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IGJ and SPATS2L immunohistochemistry sensitively and specifically identify *BCR::ABL1*+ and *BCR::ABL1*-like B-acute lymphoblastic leukaemia

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Summary

Therapeutic management and prognostication for patients with B-acute lymphoblastic leukaemia (B-ALL) require appropriate disease subclassification. *BCR::ABL1*-like B-ALL is unique in that it is defined by a gene expression profile similar to *BCR::ABL1*+ B-ALL rather than a unifying recurrent translocation. Current molecular/cytogenetic techniques to identify this subtype are expensive, not widely accessible, have long turnaround times and/or require an adequate liquid biopsy. We have studied a total of 118 B-ALL cases from three institutions in two laboratories to identify surrogates for *BCR::ABL1*+/like B-ALL. We report that immunoglobulin joining chain (IGJ) and spermatogenesis associated serine-rich 2-like (SPATS2L) immunohistochemistry (IHC) sensitively and specifically identify *BCR::ABL1*+/like B-ALL. IGJ IHC positivity has a sensitivity of 83%, a specificity of 95%, a positive predictive value (PPV) of 89% and a negative predictive value (NPV) of 90%. SPATS2L staining has similar sensitivity and NPV but lower specificity (85%) and PPV (70%). The presence of either IGJ or SPATS2L staining augments the sensitivity (93%) and NPV (95%). While these findings would need to be validated in larger studies, they suggest that IGJ and/or SPATS2L IHC may be utilized in identifying *BCR::ABL1*-like B-ALL or in selecting B-ALL cases for confirmatory molecular/genetic testing, particularly in resource-limited settings.

KEYWORDS
B-ALL, *BCR::ABL1*, *BCR::ABL1-like*, IGJ, SPATS2L

INTRODUCTION

B-acute lymphoblastic leukaemia (B-ALL) is the most common malignancy in the paediatric population globally.[1] In the United States, B-ALL comprises about 25% of paediatric cancers, and about 40% of total B-ALL cases occur in adults. Classification of B-ALL is largely based on the identification of recurrent cytogenetic abnormalities such as hyperdiploidy, hypodiploidy, t(9;22) with *BCR::ABL1*, t(12;21) with *ETV6::RUNX1*, t(1;19) with *TCF3::PBX1*, *KMT2A* rearranged (*KMT2A*-R), t(5;14) with *IGH::IL3* and intrachromosomal amplification of *RUNX1* on chromosome 21 (iAMP21). Newer subtypes recognized in the 2017 World Health Organization (WHO) classification and the 2022 proposed WHO and International Consensus Classification (ICC) classifications[2–5] are less amenable to routine

cytogenetic testing for their identification. Considering the important role of B-ALL classification in risk stratification and guiding up front or subsequent risk-adapted or targeted management decisions,[6–8] techniques to identify these new B-ALL subtypes in a rapid and cost-effective manner would be clinically significant.

BCR::ABL1-like B-ALL has a gene expression (transcriptome) profile similar to *BCR::ABL1*+ B-ALL but lacking the *BCR::ABL1* translocation.[9,10] Subsequent studies have demonstrated that about 90% of *BCR::ABL1*-like B-ALL cases harbour other genetic alterations resulting in activation of kinase and cytokine receptor signalling, most commonly rearrangements involving *CRLF2* and fewer cases with rearrangements involving *ABL1* (non-*BCR* partner), *EPOR* and other genes.[11] *BCR::ABL1*+ and *BCR::ABL1*-like B-ALL are considered high-risk B-ALL subtypes and together comprise about 10%–20% of paediatric and about 50% of adult B-ALL.[9,12,13] Classification of a new B-ALL into one of these subtypes allows identification of patients who may benefit from targeted kinase inhibitor therapy either as a part of an established standard of care (for *BCR::ABL1*+ B-ALL) or emerging treatments in the context of a clinical trial (for *BCR::ABL1*-like B-ALL).[14,15] *BCR::ABL1* identification by karyotype, fluorescence in situ hybridization (FISH) or PCR is routine in many diagnostic laboratories. However, identification of *BCR::ABL1*-like B-ALL is less straightforward. DNA- or RNA-based techniques such as FISH, RNA sequencing and gene expression arrays to identify either structural abnormalities prevalent in *BCR::ABL1*-like B-ALL or to identify its defining gene expression programme have been utilized.[16,17] However, these techniques are expensive, not widely accessible, may have long turn-around times, rely on an adequate marrow aspirate sample or the presence of circulating blasts, may be susceptible to RNA stability concerns and/or lack of correlative spatial information.

Immunohistochemistry (IHC) is a rapid, widely available and relatively low cost means of evaluating protein expression on tissue sections and thus could circumvent or mitigate many of the challenges in identifying *BCR::ABL1*-like B-ALL if sensitive/specific markers are identified. We hypothesized that genes upregulated at the protein level may serve as useful IHC markers and have recently shown that MUC4 IHC is specific for *BCR::ABL1*+/like B-ALL, albeit not very sensitive.[18] In the current study, we have tested protein expression of *IGJ* and *SPATS2L*, both previously shown to be upregulated at the mRNA level in *BCR::ABL1*+/like B-ALL relative to other subtypes,[10,19,20] which is incorporated in currently utilized or reported targeted low density array (LDA) or quantitative real-time PCR assays for identifying *BCR::ABL1*-like B-ALL.[21–26]

The *IGJ* expression in B-ALL is paradoxical for two reasons: First, *IGJ* is not appreciably expressed in normal committed B lineage precursors, and second, the known function of IGJ protein is in mature B cells/plasma cells, where it concatenates IgA and IgM monomers into multimeric mature forms and is involved in mucosal transport of these secreted immunoglobulins.[27,28] *SPATS2L* is an understudied gene with putative roles in ribosome biogenesis and response to oxidative stress[29] and no established function in the haematopoietic system. IGJ IHC is utilized as a reliable marker for neoplastic cells of nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL).[30–32] Although SPATS2L antibodies are commercially available, there is no current diagnostic use for SPATS2L IHC.

We demonstrate in this report that IHC for IGJ and SPATS2L sensitively and specifically identifies *BCR::ABL1*+/like B-ALL.

