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Early mixed chimerism-based preemptive immunotherapy in children undergoing allogeneic hematopoietic stem cell transplantation for acute leukemia

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Abstract

This retrospective analysis comprises 10-year experience with early posttransplant mixed chimerism-based preemptive intervention. Out of 104 patients, 51 received preemptive immunotherapy. Their outcomes were similar to patients achieving full donor chimerism spontaneously. Among patients receiving intervention, 5-year event-free survival was identical in patients with and without pretransplant residual disease, respectively (68% [95% confidence interval (CI) 38–98%] vs. 69% [95% CI 54–85%] log–rank = 0.4). In patients who received preemptive immunotherapy, chimerism status and residual disease prior to transplant were no longer predictors of poor outcome; however, 41% of the patients with residual disease prior to transplant relapsed early and did not benefit from this strategy.

Keywords

allogeneic stem cell transplant; immunotherapy; pediatric leukemia

1 | INTRODUCTION

Relapse of leukemia following hematopoietic stem cell transplant (HSCT) remains the major cause of transplant failure.[1,2] Two well-described risk factors for relapse include residual disease prior to transplant, even if documented by next-generation sequencing (NGS)[3,4] and residual host hematopoetic cells posttransplant, also known as mixed chimerism (MC), which can induce immunologic tolerance.[4,5] Posttransplant immunotherapy with fast

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

withdrawal of immunosuppression (FWI) and donor lymphocyte infusions (DLI) can reduce relapse in children with MC.[4–9]

In a prospective study, we validated early MC (day 28–40 posttransplant) as a marker of increased risk of relapse in children with leukemia.[8] In the subsequent prospective study, we showed that treatment with chimerism-based preemptive immunotherapy conferred 73% (95% confidence interval [CI] 55–91%) 2-year event-free survival (EFS). Due to late relapses, EFS declined to 55% [95% CI 34–76%] at 42 months posttransplant.[9]

Although efficacy of immunotherapy was documented in adults with acute myeloid (AML) and lymphoblastic leukemia (ALL) who had posttransplant minimal residual disease,[10] prior reports did not show definite benefit of preemptive immunotherapy in children with residual disease pretransplant.[4–9,11–14]

This report describes long-term outcomes of children treated with preemptive immunotherapy and addresses the efficacy of this approach in children with residual disease prior to transplant.

2 | METHODS

All patients with acute leukemia undergoing unmodified bone marrow (BM) or peripheral blood (PB) HSCT from 2005–2015 at University of California, San Francisco (UCSF) Benioff Children's Hospital are presented, including 62 new patients and 42 patients from previously published prospective studies, adding significantly to their follow-up. Consents for immunotherapy were obtained from all patients, and UCSF Institutional Review Board approved this retrospective study. Disease evaluation before and after HSCT was done with clinically available tests and are presented in Table 1. In accordance with the recent literature, any measurable disease, regardless of test sensitivity, was considered positive.[3,4]

Chimerism tests were performed at the UCSF Immunogenetics Laboratory and described previously.[8,9] The test has not changed during the study period. Early MC was defined as the presence of 1% or more host cells in whole PB, BM, or any of the tested subsets, which included CD3+, CD14/15+, CD19+ subsets from the PB and BM, and CD33+ and CD34+ subsets from the BM done on two independent tests between day 28 and 40 posttransplant. Patients who were in remission posttransplant and had early MC and absence of graft-versus-host disease (GVHD) underwent preemptive intervention (N = 51). Observation arm (N = 40) consisted of patients who were in remission, had early FDC, or MC and acute GVHD (aGVHD) or history of peri-engraftment syndrome requiring steroids. They did not receive preemptive immunotherapy. Patients with persistent or recurrent leukemia on the first posttransplant BM examination (N = 12) or early death (N = 1) were assigned to early events arm.

