original paper

haematologica 2001; 86:646-651
http://www.haematologica.it/2001_06/0646.htm

Conventional hematopoietic stem cell transplants from identical or alternative donors are feasible in recipients relapsing after an autograft

Carmela di Grazia, Anna Maria Raiola, Maria Teresa Van Lint, Teresa Lamparelli, Francesca Gualandi, Giovanni Berisso, Stefania Bregante, Alida Dominietto, Nicola Mordini, Barbara Bruno, Francesco Frassoni, Andrea Bacigalupo

Dipartimento di Ematologia, Ospedale San Martino, Genoa, Italy

Correspondence: Carmela di Grazia, M.D., Divisione Ematologia 2 (PAD 5/II), Ospedale San Martino, largo Rosanna Benzi 10, 16132 Genoa, Italy. Phone: international +39-010-355469/5552148.
Fax: international +39-010-355583 - E-mail: emato2@smartino.ge.it

Background and Objectives. The risk of relapse after autologous bone marrow transplantation (ASCT) is high and is related to the type of malignancy and phase of the disease. The outcome for the patient who relapses after an autologous transplant is poor. Some of these patients achieve a remission with conventional chemotherapy, but it is usually short-lasting. Most of them succumb to the original disease. One further therapeutic possibility is an allogeneic transplant which would confer the potential advantage of a graft-versus-leukemia effect in addition to the lack of tumor contamination of the graft and to a high-dose intensity conditioning regimen.

Design and Methods. We have studied the outcome of 31 patients with hematologic malignancies who underwent an allogeneic hematopoietic stem cell transplant (HSCT) after failing an autologous transplant because of relapse (n=29) or persistent aplasia (n=2). The median age at allograft was 36 years (18-55) and the interval from autograft to allograft was 21 months (3-141). The source of stem-cells was unmanipulated bone marrow (n=26) or growth-factor-mobilized peripheral blood (n=5). The donor was an HLA-identical sibling (n=7), or an alternative donor (n=24) (family mismatched n=11, or matched unrelated n=13). The conditioning regimen was cyclophosphamide and thiotepa (n=22), or cyclophosphamide and total body irradiation (n=9) Graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporine (CyA) + methotrexate (MTX).

Results. Acute GvHD was scored as 0-I, II, or III-IV in 39%, 48%, and 13% of the patients, respectively. Sixteen patients died of transplant-related complications and one of progressive disease. With a median follow-up of 220 days (9-2104) the actuarial 2-year transplant-related mortality (TRM) was 51%, the actuarial relapse risk 37%, the actuarial survival 46%. Fifteen patients (48%) are alive in complete remission, with a median follow-up of 32 months (range 2-71).

Interpretation and Conclusions. These data suggest that patients relapsing after an autotransplant should be screened for potential related or unrelated donors: although TRM remains high there is a definite chance of long-term disease-free survival if these patients are allografted.

Key words: autologous transplantation, relapse, allogeneic transplantation

The risk of relapse after autologous bone marrow transplantation (ASCT) is high and is related to the type of malignancy and phase of the disease. This has been regarded as the consequence of either tumor contamination of the graft,[1] persisting residual disease in the patients at the time of ASCT,[2] or a combination of the two. The outcome for the patient who relapses after an autologous transplant is poor. Some of these patients achieve a remission with conventional chemotherapy, but it is usually short-lasting. Most of them succumb to the original disease.

One further therapeutic possibility is an allogeneic transplant which would confer the potential advantage of a graft-versus-leukemia effect (GVL),[3,4] in addition to the lack of tumor contamination of the graft and to a high-dose intensity conditioning regimen. However, only a small number of patients have an HLA-identical sibling donor and the majority will need to have a search initiated for an unrelated donor. The rapid development of a registry network has extended the availability of unrelated donors to patients without a family match and in Italy this is currently 41% for a 6-antigen match and over 70% for a 5-antigen match (*unpublished data*). An additional possibility is the use of a partially mismatched related donor who can be found by extended family HLA typing. However, with or without an HLA-identical sibling donor, the role of allogeneic

transplantation after an ASCT is controversial, especially because these patients are heavily pre-treated and may be at high risk of transplant-related mortality (TRM).

We report here our single Center experience in 31 patients who relapsed after an autologous transplant and were given an allogeneic graft from related or unrelated donors.

