αβT- and B-cell-depleted HLA-haploidentical hematopoietic stem cell transplantation in children with myelodysplastic syndromes

In this study, we investigated the outcomes of pediatric patients affected by myelodysplastic syndromes (MDS) and lacking an HLA-matched donor undergoing αβT-cell receptor (TCRαβ) and CD19 B-cell depleted HLA-haploidentical hematopoietic stem cell transplantation (TBdepl-haploHSCT), showing low incidence of transplant-related mortality (TRM), acute and chronic GvHD (aGvHD and cGvHD), as well encouraging overall (OS) and event-free survival (EFS).

Pediatric MDS are a heterogeneous group of clonal disorders, accounting for less than 5% of childhood hematologic malignancies; the incidence is estimated 1-4 per million.[1] They are characterized by peripheral cytopenia, ineffective hematopoiesis, and, most important in pediatrics, an increased risk of progression to acute myeloid leukemia (AML). Up to 20-30%,[2,3] of pediatric MDS develop in the context of inherited bone marrow failure syndromes (IBMFS) or genetic predisposition syndromes.[4] The genetic somatic landscape of pediatric MDS is characterized by driver mutations in *SETBP1*, *ASXL1*, *RUNX1* and *RAS* oncogenes.[3] Allogeneic HSCT is the sole curative treatment available. Recognized indications to transplantation are: MDS with excess of blasts, MDS secondary to previously administered chemoradiotherapy, refractory cytopenia of childhood (RCC) associated with monosomy 7, complex karyotype, severe neutropenia, or erythrocyte/platelet transfusion dependence.[1] We previously demonstrated that TBdepl-haploHSCT is a suitable option for children with acute leukemia lacking a readily available, HLA-matched donor.[5] Here we present the results of this transplant platform in children with MDS.

Between 03/2013 and 09/2021, 28 children with MDS received TBdepl-haploHSCT from an HLA-partially matched relative at Ospedale Pediatrico Bambino Gesù, Rome, Italy or at Fondazione IRCCS Policlinico San Matteo, Pavia, Italy as part of a prospective study (clinicaltrials gov. Identifier: NCT01810120). The trial was approved by the local Ethics Committees and was conducted according to the Declaration of Helsinki. All patients or their parents/legal guardians provided written informed consent.

All patients were conditioned using a fully-myeloablative regimen including a combination of cytotoxic drugs, such as treosulfan, thiotepa and fludarabine, and/or total body irradiation (TBI). Anti-T-lymphocyte globulin was used before transplantation (12 mg/kg total dose, from days -5 to day -3) to modulate bi-directional donor/recipient alloreactivity. Rituximab (200 mg/m^2) was administered on day -1 to prevent post-transplantation Epstein-Barr virus-induced lymphoproliferative disorders. No patient received any post-transplant pharmacological GvHD prophylaxis. Mobilization, apheresis and graft manipulation were performed as follows[6]: i) donor CD34+ cells were mobilized by administration of subcutaneous G-CSF 10-12 μg/kg per day from day –5 until leukapheresis (day –1); ii) if the cut-off of ≥40 CD34$^+$ cells/μL was not achieved, plerixafor (Mozobil, Genzyme) 0.24 mg/kg was given; iii) Spectra Optia Cell Separator (Terumo BCT, Leuven, Belgium) was used for apheresis; iv) the fully automated CliniMACS device (Miltenyi Biotec, Bergisch-Gladbach, Germany) was used for graft manipulation.

Genomic DNA from peripheral blood was available from 21 subjects. Moreover, DNA specimens from parents were available in 17 cases, and DNA from tissues other than blood were used to discriminate mosaicism events. Genomic DNA was used to assess the mutational profile by capture-based parallel sequencing using a NextSeq550 platform (Illumina) as previously described.[7]

End-points for survival were OS and EFS. Other end-points were: i) cumulative incidence and median time of neutrophil and platelet engraftment; ii) cumulative incidence of aGvHD and cGvHD; iii) cumulative incidence of relapse and iv) TRM. OS and EFS were estimated by using the Kaplan-Meier method. TRM, relapse incidence, aGvHD and cGvHD were expressed as cumulative incidences to adjust the estimations for competing risks. Statistical analysis was performed using EZR version 1.32 (Saitama Medical Center, Jichi Medical University), a graphical user interface for R (The R Foundation, Vienna, Austria; http://www.R-project.org).

