Regular Article

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TRANSPLANTATION

Outcome of children with acute leukemia given HLA-haploidentical HSCT after αβ T-cell and B-cell depletion

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Key Points

- Children with AL given haplo-HSCT after αβ T- and B-cell depletion are exposed to a low risk of acute and chronic GVHD and NRM.
- The leukemia-free, GVHD-free survival of patients given this type of allograft is comparable to that of HLA-matched donor HSCT recipients.

Allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-haploidentical relative (haplo-HSCT) is a suitable option for children with acute leukemia (AL) either relapsed or at high-risk of treatment failure. We developed a novel method of graft manipulation based on negative depletion of αβ T and B cells and conducted a prospective trial evaluating the outcome of children with AL transplanted with this approach. Eighty AL children, transplanted between September 2011 and September 2014, were enrolled in the trial. All children were given a fully myeloablative preparative regimen. Anti–T-lymphocyte globulin from day −5 to −3 was used for preventing graft rejection and graft-versus-host disease (GVHD); no patient received any posttransplantation GVHD prophylaxis. Two children experienced primary graft failure. The cumulative incidence of skin-only, grade 1-2 acute GVHD was 30%; no patient developed extensive chronic GVHD. Four patients died, the cumulative incidence of nonrelapse mortality being 5%, whereas 19 relapsed, resulting in a 24% cumulative incidence of relapse. With a median follow-up of 46 months for surviving patients, the 5-year probability of chronic GVHD-free, relapse-free survival (GRFS) is 71%. Total body irradiation–containing preparative regimen was the only variable favorably influencing relapse incidence and GRFS. The outcomes of these 80 patients are comparable to those of 41 and 51 children given transplantation from an HLA-identical sibling or a 10/10 allelic-matched unrelated donor in the same period. These data indicate that haplo-HSCT after αβ T- and B-cell depletion represents a competitive alternative for children with AL in need of urgent allograft. This trial was registered at www.clinicaltrials.gov as #NCT01810120. (*Blood*. 2017;130(5):677-685)

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) from an HLA-haploidentical relative (haplo-HSCT) offers the option of immediate transplantation virtually to any patient in need of an allograft and lacking a suitable HLA-matched donor.[1-3] In order to remove T cells, responsible for graft-versus-host disease (GVHD), and B cells, from which posttransplant lymphoproliferative disease (PTLD) can arise,[4] positive selection of $CD34^+$ hematopoietic stem cells (HSCs) has been employed for many years in haplo-HSCT.[1-3] Although the administration of $CD34^+$ cell "megadoses" demonstrated to be a suitable approach for preventing both graft failure and severe GVHD in haplo-HSCT recipients,[5] removal of lymphoid cells and committed hematopoietic progenitors from the graft entailed prolonged lymphopenia and delayed immune reconstitution, resulting in an increased risk of nonrelapse mortality (NRM), mainly from opportunistic infections.[2,3,6]

A promising approach to circumvent this delay in immune recovery is represented by a more sophisticated method of graft manipulation that we and other groups recently developed, based on selective depletion of αβ T lymphocytes, and of B cells.[7-9] Through this approach, it is possible to transfer to the recipient not only donor HSCs but also committed hematopoietic progenitors, as well as mature natural killer (NK) and γδ T cells, both these lymphocyte subsets being

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capable of exerting a protective effect against leukemia cell regrowth and life-threatening infections.[10] Initial clinical results in children with nonmalignant disorders who received haplo-HSCT using this new method of selective T-cell depletion[11] and in small series of pediatric patients with malignancies, some of which were given additional post-transplantation immune suppression,[12,13] were promising, as the reported incidence of GVHD was low, whereas immune reconstitution was improved.

Herein, we report the long-term outcome of a cohort of 80 children with acute leukemia (AL) recruited into a prospective, single-center phase 2 trial, aimed at testing the safety and efficacy of αβ T- and B-cell–depleted haplo-HSCT. Moreover, to better define the role of this approach, we compared the outcomes of these children with those of 41 and 51 patients transplanted in our center in the same period from either an HLA-identical sibling or a 10/10 allelic-matched unrelated donor (UD), respectively.

Patients and methods

Patients

Children (aged <21 years) with acute lymphoblastic or myeloid leukemia (ALL and AML, respectively) who received a first allograft between September 2011 and September 2014 were enrolled in this prospective clinical trial, approved by the local Ethical Committee and registered at ClinicalTrial.gov Web site (#NCT01810120). Written informed consent was obtained from either patients or their legal guardians in accordance with the Helsinki Declaration.

We offered αβ T- and B-cell–depleted haplo-HSCT to all children with AL in morphological complete remission (CR) with an indication to receive an allograft and who lacked either an HLA-identical sibling or a fully allelic-matched (at the HLA A, B, C, DRB1, and DQB1 loci) UD or who needed an urgent procedure (ie, within 2 months from recognition of the indication) according to physician's judgment. Details on patient and donor characteristics are reported in Table 1. All patients received a pretransplant, myeloablative conditioning regimen; total body irradiation (TBI) was employed in patients older than 3 years affected by either ALL or very high-risk AML (ie, those with cytogenetic/molecular features predicting high risk of relapse; see Table 1 for further details). Anti–T-lymphocyte globulin (Grafalon; Neovii Biotech) was administered at a dose of 12 mg/kg over 3 days (ie, from day −5 to −3) for prevention of both graft rejection and GVHD through in vivo depletion/modulation of bidirectional alloreactivity. Moreover, to reduce as much as possible the risk of Epstein-Barr virus (EBV)–related PTLD, on day −1, patients were also given rituximab (200 mg/m^2) for in vivo depletion of both donor and recipient B cells. No patient received posttransplantation pharmacological GVHD prophylaxis.

