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

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Supplementary Methods:

1. Minimal Residual Disease (MRD)

MRD was measured by RQ-PCR for patient-specific immunoglobulin and T-cell receptor rearrangements with data interpreted according to EuroMRD guidelines.[1,2]

2. Determination of Ph-like ALL by custom Taqman Low Density Array (TLDA)

Gene expression was quantified using HTqPCR v.1.14.0 Bioconductor package.[3] Nearest shrunken centroid modelling, as implemented using prediction analysis of microarray (PAM) v.1.55 package[4], was employed on a training set of 20 known true positive and true negative samples provided by St Jude Children's Research Hospital, to derive a 9-gene signature, consisting of *BMPR1B, CA6, CHN2, GPR110, IGJ, MUC4, NRXN3, SPATS2L* and *TP53INP1* to identify Ph-like ALL patients. Genes were selected based upon prior reports[5,6] with *CRLF2, PDGFRB, ABL1, ABL2* and *EPOR* also included to aid identification of potential fusions. *EEF2* was used as a housekeeping gene. PAM results were reported as probability score between 0-1, with those over 0.5 deemed positive and indicative of a Ph-like signature.[6,7] The *CRLF2* probe was also evaluated separately for high expression ($\Delta CT < 3.7$, as determined by ROC curve), indicative of rearrangement of these genes. All analyses were performed using R v.3.0.2 statistical software.[8]

3. IKZF1 deletions

IKZF1 deletions were detected by multiplex ligation-dependent probe amplification (MLPA) (SALSA MLPA P335B1 and P202, MRC-Holland, Amsterdam, The Netherlands) and by real-time quantification polymerase chain reaction (RQ-PCR) as previously described.[9,10]

4. mRNA sequencing

mRNA seq was performed using the Truseq Stranded mRNA LT kit (Illumina, CA, USA) as per manufacturers instructions, from 1 microgram of high quality total RNA and sequenced by either the Illumina HiSeq 2000 or NextSeq 500 platforms. A read depth of 70 million reads was achieved for most samples. FusionCatcher and deFuse software were used to identify fusion transcripts from mRNA sequencing data.[11,12] Variant calling on mRNA seq data was based upon Broad Institute GATK best practice. The variants were called using GATK HaplotypeCaller (v3.4.46) and annotated by ANNOVAR software (2015-06-17) after undergoing two further filtering steps, the first using SNPiR[13] and the final filter being a set of 35 genes previously shown to be associated with Ph-like ALL.[14,15]

5. Statistical analyses

Associations between categorical variables were examined by two-tailed Fisher's exact test using GraphPad Prism (version 6). Five-year survival analyses of outcome data were estimated by Kaplan-Meier and log-rank test using XLSTAT (version 2016.02.28013). An event was defined as relapse, secondary malignancy (excluding skin cancer) or death from any cause and event free survival was calculated from the date of diagnosis to the event or date of last follow up. Overall survival was from diagnosis to death or to last follow up.

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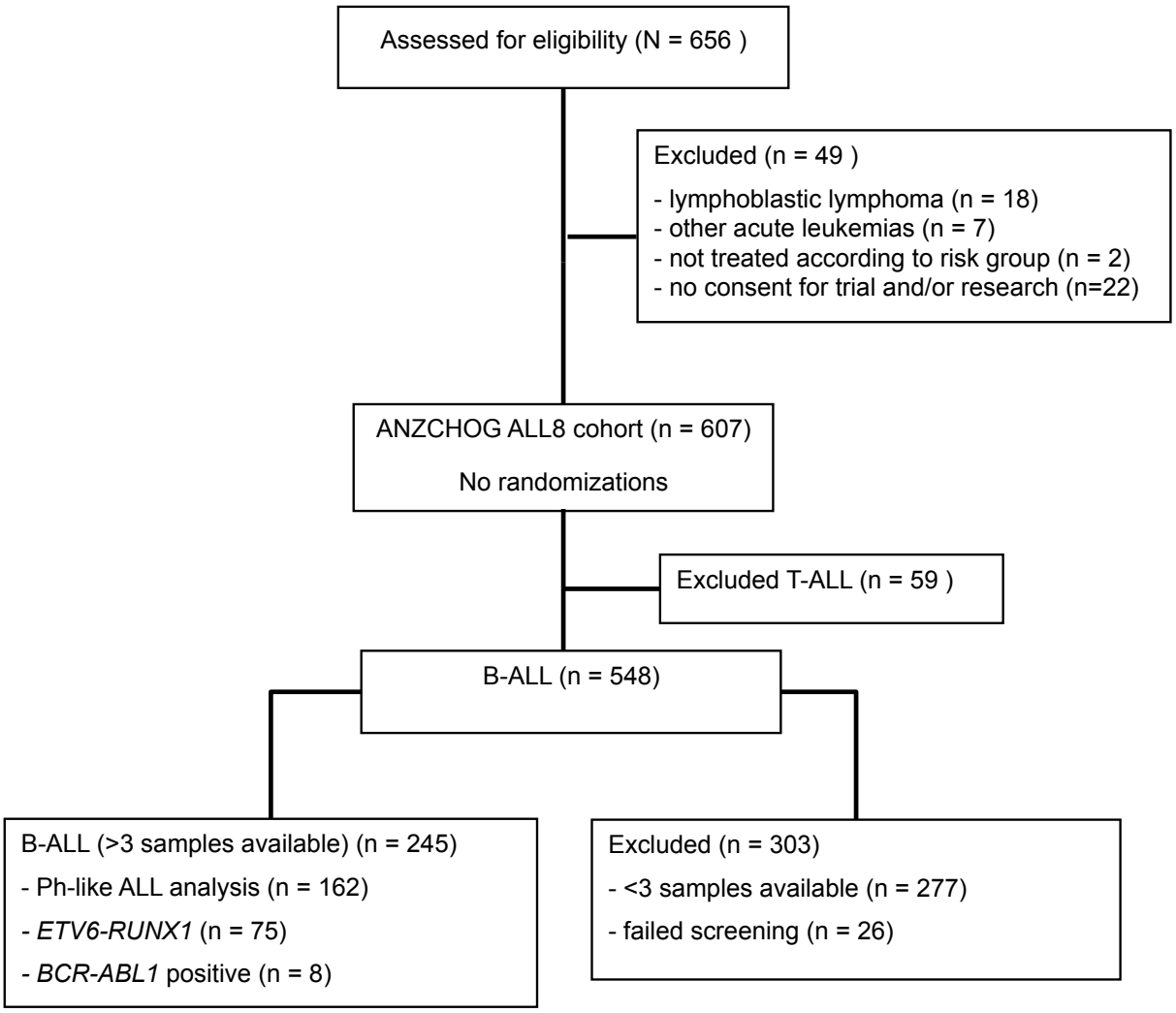
Supplementary Tables and Figures

