BCR-ABL1-like cases in pediatric acute lymphoblastic leukemia: a comparison between DCOG/Erasmus MC and COG/St. Jude signatures

Patients with pediatric B-cell precursor acute lymphoblastic leukemia (BCP-ALL) with the *BCR-ABL1* fusion gene form a small, high-risk group with a poor prognosis.[1] Approximately 15% of cases of BCP-ALL are characterized by a gene expression signature similar to that of *BCR-ABL1*-positive disease and an unfavorable prognosis.[2,3] Two independent gene expression signatures were described that identify *BCR-ABL1*-like ALL, alternatively called Philadelphia-like ALL.[2,3] The *BCR-ABL1*-like signature described by Mullighan *et al*. is based on the prediction analysis of microarrays (PAM) classifier consisting of 257 gene probe sets trained on *BCR-ABL1*-positive cases.[4,5] Genetic alterations activating kinase or cytokine receptor signaling are a hallmark of this subtype, which also has frequent deletions of *IKZF1*, and poor outcome.[4,5] The signature developed by Den Boer *et al*. is based on hierarchical clustering (HC) of 110 gene probe sets identified to predict the major pediatric ALL subtypes (T-cell ALL, *ETV6-RUNX1*, high-hyperdiploidy, *TCF3* or *MLL*-rearranged, *BCR-ABL1*).[2,6] This group of *BCR-ABL1*-like patients had frequent deletions in B-cell development genes (e.g. *IKZF1*), dic(9;20), and intrachromosomal amplification of chromosome 21 (iAMP21).[2,6] The two signatures have nine overlapping probe sets (*Online Supplementary Table S1*). Here, we compared *BCR-ABL1*-like cases identified by each of these two signatures in two independent, previously published pediatric cohorts. We found that the two *BCR-ABL1*-like signatures identify molecularly distinct but overlapping groups of patients with a poor prognosis. The tyrosine kinase fusion genes affecting *ABL1*, *PDGFRB* and *JAK2* were exclusively found in the *BCR-ABL1*-like group; no fusion-positive patients were found in the remaining cases negative by either signature.

This signature comparison study comprised children with newly diagnosed BCP-ALL negative for the known aberrations *BCR-ABL1, ETV6-RUNX1, MLL* rearrangement, *TCF3* rearrangement, and high-hyperdiploidy in consecutive Dutch Childhood Oncology Group (DCOG) and German Cooperative ALL trials (146 cases),[2,6] and the P9906 study of the US Childhood Oncology Group (COG) (143 cases).[3-5,7,8] The patients' characteristics are summarized in *Online Supplementary Table S2*. Affymetrix U133 Plus 2.0 microarray files were exchanged between Erasmus MC and St. Jude Children's Research Hospital, and *BCR-ABL1*-like cases were identified by both HC and PAM signatures following described methods (see *Online Supplementary Methods*).[2,4-6] Molecular aberrations were determined as described in the *Online Supplementary Methods*. Cumulative incidence of relapse probabilities were estimated using a competing risks model and the equality of these cumulative incidences was tested with the Gray test.

In the population-based DCOG/COALL cohort, 79 out of 146 B-other cases were identified as *BCR-ABL1*-like by HC, 33 by PAM, and 25 cases by both signatures (henceforth called double-positive cases; Figure 1A). The cases identified by each of the signatures were predominantly (72-76%) NCI-Rome[9] high-risk (*Online Supplementary Table S3*, Figure 1B). We compared the frequencies of molecular aberrations among the *BCR-ABL1*-like identified cases with the 59 B-other BCP-ALL cases negative for either of the two *BCR-ABL1*-like signatures and negative for *BCR-ABL1, ETV6-RUNX1, MLL* rearrangement, *TCF3* rearrangement, and high-hyperdiploidy (Figure 1D, *Online Supplementary Figure S2A*). The double-positive samples showed significant enrichment of *IKZF1* deletion (P=0.01), *CRLF2* high expression (P<0.001), iAMP21 (P<0.001), *RB1* deletion (P=0.03), and kinase-activating fusions (P<0.001). Among the cases that were only identified by HC, dic(9;20) was enriched (P<0.001). Among the cases that were only identified by PAM, *IKZF1* deletion, high *CRLF2* expression, and *JAK2* mutations (P≤0.01) were enriched. Deletions of B-cell development genes *CDKN2A/B, PAX5, EBF1*, and *ETV6* were frequent in all groups, including non-*BCR-ABL1*-like B-others, and not enriched in specific groups (*Online Supplementary Table S4*). Similar results were obtained when analyzing the 92 NCI-HR cases (Figure 1E, *Online Supplementary Table S5*).

