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Hematopoietic stem cell transplantation in hematologic malignancies and solid tumors: Hacettepe University Institute of Oncology experience

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ABSTRACT

Autologous and allogeneic hematopoietic stem cell transplantation (HSCT) have emerged as a frequently used treatment modality in patients with hematologic malignancies and selected solid tumors. Hematopoietic Stem Cell Unit of Hacettepe University Institute of Oncology is recently inaugurated and activated in January 2000. Until January 2003, 57 autologous HSCT and 14 non-myeloablative allogeneic transplantations (NST) have been performed. The median age of patients at the time of transplantation was 41 (range 17-75). The mean time elapsed from diagnosis to transplantation was 3.7 years and patients had received a mean number of 2.7 salvage regimens prior to transplantation. During the mean follow-up period of 12.7 (1-35) months, 9 patients relapsed and 11 died. Of the 51 (82%) surviving patients, 46 (92%) were in complete remission. The estimated 3-year overall survival (OS) of all patients was 81%. Fifty-seven autologous transplantations were performed in 55 patients and transplant-related mortality (TRM) was 5%. Three out of 55 patients died within 100 days, due to CMV pneumonia, veno-occlusive disease and infection secondary to poor graft function. Three patients died during the late post transplantation period, two due to relapse and one due to infection. The estimated 3-year disease-free survival (DFS) and OS of 42 patients with lymphoma undergoing autologous HSCT were 74% and 79%, respectively. Eighteen autologous and allogeneic transplantations were performed in 12 patients with myeloma. Six patients underwent double transplants, i.e. 3 patients double autologous, 2 patients autologous followed by NST and one patient conventional allogeneic followed by NST. As a result of these transplants in 10 evaluable patients with myeloma, 9 (90%) were in CR and 1 (10%) was in PR. All patients are alive and 3-year OS is 100% in the myeloma group. NST was performed in 13 patients with the diagnosis of relapsed Hodgkin's disease, non-

Hodgkin's lymphoma and multiple myeloma, metastatic renal cell carcinoma, relapsed AML, resistant CLL, CML, and large granular leukemia. The survival rate at day +100 was 84.6% for all patients undergoing NST. To date, with an observation period ranging between 1 to 21 months, 10 of 13 patients are alive, majority being in CR. In 11 patients in which tumor response could be evaluated, CR was achieved in 9 (82%). The estimated probability of 20-month OS in these patients was 76%, with an overall TRM of 14%; 0% in patients without previous HSCT and 25% in patients with previous HSCT. Acute GVHD (grade I-III) was observed in 5 of 13 patients all following donor lymphocyte infusion, none developing within the first 100 days. None of our patients developed grade IV acute GVHD and mortality related to GVHD was 0%. Achievement of successful outcome in autologous and allogeneic transplantation in the unit highly depends on our multidisciplinary team approach. [Turk J Cancer 2003;33(1):27-39]

KEY WORDS:

Hematopoietic stem cell transplantation, autologous, non-myeloablative

INTRODUCTION

Widespread use of autologous or allogenic hematopoietic stem cell transplantation (HSCT) over the past decade has led to cure or prolongation of survival in patients with hematologic cancers. Among these diseases, acute leukemias, chronic myelocytic leukemia, myeloma and lymphomas are the most common indications for HSCT (1-7).

Autologous and allogeneic transplantation program were activated at Hacettepe University Institute of Oncology since January 2000 and June 2001, respectively. This report summarizes the experience of the transplant team at Hacettepe University Institute of Oncology.

PATIENTS AND METHODS

Seventy-one autologous HSCT and non-myeloablative allogeneic transplantation (NST) performed in 62 patients at Institute of Oncology between January 2000 and December 2002 were analyzed. During this period, 57 autologous HSCT and 14 NST were performed. Eligibility criteria were: 1) Age 17 to 60 years (upper age limit was not applied to patients undergoing NST and in patients with myeloma); 2) normal hepatic, cardiac and pulmonary functions based on echocardiography, pulmonary function tests, and clinical and laboratory evaluations; 3) ECOG performance status less than or equal to 2. Patients were excluded if they had a positive serologic test for HIV or a serious coexisting illness. A written informed consent was obtained from all patients. Patients with chemoresistant disease except in patients with myeloma were excluded.

Autologous HSCT

From January 2000 to December 2002, 55 patients with relapsed/refractory Hodgkin's disease, relapsed/refractory/high risk non-Hodgkin's lymphoma, newly diagnosed/ relapsed/refractory multiple myeloma, acute myeloid leukemia (AML) without a matched sibling and relapsed testicular carcinoma underwent autologous HSCT following high-dose chemotherapy. Double autologous HSCT was performed in 2 patients with myeloma. Except in myeloma, patients resistant to salvage chemotherapy were considered ineligible for HSCT. Response to HSCT was determined on day +100.

Peripheral blood stem cell mobilization and preparative Regimen

Mobilization regimen consisted of cyclophosphamide (4.5 g/m^2) and granulocyte colony stimulating factor (G-CSF) (10 µg/kg/d) combination in lymphoma patients. In patients with multiple myeloma, AML and testicular carcinoma, G-CSF (10 µg/kg/d) alone was used prior to stem cell collection. Cells were collected with 2 or 3 aphereses when neutrophil count reached >5000x10^9/l. Target dose was at least 2x10^6 CD34+ cells or 5x10^8 mononuclear cells per kg body weight.