Chimerism analysis was performed biweekly for the first 3 months and monthly thereafter. Immune recovery (count of T-cell receptor [TCR] αβ CD3$^+$, TCR γδ CD3$^+$, CD4$^+$, CD8$^+$, NK, and CD19$^+$ cells) was evaluated at 1, 3, 6, and 12 months after transplantation. Immunoglobulin G (IgG) replacement therapy was continued until achievement of sustained serum levels comparable to those normal for patient's age.

The donor was mainly chosen according to immunological criteria, giving priority to NK alloreactivity, evaluated according to the killer immunoglobulin-like receptor (KIR)/KIR-Ligand model, KIR B haplotype, higher B-content score, and size of NK alloreactive subset.[14-19] A detailed description of the methods used for NK-cell genotype/phenotype characterization, of the criteria used for selecting the more appropriate donor, and of the KIR genotype asset of the 80 donors is reported in supplemental Figure 1 and supplemental Table 1, available on the *Blood* Web site.

The donor was the mother in 46 patients (57%) and the father in the remaining 34 patients (43%). Since June 2012, all recipients were tested for the presence of donor-specific antibodies,[20] and 2 out of 65 resulted to be positive.

Donor mobilization and graft manipulation procedures have been previously reported.[8,9] Briefly, donors received granulocyte-colony stimulating factor for 4 days at 12 μg/kg body weight in 2 divided doses to induce peripheral mobilization of CD34$^+$ hematopoietic progenitors. Apheresis was performed on day 5 after start of mobilization. When on day 4 the CD34$^+$ cell count was <40/μL and/or the predicted apheresis yields was ≤12.0 × 10^6 CD34$^+$ HSC/kg recipient's body weight, according to a previously reported formula,[8,9] Plerixafor (Mozobil) was given at 0.24 mg/kg with the aim of boosting mobilization of hematopoietic stem/progenitor cells. Plerixafor was usually given at midnight, 9 hours prior to collection on day 5. Large-volume apheresis was performed with the Spectra Optia Cell Separator (Terumo BCT, Leuven, Belgium). Manipulations were performed in a closed system. Clinical grade reagents, disposable kits, and instrumentation were from Miltenyi Biotec (Bergisch Gladbach, Germany). Procedures were performed with the fully automated CliniMACS device in a laminar-flow hood, located in a clean room certified for sterile manipulations.

Definitions and statistical analysis

Graft failure was defined as either lack of initial engraftment of donor cells (primary graft failure) or loss of donor cells after initial engraftment (secondary graft failure). Time to neutrophil engraftment was defined as time from haplo-HSCT to the first of 3 consecutive days with an absolute neutrophil count ≥0.5 × 10^9/L, whereas time to platelet engraftment was defined as time from transplantation to the first of 7 consecutive days with an unsupported platelet count ≥20 × 10^9/L.

Patients surviving >14 and 100 days after transplantation were evaluated for acute and chronic GVHD, respectively, which were diagnosed and graded according to previously published criteria.[21,22]

Overall survival (OS) was defined as the probability of survival, regardless of disease status, from the time of haplo-HSCT to time of death or of last follow-up (surviving patients were censored at last follow-up, whereas only death was considered an event). NRM was defined as the probability of death from any cause other than malignancy recurrence. Leukemia-free survival (LFS) was defined as the probability of survival, without evidence of disease at any time after transplantation. In estimating LFS, death and relapse were considered events, whereas patients who were alive with sustained donor engraftment and disease free were censored at last follow-up. We also evaluated event-free survival (EFS), considering graft failure as an additional event to those considered for estimating LFS and the composite endpoint of chronic GVHD-free and relapse-free survival (GRFS).[23]

Quantitative variables were reported as median value and range, whereas categorical variables were expressed as absolute value and percentage. Demographic and clinical characteristics of patients were compared using the χ^2 test or Fisher's exact test for categorical variables, whereas the Mann-Whitney rank sum test or the Student *t* test was used for continuous variables, as appropriate.

Rejection, engraftment, acute and chronic GVHD, OS, LFS, NRM, and relapse incidence were estimated from the date of transplantation to the date of an event or last follow-up.

Probabilities of OS, LFS, and EFS were calculated according to the Kaplan and Meier method.[24] Engraftment, acute GVHD and chronic GVHD, NRM, and relapse were calculated as cumulative incidence curves in order to adjust the estimates for competing risks.[25] All results were expressed as probability or cumulative incidence (%) and 95% confidence interval (95% CI).[26,27]

The significance of differences between survival probabilities was estimated by the log-rank test (Mantel-Cox), whereas Gray's test was used to assess, in univariable analyses, differences between cumulative incidences.[28] Multivariable analysis was performed using the Cox proportional hazard regression model.[29] Several patient-, donor-, and transplantation-related factors, detailed in Table 2, were evaluated for their impact on relapse incidence and LFS.

With the aim of better defining the role of haplo-HSCT, we compared the outcomes of children enrolled in this trial with those of patients with AL transplanted from either an HLA-identical sibling or a 10/10 allelic matched UD in the same period in our center. Details on clinical characteristics of patients belonging to the 3 groups and the correlated comparative analysis are reported in supplemental Table 2.

