bjh research paper

Rapid identification of *BCR/ABL1*-like acute lymphoblastic leukaemia patients using a predictive statistical model based on quantitative real time-polymerase chain reaction: clinical, prognostic and therapeutic implications

Sabina Chiaretti,[1] Monica Messina,[1] Sara Grammatico,[1] Alfonso Piciocchi,[2] Anna L. Fedullo,[1] Filomena Di Giacomo,[1,3] Nadia Peragine,[1] Valentina Gianfelici,[1] Alessia Lauretti,[1] Rohan Bareja,[4] Maria P. Martelli,[5] Marco Vignetti,[1] Valerio Apicella,[1] Antonella Vitale,[1] Loretta S. Li,[6] Cyril Salek,[7] Olivier Elemento,[4] Giorgio Inghirami,[3] David M. Weinstock,[8] Anna Guarini[9] and Robin Foà[1]

[1]Haematology, Department of Cellular Biotechnologies and Haematology, "Sapienza" University, [2]GIMEMA Data Centre, GIMEMA Foundation, Rome, Italy, [3]Department of Pathology and Laboratory Medicine, Weill Cornell Medical College, [4]Department of Physiology and Biophysics, Weill Cornell Medical College, New York, NY, USA, [5]Institute of Haematology, Centro Ricerche Onco-Ematologiche (CREO), University of Perugia, Perugia, Italy, [6]Department of Paediatric Haematology/Oncology, Dana-Farber Cancer Institute, Boston, MA, USA, [7]Institute of Haematology and Blood Transfusion, Prague,Czech Republic, [8]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA, USA, and [9]Department of Molecular Medicine, "Sapienza" University, Rome, Italy

Received 12 January 2018; accepted for publication 1 March 2018

Correspondence: Sabina Chiaretti and Robin Foà, Haematology, Department of Cellular Biotechnologies and Haematology, "Sapienza" University, Via Benevento 6, 00161 Rome, Italy.
E-mails: chiaretti@bce.uniroma1.it; rfoa@bce.uniroma1.it
SC and MM equally contributed equally to this work.

Summary

BCR/ABL1-like acute lymphoblastic leukaemia (ALL) is a subgroup of B-lineage acute lymphoblastic leukaemia that occurs within cases without recurrent molecular rearrangements. Gene expression profiling (GEP) can identify these cases but it is expensive and not widely available. Using GEP, we identified 10 genes specifically overexpressed by *BCR/ABL1*-like ALL cases and used their expression values – assessed by quantitative real time-polymerase chain reaction (Q-RT-PCR) in 26 *BCR/ABL1*-like and 26 non-*BCR/ABL1*-like cases to build a statistical "*BCR/ABL1*-like predictor", for the identification of *BCR/ABL1*-like cases. By screening 142 B-lineage ALL patients with the "*BCR/ABL1*-like predictor", we identified 28/142 *BCR/ABL1*-like patients (19·7%). Overall, *BCR/ABL1*-like cases were enriched in *JAK/STAT* mutations ($P < 0{\cdot}001$), *IKZF1* deletions ($P < 0{\cdot}001$) and rearrangements involving cytokine receptors and tyrosine kinases ($P = 0{\cdot}001$), thus corroborating the validity of the prediction. Clinically, the *BCR/ABL1*-like cases identified by the *BCR/ABL1*-like predictor achieved a lower rate of complete remission ($P = 0{\cdot}014$) and a worse event-free survival ($P = 0{\cdot}0009$) compared to non-*BCR/ABL1*-like ALL. Consistently, primary cells from *BCR/ABL1*-like cases responded *in vitro* to ponatinib. We propose a simple tool based on Q-RT-PCR and a statistical model that is capable of easily, quickly and reliably identifying *BCR/ABL1*-like ALL cases at diagnosis.

Keywords: Acute lymphoblastic leukaemia, *BCR/ABL1*-like, adults, prognosis, tyrosine kinase inhibitors.

First published online 19 April 2018
doi: 10.1111/bjh.15251

Among B-lineage acute lymphoblastic leukaemia (ALL), *BCR/ABL1*-like ALL is one of the most clinically relevant subsets because it is characterized by a poor outcome and could potentially benefit from the use of tyrosine kinase inhibitor (TKI) therapy (Ofran & Izraeli, 2017). Mullighan *et al* (2009a) identified a subgroup of paediatric B-lineage ALL (B-ALL) with a gene expression profile similar to that of *BCR/ABL1*-positive patients, frequent *IKZF1* deletions and poor outcome. Simultaneously, Den Boer *et al* (2009) performed gene expression profiling (GEP) analysis on a cohort of paediatric B-ALL cases and termed this subgroup *BCR/ABL1*-like; it represented 15–20% of B-ALL cases, showed an unfavourable outcome and was associated with *IKZF1, TCF3, EBF1, PAX5* and *VPREB1* deletions, and upregulation of *CRLF2*.

Subsequently, the *BCR/ABL1*-like ALL subgroup has been extensively evaluated, particularly in paediatric cohorts (Mullighan *et al*, 2009b,c; Harvey *et al*, 2010a,b; Yoda *et al*, 2010; Chen *et al*, 2012; Asai *et al*, 2013; van der Veer *et al*, 2013) and more recently in adults (Tokunaga *et al*, 2013; Boer *et al*, 2015a; Herold *et al*, 2017; Jain *et al*, 2017a; Roberts *et al*, 2017). In addition to the association with *IKZF1* deletions and *CRLF2* deregulation, Roberts *et al* (2012) unveiled that kinase activating alterations characterize the majority (91%) of paediatric *BCR/ABL1*-like ALL cases. The most frequent alterations involve *ABL1*, *JAK2*, *PDGFRB*, *CRLF2* and *EPOR*, activating mutations of *IL7R*, *FLT3*-internal tandem duplication mutations and deletion of *SH2B3* (Roberts *et al*, 2012, 2014a). Several of these alterations are targeted by TKIs, suggesting a potential role for tailored treatment (Roberts *et al*, 2012, 2014a). Furthermore, all authors confirmed an association with poor outcome, while the relationship with minimal residual disease (MRD) is still debated (Roberts *et al*, 2014b; Heatley *et al*, 2017).

Despite this in-depth genetic characterization, the identification of *BCR/ABL1*-like patients is still challenging: it relies on GEP and/or a multistep approach, such as a combination of next generation sequencing (NGS) and fluorescence *in situ* hybridisation (FISH) (Roberts *et al*, 2014a). However, GEP is largely cohort/experiment-dependent and a consensus diagnostic signature has not been agreed upon (Boer *et al*, 2015b). Similarly, NGS is costly and requires bioinformatic skills. Alternatively, these cases can be recognized with Low Density Assay (LDA) of selected genes, but the full methodology has not been published (Harvey *et al*, 2013). Thus, this study aimed to: (i) produce an easy, rapid and reproducible assay, based on quantitative real time-polymerase chain reaction (Q-RT-PCR) analysis, for the recognition of *BCR/ABL1*-like ALL cases; (ii) define the molecular background, clinico-biological features and outcome of the cases thus identified; and (iii) verify the *in vitro* response of primary *BCR/ABL1*-like ALL cells to the pan-TKI, ponatinib. The final goal is to provide a rapid, user-friendly and economically-viable diagnostic tool that can recognize these cases at presentation, a step towards a refined prognostic and therapeutic management of these poor prognosis patients.

Methods

Identification of BCR/ABL1-like cases and genes

To identify the core *BCR/ABL1*-like cases and *BCR/ABL1*-like specific genes (Figure S1), we used two *in house* GEP cohorts: GEP1, run on HG-U133 plus2 (Affymetrix, Santa Clara, CA, USA) – comprising 148 B-ALL cases (70 *BCR/ABL1*-positive and 78 B-NEG, i.e. negative for *BCR/ABL1*, *ETV6/RUNX1*, *TCF3/PBX1* and *KMT2A* rearrangements) (Haferlach *et al*, 2010; Messina *et al*, 2010) – and GEP2 – including 79 cases (37 *BCR/ABL1*-positive and 42 B-NEG) (Chiaretti *et al*, 2005) – evaluated with an older version of the array (HG-U95 Av2, Affymetrix). In both GEP1 and GEP2, a *t*-test between *BCR/ABL1*-positive and B-NEG cases was performed to recognize the *BCR/ABL1*-like ALL cases. B-NEG cases clustering within the *BCR/ABL1*-positive cluster were regarded as the "core" *BCR/ABL1*-like samples.

To select the *BCR/ABL1*-like genes (Figure S2), the 16 "core" *BCR/ABL1*-like ALL cases of GEP1 were compared with the remaining 62 B-NEG cases by *t*-test. The genes selected by this approach were compared with the literature (Harvey *et al*, 2010b) and the overlapping genes, together with *CRLF2* (Yoda *et al*, 2010; van der Veer *et al*, 2013; Chiaretti *et al*, 2016; Herold *et al*, 2017), were used to build the Q-RT-PCR-based *BCR/ABL1*-like predictor. GEP analyses are detailed in Data S1.

