

# Allogeneic Cell Therapy With Donor Peripheral Blood Cells and Recombinant Human Interleukin-2 to Treat Leukemia Relapse After Allogeneic Bone Marrow Transplantation

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**Allogeneic bone marrow transplantation (BMT) is the only effective treatment for hematologic malignancies resistant to conventional chemotherapy. Until recently, no cure existed for patients who relapsed post-BMT. We present our long-term observations on remission induction, after relapse post-BMT, by allogeneic cell therapy (allo-CT) and the feasibility of remission induction in allo-CT-resistant patients by activation of antileukemia effector cells with recombinant human interleukin-2 (rhIL-2) in vitro and in vivo. The longest observation of successful allo-CT (event-free survival, greater than 8 years) was made in a patient with resistant pre-B lymphoblastic leukemia who received infusions with graded increments of donor (female) peripheral blood lymphocytes (PBL) as soon as bulky hematologic and extramedullary relapse was noticed early post-BMT. The patient is currently without evidence of residual host (male) cells as determined by polymerase chain reaction (PCR). Of 17 patients with acute and chronic leukemia in relapse after BMT, 10 were reinduced into complete remission. Four patients**

**with cytogenetic relapse responded to allo-CT alone, while five of six patients with overt hematologic relapse responded only after additional activation of donor with rhIL-2. Allo-CT can, therefore, successfully reverse chemoradiotherapy-resistant relapse of both acute and chronic leukemia. Moreover, in patients resistant to donor lymphocyte infusion, remission can be accomplished by additionally activating donor PBL in vitro and/or in vivo with rhIL-2. Based on our observations, after BMT, allo-CT should be considered the treatment of choice for patients with hematologic malignancies resistant to conventional anticancer modalities. Allogeneic activated cell therapy (allo-ACT) should be considered for patients with tumor cells resistant to allo-CT. Although allo-CT, followed if indicated by allo-ACT, can be effective for patients with overt hematologic relapse, reversal of persistent minimal residual disease or documented molecular/cytogenetic relapse early after BMT may also be considered as a possible indication for allo-CT.**

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**H**IGH-DOSE chemoradiotherapy followed by bone marrow transplantation (BMT) with cells from genotypically or phenotypically matched donors has become the treatment of choice for chronic myeloid leukemia (CML), for patients with acute leukemia who have already relapsed or who are at high risk to relapse, and for those with primary resistant disease.<sup>1-3</sup> The advantage of BMT over conventional chemotherapy lies in the combined effects of the higher myeloablative dose of chemoradiotherapy given pretransplant and the ability of immunocompetent allogeneic donor T lymphocytes to react to residual tumor cells of host origin, ie, the graft-versus-leukemia (GVL) effect.<sup>4-10</sup> The possibility that allogeneic BMT eliminates leukemia through immune-mediated GVL effects has been suggested ever since the earliest days of experimental<sup>8-15</sup> and clinical BMT.<sup>4-7</sup> Recent data from murine models imply that GVL effects may also be induced by posttransplant administration of graded increments of immunocompetent allogeneic lymphocytes<sup>16-20</sup> and may be additionally increased by in vivo activation of lymphocytes with recombinant human interleukin-2 (rhIL-2).<sup>16-20</sup> Preliminary data from pilot clinical trials suggest that a similar rationale for the treatment and prevention of relapse<sup>17-23</sup> may be applicable. The present report documents the first successful induction of GVL effects by allogeneic cell therapy (allo-CT) using donor peripheral blood lymphocytes (PBL) in a patient with resistant acute lymphoblastic leukemia (ALL) who relapsed shortly after BMT. Similar cases with a variety of malignant hematologic diseases have been successfully treated at many BMT centers, including our own. The cumulative international data indicate that cell therapy using major histocompatibility complex (MHC)-matched allogeneic lymphocytes should be considered the treatment of choice for persistent disease or relapse post-BMT. Moreover, our data show that patients with tumor cells resistant to allo-CT can still respond to in vivo  $\pm$  in vitro activation of donor PBL by rhIL-2.

## MATERIALS AND METHODS

*Patient characteristics.* A total of 17 patients (age range, 2.5 to 39 years; median, 17 years) are presented: six with ALL, three with acute myeloid leukemia (AML), six with CML (two in accelerated phase), one with Burkitt's lymphoma, and one with myelodysplastic syndrome (MDS) with excess blasts. All patients gave their informed consent after approval of the proposed study by the Institutional Review Board (Helsinki Committee). Patient characteristics and details of all pretransplant and posttransplant therapies are listed in Table 1 for 13 patients with overt hematologic relapse and in Table 2 for four patients with minimal cytogenetic relapse. All patients received BMT from a serologically HLA-A,B,DR-matched, MLR nonreactive sibling. PBL were obtained from the marrow donor.