Characteristics of patients enrolled in the study are shown in Table 1 (which reports also donor and graft characteristics). Median follow-up of surviving patients was 2.9 years (range, 0.3–8.5 years). The diagnosis of pediatric MDS was confirmed in the national reference laboratory of pathology (Rome). Twenty children had RCC (3 cases occurring in the context of inherited bone marrow failure syndromes: two had *GATA2* germline pathogenic variants and one *SAMD9L* variant, associated with monosomy of chromosome 7), while two and six were affected by MDS with excess of blasts 1 (EB1) and EB2/AML (1 had *GATA2* deficiency), respectively. Regarding genetic predisposition syndromes, three additional patients had variants of un-

known significance (VUS) in *TP53*, *MECOM* and *ANKRD26*. In addition, with regards to somatic events, one patient had a pathogenic variant in the *PTPN11* gene, one patient showed two mutational events, respectively in *RUNX1* and *CBL* genes and one had a likely pathogenic variant in *KRAS*. The different detected VUS are detailed in the *Online Supplementary Table S1*. No patients had therapy-related MDS and nobody was diagnosed to be evolved from SAA.

Median time to neutrophil and platelet recovery was 15 (range, 10-19) and 11 (range, 9-14) days (Figure 1A), respectively, with four patients (3 with RCC and 1 with EB2; this latter patient received chemotherapy and 5-azacytidine before HSCT, while the others did not received any therapy before transplant) experiencing primary graft failure, the cumulative incidence of this complication being 14.2% (95% confidence interval [CI]: 5.6-33.7). This was significantly lower than that experienced by patients with SAA (details are reported in the *Online Supplementary Table S2*) transplanted at our center with the same strategy and a TBI-free conditioning regimen (58.3%, 95% CI: 33.4-84.8; *P*=0.004) (Figure 1B).[8] Two of these four GF patients had chromosomal abnormalities (1 monosomy of chromosome 7 and 1 trisomy of chromosome 8), but none had *GATA2* and *SAMD9L* germline mutations. The cumulative incidence of GF was higher in patients with chromosomal abnormalities (28.6%, 95% CI: 8.0-74.2) than in those without (9.5%, 95%: CI 2.4-33.3), although this difference was not statistically significant (*P*=n.s.; *Online Supplementary Figure S1*). All four patients were rescued with a second TBdepl-haploHSCT from either the same or the other parent. Cumulative incidence of grade I-IV and II-IV aGvHD was 21.0% (95% CI: 9.3-43.4) and 8.3% (95% CI: 0.5-29.4), respectively. One additional patient developed stage 2 skin and stage 4 gut aGvHD after the second TBdepl-haploHSCT, while for all other patients skin was the sole organ involved; no case of grade IV aGvHD was observed in patients primarily engrafting. Of the 21 patients at risk, two developed cGvHD (one mild [skin] and the other moderate [lung]; cumulative incidence 9.7% [95% CI 2.5-33.8]), which completely resolved with low-dose steroids and ruxolitinib. Another patient developed moderate cGvHD of the lung after a DLI administered for minimal residual disease reappearance at flow-cytometry analysis. Ten patients experienced Cytomegalovirus (CMV), three human Herpesvirus 6 and one adenovirus infection/reactivation, the cumulative incidence of these infectious complications being 50.6% (95% CI: 33.8-70.2). In the patient with grade IV aGvHD after the second allograft, CMV infection with central nervous system, lung and gut involvement did not respond to specific treatment (including CMV-specific donor lymphocyte infusion), leading to transplant-related death; thus, cumulative incidence of transplant-related mortality (TRM) was 4% (95% CI: 0.3-17.0). One patient developed lung aspergillosis, which resolved with specific

Table 1. Patient, donor and transplant characteristics.

