Analysis of the Role of Hematopoietic Stem-Cell Transplantation in Infants With Acute Lymphoblastic Leukemia in First Remission and *MLL* Gene Rearrangements: A Report From the Children's Oncology Group

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ABSTRACT

Purpose

Although the majority of children with acute lymphoblastic leukemia (ALL) are cured with current therapy, the event-free survival (EFS) of infants with ALL, particularly those with mixed lineage leukemia (*MLL*) gene rearrangements, is only 30% to 40%. Relapse has been the major source of treatment failure for these patients. The parallel Children's Cancer Group (CCG) 1953 and Pediatric Oncology Group (POG) 9407 studies were designed to test the hypothesis that more intensive therapy, including dose intensification of chemotherapy, and hematopoietic stem-cell transplantation (HSCT) would improve the outcome for this group of patients.

Patients and Methods

One hundred eighty-nine infants (CCG 1953, $n = 115$; POG 9407, $n = 74$) were enrolled between October 1996 and August 2000. For infants with the *MLL* gene rearrangement and an appropriate donor, HSCT was the preferred treatment on CCG 1953 and investigator option on POG 9407 after completion of the second phase of therapy. Fifty-three infants underwent HSCT.

Results

The 5-year EFS rate was 48.8% (95% CI, 33.9% to 63.7%) in patients who received HSCT and 48.7% (95% CI, 33.8% to 63.6%) in patients treated with chemotherapy alone ($P = .60$). Transplantation outcomes were not affected by the preparatory regimen or donor source.

Conclusion

Our data suggest that routine use of HSCT for infants with *MLL*-rearranged ALL is not indicated. However, limited by small numbers, this study should not be considered the definitive answer to this question.

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INTRODUCTION

Despite excellent cure rates in most children with acute lymphoblastic leukemia (ALL),^{1,2} infants with ALL continue to have poor event-free survival (EFS).³ The prognosis of patients with ALL with mixed lineage leukemia (*MLL*) gene rearrangements is particularly dismal, with failure related primarily to early relapse.⁴⁻⁸

The parallel Children's Cancer Group (CCG) 1953 and Pediatric Oncology Group (POG) 9407 studies tested whether intensification of early therapy would improve the outcome of infants with ALL. In addition to intensifying chemotherapy by calculating doses of chemotherapy by body-surface area (BSA) rather than weight, these protocols

investigated the role of hematopoietic stem-cell transplantation (HSCT) in early first complete remission (CR1) for infants with *MLL*-positive ALL.

In this report, HSCT results are compared with the outcome of infants with *MLL*-positive ALL treated with the intensified chemotherapy protocol of studies CCG 1953 and POG 9407. Our data suggest that HSCT does not improve EFS or overall survival for these children.

PATIENTS AND METHODS

Patients

The CCG 1953 and POG 9407 protocols were approved by the National Cancer Institute and by the institutional review boards at participating institutions.

Fig 1. CONSORT diagram. Eligibility and assignment of patients: Children's Cancer Group 1953 and Pediatric Oncology Group 9407. SCT, stem-cell transplantation; PI, principal investigator; MLL, mixed lineage leukemia; chemo, chemotherapy; EFS, event-free survival.

Informed consent was obtained from parents or legal guardians according to Department of Health and Human Services guidelines. CCG 1953 enrolled 115 infants and POG 9407 enrolled 74 infants (< 1 year of age) with newly diagnosed ALL between October 1996 and August 2000 (Fig 1).

The diagnosis of ALL was based on morphology, cytochemistry, and immunophenotyping performed at the individual institutions. Leukemic cell karyotype was determined at individual institutions as previously described.⁹

For CCG patients, molecular analyses were performed in a Clinical Laboratory Improvement Amendments–certified laboratory (University of Minnesota Molecular Diagnostics Lab, Minneapolis, MN). Detection of *MLL* gene rearrangement by Southern analysis and detection of t(4;11), t(11;19), or t(9;11) fusion transcripts by reverse transcriptase polymerase chain reaction were performed as previously described.¹⁰⁻¹² For POG patients, analysis for *MLL* gene rearrangement was performed in reference laboratories at St Jude

Children's Research Hospital (Memphis, TN) and the University of New Mexico (Albuquerque, NM) using marrow or peripheral blood in patients presenting with elevated WBC counts. Reverse transcriptase polymerase chain reaction was used to identify the *MLL-AF4* common translocation fusion gene partner. Southern blot hybridization was used for detecting the rarer *MLL* gene rearrangements.

