instance, high CXCR4 expression on AML blasts correlates with a poor prognosis.[26] Beyond its role on the tumor cell itself, the CXCR4/CXCL12 axis plays a role in the tumor microenvironment, since the protective BM environment is considered a major reason for treatment resistance and relapse in hematologic neoplasms such as leukemia or MM.[27] Therefore, the development of suitable vectors for theranostic purposes is of particular interest.

2.2 | CXCR4-targeted radionuclide therapy

Pentixather, the therapeutic twin of pentixafor, is a promising CXCR4 ligand that can be labeled with various radionuclides for ERT.[28] CXCR4-directed ERT has been investigated in patient-derived xenograft (PDX) mouse models of acute lymphocytic leukemia (ALL) and AML.[29] This study demonstrated the therapeutic efficacy of this approach, with significant leukemia blast kill. Of note, off-target effects of ERT to hematopoietic and mesenchymal stem cells in the BM niche were also investigated. Whereas specific targeting of CXCR4 on hematopoietic stem cells (HSC) impaired proliferative capacity, mesenchymal stem cells subjected to ERT were viable and capable of supporting the growth and differentiation of non-targeted normal hematopoietic cells ex vivo, despite the substantial in vivo cross-fire effect to the leukemia microenvironment. These encouraging results led to the translation to the human setting, with 3 patients with refractory AML after first allogeneic HSC transplantation successfully undergoing CXCR4-directed ERT.

To date, >50 chemokine receptor-directed ERT have been conducted. Given the high specificity of the therapeutic vector for human CXCR4, the therapy is generally safe and well-tolerated.[30] The largest studies so far examined the use of pentixather, labeled with beta-emitters ^{177}Lutetium or ^{90}Yttrium, for ERT of advanced stage MM patients. Although initial response rates were high, and adverse effects were limited, no overall survival (OS) benefit could be observed in this cohort of heavily pretreated MM patients.[31,32] Another pilot investigation showed encouraging results using ERT with [^{177}Lu]/[^{90}Y]-pentixather in diffuse large B cell lymphoma (DLBCL).[33] Currently, there is only one prospective trial for CXCR4-directed ERT in preparation (COLPRIT trial, EudraCT 2015-001817-28), which will investigate the activity and tolerable dose and side effects of ERT in patients with MM or lymphoma.

Due to its effect on the BM niche, ERT has been performed in addition to high-dose chemotherapy regimens, followed by subsequent HSC transplantation in all cases to date. It is noteworthy that in hematologic diseases, profound myeloablation by ERT prior to autologous or allogeneic hematopoietic transplantation is a desired effect that has already been enhanced by the addition of anticluster of differentiation 66 (CD66)-directed or anti-CD20-directed radioimmunotherapy in some cases.[33]

However, in other (solid) malignancies where hematopoietic cell transplantation is not an established and suitable approach, such myeloablation induced by binding of the radionuclide to hematopoietic progenitor cells in the BM is certainly of concern. ERT without stem cell rescue might be technically feasible for tumors with pronounced receptor overexpression, but this requires further development and prospective investigations.

3 | THERAPEUTIC USE OF CXCR4 INHIBITION IN AML

AML represents a heterogeneous group of hematopoietic malignancies characterized by dysregulated proliferation of HSC and

progenitors, with accumulation of immature myeloid cells in the BM leading to interference of normal blood cell production.[34,35] Despite advances in chemotherapy and allogeneic transplantation, long-term outcomes are poor, and most patients eventually succumb to resistant or relapsed disease.[34] Although most patients initially respond to chemotherapy, the principal cause of treatment failure is relapse due to chemotherapy resistance.[34,36]

In addition to intrinsic cellular mechanisms of drug resistance, leukemic cells have been shown to interfere with the homeostatic mechanisms of normal HSC and take refuge in the BM or stem cell "niche".[37–39] This interaction between malignant AML cells and the BM microenvironment seems to be key to the survival of residual AML cells after chemotherapy and consequent disease relapse. It has been shown that BM stromal cells can confer drug resistance in AML,[39,40] and the homing and adhesion of AML cells to the BM niche is critical to this process.

As described by Luker et al. in this issue, CXCR4 and its ligand, CXCL12, are key mediators of hematopoietic cell trafficking. CXCR4 is widely expressed on hematopoietic cells such as HSCs, T and B cells, monocytes, Mϕs, neutrophils, and eosinophils. It is highly expressed in the BM of adults and is a critical regulator of hematopoiesis; CXCR4 on HSCs controls homing and retention of these cells in the BM.[41,42]

CXCR4 expressed on myeloid cells binds the homeostatic chemokine CXCL12 that is produced by marrow stromal cells in the BM microenvironment and presented to the receptor by glycosaminoglycans (GAGs).[43–45] Once bound to ligand, these CXCR4-expressing BM myeloid cells stay tethered to GAG-bound CXCL12 in the BM. Disruption of CXCR4/CXCL12 interactions results in mobilization of hematopoietic progenitors.[46–49] This function has been targeted therapeutically by using CXCR4 inhibitors such as plerixafor (AMD3100), motixafortide (BL-8040; TN14003/BKT140), and balixafortide (POL6326) to break this tethered interaction and release CXCR4-expressing HSC from the BM into the circulation for recovery and transplantation.[46,50–54]

AML cells can promote their own survival using CXCR4/CXCL12 downstream signaling pathways such as the PI3K/AKT and MAPK/extracellular signal-regulated kinase (ERK) axes.[55] Chen et al. found that CXCR4 down-regulation of the microRNA let-7a, mediated by the transcription factor Yin Yang 1, may be responsible for chemoresistance in AML cells.[37] Overexpression of CXCR4 has been reported in up to 55–65% of AML patients, and patients with high CXCR4 expression in the $CD34^+$ subset of cells have significantly reduced survival and a greater risk of relapse.[26,56–58] CXCR4 inhibition has been shown to reduce resistance of AML cells to chemotherapy in vitro, in vivo, and in clinical trials.[59–61]

Polyphor's synthetic cyclopeptide CXCR4 inhibitors, balixafortide, was found in a murine leukemia model effectively to mobilize engrafted leukemia cells from their protective stromal microenvironment into the circulation, strongly synergizing when combined with G-CSF, and then to enhance the efficacy of the chemotherapeutic agent cytarabine, resulting in significantly reduced leukemia burden and prolonged survival of the animals.[60] In AML-PDX models, Sanofi/Genzyme's plerixafor (Mozobil, AMD3100), BioLineRx's BL-8040, and Lilly's LY2510924 also significantly reduced AML tumor burden.[55,62] Inhibition of CXCR4 has therefore emerged as an attractive therapeutic approach for mobilizing and destroying AML.

