Heme fusion assay

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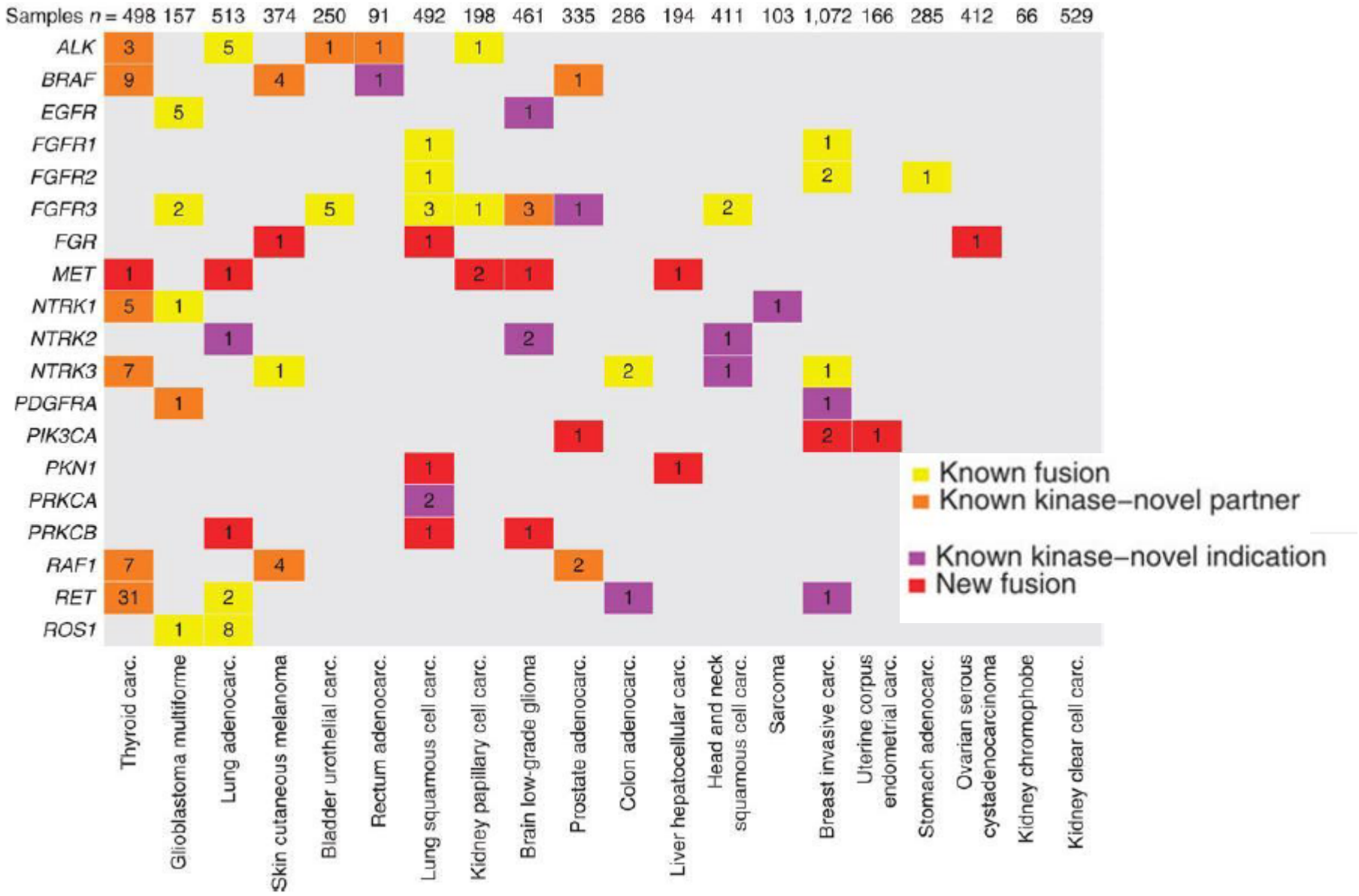
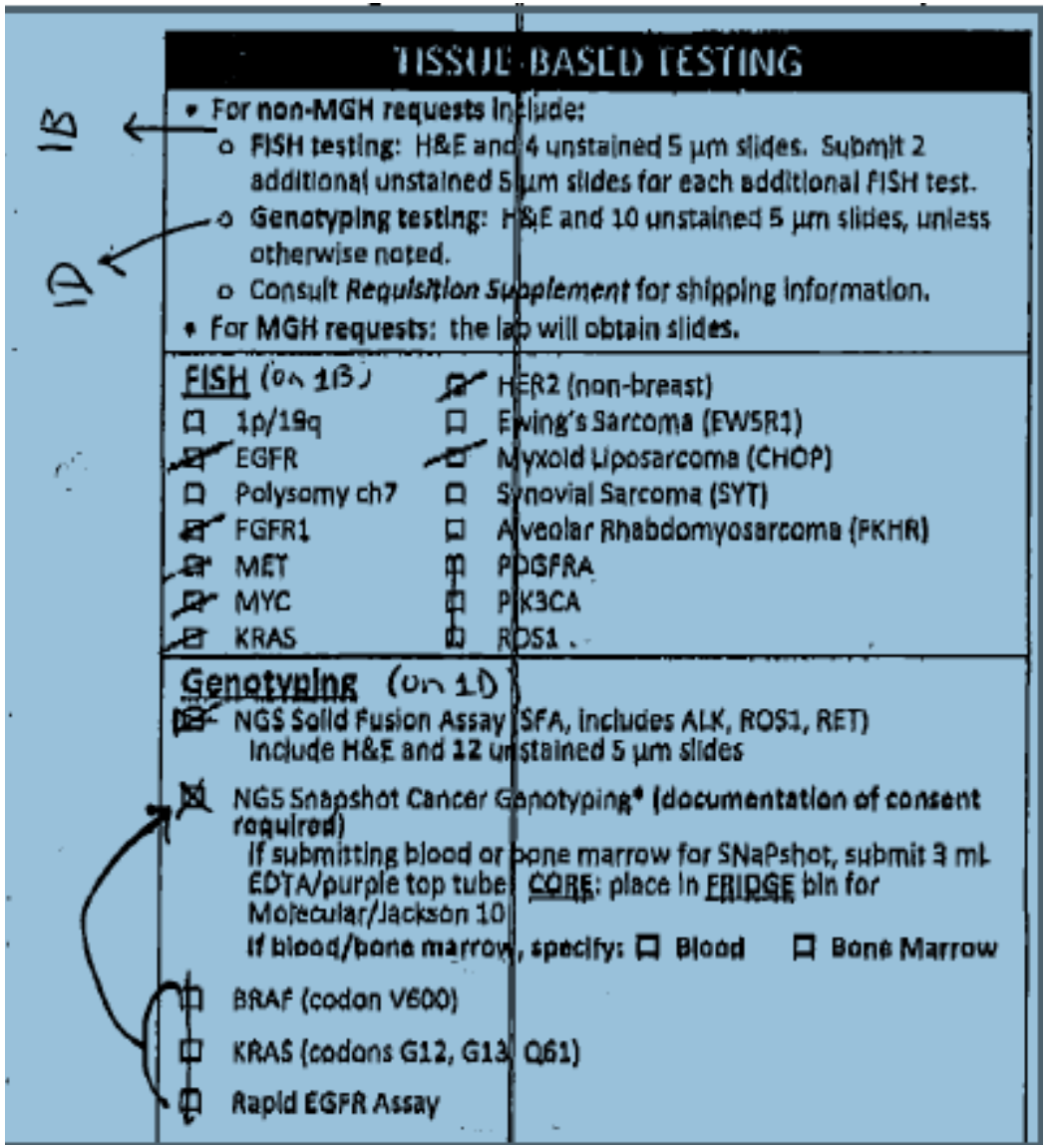
No personal disclosures/conflicts of interest

Kinase fusions in solid tumors



The landscape of kinase fusions in cancer, Nat Comm, 2014

How can we keep up ?



TISSUE BASED TESTING

- For non-MGH requests include:
  - FISH testing: H&E and 4 unstained 5 µm slides. Submit 2 additional unstained 5 µm slides for each additional FISH test.
  - Genotyping testing: H&E and 10 unstained 5 µm slides, unless otherwise noted.
  - Consult *Requisition Supplement* for shipping information.
- For MGH requests: the lab will obtain slides.

FISH (on 1B)

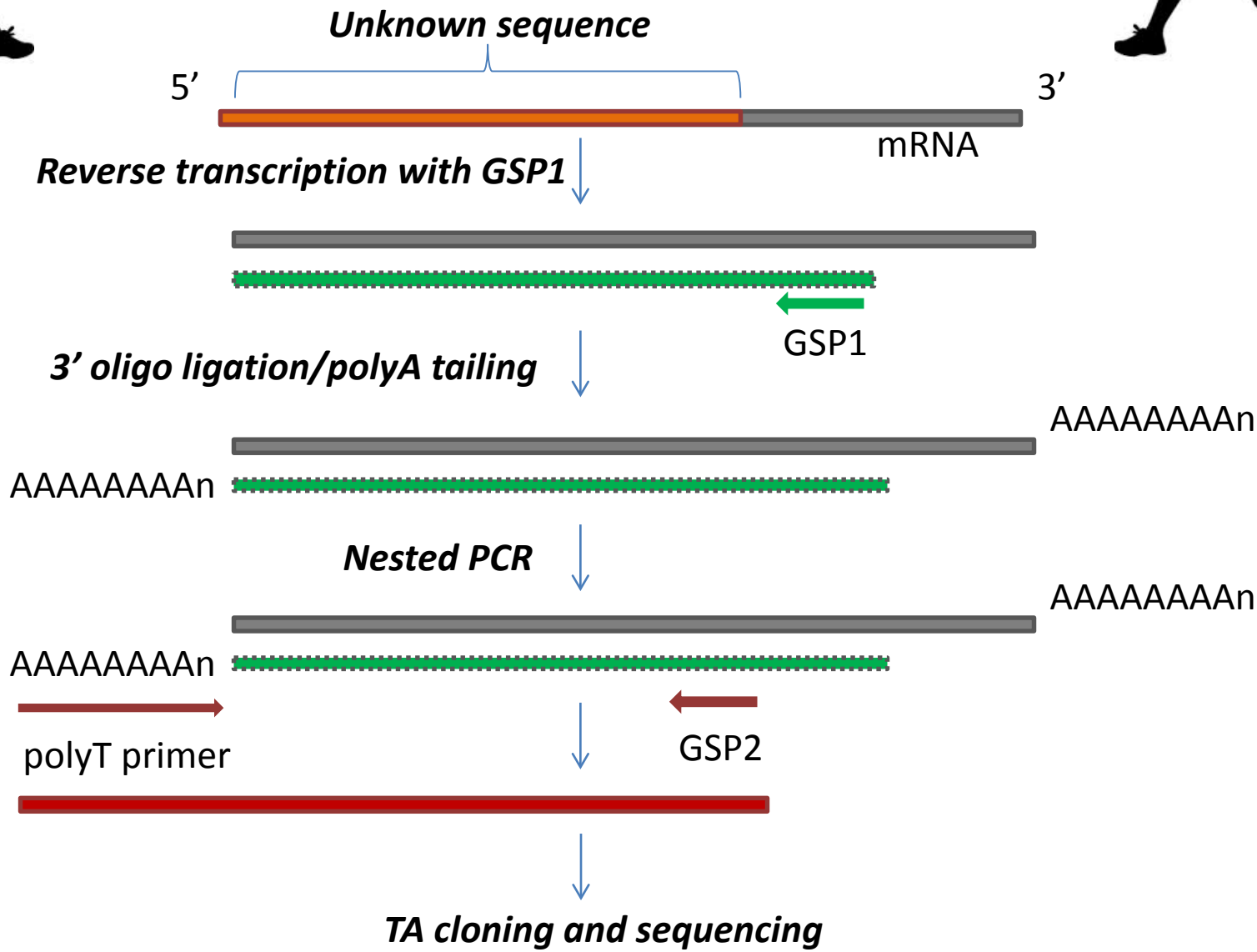
- ☐ 1p/19q
- ☑ EGFR
- ☐ Polysomy ch7
- ☑ FGFR1
- ☑ MET
- ☑ MYC
- ☑ KRAS
- ☑ HER2 (non-breast)
- ☐ Ewing's Sarcoma (EWSR1)
- ☑ Myxoid Liposarcoma (CHOP)
- ☐ Synovial Sarcoma (SYT)
- ☐ Alveolar Rhabdomyosarcoma (FKHR)
- ☐ PDGFRA
- ☐ PIK3CA
- ☐ ROS1

