Supplementary Methods

Donor Selection

When both parents (and eventually a HLA-haploidentical sibling > 18 year-old) were eligible for donation, the donor was chosen according to CMV and adenovirus serostatus (i.e., privileging donors with positive serology), as well as to immunological criteria [including, in hierarchical order: i) NK alloreactivity, evaluated according to the killer immunoglobulin-like receptor (KIR)/KIR-Ligand model; ii) KIR B haplotype, iii) higher B-content score and iv) size of NK alloreactive subset].[1-3]

Donor mobilization and graft manipulation.

Donor hematopoietic progenitors were mobilized through subcutaneous administration of 10-12 μg/kg/day granulocyte colony-stimulating factor in 2-3 divided doses from day -5 until day -1 (i.e., the day of leukapheresis). Plerixafor (Mozobil®, Genzyme) was given at a dose of 0.24 mg/kg in case of failure to achieve the cutoff of ≥40 CD34+ cells/μL and/or with a predicted apheresis yield ≤$12x10^6$ CD34+ cells/kg of recipient's body weight.[4] Apheresis was performed with the Spectra Optia® Cell Separator (Terumo BCT, Leuven, Belgium). Graft manipulation procedures were performed with the fully automated CliniMACS® device (Miltenyi Biotec, Bergisch Gladbach, Germany) as already described.[5] Clinical grade reagents, disposable kits, and instrumentation were also from Miltenyi Biotec.

Supportive therapy

All patients received antiviral prophylaxis with acyclovir, antifungal prophylaxis with agents active on both yeast and molds (i.e., liposomal amphotericin B or caspofungin) and prophylaxis against Pneumocystis jirovecii pneumonia with cotrimoxazole. G-CSF was not routinely used; it was administered during aplasia only in case of life-threatening bacterial or fungal infections.

$AAUC_{180}$

Time-averaged area-under-the-curve for the first 180 days ($AAUC_{180}$) was used to better report the overall exposure of patients to the viral burden over time and the time of positivity (thus indirectly reflecting the burden of medical care required). $AAUC_{180}$ (expressed as log10 copies*week/ml whole blood) was calculated for each patient with viremia > 500 (2.69 log10) copies/ml whole blood and divided per the number of weeks at risk (i.e., 26 weeks or time to last follow-up or death).

Statistical analysis

Engraftment was defined as time from HSCT to the first of 3 consecutive days with an absolute neutrophil count ≥$0.5x10^9$/L and reported as median and range; platelet recovery was defined as the first of 7 consecutive days with an unsupported platelet count ≥$20x10^9$/L, and reported as median and range.

Competing risks for calculation of cumulative incidences were as follows: i) for GF, death without engraftment; for GVHD (acute and chronic), GF and death without GVHD.

References

1. Moretta L, Locatelli F, Pende D, Marcenaro E, Mingari MC, Moretta A. Killer Ig-like receptor-mediated control of natural killer cell alloreactivity in haploidentical hematopoietic stem cell transplantation. *Blood*. 2011;117(3):764-771.
2. Pende D, Marcenaro S, Falco M, et al. Anti-leukemia activity of alloreactive NK cells in KIR ligand-mismatched haploidentical HSCT for pediatric patients: evaluation of the functional role of activating KIR and redefinition of inhibitory KIR specificity. *Blood*. 2009;113(13):3119-3129.
3. Meazza R, Falco M, Loiacono F, et al. Phenotypic and Functional Characterization of NK Cells in alphabetaT-Cell and B-Cell Depleted Haplo-HSCT to Cure Pediatric Patients with Acute Leukemia. *Cancers (Basel)*. 2020;12(8).
4. Merli P, Biagini S, Girolami E, et al. A new BSA-based Threshold Predicts Optimal PBSC Collection in T-cell Depleted HLA-haploidentical Stem Cell Transplantation. *Bone Marrow Transplantation*. 2020;55(SUPPL 1):681-681.
5. Li Pira G, Malaspina D, Girolami E, et al. Selective Depletion of alphabeta T Cells and B Cells for Human Leukocyte Antigen-Haploidentical Hematopoietic Stem Cell Transplantation. A Three-Year Follow-Up of Procedure Efficiency. *Biol Blood Marrow Transplant*. 2016;22(11):2056-2064.

Supplementary Table.

Supplementary Table 1. Multivariable analysis on factors influencing GF.

Variable	Hazard ratio	Lower 95%CI	Upper 95%CI	p-value
CD34+/kg>20x10E6	4.1620	0.09699	178.6000	0.4600
Gender	0.7909	0.28980	2.1590	0.6500
Gender mismatch	0.2227	0.05121	0.9682	0.0450
Infections pre-HSCT	2.76500	0.68990	11.080	0.1500
Disease at high-risk for GF	5.6320	1.91900	16.5300	0.0017

Supplementary Table 2. Characteristics of second HSCT.

