Molecular Therapy
Methods & Clinical Development
Original Article

Peripheral blood stem and progenitor cell collection in pediatric candidates for *ex vivo* gene therapy: a 10-year series

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Hematopoietic stem and progenitor cell (HSPC)-based gene therapy (GT) requires the collection of a large number of cells. While bone marrow (BM) is the most common source of HSPCs in pediatric donors, the collection of autologous peripheral blood stem cells (PBSCs) is an attractive alternative for GT. We present safety and efficacy data of a 10-year cohort of 45 pediatric patients who underwent PBSC collection for backup and/or purification of $CD34^+$ cells for *ex vivo* gene transfer. Median age was 3.7 years and median weight 15.8 kg. After mobilization with lenograstim/plerixafor (n = 41) or lenograstim alone (n = 4) and 1−3 cycles of leukapheresis, median collection was 37 × 10^6 $CD34^+$ cells/kg. The procedures were well tolerated. Patients who collected ≥7 and ≥13 × 10^6 $CD34^+$ cells/kg in the first cycle had pre-apheresis circulating counts of at ≥42 and ≥86 $CD34^+$ cells/μL, respectively. Weight-adjusted $CD34^+$ cell yield was positively correlated with peripheral $CD34^+$ cell counts and influenced by female gender, disease, and drug dosage. All patients received a GT product above the minimum target, ranging from 4 to 30.9 × 10^6 $CD34^+$ cells/kg. Pediatric PBSC collection compares well to BM harvest in terms of $CD34^+$ cell yields for the purpose of GT, with a favorable safety profile.

INTRODUCTION

Autologous haemopoietic stem and progenitor cells (HSPCs) are the source material for *ex vivo* gene therapies in pediatric monogenic diseases.[1–7] Although unmanipulated autologous haemopoietic stem cell transplantation (HSCT) requires the collection of ≥2 × 10^6 $CD34^+$ cells/kg, gene therapy (GT) collection targets are usually higher, due to purification, *ex vivo* manipulation, extensive quality testing, freezing, and thawing.[8] Furthermore, for safety purposes, an unmanipulated backup is usually stored separately before infusion of the drug product (DP).

Bone marrow (BM) harvest is the standard of care to collect HSPCs from pediatric donors.[9] We have previously reported the outcome of BM harvests in a comparable cohort of patients undergoing GT,[10] collecting a sufficient amount of cells without any major adverse event (AE). Mobilization and apheresis of HSPCs are standard procedures for adult donors and have been adapted to pediatric patients with a favorable safety profile.[4,9,11–13] However, the pediatric experience in peripheral blood stem cell (PBSC) leukapheresis remains limited and mainly reported for patients weighing >20 kg and not systematically addressed for GT so far. In our center, we progressively transitioned to use PBSCs in GT patients with the aim of increasing the amount of HSPCs collected and reducing the invasiveness associated with the BM harvest.

Here, we report a 10-year experience of PBSC collection in pediatric patients enrolled in GT protocols and provide safety and collection efficacy data. We also evaluate the process yields from harvest to infusion and compare these results with our historical cohort of disease-matched BM harvests.[10]

Received 8 April 2021; accepted 26 May 2021;
https://doi.org/10.1016/j.omtm.2021.05.013.

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Table 1. Patients' characteristics

Disease	n	Female/male	Age in years (range)	Weight in kg (range)	BM CD34$^+$ cells % (range)	Previous BM harvest (n of patients)	Designated use of HSPCs (n of patients)	
							Backup	DP manufacturing
ADA-SCID	4	3/1	5.3 (3.5–10.8)	19.8 (14.7–28.8)	2.1 (1.8–3.7)	1	4	2
β-thalassemia	7	2/5	6.6 (4.4–13.6)	20.0 (15.4–54.0)	4.1 (1.7–5.5)	0	7	7
MLD	10	2/8	2.5 (0.6–7.7)	13.2 (7.0–24.0)	3.7 (0.8–6.4)	1	8	10
MPSIH	8	2/6	1.9 (1.0–2.7)	11.8 (11.0–14.3)	3.9 (1.7–7.7)[a]	0	8	8
WAS	16	0/16	3.7 (0.9–14.4)	18.4 (7.5–54.1)	4.5 (0.5–10.8)	0	16	16
Total	45	9/36	3.7 (0.6–14.4)	15.8 (7.0–54.1)	3.8 (0.5–10.8)	2	43	43

DP, drug product; BM, bone marrow, HSPC, hematopoietic stem and progenitor cell.
[a]Data not available for 3 patients.

RESULTS

Patient population

Between April 1, 2010, and March 31, 2020, 45 consecutive patients affected by adenosine deaminase (ADA)-severe combined immunodeficiency (SCID; n = 4); β-thalassemia (n = 7); metachromatic leukodystrophy (MLD; early juvenile = 8, late infantile = 2); late infantile or early juvenile, mucopolysaccharidosis 1 Hurler (MPSIH; n = 8); or Wiskott–Aldrich syndrome (WAS; n = 16) enrolled in GT protocols were included in the study. Patients' characteristics are summarized in Table 1. β-thalassemic patients were older than MLD and MPSIH ones, as expected by the design of the trial.[14] Forty out of 45 patients performed leukapheresis upfront to collect cells for both DP manufacturing and backup, and 38/40 met this goal, whereas 2 required an additional BM harvest (Supplemental materials and methods).

Schedule of HSPC collection

All patients underwent a single mobilization with lenograstim subcutaneously (s.c.) alone (n = 4) or in combination with plerixafor s.c. (n = 41). Four required anesthesiologic support for central venous catheter (CVC) malfunction, transient malaise, or sedation for agitation. Cells were destined to backup (n = 2), manufacturing of the DP (n = 2), or both (n = 41).

A median of 2.5 days (range 2–4.5) passed between the first dose of lenograstim and the first leukapheresis cycle. Patients who did not receive plerixafor underwent apheresis 1.1 days later, but sample size was small (n = 4). As detailed in Table 2, 13 patients underwent 1 apheresis, 27 underwent 2 aphereses, and 5 underwent 3 cycles. For each patient, all aphereses took place on consecutive days (Figure S1A).