In lymphoma patients, high-dose sequential therapy (Figure 1) consisting of etoposide (2 g/m^2), mitoxantrone (60 mg/m^2) and melphalan (180 mg/m^2) was used as the conditioning regimen following successful salvage therapy. High dose melphalan at a dose of 200 mg/m^2 was administered to patients with myeloma patients prior to hematopoietic stem cell (HSC) infusion. Conditioning

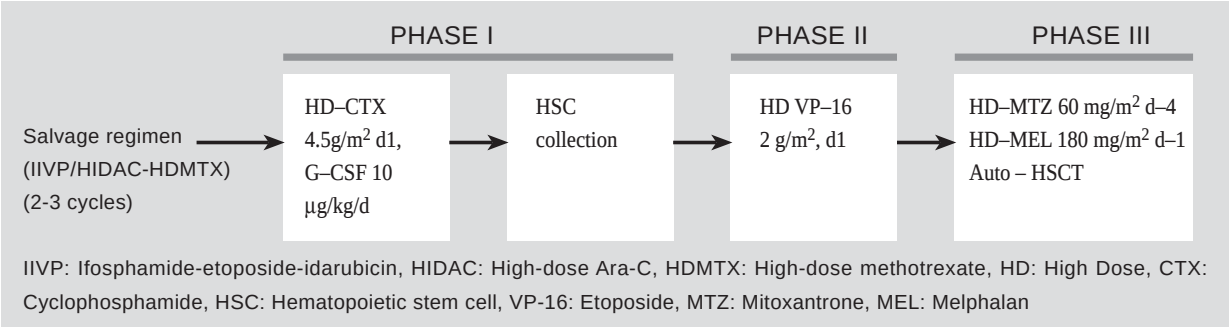


Fig 1. High-dose sequential therapy conditioning regimen administered to patients with Hodgkin's and non-Hodgkin's lymphoma

regimen was carboplatin (AUC=25)/melphalan (180 mg/m^2) in patients with testicular carcinoma and mitoxantrone (60 mg/m^2)/melphalan (180 mg/m^2) in patients with AML.

Supportive care

All patients received antifungal prophylaxis with fluconazole 200 mg per day. Acyclovir was given at a dose of 250 mg/m^2 twice daily in patients with positive HSV serology. Broad-spectrum combined intravenous antibiotics were initiated for fever greater than 38.3°C in the setting of neutropenia. Myeloma patients received intravenous immunoglobulin 400 mg/kg on day +4. G-CSF 10 µg/kg/day was administered intravenously beginning 1 day after hematopoietic stem cell (HSC) infusion and continued until engraftment. Platelet transfusions were routinely administered to keep the platelet count above 20x10^9/l. Red cell transfusions were administered to maintain the hematocrit level over 27%.

Maintenance

In lymphoma patients, post-transplant maintenance therapy included interferon-alpha administered subcutaneously at 1-5x10^6 units, three times weekly starting after day +100 when platelet count reached >100x10^9/l. Interferon therapy was usually continued for 18 months until disease progression or patient intolerance was observed. In myeloma patients, thalidomide was started at a dose of 200 mg/day as the maintenance therapy for 18 months.

Non-myeloablative allogeneic transplantation

The indications for NST were relapse following autologous transplantation in Hodgkin's disease, non-Hodgkin's lymphoma and multiple myeloma, metastatic renal cell carcinoma, relapsed AML, resistant CLL, CML, and large granular leukemia.

HLA tissue typing

In the Department of Basic Oncology, Immunogenetics and Molecular Tissue Typing Laboratory, procedures for histocompatibility were designated according to the regulations/criteria of European Federation for Immunogenetics (EFI) and/or American Society for Histocompatibility and Immunogenetics (ASHI). Strict precautions for contamination control were taken such as dedicated material and equipment as well as separated pre- and post-amplification areas.

Donor samples were collected from both siblings and parents when/if available. DNA was extracted from peripheral blood mononuclear cells using commercial DNA extraction kits as described by the manufacturer (Qiagen GmbH. Hilden, Germany). Back up of each sample was obtained by using phenol-chlorophorm extraction method. Both quantitative and qualitative evaluation of DNA samples were done by using Perkin-Elmer U.V. spectrophotometer. HLA alleles were assigned employing low resolution Sequence Specific Primers (SSP) method (Pel-Freez Clinical Systems LLC. Brown Deer, WI, U.S.A.). PCR setup and thermocycling conditions were performed as described by the manufacturer (M.J Research PTC-100. Watertown, MA, U.S.A.). Post-amplification procedure was 2% agarose gel electrophoresis followed by photography and visual evaluation. Allele assignments, and family tree studies were controlled at least by 3 observers and checked by using software provided by the manufacturer. Results and personal data were recorded on confidential basis by password in

protected files and computers (8-10).

Stem cell collection and conditioning therapy

All patients received unmanipulated HSC from matched sibling donors. Donors were mobilized with G-CSF at 10 µg/kg/d for administered 5 to 7 days. Cells were collected by 2 to 3 aphereses performed on days 5-7 after initiation of G-CSF.

Conditioning regimen before infusion of allogeneic stem cells mainly included immunosuppressive therapy with fludarabine and single dose total body irradiation (TBI). In patients with disease necessitating immediate cytoreductive therapy (patients no:1-4 and 14), cytarabine was added to the conditioning regimen before emergence of graft versus tumor effect. Two NST patients with relapsed myeloma received melphalan only instead of fludarabine/TBI regimen as they were already immunosuppressed due to prior autologous HSCT. A patient with renal cell carcinoma received cyclophosphamide/fludarabine as the preparative regimen for NST.

Prophylaxis of Graft-versus-Host disease (GVHD)

GVHD prophylaxis consisted of cyclosporin-A (CsA) administered as 24 hour infusion at 2.5 mg/kg and oral mycophenolate mofetil (MMF) at 1500 mg/d starting the day before allogeneic HSC infusion. CsA dose was adjusted daily to maintain the target blood level between 475 and 525 ng/ml. CsA was switched to oral administration at 5 mg/kg/d following engrafting and discontinuation of TPN. CsA was slowly tapered according to protocol schedule after day +60 and discontinued before day +100. MMF was discontinued on day +180 if there were no signs of GVHD.