Chemotherapy

Patients on CCG-1953 received induction/intensification (phase 1) followed by reinduction (phase 2), reintensification (phase 3), consolidation (phase 4), three cycles of an intensified maintenance (phase 5), and four cycles of routine maintenance (phase 6; Table 1). Phases 1 and 2 of the POG 9407 protocol were identical to those on CCG 1953 to ensure that patients had been treated identically before HSCT. During phases 3 and 4, patients enrolled onto

Table 1. Details of Treatment in CCG 1953 and POG 9407

Phase	Treatment
Phase 1 induction (4 weeks)	CPM 250 mg/m ² every 12 hours for four doses, days 2 and 3 DXM 10 mg/m ² PO three times a day on days 0-20, no taper VCR 0.05 mg/kg days 0 and 14, 0.03 mg/kg day 7 DNM 2 mg/kg/dose IV over 30 minutes for patients age ≤ 90 days; 2 mg/kg/d, 4 mg/kg over 48 hours, age > 90 days but < 6 months; 2.5 mg/kg/d, 5 mg/kg over 48 hours, age 6 to < 9 months; 3 mg/kg/d, 6 mg/kg over 48 hours, age ≥ 9 months, as a 48-hour continuous infusion through central venous catheter, days 0 and 1 L-ASP 6,000 U/m ² for eight doses 3 times a week ITT: methotrexate 7.5 mg, hydrocortisone 7.5 mg, and cytarabine 15 mg on days 0, 7, 14, 21, and 28 Mtx 4 g/m ² for two doses, days 21 and 28 CF 10 mg/m ² PO or IV every 6 hours for five or more doses, start 18 hours after Mtx completion VCR 0.05 mg/kg IV push
Phase 1 intensification (4 weeks)	VP-16 100 mg/m ² for five doses, days 36-40 DXM 10 mg/m ² PO three times a day, days 0-20, no taper CPM 300 mg/m ² /d IV for five doses, days 36-40
Phase 2 reinduction (4 weeks)	CPM 250 mg/m ² every 12 hours for four doses, days 2 and 3 VCR 0.05 mg/kg days 0 and 14, 0.03 mg/kg day 7 DNM 45 mg/m ² /d for 2 days DXM 10 mg/m ² divided three times a day, days 0-20, no taper L-ASP 6,000 U/m ² IM for eight doses, three times a day, days 0-20 ITT days 0 and 14 BMT for 11q23/MLL
Phase 3 reintensification (6 weeks)	VCR 0.75 mg/m ² IV days 0 and 7 Mtx: CCG, 6,000 mg/m ² in 1 hour, followed by 1,200 mg/m ² /h for 23 hours for two doses; POG, 4 g/m ² for two doses, days 0 and 14 CF: CCG, 200 mg/m ² IV over 1 hour, then 12 mg/m ² IV push every 3 hours for six doses, then 12 mg/m ² IV or PO every 6 hours until plasma Mtx level < 0.1 μM, start 12 hours after Mtx completion (36 hours after start); POG 10 mg/m ² PO or IV every 6 hours for five or more doses, start 18 hours after Mtx completion VP-16 100 mg/m ² /d IV, days 28-32 CPM 300 mg/m ² /d IV over 30 minutes, days 28-32 IT Ara-C 15 mg IT, days 7 and 2
Phase 4 consolidation	Mtx: CCG, 6,000 mg/m ² IV in 1 hour, followed by 1,200 mg/m ² /h IV for 23 hours CF: CCG, 200 mg/m ² IV over 1 hour, then 12 mg/m ² IV push every 3 hours for six doses, then 12 mg/m ² IV or PO every 6 hours until plasma Mtx level < 0.1 μM; start 12 hours after Mtx completion (36 hours after start) IT Ara-C 15 mg IT days 35 and 49 VCR 0.75 mg/m ² IV days 28 and 42 IV Ara-C 3,000 mg/m ² IV as a 3-hour infusion every 12 hours, days 0, 2, 7, and 8 for a total of eight doses L-ASP 6,000 U/m ² IM 3 hours after IV Ara-C on days 1 and 8
Phase 5 intensified maintenance (72 days for three cycles)	VCR 0.75 mg/m ² IV, days 0 and 28 DXM 10 mg/m ² divided PO three times a day, days 1-4 and 28-32 6-MP 75 mg/m ² PO, days 0-48 Mtx 20 mg/m ² IM every week, days 0, 7, 14, 21, 28, 35, and 42 VP-16 100 mg/m ² /d, days 49-53 CPM 300 mg/m ² /d IV over 30 minutes, days 49-53 IT Ara-C 15 mg IT, days 0 and 28
Phase 6 routine maintenance (four cycles, 1 year), CCG only	VCR 1.5 mg/m ² IV every 4 weeks for 12 doses, days 0, 28, and 56 PRED 40 mg/m ² PO divided into three doses for 5 days with each VCR dose Mtx 20 mg/m ² PO daily for 12 weeks per cycle IT Mtx 8 mg for ages 12-23 months, 10 mg for ages 24-35 months, day 0