Design and Methods

Patients' characteristics

Between March 1995 and December 2000, thirty-one patients underwent an allogeneic stem cell transplant after relapsing (n=29) or failing to recover (n=2) after an autologous transplant at our institution. Diagnoses included acute myeloid leukemia (n=13), acute lymphoid leukemia (n=1), chronic myeloid leukemia (n=10), non-Hodgkin's disease (n=4), multiple myeloma (n=1), myelodysplasia (n=1) and Hodgkin's disease (autograft failure) (n=1). Seventeen patients were male and fourteen patients were female. The median age of the patients at the time of allogeneic transplant was 36 years (range: 18-55). The median interval from diagnosis to ASCT was 8 months (range: 3-82). Nine patients received autologous bone marrow, eighteen granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood, one patient bone marrow plus G-CSF mobilized peripheral blood. In three patients the information is lacking. The median interval between ASCT and subsequent relapse, evaluable in 27 patients, was 9 months (range 2-17); this information was not available for two patients, in another two patients there was persistent aplasia and in one patient there was progressive disease. Patients underwent allogeneic transplant at a median of 21 months (range: 3-141) following ASCT. The donor was an HLA–identical sibling for seven patients and an alternative donor for twenty-four patients. Thirteen patients underwent matched-unrelated donor (MUD) transplants and eleven patients received mismatched marrow from related donors. The source of stem cells was bone marrow for twenty-six patients and G-CSF-mobilized peripheral blood for five patients. The median Karnofsky performance status score prior to allogeneic transplant was 50% (range: 40-80%). The disease status at the time of second transplant is shown in Table 1.

Table 1. Clinical data.

No. of patients	31
Donor	
Gender m/f	15/16
Age	36 (14-77)
Patient	
Gender m/f	17/14
Age	36 (18-55)
Disease	
Acute myeloid leukemia	13
Acute lymphocytic leukemia	1
Chronic myeloid leukemia	10
Non Hodgkin's lymphoma	4
Multiple myeloma	1
Myelodysplastic syndrome	1
Hodgkin's disease	1
Phase at HSCT	
1st CR/CP	2
2nd CR/CP	21
2nd relapse	5
3rd relapse	3
Interval dx-tx	979 (242-7935)
Transplant	
source BM/PB	26/5
cells $< \times 10^8$/kg	5 (2-20.5)
Conditioning regimens	
Cy-TBI	9
Cy-thio	22
Gvhd prophylaxis	
CyA-MTX	31

Abbreviations: CR, complete remission; CP, chronic phase; BM, bone marrow; PB, peripheral blood; Interval dx-tx, time in days between diagnosis and transplant; Cy, cyclophosphamide; TBI, total body irradiation; Thio, thiothepa; CyA, cyclosporine; MTX, methotrexate.

Transplantation programs

Autograft. The conditioning regimen for the autograft consisted of melphalan or etoposide or idarubicin plus TBI in three patients, melphalan or busulfan alone in six and high-dose combination chemotherapy in twenty. This information is lacking for two patients.

Allograft. The preparative regimens for allograft were: a) cyclophosphamide (CY) 100-150 mg/kg and thiotepa (TT) 10-15 mg/kg (n=22) with the addition of total body irradiation 50 rads (n=1), or rabbit antithymocyte globulin (ATG) (Thymoglobuline Sangstat, Merieux, France) 3.75 mg/Kg for 4 consecutive days (n=6), or fludarabine (FLU) 30mg/m^2 for 3 consecutive days (n=5); b) CY 120 mg/kg- total body irradiation (9.9-12Gy) (n= 9) plus ATG 3.75 mg/kg for 4 consecutive days (n=3). Graft-versus-host disease (GvHD) prophylaxis consisted of cyclosporine (CyA) and methotrexate (MTX) for all patients.

Statistical analyses

All analyses were performed with NCSS software. Kaplan-Meier curves for patient survival, risk of

Table 2. Outcome.

No. of patients	n=31
Day PMN > 0.5×10⁹/L	18 (13-24)
Day PLT >30×10⁹/L	24 (11-93)
PLTS day 21	15 (4-45)
PLTS day 50	55 (9.5-252)
PLTS day 100	55 (12-195)
Acute GVHD	
0-I	12
II	15
III-IV	4
Chronic GVHD	
absent	12
limited	11
extensive	8
Surviving	15
Dead	16
Cause of death	
relapse	1
infection	11
aGVHD+IP	1
rejection	1
hemorrhage	1
multiorgan failure	1
Median follow-up	
HLA id. sib	395 (9-1735)
alternative	182 (14-2104)

Abbreviations: IP, idiopathic pneumonitis.

relapse and TRM were calculated with the product limit method. Event times were measured from date of allograft to date of relapse, death or last follow-up examination.

