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Autologous bone marrow transplantation for childhood acute lymphoblastic leukemia: a novel combined approach consisting of *ex vivo* marrow purging, modulation of multi-drug resistance, induction of autograft *vs* leukemia effect, and post-transplant immuno- and chemotherapy (PTIC)

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Summary:

In an attempt to reduce the high relapse rate associated with ABMT, five children with high-risk first CR and 19 in second or subsequent CR lacking matched family allogeneic donors underwent ABMT with chemopurged bone marrow utilizing verapamil (VPL), vincristine, and VP-16. Patients were conditioned with TBI, VPL bolus and infusion with VP-16 and cyclophosphamide. The first cohort of patients (n = 4) received only cyclosporin A (CsA). The second cohort (n = 7) received CsA and alpha interferon (total = 11 with post-transplant immunotherapy alone.) The third cohort (n = 13) received CsA and six alternating cycles of αIFN and chemotherapy and six additional cycles of chemotherapy (vincristine, VP-16, Ara-C, prednisone) followed by G-CSF (post-transplant immune chemotherapy (PTIC)). The 2-year DFS is 42 ± 10% (90% confidence interval (CI) is 26.5–58.5%) and 2-year overall survival is 54 ± 10% (90% CI is 37.5–70.5%). Furthermore, patients receiving PTIC (n = 13) *vs* immunotherapy alone (CsA ± αIFN) (n = 11) had a substantially better 2 year DFS and OS: 69 ± 13% *vs* 13 ± 12% and 85 ± 10% *vs* 25 ± 15% (P = 0.008 and P = 0.06, respectively). These results suggest that the use of ABMT with chemopurging, combined with PTIC is well tolerated and may be an alternative new approach in the treatment of a subset of children with high-risk first CR or ⩾ second CR ALL who lack closely matched family-related allogeneic donors. *Bone Marrow Transplantation* (2001) **27,** 145–153.

Keywords: childhood ALL; autologous bone marrow transplant

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Among patients with acute leukemias, children with acute lymphoblastic leukemia (ALL) have an excellent prognosis following modern intensive chemotherapy.[1] However, for children who relapse, chemotherapy alone is rarely curative.[2] Allogeneic bone marrow transplant (BMT) from a matched sibling donor has been shown to be superior to chemotherapy, especially for patients with a short initial complete remission (CR).[3,4] Unfortunately, about 70% of children lack a matched family donor and it is not always possible to identify a fully compatible unrelated allogeneic donor in a timely fashion. For children without a suitable matched allogeneic donor, autologous BMT (ABMT) may offer an alternative with similar survival combined with the advantages of absence of graft-versus-host-disease (GVHD) and of an accelerated immunological recovery compared to allogeneic transplantation.[3,5] However, to date, a higher relapse rate is observed in patients receiving ABMT compared to related or unrelated allogeneic BMT.[3,6]

Leukemia relapse after ABMT may be attributed to multiple factors. The marrow graft may be contaminated with leukemic cells and therefore patients receiving unpurged ABMT may receive a high burden of leukemic cells, which could increase the probability of post-transplant relapse. Secondly, there may be a failure of conditioning regimens to successfully eradicate the residual leukemia burden *in vivo*. Thirdly, patients undergoing ABMT may not benefit from the immunologic effects of the graft-versus-leukemia phenomena which has been postulated to play a major role in decreasing the leukemia relapse rate following allogeneic BMT.[7] Lastly, the presence of multiple drug resistance (MDR) expressed within residual leukemia cells may be associated with increased drug efflux and decreased chemotherapy accumulation of certain classes of chemotherapy compounds including vinca alkaloids, anthracyclines and epipodophyllotoxins.

To improve disease-free survival (DFS) following ABMT for high-risk childhood ALL, ABMT transplant protocols need to address each of these potential sources of relapse. Verapamil inhibits drug efflux and binds to the product of the *mdr* gene, the P-glycoprotein.[8,9] During the

past couple of years our group has been investigating different methods of marrow purging in order to be able to eradicate the leukemia cells from the marrow graft.[10–12] These studies suggest that verapamil may potentiate the ability of VP-16 and vincristine to chemopurge resistant leukemia cells from human bone marrow without significantly enhancing bone marrow toxicity. The dose-limiting bone marrow cytotoxicity (CFU-GM IC_{90}) (IC = inhibitory concentration) with verapamil (10 μM) is 1.5 μM of vincristine and 50 μg/ml of VP-16.[12] Furthermore, to eradicate the residual resistant leukemia burden *in vivo*, a conditioning regimen of fractionated total body irradiation (TBI), VP-16 plus verapamil, and cyclophosphamide was developed. Preclinical data have suggested the *in vitro* synergy of these agents against HL60, and against murine L1210 *in vivo*.[13,14]

Additionally, recent data suggest the use of post-transplant immuno- or chemotherapy may reduce the risk of post-BMT leukemia relapse.[15–17] It has been demonstrated that it is possible to induce acute GVHD following ABMT in children with cyclosporin A (CsA).[16] Alpha-interferon (α-IFN) is also able to induce autologous GVHD and has been shown to enhance autologous GVHD induced by cyclosporin.[17]

To reduce the relapse rate post ABMT for poor-risk childhood ALL, we piloted the use of CsA, CsA and α-IFN, and CSA, α-IFN, and chemotherapy post-ABMT. The aims of the present study were to explore the safety, feasibility and efficacy of a novel combined approach, consisting of *ex vivo* marrow purging with verapamil, VP-16 and vincristine, modulation of the multi-drug resistance (MDR) phenomenon with continuous infusion verapamil with VP-16 during the myeloablative conditioning, and post-transplant immunochemotherapy in children with poor risk acute lymphoblastic leukemia.