Supportive care

All patients received antibacterial and antifungal prophylaxis with levofloxacin 500 mg per day, ornidazole 200 mg twice daily and fluconazole 200 mg per day. Acyclovir was given at 250 mg/m^2 twice daily to patients with positive HSV serology. Peripheral blood CMV DNA titers were monitorized weekly by PCR assays. Broad-spectrum intravenous antibiotics were initiated when fever over 38.3°C was observed during neutropenia and for documented infections. Prophylaxis against Pneumocystis carinii included trimethoprim/sulfamethaxazole p.o. qd, thrice weekly, administered during the period between engraftment and day +360. Hematocrit and platelet counts were maintained with irradiated blood products above >27% and >20x10^9/l, respectively. G-CSF at 5 µg/kg/d was administered between day +1 and time of engraftment.

Donor lymphocyte infusion (DLI)

DLIs were routinely performed on day +30 and day +100 to achieve full chimerism. Then, decision for DLI was made according to status of chimerism, disease activity or presence of GVHD. DLI was infused at a starting cell dose of 1x10^7 CD3+ cells/kg.

Analysis of chimerism

Chimerism was assessed by cytogenetic analysis using fluorescent in situ hybridization (FISH) in sex mismatched NSTs. PCR based cytogenetic analysis of various number of tandem repeats (VNTR) was performed in the setting of sex-mismatched transplants.

Ambulatory Nursing Care

Medical nurse coordinator monitored patients on out-patient basis. She was responsible for administration of blood products and routine intravenous medications, routine care of central catheters, and education activities for patient home care and self injections.

Dental care

Dental care of the patients was carried out at the Department of Oral Diagnosis in the Faculty of Dentistry of Hacettepe University.

Coordinator services

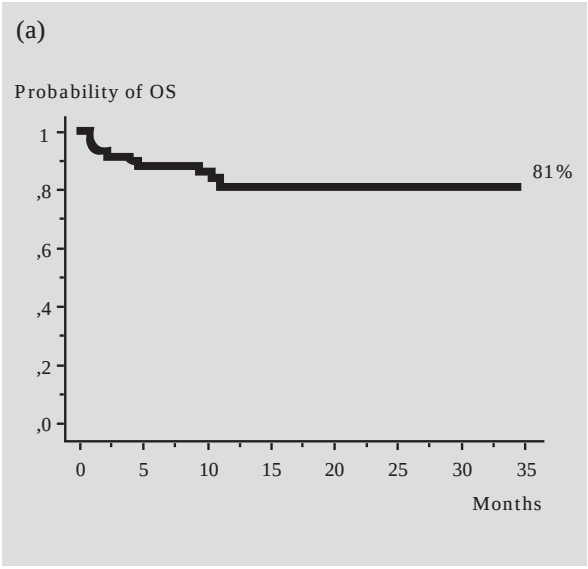
One social and one research coordinator organized the maintenance of out-patient clinic, appointments and financial reimbursement of patients with social security civil public insurance services. Coordinators reported all the patient data on a regular basis to EBMTR (CIC code:292), ABMTR and IBMTR (Center code:589) every 6 months.

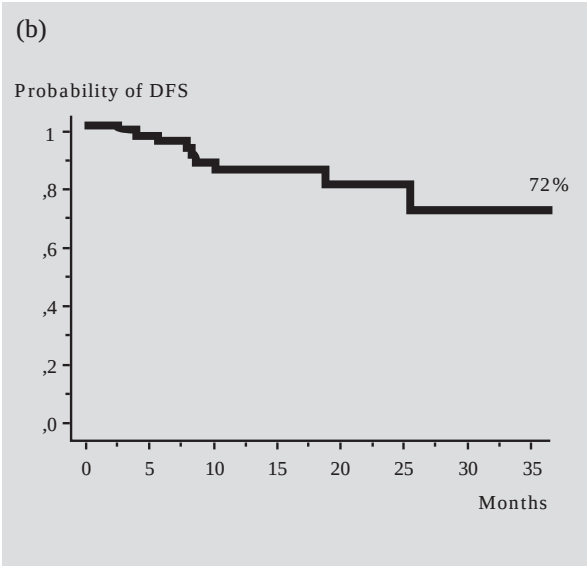
Long-term follow-up

Chronic GVHD and late effects of NST were followed and recorded during periodic outpatient visits.

Statistical Analysis

The primary end-point for this report is to determine overall survival (OS) and disease-free survival (DFS). OS was defined from the day of autologous HSCT until death from any cause or latest contact with the patient. DFS was defined as the time from the day of transplantation until disease progression, death from any cause or latest follow-up. Transplant-related mortality (TRM) was the secondary end-point and was defined as death from any cause except relapse during the first 100 days of transplantation. Kaplan-Meier method was used to calculate estimated DFS and OS (11).





RESULTS

Overall, the median age of 71 patients at the time of transplantation was 40.6 (range 17-75). The patient population comprised of 24 (34%) females and 47 (66%) males. The mean time elapsed from diagnosis to transplantation was 3.7 years and patients had received a mean number of 2.7 salvage regimens prior to transplantation. Ten patients had primary refractory disease before receiving their salvage regimen.

During the mean follow-up period of 12.7 (range 1-35) months, 9 patients relapsed and 11 died. Cause of death was primary disease in 5 patients, infection in 3 patients, poor graft function and infection in 2 patients and veno-occlusive disease in one patient. Of the 51 (82%) surviving patients, 46 (92%) were in complete remission. The estimated 3-year survival of all patients was 81%. The DFS and OS data are shown in figure 2.

