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Research paper

Plerixafor in combination with chemotherapy and/or hematopoietic cell transplantation to treat acute leukemia: A systematic review and metanalysis of preclinical and clinical studies

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ABSTRACT

Leukemia-initiating cells localize to bone marrow niches via cell surface CXCR4 binding to stromal-derived factor 1 (SDF-1). Plerixafor, a CXCR4 antagonist, can mobilize and sensitize leukemia cells to cytotoxic therapy, and/or enhance the engraftment of healthy donor stem cells in the context of hematopoietic cell transplantation (HCT). A systematic review of preclinical and clinical studies was performed (updated May 1, 2020) to inform the design of definitive clinical trials and identified 19 studies. Pooled data from 10 preclinical *in-vivo* studies of AML and ALL in mouse models of leukemia revealed significant mobilization of leukemia cells into the peripheral circulation, decreased total blast burden and increased survival with plerixafor in addition to cytotoxic treatment compared to control animals. Two of 9 clinical studies compared outcomes to a control group. Plerixafor appears well tolerated and safe and can mobilize leukemia cells into the peripheral circulation. In patients with AML undergoing HCT, plerixafor given with the conditioning regimen appears safe and well tolerated. Engraftment, relapse and survival were not different from controls after limited follow-up. Studies in high risk patients with AML with longer follow-up are needed to understand the influence on relapse following treatment and on donor cell engraftment following HCT.

1. Introduction

Acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) are heterogeneous diseases that derive from the myeloid and lymphoid hematopoietic cell populations, respectively [1,2]. Despite aggressive chemotherapy and targeted inhibition of specific genetic mutations, only 40–60 % of adult patients with acute leukemia can be cured with current treatment strategies [1]. Refractoriness to initial therapy and relapse remain common. [2]. There is an urgent need for novel therapeutic strategies to improve outcomes. The persistence of quiescent leukemic stem cells in specialized niches within the bone marrow contributes significantly to refractory and relapsed leukemia. Disrupting molecular bonds tethering leukemic stem cells in the marrow may enhance the therapeutic effects of cytotoxic treatment and facilitate greater donor cell engraftment in allogeneic hematopoietic cell transplantation.

A population of quiescent leukemic initiating cells (LICs) that are resistant to chemotherapy, and have limitless self-renewal have been identified through seminal studies in transplantation studies of human leukemia cells into immunodeficient mice [3]. Interestingly, LICs in AML are enriched within the $CD34^{+}CD38^{-}$ population of cells within the hematopoietic system and have been previously shown to hide within the protective niches of the bone marrow. Within the protective niches, LICs are surrounded by other cell types, including stromal cells, that promote their survival by enabling them to evade the host immune system as well as chemotherapy drugs, thereby leading to persistent disease and relapses in excessive numbers of patients [4]. Recent studies have demonstrated that the mechanism of stromal-mediated protection of LICs is driven by adhesion proteins, chemokines and cytokines [5]. Targeting these mechanisms is an exciting therapeutic strategy.

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CXCR4 is a G-protein-coupled chemokine receptor that is expressed on the cell surface of ~ 40 % of LICs and hematopoietic stem and progenitor cells (HSPCs). Stromal cell derived growth factor (SDF1α) is a chemokine released by stromal cells and is found on their cell surface. SDF1α is a specific ligand for CXCR4. In hematopoietic transplant studies using mice with severe combined immunodeficiency (SCID), CXCR4 binding to SDF1α acts as an anchorage for hematopoietic progenitors and LICs and allows them to home to the bone marrow. In addition, the binding of SDF1α to CXCR4 on leukemic cells activates multiple pro-survival signaling pathways including the phosphoinositide 3-kinase (PI3K)/AKT and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) axes, and the YY1-mediated transcriptional activation of myelocytomatosis. Furthermore, overexpression and activation of CXCR4 has been shown to result in the downregulation of microRNA let-7a. The down regulation of microRNA let-7a leads to the increased expression of anti-apoptotic proteins such as BCL-XL and therefore confers a refractory phenotype to leukemic cells. Consistent with these findings, patients with hematologic malignancies, including acute leukemia, that have increased expression of CXCR4 are associated with poor prognosis and increased mortality (hazard ratio 2.31 [1.33–4.01]) [6–9].

Plerixafor (AMD3100) is a CXCR4 antagonist which disrupts the interaction between HSPCs or LICs and the bone marrow stroma by inhibiting SDF1α binding to CXCR4. In both healthy patients and those with cancer, plerixafor blocks CXCR4 binding to SDF1α, disrupting the anchorage of LICs and hematopoietic progenitors to the bone marrow stromal cells, resulting in their mobilization into the peripheral blood [10]. Plerixafor is currently approved by the Food and Drug Administration in the United States and in other jurisdictions for use in stem cell mobilization for autologous transplantation in hematologic malignancies. The addition of CXCR4 antagonists to standard leukemia regimens is also being studied, with the rationale that agents such as plerixafor may mobilize LICs from the protective bone marrow niches into the peripheral blood thereby increasing LIC exposure and susceptibility to chemotherapy. While this approach appears promising in pre-clinical *in-vivo* studies [11–20], clinical trials assessing the efficacy and safety of this therapeutic approach are still in early phases [21–29].

A systematic review and meta-analysis of preclinical *in vivo* studies and clinical studies using plerixafor in combination with cytotoxic chemotherapy for the treatment of AML and ALL is required to provide insight that will accelerate the optimal use of plerixafor for the management of patients with AML and ALL. Moreover, competitive repopulation of the bone marrow by donor cells in patients undergoing hematopoietic cell transplantation (HCT) may be enhanced using plerixafor to reduce relapse in patients with AML or ALL.

2. Methods

2.1. Search strategy, study selection and data extraction

The systematic search of published literature was performed in accordance with Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [30]. Our search strategy was developed to identify studies in Medline, Embase and Cochrane. The following terms: (Mozobil or Plerixafor or AMD 3100) AND (CXCR4 or CD184) AND (Stem Cell Niche and regeneration AND Signal Transduction and regeneration) AND (Leukemia) were used as part of our search strategy. Databases were searched between 1947 to April 15, 2020. The complete electronic search strategy is presented in Supplementary Table 1. Abstracts of all the identified studies were independently screened by two investigators (AV and HM). Duplicates were excluded and all potentially relevant articles were retrieved for further review based on the eligibility criteria (see below). Studies were broadly classified into pre-clinical and clinical. Data were extracted by two individuals using standardized forms. All discrepancies were resolved through consensus. Our research protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO; registration number CRD42016052970 registered December 9, 2016).

