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Supplemental information

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

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Supplementary methods

40/45 patients were enrolled upfront to collect cells for both drug product (DP) manufacturing and backup. 38/40 met this goal by PBSC collection alone, while 2 required an additional BM harvest. For 5/45 patients, mobilization and apheresis were done to overcome potential or actual limitations of bone marrow harvest. 2/45 were enrolled in the Strimvelis hospital exemption program, which allowed to manufacture the commercial DP from PBSCs due to low $CD34^+$ cell counts in the BM. 2/45 collected only for backup purpose; another one was enrolled for a rescue procedure to collect an additional quota of cells for the DP.

Conditioning regimens varied across disease-specific protocols. Except for ADA-SCID patients, that received low-dose busulfan (2 mg/kg per day divided into 4 doses of 0.5 mg/kg on days –3 and –2), and WAS patients that received non-myeloablative busulfan and fludarabine, all patients received a myeloablative conditioning. BTHAL patients received threosulfan and thiotepa regimen, MLD patients received busulfan, and MPS1 patients received fludarabine and busulfan. The DP was infused intravenously through a CVC except for B-thal patients that received an intrabone infusion.

Neutrophil engraftment was defined as the first of three consecutive days with ≥500 neutrophils/µL. Platelet engraftment was defined as the first of three consecutive days of platelet counts >20.000/µL 7 days after the last platelet transfusion. For those that never reached platelet counts <20.000/µL, the day of platelet nadir was used instead.

Supplementary Tables

Table S1: number of $CD34^+$ cells stored for backup, averaged by weight. Median values and (range) are reported.

Disease	**ADA-SCID**	**β-thalassemia**	**MLD**	**MPSIH**	**WAS**
$CD34^+$ cells x 10^6/kg (range)	3.2 (1.2-5.0)	5.1 (4.3-6.9)	4.4 (3.0-5.5)	4.4 (3.7-6.8)	3.7 (2.8-13.8)

Supplementary Figures

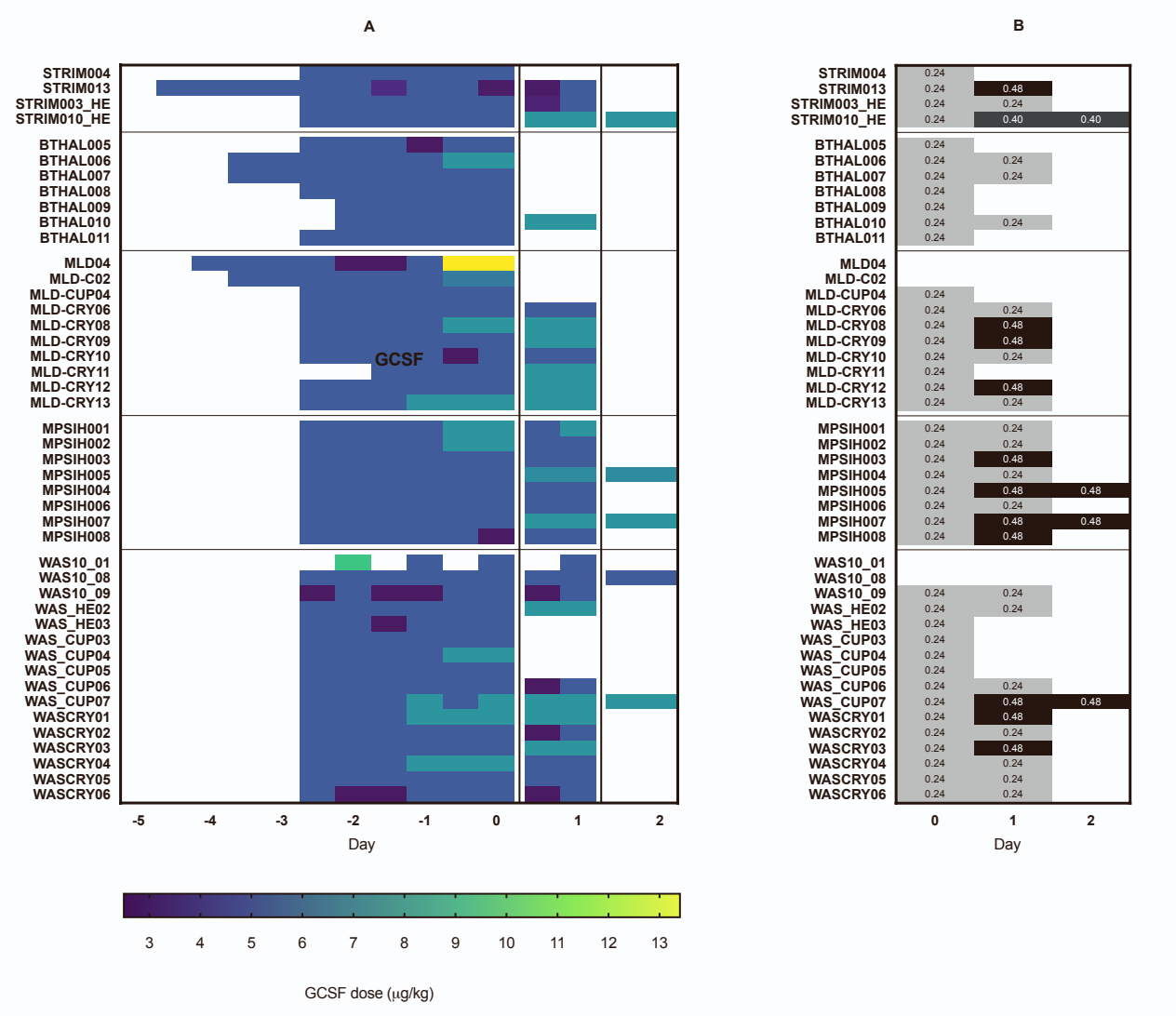

Figure S1 A: Heat map illustrating consecutive doses of lenograstim administered to each patient. Aphereses took place on Day 0, 1 and 2, corresponding to solid vertical lines. With a single exception (WAS10_01), mobilization was started with lenograstim 5 µg/kg twice daily and adjusted according to the peripheral WBC count, and continued until the morning before the last apheresis. WAS10_01 received a once daily lenograstim regimen.