*BMT procedures.* The three conditioning regimens used before BMT were (1) cyclophosphamide 60 mg/kg  $\times$  2 days followed by fractionated total body irradiation (TBI) 200 cGy  $\times$  six fractions (protocol in use for patients with CML); (2) etoposide 1,500 mg/m<sup>2</sup>  $\times$  1 day, cyclophosphamide 60 mg/kg  $\times$  1 day, melphalan 60 mg/m<sup>2</sup>  $\times$  1 day followed by TBI 200 cGy  $\times$  6 over 3 days (protocol in use for patients with acute leukemia); and (3) combination chemotherapy without TBI, consisting of busulfan 4 mg/kg  $\times$  4 days and

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**Table 1. Cell Therapy With Donor HLA-Matched Immunocompetent Blood Lymphocytes With or Without rhIL-2 Activation In Vivo and/or In Immunotherapy Protocol With Donor Blood**

UPN	Patient Characteristics and BMT Procedure								Relapse	
	Age/ Sex	Diagnosis at BMT	Disease Status at BMT	Conditioning*	Date of BMT	Donor Sex	TCD	GVHD Post- BMT	Mo Post-BMT	Type of Relapse
139	2/M	ALL (pre B)	2nd relapse	a	12/17/86	F	+	0	1	Hematologic
166	23/F	ALL (1.1)	1st CR	b	9/21/87	M	+	0	15	Hematologic
244	32/F	CML	AP	b	3/23/89	F	+	0	12	Cytogenetic
278	28/F	AML (M5)	1st relapse	b	8/9/89	M	+	0	4	Hematologic
G.R.¶	5/M	ALL (CALLA <sup>+</sup> )	2nd CR	a	1/8/90	M	-	Grade 1	1	Hematologic and cytogenetic
N.L.¶	9/F	CML	CP	c	2/21/91	M	-	0	9	Hematologic and cytogenetic
415	3/F	CML	CP	c	5/22/91	M	-	0	8	Hematologic and cytogenetic
517	14/M	ALL (CALLA <sup>+</sup> )	3rd relapse	b	7/22/92	M	+	0	3	Hematologic
545	6/F	AML (M2)	1st relapse	a	10/28/92	M	+	0	2	Hematologic
571	20/F	Burkitt's lymphoma	Resistant disease	a	1/13/93	M	-	0	1.5	Hematologic
579	2/F	MDS with excess blasts	Resistant disease	c	2/10/93	M	-	0	5	Hematologic and cytogenetic
634	17/M	CML	AP	a	7/21/93	M	-	0	4	Hematologic and cytogenetic
656	8/M	AML (M5)	2nd relapse	a	9/15/93	M	+	Grade II	1.5	Hematologic

Abbreviations: UPN, unique patient number; BMT, bone marrow transplantation; TCD, T-cell depletion; Allo-CT, allogeneic cell therapy; Allo-ACT, allogeneic activated cell therapy; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; CR, complete remission; AP, accelerated phase; CP, chronic phase; chrom, chromosome.

\* Details of conditioning at BMT: (a) total lymphoid irradiation (TLI) + chemotherapy total body irradiation (TBI); (b) chemotherapy + total body irradiation (TBI); (c) busulfan + cyclophosphamide (for further details see Materials and Methods).

† Donor PBL alone.

‡ Donor PBL further activated in vivo by rhIL-2.

§ Donor PBL preactivated in vitro with rhIL-2 (allogeneic LAK cells) with additional in vivo activation of effector cells with rhIL-2 for 3 days.

¶ Patient transplanted in Barcelona, Spain.

|| Patient transplanted in Seattle, WA.

cyclophosphamide 50 mg/kg × 4 days. Post-BMT anti-graft-versus-host disease (GVHD) prophylaxis was given to only one recipient of non-T cell-depleted allografts and consisted of standard doses cyclosporin A with or without methotrexate.<sup>7</sup>

*T-cell depletion for prevention of GVHD.* Patients receiving T cell-depleted allografts (n = 11) to prevent graft rejection were additionally conditioned by total lymphoid irradiation (TLI), with

four fractions of 150 cGy over 2 days, as detailed previously.<sup>24</sup> Before BMT, marrow cells were treated either in vitro with the monoclonal rat anti-human lymphocyte antibody CAMPATH-1M (IgM; rat anti-human-CDW52; n = 2) using fresh donor serum as the source of complement<sup>24-27</sup> or with CAMPATH-1G (IgG2b isotype switch variant, n = 9) added to the marrow collection bag to deplete T cells in vivo by Fc-mediated antibody-dependent cell-

