




# Concordant acute myeloblastic leukemia in identical twins treated with allogeneic transplantation from a younger HLA-identical sibling following a single apheresis procedure

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A concordant leukemia is that which occurs in a pair of monozygotic twins; a similar genetic background suggests an *in utero* monoclonal origin. We present the case of a pair of monozygotic infants with concordant acute myeloid leukemia who underwent a peripheral blood hematopoietic stem-cell transplant (HSCT) from a single, younger human leukocyte antigen-identical sibling donor, using a fractionated graft collected during only one apheresis procedure. Twin A relapsed at +456 and received a second haploidentical HSCT from his father, twin B has been in complete remission since the first HSCT. Both children are in complete remission and with negative minimal residual disease at +900 (after second transplant) and +1488, respectively.

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The first case report of concordant leukemia in twins appeared in the literature in 1882 and since then many such pairs of infants have been described [1,2]. By the year 2003 there had been 70 similar cases described [3]. Different molecular markers, including genomic fusion gene sequences, have provided evidence that cases of twins with leukemia have a common clonal origin [2]. The origin of infant acute myeloid leukemia (IAML) twins is an *in utero* acquired leukemic clone, which arises in one twin and is then metastasized to the other twin through the placenta. These *in utero* mutations involve in up to 80% of cases the 11q23 (*KMT2A*) gene [4].

Here we present the experience of a pair of twins with acute myeloblastic leukemia being transplanted using the same donor. **Table 1** shows patients characteristics.

## Twin A

A 17-month-old infant was referred due to a history of pallor, epistaxis and bone pain. He had a hemoglobin of 2.9 g/dl, a leukocyte count of  $7.41 \times 10^9/l$ , an absolute neutrophil count (ANC) of  $0.25 \times 10^9/l$ , a lymphocyte count of  $5.9 \times 10^9/l$  and platelets of  $105 \times 10^9/l$ . The peripheral blood smear showed blasts with myeloid lineage characteristics, later confirmed by bone marrow flow cytometry with a blast population of 22% of the total mononuclear cells. The blasts were CD33<sup>+</sup>, CD64<sup>+</sup>, CD56<sup>+</sup>, with characteristics of myelodysplasia due to an alteration in the pattern of expression of CD11b/CD13 and CD11b/CD16. Cytogenetic analysis showed a t(2;11) and an isodicentric X chromosome. The patient received the MRC acute myeloid leukemia (AML) 10 protocol with

Table 1. Patient characteristics.

Characteristics	Twin A	Twin B
Sex	Male	Male
Age at diagnosis (months)	17	17
Leukocyte count at diagnosis $\times 10^9/l$	7.41	5.77
Lymphocyte count at diagnosis $\times 10^9/l$	5.9	4.94
Platelet count at diagnosis $\times 10^9/l$	105	46
Hemoglobin count at diagnosis (g/dl)	2.9	8.2
Percentage of blasts, bone marrow (%)	22	74
Blasts immunophenotype	CD33 <sup>+</sup> , CD64 <sup>+</sup> , CD56 <sup>+</sup> , CD117 <sup>+</sup> , MPOc <sup>+</sup> , CD11b/CD13, CD11b/CD16	HLA-DR <sup>+</sup> , CD13 <sup>+</sup> , CD33 <sup>+</sup> , CD64 <sup>+</sup> , CD56 <sup>+</sup> , CD117 <sup>+</sup> , MPOc <sup>+</sup> , CD11b/CD13, CD11b/CD16
Cytogenetics	t(2;11)(p21;q23.3) idic(X)(q13)	t(2;11)(p21;q23.3) idic(X)(q13)
Treatment before HSCT	AML 10	AML 10
Postinduction MRD (%)	1.3	1.3
Pretransplant MRD (%)	1.4	0
Interval between diagnosis and HSCT (months)	9	9
Weight (kg)	12	12
Age at transplant (months)	26	26
Donor source	Related, HLA matched sibling, PBSC	Related, HLA matched sibling, PBSC
Donor weight (kg)	12	12
D/R blood group	A1/A	A1/A
D/R CMV sero status	Negative/positive	Negative/positive
CD34 <sup>+</sup> cells infused $\times 10^6/kg$	6.4	6.4
Neutrophil engraftment (days)	+25	+18
Platelet engraftment	Never below $20 \times 10^9/l$	Never below $20 \times 10^9/l$
Acute GVHD	No	No
Chronic GVHD	No	No
Chimerism day 30 (%)	90	92

