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# Feasibility and outcome of haploidentical SCT in pediatric high-risk hematologic malignancies and Fanconi anemia in Uruguay

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**In total, 17 pediatric patients with hematologic malignancies ($n = 14$) and Fanconi anemia (FA) ($n = 3$) underwent haploidentical SCT with T-cell depletion. The patients were conditioned with reduced-intensity regimens, and CYA was used for GVHD prophylaxis. Successful engraftment occurred in 16 patients (94%). One patient failed to achieve a primary engraftment. Another patient rejected the first SCT after 10 weeks and had a successful second transplant. Of all engrafted patients, only one developed severe acute GVHD. Ten patients were alive at a median follow-up of 18 months (range, 5–62 months). The 5-years' OS was 53.8%. The three patients with FA are currently well with full-donor chimerism at 16, 6 and 5 months post transplant, respectively. The OS of 14 patients with high-risk hematologic malignancies was 47.6%. Three patients died as a result of post transplant leukemia relapse. CMV infection, GVHD and organ injury were other causes of mortality. Haploidentical SCT was found to be an alternative feasible treatment in Uruguay for patients who need allogenic transplantation but lack an HLA-identical family donor. It should be considered as an early option in FA patients before transformation or significant exposure to blood products.**
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**Keywords:** haploidentical SCT; T-cell depletion; Fanconi anemia; developing countries; children

## Introduction

Allo-SCT is the only currently available curative treatment for a number of high-risk hematologic malignancies and for a range of inherited and acquired non-malignant hematologic diseases. Only 25% of children in whom an allograft is indicated have the ideal option of an HLA-identical sibling donor. However, substantial advances in the use of alternative donors (unrelated volunteer donors, haploidentical family donors and unrelated umbilical cord blood donors) have led to the development of life-saving treatment currently available to nearly all children. The choice of the donor will depend on various factors related to type of disease to be treated, urgency of transplantation, donor characteristics and center's experience.

In Uruguay, a developing country with a population of 3.3 million, 100 new cases of pediatric cancer are diagnosed yearly.[1] Previous experience provides a yearly estimate of 10 pediatric patients who are candidates for allo-SCT, of whom approximately one-third have an HLA-compatible sibling. This means that only six to seven patients, on a yearly basis, will need a transplant from an alternative donor. The majority of these patients will have developed high-risk conditions —such as acute leukemia—that require urgent transplantation. In this setting, only one type of alternative donor source is used with a view to concentrating efforts on one particular area.

Several advantages are associated with the use of haploidentical SCT, namely, all children are expected to have a suitable donor; the ready availability of the donor facilitates urgent transplantation; the possibility of re-accessing donor in the event of graft failure; the feasibility of reduced-intensity conditioning;[2] effective *in vitro* T-cell depletion (TCD) systems; rapid engraftment associated with a very short duration of neutropenia; GVL effect if the donor–recipient pair are mismatched for killer-inhibitory receptors;[3] tolerable GVHD rates; encouraging results in malignant and non-malignant diseases.[2]

In this paper, results of haploidentical SCT in children with high-risk hematologic malignancies and Fanconi

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anemia (FA) were assessed, using data from a single center that centralizes the totality of haploidentical transplants in Uruguay.

## Materials and methods

*Patients characteristics*

Between April 2005 and November 2010, 17 pediatric patients were transplanted with T-cell-depleted grafts from haploidentical family donors. Fourteen patients had high-risk hematologic malignancies and three patients FA with severe aplastic anemia. At transplantation, patients with ALL and AML were in CR. We enrolled four patients with ALL, one in CR1 after induction failure and three in CR2 after early medullary relapse. Two patients with AML were in CR1—one after induction failure and the other with FLT3-ITD-mutation-positive—and four in CR2, of which three had been previously autografted. Informed consent, as required by the national regulatory agency, was provided by parents. The median age was 6 years (range, 0.5–17 years). Patient characteristics are detailed in Table 1.

