Haematologica 2000; 85:1165-1171
original paper

Allogeneic peripheral blood stem cell transplantation with CD34$^+$-cell selection and delayed T-cell add-back in adults. Results of a single center pilot study

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ABSTRACT

Background and Objectives. **Allogeneic peripheral blood stem cell transplantation with CD34$^+$ cell-selection (CD34$^+$-PBSCT) allows rapid hematologic engraftment with a reduction in graft-versus-host disease (GVHD), although concerns exist regarding the increased risk of tumor relapse associated with T-cell depletion of the graft. Delayed T-cell add-back (TCAB) after such transplants may restore the graft-versus-tumor effect while achieving a reduced early transplant-related mortality due to less GVHD in a group of patients at high risk of early death (i.e., age ≥45 years).**

Design and Methods. **Ten patients 45 years of age or older with hematologic malignancies received a CD34$^+$-PBSCT and cyclosporin A (CyA) to prevent acute GVHD, followed by a planned delayed donor TCAB of 10^7 T-cells/kg to restore the graft-versus-tumor effect. The infused graft included a median of 6.3×10^6 CD34$^+$ cells/kg and 4.4×10^4 CD3$^+$ cells/kg.**

Results. **Engraftment was prompt in all cases. Four patients developed acute GVHD after the CD34$^+$-PBSCT and/or chronic GVHD after CyA withdrawal and did not proceed to TCAB, and two patients died early before the planned TCAB. Four patients proceeded to TCAB at a median of day +104 after CD34$^+$-PBSCT (+92 to +150). Two of these patients developed acute GVHD grades I-II (IBMTR Index B) after TCAB and all four developed chronic GVHD, which was extensive in two. With a median follow-up of 611 days (range 499-847) after transplant in the seven survivors, there have been no disease progressions, and all patients show a pattern of complete donor chimerism in bone marrow and peripheral blood.**

Interpretations and Conclusions. **The results of our pilot study suggest that this protocol produces an acceptable transplant-related morbidity and mortality in patients 45 years and older. However, there may be benefit in infusing CD34$^+$-selected PBSCT with even lower T-cell contents and further delaying the TCAB.**

Key words: allogeneic; hematopoietic stem cell transplantation; T-cell depletion; graft- versus-host disease.

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T-cell depletion (TCD) of the donor hematopoietic stem cells can successfully reduce the incidence of moderate-to-severe acute graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (HSCT).[1] TCD, however, increases the risk of graft failure and leukemic relapse, especially in patients with chronic myelogenous leukemia. In HSCT recipients, acute GVHD and its treatment are major causes of transplant-related morbidity and mortality. The risk of moderate-to-severe acute GVHD increases with age, and its occurrence contributes to the higher rate of transplant-related mortality in patients above the age of 45 years.[2] Experimental[3,4] and clinical studies have demonstrated that the administration of donor lymphocytes late after a TCD HSCT may restore the graft-versus-tumor effect with a presumably lower risk of moderate-to-severe acute GVHD. Several studies have analyzed the feasibility of using delayed donor T-cell add-back (TCAB) after a TCD HSCT,[5-12] mostly after bone marrow transplants. On the other hand, allogeneic peripheral blood stem cell transplantation with CD34$^+$-cell selection (CD34$^+$-PBSCT) leads to rapid hematopoietic engraftment with reduced incidence of acute and chronic GVHD.[13] In an effort to decrease the early morbidity and mortality from a HSCT while retaining the graft-versus-tumor effect we performed a pilot study of delayed TCAB after a CD34$^+$-PBSCT from an HLA-identical sibling in patients 45 years of age or older.