We also compared the overlap between HC- and PAM-identified *BCR-ABL1*-like cases in 143 B-other BCP-ALL cases negative for the known aberrations *BCR-ABL1*, *ETV6-RUNX1*, *MLL* rearrangement, *TCF3* rearrangement, and high-hyperdiploidy in the high-risk COG P9906 cohort. The PAM classification and molecular aberrations for these samples were described previously.[4] We identified 43 *BCR-ABL1*-like cases using HC, 40 cases using PAM, with 25 double-positive cases (Figure 1C). We compared the frequencies of molecular aberrations among the *BCR-ABL1*-like identified cases with 85 B-other BCP-ALL cases that were not identified by either of the two *BCR-ABL1*-like signatures (Figure 1F, *Online Supplementary Figure S2B*). Among the double-positive cases, translocations resulting in kinase-activating fusions, *IKZF1* deletion, *CRLF2* high expression, *JAK2* mutation, Hispanic or Latino background, *VPREB, EBF1*, and *BTG1* deletion were most enriched (P<0.01). *IKZF1* deletion, high *CRLF2* expression, *JAK2* mutation, Hispanic or Latino background and *EBF1* deletion were enriched in cases only identified by PAM (P≤0.01), while *VPREB1* deletion (P=0.04) was enriched in cases only identified by HC (*Online Supplementary Table S6*).

We evaluated the prognostic value of the *BCR-ABL1*-like signatures in DCOG cases from two recent, similar treatment protocols. In the DCOG ALL-9/10, the cases identified by HC only (P<0.05) and the total HC group (P=0.07) showed higher relapse rates compared with the non-*BCR-ABL1*-like B-other reference group (Figure 1G, *Online Supplementary Figure S3A-C*, *Online Supplementary Table S7*). Outcome analysis of the DCOG ALL-9/10 cases at high risk of treatment failure according to NCI-Rome criteria (white blood cell count ≥50x10^9/L and/or age ≥10 years) showed a similar unfavorable outcome (Figure 1H, *Online Supplementary Table S7*). In the high-risk P9906 protocol, total HC (P=0.005), total PAM (P<0.0001), PAM-only (P=0.04) and HC-and-PAM (P<0.0001) *BCR-ABL1*-like cases showed higher relapse rates compared with non-*BCR-ABL1*-like B-other (Figure 1I, *Online Supplementary Figure S3D-F*, *Online Supplementary Table S7*).

Several differences between the (genetic) composition of the discovery cohorts and the methodology to develop the signatures may contribute to the partial overlap in *BCR-ABL1*-like cases identified by the HC and PAM signatures.[2,4] Firstly, the 110 probe sets used for HC were previously selected to discriminate the major subtypes of pediatric ALL and as such those 110 probe sets were not selected to only identify *BCR-ABL1*-like ALL. In contrast, the 257 probe sets used for PAM were chosen for high discriminative value to identify *BCR-ABL1*-positive cases. Secondly, the COG discovery cohort consisted of high-