**Vitro: Allogeneic Cell Therapy (Allo-CT) for Patients With Overt Relapse Following Allogeneic BMT for Leukemia Using an Escalated Lymphocytes and rhIL-2 In Vivo and In Vitro**

Post-BMT Course, Procedures, and Outcome								
Immunotherapy With Donor PBL					Response		Outcome (mo)	
Allo-CT†	Allo-CT+ IL-2‡	Allo-ACT+ IL-2§	Total T Cells/kg	Other	Evidence for Response	GVHD Post-Cell Therapy	Alive and Well	Died (mo post-BMT)
+	-	-	$2.1 \times 10^6$	-	Hematologic response, karyotype: female, PCR Y chrom: negative	Grade II	>96	-
+	+	-	$1.3 \times 10^8$	-	Progressive disease	0	-	24
+	+	-	$2.1 \times 10^7$	$\alpha$ IFN + rhIL-2	Cytogenetic: 16% Ph <sup>+</sup> to 0%, RT-PCR: bcr/abl: negative	0	>69	-
+	+	-	$1.1 \times 10^8$	-	Progressive disease	0	-	7.5
+	+	+	$3.2 \times 10^8$	-	Cytogenetic: nonspecified aberration 16% to 0%	Grade II-III	>60	-
+	+	+	$2.9 \times 10^8$	$\alpha$ IFN	Cytogenetic: 100% Ph <sup>+</sup> to 0%, RT-PCR: bcr/abl: negative	0	>46	-
+	+	+	$3.3 \times 10^8$	$\alpha$ IFN	Progressive disease	0	Underwent 2nd allogeneic BMT	-
+	-	-	$8 \times 10^7$	-	Progressive disease	0	-	9
+	+	-	$8 \times 10^7$	-	Progressive disease	0	-	3
+	+	-	$7 \times 10^7$	-	Progressive disease	0	-	3
+	+	+	$4.6 \times 10^8$	-	Hematologic response, cytogenetic response, karyotype: male, PCR Y chrom: positive	0	-	10 (relapse)
+	+	+	$1.7 \times 10^8$	-	Hematologic response, cytogenetic: 100% Ph <sup>+</sup> to 0%, RT-PCR:bcr/abl: negative	II	>17	-
+	+	-	$3.6 \times 10^8$	-	Progressive disease	II	-	3

mediated cytotoxicity. Both CAMPATH-1 antibodies were provided by Drs H. Waldmann and G. Hale, Department of Pathology, Cambridge University School of Medicine, Cambridge, UK. Recipients of T cell-depleted allografts received neither cyclosporin A nor any other anti-GVHD prophylaxis after BMT. The outlines of the protocols used are presented schematically in Fig 1, and the detailed procedures for patients with overt hematologic or cytogenetic relapse are presented in Tables 1 and 2, respectively. No prior treatment with alpha-interferon ( $\alpha$ IFN) was given before allo-CT.

**Immunotherapy with allogeneic donor PBL.** Allo-CT was initiated by infusion with graded increments of donor PBL (Fig 1). Eligibility criteria included patients with documented relapse or no immunosuppressive agents, with no evidence of GVHD in the immediate posttransplant period. In patients with relapse resistant to infusion with donor PBL and no severe GVHD, allo-CT was combined with in vivo administration of rhIL-2. Allogeneic activated cell therapy (allo-ACT), ie, in vitro activated donor lymphocytes (ADL) precultured for 4 days with

rhIL-2 and activated in vivo by administration of rhIL-2, was given to patients with resistant relapse not responding to allo-CT (Fig 1). All allo-CT procedures, including rhIL-2 administration, were performed on an outpatient basis within 1 to 16 months (median, 4 months) after BMT, as soon as relapse was diagnosed. Donor PBL were obtained by blood aspiration (for small cell doses) or by apheresis using a Baxter CS-3000+ cell separator (Baxter, Deerfield, IL). Cells were infused without further in vitro manipulation, except for removal of red blood cells in cases of major ABO incompatibility. The cell dose given was calculated as the total number of T cells per kilogram. The cumulative number of T cells infused with donor PBL ranged from  $0.2 \times 10^8$ /kg to  $4.6 \times 10^8$ /kg; cell numbers per dose are listed in Tables 1 and 2. Escalation of post-BMT immunotherapy was considered if no measurable response was observed within 1 month or whenever disease progression was documented.