Genotyping (on 1D)

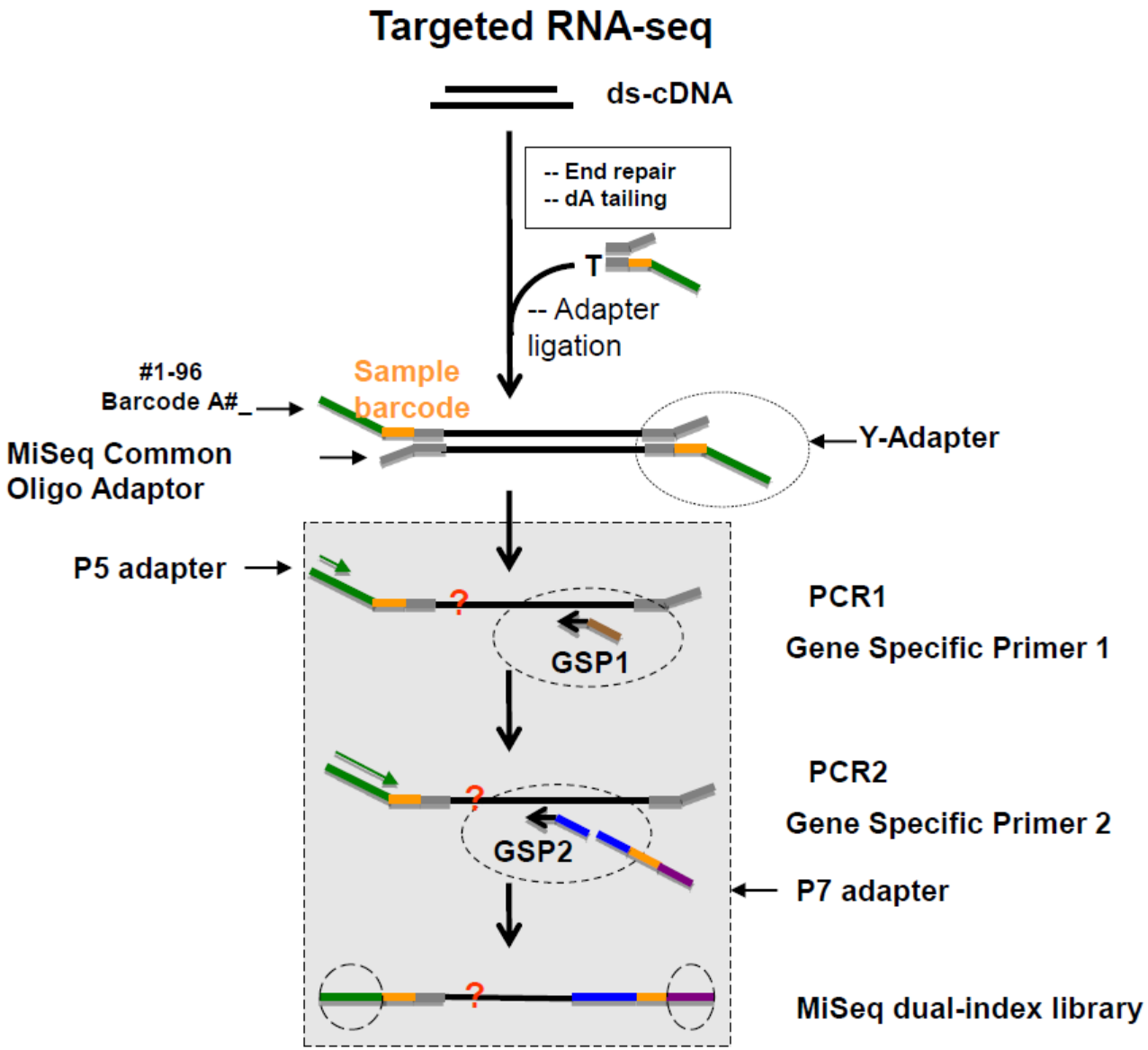
- ☑ NGS Solid Fusion Assay (SFA, includes ALK, ROS1, RET) Include H&E and 12 unstained 5 µm slides
- ☒ NGS Snapshot Cancer Genotyping* (documentation of consent required) If submitting blood or bone marrow for SNaPshot, submit 3 mL EDTA/purple top tube. CORE: place in FRIDGE bin for Molecular/Jackson 10
  - If blood/bone marrow, specify: ☐ Blood ☐ Bone Marrow
- ☐ BRAF (codon V600)
- ☐ KRAS (codons G12, G13, Q61)
- ☐ Rapid EGFR Assay

Test
FGFR1 FISH
MYC FISH
HER2 FISH
EGFR FISH
ROS1 FISH
PIK3CA FISH
KRAS FISH
MET FISH
PDGFRA FISH
CHOP FISH
Solid Fusion Assay V1
SNAPSHOT-NGS-V1

AMP as a modification of Rapid Amplification of cDNA Ends (5' RACE & 3' RACE)



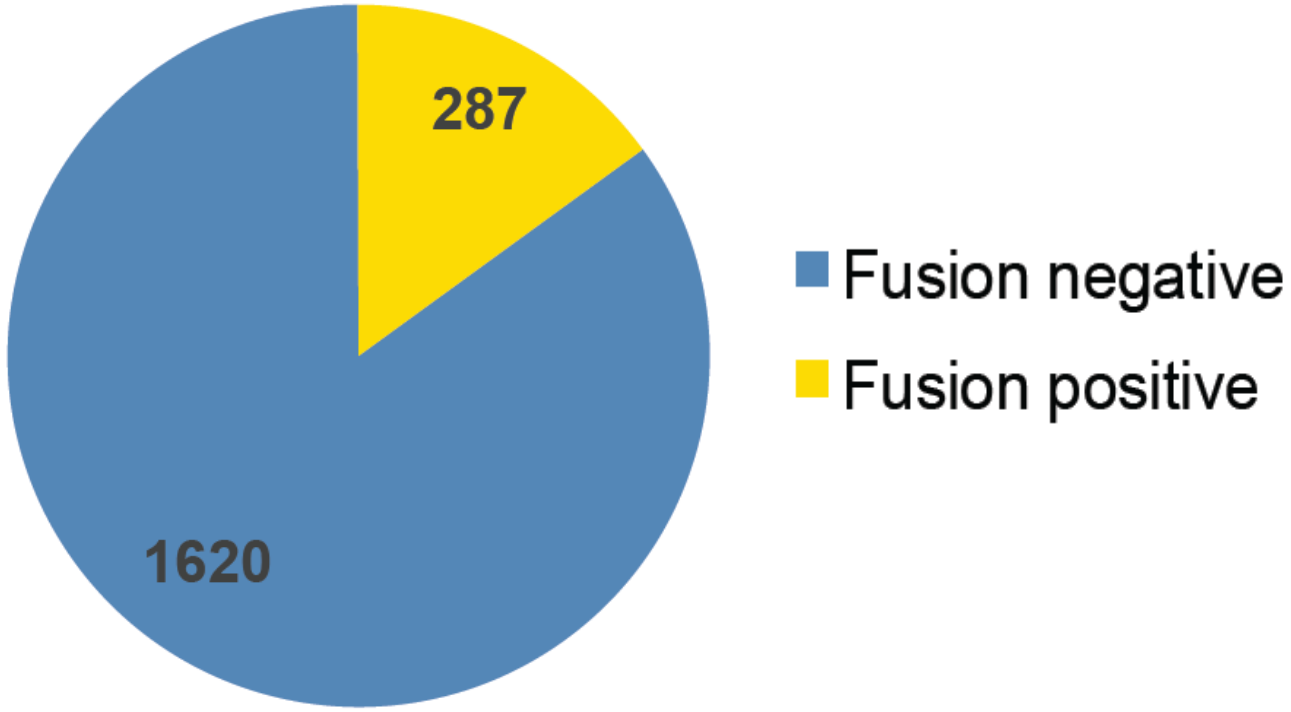
Anchored multiplexed PCR (AMP) assay



Zongli Zheng & Long Le, Nature Medicine 2014

AMP-based Solid Fusion Assay

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



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

Gene Fusions in AML and ALL - WHO 2016

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
APL with PML-RARA
t(9;11)(p21.3;q23.3);MLLT3-KMT2A
inv(3)/t(3;3)(q21.3;q26.2); GATA2, MECOM
AML with t(9;22)(q34.1;q11.2);BCR-ABL1