Preemptive immunotherapy consisted of calcineurin inhibitor (CNI) taper over 3 ± 1 weeks. If MC persisted, patients proceeded to DLI if there was no evidence of GVHD. DLI were repeated every 6–12 weeks until development of GVHD or FDC was achieved in all PB subsets. Stored stem cells or unmobilized PB were used for DLI with a starting CD3+ dose of 1×10^5 per kg, as previously described.[8,9] aGVHD and chronic GVHD (cGVHD) were

graded using the published criteria.[15,16] Clinically relevant GVHD was defined as aGVHD of grade II or higher and/or moderate or severe cGVHD. Data were analyzed using SPSS® v23.0 (IBM Corp., Armonk, NY) statistical program. Fisher exact test with two-sided *P* values was used as a test of homogeneity. Kaplan–Meier analysis and log–rank test were used in analysis of EFS. Events were defined as relapse, second malignancy or death. *P* value less than 0.05 was considered significant. Newcombe method was used for calculating CIs for the single proportion.

3 | RESULTS

Table 1 describes pretransplant characteristics of patients and outcomes by treatment arm. Median age of patients was 12 years (age 0.9–24.4). Pretransplant disease was present in 27 (26%) of the patients, with the majority (78%) having 1% or less of blasts. Fifteen out of 27 (56%) patients with pretransplant disease relapsed. Median post-HCT follow-up of living patients was 63 (range 9–131) months. Immunotherapy with FWI was started in 51 patients, and 30 (59%) proceeded to receive DLI for persistent MC. Median time of initiation and end of FWI was 47 days (range 23–85) and 74 days (range 40–125) posttransplant. First and last DLIs were given at a median of 120 days (range 79–351) and 259 days (range 79–945) posttransplant. A total of 93 DLIs were given to 30 patients, median of 3 (range 1–10). Mean EFS was similar for preemptive intervention and observation arm and significantly higher than in patients with early events (mean survival in months and 95% CI 92 [76–109], 70 [53–86], 24 [0–51]; *P* log–rank < 0.001). Patients with MC and preemptive immunotherapy and those with spontaneous FDC who were observed had similar relapse rates (11/51 vs. 14/40, $P = 0.2$), nonrelapse mortality (4/51 vs. 5/40, $P = 0.5$), evidence of disease pretransplant (10/51 vs. 6/40, $P = 0.6$), cGVHD rates (14/51 vs. 17/40, $P = 0.2$) and clinically relevant GVHD (14/51 vs. 17/40, $P = 0.18$). aGVHD rates were significantly lower in immunotherapy group (7/51 vs. 23/40, $P < 0.001$) due to assignment of patients with GVHD to the observation arm. Patients with positive disease prior to transplant were more likely to be in the early event arm than in observation or intervention arm (11/13 vs. 16/91, $P < 0.001$).

There was no difference in survival between patients with lymphoid and myeloid malignancies undergoing immune intervention (log–rank $P = 0.4$).

Figure 1A depicts EFS based on residual disease prior to transplant in the entire cohort (N = 104). Five-year EFS was significantly better in patients without documented residual disease prior to transplant than in those with disease (64% [95% CI 52–76%] vs. 40% [95% CI 20–60%], respectively; log–rank $P = 0.005$). Figure 1B indicates that in patients who received preemptive IT (N = 51), residual disease prior to transplant was no longer a predictor of poor EFS (5-year EFS 68% [95% CI 38–98%] vs. 69% [95% CI 54–85%] in patients with and without disease, respectively; log–rank $P = 0.4$). However, out of 27 patients with disease prior to transplant, 11 (41%) had an event or relapse before initiation of MC-based immunotherapy. Cumulative rate of clinically significant GVHD was 27% (95% CI 17–41%) in the preemptive IT group and 42% (95% CI 28–58%) in the observation arm. Two patients (4%) in the intervention group died due to complications of GVHD.

4 | DISCUSSION

We describe long-term follow-up of children treated with MC-based posttransplant immunotherapy. Children receiving posttransplant immunotherapy achieved stable EFS curves at approximately 3 years posttransplant. Previously described inferior outcomes related to MC[4,5] were averted with early preemptive immunotherapy.