Abbreviations: CCG, Children's Cancer Group; POG, Pediatric Oncology Group; CPM, cyclophosphamide; DXM, dexamethasone; PO, orally; VCR, vincristine; DNM, daunorubicin; IV, intravenous; L-ASP, asparaginase; ITT, intrathecal triple therapy (methotrexate, hydrocortisone, and cytarabine); Mtx, methotrexate; CF, citrovorum factor (leucovorin); VP-16, etoposide; IM, intramuscular; BMT, bone marrow transplantation; Ara-C, cytarabine; IT, intrathecal; 6-MP, mercaptopurine; PRED, prednisone.

CCG 1953 received 24-hour infusions of methotrexate 33.6 g/m², whereas patients enrolled onto POG 9407 received 24-hour infusions of methotrexate 4 g/m² given only in phases 1 and 3. Patients enrolled onto POG 9407 did not receive phase 6 maintenance therapy (Table 1). The total duration of therapy on CCG 1953 was 110 weeks, whereas therapy on POG 9407 lasted only 46 weeks.

Transplantation

Infants with documented abnormalities in chromosome band 11q23 or *MLL*-positive ALL gene rearrangement were eligible for HSCT after phase 2 if they had a suitable donor. Suitable donors included an HLA-A, HLA-B, or HLA-DRB1 genotypic identical sibling; an HLA-A, HLA-B, or HLA-DRB1 phenotypic identical relative; a single HLA-A, HLA-B, or HLA-DRB1 antigen-mismatched relative; an HLA-A, HLA-B, or HLA-DRB1 identical unrelated individual (including cord blood); or a single HLA-A, HLA-B, or HLA-DRB1 antigen-mismatched unrelated individual (including cord blood). Class I antigens were determined by standard serologic methods, and class II antigens were determined by low-resolution DNA typing.

The protocol guidelines on CCG 1953 indicated that HSCT was the preferred treatment for patients with abnormalities in chromosome band 11q23 or *MLL*-positive ALL if an appropriate donor could be identified within the allowed time frame. On POG 9407, transplantation was an investigator option. All patients were to proceed to HSCT at completion of reinduction.

The protocol-prescribed transplantation regimen included cytarabine (100 mg/kg/dose every 12 hours, days −8, −7, and −6), cyclophosphamide (45 mg/kg/dose, days −7 and −6), methylprednisolone (33 mg/kg twice per day starting at midnight on days −3, −2, and −1), and total-body irradiation (TBI; given in two 1.5-Gy fractions daily to a total of 12 Gy, on days −3 to 0; Table 2).

The recommended dose of the HSCT infusion was 1 to 3 × 10⁸ nucleated, unmanipulated bone marrow cells per kilogram of patient weight. Graft-versus-host disease (GVHD) prophylaxis was single-agent cyclosporine.

Study Amendments

Enrollment was suspended in July of 1997 because of a 25% induction mortality rate predominantly resulting from infections associated with severe

Table 2. CCG 1953 and POG 9407 Transplantation Therapy

Treatment Day	Therapy	Time	Dose
Preparative regimen			
–8	Ara-C	8:00 PM	100 mg/kg/dose (every 12 hours)
–7	Begin IV fluids	6:00 AM	2 times maintenance
	Ara-C	8:00 AM	100 mg/kg/dose
	CPM	2:00 PM	45 mg/kg/dose
	Ara-C	8:00 PM	100 mg/kg/dose
–6	Ara-C	8:00 AM	100 mg/kg/dose
	CPM	2:00 PM	45 mg/kg/dose
	Ara-C	8:00 PM	100 mg/kg/dose
	Ara-C	8:00 AM	100 mg/kg/dose
–5	Rest		
–4	TBI		1.5 Gy twice a day
–3	TBI		1.5 Gy twice a day
–2	MPDN	Midnight	33 mg/kg IV
–1	TBI		1.5 Gy twice a day
	MPDN	Noon/midnight	33 mg/kg IV
0	TBI		1.5 Gy twice a day
	MPDN	Noon	33 mg/kg IV
	MPDN	90 minutes before stem-cell infusion	6.6 mg/kg IV
	Stem-cell infusion*	1 to 24 hours after TBI	
	MPDN	8 hours after stem-cell infusion	3.3 mg/kg IV
+1	MPDN	16 hours after stem-cell infusion	3.3 mg/kg IV
GVHD prophylaxis			
+1 start	CSA		1.5 mg/kg IV every 12 hours; 100 mg/kg when tolerating PO
+60			Taper if no GVHD

Abbreviations: CCG, Children's Cancer Group; POG, Pediatric Oncology Group; Ara-C, cytarabine; IV, intravenous; CPM, cyclophosphamide; TBI, total-body irradiation; MPDN, methylprednisolone; GVHD, graft-versus-host disease; CSA, cyclosporine; PO, oral.