Table 1. Patient, donor, and transplantation characteristics

Patients	(n = 80)
Sex	
Male	55 (69%)
Female	25 (31%)
Median (range) age at diagnosis, y	6.6 (0.4-16.8)
Median (range) age at transplantation, y	9.7 (0.9-20.9)
Disease	
ALL	56 (70%)
AML	24 (30%)
ALL phenotype	
BCP	41 (73%)
T	15 (27%)
ALL recurrent molecular lesions	
t(4;11)(AF4/MLL)	3
t(9;22)(BCR/ABL)	2
SIL-TAL	1
t(12;21)(TEL/AML1)	2
Hypodiploid	1
AML recurrent molecular/cytogenetic lesions	
MLL/FLT3-ITD	5
7-	1
Complex karyotype	3
inv(16) (MYH11-CBFB)	2
Other	1
Disease status at transplantation	
ALL	
CR1*	15 (19%)
CR2†	37 (46%)
≥CR3	4 (5%)
AML	
CR1‡	16 (20%)
CR2	8 (10%)
CMV serology (donor/recipient)	
Neg/Neg	5 (6%)
Neg/Pos	7 (9%)
Pos/Neg	11 (14%)
Pos/Pos	57 (71%)
Conditioning regimens§	
TBI+TT+Flu	40 (50%)
TBI+TT+L-PAM	20 (25%)
TT+Bu+Flu	13 (16%)
Bu+Cy+L-PAM	7 (9%)
Donor characteristics	
Age, y	41.5 (27-55)
Type of donor	
Mother	46 (58%)
Father	34 (42%)
Sex mismatch	49 (61%)
Female donor → Male recipient	35/49 (71%)
NK alloreactivity (KIR/KIR-L model) yes/no	36 (45%)/44 (55%)
KIR genotype A/A vs B/X	16 (20%)/64 (80%)
Donor B content value 0-1 vs ≥2	44 (55%)/36 (45%)
Donor KIR2DS1 "educated and useful"‖ yes/no	28 (35%)/52 (65%)
Cell dose infused, median (range)	
$CD34^+$ cells × 10^6/kg	13.93 (6-40.44)
$\alpha\beta^+$ T cells × 10^6/kg	0.047 (0.002-0.099)
$\gamma\delta^+$ T cells × 10^6/kg	8.1 (0.86-56.7)
NK cells × 10^6/kg	34.6 (3.84-146.1)
$CD20^+$ B cells × 10^6/kg	0.09 (0.05-0.48)

BCP, B-cell precursors; BU, busulfan; CMV, cytomegalovirus; CY, cyclophosphamide; Flu, fludarabine; L-PAM, melphalan; Neg, negative; Pos, positive; TT, thiotepa.

*Of the 15 patients with ALL transplanted in CR1, 9 had high level of minimal residual disease at the end of induction therapy (ie, $>1 \times 10^{-3}$ at day +78 after beginning of treatment), 2 had high-risk infant ALL, 1 had t(4;11), and 3 had hyperleukocytosis T ALL with poor response to the steroid prephase.

†Of the 16 patients with AML transplanted in CR1, 3 had t(10;11), 2 a complex karyotype, 3 had FLT3-ITD with high allelic ratio, 2 had M7 AML, and 6 were not in morphological CR after the first of the 2 induction courses.

‡Of the 37 patients with ALL transplanted in CR2, 21 (57%) and 16 (43%) patients belonged to the S2 and S3/S4 Berlin-Frankfurt-Münster classification of first relapse ALL, respectively.[59]

§TBI (12 Gy over 3 d in 6 fractions of 200 cGy each) was employed in 50 children with ALL and in 10 children with AML; all these patients were older than 3 y.

‖HLA-C $C1^{pos}$ donor and HLA-C $C2^{pos}$ patient.

Statistical analysis was performed using NCSS (NCSS 10 Statistical Software [2015]; NCSS, LLC, Kaysville, UT, ncss.com/software/ncss) and R 2.5.0 software package (http://www.R-project.org). Data were analyzed as of 1 January 2017.

Results

All children received at least 6×10^6 $CD34^+$ cells/kg body weight and an αβ T-cell number lower than 1×10^5/kg body weight; details on the number of HSC and lymphocyte subsets infused are shown in Table 1. Sixteen donors (20%) were given plerixafor for optimizing mobilization of HSC.

Engraftment

Two patients did not engraft; 1 patient was successfully rescued through haplo-HSCT from the other parent, whereas the other died of disseminated adenovirus infection, despite receiving a second allograft from the same donor with engraftment and hematopoietic recovery. This patient was 1 of the 2 with donor-specific alloantibodies. The number of $CD34^+$ cells/kg body weight infused in these 2 patients was 8.9 and 24.3 × 10^6, respectively. The median time to neutrophil engraftment in the whole study population was 13 days (range, 9-19); no variable influenced the kinetics of recovery. The median time to platelet engraftment was 11 days (range, 8-20). It was 10 days (range, 8-16) and 12 days (range, 10-20) in patients given a number of $CD34^+$ cells either above or below the median value infused ($P = .03$).

Acute and chronic GVHD

Twenty-four patients (30%) developed grade 1-2, skin-only acute GVHD, whereas 56 children (70%) did not present any grade of acute GVHD. No single case of acute GVHD with visceral involvement or of grade 3-4 acute GVHD was recorded (supplemental Figure 2A). The overall 100-day cumulative incidence of grade 1-2 acute GVHD was 30% (95% CI, 21-41). No variable predicted the occurrence of acute GVHD. All patients with grade 1-2, skin-only, acute GVHD responded to treatment with either topical or systemic steroids.