Patients and methods

Patients

Patients were eligible for enrollment into the study if they had ALL and were under 21 years of age and met one of the following criteria: high-risk ALL in first complete remission (CR), defined as either age less than 1 year, or initial white blood cell count of $>250\,000/mm^2$ or, more than 6 weeks to attain first CR (induction failure) or certain chromosomal abnormalities, including the following translocations: t(4;11) or t(9;22). Patients were also eligible if they were younger than 21 and were in second or subsequent remission after relapse of ALL. Patients were excluded from the study if they had a matched sibling donor or an unrelated donor who could be accessed in a clinically relevant time period. Organ function eligibility included: $\geqslant$40% cardiac left ventricular ejection fraction, adequate pulmonary function ($\geqslant$50% diffusing carbon monoxide capacity), adequate renal function ($\geqslant$60 ml/min (1.73 m^2) corrected creatinine clearance) and adequate hepatic function (bilirubin $<$3 mg/dl). Informed consent was obtained from the patient's legally authorized guardian in accordance with institutional policies.

Ex vivo purging

For each patient, 10–20 cc/kg of marrow was harvested during complete remission ($<$5% blasts). Bone marrow was collected in sterile preservative-free heparin and RPMI-1640 (Gibco, Grand Island, NY, USA). The bone marrow was filtered once through a 300 millimicron mesh screen and twice through a 200 millimicron screen. Approximately 30% of nucleated bone marrow cells were cryopreserved without treatment as back up unpurged bone marrow. The rest of the bone marrow specimen underwent Ficoll–Hypaque gradient separation. The total purging volume and the volume of human serum albumin (HSA) was determined by the following calculations:

purging volume = total number of cells: 2.0×10^7 cells/ml
ml 5% HSA = total volume $\times$ 0.1

Vincristine (1.5 μM), VP-16 (50 μg/ml) and verapamil (10 μM) were added to 5 ml of RPMI-1640 by using an Eppendorf pipette. The marrow was added to a transfer pack using a canula and syringe fitted with a three-way stopcock. RPMI was added to make a final cell concentration of 2.0×10^7 cells/ml. Then, 5% HSA and the drug solution were added. The marrow was incubated for 1 h at 37°C in a shaking waterbath. The marrow was divided into aliquot portions, added to 50 ml conical tubes and spun at 400 *g* for 10 min. Then the cells were resuspended in S-MEM (Gibco) with 1:9 v/v HSA:S-MEM and centrifuged at 400 *g* for 10 min. After that the cells were washed with S-MEM and HSA in order to remove residual drugs and resuspended in 25–35 ml of S-MEM and HSA for cryopreservation. The bone marrow was thawed in a 37°C water bath and quickly reinfused using standard methods, on day 0 of conditioning therapy.

Conditioning therapy

Patients received fractionated TBI, 1200 cGy given in six fractions of 200 cGy, twice a day for 3 days (days −7 to −5). On day −4 verapamil (0.15 mg/kg) was administered as an i.v. bolus and was followed by a continuous infusion at 0.005 mg/kg/min. After 60 min of continuous infusion verapamil, VP-16 1800 mg/m^2 was infused over 4 h. On days −3 and −2 cyclophosphamide (60 mg/kg/day) was administered. For uroprotection, Mesna (360 mg/m^2) was started 1 h prior to each cyclophosphamide infusion and continued until 12 h after the last cyclophosphamide infusion (2800 mg/m^2/day). Routine bladder irrigation was required during the cyclophosphamide infusion on day −3 and day −2.

Supportive care

All patients were placed in protective isolation and treated in hepa-filtered rooms when their absolute neutrophil count (ANC) dropped below $500/mm^3$. They remained there until their ANC became more than $500/mm^3$ on two repeated determinations. Patients received total parenteral nutrition during the acute phase of mucositis following myeloablative therapy. All patients received *Pneumocystis carinii* prophylaxis with sulfamethoxazole and trimethoprim or

pentamidine nebulizer (days −8 to −2). Sulfamethoxazole and trimethoprim were restarted after myeloid engraftment on 2 consecutive days a week. All patients also received acyclovir prophylaxis (i.v. 250 mg/m^2/day) until myeloid engraftment. Patients received weekly intravenous gamma globulin (IVIG) (500 mg/kg/week) starting on day −1 until day +100. Episodes of fever and neutropenia were treated with broad-spectrum intravenous antibiotics. Blood product transfusions were given after irradiation with 2500 cGy.

Verapamil levels

Serum samples for verapamil levels were collected and measured at 15, 60 and 300 min after the start of the infusion.