3.1 | Clinical use of CXCR4 inhibitors in HSC mobilization

To date, the only marketed inhibitor of CXCR4 is plerixafor, which was approved by FDA in 2008 for combination with G-CSF to mobilize HSC to the peripheral blood for collection and subsequent autologous transplantation in patients with NHL and MM[2]. BL-8040 is currently being evaluated in a phase 3 clinical trial on top of G-CSF for mobilization of HSCs for autologous transplantation in MM patients (NCT03246529). Balixafortide was also shown to be safe and well-tolerated, and provided efficient mobilization of HSCs in a Phase 2a study in MM patients.[54,63]

3.2 | Clinical use of CXR4 inhibitors in AML treatment

The encouraging preclinical results in AML and the demonstrated HSC mobilizing effects of CXCR4 inhibitors prompted their clinical investigation in the treatment of AML. The hypothesis was that inhibition of the CXCR4/CXCL12 axis by CXCR4 inhibitors would disrupt the interaction of leukemic blasts with the BM environment in AML patients and increase the sensitivity of the AML blasts to chemotherapy.

3.3 | Plerixafor

Sanofi Genzyme's bicyclam-type small molecule plerixafor has been the most studied CXCR4 inhibitor for AML treatment, with 10 clinical trials completed and 1 recruiting (Table 1). Of these, 6 trials have been published, and these are summarized below.

Uy et al. at the Washington University School of Medicine in St Louis were the first to study and publish results of CXCR4 inhibitors. In a phase 1/2 study in 52 relapsed/refractory AML (rrAML) patients (NCT00512252), they showed that plerixafor, combined with cytotoxic chemotherapy (mitoxantrone, etoposide, and cytarabine [MEC]), mobilized malignant cells from the BM and increased their sensitivity to chemotherapy, resulting in increased clinical remission, with an overall complete remission (CR) and complete remission with incomplete blood count recovery (CR/CRi) rate of 46%, which compared favorably with published studies of MEC alone.[61,64]

Uy et al. also studied plerixafor in newly diagnosed AML patients, combining it with cytarabine and daunorubicin in a phase 1 study (NCT00990054). Preliminary results in 23 patients showed that plerixafor did not significantly alter the toxicity or hematopoietic recovery expected with the 7+3 regimen used. Transient mobilization of AML blasts was observed immediately following plerixafor treatment, and CR/CRi rate was 76%, despite most patients having intermediate or poor risk cytogenetics.[65]

Cooper et al. in the United States (US) centers of the Pediatric Oncology Experimental Therapeutics Investigators' Consortium were

TABLE 1 Plerixafor trials in AML treatment

Combination	Patients	Phase	Sponsor	Completion	Enrolment	Clinicaltrials.gov
MEC*	rrAML	1/2	Washington Univ. School Med.	June 2010	52	NCT00512252
MEC	rrAML	1	Washington Univ. School Med.	September 2011	6	NCT01027923
Cytarabine, daunorubicin*	Newly diagnosed AML	1	Sanofi Genzyme	March 2012	36	NCT00990054
Daunorubicin, clofarabine, cytarabine	Untreated elderly AML and MDS	1/2	Cardiff University (UK)	January 2014	113	NCT01236144
G-CSF, mitoxantrone, etoposide, cytarabine	rrAML	1/2	Washington Univ. School Med.	September 2015	39	NCT00906945
Clofarabine	Untreated elderly AML	1/2	M.D. Anderson Cancer Center	March 2016	22	NCT01160354
Cytarabine, etoposide*	Relapsed acute leukemia incl AML in children	1	Seattle Children's Hospital (POETIC study)	June 2016	20	NCT01319864
Decitabine*	Untreated elderly AML	1	Weill Med. Coll. Cornell, Genzyme	December 2016	69	NCT01352650
FLAG-Ida*	rrAML	1/2	PETHEMA (Spain)	December 2016	57	NCT01435343
G-CSF, sorafenib*	rrAML with FLT3 mutations	1	M.D. Anderson Cancer Center	March 2017	33	NCT00943943
Busulfan, cyclophosphamide	AML and ALL in CR	2	Salvador Zubiran INCMN (Mexico)	February 2021	20	NCT02605460

ALL, acute lymphocytic leukemia; AML, acute myeloid leukemia; CR, complete remission; FLAG-Ida, fludarabine, idarubicin, cytarabine; FLT3, FMS-like tyrosine kinase 3; G-CSF, granulocyte-colony stimulating factor; MDS, myelodysplastic syndrome; MEC, mitoxantrone, etoposide, cytarabine; PETHEMA, Programa Español de Tratamientos en Hematología; POETIC, Pediatric Oncology Experimental Therapeutics Investigators' Consortium; rrAML, relapsed/refractory acute myeloid leukemia; UK, United Kingdom.
*Published studies.

the first to test the concept of a "chemosensitization" approach in children using plerixafor. They recruited 19 children and young adults with rrAML (13 patients), ALL (5 patients), or myelodysplastic syndrome (MDS) (1 patient) into a phase 1 study (NCT01319864) of plerixafor combined with intensive chemotherapy (high-dose cytarabine and etoposide). Clinical responses in this heavily pretreated cohort were modest: of 18 patients evaluable for response, 3 patients, all with AML, achieved CR/CRi, giving a CR/CRi rate for AML of 23% (3/13). No responses were seen in patients with acute lymphocytic leukemia or MDS. The combination was well-tolerated.[66]

At the other end of the age spectrum, Roboz et al. combined plerixafor with the hypomethylating agent decitabine in 69 newly diagnosed AML patients aged ≥60 years in a phase 1 trial (NCT01352650). Objective response rate (ORR) was 43% (35% CR, 7% CRi, 1% partial remission [PR]), median remission duration was 4.5 months (median follow-up 9.9 months), and median OS was 11.2 months. Prior hypomethylating agent treatment was the strongest independent predictor of reduced OS (hazard ratio 3.1) and response (14% in previously treated patients, 46% in treatment-naïve). Median OS was 10.9 months in 59 patients without a *TP53* mutation and 18.1 months in the 10 patients with *TP53* mutation; although this result was not statistically significant (possibly due to the small sample size), multivariate analysis showed adverse karyotype to be a significant predictor of poor OS.[67]

