Stem Cell Research & Therapy

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Co-infusion of mesenchymal stromal cells to prevent GVHD after allogeneic hematopoietic cell transplantation from HLA-mismatched unrelated donors after reduced-intensity conditioning: a double-blind randomized study and literature review

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Abstract

Background Mesenchymal stromal cells (MSC) have immunomodulatory and hematopoiesis-supporting properties that could potentially benefit hematopoietic stem cell (HSC) engraftment and decrease the incidence and/or severity of graft-versus-host disease (GVHD).

Methods Based on our previous pilot study, we established a multicenter, prospective, randomized, double-blind trial evaluating the efficacy of co-infusing third-party MSC ($1.5–3 \times 10^6$/kg) versus placebo on the day of HSC transplantation (HCT) to prevent GVHD in recipients of HLA-mismatched unrelated donors after reduced-intensity conditioning.

Results The study planned to include 120 patients to improve 1-year overall survival (OS) from 55 to 77% but was stopped after 9 years for low recruitment (n = 38). One-year OS was 74% in the MSC group and 80% in the placebo group. In multivariate analysis, the incidence of grade II-IV acute GVHD was significantly lower in patients receiving MSC (HR 0.332, 95% CI 0.124–0.890, $p = 0.0284$). No difference was observed in the incidences of chronic GVHD, infection or relapse, overall or progression-free survival at 1 year or long-term, or hematopoietic and immune reconstitution.

Conclusions Despite premature study closure, the suggested beneficial effect of MSC co-transplantation for the prevention of acute GVHD in HLA-mismatched HCT warrants further investigation.

Keywords Allogeneic hematopoietic cell transplantation, Mesenchymal stromal cells, GVHD, Prevention, HLA mismatch

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Introduction

Allogeneic hematopoietic cell transplantation (alloHCT) offers potential curative treatment for several hematological disorders. However, its success is counterbalanced by the occurrence of acute (aGVHD) and chronic (cGVHD) graft-versus-host disease (GVHD). AGVHD occurs in 30 to 60% of alloHCT recipients despite standard prophylaxis and is more common in cases of HLA disparities between donor and recipient [1]. First-line therapy with high-dose corticosteroids provides partial or complete responses in only 30 to 50% of patients [1]. Further, non-relapse mortality (NRM) is high in patients with steroid-refractory aGVHD, for example it was reported at 49% at 18 months among patients randomized in the ruxolitinib arm of the REACH2 trial [2]. CGVHD is also a serious complication of alloHCT, typically occurring between 100 days and 2 years after transplantation and affecting around 50% of patients sometimes for many years [3]. Therefore, it is critical to further improve GVHD prophylaxis and therapy.

Mesenchymal stromal cells (MSC) are multipotent non-hematopoietic progenitor cells that can differentiate into a variety of mature cells of the mesenchymal lineage (adipocytes, chondrocytes, osteocytes, muscles, …) [4]. MSC are present not only in the bone marrow but can be cultured from multiple tissues [4]. They support bone formation [5] and hematopoiesis in the bone marrow microenvironment in vitro [6] or in preclinical models [7]. Many studies have demonstrated the immunomodulatory and anti-inflammatory properties of MSC [8–10]. Furthermore, MSC are poorly immunogenic as they express little/no HLA class I/II molecules [8]. A number of academic cell therapy labs have embarked on the production of MSC according to Good Manufacturing Practice (GMP) [11, 12]. Based on their immunomodulating characteristics and on the capacity to produce them in GMP conditions, MSC have been investigated for the prevention or treatment of solid organ transplant rejection [13–16] as well as other inflammatory disorders such as COVID-19 [17], or Crohn's disease [18, 19].

Le Blanc et al. were the first to successfully treat patients with severe aGVHD with MSC [20]. Since then, we and many others have used MSC after alloHCT for the treatment of steroid-refractory aGVHD [21–29] or poor graft function [30]. Other studies have taken a preventive approach, using MSC co-infusion at the time of or early after transplantation in order to reduce the risk of graft rejection and GVHD [23, 25, 26, 31–34]. This has been particularly investigated in settings of non-malignant diseases, such as severe aplastic anemia (SAA) [35], or alloHCT procedures associated with a higher probability of graft rejection or severe GVHD, such as cord blood (CB), HLA-mismatched unrelated or haploidentical HCT. Some meta-analyses indicated that co-transplantation of MSC facilitated hematopoietic recovery and reduced the incidence of GVHD without increasing the risk of mortality [33, 34], but several others did not [25, 32, 35]. However, few studies were randomized, and results are inconsistent due to the heterogeneity of the MSC source, culture methods, dose and timing of injection, as well as of patient characteristics (age, disease …) and type of transplant (conditioning, donor, graft …). In addition, meta-analyses had much varying paper selection strategies, sometimes including abstracts or foreign language publications.

We previously published the result of a pilot study in which 20 patients received a single dose of MSC just before infusion of HSC from HLA-mismatched peripheral blood stem cells (PBSC) after nonmyeloablative conditioning [36]. Compared to a historical group of similar patients, NRM and death from GVHD were significantly decreased and one-year overall survival (OS) was increased [36]. Based on these promising data, we initiated in 2010 a multicenter, randomized, double-blind clinical trial (MSC versus placebo) to investigate the impact of a co-infusion of MSC with the HSC graft on the incidence of GVHD and survival. The study, however, had to be stopped due to slow recruitment. We nevertheless analyzed and present here the long-term results of the study in the cohort of 38 included patients.