2.2. Eligibility criteria for systematic search

We included all controlled interventional pre-clinical studies that tested the *in-vivo* use of plerixafor in combination with chemotherapy for the management of acute leukemia. We also included clinical studies that used plerixafor for the management of acute leukemia in patients receiving chemotherapy and/or undergoing allogeneic HCT. Review articles, editorials, pre-clinical *in-vitro* or *ex-vivo* studies, conference abstracts and studies that used plerixafor for the management of malignancies other than acute leukemias were excluded. Furthermore, we also excluded clinical trials where plerixafor was used as mobilization therapy prior to autologous HCT.

2.3. Statistical analysis

For the meta-analysis involving preclinical *in-vivo* studies, raw data relating to blast percentages and survival were abstracted from the manuscripts or from published supplementary data. In studies where the raw data were not presented, the data was estimated from the presented graphs using Digitizelt (Version 2.2; Braunschweig, Germany). To minimize bias, raw data were extracted by two individuals and all discrepancies were resolved through consensus. Control and treatment arms of individual pre-clinical studies were compared using a Student's t test. Bias and significance in pooled analysis was done using DerSimonian and Laird random effects model. Key parameters such as number of animals that contributed to the study, cells used for inducing acute leukemia in mice, randomization, and assessment of investigators and/or lab personnel blinding were also presented. These key parameters have been previously identified as threats to validity in a systematic review of guidelines for preclinical studies [31].

3. Results

3.1. Identification of relevant published studies

A total of 1279 studies were identified in our systematic literature search. After excluding duplicates and screening for potential relevance, 67 studies underwent comprehensive review for assessment of eligibility. Forty-seven studies were subsequently removed for the following reasons: duplicate studies with different titles (3 reports), CXCR4 antagonist other than plerixafor (14 reports), CXCR4 antagonist used *in vitro* (3 reports), CXCR4 antagonist not used as part of the chemotherapy regimen (8 reports), CXCR4 antagonist used independent of chemotherapy (3 reports), disease studies other than acute leukemia (1 report), no control (1 report), abstract only (13 reports), and case report only (2 reports). The summary of the study selection process is provided in Fig. 1. A total of 19 studies met eligibility for analysis of which 10 were pre-clinical and 9 were clinical.

3.2. Characteristics of published preclinical studies

Pre-clinical studies addressed acute myeloid leukemia (AML, 4 studies) including 2 studies of acute promyelocytic leukemia (APL), and acute lymphoblastic leukemia (ALL, 6 studies) including 4 studies of pre-B cell ALL and 2 studies of ALL with MLL rearrangements (MLL-ALL). All AML studies utilized murine cell lines transplanted into B6129F1 mice (for APL studies) or C57Bl/6 J mice. All studies of ALL used human-derived patient samples transplanted into immunosuppressed mice (5 studies used NSG and 1 study used SCID mice) [Table 1].

Nine studies (90 %) provided information regarding the number of animals used for the control and experimental conditions, with only one study [11] lacking clear information regarding the total number of animals used in the experiments [Table 1]. Information regarding animal

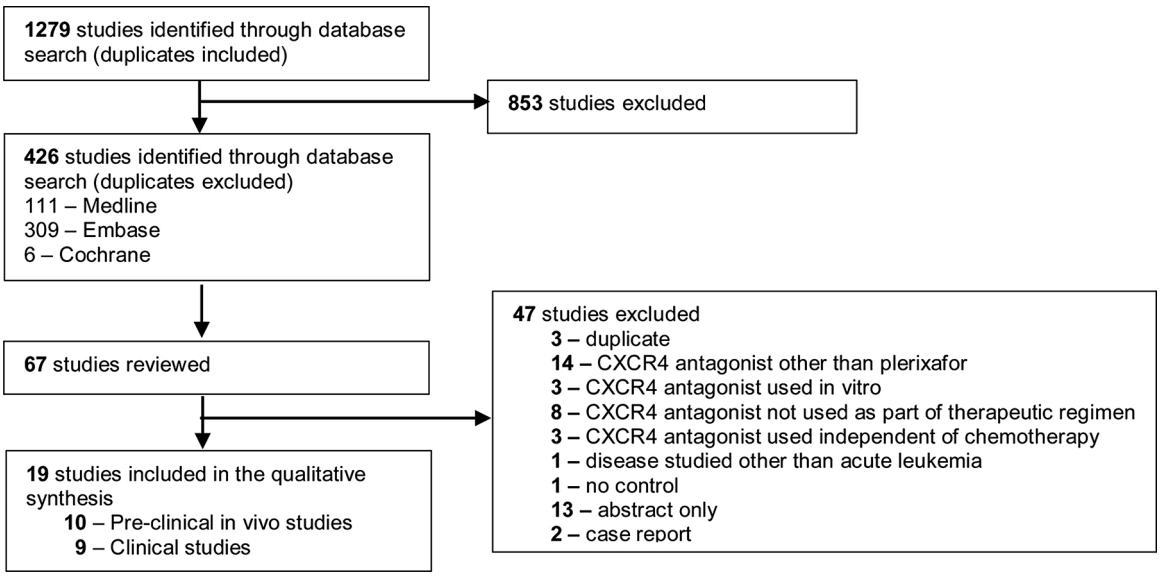


Fig. 1. Result of systematic search of the literature.

allocation to treatment groups was provided in the methods (3 studies) or in the pertinent area of the results section or figure legends where they contributed data (6 studies). None of the preclinical studies reported on randomization, allocation concealment, or provided information regarding physiological baseline characteristics to ensure the treatment and baseline groups were balanced. All included studies, however, provided information about the mice (strain and source) and specifics of the treatment regimens. No information pertaining to power calculations and sample size determination was provided in any of the studies.