Design and Methods

Patients

Between July 1998 and June 1999, 10 patients with hematologic malignancies (excluding acute leukemia in first remission) entered this protocol. Table 1 details the patients' characteristics. All patients gave written informed consent to the study. Their median age was 51 years (range 45-62). Underlying diseases were multiple myeloma (n=5), acute myelogenous leukemia, myelodysplastic syndrome, follicular lymphoma, chronic myelogenous leukemia (CML) and chronic lymphocytic leukemia (CLL). Disease stage was early phase (first chemotherapy-induced remission) in 3 cases, intermediate (second chemotherapy-induced remission or CML in accelerated phase) in 5 and advanced phase (beyond second remission) in 3 cases. All donor/recipient pairs were CMV seropositive. All patients were transplanted from HLA-identical siblings.

Preparative regimen

Two preparative regimens were used. Five patients received thiotepa (5 mg/kg/day i.v. on days -8 and -7), total body irradiation (13.5 Gy divided over 6 fractions) and cyclophosphamide (50 mg/kg/day iv. on days -6 and -5). The other 5 patients received thiotepa (250 mg/m^2/day i.v. on days -9, -8 and -7), busulphan (1 mg/kg every 6 hours p.o. for a total of 10 doses on days -6, -5 and -2, total dose 10 mg/kg) and cyclophosphamide (50 mg/kg/day i.v. on days - 3 and -2).

Stem cell mobilization and CD34$^+$ cell selection

The median age of the donors was 53 years (range 40-65). One donor required placement of a central venous catheter for PBSC harvesting, and all others underwent leukaphereses using peripheral veins. Donors were mobilized with recombinant human granulocyte colony-stimulating factor at a dose of 8 g/kg s.c. every 12 hrs for four consecutive days and leukaphereses were performed on the fifth day (and sixth if necessary) to achieve a target CD34$^+$ cell dose of >5 $\times 10^6$/kg recipient's weight. CD34$^+$ cell selection was performed with an immunomagnetic cell separator (Isolex 300i, Baxter, Deerfield, IL, USA), as previously described in detail.[14] The CD34$^+$-selected fraction was cryopreserved in all cases using standard techniques.

GVHD prophylaxis and grading

As further GVHD prophylaxis patients received cyclosporin A (CyA) at 1 mg/kg/day by continuous intravenous infusion from day -7 to -1 and 2 mg/kg/day from day -2, with a switch to the oral route when feasible, with doses adjusted to maintain a CyA plasma level of 150-300 g/mL. If the patient did not develop acute GVHD >grade I (IBMTR Index A), CyA was tapered down starting on day +30 to +45 and was stopped from day +60 to +75. Acute GVHD was graded using the updated Glucksberg grading system[15] and the IBMTR severity index.[16,17] Chronic GVHD was graded by standard criteria.[18]

T-cell add-back (TCAB)

Patients were observed for 30 to 45 days after discontinuation of CyA, and those who did not develop acute GVHD >grade I (nor IBMTR Index A) were to receive a TCAB from the original donor. The dose of TCAB was 1×10^7/kg CD3$^+$ cells, which were obtained freshly from the donor at the time of TCAB or were cryopreserved in a separate aliquot from a fraction of the harvested PBSC.

Table 1. Patient characteristics and results of CD34$^+$-cell selection.

