ALLOGENEIC STEM CELL TRANSPLANTATION FOR ADVANCED LOW-GRADE LYMPHOPROLIFERATIVE DISORDERS: REPORT OF SIX CASES

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ABSTRACT

Background. Allogeneic stem cell transplantation is being increasingly used to treat young patients with poor-prognosis low-grade lymphoproliferative disorders. We report our single-center experience.

Patients and Methods. Six adults (four with advanced chronic lymphocytic leukemia, one follicular center cell lymphoma and one mantle cell lymphoma) underwent allogeneic stem cell transplantation (SCT). Five received bone marrow while one recieved peripheral blood stem cells. Donors were HLA-identical siblings in five cases and an HLA-haploidentical sibling in one. The conditioning regimen included in five cases cyclophosphamide, TBI and high-dose chlorambucil, without the latter in the patient with follicular lymphoma.

Results. Five patients successfully engrafted, while the patient who received the haploidentical marrow suffered primary graft failure. There were two cases of grade 2 acute GVHD and one limited chronic GVHD. Four patients are alive in complete remission (CR) with a follow-up of 17+ to 118+ months. Additionally, there is no evidence of residual disease by immunologic and molecular techniques in three cases, while one patient has residual disease assessed by molecular methods.

Conclusions. These results suggest that allogeneic SCT can achieve prolonged remissions in advanced chronic lymphoproliferative disorders.

Key words: chronic lymphocytic leukemia; mantle zone lymphoma; follicular lymphoma; bone marrow transplantation

Allogeneic bone marrow transplantation (BMT) is being increasingly used to treat young patients with poor prognosis chronic lymphocytic leukemia (CLL)[1-3] and other low-grade lymphoproliferative disorders.[5,6] We report our single-center experience with this procedure in six patients with an advanced low-grade lymphoproliferative disease.

Patients and Methods

There were four cases of B-CLL, one follicular center cell lymphoma (FCL) and one mantle cell lymphoma (MCL), the latter two diagnosed according to standard criteria.[6] Disease characteristics at diagnosis and transplantation are shown in Table 1. At diagnosis patients with CLL were in stages B (II) or C (IV).[7] At transplant they were in these same stages after having relapsed or not having responded to various kind of treatment previously administered; most importantly, two refractory patients had massively enlarged liver, spleen and lymph nodes at BMT.

The patients with FCL and MCL were in leukemic-phase Ann Arbor stage IVB at diagnosis. They showed no response to first-line chemotherapy but achieved a partial remission (PR) with salvage chemotherapy IAPVP-16 before transplant (see Table 1).[8] Donors were HLA-identical siblings (n=5) or a haploidentical sibling (n=1). Five patients received bone marrow while the patient with MCL received

Table 1. Patient characteristics.

UPN	Dx	Sex/Age at SCT	Time (mo.) from Dx to SCT	Stage at Dx*	BM pattern at Dx	Prior therapy	Stage at SCT*	BM pattern at SCT	% BM involment at SCT
28	CLL	M/32	48	B (II)	M	CB+PDN (6)	B(II)	M	50
173	CLL	F/39	48	B (II)	D	COP(10) CHOP (8) CB+PDN(6) Splenectomy Local RT	B(II)	D	70
376	FCL	M/35	6	IV-B	D	CB+PDN (2) CHOP (1) IAPVP-16(2)	PR	N	30
407	CLL	M/47	14	C (IV)	M	CHOP (7)	C(IV)	M	90
488	CLL	M/42	19	C (IV)	D	CB+PDN (6)	C(IV)	D	95
524	MCL	F/44	14	IV-B	D	CHOP (6) COP (2) IAPVP-16(2)	PR	M	95

Dx, diagnosis; CLL, chronic lymphocytic leukemia; FCL, follicular cell lymphoma; MCL, mantle cell lymphoma; mo, months; BM, bone marrow; M, mixed nodular-interstitial infiltration; D, diffuse infiltration; N, nodular; CB+PDN, chlorambucil+prednisone; COP, cyclophosphamide, vincristine, prednisone; CHOP, as COP plus adriamycin; RT, radiotherapy; IAPVP-16, ifosfamide, intermediate-dose cytosine arabinoside, etoposide, methylprednisolone; PR, partial remission; SCT, stem cell transplantation.
*Staging according to the International Workshop in CLL staging system and according to the Ann Arbor staging system in the cases of FCL and MCL.

peripheral blood stem cells.[9]

Conditioning for transplantation consisted in the combination of oral chlorambucil (0.6 mg/kg daily for four days), cyclophosphamide (60 mg/kg for two days) and total body irradiation (TBI); the total delivered dose was 12.5 to 13.5 Gy except in UPN 407 whose TBI was prematurely discontinued due to a life-threatening tumor lysis syndrome, receiving a total of 9.5 Gy.[10] The patient with FCL did not receive chlorambucil. Graft-versus-host disease (GVHD) prophylaxis was done with cyclosporine A and short-course methotrexate in five cases, and complete bone marrow T-cell depletion was done in UPN 173. Table 2 shows these data in detail.

