Blood First Edition Paper, prepublished online June 6, 2017; DOI 10.1182/blood-2017-04-779769

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

Franco Locatelli,[1,2] Pietro Merli,[1] Daria Pagliara,[1] Giuseppina Li Pira,[1] Michela Falco,[3] Daniela Pende,[4] Roberto Rondelli,[5] Barbarella Lucarelli,[1] Letizia Pomponia Brescia,[1] Riccardo Masetti,[5] Giuseppe Maria Milano,[1] Valentina Bertaina,[1] Mattia Algeri,[1] Rita Maria Pinto,[1] Luisa Strocchio,[1] Raffaella Meazza,[4] Lavinia Grapulin,[6] Rupert Handgretinger,[7] Alessandro Moretta,[8] Alice Bertaina,[1*] Lorenzo Moretta.[9*]

[1] Department of Pediatric Hematology and Oncology, Istituto di Ricovero e Cura a Carattere Scientifico, Ospedale Bambino Gesù, Rome, Italy

[2] Department of Pediatric Science, Università di Pavia, Italy.

[3] Istituto di Ricovero e Cura a Carattere Scientifico Giannina Gaslini, Genoa, Italy

[4] Istituto di Ricovero e Cura a Carattere Scientifico, UOC Immunologia, Ospedale San Martino, Genoa, Italy.

[5] Department of Pediatrics, Sant'Orsola Hospital, University of Bologna, Italy

[6] Department of Radiotherapy, Policlinico Umberto I, Rome, Italy

[7] Children's University Hospital, University of Tuebingen, Tuebingen, Germany

[8] Dipartimento di Medicina Sperimentale and Centro di Eccellenza per la Ricerca Biomedica, Università di Genova, Genoa, Italy.

[9] Immunology Research Area, IRCCS Ospedale Bambino Gesù, Rome, Italy

*These authors equally contributed

Corresponding Author: Prof. Franco Locatelli, Dipartimento di Oncoematologia Pediatrica, IRCSS, Ospedale Pediatrico Bambino Gesù, Piazza Sant'Onofrio 4, 00165 Roma, Italy. Tel +39 06 6859 2678. Fax +39 06 6859 2129. E-mail: franco.locatelli@opbg.net

Running title: αβ T- and B-cell depleted haplo-HSCT in childhood leukemia

Key points:

Children with acute leukemia 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-identical donor HSCT recipients.

Abstract

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 (ClinicalTrial.gov identifier: NCT01810120). 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 post-transplantation GvHD prophylaxis. Two children experienced primary graft failure. The cumulative incidence (CI) of skin-only, grade I-II acute GvHD was 30%; no patient developed extensive chronic GvHD. Four patients died, the CI of non-relapse mortality being 5%, while 19 relapsed, resulting into a 24% CI 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.

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 post-transplant lymphoproliferative disease (PTLD) can arise,[4] positive selection of CD34+ hematopoietic stem cells (HSC) 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 into an increased risk of non-relapse 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 hematopoietic stem cells, but also committed hematopoietic progenitors, as well as mature natural killer (NK) and γδ T cells, both these lymphocyte subsets being capable of exerting a protective effect against leukemia cell re-growth and life-threatening infections.[10] Initial clinical results in children with non-malignant 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 given additional post-transplantation immune suppression,[12,13] were promising, as the reported incidence of GvHD was low, while 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-centre phase II 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 Centre 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 (age <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 website (#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 (i.e. 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 pre-transplant, myeloablative conditioning regimen; total body irradiation (TBI) was employed in patients older than 3 years affected by either ALL or very high-risk AML (i.e. those with cytogenetic/molecular features predicting high risk of relapse, see Table 1 for further details). Anti-T lymphocyte globulin (ATLG Grafalon®, Neovii Biotech) was administered at a dose of 12 mg/Kg over 3 days (i.e. from day -5 to -3) for prevention of both graft rejection and GvHD through *in vivo* depletion/modulation of bi-directional alloreactivity. Moreover, to reduce as much as possible the risk of EBV-related PTLD, on day -1 patients were given also rituximab (200 mg/m^2) for *in vivo* depletion of both donor and recipient B cells. No patient received post-transplantation pharmacological GvHD prophylaxis.