3.3. Study design and outcomes of preclinical AML studies

Plerixafor was given in combination with cytarabine for the management of AML in all 4 studies [11–14] [Table 1]. The dose of plerixafor and cytarabine [Table 1] were higher in studies addressing APL in comparison to the other studies. Plerixafor was well tolerated by the mice in all the studies. Tumor burden (reported in 2 studies) and median over-all survival (reported in 3 studies) were used as outcome measures in the preclinical studies of AML [Table 2]. In both studies reporting on tumor burden [11,12], mice in the plerixafor treated groups demonstrated significant reductions in leukemia cells in bone marrow, liver and spleen in comparison to control mice. In two of three studies reporting on survival [13,14], median OS in the treatment groups was significantly improved with the combination of plerixafor and cytarabine compared to the mice treated with cytarabine alone (30 vs 23 days, $p < 0.001$, and 21 vs 17 days, $p < 0.05$) while one study [12] reported no significant difference in survival based on extraction of data from figures presented in the publication (23 vs 24 days, p = NS).

3.4. Study design and outcomes of preclinical ALL studies

Four studies (67 %) [17–20] examined the effect of using plerixafor in combination with chemotherapy for the management of pre-B ALL, while two [15,16] studies (33 %) examined the use of plerixafor in combination with cytarabine (1 study) or in combination with GCSF and lestaurtinib (1 study) for the management of ALL with MLL rearrangements (MLL-ALL) [Table 1]. Doses of plerixafor used in the studies were broad ranging from a single injection of 5000 mcg/kg in one study to 10, 000 mcg/kg daily by continuous infusion for 28 days [Table 1]. With regard to outcomes, five studies [15–19] reported on tumor burden and 4 (80 %) observed reduced tumor burden in mice treated with plerixafor compared with controls. Three studies [17,18,20] reported survival outcomes and 2 studies [18,20] found that mice treated with plerixafor survived significantly longer than the mice within the control groups [Table 2].

3.5. Heterogeneity of outcome reporting in pre-clinical studies

A total of 7 preclinical studies reported reduction of tumor burden [11,12,15–19], and only one study [13] reported an increase in the tumor burden within the treatment cohort. However, three studies [13, 15,17] reported changes in tumor burden relative to untreated controls and did not report standard deviations, which precluded their use in pooled estimates by metanalysis. One additional study [11] was excluded from metanalysis because the number of mice within each cohort were not reported which prevented us from assigning a weighting in the pooled estimate. Pooled analysis of reduction in tumor burden was possible in the remaining four studies [12,16,18,19] which analyzed AML [12] or ALL [16,18,19], including 2 studies that reported 2 separate treatment cohorts that are reported separately and included as independent experiments in our analysis (total of 29 mice in both treatment and control groups) (see Table 3). Following a pooled random effects analysis, the mean reduction in the percentage of leukemia cells within the treatment cohort compared to the control cohort was 74.19 % [72.04–76.35, 95 % confidence interval] [Table 3]. A large portion of this pooled estimate was contributed by one study (84 % weighted contribution to overall effect) [xenograft 1908 from ref [18] [Table 3]. After removing this study from the analysis, the estimated mean reduction in tumor cells within the treatment cohort compared to the control cohort was 37.76 % [32.39–43.14, 95 % confidence interval] [Table 3].

A funnel plot of the difference of the means from these studies regarding an effect on tumor burden reveals a significant outlier effect of xenograft 1908 (93 % of heterogeneity) [Fig. 2A/B]. The pooled effects analysis using the survival data from 6 studies [12–14,17,18,20] that used 9 xenografts (total of 60 and 55 mice in treatment and control groups, respectively) suggested that mice within the treatment cohort survived significantly longer than the control cohort (mean difference of 6.0 days, 5.1–6.9 in treated compared with controls, $p < 0.00001$) [Table 4].

Table 1
Summary of Pre-clinical Studies. All studies use a mouse model of leukemia and transplanted leukemia cells on Day 1 except #11, #12, and #18 on Day 0.

Study	Disease model (Mouse Backbone)	Source of leukemia cells (Cells Transplanted - population)	Dose of Plerixafor – mcg/kg (doses/schedule)	Additional therapy (dose; timing within treatment regimen)
[11]	AML (C57Bl/6 J mice)	C1498 murine myeloid leukemia (2×10^6 - C1498 cell line)	2500 (6 doses, 2 h pre or post chemotherapy)	**Cytarabine** (100 mg/kg/day; days 7, 9, 11)
[12]	AML (C57Bl/6 J mice)	C1498 murine myeloid leukemia (2×10^6 – C1498 Cell line)	2500 (2 doses/day for 7 days, 1 dose 2 h prior to AraC and 2 h after AraC)	**AraC** (100 mg/kg, days 7–13)
[13]	APL (B6129F1)	APL cells from spleens of mCG-PML-RAR knock in mice (1×10^6 - $mCG^{PR/+}$ APL cells)	5000 (4 doses, 1 h pre and 3 h post chemo d12, 13)	**Cytarabine** (500 mg/kg/day; days 12, 13)
[14]	APL (B6129F1)	APL cells from spleens of mCG-PML-RAR knock in mice (1×10^6 - $mCG^{PR/+}$ APL cells)	5000 (4 doses, 1 h pre and 3 h post chemo, or 2 h pre radiation)	**Cytarabine** (500 mg/kg/day; days 12, 13) **Radiation** (350 cGy; day 12)
[15]	MLL ALL (NSG)	Human ALL samples with MLL rearrangement (1×10^6 – PDX expanded bulk ALL cells)	**A:** 5000 (1 dose, 4 h pre-chemo) **B:** 5000 (6 doses, 4 h pre-chemo)	**A: Cytarabine** 100 mg/kg/d day 14) **B: Cytarabine** 200 mg/kg/day, days 15−17 and 22−24
[16]	MLL ALL (NSG)	Human ALL patient samples with MLL rearrangement (NR – Primary $CD10^+CD19^+$ ALL cells)	5000 (4 doses, days 16, 23, 30, 37)	**GCSF** (2−5mcg BID; days 14,15,21,22,28,29,35,36 **Lestaurtinib** 20 mg/kg BID days 15−19, 22−26, 29−33, 26−40
[17]	Pre-B ALL/ Flt3 (SCID)	Ba/F3 pro-B lymphocytic cells with FLT3 ITD insertion ($0.5 \times 10^{6)}$	10,000 (8 doses, 2 days between day 3−17)	**Cytarabine** (50 mg/kg/day; q1 week starting on day 3) **ID11** (0.5 mg/kg q2 weeks; starting on day 3)
[18]	Pre-B-ALL (NSG)	Human pre-B ALL cells (Transplanted cell numbers not reported) Xenograft 1809 – $CD34^+$ $CD19^+$ $CD10^+$ Xenofraft 2032 – $CD19^+$ $CD10^+$	**Tumor burden experiment:** 10,000 (30 doses, continuous infusion via osmotic pump for 3 weeks) **Survival experiment:** 10,000 (daily; days 1−43)	**Tumor burden experiment:** • **Vincristine** (0.25 mg/kg q1 week for 3 weeks) **Survival experiment:** • **Vincristine** (0.05 mg/kg q1 week for 3 weeks)
[19]	Ph + B-ALL (NSG)	Human TXL-2 cells (Ph positive B-ALL) (1.5×10^6 - Primary $CD10^+$ $CD19^+$ ALL cells)	10,000 (5 doses total, twice per week and given 5−6 h prior additional therapy, starting on day ~16)	**rGel/BLyS** (2.75 mg/kg BID; twice weekly between day 6−33)
[20]	Pre B-ALL, Ph(-) / Ph(+) (NSG)	pre-B ALL (6×10^6 - transgenic mouse p190 Bcr/Abl ALL cells)	**Ph (+) ALL** 10,000 (1 dose/day for 28 days using osmotic pump) **Ph (-) ALL** 10,000 ((1 dose/day for 28 days using osmotic pump)	**Ph(+) ALL** • **Nilotinib** (60 mg/kg/day; days 17–28) **Ph(-) ALL** • **Vincristine** (0.5 mg/kg/day; days 12–40) • **Dexamethasone** (10.5 mg/kg/day; days 12–40) • **L-asparaginase** (1500IU/kg/day; days 12–40)