*Unmanipulated stem cells in a recommended dose of 1 to 3×10^8 nucleated marrow cells/kg of patient weight.

skin and mucosal breakdown. Because this was thought to be related to the dose of daunorubicin calculated by BSA rather than weight, an amendment was passed in November 1997 to calculate the dose based on weight. Despite some reduction in toxicity, the protocol was further amended in July 1998 changing the daunorubicin to an intravenous bolus on days 0 and 1 rather than a 48-hour infusion, resulting in a reduction in induction toxicity.

Statistical Analyses

Protocol compliance on the transplantation arm was a major problem, with more than half the patients receiving nonprotocol preparative regimens. In the overall study analysis, these patients were censored at the time of the off-study transplantation.¹³ Designed as a prospective trial, this lack of compliance severely hindered the analysis of the trial as prospectively defined. Therefore, two statistical approaches were used to analyze the outcome of HSCT. The first approach was life-table comparisons of infants who received transplantation in CR1 both on (HSCT-ON) and off (HSCT-OFF) protocol compared with a control, or chemotherapy-only, group using the log-rank test and giving the EFS outcome in life-table form. For this evaluation, the control group consisted of 47 patients who were identified with *MLL*-positive ALL who were treated with chemotherapy and survived 143 days, the median time to CR1 transplantation. The second approach was a statistical regression model that allowed each individual transplantation patient to remain in the chemotherapy control group up to exactly the time that they underwent HSCT and then transfer into the HSCT group for the remaining follow-up time. Life-table estimates were calculated using the Kaplan-Meier method,¹⁴ with SEs of the estimates obtained using the method of Peto et al.¹⁵ The relative hazard rate (RHR) was estimated by the ratio of observed to expected events in the comparison groups.^{15,16} EFS events included leukemic relapse at any site and death during remission, whichever occurred first.

RESULTS

Of 189 patients enrolled, 132 were *MLL* positive. Of these patients, 53 underwent HSCT, and 47 received chemotherapy alone (control arm). Thirty-two patients did not survive to day 143, with deaths primarily caused by infection (26 deaths during induction; six deaths after induction). Twenty-five of 132 patients (seven from POG institutions, 18 from CCG institutions) identified as having *MLL*-positive ALL underwent transplantation in CR1 using the protocol-specified transplantation regimen. Twenty-eight patients (eight from POG institutions, 20 from CCG institutions) underwent transplantation in CR1 using non-protocol-specified transplantation regimens. Tables 3 and 4 list the clinical information of the patients in these two groups. Table 5 lists the characteristics of the following three patient groups: HSCT-ON, HSCT-OFF, and the chemotherapy-only control group. EFS comparing the 53 patients who underwent HSCT in CR1 compared with the 47 control patients treated with chemotherapy and who survived 143 days is displayed in Figure 2A. The 5-year EFS rate is similar for the HSCT group (48.8%; 95% CI, 33.9% to 63.7%; RHR, 1.15) and the chemotherapy control group (48.7%; 95% CI, 33.8% to 63.6%; $P = .60$). There were no statistically significant differences in EFS or survival for infants treated with chemotherapy compared with HSCT in subgroups defined by age, WBC count at diagnosis, or CD10

Table 3. Patients Who Received Transplantation in First Remission Using Protocol-Specified Transplantation Regimen