Residual disease prior to transplant was not a predictor of inferior outcome in children receiving immunotherapy, indicating that timely posttransplant immunotherapy can overcome the risk of relapse. As published previously, the main challenge to this approach are patients (approximately 10% of all patients but 41% of those with pretransplant residual disease) who develop early events/relapses before immunotherapy can be initiated.[4,5,9] Additional challenges include treatment-related deaths (4%) in patients receiving intervention and overtreatment of a subgroup of patients whose mixed chimerism would have converted to full donor chimerism spontaneously.[9] The limitations of this retrospective study are that it spanned a decade during which the approach to measurement and treatment of minimal residual disease evolved. Also, the number of observed events (relapses) was relatively low. Due to a small number of patients and events, we could not evaluate if the amount of pretransplant disease affects the risk of relapse.

Although future immunotherapy strategies will be based on more sensitive and specific tests for the detection of relapse, such as NGS,[3] chimerism measurement may still provide valuable information about potential for tolerance as opposed to alloreactivity, and the potential for GVHD.[17] Until targeted cellular therapy such as CAR T-cells[18] or monoclonal antibodies are available on a clinical basis for all malignancies including AML and T-ALL, preemptive immunotherapy with FWI and DLI remains a readily available method of preventing relapse in children with leukemia.

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Abbreviations:

ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
aGVHD	acute graft-versus-host disease
BM	bone marrow
CAR	chimeric antigen receptor
cGVHD	chronic graft-versus-host disease
CI	confidence interval

CML	chronic myelogenous leukemia
CNI	calcineurin inhibitor
DLI	donor lymphocyte infusions
EFS	event-free survival
FDC	full donor chimerism
FWI	fast withdrawal of immunosuppression
HSCT	hematopoietic stem cell transplant
JMML	juvenile myelomonocytic leukemia
MC	mixed chimerism
NGS	next-generation sequencing
PB	peripheral blood
UCSF	University of California San Francisco

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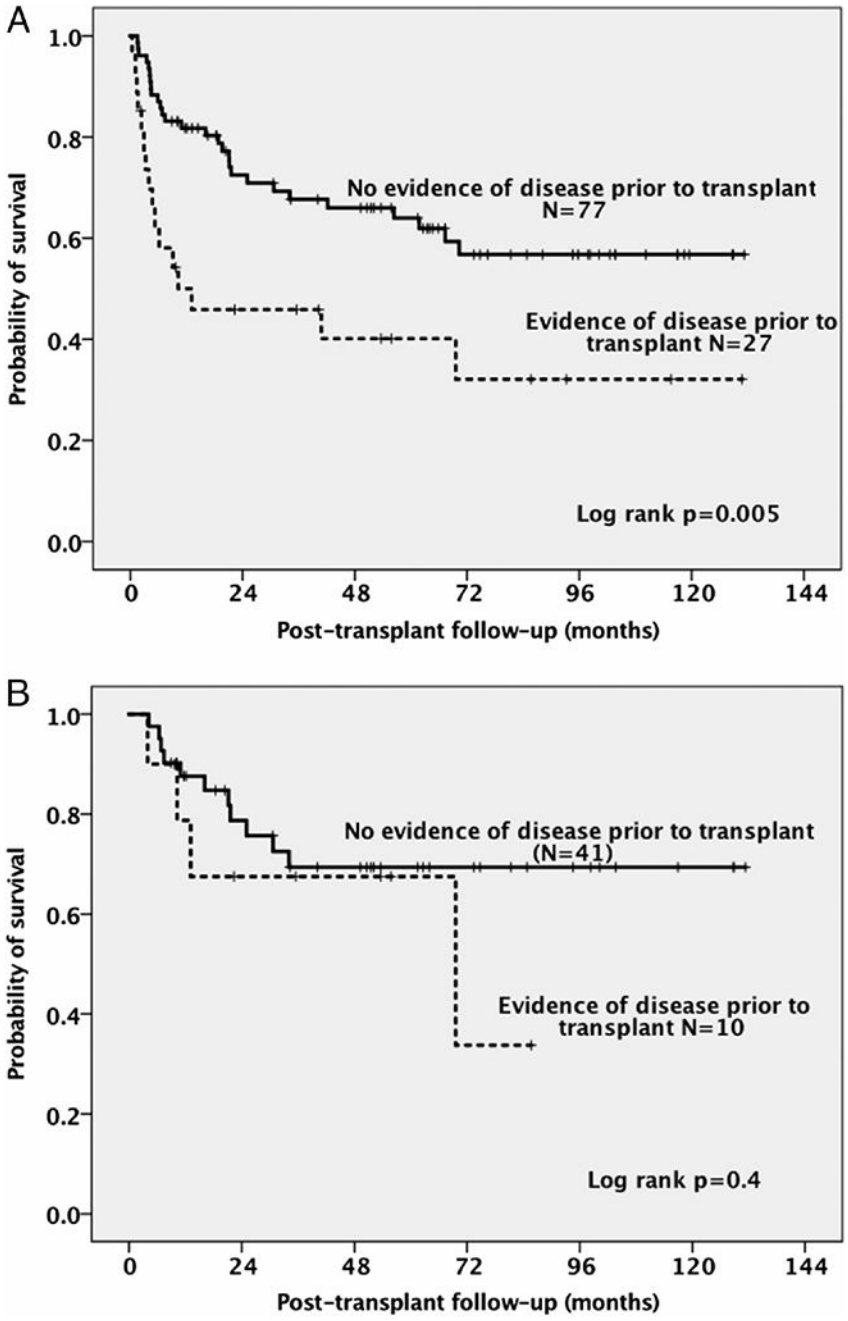

