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REVIEW

Novel strategies for improving hematopoietic reconstruction after allogeneic hematopoietic stem cell transplantation or intensive chemotherapy

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ABSTRACT

Introduction: High-dose conditioning regimens for allogeneic hematopoietic cell transplantation (allo-HCT) as well as intensive poly-chemotherapy for acute myeloid leukemia (AML) induce prolonged periods of neutropenia. The duration of the neutropenia is particularly long following umbilical cord blood transplantation (UCBT).

Areas covered: After briefly reviewing the impact of hematopoietic growth factors administration to hasten hematologic reconstitution after allo-HCT or intensive AML chemotherapy, this article summarizes recent approaches that have been investigated to prompt hematologic reconstruction after UCBT or intensive AML chemotherapy.

Expert opinion: In the allo-HCT setting, administration of G-CSF or GM-CSF shortened the duration of the neutropenia but failed to decrease infection-related mortality or to improve survival. Novel approaches to hasten hematological reconstruction after UCBT such as double UCBT with expansion of one of the 2 UCB units with Notch ligand, mesenchymal stromal cells, nicotinamide, or StemRegenin 1, co-transplanting a single UCB unit with HLA-haploidentical CD34+ cells, or increasing UCB HSC homing to marrow niches via direct intra bone UCB administration, pulse treatment with dmPGE2 or enforced fucosylation are promising and deserve further investigations in prospective phase III studies. In the AML setting, G-CSF or GM-CSF administration after intensive chemotherapy decreased the duration of the neutropenia without improving survival.

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1. Introduction

The incidences of nonrelapse mortality following allogeneic hematopoietic stem cell transplantation (allo-HCT) or intensive chemotherapy for acute myeloid leukemia (AML) have been substantially reduced in the last decades [1,2]. Nevertheless, high-dose conditioning regimen administered before myeloablative allo-HCT as well as intensive chemotherapy regimens used to treat AML still results in prolonged bone marrow aplasia and particularly prolonged neutropenia that often leads to bacterial and/or fungal infections [3–6]. This is particularly the case when umbilical cord blood (UCB) is chosen as stem cell source for allo-HCT [4], or when the intensity of the chemotherapy is increased in the induction or consolidation chemotherapy course of AML [7].

Several studies have assessed the efficacy of hematopoietic growth factors to shorten the duration of neutropenia after allo-HCT [8,9] or in the induction chemotherapy course for AML [10]. In the setting of allo-HCT, a meta-analysis including data from all randomized studies demonstrated that administration of granulocyte colony-stimulating factor (G-CSF) successfully reduced the duration of neutropenia and reduced the incidence of infection but failed to decrease infection-related mortality [9]. In AML administration of G-CSF or of granulocyte macrophage colony-stimulating factor (GM-CSF) after chemotherapy hasten neutrophil recovery and shorten hospitalization without improving survival [10,11]. More recently, several approaches have been assessed to shorten the duration of the neutropenic phase during allo-HCT, and particularly after UCB transplantation (UCBT). These approaches include direct intra bone implantation of UCB, combination of single UCBT with CD34+ cells from a G-CSF-mobilized HLA-haploidentical donor, UCB expansion with immobilized Delta-1, mesenchymal stromal cells, nicotinamide or StemRegenin 1 (SR1), or stem cell modification aimed at increasing stem cell homing such as pulse treatment with the 16,16-dimethyl prostaglandin E2 (dmPGE2) or enforced fucosylation [12,13]. Further, approaches aimed at preventing viral infections after UCBT by transfer of virus-specific T cells have been developed [14–16].

In this article, after briefly discussing the potential role of hematopoietic growth factors, we review recent approaches that are currently assessed for hasting hematologic recovery after allo-HCT or after intensive chemotherapy for AML [17].

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Article highlights

- G-CSF administration significantly prompts neutrophil recovery after allo-HCT or intensive chemotherapy for AML.
- G-CSF administration after allo-HCT or intensive chemotherapy for AML does not improve OS.
- Double UCBT allows patients without a sufficiently rich single UCB unit to benefit from UCBT.
- double UCBT failed to improve engraftment and other transplantation outcomes in patients who had a single UCB unit containing ≥2.5 x 10^7 TNC/kg recipient.
- double UCBT with expansion of one of the 2 UCB units with Notch ligand, mesenchymal stromal cells, nicotinamide, or StemRegenin 1 results in prompt neutrophil engraftment.
- co-transplanting a single UCB unit with HLA-haploidentical CD34+ cells fastens neutrophil engraftment in comparison to double UCBT.
- increasing UCB HSPC homing to marrow niches via direct intra bone UCB administration, pulse treatment with dmPGE2 or enforced fucosylation also fastens neutrophil engraftment.

This box summarizes key points contained in the article.

2. Strategies to hasten hematologic recovery after allo-HCT

2.1. Factors affecting hematologic recovery after allo-HCT

The kinetics of hematologic reconstitution after allo-HCT are influenced by several factors such as the stem cell source, graft composition, the underlying disease, the conditioning regimen and the type of GVHD prophylaxis [18–22].

Stem cell source is one of the main factors affecting engraftment kinetics [18,23–25]. As example in one large study assessing the impact of graft source on unrelated donor allo-HCT in adults with acute leukemia, median times to neutrophil (defined as achievement of an absolute neutrophil count of ≥500 cells/mm^3 for 3 consecutive days) and platelet (defined as achievement of ≥20,000 platelets/mm^3 unsupported by transfusion for 7 days) recoveries were 14 (range, 5–28) and 19 (range, 7–112) days respectively after peripheral blood stem cell transplantation (PBSCT), 19 (range 6–41) and 28 (range, 10–150) days respectively after bone marrow transplantation, and 24 (range, 12–68) and 52 (range, 22–275) days, respectively after UCBT [18].