Patients	N=28	%
Sex		
Male	14	50
Female	14	50
Median (range) age at diagnosis, yr	9.6 (1.3-17.5)	
Median (range) age at HSCT, y	10.2 (1.8-18.0)	
Median (range) time from diagnosis to HSCT, mth	7.7 (1.7-120.4)	
Initial diagnosis		
RCC	20	72
EB1	2	7
EB2/AML	6	21
IBMFS		
GATA2	2	7
*SAMD9L**	1	4
ANKRD26 (VUS)	1	4
TP53 (VUS)	1	4
MECOM (VUS)	1	4
Recurrent cytogenetic lesions		
monosomy of chr 7	6	21
trisomy of chr 8	1	4
RUNX1 + CBL	1	4
PTPN11	1	4
KRAS	1	4
Treatment before TBdepl-haploHSCT		
None§	13	47
Immunosuppressive therapy	6	21
5-azacytidine	5	18
chemotherapy	4	14
Disease status at transplantation		
Active disease	25	89
CR	3	11
Previous HSCT	2	7
Conditioning regimen		
Treo+TT+Flu	22	79
TBI+TT+L-PAM	2	7
Bu+Cy+L-PAM	4	14
CMV serology (donor/recipient)		
Neg/Neg	2	7
Neg/Pos	2	7
Pos/Neg	2	7
Pos/Pos	22	79
Donor		
Mother	11	39
Father	17	61
Sex mismatch	15	54
Female donor → Male recipient	6	40
Cell dose infused, median (range)		
CD34+ cells × 10^6/kg	14.7 (8.3-28.6)	
αβ+ T cells × 10^6/kg	0.027 (0.008-0.098)	
γδ+ T cells × 10^6/kg	8.8 (1.6-40.0)	
NK cells × 10^6/kg	23.9 (2.8-80.5)	
CD20+ cells × 10^6/kg	0.016 (0.003-0.230)	

AML: acute myelogenous leukemia; Bu: busulfan; chr: chromosome; CR: complete response; Cy: cyclophosphamide; EB: MDS with excess of blasts; Flu: fludarabine; IBMFS: inherited bone marrow failure syndromes; L-PAM: melphalan; neg: negative; pos: positive; RCC: refractory cytopenia of childhood; TBI: total body irradiation; Treo: treosulfan; TT: thiotepa; VUS: variant of unknown significance. *Although formally identified as VUS, given the young age (1.6 years) and the presence of monosomy 7 this mutation was deemed as clinically relevant. §Not including supportive therapy.

