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

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1Cancer Theme, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, SA, Australia; 2Discipline of Medicine, University of Adelaide, SA, Australia; 3Australian and New Zealand Children’s Haematology/Oncology Group (ANZCHOG); 4Molecular Diagnostics, Children’s Cancer Institute, Sydney, NSW, Australia; 5Genetics and Molecular Pathology, SA Pathology, Adelaide, SA, Australia; 6Sydney Children’s Hospital Network, Sydney, NSW, Australia; 7Women’s & Children’s Hospital, Adelaide, SA, Australia; 8Australian Genomic Health Alliance (AGHA); 9Oncology Services Group, Children’s Health Queensland Hospital and Health Service, Brisbane, QLD, Australia; 10The University of Queensland Diamantina Institute and UQ Child Health Research Centre, The University of Queensland, Brisbane, QLD, Australia; 11Mater Children’s Hospital Brisbane, QLD, Australia; 12John Hunter Children’s Hospital, Newcastle, NSW, Australia; 13Division of Haematology, SA Pathology, Adelaide, SA, Australia; 14Department of Pathology, St Jude Children’s Research Hospital, Memphis, TN, USA; 15School of Women and Children’s Health, University of New South Wales, Sydney, NSW, Australia and 16Discipline of Paediatrics, University of Adelaide, SA, Australia

Correspondence: deborah.white@saehmr.com
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High prevalence of relapse in children with Philadelphia-like acute lymphoblastic leukemia despite risk-adapted treatment

Susan L Heatley¹,²,³, Teresa Sadras¹,², Chung H Kok¹,², Eva Nievergall¹,², Kelly Quek¹, Phuong Dang¹, Barbara McClure¹, Nicola Venn⁴, Sarah Moore⁵, Jeffrey Suttle⁶, Tamara Law⁷, Anthea Ng³,⁶, Walter Muskovic⁴, Murray D Norris⁴, Tamas Revesz²,³,⁷, Michael Osborn³,⁷,⁸, Andrew S. Moore³,⁷,¹⁰, Ram Suppiah¹¹, Chris Fraser³,⁹, Frank Alvaro³,¹², Timothy P Hughes¹,²,¹³, Charles G Mullighan¹⁴, Glenn M Marshall³,⁴,⁶,⁸,¹⁵, Luciano Dalla Pozza³,⁶, David T Yeung¹,²,¹³, Rosemary Sutton³,⁴,⁸,¹⁵ and Deborah L White¹,²,³,⁸,¹⁶

1. Cancer Theme, South Australian Health & Medical Research Institute (SAHMRI), Adelaide, SA, Australia
2. Discipline of Medicine, University of Adelaide, Adelaide, SA, Australia
3. Australian and New Zealand Children’s Haematology/Oncology Group (ANZCHOG)
4. Molecular Diagnostics, Children’s Cancer Institute, Sydney, NSW, Australia
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8. Australian Genomic Health Alliance (AGHA)
9. Oncology Services Group, Children’s Health Queensland Hospital and Health Service, Brisbane, Qld, Australia
10. The University of Queensland Diamantina Institute and UQ Child Health Research Centre, The University of Queensland, Brisbane, Qld, Australia.
11. Mater Children’s Hospital, Brisbane, Qld, Australia
12. John Hunter Children’s Hospital, Newcastle, NSW, Australia
13. Division of Haematology, SA Pathology, Adelaide, SA, Australia
14. Department of Pathology, St Jude Children’s Research Hospital, Memphis, TN, USA
15. School of Women and Children’s Health, University of New South Wales, Sydney, NSW, Australia
16. Discipline of Paediatrics, University of Adelaide, Adelaide, SA, Australia
**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.\textsuperscript{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\textsuperscript{13} and the final filter being a set of 35 genes previously shown to be associated with Ph-like ALL.\textsuperscript{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.
Supplementary References

### 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).

<table>
<thead>
<tr>
<th></th>
<th>pre B-ALL n = 245</th>
<th>Excluded n = 303</th>
<th>Significance P =</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standard</td>
<td>n = 64, % 26</td>
<td>n = 83, % 27</td>
<td>0.77</td>
</tr>
<tr>
<td>Medium</td>
<td>n = 157, % 64</td>
<td>n = 202, % 67</td>
<td>0.52</td>
</tr>
<tr>
<td>High</td>
<td>n = 24, % 10</td>
<td>n = 18, % 6</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 142, % 58</td>
<td>n = 150, % 50</td>
<td>0.058</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td>n = 103, % 42</td>
<td>n = 153, % 50</td>
<td></td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td>mean = 6.4, SEM = 0.27</td>
<td>mean = 5.6, SEM = 0.23</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>WCC x 10^9/l</strong></td>
<td>mean = 45.7, SEM = 6.6</td>
<td>mean = 21.3, SEM = 3.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Supplementary Figure 1. CONSORT diagram

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