The impact of graft composition is particularly marked in the UCBT setting where transplantation of units containing total nucleated cells (TNC) ≥2.5 × 10^7 cells/kg of recipient body weight or ≥1.7 × 10^5 CD34+ cells/kg of recipient body weight have been associated faster neutrophil engraftment and reduced incidence of engraftment failure for adults with acute leukemia [19,26]. There is also a positive correlation between the CD34+ cell dose and the speed of neutrophil and platelet engraftments following myeloablative BMT and PBSCT [20], and with donor T cell engraftment after nonmyeloablative conditioning [27].

The type of the conditioning regimen also impact engraftment kinetics after allo-HCT. Specifically, the use of total-body irradiation based myeloablative conditioning has been associated with faster neutrophil and platelet engraftment than chemotherapy only based regimens after UCBT [18], while engraftment kinetics were comparable in patients receiving UCBT after myeloablative or reduced intensity conditioning (RIC) [28].

Finally, regarding GVHD prophylaxis, the use of antithymocyte globulin (ATG) in the conditioning regimen as well as postgrafting immunosuppression with methotrexate have each been associated with a delayed hematologic recovery [29–31].

2.2. Hematopoietic growth factors

Several registry and prospective randomized studies have assessed the impact of G-CSF after allo-HCT. Specifically, two registry studies, one from the European Society for Blood and Marrow Transplantation (EBMT) and one from the Center for International Blood and Marrow Transplant Research (CIBMTR) demonstrated faster neutrophil engraftment in patients given G-CSF [32,33]. This was confirmed in three prospective randomized studies (Table 1). Specifically, Ernst et al. reported the results of a randomized phase III placebo-controlled trial of G-SCF administration after allogeneic bone marrow transplantation (BMT; n = 51) [8]. G-CSF was administered from day 0 to engraftment or day +42 at the dose of 5 µg/kg. Patients randomized in the G-CSF group had significantly faster neutrophil engraftment than control patients (15 vs. 19 days, p < .001), while other transplantation outcomes were similar in the two groups of patients. Bishop et al. performed a randomized double blind trial of G-CSF administration in patients receiving PBSC from HLA-matched related donors [34]. G-CSF was administered at the dose of 10 µg/kg from day 0 to neutrophil recovery. The incidence of neutrophil recovery was significantly faster in patients randomized in the G-CSF arm (11 vs. 15 days, p = .008). Similarly, Przepiorka et al. randomized 42 adult patients given PBSC from HLA-identical sibling donors to receive or not G-CSF at 10 µg/kg from day 1 to neutrophil recovery [35]. Again patients randomized in the G-CSF arm had faster neutrophil recovery (12 vs. 15 days, p = .002) and a trend for earlier hospital discharge (16 vs. 20 days, p = .05). Taken together, these studies suggest that administration of G-CSF after allo-HCT decreases the time

Table 1. Selected randomized studies of G-CSF or GM-CSF administration after allo-HCT.

				Time to 500 neutrophils in G-CSF/control pts		Incidence of grade II-IC acute GVHD in G-CSF/control pts		1-year OS in G-CSF/control pts	
First author	Dose (µg/kg) /first day of G-CSF	Stem cell source	Number of pts	Median (range)	p-value	%	p-value	%	p-value
Bishop [34]	10/0	PBSC	44	11 (9–20) /15 (10–22)	0.008	48/61	0.4	65/58	.6
Przepiorka [35]	10/1	PBSC	42	12 (8–18)/15 (8–23)	0.002	27/34	NS	60/54	NS
Ernst [8]	5/0	BM	51	15 (1–22) /19 (15–28)	<0.001	12/20	NS	84/69	NS

PBSC: G-CSF mobilized peripheral blood stem cells; BM: bone marrow; NR: not reported; Pts: patients.

to neutrophil engraftment by 4 days and might decrease the length of hospitalization without undue toxicities. However, G-CSF administration failed to decrease the incidence of infections or to improve survival after allo-HCT [9].

GM-CSF has also been assessed after allo-HCT and also resulted in faster neutrophil engraftment than placebo [9]. Interestingly, a recent prospective randomized phase IV study suggested that GM-CSF might be more efficient than G-CSF to prevent invasive fungal disease (p = .07) and was associated with lower day 100 mortality (p = .04)[36]. Unfortunately, GM-CSF is no longer available in Europe.

Another approach to benefit from hematopoietic growth factors has consisted of *ex vivo* incubating the grafts in their presence for a few days before transplantation. Specifically, incubating 1/3 of bone marrow grafts with interleukin-3 (IL-3) and GM-CSF for 4 days enhanced hematopoietic recovery after allogeneic BMT (the remaining 2/3 of the grafts was infused unmanipulated on day 0) [37,38]. Similarly, infusion *ex vivo* expanded (10 days in the presence of stem cell factor (SCF), G-CSF and pegylated megakaryocyte growth and development factor (PEG-MGDF)) peripheral blood progenitor cells led to prompt neutrophil recovery after autologous HCT [39].

Finally, a randomized study, published in 2014, has demonstrated that recombinant human erythropoietin (Neorecormon, Roche, administered once weekly at the dose of 500 U/kg per week) hastened erythroid recovery and decreased red blood cell transfusion requirements when started 4 weeks after allo-HCT [40], a time where levels of endogenous erythropoietin are inappropriately low in regard to the degree of anemia [41].

2.3. Novel approaches to hasten hematologic recovery after UCBT

Given that hematological recovery is particularly slow after UCBT, efforts at prompting engraftment have been mostly studied in the UCBT setting. As mentioned above, there are some correlations between the number of CD34+ cells and TNC infused and the kinetics of neutrophil engraftment after UCBT [19,26]. Initial approaches aimed at expanding UCB with various cytokine cocktails met with little successes [12,13]. Fortunately, novel strategies aimed at increasing the number of hematopoietic stem and progenitor cells (HSPCs) transplanted for UCBT or at increasing their ability to home to their bone marrow niches have been much more encouraging and might lead in the future to a regrowth of adult UCBT in Europe, a transplant approach currently challenged by the development of T-cell repleted HLA-haploidentical stem cell transplantation [42–44]. These strategies have consisted of double UCBT with or without expansion of one of the 2 UCB units, cotransplanting a single UCB unit with HLA-haploidentical CD34+ cells (haplo-cord transplantation), or increasing UCB HSC homing to marrow niches via direct intrabone UCB administration, pulse treatment with dmPGE2 or enforced fucosylation [13,45] (Table 2).