Safety

A total of 108 AEs were recorded, as detailed in Table 3. The incidence of AEs was higher in WAS patients as compared to β-thalassemic, MLD, and ADA-SCID patients (Kruskal-Wallis test, $p < 0.001$, for all multiple comparisons $p < 0.05$). No significant difference in the incidence of AEs was observed in patients weighing <20 kg as compared to those weighing more ($p = 0.25$), and no correlation was found between the total number of AEs and weight or age. Infection (n = 3) was the most common grade 3 AE.

Median hemoglobin level before mobilization was 11 g/dL; after the last leukapheresis, hemoglobin decreased to 9.9 g/dL ($p = 0.015$). Excluding WAS patients due to the disease-related thrombocytopenia, median platelet values before and after mobilization were 342,000/μL and 139,000/μL, respectively ($p < 0.0001$). 13/31 patients had counts <130,000/μL and 2 of them <50,000/μL in the absence of clinical manifestations of thrombocytopenia. Cumulatively, considering the time window between the first apheresis and the 7 days following the last apheresis, patients were exposed to a total of 89 packed red blood cells (RBCs) and 20 platelet units. During leukapheresis, 69 RBCs units were administered as priming of the circuit system and 13 platelet units as transfusion support. Four patients had no exposure to blood products.

Peripheral blood cell counts and leukapheresis

The median PBSC cell count before the first apheresis was 145 CD34$^+$ cells/μL (range 28–464 CD34$^+$ cells/μL). As shown in Figures 1A and S2, patients who continued the mobilization after the first apheresis had a significantly higher CD34$^+$ cell count at day 2 (median increase of 88 CD34$^+$ cells/μL, $p < 0.0001$) compared to day 1. We observed a median increase of 114 CD34$^+$ cells/μL on day 3 compared to day 2 ($p = 0.13$) and of 53 CD34$^+$ cells/μL compared to day 1 ($p = 0.13$).

Apheresis yields are summarized in Table 2. Overall, the median collection yield of the 82 apheresis was 37.0×10^6 CD34$^+$ cells/kg, with a range of $3.3–63.8 \times 10^6$, corresponding to a median volume of 228.7 mL (range 70–891 mL).

For the first procedure, volumes ranged from 48 to 318 mL, containing $50–429 \times 10^6$ white blood cells (WBCs)/mL and 0.2%–3.5% of CD34$^+$ cells, and weight-averaged yields ranged from 0.7 to 53.2×10^6 CD34$^+$ cells/kg, with a median of 18.3×10^6 CD34$^+$ cells/kg.

Volumes (median 132.5 mL, range 44–301 mL) and WBC counts (median 205×10^6 WBC/mL, range $53–374 \times 10^6$ WBC/mL) of

Table 2. Single and total apheresis yield by weight, stratified by disease

	1st cycle		2nd cycle		3rd cycle		
Disease	n	Yield	n	Yield	n	Yield	Total yield
ADA-SCID	4	11.5 (1.8–30.9)	3	8.2 (1.5–18.8)	1	9.1	27.7 (3.3–34.7)
β-thalassemia	7	31.1 (7.5–53.2)	3	23.1 (15.5–30.9)	–		45.6 (30.6–53.2)
MLD	10	15.5 (5.2–36.7)	6	24.1 (20.4–43.6)	–		36.5 (5.2–57.9)
MPSIH	8	18.2 (1.6–24.3)	8	23.9 (12.3–34.2)	2	13.0 (6.3–19.6)	45.4 (31.0–55.3)
WAS	16	20.2 (0.7–42.0)	12	21.6 (9.0–35.4)	2	12.7 (9.3–16.2)	34.4 (18.7–63.8)
Total	45	18.3 (0.7–53.2)	32	32 (1.5–43.6)	5	9.3 (6.3–19.6)	37 (3.3–63.8)

Cell counts ($\times 10^6$ $CD34^+$ cells/kg) are reported as median and range (in parentheses). The number of patients is reported for each apheresis procedure.

the second apheresis did not differ significantly from the previous one. However, as illustrated in Figure 1B, the median percentage of $CD34^+$ cells increased by 0.55% ($p < 0.0001$); similarly, the median yield was 23.0 $\times$ 10^6 $CD34^+$ cells/kg (range 1.5–43.6 $CD34^+$ cells/kg) corresponding to a median increase of 3.75 $\times$ 10^6 $CD34^+$ cells/kg ($p < 0.0001$) as compared to the first apheresis in the same patients, as shown in Figure 1C.

As for the third apheresis, the collection parameters did not appear to differ significantly from the previous one, albeit the sample size was small (n = 5).

Volumes of the first and second apheresis correlated with age (both $p < 0.0001$) and weight (both $p < 0.0001$). Peripheral $CD34^+$ cell counts before the apheresis correlated with relative $CD34^+$ content of the apheresis bag (Spearman r 0.77, $p < 0.0001$ and 0.53, $p = 0.0016$, respectively; Figure S3A) and weight-adjusted $CD34^+$ cell yield for both the first and second day (Spearman r 0.92, $p < 0.0001$ and 0.65, $p < 0.0001$; Figure S3B). Finally, the percentage of $CD34^+$ cells in the bag correlated with weight-adjusted yield (Spearman r 0.81, $p < 0.0001$ for day 1; 0.46, $p = 0.0081$ for day 2; and 1, $p = 0.0167$ for day 3; Figure S3C). In summary, a higher peripheral $CD34^+$ cell count corresponded to a higher percentage of $CD34^+$ cells in the apheresis bag and both to an increased weight-adjusted yield, ultimately pointing to peripheral $CD34^+$ cell number as a predictor of yield.

Of note, one ADA-SCID patient was a poor mobilizer, collecting 1.76 and 1.53 $\times$ 10^6 $CD34^+$ cells/kg, corresponding to pre-apheresis of 28 $CD34^+$ cells/μL on day 1 and of 11 on day 2, respectively.