*Donor characteristics*

Patients and donors were typed for alleles at HLA-A, B, C, DRB1 and DQ by serology and intermediate resolution methods (PCR-sequence-specific oligonucleotide). Donor selection was performed on a random basis until the year 2008, when a retrospective analysis found that patients transplanted from the mother had a better outcome compared with recipients of paternal grafts.[4] The mother is, thus, considered as the first choice. PBSCs were mobilized with G-CSF at a dose of 10 μg/kg for 5 days. Additional donor characteristics are shown in Table 1.

*Transplant characteristics*

The conditioning regimen for patients with hematologic malignancies consisted of 30 mg/m$^2$ fludarabine from days −9 to −5, 5 mg/kg thiotepa every 12 h on day −4 and one daily dose of melphalan (70 mg/m$^2$) on days −3 and −2. Prevention of graft rejection was assured by means of methylprednisolone (MP) and *in vivo* 0.1 mg/kg OKT3 (Cimab, Havana, Cuba) (maximum dose 5 mg) from day +1 to +15, until September 2008 (first nine patients), after which OKT3 was substituted by 1.5 mg/kg of rabbit anti-thymocyte globulin on days −9 through −6, in conjunction with 700 cGy TLI administered in two fractions on day −1. Patients with FA received 40 mg/m$^2$ fludarabine between days −6 and −3, a daily dose of 10 mg/kg Cy between days −5 and −2, 1.5 mg/kg anti-thymocyte globulin on days −6 through −3 and 400 cGy TLI in two fractions on day −1. PBSCs collected by apheresis from mobilized donors were T-cell-depleted by CD3 negative selection using the CliniMACS system (Miltenyi Biotech, Bergisch-Gladbach, Germany) with the anti-CD3 antibody OKT3 attached to magnetic microbeads. The median TCD log was 3.11 (range, 1.44–4.0). A total of 10 out of the 18 transplants also received an enrichment of highly purified haploidentical CD34$^+$ cells obtained by a positive selection strategy. Infused cell doses in each patient are shown in Table 1. The median number of CD34$^+$ and CD3$^+$ cells infused was $11.41 \times 10^6$/kg (range, 5.75–$25.30 \times 10^6$/kg) and $15.0 \times 10^4$/kg (range, 4.8–$2500 \times 10^4$/kg), respectively. To prevent post transplant lymphoproliferative disease, all patients received 375 mg/m$^2$ Rituximab on day −1. Two daily doses of 1.5 mg/kg CsA were administered for GVHD prophylaxis. In one patient (patient 10), as TCD failed and the dose of

**Table 1** Patients, donors, and graft characteristics

| *Patient no.* | *Age (years)* | *Sex* | *Diagnosis* | *Disease status* | *Donor/age (years)* | *CMV D/R status* | *CD34+ × 10$^6$/kg infused* | *CD3+ × 10$^4$/kg infused* |
|---|---|---|---|---|---|---|---|---|
| 1 | 11 | Male | CML | CP2 | Father/40 | +/+ | 11.31 | 39.40 |
| 1[a] | 12 | Male | CML | CP2 | Mother/30 | +/+ | 11.36 | 23.00 |
| 2 | 9 | Male | AML | CR2[b] | Father/37 | +/+ | 7.80 | 45.00 |
| 3 | 5 | Male | ALL | CR1 | Father/30 | +/+ | 12.20 | 50.00 |
| 4 | 11 | Female | AML | CR2[b] | Father/45 | +/+ | 25.00[c] | 40.00 |
| 5 | 17 | Female | ALL | CR2 | Father/45 | +/+ | 25.30[c] | 36.90 |
| 6 | 6 | Male | AML | CR2[b] | Mother/34 | +/+ | 6.97 | 18.00 |
| 7 | 0.6 | Male | JMML | CR | Mother/30 | +/+ | 6.96 | 27.00 |
| 8 | 0.5 | Male | JMML | PR | Mother/25 | +/+ | 23.60 | 15.00 |
| 9 | 5 | Female | ALL | CR2 | Mother/30 | +/+ | 24.50[c] | 10.00 |
| 10 | 11 | Male | ALL | CR2 | Father/35 | +/+ | 14.80 | 2500.00 |
| 11 | 15 | Male | AML | CR1 | Mother/34 | +/+ | 10.93[c] | 4.80 |
| 12 | 5 | Male | AML | CR1 | Mother/31 | +/+ | 11.41[c] | 11.00 |
| 13 | 10 | Male | AML | CR2 | Mother/38 | +/+ | 5.75[c] | 13.00 |
| 14 | 5 | Male | FA | SAA | Mother/24 | +/+ | 12.25[c] | 11.50 |
| 15 | 1 | Male | JMML | PR | Mother/35 | +/+ | 15.10[c] | 11.80 |
| 16 | 2 | Male | FA | SAA | Mother/29 | +/+ | 8.75[c] | 12.30 |
| 17 | 9 | Male | FA | SAA | Father/40 | +/+ | 18.80[c] | 9.80 |