Patient No.	Age on Study (months)	Time to HSCT (months)	Donor*	aGVHD Sites and Maximum Grade Documented	Event	Status at Time Since Transplantation or Last Contact (months)
1	12	6	5/6 cord	Sk; grade 2		A 92
2	5	4	6/6 sibling	Sk; grade 3		A 84
3	6	4	6/6 sibling	No	Relapse (3 months)	D 8
4	9	4	6/6 sibling	Sk; grade 2		A 29
5	5	4	4/6 cord	No	VOD and respiratory failure (2 months)	D 2
6	4	5	6/6 cord	Sk, Liv, GI; grade 3	aGVHD with GI hemorrhage (1 month)	D 1
7	2	4	5/6 cord	Sk, GI; grade 3	Relapse (6 months)	A 56; S/P second HSCT
8	4	5	5/6 cord	Sk, Liv, GI; grade 3	Relapse (4 months)	D 6
9	10	5	6/6 sibling	Sk, Liv, GI; grade 2		A 59
10	6	4	6/6 sibling	Sk, GI; grade 3	Cardiopulmonary arrest at home (10 months)	D 10
11	4	4	5/6 parent	Sk, Liv, GI; grade 4	VOD and aGVHD (1 month)	D 1
12	8	4	5/6 URD	Sk; grade unknown		A 66
13	9	3	4/6 cord	Sk grade 3		A 52
14	2	5	5/6 URD	Liv, GI; grade 3		A 39
15	5	4	6/6 URD	Sk, Liv, GI; grade 4	cGVHD with sepsis and GI hemorrhage (26 months)	D 26
16	4	4	5/6 URD	Sk, GI; grade 3	Pulmonary hemorrhage (1 month)	D 1
17	10	4	6/6 sibling	No		A 57
18	9	4	6/6 URD	Sk; grade 2	cGVHD to multiorgan failure (15 months)	D 15
19	10	6	5/6 parent	Sk, Liv, GI; grade 3	Fungal pneumonia (1 month)	D 1
20	6	4	5/6 cord	Sk, GI; grade 4	Sepsis (3 months)	D 4
21	4	4	6/6 URD	Sk, Liv; grade 3	Interstitial pneumonia (0.5 month)	D 0.5
22	7	3	6/6 URD T-cell depleted	Sk; grade 1		A 60
23	5	5	5/6 URD	Sk; grade 1		A 46
24	4	5	6/6 URD	No	VOD (2 months)	D 2
25	8	7	5/6 URD	Sk, GI; grade 4	Died of cGVHD (14 months)	D 14

Abbreviations: HSCT, hematopoietic stem-cell transplantation; aGVHD, acute graft-versus-host disease; Sk, skin; A, alive; D, dead; VOD, veno-occlusive disease of the liver; Liv, liver; URD, unrelated donor; cGVHD, chronic graft-versus-host disease.

*No. of HLA-A, HLA-B, and HLA-DRB1 sites matched out of six potential sites; cord indicates unrelated cord blood unit.

expression using either the life-table analysis or the statistical regression model.

There were more events in the first 6 months in the HSCT group (20 total events; 11 deaths as a result of transplantation-related morbidity and nine relapses) compared with the chemotherapy control group (nine total events; two toxic events and seven relapses). Overall survival at 5 years was 53.08% in the HSCT group versus 59.36% in the control group ($P = .26$).

The analysis using the statistical regression model includes all 132 patients enrolled onto CCG 1953 and POG 9407 with leukemia identified as being *MLL*-positive ALL. In this analysis, the RHR for HSCT is 1.454 (90% CI, 0.911 to 2.318; $P = .19$). However, 10% of the HSCT patients received transplantation more than 209 days from study entry.

The following exploratory analyses of EFS comparing transplantation-related factors were performed using life-table analysis. The 5-year EFS rate of HSCT-ON patients (40.0%; 95% CI, 19.8% to 60.2%) compared with HSCT-OFF patients (56.9%; 95% CI, 35.8% to 78.1%) was not statistically significant (log-rank $P = .17$; Fig

2B). The 5-year EFS rate for HSCT-OFF patients (56.9%; 95% CI, 35.8% to 78.1%) compared with the chemotherapy control group (48.7%; 95% CI, 33.8% to 63.6%) was also not statistically significant (log-rank $P = .18$; Fig 2B). Further analyses of the data suggest that the protocol-specified preparative regimen was not optimal (Tables 3 and 4).

When evaluating outcomes in patients who received HSCT on and off protocol and patients who received chemotherapy alone, the toxicities observed were quite varied. The incidence of grade 3 or 4 GVHD was 58% in HSCT-ON patients versus 21% in HSCT-OFF patients. There were twice as many toxic deaths during the first 100 days after transplantation in HSCT-ON patients (12 of 25 patients) than in HSCT-OFF patients (five of 11 patients). Toxic deaths were also more common in patients who received TBI (14 of 36 patients) compared with patients who did not receive TBI (five of 17 patients), although relapse was less common in patients who received TBI (four of 36 patients) compared with those who did not (five of 17 patients). The toxic death rate in remission was quite high in HSCT patients (17 of 53 patients) compared with the control arm (five of 47 patients).

