High prevalence of relapse in children with Philadelphia-like acute lymphoblastic leukemia despite risk-adapted treatment

Acute lymphoblastic leukemia (ALL) remains a leading cause of cancer-related death in children and young adults. Since the 1960s, improvements in the treatment of children with ALL have led to 10-year survival rates now exceeding 85%.[1] Philadelphia-like (Ph-like) ALL is characterized by a gene expression profile similar to that of *BCR-ABL1* positive (Ph+) ALL but lacking the *BCR-ABL1* oncogene and similarly, patients experience poor outcome.[2-4] Ph-like ALL is associated with a range of genetic alterations, particularly rearrangements, which activate cytokine receptor and kinase signalling.[2-4] In 2011, the Australian and New Zealand Children's Haematology/Oncology Group (ANZCHOG) completed enrolment of patients to a minimal residual disease (MRD) intervention clinical trial, known as ALL8 (*clinicaltrials.gov Identifier: ACTRN12607000302459*). Children were stratified to high-risk regimens based on several criteria, including treatment failure or high MRD at day 79.[5] Overall, 46 children with precursor B-ALL relapsed, and surprisingly 72% (33/46) of these patients were classified as medium-risk.[6] In this retrospective study of ALL8, the frequency of patients with Ph-like ALL and their attendant genomic lesions were studied, and clinical outcomes were compared to those of non Ph-like B-ALL patients. The incidence of Ph-like ALL was 11.7%, where the majority of these children were reported to be of Caucasian ethnicity and stratified as being either standard- or medium-risk. Significantly, 57.8% (11/19) of Ph-like ALL patients subsequently relapsed compared to 16% (26/143) who were not Ph-like, with significantly inferior event-free and overall survival ($P< 0.0001$ and $P=0.003$, respectively).

Six hundred and fifty-six patients aged between one and eighteen years were evaluated for eligibility on the ANZCHOG ALL8 trial from 2002-2011. Ethical approval was obtained from each institutional Human Research Ethics Committee and parents or legal guardians gave written, informed consent. Two hundred and forty-five patients, selected on the basis of sample accessibility, were available for Ph-like ALL screening (*Online Supplementary Figure S1*). The mean age at diagnosis (6.4 yrs *vs.* 5.6 yrs, $P=0.02$) and higher white cell counts (WCC) ($P<0.001$), were significantly different between those available for analysis and those patients excluded, with the studied group also having a higher number of patients over ten years of age (23% *vs.* 16%) (*Online Supplementary Table S1*). Risk groups were similar in each cohort and, overall, event-free and relapse-free survival were not significantly different between both groups (*Online Supplementary Figure S2*).

The criteria for stratification to the high-risk group, based upon Berlin-Frankfurt-Münster (BFM) protocols, were the presence of *BCR-ABL1* or *MLL* t(4;11) translocation; poor prednisolone response at day eight; failure to achieve

Table 1A. Age, MRD, risk stratification, relapse status (A), rearrangements and variants of patients (B) identified with Ph-like ALL and *P2RY8-CRLF2*.

Patient code	Age at diagnosis	Final risk stratification	MRD at day 79	Relapsed	Relapse free survival (years)	SCT
A2489	5.2	High	$2x10^{-2}$	Y	1.77	Y
A5258	14.9	High	$6x10^{-3}$	N	4.56	Y
A1781	3.5	Medium	Negative	Y	4.34	N
A5243	16.3	Medium	Pos<10^{-4}	Y	2.56	N
A1516	8.2	Medium	Pos<10^{-4}	N*	1.91	N
A4513	13.2	Medium	Negative	N	5.46	N
A1725	12.7	Medium	Pos<10^{-4}	N	5.07	N
A2497	16.7	Medium	Pos<10^{-4}	Y	1.92	Y
A3019	14.9	Medium	Negative	Y	0.96	N
A5164	6.2	Medium	Negative	Y	2.26	Y
A1702	3	Medium	Negative	N	10.86	N
A1747	3.1	Medium	Pos<10^{-4}	Y	2.18	Y
A2173	8.2	Medium	Negative	Y	2.15	N
A3100	15	Medium	Negative	Y	1.06	N
A5416	5.5	Medium	Negative	Y	2.12	Y
A3086	11	Medium	Negative	N	8.07	N
A5428	1.5	Medium	Negative	Y	2.54	Y
A2481	5.9	Standard	Negative	N	2.94	N
A2005	13.4	Medium	Pos<10^{-4}	N	5.78	N
A2273	5	Medium	Negative	Y	3.54	Y
A2426	10.7	Standard	Negative	Y	4.51	N
A2517	4.9	Standard	Negative	Y	2.35	Y
A3239	2.1	Medium	Negative	N	5.15	N
A3700	2.7	Medium	Negative	N	6.90	N
A4964	12.9	High	$5x10^{-4}$	N**	1.76	Y

remission by day 33 or high MRD (>5 x10^{-4}) at day 79 (Table 1A). Standard- and medium-risk patients received the same standard BFM four-drug induction chemotherapy regimen including a prednisolone pre-phase and intrathecal methotrexate. In addition to the four-drug protocol, high-risk patients received a further three novel intensive blocks of chemotherapy followed by stem cell transplant (SCT) in most cases.[5] All *BCR-ABL1* positive patients also received imatinib.