2.3.1. Double units UCBT

Transplantation of two cord blood units, pioneered by the Minnesota group, has been a major breakthrough in the field of UCBT [54–56]. This approach allowed increasing the dose of TNC transplanted, and, as a result, overcame the cell-dose barrier that limits the feasibility of UCBT in adults. Interestingly, while the two units contributed to hematopoiesis the first months after transplantation, only one of the two transplanted unit was responsible for hematopoiesis by day 100 and beyond in 70–95% of the cases [57,58], depending of the conditioning regimen used.

Based on the feasibility of double UCBT in adult patients, the Blood and Marrow Transplant Clinical Trials Network (BMT-CTN) conducted a prospective randomized trial of single versus double UCBT in children and adolescents with hematologic cancers [59]. Two hundred and twenty-four patients were

Table 2. Outcomes of selected studies of UCBT with engineered UCB-derived HSPCs or with cotransplantation of CD34+ cells from HLA-haploidentical donors.

Study	Patients (#)	Type of engineering	CD34 fold expansion/ days of *ex vivo* expansion	Days to neutrophil engraftment: median (range) in study vs. control patients	Predominant engrafting unit
Double UCBT with HSPCs expansion of one of the two units					
Delaney et al. [46,47]	10	Notch-mediated HSPCs expansion	164/16	16 (7–34)[a,b]/26 (16–48) (p = 0.002)	Unmanipulated
de Lima et al. [48]	31	MSCs-mediated HSPCs expansion	30/14	15[c] (9–42) vs. 21 (6–45)[d] (p = 0.08) or 24 (12–52)[e] (p < 0.001)	Unmanipulated
Horwitz et al. [49]	11	Nam-mediated HSPCs expansion	72/21	13 (7–26) vs. 25 (13–38) (p < 0.001)	Manipulated
Wagner et al. [50]	17	SR1-mediated HSPCs expansion	330/NR	15 (6–30) /24 (ND) p < 0.001	Manipulated
Double UCBT with UCB chemical modification to improve homing of one of the two units					
Cutler et al. [51]	12	$dmPGE_2$ modification of 1 UCB unit	NA	18 (14–31) vs. 21 (NR) (p<.05)	Manipulated
Popat et al. [52]	22	Fucosylation of one UCB unit	NA	17 (12–34) vs. 26 (11–48) (p = 0.002)	Manipulated in half of the patients
Single UCBT with cotransplantation of CD34± cells from a G-CSF mobilized HLA-haploidentical donor (haplo cord)					
Van Besien [53]	97	Unmanipulated UCBT Cotransplantation of CD34+ cells from HLA-haploidentical G-CSF mobilized donors	NA	NR but significantly faster (HR = 1.4, p = 0.007)[f]	UCB

[a]Median of 11 days in an updated cohort of 17 patients.
[b]One of 10 patients had primary graft rejection.
[c]One patient died on day 30 without engraftment.
[d]Controls from the MD Anderson Cancer Research Center.
[e]Controls from the CIBMTR.
[f]The control group consisted of 193 double UCBT recipients.

Table 3. Impact of double versus single UCBT on outcomes.

		Relapse		Nonrelapse mortality		Treatment failure (inverse of LFS)	
Study group	Number of pts given sUCB/dUCB	HR (95% CI)	p-value	HR (95% CI)	p-value	HR (95% CI)	p-value
Studies in children and adolescents							
BMT-CTN [59]	113/111	1.2 (NR)	0.1	1.2 (NR)	0.4	1.5 (1.0–2.3)	.08
French [60]	74/77	0.7 (NR)	0.3	2.0 (NR)	0.3	1.0 (NR)	.7
Studies in adults							
CIBMTR [61]	106/303	0.9 (0.6–1.6)	.78	0.9 (0.7–1.3)	.56	1.0 (0.8–1.2)	.8
EBMT-Eurocord MAC[a][62]	88/83	0.8 (0.4–1.9)	.62	1.1 (0.6–1.9)	.75	1.0 (0.6–1.5)	1
EBMT-Eurocord RIC [63]	172/362	0.9 (0.6–1.3)	0.5	0.8 (0.5–1.2)	0.3	0.8 (0.7–1.1)	.2

[a]Single UCB recipient group restricted to patients given UCB after TBF-based conditioning. CIBMTR: Center for International Blood and Marrow Transplant Research; EBMT: European group for Blood and Marrow Transplantation; BMT-CTN: the Blood and Marrow Transplant Clinical Trials Network; Pts: patients.

included. Each patient had two UCB units (a first containing >2.5 × 10^7 TNC/kg recipient and a second containing >1.5 × 10^7 TNC/kg recipient) that were HLA-matched at ≥4/6 loci. Main observations were that double UCB failed to improve 1-year overall survival (OS; the primary end point of the study). Further, in comparison to single unit recipients, double UCB recipients had higher incidences of each grade III-IV acute (23% vs. 13%, p = .02) and extensive chronic (15% vs. 9%, p = .05) GVHD (Table 3).

Another prospective randomized study of single versus double UCB was performed by the French group in children and young adults with acute leukemia or myelodysplastic syndrome [60]. One hundred and fifty-one patients were randomized (and 137 were transplanted). Double UCBT failed to decrease the transplantation strategy failure (defined as the first of the four following events: transplant-related mortality, autologous recovery, second allogeneic transplantation, or infusion of an autologous stem cell rescue for engraftment failure). Secondary end points were also similar in the two groups of patients (Table 3).

