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ORIGINAL RESEARCH

Treatment response and outcome of children with T-cell acute lymphoblastic leukemia expressing the gamma-delta T-cell receptor

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ABSTRACT

T-cell malignancies expressing the γδ T-cell receptor (TCR) are often associated with poor prognosis. Here, we determined the clinical outcome of pediatric patients with T-cell acute lymphoblastic leukemia (T-ALL) expressing the γδ TCR. Of 100 newly diagnosed T-ALL patients, 93 had γδ TCR analysis performed at diagnosis. Repertoire was evaluated by paired sequencing of the rearranged TCR. All patients received intensified chemotherapy and those with minimal residual disease (MRD) ≥ 1% on day 42–46 became candidates for hematopoietic cell transplantation. Of the 93 T-ALL patients, 12 (13%) had γδ T-ALL and 11 (12%) had early T-cell precursor (ETP) ALL. Compared to the remaining 70 T-ALL patients, the γδ T-ALL patients were more likely to have MRD ≥ 1% on day 15–19 (67% vs. 33%, $P = 0.03$) and day 42–49 (33% vs. 7%; $P = 0.007$) of remission induction. The 10-year overall survival for γδ T-ALL patients (66.7% ± 22.2%) were lower than that of T-ALL patients (93.3% ± 7.3%, $P = 0.001$). TCR analysis demonstrated a conserved clonotype. In conclusion, the data suggest that children with γδ T-ALL may have a poor response to remission induction, based on MRD levels and decreased survival than the other T-ALL patients, despite receiving risk-directed therapy.

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Introduction

Two distinct T-cell lineages, which are based on the use of αβ or γδ heterodimers in the T-cell receptor (TCR) complex, diverge early during thymic development and during the course of the β-, γ-, and δ-chain rearrangement. Lineage commitment toward γδ over αβ T-cell and subsequent γδ thymocyte development is favored by strong TCR signaling. Recent studies have shown that the developmental and proliferative programs as well as the expression of genes regulating apoptosis differ between the γδ and αβ lineages.[1,2] Certain neoplasms derived from the γδ T cell lineage such as primary cutaneous and hepatosplenic lymphomas are clinically aggressive with bleak prognoses and are recognized as being biologically distinct.[3–8] For patients with T-cell acute lymphoblastic leukemia (T-ALL) expressing the γδ TCR, case reports have suggested that they are at higher risk for poor outcomes.[7,9–20] Recently, identification of γδ T-cell lineage-specific genetic alterations leading to the fusion transcripts, *SET-NUP214* and *CALM-AF10*, have been associated with chemotherapy resistance and poor prognosis, respectively, suggesting a biological link to the clinical outcome for patients with T-ALL.[19,20]

For patients with T-ALL, one of the most significant prognostic indicator is the level of minimal residual disease (MRD) during and after remission induction chemotherapy.[21] Despite intensification of therapy, approximately 10% of patients with T-ALL continue to have elevated MRD or fail to obtain a morphologic remission after remission induction.[21–25] Timely identification of patients with poor response to remission induction allows them to potentially benefit from early intensification treatment.[26] Risk stratification of T-ALL has greatly improved by integrating immunophenotypic, cytogenetic, and/or molecular aberration, although the time restraints of genetic profiling remain problematic.[27–30] However, flow cytometry (FCM) can rapidly detect TCR expression on lymphoblasts at diagnosis.

The TCR is formed by the recombination of the variable (*V*) gene to a joining (*J*) segment and the diversity (*D*) gene on the delta (*TRD*) or gamma (*TRG*) locus. Although similar in TCR structure, γδ T cells are not restricted by major histocompatibility complex (MHC), and their ability to recognize antigens differs remarkably.[31–33] The γδ TCR repertoire is limited by six *TRGV* and eight *TRDV* genes.[34] The two major γδ T cell subsets are determined by the expressed Vδ chain, Vδ1 and Vδ2. Vδ1 T cells are prominent in organs enriched with epithelial or mucosal surfaces and recognize stress-induced self-antigens, heat shock proteins, glycolipids, and members of the "MHC-like" superfamily.[35] Vδ2 T cells are found in peripheral blood (PB) and comprise only 1–10%

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of the lymphocyte population. Majority of the PB TCR repertoire are Vγ9Vδ2 T c ells, which recognizes nonpeptide antigens commonly expressed by microbes.[36,37] Here, we evaluated the clinical presentation and outcomes of patients with T-ALL with or without γδ TCR expression.