Chimerism analysis was performed biweekly for the first 3 months and monthly thereafter. Immune recovery (count of TCR αβ CD3+, TCR γδ CD3+, CD4+, CD8+, NK and CD19+ cells) was evaluated at 1, 3, 6, and 12 months after transplantation. IgG replacement therapy was continued till 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 supplementary file, supplementary Figure 1 and supplementary Table 1.

The donor was the mother in 46 patients (57%) and the father in the remaining 34 (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 (G-CSF) 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 $\leq 12.0 \times 10^6$ $CD34^+$ HSC/kg recipient's body weight, according to a previously reported formula,[8,9] Plerixafor (Mozobil®, MZ) 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 (Bergish-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 $\geq 0.5x10^9$/L, while time to platelet engraftment was defined as time from transplantation to the first of 7 consecutive days with an unsupported platelet count $\geq 20x10^9$/L.

Patients surviving more than 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, while 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, while 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 additional event to those considered for estimating LFS and the composite end-point of chronic GvHD-free and relapse-free survival (GRFS).[23]

Quantitative variables were reported as median value and range, while categorical variables were expressed as absolute value and percentage. Demographic and clinical characteristics of patients were compared using the Chi-square test or Fisher's exact test for categorical variables, while the Mann-Whitney rank sum test or the Student's T-test were 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), while 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 supplementary Table 2.

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 January 1st, 2017.

Results

All children received at least $6x10^6$ CD34+ cells/kg body weight and a αβ T-cell number lower than $1x10^5$/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; one of them was successfully rescued through haplo-HSCT from the other parent, while the other died of disseminated adenovirus infection, despite receiving a second allograft from the same donor with engraftment and hematopoietic recovery. This patient was one of the 2 with donor-specific allo-antibodies. The number of CD34+ cells/kg body weight infused in these two patients was 8.9 and $24.3x10^6$, 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 = 0.03).

Acute and chronic GvHD

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

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

Non-relapse mortality

Four patients died for transplantation-related causes: two because of idiopathic pneumonitis and 1 each of disseminated adenovirus infection after graft failure and cardiac insufficiency. Only one of the 4 patients who died due to transplant-related causes had received TBI as part of the preparative

regimen, while the remaining 3 had been prepared with a chemotherapy-based regimen. These fatal events occurred all 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% (95% CI, 14-37) for the 56 children with ALL and 28% (95% CI, 14-58) for the 24 with AML (P= N.S.). In univariable analysis (Table 2), a number of CD34+ cells 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 (HR) 0.36, 95% CI 0.14-0.91, P=0.03].

Survival and leukemia-free survival

Fifty-eight patients (72% of the whole population) are alive at time of the last follow-up: 57 are in continuous CR after haplo-HSCT, while 1 is alive after post-transplantation 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=N.S., Figure 1D). The 5-year probability of EFS was 69.5% (95% CI 58-78.4), while 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 (HR 0.30, 95% CI 0.12-0.72, P=0.007, see also Figure 2A). Noteworthy, in this study, we were unable to confirm any

protective any 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 analysed 41 and 51 consecutive AL children of the latter 2 cohorts given an allograft in CR in the same period (see supplementary Table 2). The three 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), GvHD prophylaxis (none of the patients of the 2 control groups was given a T-cell depleted allograft) and human cytomegalovirus (HCMV) serology (see supplementary Table 2 for details).

Haplo-HSCT had a lower incidence of grade III-IV acute GvHD, of visceral GvHD and of chronic GvHD ($P < 0.01$, < 0.01 and 0.03, respectively, supplementary Figure 2A and 2B). 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 and 2D).

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 post-transplant 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 is the first study reporting 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 II trial. Our data indicate that, despite not receiving any post-transplantation 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 III-IV or gut/liver acute GvHD and all cases of chronic GvHD were of limited severity. Moreover, we did not observe any case of 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, while T cells displaying the αβ T-cell receptor 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 anti-leukemia role.[37-40] The Vδ2 population recognizes non-peptide phospho-antigens expressed by leukemia cells, while 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, around 8 weeks after transplantation are needed to detect mature KIR+ NK cells and this gap in reconstitution may favour early leukemia relapse in case of high residual tumour 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 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 G-CSF and plerixafor),[9] may have contributed to the low risk of NRM, which we found to be comparable to that observed after transplantation from either an HLA-compatible donor, either sibling or UD. We did not document any favourable 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, non-prospective 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. analysed 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 post-transplantation pharmacological immune suppression, including tacrolimus until day +30 and methotrexate (MTX) in 21 patients, tacrolimus in 5, MTX in 2, while 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), while 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 non-malignant diseases (n=5), who received αβ T- and B-cell-depleted allografts from a haploidentical relative after reduced-toxicity regimens.[49] Grade III–IV 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 post-transplantation GvHD prophylaxis, potentially able to impair the innate immunity-mediated graft-versus-leukemia 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] display potent anti-leukemia 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 anti-leukemia 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 favourably 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 post-transplantation infusion of cyclophosphamide, have been developed. [55] While 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 two 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. Since we have been able to successfully transplant adolescent and patients with a body weight greater than 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 seven 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.