Seventy-three patients surviving >100 days after haplo-HSCT were evaluated for chronic GVHD occurrence. Clinically limited, skin-only chronic GVHD was diagnosed in 4 patients (5%). In these children, acute GVHD preceded chronic GVHD. The overall cumulative incidence of limited chronic GVHD was 5% (95% CI, 2-15; supplemental Figure 2B).

NRM

Four patients died of transplantation-related causes: 2 because of idiopathic pneumonitis and 1 each of disseminated adenovirus infection after graft failure and cardiac insufficiency. Only 1 of the 4 patients who died because of transplant-related causes had received TBI as part of the preparative regimen, whereas the remaining 3 had been prepared with a chemotherapy-based regimen. These fatal events occurred in the first 100 days after the allograft. The 5-year cumulative incidence of NRM for the whole cohort of patients is 5% (95% CI 2-13; Figure 1A).

Relapse

With a median follow-up of 46 months (range, 26-60), 19 patients (24%) relapsed at a median of 6.3 months (range 2-22), the CI of relapse being 24% (95% CI, 16-36; Figure 1B). Fifteen of these 19 relapses occurred in first year after transplantation. The CI of relapse was 23%

Table 2. Cumulative incidence of relapse and LFS: univariable analysis

		Cumulative incidence of relapse				LFS			
Outcome	**No. of patients**	**Events**	**Probability (%)**	**95% CI**	***P* value**	**Events**	**Probability (%)**	**95% CI**	***P* value**
Recipient sex									
Male	55	12	22.8	14-38	.60	12	77.2	66-89	**.031**
Female	25	7	28.6	15-53		11	56.0	37-75	
Recipient age at haplo-HSCT									
<9.7 y	41	13	31.7	20-50	.057	16	61.0	46-76	**.023**
>9.7 y	39	6	16.0	8-33		7	81.5	69-94	
Donor sex									
Male	34	5	14.7	7-33	.13	9	73.5	59-88	.88
Female	46	14	31.2	20-48		14	68.7	55-82	
Donor age, y									
<41.5	40	12	31.5	19-51	.17	12	68.5	53-84	.86
>41.5	40	7	17.5	9-34		11	72.5	59-86	
Donor-recipient sex combination									
Female donor–male recipient	49	12	25.5	16-42	.90	16	66.4	53-80	.34
Other combinations	31	7	22.6	12-43		7	77.4	63-92	
Type of leukemia									
ALL	56	13	23.2	14-37	.87	16	71.4	60-83	.98
AML	24	6	28.3	14-58		7	67.5	47-88	
Disease phase									
ALL CR1	15	2	13.3	4-48	.61	3	80.0	60-100	.62
ALL CR2	37	9	24.3	14-43		10	73.0	59-87	
AML CR1	16	5	36.5	17-77		6	53.7	29-85	
AML CR2	8	1	12.5	2-78		1	87.5	65-100	
Berlin-Frankfurt-Münster classification of CR2 ALL patients									
S2	21	3	14.3	3-33	.08	4	81.0	57-92	.18
S3-S4	16	6	37.5	15-61		6	62.5	35-81	
Donor-recipient CMV serology									
Neg/Neg	5	2	40.0	14-100	.43	2	60.0	17-100	.75
Pos/Neg	7	3	42.9	18-100		3	57.1	20-94	
Neg/Pos	11	2	18.2	5-64		3	72.7	46-99	
Pos/Pos	57	12	21.9	13-36		15	72.8	61-85	
Conditioning regimen									
TBI-based	60	10	16.7	9-29	**.014**	11	81.7	72-91	**.0002**
Chemo-based	20	9	47.5	29-77		12	37.5	15-60	
Graft composition									
$CD34^+$ cells $\times 10^6$/kg									
≥m.v.	41	6	15.1	7-32	**.045**	7	82.4	71-94	**.016**
<m.v.	39	13	33.3	21-52		16	59.0	44-74	
$\alpha\beta^+$ T cells $\times 10^6$/kg									
≥m.v.	41	12	29.3	18-47	.23	15	63.4	49-78	.11
<m.v.	39	7	18.9	10-37		8	78.5	65-92	
$\gamma\delta^+$ T cells $\times 10^6$/kg									
≥m.v.	40	11	28.2	17-47	.42	12	69.2	55-84	.82
<m.v.	40	8	21.1	11-39		11	71.4	57-86	
NK cells $\times 10^6$/kg									
≥m.v.	40	9	23.8	13-42	.73	12	68.5	54-83	.88
<m.v.	40	10	25.0	15-43		11	72.5	59-86	
NK cell alloreactivity									
Yes	36	8	22.2	12-41	.84	9	75.0	61-89	.53
No	44	11	26.4	16-44		14	66.7	52-81	
KIR genotype									
B/X	64	18	28.7	19-43	.11	22	65.0	53-77	**.045**
A/A	16	1	6.7	1-44		1	93.3	81-100	
B-content score									
≥2	36	9	25.0	14-44	.89	10	72.2	58-87	.72
0-1	44	10	23.9	14-41		13	69.1	55-83	
Donor KIR2DS1 "educated and useful"									
Yes	28	8	28.6	13-46	.48	10	64.3	44-79	.32
No	52	11	22.2	12-35		13	74.0	59-84	
Grade 1-2 acute GVHD									
Yes	24	3	12.5	4-36	.12	18	67.2	55-80	.34
No	56	16	29.2	19-44		5	79.2	63-95	
Chronic GVHD									
Yes	4	1	25.0	5-100	.94	1	75.0	33-100	1.00
No	69	15	22.0	14-35		16	76.6	66-87	

Values of $P < .05$ are significant (bold).
m.v., Median value.