Collection targets are reported in Table 4. Only one patient did not meet the target by PBSCs alone. In absolute terms and by conventional cutoffs for autologous PBSC collection,[15] 44 patients had an optimal collection, exceeding 5 $\times$ 10^6 $CD34^+$ cells/kg, one patient fell into the "low" 2–5 $\times$ 10^6 $CD34^+$ cells/kg interval, and none had a poor yield, i.e., <2 $\times$ 10^6 $CD34^+$ cells/kg.

Table 3. Summary of adverse events

	Grade				
Category	4	3	2	1	Most common event (n)
Allergic		3	4		urticarial skin rash (3)
Blood related		1	6	9	anemia (6)
Cardiovascular			1		hypertension (1)
Electrolyte disturbances		1	1	5	hypokalemia (3)
ENT			2	2	
Gastrointestinal		1	8	7	vomit (5)
Infectious		3	6	4	upper airway infection (3)
Kidney			3		
Metabolic		1	2	6	metabolic acidosis (9)
Musculoskeletal		2	6	7	arthralgia (3)
Neurological			2	2	headache (4)
Respiratory			1	3	bronchospasm (2)
Other		2	3	4	
Total	0	14	45	49	

Adverse events related to rituximab adverse events were excluded (n = 1 grade 4, n = 2 grade 3, n = 1 grade 2). ENT, ear, nose, throat.

Predictors of apheresis yield

Beyond circulating peripheral $CD34^+$ cell counts, other variables were found to be predictive of apheresis yield. Considering the entire cohort, the duration of mobilization correlated both with the yield of the first apheresis (Spearman r –0.33, $p = 0.026$) and the total yield (Spearman r –0.33, $p = 0.029$), whereas the cumulative dose of lenograstim correlated negatively with the yield of the first apheresis (Spearman r –0.65, $p < 0.0001$) but not with total yield ($p = 0.40$).

As lenograstim dosing is adjusted mostly based on peripheral WBC counts, the total lenograstim dose reflects both the WBC increase as well as the length of the mobilization (Figure S1A). Vice versa, the interval between the beginning of mobilization and the first leukapheresis reflects the increase in peripheral $CD34^+$ cell counts and thus the individual response to the drug regimen.

As for what concerns patients' characteristics, female gender was associated with lower total yield ($p = 0.015$), with a trend for lower

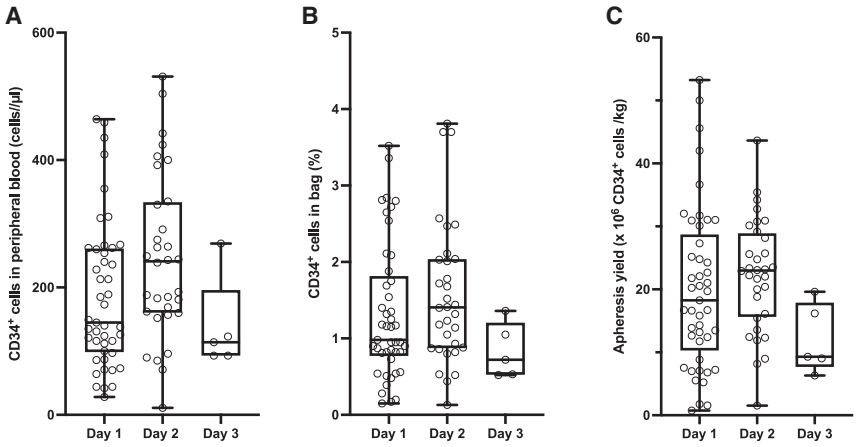

Figure 1. CD34$^+$ cell counts in peripheral blood, apheresis bags, and collection yield

Box and whiskers plots illustrating the absolute number of CD34$^+$ cells in peripheral blood before the apheresis (A) and the corresponding relative CD34$^+$ content in the apheresis bag (B) and absolute CD34$^+$ cell yield averaged by weight (C). Whiskers range from minimum to maximum.

yield of the first apheresis (p = 0.058). Furthermore, patients who were enrolled upfront to HSPC mobilization and apheresis collected more cells than the others (p = 0.0066). Instead, no correlation was found between first apheresis or overall yield and age, weight, disease, or relative percentage of CD34$^+$ cells in the BM.

Stepwise linear regression of first apheresis yield considering age, disease, gender, weight percentile, total lenograstim dose, and undergoing mobilization upfront identified total lenograstim dose, disease, and gender as independent predictors of first apheresis yield (R^2 0.531). Gender-specific differences may at least be partly due to the effect of lenograstim, which has indeed been shown to be more effective than filgrastim in males but not in females.[16] When we included the peripheral CD34$^+$ cell counts variable in the linear regression model, disease and peripheral CD34$^+$ cell counts were the strongest predictive variables (R^2 0.843).

Plerixafor was a major contributor to harvest yield. In fact, the four patients who did not receive plerixafor collected fewer cells both during the first apheresis and overall, with a median difference of 13 $\times$ 10^6 CD34$^+$ cells/kg (p = 0.0082) and 23 $\times$ 10^6 CD34$^+$ cells/kg (p = 0.0005), respectively.

Regarding the subgroup of patients who received plerixafor, all received 0.24 mg/kg/day before the first apheresis. A lower yield at the first apheresis corresponded to a higher subsequent plerixafor dose (Spearman r −0.64, confidence interval [CI] −0.82 to −0.35), and all four patients who underwent a third apheresis received a high dose (0.4−0.48 mg/kg; Figure S1B). The total plerixafor dose was not correlated with the overall yield (p = 0.69) nor was there a dose-response relation between the second plerixafor dose and the yield of the second apheresis (p = 0.4).

All patients who received plerixafor except one collected $\geq$20 $\times$ 10^6 CD34$^+$ cells/kg. Female gender was associated with lower overall yield (p = 0.012) but no significant differences in 1st apheresis yield (p = 0.073). By univariate analyses, first apheresis and total yield were not influenced by age, weight, BM CD34$^+$ counts, nor disease (p = 0.13 and p = 0.09, respectively). Stepwise linear regression of first apheresis yield considering age, disease, gender, weight percentile, undergoing mobilization upfront, total lenograstim dose, and total plerixafor dose identified total plerixafor dose and gender as negative predictors of first apheresis yield and β-thalassemia as a positive predictor of yield (R^2 0.631). Inclusion of peripheral CD34$^+$ cell counts in the model instead replaced gender as a predictor of yield (R^2 0.853).