UPN	Age/ sex	Underlying disease	Status of dis./mo. from diagnosis	Cytogenetic, molecular or IP marker(s)	Conditioning regimen	Do-Re sex mismatch	Do-Re CM serostatus	CD34$^+$ cells ($\times 10^6$/kg) at harvest	CD34$^+$ cells ($\times 10^6$/kg) after CD34$^+$ selection	CD3$^+$ cells at harvest ($\times 10^8$/Kg)	CD3$^+$ cells after CD34$^+$ selection ($\times 10^4$/kg)
842	62/M	AML	2nd CR / 18 mo.	IP	ThioBuCy	Yes	+/+	14	7.9	6.5	25
926	45/M	MM	4th PR, Prior APBSCTP	IP	ThioBuCy	No	+/-	15	5.9	2.8	0.23
971	46/M	RAEB	1st PR / 5 mo	t(1;11;21)	ThioBuCy	Yes	+/+	13	6.7	2.7	2.9
959	59/M	MM	2nd OR / 132 mo.	IP	ThioCyTBI	Yes	+/+	9.2	4.5	3.9	3.9
1009	52/F	MM	1st OR / 8 mo.	IP	ThioBuCy	No	+/+	10.8	6	7.3	4.5
1008	47/M	MM	1st OR / 6 mo.	IP	ThioCyTBI	No	+/+	11.8	5	4.9	2.8
1010	51/F	CML	AP / 6 mo.	t(9;22), bcr/abl+	ThioCyTBI	No	+/+	19	11	5.2	15
1006	47/M	CLL	2nd PR / 42 mo.	IP/IgH	ThioBuCy	No	+/-	18.1	8.3	4.1	7.5
1049	45/F	MM	1st OR / 4 mo.	IP	ThioCyTBI	No	+/+	14.6	8.4	5.6	13
1074	45/F	NHL-FL	2nd PR / 36 mo.	IP, bcl-2/IgH	ThioCyTBI	No	+/+	6.5	4.6	4.8	2.7

AML, acute myelogenous leukemia; CR, complete remission; IP, immunophenotypic marker; ThioBuCy, thiotepa, busulphan, cyclophosphamide; MM, multiple myeloma; PR, partial remission; APBSCT, autologous peripheral blood stem cell transplantation; RAEB, refractory anemia with excess blasts; OR, objective response; ThioCyTBI, thiotepa, cyclophosphamide and total body irradiation; CML, chronic myelogenous leukemia; AP, accelerated phase; CLL, chronic lymphocytic leukemia; NHL-FL, follicular lymphoma; CMV, cytomegalovirus.

Table 2. Results of CD34+-selected PBSCT.

UPN	ANC > 0.5×10^9/L	PLT > 20×10^9/L and 50×10^9/L	Grades 3-4 RRT (WHO)	CMV infection (day 1st antigenemia)	Serious infections	aGVHD GSC/IBMTR	Dis. status around day +90	Proceeded to TCAB	Status at last follow-up (day)
842	+15	–	mucositis-4	yes (+35)	CMV-IP*	IV/D (liver 4)	NA	no	Died aGVHD (liver), day +40
926	+9	+10/+14	GI-3	no	no	I/A (skin 1)	PR†	yes	A/W,† day +847
971	+11	+10/+13	mucositis-3	no	no	0	CR	yes	A/W, day +698
959	+10	+13/+43	renal-3	yes (+30)	no	I/A (skin 1)	PR‡	yes	A/W, day +723
1009	+10	+9/+14	none	yes (+38)	no	I/A (skin 1)	CR	yes	A/W, day +598
1008	+13	+11/+18	none	yes (+33)	IPS (+50)	I/A (skin 1)	NA	no	Died, IPS day +55
1010	+13	+6/+13	none	no	no	I/A (skin 1)	CR	no§	A/W, day +597
1006	+12	+18/+52	cystitis-3	yes (+34)	VHZ	II/C (skin 3) (ophthalmitis)	nodular CR	no	A/W, day +611
1049	+14	+12/+14	none	yes (+39)	no	I/B (skin 2)	CR†	no§	A/W,† day +499
1074	+11	+42/+57	GI-3	yes (+25), recurrent x3	no	II/B (GI 1)	CR	no#	Died, pneumonia day +176

*ANC, absolute neutrophil count; PLT, platelets; RRT, regimen-related toxicity (WHO grading system); CMV, cytomegalovirus; CMV-IP, CMV interstitial pneumonia; aGVHD, acute graft-versus-host disease; GSC, Glucksberg-Seattle grading criteria; IBMTR, International Bone Marrow Transplant Registry staging criteria; TCAB, T-cell add-back; CR, complete remission; PR, partial remission; GI, gastrointestinal; A/W, alive and well; cGVHD, chronic GVHD; VHZ, varicella herpes zoster; NA, not applicable; IPS, idiopathic pneumonia syndrome; *post-mortem finding; †persistent low monoclonal gammopathy in serum; ‡increase less than 50% of monoclonal gammopathy in serum and urine with respect to pre-transplant levels; §developed localized chronic GVHD (liver) after CyA withdrawal; #developed localized chronic GVHD (skin) after CyA withdrawal.*