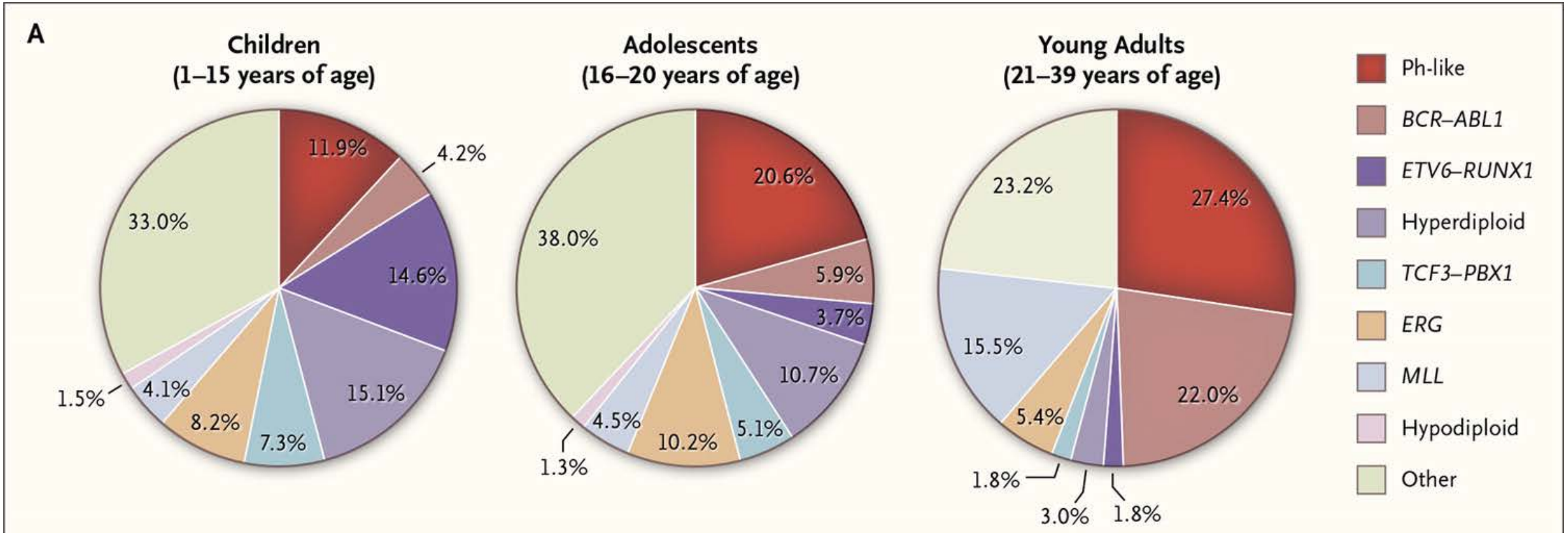
B-ALL/LBL with recurrent genetic abnormalities:

t(9;22)(q34.1;q11.2);BCR-ABL1
t(12;21)(p13.2;q22.1); ETV6-RUNX1
t(1;19)(q23;p13.3);TCF3-PBX1
t(v;11q23.3);KMT2A rearranged
t(5;14)(q31.1;q32.3) IL3-IGH
BCR-ABL1–like

Cryptic Chromosomal Translocations

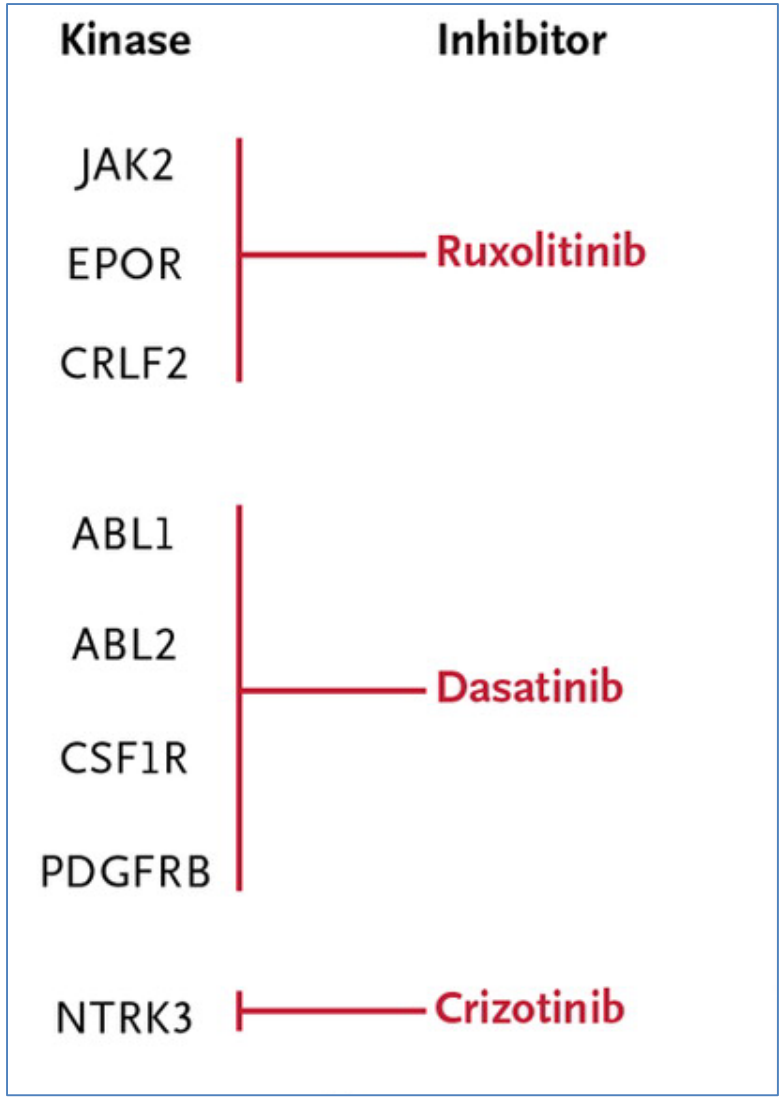
t(5;11)(q35.3;p15.5) NUP98-NSD1
PDGFRA (FIP1L1-PDGFRA)
BCR-ABL1–like fusions
t(11;12)(p15.5;p13.5) NUP98- KDM5A
inv(16)(p13.3q24.3) CBFA2T3- GLIS2

Distribution of major molecular subtypes of precursor B-cell ALL



Graubert TA. N Engl J Med 2014;371:1064-1066.

Targetable kinase Fusions Identified in Ph-like ALL



Graubert TA. N Engl J Med 2014;371:1064-1066.

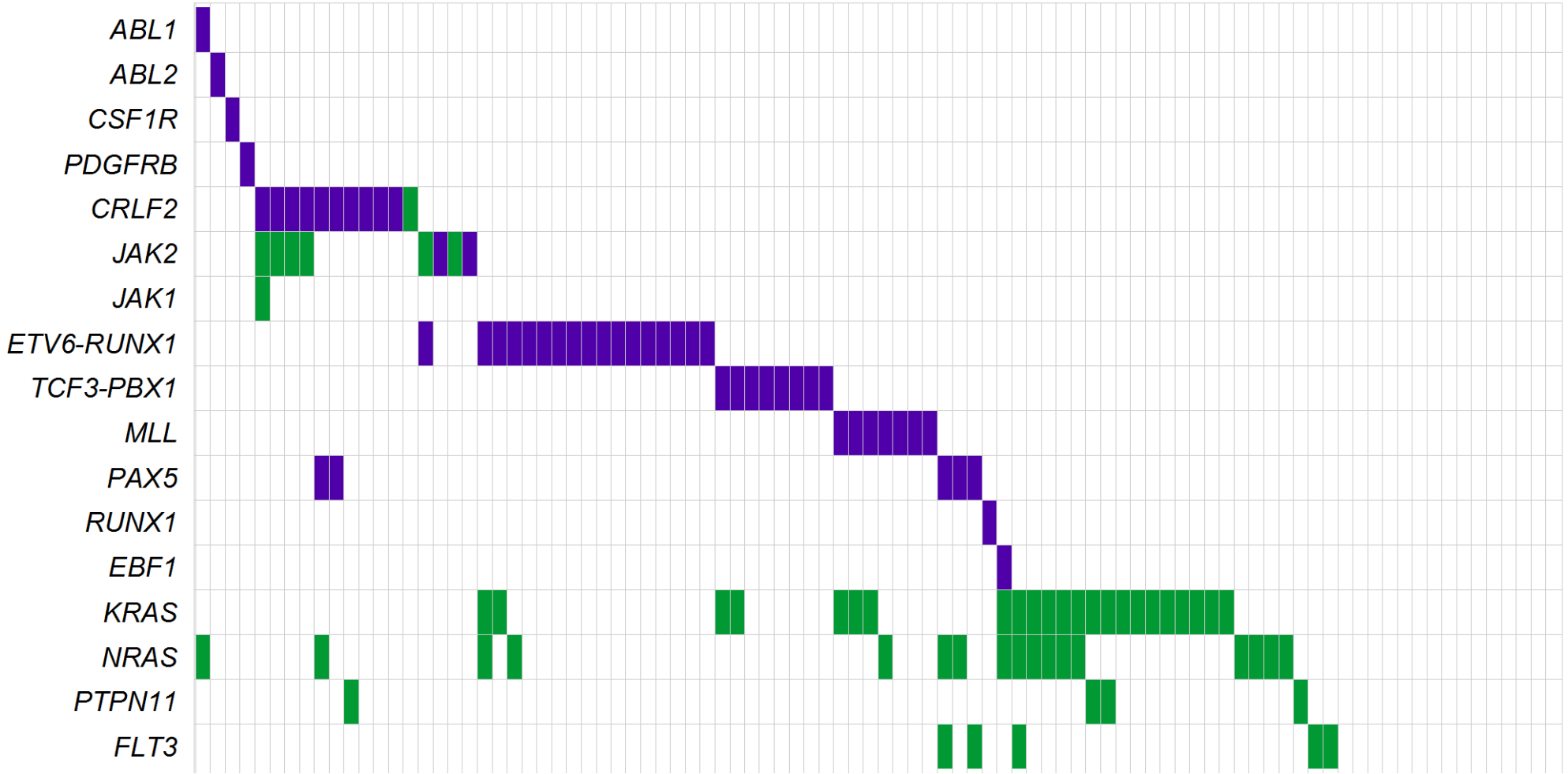
Target genes included in the MGH ArcherDx Heme fusion assay

ABL1	EPOR	NTRK3	RBM15
ABL2	ERG	NUP214	RUNX1
ALK	ETV6	NUP98	RUNX1T1
BCR	FGFR1	P2RY8	SEMA6A
CBFB	JAK2	PAX5	SETD2
CHD1	KLF2	PDGFRA	TAL1
CRLF2	KMT2A	PDGFRB	TCF3
CSF1R	MECOM	PICALM	TYK2
EBF1	MKL1	PTK2B	IKZF1
ZCCHC7	NOTCH1	RARA	FLT3

MGH ArcherDx heme fusion assay, SNV hot spots

ABL1:*Y253-E255,V299,T315-F317,M351-F359*
BRAF*:V600*
CRLF2*:F232C*
ETV6*:Y104-R105*
FLT3*:D835-S838,F590-N609*
IL7R*:S185,P240-S246*
JAK1*:R724,S703,V658*
JAK2*:F537-F547,V617-C618,L681-R683*
JAK3*:S789,R657,A572-A573,M511*
KRAS*:A146,Q61,G12-G13*
NRAS*:G60-Q61,G12-G13*
PAX5*:P80R*
PDGFRA*:T674,V824*
PTPN11*:G60-D61,E69-T73,E76,S502-G503*
SH2B3*:E208,D231-D234*

