Genetic characterisation of childhood B-other-acute lymphoblastic leukaemia in UK patients by fluorescence in situ hybridisation and Multiplex Ligation-dependent Probe Amplification

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Summary

While next-generation sequencing technologies provide excellent strategies to screen for newly defined genetic abnormalities of prognostic or therapeutic significance in patients with B-other-acute lymphoblastic leukaemia (ALL), they are not widely available. We used a dual screening approach, incorporating fluorescence in situ hybridisation (FISH) and Multiplex Ligation-dependent Probe Amplification (MLPA), to establish the frequency and long-term outcome of a representative cohort of specific subgroups of B-other-ALL recruited to the childhood ALL trial, UKALL2003. We focussed on abnormalities of known prognostic significance, including ABL-class fusions and ERG deletions, as a surrogate marker for DUX4-rearranged ALL. ABL-class fusions accounted for ~4% of B-other-ALL and were associated with high levels of minimal residual disease (MRD; 14/23 with MRD >5%) and a high relapse rate (55/7%) following treatment without tyrosine kinase inhibitor (TKI), confirming the importance of prospective screening with a view to incorporating TKI into therapy. Patients with deletions of ERG (~10% of B-other-ALL) had a 10-year event-free-survival of 97/2%, validating previous reports of their excellent outcome. Rearrangements of ZNF384, MEF2D and NUTM1 were observed at low frequencies. Here, we estimate that approximately one third of B-other-ALL patients can be reliably classified into one of the known genetic subgroups using our dual screening method. This approach is rapid, accurate and readily incorporated into routine testing.

Keywords: ALL, cytogenetics of leukaemia, childhood leukaemia.

Key points

- We estimate that one third of B-other-ALL patients can be reliably classified into genetic subgroups using FISH and MLPA.
- ABL-class fusions are associated with high MRD and a high relapse rate, while deletions of ERG are associated with an excellent outcome.

Introduction

In childhood B-progenitor acute lymphoblastic leukaemia (B-ALL), chromosomal abnormalities remain important diagnostic and prognostic markers. A number of well-established abnormalities define significant cytogenetic subgroups, now used in risk stratification to guide treatment.1 Approximately 30% of patients with B-ALL, with none of the major chromosomal abnormalities, are classified together as B-other-ALL. Recent analyses of this subgroup have revealed some distinct genetic entities.2-4 Notably, two subgroups, strongly correlated with outcome, have emerged; DUX4-rearranged (DUX4-r) ALL, which confers a good prognosis5-8 and Philadelphia chromosome (Ph)-like/BCR-ABL1-like-ALL, associated with a poor treatment response.9-15 The clinical relevance of other emerging subgroups, including fusions of ZNF38416-18, MEF2D19-21 and NUTM1,22 is less clear.
Although these subgroups display characteristic gene expression signatures, their underlying genetic profiles may be heterogeneous, cytogenetically cryptic or overlapping with other subgroups. For example, patients with Ph-like ALL harbour novel fusions involving tyrosine kinases, known as ABL-class genes: ABL1, ABL2, PDGFRB and CSF1R, or abnormalities that activate the Janus Kinase and Signal Transducer and Activator of Transcription (JAK–STAT) pathway, including CRLF2-rearrangements (CRLF2-r) and JAK2 fusions, with multiple partner genes reported for each kinase.10,11,13 Although these abnormalities, notably CRLF2-r, are enriched within Ph-like-ALL, they are not unique to this group and occur alongside other defining abnormalities.23 Thus, for precise risk assignment, accurate identification of these poor-risk abnormalities is important, particularly for patients with ABL-class fusions, who have shown successful responses to treatment with tyrosine kinase inhibitors (TKI) in otherwise refractory disease.24–27

Despite a frequency of 5% in childhood B-ALL, DUX4-r were relatively recently described, likely due to the small size and repetitive nature of DUX4, along with its location within the sub-telomeric regions of both chromosomes 4 and 10.5–7 These features render DUX4-r difficult to identify using standard techniques. Intragenic deletions of ERG provide a surrogate marker for DUX4-r-ALL, as they occur exclusively within this subgroup in >50% of cases.