Patients and methods

Study protocol

This is a prospective, multicenter, double-blind, clinical trial in which eligible patients were randomized between a single infusion of MSC or a placebo to be co-transplanted with HSC.

Key eligibility criteria included (1) a diagnosis of histologically confirmed malignant hematological disease not in rapid progression (acute leukemia in complete remission (CR); chronic myelogenous leukemia (CML) unresponsive/intolerant to TKi or other myeloproliferative neoplasms not in blast crisis; myelodysplastic syndromes (MDS) with < 5% blasts; chronic lymphocytic leukemia (CLL) or multiple myeloma not progressing rapidly; relapsed/refractory chemosensitive non-Hodgkin's or Hodgkin's lymphoma); (2) age ≤ 75 years; (3) having no HLA-identical (10/10 A-B-C-DRB1-DQB1) related or unrelated donor; and (4) not eligible to high-dose conditioning. Two conditioning regimens were selected, i.e. (1) a nonmyeloablative combination of fludarabine and 2 Gy total body irradiation (TBI), and (2) a reduced-intensity combination of fludarabine, melphalan and anti-T cell globulins (ATG). GVHD prophylaxis was based on tacrolimus combined with mycophenolate mofetil (MMF).

All patients had to receive PBSC from 1- or 2-mismatch unrelated donors.

The primary endpoint of the trial was to evaluate one-year OS in the two arms. Based on the results of our pilot study, which showed an 1-year OS of 77% in the MSC group compared to 55% in historical controls [36], we calculated that we needed to include 120 patients to demonstrate an improvement of 1-year OS from 55 (with placebo) to 77% (with MSC) at a significance level of $p < 0.05$, with a power of 0.70. Secondary endpoints comprised long-term survival, progression-free survival and relapse, recovery of the three hematological lineages and immune reconstitution, number of transfusions and transfusion independence, the incidence of aGVHD and cGVHD and their resolution rates, the incidence of various types of infection and secondary cancers.

The protocol was approved by the ethics committees of all participating centers and the study was conducted in accordance with the Declaration of Helsinki. Before participating in the study, all patients (or their legal representatives if minors) signed an informed consent form. The study was registered at ClinicalTrials.gov (NCT01045382, registered 08 Jan 2010, https://clinicaltrials.gov/study/NCT01045382) and EUDRACT (2009-014980-38).

Mesenchymal stromal cells

MSC were collected from the BM of 12 third-party healthy volunteer donors at the CHU of Liège. Written informed consent was obtained from each donor and the MSC harvest protocol was approved by the institutional ethics review board. MSC were expanded, cryopreserved and stored in our clinical-grade cell production facility (Laboratory of Cell and Gene Therapy, CHU and University of Liège, Liège, Belgium). The whole process for donor screening, BM collection, mononuclear cell isolation, MSC expansion, harvesting, cryopreservation, batch selection and thawing procedure, as well as quality control criteria has been described in detail elsewhere [12, 37]. Briefly, MSC were cultured in fetal bovine serum (FBS)-supplemented medium in a normoxic and humidified atmosphere, harvested after 3 passages and cryopreserved in a 10% dimethyl sulfoxide (DMSO)-containing solution. MSC were compliant with all criteria defined by the International Society for Cellular Therapy (ISCT) [4]. Placebo consisted of the same freezing solution without MSC.

MSC and placebo were thawed at the laboratory of cell therapy at each participating hospital and diluted by adding a 75% volume of PBS. MSC were administered as a single i.v. infusion at a post-thaw dose of $1.5–3 \times 10^6$ cells/kg body weight, through a central venous catheter and within 1 h of thawing, before PBSC. Patients were premedicated with 2 mg/kg methylprednisolone and an anti-histaminic drug.

Statistics

Patients were randomized 1:1 between MSC and placebo, with stratification per center. Results are presented as medians for continuous variables and as frequency tables for qualitative variables. Comparisons of variables between the two groups (placebo and MSC) were made using the Kruskal–Wallis test for continuous variables and the Fisher exact test for qualitative variables. Survivals are represented by Kaplan–Meier curves and compared between the two groups using the log-rank test. The cumulative incidences of different events (relapse, GVHD, infections, etc.) in competition with death and progression ("competing risks") were reported graphically and compared between groups using the Gray test. GVHD and survival outcomes were also studied after adjustment for the administration or not of pre-transplant ATG using a Cox regression model. In this case, we present the hazard ratio (HR) and its 95% confidence interval. No other multivariate analysis could be performed given the low number of patients.

The results are considered significant at the 5% uncertainty level ($p < 0.05$). The calculations were carried out using SAS version 9.4 and the figures using R version 4.2.2.