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

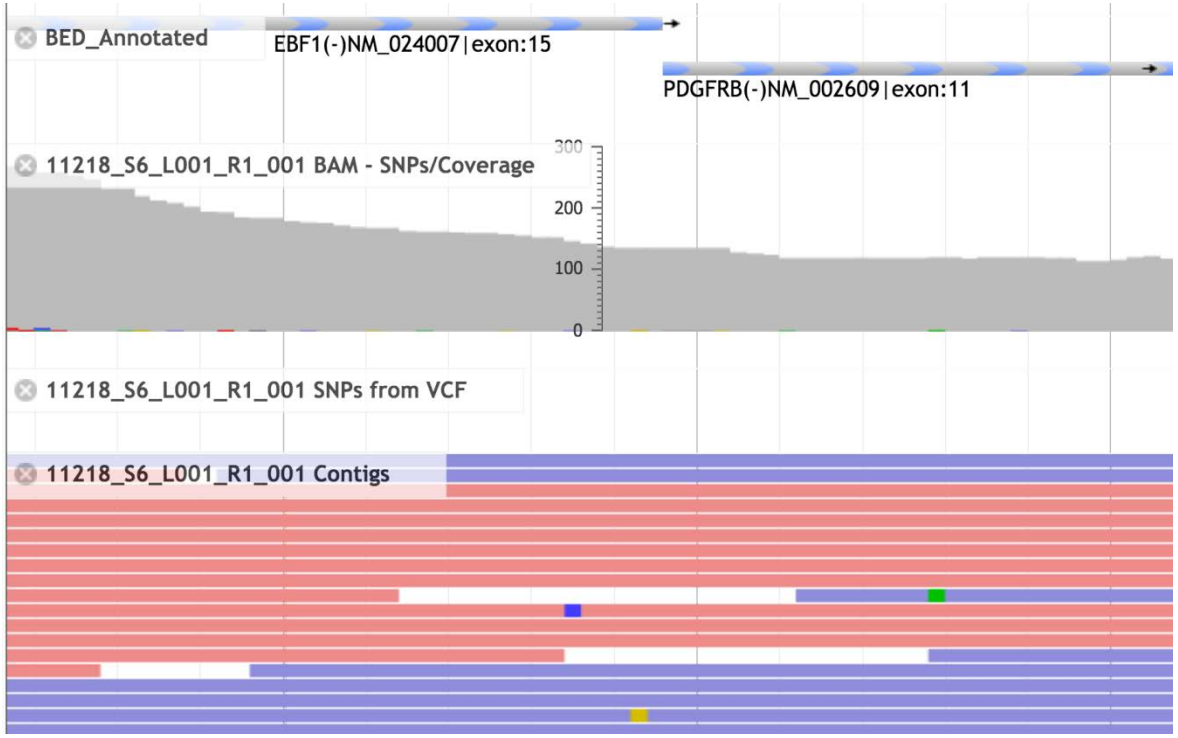
FISH testing: Negative for ETV6/RUNX1, KMT2A rearrangements

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 1. Kinase Fusions Identified in Ph-like Acute Lymphoblastic Leukemia.

Kinase Gene	Tyrosine Kinase Inhibitor	Fusion Partners	Patients	5′ Genes
		number		
ABL1	Dasatinib	6	14	*ETV6*,[11] *NUP214*,[11] *RCSD1*,[11] *RANBP2*,[11] *SNX2*,[19] *ZMIZ1*[20]
ABL2	Dasatinib	3	7	*PAG1*,* *RCSD1*,* *ZC3HAV1**
CSF1R	Dasatinib	1	4	*SSBP2**
PDGFRB	Dasatinib	4	11	*EBF1*,[11-13] *SSBP2*,* *TNIP1*,* *ZEB2**
CRLF2	JAK2 inhibitor	2	30	*IGH*,[21] *P2RY8*[22]
JAK2	JAK2 inhibitor	10	19	*ATF7IP*,* *BCR*,[11] *EBF1*,* *ETV6*,[23] *PAX5*,[11] *PPFIBP1*,* *SSBP2*,[24] *STRN3*,[11] *TERF2*,* *TPR**
EPOR	JAK2 inhibitor	2	9	*IGH*,[11] *IGK**
DGKH	Unknown	1	1	*ZFAND3**
IL2RB	JAK1 inhibitor, JAK3 inhibitor, or both	1	1	*MYH9**
NTRK3	Crizotinib	1	1	*ETV6*[25-27]†
PTK2B	FAK inhibitor	2	1	*KDM6A*,* *STAG2**
TSLP	JAK2 inhibitor	1	1	*IQGAP2**
TYK2	TYK2 inhibitor	1	1	*MYB**

* 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]

Roberts KG et al. N Engl J Med 2014;371:1005-1015.

Case 2 (retrospective analysis)

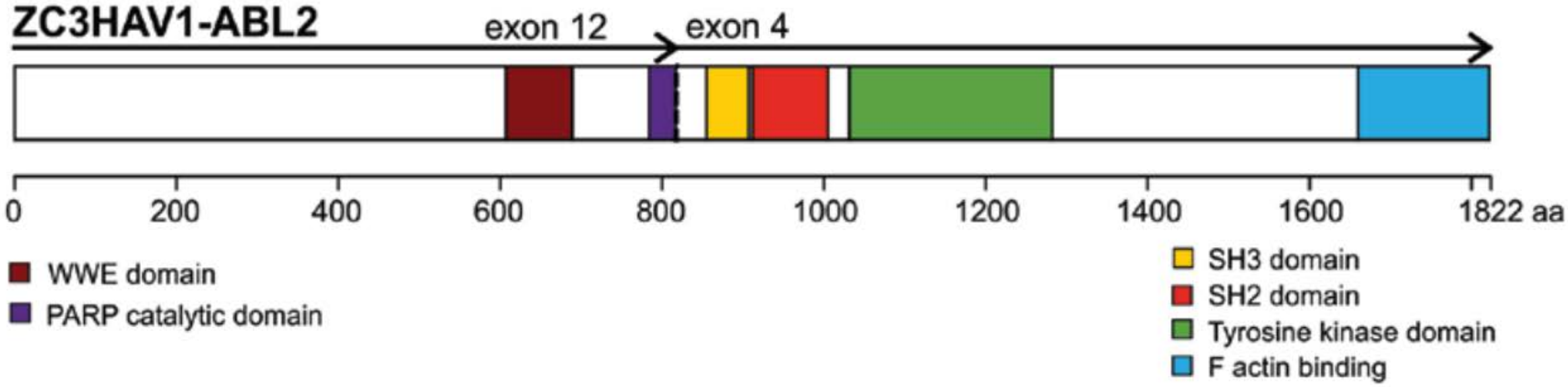
15 yo boy with precursor B- acute lymphoblastic leukemia

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

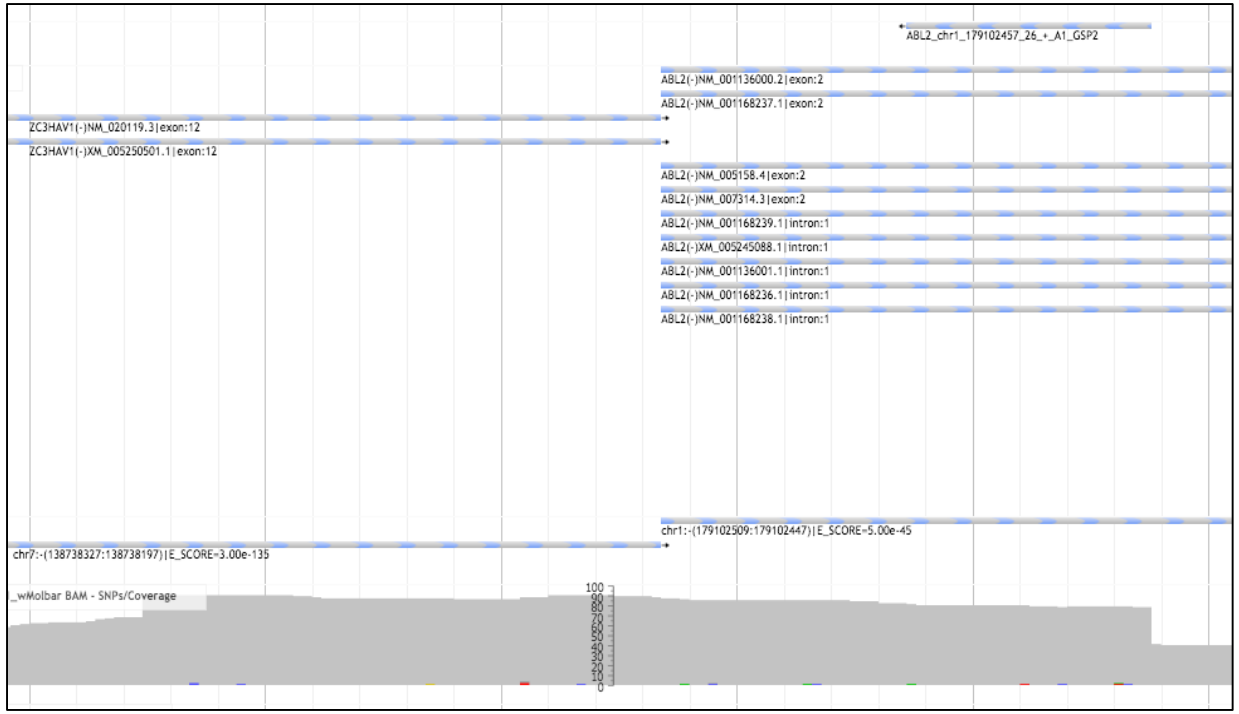
Case 2- (retrospective analysis)