Manipulation and engraftment

A backup was stored for 43 patients; all but one were above the minimum 2 $\times$ 10^6 CD34$^+$ cell/kg threshold for a rescue autologous HSCT,[17] as shown in Table S1.

Table 5 shows the median CD34$^+$ cell count at each step in the production process, not accounting for cells that were withdrawn for research or quality control. CD34$^+$ selection yield was in line with our historical BM cohort and previous studies.[10,18] In some cases, the number of cells that was manufactured exceeded the upper infusion limits defined for each protocol. Table 5 also illustrates the infused DP dose and the predetermined dose range. One patient received a fresh formulation of the DP; 14 patients received a DP that was cryopreserved before transduction, and 28 received a DP that had been frozen after manipulation. Of note, three patients also received transduced BM cells (3.8, 6.7, and 3.66 $\times$ 10^6 CD34$^+$ cell/kg; data not shown). 41/42 patients received a DP dose within the reference infusion range.

The DP dose ranged from 4 to 30.9 $\times$ 10^6 CD34$^+$ cell/kg, and all patients engrafted. For patients who received a DP uniquely sourced from PBSCs, the median day of neutrophil engraftment was 24.5 (range 15−77), whereas the median day of platelet engraftment was 21 (range 10−76). No patient required reinfusion of the unmanipulated backup. No correlation was observed between the DP dose and the number of days to neutrophil or platelet engraftment.

DISCUSSION

Autologous HSPC-based GT is becoming a new paradigm for the treatment of inborn errors of immunity,[6,7,19] metabolism,[2,3,20] and haemopoiesis.[1,4,14] Three medicinal products based on HSPC have been authorized in the European Union (EU), and others are in advanced clinical development.[1] HSPCs may be collected by BM

Table 4. Minimum collection targets by protocol

Protocol	DP manufacturing	Backup	Total
ADA-SCID	0−NA	1	1−NA
β-thalassemia	5	2	7
MLD	8−10	0−3	8−13
MPSIH	8	3	11
WAS	5−10	3	8−13

Numbers are reported as $\times 10^6$ $CD34^+$ cells/kg. DP, drug product.

harvest or mobilization and leukapheresis for DP manufacture; however, no standards or guidelines are available for their collection in pediatric patients for the purpose of GT.

Previous experience with mobilization was reported in healthy pediatric donors for allogeneic transplantation or autologous HSPC transplantation for malignancies.[9,12,21–23] Our analysis focuses on a large cohort of pediatric subjects with nonmalignant diseases with various comorbidities related to the underlying disorder and include also infants <1 year of age and weighing less than 10 kg.

We show that mobilization and leukapheresis in the context of autologous GT for pediatric subjects have a favorable short-term safety profile and result in adequate cell collection for backup and DP manufacturing. All patients received a DP that respected the specifications in terms of minimum $CD34^+$ cells/kg content, and all eventually engrafted.

The vast majority of patients was fully compliant, and about one-half of them experienced no or minimal adverse effects during the mobilization and collection procedure. A number of AEs have already been reported to be related to the mobilization or apheresis procedure.[9,21,24] We found some AEs to be confined to specific diseases, e.g., metabolic acidosis in MLD,[25] despite the fact that leukapheresis and plerixafor rather carry a risk of alkalosis.[26,27] The frailty of WAS patients, who showed the highest rate of AEs, is not surprising, as thrombocytopenia, immunodeficiency, immune dysregulation, and auto-inflammatory manifestations can understandingly be exacerbated by mobilizing drugs and other procedures. Although it is not possible to exclude that procedure-related AEs also occurred at later time points, extending the time frame would have reduced specificity of the analysis and suffered from the impact of major confounding factors, i.e., chemotherapy and GT.

The number of circulating $CD34^+$ cells is known to be a reliable indicator of the expected apheresis yield.[28] Indeed, we found a clear linear relation between the number of circulating $CD34^+$ cells and relative number of $CD34^+$ cells in the bag. As volumes instead correlated with age and weight, the weight-averaged $CD34^+$ cell yield resulted directly proportional to the percentage of $CD34^+$ cells in the bag. In our cohort, apheresis yield was influenced by gender, possibly due to suboptimal efficacy of lenograstim in females, and underlying disease; drug dosages were increased in patients with initial lower responses.

There is no consensus on the definition of "poor pediatric mobilizer."[29,30] Our GT protocols require the collection of $\geq 7-13 \times 10^6$ $CD34^+$ cells/kg, significantly higher than conventional cutoffs.[28] This is due to the fact that autologous HSPC GT requires higher numbers for cell manipulation for drug manufacturing and unmanipulated backup. Therefore, the traditional definition of poor mobilizer may be too loose; in our series, patients who collected $\geq 7 \times 10^6$ $CD34^+$ cells/kg in the first cycle had pre-apheresis circulating counts of ≥ 42 $CD34^+$ cells/μL, and those who collected $\geq 13 \times 10^6$ $CD34^+$ cells/kg had ≥ 86 $CD34^+$ cells/μL.

As compared to the historical BM cohort,[10] patients who collected HSPCs weighed more (median 15.8 kg versus 10.6 kg, p = 0.0003) and were older (median 3.7 versus 1.5 years, p = 0.0013), whereas gender distribution was similar (p = 0.63 excluding WAS, p = 0.12 including WAS).