Abbreviations: CP2 = second chronic phase; CR1 = first complete remission; CR2 = second CR; D = donor; FA = Fanconi anemia; JMML = juvenile myelomonocytic leukemia; PR = partial response; R = recipient.
[a]Second transplant.
[b]Previous auto-SCT.
[c]CD34$^+$ enrichment.

CD3+ infused was too high, GVHD prophylaxis was achieved by 50 mg/kg Cy on day +3 followed by CsA and mycophenolate mofetil.

*Supportive care*

Each patient was isolated in a laminar airflow room, and received antifungal (fluconazole), antiviral (acyclovir), anti-*Pneumocystis jirovecii* (cotrimoxazole) prophylaxis and regular Ig infusions until evidence of immune reconstitution was observed. Intravenous G-CSF (Filgastrim, 10 mcg/k/day) was given to recipients from day 6 after transplantation, until engraftment. All patients were monitored weekly by PCR assay for CMV, as well as by galactomannan assay for aspergillus. Preemptive treatments, including ganciclovir and amphotericin B, respectively, were used if the surveillance tests became positive. All blood products were leucodepleted and irradiated.

*Definitions*

Engraftment was defined as achieving an ANC greater than 500/mm$^3$ for three consecutive days, with evidence of donor hematopoiesis. Platelet recovery was defined as the first day on which the platelet count (unsupported by platelet transfusions for 7 days) was greater than 20 000/mm$^3$. Primary graft failure was defined as failing to achieve an ANC >500/mm$^3$ by day +30, and secondary graft failure as sustained graft loss (drop in ANC to <500/mm$^3$) for ⩾5 days after the initial engraftment. Chimerism was assessed from unseparated peripheral blood using DNA polymorphism based on PCR amplification of STR loci in recipients, twice monthly, from day +14 to day +100, and later less frequently in patients with stable full-donor chimerism. Acute GVHD was diagnosed and graded according to the published criteria.[5] Chronic GVHD was diagnosed according to the standard criteria.[6] Non-relapse mortality was defined as any death without evidence of relapse. Relapse for patients with ALL or AML was defined on the basis of morphological evidence of leukemia in BM or other sites. OS was calculated from transplantation to death due to any cause, and EFS was defined as time from transplantation to the last follow-up or first event, death or relapse, whichever occurred first.

**Table 3** Clinical outcomes of haploidentical SCT recipients

| | |
|---|---|
| *Engraftment (n=17)* | |
| Median day (range) | 11 (9–12) |
| *Platelet recovery* | |
| Median day (range) | 16 (12–20) |
| *Acute GVHD* | |
| No | 13 |
| Grade I | 1 |
| Grade II | 2 |
| Grade III | 0 |
| Grade IV | 1 |
| *Chronic GVHD* | |
| No | 10 |
| Limited type | 0 |
| Extensive type | 1 |
| NA | 6 |
| *CMV viremia* | 13 |
| *Relapse (n=3)* | |
| Median month (range) | 3 (2.5–3) |
| *Survival* | |
| Alive | 10 |
| Median month (range) | 18 (15–62) |
| Death | 7 |
| Median month (range) | 4 (2–10) |
| *Cause of death (n=7)* | |
| Relapse | 3 |
| GVHD | 1 |
| CMV IP | 2 |
| Heart failure | 1 |

Abbreviations: CMV IP = CMV interstitial pneumonia; NA = not applicable.