Development and validation of the "BCR/ABL1-like predictor"

To generate the *BCR/ABL1*-like predictor, we validated the expression levels of the selected genes in the discovery cohort, including 26 core *BCR/ABL1*-like samples (16 from GEP1 and 10 from GEP2) and 26 non-*BCR/ABL1*-like selected from GEP1. The quantification of transcript levels was performed by Q-RT-PCR (Data S1 and Table SI) and computed as $2^{-\Delta Ct}$.

Q-RT-PCR results were used to build the "*BCR/ABL1*-like predictor", extensively described in the Data S1. Briefly, expression values were shrunk into principal components (PCs) and a logistic regression model was used to examine the association among the PCs and *BCR/ABL1*-like ALL cases. Subsequently, a score on PCs was built and used to classify 142 additional B-NEG ALL cases, representing the screening panel.

The analysis of the genetic and clinical features was performed on a total of 194 B-NEG ALL, 52 belonging to the discovery and 142 to the screening panels (Table SII and Data S1). Patients were enrolled in Gruppo Italiano Malattie EMatologiche dell'Adulto (GIMEMA) and Associazione

Italiana di Ematologia ed Oncologia Pediatrica (AIEOP) protocols (Table SIII).

The study was approved by the local IRB, in accordance with the Helsinki Declaration.

Analysis of genetic features

Recurrently mutated JAK/STAT and RAS pathways genes (Messina *et al*, 2016) were sequenced in 182/194 samples (Table SIV). Copy number aberrations (Messina *et al*, 2017) were assessed by multiplex ligation-dependent probe amplification (MLPA) in 111/194 samples. RNA-sequencing was performed in 54 samples by the TruSeq RNA Sample Preparation Kit (Illumina, San Diego, CA). RNA libraries were paired-end sequenced (2 × 100 bp) using the Illumina HiSeq2500 platform. Identification of fusion transcripts was performed by STAR-fusion (STAR-Fusion_v0.5.1) and Fusioncatcher (v0.99.3e) (Nicorici *et al*, 2014).

Statistical analysis of clinical features and event-free survival (EFS)

Patients' characteristics were compared by chi-squared or Fisher's exact test for categorical variables and by Wilcoxon test for continuous data. Event-free survival (EFS) and overall survival (OS) was estimated by Kaplan–Meier: EFS was estimated from the time of diagnosis to the occurrence of refractoriness, relapse or death, while OS was estimated from the time of diagnosis to death. Multivariate analysis was performed using the Cox proportional model to adjust the effect of *BCR/ABL1*-like ALL cases to white blood cell (WBC) count, age and *CRLF2* on EFS. All tests were two-sided and P values <0·05 were considered statistically significant. Analyses were performed using the SAS software (release 9.4; SAS Institute, Cary, NC, USA).

In vitro experiments

To assess the sensitivity to ponatinib, the annexin V/7-aminoactinomycin D (7AAD) apoptotic test (BD Bioscience, San Josè, CA) and ^{3}H-thymidine (Perkin Elmer, Waltham, MA) proliferation assays were performed on primary cells from 7 *BCR/ABL1*-like, 6 non-*BCR/ABL1*-like and 4 *BCR/ABL1*-positive cases. Ponatinib (Selleck Chemicals, Houston, TX) at 0 μmol/l (dimethyl sulfoxide only) or at increasing doses (0·01–10 μmol/l) was added at time 0 and viability was measured after 48 and 72 h (Data S1).

Results

Identification and quantification of BCR/ABL1*-like predictor genes in the discovery cohort*

The comparison of *BCR/ABL1*-positive and B-NEG cases of GEP1 (Haferlach *et al*, 2010; Messina *et al*, 2010) using a *t*-test recognized 16 B-NEG cases that clustered with the *BCR/ABL1*-positive cases: these 16 misclustered B-NEG cases were regarded as the "core" *BCR/ABL1*-like samples (Figure S2).

To select the *BCR/ABL1*-like genes (Figure S2) the "core" *BCR/ABL1*-like ALL cases were compared with the remaining B-NEG cases by *t*-test, that resulted in the identification of 285 genes (Table SV), of which 9 had been previously reported as part of the *BCR/ABL1*-like signature (Harvey *et al*, 2010b). These 9 genes (*SOCS2*, *IFITM1*, *CD99*, *TP53INP1*, *IFITM2*, *JCHAIN*, *NUDT4*, *ADGRE5* and *SEMA6A*) together with *CRLF2* (Yoda *et al*, 2010; van der Veer *et al*, 2013; Herold *et al*, 2017) were used to build the Q-RT-PCR-based *BCR/ABL1*-like predictor.

By analysing GEP2 (Chiaretti *et al*, 2005) we selected 10 additional *BCR/ABL1*-like ALLs, leading to a total of 26 *BCR/ABL1*-like cases that were included in the discovery panel of this study.

Next, the expression levels of these 10 genes were quantified by Q-RT-PCR in the discovery cohort (n = 52), comprising 26 *BCR/ABL1*-like and 26 non-*BCR/ABL1*-like ALLs, classified according to GEP (Chiaretti *et al*, 2005; Haferlach *et al*, 2010; Messina *et al*, 2010). All genes were significantly overexpressed in *BCR/ABL1*-like ALL samples (Figure S3).

Development of the "BCR/ABL1-like predictor"

The Q-RT-PCR expression values of the 10 genes were used to build the "*BCR/ABL1*-like ALL predictor". First, we verified that in univariate analysis, all genes were risk factors for a higher *BCR/ABL1*-like ALL probability (Table SVI). As a high correlation was detected among the expression levels of the 10 genes (Figure S4), it was possible to summarize the variability by means of PC analysis; by definition, the PCs thus identified are uncorrelated. In detail, expression values were shrunk into 3 PCs (accounting for >80% of the variability): the contributions of each gene to each component are expressed by factor loadings, a measure of their relationship (Table SVII). Each component is mainly explained by genes with the highest loadings: PC1 is explained by the expression values of *NUDT4*, *SEMA6A*, *ADGRE5*, *SOCS2* and *JCHAIN*, PC2 by *CRLF2*, *TP53INP1*, *CD99* and PC3 by *IFITM1* and *IFITM2*.

Second, a logistic regression model was used to estimate the probability of a case being *BCR/ABL1*-like using the first 3 PCs; all components were statistically significant in multivariate analysis (Table SVIII).

Finally, to generate a predictive model, a score was computed by means of a linear combination of the PCs, as a result of the above mentioned multivariate logistic regression model. Figure S5 illustrates the generation of the score. The optimal cut-off was set at −0·30: cases with a score ≥−0·30 were defined as *BCR/ABL1*-like. This cut-off provides the best distinction between *BCR/ABL1*-like and non-*BCR/ABL1*-

like cases and ensures the optimal compromise between sensitivity and specificity (88·5% and 100%, respectively). The mathematical equation is provided in Data S1.

An online *BCR/ABL1*-like score calculator was implemented into the web-based application GIMEMA REDCap system hosted at GIMEMA Foundation (Harris *et al*, 2009).

Identification of the BCR/ABL1-like ALL cases and genomic characterization

The predictive model was then validated in a screening cohort of 142 B-NEG ALL patients: 28 cases (19·7%) were classified as *BCR/ABL1*-like (min score −0·279, max score 2·176) and 114 as non-*BCR/ABL1*-like ALL (min score −1·810, max score −0·353). The comparison of the clinico-biological features of *BCR/ABL1*-like and non-*BCR/ABL1*-like ALL cases at diagnosis, carried out in the whole cohort, showed that *BCR/ABL1*-like ALLs were associated with a significantly higher WBC count at diagnosis (22·9 vs. 12·6 × 10^9/L, $P = 0·013$) while no differences were observed for the other parameters (Table I).

Recurrently mutated genes were investigated in 182/194 cases. Mutations of the JAK/STAT pathway (i.e. *JAK1/2*, *CRLF2* and *IL7R*) were detected in 44·2% (23/52) of *BCR/ABL1*-like cases and only in 7·7% (10/130) of the non-*BCR/ABL1*-like cases ($P < 0·001$), RAS pathway mutations were more frequent in non-*BCR/ABL1*-like ($n = 38/130$, 29·2%) than in *BCR/ABL1*-like cases ($n = 9/52$, 17·3%, $P = 0·068$). Details are provided in Table II.