Results

Patients

Of the 93 T-ALL patients, 12 (13%) had γδ T-ALL and 11 (12%) had ETP ALL. Table 1 shows the presenting clinical and laboratory features of γδ T-ALL and the other T-ALL. There were no significant differences between γδ T-ALL and the other T-ALL in regards to age, gender, presenting leukocyte count, and CNS involvement at diagnosis. The γδ T-ALL group had a significantly higher percentage of African Americans (42% vs. 16%; $P = 0.03$) and presence of mediastinal mass (50% vs. 20%; $P = 0.03$) compared to the other T-ALL group. A higher percentage of γδ T-ALL patients were classified to have high-risk disease compared to other T-ALL patients (33% vs. 7%; $P = 0.007$) (Table 1).

Phenotypic alterations of γδ T-ALL

All of the lymphoblasts expressed γδ TCR, CD3, cyCD3, CD45, and CD7 and lacked αβ TCR, cyMPO, CD19, CD20, CD61, CD64, CD65, CD117, and CD45RA expression. The diverse maturation stages were reflected by the heterogeneous profile of CD2, CD1a, and CD34. The expression of CD4 and CD8 included double positive $CD4^+CD8^+$ (18%), double negative $CD4^-CD8^-$ (27%), single positive $CD4^+$ (18%), and $CD8^+$ (36%) lymphoblasts. Patients with CD56 expression (5) had variable coexpression of CD4 (1), CD8 (3), or CD4/CD8 (1). One patient (No. 9) fulfilled criteria for both γδ T-ALL and ETP ALL, with full immunophenotype details provided in Table 2.

TCR repertoire in γδ T-ALL lymphoblast

The *V-(D)-J* gene rearrangement for the *TRG* and *TRD* loci expressed by the lymphoblast was identified using single-cell PCR as previously described.[38,39] Appropriate consents and samples were available for 9 of the 12 patients with γδ T-ALL (Table 3). A dominant clonal population of lymphoblast was identified for all patients. A biclonal population was observed in one patient where one *TRDV* region paired with two unique *TRGV* regions. The combinatory diversity of the *TRG* genes showed a bias toward *TRGJ* segments from the distal region 2 *(JP2 and J2)* (89%) joining to the terminal constant region (*TRGC2*). The use of the proximal region 1 *(TRGJ1* and *TRGC1*) was rare. The *TRGV* regions detected were *TRGV*9 (33%), *TRGV5* (33%), *TRGV4* (20%), *TRGV2* (10%), and *TRGV8* (10%). Despite variability in the *TRGV* regions the CDR3γ regions were similar. The CDR3γ region contained an average of 14.2 ± 2.2 amino acids and each patient had an average of 3.3 ± 2.6 unique amino acids and shared an average of 11 ± 0.5 amino acids (88%). In contrast, the *TRDV* regions expressed were predominantly *TRDV1* (67%). Non-*TRDV1* included *TRDV3* (11%), *TRDV5* (11%), and *TRDV8* (11%). All in frame sequences used the *TRDJ1* segment.

Cytogenetic alterations

Majority of patients with γδ T-ALL had complex cytogenetic abnormalities (91%) and one patient had normal karyotype (Table 4). Hyperdiploidy was common (50%), but only one patient had high hyperdiploidy with a DNA index of 1.17. Chromosomal abnormalities involving the *TRD* locus on chromosome 14 (14q11.2) or *TRG* locus on chromosome 7 (7p14) were not detected by conventional cytogenetics. Cytogenetic aberrations were detected on chromosome 1 (1q23 and 1p36.1), 6q (6q13q21, 6q13q23, 6q21q23, and 6q21), 11 (11p11.2, 11p13, and 11q22), 12p (12p11.2 and 12p13,), and 14q (14q13, 14q32, and 14q32.1). Deletions involving 6q (q13-23) and/or 12p11-13 were observed in over half the patients (60%). Translocation included *t*(11;14)(p13;q32), *t*(10;11)(p12;q22), and *t*(11;14)(p11.2;q32.1), with the breakpoint on chromosome 14 centromeric to the *IGH* locus.

Response to remission induction and overall survival

MRD was monitored during induction treatment. A higher portion of γδ T-ALL patients had MRD ≥ 1% compared to other T-ALL patients on day 15–19 (67% vs. 33%, $P = 0.03$) and on day 42–49 (33% vs. 7%; $P = 0.007$) of remission induction. There was no difference in the percent of patients with γδ T-ALL and ETP ALL with MRD ≥ 1% on day 15–19 (67% vs. 82%; $P = 0.4$) and on day 42–46 (33% vs. 27%; $P = 0.7$) of remission induction.