Acknowledgments: This work was supported by grants from: AIRC (Special Grant “5xmille”-9962 to F.L., A.M. and L.M; “My first AIRC” grant 15925 to A.B.; AIRC IG-16764 to D.P.), Ministero della Salute (RF-2010-2316606 to F.L.; Ricerca Corrente to F.L.), Regione Lazio (Grant FILAS to F.L.) and Ministero dell’Istruzione, Università e Ricerca (Grant Progetto di Rilevante Interesse Nazionale, PRIN 2010, to F.L.).

Authors' contribution

Conception and design: F. Locatelli, A. Bertaina, P. Merli, R. Handgretinger, A. Moretta, and L. Moretta

Donor NK cell characterization and donor selection: M. Falco, D. Pende, R. Meazza

Transplantation of patients and collection of data: F. Locatelli, P. Merli, L. P. Brescia, D. Pagliara, B. Lucarelli, G.M. Milano, M. Algeri, R.M. Pinto, L. Strocchio, and A. Bertaina

Graft manipulation: G. Li Pira

Radiotherapy: L. Grapulin

Study on immune reconstitution: V. Bertaina, G. Li Pira

Analysis and interpretation of data (e.g. statistical analysis, computational analysis): R. Rondelli, R. Masetti, P. Merli, and F. Locatelli

Writing, review and/or revision of the manuscript: All co-authors

Study supervision: F. Locatelli, A. Bertaina and L. Moretta

Disclosure: None of the authors has any relevant conflict of interest to disclose.

Table 1. Patient, donor and transplantation characteristics.

Patients	**(n=80)**
Gender	
M	55 (69%)
F	25 (31%)
Median age at Diagnosis, years (range)	
	6.6 (0.4-16.8)
Median age at Transplantation, years (range)	
	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 (years)	41.5 (27-55)
Type of donor	
Mother	46 (58%)
Father	34 (42%)
Gender 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 $\geq$2	44 (55%) / 36 (45%)
Donor KIR2DS1 "educated and useful"# YES/NO	28 (35%) / 52 (65%)
Cell dose infused, median (range)	
CD34+ cells x 10^6/kg	13.93 (6-40.44)
αβ+ T cells x 10^6/kg	0.047 (0.002-0.099)
γδ+ T cells x 10^6/kg	8.1 (0.86-56.7)
NK cells x 10^6/kg	34.6 (3.84-146.1)
CD20+ B cells x 10^6/kg	0.09 (0.05-0.48)

M = male; F = female; ALL = acute lymphoblastic leukemia; AML = acute myeloid leukemia; BCP = B-cell precursors; TBI = total body irradiation; TT = thiotepa; Flu = fludarabine; L-PAM = melphalan; BU = busulfan; CY = cyclophosphamide; CR = complete remission; CMV = cytomegalovirus; NK = natural killer; KIR = killer inhibitory receptors.

§ Of the 15 patients with ALL transplanted in CR1, 9 had high level of minimal residual disease at end of induction therapy (i.e. $>1x10^{-3}$ 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 pre-phase.