**Augmentation of cell therapy by administration of rhIL-2.** For both in vivo and in vitro activation of donor PBL, rhIL-2 was used.

**Table 2. Allogeneic Cell Therapy (Allo-CT) for Patients With Minimal Residual Disease After Allogeneic BMT Using Donor Peripheral Blood Lymphocytes**

Patient Characteristics and BMT Procedure										Post-BMT Course, Procedure, and Outcome							
UPN	Age/ Sex	Diagnosis at BMT	Disease Status	Con-dition-ing*	Date of BMT	Donor Sex	TCD	GVHD Post-BMT	Mo Post-BMT	Relapse	Donor PBL Response					Outcome	
			1st CR	a	11/20/91	F	+	0	16	Type of Relapse	10 <sup>5</sup> T Cells/kg	10 <sup>6</sup> T Cells/kg	10 <sup>7</sup> T Cells/kg	Evidence for Response	Post-Cell Therapy GVHD	Alive and Well (mo)	Died (mo from cell therapy)
451	17/M	ALL (L1-L2)		a	11/20/91	F	+	0	16	residual male cells	-	+	+	Karyotype: female, PCR Y chrom: negative	0	>37	-
564	21/M	ALL (T-type)	2nd CR slow response	a	12/13/92	F	+	Grade I	4	residual male cells	-	+	5 × 10 <sup>7</sup>	Karyotype: female, PCR Y chrom: negative	Grade III	>24	-
613	36/M	CML	CP	b	5/27/93	F	+	0	3	residual male cells	+	+	-	Karyotype: female, PCR Y chrom: negative RT-PCR bcr/abl: negative	Grade II-III	>19	-
622	39/M	CML	CP	b	6/23/93	F	+	0	5	residual male cells	+	+	+	Karyotype: female, PCR Y chrom: negative; RT-PCR bcr/abl: negative	Grade IV	-	15 (GVHD)

Abbreviations: UPN, unique patient number; BMT, bone marrow transplantation; TCD, T-cell depletion; Allo-CT, allogeneic cell therapy; PBL, peripheral blood lymphocytes; PCR, polymerase chain reaction; RT-PCR, reverse transcriptase PCR; ALL, acute lymphoblastic leukemia; CML, chronic myeloid leukemia; CP, chronic phase; chrom, chromosome.  
 \* Details of conditioning at BMT: (a) total lymphoid irradiation (TLI) + chemotherapy total body irradiation (TBI); (b) chemotherapy + total body irradiation (TBI) (for further details see Materials and Methods).

It was purchased from EuroCetus/Chiron (Amsterdam, The Netherlands) and was provided as 1 mg Proleukin, equivalent to 18 × 10<sup>6</sup> international units (IU). In vivo rhIL-2 was given subcutaneously on an outpatient basis at a dose of 6 × 10<sup>6</sup> IU/m<sup>2</sup>/d for 3 consecutive days, starting on the day of administration of donor PBL (allo-CT) or ADL (allo-ACT).

*In vitro activation of donor PBL by rhIL-2 (ADL).* ADL were prepared by culturing donor PBL at a concentration of 2 × 10<sup>6</sup> mononuclear cells per milliliter in RPMI 1640 medium (Biological Industries, Beit Haemek, Israel) containing 100 U/mL penicillin and 100 µg/mL streptomycin in 750-mL sterile culture flasks (Corning, Corning, NY). The culture medium was supplemented with 4% heat inactivated human AB serum (after screening for hepatitis A, B, and C and human immunodeficiency virus-1 [HIV-1]). Cells were cultured in rhIL-2 at a concentration of 6,000 IU/mL for 4 days in a humidified 5% CO<sub>2</sub> incubator at 37°C. Cells were harvested, centrifuged, washed twice with Hank's balanced salt solution, and adjusted to a concentration of 2 × 10<sup>6</sup>/mL. ADL were administered by infusion with a nonfiltered intravenous set. Activation of cells was confirmed by immunophenotyping, measuring <sup>3</sup>H-thymidine uptake and in vitro microcytotoxic activity using chromium-labeled natural killer cell (NK)-sensitive (K562) and NK-resistant (Daudi) target cell lines (data not shown) as previously described.<sup>25</sup>