Supportive care and post-transplant follow-up

Antimicrobial prophylaxis consisted in norfloxacin and fluconazole during neutropenia, followed by trimethoprim-sulphamethoxazole (2 double strength tablets twice weekly) until day +180. Weekly intravenous immunoglobulin (200 mg/kg) was given from day -3 until day +90. Monitoring for CMV consisted in twice weekly pp65 antigenemia testing and CMV blood cultures until day +120, with pre-emptive treatment with gancyclovir in case of a positive result.[19] All patients were cared for in single rooms with positive pressure filtered air until discharge. Acute GVHD grade ≥ II or IBMTR Index ≥ B was treated with prednisone (2 mg/kg/day), while chronic GVHD was treated with CyA (daily or on alternate days) with prednisone. Chimerism studies and disease status were monitored by bone marrow examinations on days +21, +100 and thereafter every 3 to 6 months for the first 2 years. Chimerism studies were performed in nucleated cells of marrow and peripheral blood by polymerase chain reaction (PCR)-based analysis of single strand conformation polymorphism (SSCP) which differed between the donor and recipient. The sensitivity of this technique for detecting mixed chimerism is below 5 %, as previously described.[20]

Results

CD34+ cell selection

The median number of CD34+ and CD3+ cells/kg recipients' weight harvested were 13.5 $\times10^6$ (range 6.5-19) and 4.9×10^8 (range 2.7-7.3), respectively. After immunoselection, the medians were 6.3×10^6 (range 4.5-11.4) and 4.4×10^4/kg (range 0.23-25), respectively.

Procedure-related complications and hematologic recovery

Table 2 details the post-PBSCT course of the 10 patients included in the study. All patients showed leukocyte engraftment, while one patient did not show platelet recovery. There were five cases of grades 3-4 (WHO grading system) regimen-related toxicity. There were three transplant-related deaths, one on day +40 from progressive hepatic failure (consistent with acute liver GVHD at autopsy), one from idiopathic pneumonia syndrome on day +55 and one from *Escherichia coli* pneumonia on day +176. Significant infectious complications included CMV infection in seven cases with the first positive antigenemia on day +34 (range +25 to +39) and one case of CMV pneumonia found at autopsy. Three patients were found to have adenovirus infection of the colon and one developed herpes zoster ophthalmitis. Two patients died from infectious complications, as specified above.

GVHD

GVHD following CD34+-PBSCT

Six patients developed acute GVHD grade 0-I (IBMTR Index 0-A) after transplant, while four developed grades II-IV and/or IBMTR Index B-D. Table 2 shows the details on acute GVHD after CD34+-PBSCT.

GVHD after TCAD

Two patients died early before the planned

Table 3. Details of T-cell add-back (TCAB) for the 4 patients who received it.

UPN	*Before TCAB* Chimerism in BM and PB by PCR-SSP	Disease status	days post-PBSCT of TCAB	*8-12 weeks after TCAB* Chimerism in BM and PB by PCR-SSP	Disease status	GVHD after TCAB	Treatment for GVHD and last status of GVHD
926	MC	PR*	+93	CDC[†]	PR*	Limited cGVHD (liver)	CyA+PDN, CR
971	MC	CR	+92	CDC[†]	CR	Grade I/Index B aGVHD (skin 2)	CyA+PDN, CR
959	CDC	PR[‡]	+120	CDC	CR	Extensive cGVHD (liver, mucosa)	CyA+PDN, CR
1009	CDC	CR	+150	CDC	CR	Grade II/Index B aGVHD (GI 1, skin 2)	CyA+PDN, PR
						Limited cGVHD (skin)	CyA+PDN, PR