We also examined copy number aberrations in 111 cases: *IKZF1* deletions were significantly more frequent in *BCR/ABL1*-like than non-*BCR/ABL1*-like cases (82·8% vs. 40·8%, $P < 0·001$). We found that *EBF1* and *BTG1* deletions were significantly more frequent in *BCR/ABL1*-like cases (Table II).

Finally, *CRLF2* levels were significantly higher ($P \leq 0·001$) in *BCR/ABL1*-like (median ΔCt = 6·7, range 0·6–16·6) than in non-*BCR/ABL1*-like cases (median ΔCt = 11·3, range 3·2–17·9). Complete results are detailed in Tables SIX and SX. Comparable results were obtained when separating the discovery (Table SXI) and screening cohorts (Table III).

RNA-sequencing, performed in 54 samples (28 *BCR/ABL1*-like and 26 non-*BCR/ABL1*-like), identified 13 fusion transcripts targeting tyrosine kinases (TKs)/cytokine receptors, of which 12 were detected in *BCR/ABL1*-like cases (12/28 vs. 1/26 $P = 0·001$). The most recurrent fusion was *EBF1/PDGFRB* found in 3 cases; *JAK2* rearrangements with different partners (i.e. *PAX5*, *EBF1*, *SSBP2*) were detected in 3 other cases, *P2RY8/CRLF2* and *TSLP*-fusion genes in 2 samples; finally, *RCSD1/ABL1* and *TRIM24/FGFR1* were found in 1 case each. Within non-*BCR/ABL1*-like cases, 1 case harboured *P2RY8/CRLF2* while no fusion genes targeting TKs were documented. Figure 1 shows that 27/28 (96·4%) had at least one lesion typical of the *BCR/ABL1*-like profile and suggests that the *BCR/ABL1*-like profile is sustained by at least 2 different mechanisms: one represented by TK-rearrangements only and the other by *CRLF2* overexpression plus *JAK/STAT* mutations.

Outcome of the BCR/ABL1-like ALL samples

We analysed the complete remission (CR) rate and survival in the adolescents and adults with clinical data available ($n = 142$, Table SIII).

In the whole cohort, the CR rate was lower in *BCR/ABL1*-like than in non-*BCR/ABL1*-like cases (77·8% vs. 89·6%, $P = 0·06$).

Consistently, EFS at 36 months was significantly inferior for *BCR/ABL1*-like cases compared to non-*BCR/ABL1*-like cases (21·6% vs. 47·2%, $P < 0·0001$; Fig 2A).

When we considered the screening cohort only ($n = 95$), we confirmed that the CR rate was significantly lower in *BCR/ABL1*-like than in non-*BCR/ABL1*-like cases (71·4% vs. 91·8%, $P = 0·014$). Similarly, EFS at 36 months was significantly inferior for *BCR/ABL1*-like cases compared to non-*BCR/ABL1*-like cases (21·3% vs. 43·3%, $P = 0·0009$; Fig 2B).

In line with EFS estimates, OS at 36 months was significantly inferior for *BCR/ABL1*-like cases compared to non-*BCR/ABL1*-like cases (37·3% vs. 60·7%, $P = 0·05$; Figure S6A) in the whole cohort and a similar trend was observed in the screening cohort only (Figure S6B). The

Table I. Comparison between *BCR/ABL1*-like and non-*BCR/ABL1*-like clinico-biological features.

	BCR/ABL1-like $n = 54$	Non-*BCR/ABL1*-like $n = 140$	*P*-value
Gender (male/female)	36/18	78/62	P = ns
Median age (range), years	32 (6–72)	28 (0–78)	P = ns
Age cohort 0–15 years ($n = 21$)	2 (9·5%)	19 (90·5%)	
Age cohort 15–35 years ($n = 98$)	29 (29·5%)	69 (70·4%)	P = ns
Age cohort >35 years ($n = 75$)	23 (30·6%)	52 (69·3%)	
Median (range) WBC count, x10^9/l	22·6 (1·89–239)	12·4 (0·6–425)	$P = 0·023$
Median (range) platelet count, x10^9/l	47 (0·15–283)	47 (1–308)	P = ns
Median (range) Hb g/l	97 (41–153)	89 (37–158)	P = ns

ns, not significant; WBC, white blood cell.

Table II. Comparison between *BCR/ABL1*-like and non-*BCR/ABL1*-like genetic features.

	BCR/ABL1-like	Non-*BCR/ABL1*-like	*P*-value
JAK/STAT pathway members mutated cases	23/52 (44·2%)*	10/130 (7·7%)	$P < 0·001$
JAK1/2 mutations	14/52 (26·9%)	4/130 (3·1%)	$P < 0·001$
CRLF2 mutations	6/52 (11·5%)	2/130 (1·5%)	$P = 0·007$
IL7R mutations	6/52 (11·5%)	4/130 (3·1%)	$P = 0·033$
RAS pathway members mutated cases	9/52 (17·3%)	38/130 (29·2%)†	$P = 0·068$
FLT3 mutations	0/52 (0%)	10/130 (7·7%)	$P = 0·031$
KRAS/NRAS mutations	9/52 (17·3%)	30/130 (23·1%)	P = ns
IKZF1 deletions	29/35 (82·8%)	31/76 (40·8%)	$P < 0·001$
EBF1 deletions	14/35 (40%)	5/76 (6·6%)	$P < 0·001$
BTG1 deletions	10/35 (28·6%)#	5/76 (6·6%)	$P = 0·003$
CRLF2 overexpressing cases‡	33/54 (61·1%)	25/140 (17·8%)	$P < 0·001$
CRLF2 expression levels	6·7 (0·6–16·6)	10·9 (3·2–17·9)	$P < 0·001$
TK/cytokine receptor fusions	*EBF1/PDGFRB* ($N = 3$)	*P2RY8/CRLF2* ($N = 1$)	
	JAK2-fusions ($N = 3$)		
	RCSD1/ABL1 ($N = 1$)		
	TRIM24/FGFR1 ($N = 1$)		
	P2RY8/CRLF2 ($N = 2$)	1/26 (3·8%)	$P = 0·001$
	TSLP-fusions ($N = 2$)		
	12/28 (42·8%)		

*Three cases carried 2 concomitant JAK/STAT pathway mutations: 2 cases harboured *JAK2* and *CRLF2* mutations, 1 case harboured *IL7R* and *CRLF2* mutations.

†Two cases carried 2 concomitant RAS pathway mutations: 1 case harboured *NRAS* and *KRAS* mutations, 1 case *FLT3* and *NRAS* mutations.

‡Overexpression was defined at $\Delta Ct < 8$ as previously described by Chiaretti *et al* (2016).

#Correction added on 17 May 2018, after first online publication: The percentage has been corrected from 2064% to 28·6%.

Table III. Comparison between *BCR/ABL1*-like and non-*BCR/ABL1*-like ALL cases included in the screening panel.

	BCR/ABL1-like	Non-*BCR/ABL1*-like	*P*-value
JAK/STAT pathway members mutated cases	12/27 (44·4%)*	10/107 (9·3%)	$P < 0·001$
JAK1/2 mutations	7/27 (25·9%)	4/107 (3·7%)	$P = 0·001$
CRLF2 mutations	2/27 (7·4%)	2/107 (1·9%)	ns
IL7R mutations	4/27 (14·8%)	4/107 (3·7%)	$P = 0·05$
RAS pathway members mutated cases	6/27 (22·2%)	32/107 (29·9%)†	ns
FLT3 mutations	0	9/107 (8·4%)	ns
KRAS/NRAS mutations	6/27 (22·2%)	24/107 (22·4%)	ns
IKZF1 deletions	14/18 (77·7%)	23/62 (37·1%)	$P = 0·029$
EBF1 deletions	6/18 (33·3%)	4/62 (6·5%)	$P = 0·007$
BTG1 deletions	4/18 (22·2%)	4/62 (6·5%)	$P = 0·071$
CRLF2 overexpressing cases	16/28 (57·1%)	21/114 (18·4%)	$P < 0·001$
CRLF2 median expression levels (range)	7·6 (2–16·6)	11·3 (3·2–17·5)	$P < 0·001$
TK/cytokine receptor fusions	*JAK2*-fusions ($N = 1$)	*P2RY8/CRLF2* ($N = 1$)	$P = 0·037$
	TRIM24/FGFR1 ($N = 1$)	1/21 (4·7%)	
	TSLP-fusions ($N = 2$)		
	4/13 (30·7%)		

ns, not significant.

*One case harboured *IL7R* and *CRLF2* mutations.

†One 1 case harboured *NRAS* and *FLT3* mutations.

impact of *BCR/ABL1*-like prediction retained statistical significance on EFS in multivariate analysis (Hazard ratio: 2·12, 95% confidence interval: 1·18–3·82, $P = 0·01$) in a model adjusted for age and WBC count. We also evaluated the interaction on EFS between the *BCR/ABL1*-like signature and *CRLF2* overexpression: *BCR/ABL1*-like prediction retained statistical significance on EFS ($P = 0·05$) in the bivariate model adjusted by *CRLF2* overexpression.