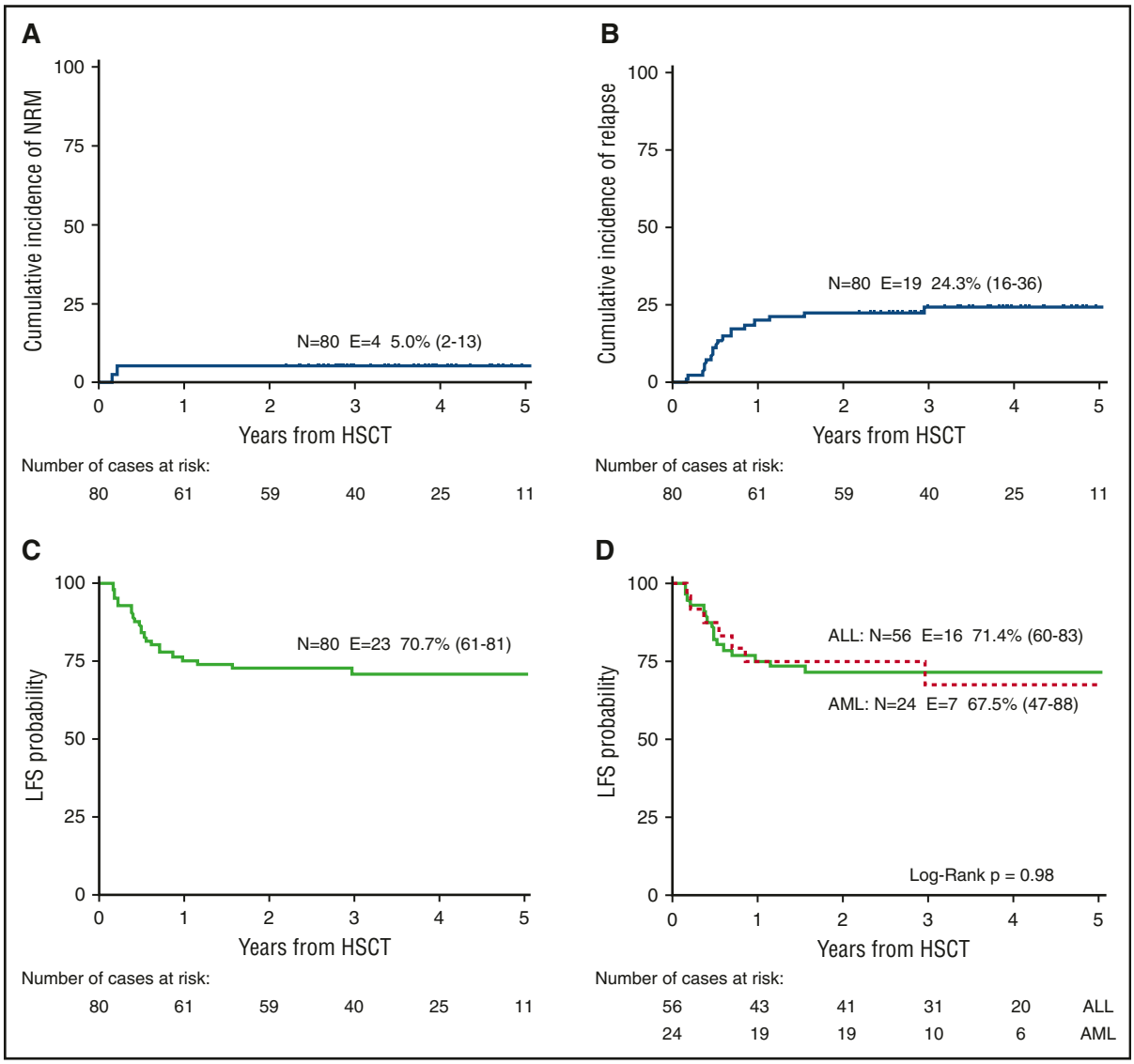

Figure 1. Outcomes of TCRαβ/CD19-depleted haplo-HSCT. (A) NRM of the whole cohort of patients enrolled in the trial. (B) Cumulative incidence of relapse of the whole cohort of patients enrolled in the trial. (C) LFS of the whole cohort of patients enrolled in the trial. (D) LFS according to the disease type (ALL [continuous green line] vs AML [dotted red line]). E, events; N, number.

(95% CI, 14-37) for the 56 children with ALL and 28% (95% CI, 14-58) for the 24 children with AML (P = not significant). In univariable analysis (Table 2), $CD34^+$ cell number above the median value and a TBI-containing regimen were associated with lower risk of leukemia recurrence; use of TBI remained significant in multivariable analysis (hazard ratio 0.36; 95% CI 0.14-0.91; P = .03).

Survival and LFS

Fifty-eight patients (72% of the whole population) were alive at time of the last follow-up: 57 were in continuous CR after haplo-HSCT, whereas 1 was alive after posttransplantation leukemia relapse.

The 5-year OS probability was 72% (95% CI, 62-82) for the whole study population. Overall, the 5-year LFS was 71% (95% CI, 61-81; Figure 1C); it was 71% (95% CI, 60-83) and 68% (95% CI, 47-88) in patients transplanted for ALL and AML, respectively (P = not significant; Figure 1D). The 5-year probability of EFS was 69.5% (95% CI 58-78.4), whereas the 5-year probability of GRFS was 71% (95% CI, 61-81; Figure 2D).

