Heme fusion assay

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No personal disclosures/conflicts of interest
Kinase fusions in solid tumors

The landscape of kinase fusions in cancer, Nat Comm, 2014
How can we keep up?
AMP as a modification of Rapid Amplification of cDNA Ends (5' RACE & 3' RACE)

Reverse transcription with GSP1

3' oligo ligation/polyA tailing

 Nested PCR

TA cloning and sequencing
Anchored multiplexed PCR (AMP) assay

Targeted RNA-seq

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ds-cDNA

---

-- End repair
-- dA tailing

T

-- Adapter ligation

Sample barcode

#1-96 Barcode A#

MiSeq Common Oligo Adaptor

P5 adapter

GSP1
Gene Specific Primer 1

GSP2
Gene Specific Primer 2

PCR1

PCR2

P7 adapter

MiSeq dual-index library

AMP-based Solid Fusion Assay

- Driver gene fusions (detected as RNA rearrangements) in >10% of solid tumors
- 10-15% of rearrangements involve novel partners

### Driver Genes

- ALK: 52
- ARHGAP26: 4
- BRAF: 8
- BRD4: 1
- EGFR: 13
- ERBB2: 5
- ERG: 1
- ETV6: 1
- EWSR1: 3
- FGFR2: 8
- FGFR3: 4
- FGR: 1
- JAK1: 2
- JAZF1: 1
- MAML2: 7
- MAST1: 1
- MAST2: 1
- MET: 70
- MUSK: 1
- NOTCH2: 1
- NRG1: 3
- NTRK1: 5
- NTRK2: 1
- NTRK3: 6
- NUTM1: 7
- PLAG1: 1
- PRKCA: 2
- PRKCB: 1
- RAF1: 2
- RELA: 1
- RET: 34
- RHOA: 1
- ROS1: 19
- TMPRSS2: 1

Fusion negative
Fusion positive

- Total samples: 1620
- Fusion-negative cases: 287
AML with recurrent genetic abnormalities

- t(8;21)(q22;q22.1);RUNX1-RUNX1T1
- inv(16)/t(16;16)(p13.1;q22);CBFB-MYH11
- t(6;9)(p23;q34.1);DEK-NUP214
- t(1;22)(p13.3;q13.3);RBM15-MKL1

- t(9;11)(p21.3;q22.3);MLLT3-KMT2A
- inv(3)/t(3;3)(q21.3;q26.2); GATA2, MECOM
- t(1;19)(p23;p13.3);TCF3-PBX1

B-ALL/LBL with recurrent genetic abnormalities:

- t(9;22)(q34.1;q11.2);BCR-ABL1
- t(12;21)(p13.2;q22.1); ETV6-RUNX1
- t(1;22)(p13.3;q13.3);RBM15-MKL1

- t(v;11q23.3);KMT2A rearranged
- t(5;14)(q31.1;q32.3) IL3-IGH
- t(1;19)(p23;p13.3);TCF3-PBX1

Cryptic Chromosomal Translocations

- t(5;11)(q35.3;p15.5) NUP98-NSD1
- PDGFRA (FIP1L1-PDGFR)

- t(11;12)(p15.5;p13.5) NUP98- KDM5A
- inv(16)(p13.3q24.3) CBFA2T3- GLIS2
Distribution of major molecular subtypes of precursor B-cell ALL

Targetable kinase Fusions Identified in Ph-like ALL

<table>
<thead>
<tr>
<th>Kinase</th>
<th>Inhibitor</th>
</tr>
</thead>
<tbody>
<tr>
<td>JAK2</td>
<td>Ruxolitinib</td>
</tr>
<tr>
<td>EPOR</td>
<td></td>
</tr>
<tr>
<td>CRLF2</td>
<td></td>
</tr>
<tr>
<td>ABL1</td>
<td>Dasatinib</td>
</tr>
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<td></td>
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<tr>
<td>CSF1R</td>
<td></td>
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<tr>
<td>PDGFRB</td>
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<td>NTRK3</td>
<td>Crizotinib</td>
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<tr>
<th>Target genes included in the MGH ArcherDx Heme fusion assay</th>
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<tbody>
<tr>
<td><strong>ABL1</strong></td>
</tr>
<tr>
<td><strong>ABL2</strong></td>
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<tr>
<td><strong>ALK</strong></td>
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<tr>
<td><strong>BCR</strong></td>
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<tr>
<td><strong>CBFB</strong></td>
</tr>
<tr>
<td><strong>CHD1</strong></td>
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<td><strong>CRLF2</strong></td>
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<td><strong>CSF1R</strong></td>
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<tr>
<td><strong>EBF1</strong></td>
</tr>
<tr>
<td><strong>ZCCHC7</strong></td>
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### MGH ArcherDx heme fusion assay, SNV hot spots