As illustrated in Figure 2, the leukaphereses of MLD and WAS patients yielded more cells as compared with BM collections (p = 0.047 and

Table 5. $CD34^+$ cell counts across the manufacturing process

Protocol	n	Starting material ($\times 10^6$ $CD34^+$ cells/kg)	Recovery from selection ($\times 10^6$ $CD34^+$ cells/kg)	Destined to transduction ($\times 10^6$ $CD34^+$ cells/kg)	Recovery from transduction ($\times 10^6$ $CD34^+$ cells/kg)	Predefined DP infusion range ($\times 10^6$ $CD34^+$ cells/kg)		DP dose ($\times 10^6$ $CD34^+$ cells/kg)
						MIN	MAX	
β-thalassemia	7	44.7 (18.3−50.1)	29.6 (15.1−33)	17.5 (14.2−18.6)	25.2 (20.1−36.9)	2	20	19.6 (16.3−20)
MLD	10	33.9 (4−53.9)	23.3 (3.5−33.8)	23.1 (2.6−33.3)	30.3 (4−48.3)	2−3	20−30	26.7 (4−30)
MPSIH	8	38 (27.3−45)	21.9 (18.7−27.1)	21.5 (18.3−26.7)	19.8 (12.8−30.6)	4	35	19.8 (12.8−30.6)
ADA-SCID	2	40.6 (36.2−44.9)	18.1 (15.8−20.4)	11.1 (8.0−14.1)[a]	17.9 (10.1−25.7)	2	30	17.9 (10.1−25.7)
WAS	16	38.1 (11.9−67.5)	22.0 (8.8−38.5)	21.1 (8.8−37.2)	18.0 (5.25−61.8)	2−3	20−30	18.0 (5.3−30.9[b])

Variables are reported as median and range (in parentheses). DP, drug product. The interval between the last apheresis and DP infusion ranged from 4 to 163 days.
The interval between the last apheresis and DP infusion ranged from 4 to 163 days
[a]Total nucleated cells.
[b]One patient received slightly more than 30×10^6 $CD34^+$ cells/kg due to high busulfan exposure.

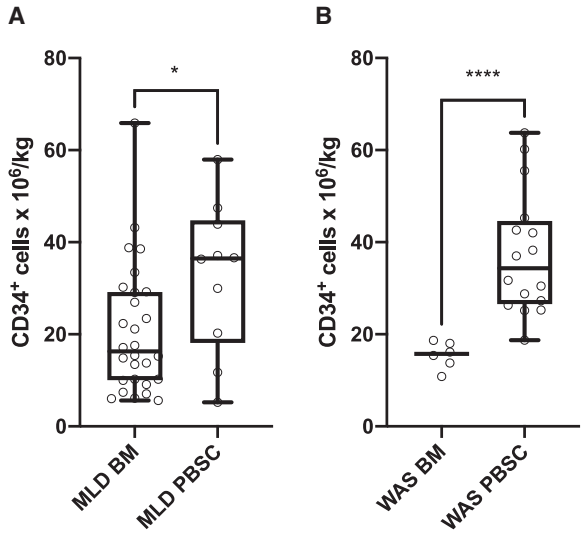

Figure 2. Comparison of CD34⁺ cell yield of mobilization and leukapheresis versus bone marrow harvest

(A) MLD patients. (B) WAS patients. Whiskers range from minimum to maximum. * $p<0.05$ **** $p<0.0001$.

$p < 0.0001$, respectively). A similar difference was found between the pooled BM harvests and the pooled leukaphereses (median of 17 versus 37×10^6 CD34⁺ cells/kg, $p < 0.0001$). Indeed, linear regression analysis on the whole patient population revealed that neither age nor weight influenced the HSPC yield and that the difference between BM and PBSC yield was explained entirely by the different procedure.

Among the 40 patients who underwent mobilization upfront, 37 did not require a second HSPC collection from either PBSC or BM. Instead, 57/57 patients of the BM harvest cohort required a separate backup collection, either by BM harvest (n = 54) or mobilization and leukapheresis (n = 3).

Thus, mobilization and leukapheresis allowed the collection of more HSPCs for the purpose of GT, in a shorter period of time, irrespectively of weight; additional harvests may be required for a minority of patients. With the consideration of the complexity of the GT treatment path, the urgency of treating diseases such as MLD, MPSIH, and WAS; the simultaneous collection of backup; and cells for GT manufacturing represents an important advantage over BM harvest. Shorter duration of anesthesia, lower intravascular fluctuation, and reduced pain represent additional advantages over BM collections. Indeed, the most recent GT trials have exploited the collection of PBSCs to allow for large DP infusions;[2,13,14,31] however, methods and goals of collection in children have not been addressed systematically. We believe our data may be useful for the optimization of the collection procedures and for the drafting of guidelines on PBSC collection in pediatric patients for the purpose of GT.

Furthermore, we show that the collection of very large amounts of CD34⁺ cells is feasible and may prospectively allow the implementation of strategies to enrich specific subpopulations of genetically modified HSPC,[32–36] whereas compensating for the cell loss expected from the purification procedures. Our results are also relevant for collection strategies in the context of allogeneic HSCT. Harvesting large amounts of PBSCs with lenograstim and plerixafor may in fact allow us to overcome significant weight discrepancies between pediatric family donors and HSCT recipients. In any event, the expected benefits of collecting and manipulating large cell doses must be weighed against the procedural risks and the increase in marginal cost.

The first limitation of this work is its retrospective nature, which is partly mitigated by prospective data collection. The second limitation is the potential variability due to the different disease background, coupled with the small size of patient subgroups. One cannot exclude that increased data accrual will lead to new or slightly different conclusions.

In summary, our work provides the necessary basis for an informed decision on the benefits and drawbacks of PBSC collection in pediatric patients.

MATERIALS AND METHODS

Patient population

We included all consecutive patients <18 years who underwent mobilization between April 1, 2010, and March 31, 2020, at Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) Ospedale San Raffaele (OSR). Legal guardians provided written, informed consent according to Italian law. Patients were affected by ADA deficiency (ADA-SCID), β-thalassemia, MLD, MPSIH, or WAS. Patients were enrolled in GT clinical trials (n = 32; ClinicalTrials.gov: NCT02453477,[14] NCT03392987, NCT01560182,[3] NCT03488394,[20] NCT01515462,[5] and NCT03837483[19]) and treated under compassionate use (n = 7), hospital exemption (n = 4), or Strimvelis (n = 2, included in this study only for backup collection). All studies were approved by the OSR Ethical Committee and Italian competent authorities.