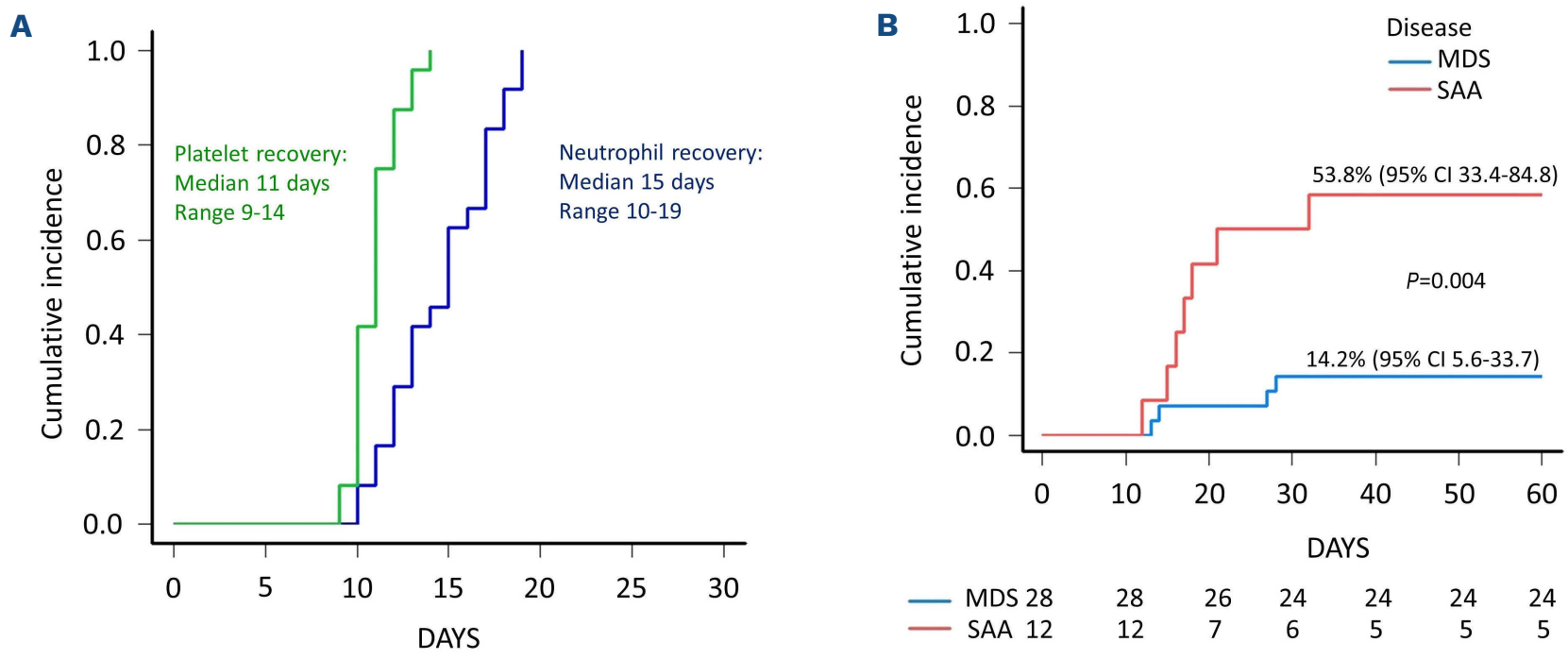

Figure 1. Engraftment. (A) Neutrophil (purple line) and platelet (grey line) recovery over time. (B) Comparison of cumulative incidence of graft failure in patients affected by myelodysplastic syndromes (MDS, blue line) and severe aplastic anemia (SAA, red line) transplanted from a haploidentical donor after TCRαβ/CD19 depletion.

treatment. No patient experienced VOD/SOS, while one patient developed, 3 month after HSCT, TA-TMA which resolved after treatment with eculizumab. Two patients, both affected by EB2, one in remission and one not at time of transplant, relapsed at 3 and 27 months after HSCT, respectively. The 5-year cumulative incidence of relapse was 9.4% (95% CI: 1.6-26.1) for the whole cohort, while it was 42.9% (95% CI: 11.4-92.4) for patients with EB1 and EB2. The patient with the early relapse was rescued with DLI and 5-azacytidine, being now alive and disease-free 3 years after HSCT; the other patient died of disease progression after a second HSCT failed. The 5-year probability of OS and EFS were 88.6% (95% CI: 59.5-97.2; Figure 2A) and 76.2% (95% CI: 53.8-88.8; Figure 2B), respectively. In line with previously reported data,[9] monosomy of chromosome 7 was associated with a reduced OS (53.3% vs. 100%, *P*=0.007, Figure 2C) and EFS (33.3% *vs*. 85.9%, *P*=0.03, Figure 2D); although MDS variant had no influence on the patient's outcome, this may be due to the small sample size; moreover, since three of eight patients with advanced MDS had monosomy of chromosome 7, this may have had an impact on survival analysis. However, multivariable analysis was inconclusive due to small sample size (not shown). No other variable had an impact on survival. The median cell counts on day +30, +90, +180 and +360 were: i) for CD3+, 246, 274, 556 and 1,242/mcL, respectively; ii) for CD4+, 15, 42, 121, 410/mcL, respectively; iii) for CD8+, 23, 106, 128, 486/mcL, respectively.

Although data on HSCT in this setting are scarce, these data compare favorably with those reported in the literature. In particular, OS and EFS are comparable to those of single-[10] and multicenter studies,[11] including HSCT from matched family donor, cord blood or matched unrelated donor[12] in children with RCC[13] or advanced diseases.[14] Moreover, TRM remains low, as already reported in patients with acute leukemia[5] or non-malignant disorders.[8] Graft failure, a risk which is notoriously increased in T-cell depleted transplants, seems less frequent than in patients with SAA, highlighting once more the importance of a correct diagnosis (which can be difficult in the pediatric setting). This could be due to a higher "degree of activation" of the immune system, as well as higher circulating levels of Iinterferon γ (which has deleterious effects on hematopoietic progenitors), in patients with SAA.[15] The observation that GF seems more frequent in patients with chromosomal abnormalities suggests that this group might require intensified monitoring and immune prophylaxis for rejection either pre- and/or post-transplant.