Results

Patients

The characteristics of the 38 included patients are described in Table 1. Median follow-up was 5.7 years. The placebo (n = 15) and MSC (n = 23) groups were mostly well balanced (no significant difference between groups, Table 1) but there was a trend for a higher Disease Risk Index (DRI) in the MSC group. Median age was 63 years (range 27 to 75 years), with 17 men and 21 women. Patients had acute leukemia (n = 16), myeloproliferative neoplasms or myelodysplastic syndromes (n = 9) or malignant lymphopathies (n = 13). Their DRI [38] was intermediate for 23 patients, low for 10 patients, and high for 5 patients (all 5 randomized in the MSC arm), and their co-morbidity index (HCT-CI) [39] was 0–2 in 16 patients and 3–8 in 12. All patients had a performance status ≤ 2. The conditioning regimen was fludarabine and 2 Gy of TBI in 68% and fludarabine, melphalan and ATG in 32%. Thirteen % of transplants were in male patients received HSC from a female donor, 45% had a major ABO incompatibility with their donor, and 45% were at high risk for cytomegalovirus (CMV) reactivation based on the serological status of the patient and donor. All patients had HLA disparities in the rejection direction (61% with 1 and 29% with 2 disparities) and/or

Table 1 Patient characteristics

A. General patient characteristics

	Total	Placebo	MSC	*P* value
Total number	38	15	23	
Age (median)	62.7	62.7	63	0.75
Gender (M/F)	17/21	6/9	11/12	0.74
Diagnosis				0.80
Acute lymphoblastic leukemia	2	1	1	
Acute myeloblastic leukemia	14	4	10	
Myeloproliferative neoplasm	5	3	2	
Myelodysplastic syndrome	4	1	3	
Chronic lymphocytic leukemia	4	2	2	
Non-Hodgkin's lymphoma	5	3	2	
Hodgkin's lymphoma	1	0	1	
Multiple myeloma	3	1	3	
Treatment lines (0–1/2/ ≥ 3)	10/7/11	5/3/6	5/4/5	0.57
Disease risk index DRI (low, intermed, high)	10/23/5	3/12/0	7/11/5	0.075
Comorbidity index HCT-CI (0/1–2/ ≥ 3)	8/8/12	5/2/7	3/6/5	0.52
ECOG performance status (0/1/2)	12/23/3	3/11/1	9/12/2	0.46
HLA mismatches, rejection direction (0/1/2)	4/23/11	1/10/4	3/13/7	0.76
HLA mismatches, GVHD direction (0/1/2)	1/25/12	0/10/5	1/15/7	0.71
Don-Rec sex matching (F—> M/other)	5/33	2/13	3/20	1
Don-Rec ABO compatibility (id/minor MM/major MM)	16/4/17	6/2/7	10/2/10	0.41
Don-Rec CMV status (Rec + /Don + /Don & Rec -)	14/3/21	7/0/8	7/3/13	0.32
Conditioning				0.37
Fludarabine + TBI (2 Gy)	26	9	17	
Fludarabine + Busulfan + ATG	12	6	6	
ATG (no/yes)	26/12	9/6	17/6	0.48

B Number of patients with 0, 1 or 2 donor/recipient HLA mismatches in the rejection and/or GVHD direction among 10 (A-B-C-DQB1-DRB1) alleles

Group	N mismatches (rejection direction)	N mismatches (GVHD direction)			Total
		0	1	2	
Placebo	0	0	1	0	1
	1	0	9	1	10
	2	0	0	4	4
	Total	*0*	*10*	*5*	*15*
MSC	0	0	3	0	3
	1	1	11	1	13
	2	0	1	6	7
	Total	*1*	*15*	*7*	*23*

in the GVHD direction (66% with 1 and 32% with 2 disparities) (Table 1B).

Patient outcomes

Survival and relapse

The primary objective of the study was one-year OS, which was 80% in the placebo arm and 74% in the MSC arm (Table 2 and Fig. 1A) (NS). The 5-year OS rate was 58% versus 44% (Fig. 1B). There was no significant difference in OS, progression-free survival (PFS), or relapse at day 100, 1 year, or 5 years post-transplant between the groups (Table 2). This remained true when we accounted for ATG use in bivariate Cox models where neither MSC nor ATG had a significant impact on OS, PFS or relapse (Supplementary Table 1). There was also no significant difference in causes of death

Table 2 Patient outcomes after transplantation

			Placebo	MSC	P value
Kaplan–Meier overall survival	1 year	Rate	80%	74%	0.64
	5 years	Rate	58%	44%	
Kaplan–Meier progression-free survival	1 year	Rate	80%	65%	0.41
	5 years	Rate	42%	33%	
Cumulative incidence of relapse	1 year	Rate	21%	23%	0.59
	5 years	Rate	32%	41%	
Cause of death (hemopathy/GVHD/other)		Number	3/3/0	4/3/5	0.63
aGVHD					
Grade 1/2/3/4		Number	1/4/2/2	2/6/2/1	0.87
100-d cumulative incidence of aGVHR, grades II-IV		Rate	53%	30%	0.15
100-d cumulative incidence of aGVHR, grades III-IV		Rate	27%	9%	0.13
aGVHD resolution (CR/no CR)		Number	6/3	6/5	0.67
cGVHD					
Mild/Moderate/Severe		Number	2/2/4	4/5/3	0.71
5-yr cumulative incidence of cGVHR, overall		Rate	67%	48%	0.27
5-yr cumulative incidence of cGVHR, moderate/severe		Rate	40%	30%	0.56
cGVHD resolution (CR/no CR)		Number	2/6	5/7	0.44
Donor engraftment (no/yes)		Number	0/15	0/23	1
Chimemism (full donor/other)*		Number	13/1	22/1	0.72
T lymphocyte count on day 30, median		/µL	530	363	0.56
Neutrophil recovery (complete/partial)		Number	15/0	23/0	0.19
Median time to 1,000 PMN/µL		Days	13	17	0.0174
Red blood cell recovery (complete/partial or none)*		Number	11/3	14/4	0.96
Median time to hemoglobin > 10 g/dl		Days	59	73	0.94
Red blood cell transfusions days 0–30, median		Number	1	1	0.57
Median time to RBC transfusion independence		Days	16	22	0.57
Platelet recovery (complete/partial or none)		Number	13/2	22/1	0.32
Median time to 50,000 platelets/µL		Days	11	11	0.93
Median time to 100,000 platelets/µL		Days	14	16	0.54
Platelet count on day 30, median		10^3/µL	100	134	0.14
Platelet transfusions days 0–30, median		Number	0	0	0.31
Infections					
Cumulative incidence of infection		Rate	80%	91%	0.29
Cumulative incidence of sepsis		Rate	33%	44%	0.37
Cumulative incidence of CMV infection		Rate	33%	35%	0.82
Cumulative incidence of other infections		Rate	80%	87%	0.59
Second cancer (no/yes)		Number	14/1	20/3	0.53