AML: Acute myeloid leukemia; CMV: Cytomegalovirus; D/R: Donor/recipient; GVHD: Graft-versus-host disease; HSCT: Hematopoietic stem-cell transplant; MRD: Minimal residual disease; PBSC: Peripheral blood stem cells.

a postinduction minimal residual disease (MRD) of 1.3% and an MRD of 1.4% before hematopoietic stem-cell transplant (HSCT).

An HLA-identical peripheral blood HSCT was performed with his 12-month-old sister, the conditioning regimen consisted of Cy 350 mg/m<sup>2</sup>/d and Flu 25 mg/m<sup>2</sup>/d from days -7 to -5 and busulfan 3.2 mg/kg/d from days -4 to -2. Graft-versus-host disease (GVHD) prophylaxis was cyclosporine A at 6 mg/kg/d starting at day -1 and methotrexate 5 mg/m<sup>2</sup>/d on days +1, +3 and +5. The donor was mobilized with granulocyte-colony stimulating factor at 10 mg/kg/d for 4 days and after a single apheresis a total of  $12.8 \times 10^6/kg$  CD34<sup>+</sup> were harvested. The graft was divided in two equal fractions, one was infused immediately to the patient and the other was cryopreserved.

Neutrophil engraftment was on day +25 and platelets were never below  $20 \times 10^9/l$ . On days +30 and +100 chimerisms were at 90 and 96%, respectively. The patient did not present GVHD at any time. At day +463 the patient relapsed, with 66% of myeloid blasts with the same immunophenotype as at diagnosis.

The patient received 5+2 (Cytarabine + mitoxantrone), achieving negative MRD, and was followed by a second 5+2 regimen as consolidation. A haploidentical HSCT was performed, with his father as the donor. Conditioning regimen was the same as the first transplant, but GVHD prophylaxis was Cy 50 mg/kg/d on days +3 and +4, followed by cyclosporine A 6 mg/kg/d and mycophenolate mofetil 30 mg/kg/d starting at day +5. Currently this boy is in complete remission with negative MRD at +900 days, and without GVHD.

### Twin B

The monozygotic twin of the previous patient was asymptomatic and was evaluated 7 days after the diagnosis of his brother. A complete blood count showed a hemoglobin of 8.2 g/dl, leukocytes of  $5.77 \times 10^9/l$ , an ANC of

$0.13 \times 10^9/l$ , a lymphocyte count of  $4.44 \times 10^9/l$  and platelets of  $46 \times 10^9/l$ . A bone marrow aspirate was performed and a population of blasts comprising 74% of nucleated cells with the same immunophenotype and cytogenetic alterations of his twin was observed. The patient received the same chemotherapy regimen of his twin, having a postinduction MRD of 1.2%, but a negative pretransplant MRD. The patient received the cryopreserved fraction of the graft harvested from their sister 10 days after his brother. The same conditioning regimen and GVHD prophylaxis were used.

Neutrophil engraftment was on day +18 and platelets were never below  $20 \times 10^9/l$ . On days +30 and +100 chimerism were 92 and 84%, respectively. The patient has not presented GVHD since the transplant. His last evaluation was performed on +1488 and is still in remission.

## Discussion

AML represents 20% of childhood leukemia, and has a higher incidence in infants with characteristic cytogenetic abnormalities [5]. IAML has a higher prevalence of unfavorable risk factors and an increased susceptibility to therapy-related toxicity [6].