** Of the 16 patients with AML transplanted in CR1, 3 had t(10;11), 2 a complex karyotype, 3 FLT3-ITD with high allelic ratio, 2 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 BFM classification of 1st relapse ALL, respectively. [59]

^ TBI (12 Gy over 3 days in 6 fractions of 200 cGy each) was employed in 50 children with ALL and in 10 with AML, all these patients being older than 3 years;

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

Table 2. Cumulative Incidence of relapse and Leukemia-Free Survival: univariate analysis

		Cumulative Incidence of relapse					Leukemia-Free Survival			
Outcome	**N. of patients**	**Events**	**Probability (%)**	**(95% CI)**	**P value**		**Events**	**Probability (%)**	**(95% CI)**	**P value**
Recipient gender										
Male	55	12	22.8	14-38	0.60		12	77.2	66-89	**0.031**
Female	25	7	28.6	15-53			11	56.0	37-75	
Recipient age at haplo-HSCT										
< 9.7 years	41	13	31.7	20-50	0.057		16	61.0	46-76	**0.023**
> 9.7 years	39	6	16.0	8-33			7	81.5	69-94	
Donor gender										
Male	34	5	14.7	7-33	0.13		9	73.5	59-88	0.88
Female	46	14	31.2	20-48			14	68.7	55-82	
Donor age										
< 41.5 years	40	12	31.5	19-51	0.17		12	68.5	53-84	0.86
> 41.5 years	40	7	17.5	9-34			11	72.5	59-86	
Donor-recipient gender combination										
Female donor- male recipient	49	12	25.5	16-42	0.90		16	66.4	53-80	0.34
Other combinations	31	7	22.6	12-43			7	77.4	63-92	
Type of leukemia										
ALL	56	13	23.2	14-37	0.87		16	71.4	60-83	0.98

AML		24	6	28.3	14-58			7	67.5	47-88	
Disease phase											
ALL CR1		15	2	13.3	4-48	0.61		3	80.0	60-100	0.62
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	
BFM classification of CR2 ALL patients											
S2		21	3	14.3	3-33	0.08		4	81.0	57-92	0.18
S3-S4		16	6	37.5	15-61			6	62.5	35-81	
Don./Rec. CMV serology											
Neg/Neg		5	2	40.0	14-100	0.43		2	60.0	17-100	0.75
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	**0.014**		11	81.7	72-91	**0.0002**
Chemo-based		20	9	47.5	29-77			12	37.5	15-60	
Graft composition											
CD34+ cells x 10^6/kg	$\geq$ m.v.	41	6	15.1	7-32	**0.045**		7	82.4	71-94	**0.016**
	< m.v.	39	13	33.3	21-52			16	59.0	44-74	
$\alpha\beta$+ T cells x 10^6/kg	$\geq$ m.v.	41	12	29.3	18-47	0.23		15	63.4	49-78	0.11
	< m.v.	39	7	18.9	10-37			8	78.5	65-92	
$\gamma\delta$+ T cells x 10^6/kg	$\geq$ m.v.	40	11	28.2	17-47	0.42		12	69.2	55-84	0.82
	< m.v.	40	8	21.1	11-39			11	71.4	57-86	
NK cells x 10^6/kg	$\geq$ m.v.	40	9	23.8	13-42	0.73		12	68.5	54-83	0.88

	< m.v.	40	10	25.0	15-43			11	72.5	59-86	
NK cell alloreactivity											
Yes		36	8	22.2	12-41	0.84		9	75.0	61-89	0.53
No		44	11	26.4	16-44			14	66.7	52-81	
KIR genotype											
B/X		64	18	28.7	19-43	0.11		22	65.0	53-77	**0.045**
A/A		16	1	6.7	1-44			1	93.3	81-100	
B-content score											
≥2		36	9	25.0	14-44	0.89		10	72.2	58-87	0.72
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	0.48		10	64.3	44-79	0.32
No		52	11	22.2	12-35			13	74.0	59-84	
Grade I-II acute GvHD											
Yes		24	3	12.5	4-36	0.12		18	67.2	55-80	0.34
No		56	16	29.2	19-44			5	79.2	63-95	
Chronic GvHD											
Yes		4	1	25.0	5-100	0.94		1	75.0	33-100	1.00
No		69	15	22.0	14-35			16	76.6	66-87	

m.v.= median value

Table 3. Details on immune reconstitution of the 80 patients given haplo-HSCT

	1 Month	3 Months	6 Months	12 Months
$CD3^+$ 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)

Figure legends

Figure 1. 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 vs. AML).

Figure 2. A: LFS probability according to the use of total body irradiation (TBI) in the conditioning regimen. B: LFS probability according to NK alloreactivity (evaluated using the KIR-KIR-L model of prediction). C: Comparison of LFS probability among patients transplanted from different donors (HLA-haploidentical relative, HLA-identical sibling, and unrelated donor) during the same period. D: Comparison of GRFS probability among patients transplanted from different donors (HLA-haploidentical relative, HLA-identical sibling, and unrelated donor) during the same period.