Patient monitoring

CBC with differential and platelet counts were obtained everyday and serum chemistries were done twice a week. Prior to each chemotherapy cycle and once a week during α-IFN treatment CBC with differential, platelet count and serum chemistries were obtained. The National Cancer Institute toxicity scale was utilized to grade toxicity. Engraftment was defined as absolute neutrophil count (ANC) of $\geqslant$500/mm^3 and untransfused platelet count of $\geqslant$20 000/mm^3 for 3 days. On days +28, +90, +180, +360 and +730 bone marrow aspirates were obtained. Lumbar puncture was done on days +90 and +180.

Post-transplant immunochemotherapy (PTIC)

Three separate cohorts of patients were evaluated for toxicity, safety and efficacy of post-transplant immuno- $\pm$ chemotherapy: *Cohort I*, CsA therapy alone: (day +1 until day +29) patients received 1.0 mg/kg/day CsA intravenously (four patients). *Cohort II*, CsA therapy (similar to phase one) (days +1 to +29) plus α-IFN therapy: α-IFN (kindly provided by Roche Pharmaceuticals, Nutley, NJ, USA) (Roferon) (1×10^6 U/m^2) was given subcutaneously three times a week for a total of six cycles of therapy beginning when the ANC was $\geqslant$500/mm^3 and platelet count $\geqslant$50 000/mm^3 post transplantation (seven patients). *Cohort III*, CsA (day +1 to day +29) similar to cohort I and alternating chemotherapy and α-IFN (Roferon-a; Roche Pharmaceuticals): following 28 days of CsA post ABMT, patients received six alternating cycles, consisting of 4 weeks of α-IFN followed by a 4 week course of chemotherapy. α-IFN (1×10^6 units/m^2) was given three times a week, subcutaneously. Chemotherapy consisted of vincristine (i.v. 1.5 mg/m^2 × 1), VP-16 (i.v. 50 mg/m^2/day × 5 days), Ara-C (i.v. 50 mg/m^2/day × 5 days), prednisone (p.o. 40 mg/m^2/day × 5 days) followed by G-CSF (s.c. 5 μg/kg/day). Patients also received intrathecal therapy on the first day of chemotherapy with Ara-C and hydrocortisone in age-dependent doses (age <1 year: 20 mg Ara-C and 15 mg hydrocortisone, age 1–2 years: 30 mg Ara-C and 20 mg hydrocortisone, age 2–3 years: 50 mg Ara-C and 30 mg hydrocortisone, age >3 years: 70 mg Ara-C and 50 mg hydrocortisone). After completion of six alternating cycles of α-IFN and chemotherapy, patients received six additional cycles of chemotherapy alone. Each cycle consisted of 4 weeks of vincristine (i.v. 1.5 mg/m^2 × 1), VP-16 (i.v. 50 mg/m^2/day × 5), Ara-C (i.v. 50 mg/m^2/day × 5), prednisone (p.o. 40 mg/m^2/day × 5) and G-CSF (s.c. 5 μg/kg/day) (13 patients).

Statistical methods

The first cohort of patients received CsA only after the preparative regimen and ABMT starting on day +1 to day +29 (four patients). Since there was no evidence of grade IV non-hematological toxicity attributed to post-ABMT CsA, the next cohort of patients received both CsA and α-IFN (seven patients). Since there was no evidence of grade IV non-hematological toxicity secondary to CsA and α-IFN, the next cohort of patients (n = 13) received post-transplant CsA, α-IFN, and chemotherapy (n = 13 patients).

Survival curves for disease-free survival (DFS), overall survival (OS) and times to ANC and platelet engraftment were calculated using the method of Kaplan-Meier.[18] Differences between survival curves within the prognostic factors were tested using the log-rank test.[19] Late relapse was defined as occurring >6 months after completing frontline chemotherapy. Early relapse was defined as occurring $\leqslant$6 months after or during frontline chemotherapy. Patients who failed to engraft or who were too sick to receive post-transplant chemotherapy at day 28 were excluded from the post-transplant (PT) chemotherapy DFS but not overall survival subgroup analysis. This was done to avoid the confounding caused by poorer prognosis patients being included in the 'no PT-chemotherapy' group solely because of lower disease status. The numbers, proportions, and number of deaths were reported for each prognostic factor by chemotherapy subgroups, however, no tests were performed due to the small sample sizes.

Results

From August 1990 to November 1996, 24 children underwent ABMT at the Children's Hospital of Orange County, California. Table 1 summarizes patient characteristics. Table 2 gives the distribution of patient characteristics and post-transplant chemotherapy distribution. Five patients were classified as having high-risk ALL in first complete remission (CR), two patients in third CR, and the remaining 17 were in second CR (see Table 1). The patients in CR1 included two patients less than 1 year old at the time of diagnosis, two patients with a white blood cell count above 250 000/mm^3 and one patient who was positive for t(9;22). Seventeen patients had cytogenetics reported, of which nine were abnormal.

Verapamil levels

Seventeen patients were available for evaluation of verapamil levels. Mean $\pm$ (s.e.m.) serum verapamil levels were 432 $\pm$ 187 ng/ml, 466 $\pm$ 154 ng/ml and 442 $\pm$ 309 ng/ml at 15, 60 and 300 min of infusion, respectively. No patient had verapamil-related toxicities.

