

TESTICULAR RELAPSE OF AML DURING CHRONIC GRAFT-VERSUS-HOST DISEASE INDUCED BY DONOR LEUKOCYTE INFUSION

Stefania Tringali, Stefania Vasta, Rosanna Scimè, Patrizia Catania, Anna Maria Cavallaro, Ignazio Majolino

Department of Hematology and Bone Marrow Transplant Unit, Ospedale Cervello, Palermo, Italy

ABSTRACT

Treatment options for acute leukemia relapsing after allogeneic BMT include conventional chemotherapy or a second transplant; however, results are rather discouraging, the first option being associated with poor survival and the second with a high mortality rate. More recently, donor leukocyte infusion (DLI) from the original donor has been used for relapsed patients in an attempt to induce a graft-versus-leukemia (GVL) effect. This procedure is partially devoid of the toxicity inherent to a second BMT. At our Institution, a 36-year-old patient with biphenotypic AML in second complete remission after relapse following allogeneic BMT was treated with peripheral blood stem cell (PBSC)-enriched donor leukocytes, obtained after *in vivo* priming with rhG-CSF. The patient experienced extensive cGVHD but developed a testicular relapse while in full hematologic remission. After irradiation of the sanctuary site he remains free of disease, still with chronic GVHD, 21 months after bone marrow relapse. This case suggests that immunologically privileged sites are inaccessible to GVHD/GVL effect. This should be considered when planning salvage transplants procedures in patients at risk for extramedullary involvement.

Key words: GVHD, GVL, testis, AML, donor leukocyte infusion

The anti-leukemic effect of allogeneic BMT is due in part to the conditioning regimen and in part to the immunoreactive donor cells directed against residual leukemic clones, the so called *graft-versus-leukemia* (GVL) effect.

There is clinical evidence to support the GVL effect. Development of GVHD has a protective action against relapse following BMT. In CML patients, T cell depletion is associated with a low incidence of GVHD and an increase of the relapse rate. In some cases of relapse occurring after BMT, the discontinuation of immunosuppressive therapy has allowed onset of clinically significant GVHD associated with restoration of hematologic remission.¹ In CML relapsed after BMT, the GVL effect has been induced using interferon-alpha and infusions of leukocytes from the original donor.² The same treatment was applied in acute leukemia patients and pro-

duced complete remissions.^{3,4} A GVL effect, however, seems unable to control disease recurrence in the so-called *sanctuary sites*, namely the central nervous system and the testis.⁵

We report here a case of relapsed biphenotypic AML in remission after salvage chemotherapy treated with donor leukocyte infusions. The cells were collected from the original donor after mobilization with rhG-CSF.^{6,7} Despite extensive chronic GVHD involving skin, oral mucosae, liver and eyes, the patient developed an isolated testicular relapse. This supports the idea that GVHD/GVL is not able to control the disease in immunologically privileged sites.

Case report

A 36-year-old male was diagnosed with biphenotypic AML in January 1993. The leukemia cell phenotype was Tdt⁺, DR⁺, CD10⁺, CD19⁺,

CD34⁺, CD38⁺, CD24⁺, CD16⁺, CD33⁺, CD11b⁺, CD42b⁺. He was treated with two chemotherapy courses consisting of Ara-C 100 mg/m²/12h, i.v. for 10 days, etoposide 100 mg/m²/day, i.v. for 5 days, idarubicin 10 mg/m²/day, i.v. for 3 days (GIMEMA AML 10 protocol) and achieved hematologic remission. He relapsed in June 1993 and underwent allogeneic BMT from an HLA-identical sibling without any attempt to induce remission. BMT was given after conditioning with busulphan 16 mg/kg orally and cyclophosphamide 120 mg/kg i.v. GVHD prophylaxis consisted of CSA 3 mg/kg and short-course MTX. The patient developed grade II acute GVHD and was successfully treated with 6-methylprednisolone 0.5 mg/kg/day i.v. CMV pneumonitis was treated with gancyclovir and high-dose i.v. immunoglobulins. No chronic GVHD was observed.

In January 1994, 7 months after BMT, the patient suffered a marrow relapse showing the same phenotypic pattern detected at diagnosis. CSA was stopped and the patient began treatment with prednisone 75 mg/day orally, three weekly administrations of VCR 2 mg i.v. and a single dose of DNR 70 mg i.v. CNS prophylaxis consisted of MTX 12 mg i.t., two administrations; rhG-CSF 300 (µg/day s.c. was also given in an attempt to stimulate residual donor hematopoiesis.⁸ Bone marrow evaluations performed

weekly from day +22 after salvage chemotherapy showed a progressive reduction of leukemic cells. Complete remission was achieved five weeks after starting the treatment.