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Figure 1.

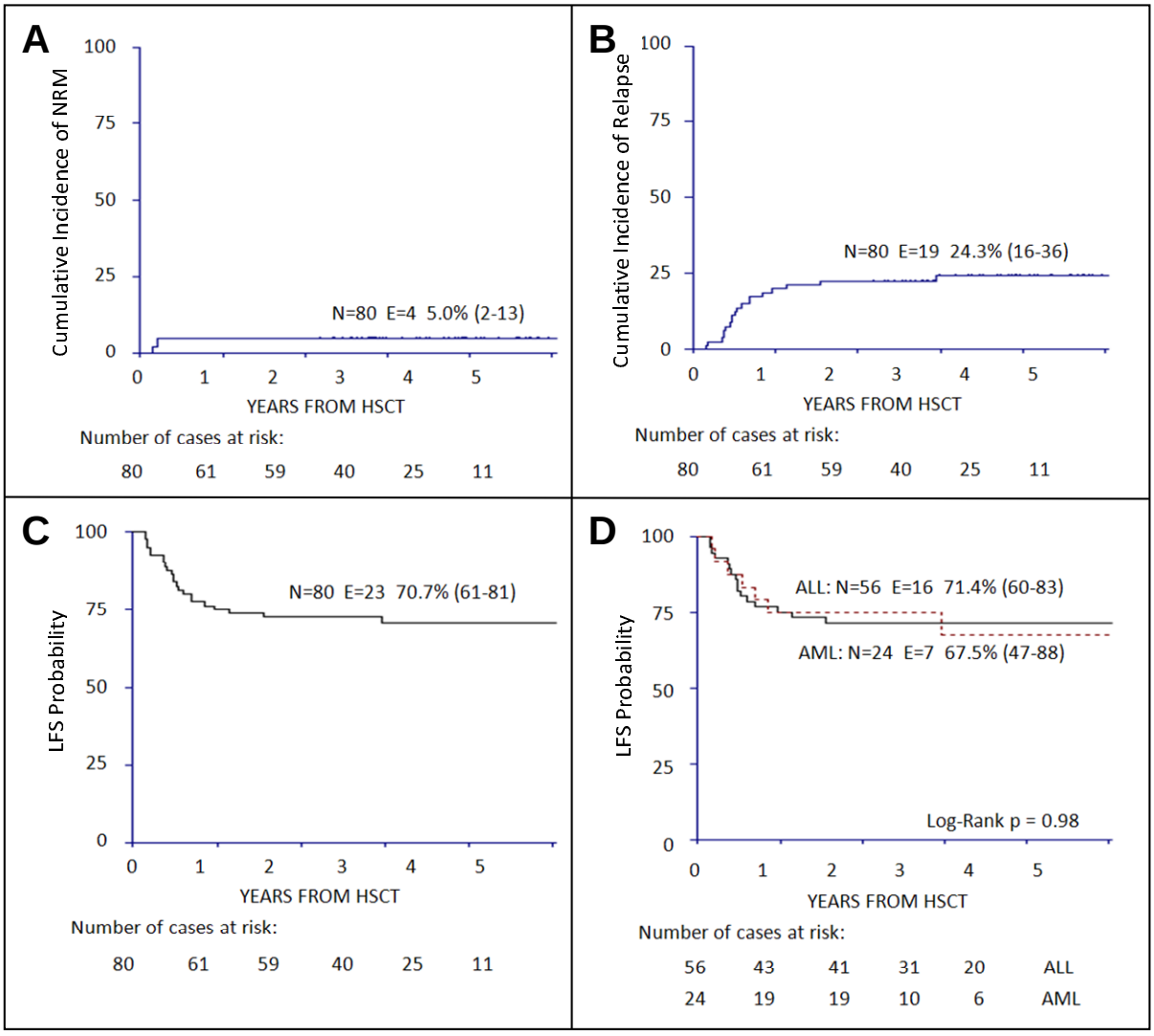
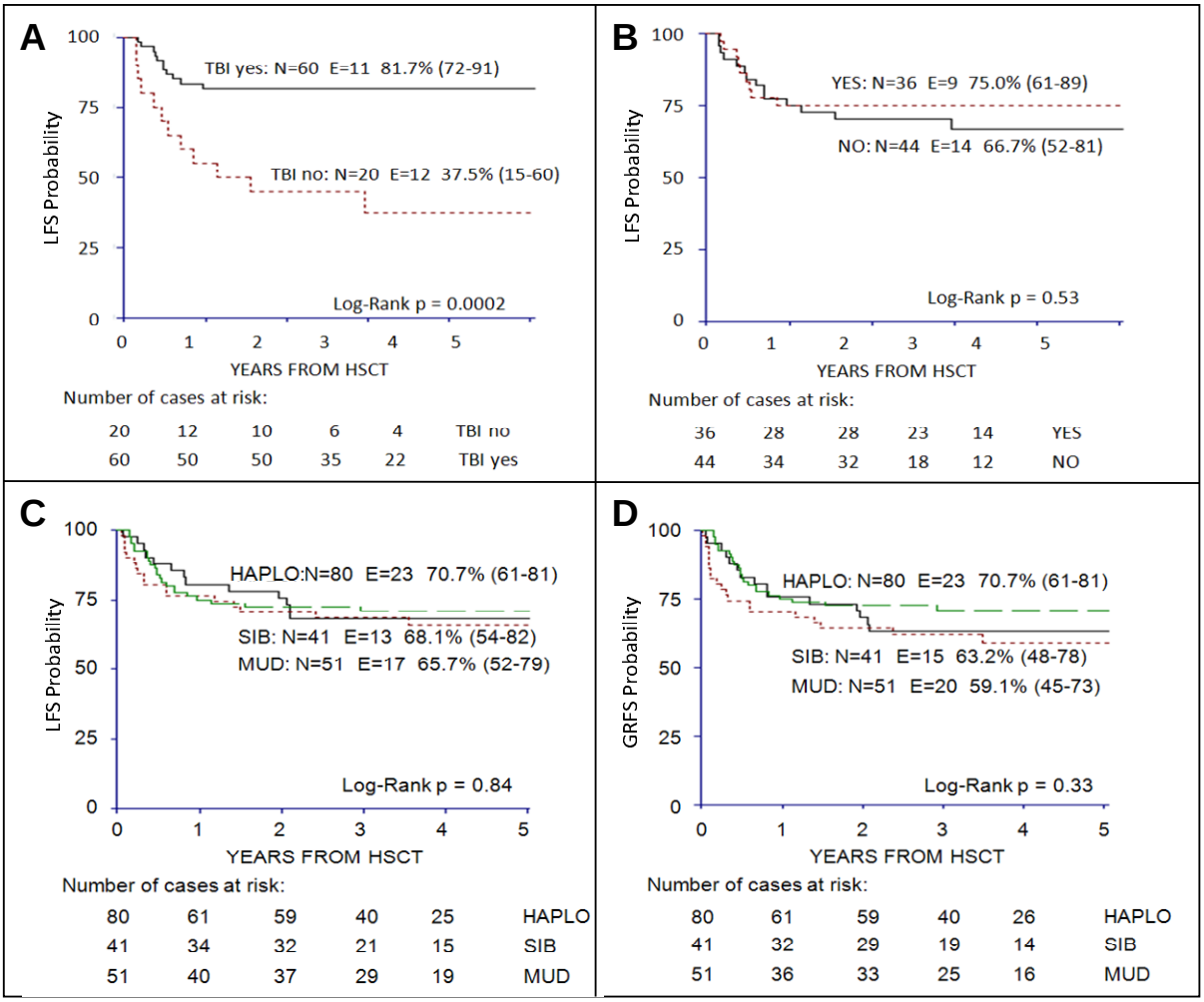


Figure 2.



Prepublished online June 6, 2017;
doi:10.1182/blood-2017-04-779769

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

Franco Locatelli, Pietro Merli, Daria Pagliara, Giuseppina Li Pira, Michela Falco, Daniela Pende, Roberto Rondelli, Barbarella Lucarelli, Letizia Pomponia Brescia, Riccardo Masetti, Giuseppe Maria Milano, Valentina Bertaina, Mattia Algeri, Rita Maria Pinto, Luisa Strocchio, Raffaella Meazza, Lavinia Grapulin, Rupert Handgretinger, Alessandro Moretta, Alice Bertaina and Lorenzo Moretta

Blood (print ISSN 0006-4971, online ISSN 1528-0020), is published weekly by the American Society of Hematology, 2021 L St, NW, Suite 900, Washington DC 20036.