T-ALL patients with relapse/refractory disease or MRD ≥ 1% at the end of induction were referred to HSCT. The proportion γδ T-ALL patients who received a HSCT (42%) was significantly higher than other T-ALL patients (10%; $P = 0.004$). The γδ T-ALL patients referred for an allogeneic HSCT were due to elevated MRD ≥ 1% at the end of induction (*n*; No. 1, 7, and 11), relapse after induction (No. 2), refractory disease (No. 4), or γδ/ETP ALL diagnosis (No. 9). Patient No. 9 was stratified as high-risk, underwent a HSCT, and remains in CR. The one patient with refractory disease (No. 4) died from disease progression prior to undergoing an HSCT. Three patients (No. 1, 9, and 11) are in complete remission 10, 3, and 2.5 years post-HSCT, respectively and two patients relapsed (No. 2 and 7) on day 70 and day 90 after HSCT.

With a median follow-up of 9.3 ± 1.4 years (range; 7.1–15.5 years), the 10-year OS for patients with γδ T-ALL was less compared to the other T-ALL patients (66.7% ± 22.2% vs. 93.3% ± 7.3%) ($P = 0.001$). The 10-year EFS for patients with γδ T-ALL was lower as compared to the other T-ALL patients (66.7% ± 22.2% vs. 81.2% ± 12.4%), albeit not significant ($P = 0.11$) (Figure 1). The cumulative incidence of relapse was not significantly different for patients with γδ T-ALL (18.2% ± 12.3%) compared to the other T-ALL patients (13.3% ± 4.2%) ($P = 0.58$). In multivariable analyses, adjusting for age, race, presenting leukocyte count, and MRD level on day 15–19 or day 42–46, γδ T-ALL was independently associated with poor survival (hazard ratio, 6.95; 95% CI, 1.2–40.2; $P = 0.03$; Table 5).

Table 1. Comparison of clinical and biologic variables between patients with γδ T-ALL, T-ALL and ETP.

		γδ T-ALL	T-ALL	vs. γδ T-ALL	ETP ALL	vs. γδ T-ALL
Category	Group	*N* = 12 (13%)	*N* = 70 (75%)	*P*	*N* = 11 (12%)	*P*
Age	1–10 years	8 (67%)	44 (63%)	0.8	6 (55%)	0.6
	>10 years	4 (33%)	26 (37%)		5 (45%)	
	Mean ± sem	8.7 ± 1.3	9.3 ± 0.6		11.7 ± 1.1	
	Median (Range)	8.5 (1.4–15.1)	8.2 (1.1–18.6)		10.6 (6.1–18.7)	
Gender	Male	9 (75%)	49 (70%)	0.7	7 (67%)	0.6
	Female	3 (25%)	21 (30%)		4 (33%)	
Race	White	7 (58%)	59 (80%)	0.03	7 (64%)	0.8
	Black	5 (42%)	11 (16%)		4 (36%)	
Leukocyte count $10^3/mm^3$	<10	2 (17%)	12 (17%)	0.8	2 (18%)	0.8
	10–50	4 (33%)	15 (21%)		4 (36%)	
	≥50–100	2 (17%)	10 (14%)		3 (27%)	
	≥100	4 (33%)	33 (47%)		2 (18%)	
	Mean ± sem	107 ± 39	171 ± 22	0.1	50 ± 17	0.6
	Median (Range)	46 (4.2–401)	90 (1.9–657)		27 (3.9–182)	
CNS	CNS1	5 (42%)	35 (50%)	0.7	7 (64%)	0.6
	CNS 2	5 (42%)	22 (31%)		3 (27%)	
	CNS 3	0	5 (7%)		0	
	Traumatic	2 (17%)	8 (11%)		1 (9%)	
Mass	Present	6 (50%)	14(20%)	0.03	2 (18%)	0.1
	Absent	6 (50%)	56 (80%)		9 (82%)	
Risk	Standard	7 (58%)	65 (93%)	0.0007	4 (36%)	0.3
	High	5 (42%)	5 (7%)		7 (64%)	
MRD Day 15–19	<1% vs .≥ 1%	33% vs. 67%	67% vs. 33%	0.03	18% vs. 82%	0.4
	<.01%	1 (8%)	18 (26%)		0	
	.01–0.1%	3 (25%)	10 (14%)		0	
	0.1–1%	0	18 (26%)		2 (18%)	
	≥ 1%	8 (67%)	23 (33%)		9 (82%)	
	Mean ± sem	27 ± 8.5	6 ± 1.8		28 ± 7.6	
	Median (Range)	23 (0.05–79)	0 (≤.001–69)		28 (0.83–87)	
	<1% vs. ≥ 1%	67% vs. 33%	93% vs 7%	0.007	73% vs 27%	0.7
MRD Day 42–46	<.01%	7(58%)	55 (79%)		3 (27%)	
	.01–0.1%	1 (8%)	5 (7%)		3 (27%)	
	0.1–1%	0	4 (6%)		2 (18%)	
	≥ 1%	4 (33%)	5 (7%)		3 (27%)	
	Mean ± sem	1.9 ± 0.9	0.13 ± 0.05		0.63 ± 0.4	
	Median (Range)	0 (≤.001–7.1)	0 (≤.001–2.1)		0.03 (≤.001–3.8)	
HSCT	Yes	5 (42%)	7 (10%)	0.004	8 (73%)	0.1
	No	7 (58%)	63 (90%)		3 (27%)	
STATUS	Alive	8 (67%)	66 (94%)	0.003	7 (64%)	0.9
	Expired	4 (33%)	4 (6%)		4 (36%)	