*TCAB, T-cell add-back; BM, bone marrow; PB, peripheral blood; MC, mixed chimerism; CDC, complete donor chimerism; PR, partial remission; CR, complete remission; CyA, cyclosporin A; PDN, prednisone; *persistent low monoclonal gammopathy in serum; [†]these patients reverted from MC in bone marrow and peripheral blood leukocytes pre-TCAB to CDC after TCAB and development of GVHD; [‡]increase less than 50% of monoclonal gammopathy in serum and urine with respect to pre-transplant levels.*

TCAB, four patients did not proceed to TCAB due to previous acute GVHD and/or development of chronic GVHD after CyA withdrawal. Four patients proceeded to TCAB at a median of 104 days after PBSCT (range +92 to +150). Table 3 details the results in these cases. Two patients developed acute GVHD grades I-II (IBMTR Index B) after TCAB and all four developed chronic GVHD, which was extensive (liver+mucosa) in two and localized (liver) in the other two patients. All patients who developed GVHD after TCAB required systemic immunosuppression with CyA ± steroids. Of these four cases, one patient with myeloma had an increasing monoclonal paraprotein in blood and urine after $CD34^+$-PBSCT, which disappeared after TCAB and chronic GVHD (UPN 959), while the other three had no evidence of disease progression at TCAB.

Changes in lymphocyte subsets after TCAB

B-cells ($CD19^+$), total T-cells ($CD3^+$), $CD4^+$ T-cells, $CD8^+$ T-cells and NK-cells ($CD56^+$) in peripheral blood were studied before TCAB and at 2-4 weeks and 8-12 weeks afterwards. B-cells increased in all four cases from a median of 0.024×10^9/L (0.020-0.281) to 0.198 (0.031-0.533) and 0.203 (0.057-0.364), respectively. NK-cells decreased in all cases from a median of 0.577×10^9/L (0.517-0.684) to 0.261 (0.234-0.388) and 0.215 (0.202-0.327), respectively. $CD8^+$ and $CD4^+$ cells showed no significant changes, although the latter were greater than 0.2×10^9/L in only one case at all three time points.

Disease status and outcome

With a median follow-up of 611 days (range 499 to 847) after transplant in the seven survivors, there have been no disease progressions. Of the four survivors with multiple myeloma, two were in complete remission and two in partial remission at last follow-up. The patient with CLL and the patient with myelodysplastic syndrome are in complete remission, while the patient with CML-acute phase was in hematologic, cytogenetic and molecular remission. All patients show a pattern of complete donor chimerism in bone marrow and peripheral blood.

Discussion

TCD allogeneic HSCT has been largely abandoned because of the increased risk of graft failure and disease relapse and delayed immune reconstitution. However, TCD undoubtedly leads to a reduction in acute and chronic GVHD.[1] $CD34^+$-PBSCT is a novel method of performing TCD HSCT with a large content of $CD34^+$ cells. Preliminary experience in our country suggests that the rates of graft failure and moderate-to-severe acute GVHD after a $CD34^+$-PBSCT from an HLA-identical sibling are low.[13] We thus chose this TCD technique in order to obtain fast hematopoietic reconstitution after a myeloablative conditioning regimen and tested a delayed TCAB protocol in order to restore the graft-versus-tumor effect present in an unmanipulated HSCT. Our results suggest that this protocol carries an acceptable transplant-related morbidity and mortality in patients 45 years and older. Engraftment is rapid, but there is a high rate of viral infections. However, half the eligible patients did not reach the planned TCAB due to prior GVHD, suggesting that a lower T-cell content in the final product (well below 0.5×10^5 $CD3^+$ cells/kg) should be incorporated in further studies. Additionally, all four patients who received TCAB developed GVHD (acute and/or chronic) which required systemic immunosuppression, confirming that this "low" T-cell dose selected for add-back (1×10^7 $CD3^+$ cells/kg) is sufficient for a graft-versus-tumor

Table 4. Results of published studies using T-cell depleted allogeneic HSCT from HLA-identical siblings followed by planned delayed T-cell add-back.