Patients	**(n = 18)**	**%**
Sex		
Male	11	61
Female	7	39
Donor characteristics		
Age (years)*, median (range)	40.5 (24-52)	
Type of donor		
Mother	13	72
Father	3	17
mismatched UCBT	2	11
Conditioning regimen used#		
Cyclophosphamide+Fludarabine (±TBI§)	10	55.5
Cytarabine+Fludarabine	5	27.5
Treosulfan+Fludarabine	3	17
Cell dose infused*, median (range)		
CD34+ cells × 10^6/kg	22.1 (15.8-37.4)	
αβ+ T cells × 10^6/kg	0.022 (0.002-0.066)	
γδ+ T cells × 10^6/kg	12.8 (4.0-38.9)	
NK cells × 10^6/kg	44.2 (16.2-116.5)	
CD20+ cells × 10^6/kg	0.035 (0.003-0.12)	
Engraftment	16	89
Time to engraftment (days), median (range)	14 (11-21)	
Infections		
CMV	6	33.5
ADV	5	27

Bacterial	1	5.5
Fungal	1	5.5
acute GvHD (grade I)	3(/16)	18.7
* for haplo HSCT		
# +ATLG Genzyme® 1.5-2 mg/kg for 2 days (-3,-2) and rituximab 200 mg/sm on day -1		
§ single dose 200 cGy		

Figure legends

Supplementary Figure 1. Engraftment. A. Kinetics of recovery of neutrophils (green line) and platelets (purple line). **B.** Cumulative incidence of platelet recovery for patients who received a dose of CD34+ cells in the first quartile (i.e., 8.5-18 x 10^6/kg; blue line) or in the 2^{nd}-4^{th} quartiles.

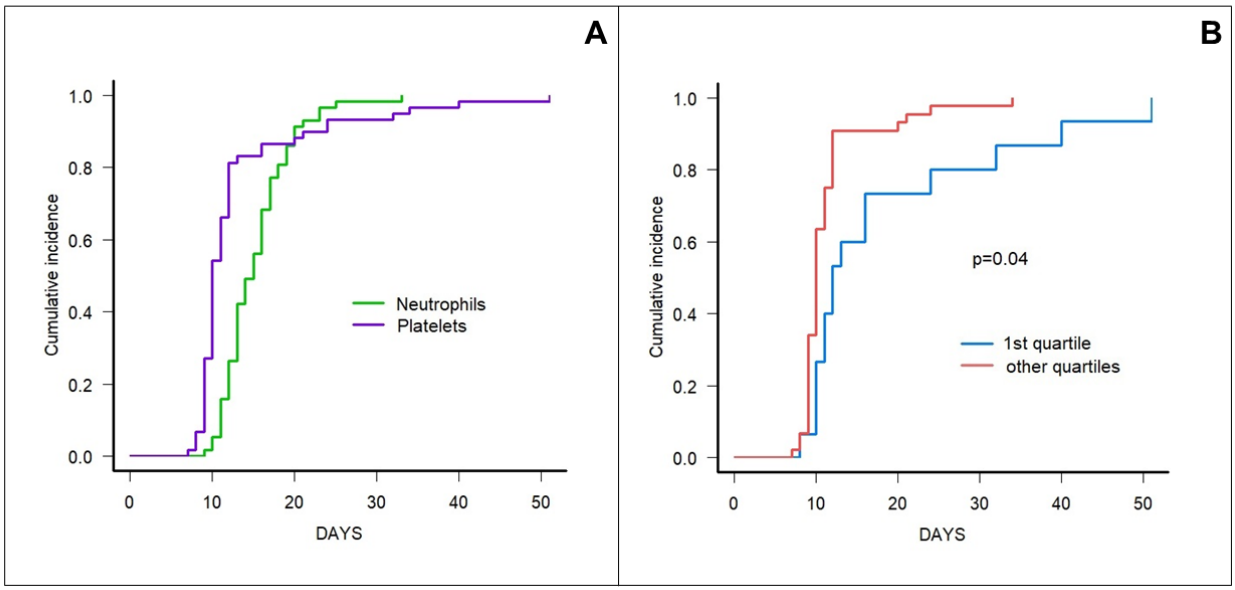
Supplementary Figure 2. Graft failure. Cumulative incidence of GF according to the presence (red line) or absence (blue line) of gender mismatch.

Supplementary Figure 3. Chimerism analysis. A. Pie chart representing chimerism of patients at 1 year after HSCT. **B.** Details on patients with mixed chimerism (percentage of donor chimerism is reported).