Three large registry studies have compared UCBT outcomes in adult patients with acute leukemia transplanted with one (containing >2.5 × 10^7 TNC/kg) versus two UCB units [61]. The first study was reported by the CIBMTR and the National Cord Blood Program New York Blood Center and included mainly patients transplanted after myeloablative conditioning regimen [61]. The authors observed similar engraftment kinetics, relapse, nonrelapse mortality (NRM), leukemia-free survival (LFS) and OS in patients given one (n = 106) versus two (n = 303) UCB units (Table 3). Eurocord and the acute leukemia working party of the EBMT performed two separate analyses, one in patients given UCBT after myeloablative and a second in patients receiving UCBT following RIC conditioning. In the study reporting data of patients receiving UCB after myeloablative conditioning (n = 239), among patients transplanted with one single UCB unit, those receiving a thiothepa, busulfan and fludarabine (TBF) regimen had better LFS than those transplanted with busulfan- or TBI-based regimens [62]. When the single UCBT group was restricted to patients given TBF-based conditioning, transplantation outcomes were comparable between patients receiving single or double UCBT, with the exception for a higher incidence of grade II–IV acute GVHD in double UCBT recipients. Similarly, in the study reporting data from patients receiving grafts after RIC, engraftment kinetics, relapse, NRM, LFS and OS were similar in patients given one (n = 172) versus two (n = 362) UCB units, while there was a suggestion for higher incidence of grade II-IV aGVHD in double UCB recipients (36 vs. 28%, p = .08) [63] (Table 3).

Taken together these results demonstrate that although double UCBT achieved the aim of allowing patients without a sufficiently rich single UCB unit to benefit from UCBT, it failed to improve engraftment and other transplantation outcomes in patients who had a single UCB unit containing ≥2.5 × 10^7 TNC/kg recipient. This is probably due to the development of graft-versus-graft reactions, as recently evidenced by Lamers et al. [64]

2.3.2. Double units UCBT with expansion of one of the two units

In addition of allowing successful UCBT in patients who lack a single unit containing >2.5 × 10^7 TNC/kg, the development of double UCBT provided a great platform for assessing strategies of HSPCs expansion. Indeed, it offered the possibility of cotransplanting an unmanipulated UCB unit containing a sufficient number of TNC to secure long-term engraftment with a second fully expanded unit. Further, this experimental setting allowed quantifying the proportion of hematopoiesis originating from the unmanipulated versus the expanded unit by assessment of blood or bone marrow chimerism [65]. Potential limitations of all these *ex vivo* UCBT expansion approaches are their cost, the need for a GMP production facility, and the difficulty of expanding HSPCs without inducing their differentiation.

Indeed, the proliferation, expansion and differentiation of HSPCs can be stimulated by several growth factors and cytokines that are expressed in HSC niches. Most potent cytokines for HSPCs expansion include SCF, thrombopoietin (TPO), and flt3 ligand (flt3l) [66]. On the other hand IL-3, IL-6, IL-11 and G-CSF have a tendency to generate differentiated cells [66]. Nevertheless, there are strong synergistic effects for HSPCs expansion between SCF and flt3l, and between IL-6 and both SCF and flt3l [66].

2.3.2.1. Notch-mediated expansion. Notch proteins impact cell-fate decisions in many developmental systems. Notch receptors 1 (Notch-1) and 2 (Notch-2) are expressed by

human HSPCs, while Notch ligands Delta-1 and Jagged-1 are expressed by human BM stromal cells, endothelial cells and osteoblasts [67]. Preclinical studies by Delaney et al. demonstrated that *ex vivo* expansion of UCB with low density (2.5 µg/mL) of an engineered form of the Notch ligand Delta1 in immobilized form and a cytokine cocktail combining SCF, TPO, flt3l, IL-6, and IL-3 allowed a >100 fold increase in the absolute number of HSPCs, including those capable of repopulating NOD/SCID mice [46,68]. Based on these observations, the authors assessed the feasibility and safety of coinfusing a first unmanipulated UCB unit with a second UCB unit that was CD34-selected and then expanded *ex vivo* for 16 days on low-density immobilized Delta-1, as described above [46]. The authors evidenced durable engraftment in nine of the 10 included patients, while the remaining patient experienced primary graft rejection. Median time to neutrophil engraftment was 16 days (range, 7–34 days) in study patients versus 26 days (range, 16–48 days) in historical ones ($p = .002$). Unfortunately, OS from study and historical patients was not compared in this report. Interestingly, while long-term engraftment originated mostly from the nonexpanded unit, the expanded graft contributed almost exclusively to initial myeloid engraftment [47]. The relatively weak contribution of the Notch-mediated expanded unit to long-term hematopoiesis might suggest a deficiency in true stem cell expansion with this technique and that the improved engraftment observed was due mainly to expansion of short-term repopulating progenitor cells. However, another potential explanation might be that the unmanipulated (T-cell-replete unit) developed an immune response against the expanded graft leading to its subsequent rejection [45,64].

2.3.2.2. MSC-mediated expansion. Mesenchymal stromal cells (MSCs) are fibroblast-like multipotent cells that have the ability to support hematopoiesis on one hand, and to regulate immune reactions such as GVHD on the other hand [69–72]. In a preclinical study, investigators from the M.D. Anderson Cancer Center observed that coculture of unmanipulated UCB with bone marrow-derived MSCs in a culture media supplemented with SCF, flt3l, TPO and G-CSF resulted in a CD34+ and CD133+ expansions of eight and 31 fold after 14 days [73].