Table 2 details the univariable analysis of factors potentially influencing the LFS probability. Only a TBI-containing regimen remained significant in multivariable analysis (hazard ratio 0.30; 95% CI 0.12-0.72; P = .007; see also Figure 2A). Noteworthy, in this study, we were unable to confirm any protective advantage for LFS in patients transplanted from an NK alloreactive donor (evaluated using the KIR-KIR-L model of prediction; Figure 2B).

Comparison of outcomes of haplo-HSCT recipients with those of children given HLA-identical sibling or UD transplantation

In order to compare differences between haplo-HSCT and transplantation from either an HLA-identical sibling or a 10/10 allelic-matched UD, we also analyzed 41 and 51 consecutive AL children of the latter 2 cohorts given an allograft in CR in the same period (see supplemental Table 2). The 3 groups were comparable for many relevant features considered, with the exception of source of stem cells employed (98% and 78% of HLA-identical siblings or UD HSCT recipients were transplanted with bone marrow cells),

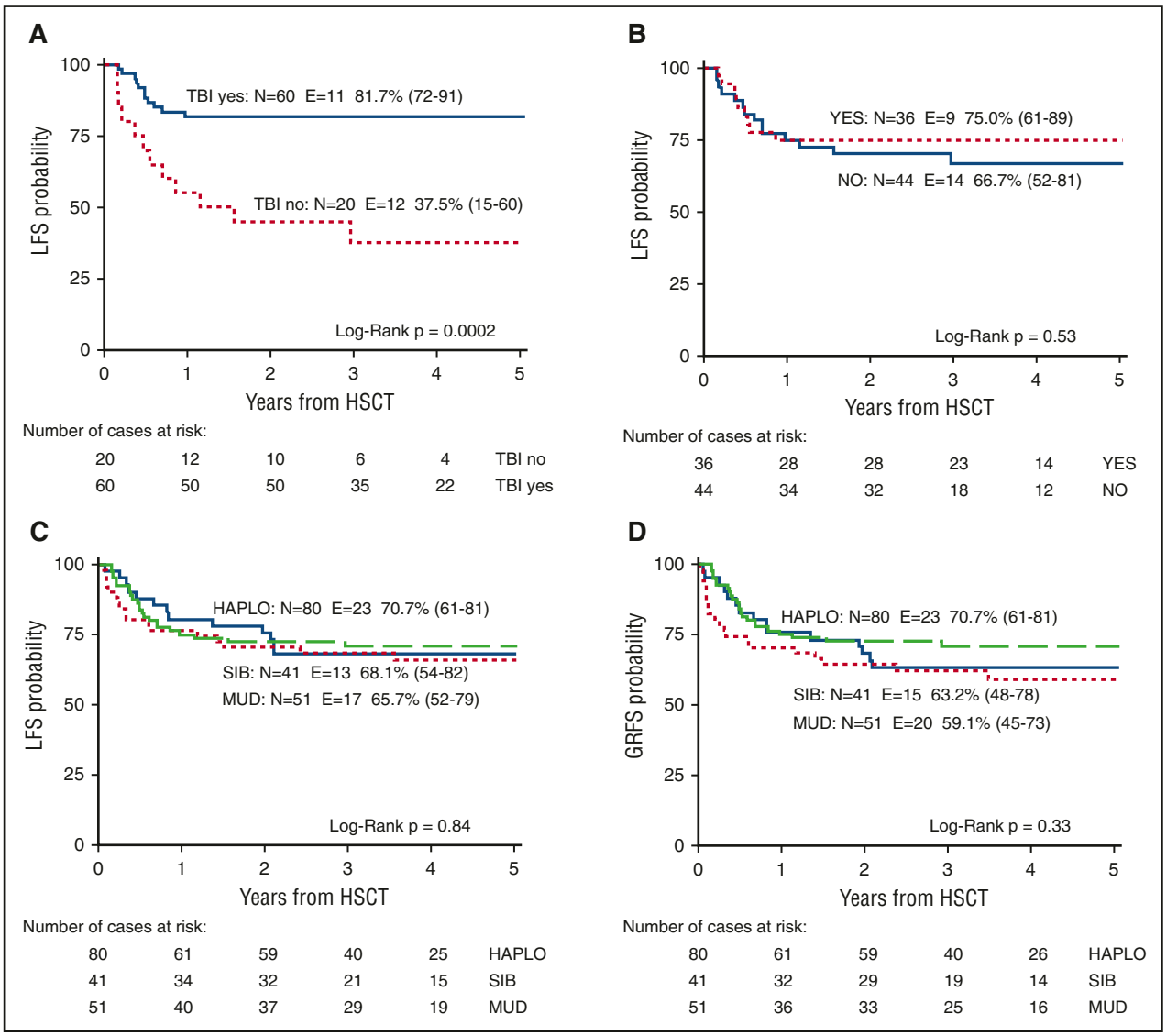

Figure 2. Outcomes of TCRαβ/CD19-depleted haplo-HSCT and comparison with transplants from HLA-identical sibling and MUD. (A) LFS probability according to the use of TBI in the conditioning regimen (yes, continuous blue line; no, dotted red line). (B) LFS probability according to NK alloreactivity (evaluated using the KIR-KIR-L model of prediction) (yes, dotted red line; no, continuous blue line). (C) Comparison of LFS probability among patients transplanted from different donors (HLA-haploidentical relative [dashed green line], HLA-identical sibling [continuous blue line], and UD [dotted red line]) during the same period. (D) Comparison of GRFS probability among patients transplanted from different donors (HLA-haploidentical relative [dashed green line], HLA-identical sibling [continuous blue line], and UD [dotted red line]) during the same period. MUD, matched unrelated donor; SIB, HLA-identical sibling.