MATERIALS AND METHODS

Patient selection

Patients diagnosed with de novo B-ALL at University Hospitals (UH; 100 cases) and with available bone marrow (BM) biopsies in formalin fixed paraffin embedded (FFPE) blocks were identified as previously described.[18] Additional cases from Boston Children Hospital (BCH; 9 cases) and the University of Pittsburgh Medical Center (UPMC; 9 cases), most *BCR::ABL1*-like, were also included. In all but seven cases, the initial diagnostic BM biopsy was utilized. For seven cases, the initial biopsy was not available or was inadequate, and a relapse/refractory disease sample was used instead. The study was performed in accordance with respective Institutional Review Board approvals.

B-ALL diagnosis and classification

B-ALL diagnosis and classification were made by board-certified hematopathologists based on morphological, immunophenotypic and molecular/genetic characteristics as described previously.[18] Targeted archer-based RNA next generation sequencing (NGS), DNA NGS and/or single nucleotide polymorphism microarray (SNP array) to identify *BCR::ABL1*-like translocations or reveal gene copy number variants/mutations was performed in a subset of patients. Although all *CRLF2*-rearranged (*CRLF2*-R) cases evaluated showed CRLF2 overexpression by flow cytometry or at the mRNA level, overexpression of CRLF2 alone without a corroborating genetic abnormality was not used to designate a case as *BCR::ABL1*-like since overexpression can rarely be observed in non-*CRLF2*-R/non-*BCR::ABL1*-like B-ALL.[33,34]

Of the 118 B-ALL cases evaluated, 95 were classifiable into categories conforming to WHO 2017, WHO 2022 and ICC 2022 criteria after standard cytogenetics and *BCR::ABL1*-like testing. Sophisticated DNA/RNA sequencing, transcriptome evaluation and specialized FISH testing to identify molecular/genetic abnormalities defining newly described definitive and provisional rare B-ALL subtypes in the most recently proposed WHO 2022 & ICC 2022 classifications,[3–5] many of which are cryptic to routine testing, were not performed for most cases. Also, since *BCR::ABL1* FISH was performed on total bone marrow cells and not on

separately sorted myeloid and lymphoid cells, *BCR::ABL1*+ B-ALL were not further subtyped into those with 'lymphoid only' versus 'multilineage' involvement (ICC 2022[3,4]). The 23 patients who could not be classified into a specific B-ALL type were designated 'Not Further Specified' (NFS). The NFS terminology is employed to avoid confusion with the WHO 2022/ICC 2022 B-ALL, NOS category, since such designation depends on the exclusion of other defined types of B-ALL, which in turn depends on the degree of comprehensive molecular testing performed.

Immunohistochemistry

IHC for IGJ and SPATS2L were performed at UH on BM FFPE sections as previously described.[18] For IGJ, a mouse monoclonal antibody (clone OTI3B3; Invitrogen; 1:3200 dilution) was used as the primary antibody. Reactive tonsil or lymph nodes involved by NLPHL[30] were used for antibody validation and as technical controls. IGJ IHC at UPMC was performed as previously described (Clone 3B3, Lifespan Biosciences, 1:3000 dilution).[30] For SPATS2L, a rabbit polyclonal antibody (Invitrogen; 1:250 dilution) was used as a primary. Hippocampus sections were used for antibody validation and for technical controls.

For IGJ, cellular staining of lymphoblasts was distinguishable from occasional background staining (possibly attributable to serum immunoglobulins as occurs frequently in immunoglobulin heavy or light chain IHC) and scored in a blinded manner by two board-certified haematopathologists or one board-certified haematopathologist and one to two pathologists in training as diffusely positive, partially positive, or negative. The intensity of staining was also recorded as weak or strong. Staining was considered diffuse when over 50% of the blasts were stained. Most negative cases showed no staining. Few cases with very rare cells (less than 10%) showing weak staining were also scored as negative (i.e. partial staining was 10%–50% of blasts).

Additional Materials and Methods are provided in a Supplementary document.

RESULTS

Sensitive and specific identification of *BCR::ABL1*+/like B-ALL by IGJ IHC

To determine whether *BCR::ABL1*+/like express IGJ protein and explore the utility of IGJ IHC in identifying these B-ALL subtypes, we stained BM biopsy samples from 116 cases from three institutions and across a wide age range (1–82 years), including 64 paediatrics (<18 years), 20 adolescent-young adults (AYA; 18–39 years) and 32 older adults (>39 years). The cohort was comprised of 23 *BCR::ABL1*-like, 17 *BCR::ABL*+ and 75 non-*BCR::ABL1*+/like (including 21 hyperdiploid, 13 *ETV6::RUNX1*, 5 *TCF3::PBX1*, 4 hypodiploid, 7 *KMT2A*-rearranged, 2 iAMP21, 1 t(5;14) and 23 NFS). We also stained 11 normal bone marrow cases. IGJ IHC was positive in 20/23 (87%) of *BCR::ABL1*-like, 13/17 (76%) *BCR::ABL1*+ B-ALL and 4/75 (5%) non-*BCR::ABL1*+/like (Figure 1, Table 1).