In vitro sensitivity to ponatinib

After 72 h of incubation with ponatinib (0·01 μmol/l), a ^{3}H-thymidine uptake assay showed that the proliferation

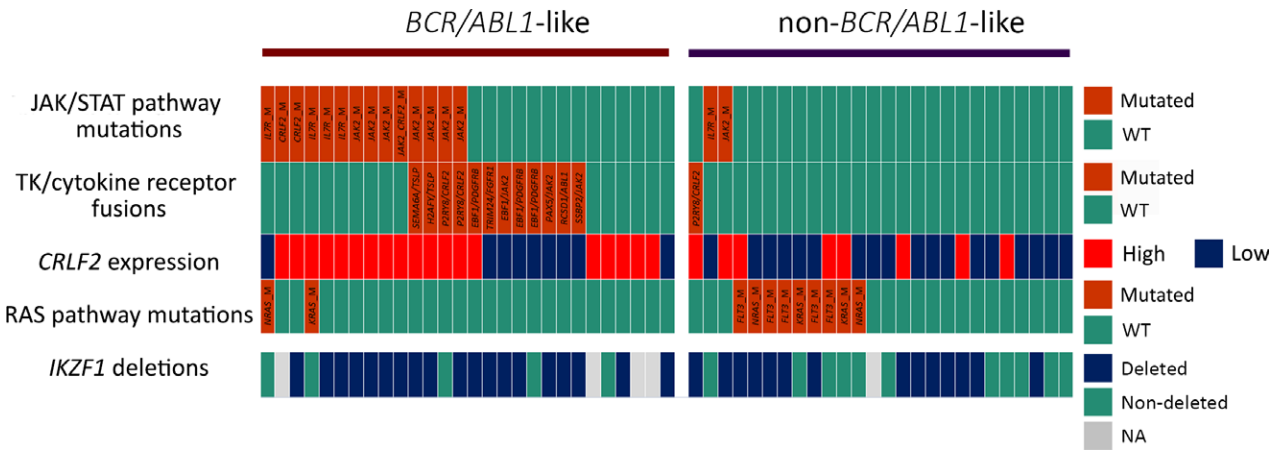

Figure 1. Distribution of *BCR/ABL1*-like specific genetic lesions in the samples with a complete molecular characterization. Legend: green boxes for JAK/STAT pathway mutations: wild-type; red boxes: mutation detected. The mutated gene name is provided in the figure; green boxes for RAS pathway mutations: wild-type; red boxes: mutation detected; the mutated gene name is provided in the figure; *CRLF2* expression; red boxes: overexpression; TK/cytokine fusions: green boxes: no rearrangement detected; red boxes: rearrangement detected. The fusion gene is specified in the figure; *IKZF1* deletions: green boxes: no deletions; blue boxes: presence of deletions; grey boxes: sample not evaluated.

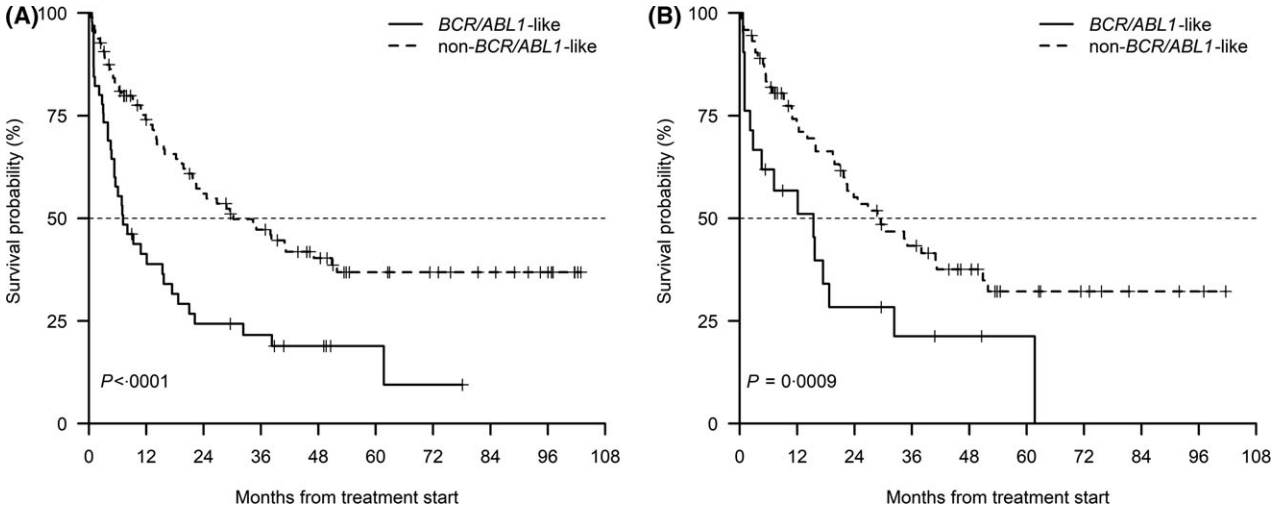

Figure 2. Event-free survival at 36 months of adolescents and adults classified as *BCR/ABL1*-like and non-*BCR/ABL1*-like belonging to the whole cohort (A) and the screening panel only (B).

rate of primary cells from 7 *BCR/ABL1*-like cases (2 *EBF1/PDGFRB*-positive, 1 *JAK2*-mutated and *P2RY8/CRLF2*-positive, 1 *RCSD1/ABL1*, 3 WT for JAK/STAT and RAS mutations) decreased to 33·6% ± 15%, comparable to the sensitivity observed in 4 *BCR-ABL1*-positive cases (44·3-% ± 12·4%). Contrarily, in the non-*BCR-ABL1*-like (non-*BCR-ABL1*-positive) samples ($n = 6$) – all WT for JAK/STAT and RAS hotspot mutations – the proliferation rate upon ponatinib treatment decreased only to 71·5-% ± 28·7% ($P = 0·0007$) (Fig 3A). Ponatinib (0·01 μmol/l) also increased the apoptotic rate in both *BCR/ABL1*-like and *BCR/ABL1*-positive primary ALL cells (22·1% ± 10%, 19·9% ± 8·5%, respectively), while the apoptotic response in non-*BCR/ABL1*-like/non-*BCR/ABL1*-positive ALL was significantly inferior (6·1% ± 8·4%, $P = 0·023$, Fig 3B).

Discussion

Although the recognition of the *BCR/ABL1*-like ALL subset dates back to 2009, a consensus on a *BCR/ABL1*-like signature has not been reached (Den Boer *et al*, 2009; Mullighan *et al*, 2009a) and a standardized tool to identify these cases is currently not available (Ofran & Izraeli, 2017). Roberts *et al* (2014a) and Fasan *et al* (2015) proposed a combination of different methods: analysis of *CRLF2* expression, FISH targeting *ABL1* and *JAK* activating rearrangements, fusion-specific RT-PCR for the identification of the *ABL* and *JAK* partners

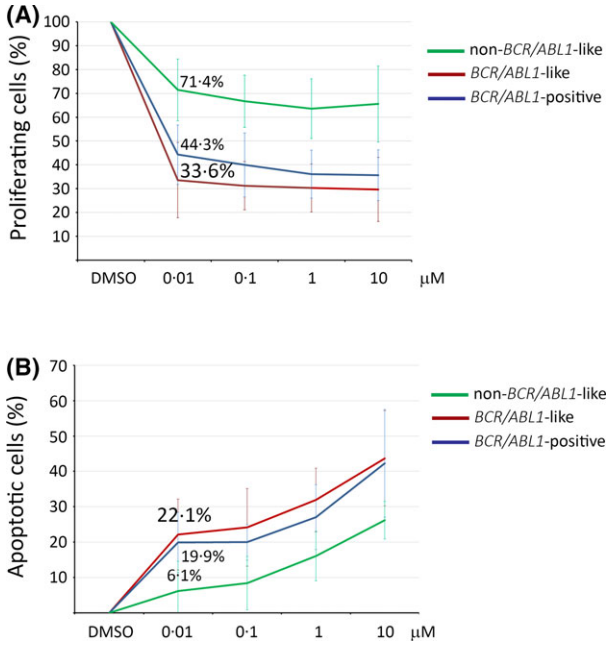

Figure 3. *In vitro* response to ponatinib in *BCR/ABL1*-like and non-*BCR/ABL1*-like primary cells; *BCR/ABL1*+ cells were used as control. (A) Average values of ^{3}H-thymidine incorporation of 16 primary B-ALL cells samples after 72 h of treatment with ponatinib (0·01 μmol/l); (B) Average values of Annexin V positive primary B-ALL cells samples after 72 h of treatment with ponatinib (0·01 μmol/l). Samples are grouped according to *BCR/ABL1*-like (n = 7), *BCR/ABL1*-positive (n = 4) and non-*BCR/ABL1*-like (n = 6).

and MRD monitoring. However, this approach relies on multiple techniques and can only recognise cases carrying already known fusion transcripts.