Most recently, Martinez-Cuadron et al. in the Programa Español de Tratamientos en Hematología (PETHEMA) group published the results of a phase 1/2 trial (NCT01435343) that studied FLAG-Ida (fludarabine, idarubicin, cytarabine, and G-CSF) plus plerixafor in adult patients (median age 52 years) with early-relapsed (first CR/CRi < 12 months) or primary refractory AML. Of 57 patients enrolled, 41 received the recommended phase 2 dose, of whom 20 (49%) achieved CR/CRi. Median OS and disease-free survival were 9.9 and 13 months, respectively. This "PERIFLAG" combination led to a relatively high CR/CRi rate in adult patients with primary refractory or early relapsed AML and bridged the majority of patients to allogeneic stem cell transplantation.[68]

The multikinase inhibitor sorafenib (Nexavar) was also studied in combination with plerixafor, in FMS-like tyrosine kinase 3 (FLT3)-internal tandem duplication (ITD) mutated AML. Mutations of the FLT3 gene carry a poor prognosis and occur in ~30% of all AML cases, with FLT3-ITD representing the most common type of FLT3 mutation (25% of all AML cases).[69]

Zeng et al. had previously shown that CXCR4 inhibition by a plerixafor analog (AMD3465) increased the sensitivity of FLT3-mutated leukemic cells to the apoptogenic effects of sorafenib.[59] Zeng et al. therefore designed a Phase 1 trial (NCT00943943) testing the combination of plerixafor, sorafenib, and G-CSF in patients with FLT3-ITD mutated rrAML. A preliminary report on the first 13 patients showed CR with incomplete platelet recovery in 4 of 13 (31%) patients and PR in 6 of 13 (46%) patients for an ORR of 77%. They also noted a massive mobilization of blasts (41×) and stem/progenitor cells (68–231×) compared with plerixafor alone (blast increase 2.1×).[61,70] In summary,

the published data from these phase 1/2 clinical trials suggest that the combination of plerixafor with chemotherapy has an acceptable safety profile, mobilizes leukemic cells into the peripheral circulation, and results in encouraging remission rates.

3.4 | Motixafortide (BL-8040)

Based on encouraging preclinical data showing long receptor occupancy and extended CXCR4 inhibition as well as direct proapoptotic activity against AML cells,[55,71] and clinical demonstration of HSC mobilization, BioLineRx's peptidic CXCR4 inhibitor motixafortide (BL-8040) is also being developed for the treatment of AML (Table 2).

Motixafortide in combination with high-dose cytarabine was studied in a phase 2a trial (NCT01838395) in 42 adult patients with relapsed/refractory AML in the United States and Israel, using a dose escalation (3+3) design, followed by an expansion at the selected dose of 1.5 mg/kg. Data were presented at the 2018 European Hematology Association conference.[72] The combination was safe and well tolerated. In the 23 patients receiving the expansion dose, the remission rate (CR/CRi) was 39% (9/23) and median OS was 9.2 months, comparing favorably with historical data on OS of 6.1 months for high-dose cytarabine (HiDAC) alone,[73] with 1- and 2-year survival rates of 31.6% and 21.1%, respectively.

Motixafortide is also being tested in combination with HiDAC as consolidation AML therapy in a phase 2b study (NCT02502968) of the German Study Alliance Leukemia Group. This double-blind, placebo-controlled, randomized, multicenter study aims to enroll up to 194 patients. The primary endpoint is relapse-free survival during a minimum follow-up time of 18 months. Interim results are expected in late 2019.

A third trial (NCT03154827) is studying the safety, tolerability, and efficacy of Motixafortide combined with the programmed death-ligand 1 (PD-L1) inhibitor atezolizumab as maintenance treatment in 60 AML patients aged 60 years or older in the United States, Israel, Czech Republic, Poland, Slovakia, and Spain (the BATTLE Study).

3.5 | LY2510924

In preclinical AML models, the peptidic CXCR4 inhibitor LY2510924 rapidly and durably blocked surface CXCR4 and inhibited CXCL12–induced chemotaxis and pro-survival signals of AML cells more effectively than plerixafor, and, in mice with AML, demonstrated antileukemia effects as monotherapy and in combination with chemotherapy.[62] LY2510924 is being studied in a phase 1 trial (NCT02652871) combined with idarubicin and cytarabine in 36 adult patients with relapsed or refractory AML (Table 3).

Initial results on the first 11 patients enrolled were published in 2018.[74] LY2510924 was administered daily for 7 days followed by chemotherapy from day 8. Two dose escalation levels of LY2510924 (10 and 20 mg) were evaluated, with a plan to enroll up to 12 patients in the phase 1 portion. Six patients received 10 mg, of whom 3 had CR, while 1 of 5 patients receiving 20 mg had a CR, giving an ORR of 36% (4 of 11 patients). The combination appeared to be safe in this study. Flow cytometry indicated incomplete suppression of CXCR4-receptor occupancy in some patients, and so dose-escalation to a 30 mg LY2510924 dose was planned in order to increase blockade of CXCR4 receptors, followed by an expansion phase at the recommended phase 2 dose-level.

3.6 | Ulocuplumab

The fully human immunoglobulin G4 (IgG4) anti-CXCR4 mAb ulocuplumab (BMS-936564/MDX-1338) was shown to induce apoptosis in vitro and showed in vivo antitumor activity as monotherapy in AML xenograft models.[75] A phase 1 dose escalation/expansion trial (NCT01120457; see Table 4) in 9 US centers determined the maximum tolerated dose (MTD) of ulocuplumab and assessed safety and tolerability of the drug combined with chemotherapy in 96 patients with rrAML or selected B-cell cancers. Results for the 73 patients with rrAML were published in 2014.[76] The chemotherapy used was MEC (mitoxantrone, etoposide, cytarabine). Thirty subjects in escalation received a single infusion of ulocuplumab (0.3, 1, 3, or 10 mg/kg) 1 week prior to starting MEC and 3 additional weekly doses per MEC cycle thereafter. Ulocuplumab was escalated to a maximum of 10 mg/kg without any dose-limiting toxicity during monotherapy or in combination with MEC in the first cycle. In the expansion phase, 43 patients received 10 mg/kg ulocuplumab and MEC. The overall CR/CRi was 51%, comparing favorably with the historic response rate for MEC alone (24–28%).[64] Of note, 4 subjects had CR/CRi after a single dose of ulocuplumab monotherapy. A 5-fold mobilization of leukemic blasts into the peripheral circulation was seen at Day 8. The safety profile in combination with MEC was similar to MEC alone.