Table 1 Summary of patient characteristics

UPN	*Age at BMT (years)*	*Sex*	*Ds status at BMT*	*Length of 1st CR (months)*	*Site/Type relapse*	*Timing of relapse*	*Phenotype*	*WBC K/mm³ at DX*	*Cytogenetics*	*CsA*	*α-IFN*	*Chem Tx*	*Survival*
1	19	F	2nd CR	9	BM	Early	Pre B	6.1	t(1;19)	Yes	No	No	Dead (no engraft)
2	12	M	2nd CR	13	Test/BM	Early	Pre B	1	NL	Yes	No	No	Dead (Rel)
3	5	F	2nd CR	40	BM	Late	NA	76.1	NA	Yes	No	No	Dead (Rel)
4	9	M	2nd CR	62	Test/BM	Late	NA	44.9	NA	Yes	No	No	Dead (no engraft)
5	12	M	1st CR	N/A	N/A	N/A	NA	NA	PH+	Yes	Yes	No	Dead (Rel)
6	10	M	2nd CR	12	BM	Early	NA	600	Multi Abnl	Yes	Yes	No	Dead (Rel)
7	0	M	2nd CR	34	BM	Early	NA	190	NL	Yes	Yes	No	Dead (Rel)
8	11	M	2nd CR	42	CNS/Test	Early	NA	8.7	NA	Yes	Yes	No	Alive
9	5	F	2nd CR	49	BM	Late	NA	15.6	Multi Abnl	Yes	Yes	No	Dead (Rel)
10	10	M	3rd CR	34	CNS	N/A	Pre B	NA	NA	Yes	Yes	No	Dead (Rel)
11	1	M	1st CR	N/A	N/A	N/A	Pre B	22	NL	Yes	Yes	No	Alive
12	10	M	2nd CR	16	Test/BM	Early	T cell	314	NA	Yes	Yes	Yes	Dead (Rel)
13	5	M	1st CR	N/A	N/A	N/A	Pre B	336	NA	Yes	Yes	Yes	Alive
14	2	M	1st CR	N/A	N/A	N/A	Pre B	96	t(1;14) + others	Yes	Yes	Yes	Dead (Rel)
15	0.67	M	1st CR	N/A	N/A	N/A	Pre B	502	t(4;11)	Yes	Yes	Yes	Alive
16	10	F	2nd CR	19	BM	Early	Pre B	2	NL	Yes	Yes	Yes	Dead (Rel)
17	3	F	2nd CR	20	BM	Early	NA	42	NL	Yes	Yes	Yes	Alive
18	9	M	2nd CR	21	BM	Early	NA	1.8	NA	Yes	Yes	Yes	Dead (Rel)
19	11	M	2nd CR	29	lymph node	Early	T cell	NA	NA	Yes	No[a]	Yes	Dead (Rel)
20	5	F	2nd CR	29	BM	Early	NA	47	Multi Abnl	Yes	Yes	Yes	Alive
21	10	F	2nd CR	40	BM	Late	NA	4.6	NL	Yes	Yes	Yes	Alive
22	11	M	2nd CR	41	Test/BM	Late	NA	6.7	Multi Abnl	Yes	Yes	Yes	Dead (Rel)
23	7	M	2nd CR	42	BM/CNS/Test	Late	NA	NA	NA	Yes	Yes	Yes	Alive
24	15	M	3rd CR	55	BM	N/A	Pre B	NA	Triploid	Yes	Yes	Yes	Alive

N/A = not applicable; NA = not available; Ds = disease; UPN = unique patient number; Test = testicular; BM = bone marrow; CNS = central nervous system; Multi Abnl = multiple abnormalities; NL = normal karyotype; MDS = myelodysplastic syndrome; Rel = relapse; Dx = diagnosis; CsA = cyclosporine A; Chem Tx = chemotherapy.
[a]Parental refusal.

Table 2 Distribution of patient characteristics for the 21 ALL pediatric patients included in the post-transplant (PT) chemotherapy subgroup analysis

Characteristic	*Total n (%)*	*PT chemotherapy*		*No PT chemotherapy*	
		n (%)	*Deaths*	*n (%)*	*Deaths*
All patients	21 (100)	13 (100)	5	8 (100)	6
Age					
0–9 years	11 (52)	8 (62)	2	3 (38)	3
10+ years	10 (48)	5 (38)	3	5 (63)	3
Sex					
Male	15 (71)	9 (69)	4	6 (75)	4
Female	6 (29)	4 (31)	1	2 (25)	2
WBC					
100+	4 (19)	2 (15)	0	2 (25)	2
0–99	17 (81)	11 (85)	5	6 (75)	4
Complete response (CR)					
First	5 (24)	4 (31)	1	1 (13)	1
Later	16 (76)	9 (69)	4	7 (87)	5
Length of first remission[a]					
$<$2 years	5 (24)	3 (23)	2	2 (25)	2
$\geqslant$2 years	11 (52)	6 (46)	2	5 (63)	3
Site of prior relapse[a]					
Bone marrow	12 (57)	8 (62)	3	4 (50)	4
Not bone marrow	4 (19)	1 (8)	1	3 (38)	1
Timing of relapse[b]					
$<$6 months after treatment	9 (64)	5 (63)	3	4 (67)	3
$\geqslant$6 months after treatment	5 (36)	3 (38)	1	2 (33)	2

[a]Totals for these factors are 16, 9, 4, 7 and 5 corresponding to the 16 patients in later than first CR.
[b]Totals for this factor are 14, 8, 4, 6 and 5 corresponding to the 14 patients in their second CR.