Autologous HSCT

Fifty-seven autologous transplantations were performed in 55 patients with a median age of 40 (range 17-75). Double autologous HSCT was performed in 2 patients with myeloma. Majority of patients had a diagnosis of lymphoma, 55% being NHL and 22% being HD. Tables 1 and 2 give an overview of characteristics and outcome of the patient population, grouped according to disease, respectively.

Fig 2. The estimated (a) 3-year overall survival (OS) and (b) 3-year disease-free survival (DFS) of all patients undergoing autologous and allogeneic HSCT

Table 1
Characteristics of patients undergoing autologous HSCT

	All HSCT (n=57)	NHL* (n=24)	LL & BL (n=6)	HD (n=12)	MM (n=13)	Testis (n=1)	AML (n=1)
Age							
Median	40	45.5	24.5	32.5	53	25	31
Range	17-75	18-61	17-36	20-51	33-75		
Sex							
Female	18	8	3	1	5	-	1
Male	39	16	3	11	8	1	-
Time to HSCT (year)	1.8 (0.4-22)	1.7 (0.9-11.4)	0.9 (0.5-1.9)	4.8 (1.2-22)	1 (0.3-7)	-	-
Presalvage status							
1st relapse	21	10	2	6	3	-	-
2nd relapse	8	3		3	1	1	-
>2 relapse	4	2		2	-	-	-
Primary refractory	10	5		1	4	-	-
Newly diagnosed	14	4	4	-	5	-	1
Median follow-up (mo)	12.5 (0.8-34)	19 (1-33)	10 (4-14)	13 (1-35)	9 (3-32)	-	-
NST after autologous HSCT	6	2	-	1	3	-	-

** All subtypes except lymphoblastic lymphoma and Burkitt lymphoma*

HSCT: Hematopoietic stem cell transplantation, NHL: Non-Hodgkin's lymphoma, LL: Lymphoblastic lymphoma, BL: Burkitt lymphoma, HD: Hodgkin's disease, MM: Multiple myeloma, AML: Acute myeloblastic leukemia, NST: Non-myeloablative allogeneic transplantation

Table 2
Overview of outcome of all patients after autologous HSCT

Diagnosis	n*	TRM	CR at day +100	Outcome			3-year OS
				Relapse	CR	Death	
Lymphoma	42	3 (7%)	38 (90%)	6 (14%)	32 (76%)	8 (19%)	79%
NHL	30	1	28 (93%)	5 (17%)	23 (77%)	6 (20%)	77%
HD	12	2	10 (83%)	1 (8%)	9 (75%)	2 (17%)	83%
Myeloma (n=11)	13	0	6 CR, 4 PR,1 NR	3 (23%)	5 (39%)	0	100%
Testicular cancer	1	0	1	-	1	0	-
AML	1	0	1	-	1	0	-
All patients (n=55)	57	(%5)	46 (81%)	9 (16%)	39 (68%)	8 (14%)	83%

** n: Number of transplantations*

TRM: Transplantation related mortality, CR: Complete remission, OS: Overall survival, PR: Partial remission, NR: No response, NHL: Non-Hodgkin's lymphoma, HD: Hodgkin's disease, AML: Acute myeloblastic leukemia

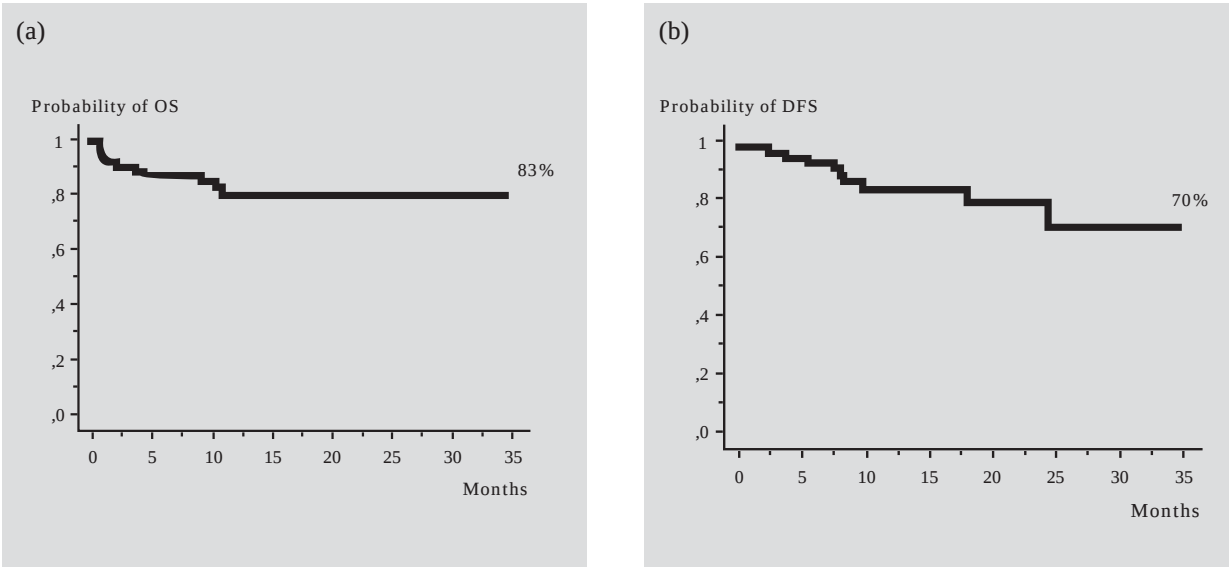


Fig 3. The probability of (a) overall survival (OS) and (b) disease-free survival (DFS) of patients undergoing autologous HSCT

Lymphoma

The median age of 42 patients with Hodgkin's and non-Hodgkin's lymphoma was 39 years (range 17-61). Outcome of autologous HSCT in lymphoma patients are shown in table 3, figures 4 and 5.