**Table 2** Results after haploidentical SCT

| *Patient no.* | *ANC>500 (days)* | *Graft failure* | *Rejection (days)* | *aGVHD grade* | *cGVHD* | *Infections* | *Relapse (months)* | *Follow-up (months)* |
|---|---|---|---|---|---|---|---|---|
| 1 | 11 | N | Y (78) | N | NA | N | N | — |
| 1[a] | 11 | N | N | N | N | N | N | Alive (62) |
| 2 | 10 | N | N | 2 | N | CMV IP | N | Died (4) |
| 3 | 10 | N | N | 1 | NA | N | Y (2.5) | Died (3) |
| 4 | 9 | N | N | N | N | N | N | Alive (58) |
| 5 | 11 | N | N | N | Y | CMV IP | N | Died (10) |
| 6 | 12 | N | N | N | N | N | N | Alive (33) |
| 7 | 11 | N | N | N | N | N | N | Alive (31) |
| 8 | 9 | N | N | 4 | NA | N | N | Died (2) |
| 9 | 10 | N | N | N | NA | N | Y (3.0) | Died (5) |
| 10 | 12 | N | N | 2 | N | N | N | Alive (18) |
| 11 | 10 | N | N | N | NA | N | Y (3.0) | Died (4) |
| 12 | 10 | N | Y (28) | N | NA | N | N | Died (2) |
| 13 | 11 | N | N | N | N | N | N | Alive (10) |
| 14 | 10 | N | N | N | N | N | N | Alive (16) |
| 15 | NA | Y | N | N | N | N | N | Alive (8) |
| 16 | 12 | N | N | N | N | N | N | Alive (6) |
| 17 | 11 | N | N | N | N | N | N | Alive (5) |

Abbreviations: aGVHD = acute GVHD; cGVHD = chronic GVHD; CMV IP = CMV interstitial pneumonitis; N = no; NA = not applicable; Y = yes.
[a]Second transplant.

The probabilities of EFS and OS are estimated using the Kaplan–Meier method.

## Results

*Engrafment and hematopoietic recovery*

Engraftment was observed in 17 of the 18 transplants (94.4%) (Tables 2 and 3). The median time required to reach a neutrophil count above 500/mm$^3$ was 11 days (range, 9–12 days), and that necessary to attain a platelet count of 20 000/mm$^3$ without transfusion was 16 days (range, 12–20 days). Complete donor chimerism on day +14 was observed in all patients who engrafted, except for one patient with FA, whose initial donor chimerism was 80%. Only one patient—transplanted for juvenile myelomonocytic leukemia—failed to achieve primary engraftment presumably because of rejection. This patient had an autologous hematopoietic recovery and is currently alive in remission with normal blood counts. Two patients—with AML and CML, respectively—rejected the graft on day +28 and +78, respectively. The patient with CML received a second transplant from a different parent using a preparative regimen consisting of 30 mg/m$^2$ fludarabine, days −6 to −2, 60 mg/kg Cy on day −6 and anti-rejection therapy with methylprednisolone and OKT3. This patient engrafted and recovered hematopoiesis following the second transplant. The other patient died on day +60 because of congestive heart failure, probably because of anthracyclines.

*Immune cell resconstitution*

T, B and natural killer reconstitution were measured monthly by flow cytometry, results of which are detailed in Table 4. The patients experienced profound impairment of immune function mainly during the 3 months following the allograft. Finally, all patients showed evidence of full immune reconstitution.

*Regimen-related toxicity*

The preparative regimen was fairly well tolerated and led to limited regimen-related toxicity, the gastrointestinal type being the most common. Most of the patients had nausea, vomiting and diarrhea. Grade II and III mucositis was observed in 13 of the 18 patients (72%), 11 of whom (61%) received total parenteral nutrition for a median of 10 days (range of 8–16 days). Posterior reversible encephalopathy syndrome occurred in one patient with FA at 31 days post transplant. No significant toxicity was observed in children who had received previous auto-SCT.

*Acute and chronic GVHD*

Acute GVHD was grade ⩽1 in 14 children, grade 2 in 2 children and grade 4 in 1 child (resulting in death). Chronic GVHD with extensive manifestations was observed in only one of the 11 patients at risk.

*Infections*

In total, 13 of the 18 patients showed CMV reactivation in one or more opportunities. Despite the preemptive therapy, two patients with acute and chronic GVHD, respectively, developed CMV interstitial pneumonitis and as a result of which they died. Significant bacterial or fungal complications were not observed in this group.