* = not all patients are evaluable

between patients who received MSC or a placebo (Table 2).

aGVHD

Twenty patients (53%), 9 (60%) in the placebo and 11 (48%) in the MSC groups, developed aGVHD, including 17 (8 placebos (53%) and 9 MSC (39%)) who had grades II-IV aGVHD (Table 2). There was a trend for a reduced cumulative incidence of grade II-IV aGVHD with MSC compared to placebo (30% vs 53%) but this did not reach statistical significance, neither in raw proportions nor in cumulative incidences, considering all grades or only grades II-IV aGVHD (Table 2). The majority of patients in both groups had complete resolution of their aGVHD (Table 2).

As mentioned above, 32% of patients received ATG, a treatment associated with a significant reduction in the risk of aGVHD and cGVHD [40]. We therefore performed bivariate analyzes with Cox regression models to determine the relative impact of group (MSC vs placebo)

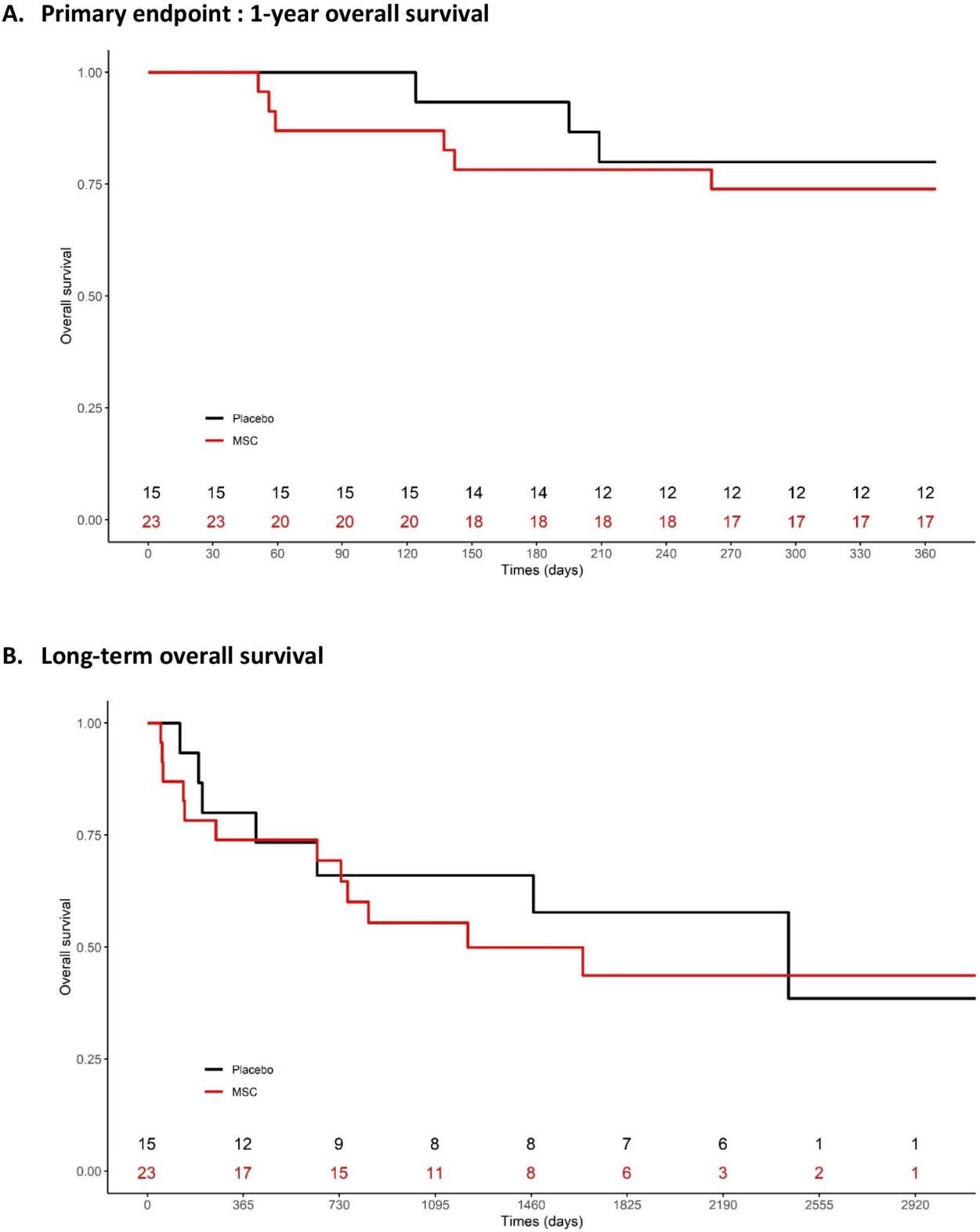

Fig. 1 Overall survival in MSC compared to placebo patients

and ATG administration (yes vs no) on the incidence of aGVHD (Supplementary Table 1). These analyzes showed a significant reduction in the incidence of grade II-IV aGVHD on day 100 in patients treated with MSC (HR 0.332, CI 0.124–0.890, $p=0.0284$), independently of the effect of ATG which also had a preventive effect (HR 0.085, CI 0.013–0.545, $p=0.0094$).

cGVHD

Twenty patients (53%) developed cGVHD, 8 in the placebo group (53%) and 12 in the MSC group (52%), including 14 (37%) with moderate to severe form (Table 2). The cumulative incidence of cGVHD was comparable in the MSC and placebo groups (Table 2), whether considering all grades (48% vs 67%) of cGVHD or only moderate to severe forms (30% vs. 40%). The majority of patients (65% in both groups) did not completely resolve their cGVHD.

We also performed bivariate analyzes in Cox models to determine the relative impact of MSC and ATG administration on the incidence of cGVHD (Supplementary Table 1). MSC were only associated with a non-significant trend towards a reduction in the overall risk of cGVHD (HR 0.484, CI 0.211–1.112, $p=0.0874$), and not in the risk of moderate to severe cGVHD (HR 0.637, CI 0.210–1.930, $p=0.4254$). On the other hand, the use of ATG was associated with a significant reduction in the overall risk of cGVHD (HR 0.298, CI 0.104–0.849, $p=0.0234$), although this was no longer significant when analyzing only moderate to severe cGVHD (HR 0.490, CI 0.137–1.749, $p=0.2721$).

Hematopoietic recovery

There were no differences between groups for the numbers of CD34+or CD3+cells transplanted and these numbers did not influence the incidence or severity of aGVHD nor cGVHD. Engraftment was excellent in both groups with no graft rejection and full donor chimerism was achieved in 89% of patients (Table 2). The time required to reach 500 neutrophils/µL or 20,000 platelets/µL (and therefore platelet transfusion independence) was not analyzable because many patients never fell below these thresholds.

Neutrophil recovery was complete (2000 neutrophils/µL) in 100% of patients (Table 2). The time to recovery of 1,000 neutrophils/µL was slightly longer in the MSC than in the placebo group (13 vs 17 days, $p=0.0174$) (Table 2). Immune recovery was identical in the two groups, whether in terms of the number of T lymphocytes on day 28 and of complete chimerism (Table 2), or the different lymphocyte populations at various post-transplant times (data not shown).