Simultaneously, Harvey *et al* (2013) developed a method based on the quantification of 15 transcripts by LDA and several groups adopted this method (Heatley *et al*, 2017; Reshmi *et al*, 2017). However, the mathematical equations were not provided. Our approach took advantage of previously reported GEP data (Chiaretti *et al*, 2005; Messina *et al*, 2010) to identify a narrow list of 10 transcripts (*CRLF2*, *SOCS2*, *IFITM1*, *CD99*, *TP53INP1*, *IFITM2*, *JCHAIN*, *NUDT4*, *ADGRE5*, *SEMA6A*), capable of accurately identifying the *BCR/ABL1*-like ALLs.

The genes chosen to build the predictive model are also in common with other algorithms recently used to identify *BCR/ABL1*-like cases (Roberts *et al*, 2012; Harvey *et al*, 2013).

Using Q-RT-PCR of these 10 transcripts, we built an algorithm capable of identifying *BCR/ABL1*-like cases with a high sensitivity and specificity. Furthermore, we generated a user-friendly tool, which requires only the upload of gene expression values to assess whether a sample is *BCR/ABL1*-like or non-*BCR/ABL1*-like.

Next, the screening of 142 B-NEG ALL samples by the *BCR/ABL1*-like predictor assigned 28 cases (19·7%) to the *BCR/ABL1*-like ALL subset, in line with the reported incidence (Ofran & Izraeli, 2017).

The prediction accuracy was indirectly corroborated by the analysis of the genetic features of *BCR/ABL1*-like ALL cases. As reported in both paediatric and adult cohorts, *BCR/ABL1*-like ALL is associated with *IKZF1* deletions, *CRLF2* deregulation/rearrangements, *JAK1/2* mutations, rearrangements of genes coding for TKs and cytokine receptors (Mullighan *et al*, 2009b,c; Harvey *et al*, 2010a,b; Yoda *et al*, 2010; Chen *et al*, 2012; Asai *et al*, 2013; Tokunaga *et al*, 2013; van der Veer *et al*, 2013; Boer *et al*, 2015a; Ge *et al*, 2016; Herold *et al*, 2017; Jain *et al*, 2017a; Roberts *et al*, 2017). Consistently, our *BCR/ABL1*-like cases frequently carried JAK/STAT pathway mutations, the most recurrent targeting *JAK2*, followed by *CRLF2* and *IL7R*. In addition, *BCR/ABL1*-like cases were enriched in *IKZF1* deletions, detected in 80% of cases, in line with Herold and colleagues (Herold *et al*, 2017).

More importantly, RNA-sequencing – performed in 28 *BCR/ABL1*-like cases - revealed that 7 carried TK rearrangements, 2 *P2RY8/CRLF2* and 2 *TSLP*-rearrangements. TK-activating fusion genes are specific to *BCR/ABL1*-like cases and may be targeted by TKIs, as shown in pre-clinical models and sporadic case reports (Maude *et al*, 2012; Roberts *et al*, 2012, 2014a,c; Tasian *et al*, 2012; Lengline *et al*, 2013; Weston *et al*, 2013; Shi *et al*, 2014, 2015; Francis *et al*, 2016).

The integration of all the molecular features, feasible in 28 *BCR/ABL1*-like, demonstrated that 96·4% had at least one lesion typical of the *BCR/ABL1*-like profile and suggested that the *BCR/ABL1*-like profile is sustained by at least two mechanisms, either a TK-activating fusion or *CRLF2* overexpression with a concomitant JAK/STAT mutation; at variance, *CRLF2* overexpression alone seems insufficient to induce a *BCR/ABL1*-like profile.

From a clinical standpoint, the features of the *BCR-ABL1*-like cases hereby identified were in line with a *BCR-ABL1*-like profile: they displayed a significantly higher WBC count at diagnosis, a lower CR rate and a significantly worse EFS than non-*BCR-ABL1*-like patients; we observed no differences related to gender, in agreement with other reports (Boer *et al*, 2015b; Herold *et al*, 2017). An association with MRD levels was not feasible since this parameter was not available for a large set of patients. Furthermore, the incidence in paediatric cases was lower than in adolescents and adults (9·5% vs. 29·5% and 30·6%, respectively) in our cohort.

Finally, *in vitro* experiments showed that the pan-TKI, ponatinib, the most potent inhibitor in *BCR/ABL1*-positive ALL (Jabbour *et al*, 2015), was able to reduce the proliferative rate in *BCR-ABL1*-like samples and to increase apoptosis, similarly to that observed in *BCR-ABL1*-positive ALL. Notably, the *BCR-ABL1*-like samples analysed by *in vitro* experiments comprised ABL class lesions, JAK/STAT mutated and WT cases, indicating that ponatinib is active in all cases regardless of the underlying lesion and may represent an alternative to ruxolitinib whose clinical activity remains to be determined (Jain *et al*, 2017b).

In conclusion, we hereby describe a Q-RT-PCR based assay capable of singling out *BCR-ABL1*-like patients from the B-NEG ALL cohort. This approach has many advantages: first, it requires minimal amounts of diagnostic RNA; second, it is simple and cost-effective, being based on Q-RT-PCR; third, it is rapid, because the screening can be completed within a few days. This is essential, considering the high rate of refractory cases that – if promptly recognized – could benefit from an alternative approach, contemplating the use of the pan-TKI, ponatinib. These advantages make this tool suitable to be introduced in the diagnostic workflow and appears applicable to many haematology centres, followed by further genomic screens that comprise RNA-sequencing characterization to fully elucidate the underlying molecular lesion in the patients thus identified. Finally, because this assay is not based on a specific target identification, it allows the potential recognition of all *BCR/ABL1*-like cases, including those carrying novel genetic lesions.

Acknowledgements

The authors wish to thank Associazione Italiana per la Ricerca sul Cancro (AIRC), Special Program Molecular Clinical Oncology-Extension program, 5 × 1000 (10007), Milan (Italy) to RF; Finanziamento per l'avvio alla ricerca 2015 (Sapienza University of Rome) to MM; Finanziamento Medi Progetti Universitari 2015 to SC (Sapienza University of Rome); Fondazione Le Molinette Onlus, Turin (Italy); MM was partly supported by Associazione Cristina Bassi Onlus (Genova). DMW is supported by NCI 5R01CA151898 and 5R01CA17238. SC designed research, analysed data and wrote the manuscript; MM performed experiments, analysed data and wrote the manuscript; SG performed experiments and analysed data; AP designed the *BCR/ABL1*-like predictor model and performed statistical analyses; ALF, VG, AL, NP performed experiments; FDG performed RNA sequencing experiments; MV performed statistical analyses; MPM, VA, AV and CS provided samples and clinico-biological data; OE and RB analysed RNA-sequencing data; LSL and DW provided clinical samples; GI analysed data and critically revised the manuscript; AG and RF designed the study, analysed data and critically revised the manuscript.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Supplementary materials and methods.

Table SI. Genes and primers for Q-RT-PCR experiments.

Table SII. Characteristics of the entire study population, further subdivided in the discovery and screening panels separately.

Table SIII. List of adult and paediatric clinical trials, relative NCT, and number of patients with follow-up data available, further subvided in c

Table SIV. Genes and primers for PCR experiments.

Table SV. Results of the *t*-test between *BCR-ABL1*-like and the remaining B-NEG cases.

Table SVI. Univariate logistic regression analyses of the selected *BCR/ABL1*-like genes.

Table SVII. Results of principal component analysis on the 10 selected genes.

Table SVIII. Results of a multivariate logistic regression (odds ratio, 95% CI, *P*-value) where the response variable is *BCR/ABL1*-like and the three principal components (PC) are the covariates.

Table SIX. Detailed list of the genetic features of the discovery panel.

Table SX. Detailed list of the genetic features of the screening panel.

Table SXI. Summary of the genetic features of *BCR/ABL1*-like and non-*BCR/ABL1*-like ALL cases included in the discovery panel.

Figure S1. Flowchart of the identification of *BCR-ABL1*-like specific genes.

Figure S2. Stages of the study and cohorts of study analyzed.

Figure S3. Box plots of the expression levels, reported as $2^{-\Delta Ct}$, of the 10 genes tested showed a significant overexpression in *BCR-ABL1*-like cases than in non-*BCR-ABL1*-like cases for all genes ($P = 0{\cdot}005$ for *CRLF2* and $P < 0{\cdot}001$ for the other genes).