A subsequent ongoing phase 1/2 study (NCT02305563; see Table 4) in 45 centers across 10 countries is studying ulocuplumab combined with low-dose cytarabine in 68 patients with newly diagnosed AML who are considered inappropriate for intensive remission induction therapy and who are not eligible for stem cell transplantation.

3.7 | PF-06747143

A phase 1 clinical trial (NCT02954653) evaluating Pfizer's CXCR4 inhibitor IgG1 Ab PF-06747143 alone and in combination with chemotherapy in AML patients enrolled 8 patients but was terminated due to change in sponsor prioritization (see Table 5). In January 2018, Pfizer announced the discontinuation of PF-06747143 development.[77]

3.8 | Summary and future perspectives

AML remains a disease with a dismal prognosis, and novel treatment approaches are particularly welcome. The concept of coaxing malignant cells out of the protective BM microenvironment to allow more effective antitumor therapy seems to be translating into clinical effect at the bedside. These early stage trials provide encouraging clinical evidence supporting the preclinical rationale showing that CXCR4 inhibitors can mobilize leukemic cells into the peripheral circulation

TABLE 2 Motixafortide trials in AML treatment

Combination	Patients	Phase	Sponsor	Completion	Enrolment	Clinicaltrials.gov
Cytarabine	rrAML	2a	BioLineRx	June 2016	42	NCT01838395
Cytarabine	AML consolidation	2b	Dr. Petra Tschanter	July 2018	194	NCT02502968
Atezolizumab	AML in CR/CRi	1b/2	BioLineRx	March 2022	60	NCT03154827

AML, acute myeloid leukemia; CR, complete remission; CRi, CR incomplete blood count recovery; rrAML, relapsed/refractory AML.

TABLE 3 LY2510924 trial in AML treatment

Combination	Patients	Phase	Sponsor	Completion	Enrolment	Clinicaltrials.gov
Idarubicin + Cytarabine	rrAML	1	M.D. Anderson Cancer Center	May 2022	36	NCT02652871

AML, acute myeloid leukemia; rrAML, relapsed/refractory acute myeloid leukemia.

TABLE 4 Ulocuplumab trials in AML treatment

Combination	Patients	Phase	Sponsor	Completion	Enrolment	Clinicaltrials.gov
MEC	rrAML and selected B cell cancers	1	Bristol-Myers Squibb	November 2014	96	NCT01120457
Cytarabine	Newly diagnosed AML, inappropriate for intensive induction	1/2	Bristol-Myers Squibb	September 2021	68	NCT02305563

AML, acute myeloid leukemia; MEC, mitoxantrone, etoposide, cytarabine; rrAM, relapsed/refractory acute myeloid leukemia.

TABLE 5 PF-06747143 trial in AML treatment

Combination	Patients	Phase	Sponsor	Completion	Enrolment	Clinicaltrials.gov
Cytarabine, daunorubicin, azacitidine, decitabine	rr/mrd AML	1	Pfizer	December 2017	8	NCT02954653

AML, acute myeloid leukemia; mrd, minimal residual disease; rrAML, relapsed/refractory acute myeloid leukemia.

and enhance their killing by chemotherapy. CR/CRi rates seem promising compared with historic rates for chemotherapy alone, but this will need to be proven in larger randomized trials. The new drugs appear to be reasonably safe and well-tolerated in monotherapy and in combination with chemotherapy.

Of these 18 AML clinical trials, the majority have addressed areas of particular medical need, with 10 trials in relapsed/refractory disease, and 3 in untreated elderly patients. The remaining trials have explored CXCR4 inhibitors as chemosensitizers in newly diagnosed patients, or to consolidate or maintain CR/CRi after chemotherapy. Only one small study, in relapsed AML, included children. The observation that BMS's ulocuplumab induced CR/CRi in some patients after a single dose as monotherapy suggests potential additional mechanisms of action for human IgG1 Abs, such as Ab-dependent cell mediated cytotoxicity (ADCC)/phagocytosis of malignant cells,[78] with relevance to patients resistant to, or unable to, tolerate intensive chemotherapy.

It is encouraging to see approvals of three new AML drugs in late 2018: Pfizer's glasdegib and Roche's venetoclax, both in combination with low dose cytarabine in newly diagnosed AML patients, and Astellas' gilteritinib for rrAML patients who have FLT3 mutations (approximately the 25–30% of AML patients).[69] However, there remains a significant need for better treatment approaches for the high proportion of patients whose disease relapses, is refractory to treatment, or is associated with other mutations. Future clinical trials of small molecule and antibody CXCR4 inhibitors should explore additional combinations, including those with targeted therapies, and schedules to optimize efficacy/safety benefit, especially in the youngest and oldest patients.

4 | THERAPEUTIC USE OF CXCR4 INHIBITION IN WM, NHL, AND MM

Waldenstrom macroglobulinemia (WM) is a B-cell neoplasm representing ~2% of all cases of NHL. WM is characterized by the accumulation of malignant IgM-secreting lymphoplasmacytic cells in the BM, lymph nodes, and spleen. The excess presence of serum IgM leads to symptoms related to autoimmune-related reactions, tissue infiltration, and hyperviscosity.

On the genetic level, the lymphoplasmacytic cells of WM patients frequently (>90%) carry the L265P mutation in the protein myeloid differentiation primary response 88 (MYD88) gene. In addition, mutations in CXCR4 are present in 30–35% of all WM cases.

Mutations in CXCR4 are related to similar mutations, mostly located in the C-terminus, in patients with warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM) syndrome. Both

nonsense (CXCR4WHIM/NS) and frameshift CXCR4 mutations (CXCR4WHIM/FS) occur in WM patients with approximately equal number of cases; however, the clinical manifestations are different.