<table>
<thead>
<tr>
<th>Gene</th>
<th>Hot Spots</th>
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</thead>
<tbody>
<tr>
<td>ABL1</td>
<td>Y253-E255, V299, T315-F317, M351-F359</td>
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<tr>
<td>BRAF</td>
<td>V600</td>
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<tr>
<td>CRLF2</td>
<td>F232C</td>
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<tr>
<td>ETV6</td>
<td>Y104-R105</td>
</tr>
<tr>
<td>FLT3</td>
<td>D835-S838, F590-N609</td>
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<tr>
<td>IL7R</td>
<td>S185, P240-S246</td>
</tr>
<tr>
<td>JAK1</td>
<td>R724, S703, V658</td>
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<tr>
<td>JAK2</td>
<td>F537-F547, V617-C618, L681-R683</td>
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<tr>
<td>JAK3</td>
<td>S789, R657, A572-A573, M511</td>
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<tr>
<td>KRAS</td>
<td>A146, Q61, G12-G13</td>
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<tr>
<td>NRAS</td>
<td>G60-Q61, G12-G13</td>
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<tr>
<td>PAX5</td>
<td>P80R</td>
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<tr>
<td>PDGFRA</td>
<td>T674, V824</td>
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<tr>
<td>PTPN11</td>
<td>G60-D61, E69-T73, E76, S502-G503</td>
</tr>
<tr>
<td>SH2B3</td>
<td>E208, D231-D234</td>
</tr>
</tbody>
</table>
MGH ArcherDx heme fusion assay, validation samples results

Samples (n=92)

- = point mutations
- = gene fusions

Slide Courtesy of Max Jan
MGH ArcherDx heme fusion assay, selected cases

- Inform treatment
- Help monitoring the patient’s disease
- Clarify a karyotype result
- Help reaching a diagnosis
MGH ArcherDx heme fusion assay, selected cases

- Inform treatment
- Help monitoring the patient’s disease
- Clarify a karyotype result
- Help reaching a diagnosis
Case 1 (retrospective analysis)

17 yo boy with precursor B- acute lymphoblastic leukemia

Karyotype analysis: 59XY +X +X +X dup(1)(q21q43)  
+4 +6 +10 +14 +14 +17 +18  
+der(18)t(11;18)(q13;q23) +19 +21 +21 +mar +mar

FISH testing: Negative for ETV6/RUNX1, KMT2A rearrangements

Clinical history: Low risk- Reached CR- Alive
Case 1

fusion reads from EBF1 exon 15 primer →

← fusion reads from PDGFRB exon 11 primer
## Case 1

<table>
<thead>
<tr>
<th>Kinase Gene</th>
<th>Tyrosine Kinase Inhibitor</th>
<th>Fusion Partners</th>
<th>Patients</th>
<th>5’ Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABL1</td>
<td>Dasatinib</td>
<td>6</td>
<td>14</td>
<td>ETV6, 11 NUP214, 13 RCSD1, 11 RANBP2, 11 SNX2, 10 ZMIZ1, 20</td>
</tr>
<tr>
<td>ABL2</td>
<td>Dasatinib</td>
<td>3</td>
<td>7</td>
<td>PAG1, * RCSD1, * ZC3HAV1 *</td>
</tr>
<tr>
<td>CSF1R</td>
<td>Dasatinib</td>
<td>1</td>
<td>4</td>
<td>SSBP2 *</td>
</tr>
<tr>
<td>PDGFRB</td>
<td>Dasatinib</td>
<td>4</td>
<td>11</td>
<td>EBF1, 13-13 SSBP2, * TNIP1, * ZEB2 *</td>
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<tr>
<td>CRLF2</td>
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<td>30</td>
<td>IGH, 21 P2RY82</td>
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<tr>
<td>JAK2</td>
<td>JAK2 inhibitor</td>
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<td>19</td>
<td>ATF7IP, * BCR, 11 EBF1, * ETV6, 21 PAX5, 13 PPFIBP1, * SSBP2, 24 STRN3, 11 TERF2, * TPR *</td>
</tr>
<tr>
<td>EPOR</td>
<td>JAK2 inhibitor</td>
<td>2</td>
<td>9</td>
<td>IGH, 11 IGK *</td>
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<tr>
<td>DGKH</td>
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<td>1</td>
<td>ZFAND3 *</td>
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<tr>
<td>IL2RB</td>
<td>JAK1 inhibitor, JAK3 inhibitor, or both</td>
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<td>MYH9 *</td>
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<tr>
<td>TSLP</td>
<td>JAK2 inhibitor</td>
<td>1</td>
<td>1</td>
<td>IQGAP2 *</td>
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<tr>
<td>TYK2</td>
<td>TYK2 inhibitor</td>
<td>1</td>
<td>1</td>
<td>MYB *</td>
</tr>
</tbody>
</table>