Table 4. Patients Who Received Transplantation in First Remission Using Non-Protocol-Specified Transplantation Regimen

Patient No.	Age on Study (months)	Time to HSCT (months)	Preparative Regimen	Donor*	GVHD Prophylaxis	aGVHD Sites and Maximum Grade Documented	Event	Status at Time Since Transplantation or Last Contact (months)
1	10	9	Bu/Mel/ATG	4/6 cord	CSA/corticosteroid	No		A 98
2	6	3	Bu/VP/Cy	6/6 sibling	CSA/Mtx	No	Relapse (3 months)	D 5
3	6	6	Bu/Ara/Cy	5/6 URD	CSA/Mtx	No		A 86
4	4	7	TBI/Cy	6/6 URD	CSA/Mtx/corticosteroid	No	Relapse (3 months)	D 7
5	7	7	TBI/VP/Cy	6/6 URD	No information	Sk; grade unknown		A 65
6	4	3	TBI/Cy	5/6 grandfather	CSA/Mtx/corticosteroid	Sk; mild		A 82
7	12	6	Cy/TBI	5/6 cousin	CSA/Mtx	Sk, GI; grade 1		A 61
8	10	5	TBI/Mel/ATG	4/6 cord	CSA/Mtx	Sk, Liv, GI; grade 3	VOD (1 month)	D 1
9	4	6	Bu/Cy/Mel	5/6 URD	CSA/Mtx	Sk; grade 2	Bronchiolitis obliterans (14 months)	D 14
10	11	7	Bu/Mel	4/6 cord	CSA	Sk; grade 1	Graft failure (2 months)	D 2
11	5	3	Bu/Mel/ATG	6/6 sibling	CSA/Mtx	No	VOD (1.5 month)	D 1.5
12	6	5	Bu/Mel	5/6 cord	CSA/ATG/corticosteroid	No		A 70
13	8	13	Bu/Cy	6/6 URD	CSA	Sk, GI; grade unknown		A 45
14	3	6	Bu/VP/Cy/ATG	URD	CSA/Mtx	No information	Relapse (3 months)	D 5
15	1	4	TT/Bu/Cy	5/6 cord	Tac/Mtx	Sk, GI; grade 3	Relapse (6 months)	D 30
16	2	4	Bu/Mel	5/6 sibling	CSA/Mtx	No		A 66
17	4	6	Bu/VP/Cy	6/6 URD	CSA/Mtx	No	Relapse (6 months)	A 55
18	7	6	TBI/Cy	6/6 sibling	CSA/Mtx	Sk; grade unknown		A 52
19	5	4	Cy/VP/TBI	6/6 URD	ATG	No		A 60
20	11	10	TT/Bu/Cy	5/6 cord	Tac/Mtx	Sk; grade 3		A 56
21	1	8	Bu/Mel	5/6 cord	ATG/corticosteroid	No		A 18 LTFU
22	1	6	Bu/Mel	4/6 cord	CSA/corticosteroid	GI; grade 3		A 26
23	9	4	Cy/TBI	6/6 URD	CSA/Mtx	No	Pulmonary hemorrhage (1 month)	D 1
24	8	5	Bu/Mel/ATG	6/6 cord	CSA/corticosteroid	No	Relapse (4 months)	D 6
25	1	4	Bu/Cy	6/6 sibling	Tac/Mtx	No		A 47
26	6	7	TBI/Cy	6/6 URD	CSA/Mtx	Sk, GI; grade 3		A 51
27	9	6	TBI/Cy	5/6 URD	CSA/Mtx	Sk, GI; grade 2		A 36
28	5	7	TBI/Cy	6/6 URD	CSA/Mtx	No		A 47

Abbreviations: HSCT, hematopoietic stem-cell transplantation; GVHD, graft-versus-host disease; aGVHD, acute graft-versus-host disease; Bu, busulfan; Mel, melphalan; ATG, antithymocyte globulin; CSA, cyclosporine; A, alive; VP, etoposide; Cy, cyclophosphamide; Mtx, methotrexate; D, dead; Ara, cytarabine; URD, unrelated donor; TBI, total-body irradiation; Sk, skin; ATG, antithymocyte globulin; Liv, liver; VOD, veno-occlusive disease of the liver; TT, thiopeta; Tac, tacrolimus; LTFU, long-term follow-up.

*No. of HLA-A, HLA-B, and HLA-DRB1 sites matched out of six potential sites; cord indicates unrelated cord blood unit.

There was no significant difference in EFS rate between patients who received TBI (50.0%; 95% CI, 31.6% to 68.4%) compared with patients who did not (46.3%; 95% CI, 21.2% to 71.4%; log-rank $P = .91$; Fig 2C) and no statistical difference in 5-year EFS based on the source of donor cells. The 5-year EFS rate was 60.0% (95% CI, 29.6% to 90.4%) for patients who received transplantation using a family donor compared with 50.0% (95% CI, 15.4% to 84.7%) for patients who received transplantation with unrelated donor bone marrow and 36.5% (95% CI, 3.6% to 69.4%) for patients who received transplantation with cord blood (log-rank $P = .51$; Fig 2D). All of these subgroup analyses are underpowered, and interpretation of the data should be made with caution.

DISCUSSION

The parallel CCG 1953 and POG 9407 studies were designed to test the hypothesis that intensification of early therapy would improve the outcome of infants with ALL. This was achieved by two approaches,

dose intensification of chemotherapy and HSCT for infants with *MLL*-positive ALL early in CR1.