Figure S4. Correlation matrix of genes. Blue indicates a positive relationship, red a negative one; color intensity is proportional to Pearson's correlation coefficient among genes.

Figure S5. Nomogram is a graphical representation of Table SVII and explains how the score was derived from the discovery panel data.

Figure S6. Overall survival at 36 months of adolescents and adults classified as *BCR/ABL1*-like and non-*BCR/ABL1*-like belonging to the whole cohort (A), and the screening panel only (B).

References

Asai, D., Imamura, T., Suenobu, S., Saito, A., Hasegawa, D., Deguchi, T., Hashii, Y., Matsumoto, K., Kawasaki, H., Hori, H., Iguchi, A., Kosaka, Y., Kato, K., Horibe, K., Yumura-Yagi, K., Hara, J. & Oda, M. (2013) IKZF1 deletion is associated with a poor outcome in pediatric B-cell precursor acute lymphoblastic leukemia in Japan. *Cancer Medicine*, **2**, 412–419.

Boer, J.M., Koenders, J.E., van der Holt, B., Exalto, C., Sanders, M.A., Cornelissen, J.J., Valk, P.J., den Boer, M.L. & Rijneveld, A.W. (2015a) Expression profiling of adult acute lymphoblastic leukemia identifies a BCR-ABL1-like subgroup characterized by high non-response and relapse rates. *Haematologica*, **100**, e261–e264.

Boer, J.M., Marchante, J.R., Evans, W.E., Horstmann, M.A., Escherich, G., Pieters, R. & Den Boer, M.L. (2015b) BCR-ABL1-like cases in pediatric acute lymphoblastic leukemia: a comparison between DCOG/Erasmus MC and COG/St. Jude signatures. *Haematologica*, **100**, e354–e357.

Chen, I.M., Harvey, R.C., Mullighan, C.G., Gastier-Foster, J., Wharton, W., Kang, H., Borowitz, M.J., Camitta, B.M., Carroll, A.J., Devidas, M., Pullen, D.J., Payne-Turner, D., Tasian, S.K., Reshmi, S., Cottrell, C.E., Reaman, G.H., Bowman, W.P., Carroll, W.L., Loh, M.L., Winick, N.J., Hunger, S.P. & Willman, C.L. (2012) Outcome modeling with CRLF2, IKZF1, JAK, and minimal residual disease in pediatric acute lymphoblastic leukemia: a Children's Oncology Group study. *Blood*, **119**, 3512–3522.

Chiaretti, S., Li, X., Gentleman, R., Vitale, A., Wang, K.S., Mandelli, F., Foà, R. & Ritz, J. (2005) Gene expression profiles of B-lineage adult acute lymphocytic leukemia reveal genetic patterns that identify lineage derivation and distinct mechanisms of transformation. *Clinical Cancer Research*, **11**, 7209–7219.

Chiaretti, S., Brugnoletti, F., Messina, M., Paoloni, F., Fedullo, A.L., Piciocchi, A., Elia, L., Vitale, A., Mauro, E., Ferrara, F., De Fabritiis, P., Luppi, M., Ronco, F., De Propris, M.S., Raponi, S., Kronnie, G.T., Vignetti, M., Guarini, A. & Foà, R. (2016) CRLF2 overexpression identifies an unfavourable subgroup of adult B-cell precursor acute lymphoblastic leukemia lacking recurrent genetic abnormalities. *Leukemia Research*, **41**, 36–42.

Den Boer, M.L., van Slegtenhorst, M., De Menezes, R.X., Cheok, M.H., Buijs-Gladdines, J.G., Peters, S.T., Van Zutven, L.J., Beverloo, H.B., Van der Spek, P.J., Escherich, G., Horstmann, M.A., Janka-Schaub, G.E., Kamps, W.A., Evans, W.E. & Pieters, R. (2009) A subtype of childhood acute lymphoblastic leukaemia with poor treatment outcome: a genome-wide classification study. *The Lancet. Oncology*, **10**, 125–134.

Fasan, A., Kern, W., Nadarajah, N., Weber, S., Schindela, S., Schlenther, N., Schnittger, S., Haferlach, T. & Haferlach, C. (2015) Three steps to the diagnosis of adult Ph-like ALL. 57th ASH meeting, Orlando, December 5-8, 2015. *Blood*, **126**, 2610.

Francis, O.L., Milford, T.A., Martinez, S.R., Baez, I., Coats, J.S., Mayagoitia, K., Concepcion, K.R., Ginelli, E., Beldiman, C., Benitez, A., Weldon, A.J., Arogyaswamy, K., Shiraz, P., Fisher, R., Morris, C.L., Zhang, X.B., Filippov, V., Van Handel, B., Ge, Z., Song, C., Dovat, S., Su, R.J. & Payne, K.J. (2016) A novel xenograft model to study the role of TSLP-induced CRLF2 signals in normal and malignant human B lymphopoiesis. *Haematologica*, **101**, 417–426.

Ge, Z., Gu, Y., Zhao, G., Li, J., Chen, B., Han, Q., Guo, X., Liu, J., Li, H., Yu, M.D., Olson, J., Steffens, S., Payne, K.J., Song, C. & Dovat, S. (2016) High CRLF2 expression associates with IKZF1 dysfunction in adult acute lymphoblastic leukemia without CRLF2 rearrangement. *Oncotarget*, **7**, 49722–49732.

Haferlach, T., Kohlmann, A., Wieczorek, L., Basso, G., Kronnie, G.T., Béné, M.C., De Vos, J., Hernández, J.M., Hofmann, W.K., Mills, K.I., Gilkes, A., Chiaretti, S., Shurtleff, S.A., Kipps, T.J., Rassenti, L.Z., Yeoh, A.E., Papenhausen, P.R., Liu, W.M., Williams, P.M. & Foà, R. (2010) Clinical utility of microarray-based gene expression profiling in the diagnosis and subclassification of leukemia: report from the International Microarray Innovations in Leukemia Study Group. *Journal of Clinical Oncology*, **28**, 2529–2537.

Harris, P.A., Taylor, R., Thielke, R., Payne, J., Gonzalez, N. & Conde, J.G. (2009) Research electronic data capture (REDCap)–a metadata-driven methodology and workflow process for providing translational research informatics support. *Journal of Biomedical Informatics*, **42**, 377–381.

Harvey, R.C., Mullighan, C.G., Chen, I.M., Wharton, W., Mikhail, F.M., Carroll, A.J., Kang, H., Liu, W., Dobbin, K.K., Smith, M.A., Carroll, W.L., Devidas, M., Bowman, W.P., Camitta, B.M., Reaman, G.H., Hunger, S.P., Downing, J.R. & Willman, C.L. (2010a) Rearrangement of CRLF2 is associated with mutation of JAK kinases, alteration of IKZF1, Hispanic/Latino ethnicity, and a poor outcome in pediatric B-progenitor acute lymphoblastic leukemia. *Blood*, **115**, 5312–5321.

Harvey, R.C., Mullighan, C.G., Wang, X., Dobbin, K.K., Davidson, G.S., Bedrick, E.J., Chen, I.M., Atlas, S.R., Kang, H., Ar, K., Wilson, C.S., Wharton, W., Murphy, M., Devidas, M., Carroll, A.J., Borowitz, M.J., Bowman, W.P., Downing, J.R., Relling, M., Yang, J., Bhojwani, D., Carroll, W.L., Camitta, B., Reaman, G.H., Smith, M., Hunger, S.P. & Willman, C.L. (2010b) Identification of novel cluster groups in pediatric high-risk B-precursor acute lymphoblastic leukemia with gene expression profiling: correlation with genome-wide DNA copy number alterations, clinical characteristics, and outcome. *Blood*, **116**, 4874–4884.

Harvey, R.C., Kang, H., Roberts, K.G., Atlas, S.R., Bedrick, E.J., Gastier-Foster, J.M., Zhang, J., Gerhard, D.S., Smith, M.A., Larsen, E.C., Raetz, E.A., Winick, N.J., Carroll, W.L., Stonerock, E., Heerema, N.A., Carroll, A.J., Chen, S., Song, G., Becksfort, J., Rusch, M., Li, Y., Ma, J., Ell, D., Reshmi, S.C., Loh, M.L., Davidas, M., Hunger, S.P., Mullighan, C.G. & Willman, C.L. (2013) Development and validation of a highly sensitive and specific gene expression classifier to prospectively screen and identify B-precursor acute lymphoblastic leukemia (all) patients with a Philadelphia chromosome-like ("Ph-like" or "BCR-ABL1-Like") signature for therapeutic targeting and clinical intervention. 55th ASH meeting, New Orleans, December 7-10, 2013. *Blood*, **122**, 826.