Adenopathy is frequent in patients with CXCRWHIM, regardless of NS or FS mutation. CXCR4WHIM/NS is characterized by more aggressive disease features, such as hyperviscosity syndrome that requires therapy, higher serum IgM levels, and stronger BM disease burden, suggesting a more pronounced BM tropism and stronger adhesion of CXCR4WHIM-mutated WM cells. Surprisingly, the OS for CXCR-WHIM/NS patients is not adversely impacted despite the more aggressive disease manifestation, suggesting that not all roles and functions of CXCR4 in WM are completely understood. In addition, the CXCR4 biology in WM and WHIM is likely to be different, because very few patients with WHIM syndrome have a FS mutation, while nearly all have NS mutations (R334X or S338X).

Two recently launched clinical studies in WM patients, 1 interventional at the Centre Henri Becquerel in France (NCT03952052) and the other observational in 300 patients in multiple centers across Italy (NCT03521596), may help to get a better understanding about genetic status of CXCR4 and clinical manifestation in WM. Treatment with rituximab is an established therapy for CD20-positive B-cell malignancies, including WM. Preclinical studies suggested a close functional link of CXCR4 to Bruton's tyrosine kinase (BTK) in WM.[79] WM cells carrying the S338X NS CXCR4 mutation were resistant to the BTK inhibitor ibrutinib (Imbruvica)-dependent AKT and ERK1/2 signaling.[80] The resistance could be resolved by plerixafor. In addition, BTK is activated by CXCR4. Based on impressive activity in relapsed and refractory WM,[81] the FDA approved ibrutinib as a breakthrough therapy for WM in January 2015 and for use in combination with rituximab as a treatment option across all lines of therapy for WM patients in 2018.[82] The 30-month progression-free survival (PFS) was 82% for ibrutinib-rituximab versus 28% for placebo-rituximab, and the benefit of the combination was observed across key subgroups. The 24-month PFS rate in treatment-naive patients was 84% in the combination arm versus 59% in the control arm. In relapsed patients, the 30-month PFS rates were 80% versus 22%, respectively. In the overall population, the ORR was 92% with the ibrutinib combination versus 47% with rituximab alone. The major response rate (defined as CR, very good PR or PR) was 72% versus 32%, respectively. At a median follow-up of 26.5 months, 75% of patients in the combination arm remained on treatment. The OS rate at 30 months was 94% versus 92%, in the combination versus control arms, respectively.

Despite plentiful evidence of ibrutinib activity in WM, clinical progression occurs while on therapy, and new therapy options are being studied, including venetoclax, a B-cell CLL/lymphoma 2 (BCL2) inhibitor (NCT02677324), and ulocuplumab (BMS-936564/MDX1338), a fully human mAb that targets CXCR4 (NCT03225716). This phase 1/2 single center study with ulocuplumab in combination with ibrutinib was launched in October 2017 and the expected primary completion date is January 2021. The study will include 38 WM patients with mutations in both MYD88 and CXCR4. Patients will receive ibrutinib oral once daily (QD) and ulocuplumab administered intravenously 2–4 times per cycle for cycles 1–6. Ulocuplumab was lacking efficacy and was discontinued in other cancer indications such as AML (NCT01120457) and solid tumors (NCT02472977). However, small molecule (e.g., from X4 Pharmaceuticals) or peptidic inhibitors (e.g., from Polyphor and BioLineRx), which are still in clinical studies, may offer promising treatment options for the CXCR4 target. X4 Pharmaceuticals recently announced plans to conduct a multinational Phase 1/2 clinical study to evaluate the safety and assess the preliminary antitumor activity of mavorixafor in combination with ibrutinib in WM patients. The study is planned to commence in 2019.

CXCR4 inhibitors have also been studied in hematological indications other than ALL and WM. Based on promising in vitro, ex vivo, and in vivo data in animal models,[83] BMS started trials of ulocuplumab in NHL including follicular lymphoma (FL), DLBCL, and CLL. A phase 1, open-label, multicenter study of ulocuplumab monotherapy study began in 2010 (NCT01120457); 96 AML patients were enrolled receiving 0.3–10 mg/kg ulocuplumab infusions for 7 days in cycle 1 and a combination with chemotherapy for subsequent 28 days cycles. FL, DLBCL, and CLL patients received weekly 60-min ulocuplumab infusions on the basis of the AML patient dose in the first cycle up to 56 days and in combination with chemotherapy for subsequent 28-day cycles. First clinical data from 3 subjects of the CLL patient group were disclosed at the 2013 American Society of Hematology (ASH) conference.[84] Leukocytosis was present during the entire 4 weeks of monotherapy, primarily driven by an increase in absolute counts of CLL cells (median increase of 129.6%; range: 95.3–324.8%). Surprisingly, although reported mostly for peptidic CXCR4 inhibitors, there was no increase in the absolute number of normal lymphocytes, and only 1 subject had increased neutrophil counts. Additional data from the CLL patient group in NCT01120457 were published in 2016.[85] Data in AML, FL, or DLBCL were not disclosed, but mechanistic studies on ulocuplumab-induced apoptosis in CLL were described. Apoptosis was driven not through complement-dependent cytotoxicity ADCC, but rather through caspase-independent induction of reactive oxygen species. Unfortunately, there was no information disclosed about ulocuplumab-induced apoptosis in lymphocytes or other CXCR4-positive normal cells.