Author[ref.]	No. Pts.	Median age in yrs (range)	No. CMV positive (%)	Source of stem cells	Method of TCD	Median CD3+ cells/kg infused	GVHD proph.	Day of 1st TCAB	$CD3^+$ cells/kg in 1st TCAB	Grades II-IV aGVHD pre-TCAB	No. of pts. who received TCAB (%)	Grades II-IV aGVHD after TCAB	cGVHD after TCAB	TRM
Naparstek[5]	54	19 (1-48)	NS	BM	Campath-1G	NA	none	28	10^5*	17%	43 (80)	53%	40%	NS
Barrett[6]	38	34 (17-58)	32 (84)	BM	elutriation	2.1×10^5	CyA	30	2×10^6 (n=26)† 1×10^7 (n=12)	16%	29(76)	38%	46%	39%
Redei[7]	8	33 (21-51)	NS	PBSC	$CD34^+$ sel.	1.6×10^5	CyA/MP	45	1×10^7	3/8 (38%)	3 (38)	3/3 (100%)	NS	2/8 (25%)
Schaap[8]	100	NS	NS	BM	elutriation	NS	CyA	180	7×10^7 (n=6) 1×10^7 (n=25)	47%	31 (31)	42%	NS	NS
Baron[9]	13	NS	8(62)	PBSC	$CD34^+$ sel.	2.8×10^5	CyA±MTX	60	2×10^6‡	4/13 (31%)	8 (62)	0	NS	3/13 (23%)
Wassmann[10]	10	39 (25-57)	NS	PBSC	$CD34^+$ sel.	3.1×10^3	none	30	5×10^4§	0	10 (100)	74%	NS	2/10 (20%)
Alyea[11]	22¶	45	NS	BM	CD6+ depletion	NS	none	180	3×10^7 $CD4^+$ cells#	23%	11 (50)	6/11 (55%)	4/11 (36%)	NS
Lee[12]	48**	44†† (18-60)	NS	BM	MoAb+C	0.8×10^5	CyA	21	10^5‡‡	8%	34 (71)	60%	58%	50%††
This study	10	51 (45-62)	10 (100)	PBSC	$CD34^+$ sel.	0.4×10^5	CyA	104	10^7	3/10 (30)	4 (40)	1/4 (25%)	4/4 (100%)	3/10 (30%)

*CMV, cytomegalovirus; NS, not specified; BM, bone marrow; PBSC, peripheral blood stem cells; TCD, T-cell depletion; MoAb+C, monoclonal antibodies plus complement; CyA, cyclosporin A; MP, methylprednisolone; MTX, methotrexate; aGVHD, acute GVHD; cGVHD, chronic GVHD; TRM, transplant-related mortality; *this protocol included planned graded increments of T-cell add-back of 10^5/kg, 10^6/kg and 10^7/kg cells on days +28, +56 and +84, respectively, which were completed in 27 (50%) of cases; †these 26 patients were planned to receive 2×10^6/kg T-cells on day +30 and $5x10^7$/kg on day +45, which were completed in 18 (69%) of cases; ‡this protocol included planned graded increments of $CD8^+$-depleted T-cell add-back of 2×10^6/kg, 10^7/kg and 5×10^7/kg cells on days +60, +100 and +140, respectively, which were completed in 7 (54%) of cases; §this protocol included planned graded increments of T cell add-back of 5×10^4/kg, 5×10^5/kg and 2×10^6/kg cells on days +30, +60 and +112, respectively, which were completed in 6 (60%) of cases; ¶all patients suffered from multiple myeloma; #$CD8^+$-depleted donor lymphocytes were used for T-cell add-back in this study; **a total of 144 patients who received a BMT from related and unrelated donors were included in this study, but only 48 were planned to receive a delayed TCAB; another 96 patients received the 1st TCAB on day 0 along with the TCD-BMT; ††refers to all 144 included in the study; ‡‡this protocol included planned graded increments of T-cell add-back of 10^5/kg, 2.5 x 10^5/kg and 7.5 x 10^5/kg cells after leukocyte recovery and 6-8 and 12-14 weeks thereafter, which were completed in 9 (20%) of cases.*