GVHD prophylaxis (none of the patients of the 2 control groups was given a T-cell–depleted allograft), and human cytomegalovirus (HCMV) serology (see supplemental Table 2 for details).

Haplo-HSCT had a lower incidence of grade 3-4 acute GVHD, of visceral GVHD, and of chronic GVHD ($P < .01$, $<.01$, and .03, respectively; supplemental Figure 2A-B). There was no significant difference in the risk of any cause-specific death (GVHD, infection, regimen-related toxicity, and leukemia relapse) among these 3 groups (data not shown). The 5-year probability of LFS and GRFS did not differ between haplo-HSCT recipients and the other 2 cohorts of patients (Figure 2C-D).

Immune recovery of haplo-HSCT recipients

Recovery of lymphoid subsets and of immunoglobulin serum levels is detailed in Table 3, showing prompt recovery of γδ T lymphocytes and NK cells in the early posttransplant period and progressive emergence over time of αβ T lymphocytes. Noteworthy, notwithstanding a dose of rituximab administered before transplantation and depletion of donor B cells from the graft, all children had B-cell engraftment.

Discussion

This study is the first to report long-term outcome of a large population of children with AL given αβ T- and B-cell–depleted haplo-HSCT after a myeloablative regimen and enrolled into a prospective, registered phase 2 trial. Our data indicate that, despite not receiving any posttransplantation pharmacological prophylaxis, these patients benefited from a high engraftment rate (98%) and experienced a low incidence of both acute and chronic GVHD, which contributed to the reduced risk of NRM. Remarkably, none of our patients had either grade 3-4 or gut/liver acute GVHD and all cases of chronic GVHD were of limited severity. Moreover, we did not observe any case of

Table 3. Details on immune reconstitution of the 80 patients given haplo-HSCT, median (range)

	1 mo	3 mo	6 mo	12 mo
$CD3^+$ T cells/μL	231 (1-1618)	254 (23-1472)	668 (153-2596)	1379 (407-3449)
$CD4^+$ T cells/μL	19 (0-442)	89 (4-397)	264 (87-1055)	599 (174-1421)
$CD8^+$ T cells/μL	24 (0-910)	96 (9-1108)	301 (25-1581)	574 (112-2301)
αβ T cells/μL	47 (1-672)	186 (12-1340)	573 (135-2146)	1291 (259-2795)
γδ T cells/μL	181 (1-1335)	49 (4-388)	84 (4-752)	94 (10-660)
$CD3^-CD56^+$ NK cells/μL	236 (47-1813)	196 (29-1448)	283 (48-5441)	269 (79-3116)
$CD19^+$ B cells/μL	0 (0-20)	2 (0-473)	160 (2-1609)	291 (40-1616)
IgG, mg/dL	973 (104-3153)	580 (340-1041)	597 (213-1487)	711 (377-1351)
IgA, mg/dL	32 (0-190)	26 (0-186)	33 (0-284)	41 (0-221)
IgM, mg/dL	10 (0-66)	10 (0-44)	47 (0-496)	64 (0-469)

EBV-related PTLD, which may occur in immune-compromised individuals in the absence of virus-specific, adaptive T-cell immunity.[4] Administration of rituximab before transplantation may have helped prevent EBV-PTLD, and recipient B-cell depletion may have contributed to the low GVHD incidence and severity.[30]

In the past, ex vivo T-cell–depleted haplo-HSCTs have been performed either through positive selection of $CD34^+$ cells[31,32] or through removal of $CD3^+$ T cells in combination with $CD19^+$ B cells.[33,34] Unfortunately, both approaches result in loss of certain cell subsets that may play a positive role in the recipient. In fact, although T cells displaying the αβ TCR are responsible for GVHD, T cells carrying the γδ receptor chains have no alloreactive capacity, but contribute an important anti-infectious activity,[35,36] in addition to a possible antileukemia role.[37-40] The Vδ2 population recognizes nonpeptide phosphoantigens expressed by leukemia cells, whereas Vδ1 cells expand in response to HCMV reactivation,[39] and their presence was associated with complete responses observed in patients with B-cell ALL after T-cell–depleted allogeneic HSCT.[38] It is conceivable that the high number of γδ T cells adoptively transferred with the graft in our patients may have contributed to prevent disease recurrence and severe infections. Also donor-derived, mature NK cells, lost in the procedure of positive selection of $CD34^+$ cells and spared in our αβ T-cell–depleted graft, exhibit a graft-versus-leukemia (GVL) effect[6,10,14-16,41,42] and participate in the control of opportunistic infections, including HCMV.[43-45] In previous studies, we documented that in haplo-HSCT recipients given positively selected $CD34^+$ cells, ~8 weeks after transplantation are needed to detect mature KIR^+ NK cells, and this gap in reconstitution may favor early leukemia relapse in the case of high residual tumor burden and/or rapidly proliferating blasts.[14,16] Through the approach of selective αβ T- and B-cell depletion, the recipient immediately benefits from high numbers of donor mature NK cells that can fully display their activity, because the recipient is not exposed to the effect of pharmacological prophylaxis of GVHD, which can impair differentiation/expansion of this lymphocyte subset.[46] Altogether, the infusion of cells belonging to innate immunity, together with that of high numbers of committed hematopoietic progenitors and monocyte/dendritic cells (in particular, in patients whose donor was mobilized with granulocyte-colony stimulating factor and plerixafor)[9] may have contributed to the low risk of NRM, which we found to be comparable to that observed after transplantation from an HLA-compatible donor, either a sibling, or a UD. We did not document any favorable influence of NK alloreactivity[42,47] and of donor KIR B-haplotype[15] reported in other studies mainly based on infusion of $CD34^+$ cells, likely because the NK-mediated GVL effect was partially obscured by other cells present in the graft, including γδ T cells.[48]