Determination of Ph-like ALL has differed between cohorts. European studies have favored the term *BCR-ABL1*-like ALL and have used hierarchical clustering (HC) of an Affymetrix gene expression array based on a probe set of 110 genes designed to detect major pediatric ALL subtypes.[3] In contrast, US studies have used a TaqMan Low Density Arrays (TLDA) based approach consisting of either eight or fifteen genes selected by Prediction Analysis for Microarrays (PAM) analysis.[7,8] While there is overlap, the HC model identifies a greater proportion of patients as having a *BCR-ABL1*-like signature, but this approach does not directly identify causative fusions.[9,10] Based on the US approach, we have designed a custom TLDA using nine genes to identify patients with Ph-like ALL.[7,11]

The TLDA was used according to manufacturer's instructions (Thermo Fisher Scientific, MA, USA) to determine Ph-like status. Genes were selected based upon prior reports[8] with *CRLF2, PDGFRB, ABL1, ABL2* and *EPOR* also included to aid identification of potential fusions (*Online Supplementary Methods*). Reverse transcription polymerase chain reaction (RT-PCR) followed by Sanger sequencing was performed using a panel of 30 known fusions on all TLDA positive cases and those with high *CRLF2* gene expression.[4] Cases with high *CRLF2* expression were also subjected to fluorescent *in situ* hybridization (FISH) to confirm *IGH-CRLF2* fusions. Illumina TruSeq stranded library preparation for messenger ribonucleic acid sequencing (mRNA seq) on the Illumina NextSeq or HiSeq platforms was performed on all TLDA positive and high *CRLF2* cases, with the sole exception being that of a case with low RNA quality (*Online Supplementary Methods*). Patients were classified as having Ph-like ALL if a sample was TLDA positive.

Cytokine or kinase activating lesions have been identified in the majority of childhood and adolescent/young adults (AYA) with Ph-like ALL.[12] Of the 245 childhood B-ALL patients evaluated, eight patients were identified as being *BCR-ABL1* positive and 75 had an *ETV6-RUNX1* fusion, leaving 162 available for Ph-like screening. Nineteen patients (11.7%) were identified as having Ph-like ALL, as determined by TLDA. Rearrangements were identified in 17/19 patients (Table 1B).

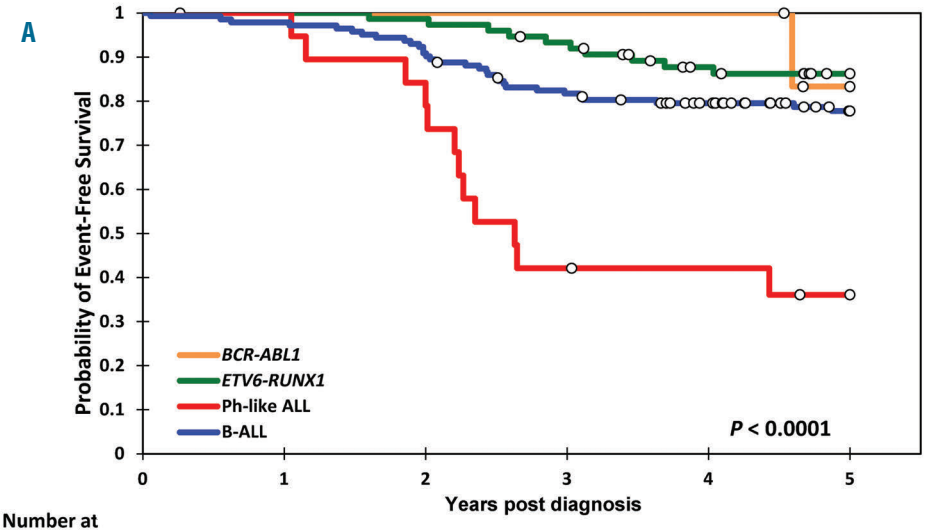
Table 1B. Age, MRD, risk stratification, relapse status (A), rearrangements and variants of patients (B) identified with Ph-like ALL and *P2RY8-CRLF2*.