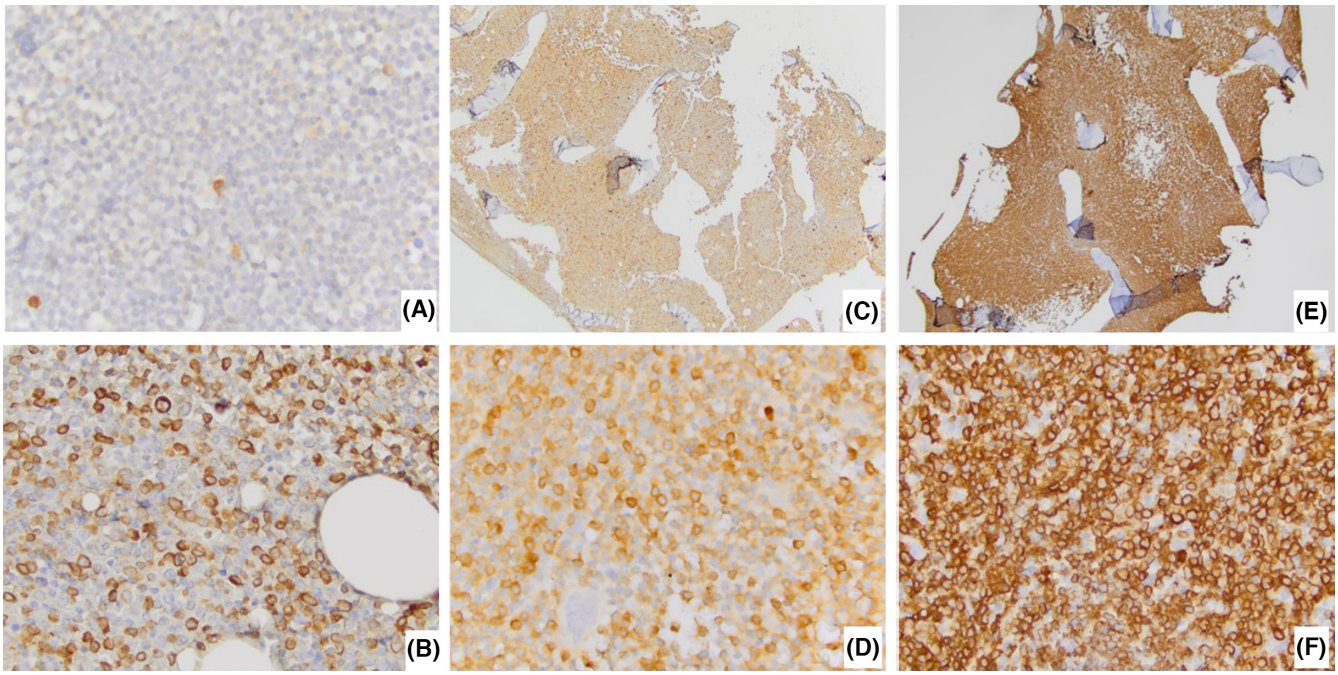

FIGURE 1 Variable expression of IGJ protein in B-ALL. Representative images of the IgJ IHC. (A) Negative, 400×, UH-077. A rare positive plasma cell is present. (B) Partial positive, 400×, UH-005. (C) Diffuse weak positive, 40×, UH-025. (D) Diffuse weak positive, 400×, UH-025. (E) Diffuse strong positive, 40×, UH-012. (F) Diffuse strong positive, 400×, UH-012.

TABLE 1 Summary of IgJ IHC results by B-ALL subtype.

Subtype	Total # of cases	IgJ+	Diffuse staining	Partial staining
BCR::ABL1-like	23	20 (87%)	13	7
BCR::ABL1+	17	13 (76%)	10	3
Hyperdiploidy	21	0	0	0
ETV6::RUNX1	13	2 (15%)	0	2
TCF3::PBX1	5	0	0	0
Hypodiploidy	4	0	0	0
KMT2A-rearranged	7	1 (14%)	0	1
iAMP21	2	0	0	0
t(5;14)	1	0	0	0
NFS	23	1 (4%)	0	1

Abbreviations: B-ALL, B-acute lymphoblastic leukaemia; NFS, not further specified.

Diffuse positive staining was only present in the *BCR::ABL1*-like (13/20) and *BCR::ABL1*+ (10/13) groups. All the control normal bone marrows were negative.

The *BCR::ABL1*-like cases (Table 2) were mostly *CRLF2*-R, and IGJ staining was independent of CRLF2 partner—IGJ+ in 8/9 *IGH::CRLF2* (7 diffuse, 1 partial staining) and 8/10 *P2RY8::CRLF2* (4 diffuse, 4 partial staining). Importantly, there were three non-CRLF2-R *BCR::ABL1*-like cases—one with a *JAK2* rearrangement (BCH-9), one with an *IGH::EPOR* rearrangement (UPMC-4) and one with an *ETV6::ABL1* rearrangement (BCH-10)—and they were all IGJ+. All three *BCR::ABL1*-like cases, which were IGJ− (UH-006, BCH3, BCH4) and *CRLF2*-R, also had a *CDKN2A* loss, but *CDKN2A* loss was also seen in a subset (4/14, 29%) of IGJ+ *BCR::ABL1*-like cases. For *BCR::ABL1*+ (Table 3), all 5/5 (100%) cases with a major breakpoint were diffuse IGJ+, while staining in the 10 cases with a minor breakpoint was more variable (4 diffuse IGJ+, 3 partial IGJ+, 3 IGJ−; $p = 0.04$). Nevertheless, the prevalent IGJ+ result in *BCR::ABL1*+ B-ALL further indicates that IGJ+ is not restricted to *CRLF2*-R or JAK–STAT-activated B-ALL, despite the few non-CRLF2-R *BCR::ABL1*-like cases in our cohort. No consistent distinguishing features were seen in the *BCR::ABL1*+/like cases negative for IGJ IHC.

Intriguingly, four non-*BCR::ABL1*+/like cases were also IGJ+, all with partial staining. These included two *ETV6::RUNX1* cases (UH-047 & BCH-2), one *KMT2A*-R case (UH-072) and one NFS case (UH-100). *BCR::ABL1*-like testing in two of these cases revealed a concomitant *P2RY8::CRLF2* in BCH-2, which has been reported before,[35,36] and no rearrangements in UH-100. *BCR::ABL1*-like testing performed on 24 other non-*BCR::ABL1*-like cases showed three additional cases with a co-occurring *P2RY8::CRLF2* rearrangement—BCH-6 (hyperdiploidy), UPMC-7 (hyperdiploidy) and UH-076 (iAMP21)—all of which were IGJ−. Therefore, co-occurring *CRLF2*-R or other *BCR::ABL1*-like genetic abnormality does not appear to explain the few non-*BCR::ABL1*+/like cases with IGJ+ staining. UH-072 differed from the other *KMT2A*-R cases in lacking the characteristic CD10−, CD15+ phenotype of most *KMT2A*-R B-ALL[37] and instead having a mature immunophenotype (Tdt-, CD34−, CD20+, dim surface lambda light chain) as described in a minority of *KMT2A*-R B-ALL cases.[38–41] No distinguishing features were seen between the IGJ+ and the respective IGJ− *ETV6::RUNX1* and NFS cases. We also did not identify any unifying features in IGJ+ non-*BCR::ABL1*+/like cases or between these cases and the *BCR::ABL1*+/like cases.