The recovery rate of a Hb at 10 gr/dL on day 100 (61% vs. 60%) and the time necessary to reach it was also similar (Table 2). The recovery of the red blood cell (RBC) lineage was complete (normalized Hb) in the vast majority of patients, with no difference between MSC or placebo (Table 2). Platelet counts on day 30, recovery rates of 50,000 (96% vs. 100%) or 100,000 (87% vs. 80%) platelets/µL on day 100, and times to reach them were similar in the MSC and placebo groups (Table 2). RBC (median 1) and platelet (median 0) transfusion requirements in the first 30 days post-transplant were very moderate and similar in the two groups (Table 2). The rate of transfusion independence on day 100 (87% vs. 87% for RBC, 87% vs. 93% for platelets) and the time necessary to achieve RBC transfusion independence was also comparable in the two groups (Table 2).

Miscellaneous

There was no acute toxicity observed during MSC administration. The risk of secondary cancer was not different in the two groups (Table 2). During post-transplant follow-up, 87% of patients presented at least one infection, within a time frame and with a comparable incidence in the two groups (Table 2). There was also no difference between the two groups in the incidence or time to onset of sepsis, CMV infection or disease, or other types of infections (Table 2).

Discussion

AlloHCT is a standard treatment for many serious hematological malignancies, but its success is still limited by a significant rate of morbidity and mortality linked to relapse and GVHD, infections or toxic complications, as well as graft rejection or dysfunction. Many strategies have been evaluated to reduce such morbidity and mortality and make alloHCT a better tolerated therapy. Cell therapy based on MSC appears to be a potentially promising option, based on their immunomodulatory, regenerative and hematopoietic supportive properties, as reported in several preclinical studies [41, 42] as well as in case reports and pilot studies in the context of alloHCT [23, 25, 26, 31–35].

Based on the promising results of our pilot study in the same setting [36], we conducted a prospective, randomized, placebo-controlled, comparative clinical trial that evaluated the efficacy and safety of co-infusing MSC with the HSC graft in the context of alloHCT with a non-HLA-identical unrelated donor (1 or 2 HLA mismatches between donor and recipient) after attenuated or non-myeloablative conditioning. Whereas improvement in OS, if any, would probably come from reduced GVHD incidence and/or severity and from improved graft function, we chose one-year OS as the primary endpoint of the trial as a more compelling endpoint. Our study unfortunately had to be stopped without having achieved its

recruitment objective because enrolment was much too slow to hope to complete it within a reasonable timeframe. The main reason for the low rate of inclusions in the study was the rise of HLA-haploidentical donor transplants that have nowadays become the first option for patients who do not have an HLA-identical donor.