Patient code	Rearrangement	TLDA	mRNA sequencing	Variants detected by mRNA and/or Sanger sequencing	*IKZF1* deletion
A2489	*EBF1-PDGFRB*	Pos	Y	no variants detected	del 4-7
A5258	*EBF1-PDGFRB*	Pos	Y	no variants detected	del 4-7
A1781	*SSBP2-JAK2*	Pos	Y	no variants detected	del 4-8
A5243	*PAX5-JAK2*	Pos	Y	no variants detected	del 2-7
A1516	*IGH-EPOR*	Pos	Y	*SREBF* pV580M	del 2-7
A4513	*IGH-EPOR*	Pos	Y	*ABL2* pP608S, *SREBF1* pV580M, *TYK2* pG363S, *RUNX1* pL29S	del 4-7
A1725	*IGH-CRLF2*	Pos	Y	*CRLF2* pF232C, *CDKN2A* pA148T	del 2-8
A2497	*IGH-CRLF2*	Pos	Y	*CREBBP* pN1940S	del 2-8
A3019	*IGH-CRLF2*	Pos	Y	*JAK2* pR683S	del 4-7
A5164	*IGH-CRLF2*	Pos	Y	*CRLF2* pF232C	del 4-7
A1702	*P2RY8-CRLF2*	Pos	Y	*JAK2* pI682F	del 4-7
A1747	*P2RY8-CRLF2*	Pos	Y	*JAK2* pT875N, *FLT3* pD324N	del 2-7
A2173	*P2RY8-CRLF2*	Pos	Y	*JAK2* pR683S	del 4-7
A3100	*P2RY8-CRLF2*	Pos	Y	*NRAS* pG13D, *JAK3* pP132T	del 4-7
A5416	*P2RY8-CRLF2*	Pos	Y	*JAK2* pR683S, *SET2D* pM1080I	del 2-8
A3086	*PSMG1-ERG*	Pos	Y	*PAX5* pG266E, *BRAF* pA31V	None
A5428	*PAX5-ZNF521*	Pos	Y	*ABL1* pS972L, *ABL2* pK909R, *CREBBP* pV1243I, *SREBF1* pV580M, *TYK2* pI684S, *RUNX1* pE395A	del 4-7
A2481	Unknown	Pos	Y	no variants detected	None
A2005	Unknown	Pos	Y	*IKZF1* pN159Y, *PTK2B* pT65R, *BRAF* pD594G	None
A2273	*P2RY8-CRLF2*	Neg	N	Poor RNA	del 4-7
A2426	*P2RY8-CRLF2*	Neg	Y	*KRAS* pA146P	None
A2517	*P2RY8-CRLF2*	Neg	Y	*TYK2* pR568W, *RUNX1* pL29S	del 4-7
A3239	*P2RY8-CRLF2*	Neg	Y	*CREBBP* pN1940S	None
A3700	*P2RY8-CRLF2*	Neg	Y	no variants detected	None
A4964	*P2RY8-CRLF2*	Neg	Y	*NRAS* pG12D, *SET2D* pM1080I	del 2-8

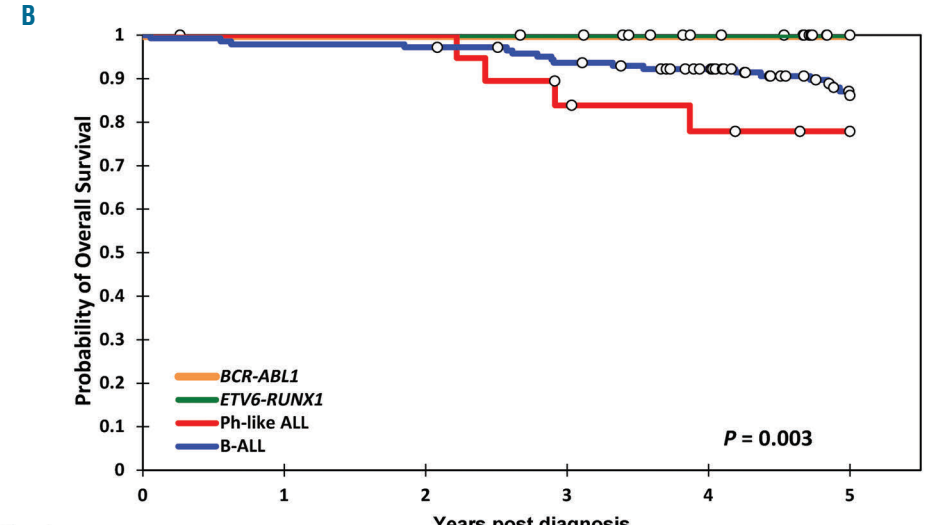
*Secondary malignancy (AML) at 1.91 years; **died in remission. MRD: minimal residual disease; TLDA: Taqman low density array; mRNA: messenger ribonucleic acid; Pos: positive result; Neg: negative result; Y: yes; N: no; del: deletion of exons; SCT: stem cell transplant.