To reinforce donor chimerism and to induce a GVL effect, the patient then received rhG-CSF-primed donor leukocytes from the original HLA-identical donor, without any prior conditioning treatment (Majolino I, *et al. High incidence of chronic graft-versus-host disease after primary allogeneic peripheral blood stem cell transplantation in patients with hematologic malignancies*. Bone Marrow Transplant 1996; in press) (Figure 1). The donor received rhG-CSF 18 (µg/kg/day for 4 days, and donor leukocytes were collected by apheresis on the fourth and fifth days of treatment using a Fresenius AS 104 cell separator (Fresenius St. Wendel, Germany). A total of 9.1×10^8 /kg nucleated cells, 2.7×10^8 /kg CD3⁺ and 8.4×10^8 /kg CD34⁺ cells were infused on two consecutive days. No GVHD prophylaxis was given. Following primed DLI, blood count remained steady, but on day +51 grade II acute GVHD (skin, gut) again developed; it responded to methylprednisolone 2 mg/kg i.v.

The subsequent clinical course was characterized by extensive chronic GVHD affecting the skin, eyes, liver and oral mucosae, with onset on day +189 after DLI, that required steroid and azathioprine therapy; it was accompanied by

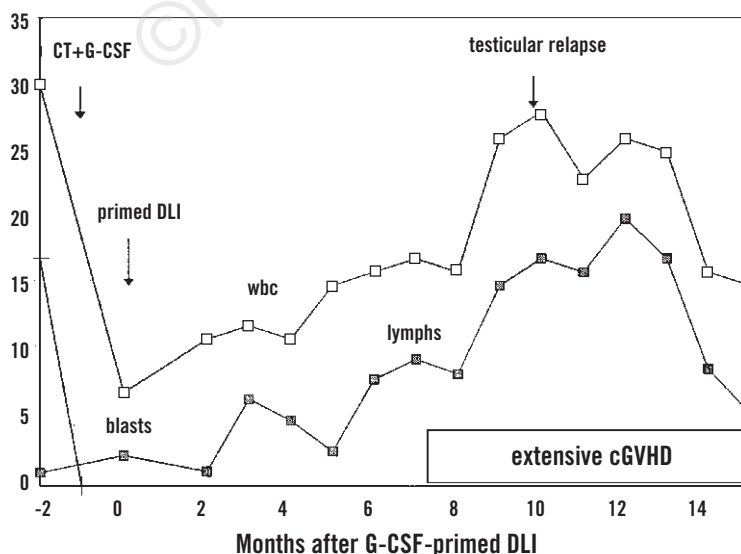


Figure 1. Infusion of donor leukocytes primed with rh-G-CSF as consolidation treatment of recurrent leukemia after allogeneic BMT. Intensive cGVHD and conspicuous CD8⁺ lymphocytosis did not prevent testicular relapse. CT: chemotherapy; DLI: donor leukocyte infusion.

non-infectious fever and by

conspicuous CD8⁺ lymphocytosis (absolute count up to $10.8 \times 10^9/L$ with a CD4/CD8 ratio of 0.12).

In December 1994, 9 months after primed DLI, while still in hematologic remission, the patient experienced a testicular relapse and received local radiotherapy (25G). At the time of this report, 19 months after primed DLI the patient is still alive and in complete remission with extensive chronic GVHD.

Discussion

Patients relapsing after allogeneic BMT have a very poor prognosis and little prospect of prolonged survival after salvage chemotherapy or a second transplant. Front-line therapeutic strategies include selective stimulation of residual donor hemopoiesis with filgrastim⁸ and induction of a GVL reaction by donor leukocyte infusion.⁹ DLI induced haematological and molecular remission in relapsed CML and acute leukemia patients^{3,4} but marrow aplasia remains a potentially fatal complication probably related to the development of acute GVHD.²

In order to reduce the risks of marrow aplasia and to reinforce donor chimerism, we modified the standard DLI procedure by collecting donor mononuclear cells after priming with rhG-CSF, thereby harvesting peripheral blood stem cells (PBSC) as well.