* The gene is a previously unreported fusion partner.
† ETV6–NTRK3 has been reported in multiple cancers, including congenital fibrosarcoma 25, 26 and secretory breast carcinoma, 27 but it has not previously been described in acute lymphoblastic leukemia. 28, 29
Case 2 (retrospective analysis)

15 yo boy with precursor B- acute lymphoblastic leukemia

Karyotype analysis: 46,XY,add(1)(q21),add(7)(q36)

FISH testing: Negative for ETV6/RUNX1, KMT2A, BCR-ABL1 rearrangements

Clinical history: Reached CR- Relapsed- Died of infection
Case 2- (retrospective analysis)

Case 2
<table>
<thead>
<tr>
<th>Kinase Gene</th>
<th>Tyrosine Kinase Inhibitor</th>
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<td>PAG1,* RCSD1,* ZC3HAV1*</td>
</tr>
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<td>ATF7IP,* BCR,11 EBF1,* ETV6,13 PAX5,13 PPFIBP1,* SSBP2,24 STRN3,11 TERF2,* TPR*</td>
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<td>1</td>
<td>ETV625-27*</td>
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<tr>
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<td>KDM6A,* STAG2*</td>
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<tr>
<td>TSLP</td>
<td>JAK2 inhibitor</td>
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<td>1</td>
<td>IQGAP2*</td>
</tr>
<tr>
<td>TYK2</td>
<td>TYK2 inhibitor</td>
<td>1</td>
<td>1</td>
<td>MYB*</td>
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Case 3- retrospective analysis

16 yo boy with precursor B- acute lymphoblastic leukemia

Karyotype analysis: 46XY del(4)(q?31.1)

FISH testing: Negative for ETV6/RUNX1, KMT2A, BCR-ABL1 rearrangement, Trisomy 4 and 10

Clinical history: Failed induction- BMT-
Died of transplant related complications
Case 3

PAX5-JAK2

Exon 4  exon 19

Tyrosine kinase domain

395 fusion reads
Case 3
## Case 3

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</tr>
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<td>JAK2 inhibitor</td>
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<td>IGH,21 P2RY8*2</td>
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<td>KDM6A,* STAG2*</td>
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<td>IQGAP2*</td>
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Conclusions (cases 1-3)

- The heme fusion assay detected cytogenetically cryptic gene fusions characteristic of BCR-ABL1-like ALL

- The fusions identified in the three cases activate kinases and could be sensitive to targeted treatment with kinase inhibitors
Inform treatment

Help monitoring the patient’s disease

Clarify a karyotype result

Help reaching a diagnosis
Case 4

50 yo man with Ph+ B-ALL

Karyotype analysis: 46,XY,t(9;22)(q34;q11.2)[9]/46,XY[11]

RT-PCR for BCR-ABL1 chimeric transcripts: negative

-- BCR ex13/14-ABL ex2 (B2A2 and B3A2): negative (p210)
-- BCR ex1-ABL ex2 (E1A2): negative (p190)
Case 4

Exon 13  BCR
Exon 3  ABL1

459 fusion reads

BCR-ABL fusion transcripts in B-ALL

typical BCR-ABL fusion transcripts

e1 a2 a3 a11  e1a2  (p190)
e1 e2 3 4 5 8 9 10 a2 a3 a11  e13a2  (p210)
e1 e2 3 4 5 8 9 10 a2 a3 a11  e14a2  (p210)

atypical BCR-ABL fusion transcripts

e1 a3 a11  e1a3  (p210)
e1 e2 3 4 5 8 9 10 a3 a11  e13a3

e1 e2 3 4 5 a2 a3 a11  e6a2

Burmeister T. et al, Haematologica 2007
Conclusions (case 4)