Dosing of chemotherapy for infants in previous protocols was determined by weight, not BSA, resulting in significantly lower doses of drugs for infants. To intensify the protocol therapy, BSA was used to calculate doses of all agents except vincristine and, after protocol amendment, daunorubicin. Dexamethasone was chosen as the corticosteroid based on reports of improved outcome with dexamethasone.¹⁷

At the time these protocols were developed, there were no reported large prospective trials of HSCT in infants with ALL. The promising results from the Fred Hutchinson Cancer Research Center (FHCRC) of 11 infants (seven of 11 infants were *MLL* positive) who received transplantation in first or second remission were the basis for evaluating this treatment modality. On the basis of this experience, a major objective of the CCG 1953 and POG 9407 studies was to prospectively evaluate the role of HSCT in the highest risk infants with *MLL*-positive ALL.^{4,18,19}

Table 5. Clinical Characteristics by Treatment Group

Characteristic	HSCT ON		HSCT OFF		Control*		Total No. of Patients	P
	No. of Patients	%	No. of Patients	%	No. of Patients	%		
Age at diagnosis								
< 90 days	3	15.8	6	31.6	10	52.6	19	.59
≥ 90 days	22	27.2	22	27.2	37	45.7	81	
< 6 months	16	26.7	15	25.0	29	48.3	60	.70
≥ 6 months	9	22.5	13	32.5	18	45.0	40	
WBC at diagnosis								
< 50,000	3	18.8	7	43.8	6	37.5	16	.31
≥ 50,000	22	26.2	21	25.0	41	48.8	84	
< 100,000	5	15.2	13	39.4	15	45.5	33	.12
≥ 100,000	20	29.9	15	22.4	32	47.8	67	

Abbreviations: HSCT, hematopoietic stem-cell transplantation; HSCT ON, protocol-specific preparative regimen; HSCT OFF, non-protocol-specific preparative regimen.

*Control group includes patients who received chemotherapy alone and were alive at day 143.

The decision to include TBI in the pretransplantation preparative regimen was based on the favorable outcome of the infants treated at the FHCRC. Two reports of acceptable neurocognitive outcomes for infants treated with TBI-containing transplantation regimens were

available at the time, although more recent reports do cite neurocognitive and hormonal disturbances as late effects associated with TBI.²⁰⁻²² Cytarabine was incorporated based on superior results with regimens incorporating either hyperfractionated TBI (1.2 Gy three

Fig 2. (A) Event-free survival (EFS) comparing on- and off-study remission bone marrow transplantation (RBMT) groups versus control chemotherapy group (*MLL*-positive subset only). (*) Baseline time for RBMT patients is from date of bone marrow transplantation (BMT); for control chemotherapy patients, baseline time is from the median time to transplantation, which was 143 days from study entry. (B) EFS comparing on-study remission hematopoietic stem-cell transplantation (HSCT) group, off-study remission HSCT group, and chemotherapy control group (*MLL*-positive subset only). (†) Baseline time for remission HSCT patients is from date of HSCT; for control chemotherapy patients, baseline time is from the median time to transplantation, which was 177 days from study entry. (C) EFS outcome for RBMT by total-body irradiation (TBI) status (*MLL*-positive subset only). (‡) Baseline time for RBMT patients is from date of BMT. (D) EFS outcome for RBMT by donor type (*MLL*-positive subset only). (§) Baseline time for RBMT patients is from date of BMT. RHR, relative hazard rate.

times daily for 4 days)²³ or high-dose cytarabine.²⁴ The combination of cytarabine with cyclophosphamide and TBI had been demonstrated to be tolerable in older children.²⁵

A major impediment to successful transplantation in these patients was the lack of suitable family donors. To increase the number of patients with suitable donors, acceptable stem-cell sources included family or unrelated donors with up to a one antigen mismatch including unrelated cord blood units. Single-agent cyclosporine was the protocol-specified GVHD prophylactic regimen with the hope of an enhanced graft-versus-leukemia effect.

To our knowledge, this experience represents the largest evaluation of infants with *MLL*-rearranged ALL who received transplantation in CR1 compared with a concomitantly treated control group who received intensive chemotherapy. The results suggest that HSCT offered no advantage compared with intensive chemotherapy regardless of the choice of preparative regimen. The 5-year EFS rates for the 53 infants who received transplantation in CR1 and for the 47 infants treated with intensive chemotherapy were not dissimilar. Potential reasons for the lack of a difference in outcome between patients who did and did not receive transplantation were the improved outcome of patients treated with the chemotherapy regimens and the suboptimal transplantation regimen.