Results

Engraftment

One patient rejected the graft and died. Twenty-five patients were evaluable for neutrophil engraftment and twenty-three for platelet engraftment. Six patients died prior to neutrophil recovery on a median of day +16 (range 9-106). The median time to achieve an absolute neutrophil count of > 0.5×10⁹/L was 18 days (range 13-24) in twenty-five patients. The median time to achieve a platelet count of 30×10⁹/L was 24 days (range 11-93) in twenty-three patients. Table 2 shows the patients' clinical outcome.

Graft–versus-host disease

Twenty-nine patients were evaluable for acute GVHD (aGVHD) scored as grades 0-I, II, or III-IV in 39%, 48%, and 13% of patients, respectively. Twenty patients were alive on day +100 and evaluable for chronic GVHD (cGVHD) (11 patients died before 100 days) which was scored as absent (n=1), limited (n=11) or extensive (n=8).

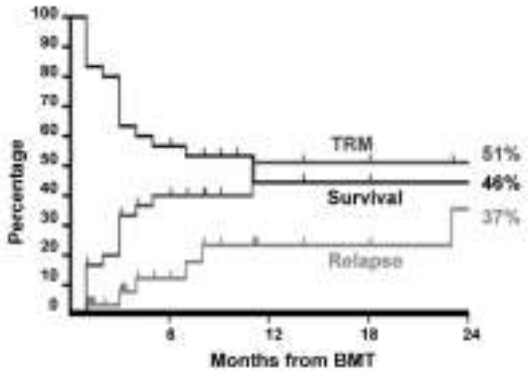

Figure 1. Two-year actuarial probability of transplant-related mortality, survival and relapse rate.

Survival, relapse and cause of death

The Kaplan-Meier actuarial survival, relapse risk (RR) and transplant-related mortality (TRM) at 2 years were 46%, 37% and 51%, respectively (Figure 1). When we evaluated patients according to donor type, HLA identical siblings and alternative donors, there was no significant difference in OS , RR and TRM in the two groups. Sixteen patients died (52%) at a median of interval of 65 days after transplant (range: 30-330). One patient died of progressive disease. Fifteen patients died of transplant-related mortality. The causes of death were rejection of the graft in one patient, infections in eleven, aGVHD with idiopathic pneumonitis in one, multiorgan failure (MOF) in one, and hemorrhage in one. Three patients relapsed: of these one obtained complete remission (CR) after reduction of immunosuppressive therapy, one received donor lymphocyte infusions (DLI) and is currently alive and in complete remission, one obtained CR with additional chemotherapy DLI and has now relapsed with extramedullary disease. Fifteen patients (48%) are alive and in CR after a median follow-up of 32 months (range: 2-71).

Discussion

We have shown in the present study that allogeneic transplant is a therapeutic option for patients up to

55 years of age who relapse after an autograft. This is true even for patients who lack an HLA identical sibling, and for whom an alternative donor can be identified. These findings have two important clinical implications: a) relapse after an auto-transplant should not be regarded as a terminal event, and b) a search for an alternative donor for patients who lack an HLA identical sibling should be initiated soon after the post-autograft relapse.

Transplant-related mortality was not negligible in our study: 58% in alternative donor recipients and 14% for HLA identical recipients for an overall rate of 48%. TRM has been reported to be as high or greater in other similar studies of allografts performed after a failed autograft: Tsai *et al.*[5] reported a TRM of 85% and the median survival of 2 months. Metha *et al*.[6] reported a 72% mortality in 42 myeloma patients. Ringden *et al.*[7] reported a 51% TRM in 94 patients.