The baseline BM aspirate CD34⁺ cell count was considered for predictive analysis. HSPCs were mobilized with lenograstim s.c. alone or with plerixafor s.c. Dosing was adjusted according to peripheral WBC and CD34⁺ counts. Leukapheresis was performed with Spectra COBE or the Optia Apheresis System (Terumo Blood and Cell Technologies [BCT]) and the WBC set or Spectra Optia IDL Set, respectively, through a percutaneous CVC; $\lesssim$3 blood vol was processed. During the collection, venous blood gas analyses were checked serially, and calcium gluconate and sodium bicarbonate were administered accordingly. For patients weighing <25 kg or with low hematocrit, the extra-corporeal circuit was primed with irradiated allogenic-packed RBCs.

Collection targets are reported as stated in the corresponding trial protocol[5,14,19,20] (and ClinicalTrials.gov: NCT03392987), Strimvelis summary of product characteristics,[37] or in the individualized patient's treatment plans. The number of CD34⁺ cells was determined

by flow cytometry (International Society of Hematotherapy and Graft Engineering [ISHAGE]); $CD34^+$ cell content of apheresis bags was normalized by patients' weight. Leukapheresis were enriched for $CD34^+$ cells by Miltenyi CliniMACS and used as starting material for manufacturing of DPs by transduction with viral vectors at Molmed (currently AGC Biologics). The leukapheresis fractions dedicated to backup were cryopreserved.

AEs were recorded in the case report forms or clinical charts and graded according to the Common Terminology Criteria for Adverse Events. AEs occurring between the beginning of the mobilization and the 14 days following the last apheresis or, if occurring earlier, the first dose of conditioning chemotherapy were considered for analysis.

Statistical analysis

Statistical analysis was done with Prism version 8 (GraphPad Software, San Diego, CA, USA) or SPSS Statistics version 24 (IBM, Armonk, NY, USA). Continuous variables are summarized with median and range; correlation was assessed with Spearman r coefficient and linear regression, whereas differences were assessed by two-tailed Mann-Whitney test or Wilcoxon matched-pairs signed-rank test. Comparison among three or more groups was performed by Kruskal-Wallis and Dunn's multiple comparison tests. Relations between categorical variables were assessed with Fisher's exact test. Relationship with multiple independent variables was assessed with stepwise multiple linear regression, provided no significant outliers were present.

Groups with at least 5 data points were considered for statistical analysis; however, the four ADA-SCID patients were not *a priori* excluded from multiple comparison analyses. Significant p values are summarized on figures as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$; ns, not significant.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.omtm.2021.05.013.

ACKNOWLEDGMENTS

The authors would like to thank Fondazione Telethon for support. We thank the medical and nursing team of the Pediatric Immunohematology Unit and Stem Cell Transplant Program of the IRCCS San Raffaele Scientific Institute for their professional care of patients during hospitalization; Laura Castagnaro and the OSR quality team; Stefano Zancan, data managers, study coordinators, research nurses, and administrative personnel of the San Raffaele Telethon Institute for Gene Therapy (SR-TIGET) Clinical Trial Office (TCTO); and Alessandro Nonis for statistical support. Orchard Therapeutics is the current sponsor of GT studies for ADA-SCID, WAS, β-thalassemia, and MLD. The graphical abstract was created with BioRender.com. Several authors are members of the European Reference Network for Rare Immunodeficiency, Autoinflammatory and Autoimmune Diseases (ERN-RITA); Inborn Error Working Party of EBMT and Italian Primary Immunodeficiencies Network (IPINET); and Associazione Italiana Ematologia e Oncologia Pediatrica (AIEOP). A.A. is the recipient of the Else Kröner Fresenius Prize for Medical Research 2020. This work was supported by Fondazione Telethon.

AUTHOR CONTRIBUTIONS

Conceptualization, D.C. and F.T.; data curation, D.C., F.T., E.A., and P.M.; formal analysis, D.C. and F.T.; funding acquisition, M.P.C., M.E.B., and A.A.; investigation, D.C., F.T., V.C., B.G., Francesca Ferrua, S.M., M.M., F.B., G.C., Francesca Fumagalli, G.V., P.S., R.M., and L.S.; methodology, S.G., M.Z., V.G., C.P., and M.P.C.; resources, A.A.; supervision, A.A., F.C., M.E.B., and M.P.C.; visualization, D.C.; writing – original draft, D.C. and F.T.; writing – review & editing, M.P.C., M.E.B., and A.A.

DECLARATION OF INTERESTS

SR-TIGET is a joint venture between Fondazione Telethon and OSR. Gene therapies for ADA-SCID, WAS, MLD, β-thalassemia, and MPSIH developed at SR-TIGET were licensed to Orchard Therapeutics (OTL) in 2018 and 2019. A.A. is the principal investigator (PI) of the above clinical trials. M.E.B. is the current PI of the MPSIH clinical trial.

