# Donor lymphocyte infusion to eradicate recurrent host hematopoiesis after allogeneic BMT for sickle cell disease

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**BACKGROUND:** Donor lymphocyte infusion (DLI) is currently standard therapy for relapse of malignancies after allogeneic BMT. Several observations suggest that both normal and leukemic progenitor cells of host origin constitute effective target cells for donor-derived lymphocytes. To prevent relapse of sickle cell disease (SCD), a child with evidence of decreasing mixed chimerism received DLIs 8 months after allogeneic BMT for SCD.
**CASE REPORT:** A 4-year-old child who was homozygous for SCD underwent a transplantation of bone marrow from his fully HLA-matched sister. Routine detection of sex chromosomes in bone marrow cells evidenced decreasing mixed chimerism, which heralded a probably imminent recurrence of the disease. The patient received two DLIs in graded incremental doses on Days 234 and 267. One month later, he developed grade 2 acute GVHD that responded well to corticosteroids and cyclosporine.
**RESULTS:** DLI resulted in complete donor chimerism within 2 months of the second infusion. Now, 2 years after the second DLI, the patient is in excellent condition, with normal Hb and excellent growth and development.
**CONCLUSION:** This is the first report of successful use of DLI in a patient with probable imminent SCD recurrence after allogeneic BMT. It shows that DLI can displace residual host HPCs in case of recurrence of nonmalignant disease after allogeneic BMT.

**ABBREVIATIONS:** AML = acute myelogenous leukemia; CML = chronic myelogenous leukemia; DLI(s) = donor lymphocyte infusion(s); FISH = fluorescent in situ hybridization; SCD = sickle cell disease.

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Sickle cell disease (SCD) may be associated with considerable morbidity and mortality, particularly as patients reach adulthood.[1] Among the various therapeutic options, allogeneic HPC transplantation is the only treatment with proven curative potential.[1,2] However, the underlying disease recurs in about 10 percent of patients.[2]

Donor lymphocyte infusions (DLIs) are increasingly used to treat relapses of malignant diseases after allogeneic HPC transplantations,[3-5] inducing a complete remission in about 65 percent of the patients with chronic myelogenous leukemia (CML) and in 20 to 30 percent of the those with acute myelogenous leukemia (AML) or myelodysplastic syndromes.[4] Persistence of donor chimerism at the time of DLI was shown to improve the rate of complete remission after DLI[6] and to reduce the risk of marrow aplasia,[5] which justified early use of DLI in case of unstable chimerism or cytogenetic relapse. Because several observations suggest that both normal and leukemic host-derived HPCs constitute effective target cells for lymphocytes of donor origin,[7] we infused donor lymphocytes to a 4-year-old child with evidence of unstable mixed chimerism 8 months after an allogeneic bone marrow for SCD. This observation reports the first successful use of DLI in a patient with probable imminent SCD recurrence after allogeneic HPC transplantation.

## CASE REPORT

A 1-year-old black African child (Congolese) was diagnosed in December 1992 as being homozygous for the SCD gene. Between December 1992 and August 1996, he underwent multiple recurrent vaso-occlusive crises and had two splenic crises with a drop in Hb of around 5 g per dL. After the failure of hydroxyurea therapy and splenectomy, he was referred to us at age 4 for the transplantation of bone marrow from an HLA-identical sister who was heterozygous for the SCD gene. After conditioning with oral busulfan (16 mg/kg), IV cyclophosphamide (200 mg/kg), and antithymocyte globulin (90 mg/kg), the patient received unmanipulated marrow in August 1996. GVHD prophylaxis was carried out with cyclosporine alone because of his young age. The immediate posttransplant course was uneventful and the pa-

tient was discharged on Day 22 on daily cyclosporine. He regularly attended the outpatient clinic and did not experience significant complications (except urinary CMV excretion treated by IV gancyclovir 10 mg/kg 3 × week) or acute GVHD. After tapering, cyclosporine was discontinued on Day 180.

Detection of sex chromosomes in bone marrow cells was performed by standard cytogenetics and fluorescent in site hybridization (FISH). Cytogenetic analysis of bone marrow cells on Day 73 showed a mixed chimera with 6 percent of the mitoses of host origin, while FISH evidenced 14 percent residual host cells (Fig. 1). On Day 200, a new examination of bone marrow cells found a decrease in donor chimerism, with 18 percent residual host cells by standard cytogenetics and 37 percent by FISH. In an attempt to prevent recurrence of the underlying disease, we decided to use DLI to eradicate host-derived HPCs. A first injection of 1 $\times 10^7$ CD7+ ($0.6 \times 10^7$ CD4+, $0.4 \times 10^7$ CD8+, $0.2 \times 10^7$ CD56+, and $0.4 \times 10^7$ CD19+) donor cells per kg of recipient body weight was performed on Day 234 in the form of 245 mL of whole blood. Chimerism was further decreased to 34 percent host cells by cytogenetics and to 44 percent by FISH on Day 243 (Fig. 1). Moreover, the patient became anemic (Hb 7.4 g/dL) and required an RBC transfusion, while his WBC and platelet counts remained normal. The percentage of Hb S was not informative, because the donor was heterozygous for the SCD gene. In the absence of GVHD, a second infusion of $2 \times 10^7$ CD7+ donor cells per kg was performed on Day 267 as 436 mL of whole blood. One month later, the patient experienced stage 2 cutaneous and stage 1 liver GVHD (overall stage 2), which responded to treatment with corticosteroids and cyclosporine. Examination of bone marrow cells by standard cytogenetics alone on Day 297 and in combination with FISH on Day 367 evidenced full donor chimerism (Fig. 1) and the patient's Hb value normalized. Cyclosporine and corticosteroids were discontinued on Days 367 and 451, respectively, without any further evidence of chronic GVHD over the next 2 years. Now, 2 years after the second DLI, the patient is in excellent condition, with adequate growth and development, normal Hb values, and he is heterozygous for the SCD gene.