Table 3
Results of autologous HSCT in patients with lymphoma

Diagnosis	3-year OS	3-year DFS
All patients (HD and NHL, n=42)	79%	74%
Non-Hodgkin's lymphoma		
All patients (n=30)	77%	69%
All patients except lymphoblastic and Burkitt lymphoma (n=24)	87%	79%
Lymphoblastic and Burkitt lymphoma (n=6)	25%	28%
Hodgkin's Disease (n=12)	83%	88%

HD: Hodgkin's disease, NHL: Non-Hodgkin's lymphoma, OS: Overall survival, DFS: Disease-free survival

Multiple myeloma

A total of 18 autologous (n=12) or allogeneic transplantations (n=6) were performed in 12 patients with myeloma. Median age of the patient group was 51 years (range: 33-75). Six patients underwent double transplants, i.e. 3 patients double autologous, 2 patients autologous followed by NST and one patient conventional allogeneic followed by NST. As a result of these transplants in 10 evaluable patients, 9 (90%) were in CR and 1 (10%) was in PR. At the time of this report, all patients are alive and 3-year OS is 100% in the myeloma group.

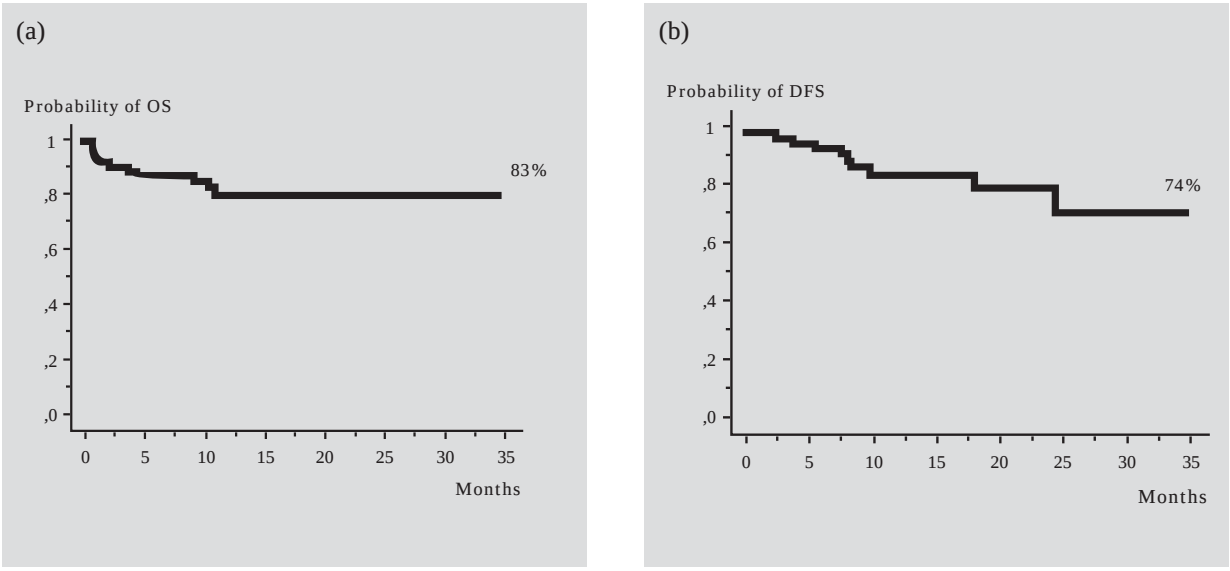
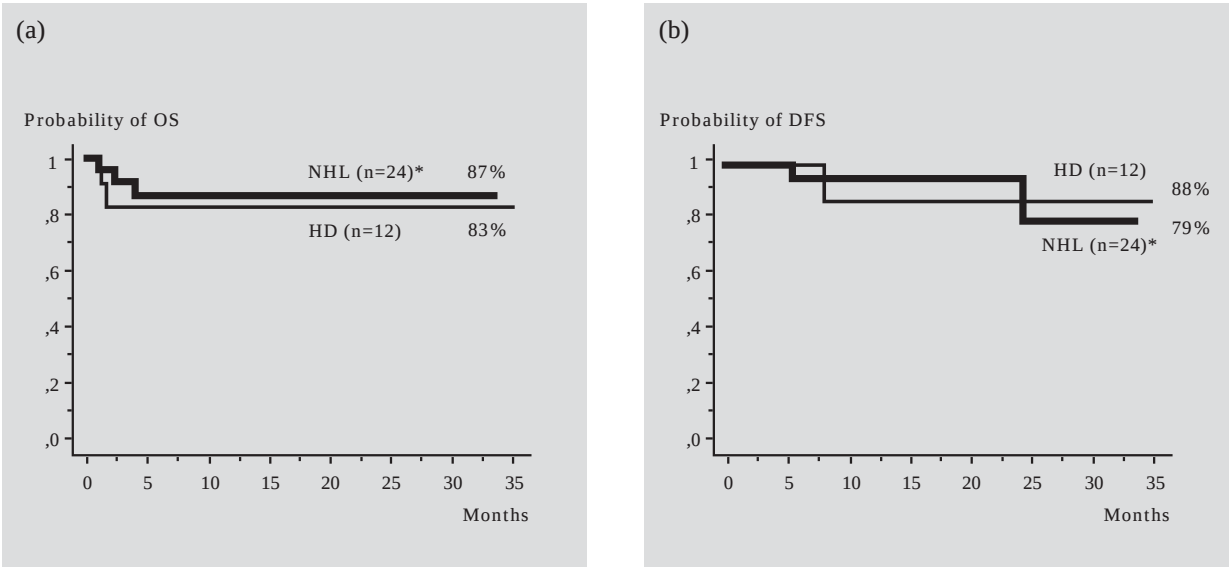