Abbreviations: T-ALL: T-cell acute lymphoblastic leukemia; γδ: γδ T-All; ETP: early T-cell progenitor; WBC: white blood count; CNS: central nervous system; MRD: minimal residual disease; sem: standard error of mean; Med.: media

Table 2. Immunophenotype of γδ T-ALL.

PT	γδ TCR	CD 45	CD 3	cyCD 3	CD 7	CD 5	TDT	CD 1a	CD 2	CD 4	CD 8	CD 56	CD 57	CD 34	CD 45RO	CD 10	CD 133	CD 21	CD 22	HLA-DR	cyCD 79a	CD 1b	CD 13	CD 15	CD 33
1	S	M	S	M	S	-	-	-	S	S	-	C	-	C	-	-	C	-	-	-	-	C	M	-	-
2	C	M	C	M	S	M	C	W	C	-	C	M	-	-	S		-		-	-	-	C	-		
3	C	M	M	M	S	M	C	C	C	C	C	W	-	C	M	-	-	-		-	-	C	-	-	C
4	C	M	M	W	S	M	W	-	W	-	-	-	-	C	W	-	-	-	-	M	W	C	-	-	-
5	M	M	M	M	B	M	C	D	B	-	M	D	-	-	M	-	-	D	-	-	-	-	-	-	-
6	D	M	M	M	B	M	D	-	M	-	D	-	-	-	-	M	-	D	-	-	-	M	-	-	D
7	M	M	B	B	B	M	-	-	-	-	-	-	-	M	-	-	-	D	-	-	-	-	-	D	-
8	D	M	M	D	B	M	D	M	M	D	M	-	D	-	D	D	-	D	-	-	D	-	-	-	-
9*	D	M	C	D	B	D	D	-	M	-	-	-	-	-	-	-	D	-	C	D	D	C	-	-	D
10	C	M	M	M	B	M	D	C	-	C	-	-	-	-	D	-	-	C	-	-	-	C	C	C	-
11	M	B	M	B	B	B	D	-	B	-	C	D	-	-	M	-	-	-	-	-	-	C	-	-	-
12	M	M	M	M	B	B	D	M	B	C	C	-	–	-	M	M	M	M	-		D	-		D	D

Abbreviations: S: song; B: bright; M: moderate; C: complex; D: dim; W: weak; *ETP: early T-cell progenitor, cy: cytoplasmic

Table 3. V-(D)-J rearrangement and CDR3 region.