The best method(s) for detecting these abnormalities, to guide real-time therapeutic decisions, remain a matter of debate. Here, we have carefully defined the B-other-ALL cohort and applied a dual screening approach, using FISH (fluorescence in situ hybridisation) and MLPA (Multiplex Ligation-dependent Probe Amplification), to clarify the frequency and clinical significance of these rearrangements within a single childhood ALL treatment trial, UKALL2003, to assist in improving risk stratification within future childhood ALL trials.

Patients and methods

Patients in this study were diagnosed with B-ALL and registered onto the UK childhood treatment trial, UKALL2003 (2003–01; ages 1–24 years), with ethical approval and consent in accordance with the Declaration of Helsinki.28 Demographic, clinical and treatment details were collected by the Clinical Trial Service Unit (CTSU), Oxford, UK. Cytogenetic analyses and FISH for detection of established chromosomal abnormalities of prognostic significance were performed in the regional cytogenetics laboratories and collated by the Leukaemia Research Cytogenetics Group.

B-other-ALL patients were screened by FISH for rearrangements associated with Ph-like-ALL, including: ABL-class genes: ABL1, ABL2, PDGFRB/CSF1R; JAK–STAT pathway genes: CRLF2, JAK2; and other newly defined subgroups: ZNF384, MEF2D and NUTM1; using commercial or home-grown break-apart FISH probes (Cytocell Ltd., Cambridge, UK; Leica Microsystems, Milton Keynes, UK). Where possible, partner genes were identified by cytogenetics, FISH, reverse transcription polymerase chain reaction (RT-PCR) and/or single nucleotide polymorphism (SNP) arrays, as previously described.26

Multiplex Ligation-dependent Probe Amplification was performed on DNA extracted from diagnostic bone marrow, using the P335-ALL-ikz41 and P327-iamp21-erg-MLPA kits (MRC Holland, Amsterdam, The Netherlands) and analysed as previously described.29 The P335 kit includes probes for identification of deletions within genes involved in B-cell development and cell cycle control: IKZF1, PAX5, EBF1, CDKN2A/B, RB1, BTG1 and ETV6, as well as the PAR1 region (resulting in P2RY8–CRLF2 fusion). The P327 kit comprises probes for ERG (n = 13) and RUNX1 (n = 6), alongside others mapping to chromosome 21.

Event-free survival (EFS) was defined as time to relapse, second tumour or death, censoring at date of last contact. Relapse rate (RR) was defined as time to relapse for those achieving complete remission, censoring at date of death in remission or last contact. The median follow-up time for the whole cohort was 9–4 years. Overall survival (OS) was defined as time to death, censoring at date of last contact. Kaplan–Meier methods were used to estimate survival rates and two-sided log-rank testing was employed to evaluate the equality of the survivorship functions in different subgroups. Univariate and multivariate Cox regression models were used to determine hazard ratios. Log transformation of minimal residual disease (MRD) used for the adjusted hazard ratios was applied, as previously described.30 Other comparisons were performed using the chi-squared or Fisher’s exact tests as appropriate. All P values were two-sided and, because of multiple testing, values <0.01 were considered statistically significant. All analyses were performed using Intercooled Stata (Stata Statistical Software, StataCorp, College Station, TX, USA).

Results

Refining the B-other-ALL cohort

In this study, we focussed on B-other-ALL patients 1–25 years old, thus infants, patients with Down Syndrome, T-ALL or positive for the cytogenetic abnormalities ETV6–RUNX1, BCR–ABL1, TCF3–HLF and TCF3–PBX1 fusions, high hyperdiploidy, near haploidy, low hypodiploidy, KMT2A rearrangements, and intrachromosomal amplification of chromosome 21 (iAMP21-ALL), were excluded (Fig S1).