Thus the major problem for a patient undergoing an allograft after failed autograft is transplant-related mortality. There are at least three 3 ways to reduce TRM: select patients, reduce the intensity of the conditioning regimen, reduce the complications of a conventional conditioning regimen. Selection of donors may imply both restricting the procedure to younger patients with excellent performance status and improving donor/recipient match. Recently Frumento *et al.*[8] tested the hypothesis that specific amino acid substitutions within class I molecules would have a different impact on aGVHD and TRM in patients receiving unmanipulated marrow from an unrelated donor. In 100 donor/recipient pairs, otherwise matched by PCR-SBT for DRB1/3/4/5, DQA1, and DQB1 loci, non-conservative substitutions at position 116 (n=29 patients) were associated with significantly more aGvHD (p=0.003) and TRM (p=0.002) than conservative substitutions or no substitutions. Reduced intensity conditioning regimens have received some attention,[9] but are probably not suitable for patients with advanced disease, as indicated by the recent report:[10] indeed, it is unlikely that patients with advanced disease, as indicated by recurrence after an autograft, would be cured by reduced intensity transplants. As to improving tolerance of conventional conditioning regimens, we should keep in mind that high-dose chemo-radiotherapy does not produce tissue damage if followed by syngeneic cells.[11] This is the case also in man as proven by no case of TRM in 36 twin transplants in patients with chronic myeloid leukemia receiving high-dose TBI, busulphan and cyclophopshamide.[12] Therefore one might consider that the alternative to reducing the intensity of the conditioning would be to prevent allo-reactivity and this would be of particular importance in heavily pretreated patients, who have failed to benefit from chemotherapy and also an autograft. The same hypothesis is sustained by others authors. Aschan *et al.*[13] who analyzed the outcome of seven patients submitted to transplant from unrelated donors after failing an ASCT which resulted in a 5-year probability of survival of 57% ascribed the success, in part, to the use of anti-T-cell antibodies in the preparatory regimen, because these cells improve engraftment and reduce the risk of aGVHD. An ATG schedule was not reported in the study previously reported. In our series , nine patients received ATG at a dose of 15 mg/kg in the conditioning regimen, four of them died of infection . An alternative approach is early identification of patients at risk of GvHD. Bacigalupo *et al.*[14] showed that GvHD and TRM can be predicted on day +7 post-transplant on the basis of blood nitrogen urea (BUN) and serum bilirubin levels. These high-risk patients would be eligible to receive pre-emptive treatment of GvHD.

Infections caused 68% of all deaths, despite prophylaxis with ciprofloxacin (500 mg/12h/ day) and fluconazole (100 mg/12h/day) and early treatment of fever of unknow origin with intravenous antibiotics and amphotericin B. In the present series all patients who received an alternative donor transplant were treated with foscarnet for cytomegalovirus prophylaxis on a daily basis for 100 days. This is probably still not sufficient to protect patients from lethal infections, and we should aim to improve hematologic and immunologic reconstitution. One approach could be the use of a high cell dose at transplant which has been shown to reduce the risk of transplant-related complications[15] and improve hematologic reconstitution.[16]

The actuarial risk of relapse at two years in our study was 37% in keeping with results from other studies which reported relapse rates between 30% and 70%.[17,20] This is clearly inferior to the risk of dying of a transplant complication, suggesting that particular attention needs to be given to the procedure and ways to improve it.

The largest study evaluating the impact of an allograft after failing an autograft was published very recently by Ringdén *et al.*[21] Ninety patients underwent an allograft, 74 an autograft and 2,584 were treated with chemotherapy. The 2-year survival was 32%, 42% and 11% in the three groups, respectively, but when they compared patients receiving matched related or unrelated marrow with patients receiving graft from mismatched marrow 2-year survival was 37% vs 13%.

In conclusion screening of potential donors should be undertaken at the time a patient relapses after an autograft: despite the high-transplant mortality there is significant chance of long-term disease-free survival if a suitable donor is identified.

Contributions and Acknowledgments

CdG is a recipient of an educational grant from the Department of Hematology, San Martino Hospital, Genoa. CdG was responsible for data analysis and writing the manuscript. AMR, MTVL, TL, FG, GB, SB, AD, NM and FF were responsible for the patients' care. BB collaborated in the statistical analyses. AB was responsible for the analysis of the results and writing the paper.

The excellent work of our nursing staff is gratefully acknowledged.

Funding

This work was supported by Associazione Italiana Ricerca contro il Cancro (A.I.R.C.) Milan, and the Associazione Ricerca Trapianto Midollo Osseo (A.RI.T.M.O.), Genoa, Italy.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Claudio Anasetti, who acted as an Associate Editor. The final decision to accept this paper was taken jointly by Dr. Anasetti and the Editors. Manuscript received February 23, 2001; accepted May 2, 2001.

Potential implications for clinical practice

Relapse after an ASCT is not a terminal event; encourage results to search an alternative donor; a conventional conditioning regimen is feasible in patients relapsing after an ASCT; transplant-related mortality needs to be reduced; long-term disease-free survival can be achieved after an allogeneic graft.