In summary, IGJ protein expression was significantly associated with *BCR::ABL1*+/like B-ALL, identifying these subtypes with an 83% sensitivity, 95% specificity, 89% PPV and 90% NPV ($p < 0.0001$). For cases that were unclassifiable after routine cytogenetic testing, the PPV was even better (19/20 IGJ IHC-positive cases were *BCR::ABL1*-like; PPV of 95%), indicating IGJ IHC could provide added value to routine cytogenetics for the identification of *BCR::ABL1*-like cases. Importantly, the data also indicates that *CRLF2* rearrangement is neither necessary nor sufficient for IGJ protein expression.

SPATS2L IHC augments the sensitivity of identifying *BCR::ABL1*+/like B-ALL

We tested SPATS2L protein expression by IHC in 104 B-ALL cases from UH and BCH (Figure 2, Table 4). SPATS2L was positive in 14/17 (82%) *BCR::ABL1*+ B-ALL and 12/15 (80%) *BCR::ABL1*-like B-ALL, including both non-*CRLF2*-R cases tested (BCH-9 with *JAK2*-R and BCH-10 with *ETV6::ABL1*). SPATS2L IHC was positive in 11/72 (15%) non-*BCR::ABL1*-like cases, which included 6 NFS, 2 *ETV6::RUNX1*, 2 hypodiploidy, 1 *KMT2A*-R. Most of the SPATS2L-positive cases (34/37) showed diffuse staining. Diffuse staining in *BCR::ABL1*+/like cases predominantly (21/23 cases) showed a strong intensity, while diffuse staining in non-*BCR::ABL1*+/like cases (6/11 cases) was mostly weak ($p = 0.007$). None of the B-ALL cases with hyperdiploidy (0/19), *TCF3::PBX1* (0/5), iAMP21 (0/2) or t(5;14) (0/1) were SPATS2L+. No SPATS2L staining was seen in all three normal bone marrows evaluated.

Although IGJ and SPATS2L stain results were mostly concordant (83% concordance, $p < 0.0001$), there was some discordant staining (Figure 3). Specifically, 6/31 *BCR::ABL1*+/like (2 IGJ+SPATS2L−, 4 IGJ−SPATS2L+) and 11/72 non-*BCR::ABL1*+/like (2 IGJ+SPATS2L−, 9 IGJ−SPATS2L+) cases showed discordant staining. Concordant cases included *BCR::ABL1*+/like cases (UH-006, UH-011, BCH-4) negative for both IGJ and SPATS2L and 2 non-*BCR::ABL1*+/like cases (UH-047 and UH-100) positive for both stains. The other two IGJ+ non-BCR::ABL1+/like cases (BCH-2 and UH-072) were SPATS2L negative. All non-*BCR::ABL1*-like cases with a concomitant *P2RY8::CRLF2* rearrangement evaluated—UH-076 (iAMP21), BCH-2 (*ETV6::RUNX1*) and BCH-6 (hyperdiploidy)—were negative for SPATS2L.

Overall, IGJ and SPATS2L IHC have comparable sensitivity (81%–83%) and negative predictive value (91%) for identifying *BCR::ABL1*+/like cases. However, IGJ has far superior

TABLE 2 *BCR::ABL1*-like cases.

Case #	Age (years)	ICC 2022 BCR::ABL1-like class	Positive BCR::ABL1-like genetic/molecular testing	CRLF2 overexpression?	Karyotype	Downs?	Additional positive genetics findings
UH-001	49	JAK–STAT	CRLF2-R (FISH)	Y (flow)	46,XY,del(6)(q13q23),add(19)(q13.4)[12]/46,XY[10]	N	CRLF2 gain (FISH)
UH-002	80	JAK–STAT	CRLF2-R (FISH)	Y (flow)	CF	N	None
UH-003	44	JAK–STAT	Loss of 5′ (FISH)	Y (flow)	46,XY[20]	N	None
UH-004	41	JAK–STAT	CRLF2-R (FISH), JAK2 L681_I682insGQD (NGS)	Y (flow, mRNA)	CF	N	Non-productive KMT2A-R (FISH). IKZF1 loss (NGS).
UH-005	37	JAK–STAT	CRLF2-R (FISH)	Y (flow)	46,Y,t(X;20)(p22;q13.3),del(1)(q32q42),i(7)q10)[15]/46,XY[5]	N	None
UH-006	31	JAK–STAT	CRLF2-R (FISH)	Y (flow)	47,XY,+21c[25]	Y	CRLF2 gain and CDKN2A loss (FISH)
UH-007	19	JAK–STAT	Loss of 5′ (FISH), Xp22.3 del (SNP array)	Y (flow)	47,XY,+21c[8]/48,idem,+X[12]	Y	IKZF1 and CDKN2A losses (SNP array)
UH-008	4	JAK–STAT	P2RY8-CRLF2+ (PCR), JAK2 p.R683S (NGS), Xp22.3 del (SNP array), LDA+	Y (flow, mRNA)	46,XX,dic(9;20)(p13.2;q11.2),+21[5]/47,idem,+20[3]/46,XX[2]	N	CDKN2A loss (FISH)
UH-009	2	JAK–STAT	Loss of 5′ (FISH), P2RY8-CRLF2+ (PCR), JAK2 p.R683G (NGS), Xp22.3 del (SNP array), LDA+	Y (flow)	46,XX,dic(9;20)(p13.2;q11.21),+21[18]/46,XX[2]	N	CDKN2A loss (FISH)
UPMC-1	2	JAK–STAT	Loss of 5′ (FISH)	N/A	46,XX,i(9)(q10)[7]/46,XX[1]	N	PAX5 loss, ABL1 gain and sublconal IGH-R (FISH)
UPMC-2	52	JAK–STAT	CRLF2-R (FISH)	N/A	47,XX,+21[9]/46,XX[1]	N	IGH-R (FISH)
UPMC-3	16	JAK–STAT	CRLF2-R (FISH), JAK2 p.R683S (NGS)	N/A	46,XY,del(6)(q15q24),del(12)(p12p13),add(15)(q24)[8]/46,XY[12]	N	IGH-R and ETV6 loss (FISH). EBF1, ETV6, and BTG1 losses (SNP array)
UPMC-4	22	JAK–STAT	EPOR-IGH (NGS)	N/A	46,XY,der(19)t(1;19)(q21;p13)[10]	N	PBX1 gain and TCF3 loss (FISH). IKZF1 loss (SNP array). No PBX1::TCF3 by FISH.
UPMC-5	4	JAK–STAT	Loss of 5′ (FISH), Xp22.3 del (SNP array)	N/A	48,XY,+X,+21[5]/46,XY[15]	N	IKZF1, PAX5 and ETV6 losses (SNP array)
UPMC-6	12	JAK–STAT	CRLF2-R (FISH)	N/A	47,XY,+21c[20]	Y	EBF1, SETD2 and FBXW7 losses (SNP array)
UPMC-8	2	JAK–STAT	Loss of 5′ (FISH), Xp22.3 del (SNP array)	N/A	49,XY,+X,+17,+21[3]/46,XY[18]	N	PAX5 loss (SNP array)
UPMC-9	12	JAK–STAT	CRLF2-R (FISH)	N/A	49,XY,+X,der(1)t(1;8)(q44;q11.2),del(3)(p11.2),ins(4;3)(p14;p24.3p26.3),+7,del(8)(p12p23.2),+del(8)(p12p23.2)[cp8]/46,XY[4]	N	IKZF1 loss (SNP array)
BCH-1	5	JAK–STAT	P2RY8-CRLF2 (PCR), LDA+	N/A	46,XY[11]	N	None