Engraftment

Total nucleated cell counts of purged bone marrow were available on 23 patients. Median nucleated cell dose infused was 3.44 $\times$ 10^8/kg (range 1.26–8.24 $\times$ 10^8/kg). $CD34^+$ counts were available for nine patients, median $CD34^+$ cell dose infused was 5.33 $\times$ 10^6/kg (range 2.31–46.0 $\times$ 10^6/kg). Figure 1 provides time to ANC engraftment and time to platelet engraftment. Successful engraftment was achieved in 22 patients. Myeloid engraftment, defined as a neutrophil count above 500/mm^3 for 2 days was reached at a median time of 19 days (range 12–33). Platelet engraftment, defined as a platelet count above 20 000/mm^3 for 3 continuous days without platelet transfusions was reached at a median of 30 days (range 11–67). One patient with a history of myelofibrosis (UPN 3) who had prior bone marrow fibrosis did not engraft and died on day +58. Another patient (UPN 16) never engrafted with platelets and died on day +59. Back up marrow was not used for either of these patients.

BMT-related toxicity

All 24 patients were assessable for toxicity. Fourteen patients developed severe (grade III/IV) mucositis. Eleven patients became septic due to bacterial infections. Eight patients developed a skin rash and three had a biopsy, of which one was consistent with acute GVHD. There were three toxic deaths. Three patients died before day +100. One patient (UPN 3) died on day +58 of an *Aspergillus* infection and veno-occlusive disease. Another patient (UPN 4) died on day +89 of progressive disease. The third patient (UPN 16) never achieved platelet engraftment, was refractory to platelet transfusions, and died on day +59 of an intracranial and pulmonary hemorrhage.

Alpha interferon toxicity

Of our 24 patients, 19 received α-IFN. Mildly toxic reactions were reported in four. Two patients became febrile and complained of either upper chest or generalized pain. One other patient became febrile and one suffered from decreased appetite and weight loss. No dose alterations or treatment interruptions were required because of toxicity.

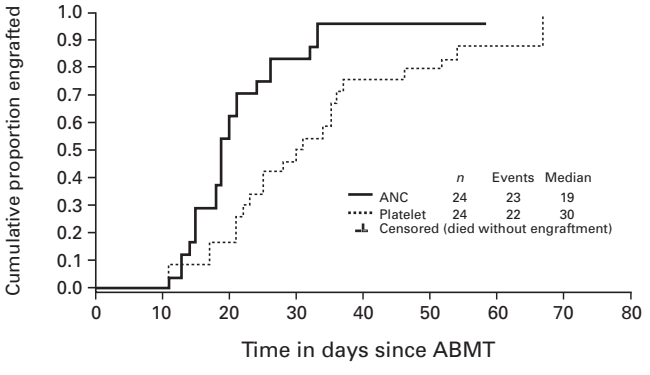

Figure 1 Time to ANC $\geq$1000/mm^3 and platelet count $\geq$100 000 after ABMT calculated by the method of Kaplan–Meier.

Treatment outcome

Figure 2 provides the overall survival (OS) and disease-free survival (DFS) curves. At 2 years, the estimated percentage of patients disease-free and surviving is 42 $\pm$ 10% (90% confidence interval (CI) is 26.5–58.5%) and overall survival is 54 $\pm$ 10% (90% CI is 37.5–70.5%). The median follow-up time of the surviving patients is 41 months (range 23–65 months). All patients with censored observations were alive or disease-free at the end of the study. No patients were lost to follow-up.

Overall, 14 patients relapsed a median time of 9 months (range 2–29 months), 14 patients died at a median of 16 months (range 2–36 months) and 10 are alive and doing well today. The median time to death after progression was 4 months (range 0–42). Three patients were excluded from the overall survival analysis, two (UPN 3 and 16) due to non-engraftment and one (UPN 12) did not start post-transplant immunochemotherapy due to treatment-related complications. This patient relapsed within 12 months after transplant and died of progressive disease. One patient in third remission with intensive prior therapy developed a secondary myelodysplastic syndrome (MDS) malignancy and is still alive.

Post-transplant immunochemotherapy

Table 3 gives the % DFS and % OS at 2 years categorized by prognostic factors and post-transplant immunochemotherapy. The only factor demonstrating a significant relationship to DFS or OS is receiving post-transplant immunochemotherapy. Patients receiving immunochemotherapy have a better DFS (69% *vs* 13%; $P = 0.008$) (Figure 3) and better OS (85% *vs* 25%; $P = 0.06$) compared to those patients without the additional chemotherapy. Table 3 gives the breakdown of the chemotherapy groups by various prognostic factors. Of the eight patients who only received post-ABMT immunotherapy, seven relapsed, six within 12 months after transplant. One of them was reinduced and remains in remission; the remaining six died. Of the 13 patients who received post-transplant immunochemotherapy, eight are alive.