PT	T-cell receptor gamma (TRG) rearrangement							T-cell receptor delta (TRD) rearrangement						
#	V	J	C		CDR3γ		AA	V	J	D		CDR3δ		AA
2	5*0	1*0	2*0	CAT	WDRH	YKKLF	12	8*0	1*0	1*0	CA	YRSAR LPDDT	DKLIF	17
4	5*0	2*0	2*0	CAT	WDRR	YKKLF	12	1*0	1*0	1*0	CA	LGELNTLRGGEVT	DKLIF	20
	3*0	2*0	2*0	CAT	WDRRDY	YKKLF	14							
5	9*0	2*0	2*0	CAL	WEVHY	YKKLF	13	5*0	1*0	3*0	CA	ASE LYWGNSTAQL	DKLIF	15
6	9*0	1*0	2*0	CAL	WEVHVGAQLD	KKLF	17	5*0	2*0	3*0	CA	A LPTGLGGVGDSAA	QLFF	20
7	4*0	P2*0	2*0	CAT	WDDG	SDWIKTF	14	1*0	1*0	3*0	CA	LGDSTGGYT	DKLIF	14
9	8*0	P2*0	2*0	CAT	WDMG	SDWIKTF	14	1*0	1*0	2*0	CA	LGELNPSKLGDMG	LIF	18
10	2*0	2*0	2*0	CAT	WDGHLKTKNY	YKKLF	18	1*0	1*0	3*0	CA	LGKGGFS	DKLIF	14
11	5*0	P2*0	2*0	CAT	WAYSL	SDWIKTF	15	3*0	1*0	2*0	CA	FP LSYNSGGRKC	DKLIF	19
12	9*0	2*0	2*0	CAL	WEG	YKKLF	11	1*0	1*0	2*0	CA	LGEQPSPWGIRN	KLIF	18

Abbreviations: TRGV: T-cell receptor gamma variable; TRGJ: T-cell receptor gamma joining; TRGC: T-cell receptor gamma constant; CDR3: complementarity determining region 3; TRDV: T-cell receptor delta variable, TRDJ: T-cell receptor delta joining; TRDD: T-cell receptor delta diversity; AA: amino acid

Discussion

We found the incidence of γδ T-ALL to be ~15% in childhood T-ALL, which is within the range of 10–26% reported in other studies.[9,40] The proportion of African Americans and patients with mediastinal mass was higher in the γδ T-ALL group compared to the other T-ALL group. Ethnic differences in the frequency of γδ T cells, with a higher proportion of Vδ1 T cells in healthy African Americans compared to Caucasians have been reported.[41–43] Furthermore, MICA genetic polymorphisms in African Americans have significant linkage disequilibria with HLA-B and has been implicated in various disease susceptibilities, but its role in T-ALL remains elusive.[44]

Patients with γδ T-ALL are more likely to have a poor response to treatment as reflected by the high MRD levels during remission induction therapy. A large proportion (67%) of patients with γδ T-ALL had MRD levels ≥ 1% after 15–19 days of induction therapy and received additional chemotherapy. Despite intensification of therapy, a third of the γδ T-ALL patients still had elevated MRD levels ≥ 1% at the end of induction therapy and became candidates for HSCT. Patients with T-ALL expressing the γδ TCR demonstrated a poor response to remission induction treatment, which has been highly predictive for extremely poor outcomes.[23,24] The 10-year OS for patient with γδ T-ALL was lower compared to other T-ALL patients (66.7% vs. 93.3%; $P = 0.001$). The lack of significant difference in the cumulative risk of relapse between the two groups may be due to a higher proportion of γδ T-ALL patients undergoing HSCT.

Deletion of 6q is observed in approximately 10–20% of T-ALL patients and was frequently observed in patients with γδ T-ALL. However, the *t*(11;14)(p11;q32) translocation observed are more often reported in patients with relatively aggressive splenic marginal zone B cell lymphoma but is uncommon in T-ALL. The specific breakpoint on chromosome 14 centromeric to the *IGH* locus occurs at high frequency in patients with ataxia telangiectasia and mature T cell diseases but less common in T-ALL. Guiterrez *et al.*[30] showed that absence of biallelic deletion (ABD) of the *TCR*γ locus was a robust predictor of induction failure and associated with poor overall survival. Given that TCRγ rearrangements occur early in development, the authors found an overlap between patient with ABD and ETP. Patients with γδTCR have VJ recombination on at least one *TCR*γ allele occurring later in development and would not fulfill the definition of ABD. In summary, majority of patients with γδ T-ALL had complex cytogenetic abnormalities and additional studies are needed to detect the cryptic lesions associated with γδ T-ALL.

Here, we describe the outcomes for the largest cohort of γδ T-ALL patients reported to date. The incidence of γδ T-ALL ~10–15% was comparable to other reports. Similarly, we observed that a higher proportion of γδ T-ALL patients had aggressive disease. Patients who relapsed were refractory to

Table 4. Clinical and laboratory characteristics of the 12 patients with γδ T-ALL.