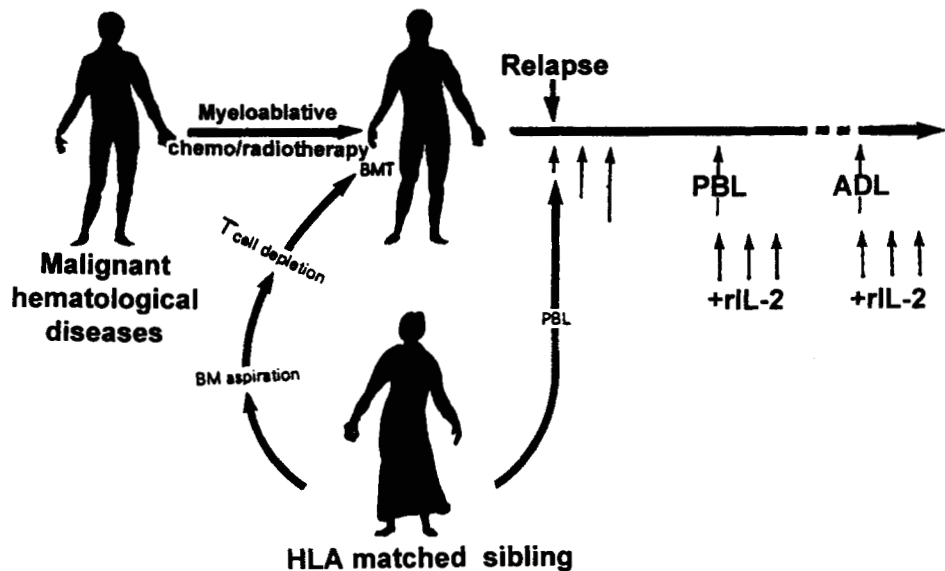
*Assessment of response to cell therapy.* The time interval from initiation of allo-CT to administration of rIL-2 together with the subsequent dose of donor-derived PBL or allo-ACT ranged between 52 and 206 days (median, 60 days; a median of 30 days from allo-CT to allo-CT + rhIL-2 and a median of 30 days from allo-CT + rhIL-2 and allo-ACT + rhIL-2). The effect of allo-CT and allo-ACT ± rhIL-2 on relapse was assessed by hematologic evaluation of disease-specific parameters, including blood and bone marrow morphology, cytogenetics (disease-specific translocations), and disease-specific transcripts (bcr/abl by the reverse transcriptase-polymerase chain reaction [RT-PCR]).<sup>28</sup> In addition, whenever applicable, host- and donor-specific markers were determined (eg, presence of male cells in female-to-male chimeras) by cytogenetic analysis of phytohemagglutinin-stimulated PBL and spontaneous metaphases in bone marrow aspirates and/or by detection of male-specific molecular markers by PCR, using SRY or amelogenin-specific (AMG) oligonucleotide primers.<sup>29,30</sup> Disease-free survival was reported for all assessable cases.

*Molecular analysis of minimal residual disease.* Minimal residual disease was determined by detection of both disease and host-specific markers. Disappearance of previously positive RT-PCR or Y-specific host markers for a minimum of two consecutive tests at ≥1-month intervals after cell therapy was interpreted as evidence of elimination of minimal residual disease. RT-PCR for detection of bcr/abl was performed according to published methods.<sup>28</sup> Detection of Y-specific markers was performed either by PCR of SRY-specific regions, as previously described,<sup>29</sup> or by PCR of part of the AMG gene on the X-chromosome and its shorter copy on the Y-chromosome.<sup>30</sup>

**RESULTS**

*Treatment of relapse after BMT with allo-CT.* Allo-CT with graded increments of donor PBL was pioneered in a 30-month-old boy referred to the BMT Center at the Hadassah University Hospital (Jerusalem, Israel) in November 1986 for resistant pre-B ALL. Pertinent clinical details and procedures are listed in Table 1.

At 1 month post-BMT, his peripheral white blood cell count rose to 11 × 10<sup>9</sup>/L with 20% lymphoblasts, and the bone marrow aspirate showed massive infiltration with lymphoblasts. The patient presented with four visible subcu-



**Fig 1. Recommended allo-CT and allo-ACT protocol for treatment of overt hematologic relapse after T cell-depleted (or non-T cell-depleted) BMT. Immunotherapy for relapse can be intensified after documentation of resistant tumor cells and provided no GVHD ( $\geq$  grade II) is diagnosed: (1) graded increments of donor PBL; (2) infusion of donor PBL with in vivo administration of rhIL-2; and (3) administration of in vitro ADL combined with in vivo administration of rhIL-2.**