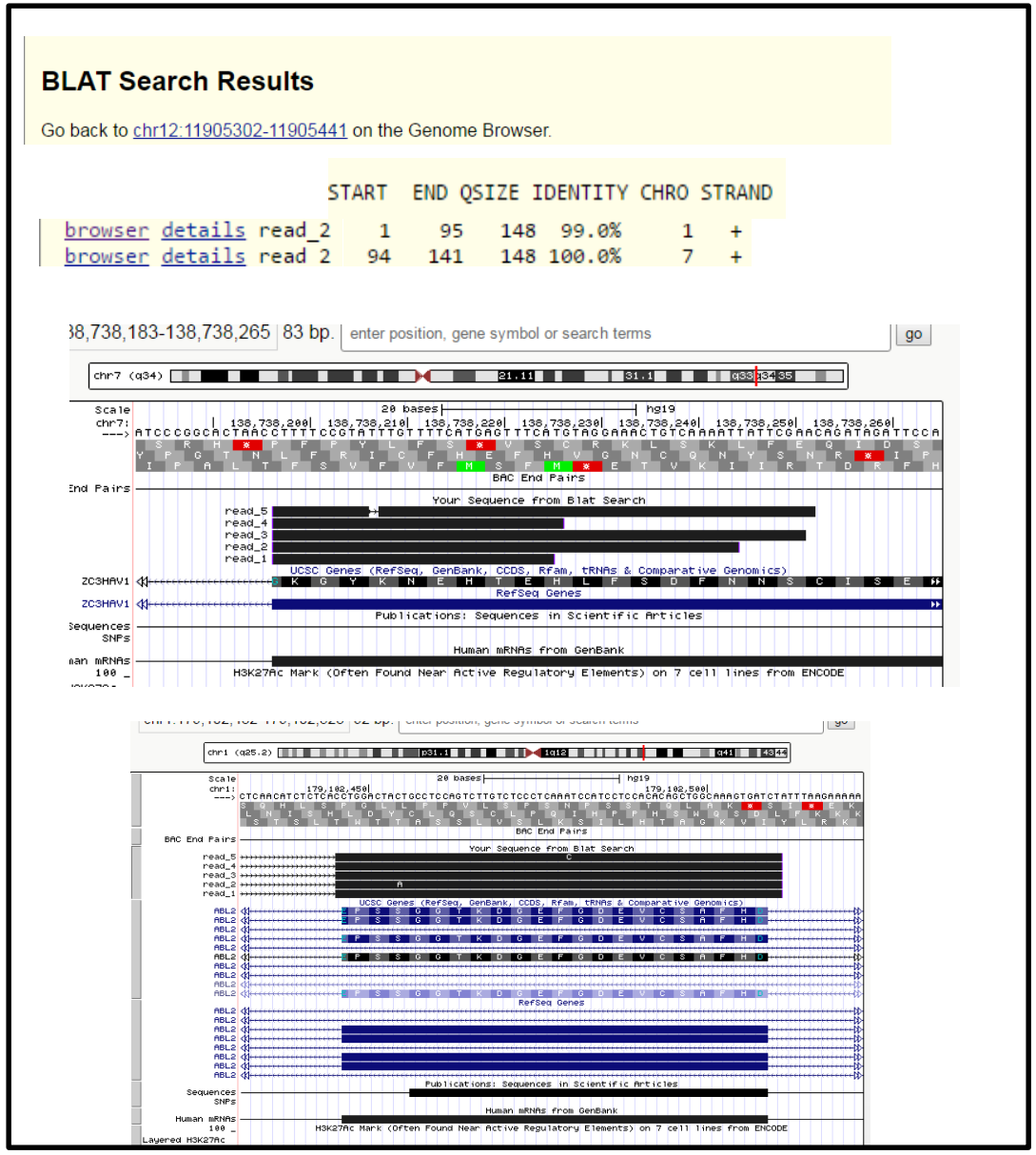


>1000 fusion reads

Roberts KG et al. N Engl J Med 2014;371:1005-1015.

Case 2


ABL2_chr1_179102457_26_+_A1_GSP2
ABL2(-)NM_001136000.2|exon:2
ABL2(-)NM_001168237.1|exon:2
ZC3HAV1(-)NM_020119.3|exon:12
ZC3HAV1(-)XM_005250501.1|exon:12
ABL2(-)NM_005158.4|exon:2
ABL2(-)NM_007314.3|exon:2
ABL2(-)NM_001168239.1|intron:1
ABL2(-)XM_005245088.1|intron:1
ABL2(-)NM_001136001.1|intron:1
ABL2(-)NM_001168236.1|intron:1
ABL2(-)NM_001168238.1|intron:1
chr1:-(179102509:179102447)|E_SCORE=5.00e-45
chr7:-(138738327:138738197)|E_SCORE=3.00e-135
_wMolbar BAM - SNPs/Coverage


BLAT Search Results
Go back to chr12:11905302-11905441 on the Genome Browser.
START END QSIZE IDENTITY CHRO STRAND
browser details read_2 1 95 148 99.0% 1 +
browser details read_2 94 141 148 100.0% 7 +
8,738,183-138,738,265 83 bp.
enter position, gene symbol or search terms
go
chr7 (q34)
BAC End Pairs
Your Sequence from Blat Search
read_5
read_4
read_3
read_2
read_1
UCSC Genes (RefSeq, GenBank, CCDS, Rfam, tRNAs & Comparative Genomics)
ZC3HAV1
RefSeq Genes
Publications: Sequences in Scientific Articles
Human mRNAs from GenBank
H3K27Ac Mark (Often Found Near Active Regulatory Elements) on 7 cell lines from ENCODE

Case 2

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Kinase Gene	Tyrosine Kinase Inhibitor	Fusion Partners	Patients	5′ Genes
		number		
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TSLP	JAK2 inhibitor	1	1	*IQGAP2**
TYK2	TYK2 inhibitor	1	1	*MYB**

* 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]

Roberts KG et al. N Engl J Med 2014;371:1005-1015.

Case 3- retrospective analysis

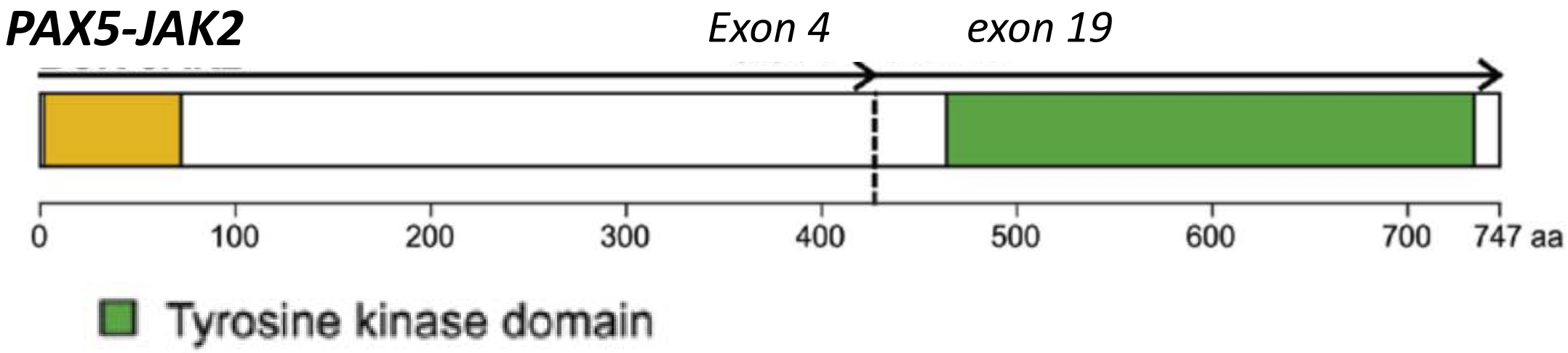
16 yo boy with precursor B- acute lymphoblastic leukemia

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

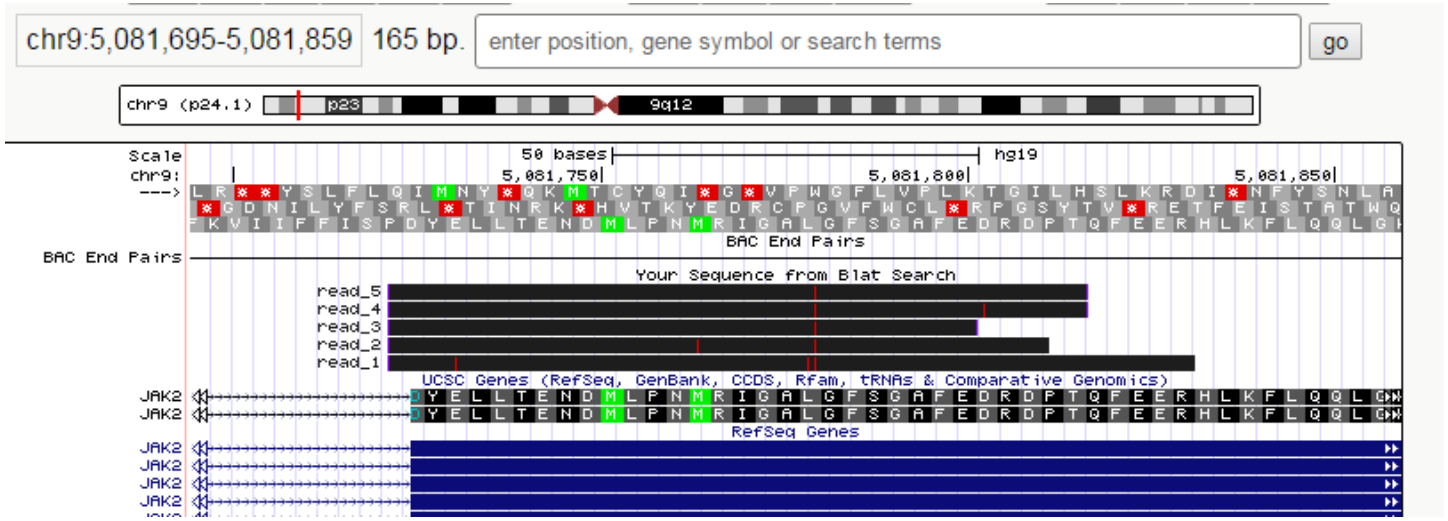
Case 3

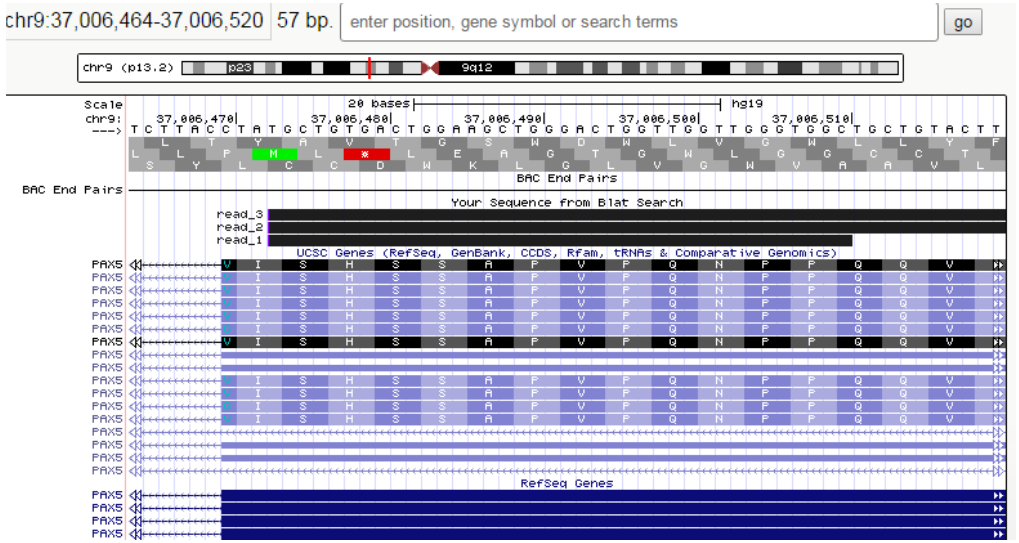


395 fusion reads

Case 3

	START	END	QSIZE	IDENTITY	CHRO	STRAND
browser details read_2	39	148	148	97.3%	9	+
browser details read 2	1	38	148	100.0%	9	-





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Roberts KG et al. N Engl J Med 2014;371:1005-1015.