AML = acute myeloid leukemia, APL = acute promyelocytic leukemia, ALL = acute lymphoblastic leukemia, ALI = allogeneic lymphocyte infusion, IP = intraperitoneal, GFP = green fluorescent protein, NR = not reported, AraC = cytarabine, MLL = mixed lineage leukemia, NSG – non-obsese severe combined immunodeficient gamma (mouse strain), Ph – Philadelphia chromosome, t(9;22), PML-RAR = promyelocytic leukemia – retinoic acid receptor alpha, ID11: TGF-beta-Neutralizing Antibody, rGel/BLyS: is a toxin-cytokine fusion protein used for selective killing of malignant B-cells expressing receptors for B-cell Activating Factor (BAFF/BLyS) by receptor-targeted delivery of the toxin, Gelonin.

3.6. Characteristics of clinical studies

A total of 289 patients with acute leukemia from 9 published clinical trials were included in our analysis (summarized in Table 5). With regard to disease risk, 15 patients harbored leukemia with adverse cytogenetics, 137 patients had intermediate cytogenetics and 20 patients had favorable cytogenetic profiles (cytogenetic results were not reported in 17 patients). Most patients (n = 273, 94 %) had AML, while 9 patients were treated for ALL, and 7 patients had myelodysplastic syndrome. The studies enrolled patients with de novo AML (3 studies), relapsed or refractory disease (4 studies) and/or a mixture of patients (2 studies). The median ages of patients enrolled across the 9 clinical trials was 50.5 years (range, 9–73) [Table 5]. Our analysis included studies that are Phase I (5 studies), Phase I/II (3 studies) and Phase II (1 study). All the studies were unblinded and only two studies [21,27] contained control cohorts. One study [27] used historical controls and one study [21] enrolled patients receiving plerixafor in alternating treatment cycles, providing internal controls for each patient. Six studies [22,23,26–29] treated patients with 240 mcg/kg/day (the dose used in previous studies of autologous hematopoietic cell mobilization prior to autologous stem cell transplant) [32] and the remaining three studies [21,24,25], administered higher doses, ranging from 320 to 810 mcg/kg or 6000–15000 mcg/m2.

3.7. Clinical outcomes and scientific correlatives

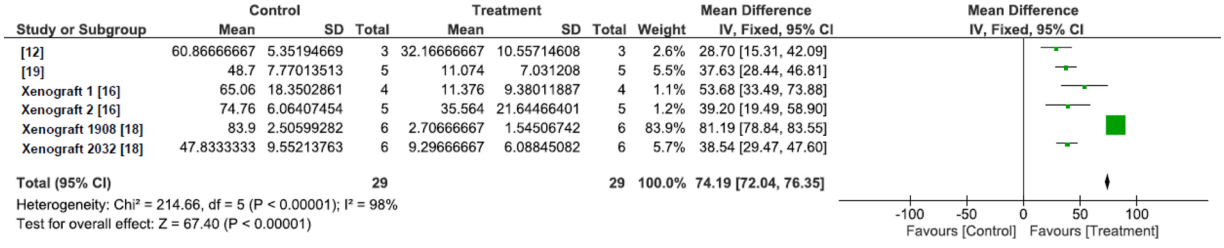
Plerixafor treatment was administered in the context of induction therapy (2 studies), salvage therapy (4 studies), or conditioning therapy prior to hematopoietic cell transplantation (HCT) (3 studies). Studies of induction or salvage treatment reported complete remission rates, however, only six out of the nine studies reported data pertaining to relapse free survival, relapse related mortality, or overall survival (Supplemental Table 3). In the two controlled studies, differences in outcomes between plerixafor-treated patients and controls were not observed. All four studies reporting on changes in leukemia cell surface expression of CXCR4 reported significant increases following treatment with plerixafor. CXCR4 levels and $CD34^+CD38^-$ cell [21] mobilization increased in a dose dependent manner when plerixafor is administered

Table 2
Assessment of tumor burden and overall survival in pre-clinical studies.