taneous masses of 2 cm in diameter and one additional retro-tracheal mass of 3 cm in diameter that restricted the larynx, with symptomatic respiratory distress requiring tracheotomy. Cytogenetic analysis of the marrow and cells obtained from one of the masses showed male cells with the original clonal cytogenetic abnormality, with no evidence of Epstein-Barr virus (EBV), thus excluding post-BMT EBV-induced lymphoma. Emergency palliative systemic treatments included vincristine and prednisone, with low-dose methotrexate ( $20 \text{ mg/m}^2$ ) and local irradiation ( $2,400 \text{ cGy}$ ) of the retro-tracheal lesion and of one of the masses. Due to progressive hematologic relapse and extramedullary lesions, the patient received a total of six doses of graded increments of donor (female) PBL equivalent to  $10^3$ ,  $10^4$ ,  $10^5$ ,  $5 \times 10^5$ ,  $5 \times 10^5$ , and  $1 \times 10^6$  T cells per kilogram to induce GVL. On day +102 post-BMT, the patient developed mild grade I acute cutaneous GVHD, which gradually progressed within 2 weeks to grade II with involvement of the skin and liver with rapid response to prednisone ( $2 \text{ mg/kg}$ ). Surprisingly, a visible response was noted within 2 to 3 weeks; all masses gradually disappeared and blasts could no longer be detected. The patient was gradually tapered off steroids as soon as all cutaneous manifestations of GVHD regressed and liver function tests normalized. Remission was confirmed by normal bone marrow morphology and by cytogenetic analysis featuring normal female karyotype in 50 of 50 metaphases investigated. Continuous follow up of the patient showed normal growth and development. To date (more than 8 years after allo-CT), no residual male cells have been detected by PCR analysis with either SRY-specific or AMG-specific primers (sensitivity,  $1:10^6$  male cells).

On the basis of the successful outcome with the first patient, allo-CT given as graded increments of donor PBL (Fig 1) was administered to an additional cohort of 16 patients who had relapsed 1 to 16 months (median, 4 months) after BMT. As shown in Tables 1 and 2, only 5 of 17 patients (four with cytogenetic relapse and one with hematologic

disease) had no detectable leukemic cells after allo-CT. Of 13 patients with overt hematologic relapse (four with CML, four with ALL, three with AML, one with Burkitt's lymphoma, and one with MDS with excess blasts), only one patient responded to allo-CT. Hence, cell therapy was escalated by rhIL-2 (see below). In contrast, all four patients with minimal cytogenetic relapse responded to donor PBL alone (Table 2).

**Intensification of cell therapy with rhIL-2.** Based on the cumulative preclinical data in murine models of acute lymphoid and myeloid leukemias,<sup>18-20,31-33</sup> we investigated the use of rhIL-2 administered in vivo and in vitro to increase GVL effects, as presented in Fig 1. Eleven patients who had not responded to allo-CT (excluding one patient, UPN 517 in Table 1, where disease progression occurred before therapy could be initiated) were given rhIL-2 in vivo for 3 days after infusion with donor PBL. Allo-ACT, ie, combining in vitro activation of donor PBL (ADL) with additional in vivo activation of GVL effects by rhIL-2 for 3 consecutive days after infusion with ADL, was tested in five patients who did not respond to infusion with donor PBL and rhIL-2 alone and who had not developed GVHD (Table 1). As can be seen in Tables 1 and 2, relapse after BMT was successfully reversed in 10 of the 17 patients: in four of six with ALL, none of three with AML, five of six with CML, and one patient with MDS with excess blasts (one of two cases with other syndromes). Of six patients with overt hematologic relapse who responded to cell therapy, five patients were induced into remission only after additional activation of donor PBL with rhIL-2. As detailed above, the time interval from induction of immunotherapy by donor-derived T cells alone and donor T cells activated by rhIL-2 (in vivo, in vitro, or both) ranged between 52 and 206 days (median, 60 days).

At present, all four responders with ALL and four of the five patients with CML (one of whom was transplanted in accelerated phase) are alive and well, free of disease 17 to 96 months (median, 38 months) after BMT and more than

13 to 95 months (median, greater than 2 years) after cell therapy. One of the responders with CML died of GVHD grade IV with no evidence of disease (Table 2), while another responder with MDS treated in transition to overt leukemia died of late relapse (Table 1). Two of the four patients with CML with extremely resistant relapse received additional posttransplant immunotherapy to maintain remission; one patient treated at accelerated phase (UPN 244) received additional rhIL-2 and  $\alpha$ IFN therapy for 2 months. Patient N.L. with adult-type CML at the age of 9 years, who was originally treated with a non-T cell-depleted graft in Seattle, WA, received  $\alpha$ IFN after completing allo-CT and allo-ACT. Currently, all four patients are persistently negative for bcr/abl, according to RT-PCR with no evidence of GVHD and a Karnofsky score of 100%. Of the four responding patients with ALL, the first (UPN 138), whose case report is described here in detail, has no evidence of GVHD, while two patients (G.R. and UPN 564) have moderate and mild chronic GVHD, respectively. All are free of disease.