effect. Since the rate of GVHD appears high when TCAB is given within five months after a $CD34^+$-PBSCT, delaying TCAB until six months after transplant may be appropriate for further studies. In a previous study by Barrett *et al.*[6] the timing and dose of add-back were found to be critical for the development of GVHD. On day +30 a TCAB of 2×10^6 T-cells/kg produced no significant GVHD, while 10^7 T-cells/kg resulted in 8/10 patients developing grade II-IV acute GVHD. Conversely, only 3/18 who received 5×10^7 T-cells/kg on day +45 developed grades II-III acute GVHD, although 10 developed chronic GVHD. Several studies have analyzed TCD HSCT followed by planned delayed TCAB. Table 4 summarizes the results of these studies, most of which have been reported only in abstract form. These studies varied in the patient populations included, the method of TCD and post-transplant immunoprophylaxis, the source of the stem cells and the dose and schedule of TCAB. The percentage of patients who reached the planned TCAB varied from 31 to 100% and was mainly dependent on the precocity of the planned add-back (the earlier it was planned the higher the percentage of patients who received it) and the rate of acute GVHD after the HSCT, since of course patients who developed significant acute GVHD were excluded from TCAB. The rate of significant acute GVHD after TCAB ranged from 38% to 100% and that of chronic GVHD from 36% to 58%, and the transplant-related mortality varied from 20% to 50%. These figures appear similar to the rates after unmanipulated HSCT from HLA-identical siblings. Whether the morbidity and mortality directly due to GVHD can be reduced with TCD HSCT followed by delayed TCAB cannot be determined without randomized or matched cohort studies, which are currently not available. However, in patients with CML unmanipulated BMT has been compared with a strategy of TCD BMT followed by donor lymphocyte infusions in case of relapse, with similar 5-year overall and current disease-free survivals of 65-80%.[21,22] These studies suggest that planned delayed TCAB may indeed have a role in improving the results of TCD HSCT in other diseases in which a graft-versus-tumor effect is operative. However, further studies should focus on reducing the risk of GVHD after the TCD HSCT and thus increasing the number of patients who reach the planned TCAB and developing a schedule of TCAB that will reduce the risk of moderate-to-severe acute GVHD and extensive chronic GVHD to well below that seen after an unmanipulated HSCT. In conclusion, our results suggest that this protocol produces acceptable transplant-related morbidity and mortality in patients 45 years and older. Further studies could investigate infusing CD34$^+$-selected PBSCT with an even lower number of CD3$^+$ cells/kg and further delaying TCAB after transplant.

Contributions and Acknowledgments

RM designed the study, was responsible for data management and prepared the manuscript. CC collaborated in data management. JS is the head of the Division and participated in writing the paper. AA, AS and SB collaborated in patient care and in preparation of the manuscript.

Funding

Supported in part by a grant from AMGEN España (Beca de Terapia Celular 1998).

Disclosures

Conflict of inerest: The main author (RM) was the direct recipient of a grant from AMGEN España (Beca de Terapia Celular 1998).

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

Manuscript received August 7, 2000; accepted October 4, 2000.

Potential implications for clinical practice

- Planned T-cell add-back after a CD34$^+$ PBSC allograft can be safely accomplished in around 40-50% of transplant recipients.
- Early transplant-related mortality can be kept low in patients at high-risk of early severe morbidity following a standard allograft.
- In order for a significant proportion of patients to be able to receive a planned T-cell add-back, the risk of moderate-to-severe GVHD and severe opportunistic infections after a T-cell depleted transplant must be kept low.

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