Recently, Suo and colleagues reported on the results of a T-cell replete haploHSCT approach (based on G-CSF priming, ATLG, Cyclosporine-A, mofetil-mycophenolate and short-term methotrexate) in 27 children with MDS (17 with advanced disease).[16] Although the two cohorts are not fully comparable, especially with regards to the cumulative incidence of relapse (7.4%), EFS (81.9%) and OS (81.9%) (because of the "more advanced" population"), the cumulative incidence of both aGvHD (52.6% for grade II-IV) and cGvHD (21.1% for extensive) are lower with the strategy of *ex-vivo* TBdepl-haploHSCT. Yoo and co-authors, describing their cohort of patients transplanted for pediatric MDS, reported nine patients who received a T-cell depleted haploHSCT, with comparable outcomes.[17] In details: i) one patient died of transplant-related cause (14%); ii) three patients experienced grade II aGvHD (33.3%) and one patient extensive cGvHD (14%), while iii) 5-year EFS was 78%. Notably, before a targeted dose of αβ+ T cells <5×10^4/kg was implemented at the Center, five of these patients received, differently from our cohort,

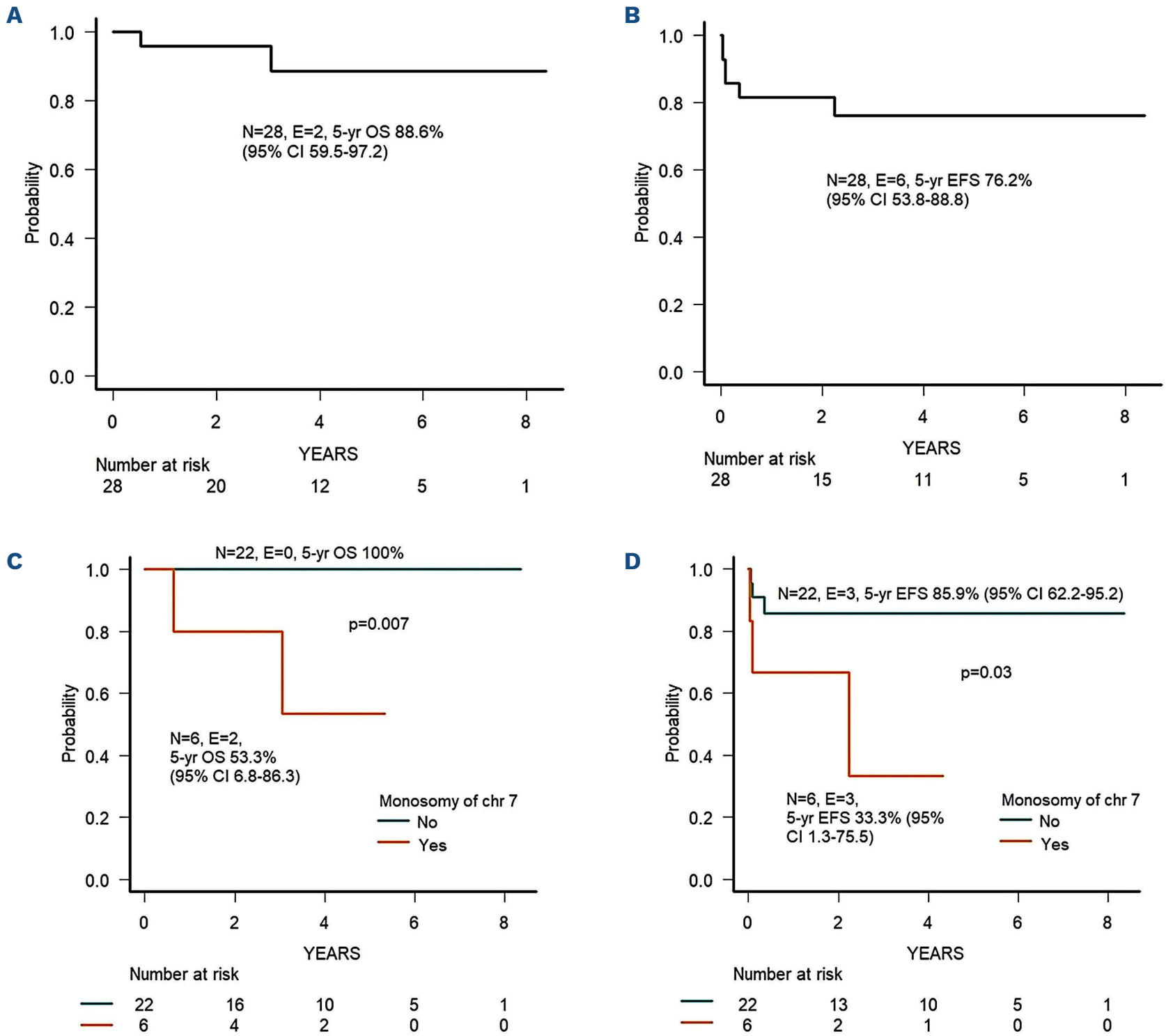

Figure 2. Survival outcomes. (A) Kaplan-Meier curve of overall survival (OS). (B) Kaplan-Meier curve of event-free survival (EFS). (C) Kaplan-Meier curve of OS according to monosomy of chromosome 7 (chr 7). (D) Kaplan-Meier curve of EFS according to monosomy of chr 7. CI: confidence interval.

post-transplant GvHD pharmacological prophylaxis with tacrolimus and mycophenolate mofetil. Relapse, especially in patients with EB1-EB2 MDS, remains the main cause of treatment failure; thus, strategies aimed at preventing/pre-emptive treating impending relapse are desirable. In this regard, this haploHSCT platform, characterized by the absence of post-transplant pharmacological GvHD prophylaxis is the ideal setting for implementing post-transplant adoptive cell therapies. Another open issue concerns the outcome of patients with MDS due to genetic predisposition syndromes; in our cohort (in which the percentage of patients with proven or suspected [VUS] genetic predisposition syndromes was in line with the literature),[2,3] the six patients with this type of MDS (proven or suspected because of VUS) are all alive