Safety and tolerability of ulocuplumab was further evaluated in another phase 1b study launched in 2011 in 44 patients with relapsed/refractory multiple myeloma alone and in combination with lenalidomide/dexamethasone (U-Len-Dex) or bortezomib/dexamethasone (U-Bor-Dex) (NCT01359657). The dose regimen in cycle 1 was more complex. Ulocuplumab (1, 3, and 10 mg/kg) was administered as monotherapy on days 1 and 8. Starting on Day 15, ulocuplumab was administered in combination with lenalidomide at 25 mg/day for 21 days of a 28-day cycle plus low dose dexamethasone 40 mg/week. For the U-Bor-Dex group, also starting on Day 15, ulocuplumab was administered in combination with Bor at 1.3 mg/m^2 on days 1, 4, 8, and 11 of a 21-day cycle plus dexamethasone on days 1, 2, 4, 5, 8, 9, 11, and 12 and subjects were monitored for incidence of dose limiting toxicity within cycle 1 of study treatment. For the expansion phase, subjects received 10 mg/kg ulocuplumab monotherapy on days 1 and 8 followed by weekly

doses in combination with Len-Dex in 28-day cycles. Interestingly, ulocuplumab was escalated to a maximum of 10 mg/kg without reaching MTD in monotherapy or in combination therapy. The safety profile of ulocuplumab in U-Len-Dex or U-Bor-Dex was similar to that of either combination alone. The ORR by group was 55% and 40% for U-Len-Dex and U-Bor-Dex, respectively, and 57% in the U-Len-Dex 10 mg/kg expansion. A rather limited 2-fold mobilization of leukocytes into the peripheral circulation was reported after each infusion of ulocuplumab at 3 and 10 mg/kg. Based on promising data that were disclosed at the ASH conference in 2018,[86] BioLineRx started a phase 2a study in 2016 with 20 patients on BL-8040 in combination with nelarabine for relapsed or refractory T-ALL/lymphoblastic lymphoma (NCT02763384). In cycle 1, BL-8040 was injected subcutaneously daily from day 1 to 6, and nelarabine intravenously on days 2, 4, and 6. In subsequent 21-day cycles 2–4, BL-8040 was injected only from day 1 to 5, and nelarabine intravenously on days 1, 3, and 5. In addition to safety and tolerability, induction of apoptosis, inhibition of CXCR4 intracellular signaling, cell cycle status, neutrophil, and lymphoblast counts will be assessed. The study is expected to be completed in 2021.

5 | THERAPEUTIC USE OF CXCR4 INHIBITION IN SOLID TUMORS

5.1 | Introduction

The enhancement by CXCR4 of tumor growth, invasion, and metastasis in multiple solid tumors, and the correlations between CXCR4 expression and disease progression described in the Bench review, have led to CXCR4 inhibitors being studied therapeutically in several cancers, either as monotherapy or in combination with chemotherapies or immunotherapies. Eleven drugs across three classes (small molecule, peptide, and Ab) have been tested in patients with advanced cancers, and 1 drug only in healthy volunteers.

5.2 | Small molecule CXCR4 inhibitors

As can be seen in Table 6, Sanofi's marketed drug plerixafor (Mozobil, AMD3100) leads the so far largest group of drugs, the small molecule CXCR4 inhibitors, with 5 clinical studies in solid tumors. All studies with exception of NCT00591682 are ongoing or were recently concluded.

There have been two phase 1 studies in advanced pancreatic, ovarian, and CRC. Both trials are aiming at a better understanding of changes of the immune system in the tumor microenvironment. One trial is still active but not recruiting (NCT03277209), and the other trial ("CAM-PLEX," NCT02179970) at the CRUK Cambridge Institute and the University of Cambridge, UK was recently completed with 26 recruited patients. Cancer tissue (biopsies) and blood samples from subjects were taken before and after they received plerixafor. Based on the pharmacokinetics and safety profile from CAM-PLEX, a recommended infusion rate was identified for further study and translational studies identified changes in the tumor microenvironment, which have led to the design of a phase 2 trial combining plerixafor with an immune-checkpoint inhibitor in pancreatic cancer patients.[87]

A phase 1 study of plerixafor, combined with bevacizumab, in 26 patients with recurrent high-grade glioma (NCT01339039) showed that the combination plerixafor 320 μg/kg on days 1–21 and bevacizumab 10 mg/kg on days 1 and 15 of each 28-day cycle was well-tolerated, while plerixafor distributed to both the cerebrospinal fluid and brain tumor tissue, and treatment was associated with biomarker changes consistent with vascular endothelial growth factor (VEGF) and CXCR4 inhibition (increase in plasma SDF-1α). Interestingly, PFS correlated with pretreatment plasma soluble mesenchymal–epithelial transition receptor and sVEGFR1, and OS with the change during treatment in $CD34^+$ progenitor/stem cells and CD8 T cells.[88]

Twenty-nine patients with newly diagnosed glioblastoma were treated with plerixafor after surgical resection, temozolomide, and radiotherapy in a single group phase 1/2 study at Stanford University (NCT01977677). Plerixafor was given IV continuously for 2–4 weeks (3 patients received 200 ug/kg/day, and 26 patients received 400 ug/kg/day), beginning 8 days prior to completion of chemoradiotherapy. Although patient numbers are too small to draw statistically significant conclusions, the authors reported in their www.clinicaltrials.gov entry that 19 of 20 evaluable patients in the dose expansion cohort had PFS at 6 months. A large study of newly diagnosed patients by Stupp et al. had previously shown median PFS of 6.9 months and 6-month PFS rate of ~60% in the temozolomide plus radiotherapy arm.[89] Magnetic resonance imaging showed a marked decrease in relative cerebral blood volume in the radiation treatment field, suggesting enhanced local treatment effect in support of the investigators' hypothesis that inhibition of the CXCR4/CXCL12-mediated vasculogenesis pathway in the post-radiotherapy period enhances radiation.[90] The same center has expanded this study into a phase 2 study (NCT03746080) with the addition of whole brain irradiation. Plerixafor was given by continuous infusion (400 μg/kg/day) for 4 weeks, beginning 7 days before the completion of whole brain radiation therapy.

X4 Pharmaceuticals' mavorixafor (X4P-001, AMD11070, AMD070) was studied in 16 patients with advanced melanoma as monotherapy and in combination with pembrolizumab (NCT02823405). Results for single agent mavorixafor were presented at SITC 2018; tumor biopsies showed that mavorixafor modulated the immune cell profile in the tumor microenvironment and increased $CD8^+$ T cell infiltration.[91] Mavorixafor was also studied in 2 clinical studies in clear-cell renal cell carcinoma (RCC). Vaishampayan et al. reported results from the phase 1 portion of a study in combination with axitinib (NCT02667886); the combination was well tolerated at a dose of 400 mg QD of mavorixafor with preliminary evidence of clinical activity.[92] The phase 2 portion of the study is complete. A small study (9 patients) of mavorixafor combined with nivolumab (NCT02923531) also showed potential antitumor activity, with 1 patient showing a partial response to the combination after stable disease with nivolumab alone, and a manageable safety profile.[93]