Given the low number of patients (38 included/120 planned in the trial) and their heterogeneity in terms of baseline disease (for example all 5 patients with a high DRI risk were randomized in the MSC arm), it is difficult to analyze the effect of MSC co-transplantation on overall survival (primary objective of the study). In addition, 1-year overall survival in the control arm (80%) was much higher than the initial hypothesis (55%), probably reflecting improvements in supportive care and patient management over the last decade, rendering the primary objective unreachable even if 120 patients had been included. Among 8 other randomized [27, 43–49] and 11 historical control prospective or retrospective [36, 50–59] studies we identified (Table 3), only our pilot trial was associated with an apparently improved OS, while another small study observed a decreased OS, and all other controlled trials as well as meta-analyses showed no impact of MSC co-transplantation on survival [23, 25, 26, 31–35].

Nevertheless, the results of our study suggest a reduced risk of grade II-IV aGVHD in patients who received MSC. Whereas crude rates and cumulative incidences indicated a trend toward better aGVHD outcomes with MSC, our bivariate analysis demonstrated that co-injection of MSC with the HSC graft provided significant protection from grade II-IV aGVHD ($p=0.0284$), complementary to that of ATG, in this high-risk population (patients receiving PBSC from donors with 1–2 HLA mismatches). Number of HLA mismatches in the GVHD direction did not predict for aGVHD nor cGVHD (data not shown). Among 7 other randomized trials (Table 3) assessing aGVHD [27, 43–45, 47–49], none observed a significant impact on overall aGVHD but 3 evidenced a significantly decreased rate of grade II-IV aGVHD [44, 45, 47]. Similarly, meta-analyses yielded contradictory outcomes, with some demonstrating a reduced incidence of grade II-IV aGVHD [25, 33, 34] and others not [32, 35]. It should be underlined that other factors potentially affecting the risk of aGVHD, such as the use of ATG, were never taken into consideration in these analyses.

Regarding cGVHD, we did not observe any difference between the MSC and placebo groups. Our results must, however, be interpreted with caution given the low number of patients included and the low statistical power. Non-randomized comparative studies were generally negative as well (Table 3) [23, 25, 26, 31–35]. Among 6 randomized trials assessing cGVHD [27, 45–49], 2 detected a significant reduction in the incidences of overall as well as extensive/severe cGVHD while the other 4 did not (Table 3). Here again, the same meta-analyses yielded identically contradictory outcomes, possibly reflecting discrepancies in other co-factors [25, 32–35]. However, the largest, most recent, randomized trial aiming at limiting the risk of cGVHD by multiple MSC infusions starting at day 45 after haplo-identical HCT was positive [47].

Concerning engraftment, hematological recovery and transfusions, we did not observe any notable difference between MSC and placebo other than a slightly delayed recovery of 1000 neutrophils/µL in the MSC group, possibly in relation with the trend for a higher DRI in this group. However, several other studies observed an acceleration of platelet and/or neutrophil recovery after MSC co-transplantation [27, 44, 48, 51, 58] (Table 3) and a meta-analysis of previous trials in the context of alloHCT has indicated an acceleration of hematological reconstitution [34]. No impact on graft rejection has been observed (Table 3).

Given their immunomodulatory effects, a theoretical fear of treatment with MSC would be that their use could promote the occurrence of infections, relapse of the initial malignancy or the emergence of secondary tumors. Whereas numerous publications point to the tumor-supporting properties of tumor-associated MSC [60], some data suggest that these may differ for MSC uneducated by the tumor [61] such as allogeneic MSC used in the vast majority to co-transplantation studies in the context of HCT. Only one very small randomized trial [45] reported an apparently increased risk of disease relapse with MSC co-transplantation, but no other comparative study suggested so (Table 3). No study reported an elevated risk of infection and this has also been confirmed in a large meta-analysis examining the use of MSC in multiple indications [62]. MSC have even been used extensively to help control the systemic inflammatory response to sepsis, ARDS and COVID-19 [17]. Our study did not provide signals for these complications, but its statistical power is certainly not sufficient to exclude them. Furthermore, no unwanted reactions were reported following infusion of MSC, which was very well tolerated by patients. This apparent safety has also been confirmed by meta-analyses in the context of alloHCT [23, 25, 26, 31–35] but also in other settings [62].

