Stem Cell Transplantation

Reduced intensity conditioning allogeneic transplant for advanced chronic lymphocytic leukemia

We report the preliminary results of 12 patients with advanced stage chronic lymphocytic leukemia (CLL) transplanted following reduced intensity conditioning (RIC. With a median of 22 months of follow-up, 9 patients are alive and 3 have died of progressive disease, graft-versus-host disease (GVHD) or toxic hepatitis. Acute grade I-III GVHD occurred in 33% of patients and chronic GVHD in 50%. Eight of the 12 patients achieved a complete remission (CR) and 2 patients a partial remission (PR). Donor lymphocyte infusion was effective in 6 patients. Event-free survival, progression-free survival and non-relapse mortality at 3 years were 68%, 42% and 16%, respectively. Our results show successful immunomodulation and reduction in tumor burden in high risk CLL.

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In recent years, a number of prognostic factors have been found in patients with chronic lymphocytic leukemia (CLL). Some of these factors identify patients with a poor-risk who are resistant to conventional treatments and have a shorter overall survival.[1,2] An allogeneic transplant, supported by clear evidence of a graft-versus-leukemia (GVL) effect, has been restrained by treatment-related mortality of up to 50%.[3,4] In recent years reduced-intensity conditioning regimens (RIC) have replaced myeloablative regimens for some elderly patients with CLL. After the first report by Khouri in 1998,[5] several RIC regimens have been developed and proven effective in lymphoproliferative disorders including CLL.[6-8] We now report on 12 patients with advanced-stage CLL transplanted with grafts from sibling HLA identical donors and conditioned with two cycles of fludarabine 30 mg/m^2/day for 3 days and cyclophosphamide 300 mg/m^2/day for 3 days followed by 200cGy total body irradiation (TBI) 14 days after the second cycle. Graft-versus- host disease (GVHD) prophylaxis included cyclosporine-A and mycophenolate mofetil. Patients were infused with growth factor-mobilized donor peripheral blood stem cells (>5×10^6 CD34$^+$ cells).

Donor-recipient chimerism was assessed by microchimerism[9] on sorted peripheral blood CD3$^+$ T cells (MiniMACS, Miltenyi Biotec, Germany). Analysis of minimal residual disease (MRD) and the expression of co-stimulatory molecules was performed by flow cytometry (FACSCalibur, Becton Dickinson). Samples with fewer than 1×10^{-4} CD23/CD5/CD19 cells with a κ/λ ratio not shifted to more than 2:1 were considered negative for MRD, in agreement with the initial phenotype.[10]

The patients' main characteristics and the outcome of the transplant are summarized in Table 1. Myeloid and platelet engraftment was always rapid and complete donor chimerism was seen in 10/12 patients. Treatment-related toxicity was very mild. Five patients showed a cytomegalovirus (CMV) reactivation but only 1 developed CMV infection of the upper gastrointestinal tract (day +40). Following the reactivation of a pulmonary aspergillosis, treated with voriconazole, one patient in CR died of toxic hepatitis 20 months after the transplant. The overall inci-

Table 1. Main patients characteristics and the outcome of the transplant.

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*	*18*	*19*	*20*
1	M	59	B	3	72	multiple rearrang.	PB	100	107	56	60	0	0	never	yes	4 mo	PD	19	Richter trans.
2	F	48	B	3	86	46xx	PB	0	0	no	15 (mosDLI)	0	mild	15	yes	38 mos (DLI)	PR	45+	
3	F	56	B	3	132	trisomy 12	PB	0	15	23	210	0	mild	never	no	28	CR	38+	
4	M	57	C	1	18	trisomy 12	PB	0	16	60	14	I	0	10	yes	11mo (DLI)	CR	20	toxic hepatitis
5	M	55	B	2	80	n.a.	PB	1	0	40	20	II	ext	15	no	30	CR	16	infection
6	M	45	B	1	161	11q-	PB	0	0	33	28	III	mild	never	no	30	CR	27+	
7	M	52	C	2	112	n.a.	BM	10	20	no	60	0	0	11	yes	60	CR/PD	29+	
8	M	52	B	3	141	-3, -17	PB	0	141	19 mo	60	0	ext	26	yes	7mo (DLI)	CR	26+	
9	M	51	B	1	101	46XY	PB	0	13	no	28	0	mild	never	no	28	CR	22+	
10	M	55	C	3	140	n.a.	PB	12	120	no	mix	II	0	17	no	30	PR	22+	
11	M	58	C	2	45	46XY	PB	0	13	no	mix	0	0	5	yes	50	PR	15+	
12	M	55	C	2	24	13q-	PB	0	0	no	6 mos	0	0	never	no	30	CR	9+	

1: patients; 2: sex; 3: age; 4: Stage (Binet); 5: lines of therapy; 6: months form diagnosis; 7: cytogenetics; 8: SC source; 9: PMN>1×10^9/L (days); 10: PLT>50×10^9/L (days); 11: CMV react: (days); 12: chimerism (day of FDC); 13: acute GVHD; 14: chronic GVHD; 15: CsA tapering (months); 16: DLI; 17: max response (days); 18: current status; 19: follow-up months; 20: cause of death.