*Outcome of patients with hematologic malignancies*

With a median follow-up of 31 months (range, 8–62 months), the estimated 5-year EFS was 47.6% (±14.0) (Figure 1). Three patients—two with ALL and one with AML—relapsed 2.5–3 months post transplant.

*Outcome of patients with Fanconi anemia*

With a follow-up of 16, 6 and 5 months after SCT, the three patients are alive and have shown no evidence of GVHD. All three remain transfusion-independent and have discontinued all immunosuppressive drugs. All of them showed full chimerism.

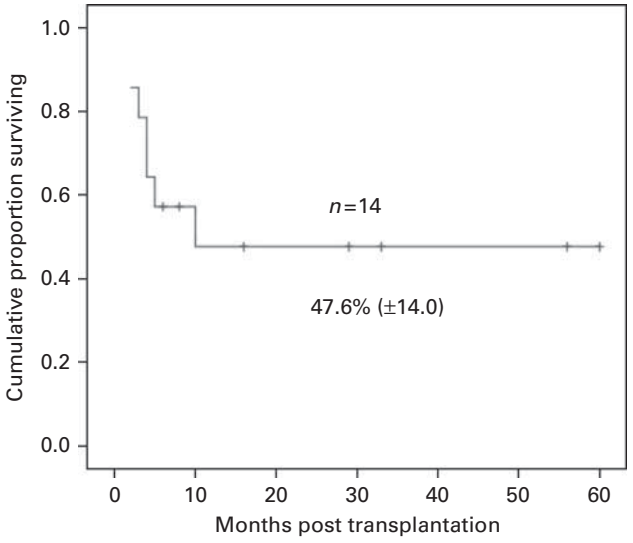


**Figure 1** OS in patients with high-risk hematologic malignancies.

**Table 4** Evaluation of immune reconstitution after TCD haploidentical SCT in survivors

| | *3 months after SCT* | *6 months after SCT* | *9 months after SCT* | *12 months after SCT* | *Reference values* |
|---|---|---|---|---|---|
| *CD3*+ cells/μL, median (range) | 459 (76–1561) | 1140 (322–3966) | 1560 (809/6528) | 1485 (1322–6084) | 1000–3900 |
| *CD4*+ cells/μL, median (range) | 110 (22–877) | 309 (176–1519) | 774 (320–1040) | 999 (523–1618) | 560–2700 |
| *CD8*+ cells/μL, median (range) | 113 (21–1068) | 679 (113–2019) | 780 (342–2534) | 634 (486–3721) | 330–1400 |
| *CD19*+ cells/μL, median (range) | 114 (1–1940) | 304 (138–1811) | 346 (208–1255) | 999 (415–1561) | 200–1300 |
| *CD16*+ cells/μL, median (range) | 189 (74–2146) | 437 (93–1929) | 490 (55–569) | 469 (155–715) | 120–1040 |

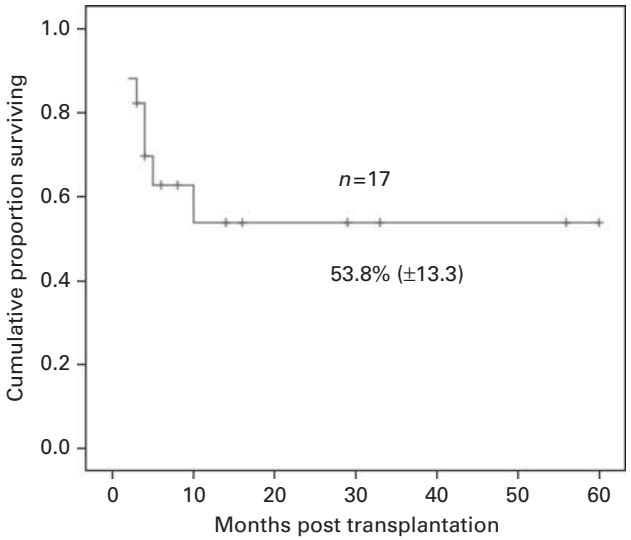


**Figure 2** OS of all patients.

*Survival and causes of death*

With a median follow-up of 18 months (range, 5–62 months), the estimated probability of OS at 5 years was 53.8% (±13.3) for the whole cohort (Figure 2). Out of seven deceased patients, three died as a result of the recurrence of the disease, two as a result of infections, one of GVHD and the remaining one of congestive heart failure.