DLI has been used successfully to stimulate a GVL effect in leukemia patients relapsing after BMT since the procedure is partially devoid of the morbidity and toxicity inherent in a second transplantation. Sullivan *et al.*¹⁰ infused donor buffy coat to advanced acute leukemia patients shortly after BMT to reduce relapse rate, but observed more toxicity from GVHD and no reduction in relapse rate. Kolb *et al.* initiated a pilot study of DLI for the treatment of relapse after BMT and successfully treated 3 relapsed CML with IFN and DLI, confirming the efficacy of the adoptive immunotherapy even without chemotherapy.⁹

Despite the ability of DLI to induce and maintain hematologic remission, the *sanctuary sites*, namely the central nervous system and the testis, do not seem to be protected by the

immunologic reactions that characterize cGVHD/GVL.

This report confirms the efficacy of DLI in inducing a potent GVL response. Our patient was in complete hematologic remission after salvage chemotherapy and rhG-CSF at the time of DLI; he was given donor leukocytes mobilized with rhG-CSF. No pancytopenia was observed, while an extensive cGVHD developed, accompanied by conspicuous CD8⁺ lymphocytosis. It is noteworthy that the leukemia-free interval after DLI was longer than after BMT. Unfortunately, an isolated testicular relapse occurred on day 289 after DLI, despite the persistence of hematologic remission and evidence of cGVHD.

Some reports suggest that cGVHD may be responsible for rare CNS pathologic pictures observed after BMT.^{11,12} This would seem to be in contrast with our observation that the CNS and testis represent immunologic sanctuary sites with respect to GVL. However, different humoral and cellular reactions as well as distinct leukemia-specific factors may explain these apparently conflicting interpretations.

Note added in proof

The patient relapsed and died on June 1996.

References

1. Odom IF, August CS, Githens JH, et al. Remission of relapsed leukemia during a graft-versus-host reaction: a graft-versus-host reaction in man? *Lancet* 1978; ii:537-40.
2. Porter DL, Roth MS, Mc Garigle C, Ferrara JLM, Antin JH. Induction of a graft-versus-host disease as immunotherapy for relapsed chronic myeloid leukemia. *N Engl J Med* 1994; 330:100-6.
3. Szer J, Grigg AP, Phillips GL, Sheridan WP. Donor leucocyte infusion after chemotherapy for patients relapsing with acute leukemia following allogeneic BMT. *Bone Marrow Transplant* 1993; 11:109-11.
4. Ferster A, Bujan W, Moureaux T, Devalk Ch, Heimann P, Sariban E. Complete remission following donor leucocyte infusion in ALL relapsing after haploidentical bone marrow transplantation. *Bone Marrow Transplant* 1994; 14:331-2.
5. Goldberg SL. Lack of a graft-versus-leukemia effect in an immunologically privileged sanctuary site (letter). *Bone Marrow Transplant* 1994; 12:180-1
6. Majolino I, Aversa F, Bacigalupo A, Bandini G, Arcese W, Reali G for GITMO. Allogeneic transplants of rhG-CSF mobilized peripheral blood stem cell from normal donors.

- Haematologica 1995; 80:40-3.
7. Majolino I, Buscemi F, Scimè R, et al. Treatment of normal donors with rhG-CSF 16 µg/kg for mobilization of peripheral blood stem cell and their apheretic collection in view of allogeneic transplantation. *Haematologica* 1995; 80:219-26.
 8. Giralt S, Escudier S, Kantarijan H, et al. Preliminary results of treatment with filgrastim for relapse of leukemia and myelodysplasia after allogeneic bone marrow transplantation. *N Engl J Med* 1993; 329:757-61.
 9. Kolb HJ, Mittermuller J, Clemm CH, et al. Donor leukocyte transfusion for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. *Blood* 1990; 76:2462-75.
 10. Sullivan KM, Storb R, Bucner CD, et al. Graft-versus-host as adoptive immunotherapy in patients with advanced hematologic neoplasm. *N Engl J Med* 1989; 320:828-34.
 11. Rouah E, Gruber R, Shearer W, Armstrong D, Hawkins E. Graft-versus-host disease in the central nervous system. A real entity? *Am J Clin Pathol* 1988; 89:543-6.
 12. Marosi C, Budka H, Grimm G, et al. Fatal encephalitis in a patient with chronic graft-versus-host disease. *Bone Marrow Transplant* 1990; 6:53-7.