REFERENCES

1. Ferrari, G., Thrasher, A.J., and Aiuti, A. (2021). Gene therapy using haematopoietic stem and progenitor cells. Nat. Rev. Genet. *22*, 216–234.
2. Eichler, F., Duncan, C., Musolino, P.L., Orchard, P.J., De Oliveira, S., Thrasher, A.J., Armant, M., Dansereau, C., Lund, T.C., Miller, W.P., et al. (2017). Hematopoietic stem-cell gene therapy for cerebral adrenoleukodystrophy. N. Engl. J. Med. *377*, 1630–1638.
3. Sessa, M., Lorioli, L., Fumagalli, F., Acquati, S., Redaelli, D., Baldoli, C., Canale, S., Lopez, I.D., Morena, F., Calabria, A., et al. (2016). Lentiviral haemopoietic stem-cell gene therapy in early-onset metachromatic leukodystrophy: an ad-hoc analysis of a non-randomised, open-label, phase 1/2 trial. Lancet *388*, 476–487.
4. Thompson, A.A., Walters, M.C., Kwiatkowski, J., Rasko, J.E.J., Ribeil, J.A., Hongeng, S., Magrin, E., Schiller, G.J., Payen, E., Semeraro, M., et al. (2018). Gene therapy in patients with transfusion-dependent β-thalassemia. N. Engl. J. Med. *378*, 1479–1493.
5. Ferrua, F., Cicalese, M.P., Galimberti, S., Giannelli, S., Dionisio, F., Barzaghi, F., Migliavacca, M., Bernardo, M.E., Calbi, V., Assanelli, A.A., et al. (2019). Lentiviral haemopoietic stem/progenitor cell gene therapy for treatment of Wiskott-Aldrich syndrome: interim results of a non-randomised, open-label, phase 1/2 clinical study. Lancet Haematol. *6*, e239–e253.
6. Gaspar, H.B., Cooray, S., Gilmour, K.C., Parsley, K.L., Zhang, F., Adams, S., Bjorkegren, E., Bayford, J., Brown, L., Davies, E.G., et al. (2011). Hematopoietic Stem Cell Gene Therapy for Adenosine Deaminase-Deficient Severe Combined Immunodeficiency Leads to Long-Term Immunological Recovery and Metabolic Correction. Sci. Transl. Med. *3*, 97ra80.
7. Cicalese, M.P., Ferrua, F., Castagnaro, L., Pajno, R., Barzaghi, F., Giannelli, S., Dionisio, F., Brigida, I., Bonopane, M., Casiraghi, M., et al. (2016). Update on the safety and efficacy of retroviral gene therapy for immunodeficiency due to adenosine deaminase deficiency. Blood *128*, 45–54.
8. Purtill, D., Antonenas, V., Chiappini, P., Tong, D., O'Flaherty, E., Bajel, A., Kabani, K., Larsen, S., Tan, S., Hutchins, C., et al. (2020). Variable CD34+ recovery of cryopreserved allogeneic HPC products: transplant implications during the COVID-19 pandemic. Blood Adv. *4*, 4147–4150.
9. Styczynski, J. (2018). Young child as a donor of cells for transplantation and lymphocyte based therapies. Transfus. Apheresis Sci. *57*, 323–330.
10. Tucci, F., Frittoli, M., Barzaghi, F., Calbi, V., Migliavacca, M., Ferrua, F., Fumagalli, F., Lorioli, L., Castagnaro, L., Facchini, M., et al. (2019). Bone marrow harvesting from

paediatric patients undergoing haematopoietic stem cell gene therapy. Bone Marrow Transplant. *54*, 1995–2003.

11. Rettig, M.P., Ansstas, G., and DiPersio, J.F. (2012). Mobilization of hematopoietic stem and progenitor cells using inhibitors of CXCR4 and VLA-4. Leukemia *26*, 34–53.

12. Karow, A., Wilhelm, A., Ammann, R.A., Baerlocher, G.M., Pabst, T., Mansouri Taleghani, B., Roessler, J., and Leibundgut, K. (2019). Peripheral blood progenitor cell collection in pediatric patients optimized by high pre-apheresis count of circulating CD34+ cells and high blood flow. Bone Marrow Transplant. *54*, 885–893.

13. Tisdale, J.F., Pierciey, F.J., Jr., Bonner, M., Thompson, A.A., Krishnamurti, L., Mapara, M.Y., Kwiatkowski, J.L., Shestopalov, I., Ribeil, J.A., Huang, W., et al. (2020). Safety and feasibility of hematopoietic progenitor stem cell collection by mobilization with plerixafor followed by apheresis vs bone marrow harvest in patients with sickle cell disease in the multi-center HGB-206 trial. Am. J. Hematol. *95*, E239–E242.

14. Marktel, S., Scaramuzza, S., Cicalese, M.P., Giglio, F., Galimberti, S., Lidonnici, M.R., Calbi, V., Assanelli, A., Bernardo, M.E., Rossi, C., et al. (2019). Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent ß-thalassemia. Nat. Med. *25*, 234–241.

15. Duong, H.K., Savani, B.N., Copelan, E., Devine, S., Costa, L.J., Wingard, J.R., Shaughnessy, P., Majhail, N., Perales, M.-A., Cutler, C.S., et al. (2014). Peripheral blood progenitor cell mobilization for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation. Biol. Blood Marrow Transplant. *20*, 1262–1273.

16. Fischer, J.C., Frick, M., Wassmuth, R., Platz, A., Punzel, M., and Wernet, P. (2005). Superior mobilisation of haematopoietic progenitor cells with glycosylated G-CSF in male but not female unrelated stem cell donors. Br. J. Haematol. *130*, 740–746.

17. Panch, S.R., Szymanski, J., Savani, B.N., and Stroncek, D.F. (2017). Sources of hematopoietic stem and progenitor cells and methods to optimize yields for clinical cell therapy. Biol. Blood Marrow Transplant. *23*, 1241–1249.

18. Gaipa, G., Dassi, M., Perseghin, P., Venturi, N., Corti, P., Bonanomi, S., Balduzzi, A., Longoni, D., Uderzo, C., Biondi, A., et al. (2003). Allogeneic bone marrow stem cell transplantation following CD34+ immunomagnetic enrichment in patients with inherited metabolic storage diseases. Bone Marrow Transplant. *31*, 857–860.

19. Ferrua, F., Cicalese, M.P., Galimberti, S., Giannelli, S., Dionisio, F., Barzaghi, F., Migliavacca, M., Bernardo, M.E., Calbi, V., Tucci, F., et al. (2019). Lentiviral Hematopoietic Stem and Progenitor Cell Gene Therapy for Wiskott-Aldrich Syndrome (WAS): Up to 8 Years of Follow up in 17 Subjects Treated Since 2010. Blood *134*, 3346.

20. Gentner, B., Bernardo, M.E., Tucci, F., Zonari, E., Fumagalli, F., Pontesilli, S., Acquati, S., Silvani, P., Ciceri, F., Rovelli, A., et al. (2019). Extensive Metabolic Correction of Hurler Disease By Hematopoietic Stem Cell-Based Gene Therapy: Preliminary Results from a Phase I/II Trial. Blood *134*, 607.