The karyotypes of the included patients were reviewed to accurately verify the B-other-ALL cohort. Patients with successful abnormal karyotypes, with none of the subgroup-defining abnormalities listed above, were included. Those with normal or failed cytogenetic results were included if KMT2A rearrangements, BCR–ABL1 and ETV6–RUNX1 fusions had been excluded by FISH and/or RT-PCR. Patients (n = 37) with two or more additional copies (≥4) of RUNX1...
by interphase FISH were excluded, as this signal pattern likely indicated additional copies of chromosome 21 within undiagnosed high hyperdiploidy or resulting from amplification of RUNX1 as seen in iAMP21-ALL. None of these 37 cases were classified as iAMP21-ALL. FISH testing identified 21 cases, with available material, to be high hyperdiploidy.

The refined B-other-ALL cohort comprised 796 patients, constituting 29-2% of B-ALL patients. Material for additional screening was available for 640 (80%) patients. However, partial testing only was possible for many of the historical samples (n = 411) due to them being used up for chromosomal analysis, explaining the variable denominators for each test. Importantly, we showed that the samples available for screening were representative of the total trial cohort in relation to clinical and demographic features (Table SI). A breakdown of these data is given in Figure 1 and Figure S1, with detailed genetic data and testing for each case provided in Table SII.

**Ph-like/BCR-ABL1-like-ALL**

Among those genetic abnormalities associated with Ph-like-ALL, CRLF2-r were the most common, observed in 9% of samples tested (49/563), comprising both P2RY8–CRLF2 fusion (n = 31, 63%) and IGH-CRLF2 (n = 18, 37%). Patients with CRLF2-r did not show significantly higher RR compared to the remainder of the B-other-ALL cohort (15-5% vs. 10-8%, P = 0.6626; Table I). Two patients had JAK2 rearrangements, both fused to PAX5.

Rearrangements involving ABL-class genes were rare and mutually exclusive: PDGFRB/CSF1R abnormalities were found in 4% of the cohort (18/446), while ABL1 (5/447) and ABL2 (1/412) rearrangements occurred in ≤1% each. Partner genes were identified in 19 of these cases, using a variety of techniques (Table SII).

**EBF1–PDGFRB** was the most common fusion (n = 11), arising from interstitial deletions (n = 8) or balanced translocations (n = 3) involving 5q31, in line with our previous publication of the genetic mechanisms giving rise to **EBF–PDGFRB** fusion.26

Both patients with rearrangements of CSF1R showed corresponding translocations of 5q33 from cytogenetic analysis: t(1;5)(q21;q33) and t(5;5)(q14;q33), resulting in MEF2D–CSF1R and SSBP2–CSF1R fusions, respectively. The single patient with ABL2 rearrangement showed the translocation, t (1;7)(q25;q34), indicating a ZC3HAV1–ABL2 fusion.

**ETV6–ABL1** fusions (n = 2) were identified from aberrant signal patterns seen while testing with FISH probes for BCR–ABL1 and ETV6–RUNX1 fusions. Metaphase FISH of both patients showed insertion of ABL1 into the ETV6 gene. Other fusions detected by sequential FISH included ZMIZ1–ABL1 (n = 2) and ATTF1P–PDGFRB (n = 1).

Collectively, cases with ABL-class fusions showed higher levels of MRD at the end of induction (EOI; 14/23 with MRD >5%) than other B-other-ALL patients, associated with a high RR (59-5% at 10 years; Table I and Fig 2). Numbers were too small to examine outcome for individual fusions; however, as previously reported,26 all patients with EBF1–PDGFRB fusions (n = 11) were MRD-positive at EOI (Table SIII).