References

1. Brenner MK, Rill DR, Moen RC, et al. Gene-marking to trace origin of relapse after autologous bone-marrow transplantation. Lancet 1993; 341:85-6.
2. Hagenbeek A, Martens AC. Reinfusion of leukemic cells with the autologous marrow graft: preclinical studies on lodging and regrowth of leukemia. Leuk Res 1985; 9: 1389-95.
3. Weiden PL, Flournoy N, Thomas ED, Fefer A, Buckner CD, Storb R. Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts. N Engl J Med 1979; 300:1068-73.
4. Horowitz MM, Gale RP, Sondel PM, et al. Graft–versus-leukemia reactions after bone marrow transplantation. Blood 1990; 75:555-62.
5. Tsai T, Goodman S, Saez R, et al. Allogeneic bone marrow transplantation in patients who relapse after autologous transplantation. Bone Marrow Transplant 1997; 20:859-63.
6. Mehta J, Tricot G, Jagannath S, et al. Salvage autologous or allogeneic transplantation for multiple myeloma refractory to or relapsing after a first-line autograft? Bone Marrow Transplant 1998; 21:887-92.
7. Ringden O, Labopin M, Frassoni F, et al. Allogeneic bone marrow transplant or second autograft in patients with acute leukemia who relapse after an autograft. Acute Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplant 1999; 24:389-96.
8. Frumento G, Morabito A, Pozzi S, et al. Relevance of amino acid substitution at position 116 of HLA class I molecules for unrelated bone marrow transplantation. Hematol J 2000; 6(Suppl 1):210.
9. Barrett A J, Locatelli F, Treleaven JG, Gratwohl A, Szydlo R, Zwaan FE. Second transplants for leukaemic relapse after bone marrow transplantation: high early mortality but favourable effect of chronic GVHD on continued remission. A report by the EBMT Leukaemia Working Party. Br J Haematol 1991; 79:567-74.
10. Uberti J, Ayash L, Reynolds C, et al. Lower intensity of preparation regimen for allogeneic stem cell transplantation from related donor in patients with advanced hematologic malignancy is associated with poor outcome. Abstract n°858; 42nd annual meeting of American Society of Hematology.
11. Hill GR, Cooke KR, Brinson YS, Bungard D, Ferrara JL. Pretransplant chemotherapy reduces inflammatory cytokine production and acute graft-versus-host disease after allogeneic bone marrow transplantation. Transplantation 1999; 67:1478-80.
12. Frassoni F, Niederwieser D, Gratwohl A, et al. The Chronic Leukemia Working Party of EBMT. Abstract 2846; 41st Annual Meeting of American Society of Hematology.
13. Aschan J, Remberger M, Carlens S, et al. Unrelated donor stem cell transplantation after autologous transplantation: experience of a single center. Bone Marrow Transplant 1999; 24:279-82.
14. Bacigalupo A, Oneto R, Bruno B, et al. Early predictors of transplant-related mortality (TRM) after allogeneic bone marrow transplants (BMT): blood urea nitrogen (BUN) and bilirubin. Bone Marrow Transplant 1999; 24: 653-9.
15. Bacigalupo A, Dominietto A, Lamparelli T, et al. Marrow cell dose is a significant predictor of outcome in patients undergoing allogeneic hematopoietic stem cell transplantation. Abstract 850; 42nd Annual Meeting of American Society of Hematology.
16. Sierra J, Storer B, Hansen JA, et al. Transplantation of marrow cells from unrelated donors for treatment of high-risk acute leukemia: the effect of leukemic burden, donor HLA-matching, and marrow cell dose. Blood 1997; 89:4226-35.
17. Mrsìc M, Horowitz MM, Atkinson K et al. Second HLA-identical sibling transplants for leukemia recurrence-Bone Marrow Transplant 1992; 9:269-75.

18. Chiang KY, Weisdorf DJ, Davies SM, et al. Outcome of second bone marrow transplantation following a uniform conditioning regimen as therapy for malignant relapse. Bone Marrow Transplant 1996; 17:39-42.
19. Radich JP, Gooley T, Sanders JE, Anasetti C, Chauncey T, Appelbaum FR. Second allogeneic transplantation after failure of first autologous transplantation. Biol Blood Marrow Transplant 2000; 6:272-9.
20. Blau IW, Basara N, Bischoff M, et al. Second allogeneic hematopoietic stem cell transplantation as treatment for leukemia relapsing following a first transplant. Bone Marrow Transplant 2000; 25:41-5.
21. Ringden O, Labopin M, Gorin NC, et al. The dismal outcome in patients with acute leukaemia who relapse after an autograft is improved if a second autograft or a matched allograft is performed. Acute Leukaemia Working Party of the European Group for Blood and Marrow Transplantation (EBMT). Bone Marrow Transplant 2000; 25:1053-8.