Ref	Method used to assess tumour burden	Timing of assessment	Tumour burden – Control	Tumour burden – Intervention	Median OS Control - (days)	Median OS Treatment - (days)	P value
[11]	**H&E staining**	d ~25–30 post leukemia	**BM:** 50.4 ± 4.5% **Liver:** 58.7 ± 2.9% **Spleen:** 47.4 ± 3.5%	**BM:** 36.1 ± 4.4% **Liver:** 23.1 ± 2.8% **Spleen:** 26.0 ± 2.7%	NR	NR	NR
[12]	**H&E staining**	Upon humane endpoint until d 26	**BM:** 60.7 ± 5.1% **Liver:** 50.2 ± 4.2% **Spleen:** 51.8 ± 3.7%	**BM:** 31.1 ± 11.9% **Liver:** 39.2 ± 5% **Spleen:** 43%± 7%	~23†	~24.1†	NS
[13]	**BLI**	D 24 post leukemia	**~3**†	**~12**†	~22†	~27†	
	NR	NR	NR	NR	~23†	~30†	<0.001
[14]	NR	NR	NR	NR	~17†	~21†	<0.05
	NR	NR	NR	NR	~27†	~29†	NS
[15]	**Flow cytometry** *(fold change in human CD49+ blasts)*	d 28	**BM** ~0.8† **Blood** ~1† **Spleen** ~1.4†	**BM** ~0.6† **Blood** ~0.9† **Spleen** ~0.5†	NR	NR	NR
			BM ~0.5† **Blood** ~0.6† **Spleen** ~0.3†	**BM** ~0.3† **Blood** ~0.5† **Spleen** ~0.2†	NR	NR	NR
[16]	**Flow cytometry** *(% BM human/ murine CD45+ cells)*	10 weeks after treatment	**Patient 1** ~65%† **Patient 2**~75%†	**Patient 1** ~11.5%† **Patient 2** ~36%†	NR	NR	NR
[17]	**BLI**	d 13 & 17	**~3.5**†	**~2**†	19 (range 16–21)	19 (range 17–22)	NS
[18]	**Flow cytometry** *(% human CD45+/CD19 + ALL cells)*	3 weeks after treatment	**BM** ~84%† **Blood** ~40%† **Spleen** ~10%†	**BM** ~3%† **Blood** ~10%† **Spleen** ~30%†	**Xenograft 1809** 60 (CI 57–63)	97 (CI 89–105)	<0.002
			BM ~48%† **Blood** ~40%† **Spleen** ~10%†	**BM** ~9%† **Blood** ~10%† **Spleen** ~30%†	**Xenograft 2032** 72 (CI 66–78)	126 (CI 94–158)	0.027
[19]	**Flow cytometry** *(% human CD19+/CD10 + ALL cells)*	d 46	**BM** ~48%† **Blood** ~0%† **Spleen** ~60%†	**BM** ~11%† **Blood** ~0%† **Spleen** ~15%†	NR	NR	NR
[20]	NR	NR	NR	NR	~27†	~35†	0.047
	NR	NR	NR	NR	54	61.5	0.015

BM = bone marrow, BLI = bioluminescence imaging, photons/sec x10^7; BM = bone marrow; VDL = combination of vincristine, dexamethasone, L-asparaginase, NR = not reported, OS = overall survival, †Data extracted from figures in the report. ††Dosing details are provided in Table 1.
*Study included multiple experimental arms, the control group pertaining to experiment that generated the outcomes listed is shown here. **Mean overall survival (in days).
H&E staining *(visual estimation of blasts %).*

Table 3
Forrest Plot analysis assessing the change in peripheral blast percentages after treatment in the control and treatment groups of pre-clinical studies.



to patients (Supplementary Table 4). All studies measured blast mobilization within the peripheral blood. As summarized in Supplementary Table 4, most studies report peak mobilization of blasts between 4–6 h post administration of plerixafor. Flow cytometry was used to quantify blast mobilization and the expression (reported as mean flourescence intensity) of the CXCR4 receptor.

3.8. HCT studies

Three studies used plerixafor as part of conditioning therapy prior to HCT for patients with AML, ALL and myelodysplastic syndrome (Table 5). While one study [28] does not provide the cytogenetics of the patients enrolled in the clinical trial, they report on pediatric patients undergoing second allogeneic HCT for relapsed disease Two studies [27, 29] provide cytogenetics and/or remission status of the patients enrolled (Table 5). While one study [27] was phase I/II, the other two studies [28, 29] were Phase I. The phase I/II study [27] used historical controls for comparison. All three studies administered 240 mcg/kg/day of plerixafor with each dose, however the timing within regimens varied (Table 5). The median ages of patients enrolled in the transplantation studies was 49 years (range: 9–54). The median complete remission status at time of transplant across the three studies was 50 % (range 17–52.5) (Supplementary Table 3). The median relapse related mortality at last follow-up across the three studies was 33 % (range 17–84) (Supplementary Table 3). While plerixafor did not impact rates of initial engraftment, relapse or survival in the studies compared with control groups, complete myeloid donor chimerism at day +30 and day +100 was increased in the treatment group in one study [27].