Based on these observations, de Lima, Shpall et al. launched a pilot trial of double UCBT with the largest unit transplanted unmanipulated (on day 0) and the second unit transplanted also on day 0 after a 14-day *ex vivo* expansion in coculture with MSCs. Results of the first 31 patients included have been reported in the New England Journal of Medicine [48]. Median time to neutrophil engraftment was 15 days (range, 9–42 days) in the MSC group, compared with 24 days (range, 12–52 days) in matched controls from the CIBMTR ($p < .001$). Further, the median time to platelet engraftment was 42 days (range, 15–62 days) in expanded UCB recipients versus 49 days (range, 18–264 days) in controls ($p = .03$). Interestingly, engraftment beyond 1 year originated primarily from the unmanipulated UCB unit in all patients while cells from the expanded unit persisted in 13% of the patients at 6 months. The relatively low contribution of the MSC-mediated expanded unit to long-term hematopoiesis suggest a deficiency in true stem cell expansion with this technique and that the improved engraftment observed was due to expansion of mainly short-term repopulating progenitors cells. Unfortunately, the authors did not compare OS between MSC and matched control patients.

2.3.2.3. Nicotinamide-mediated expansion. Nicotinamide (NAM) is a potent sirtuin 1 (SIRT1) inhibitor that facilitates HSPCs expansion and inhibits HSPCs differentiation *in vitro* [74]. Further, NAM increased both HSPCs migration toward stromal cell derived factor-1 (SDF1) in transwell migration assay and HSPCs homing/engraftment in NOD/SCID mice. These observations prompted Horwitz et al. to conduct a phase I trial of double UCBT with one unit infused unmanipulated and the second infused after *ex vivo* expansion with NAM (this expanded UCB product was termed NiCord) for 21 days [49]. The T cell containing fraction of the expanded unit was refrozen following thaw and injected with the expanded HSPCs fraction in order to retain immunologic properties and favor long-term engraftment of the expanded unit through graft-versus-graft interactions (Figure 1). Neutrophil engraftment was achieved after a median of 13 days (range, 7–26 days) in NiCord recipients ($n = 11$), versus 25 days (range, 13–38 days) in historical control patients ($n = 17$, $p < .001$). In contrast, median time to platelet engraftment was comparable in NiCord (33 days [range, 26–49 days]) and control (37 days [range, 20–66 days]) patients ($p = .09$). Interestingly, in contrast to what has been observed with Notch-ligand or MSC UCB expansion, the NiCord expanded unit was responsible for long-term hematopoiesis in seven (and predominant in six) of nine assessable patients. Further, the NiCord expanded unit also contributed to T-cell chimerism in six out of nine evaluable patients. These preliminary results indicate that NAM-mediated HSPCs expansion preserved or even expanded a proportion of true stem cells while reinfusion of the T cell containing fraction of the manipulated unit prevented its immune-mediated rejection by residual immune cells from the patient or by immune cells from the unmanipulated unit.

Based on these promising results, a prospective trial of transplantation of a single NiCord expanded UCB after myeloablative conditioning has been recently launched (Clinicaltrials.gov NCT01816230).

2.3.2.4. SR1-mediated expansion. Boitano et al. performed an unbiased screen of 100,000 compounds to identify potential molecules that promote HSPCs expansion. The authors identified a purine derivative, the StemRegenin 1 (SR1), as a potent inhibitor of HSPCs differentiation [75]. SR1 acts by antagonizing the aryl hydrocarbon receptor (AhR) and allows dramatic HSPCs expansion in serum-free culture media supplemented with SCF, flt3l, TPO and IL-6. Notably, culture with SR1 for 3 weeks let to an 11-fold TNC increase and a 73-fold increase for CD34+ cells in comparison to control cultures (without SR1) with a >1000-fold CD34+ cell increase in comparison to input cells.

These impressive results led to the development of a phase I and II study [50]. CD34+ selected cells from one UCB were expanded in the presence of SR1, SCF, flt3l, IL-6

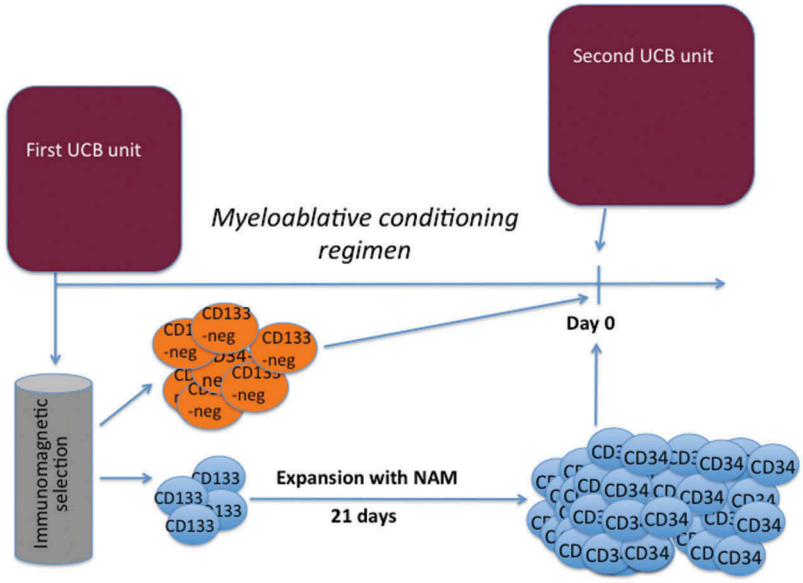

Figure 1. Scheme of the study investigating NAM-mediated hematopoietic stem and progenitor cells (HSPCs) expansion [49] (the figure is reproduced from Baron, Ruggeri and Nagler, ref [12] with permission). One UCB unit was CD133-selected and expanded ex vivo with NMA (this expanded UCB product was termed NiCord) for 21 days. The T cell containing fraction of the expanded unit was re-frozen following thaw and injected with the expanded HSPCs fraction (in order to retains immunologic potency and favor long-term engraftment of the manipulated unit) and with the unmanipulated UCB unit after myeloablative conditioning. Neutrophil engraftment was very prompt and was achieved after a median of 13 days (range, 7–26 days) in NiCord recipients (n = 11), versus 25 days (range, 13–38 days) in historical control patients (n = 17, P < 0.001). Further, the NiCord expanded unit was responsible for long-term hematopoiesis in 7 (and predominant in 6) of 9 assessable patients.