	pre B-ALL n = 245		Excluded n = 303		Significance *P* =
Risk	**n**	**%**	**n**	**%**	
Standard	64	26	83	27	*0.77*
Medium	157	64	202	67	*0.52*
High	24	10	18	6	*0.1*
	n	**%**	**n**	**%**	
Males	142	58	150	50	*0.058*
Females	103	42	153	50	
	mean	**SEM**	**mean**	**SEM**	
Age	6.4	0.27	5.6	0.23	***0.02***
	mean	**SEM**	**mean**	**SEM**	
WCC x 10^9/l	45.7	6.6	21.3	3.7	***<0.001***

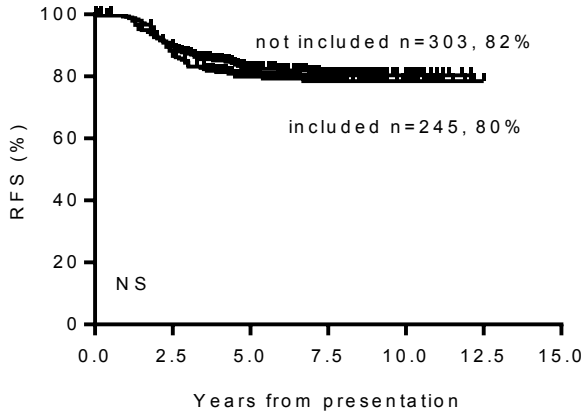
Supplementary Table 1. Comparison of characteristics between eligible patient cohorts enrolled on ANZCHOG ALL8. Patient cohorts are based upon the final numbers of patients eligible for Ph-like analysis in each group of the CONSORT diagram (Supplementary Figure 1).



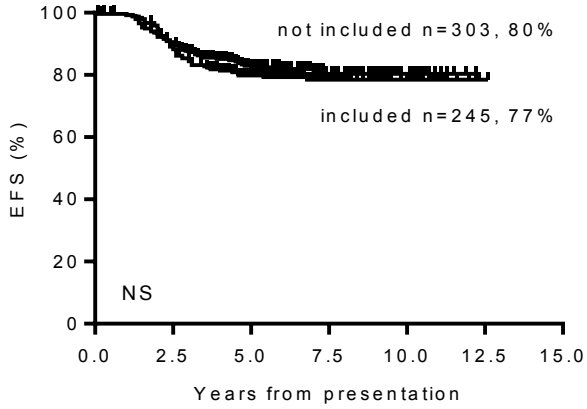
Supplementary Figure 1. CONSORT diagram

CONSORT diagram depicting the patient cohort selected for Ph-like analysis.

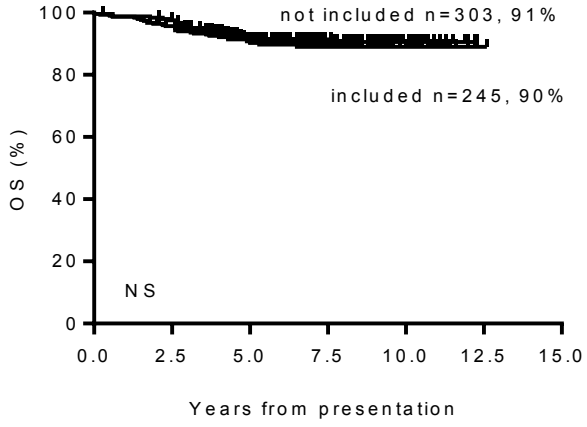
A



B



C



Supplementary Figure 2. There were no differences in survival outcomes between patients that were included or not included for Ph-like ALL analysis from ALL8.

Kaplan Meier analysis with log rank statistic of **A)** relapse free survival, **B)** event free survival and **C)** overall survival from diagnosis. Patients were grouped according to the CONSORT diagram (Supplementary Figure 1).