#### DISCUSSION

Although relapse after BMT is generally considered incurable, we present a successful treatment for resistant, relapsing acute and chronic leukemia by posttransplant immunotherapy with donor immunocompetent PBL with a follow-up period of greater than 8 years. Interestingly, in agreement with preclinical experiments in murine models of ALL and AML,<sup>17,19,30-33</sup> the antileukemic effects induced by donor PBL were amplified *in vivo* by a short course of rhIL-2 administered subcutaneously with no severe side reactions. With a standard BMT protocol, even in patients at risk of developing GVHD, the incidence of relapse may reach 25% when patients are transplanted in first complete remission, nearly 50% at more advanced disease, and greater than 75% in patients transplanted in overt relapse or with resistant disease.<sup>1-7,34,35</sup> Hence, GVL effects induced by immunocompetent T lymphocytes present in the donor marrow aspirate may be insufficient to prevent relapse when conventional anti-GVHD prophylaxis is administered.<sup>34-39</sup> Indeed, it was previously documented that posttransplant immunosuppression for prevention, attenuation, or treatment of GVHD, unavoidable after non-T cell-depleted BMT, may also abrogate the T cell-dependent GVL effects<sup>36-41</sup> in experimental animals<sup>42</sup> and humans.<sup>36-39</sup> Conversely, it was also shown that discontinuation of cyclosporin A as soon as relapse is diagnosed can reinduce remission.<sup>37-41</sup>

In our own study, we found that of the 17 patients treated by cell therapy, 6 of the 10 responders developed GVHD, whereas in the remaining four responders, GVL was independent of GVHD. Of the seven nonresponders, only one developed GVHD, pointing to the close relationship between GVL and GVHD. Furthermore, GVL can occur independently of GVHD, whereas GVHD may not be sufficient to induce effective GVL. The 40% success rate among responders without GVHD indicates that GVL can be induced by increasing the intensity of allo-CT.<sup>17-23</sup> Nonetheless, as the time to remission induction in patients relapsing after BMT in response to allo-CT may take longer than the median of 60 days elapsed between administration of donor-derived

PBL and rhIL-2-dependent immunotherapy (range, 52 to 206 days), the conclusion that remission was induced by rIL-2-activated donor T lymphocytes rather than being a late response to allo-CT alone must be kept in mind.

The cumulative international experience with allo-CT in a total of 163 patients<sup>43-65</sup> confirms our initial observations. Complete responses (molecular, cytogenetic; or hematologic) were observed in 98 of 158 (62.8%) assessable patients (72% among patients with CML and 45% among patients with other hematologic malignancies). Allo-CT proved effective in treating relapse after both unmanipulated and T cell-depleted BMT for different hematologic malignancies<sup>43-65</sup> independently of prior  $\alpha$ IFN therapy. Remission in most, but not all, cases successfully treated with allo-CT was linked to GVHD, which was observed in 63% of assessable patients with CML and 39% of assessable patients with other hematologic malignancies,<sup>43,47,48,51,52</sup> suggesting that remission may be induced with no GVHD.<sup>43,60</sup>

According to our own data and in agreement with other centers,<sup>66,67</sup> relapse was less successfully reversed in acute leukemia when compared with CML: 45.4% versus 83.3%, respectively. However, effective treatment of 6 of the 13 patients in advanced hematologic relapse, five of whom received rhIL-2 after failing allo-CT alone, indicates that the success rate may be increased in patients with acute leukemia as well as in patients with CML by additional activation of donor PBL with rhIL-2 *in vivo* and/or *in vitro*.

Based on earlier animal data<sup>68</sup> and on the results of this study, infusion with graded increments of donor PBL may be an individually adaptable, safe, simple, and cost-effective method of inducing GVL while controlling the incidence, intensity, and severity of GVHD. At early evidence of molecular or cytogenetic relapse, or to prevent relapse in high-risk cases, allo-CT may be considered with a low, relatively safe, initial cell dose of  $10^5$  T cells per kilogram to avoid severe GVHD. A 10-fold increase can then be given at 2 to 4-week intervals to patients receiving no anti-GVHD prophylaxis who do not develop GVHD.<sup>69</sup> As shown in Table 2, patients with minimal residual disease responded very effectively to small increments of donor PBL without any need for more aggressive immunotherapy (eg, high donor cell doses or rhIL-2) and with no signs of marrow aplasia.