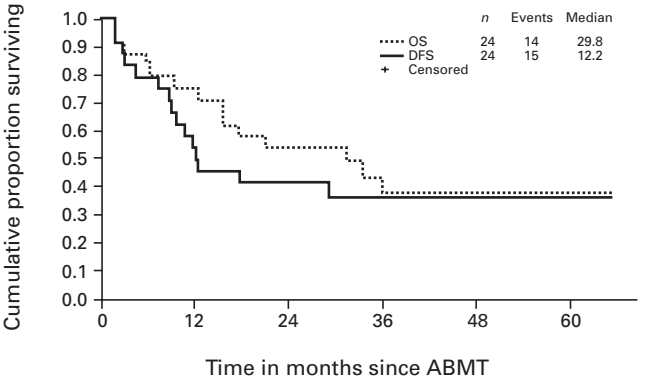

Figure 2 Probability of overall and disease-free survival among 24 children with high-risk ALL after ABMT estimated by the method of Kaplan–Meier.

Table 3 Distribution of patient characteristics and estimates of disease-free survival (DFS) and overall survival (OS) for 24 consecutive ALL pediatric patients

Characteristics	*n (%)*	*% DFS at*			*% OS at*		
		Relapses	*2 year (S.E.)*[a]	*P value*	*Deaths*	*2 year (S.E.)*[a]	*P value*
All patients	24 (100)	15	42 (10)	0.31	14	54 (10)	0.47
Age							
0–9 years	12 (50)	6	50 (14)		6	58 (4)	
10+ years	12 (50)	9	33 (14)		8	50 (14)	
Sex				0.99			0.96
Male	17 (71)	11	41 (12)		10	59 (12)	
Female	7 (29)	4	43 (19)		4	43 (19)	
WBC				0.88			0.68
100+	5 (21)	3	40 (22)		3	40 (22)	
0–99	19 (79)	12	42 (11)		11	58 (11)	
Complete remission (CR)				0.14			0.19
First	5 (21)	2	60 (22)		2	80 (18)	
Later	19 (79)	13	37 (11)		12	47 (11)	
Length of first remission[b]				0.17			0.09
$<$2 years	7 (29)	6	14 (13)		6	14 (13)	
$\geqslant$2 years	12 (50)	7	50 (14)		6	67 (14)	
Site of prior relapse[b]				0.70			0.27
Bone marrow	15 (63)	10	40 (13)		10	47 (13)	
Not bone marrow	4 (17)	3	25 (22)		2	75 (22)	
Timing of relapse[c]				0.80			0.68
$<$6 months after treatment	11 (65)	8	27 (13)		8	27 (13)	
$\geqslant$6 months after treatment	6 (35)	4	50 (20)		4	50 (20)	
Chemotherapy[d]				0.008			0.06
Yes	13 (54)	5	69 (13)		5	85 (10)	
No	8 (33)	7	13 (12)		6	25 (15)	

[a]Standard error in percent.
[b]Totals for these factors are 19 corresponding to the 19 patients in their later than first CR.
[c]Total for this factor is 17 corresponding to the 17 patients in their second CR.
[d]Total for this factor is 21 corresponding to the 21 patients included in the chemotherapy subgroup analyses.

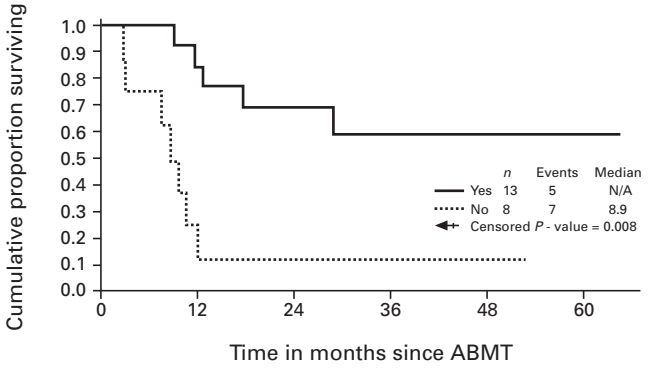

Figure 3 Probability of disease-free survival among children with high-risk ALL after ABMT with (yes) or without (no) post-transplant immunochemotherapy estimate by the method of Kaplan–Meier.

Prognostic factors

Table 3 gives the 2-year DFS and OS categorized by prognostic factors. While none of the prognostic factors demonstrated statistical significance, the factors relating to disease status showed large differences that are of interest for future research. Patients who were in their first complete remission (CR) performed better than those in second or subsequent CR. The 2-year DFS for patients in first CR was 60 $\pm$ 22% compared to 37 $\pm$ 11% for others (P = 0.14). The 2-year OS for these patients is 80 $\pm$ 18% *vs* 47 $\pm$ 11% (P = 0.19). Of the 19 patients in second or subsequent CR, those with a first remission of more than 2 years had an estimated 50 $\pm$ 14% DFS at 2 years compared to 14 $\pm$ 13% (P = 0.17) for shorter first remissions. The 2-year OS is 67 $\pm$ 14% *vs* 14 $\pm$ 13% (P = 0.09) for these patients. Patients who had relapsed on, or within 6 months of completing front line therapy had a 2-year DFS of 27 $\pm$ 13%, no different than patients who relapsed after 6 months of 50 $\pm$ 20% (P = 0.8).