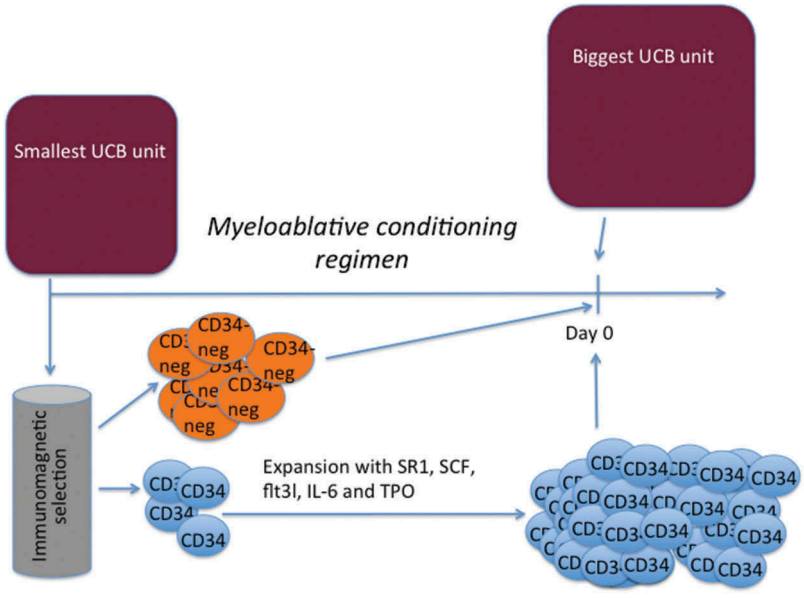

Figure 2. Scheme of the study investigating (SR1)-mediated hematopoietic stem and progenitor cells (HSPCs) expansion [50](the figure is reproduced from Baron, Ruggeri and Nagler, ref [12] with permission). CD34+ selected cells from the smallest UCB were expanded in the presence of SR1, SCF, flt3l, IL-6 and TPO (the product was referred as 'HSC835'). Preliminary results including data from 19 patients were presented at the 2014 annual meeting of the American Society of Hematology [76]. Seventeen patients were transplanted with HSC835 along with its CD34-depleted fraction (that was re-frozen after the CD34-selection) and an unmanipulated UCB unit. Expansion with SR1 was impressive with the SR1 expansion culture yielding a median of 1,440 x 10^6 CD34+ cells (range, 140–6,362 x 10^6 CD34+ cells) after a median CD34+ cell enrichment of 328 fold (range, 66–844 fold). Consequently, HSC835 patients received an impressive median of 12.3 x10^6 CD34+ cells/kg (range, 2.3–48.5 x10^6 CD34+ cells/kg). This led to a very prompt engraftment with neutrophil engraftment occurring after 11 days (range, 6–23 days) in the 11 of 17 patients in whom the HSC835 unit predominated, versus 23 days (range, 14–30 days) for those in whom the unmanipulated unit predominated. Long- term chimerism was derived exclusively from the unmanipulated or the HSC835 unit in 6 patients each, while 5 patients experienced dual chimerism (CD3 chimerism from the unmanipulated unit and myeloid chimerism from the HSC835 unit).

and TPO (the product was referred as 'HSC835') (Figure 2). Twenty patients were recruited and 17 completed the prescribed treatment plan. These 17 patients were transplanted with HSC835 along with its CD34-depleted fraction (as done in the NiCord study to prevent immune rejection of the expanded unit) and an unmanipulated UCB unit. As expected, HSC835 graft contained significantly more CD34 cells than unmanipulated units, with a median of 17.5 × 10^6

CD34 (range, 1.4–48.3) per kilogram body weight for HSC835 units versus 0.2 × 10^6 CD34+ cells/kg present in unmanipulated UCB units. In contrast, HSC835 graft contained significantly less CD3+ T cells because it was derived from the smaller unit and because of recryopreservation nonspecific losses. The high numbers of CD34+ cells infused led to a prompt engraftment with 100% of HSC835 patients achieving neutrophil recovery a median of 15 days (range, 6–30 days) after UCBT. Median time to platelet recovery was 49 days (range, 28–136 days). Interestingly, neutrophil engraftment occurred after at a median of 11 days (range, 6–23 days) in the 11 of 17 patients in whom the HSC835 unit predominated, versus 23 days (range, 14–30 days) for those in whom the unmanipulated unit predominated. Long-term chimerism was derived predominantly from the unmanipulated or the HSC835 unit in six patients each, while five patients experienced dual chimerism (CD3 chimerism from the unmanipulated unit and myeloid chimerism from the HSC835 unit). Importantly, the authors demonstrated the presence of interferon-γ producing T cells directed against the losing graft, suggesting that unit predominance was due to graft-versus-graft immune reactions.

Based on these data, the investigators launched a pilot trial aimed at evaluating the safety and efficacy of transplanting HSC835 as sole stem cell source [76]. Of note, the two first patients included achieved neutrophil engraftment on days 12 and 8, respectively. The trial that is still ongoing at the university of Minnesota plans to recruit a total of 10 patients (Clinicaltrials.gov: NCT01930162).

2.3.3. Cotransplantation a single UCB unit with HLA-haploidentical CD34+ cells

Another approach to prompt hematologic recovery after single unit UCBT in adults has consisted of coinfusion mobilized stem cells from an HLA-haploidentical donor (haplo-cord transplantation)[77]. This approach has been pioneered by Magro et al. in a phase I and II study including 27 consecutive patients with high-risk malignancies. These patients received a single UCBT coinfused with CD34- (or CD133) selected PBSC [77]. Neutrophil engraftment occurred 10 days (range, 9–36 days) after transplantation and was initially of PBSC origin in 23 out of 27 patients. In contrast, full UCB-derived chimerism was achieved in 93% of the patients. Recently, van Besien et al. compared transplantation outcomes of a group 97 adult patients who underwent haplo-cord transplantation at the University of Chicago or at the Well Cornell Medical College and a group of 193 patients from the CIBMTR database given double UCBT [53]. Conditioning regimen consisted of fludarabine, melphalan and ATG in the patients that were transplanted with the haplo-cord graft vs. low-dose TBI, cyclophosphamide and fludarabine (TCF) in double UCB recipients. Neutrophil and platelet cumulative incidences of engraftment were significantly faster in haplo-cord than in double UCBT patients ($p < .01$). Further, haplo-cord patients had also a lower incidence of grade II–IV acute (HR = .3, $p < 0.001$) and chronic (HR = .1, $p < 0.001$) GVHD, a lower risk of relapse (HR = 0.5, $p = .001$) but had comparable OS (HR = 1.0) than double UCB recipients.