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*

MGH ArcherDx heme fusion assay, selected cases

- ❑ *Inform treatment*
- ❑ ***Help monitoring the patient's disease***
- ❑ *Clarify a karyotype result*
- ❑ *Help reaching a diagnosis*

Case 4

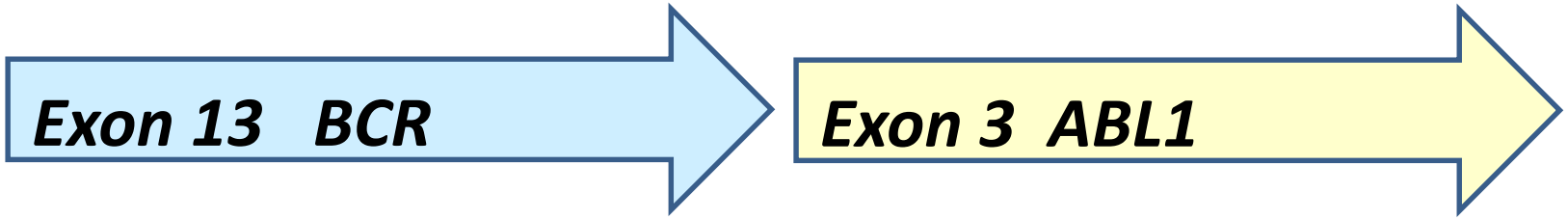
50 yo man with Ph+ B-ALL

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

RT-PCR for BCR-ABL1 chimeric transcripts: negative

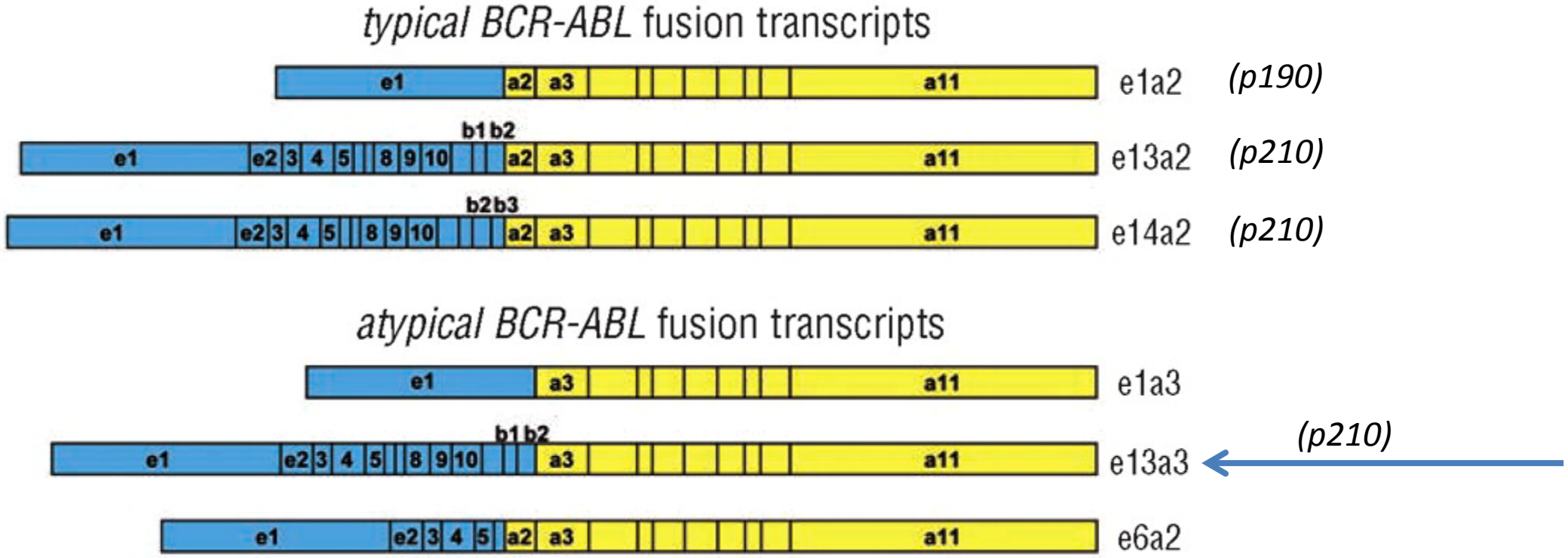
-- BCR ex13/14-ABL ex2 (B2A2 and B3A2): negative (p210)
-- BCR ex1-ABL ex2 (E1A2): negative (p190)

Case 4



459 fusion reads

BCR-ABL fusion transcripts in B-ALL



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*

MGH ArcherDx heme fusion assay, selected cases

- ❑ *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**.*

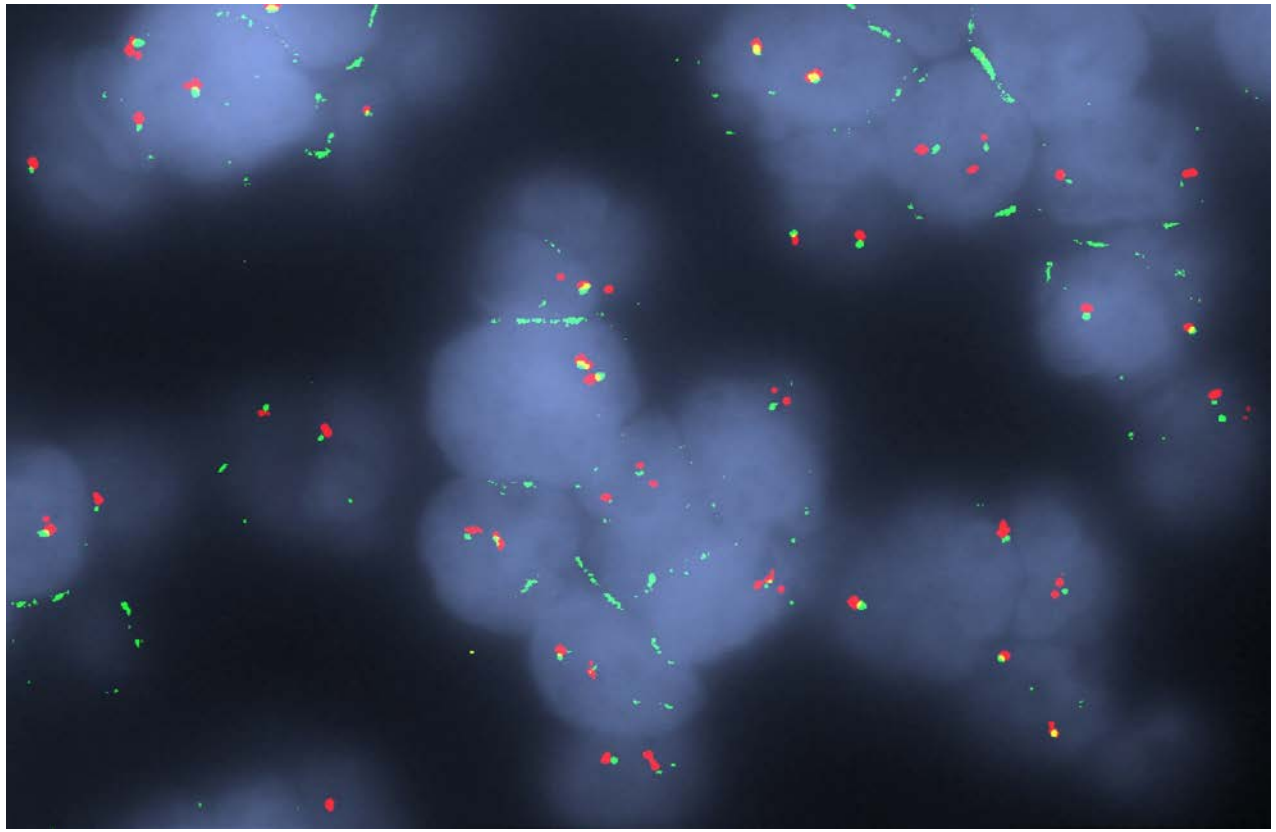
*Der(9;12) for either dic(9;12)(p13;p13) (**PAX5/ETV6** rearrangement) or dic(9;12)(p13;p12) (**PAX5/SLCO1B3** rearrangement).*

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.*

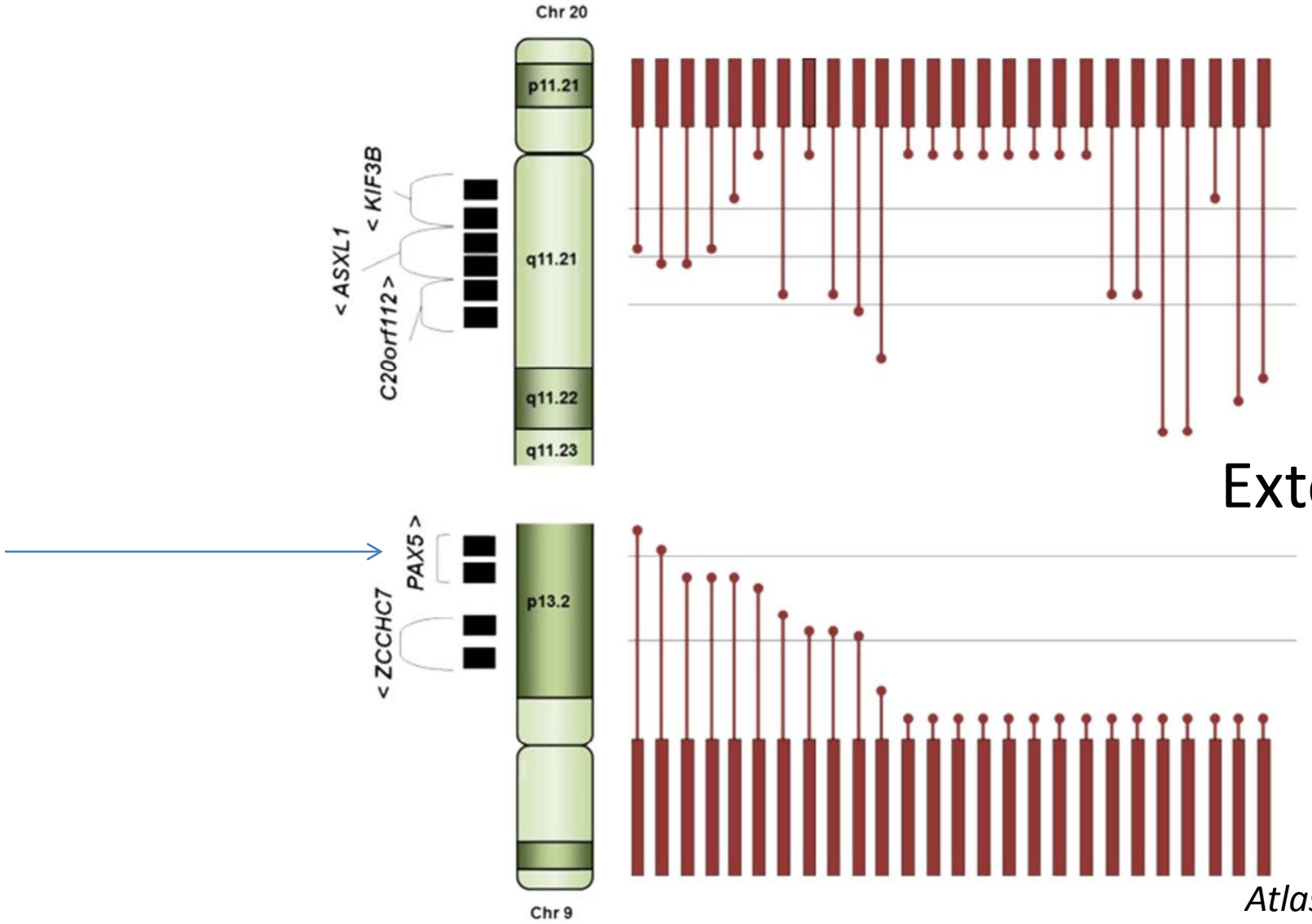
- *Identification of a novel fusion gene (MEF2D-DAZAP1) in a B acute lymphoblastic leukemia with t(1;19)(q23;p13).*