Fig 4. Probability of (a) overall survival (OS) and (b) disease-free survival (DFS) in patients with lymphoma undergoing autologous HSCT



** Burkitt lymphoma and lymphoblastic lymphoma are excluded*

Fig 5. Probability of (a) overall survival (OS) and (b) disease-free survival (DFS) in patients with Hodgkin's disease and non-Hodgkin's lymphoma following autologous HSCT

Non-myeloablative allogeneic transplantation (NST)

Between June 2001 and December 2002, 14 NSTs were performed in 13 patients. Median age was 42.5 years (range, 20-60). A patient with resistant high-grade lymphoma following renal transplantation underwent NST twice due to graft rejection (transplantation no: 4 and 5). Six patients have had prior high-dose chemotherapy with autologous HSCT. Patient characteristics and clinical outcomes are summarized in table 4.

Total number of CD34+ cells infused on day 0 ranged between 5.2 and 11x10^6/kg (mean 7.2 x10^6/kg). Chimerism was observed in all patients, except a patient with

Table 4
The patient characteristics and clinical outcome of non-myeloablative allogeneic transplantations

No	Age/sex	Diagnosis	Previous trans.	Conditioning regimen	DLI #	Actual Status*
1	43/M	Relapsed HD	Autologous HSCT	Ara-C+Fludarabine+TBI	-	Died before day +100
2	20/F	Relapsed NHL	Autologous HSCT	Ara-C+Fludarabine+TBI	2	CR
3	54/M	Relapsed NHL	Autologous HSCT	Ara-C+Fludarabine+TBI	-	Died before day +100
4	50/F	Relapsed NHL+ Renal Transplantation		Ara-C+Fludarabine	2	Resistant disease, died
5	50/F	Relapsed NHL+ Renal Transplantation	NST	Fludarabine +TBI + Rituximab	1	Resistant disease, died
6	59/M	Myeloma, IgGκ	Autologous HSCT	Fludarabine +TBI	2	CR
7	36/F	Myeloma, IgGκ	Autologous HSCT	Fludarabine +TBI	2	CR
8	48/M	Myeloma, IgGκ	Autologous HSCT	Melphalan	2	CR
9	51/M	Myeloma, IgAκ	Classic Allogeneic	Melphalan	1	CR
10	38/F	Renal Cell Ca		Cyclophosphamide + Fludarabine	4	Stable disease
11	32/M	LGL (NK)		Fludarabine+TBI	2	CR
12	52/M	CLL		Fludarabine+TBI	2	CR
13	31/M	CML		Busulfan+Fludarabine+ TBI	2	CR
14	23/F	Relapsed AML+ MDS		Ara-C+Fludarabine+TBI	2	CR

**Actual status at the time of analysis on December 31st, 2002*

DLI: Donor lymphocyte infusion, HSCT: Hematopoietic stem cell transplantation, NST: Non-myeloablative allogeneic transplantation, NHL: Non-Hodgkin's lymphoma, HD: Hodgkin's disease, AML: Acute myeloblastic leukemia, LGL: Large granular cell leukemia, AML: Acute myeloblastic leukemia, MDS: Myelodysblastic syndrome, CLL: Chronic lymphocytic leukemia, CML: Chronic myelocytic leukemia, TBI: Total body irradiation, Ara-C: Cytarabine, CR: Complete remission

high grade lymphoma who developed graft rejection and underwent a second NST. In 4 patients, absolute neutrophil count did not fall below $0.5x10^9$/l. Fifty percent of our patients did not develop any documented infection. The most frequent infections encountered in the remaining group were Staphylococcus Epidermidis and CMV activation. Only 3 of 5 patients with CMV viremia developed CMV disease which was subsequently treated successfully and without mortality.

Acute GVHD (grade I-III) was observed in 5 of 13 patients all following DLI, none developing within the first 100 days. None of our patients developed grade 4 acute GVHD and only 2 (14%) patients developed grade III acute GVHD. Patients with grade II-III acute GVHD responded to immunosuppressive therapy. Two patients with grade I acute GVHD recovered without intervention. Extensive chronic GVHD was observed in 2 patients (14%) who had prior grade III acute GVHD and mortality related to GVHD was 0%.

The survival rate at day +100 was 84.6% for all patients undergoing NST. To date, with an observation period ranging between 1 to 21 months, 10 of 13 patients

are alive, majority being in CR. In 11 patients in which tumor response could be evaluated, CR was achieved in 9 (82%), Stable disease was achieved in a patient with renal cell cancer with DLIs. Estimated probability of 20-month OS was 76% (Figure 6), with an overall TRM of 14%; 0% in patients without previous HSCT and 25% in patients with previous HSCT (2/8 patients). Two early deaths within the first 100 days were due to bacterial sepsis and viral pneumonia, and was observed in two high risk patients relapsing after an autologous transplant. Cause of death in a third patient with resistant high-grade lymphoma was progression of her disease at 7 months post-transplant.