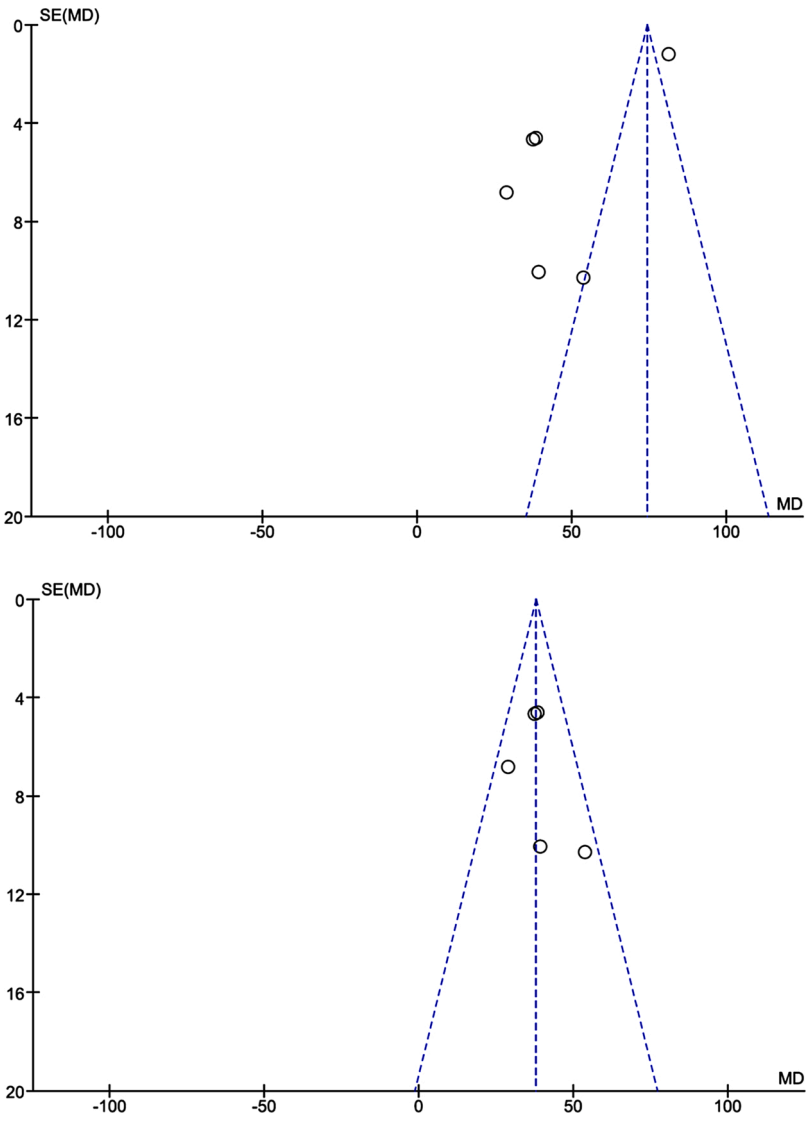
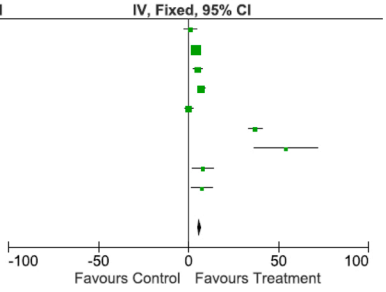


Fig. 2. (A) Funnel Plot analysis of pre-clinical studies of mice with Acute Leukemia – Control Vs Treatment. Difference in the means of blast % between Control and Treatment group are plotted against the standard error.
B) Xenograft 1809 (Study# 18) contributes to the 93 % of the heterogeneity. Removal of the study makes the meta-analysis homogeneous and unbiased.

Table 4
Forrest Plot analysis assessing the mean difference in overall survival in pre-clinical studies.

	Treatment			Control				Mean Difference	Mean Difference
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Fixed, 95% CI	IV, Fixed, 95% CI
[12]	24.1	2.066398	3	23	2.510644	3	6.2%	1.10 [-2.58, 4.78]	
[14]	21	1.82574186	8	17	1.29099445	7	33.6%	4.00 [2.41, 5.59]	
Xenograft 1 [13]	27	3.50713558	8	22	1.2234	8	12.8%	5.00 [2.43, 7.57]	
Xenograft 2 [13]	30	3.14112506	10	23	1	10	20.3%	7.00 [4.96, 9.04]	
[17]	19	1.9872	7	19	2.1452	6	16.5%	0.00 [-2.26, 2.26]	
Xenograft 1908 [18]	97	2.47302109	6	60	4.277071	6	5.4%	37.00 [33.05, 40.95]	
Xenograft 2032 [18]	126	17.42492468	6	72	13.15788737	6	0.3%	54.00 [36.53, 71.47]	
Xenograft 1 [20]	35	6.08876014	6	27	4.19642705	6	2.4%	8.00 [2.08, 13.92]	
Xenograft 2 [20]	61.5	6.64620192	6	54	2.2627417	3	2.4%	7.50 [1.60, 13.40]	
Total (95% CI)			**60**			**55**	**100.0%**	**6.00 [5.08, 6.92]**	

Heterogeneity: Chi² = 307.37, df = 8 (P < 0.00001); I² = 97%
Test for overall effect: Z = 12.78 (P < 0.00001)



3.9. Safety

Safety and adverse events are summarized for all clinical studies in Supplementary Table 3. Multiple grade 3, grade 4 and grade 5 toxicities were reported. The adverse events were expected given the age of the patients and concomitant cytotoxic chemotherapy agents that were administered. A total of two patients had dose limiting toxicity; one due to renal insufficiency that was not clearly attributed to plerixafor at a dose of 810 mcg/kg [21], and the second had grade 4 neutropenia lasting >42 days that ultimately resolved at a dose of 420 mcg/kg that was not clearly related to plerixafor [22]. None of the grade 3 or 4 adverse events were attributable to plerixafor. The most common grade

Table 5
Summary of Clinical Studies.

Ref	Disease	Therapy	Plerixafor dose mcg/kg/day (timing in regimen)*	Additional treatment intervention	Control group	Sample size (n)**	Cytogenetic Risk (Adv/Int/Fav)	Median age, years (range)	Median follow up Months (range)
[21]	AML, de novo	Induction	320, 540, or 810 (d 1−5)	**Decitabine** (20 mg/m2 IV d 1−10)	Yes†	69	37/32/0	73 (56−87)	9.9 (5.4–24.8)
[22]	AML, de novo	Induction	240 (7 patients) and 320 (3 patients)	**GCSF** (5 ug/kg days 1–10) **Daunorubicin** (60 mg/m^2 on day 1−3) **Cytarabine** (500 mg/m^2 on day 1−3)	No	10	1/4/3 NR = 2	49 (36−64)	NR
[23]	AML, rel/ref	Salvage	240 (d 0−5)	**MEC**	No	52	16/29/7	52 (18−70)	19.8 (NR)
[24]	AML, B-ALL, rel/ref	Salvage	6000, 9000, 12000, or 15,000 mcg/m2(d 1 −5)	**Cytarabine** (1 g/m2 IV q12 h days 1–5) **Etoposide** (150 mg/m2 IV days 1–5)	No	18	12/3/3	AML, 13 (3−17)ALL 14 (12 −21)	NR
[25]	AML, rel/ref	Salvage	750 (d 3−8)	**MEC** + **GCSF** (10mcg/kg SC days 1–8)	No	35	13/19/3	56 (29−70)	34.6 (NR)
[26]	AML, rel/ref	Salvage	240 (d 1−4)	**FLAG-Ida**	No	41	19/19/2 NR = 1	52 (18−64)	21 (6−38)
[27]	AML, MDS	HCT	240 (d -7 to -4 of conditioning)	**Busulfan** (130 mg/m2 IV days -6 to -3) **fludarabine** (40 mg/m2 IV days -6 to -3) **GCSF** (10mcg/kg/d SC days -9 to -4) **ATG** if unrelated donor	Yes***	40	16/22/2	54 (25−65)	11.6 (0.5−36.4)
[28]	AML, ALL, rel	HCT	240 (d -4 for 1−3 d with conditioning)	**fludarabine** (30 mg/m2 IV days -9 to -5) **thiotepa** (5 mg/kg/dose IV q12 h on day -4) **melphalan** (70 mg/m2 IV days -3 and -2) **rabbit ATG** (3 mg/kg/dose IV day -3 to -1	No	12	NR	9 (6−15)	10.9 (0.5−24.7)
[29]	AML, de novo	HCT	240 (d -4 to -2 of conditioning)	**Fludarabine** (50 mg/m2 – 1–4) **Busulfan** (3.2 mg/kg/day on day 1–4)	No	12	1/9/2	49 (38−55)	67 (53−82)