T cell-dependent GVL effects independent of GVHD have previously been reported in experimental animals<sup>11-17,32</sup> and humans. The capacity of lymphocytes fully tolerant to host-type alloantigens to mediate GVL independently of GVHD is strongly supported by data in mice.<sup>12,16,32,70</sup> Moreover, we have recently documented that high-dose rhIL-2 may induce GVL-like effects even after syngeneic BMT.<sup>71</sup> Interestingly, T cells with potential specific reactivity to tumor cells rather than normal host cells were documented in different experimental systems,<sup>72-74</sup> supporting a possible cellular basis for GVL independently of GVHD.<sup>70</sup>

In support of our concept that amplified GVL while controlling for GVHD may be accomplished by administration of graded increments of donor cells late after BMT, we have previously documented the safety of graded increments of immunocompetent allogeneic donor-type T cells in stable chimeras after non-T cell-depleted,<sup>68</sup> as well as after T cell-

depleted, BMT.<sup>32</sup> In clinical practice, a threshold of  $10^5$  donor T cells per kilogram is considered sufficient to cause acute GVHD during the immediate post-BMT period without anti-GVHD prophylaxis, but much higher numbers of donor T cells can be safely administered if the time interval between BMT and PBL administration is prolonged, as shown schematically in Fig 1. Similar data confirming our original concept were reported in one additional preclinical murine model of T-cell leukemia.<sup>75</sup> Graded increments of donor PBL administered late after BMT may account for improved outcome in our study as compared with data reported by Sullivan et al.<sup>76</sup> These investigators could not document any benefit in response to early administration of large inocula of donor PBL to recipients of non-T cell-depleted allografts in patients receiving posttransplant immunosuppression.

Based on our observations, we would like to hypothesize that transplant-related complications due to severe GVHD and postgrafting immunosuppressive agents may be partly prevented by combining T cell depletion at BMT with avoiding posttransplant immunosuppressive therapy, which is mandatory after a non-T cell-depleted BMT procedure. Late administration of graded increments of donor PBL, while controlling for GVHD, may be used at a later stage for prevention of relapse.

As shown in experimental animals and as suggested by the cumulative clinical experience, induction of optimal cell-mediated immunotherapy to treat occult residual tumor cells escaping chemoradiotherapy or relapsing disease may be best accomplished under no cover of post-BMT immunosuppressive agents, with control of GVHD by optimal timing, starting as late as possible, followed by modest increments of donor T cell number, with both procedures adapted to the unique sensitivity and needs of each individual. For patients in complete remission, T cell-depleted BMT (avoiding GVHD and the need for post-BMT immunosuppression) with compensatory T-cell repletion by a safe allo-CT regimen should be further investigated prospectively as a possible alternative to the conventional BMT protocol. For patients with primary resistant disease or overt relapse post-BMT, a non-T cell-depleted allograft may be preferable, despite the risk of GVHD, to prevent early and irreversible progression of leukemia. In principle, allo-CT may be also considered for recipients of non-T cell-depleted allografts who are off immunosuppressive therapy with stable condition and no evidence of GVHD, thus further increasing the chance of elimination of host-derived tumor cells as well as residual normal hematopoietic cells. Increments of donor PBL should be considered until elimination of all measurable tumor cells or residual host cells as determined by sensitive molecular tools (eg, PCR, RT-PCR, or PCR-variable number tandem repeats [VNTR]) or until GVHD is imminent. As a rule, aggressive, thus more risky, posttransplant immunotherapy should be considered only if disease or host-specific markers persist or reappear. Nonetheless, based on the above concepts, the clinical application of a nonaggressive allo-CT protocol, using a low and slow donor T-cell therapy regimen for prevention rather than for treatment of measurable disease, may be justified in a prospective randomized clinical trial in patients at risk to relapse, as has recently been successfully pioneered at our center.<sup>69</sup>

In conclusion, although patients with a variety of hematologic malignancies relapsing after BMT, especially CML, may be successfully treated with allo-CT, patients resistant to therapy with donor cells alone might still respond to allo-CT and allo-ACT enhanced by rhIL-2 administration *in vivo*. Alloimmune-mediated interactions between immunocompetent donor T cells and residual tumor cells of host origin should be used for patients receiving no immunosuppressive agents to prevent GVHD. The efficacy of immunotherapy as described here and the lack of a safe alternative modality for treating relapse after BMT suggest that allogeneic cell therapy with matched donor PBL may become an important tool for the treatment of hematologic malignancies, based on alloimmune recognition of host tumor cells as minor histocompatibility-mismatched allografts. The possible use of allogeneic cell therapy for prevention rather than treatment of relapse for a wider range of malignancies should be further investigated.

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