**ERG deletions**

Among 372 B-other-ALL patients screened, ERG deletions were identified in 10% (n = 37; Table I). Their outcome was...
Table I. Demographics and outcome of patients testing positive for an ABL-class fusion, CRLF2 rearrangement (CRLF2-r), deletion of ERG (ERG-d) and ZNF384 rearrangement (ZNF384-r).

|                | B-other | ABL class fusion | P value* | CRLF2-r | P value | ERG-d | P value | ZNF384-r | P value |
|----------------|---------|------------------|----------|---------|---------|-------|---------|----------|---------|
| Total          | 796     | 24               | 49       | 37      | 19      |
| Number tested positive | 24   | 49               | 37       | 19      |
| Age            |         |                  |          |         |         |       |         |          |         |
| 1–4            | 310 (38.9) | 6 (25.0)        | 24 (49.0)| 12 (32.4)| 5 (26.3)|
| 5–9            | 178 (22.4) | 4 (16.7)        | 13 (26.5)| 12 (32.4)| 3 (15.8)|
| 10–15          | 204 (25.6) | 7 (29.2)        | 5 (10.2)| 10 (27.0)| 6 (31.6)|
| 16+            | 104 (13.1) | 7 (29.2)        | 0.138    | 7 (14.3)| 0.024  | 3 (8.1)| 0.223   | 5 (26.3)| 0.212  |
| Sex            |         |                  |          |         |         |       |         |          |         |
| Female         | 342 (43.0) | 12 (50.0)       | 19 (38.8)| 12 (32.4)| 11 (57.9)|
| Male           | 454 (57.0) | 12 (50.0)       | 0.483    | 0.386   | 25 (67.6)| 0.303 | 8 (42.1)| 0.172   |
| WCC            |         |                  |          |         |         |       |         |          |         |
| <50            | 640 (80.4) | 15 (62.5)       | 33 (67.4)| 35 (94.6)| 13 (68.4)|
| >50            | 156 (19.6) | 9 (37.5)        | 0.044    | 0.029   | 2 (5.4)| 0.005  | 6 (31.6)| 0.345   |
| MRD            |         |                  |          |         |         |       |         |          |         |
| 0%             | 150 (21.9) | 1 (44)          | 7 (17.5)| 7 (21.2)| 1 (5.9)|
| 0–0.005%       | 137 (20.0) | 1 (44)          | 7 (17.5)| 8 (24.2)| 4 (23.5)|
| 0.005–0.01%    | 54 (7.9)  | 0               | 4 (10.0)| 1 (3.0)| 0      |
| 0.01–0.1%      | 158 (23.1) | 0               | 10 (25.0)| 9 (27.3)| 5 (29.4)|
| 0.1–1.0%       | 112 (16.4) | 3 (13.0)        | 7 (17.5)| 5 (15.2)| 3 (17.7)|
| 1–5%           | 38 (5.6)  | 4 (17.4)        | 2 (5.0)| 1 (3.0)| 4 (23.5)|
| >5%            | 35 (5.1)  | 14 (60.9)       | <0.001   | 3 (7.5)| 0.984  | 2 (6.1)| 0.944   | 0       | 0.057  |
| NA             | 112      | 1               | 9       | 4       | 2      |
| CR              |         |                  |          |         |         |       |         |          |         |
| No             | 11 (1.4)  | 2 (83)          | 0       | 0       | 0 (0)  |
| Yes            | 785 (98.6)| 22 (91.7)       | 0.017    | 0.301   | 0.504  | 19 (100)| 0.692   |
| Relapse Time   |         |                  |          |         |         |       |         |          |         |
| Very Early     | 25 (24.8) | 3 (23.1)        | 1 (12.5)| 0       | 1 (33.3)|
| Early          | 31 (30.7) | 5 (38.5)        | 2 (25)  | 0       | 1 (33.3)|
| Late           | 45 (44.6) | 5 (38.5)        | 0.765    | 0.632   | 1 (100.0)| 0.522 | 1 (33.3)| 0.943   |
| RR@5years      | 11:1 (9.0–13.5)| 54:5 (34.8–76.5)| <0.0001 | 15:5 (7.7–29.7)| 0.6499 | 2:8 (0.4–8.1)| 0.0447 | 15:8 (5.4–41.4)| 0.437 |
| EFS@5years     | 84:7 (82.0–87.0)| 39:8 (20.4–58.7)| <0.0001 | 76:9 (62.2–86.5)| 0.5339 | 97:2 (81.9–99.6)| 0.0116 | 84:2 (58.7–94.6)| 0.703 |
| OS@5years      | 90:3 (88.0–92.1)| 61:6 (39:1–77.9)| <0.0001 | 91:7 (79:3–96:8)| 0.8167 | 100       |
| RR@10years     | 13:4 (11.1–16.1)| 59:5 (39:5–80:4)| <0.0001 | 15:5 (7:7–29:7)| 0.6499 | 2:8 (0:4–8:1)| 0.0447 | 15:8 (5:4–41:4)| 0.437 |
| EFS@10years    | 81:8 (78:8–84:4)| 35:4 (17:1–54:4)| <0.0001 | 76:9 (62:2–86:5)| 0.5339 | 97:2 (81:9–99:6)| 0.0116 | 78:2 (51:7–91:3)| 0.703 |
| OS@10years     | 86:8 (84:1–89:1)| 52:4 (30:6–70:3)| <0.0001 | 85:6 (70:1–93:4)| 0.8167 | 100       |
| Hazard ratio RFS| 8:0 (4:2–15:2)| <0.001 | 1:2 (0:5–2:6)| 0:65 | 0:2 (0:02–1:2)| 0:078 | 1:60 (0:48–5:32) | 0:442 |
| Adjusted HR RFS**| 1:8 (0:6–4:2) | 0:189 | 1:1 (0:5–2:5) | 0:78 | 0:2 (0:03–1:5) | 0:119 | 0:67 (0:16–2:87) | 0:589 |