(Continues)

TABLE 2 (Continued)

Case #	Age (years)	ICC 2022 BCR::ABL1-like class	Positive BCR::ABL1-like genetic/molecular testing	CRLF2 overexpression?	Karyotype	Downs?	Additional positive genetics findings
BCH-3	5	JAK–STAT	P2RY8-CRLF2+ (NGS), CRLF2 p.F232C	Y (flow)	46,XY,del(9)(p21)[2]/46,sl,del(11)(q23)[1]/46,XY[19]	N	CDKN2A and KMT2A losses (FISH). IKZF1, CDKN2B, ATM losses (NGS).
BCH-4	3	JAK–STAT	P2RY8-CRLF2 (NGS)	Y (flow); 4% of blasts	46,XY[20]	N	CDKN2A loss (FISH). CDKN2B loss (NGS). PAX5::ZCCHC7, ZCCHC7::PAX5 (NGS)
BCH-7	15	JAK–STAT	P2RY8-CRLF2 (PCR), LDA+	Y (flow)	46,X,-X,add(2)(p21),-9,add(16)(p13.3),add(21)(q21),+2mar[6]/46,XX[14]	N	RUNX1 gain (FISH). CREBBP loss and SH2B3 c.519_520insGCCCG p.P173fs* (NGS)
BCH-9	6	JAK–STAT	JAK2-R (FISH)	N (flow)	46,XX[20]	N	IKZF1 loss (NGS)
BCH-10	5	ABL	ETV6::ABL1 (unknown), LDA+	N (flow)	46,XY[20]	N	IKZF1 loss and ABL1 gain (NGS)
BCH-11	2	JAK–STAT	JAK2 p.R683G (NGS), LDA+	Y (flow)	45,XX,add(9)(p13),-20,der(21)t(20;21)(p11.2;p13)[5]/46,sl,+X[3]/46,XX[12]	N	CDKN2A loss (FISH). JAK2 loss

Note: CRLF2 FISH was performed using break-apart probes. "CRLF2-R" in the table indicates separation of the 5′ and 3′ FISH probes which typically correlates with *IGH::CRLF2*. "Loss of 5′" in the table indicates loss of the 5′ FISH probe, which is typically due to an intrachromosomal deletion event resulting in *P2RY8::CRLF2*.

For BCH11, No CRLF2 FISH, PCR or RNA sequencing was performed to directly identify the CRLF2-R. Therefore whether this case is *IGH::CRLF2* or *P2RY8::CRLF2* is unknown.

No assessment for CDKN2A loss by FISH or SNP microarray performed for UH-001, UH-002, UPMC-1, UPMC-2 and BCH-7.

Abbreviations: CF, culture failure; -R, rearranged; N/A, not available.

TABLE 3 BCR::ABL1+ cases.

Case #	Karyotype	Additional FISH	BCR-ABL1 Breakpoint
UH-010	CF	BCR::ABL1, subclonal −17 and +17	Minor
UH-011	47,XX,+4,t(9;22)(q34;q11.2)[9]/47,idem,del(1)(q42),der(7)t(7;21)(q22;q11.2)[14]/46,XX[2]	BCR::ABL1, RUNX1 gain	Minor
UH-012	45,XX,-7,t(9;22)(q34;q11.2)[11]/46,XX,idem,+6[2]/46,XX[2]	BCR::ABL1	Neither
UH-013	46,XX,t(9;22)(p24;q11)[6]/46,idem,-11,+mar[9]/46,XX[5]	BCR::ABL1	Minor
UH-014	45,XX,del(7)(p15),t(9;22)(q34;q11.2),-16[11]/46,XX[9]	BCR::ABL1	Minor
UH-015	46,XX,t(9;22)(q34;q11.2)[18]/46,XX[2]	BCR::ABL1	Minor
UH-016	CF	BCR::ABL1	Minor
UH-017	46, XX[5]	BCR::ABL1	Neither
UH-018	N/A	BCR::ABL1	Major
UH-019	46,XX,t(9;22)(q34;q11.2)[11]/48,idem,-der(9)t(9;22)(q34;q11.2),-14,+22,+der(22)t(9;22)(q34;q11.2),+der(22)t(9;22)(q34;q11.2),+mar[9]	BCR::ABL1	Major
UH-020	46,XY,t(9;22)(q34;q11.2)[4]/46,XY[16]	BCR::ABL1	Major
UH-021	46,XX,t(9;22)(q34;q11.2)[20]	BCR::ABL1	Minor
UH-022	45,XX,-7,dup(8)(q24.1q13),t(9;22)(q34;q11.2)[14]/46,XX[11]	BCR::ABL1, subclonal C-MYC gain	Major
UH-023	46,XY,t(9;22)(q34;q11.2)[1]/46,idem,t(9;14)(p21;q13),del(12)(p11.2)[14]/49,idem,+8,+17,+21[3]/46,XY[2]	BCR::ABL, subclonal ETV6 loss and KMT2A gain	Major
UH-024	45,XY,-3,der(7;9)t(7;9)(q10;q10)t(9;22)(q34;q11.2),+mar[5]/46,XY[6]	BCR::ABL1, CDKN2A loss, subclonal +7	Minor
UH-025	48,XX,t(9;22)(q34;q11.2),+19,+mar[3]/46,XX[22]	BCR::ABL1	Minor
UH-026	CF	BCR::ABL1 +4	Minor