NR = not reported, Adv = Adverse, Int = Intermediate, Fav = Favorable.
Given 4–8 h prior to chemo in most studies.
MEC, Mitoxantrone (8 mg/m2 IV days 1–5), Etoposide (100 mg/m2 IV days 1–5), Cytarabine (1 g/m2 IV days.1–5).
FLAD-Ida, Fludarabine (30 mg/m2 IV days 1–4), Cytarabine (2 g/m2 IV days 1–4), GCSF (5mcg/kg SC days 1–4), Idarubicin (10 mg/m2 IV days.1–3).
GCSF, granulocyte colony-stimulating factor.
ATG, antithymocyte globulin.
HCT, hematopoietic cell transplantation.
Rel : Relapse.
Ref: refractory.
* In studies that reported phase 1 and phase 2 data, the recommended phase 2 dose is reported here.
** In studies that reported phase 1 and phase 2 data the number of patients in the phase 2 component is reported here.
*** Historical control group was treated with same regimen of busulfan, fludarabine, GCSF, +/- ATG, but did not receive AMD3100.
† plerixafor was administered during alternating treatment cycles, therefore each patient served as his/her own control for correlative scientific studies.

1–2 toxicities attributed to plerixafor included gastrointestinal complaints such as nausea, vomiting, and diarrhea. Except for 1 patient (described above), there were no delays in engraftment and/or recovery of blood counts following treatment. None of the studies reported significant leukostasis due to peripheral mobilization of blasts, however 6 of the 8 studies specifically excluded patients with a high baseline white blood cell count greater than 20−50 × 10^9/L.

4. Discussion

Our systematic review of preclinical and clinical studies provides a summary of evidence that highlights the potential role of using plerixafor in combination with chemotherapy for the management of acute leukemia. Plerixafor inhibits the binding of SDF1α to CXCR4, disrupting the interaction between leukemia cells and the bone marrow stroma, and was given to induce the mobilization of leukemia cells into the circulation and enhance their elimination by cytotoxic chemotherapy. The preclinical animal studies summarized in this systematic review confirmed that leukemia cell mobilization can be achieved with a significant reduction of leukemic burden and improved animal survival across a range of strategies that were used to induce leukemia in mice, including the use of gene knock-in methods, murine leukemia cell lines, and the use of primary patient samples transplanted into immunosuppressed mice to induce AML and ALL. In addition, clinical studies demonstrated the safety and feasibility of using plerixafor in combination with induction chemotherapy, salvage treatment for relapsed or refractory leukemia, and as part of the conditioning therapy prior to HCT.

The role of mouse genetics in animal leukemia models may have impacted treatment outcomes in the studies included in our analysis. For example, unlike C57Bl/6 J mice, NSG and SCID mice have impaired natural killer, macrophage, B cell and T cell function that enables better engraftment of human cells [33]. Furthermore, the scid mutation (Prkdc^{scid}) found within SCID and NSG mice make them highly

susceptible to high dose radiation and chemotherapy drugs such as cytarabine [34]. The pre-clinical studies summarized within our systematic review used a variety of genetic strains that included NSG, SCID and C57Bl/6 J which may not translate into similar treatment responses or tolerance in humans. Furthermore, we recognize that acute leukemia is a heterogenous disease and not all leukemic cells have the capacity to engraft and induce leukemia when transplanted into genetically-modified animals. While all the studies transplanted leukemic cells into mice, different cell numbers were transplanted and the timing of treatment with plerixafor varied significantly between studies. Furthermore, one study [15] used a secondary transplant model that significantly enriched for leukemia initiating clones (LICs). Interestingly, none of the studies provided information regarding the percentage of leukemic blasts observed within the peripheral blood at the time of treatment. Taken together, these observations suggest that mice cohorts between studies had different proportions of LICs and differences in response to treatment may be partly explained by these genetic host factors. Our pooled analysis revealed a high degree of heterogeneity observed within treatment outcomes, including *in vivo* tumor burden and overall survival. The observed heterogeneity associated with our pooled estimate of overall survival was likely due to differences in the number of engrafted LICs, whereas heterogeneity observed within the reduction of tumor burden was likely due to differences in specific cell populations transplanted into the mice. For instance, all xenografts analyzed for tumor burden were transplanted with human $CD10^+CD19^+$ ALL cells, and one xenograft model that accounted for 93 % of the heterogeneity was transplanted with human $CD34^+CD19^+CD10^+$ cells which may have been more restricted in the number of LICs transplanted into mice, yielding more favorable responses. A consideration for future studies would be to select mice with similar levels of leukemic burden, either by transplanting similar cell populations (enriched for LICs) from human leukemia samples into mice or selecting transplanted mice that display a similar degree of LIC engraftment. Utilizing mice from secondary transplants that have developed leukemia from LICs is another consideration that could enhance consistency amongst the animal models used in preclinical leukemia research.