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

Boomer T. eat 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

Atlas Genet Cytogenet Oncol Haematol. 2010

Case 6

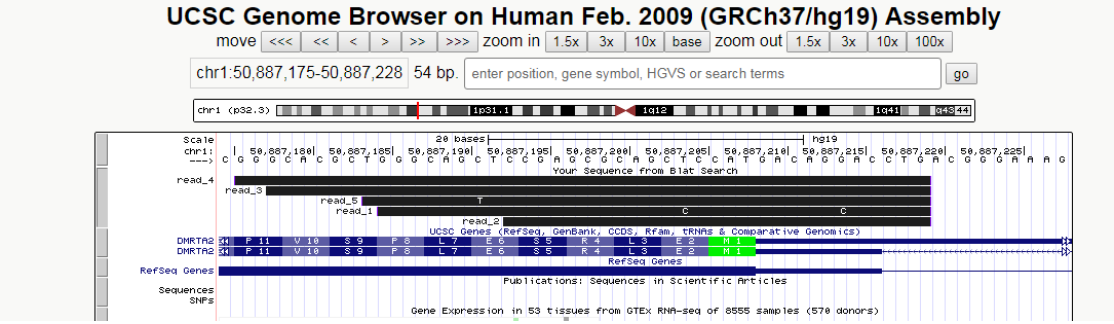
61 yo female with B-ALL and an NRAS p.Gly12Ser mutation

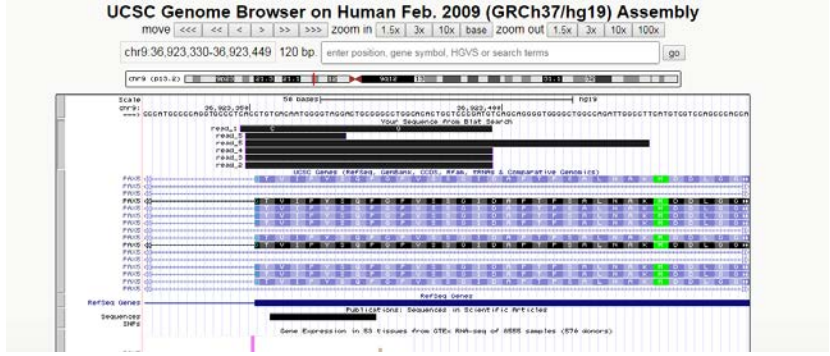
Karyotype analysis:
45,X,- X,der(7;12)(q10;q10),add(9)(p13),+mar[cp17]/46,XX[3]

Diagnosis: BCR-ABL1-like B- ALL

Case 6

Heme fusion assay: PAX5 ex 6 and DMRTA2 exon1



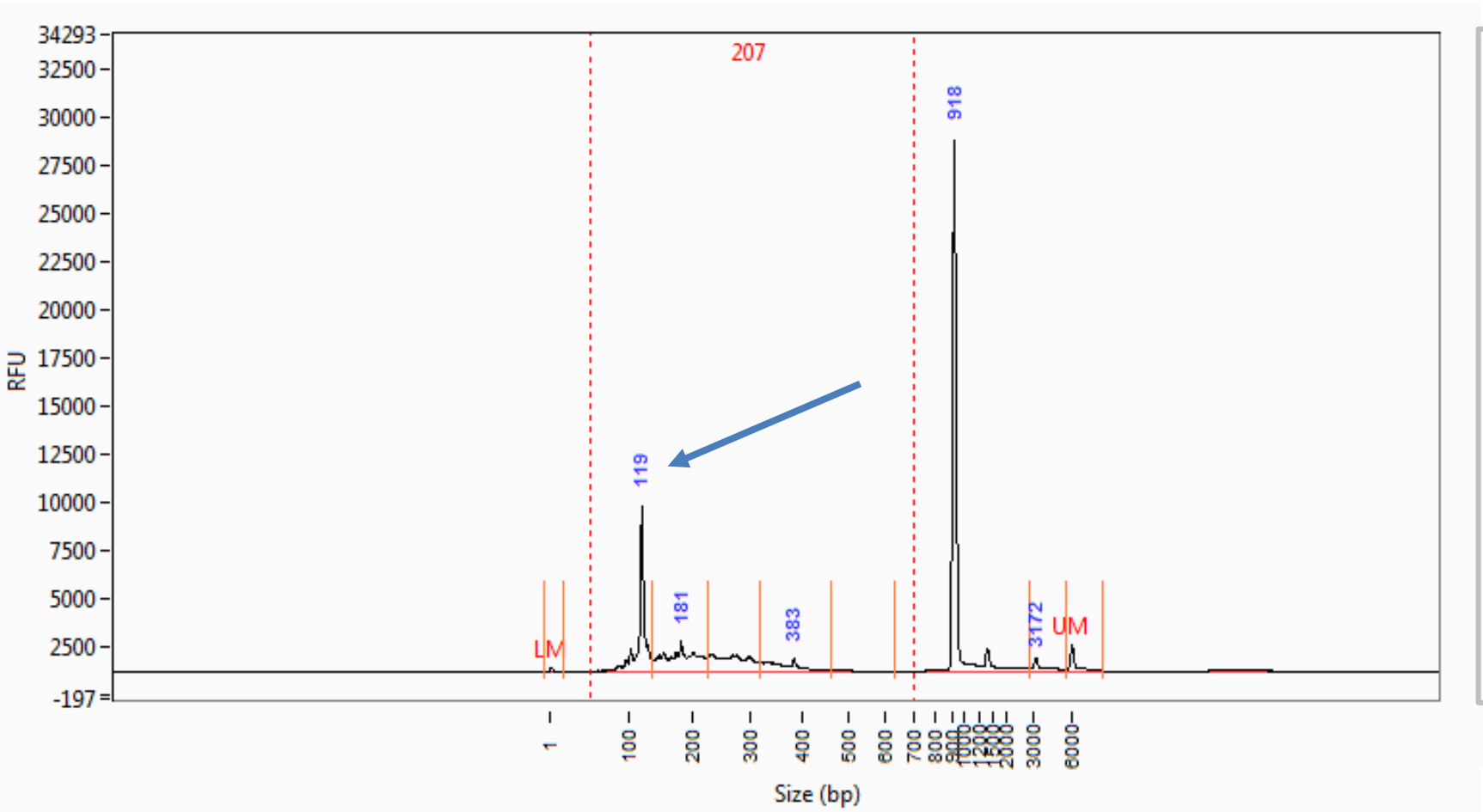


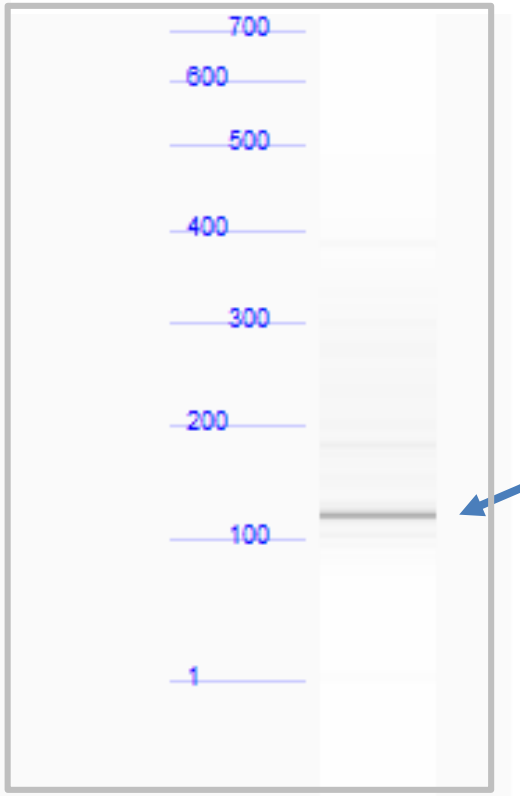
DMRTA2 ex 1

PAX5 ex 6

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*

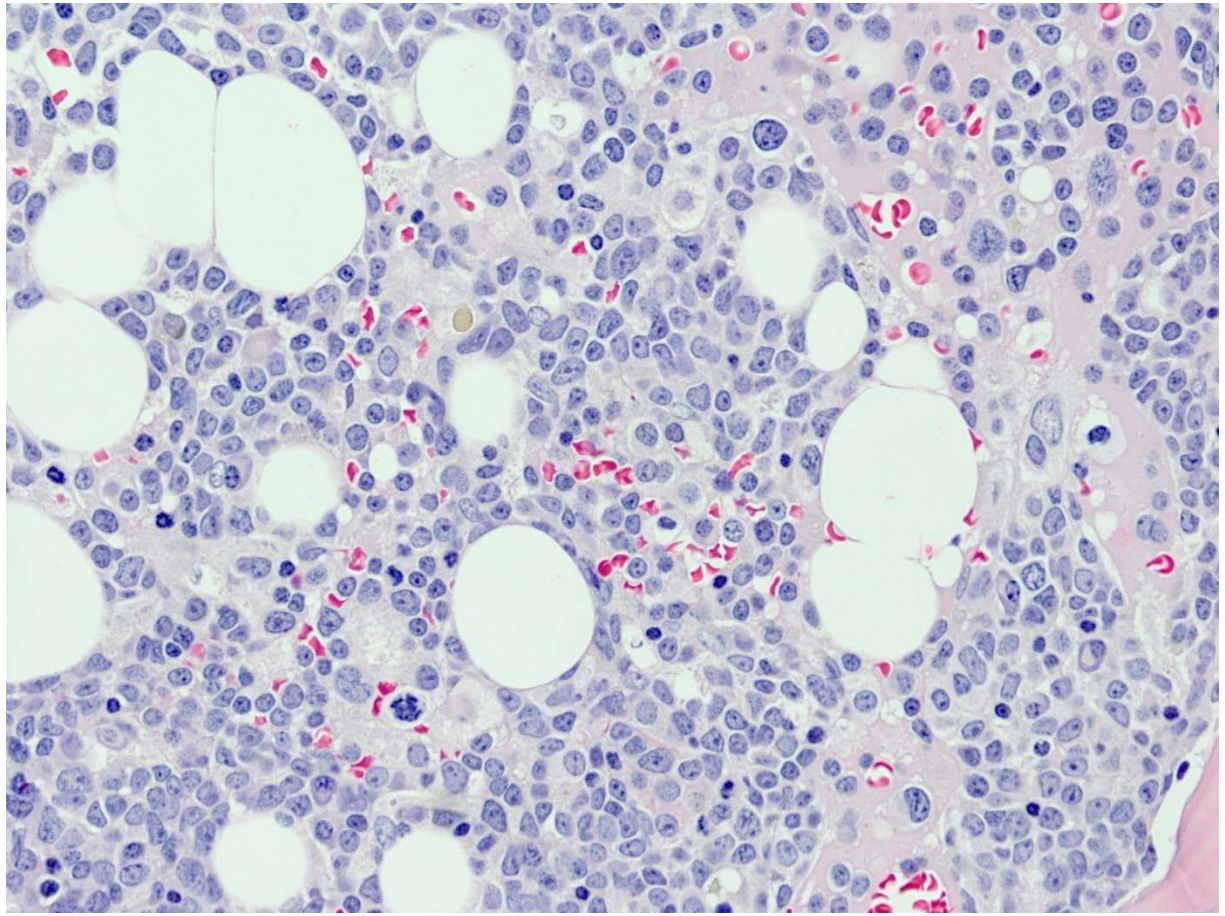
  -- *detected a novel cryptic PAX5 rearrangement*