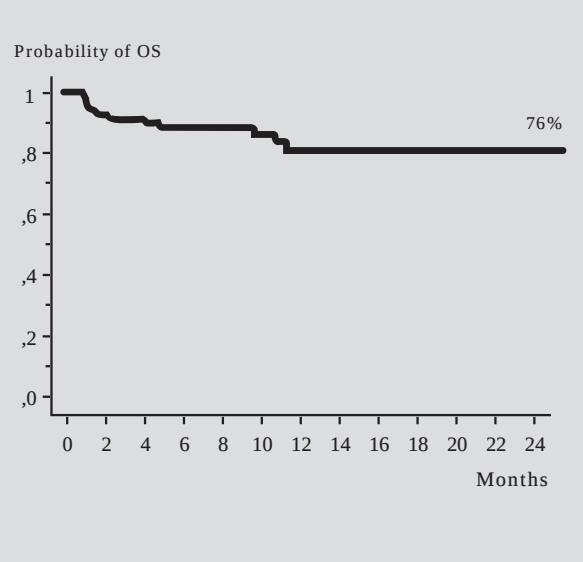


Fig 6. Probability of overall survival (OS) in patients undergoing non-myeloablative allogeneic transplantation

DISCUSSION

Hematopoietic Stem Cell Transplantation Unit of Hacettepe University Institute of Oncology is a new transplantation center inaugurated in January 2000. As a consequence of the realization of the importance of the concept of organized team approach to be successful in transplantation, our unit works in close collaboration with Basic Oncology Stem Cell Research Laboratory including Apheresis Unit, Cryopreservation, Molecular Biology, Immunocytogenetic and HLA-typing, Hematopathology and Molecular Pathology and Flow Cytometry Laboratories. The unit also has very close collaboration and prompt consultations with several clinical departments including Radiation Oncology, Infectious Diseases, Gastroenterology, Pulmonary Diseases, Radiology, Nuclear Medicine, Total Parenteral Nutrition Unit and Department of Dental Diagnosis. This organized team approach has definitely played a significant role in our low transplant-mortality rates.

Autologous HSCT has emerged as a frequently used treatment modality in patients with hematologic malignancies and selected solid tumors. Published data from various transplantation centers indicate long-term disease-free survival rates varying from 20% to 80% depending on diagnosis and remission status prior to autologous HSCT (12). Relapse of underlying disease is the main cause of treatment failure in all reported series, to date (12). However, according to the unpublished data of Turkish Oncology Group (TOG) the main cause of death in Turkey is infection (TOG Annual Meeting, 2002). The median age of our patients undergoing autologous HSCT is 40, similar to the major transplant centers in the United States. Our patient population represents a high-risk group, as the mean time elapsed from diagnosis to transplantation is 3.7 years and the mean number of salvage regimens prior to transplantation is 2.7. Despite inclusion of high-risk patients to our transplant protocols, mortality and survival rates were similar to the rates observed at experienced transplant centers listed in International Bone Marrow Transplant Registries (13).

High-dose therapy with autologous HSCT results in relatively high survival and durable remission rates in patients with HD, NHL and MM compared to conventional chemotherapy (1-7,14). In our series 3-year OS in HD and NHL were 83% and 77%, respectively. As relapse rate after 3 years is rare in the setting of autologous transplantation, we can assume that most patients probably were cured. The relatively high 3-year OS rates in lymphoma patients at our center are higher than expected (79% versus 36-55%) (15,16). This may be due to use of high-dose sequential chemotherapy approach leading to better cytoreduction prior to autologous transplantation. Although most centers adapting high-dose sequential chemotherapy have reported similar high response and overall survival rates, this has not been tested against conventional preparative regimens used

in many centers (17).

The major cause of treatment failure following autologous HSCT remains relapse of the underlying disease, which occurs in approximately 40% of patients with lymphoma. Patients with myeloma also have a continuous risk of relapse without a chance for cure. Patients who relapse following autologous HSCT have limited treatment options and the ones with chemosensitive disease may benefit from a second HSCT. However, a second autologous HSCT can be palliative for a minority of patients and is unlikely to be curative. Allogeneic HSCT is theoretically preferable as it can induce a graft versus tumor effect that is not possible following autografting and can be curative, but at the expense of extensive morbidity and mortality. Conventional allogeneic transplantation approach leads to a regimen-related toxicity and GVHD resulting in a transplant-related mortality between 25% and 50%, with less than 25% to 35% of patients achieving a durable DFS (15,16). Conventional allogeneic transplantation has a very high mortality rate (>70%) when applied following a failed autologous transplantation even at experienced transplant centers (18). Non-myeloablative allogeneic transplantation approach is an alternative approach to achieve a durable antitumor response while reducing transplant-related mortality (19,20). This has also been our major approach to relapsed patients following autologous transplantation at the Institute of Oncology.

In myeloma, high-dose therapy with melphalan leads to CR in 50% of newly-diagnosed patients, which can be durable in low risk patients with absence of chromosome 13 abnormalities and presenting with low b2-microglobulin (21). The role of conventional allogeneic HSCT remains controversial. TRM rates up to 55% make this approach unpreferable for most patients (22-24). However, durable remissions attributed to graft versus myeloma effect is documented in patients surviving allogeneic transplantation (25,26). Recently, non-myeloablative approach for allogeneic transplants has emerged as a new option for patients with relapsed or high-risk myeloma because of lower morbidity and mortality. In our patient group with myeloma TRM was 0% and all patients achieved CR, majority being in molecular remission.

Our preliminary results show that elimination of malignant cells can be achieved with a well-tolerated non-myeloablative conditioning regimen in patients with high-risk hematological malignancies. No procedure-related mortality was reported in patients undergoing NST without prior transplantation. As expected, TRM was higher in patients undergoing second HSCT. All surviving patients except one with renal cell carcinoma achieved CR. Patients with myeloma undergoing NST have responded to DLI infusions with molecular remission (n=4), majority occurring after an episode of acute or chronic GVHD (75%).

The low incidence of acute GVHD can be attributed to intensive, early and effective immunosuppression. Acute GVHD was not observed during the first 100 days due to administration of CsA at high doses and addition of MMF to the immunosuppressive regimen. We observed acute GVHD only after DLIs were administered to improve chimerism or induce graft versus tumor effect. Patients with GVHD were successfully treated with high dose methyl-prednisolone in addition to MMF and CsA, without mortality.