specificity (95%) and PPV (89%). Combining both stains improved performance for detection of *BCR::ABL1*+/like cases; for example, the presence of either IGJ *or* SPATS2L staining improved sensitivity (from 83% to 93%) and NPV (from 90% to 95%) (Table 5).

DISCUSSION

The fields of diagnostic pathology and oncology are in dire need of facile, accessible and inexpensive methods of identifying *BCR::ABL1*-like B-ALL, a high-risk B-ALL subtype with the potential for emerging targeted therapy. We report here for the first time IHC markers with high sensitivity *and* specificity for identifying *BCR::ABL1*+/like B-ALL.

The IHC results corroborate prior mRNA-based gene expression data that have demonstrated preferential upregulation of *IGJ* and *SPATS2L* genes in *BCR::ABL1*+/like B-ALL and the incorporation of their overexpression in LDA/qRT-PCR screeners for *BCR::ABL1*-like B-ALL.[10,19–24] This is noteworthy because many upregulated genes from transcriptome analyses either have limited known function (e.g. *SPATS2L*), have previously known function/properties that would not predict protein expression in haematopoietic cells (e.g. *MUC4*), or in immature precursors (e.g. *IGJ*). Preferential upregulation at the mRNA level does not always translate into a diagnostically useful IHC test for *BCR::ABL1*+/like identification, however. For instance, even between IGJ and SPATS2L, SPATS2L had a much lower PPV (70%) than IGJ (89%). Also, in contrast to IGJ and SPATS2L IHC, sensitivity for MUC4 IHC is only about 30%, even though it has 100% specificity.[18] In unpublished work, we have failed to detect some mRNA-overexpressed genes at the protein level by IHC. These differences justify empiric evaluations to identify optimal IHC markers for *BCR::ABL1*-like and other newer subtypes of B-ALL. Mechanistic studies would also be important to determine the contribution of these genes to the biology of *BCR::ABL1*+/like B-ALL.

One limitation of the study is the fact that most *BCR::ABL1*-like cases included in the study were *CRLF2*-R, by far the most prevalent genetic abnormality in *BCR::ABL1*-like B-ALL. This raises the question of whether IGJ & SPATS2L IHC are simply recognizing *CRLF2*-R B-ALL, which will diminish their utility since CRLF2 flow cytometry already provides a robust method for identifying *CRLF2*-R B-ALL. However, we do not think this is the case because, first, IGJ & SPATS2L IHC stains *BCR::ABL1*+ B-ALL (which lack *CRLF2* rearrangement) with a similar frequency as *BCR::ABL1*-like B-ALL, and second, all three non-*CRLF2*-R *BCR::ABL1*-like cases (1 with *JAK2*-R, 1 with *EPOR*-R and 1 with *ETV6::ABL1*) were also IHC positive. These results contrast with MUC4 IHC, which appeared to show preferential expression in B-ALLs with *ABL1* class rearrangement versus those with *CRLF2*-R and other *JAK–STAT* pathway activation.[18,42] Our findings suggest that IGJ and SPATS2L would be useful in identifying both *JAK–STAT*-activated and *ABL1*-rearranged classes of *BCR::ABL1*-like B-ALL recognized by the ICC 2022 classification,[3,4] although direct