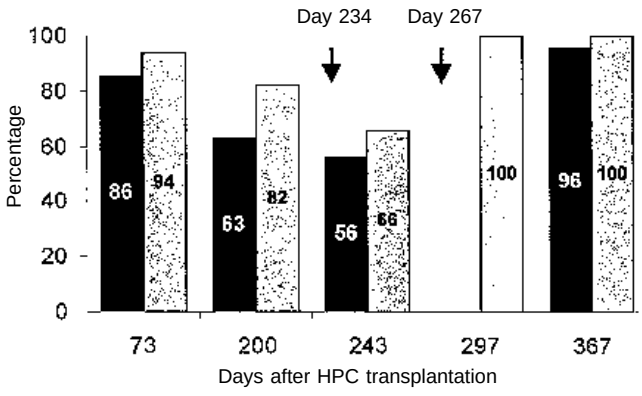


**Fig. 1. FISH (■) and standard cytogenetic analysis (□) of chimerism. Arrows indicate DLIs on Days 234 and 267.**

## DISCUSSION

Allogeneic HPC transplantation is the only treatment for SCD with proven curative potential. It is associated with a significant risk of early death and complications, including GVHD, graft failure, chemotherapy-related toxicity, infections, gonadal failure, and growth impairment. However, the risk for SCD patients of serious complications (stroke, organ failure, acute chest syndrome, and recurrent pain crises) and mortality with conventional treatment leads many centers to perform related-donor or even unrelated-donor HLA-identical HPC transplantation in patients with particularly aggressive disease.[2] The patient reported here had undergone numerous recurrent vaso-occlusive crises and two splenic crises in his first 5 years of life. After the failure of hydroxyurea therapy, we decided to perform a transplantation of bone marrow from his fully HLA-matched sister.

Unfortunately, relapse of SCD occurs in about 10 percent of the patients who undergo transplantion for SCD.[2] Patients with CML,[6] AML,[7] or various hematologic malignancies[10] and in whom mixed chimerism increases after transplant have a high risk of relapse. For patients with thalassemia major, it has been shown that disease will recur in those with more than 20 percent residual host cells.[11] Our patient harbored 40 percent host cells in the bone marrow (in progressive increase, despite cyclosporine discontinuation), which heralded a probable, imminent recurrence of the disease. It is possible that the relatively low dose of busulfan used (16 mg/kg) favored the occurrence of mixed chimerism.

It is now well demonstrated that DLIs can induce complete remission of relapsing malignant disease after allogeneic HPC transplantation.[5] Moreover, the persistence of donor chimerism was shown to improve the rate of complete remission after DLI[6] and to reduce the risk of marrow aplasia,[5] which justifies the early use of DLIs in case of unstable chimerism or cytogenetic relapse.

Several observations suggest that both normal and leukemic HPCs of host origin should constitute equally effective target cells for donor-derived lymphocytes. First, in vitro, minor histocompatibility antigen-specific cytotoxic T-lymphocytes implicated in the response to DLI cannot discriminate between leukemic HPCs from AML or CML patients and HPCs from bone marrow during their remission.[7] Second, in vivo, tumor response to DLI is associated with increasing donor chimerism.[3] Third, the use of DLIs in patients who do not show residual donor hematopoiesis before DLI induces severe marrow aplasia that can be reversed by the transfusion of donor HPCs, which suggests that host residual HPCs may be targets of DLI.

Complications of DLI include pancytopenia (in about 20% of the cases) and acute or chronic GVHD (in about 60% of cases).[4] Pancytopenia occurs only if a large proportion of hematopoiesis is of host origin at the time of DLI, and it can be reversed with the administration of G–CSF or donor HPC transfusion in the most severe cases.[4] On the other hand, transfusing very low numbers of T cells and increasing the dose in a stepwise fashion was shown to prevent GVHD while preserving the graft-versus-leukemia effect.[12,13] T-cell doses as low as $1 \times 10^7$ per kg can induce complete remission of malignant diseases with a low risk of GVHD.[12]

Therefore, we decided to give low doses of donor lymphocytes ($1 \times 10^7$ CD7+ followed 1 month later, in the absence of GVHD, by a second infusion of $2 \times 10^7$ CD7+ cells/kg) to displace residual host HPCs and prevent overt relapse of SCD. We cannot exclude that the first DLI alone would have been sufficient, but we did not want to risk donor marrow rejection while waiting for the effect of that DLI. We divided the target dose into two incremental doses to reduce the risk of GVHD and to allow the drawing of a reasonable amount of blood from the donor. We did not use leukapheresis to collect donor lymphocytes, because we calculated that enough cells could be obtained in 2 whole-blood units to reach our target cell dose and because that would also provide RBCs to correct the anemia. DLI effectively displaced residual bone marrow host cells and was accompanied by only moderate GVHD, which responded well to treatment with corticosteroids and cyclosporine. There was no sign of even transient marrow hypoplasia. The patient returned to full donor chimerism and had a very favorable course over the next 2 years. In the meantime, a similar approach has been reported in a patient with thalassemia major who also showed unstable chimerism and responded to three DLIs (total dose $7 \times 10^7$ T cells/kg).[14]

In the presence of unstable mixed chimerism after allogeneic BMT for SCD or other nonmalignant disease, DLIs can eliminate the residual and/or recurrent host cells and prevent relapse of the underlying disease without undue complications.

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