Follow-up studies after transplantation

Response to SCT was periodically evaluated after SCT and classified according to standard criteria,[11] with the addition of nodular CR (nCR) based on the presence of residual nodular lymphoid infiltrates in BM biopsies as the only sign of disease, as previously suggested by other authors.[1]

Immunophenotype analysis was performed by standard flow-cytometry techniques on PB and BM samples at diagnosis, before transplantation and at regular intervals postSCT as previously described.[12] The presence of greater than 5% of the total lymphocyte population coexpressing CD5 with CD19 with immunoglobulin light chain restriction was considered positive for residual disease,[1,12] except in the case of FCL.

Pretransplantation BM samples were available from all patients, as well as post-SCT samples at different intervals. The presence of immunoglobulin gene rearrangements (IGR) in these samples was studied by standard Southern blot hybridization techniques using the XbaI and BglII restriction endonucleases and an immunoglobulin heavy chain joining region probe.[13,14] Pretransplantation bone marrow samples from all patients showed rearranged bands along with a germline configuration of the immunoglobulin heavy genes indicating the presence of a clonal population of cells.

Donor-recipient chimerism studies were regularly performed after transplantation using DNA-based methods, either by Southern blot or polymerase chain reaction (PCR)-based techniques, as previously described.[12] These studies establish the presence of a complete donor chimerism (CDC) or mixed donor-recipient chimerism in the samples studied after transplantation.

Results

Results of SCT and follow-up evaluations are shown in Table 2. Five patients showed complete hematologic engraftment, while the

Table 2. Patient outcome after transplantation.

UPN	Conditioning	GVHD prophylaxis	ANC >0.5x10⁹/L (days)	PLT >20x10⁹/L (days)	GVHD A	GVHD C	Dis. status/ chimerism at 3 mo.	Dis. status/ chimerism at 6 mo.	Dis. status/ chimerism at 12 mo.	Dis. status/ chimerism at last follow-up (mo.)	OS(mo.)/ KfS
28	CB/CTX/TBI	CSA/MTX	+26	+26	NO	NO	nCR/NS	nCR/NS	CR/CDC	CR/CDC [d] (104)	118+ 100%
173	CB/CTX/TBI	CSA/MTX/ T cell depletion [a]	—	—	PGF	—	—/—	—/—	—/—	RLN(3)	3+/–
376	CTX/TBI	CSA/MTX	+27	+30	NO	YES	nCR/CDC	CR/CDC	CR/CDC	CR/CDC [d] (19)	34+/100%
407	CB/CTX/TBI	CSA/MTX	+37	+52	NO	NO	nCR/MC [b]	nCR/MC	nCR/MC	CR/MC [e] (24)	35+/100%
488	CB/CTX/TBI	CSA/MTX	+26	+25	II (S)	NO	nCR/MC [b]	CR/CDC	CR/CDC	CR/CDC [d] (9)	17+/80%
524[c]	CB/CTX/TBI	CSA/MTX	+13	+13	II (S)	NO	CR/CDC	CR/CDC	—/—	CR/CDC [d] (6)	7+/–

CB, chlorambucil; CTX, cyclophosphamide; TBI, total body irradiation; CSA, cyclosporine; MTX, short-course methotrexate; ANC, absolute neutrophil count; PLT, platelet count; GVHD, graft-versus-host disease [A, acute (overall grade); C, chronic]; S, skin; PGF, primary graft failure; Dis., disease; CR, complete remission; nCR, nodular CR; CDC, complete donor chimerism; MC, mixed chimerism; NS, not studied; OS, overall survival after allogeneic stem-cell transplantation; KfS, current Karnofsky performance status; RLN, residual lymphoid nodules in BM and lymph nodes at autopsy.
[a]Haploidentical BMT. [b]At this time point 15% (UPN 407) and 13% (UPN 488) of marrow cells were monoclonal CD5⁺/CD19⁺ lymphocytes, which disappeared in later controls.
[c]Allogeneic peripheral blood stem cell transplantation. [d]Immunoglobulin gene rearrangement studies showed only a germline pattern in these samples. [e]Immunoglobulin gene rearrangement studies showed rearranged bands identical to those observed preBMT in this marrow sample.

patient who received a haploidentical T-cell depleted BMT did not engraft. Two patients developed grade II acute GVHD and one limited chronic GVHD. DNA-based molecular chimerism studies revealed a pattern of CDC at last follow-up in all but one patient. One patient showed transient mixed chimerism in BM at three months and one has been in persistent mixed chimerism since BMT (UPN 407).