Discussion

Autologous bone marrow transplantation may offer a reasonable alternative for children with relapsed or high-risk ALL who lack a suitable allogeneic donor. Although morphologically normal marrow is used for ABMT, there may still be the risk of contamination of the marrow graft with residual leukemia cells. Different methods of purging have been studied, utilizing either monoclonal antibodies plus complement,[20] antibody-bead immunoconjugates[21] or pharmacological agents.[22,23] Although the use of marrow purging may remain controversial, several studies showed a positive effect on clinical outcome of patients with hema-

tologic malignancies.[24,25] One factor that could improve the success of purging is the inhibition of multiple drug resistance (MDR). We have previously investigated the effects of chemopurging utilizing vincristine and VP-16 incubated with verapamil, a P-glycoprotein inhibitor, in Nalm-6_{500}, an atypical drug resistant B-ALL cell line, and L-$_{100}$, an *mdr*-1 positive typical resistant T-ALL cell line.[11] Verapamil significantly reduced the concentration of vincristine and VP-16 that inhibited growth of L-$_{100}$ by 50% (IC_{50}). Based on these results, we selected verapamil, vincristine and VP-16 to purge the bone marrow. The results of myeloid (19 days) and platelet recovery (30 days) using triple purged marrow are similar to the results reported in other studies utilizing other forms of purging for ABMT for ALL.[21,26,27]

One of the factors that may contribute to a higher relapse rate in autologous bone marrow transplantation is failure of the conditioning regimen to completely eradicate the residual leukemia burden *in vivo*. It has been demonstrated that the quantity of residual leukemic progenitor cells is a powerful predictor of relapse after autologous bone marrow transplantation.[28] We have previously studied a group of children with recurrent tumors utilizing the combination of continuous infusion of verapamil, bolus vinblastine and continuous infusion of VP-16.[10] We concluded, that in children steady-state levels of verapamil (468 ng/ml) could be safely maintained *in vivo* and were also demonstrated to be effective in reversing P-glycoprotein *in vitro*.[10] The results in this study reconfirm our previous study. Verapamil was safely administered without cardiac toxicites. Furthermore, serum verapamil levels were within the range required to circumvent pleiotropic drug resistance *in vitro*.

Patients undergoing ABMT lack the additive effect of the GVL phenomenon associated with allogeneic transplantation which may account for a higher relapse rate seen following ABMT. As has been previously demonstrated in animal and human clinical studies, an autologous GVHD-like reaction (aGVHD) may be induced by CsA.[16,29] Of our 24 patients, eight developed a skin rash, three were biopsied and one was positive for aGVHD. The other seven rashes were consistent with grade II clinical skin aGVHD. Recently, the results of a study on CsA-induced aGVHD following ABMT in childhood malignancies suggested that aGVHD can be safely and reproducibly induced by 1.5 mg/kg/day of CsA administered intravenously for 28 days.[16,29,30] Our results confirm this finding since CsA was well tolerated and no grade III/IVtoxicity was reported with the use of CsA. Furthermore, hematopoietic recovery was not delayed by the administration of CsA during the first 28 days post-myeloablation and ABMT.

The enhancement of CsA-induced aGVHD by α-IFN has been suggested by Ratanatharathorn *et al*.[17] α-IFN interacts directly on T cells to stimulate production of lymphokines and increases tumor cell recognition by stimulating the expression of tumor-associated antigens. In this study, α-IFN was well tolerated but did not enhance the development of clinically detectable GVHD. Our patients who received α-IFN alone post transplant relapsed and died early possibly secondary to lack of GVL.

However, the addition of post-transplant chemotherapy to post-transplant immunotherapy led to a significant improvement of the 2-year DFS. Few studies have reported the results of post-ABMT chemotherapy although maintenance chemotherapy is a common post-remission therapy after induction chemotherapy in the treatment of childhood leukemia.[15] However, post-ABMT chemotherapy may be a method to suppress and eradicate residual leukemic disease and thus decrease the high relapse rate associated with ABMT.

Following marrow relapse, second remission might be induced with intensive chemotherapy regimens, although BMT increases DFS for certain groups of patients. Results of ABMT in children with poor risk ALL vary, depending on the time of relapse and age at time of ABMT (Table 4). We selected high-risk patients in both first and in second or subsequent remissions. Although our results did not show a statistically significant difference between patients in first remission and those in second or subsequent remission, patients in first remission tended to have an improved DFS and OS. Although criteria for high-risk first remission vary, our results compare favorably with the 2-year DFS percentages (30–60%), reported in other studies.[31–34]