Patient	Gender	Age (Yrs)	Race	WBC 10^3/ mm^3	Mass	CNS	°Risk	MRD 15	MRD D42	HSCT	Status	Years	Disease	Ploidy	Chrom. #	DNA Index	Cytogenetics
1	M	9.1	W	5.1	N	T	H	63	5.82	MSD	A	10	CR	QNS	QNS	1	2 Metaphases Appear Normal
2	M	3.5	AA	102	Y	2	S	1.6	<0.01*	URD	E	0.2	R	PD	46	1	46,XY,del(6)(q21q23),del(11)(p13) 46,add(1)(q23),add(14)(q13)
3	F	13.9	W	10	N	1	S	0.01	<0.01		A	8.8	CR	HD	47–50	1	48,XX,+17,add(18)(p11.3),+19;48,idem,-17,+i(17)(q10)
4	F	6.1	W	64	Y	T	H	31	>1%**	#	E	1.3	IF	HD	47–48	1	47,XX,del(6)(q13q23),t(10;11)(p12;q22),del (12)(p13),der(17); +del(22)(q11.2); 48,idem,+del(22)(q11.2)
5	M	5.5	AA	266	Y	1	S	0.02	<0.01		A	4.6	CR	HD	47–50	1	47,XY,del(6)(q13q21),t(11;14)(p13;q32),+20
6	M	9.7	AA	8.5	N	1	S	19	0.014		E	0.9	CR	PD	46		46,XY,del(7)(q22),del(12)(p11.2)
7	M	1.4	AA	401	Y	2	H	28	7.1	UCB	E	0.3	R	HD	47		47,XY,add(7)(p22),+10
8	M	13.6	W	9	Y	2	S	<0.01	<0.01		A	2.7	CR	HD	47		47,XY,del(10)(q26),del(12)(p13),+mar
9	M	8.0	W	15	N	2	H	70.8	<0.01	URD	A	3.0	CR	HD	53	1.17	53,XY,+7,+8,+10,+11,+13,+17,+21
10	M	12.9	W	30	Y	1	S	24	<0.01		A	1.8	CR	PD	46	1	46,XY,del(6)(q21)
11	F	15.2	AA	284	N	1	H	80	3.8	URD	A	2.5	CR	PD	46	1	46,XX,t(11;14)(p11.2;q32.1)
12	M	6.1	W	87	N	1	S	0.02	<0.01		A	9.5	CR	PD	46	1	46,XY,add(1)(p36.1),del(12)(p11.2)

Abbreviations: M: male; F: female; W: white; AA: African American; N: no; Y: yes; 1: CNS1; 2: CNS2; 3:CNS3; T: traumatic tap with blast; R: relapse; MSD: matched sibling donor; URD: unrelated donor; UCB: umbilical cord blood; HSCT: hematopoietic stem cell; PD: pseudodiploidy; HD: hyperdiploidy; DP: double positive $CD4^+CD8^+$; DN: double negative $CD4^-CD8^-$; A: alive; E: expired; CR: clinical remission; IF: induction failure. Risk group based on MRD levels measured on day 15 and 42; *MRD < 0.01 but relapsed after induction; ** MRD by PCR; # Expired prior to HSCT.

chemotherapy and often died from disease progression. Due to the rare occurrence and limited cases of γδ T-ALL, the adverse prognostic significance should be confirmed in an independent cohort of children with T-ALL.

For certain lymphomas, the biologic behavior of neoplasms derived from γδ T cells have been recognized as being distinct according to the World Health Organization (WHO) classification of lymphoid neoplasms. Comparably for patients with T-ALL, recent studies have identified γδ T cell specific genetic alterations that is predictive of poorer response to therapy or outcomes. Here, we present corroborating data suggesting that patients with γδ T-ALL are also at risk for poorer response to therapy or outcomes. Understanding the mechanism driving leukemic transformation and identifying factors contributing to the poor treatment response may help to develop treatment strategies to improve clinical outcome of these patients.

Patient and methods

Patient selection

Between 2000 and 2014, 100 consecutive newly diagnosed T-ALL patients were enrolled onto the Total Therapy XV (NCT00137111) or XVI (NCT00549848) study at St. Jude Children's Research Hospital (SJCRH). Of the 100 patients, 93 had diagnostic samples with γδ TCR data and were evaluable for follow up. The studies were approved by the SJCRH Institutional Review Board and are in accordance with the Helsinki Declaration of 1975; written informed consents were obtained from the parents, guardians, or the patients, and assent from the patients, as appropriate.