21. Styczynski, J., Balduzzi, A., Gil, L., Labopin, M., Hamladji, R.M., Marktel, S., Yesilipek, M.A., Fagioli, F., Ehlert, K., Matulova, M., et al.; European Group for Blood and Marrow Transplantation Pediatric Diseases Working Party (2012). Risk of complications during hematopoietic stem cell collection in pediatric sibling donors: a prospective European Group for Blood and Marrow Transplantation Pediatric Diseases Working Party study. Blood *119*, 2935–2942.

22. Doberschuetz, N., Soerensen, J., Bonig, H., Willasch, A., Rettinger, E., Pfirrmann, V., Salzmann-Manrique, E., Schäfer, R., Klingebiel, T., Bader, P., and Jarisch, A. (2019). Mobilized peripheral blood stem cell apheresis via Hickman catheter in pediatric patients. Transfusion *59*, 1061–1068.

23. Pulsipher, M.A., Levine, J.E., Hayashi, R.J., Chan, K.W., Anderson, P., Duerst, R., Osunkwo, I., Fisher, V., Horn, B., and Grupp, S.A. (2005). Safety and efficacy of allogeneic PBSC collection in normal pediatric donors: the pediatric blood and marrow transplant consortium experience (PBMTC) 1996-2003. Bone Marrow Transplant. *35*, 361–367.

24. Pulsipher, M.A., Chitphakdithai, P., Logan, B.R., Navarro, W.H., Levine, J.E., Miller, J.P., Shaw, B.E., O'Donnell, P.V., Majhail, N.S., and Confer, D.L. (2014). Lower risk for serious adverse events and no increased risk for cancer after PBSC vs BM donation. Blood *123*, 3655–3663.

25. Lorioli, L., Cicalese, M.P., Silvani, P., Assanelli, A., Salvo, I., Mandelli, A., Fumagalli, F., Fiori, R., Ciceri, F., Aiuti, A., et al. (2015). Abnormalities of acid-base balance and predisposition to metabolic acidosis in Metachromatic Leukodystrophy patients. Mol. Genet. Metab. *115*, 48–52.

26. Lee, G., and Arepally, G.M. (2012). Anticoagulation techniques in apheresis: from heparin to citrate and beyond. J. Clin. Apher. *27*, 117–125.

27. Karres, D., Ali, S., van Hennik, P.B., Straus, S., Josephson, F., Thole, G., Glerum, P.J., Herberts, C., Babae, N., Herold, R., et al. (2020). EMA Recommendation for the pediatric indications of plerixafor (Mozobil) to enhance mobilization of hematopoietic stem cells for collection and subsequent autologous transplantation in children with lymphoma or malignant solid tumors. Oncologist *25*, e976–e981.

28. Armitage, S., Hargreaves, R., Samson, D., Brennan, M., Kanfer, E., and Navarrete, C. (1997). CD34 counts to predict the adequate collection of peripheral blood progenitor cells. Bone Marrow Transplant. *20*, 587–591.

29. Morland, B., Kepak, T., Dallorso, S., Sevilla, J., Murphy, D., Luksch, R., Yaniv, I., Bader, P., Rößler, J., Bisogno, G., et al. (2020). Plerixafor combined with standard regimens for hematopoietic stem cell mobilization in pediatric patients with solid tumors eligible for autologous transplants: two-arm phase I/II study (MOZAIC). Bone Marrow Transplant. *55*, 1744–1753.

30. Sevilla, J., Guillén, M., Castillo, A., Prudencio, M., González-Vicent, M., Lassaletta, Á., Cormenzana, M., Ramírez, M., Pérez-Martínez, A., Madero, L., and Díaz-Pérez, M.Á. (2013). Defining "poor mobilizer" in pediatric patients who need an autologous peripheral blood progenitor cell transplantation. Cytotherapy *15*, 132–137.

31. Esrick, E.B., Lehmann, L.E., Biffi, A., Achebe, M., Brendel, C., Ciuculescu, M.F., Daley, H., MacKinnon, B., Morris, E., Federico, A., et al. (2021). Post-Transcriptional Genetic Silencing of *BCL11A* to Treat Sickle Cell Disease. N. Engl. J. Med. *384*, 205–215.

32. Agudelo, D., Duringer, A., Bozoyan, L., Huard, C.C., Carter, S., Loehr, J., Synodinou, D., Drouin, M., Salsman, J., Dellaire, G., et al. (2017). Marker-free coselection for CRISPR-driven genome editing in human cells. Nat. Methods *14*, 615–620.

33. Dever, D.P., Bak, R.O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C.E., Pavel-Dinu, M., Saxena, N., Wilkens, A.B., Mantri, S., et al. (2016). CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. Nature *539*, 384–389.

34. Zonari, E., Desantis, G., Petrillo, C., Boccalatte, F.E., Lidonnici, M.R., Kajaste-Rudnitski, A., Aiuti, A., Ferrari, G., Naldini, L., and Gentner, B. (2017). Efficient Ex Vivo Engineering and Expansion of Highly Purified Human Hematopoietic Stem and Progenitor Cell Populations for Gene Therapy. Stem Cell Reports *8*, 977–990.

35. Ciceri, F., Bonini, C., Stanghellini, M.T.L., Bondanza, A., Traversari, C., Salomoni, M., Turchetto, L., Colombi, S., Bernardi, M., Peccatori, J., et al. (2009). Infusion of suicide-gene-engineered donor lymphocytes after family haploidentical haemopoietic stem-cell transplantation for leukaemia (the TK007 trial): a non-randomised phase I-II study. Lancet Oncol. *10*, 489–500.

36. Vavassori, V., Mercuri, E., Marcovecchio, G.E., Castiello, M.C., Schiroli, G., Albano, L., Margulies, C., Buquicchio, F., Fontana, E., Beretta, S., et al. (2021). Modeling, optimization, and comparable efficacy of T cell and hematopoietic stem cell gene editing for treating hyper-IgM syndrome. EMBO Mol. Med. *13*, e13545.

37. European Medicines Agency. Strimvelis. Product Information. https://www.ema.europa.eu/en/documents/product-information/strimvelis-epar-product-information_en.pdf.