In the above cases, the decision to perform an HSCT in CR1 was justified because the patients had high-risk leukemias [7,8]. Both patients had a  $t(2;11)$  and an  $idic(X)$  – which are poor-outcome cytogenetics [9] – and had a postinduction MRD above 1%, which is also considered as an independent negative prognostic factor [10,11].

The use of bone marrow as a source if the donor is smaller than the recipient can be difficult because 10 to 15 ml/kg of the recipient's weight is required to obtain an adequate number of stem cells. However, pediatric peripheral blood stem-cell donors can represent a significant challenge because of catheter placement and low blood volumes.

By the time the twins' donor was harvested, our group had already published our experience with 27 PBSC collections performed on 22 children weighing 20 kg or less. One apheresis procedure was enough to obtain at least  $2 \times 10^6$  CD34<sup>+</sup> cells per kg of recipient bodyweight in 78.5% of donors and the median number of CD34<sup>+</sup> collected was  $5.79 \times 10^6$  per kg [12].

There is only one other case report where a fractionated graft from a single procedure was used as a stem-cell source in twins with IAML [13]. The similarities with our case report are that an HLA-matched sibling was the donor and that one graft fraction was cryopreserved. The main differences with our report are that the donor of our patients was younger and smaller than the recipients and that the source of stem cells was peripheral blood. The conditioning regimens were different; we used a combination of fludarabine, cyclophosphamide and busulfan IV, which we have shown in a previous study to be effective for the achievement of engraftment in 92% of the cases [14].

Our patients were the same age at the time of diagnosis, received the same induction and consolidation protocols and had a simultaneous HSCT with the same donor and conditioning regimens; however, only one of them relapsed. The only significant difference that we believe explains this is that the twin that relapsed had a positive MRD at the time of transplant, which has been shown in multiple studies to be an independent adverse prognostic factor [10,11]. However, the difference in MRD can reflect underlying differences in identical twins. Several studies have shown identical twins can have differences in epigenetic variation, somatic genetic variants, including copy number variants and mitochondrial changes, resulting from unequal distribution of molecular factors [15–17].

We decided not to use the same donor for the second transplant of twin A, because the presence of a mismatched haplotype in the new allograft in comparison with the first transplant has shown to improve survival [18].

This case report exemplifies that a pediatric donor can be used safely to obtain more than one graft in a single apheresis procedure, even for recipients that have higher weight; it also demonstrates that even patients with the exact same genetics, disease origin and environment, can have a different evolution and that pre-transplant MRD as a prognostic factor still applies in such cases.

## Future perspective

Since achievement of negative MRD is an important pretransplant prognostic factor, reaching this goal is essential for all leukemic patients; currently the combination of venetoclax and azacitidine has proven to be effective for achieving complete remission in elderly patients with AML, accomplishing sustained remissions and responses even in chemotherapy refractory disease. There are a few uplifting reports of pediatric cases with AML treated with this drug combination. If proven to be safe in children, this targeted therapy could offer the means to obtain negative pretransplant MRD in patients that do not reach this threshold with conventional chemotherapy.

### Summary Points

- Infant acute myeloid leukemia (IAML) is characterized by a high rate of genetic abnormalities, next generation sequencing, if available or cytogenetics with a molecular abnormalities panel should always be performed.
- A total of 10% of IAML cases are myelodysplastic syndrome related.
- All monozygotic twins of an IAML patient should have a clinical evaluation and a complete blood count assessed by a pediatric hematologist, as soon as possible after diagnosis.
- Postinduction positive minimal residual disease by flow cytometry is an independent risk factor for relapse and mortality in pediatric acute myeloid leukemia.
- Pretransplant minimal residual disease in IAML is an independent prognostic factor for event-free and overall survival.
- Peripheral blood stem cell harvest in donors weighing under 20 kg is safe and effective.

### Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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### Informed consent disclosure

The authors state that they have obtained verbal and written informed consent from the patient's legal guardian for the inclusion of their medical and treatment history within this case report.

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