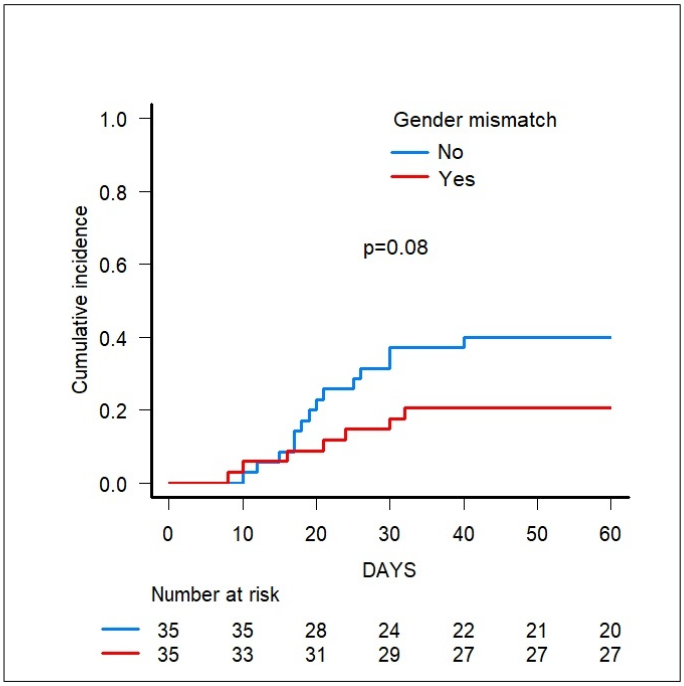
Supplementary Figure 4. Transplant-related mortality. TRM according to the infectious state at time of transplant. "Recent" infection was defined as having an infection within the month preceding TCRαβ+/CD19-depleted haploHSCT. Recent/active infection, red line; no infection at time of HSCT, blue line.

Supplementary Figure 5. Survival: Subgroup analysis on patients with PIDs. OS according to the type of primary immunodeficiency (i.e., SCID patients versus non-SCID patients).

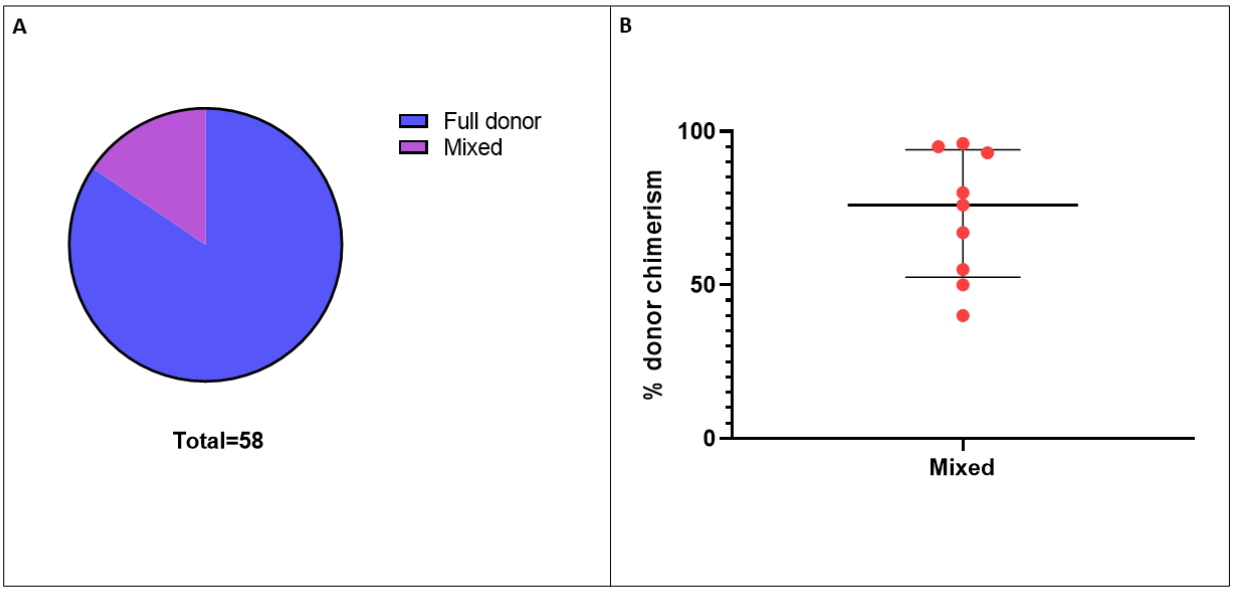
Supplementary Figure 1



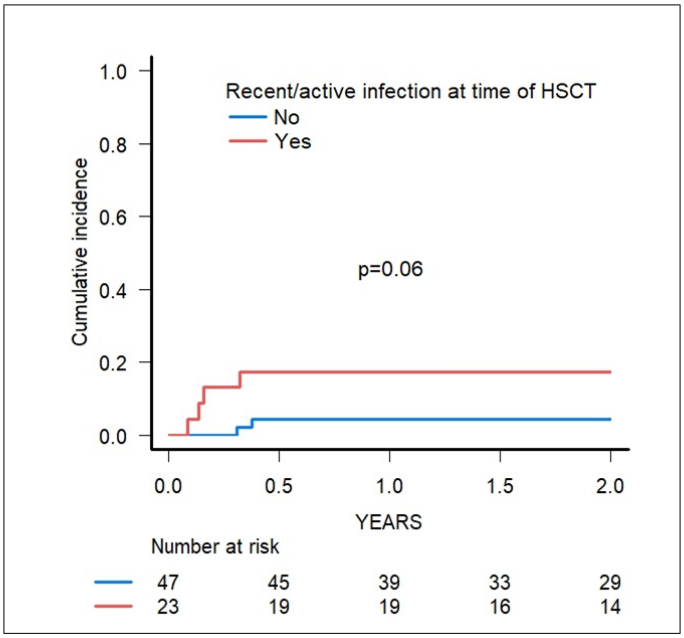
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5