In summary, our experience shows that reduced toxicity of the NST preparative regimen has a low early mortality compared to conventional intensive conditioning regimens. Non-myeloablative approach may replace the conventional myeloablative regimens in selected hematological malignancies, particularly in elderly population and in the ones with indolent nature, like CML, low grade NHL, CLL and myeloma. The success rate of autologous and allogeneic transplantations are highly dependent on excellent collaboration of basic science laboratories, clinical and pathology facilities, clinical services with high-level organization, prompt decision making, strong supportive care, close follow-up and multidisciplinary team approach. Every attempt should be taken to make high-dose therapy a safe and cost-beneficial therapeutic procedure in hematology and oncology practice.

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References

1. Linch D, Winfield D, Goldstone AD, et al. Dose intensification with autologous bone-marrow transplantation in relapsed and resistant Hodgkin's disease: Results of a BNLI randomised trial. Lancet 1993;341:1051-4.
2. Armitage JO. Bone marrow transplantation. N Engl J Med 1994;330:827-38.
3. Yuen A, Rosenberg SA, Hoppe RT, et al. Comparison between conventional salvage therapy and high-dose therapy with autografting for recurrent or refractory Hodgkin's disease. Blood 1997;89:814-22.
4. Philip T, Armitage JO, Spitzer G, et al. High-dose therapy and autologous bone marrow transplantation after failure of conventional chemotherapy in adults with intermediate-grade or high-grade non-Hodgkin's lymphoma. N Engl J Med 1987;316:1493-8.
5. Bosly A, Coiffier B, Gisselbrecht C, et al. Bone marrow transplantation prolongs survival after relapse in in agressive-lymphoma patiets treated with LNH-84 regimen. J Clin Oncol 1992;10:1615-23.
6. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med 1995;333:1540-5.
7. Attal M, Harousseau JL, Stoppa AM, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. Intergroupe Francais du Myeloma. N Engl J Med 1996;335:91-7.
8. Bodmer JG, Marsh SGE, Ekkehard DA, et al. Nomenclature for factors of the HLA system, 1996. Human Immunol 1997;53:98-129.
9. Arnett KL, Parham P. HLA Class I nucleotide sequences, 1995. Tissue Antigens 1995;46:217-57.
10. Sambrook J, Fritsch EF, Maniatis T. Molecular Cloning. A Laboratory Manual. Vol. 3, 2nd ed. New York: Cold Spring Harbor Laboratory Press; 1989.
11. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 1958;53:457-81.
12. Blume KG. Improve the outcome of autologous Hematopoietic Cell Transplantation. Biol Blood Mar Trans 2001;7:363-7.
13. The National Marrow Donor Program. Transplant Center Access Directory 2001-2002. 18th ed, 2002.
14. Barlogie B, Jagannath S, Vesole DH, et al. Superiority of tandem autologous transplantation over standard therapy for previously untreated multiple myeloma. Blood 1997;89:789-93.

15. Girourard C, Dufresne J, Imrie K, et al. Salvage chemotherapy with mini-BEAM for relapsed or refractory non-Hodgkin's lymphoma prior to autologous bone marrow transplantation. Ann Oncol 1997;8:675-80.
16. Chopra R, McMilian AK, Linch DC, et al. The place of high-dose BEAM therapy and autologous bone marrow transplantation in poor risk Hodgkin's disease. A single-center eight-year study of 155 patients. Blood 1993;81:1137-45.
17. Cortelazzo S, Rambaldi A, Rossi A, et al. Intensification of salvage treatment with high-dose-sequential chemotherapy improves the outcome of patients with refractory or relapsed agressive non-Hodgkin's lymphoma. Br J Haematol 2001;114:333-41.
18. Radish J, Gooley T, Snaders J, et al. Second allogeneic after failure of first autologous transplantation. Biol Blood Marrow Transplant 2000;6:272-9.
19. Porter DL, Luger SM, Duffy KM, et al. Allogeneic cell therapy for patients who relapse after autologous stem cell transplantation. Biol Blood Marrow Transplant 2001;7:230-8.
20. Dey BR, McCfee S, Sackstein R, et al. Successful allogeneic stem cell transplantation with nonmyeloablative conditioning in patients with relapsed hematologic malignancy following autologous stem cell transplantation. Biol Blood Marrow Transplant 2001;7:604-12.
21. Desikan R, Barlogie B, Sawyer J, et al. Results of high-dose therapy for 1000 patients with multiple myeloma: durable complete remissions and superior survival in the absence of chromosome 13 abnormalities. Blood 2000;95:4008-10.
22. Cavo M, Benni M, Cirio TM, et al. Allogeneic bone marrow transplantation for the traetment of multiple myeloma. An overview of published reports. Stem Cells1995;13(suppl2):126-31.
23. Bensinger WI, Buckner CD, Anasetti J, et al. Allogeneic bone marrow transplantation for the treatment of multiple myeloma: an analysis of risk factors on outcome. Blood 1996;88:2787-93.
24. Bensinger WI, Buckner CD, Gahrton G. Allogeneic stem cell transplantation for multiple myeloma. Hematol Oncol Clin North Am 1997;11:147-57.
25. Bjorkstrand BB, Ljungman P, Svensson H, et al. Allogeneic bone marrow transplantation versus autologous stem cell transplantation in multiple myeloma: a retrospective case-matched study from European Group for Blood and Marrow Transplantation. Blood 1996;88:4711-8.
26. Martinelli G, Terragna C, Zamagni E, et al. Polymerase chain reaction-based detection of minimal residuel disease in multiple myeloma patients receiving allogeneic stem cell transplantation. Haematologica 2000;85:930-4.