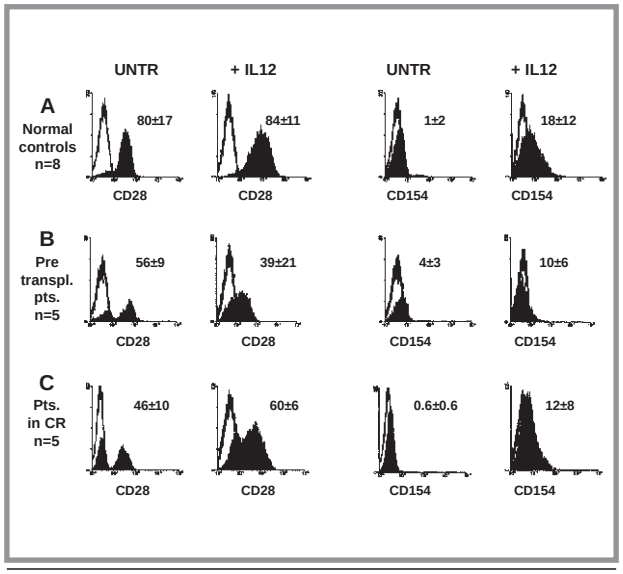

Figure 1. Representative FACS analysis showing the expression of co-stimulatory molecules (CD28 and CD154) on gated CD3⁺ cells with and without 100 U/mL interleukin-2 for 1 week in a) normal control b) pre-transplant and c) post-transplant in immunophenotypic complete remission. The upper right of each histogram indicates the% of CD3⁺ expressing co-stimulatory molecules (±SD of 8 and 5 samples). Open histograms show negative controls.

dence of acute GVHD grades I-III was 33%. One patient developed acute grade II cutaneous GVHD on day +23, which evolved into chronic sclerodermal-like GVHD, and died of hypovolemic septic shock 20 months after the transplant. One patient developed grade III hepatic GVHD on day +100, which responded completely to prednisone and antithymocyte globulin. Chronic GVHD involving either skin and mucosa or liver was present in 6 patients (50%) (4 mild and 2 extensive) (1 after DLI infusion), but five patients are still on cyclosporine treatment. Eight patients (66%) are in CR, as evaluated by flow cytometry, after a median follow-up of 22 months (range 9-45). Clearance of CD23/CD5/CD19 below the threshold of 1×10^4 peripheral blood cells was reached in 5 patients at a median time of 29 days after transplantation (range 28-30). Three patients reached CR later, in two cases after DLI. Two patients are in a good PR and one patient had stable disease controlled by DLI infusions although Richter's transformation occurred 19 months after the transplant. Overall, six out 12 patients (50%) were treated with DLI. After a median observation period of 22 months (range 9-45) 9 patients are alive (75%) and 3 patients have died (25%). The 3-year survival probability was estimated as 68%, while progression-free survival was estimated as 42%. There were no transplant-related deaths at day 100+ and 1 year. At 22 months probability of transplant related and relapse mortality were 16% and 8% respectively.

These data are comparable to those reported for other series of CLL patients treated with reduced-intensity allografts.[7,8] The incidence of both acute and chronic GVHD (33% and 50%, respectively) was comparable to that described in the EBMT study[7] and lower than that reported by Schetelig *et al.*,[8] both including some form of T-cell depletion. In addition, we, in contrast to other authors,[8] found that donor lymphocyte infusions were highly effective in half of the cases.

Since immune functions are known to be defective in CLL, lymphocyte phenotypic analyses were performed before and a mean time of 18 months after-transplant in order to detect the host-immune reconstitution. As shown in Figure 1, after interleukin-2 stimulation the percentage of cells expressing the co-stimulatory molecules CD28 and CD154 (CD40 ligand) on T cells increased in 5 patients in complete immunophenotypic remission.

These data suggest that RIC transplantation for advanced stage CLL can achieve successful immunomodulation, a reduction in tumor burden and, possibly, long-lasting complete remission.

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