FIGURE 1.

Probability of EFS in patients with and without residual disease prior to transplant, (A) the entire cohort; (B) patients receiving preemptive immunotherapy

TABLE 1

Characteristics of patients and outcomes by treatment arm

	Intervention arm		Observation arm		Early events		Total	
	N = 51	**%**	**N = 40**	**%**	**N = 13**	**%**	**N = 104**	**%**
Gender								
Male	**26**	51	**29**	72	**7**	64	**62**	60
Female	**25**	49	**11**	28	**6**	46	**42**	40
Diagnoses								
Lymphoid malignancies								
B- or T-ALL, 1st remission	**10**	40	**6**	27	**1**	14	**17**	31
B- or T-ALL, 2nd or higher remission	**8**	32	**12**	55	**1**	14	**21**	39
B- or T-ALL, not in remission	**7**	28	**4**	18	**5**	71	**16**	30
Biphenotypic leukemia								
1st or 2nd remission	**1**	33	**2**	67			**3**	100
Myeloid malignancies								
AML 1st remission	**11**	44	**7**	44	**1**	17	**19**	40
AML 2nd remission	**7**	28	**3**	19	**0**		**10**	21
t-AML or ≥2nd transplant	**2**	8	**4**	25	**0**		**6**	13
CML or JMML, s/p blast crisis	**2**	8	**0**		**0**		**2**	4
Myeloid malignancy, not in remission	**3**	12	**2**	12	**5**	83	**10**	21
Stem cell source								
Bone marrow	**14**	27	**12**	30	**5**	38	**31**	30

	Intervention arm		Observation arm		Early events		Total	
	N = 51	%	N = 40	%	N = 13	%	N = 104	%
Peripheral blood	**37**	73	**28**	70	**8**	62	**73**	70
Donor type								
Matched related	**27**	53	**6**	15	**5**	38	**38**	36.5
Matched unrelated	**18**	35	**16**	40	**5**	38	**39**	37.5
Mismatched unrelated[a]	**5**	10	**18**	45	**2**	15	**25**	24
Mismatched related[a]	**1**	2	**0**	0	**1**	8	**2**	2
Conditioning regimen								
Lymphoid/biphenotypic leukemia								
Radiation-based	**17**	65	**21**	88	**4**	57	**42**	74
Busulfan-based	**6**	23	**3**	12	**2**	29	**11**	19
Reduced intensity	**3**	12	**0**		**1**	14	**4**	7
Myeloid leukemia								
Radiation-based	**3**	12	3	19	1	17	7	15
Busulfan-based	**22**	88	13	68	5	83	40	85
Serotherapy (ATG or alemtuzumab)								
Yes	**39**	76	36	90	8	62	83	80
No	**12**	24	4	10	5	38	21	20
Residual disease prior to transplant								
No	**41**	80	34	85	2	15	77	74
Yes	**10**	20	6	15	11	85	27	26