TABLE 6 Small molecule CXCR4 inhibitors – clinical studies in solid tumors

CXCR4 inhibitor	Indications	Phase	Completion	Study identifier
Plerixafor (Mozobil, AMD3100)	Pancreatic, ovarian or colorectal cancer, advanced	1	December 2018	NCT02179970*
	Pancreatic, ovarian and colorectal adenocarcinomas	1	December 2020	NCT03277209*
	Glioma, recurrent high-grade	1	April 2017	NCT01339039*
	Glioma, newly diagnosed high-grade	1/2	September 2018	NCT01977677*
	Glioblastoma, newly diagnosed	2	January 2027	NCT03746080*
Mavorixafor (X4P-001, AMD11070, AMD070)	Melanoma, advanced	1	March 2018	NCT02823405
	Clear cell renal cell carcinoma	1/2	March 2019	NCT02667886
	Clear cell renal cell carcinoma	1/2	August 2018	NCT02923531
Burixafor (TG-0054)	Prostate cancer, metastasized to bone	1	May 2017	NCT02478125*
MSX-122	Solid tumors, refractory metastatic, or locally advanced	1	March 2009	NCT00591682
GMI-1359	Target indication to be confirmed in 2020	1 (HV)	November 2018	NCT02931214
	HER2 positive metastatic breast cancer	1	December 2019	NCT04197999
USL311 (PRX177561)	Solid tumors, advanced; GBM, relapsed/recurrent	1/2	September 2022	NCT02765165

CXCR4, C-X-C chemokine receptor type 4; GBM, glioblastoma multiforme; HER2, human epidermal growth factor receptor 2; HV, healthy volunteers.
*Investigator-initiated trial.

TaiGen's burixafor phase 1 study (NCT02478125) in PCa closed early due to poor accrual, while Metastatix's MSX-122 phase 1 in solid tumors (NCT00591682) was suspended for unknown reasons.

GlycoMimetics completed a Phase 1 (NCT02931214) in healthy volunteers of their E-selectin- and CXCR4-inhibitor GMI-1359, and in 2019, the company expects to announce the first patient population for further clinical testing. Preclinical data suggest potential in BC, AML, and colon cancer. In December 2019, GlycoMimetics launched a Phase 1 study in six metastatic human epidermal growth factor receptor 2 (HER2)-positive BC patients.

Finally, Upsher Smith have started recruitment of 120 patients into a phase 1/2 study (NCT02765165) of USL311, with or without lomustine, in advanced solid tumors and relapsed/recurrent glioblastoma.

5.3 | Peptide CXCR4 inhibitors

The peptide inhibitors, summarized in Table 7, represent the most advanced programs of CXCR4 inhibition in solid tumors, including the first phase 3 study.

Eli Lilly's cyclic peptide LY2510924 has not demonstrated efficacy in solid tumors. An initial dose-escalation phase 1 demonstrated $CD34^+$ cell mobilization and defined the MTD,[94] but a phase 1 study in combination with durvalumab (NCT02737072) was terminated, as was a phase 2 in RCC as first-line treatment in combination with sunitinib (NCT01391130), which showed no benefit of LY2510924 over sunitinib alone.[95] An additional Eli Lilly phase 2 study in SCLC (NCT01439568) also showed no benefit of adding LY2510924 to standard of care (SOC) chemotherapy.[96] Median PFS was 5.88 months for LY+SOC versus 5.85 months for SOC. Median OS was 9.72 months for LY+SOC versus 11.14 months for SOC. ORR was 74.5% for LY+SOC versus 81% for SOC. Safety results between arms were similar.

TCM Biotech is expected to start recruiting in August 2019 for a phase 1/2 study in Taiwan of PTX-9908 (CTCE-9908) given intravenously following transarterial chemoembolization to 50 patients with non-resectable hepatocellular carcinoma.

BioLineRx is focusing on checkpoint inhibitor combinations for their peptide CXCR4 inhibitor motixafortide, running 2 phase 2 studies in advanced pancreatic cancer. The COMBAT/KEYNOTE-202 study in collaboration with Merck Sharp & Dohme (NCT02826486) is recruiting 80 patients in the United States, Israel, and Spain: 40 patients with unresectable metastatic pancreatic adenocarcinoma are receiving motixafortide followed by motixafortide combined with the anti-PD-1 Ab pembrolizumab; and 40 patients with metastatic pancreatic adenocarcinoma that has progressed following first-line treatment with gemcitabine-based chemotherapy receive motixafortide monotherapy followed by a combination of BL-8040, pembrolizumab, and chemotherapy. The primary endpoint is ORR. A separate study at MD Anderson Cancer Center (NCT02907099) is also evaluating ORR to motixafortide, followed by combination with pembrolizumab, in 23 patients with metastatic pancreatic cancer. In addition to these 2 BioLineRx-sponsored studies, the combination of motixafortide and the anti-PD-L1 antibody atezolizumab is one of multiple regimens being tested in 2 international umbrella studies being run by F. Hoffmann-La Roche in metastatic cancers of the pancreas (NCT03193190), and stomach and gastro-esophageal junction (NCT03281369).

Polyphor's synthetic cyclopeptide balixafortide has meanwhile become the first CXCR4 inhibitor to enter phase 3 clinical testing as a cancer treatment, and is also the only such drug being studied in patients with breast cancer. A phase 1b study of balixafortide combined with the chemotherapy agent eribulin (NCT01837095) enrolled 56 women with HER2-negative metastatic breast cancer

TABLE 7 Peptide CXCR4 inhibitors – clinical studies in solid tumors

CXCR4 inhibitor	Indication	Phase	Completion	Study identifier
LY2510924	Advanced cancer, refractory to standard therapy	1	April 2013	–
	Solid tumors, advanced refractory	1	September 2017	NCT02737072
	RCC, metastatic	2	February 2017	NCT01391130
	SCLC, extensive-stage	2	August 2016	NCT01439568
PTX-9908 (CTCE-9908)	HCC	1/2	December 2021	NCT03812874
Motixafortide (BL-8040; BKT-140, 4F-benzoyl-TN14003)	Pancreatic carcinoma, unresectable metastatic	2	December 2021	NCT02826486
	Pancreatic carcinoma, metastatic	2	December 2019	NCT02907099*
	Pancreatic ductal adenocarcinoma, metastatic	1b/2	September 2020	NCT03193190
	Gastric or GEJ cancer, locally advanced unresectable or metastatic	1b/2	November 2021	NCT03281369
Balixafortide (POL6326)	Breast cancer, relapsed, TN and hormone-refractory ER+ metastatic	1b	August 2018	NCT01837095
	Breast cancer, HER2-negative, locally recurrent or metastatic	3	March 2022	NCT03786094