The overall 3-year EFS rates for CCG 1953 (33.6%)¹³ and POG 9407 (40%)²⁶ are comparable to those reported in the multi-institution Japan Infant Leukemia Study Group trial in which the 3-year EFS rate for infants with *MLL*-positive ALL was 44% (95% CI, 28.5% to 58.7%). However, for the 29 infants who underwent HSCT, the EFS rate was 64%.²⁷

The CCG 1953/POG 9407 results contrast somewhat with the results of selected single-center experiences of HSCT in infants. The FHCRC reported a 3-year disease-free survival rate of 76% among 17 infants (three without documented *MLL* rearrangement) who received transplantation in CR1.²² The Children's Memorial Hospital experience included 16 infants who received transplantation (age, 1 to 15 months at diagnosis; five infants without *MLL* rearrangement) and demonstrated a 1-year EFS rate of 64% (95% CI, 40% to 88%) and a 3-year overall survival rate of 75% (95% CI, 47% to 92%).²⁸ Differences in patient characteristics, such as older age, lower WBC count, and inclusion of infants without the *MLL* rearrangement, preclude strict comparison with our data.

The recently published international study Interfant-99 demonstrated a disease-free survival advantage for HSCT ($n = 16$) over chemotherapy alone ($n = 71$) in patients with *MLL*-positive ALL identified as high risk based on age less than 6 months and either poor response to corticosteroid at day 8 or WBC greater than $300 \times 10^3/\mu\text{L}$. This advantage was more evident with each successive year.³ However, an earlier review of 497 children, including infants and noninfants, with ALL and 11q23 rearrangements failed to prove an advantage for HSCT over intensified chemotherapy.⁴

More than half the infants included in this report did not receive transplantation according to the protocol specifications. When comparing HSCT with chemotherapy, bias is introduced when patients receive transplantation after prolonged periods in remission, resulting in unbalanced allocation of patients who experience relapse early to the chemotherapy arm. To address this problem, the protocol specified that infants with *MLL*-positive ALL and suitable donors were eligible for transplantation, which was to be scheduled within 4 months of enrollment. However, 60% of patients treated with the

protocol-specified preparative regimen and only 29% of the patients treated with non-protocol-specified preparative regimens received transplantation within 4 months of enrollment (Tables 3 and 4).

Additionally, more than half of the patients were not treated with the protocol-specified preparative regimen. Five-year EFS rate was 56.9% (95% CI, 35.8% to 78.1%) for HSCT-OFF patients compared with 40.0% (95% CI, 19.8% to 60.2%) for HSCT-ON patients ($P = .17$). The median time to transplantation was 6 months for the HSCT-OFF group compared with 4 months for the HSCT-ON group. This difference in time to transplantation may partially explain the nonsignificantly better outcome of the off-protocol transplantation cohort.

Decreased transplantation-related morbidity also may have contributed to the better outcome of the off-protocol cohort. Grade 3 or 4 GVHD occurred more often in the HSCT-ON group. A more intensive GVHD prophylactic regimen may have improved EFS for this group. The preparative regimen, which incorporated high-dose cytarabine and corticosteroids, may have contributed to the higher transplantation-related morbidity and mortality of the protocol-specified transplantation regimen. TBI does not seem to have affected EFS (Fig 2C). The transplantation-related morbidity was quite high in this study compared with the chemotherapy-only control group and compared with other studies of HSCT in infants. With a less morbid transplantation preparative regimen, the EFS in the patients who received transplantation may have been more favorable compared with the control group.

Additional limitations to this study include the broad heterogeneity of preparative regimens and GVHD prophylaxis in patients who received transplantation with non-protocol-specified therapy. In addition, because the decision for HSCT was investigator option on POG 9407, investigator bias must be included as a potential limitation.

The conclusions of our study and the recent report of Interfant-99 establish several important points regarding the treatment of infant ALL. First, there is no role for the routine use of HSCT in infant ALL, and most infants clearly do not benefit from this approach. Interfant-99 demonstrated a modest advantage for a small subgroup of particularly high-risk infants. We did not see an advantage for HSCT in a similar population in our trial, but numbers were limited, making subgroup analyses difficult. Second, in both studies, there is a high early failure rate for the highest risk group, particularly those younger than 3 months of age. A primary goal of future trials must be to improve complete remission rates, decrease early relapse, and lessen the toxic death rate for this patient population.

Additional studies, such as gene expression profiles or minimum residual disease, might be useful for identification of subgroups most likely to benefit from HSCT (or, conversely, who would have a high cure rate with chemotherapy alone). As chemotherapy and transplantation regimens change, the comparison of HSCT and chemotherapy in infants with *MLL*-rearranged ALL will require re-evaluation.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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