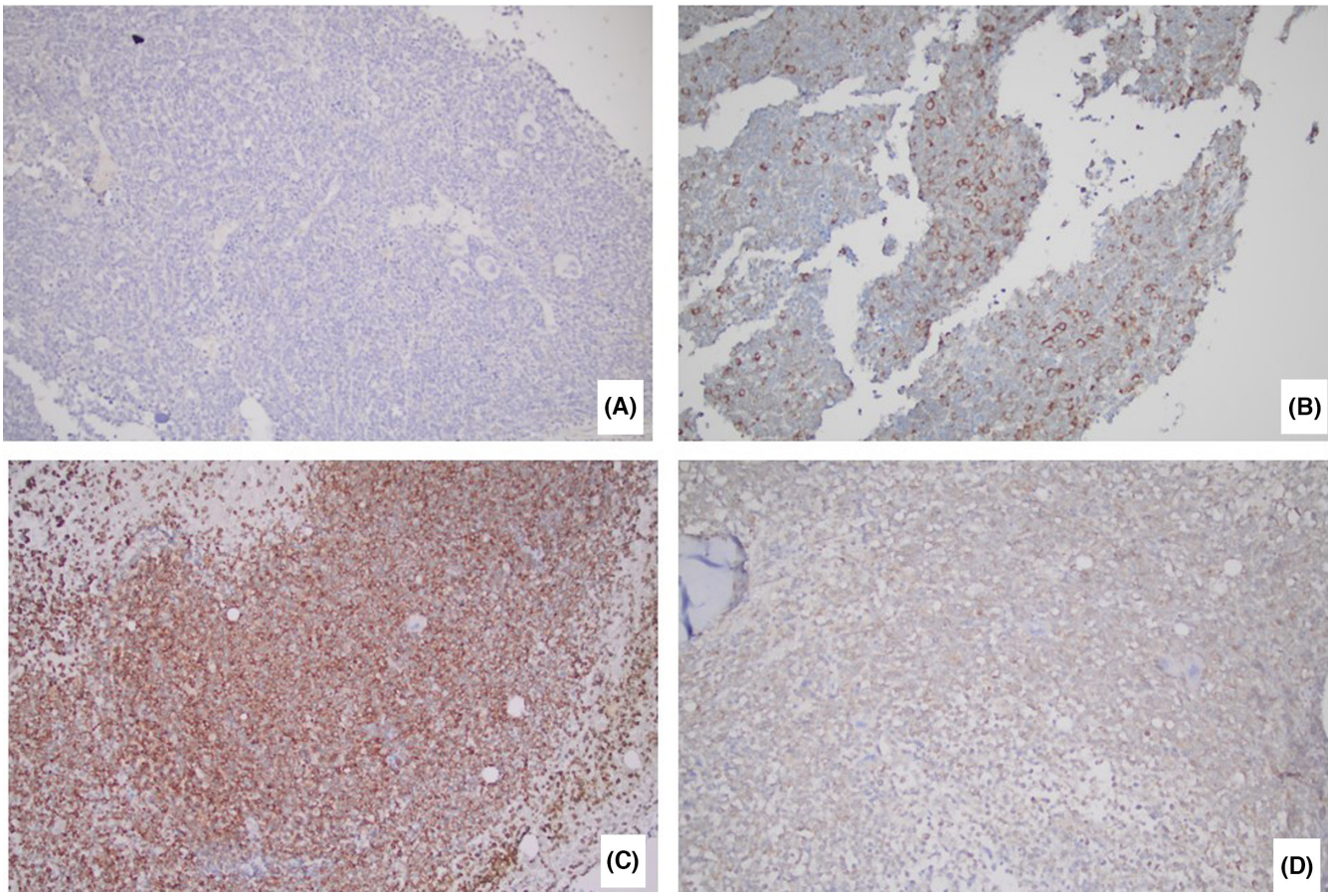

FIGURE 2 Variable expression of SPATS2L protein in B-ALL. Representative images of SPATS2L IHC. (A) Negative, 200×, UH-032. (B) Partial positive, 200×, UH-008. (C) Diffuse strong positive, 200×, UH-001. (D) Diffuse weak positive, 200×, UH-064.

TABLE 4 Summary of SPATS2L IHC results by B-ALL subtype.

Subtype	Total # of cases	SPATS2L+	Diffuse staining	Partial staining
BCR::ABL1-like	15	12 (80%)	9	3
BCR::ABL1+	17	14 (82%)	14	0
Hyperdiploidy	19	0	0	0
ETV6::RUNX1	12	2 (17%)	2	0
TCF3::PBX1	5	0	0	0
Hypodiploidy	4	2 (50%)	2	0
KMT2A-rearranged	7	1 (14%)	1	0
iAMP21	2	0	0	0
t(5;14)	1	0	0	0
NFS	22	6 (27%)	6	0

Abbreviations: B-ALL, B-acute lymphoblastic leukaemia; NFS, not further specified.

testing of the rare *ABL1*-class, non-*CRLF2*-R *JAK–STAT* class and *BCR::ABL1*-like NOS in larger cohorts would need to be performed in the future for validation.

Although genetic identification of rearrangements in tyrosine kinase receptor genes has proven invaluable in the identification of *BCR::ABL1*-like B-ALL,[11,21] one of the challenges in interpretation is the occasional co-occurrence of *CRLF2*-R (especially *P2RY8::CRLF2*) with other class-defining genetic abnormalities. *CRLF2* rearrangements occur in about 20%–25% of iAMP21 B-ALL and at a lower frequency in B-ALL with hyperdiploidy, *ETV6::RUNX1* and the novel subtypes of B-ALL with *PAX5alt* or *ETV6::RUNX1*-like gene expression.[7,35,43–45] In many of these cases, the *CRLF2*-R is thought to be subclonal, not a driver of disease biology or clinical behaviour, and may be lost at relapse.[36,46] Furthermore, *CRLF2*