Diagnosis and risk classification

ALL diagnosis was based on morphologic, immunophenotypic, and genetic features of leukemic blast cells while early T-cell precursor (ETP) ALL, a subtype of T-cell ALL generally associated with poor prognosis, was diagnosed by immunophenotype as previously described.[45] MRD was determined by FCM and/or PCR, as previously described.-[21] All T-ALL patients were provisionally classified to have standard-risk ALL to receive intensive chemotherapy. Those with MRD levels ≥1% at the end of remission induction or relapsed during therapy were candidates for hematopoietic stem-cell transplantation (HSCT). Patients with ETP ALL treated in Total Therapy XVI were all considered to have high-risk disease and were candidates for HSCT.

Risk-adapted treatment

Details of the treatment regimen for the protocols have been described previously.[45,46] Treatment consisted of remission induction, consolidation, and continuation. In brief, patients with MRD ≥ 1% in the bone marrow (BM) on day 15–19 of induction were given three additional doses of native *E. coli* asparaginase in Total Therapy XV or one dose of peg-

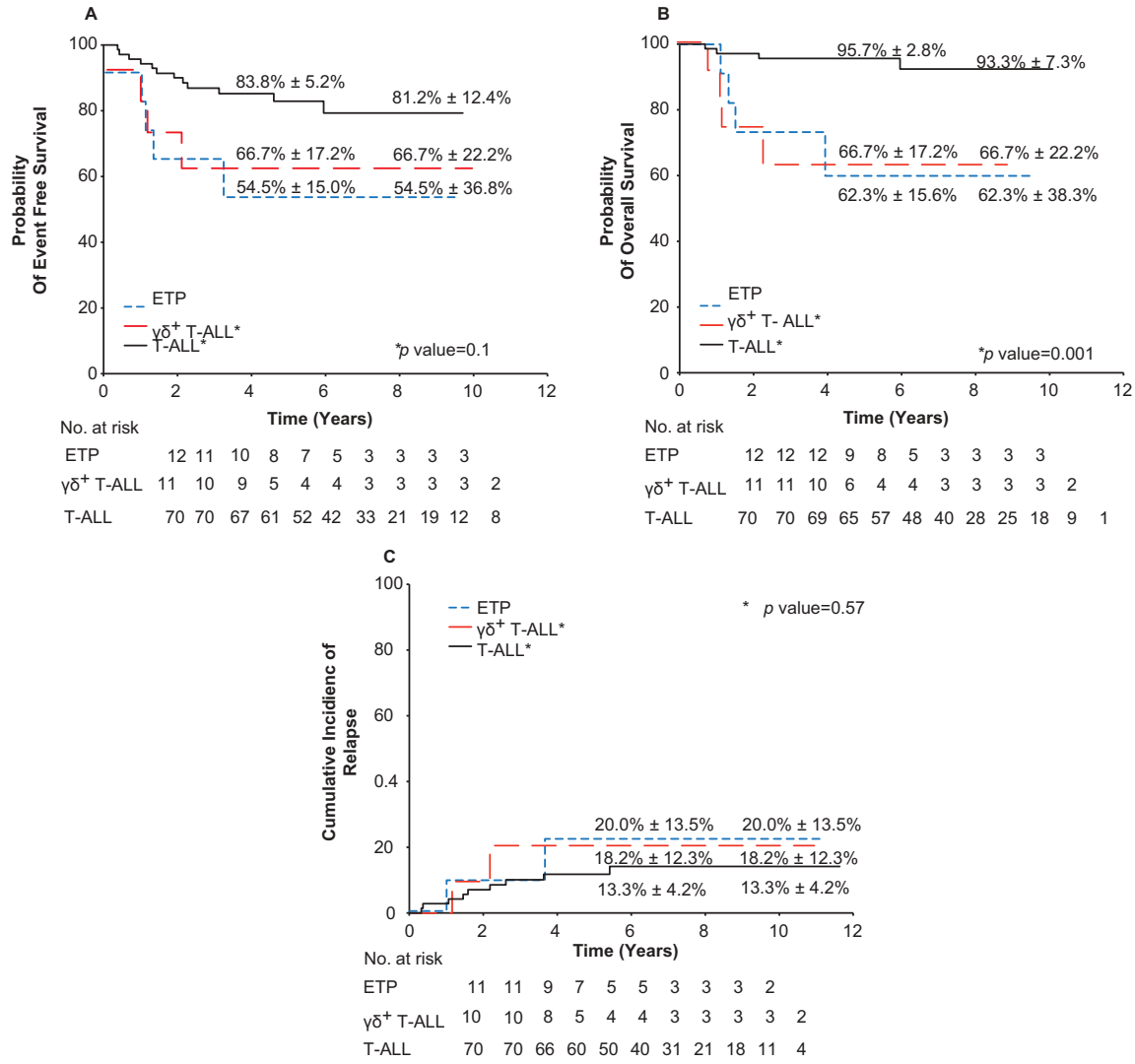

Figure 1. Probability of event-free survival, overall survival, and cumulative incidence of relapse for patients with γδ T-ALL. Kaplan-Meier estimates of (a) event-free survival, (b) overall survival, and (c) cumulative incidence of relapse for patients with γδ T-ALL, early T cell progenitor (ETP) ALL and T-ALL are shown. *P values are for γδ T-ALL vs. T-ALL. Five- and 10-year rates reported as means ± standard error.