CR, complete remission; EFS, event-free survival; HR, hazard ratio; MRD, minimal residual disease; OS, overall survival; RFS, relapse-free survival; RR, relapse rate; WCC, white cell count.

*Chi-squared test comparing positive cases to negative cases.

**Adjusted for age, log-transformed MRD and WCC.
excellent (10-year EFS 97.2%, OS 100%), with relapse in only a single patient, despite 52% of cases being MRD-positive (>0.01%) at EOI (17/33 with data available). This outcome was comparable to that of patients within the good-risk cytogenetic sub-groups, ETV6–RUNX1 and high hyperdiploidy, treated on UKALL2003 (Fig 3 and Table SIV).

Hidden iAMP21-ALL

Screening with the P327 MLPA kit revealed two patients with copy number profiles of chromosome 21 similar to those seen in iAMP21-ALL patients (Fig 4). Currently the accepted definition of iAMP21-ALL is the finding of three or more extra copies of RUNX1 on a single abnormal chromosome 21 (≥5 RUNX1 signals per interphase nucleus). As both patients showed only one additional copy of RUNX1 (three copies in total) identified by FISH, their diagnosis as iAMP21-ALL had not been considered. Subsequent SNP array profiling provided convincing evidence to redefine these cases as iAMP21-ALL (Fig 4).

Other subtypes of B-other

ZNF384 rearrangements were identified in ~7% of patients (19/263): EP300–ZNF384 (n = 8), TCF3–ZNF384 (n = 6), CREBBP–ZNF384 (n = 2), TAF15–ZNF384 (n = 2) and EWSR1–ZNF384 (n = 1). These patients showed similar outcomes to B-other-ALL patients overall (five-year EFS 84.2% vs. 86.1%; OS 89.5% vs. 90.6%).

MEF2D rearrangements were observed at a low frequency of 1.4% (3/206), including one with MEF2D–CSFIR among the ABL-class mentioned above and two with MEF2D–BCL9...
Recent studies have described copy number abnormalities (CNA), which identify good and high-risk profiles among patients with B-other-ALL, e.g. UKALL-CNA or IKZF1-plus. Both are based on those genes included within the P335-IKZF1-MLPA kit. Briefly, UKALL-CNA classifies patients as good risk (CNA-GR) if they have no deletions among the genes tested for, isolated deletions of ETV6, PAX5, BTG1 or ETV6 with a single additional deletion of BTG1, PAX5, or CDKN2A/B. All other profile combinations are classified as intermediate/poor risk (CNA-IR/PR). The IKZF1-plus profile defines patients with an IKZF1 deletion and at least one additional deletion of PAX5, CDKN2A/B or PAR1, in the absence of an ERG deletion, as poor risk.