B: Heat map illustrating consecutive doses of plerixafor on corresponding days. Plerixafor was introduced in 2015, and systematically administered from the fifth patient onwards, at the dose of 0.24 mg/kg approximately 6 hours before each apheresis. Since 2018, the second and third dose of plerixafor have been adjusted up to a maximum of 0.48 mg/kg.

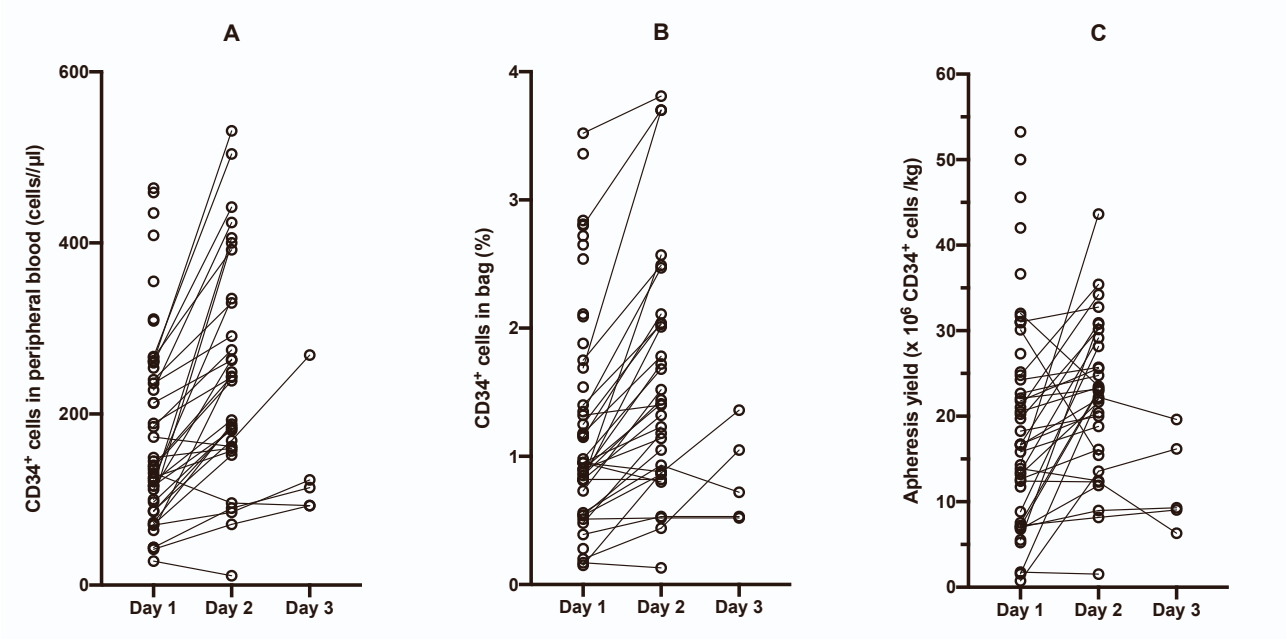

Figure S2: Dot plots illustrating the absolute number of CD34⁺ cells in peripheral blood before the apheresis (A) and the corresponding relative CD34⁺ content in the apheresis bag (B) and absolute CD34⁺ cell yield averaged by weight (C). Lines connect values belonging to the same patient.

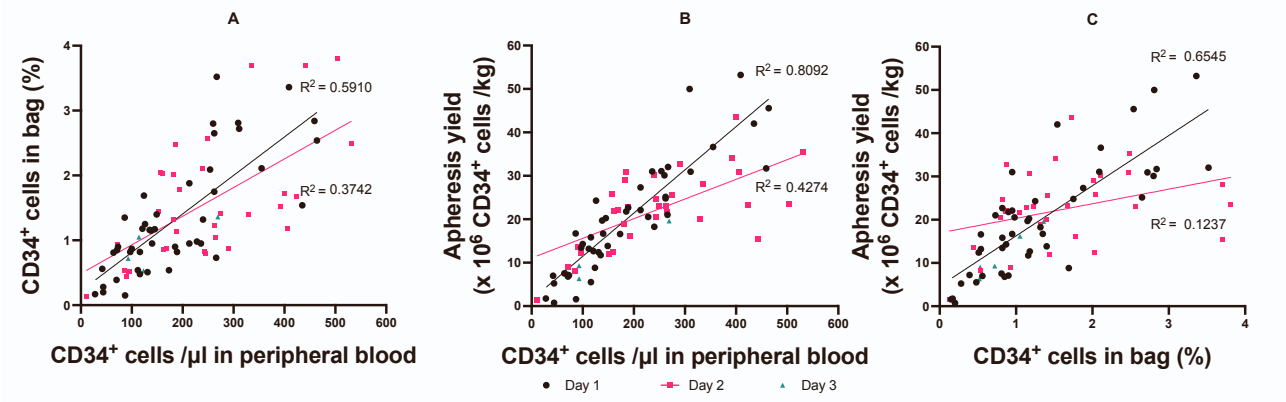

Figure S3: Dot plots illustrating the correlation between the CD34⁺ cell counts in peripheral blood and relative CD34⁺ counts in the apheresis bag (A), weight-adjusted CD34⁺ yield (B) and between the latter two (C). Data points are coded according to the day of apheresis.