Results of post-relapse therapy depend on the time and site of relapse. According to reports from the BFM study group, 60% of the children with either a late isolated central nervous system or an isolated testicular relapse may be cured with intensive chemotherapy regimens alone.[32] However, isolated extramedullary relapse in children can be associated with a more favorable outcome than bone marrow relapse, after which survival is only 15%. Few of the children enrolled in the current study would be considered favorable by these standards. Our overall survival results for children with late and even early relapse, 50% and 27%, respectively, compare favorably with those achieved by conventional polychemotherapy of 24–60% and 1–19% according to recent BFM relapse protocols.[32]

The great majority of patients with ALL undergo autologous BMT in second or subsequent remission. For these patients, our results with post-transplant immunochemotherapy are similar, or compare favorably to the results reported in previous studies, which vary from a 15–40% 2-year disease free survival.[31] Billett *et al*[5] have reported the best results in children. They have treated 51 children with relapsed ALL with a conditioning regimen of Ara-C, teniposide, cyclophosphamide and TBI followed by infusion of antibody purged autologous bone marrow. They reported a 3-year DFS of 58%, substantially better than our results without post-transplant immunochemotherapy. However, the addition of PTIC yields similar results (69% 2-year DFS). Both regimen and patient selection may impact on these results. For pediatric patients transplanted in second or subsequent remission, duration of first remission is the most important predictor of outcome. Rivera *et al*[35] suggested that success in curing childhood ALL in first marrow relapse may depend as much on how long initial remission has lasted as on whether bone marrow transplantation or intensive chemotherapy is selected as mode of therapy. Although the difference was not significant, our patients with a first remission of more than 2 years had a better estimated 2-year DFS and OS (50% for both) than did patients with a shorter first remission (20% for both). This is consistent with the BFM results who reported a 50% EFS

Table 4 Disease-free survival among ABMT regimens

Author (Ref.)	*No. of patients*	*Median age (range)*	*Status at BMT*		*BMT regimen*	*PT-Tx*	*Purged*	*DFS (years)*
			CCR1	*≥CR1*				
Houtenbos *et al* (this study)	13	6.9 (0.7–15)	4	9	CY, TBI, VP16, VPL	PTIC	Chemo	69% (2)
Buhrer *et al*[32]	326	7.8 (1.5–18)		326	ALL-REZ BFM (no BMT)	N/A	N/A	42% (7)
Billett *et al*[5]	51	9 (3–18)		51	TBI, CY, Ara-C, teniposide	No	MoAb	58% (3)
Parsons *et al*[3]	57	4.2 (0.9–14.2)	0	57	CY, TBI, teniposide, Ara-C or CY, TBI, VP16	No	None	47% (3)
Barrett *et al*[4]	255	7 (0.5–18.4)	0	255	Multiple (alloBMT)	N/A	N/A	40% (5)
Barrett *et al*[4]	255	6 (0.4–18.1)		255	Multiple POG (no BMT)	N/A	N/A	17% (5)
Tiley *et al*[15]	38	21 (3–41)	38	0	TBI, melphalan	Chemo	MoAb (29%)	50% (2)
Colleselli *et al*[33]	56	11	0	56	CY, TBI, vincristine	No	None	21% (4)
Uckun *et al*[28]	83	10 (1–48)	83	0	TBI/Ara-C or VP-16/CY	No	MoAb	17% (2)
Herve *et al*[34]	177	<16	0	177	BuCY or CY TBI	No	Chemo or MoAb	33% (4)
Herve *et al*[34]	66	<16	66	0	BuCY or CY TBI	No	Chemo or MoAb	46% (4)

VPL = verapamil; CY = cyclophosphamide; VP-16 = etoposide; Ara-C = cytarabine; Bu = busulfan; TBI = total body irradiation; MoAb = monoclonal antibody; Chemo = chemotherapy; N/A = not applicable; BMT = bone marrow transplant; CR = complete remission; DFS = disease-free survival; PT-Tx = post-transplant therapy; PTIC = post-transplant immunochemotherapy.

in patients whose first remission was at least 2 years *vs* 20% in patients whose first remission was less than 2 years.[32]

This is the first report of the results of post-transplant therapy consisting of a combination of immunotherapy and chemotherapy in childhood ALL. Tiley *et al*[15] reported the feasibility and efficacy of maintenance chemotherapy after ABMT for adults and children with ALL. Although the contribution of chemotherapy could not accurately be assessed, markedly higher relapse rates occurred in patients who did not receive post-transplant maintenance chemotherapy. A follow-up study done by Powles *et al*[36] confirms the finding that the use of post-transplant maintenance chemotherapy in adults with recurrent ALL may reduce the relapse rate post ABMT.

While this study was designed to test the toxicity and feasibility of administering post-ABMT immunotherapy and chemotherapy, the pilot results suggest a substantial improvement in both DFS and OS for children who received both post-ABMT immunotherapy and chemotherapy *vs* immunotherapy alone. Our results indicate that the use of ABMT with chemopurging, combined with post-ABMT chemotherapy may be a promising new approach in the treatment of a subset of children with ALL in high-risk first CR or subsequent CR who lack closely matched family-related allogeneic donors. The benefit of post-transplant immunotherapy alone appears to be negligible. A larger randomized prospective study in children with high-risk ALL who lack a family-related allogeneic donor is required to determine if this approach is better and/or less toxic than chemotherapy alone or unrelated and/or HLA disparate (mismatched)-related allogeneic transplants.

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