Previous reports have suggested 27-60% of Ph-like ALL patients harbor rearrangements of *CRLF2*, with 50% of these demonstrating concomitant mutations in *JAK1* or *JAK2*.[4,13] Similarly, the majority of ALL8 Ph-like ALL cases (9/19, 47%) harbored *CRLF2* rearrangements (*CRLF2*r), with 77.7% (7/9) demonstrating concomitant *JAK2* or *CRLF2* mutations. While there is some conjecture, the majority of studies have demonstrated *CRLF2* overexpression is significantly associated with poor outcome.[14,15] Importantly, 77.7% (7/9) of ALL8 Ph-like ALL patients with a *CRLF2*r, subsequently relapsed. Interestingly, a further six TLDA negative patients harbored *CRLF2*r. Of these patients three relapsed, and two subsequently underwent SCT. A fourth patient received a SCT but died in remission. The remaining identified fusions included *EBF1-PDGFRB* and *IGH-EPOR* (n=2), *PAX5-JAK2, SSBP2-JAK2, PAX5-ZNF521* and *PSMG1-ERG* in one patient each (Table 1B).

IKZF1 deletions are shown to be significantly associated with relapse risk in both Ph+ and Ph-like ALL, with the frequency reported to be between 27% and 69% in Ph-like ALL cases.[3,12,13] Of note, the ALL8 cohort included B-ALL patients in all risk stratifications, whereas other studies only included high-risk patients, potentially limiting comparisons between groups.[3,12,13] Herein, *IKZF1* deletions



Number at risk						
BCR-ABL1	8	8	8	8	8	4
ETV6-RUNX1	75	75	75	70	60	51
Ph-like ALL	19	19	16	9	8	5
B-ALL	143	141	132	116	105	84



Number at risk						
BCR-ABL1	8	8	8	8	8	5
ETV6-RUNX1	75	75	75	75	69	59
Ph-like ALL	19	19	19	16	14	11
B-ALL	143	141	140	133	123	95

Figure 1. Children with Ph-like ALL have inferior survival outcomes compared to other B-ALL patients. Kaplan-Meier analysis with log-rank statistic of A) event free survival and B) overall survival at five years post diagnosis. Children with Ph-like ALL are shown in red, *BCR-ABL1*+ patients in orange, *ETV6-RUNX1* in green and the remaining B-ALL children in blue. The number of patients at risk for the different B-ALL subtypes is shown below the graph at each year.

were significantly associated with Ph-like ALL (84% *vs.* 14%, *P*<0.0001). One Ph-like ALL patient, for whom a fusion was not identified, harbored an *IKZF1* p.N159Y mutation detected by mRNA seq and validated in genomic DNA by PCR and Sanger sequencing.

Most studies of patients with Ph-like ALL have demonstrated significantly inferior outcomes, which may be improved with treatment intensification.[3,4,12] In contrast to patients enrolled on Total Therapy XV, a study of risk-directed therapy based on MRD wherein no significant differences in outcome were reported,[13] the ALL8 Ph-like ALL cohort demonstrated significantly inferior event-free (*P*<0.0001) and overall survival (*P*=0.003; Figure 1).

On the ALL8 protocol, only two patients with Ph-like ALL had a final high-risk classification (both *EBF1-PDGFRB*) as a result of high MRD at day 79. One non Ph-like *P2RY8-CRLF2* patient was also restratified to high-risk as a result of day 79 MRD. All three cases had *IKZF1* deletions; one relapsed and a second died in remission. On ALL8, 72% (8/11) of patients classified as Ph-like ALL relapsed within six months of completing their two years of maintenance therapy (average time to relapse 2.1 years). At five years, the overall survival of Ph-like cases was 78% (15/19), indicating that many patients were salvaged by further therapy or SCT,[5] but their survival rate was still significantly inferior to other B-ALL sub-groups. Similar to that observed in Total Therapy XV, ALL8 patients with Ph-like disease were twice as likely to undergo SCT.[13]

Herein, we demonstrate that despite a risk adjusted treatment approach, there remained a high rate of relapse among children in the ANZCHOG ALL8 study who were retrospectively identified as Ph-like. Of note, the MRD risk stratification used in this protocol did not identify all Ph-like ALL cases as high-risk. Finally, rapid identification of Ph-like disease may guide therapeutic intervention with rationally targeted therapies based on patient specific driving genomic lesions. Tyrosine kinase inhibitors are increasingly utilized in patients with ABL-class fusions, with current and future trials likely to inform drug efficacy in the case of other targets.

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