Copy number profiles

We examined the distribution of ABL-class, CRLF2-r, ERG-deleted and ZNF384 fusion cases according to CNA profile (Table SV). Despite their excellent outcome, only 40% of patients with ERG deletions qualified as good risk according to the UKALL-CNA classifier, likely due to the co-occurrence of IKZF1 deletions in 42% of them (15/35 tested). Patients with ABL-class fusions were evenly distributed between CNA-GR (n = 7) and CNA-IR/PR (n = 8), while only 3/14 ABL-class patients qualified as IKZF1-plus. Unsurprisingly, the majority of patients with JAK-STAT-activating abnormalities were classified as CNA-IR/PR, as all patients with a PAR1 deletion by MLPA are assigned to this group.

The prognostic effect of both copy number profiles is modulated by MRD levels measured at EOI. We stratified patients based on integrated MRD and CNA groups derived from previous publications (Table SV). As expected, the majority of ABL-class patients had refractory disease (MRD ≥5%). Interestingly the ERG-deleted group showed no correlation with MRD, CNA profile or risk group. Patients with UKALL-CNA-GR profile and a good MRD response had an outcome equivalent to those with undetectable MRD. Both classifiers identified a small number of patients with poor-risk genetics and high MRD related to a very poor outcome (RR >30% at 10 years).

Discussion

In this study, we have accurately defined the B-other-ALL subgroup within a single trial, UKALL2003, and successfully screened a large representative cohort within this subgroup for abnormalities associated with emerging subgroups of ALL. Of those abnormalities associated with Ph-like-ALL, CRLF2-r were present in approximately 9% of patients and were associated with an intermediate outcome. This is in line with our previous study, which showed that patients treated on the UKALL97 trial had a similar outcome to those within the intermediate cytogenetic risk group (five-year OS 81% vs. 85%).

ABL-class fusions were less common (~4%); however, positive cases were associated with high levels of MRD and high RR compared to the remainder of the cohort, as we
previously reported.36 Due to the small number of patients, we were unable to examine outcome for each fusion individually. However, we have previously shown that the EBF1–PDGFRB fusion is associated with high levels of MRD and high RR,28 whereas SSBP2–CSF1R fusions relate to a more variable outcome.38 A recent collaborative study has shown that patients with PDGFRB and ABL2 fusions have an inferior five-year EFS compared to those with CSF1R and ABL1 fusions.39 However, in the context of childhood ALL, ABL-class fusions overall have been associated with an unfavourable outcome.39

These data confirm the importance of prospective screening of patients for ABL-class rearrangements, with a view to treatment with TKI during induction to improve remission rates. Patients with B-other-ALL entered to the subsequent UK trial, UKALL2011, with induction failure, or MRD positivity at day 28 (>1%) or at week 14 (>0.5%), were routinely screened for the presence of ABL-class fusions. Several patients who tested positive successfully responded to TKI therapy, as previously reported.25,26 Screening for ABL-class fusions is now carried out at the time of diagnosis within several protocols, with a view to early TKI therapeutic intervention, further emphasising the importance of routine screening for these abnormalities.

Deletions of ERG were observed at the highest incidence (~10%) amongst abnormalities investigated here. ERG deletions were associated with excellent long-term outcomes, with only one relapse reported among 37 patients, indicating that they could potentially be treated as good risk alongside other good-risk cytogenetic subgroups, ETV6–RUNX1 and high hyperdiploidy, in line with suggestions from other study groups.8,40,41 ERG deletions have been exclusively observed in patients with DUX4-r-ALL, at a frequency of 55%.7 Currently, the most reliable method to identify DUX4-r is RNA sequencing, using a range of pipelines. In this study, we chose to screen for ERG deletions using a commercial MLPA kit, a relatively inexpensive technique already employed in many laboratories. While this approach will only identify a subset of patients with DUX4-r-ALL, ERG deletions provide a useful interim surrogate marker until reliable tests for the routine detection of DUX4-r are developed.