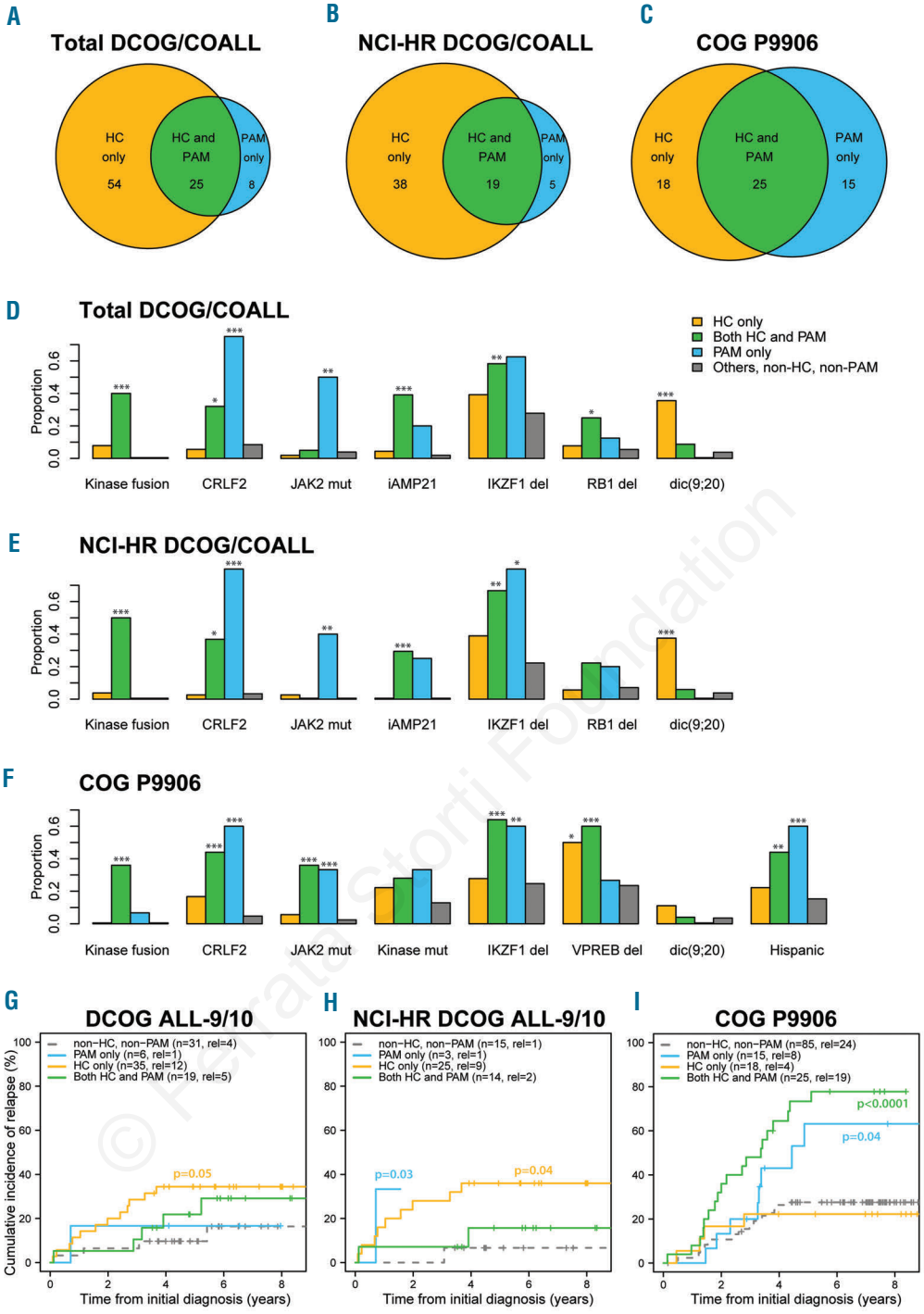

Figure 1. Comparison of *BCR-ABL1*-like cases identified by the HC and PAM *BCR-ABL1*-like signatures. (A-C) Venn diagrams showing the overlap in *BCR-ABL1*-like cases identified by the HC and PAM signatures in (A) the total DCOG/COALL cohort (B), the NCI-Rome high risk cases of the DCOG/COALL cohort, and (C) the high risk COG P9906 cohort. (D-F) Bar plots for (D) the total DCOG/COALL cohort (D), the NCI-Rome high risk cases of the DCOG/COALL cohort (E), and the high risk COG P9906 cohort (F) showing the proportions of the indicated aberrations in, respectively, the samples identified only by the HC signature (orange), double-positive samples identified by both signatures (green), samples identified only by the PAM signature (blue), and the remaining, non-*BCR-ABL1*-like B-other cases (gray). Fisher exact test results comparing proportions in each group to the remaining B-other cases are indicated as **P*-value ≤0.05; ***P*-value ≤0.01 and ****P*-value ≤0.001. mut: mutation; del: deletion. For aberration frequencies and *P*-values see *Online Supplementary Tables S4-6*. (G-I) Cumulative incidences of relapse for (G) the DCOG cases who were treated on ALL-9/10 protocols, (H) the NCI-Rome high risk cases treated on DCOG ALL-9/10 protocols, and (I) the high-risk cases treated on the COG P9906 protocol. The total numbers of patients and relapses and Gray *P*-values ≤0.1 are indicated in each plot. Additional outcome results are presented in *Online Supplementary Table S7*.