2.3.4. Promotion of UCBT HSC homing

Although the technologies discussed above of *ex vivo* HSPC expansion are becoming more and more successful, they remain costly and technically challenging. Thus, investigators are also developing easier approach to address the low HSPC content of one UCB unit: improving their homing to bone marrow niches. Most promising approaches have consisted of direct intra bone UCB injection, pulse treatment of UCB with dmPGE2, and UCB enforced fucosylation [12,13].

2.3.4.1. Direct intra bone UCB injection. Direct intra bone UCB injection has been investigated in order to prompt engraftment. This technique has been pioneered by the Genova group who demonstrated the feasibility of this approach using a single cord blood unit injected intra bone in 32 patients with acute leukemia [78]. A retrospective study by the Eurocord group has compared outcomes of 87 patients given intra bone UCBT to those of 149 double UCBT recipients [79]. All patients received UCBT after a myeloablative conditioning regimen. Median TNC infused were 2.5 × 10^7/kg for intra bone UCBT and 3.9 × 10^7/kg for double UCBT ($p < 0.001$). In comparison to double UCB recipients, intra bone UCB patients had faster neutrophil engraftment (23 vs. 28 days, $p = .001$) while a higher proportion of intra bone patients achieved platelet engraftment at 6 months (74% vs. 64%, $p = .003$). Interestingly, intra bone patients had also a lower incidence of grade II–IV acute GVHD ($p < .01$). However, importantly, OS was superimposable in the two groups (47% vs. 45% at 2 years).

2.3.4.2. Pulse treatment of UCB with dmPGE2. 16, 16-dimethyl prostaglandin E2 (dmPGE2) increases HSPC numbers *in vivo* without affecting their self-renewal and differentiation potential [80]. This is achieved through cAMP-mediated regulation of the Wnt signaling pathway that controls HSPC proliferation and apoptosis, and through increased expression cyclinD1 and surviving [80,81]. Further, dmPGE2 is able to enhance HSPC homing to bone marrow niches through upregulation of CXCR4 surface expression [81].

Based on these observations, Cutler et al. conducted a phase I study that assessed the safety and therapeutic potential of *ex vivo* modulation of a single UCB unit using dmPGE2 before reduced-intensity, double UCBT [51]. Twelve patients were treated according to an optimized *ex vivo* $dmPGE_2$ modulation protocol. The largest UCB unit was incubated with 10 μM of $dmPGE_2$ for 2-h at 37°C and then infused to the patients. The second UCB unit was infused unmanipulated 4 h later. Median time to neutrophil engraftment was 17.5 days (range, 14–31 days), significantly faster than in historical patients ($p = .04$). Further, 10 of 12 patients had early and sustained engraftment of the $dmPGE_2$-UCB unit that contributed 100% to hematopoiesis.

The demonstration that the dmPGE2 unit was predominant in 10 of 12 patients is encouraging, although this might also be partly attributed to the fact that the unit that was modulated with $dmPGE_2$ was the biggest one. Nevertheless, based on these encouraging results, dmPGE2 modulation of UCB is currently being studied in a randomized phase II study in the

double UCBT setting (Clinicaltrials.gov #NCT01627314). The study plans to include up to 60 patients. The primary end point is neutrophil engraftment and chimerism.

2.3.4.3. Enforced UCB fucosylation. Preclinical studies by Xia et al. demonstrated that alpha1-3 fucosylation of UCB HSPCs improved their homing and engraftment in NOD/SCID mice [82]. Based on these findings, Popat et al. conducted a pilot trial aimed at assessing the feasibility, safety, and efficacy of enforced UCB cell surface fucosylation of a single UCB unit in double UCBT setting [52]. Twenty-two patients with advanced hematological malignancies were included. The smallest UCB unit, containing a median of 2.4×10^7 TNCs/kg (range, 1.8–3.3 TNCs/kg), was treated *ex vivo* for 30 min with the enzyme fucosyltransferase-VI and guanosine diphosphate fucose, while the largest unit, containing a median of 3.1×10^7 TNCs/kg (range, 2.2–5.9 TNCs/kg), was infused unmanipulated. One patient experienced secondary graft failure and one patient died before engraftment. The median time to neutrophil engraftment in the 20 assessable patients was 17 days (range, 12–34 days), compared with 26 days (range, 11–48 days) for a group of 31 historical controls ($p = .002$). Similarly, platelet engraftment was also faster in study patients than in historical controls ($p = 0.05$). Interestingly, at day 30, hematopoiesis originated solely from the unmanipulated UCB in 40% of patients, solely from the fucosylated UCB unit in another 40%, and from both units in the remaining 20% of patients. Unfortunately, the impact of UCB fucosylation on OS was not reported.

3. Strategies to hasten hematologic recovery after intensive chemotherapy for AML

3.1. Hematopoietic growth factors

The use of hematopoietic growth factors such as G-CSF or GM-CSF has been extensively studied in AML patients. On the one hand, hematopoietic growth factors have the ability to enhance the killing of leukemic blasts by cytotoxic drugs *in vitro*. Specifically, exposure of leukemic cells to cytarabine in the context of growth factor stimulation increased the formation of cytarabine-triphosphate, increased DNA uptake of radiolabeled cytarabine in leukemic cells, and enhanced leukemic cell cytotoxicity [83]. This prompted several group of investigators to conduct prospective randomized trials of leukemic blast priming by G-CSF or GM-CSF during intensive chemotherapy. Several of these trials demonstrated that administration of G-CSF or GM-CSF during induction-remission chemotherapy increased the proportion of patients who achieve a complete remission [11,84], while others failed to find such an association [83].