CXCR4: C-X-C chemokine receptor type 4; ER: estrogen receptor; GEJ: gastro-esophageal junction; HCC: hepatocellular carcinoma; HER2: human epidermal growth factor receptor 2; NSCLC: non-small cell lung carcinoma; RCC: renal cell carcinoma; SCLC: small cell lung carcinoma; TN: triple-negative.
*investigator-initiated study

(MBC) across 11 sites in the United States and Spain. Patients had to have evidence of tumor cell CXCR4 expression and were heavily pretreated (between 1 and 3 chemotherapy regimens for MBC, and at least 1 endocrine therapy if they had hormone receptor-positive disease, unless considered unsuitable for endocrine therapy). An initial dose escalation phase did not identify any dose-limiting toxicities, and 25 patients were recruited into an Expanded Cohort (EC) using the highest dose established for balixafortide (5.5 mg/kg). Patients in the EC had an ORR of 38%, a 1-year OS of 75%, and median PFS of 6.2 months. These response rates are considerably higher than those reported for eribulin monotherapy in similar MBC populations (ORR 9–12%), and the OS and PFS are also higher than reported for eribulin monotherapy.[97] Subsequent landmark analyses[98] showed OS for EC patients to be 50% at 18 months and 33.3% at 24 months when study medication was given as second line or later therapy, dropping only moderately to 40% at 18 months and 25% at 24 months when given as third line or later. Based on these encouraging results, an international Phase 3 study (NCT03786094) comparing eribulin with eribulin plus balixafortide has been launched and is recruiting patients.

5.4 | Ab CXCR4 inhibitors

This last group, comprising 2 fully humanized anti-CXCR4 Abs, summarized in Table 8, has unfortunately not yielded any clinical successes.

A phase 1 study of Eli Lilly's fully humanized antibody LY2624587 in 56 patients with advanced cancer (NCT01139788) was completed in 2011, but no results have been published to date.

BMS's ulocuplumab was combined with nivolumab in a phase 1/2 study in advanced or metastatic pancreatic and SCLC (NCT02472977); the study was terminated early with 61 patients recruited, due to a reported lack of efficacy.

5.5 | Summary and future perspectives

CXCR4 inhibitor studies have generally addressed solid tumors more recently than hematological malignancies, with 8 of the 25 studies still ongoing. Results from these are awaited with great interest. In contrast to the experience in hematology, CXCR4 Ab approaches do not appear to have been successful in treating solid tumors so far, although the ulocuplumab study targeted patients with particularly challenging cancers, and it would be premature to assume that Ab approaches are not effective.

Few conclusions can yet be drawn from the small molecule studies. However, the early results of plerixafor in glioma are intriguing. Longer follow-up, and potentially comparative studies, would help in understanding whether there is a treatment effect for plerixafor. The most encouraging avenue of research seems to be with the peptide CXCR4 inhibitors. While Eli Lilly's peptide LY2510924 did not provide benefit in RCC and SCLC, and it remains to be seen whether motixafortide has more success than ulocuplumab in combination with checkpoint inhibitors against immunologically "cold" tumors like pancreatic carcinoma, it is encouraging to note the inclusion of tumors more amenable to immunotherapy (gastric cancers).

Although single-arm studies need to be interpreted with caution, balixafortide, combined with eribulin, in patients with advanced BC produced rates and durations of response that certainly merited the initiation of a phase 3 study to test this combination further. Depending on the results of these ongoing studies with CXCR4 inhibitors, as well as follow-up results on the completed studies, further exploration

TABLE 8 Antibody CXCR4 inhibitors – clinical studies in solid tumors

CXCR4 inhibitor	Indication	Phase	Completion	Study identifier
LY2624587	Solid tumor, lymphoma or CLL, advanced and/or metastatic	1	November 2011	NCT01139788
Ulocuplumab (BMS-936564, MDX1338)	Pancreatic cancer and SCLC	1/2	January 2017	NCT02472977

BMS, Bristol-Myers Squibb; CLL, chronic lymphocytic leukemia; CXCR4, C-X-C chemokine receptor type 4; SCLC, small cell lung carcinoma.

of this exciting approach in additional cancers and drug combinations can be expected.

6 | CONCLUSIONS

The role of CXCR4/CXCL12 in the proliferation of tumor cells, immune evasion, induction of angiogenesis, and invasive tumor growth has been recognized for over 2 decades. Disrupting this axis may also prevent the development of cancer metastases by blocking migration of tumor cells toward CXCL12-rich secondary organs. CXCR4/CXCL12 is thus a promising target for imaging and therapy of both hematologic and solid tumors.

Therapeutic strategies have focused on the potential of CXCR4 antagonists to enhance the cytotoxic effect of chemotherapy and immunotherapy. CXCR4 inhibition might also counteract immune evasion of tumor cells by altering the distribution of immune cells and/or activity in the tumor microenvironment.

Inhibition of CXCR4 has emerged as an attractive therapeutic approach for AML, WM, NHL, and MM, as well as some solid tumors. Early clinical study results have been encouraging, and CXCR4 inhibitors appear to be safe and well tolerated.

Polyphor's balixafortide is the first such agent to reach phase 3 clinical testing and is the only CXCR4 inhibitor being studied in BC to date. A phase 1b study in advanced or HER2-negative MBC demonstrated an ORR for balixafortide combined with eribulin, as second line or later therapy, of 38% compared with 9–12% for eribulin alone in prior studies with similar patient populations. Encouraging OS was also seen in this heavily pretreated patient population (50% at 18 months and 33.3% at 24 months). The subsequent phase 3 study is currently recruiting and is due to complete in 2022. Together, these preclinical and clinical studies strongly support CXCR4 inhibition as a promising new therapeutic approach for cancer.

ORCID

Alexandra Bobirca https://orcid.org/0000-0001-5565-2450

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