Significant procedure-related toxicities included grade II mucositis in all cases[15] and two cases of grade II bladder toxicity (hemorrhagic cystitis). One patient (UPN 524) developed disseminated cytomegalovirus infection and died on day +217, and one died of aspergillosis on day +99 (UPN 173). Five patients survived more than three months after the procedure and are thus evaluable for response to transplantation. One patient died in complete remission on day +217. Two patients (UPN 488 and UPN 376) were in nCR at three months and in CR at six months post-BMT, and both remain in CR at +17 and +34 months, respectively. Immunophenotype analysis of BM samples showed transient evidence of residual CLL cells in one patient (UPN 488) at three months postBMT, with negative results in later studies. Two other

cases never showed evidence of residual CLL cells (UPN 28 and 524). UPN 28 was in a nCR up to six months post-BMT but remained in CR for 12 months after the procedure, with a current disease-free follow-up of +118 months. On the other hand, UPN 407 was in nCR up to 19 months and in CR since then; immunophenotype analyses showed residual CLL cells in a BM sample at three months, but later studies have been negative, and he is currently in CR at +35 months. IGR were studied in a single BM sample obtained from each patient at a different interval post-BMT: UPN 28 at 104 months; UPN 376 at 19 months; UPN 407 at 24 months; UPN 488 at nine months; and UPN 524 at four months. All samples studied had no evidence of IGR, showing only germline configurations for the immunoglobulin heavy chain genes except in UPN 407. In this case, Southern blot hybridization revealed a pattern of clonal rearrangement identical to the pre-BMT pattern, despite being in CR at last follow-up.

Discussion

A significant proportion of patients with chronic lymphoproliferative disorders are under

50 years of age, and in these subjects novel treatment approaches are being investigated to prolong their survival. Allogeneic SCT, mainly BMT, has been considered an alternative therapy for such patients.[1-5]

Our four patients with CLL had poor-prognosis features at BMT,[16] mainly advanced stage and lack of response to standard chemotherapies, while patients with FCL and MCL were in PR after salvage chemotherapy. All but one patient successfully engrafted with complete hematologic recovery. Graft failure is a frequent complication of T-cell depleted grafts, especially in HLA nonidentical donor-recipient pairs.[17] Response to transplantation is not evaluable in this case, but postmortem examination revealed small foci of residual CLL in BM and lymph nodes. It is uncertain whether this finding represents refractoriness to the conditioning regimen, as would be the case in an acute leukemia, since it is frequent to observe residual CLL cells in BM by cytohistological, immunological and molecular methods during the first six months after BMT.[1,2,18] These cells could represent in some cases the maturing CLL cells that were not killed by the conditioning regimen, and once they disappear the CLL clone would be eradicated. In our experience, the three patients with CLL who survived the procedure had residual lymphoid nodules in BM (nCR) from three to 12 months after BMT (see Table 2), and two had evidence of residual CD5+/CD19+ CLL cells at three months, with disappearance in later studies. Two of these patients (UPN 28 and 488) had no evidence of IGR in BM at 104 and nine months postBMT, respectively. In these same samples chimerism studies show a pattern of CDC when studied by PCR-based techniques in both cases, although one (UPN 488) was in mixed chimerism at three months, concordant with the fact that he showed a nCR in BM and had positive CD5+/CD19+ cells at that time point; this patient later lost all evidence of residual CLL. UPN 407, on the other hand, has shown both donor and recipent cells in both PB and BM samples since BMT (mixed chimerism). Although by immunophenotype of PB and BM lymphoid cells there is no evidence of persistent monoclonal CD5+/CD19+ cells for six months post-BMT, IGR

bands identical to those observed pre-BMT were detected in the last marrow sample studied (24 months post-BMT), thus indicating the persistence of residual clonal cells. Additionally, many of the residual recipient-derived cells are normal (non-leukemic) lymphohematopoietic cells, as may occur in patients with acute leukemia in long-term mixed chimerism after BMT.[19] This conclusion derives from the fact that he has a mixed erythocyte chimerism and purified bone marrow cellular subpopulations (granulocytes, B lymphocytes, T lymphocytes) show a pattern of mixed chimerism when studied separately (data not shown). Since CLL has been reported to relapse up to 54 months after BMT,[3] this patient will be closely monitored to detect a possible future relapse. The patients with FCL and MCL were also in CR with no evidence of residual lymphoma by clinical, morphological, immunological (in the MCL) and molecular techniques at last follow-up or death. The patient who received an allogeneic PBSCT showed rapid donor lymphohematopoietic engraftment.

Five of our patients received a previously unreported conditioning regimen which includes cyclophosphamide, TBI and high-dose chlorambucil. Chlorambucil was added in an effort to increase the antitumor efficacy of the conditioning regimen, since this agent has shown a dose-dependent effect in the treatment of CLL.[20] Apparently, no excess conditioning-related toxicities were observed. The fact that these patients were in advanced stages of their disease is important, since allotransplantation has been reported to be more effective in early stages of these disorders, with poor results in advanced and refractory patients.[1-5] However, our four survivors demonstrate that advanced cases may also achieve long-term disease-free survivals.

Allogeneic-SCT is associated with higher procedure-related complications than other therapeutic approaches. Although it seems an effective treatment option for young patients with CLL or low-grade lymphomas and a predicted low short-term survival with standard treatment, alternative novel treatment options, such as the new nucleoside analogues, and results of autologous BMT,[21,22] show very promising results for long-term disease-free and overall-survival,

and thus the best treatment option for these patients cannot be currently determined with certainty.

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