and disease-free. A recent study conducted by the EWOG-MDS study group demonstrated that transplant outcomes are not influenced by *GATA2* germline variants; this finding supports the application of standard treatment algorithms also to this group of patients, advising to consider HSCT early in the course of *GATA2* deficiency in order to avoid complications.[18] Thus, HSCT from a haploidentical donor has the advantage of being available for almost all patients. However, in case an IBMFS/genetic predisposition syndrome is suspected, an accurate familiar study (especially regarding possible related donors) is mandatory.

In summary, these data indicate that TBdepl-haploHSCT is a safe and effective transplant option also in children with MDS. The low risk of both non-relapse mortality and a/cGvHD makes this approach particularly attractive in the pediatric setting.

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Disclosures

PM is on the advisory board of Sobi and is part of the speaker's bureau of Bellicum; he has receieved honoraria from Jazz. MZ is on the advisory board of Amgen, Jazz, Novartis and Sanofi. FL has received research support from Bellicum; he is part of the speaker's bureau of Miltenyi, Bellicum, Amgen, Medac, Neovii, Novartis, Sanofi, Gilead and bluebird bio; he is on the advisory board of Bellicum, Amgen, Neovii, Novartis and Sanofi. All other authors have no conflicts of interest to disclose.

Contributions

FL designed the study and supervised the project. PM, DP, TM, FG, LS, EB, RC and FdB collected the data. PM, LS and FL analyzed and interpreted the data. PM, DP, TM, FG, LS, MLC ,RC, FQ, MB, EB, FdB, AP, AA, MA and FL were involved in the clinical management of patients. SL performed donor apheresis. GLP, SB and CP performed graft manipulation and graft characterization. VB performed immune monitoring. LP and SP performed genetic analysis. PM, LS, MZ and FL wrote and edited the manuscript. All authors had access to primary clinical trial data, contributed to the intellectual content of this article, and reviewed and approved the final manuscript.

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Data-sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

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