- The heme fusion assay detected an unusual BCR-ABL fusion transcript and could be used to monitor the patient’s disease
Inform treatment
Help monitoring the patient’s disease
Clarify a karyotype result
Help reaching a diagnosis
Case 5

24 yo female with B-ALL and 2 possible gene fusions

Karyotype analysis:
45,XX, t(1;19)(q23;p13), der(9;12)(q10;q10)[cp15]/46, idem, +mar[cp2]/
46,XX[3]

Note: Translocation t(1;19) is a recurrent aberration in pre-B ALL which is
the cytogenetic hallmark of TCF3-PBX1 fusion.

Der(9;12) for either dic(9;12)(p13;p13) (PAX5/ETV6 rearrangement) or
dic(9;12)(p13;p12) (PAX5/SLCO1B3 rearrangement).
Case 5

Heme fusion assay: negative for gene fusions

RT-PCR for TCF3-PBX1 fusion: negative for gene fusion

PAX5 breakapart FISH
no rearrangement
Few notes on the t(1;19) translocation.

- 5-10% of cases have translocations that do not affect TCF3 or PBX1.

- Identification of a novel fusion gene (MEF2D-DAZAP1) in a B acute lymphoblastic leukemia with t(1;19)(q23;p13).

- Because more intensive therapy improves the outcome of patients with TCF3-PBX1\textsuperscript{positive} (1;19) translocations, it is critical to identify this subset of patients so that appropriate therapy can be administered.

Boomer T. et al., Leukemia 2001; Yuki Y. et al., Cancer Sci. 2004
Few notes on the PAX rearrangements

Break-point heterogeneity for dic(9;20)

Extent of deleted region
Case 6

61 yo female with B-ALL and an NRAS p.Gly12Ser mutation

Karyotype analysis:
45,X,- X,der(7;12)(q10;q10),add(9)(p13),+mar[cp17]/46,XX[3]

Diagnosis: BCR-ABL1-like B- ALL
Case 6

Heme fusion assay: PAX5 ex 6 and DMRTA2 exon1
Case 6

RT PCR confirmation of PAX5-DMRTA2 fusion
Conclusions (cases 5 and 6)

- The heme fusion assay
  - showed that rearrangements seen on karyotype do not necessarily correspond to presumed/expected gene fusions
  - detected a novel cryptic PAX5 rearrangement
MGH ArcherDx heme fusion assay, selected cases

- Inform treatment
- Help monitoring the patient’s disease
- Clarify a karyotype result
- Help reaching a diagnosis
Case 7

22 yo man with AML, possibly APML
Case 7

22 yo man with AML, possibly APML
Case 7

22 yo man with AML, possibly APML

Karyotype analysis:
46,XY,t(4;17)(q21;q21),add(11)(p15)[9]/48,idem,+der(4)t(4;17),+21[cp11].ishder(4)t(4;17)(FIP1L1+,LNX+,PDGFRA+;TP53+,D17Z1+,
RARA+)x1~2,+der(17)t(4;17) (TP53+,D17Z1+,dim3'RARA+)

FISH testing: FISH with the RARA probe showed that the chromosome 17q breakpoint of the t(4;17) was very close to the 3'end of RARA. Whether this rearrangement led to RARA de-regulation was unclear.
Case 8

\[ t(4;17)(q21;q21) \neq \text{recurrent} \]
\[ t(4;17)(q12;q21) \ (\text{FIP1L1/RARA}) \ (\text{JMML}) \]

NUP98-LAMC3 (novel) fusion
\[ t(9;11) (q34 ;p15) \]
319 fusion reads
NUP98 fusion partners

- **Homeodomain proteins** (e.g. HOX proteins)

- **Non homeodomain proteins**
  
  -- proteins that contain a coiled-coil domain
  (oligomerization of proteins)

  -- histone “reading” or “writing” domain
Case 8

51yo man with AML, ? CBFB rearranged AML

Karyotype analysis:
46,XY,der(16)t(16;18)(p1?2;p11.3)del(16)(q22q24),der(18)t(16;18)(p12;p11.3)[20] .ish der(16)(18pter+,5'CBFB+),der(18)(pter-)

**FISH testing:** FISH assay showed 5'CBFB on the rearranged chromosome 16, however, 3'CBFB was lost.
“This FISH result is consistent with either an unbalanced CBFB rearrangement and/or a deletion of 16q.”
Case 8