Previously published, nonprospective studies enrolling smaller cohorts of patients with shorter follow-up have analyzed the outcome of children given αβ T- and B-cell–depleted haplo-HSCT. Maschan et al analyzed the outcome of children with high-risk AML, who received transplantation from UD (n = 20) and haploidentical donors (n = 13) after this graft manipulation. Twenty-eight patients were given posttransplantation pharmacological immune suppression, including tacrolimus until day +30 and methotrexate in 21 patients, tacrolimus in 5, methotrexate in 2, whereas 5 patients did not receive prophylaxis.[13] Notably, recipients of haploidentical grafts more commonly developed isolated skin GVHD, whereas gastrointestinal involvement was more common in UD HSCT. Cumulative incidence of relapse at 2 years in the 13 haplo-HSCT recipients was 40% (95% CI: 20-80), whereas the LFS probability was 59% (95% CI: 31-87). Lang et al recently published the retrospective analysis of immune recovery in a cohort of 41 pediatric patients, with AL, myelodysplastic syndrome, and nonmalignant diseases (n = 5), who received αβ T- and B-cell–depleted allografts from a haploidentical relative after reduced-toxicity regimens.[49] Grade 3-4 acute GVHD occurred in 15% of patients; with a median follow-up of 1.6 years, 21 of the 41 patients were alive and relapse was the major cause of death (n = 17). Also in our cohort of patients, disease recurrence was the main cause of treatment failure, the CI of relapse being 24%. The lower incidence of relapse in our patients can be attributed, at least partly, to the use of fully myeloablative conditioning regimens and to the lack of posttransplantation GVHD prophylaxis, potentially able to impair the innate immunity-mediated GVL effect. Support to the former interpretation is given by the observation that a better outcome was observed when we used conditioning regimens including TBI, which, albeit more toxic in the long term for children,[50-52] displays potent antileukemia activity[53] potentially compensating for the lack of αβ T-cell–mediated GVL effect. In addition, we hypothesize that the accurate identification and determination of alloreactive NK cells, as well as a refined analysis of the main activating NK receptors, allowed selecting donors with high antileukemia activity, thus contributing to reduce the risk of relapse. In view of all these considerations, we cannot dissect the relative contribution of the different components of transplant package to the good outcome of our patients.

Our results document that this type of haplo-HSCT offers comparable risks of NRM and relapse with respect to transplantation from HLA-identical siblings or allelic-matched UDs. This finding is corroborated by the observation that the 71% probability of LFS at 5 years observed in our 54 children with ALL is superimposable to that recently reported by Peters et al[54] in 306 children transplanted from an UD using a standardized protocol for transplantation/GVHD prophylaxis (71% at 4 years). Moreover, our results compare favorably with the 5-year LFS of 30% and 34% in the larger cohort of 22 CR1 and 48 CR2 ALL patients given haplo-HSCT with $CD34^+$ cells reported so far.[31]

In the last few years, alternative platforms, such as that based on posttransplantation infusion of cyclophosphamide, have been developed.[55]

Although largely used in adults, few studies have been published on the use of this approach for modulating alloreactivity in AL children.[56,57] Although certainly cheaper than the αβ T- and B-cell depletion, the use of post-transplantation cyclophosphamide seems to be associated with a risk of leukemia recurrence higher than that observed in our cohort.[57] Future studies will further clarify the relative advantages and limitations of these 2 different haplo-HSCT platforms.

In summary, our data indicate that, through more refined approaches of graft manipulation, haplo-HSCT offers the opportunity to transplant virtually every child in need of an allograft, with an expected outcome comparable to that obtained when the donor is an HLA-matched sibling or an allelic-matched volunteer. Because we have been able to successfully transplant adolescents and patients with a body weight >40 kg, it is reasonable to hypothesize that this approach is feasible to be translated to adults, maybe through repeated apheretic collections of the donor, employed in 7 cases in our cohort. We are now running a new prospective trial, based on post–haplo-HSCT infusion of titrated numbers of donor-derived αβ T cells transduced with a suicide gene for controlling possible alloreactive reactions,[58] with the aim of accelerating recovery of adaptive immunity and, possibly, reducing the risk of leukemia recurrence.

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Authorship

Contribution: F.L., A.B., P.M., R.H., A.M., and L.M. designed the study and the concept; M.F., D. Pende, and R. Meazza provided the donor NK cell characterization and donor selection; F.L., P.M., L.P.B., D. Pagliara, B.L., G.M.M., M.A., R.M.P., L.S., and A.B. supervised the transplantation of patients and collection of data; G.L.P. oversaw the graft manipulation; L.G. conducted the radiotherapy; V.B. and G.L.P. studied the immune reconstitution; R.R., R. Masetti, P.M., and F.L. analyzed and interpreted the data (eg, statistical analysis, computational analysis); F.L., A.B., and L.M. were in charge of the study supervision; and all authors contributed to the writing, review, and/or revision of the manuscript.

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