## Discussion

The definition of an optimal donor source when an HLA-identical sibling is not available is debatable.[7,8] Unrelated volunteer donor SCT has been used successfully for over 40 years in thousands of children without an HLA-identical sibling.[9] However, the amount of time usually required from the decision to perform an unrelated volunteer donor search to the actual acquisition of cells is, in most cases, excessively long for patients who are in urgent need of transplantation.[10,11] On the other hand, both haploidentical SCT and umbilical cord blood transplantation have merits and limitations, yet, they have never been compared in a randomized study. Over the past 20 years, there has been substantial progress in the use of haploidentical SCT in children with a wide range of diseases. Obstacles like graft failure or rejection and high incidence of GVHD have been largely overcome, and encouraging disease-free survival results are increasingly being reported for selected malignant and non-malignant diseases.[2] However, no studies of its feasibility in less developed countries have been reported.

As this report is based on a limited and heterogeneous number of patients, no reliable conclusions may be drawn on the role of haplo-SCT in specific pediatric hematologic disorders. Nevertheless, these results suggest that haplo-SCT may be an effective option for patients with malignant and non-malignant diseases who need an alternative donor.

Using three different reduced-intensity conditioning regimens—one with OKT3, previously reported by Handgretinger *et al*.[2] a similar approach with anti-thymocyte globulin and TNI, instead of OKT3, and a fludarabine-based regimen for FA patients—regimen-related toxicity was limited, and the major cause of non-relapse mortality was infectious complications, most likely related to the post transplant immune deficiency intrinsic to T-cell-depleted allo-SCT.[12] The incidence of CMV reactivation was higher than reported by other authors,[13,14] leading to lethal infection in two cases, a fact that may be ascribed to the high incidence of CMV in the donor–recipient pair (Table 1). To ensure early detection of CMV infections, intensive surveillance must be considered as an essential component of haplo-SCT management. TCD was performed by negative selection in order to maintain natural killer cells, monocytes and APCs in the graft, and obtain a more rapid immune reconstitution.[2,15] The log TCD was initially lower than expected, resulting in more CD3+ cells and fewer CD34+ cells than desired in the product infused. However, engraftment occurred promptly in 94% of the patients, and the incidence of GVHD was low probably because all patients received post transplant pharmacological immunosuppression. Most patients also received purified CD34+ stem cells obtained by positive selection in order to achieve the 'megadose' concept.[16] The rate of depletion increased over time up to the currently used number of 4 logs TCD.

Improvements in the outcome of depletion were mainly because of a reduction to below 1 million $\times 10^6$/L in the number of platelets in the product to deplete, and a twofold higher amount of anti-CD3 antibody used in the process. The patient in whom TCD failed and who received high-dose post transplantation Cy presented mild GVHD, which resolved successfully. Such an interesting approach has not yet been adequately explored in children.[17]

An advantage of great impact was the short duration of the neutropenia. In fact, there were no significant bacterial or fungal infections, and the empirical use of amphotericin was not necessary. Also galactomannan antigen was used as a complementary tool.

In terms of survival—considering that the majority of cases were associated with very high-risk leukemia—these results are highly encouraging for patients with malignant diseases who are transplanted in remission, as opposed to patients undergoing transplantation with active disease, who have shown a very poor prognosis.[18]

FA patients included in this study were the first FA patients with severe aplastic anemia to have survived in Uruguay. These patients had neither an HLA-identical sibling nor the possibility of an alternative donor.

In summary, haploidentical SCT is an alternative option—feasible in Uruguay—for the treatment of children with high-risk hematologic malignancies and FA. The low toxicity of conditioning regimens and the rapid hematologic recovery constitute remarkable results, especially in the setting of heavily pretreated patients. On the other hand, it is a risky procedure demanding a high degree of care during and after transplant. Outcomes are comparable with those of transplants from other alternative donors.

## Conflict of interest

The authors declare no conflict of interest.

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