The results of studies of MSC co-injections at the time of HSC transplantation remain inconsistent, notably due to different methodologies being used [63]. Indeed, there is currently no consensus on the type (tissue origin, culture method…), dose (dose of each infusion, number of infusions…) and timing (just before MSC, days/weeks after HSC…) of MSC infusions. For instance, it has been

Table 3 Previous controlled studies of MSC for the prevention of GVHD and/or graft rejection

References	MSC product			Study			Patients					
	Source	Dose (10E6/kg)	Timing	Design	Controls	N (MSC)	Age	Disease	Conditioning	ATG (or similar)	Donor	Graft
Shipounova [43]	BM	0.9–1.65	Engraftment	Randomized	Randomized	39	Adults	Various	MAC—RIC	No > Yes	MRD	BM
Rostami [49]	BM	2 (median)	D0	Randomized	Randomized	47	Children	Thalassemia	MAC	Yes	MRD	BM or PBSC or CB
Ning [45]	BM	0.03–1.5	D0	Randomized	Randomized	10	Adults	Various	MAC	No	MRD	BM or PBSC
Wu [48]	UC	2.4–10.1	D0	Randomized	Randomized	8	Children	AL	MAC	Yes	MMUD	CB
Lombardo 2024 (current)	***BM***	***1.5–3***	***D0***	***Randomized (DB)***	***Randomized***	***23***	***Adults***	***Various***	***RIC***	***No > Yes***	***MMUD***	***PBSC***
Wang [44]	UC	1	D0	Randomized	Randomized	25	Ad & children	Various	MAC	Yes	Haplo	PBSC
Liu [27]	BM	0.3–0.5	D0	Randomized	Randomized	27	Ad & children	Various	MAC	Yes	Haplo	PBSC + BM
Gao [46]	UC	4 × 3 (10E7)	D120, Q4W	Randomized (DB)	Randomized	62	Ad & children	Various	MAC	Yes	Haplo	PBSC + BM ?
Huang [47]	UC	4 × 1	D45, Q2W	Randomized	Randomized	74	Adults	AL	MAC	Yes	Haplo	PBSC + BM ?
Baron [36]	BM	1.5–3	D0	Phase 1–2	Historical	20	Adults	Various	RIC	No	MMUD	PBSC
Gonzalo-Daganzo [59]	BM	1–2.2	D0	Phase 1–2	Historical	9	Adults	Various	MAC	Yes > No	MMUD	CB + PBSC
Lee [51]	UCB	1	D0	Phase 1–2	Historical	7	Children	Various	MAC	No	MMUD	CB
Goto [54]	BM	0.3–1.8	D0	Phase 1	Historical	5	Adults	Various	MAC	No	MMUD	CB *(intra-bone)*
Wu [58]	UC	3.1–8.2	D0	Retrospective	Historical	5	Children	Various	MAC—RIC	Yes	MMUD	CB
Bernardo [50]	BM	1–3.9	D0	Phase 1–2	Historical	13	Children	Various	MAC	Yes > No	MRD-MUD-MMUD	CB
Zhang [57]	BM or placenta	0.5–3	D1	Retrospective	Historical	79	Ad & children	AL	MAC	Yes	MRD-MMRD MUD-MMUD	PBSC
Han [65]	UC	0.27–2.5 ?	D0	Retrospective	Historical	41	Ad & children	Various	MAC	Yes	Haplo	PBSC + BM
Ding [53]	UC	1	D0	Retrospective	Historical	110	Ad & children	ALL	MAC	Yes	Haplo	PBSC + BM
Ding [52]	UC	1	D0	Phase 1–2 ?	Historical *(Children)*	25	Adults	SAA	MAC	*Basiliximab*	Haplo	PBSC + BM
Liu [55]	BM or UC	2.9–7.1	D0	Retrospective	Historical *(MRD)*	91	Ad & children	SAA	RIC	Yes	Haplo	PBSC + BM
Sheng [56]	UC	1	D0	Retrospective	Historical	47	Ad & children	SAA	RIC	Yes	Haplo	PBSC + BM

Author	Outcomes							
	AGVHD	AGVHD 2(3)-4	CGVHD	CGVHD (2)-3/extensive	Engraftment	Rejection	Relapse	OS
Shipounova [43]	NR	=	NR	NR	NR	=	=	=
Rostami [49]	=	NR	=	NR	=	=	NA	=

Table 3 (continued)

Author	Outcomes							
	AGVHD	**AGVHD 2(3)-4**	**CGVHD**	**CGVHD (2)-3/extensive**	**Engraftment**	**Rejection**	**Relapse**	**OS**
Ning [45]	=	↓ ($p=0.04$)	=	=	=	NR	↑ ($p=0.02$)	↓ ($p=0.035$)
Wu [48]	=	=	=	=	↑ PMN ($p=0.003$)			
↑ Plt ($p=0.004$)	NR	NR	=					
Lombardo 2024 (current)	=	↓ *($p=0.03$)*	=	=	↓ *PMN ($p=0.02$)*	=	=	=
Wang [44]	=	↓ ($p<0.05$)	NR	NR	↑ Plt ($p<0.05$)	NR	=	=
Liu [27]	=	=	=	=	↑ Plt ($p=0.036$)	NR	=	=
Gao [46]	NA	NA	↓ ($p=0.021$)	↓ ($p=0.047$)	NA	NR	=	=
Huang [47]	NR	↓ ($p=0.01$)	↓ ($p=0.04$)	↓ ($p=0.03$)	NR	NR	=	=
Baron [36]	NR	=	NR	NR	=	=	=	↑ ($p=0.02$)
Gonzalo-Daganzo [59]	=	=	NR	NR	=	NR	=	=
Lee [51]	=	=	NR	=	↑ PMN ($p=0.03$)	=	=	=
Goto [54]	NR	=	=	NR	=	NR	=	=
Wu [58]	=	=	=	=	↑ PMN ($p=0.02$)			
↑ Plt ($p=0.01$)	NR	NA	=					
Bernardo [50]	NR	↓ ($p=0.05$)	=	NR	=	=	=	=
Zhang [57]	↓ ($p<0.006$)	↓ ($p<0.006$) (AML)						
↓ ($p<0.008$) (ALL)	=	NR	NR	NR	NR	NR		
Han [65]	NR	=	NR	=	↑ PMN ($p=0.03$)	=	=	=
Ding [53]	=	*3–4* ↓ ($p=0.03$)	=	↓ ($p=0.04$)	=	NR	=	=
Ding [52]	NR	=	=	NR	=	NR	NA	=
Liu [55]	↑ ($p=0.000$)	↑ ($p=0.001$)	↑ ($p<0.05$) *	=	=	=	NA	=
Sheng [56]	=	=	↓ ($p=0.048$)	=	=	=	NA	=