From the P327-ERG-MLPA screening, supported by SNP array, we identified two patients with otherwise undiagnosed iAMP21-ALL. Although these patients presented with only three copies of RUNX1 by FISH, instead of the five copies usually required to fit the current accepted definition of iAMP21-ALL, the copy number profile of chromosome 21 in both cases provided conclusive evidence to reclassify these cases. As shown in Figure 4, both cases had loss of the 21q sub-telomeric region, a feature observed in ~80% of patients with iAMP21-ALL (Fig 4). In both cases, RUNX1 was not located within the most highly gained region of chromosome 21. Increasing use of SNP arrays in ALL is likely to identify additional patients with similar profiles of chromosome 21, where RUNX1 is not present within the most highly amplified region; therefore, we recommend that the definition of iAMP21-ALL should be expanded to include such cases.

Rearrangements of ZNF384, MEF2D and NUTM1 were observed at relatively low frequencies within this cohort. In particular, the observed number of MEF2D-r was lower than expected from other reports in the literature. Studies using RNA sequencing have reported MEF2D fusions in approximately 5–7% of B-other-ALL,6,19,42 whereas our FISH approach identified rearrangements in <2% of patients screened. It was difficult to draw significant conclusions regarding outcome and other clinical associations for patients with rare fusions from this small study; thus pooled data from several groups into large collaborative studies are needed.

Fig 5. Fluorescence in situ hybridisation (FISH) and single nucleotide polymorphism (SNP) profile of MEF2D–BCL9 patients. FISH using break-apart probe for MEF2D shows rearrangements (one red, one green, one fusion signal) in patients 10419 (A) and 11549 (D). FISH using a break-apart probe for BCL9 shows gain (three fusions) in 10419 (B) and a partial gain (one green, two fusions) in 11549 (E). These gains were confirmed by SNP array analysis in both patients. Both profiles show a complex copy number pattern at 1q21-1q23 with telomeric break points close to MEF2D. The centromeric break points differ between the patients. The BCL9 gene is included in a region of gain at 1q21-1 in 10419 (C). In 11549 (F), the centromeric break-point is within the BCL9 gene. These results are similar to those previously reported in patients with MEF2D–BCL9 fusion.19,21 [Colour figure can be viewed at wileyonlinelibrary.com]
In future trials, CNA profiles and MRD will play an important role in risk stratification of patients with B-other-ALL. We have shown here that both the UKALL-CNA classifier and IKZF1plus alongside MRD successfully highlighted groups of patients with good or poor outcomes. The UKALL-CNA classifier, when combined with MRD, identified approximately one third of B-other-ALL patients with excellent long-term survival, who could be considered for treatment de-escalation in future trials. However, it was surprising, given their excellent outcome, that less than half of patients with an ERG deletion were classified into good-risk groups according to this classifier (Table SIV). Use of the IKZF1plus profile with MRD highlighted a small group of patients with a very poor outcome, who may have benefited from intensive or targeted therapies; however, only two of the patients identified with ABL-class fusions in this cohort were classified into this high-risk group. Therefore, caution is required in reinterpretation of risk among cases already identified as poor or good (ABL-class and DUX4-r, respectively) based on their primary genetic change. However, such profiles will likely be valuable among those unclassified patients remaining within intermediate-risk groups.

For many years, FISH alongside karyotyping has been the gold standard for cytogenetic testing of B-ALL in the UK. Latterly, MLPA and SNP array have been added, particularly for retrospective screening of historical cohorts. A number of groups have incorporated targeted or whole transcriptome RNA sequencing into routine practice. Their validation studies have indicated, from comparison of RNA sequencing against gold-standard approaches, that while each technique has indicated, from comparison of RNA sequencing against gold-standard approaches, that while each technique

Author contributions

CS, AM and CH designed the study; CS, EW, RC, EB, SR, ZH and JM carried out the cytogenetic and molecular testing; CS, DM, EB, AE, GA, AV, CH and AM analysed and interpreted the data; all authors approved the final manuscript.

Conflicts of interest

The authors declare no competing financial interests.

Supporting Information

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

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