Heatley, S.L., Sadras, T., Kok, C.H., Nievergall, E., Quek, K., Dang, P., McClure, B., Venn, N., Moore, S., Suttle, J., Law, T., Ng, A., Muskovic, W., Norris, M.D., Revesz, T., Osborn, M., Moore, A.S., Suppiah, R., Fraser, C., Alvaro, F., Hughes, T.P., Mullighan, C.G., Marshall, G.M., Pozza, L.D., Yeung, D.T., Sutton, R. & White, D.L. (2017) High prevalence of relapse in children with Philadelphia-like acute lymphoblastic leukemia despite risk-adapted treatment. *Haematologica*, **102**, e490–e493.

Herold, T., Schneider, S., Metzeler, K., Neumann, M., Hartmann, L., Roberts, K.G., Konstandin, N.P., Greif, P.A., Bräundl, K., Ksienzyk, B., Huk, N., Schneider, I., Zellmeier, E., Jurinovic, V., Mansmann, U., Hiddemann, W., Mullighan, C.G., Bohlander, S.K., Spiekermann, K., Hoelzer, D., Brüggemann, M., Baldus, C.D., Dreyling, M. & Gökbuget, N. (2017) Philadelphia chromosome-like acute lymphoblastic leukemia in adults have frequent IGH-CRLF2 and JAK2 mutations, persistence of minimal residual disease and poor prognosis. *Haematologica*, **102**, 130–138.

Jabbour, E., Kantarjian, H., Ravandi, F., Thomas, D., Huang, X., Faderl, S., Pemmaraju, N., Daver, N., Garcia-Manero, G., Sasaki, K., Cortes, J., Garris, R., Yin, C.C., Khoury, J.D., Jorgensen, J., Estrov, Z., Bohannan, Z., Konopleva, M., Kadia, T., Jain, N., DiNardo, C., Wierda, W., Jeanis, V. & O'Brien, S. (2015) Combination of hyper-CVAD with ponatinib as first-line therapy for patients with Philadelphia chromosome-positive acute lymphoblastic leukaemia: a single-centre, phase 2 study. *The Lancet. Oncology*, **16**, 1547–1555.

Jain, N., Roberts, K.G., Jabbour, E., Patel, K., Eterovic, A.K., Chen, K., Zweidler-McKay, P., Lu, X., Fawcett, G., Wang, S.A., Konoplev, S., Harvey, R.C., Chen, I.M., Payne-Turner, D., Valentine, M., Thomas, D., Garcia-Manero, G., Ravandi, F., Cortes, J., Kornblau, S., O'Brien, S., Pierce, S., Jorgensen, J., Shaw, K.R., Willman, C.L., Mullighan, C.G., Kantarjian, H. & Konopleva, M. (2017a) Ph-like acute lymphoblastic leukemia: a high-risk subtype in adults. *Blood*, **129**, 572–581.

Jain, N., Jabbour, E., Zweidler-McKay, P., Ravandi, F., Takahashi, K., Kadia, T., Wierda, W.G., Rytting, M.E., Nunez, C., Patel, K., Lu, X., Tang, G., Konoplev, S., Wang, S.A., Han, L., Thakral, B., Deshmukh, A., Garris, R., Jorgensen, J.L., Cavazos, A., Verstovsek, S., Garcia-Manero, G., O'Brien, S., Cortes, J., Mullighan, C.G., Kantarjian, H. & Konopleva, M. (2017b) Ruxolitinib or dasatinib in combination with chemotherapy for patients with relapsed/refractory philadelphia (Ph)-like acute lymphoblastic leukemia: a phase I-II trial. *Blood*, **130**, 1322.

Lengline, E., Beldjord, K., Dombret, H., Soulier, J., Boissel, N. & Clappier, E. (2013) Successful tyrosine kinase inhibitor therapy in a refractory B-cell precursor acute lymphoblastic leukemia with EBF1-PDGFRB fusion. *Haematologica*, **98**, e146–e148.

Maude, S.L., Tasian, S.K., Vincent, T., Hall, J.W., Sheen, C., Roberts, K.G., Seif, A.E., Barrett,

D.M., Chen, I.M., Collins, J.R., Mullighan, C.G., Hunger, S.P., Harvey, R.C., Willman, C.L., Fridman, J.S., Loh, M.L., Grupp, S.A. & Teachey, D.T. (2012) Targeting JAK1/2 and mTOR in murine xenograft models of Ph-like acute lymphoblastic leukemia. *Blood*, **120**, 3510–3518.

Messina, M., Chiaretti, S., Tavolaro, S., Peragine, N., Vitale, A., Elia, L., Sica, S., Levis, A., Guarini, A. & Foà, R. (2010) Protein kinase gene expression profiling and *in vitro* functional experiments identify novel potential therapeutic targets in adult acute lymphoblastic leukemia. *Cancer*, **116**, 3426–3437.

Messina, M., Chiaretti, S., Wang, J., Fedullo, A.L., Peragine, N., Gianfelici, V., Piciocchi, A., Brugnoletti, F., Di Giacomo, F., Pauselli, S., Holmes, A.B., Puzzolo, M.C., Ceglie, G., Apicella, V., Mancini, M., Te Kronnie, G., Testi, A.M., Vitale, A., Vignetti, M., Guarini, A., Rabadan, R. & Foà, R. (2016) Prognostic and therapeutic role of targetable lesions in B-lineage acute lymphoblastic leukemia without recurrent fusion genes. *Oncotarget*, **7**, 13886–138901.

Messina, M., Chiaretti, S., Fedullo, A.L., Piciocchi, A., Puzzolo, M.C., Lauretti, A., Gianfelici, V., Apicella, V., Fazi, P., Te Kronnie, G., Testi, A.M., Vitale, A., Guarini, A. & Foà, R. (2017) Clinical significance of recurrent copy number aberrations in B-lineage acute lymphoblastic leukaemia without recurrent fusion genes across age cohorts. *British Journal of Haematology*, **178**, 583–587.

Mullighan, C.G., Su, X., Zhang, J., Radtke, I., Phillips, L.A., Miller, C.B., Ma, J., Liu, W., Cheng, C., Schulman, B.A., Harvey, R.C., Chen, I.M., Clifford, R.J., Carroll, W.L., Reaman, G., Bowman, W.P., Devidas, M., Gerhard, D.S., Yang, W., Relling, M.V., Shurtleff, S.A., Campana, D., Borowitz, M.J., Pui, C.H., Smith, M., Hunger, S.P., Willman, C.L. & Downing, J.R.; Children's Oncology Group. (2009a) Deletion of IKZF1 and prognosis in acute lymphoblastic leukemia. *New England Journal of Medicine*, **360**, 470–480.

Mullighan, C.G., Collins-Underwood, J.R., Phillips, L.A., Loudin, M.G., Liu, W., Zhang, J., Ma, J., Coustan-Smith, E., Harvey, R.C., Willman, C.L., Mikhail, F.M., Meyer, J., Carroll, A.J., Williams, R.T., Cheng, J., Heerema, N.A., Basso, G., Pession, A., Pui, C.H., Raimondi, S.C., Hunger, S.P., Downing, J.R., Carroll, W.L. & Rabin, K.R. (2009b) Rearrangement of CRLF2 in B-progenitor and Down syndrome-associated acute lymphoblastic leukemia. *Nature Genetics*, **41**, 1243–1246.

Mullighan, C.G., Zhang, J., Harvey, R.C., Collins-Underwood, J.R., Schulman, B.A., Phillips, L.A., Tasian, S.K., Loh, M.L., Su, X., Liu, W., Devidas, M., Atlas, S.R., Chen, I.M., Clifford, R.J., Gerhard, D.S., Carroll, W.L., Reaman, G.H., Smith, M., Downing, J.R., Hunger, S.P. & Willman, C.L. (2009c) JAK mutations in high-risk childhood acute lymphoblastic leukemia. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 9414–9418.

Nicorici, D., Satalan, M., Edgren, H., Kangaspeska, S., Murumagi, A., Kallioniemi, O., Virtanen, S. & Kilkku, O. (2014). FusionCatcher – a tool for finding somatic fusion genes in paired-end RNA-sequencing data. *bioRxiv*, 011650. https://doi.org/10.1101/011650

Ofran, Y. & Izraeli, S. (2017) BCR-ABL (Ph)-like acute leukemia-pathogenesis, diagnosis and therapeutic options. *Blood Reviews*, **31**, 11–16.