Ad: adult; AL: acute leukemia; ALL: acute lymphoblastic leukemia; AML: acute myeloblastic leukemia; ATG: anti-thymocyte globulins; BM: bone marrow; CB: cord blood; D: day; DB: double blind; Haplo: haplo-identical donor; MAC: myeloablative conditioning; MMRD: mismatched related donor; MMUD: mismatched related donor; MRD: matched related donor; MUD: matched unrelated donor; N: number; NA: not applicable; NR: not reported; PI: placenta; Plt: platelets; PBSC: peripheral blood stem cells; PMF: primary myelofibrosis; PMN: neutrophils; RIC: reduced intensity conditioning; OS: overall survival; SAA: severe aplastic anemia; UC: umbilical cord; ↑: increased; ↓: decreased; = : not significantly different

demonstrated by our team that MSC of different origins (BM, umbilical cord or adipose tissue) do not have the same impact on xenogeneic GVHD depending on their interaction with T lymphocytes in vitro [42]. Among all previous studies (Table 3), only two [46, 47] explored multiple late MSC infusions instead of a single administration on the day of HCT, and one of them aimed at reducing only the rate of cGVHD. There is also the possibility of a donor effect, but we could not assess it in our study given the large number of different donors providing their BM. The studies also differ in their respective objectives, such as survival, hematological recovery or the incidence and/or severity of aGVHD or GVHD. It is therefore currently difficult to make a direct comparison of the different studies and to draw clear conclusions from the use of MSC in the field of alloHCT.

Whereas the search for biomarkers capable of predicting response to MSC in patients with steroid-refractory GVHD has explored various cytokines, chemokines and immune cell subsets without identifying a reliable predictor of response [23], finding biomarkers in preventive trials is much more difficult and has not been pursued in the numerous trials analyzed for our review. It would also be interesting to identify a quantitative potency assay that would help selecting the best MSC product available [28]. In the meantime, analysis of patient characteristics such as disease type, age and HLA matching has not yielded useful predictive information [34].

Finally, the data observed with MSC must be put into perspective with new methods of GVHD prevention in patients given grafts from HLA-mismatched unrelated donors. Indeed, recent studies have reported improved outcomes with posttransplant cyclophosphamide-based GVHD prophylaxis in this subgroup of patients while administration of the CTLA4 agonist abatacept was particularly effective at preventing acute GVHD in that setting although it failed at preventing chronic GVHD [64]. Ideally, their respective validity should be evaluated in prospective comparative trials.

Conclusions

In conclusion, our study is the only randomized trial exploring the use of MSC co-infusion at the time of HSC in alloHCT recipients given PBSC from mismatched unrelated donors. MSC administration was well tolerated. Its effectiveness in terms of reducing the incidence of aGVHD and improving survival unfortunately could not be formally established, due to insufficient patient recruitment. However, the study suggests a reduction in the incidence of grade II-IV aGVHD that warrants further evaluation in future prospective trials in the context of alloHCT with a high-risk of GVHD such as transplantation from HLA-mismatched or haploidentical donors, especially given their good safety profile compared to alternative immunosuppressive treatments.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13287-024-04064-w.

Additional file 1.

Acknowledgements
The authors thank all the personnel of the Laboratory of Cell and Gene Therapy at the CHU of Liège for their help to the production of MSC.

Author contributions
Conception and design: FB, YB; Provision of study material or patients: All authors; Collection and assembly of data: GL, JH, AJ, YB; Data analysis and interpretation: GL, LS, YB; Manuscript writing: GL, YB. All authors contributed to the article and approved the submitted version.

Funding
FB is Senior Research Associate at the Fonds National de la Recherche Scientifique (FNRS). SS was Postdoctoral Researcher at the Belgian Foundation against Cancer (FBC). JH and AJ were supported by the Belgian Hematology Society (BHS). The study was supported by grants of the FNRS, the FBC, the University of Liège, the CHU of Liège and the Fondation Léon Frédéricq. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials
All supporting data can be made available upon reasonable request.

Declarations

Ethics approval and consent to participate
The protocol "Co-transplantation of mesenchymal stem cells and HLA-mismatched allogeneic hematopoietic cells after nonmyeloablative conditioning: a phase II randomized double-blind study" was approved centrally by the "Comité d'Ethique Hospitalo-Facultaire Universitaire de Liège" on 6 July 2010 (file 2010/16) as well as by the ethics committees of all participating centers and the study was conducted in accordance with the Declaration of Helsinki. Before participating in the study, all patients (or their legal representatives if minors) signed an informed consent form.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 17 September 2024 Accepted: 14 November 2024
Published online: 03 December 2024

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