risk patients according to the COG criteria, whereas the DCOG/COALL discovery cohort represented all risk groups.[4,5] Thirdly, differences in genetic ancestry between the American COG and European DCOG/COALL cohorts could have affected the signatures. Several of the hallmarks of *BCR-ABL1*-like ALL identified in the COG high-risk cohorts, including increased frequencies of *CRLF2* aberration and *JAK2* mutation, have been associated with Hispanic/Latino ethnicity.[7,10] The lack of Hispanic/Latino patients in the DCOG/COALL cohort may have contributed to the lower frequency of *CRLF2* and *JAK2* abnormalities in this cohort, and the lower number of *BCR-ABL1*-like cases identified by the PAM signature. Likely due to these cohort differences, the prognostic value of the HC and PAM signatures is most discriminative in the cohort of patients in whom these signatures were identified, DCOG/COALL and COG P9906, respectively.

The biggest challenge is how to treat *BCR-ABL1*-like cases in clinical practice. *BCR-ABL1*-like ALL identified by the HC signature comprise approximately 15% of BCP-ALL cases in the population-based DCOG/COALL cohorts, and constitute approximately 50% of the B-other cases negative for *BCR-ABL1*, *MLL* or *TCF3* rearrangements, *ETV6-RUNX1*, and high-hyperdiploidy.[6] *BCR-ABL1*-like ALL identified by the PAM signature reflected 11% of BCP-ALL and 29% of B-other cases in a US population-based cohort.[11] Both in the St. Jude Total Therapy XV study and in the DCOG ALL-10 study, patients with *BCR-ABL1*-like ALL were more likely to have higher levels of minimal residual disease after induction therapy.[6,12] In the Total Therapy XV study, *BCR-ABL1*-like cases were responsive to risk-oriented increase of treatment intensity, showing similar outcomes to other BCP-ALL cases.[12] However, in the ALL-10 study the majority of *BCR-ABL1*-like cases had intermediate minimal residual disease levels at the end of induction therapy, indicating that these patients are not identified as high-risk by minimal residual disease diagnostics and need additional molecular diagnostics.[6]

In the P9906 cohort, sequencing analyses identified kinase-activating gene fusions including *ABL1, PDGFRB* and *JAK2*.[4,5] These fusions were present in 25% of PAM-identified *BCR-ABL1*-like cases, and in 21% of HC-identified cases. Kinase-activating gene fusions resulted in a constitutive activation of kinase signaling, and gave sensitivity to kinase inhibitors in *ex vivo*-cultured primary leukemic blasts and xenograft mouse models.[4,11] Recent studies in patients with tyrosine kinase gene rearrangements and a poor response to induction chemotherapy showed rapid and sustained responses upon tyrosine kinase inhibitor therapy.[11,13,14] A substantial part of the *BCR-ABL1*-like patients (75-80%) are negative for *ABL* and *JAK* class fusions whereas their prognosis is still poor. Within the P9906 cohort, the majority of these cases had abnormalities in *CRLF2* and/or activating kinase mutations, thus differing from the DCOG/COALL cohort, which may be explained by the higher frequency of Hispanic/Latino ancestry because this coincides with a higher frequency of *CRLF2/JAK2* aberrations.[3-5,7,8] Studies addressing the pathobiology of the remaining cases are, therefore, of great importance to find other targets for treatment in the European population of patients.

The scope of the current study was to compare the two *BCR-ABL1*-like signatures. In the USA, a newly developed low-density array measuring the expression of 15 genes representative of the heterogeneous genomic lesions associated with *BCR-ABL1*-like ALL is currently being used in a first step, combined with genomic aberrations, to identify *BCR-ABL1*-like cases.[15] This assay is not currently available for world-wide use, nor can gene expression signatures be easily obtained and interpreted in ordinary diagnostic laboratories. We, therefore, expect that the identification of genomic aberrations strongly associated with *BCR-ABL1*-like ALL is crucial for diagnostic procedures. To this aim it is also important to understand the overlap and differences between the two sentinel studies on *BCR-ABL1*-like ALL.[2,3]

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Key words: BCR-ABL1*-like ALL,* IKZF1*, iAMP21, dic(9;20),* CRLF2*,* JAK2*, prognosis, translocation, kinase.*

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