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 $37 \times 10^6$ CD34+ cells/kg. The procedures were well tolerated. Patients who collected $\geq 7 \times 10^6$ and $\geq 13 \times 10^6$ CD34+ cells/kg in the first cycle had pre-apheresis circulating counts of at $\geq 42$ and $\geq 86$ 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 $30.9 \times 10^6$ CD34+ cells/kg. Pediatric PBSC collection compared 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 $\geq 2 \times 10^6$ 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.4,8,11–13 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

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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 lenograsitum 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 lenograsitum 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 × 106 CD34+ cells/kg, with a range of 3.3–63.8 × 106, 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 × 106 white blood cells (WBCs)/mL and 0.2%–3.5% of CD34+ cells, and weight-averaged yields ranged from 0.7 to 53.2 × 106 CD34+ cells/kg, with a median of 18.3 × 106 CD34+ cells/kg.

Volumes (median 132.5 mL, range 44–301 mL) and WBC counts (median 205 × 106 WBC/mL, range 53–374 × 106 WBC/mL) of

### 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) | 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                                  | 2                      |
| β-thalasemia  | 7  | 2/5         | 6.6 (4.4–13.6)       | 20.0 (15.4–54.0)     | 4.1 (1.7–5.5)            | 0                                  | 0                      |
| MLD           | 10 | 2/8         | 2.5 (0.6–7.7)        | 13.2 (7.0–24.0)      | 3.7 (0.8–6.4)            | 1                                  | 8                      |
| MPSIH         | 8  | 2/6         | 1.9 (1.0–2.7)        | 11.8 (11.0–14.3)     | 3.9 (1.7–7.7)            | 0                                  | 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                     |
| Total         | 45 | 9/36        | 3.7 (0.6–14.4)       | 15.8 (7.0–54.1)      | 3.8 (0.5–10.8)           | 2                                  | 43                     |

DP, drug product; BM, bone marrow; HSPC, hematopoietic stem and progenitor cell.

*Data not available for 3 patients.
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 × 10^6 CD34+ cells/kg (range 1.5–43.6 CD34+ cells/kg) corresponding to a median increase of 3.75 × 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 < 0.0001). 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 < 0.0001; 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 × 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 × 10^6 CD34+ cells/kg, one patient fell into the “low” 2–5 × 10^6 CD34+ cells/kg interval, and none had a low yield, i.e., <2 × 10^6 CD34+ cells/kg.

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

| Disease          | 1st cycle | YIELD | 2nd cycle | YIELD | 3rd cycle | 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 (×10^6 CD34+ cells/kg) are reported as median and range (in parentheses). The number of patients is reported for each apheresis procedure.

### Table 3. Summary of adverse events

| Category          | Grade | Most common event (n)          | Grade | Most common event (n)          | Grade | Most common event (n)          |
|-------------------|-------|--------------------------------|-------|--------------------------------|-------|--------------------------------|
|                   | 4     | 3                              | 2     |                               |
| Allergic          | 3     | 4                              |       | urticarial skin rash (3)       |
| Blood related     | 1     | 6                              | 5     | anemia (6)                     |
| Cardiovascular    | 1     |                               | 1     | hypertension (1)               |
| Electrolyte       | 1     |                               | 1     | hypokalemia (3)                |
| Disturbances      | 2     |                               | 2     |                              |
| ENT               | 2     |                               | 2     |                              |
| Gastrointestinal  | 1     |                               | 1     | vomit (5)                      |
| Infectious        | 3     |                               | 6     | upper airway infection (3)    |
| Kidney            | 3     |                               | 3     |                              |
| Metabolic         | 1     |                               | 2     | metabolic acidosis (9)         |
| Musculoskeletal   | 2     |                               | 6     | arthritis (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.
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 (R² 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 (R² 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 × 10⁶ CD34+ cells/kg (p = 0.0082) and 23 × 10⁶ 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 ≥ 20 × 10⁶ 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 (R² 0.631). Inclusion of peripheral CD34+ cell counts in the model instead replaced gender as a predictor of yield (R² 0.853).

**Manipulation and engraftment**

A backup was stored for 43 patients; all but one were above the minimum 2 × 10⁶ 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.10,16 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 × 10⁶ 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 × 10⁶ 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,6,19 metabolism,2,3,20 and haemopoiesis.14,14 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...
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.6,24 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 understandably 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.”25,30 Our GT protocols require the collection of ≥7–13 × 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 ≥7 × 10^6 CD34+ cells/kg in the first cycle had pre-apheresis circulating counts of ≥42 CD34+ cells/μL, and those who collected ≥13 × 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

| Protocol | n | Starting material (×10^6 CD34+ cells/kg) | Recovery from selection (×10^6 CD34+ cells/kg) | Destined to transduction (×10^6 CD34+ cells/kg) | Recovery from transduction (×10^6 CD34+ cells/kg) | Predefined DP infusion range (×10^6 CD34+ cells/kg) | DP dose (×10^6 CD34+ cells/kg) |
|----------|---|--------------------------------------|---------------------------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------|-----------------|
| 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) | 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) |

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.

One patient received slightly more than 30 × 10^6 CD34+ cells/kg due to high busulfan exposure.
CD34+ cells is feasible and may prospectively allow the implementation of strategies to enrich specific subpopulations of genetically modified HSPC, 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, NCT03392987, NCT01560182, NCT03488394, NCT01515462, and NCT03837483) 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; ≤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 allogeneic-packed RBCs.

Collection targets are reported as stated in the corresponding trial protocol (ClinicalTrials.gov: NCT03392987). Strimvelis summary of product characteristics, 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. Leukaphereses 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.

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**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.

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