Reshmi, S.C., Harvey, R.C., Roberts, K.G., Stonerock, E., Smith, A., Jenkins, H., Chen, I.M., Valentine, M., Liu, Y., Li, Y., Shao, Y., Easton, J., Payne-Turner, D., Gu, Z., Tran, T.H., Nguyen, J.V., Devidas, M., Dai, Y., Heerema, N.A., Carroll, A.J. 3rd, Raetz, E.A., Borowitz, M.J., Wood, B.L., Angiolillo, A.L., Burke, M.J., Salzer, W.L., Zweidler-McKay, P.A., Rabin, K.R., Carroll, W.L., Zhang, J., Loh, M.L., Mullighan, C.G., Willman, C.L., Gastier-Foster, J.M. & Hunger, S.P. (2017) Targetable kinase gene fusions in high-risk B-ALL: a study from the Children's Oncology Group. *Blood*, **129**, 3352–3361.

Roberts, K.G., Morin, R.D., Zhang, J., Hirst, M., Zhao, Y., Su, X., Chen, S.C., Payne-Turner, D., Churchman, M.L., Harvey, R.C., Chen, X., Kasap, C., Yan, C., Becksfort, J., Finney, R.P., Teachey, D.T., Maude, S.L., Tse, K., Moore, R., Jones, S., Mungall, K., Birol, I., Edmonson, M.N., Hu, Y., Buetow, K.E., Chen, I.M., Carroll, W.L., Wei, L., Ma, J., Kleppe, M., Levine, R.L., Garcia-Manero, G., Larsen, E., Shah, N.P., Devidas, M., Reaman, G., Smith, M., Paugh, S.W., Evans, W.E., Grupp, S.A., Jeha, S., Pui, C.H., Gerhard, D.S., Downing, J.R., Willman, C.L., Loh, M., Hunger, S.P., Marra, M.A. & Mullighan, C.G. (2012) Genetic alterations activating kinase and cytokine receptor signaling in high-risk acute lymphoblastic leukemia. *Cancer Cell*, **22**, 153–166.

Roberts, K.G., Li, Y., Payne-Turner, D., Harvey, R.C., Yang, Y.L., Pei, D., McCastlain, K., Ding, L., Lu, C., Song, G., Ma, J., Becksfort, J., Rusch, M., Chen, S.C., Easton, J., Cheng, J., Boggs, K., Santiago-Morales, N., Iacobucci, I., Fulton, R.S., Wen, J., Valentine, M., Cheng, C., Paugh, S.W., Devidas, M., Chen, I.M., Reshmi, S., Smith, A., Hedlund, E., Gupta, P., Nagahawatte, P., Wu, G., Chen, X., Yergeau, D., Vadodaria, B., Mulder, H., Winick, N.J., Larsen, E.C., Carroll, W.L., Heerema, N.A., Carroll, A.J., Grayson, G., Tasian, S.K., Moore, A.S., Keller, F., Frei-Jones, M., Whitlock, J.A., Raetz, E.A., White, D.L., Hughes, T.P., Guidry Auvil, J.M., Smith, M.A., Marcucci, G., Bloomfield, C.D., Mrózek, K., Kohlschmidt, J., Stock, W., Kornblau, S.M., Konopleva, M., Paietta, E., Pui, C.H., Jeha, S., Relling, M.V., Evans, W.E., Gerhard, D.S., Gastier-Foster, J.M., Mardis, E., Wilson, R.K., Loh, M.L., Downing, J.R., Hunger, S.P., Willman, C.L., Zhang, J. & Mullighan, C.G. (2014a) Targetable kinase-activating lesions in Ph-like acute lymphoblastic leukemia. *New England Journal of Medicine*, **371**, 1005–1115.

Roberts, K.G., Pei, D., Campana, D., Payne-Turner, D., Li, Y., Cheng, C., Sandlund, J.T., Jeha, S., Easton, J., Becksfort, J., Zhang, J., Coustan-Smith, E., Raimondi, S.C., Leung, W.H., Relling, M.V., Evans, W.E., Downing, J.R., Mullighan, C.G. & Pui, C.H. (2014b) Outcomes of children with BCR-ABL1–like acute lymphoblastic leukemia treated with risk-directed therapy based on the levels of minimal residual disease. *Journal of Clinical Oncology*, **32**, 3012–3020.

Roberts, K.G., Yang, Y., Payne-Turner, D., Harvey, R.C., Chen, I.M., Reshmi, S.C., Gastier-Foster, J., Loh, M.L., Willman, C.L., Hunger, S.P. & Mullighan, C.G. (2014c) Functional analysis of kinase-activating fusions in Ph-like acute lymphoblastic leukemia. *Blood*, **124**, 786.

Roberts, K.G., Gu, Z., Payne-Turner, D., McCastlain, K., Harvey, R.C., Chen, I.M., Pei, D., Iacobucci, I., Valentine, M., Pounds, S.B., Shi, L., Li, Y., Zhang, J., Cheng, C., Rambaldi, A., Tosi, M., Spinelli, O., Radich, J.P., Minden, M.D., Rowe, J.M., Luger, S., Litzow, M.R., Tallman, M.S., Wiernik, P.H., Bhatia, R., Aldoss, I., Kohlschmidt, J., Mrózek, K., Marcucci, G., Bloomfield, C.D., Stock, W., Kornblau, S., Kantarjian, H.M., Konopleva, M., Paietta, E., Willman, C.L. & Mullighan, C.G. (2017) High frequency and poor outcome of philadelphia chromosome-like acute lymphoblastic leukemia in adults. *Journal of Clinical Oncology*, **35**, 394–401.

Shi, C., Han, L., Tabe, Y., Hong, Mu, Wu, S., Zhou, J., Zeng, Z., Fruman, D.A., Tasian, S.K., Weinstock, D.M. & Konopleva, M. (2014) Dual targeting of JAK2 signaling with a type II JAK2 inhibitor and of mTOR with a TOR kinase inhibitor induces apoptosis in CRLF2-rearranged Ph-like acute lymphoblastic leukemia. *Blood*, **124**, 3706.

Shi, C., Han, L., Zhang, Q., Roberts, K.G., Park, E., Tabe, Y., Jacamo, R.O., Mu, H., Wu, S., Zhou, J., Ma, H., Zeng, Z., Jain, N., Jabbour, E.J., Muschen, M., Tasian, S.K., Mullighan, C.G., Weinstock, D.M., Fruman, D. & Konopleva, M. (2015) Combined targeting of JAK2 with a type II JAK2 inhibitor and mTOR with a TOR kinase inhibitor constitutes synthetic activity in JAK2-driven Ph-like acute lymphoblastic leukemia. *Blood*, **126**, 2529.

Tasian, S.K., Doral, M.Y., Borowitz, M.J., Wood, B.L., Chen, I.M., Harvey, R.C., Gastier-Foster, J.M., Willman, C.L., Hunger, S.P., Mullighan, C.G. & Loh, M.L. (2012) Aberrant STAT5 and PI3K/mTOR pathway signaling occurs in human CRLF2-rearranged B-precursor acute lymphoblastic leukemia. *Blood*, **120**, 833–842.

Tokunaga, K., Yamaguchi, S., Iwanaga, E., Nanri, T., Shimomura, T., Suzushima, H., Mitsuya, H. & Asou, N. (2013) High frequency of IKZF1 genetic alterations in adult patients with B-cell acute lymphoblastic leukemia. *European Journal of Haematology*, **91**, 201–208.

van der Veer, A., Waanders, E., Pieters, R., Willemse, M.E., Van Reijmersdal, S.V., Russell, L.J., Harrison, C.J., Evans, W.E., van der Velden,

V.H., Hoogerbrugge, P.M., Van Leeuwen, F., Escherich, G., Horstmann, M.A., Mohammadi Khankahdani, L., Rizopoulos, D., De Groot-Kruseman, H.A., Sonneveld, E., Kuiper, R.P. & Den Boer, M.L. (2013) Independent prognostic value of BCR-ABL1-like signature and IKZF1 deletion, but not high CRLF2 expression, in children with B-cell precursor ALL. *Blood*, **122**, 2622–2629.

Weston, B.W., Hayden, M.A., Roberts, K.G., Bowyer, S., Hsu, J., Fedoriw, G., Rao, K.W. & Mullighan, C.G. (2013) Tyrosine kinase inhibitor therapy induces remission in a patient with refractory EBF1-PDGFRB-positive acute lymphoblastic leukemia. *Journal of Clinical Oncology*, **31**, e413–e416.

Yoda, A., Yoda, Y., Chiaretti, S., Bar-Natan, M., Mani, K., Rodig, S.J., West, N., Xiao, Y., Brown, J.R., Mitsiades, C., Sattler, M., Kutok, J.L., DeAngelo, D.J., Wadleigh, M., Piciocchi, A., Dal Cin, P., Bradner, J.E., Griffin, J.D., Anderson, K.C., Stone, R.M., Ritz, J., Foà, R., Aster, J.C., Frank, D.A. & Weinstock, D.M. (2010) Functional screening identifies CRLF2 in precursor B-cell acute lymphoblastic leukemia. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 252–257.