The use of human leukemia samples compared to cell lines or murine leukemia cells introduces additional significant heterogeneity albeit greater clinical relevance. Clinical studies enrolling patients with leukemia further augments heterogeneity in treatment responses. This may contribute to overestimation of favorable outcomes in preclinical studies. In our systematic review of clinical trials that investigated the therapeutic benefit of using plerixafor in combination with standard therapy for the management of acute leukemia, we observed that the majority of studies addressed AML. The lack of control cohorts within most of these early phase clinical trials precluded us from performing a meta-analysis to measure the additive benefit of using plerixafor in combination with standard regimens. Within the two studies [21,27] that included controls, no difference in overall survival was reported, however, followup was limited in these studies and disease risk was variable and may have dominated the determination of outcomes. While there is potential concern that plerixafor may mobilize excessive numbers of leukemic blasts into the peripheral blood, no study reported instances of leukostasis, however patients enrolled in the studies had median baseline WBC less than 20 × 10/L which may have allowed selection of patients at lower risk of developing this complication. Moreover, the risk of bone marrow aplasia or delayed bone marrow reconstitution post chemotherapy due to destruction of mobilized normal HSCs by chemotherapy was not observed in the clinical studies. Overall, plerixafor was well tolerated, and the main side effects attributable to plerixafor were gastrointestinal. The safety findings of these clinical studies are in keeping with those reported in the phase 3 studies using plerixafor as mobilization therapy [32].

AML is a heterogeneous disease in which the characteristics of LICs drive leukemic progression. A recent preclinical study has shown that targeting LICs enriched within the $CD34^+CD38^-$ population can greatly reduce relapse and attenuate AML progression [35]. The strategy behind using plerixafor for the management of AML is to mobilize HSPCs/LICs from their protective niche in combination with induction chemotherapy regimens to target the mobilized cells. For this strategy to work, it is essential to identify the optimal dose of plerixafor required to mobilize sufficient numbers of LICs within the pool of $CD34^+CD38^-$ cells from the bone marrow of a patient with AML [21]. Surprisingly, only one clinical study [21] reported the mobilization of $CD34^+CD38^-$ cells upon the administration of plerixafor. The remaining studies measured only fold changes in blast or white blood cell (WBCs) mobilization. Considering blasts ($CD45^+$ or $CD45^+$ $CD33^+$) and WBCs have high expression of CXCR4 receptor in comparison to $CD34^+CD38^-$ cells, one cannot assume that the mobilization of WBCs and/or blasts guarantees the mobilization of $CD34^+CD38^-$ cells in sufficient numbers. To assess the efficacy of chemosensitization of LICs with plerixafor, clinical studies need to assess the degree of $CD34^+CD38^-$ mobilization with CXCR4 antagonists to ensure LICs are being mobilized from bone marrow niches. One study [21] reported that in an elderly patient cohort (median age 73) administering 810 mcg/kg/day of plerixafor was able to mobilize the most number of LICs from the bone marrow niche, and that the mobilizing effect of plerixafor was highly significant among clinical responders versus non-responders. Furthermore, the same study [21] reported that within responders, plerixafor forced $CD34^+CD38^-$ LICs into cell cycle. Since cytotoxic induction therapy targets cycling cells, it may be worth testing higher doses of plerixafor in combination with induction therapy within younger AML patient cohorts.

Recent single cell RNA-sequencing studies have demonstrated that CXCR4 expression varies significantly between hematopoietic stem and progenitor cells (HSPCs) [36]. Considering plerixafor binds to the CXCR4 receptor, it is unlikely that HSPCs with low levels of CXCR4 are mobilized by plerixafor to the same extent as high-expressing HSPCs. Variable expression of CXCR4 by LICs is also anticipated. One study [21] reported that patients with leukemia responding to decitabine treatment contained variable levels of cell surface CXCR4 and that plerixafor only mobilized $CD34^+CD38^-$ LICs in patients with high levels of CXCR4, leading to LIC exhaustion. Several studies [23–25,27] have also reported that administration of chemotherapy or plerixafor can result in increased CXCR4 expression. Therefore, pre-screening for CXCR4 expression levels within $CD34^+CD38^-$ cells isolated from bone marrow aspirates of AML patients at diagnosis may aid clinicians in identifying target patient populations that will respond most favorably to plerixafor.

Allogenic HCT is potentially curative for AML. Three studies [27–29] administered plerixafor as part of conditioning therapy prior to HCT. One study [27] reported that administering plerixafor and GCSF as part of conditioning therapy followed by HCT had no benefits. In terms of rates of achieving donor chimerism after transplantation, this study [27] used historical controls that reported lymphoid chimerism, whereas the treatment cohort reported myeloid chimerism. The observation that rates of GvHD were lower in the treatment group compared to historical patients is interesting. It is possible that risk factors for GVHD, including HLA matching were improved in the more recent treatment group, however, GCSF has been shown to modulate both the microenvironment and T cells in a way that promotes the expansion of T regulatory cells and plerixafor can mobilize T regulatory cells out of the protective bone marrow niche [37,38]. Taken together, the combination of GCSF and plerixafor may attenuate GvHD and should be addressed in future studies. Moreover, GCSF has been shown to affect the bone marrow niche making it hospitable for HSC engraftment in the context of AML and allogenic HCT [39]. Moving forward more studies need to be done where GCSF and plerixafor are administered separately or perhaps at different doses.

In conclusion, our systematic review provides an unbiased and robust summary of the preclinical and clinical experience using plerixafor in the management of acute leukemia and has identified several aspects of study design that should be considered to accelerate further progress in our understanding of how plerixafor can be used to improve

outcomes for patients with acute leukemia. Our analysis of preclinical studies confirms that leukemia cells can be mobilized from the protected bone marrow environment and in combination with cytotoxic treatment, plerixafor is associated with a marked reduction in tumor burden and improved survival of animals. This insight can be used to rationalize a greater focus on clinical studies. Our summary of clinical studies demonstrates relative safety and feasibility of combining plerixafor with conventional therapy for acute leukemia, including induction regimens and salvage treatment, providing further support for continued study of plerixafor in the clinical setting. Moreover, plerixafor can be included in the conditioning regimen prior to HCT. While clinical outcomes were not clearly improved with plerixafor, larger studies in more patients with higher risk disease and/or in patients with leukemia that have high CXCR4 expression appear worthwhile to assess the potential impact on reducing relapse. Identifying patients and/or creating animal models of leukemia using LICs that have high levels of surface CXCR4 may be worthwhile and use of higher doses of plerixafor appear worthy of future study. Given the modest benefits observed in early clinical studies identified in our analysis, it is possible that the significant clinical benefits from plerixafor may not be realized and it remains premature to consider plerixafor part of standard treatment for acute leukemia

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

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