On the other hand, given that infections have been the leading cause of mortality, the first month after induction chemotherapy for AML, adminsitration G-CSF and GM-CSF have also been assessed after administration of intensive chemotherapy in an effort at enhancing neutrophil recovery and preventing infections. Data from 19 randomized trials performed between 1990 and 2003 and including a total of 5256 patients have been systematically reviewed in a meta-analysis [10]. Main findings of the meta-analysis were that the administration of hematopoietic growth factors failed to decrease the incidence of febrile neutropenia, bacteremias or fungal infections and did not impact overall survival. Several of these randomized trials reported shorthened duration of neutropenia with administration of hematopoietic growth factors, as well as shorter hospitalization. As example, in one of the largest trial conducted by the EORTC/GIMEMA, patients who received G-CSF after chemotherapy had shorter time to neutrophil recovery (median, 20 vs. 25 days, $p < .01$) and slightly lower hospitalization duration (mean, 27.2 vs. 29.7 days, $p < .01$) [11]. However, there was no benefit of G-CSF administration in term of infection incidence or overall mortality. Their use should thus be restricted to patients who are expected to have a prolonged period of neutropenia such as patients who received an intensified form of chemotherapy, those who are neutropenic at diagnosis [6], as well as to patients with life-threatening infections. Further, it should be stressed that the use of G-CSF or of GM-CSF might increase the risk of secondary leukemia [85].

3.2. Infusion of a non-HLA-matched ex vivo expanded UCB

Based on the very encouraging observed in the UCBT setting, Delaney et al. conducted a phase I trial investigating the administration of non-HLA-matched ex vivo expanded UCB to accelerate hematopoietic recovery after intensive AML chemotherapy [17]. Twenty-nine patients were included. UCB were ex vivo expanded after CD34-selection in the presence of the Notch ligand Delta1 as described above. There were no unexpected toxicities associated with expanded UCB administration, and specifically no cases of GVHD, although there was more episode of febrile neutropenia than in study patients than in historical ones perhaps translating an 'engraftment syndrome'. However neutrophil recovery and infection incidence were similar in patients given expanded UCB and in historical controls. The potential utility of transplanting *ex vivo* expanded UCB to accelerate hematopoietic recovery (and more importantly improve OS) after intensive AML chemotherapy deserves further evaluation in prospective phase II/III trials.

4. Conclusions

In the allo-HCT setting or in the setting of intense chemotherapy for AML, administration of G-CSF shortened the duration of the neutropenic phase without improving OS. Several novel approaches aimed at prompting neutrophil recovery after UCBT such as double UCBT with expansion of one of the two UCB units, cotransplanting a single UCB unit with HLA-haploidentical CD34+ cells, or increasing UCB HSPC homing to marrow niches are encouraging but should be assessed in phase III studies.

5. Expert opinion

High-dose conditioning regimen administered before myeloablative allo-HCT as well as intensive chemotherapy regimens used to treat AML result in prolonged bone marrow aplasia and particularly prolonged neutropenia that often leads to bacterial and/or fungal infections [3–6]. This is particularly the case in the UCBT setting [4], or when the intensity of the

chemotherapy is increased in the induction or consolidation chemotherapy course of AML.

In the allo-HCT setting, administration of G-CSF or GM-CSF shortened the duration of the neutropenic phase by approximately 4 days, decreased the length of hospitalization but failed to decrease infection incidence or to improve OS. They should thus not be systematically administered in that setting although their use appeared to be safe and that they are relatively cheap.

Several novel approaches to hasten hematological reconstruction after UCBT are promising. Data from phase I and II studies demonstrated that double UCBT with expansion of one of the two UCB units with Notch ligand, MSCs, nicotinamide, or SR1 all shortened the duration of the neutropenic phase and accelerated platelet recovery. Although notch-mediated and MSC-mediated expansion techniques provided mainly short-term hematopoiesis, UCB expansion with nicotinamide or with SRI provided both short-term and long-term hematopoimesis. Phase III trials are needed to assess the impact of these new approaches on nonrelapse mortality and OS. Indeed, up to now none of these novel approaches have demonstrated improving OS. Further, other phase III studies are needed to compare neutrophil engraftment with these novel approaches (that are complicated, costly and require GMP infrastructure) to systematic G(M)-CSF administration.

Another (less costly) approach to provide short time hematopoiesis and decrease the duration of the neutropenia and thrombocytopenia has consisted of cotransplanting UCB with CD34+ selected cells isolated from an apheresis product obtained in a HLA-haploidentical donor mobilized with G-CSF. Preliminary encouraging results with this approach have been confirmed by several independent groups of investigators although there was no demonstration that this approach improved OS. Thus, here again, randomized are needed to compare haplocord transplantation to UCBT or haplo-identical transplantation alone.

Prompt engraftment has also been achieved with recent approaches aimed at improving UCB-derived HPSCs homing to BM niches such as direct intrabone UCB administration, PGE2-priming of UCB or forced UCB fucosylation. Confirmation of long-term safety as well as determination of the impact of these approaches on the incidence of primary and secondary graft failure and on OS will require longer follow-up and larger studies.

In the AML setting, G-CSF or GM-CSF administration after intensive chemotherapy decreased the duration of the neutropenia without increasing the incidence of relapse but unfortunately also without improving survival. Their use should thus be restricted to patients who are expected to have a prolonged period of neutropenia such as patients who received an intensified form of chemotherapy or of those who are neutropenic at diagnosis [6], as well as to patients with life-threatening infections. Finally, administration of Notch ligand expanded UCB to hasten hematological reconstitution in AML patients receiving intensive chemotherapy is a novel interesting approach that deserve further assessment in phases II/III trials.

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Declaration of interest

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