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	Intervention arm		Observation arm		Early events		Total	
	N = 51	%	N = 40	%	N = 13	%	N = 104	%
Test used for detection of disease								
Single institution MCFC	**29**	57	**22**	55	**8**	61	**59**	57
NGS	**4**	8	**1**	2	**3**	23	**8**	8
Polymerase chain reaction	**2**	4	**0**		**1**	8	**3**	3
Immunoflow	**16**	31	**17**	43	**1**	8	**34**	33
Amount of residual disease								
>1%	**2**	4	**1**	3	**3**	23	**6**	6
0.01-1%	**5**	10	**5**	12	**4**	31	**14**	13
<0.01%	**3**	6	**0**		**4**	31	**7**	7
No residual disease	**41**	80	**34**	85	**2**	15	**77**	74

	Intervention Arm		Observation Arm		Early Events		Total	
KM estimates of EFS	**%**	**[95% CI]**	**%**	**[95% CI]**	**%**	**[95% CI]**	**%**	**[95% CI]**
Lymphoid/biphenotypic malignancies								
2-year EFS	**70**	[51–89]	**74**	[56–92]	**18**	[0–49]	**66**	[54–79]
5-year EFS	**65**	[45–85]	**69**	[50–88]	**18**	[0–49]	**61**	[47–75]
Myeloid malignancies								
2-year EFS	**83**	[68–98]	**50**	[26–74]	**33**	[0–70]	**65**	[51–79]
5-year EFS	**73**	[55–91]	**44**	[20–68]	**17**	[0–46]	**55**	[41–70]
All patients								
2-year EFS	**74**	[61–87]	**64**	[49–79]	**26**	[1–52]	**65**	[55–74]
5-year EFS	**69**	[55–83]	**58**	[42–74]	**13**	[0–45]	**58**	[48–68]

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	Intervention arm		Observation arm		Early events		Total	
	N = 51	%	N = 40	%	N = 13	%	N = 104	%
Cumulative incidences	**N**	**%[95% CI]**	**N**	**%[95% CI]**	**N**	**%[95% CI]**	**N**	**%[95% CI]**
Relapse, death or 2nd malignancy	**15**	29 [19–43]	**19**	48 [33–63]	**10**	77 [50–92]	**44**	42 [33–52]
Relapse, 2nd hematologic malignancy	**11**	22 [13–35]	**14**	35 [22–50]	**9**	69 [42–87]	**34**	33 [24–42]
GVHD-related death	**2**	4 [1–13]	**4**	10 [4–23]			**6**	6 [3–12]
aGVHD any grade	**7**	14 [7–26]	**23**	58 [42–71]	**1**	8 [1–33]	**31**	30 [22–39]
aGVHD grade II-IV	**3**	6 [2–16]	**18**	45 [31–60]	**1**	8 [1–33]	**22**	21 [14–30]
cGVHD mild-severe	**14**	27 [17–41]	**17**	43 [29–58]	**3**	23 [6–54]	**34**	33 [24–42]
II–IV aGVHD or moderate/severe cGVHD	**14**	27 [17–41]	**25**	63 [47–76]	**3**	23 [6–54]	**42**	40 [31–50]

ALL, acute lymphoblastic leukemia of T- or B- cell origin; ATG, antithymocyte globulin; KM, Kaplan–Meier; MCFC, multichannel flow cytometry; tAML, treatment-related AML.

[a]Matching was done by high resolution at A, B, C and DrB1 loci, one allele or antigen mismatch at A, B or C loci was accepted.

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