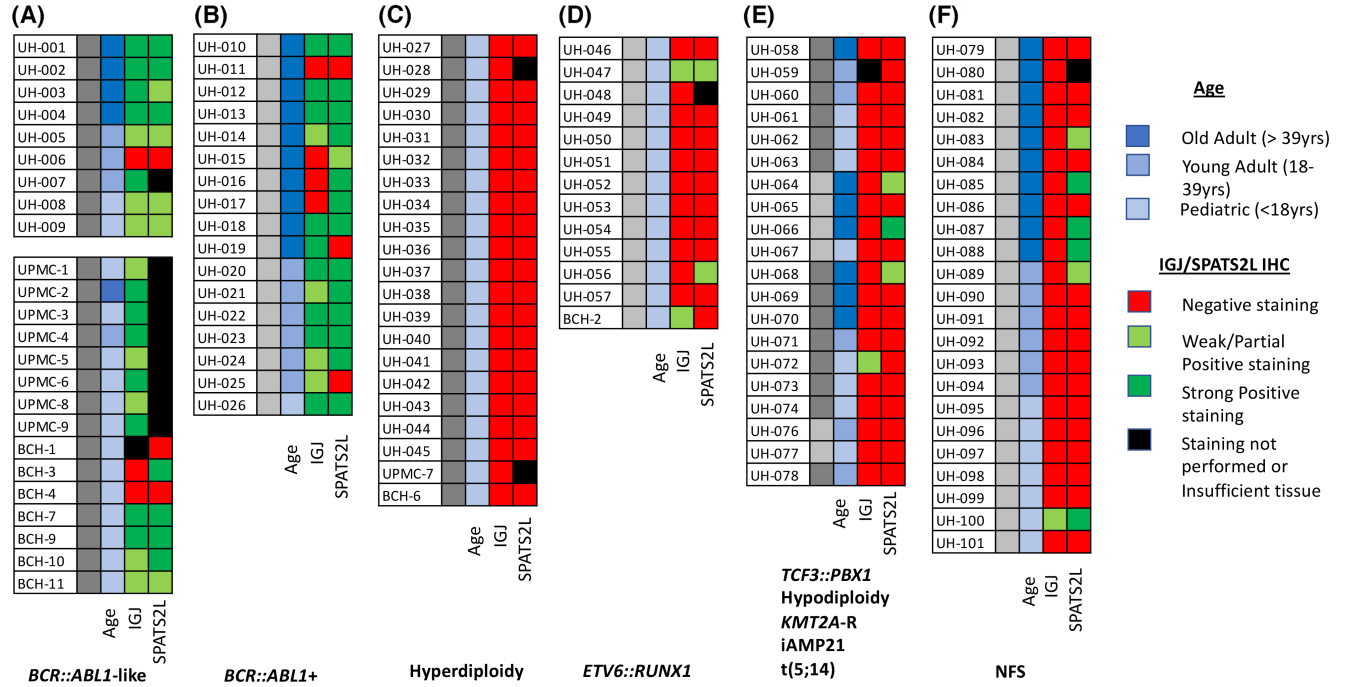

FIGURE 3 Heat map display of IGJ and SPATS2L IHC in individual B-ALL cases. IgJ and SPATS2L IHC results for each case evaluated in this study organized by B-ALL subtype. (A) *BCR::ABL1*-like. (B) *BCR::ABL1*+. (C) Hyperdiploidy. (D) *ETV6::RUNX1*. (E) *TCF3::PBX1*, hypodiploidy, *KMT2A*-R, iAMP21, t(5;14). (F) NFS (not further specified). Each row is an individual case. Columns as follows: Column 1: Case number. UH, University Hospital cases; UPMC, University of Pittsburgh Medical Center cases; BCH, Boston Children's Hospital cases. Column 2: Alternating dark grey and light grey coding are used to designate different B-ALL subtypes. In Figure 3E, there are 5 *TCF3::PBX1*, 4 hypodiploidy, 7 *KMT2A*-R, 2 iAMP21 and 1 t(5;14) in that order. Column 3: Age at diagnosis. Dark blue is older adult (>39 years old). Intermediate blue is AYA (18–39 years old). Lightest blue is paediatric (<18 years old). Column 4: IGJ IHC results. Dark green, strong diffuse positive staining. Light green, partial or weak positive staining. Red, negative staining. Black, not assessed or inadequate tissue. Column 5: SPATS2L IHC results. Dark green, strong diffuse positive staining. Light green, partial or weak positive staining. Red, negative staining. Black, not assessed or inadequate tissue.

TABLE 5 Summary of test performance of IgJ and SPATS2L IHC.

	Sensitivity	Specificity	PPV	NPV	*p* value
IGJ	83%	95%	89%	90%	<0.0001
SPATS2L	81%	85%	70%	91%	<0.0001
IGJ or SPATS2L	93%	82%	74%	95%	<0.0001
IGJ & SPATS2L	71%	97%	92%	87%	<0.0001

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.

rearrangement without *BCR::ABL1*-like gene expression does occur (about 1% of paediatric and AYA B-ALL in 1 study[47]), and false positive LDA screen due to *CRLF2* overexpression resulting from *CRLF2* genetic alterations has been described.[26] Therefore, having another assay such as IGJ/SPATS2L IHC, which is not reliant on *CRLF2* alterations, holds promise for guiding appropriate disease classification. Although our cohort only included a few cases of B-ALLs with co-occurring *CRLF2*-R and other class-defining genetic alteration, IGJ/SPATS2L IHC was different from the vast majority of other *CRLF2*-R cases. Specifically, one case with iAMP21 + *P2RY8::CRLF2* and two cases with hyperdiploidy + *P2RY8::CRLF2* were all negative for both IGJ and SPATS2L IHC, while one case with *ETV6::RUNX1* + *P2RY8::CRLF2* was partially positive for IGJ and negative for SPATS2L. Future studies on additional cases are needed to fully understand the performance and significance of IGJ/SPATS2L IHC staining in B-ALL cases that have co-occurring class-defining rearrangements, mutations or gene expression profiles in addition to *CRLF2* rearrangements.

Our work highlights the undying relevance of IHC, even in an era of technological advancement in diagnostic pathology, that may be applicable to newer subtypes of B-ALL. Current methods for *BCR::ABL1*-like identification such as LDA screen, large-scale RNA sequencing panels or multiprobe FISH panels cost several thousand dollars and are either proprietary or require sophisticated/expensive equipment. In contrast, IHC is more than an order of magnitude cheaper, utilizes commercially available

reagents and can be performed with minimal equipment. Therefore, IGJ/SPATS2L IHC would likely be more amenable to adoption in developing countries[48,49] and should also prove invaluable in the United States/other developed countries where there are increasing concerns related to decreasing individual/institutional medical care costs, the levels of insurance reimbursements or where access to current techniques may be limited. Even in settings with unimpaired access to advanced molecular testing, IGJ/SPATS2L IHC, in combination with standard cytogenetics testing, may be utilized to triage and/or justify which patient samples are more likely to benefit from advanced testing. Future studies could address optimal approaches for combining IHC, routine cytogenetics and next-generation testing for improved cost-effectiveness and speed of B-ALL subclassification.

AUTHOR CONTRIBUTIONS

Kwadwo A. Oduro Jr conceived of the project. Catherine K. Gestrich, Shanelle J. De Lancy, Christopher Ryder, Shashirekha Shetty, Jacob Bledsoe, Erika M. Moore, Kwadwo A. Oduro Jr identified cases and collected relevant clinicopathologic data. Catherine K. Gestrich, Shanelle J. De Lancy, Adam Kresak, Erika M. Moore, Kwadwo A. Oduro Jr performed IHC. Catherine K. Gestrich, Shanelle J. De Lancy, Erika M. Moore, Kwadwo A. Oduro Jr analysed the IHC data. Howard Meyerson developed UH-CRLF2 flow cytometry. Shashirekha Shetty performed and interpreted cytogenetics. Irina Pateva and Akua K. Yalley provided important discussions for the project. Kwadwo A. Oduro Jr wrote the manuscript with significant contributions from Catherine K. Gestrich, Shanelle J. De Lancy, Akua K. Yalley, Christopher Ryder, Jacob Bledsoe and Erika M. Moore.

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CONFLICT OF INTEREST STATEMENT

The authors do not report any potential conflicts of interest.

DATA AVAILABILITY STATEMENT

Abundant and relevant data and staining protocol are included in the manuscript/supplementary materials. Contact the corresponding author for further inquiries regarding original data or protocols.

ETHICS STATEMENT

The study was performed in accordance with respective Institutional Review Board approvals.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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