Table 5. EFS, OS, and risk of relapse for patients with γδ T-ALL compared to T-ALL and ETP ALL.

Factor	Year	γδ T-ALL $N = 12$ Mean ± SE (%)	T-ALL $N = 70$ Mean ± SE (%)	γδ T-ALL vs T-ALL P	ETP ALL $N = 11$ Mean ± SE (%)	γδ T-ALL vs ETP ALL P
EFS	5	66.7 ± 17.2	83.8 ± 5.2	0.11	54.5 ± 15.0	0.63
	10	66.7 ± 22.2	81.2 ± 12.4		54.5 ± 36.8	
OS	5	66.7 ± 17.2	95.7 ± 2.8	0.001	62.3 ± 15.6	0.98
	10	66.7 ± 22.2	93.3 ± 7.3		62.3 ± 38.3	
Relapse	5	18.2 ± 12.3	13.3 ± 4.2	0.58	20.0 ± 13.5	0.98
	10	18.2 ± 1 2.3	13.3 ± 4.2		20.0 ± 13.5	

Abbreviations: EFS: event-free survival; SE: standard error; OS: overall survival; ETP: early T-cell progenitor; SE: standard error

asparaginase in Total Therapy XVI. At the end of induction (days 42–46), BM aspiration was performed to assess MRD level, and consolidation therapy with high-dose methotrexate (5 g/m^2 per dose) and daily mercaptopurine was given for four courses. Standard-risk patients then received intensive continuation chemotherapy, and high-risk patients were offered the option of HSCT. All patients received triple intrathecal therapy administered early during remission induction and throughout the first 2 years of continuation treatment.

TCR repertoire by single-cell-nested PCR

Nested PCR was carried out as previously described.[38,39] Briefly, γδ T cells ($CD3^+TCR\gamma\delta^+CD14^-CD19^-$) were single cell sorted using a BD FACSAria III or Sony iCytSy3200 (Sony Biotechnology) into 96-well PCR plates (Eppendorf) preloaded with 2.5 μL of reverse-transcription master mix (iScript cDNA Synthesis Kit, Bio-Rad Laboratories/SuperScript VILO cDNA Synthesis Kit, Life Technologies) containing 0.5 μL 5X iScript reaction mix, 0.5 μL iScript reverse transcriptase, and 0.1% Triton X-100 (Sigma-Aldrich). Columns 11 and 12 of each PCR plate were left empty to serve as controls. Plates were sealed, spun down, and reverse transcription was performed on a Bio-Rad C1000 Thermo Cycler using the following protocol: 5 min at 25°C, 60 min at 42°C, 5 min at 85°C, hold 4°C. First round PCR was then carried out with 5 μM of all forward TCRγ variable (TRGV) and δ variable (TRDV) external (ext) primers and 20 μM all reverse external primers (EuroFins Genomics) using the Taq DNA Polymerase Kit (QIAGEN) according to manufacturer's instructions. Second-round reactions were then electrophoresed on a 2% agarose gel (Bio-Rad) to confirm presence of amplicons. Prior to sequencing, all PCR reactions were purified with exonuclease I-shrimp alkaline phosphatase according to the manufacturer's instructions (Affymetrix USB). A total of 20 μM of the γ or δ internal reverse primers were added to the appropriate purified PCR products, and sequenced using a ABI Big Dye sequencer (Applied Biosystems). Sequences were then blasted using the IMGT database.

Statistical analysis

Event-free survival (EFS) and overall survival (OS) were estimated by the method of Kaplan-Meier, with associated standard errors calculated by the method of Peto and Pike.[47] The cumulative incidence functions of relapse were estimated according to Kalbfleisch and Prentice, and compared with Gray's test.[48,49] Deaths in remission were considered competing events in the estimation of cumulative incidence of relapse. Statistical analyses were performed with SAS software (v9.3).

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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