Exon 1-5  CBFB  Exon 33/34-42  MYH11

CBFB-MYH11
t(16;16) (p13 ;q22)
or inv(16)(p13q22)

423 fusion reads
Conclusions (cases 7 & 8)

- The heme fusion assay clarified an equivocal cytogenetic finding

- The results of the heme fusion assay affected patient’s management:
  -- no APML treatment and consideration of BMT (case 5)
  -- good prognosis with no need of BMT (case 6)
MGH ArcherDx heme fusion assay, selected cases

- Inform treatment
- Help monitoring the patient’s disease
- Clarify a karyotype result
- Help reaching a diagnosis
Case 9

55 yo man with metastatic Ewing sarcoma. Bone marrow involvement (SH 2017, case 288)

AE1/AE3-, CD99+-, lysozyme-
CD138-, CD34-, CD117-, CD19-, Tdt-, CD64-, CD56-, Cd11c-, CD4+, CD3-CD2-
EWSR1 FISH-

KARYOTYPE:
46,XY,del(4)(q31q33), der(9)t(1;9)(q21;q13), t(11;20)(q12;q13.1)[17]/46,XY[3].ish
t(11;20)((MLL-;MLL+),22q12(EWSR1+)x2

Initial diagnosis: High-grade malignant neoplasm
Fusion of ZMYND8 and RELA Genes in Acute Erythroid Leukemia

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Abstract

Acute erythroid leukemia was diagnosed in a 4-month-old boy. Cytogenetic analysis of bone marrow (BM) cells showed a t(1;19)(p11;q11) translocation. RNA extracted from the BM was sequenced and analyzed for fusion transcripts using the software FusionMap. A ZMYND8-RELA fusion was ranked first. RT-PCR and direct sequencing verified the presence of an in-frame ZMYND8-RELA chimeric transcript. Fluorescence in situ hybridization showed that the ZMYND8-RELA was located on the p12 band of chr(11); therefore a cytogenetically invisible pericentric inversion in chromosome 11 must have taken place besides the translocation. The putative ZMYND8-RELA fusion protein contains the Zinc-Finger domain, a bromodomain, a PWWP domain, a MYND type of zinc finger of ZMYND8, and the entire RELA protein, indicating that it might act leukemogenetically by influencing several cellular processes including the NF-kappaB pathway.
Case 9

Glycophorin

Final diagnosis: Therapy-related pure acute erythroid leukemia
Conclusions (case 9)

- The solid fusion assay detected a gene fusion which has been reported once in a pure erythroid leukemia and helped reaching an otherwise difficult/unsuspected diagnosis
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Case 1 (retrospective analysis)

16 yo boy with precursor B- acute lymphoblastic leukemia

Karyotype analysis: 54XXY +X +X +4 +9 add(9)(p24) +14 +18 +21

FISH testing: Negative for ETV6/RUNX1 and KMT2A rearrangements

Clinical history: Low risk- Reached CR- Alive
Case 1

EBF1-PTK2B

Exon 4

Exon 6

90 fusion reads
# Case 1

<table>
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<tr>
<th>Kinase Gene</th>
<th>Tyrosine Kinase Inhibitor</th>
<th>Fusion Partners</th>
<th>Patients</th>
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<td>ABL1</td>
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<td>ETV6,11 NUP214,13 RCSD1,11 RANBP2,11 SNX2,19 ZMIZ120</td>
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<td>1</td>
<td>MYB*</td>
</tr>
</tbody>
</table>

* The gene is a previously unreported fusion partner.
† ETV6–NTRK3 has been reported in multiple cancers, including congenital fibrosarcoma25,26 and secretory breast carcinoma,27 but it has not previously been described in acute lymphoblastic leukemia.28,29
Case 9

3 yo child with down syndrome and B-ALL

Karyotype analysis: 46,XX,dic(9;20)(p11-13;q11),+21c[17]/47,XX,+21c[3].nuc ish (D4Z1,D10Z1, D17Z1)x2,(ABL1,BCR)x2[100],(MLLx2)[100],(ETV6x2,RUNXx3)[93/100]

Note: dic(9;20) targets PAX5 gene on chromosome 9p and is a recurrent aberration in a subgroup of B-ALL.
Case 9

Heme fusion assay: negative for gene fusions

PAX5 breakapart FISH: no rearrangement