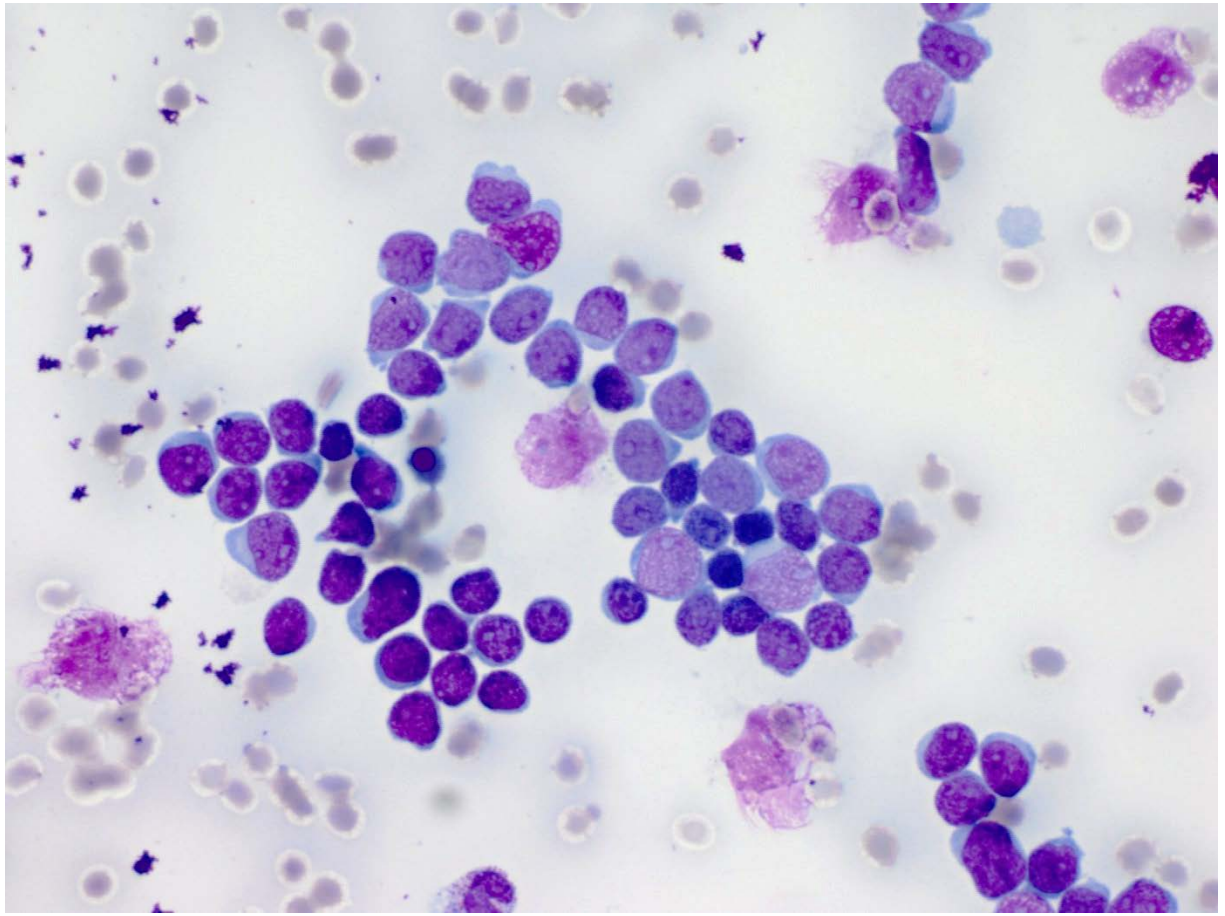
MGH ArcherDx heme fusion assay, selected cases

- ❑ *Inform treatment*
- ❑ *Help monitoring the patient's disease*
- ❑ ***Clarify a karyotype result***
- ❑ ***Help reaching a diagnosis***

Case 7

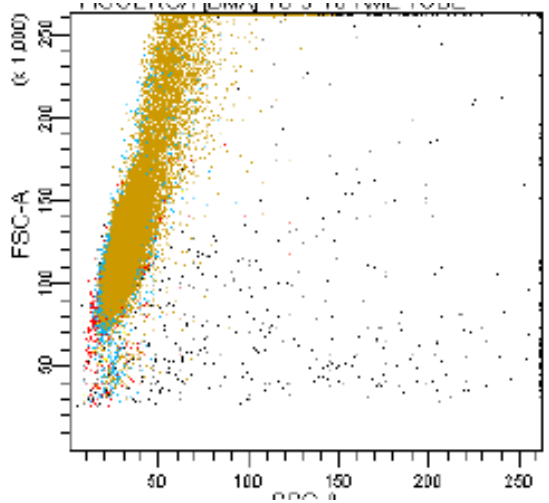
22 yo man with AML, possibly APML

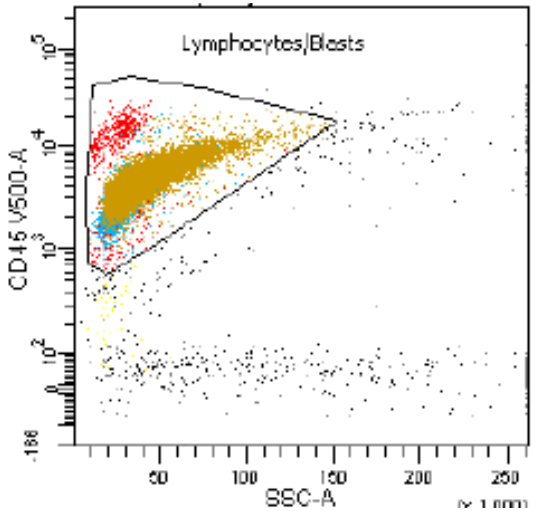


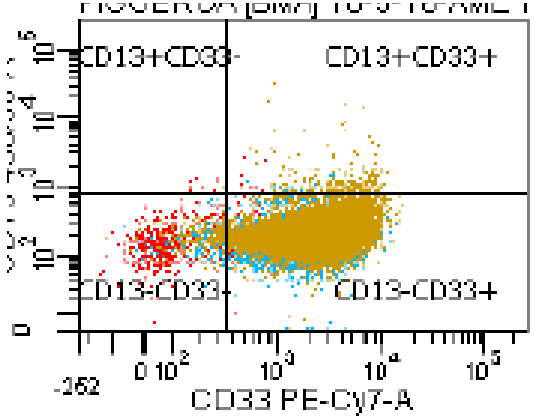


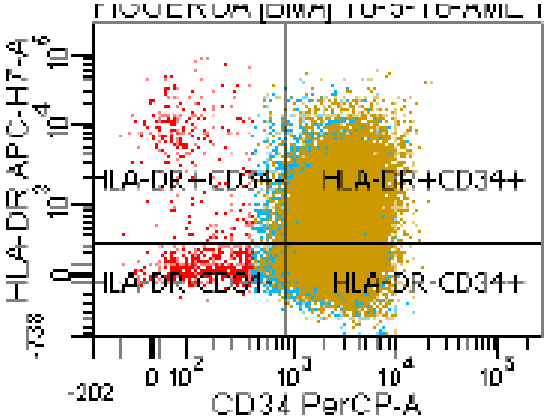
Case 7

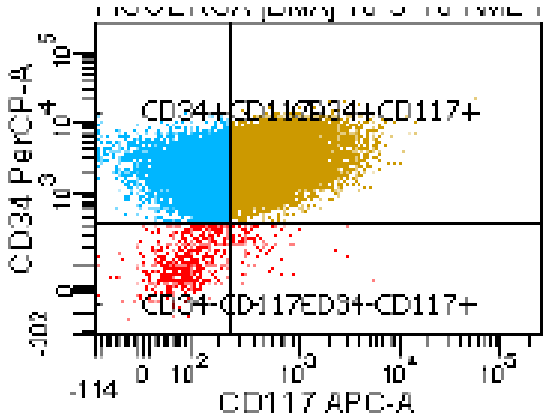
22 yo man with AML, possibly APML

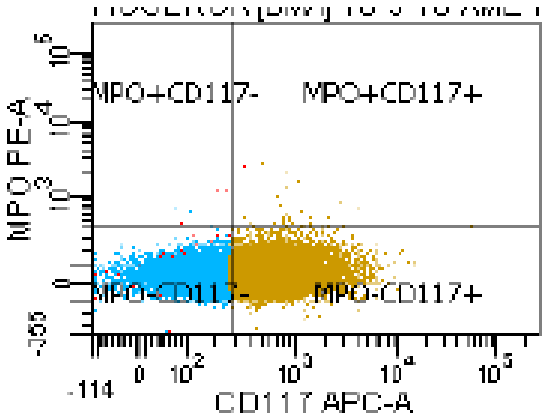












Case 7

22 yo man with AML, possibly APML

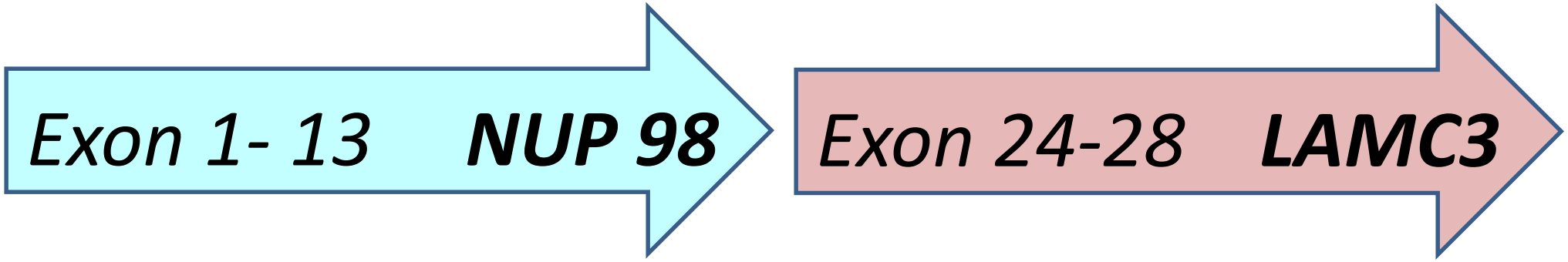
Karyotype analysis:
*46,XY,**t(4;17)(q21;q21),**add(11)(p15)[9]/48,idem,+der(4)t(4;17),+21[cp11].ishder(4)t(4;17)(FIP1L1+,LNX+,PDGFRA+;TP53+,D17Z1+,RARA+)x1~2,+der(17)t(4;17) (TP53+,D17Z1+,dim3'RARA+)*

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) ≠ *recurrent t(4;17)(q12;q21) (FIP1L1/RARA) (JMML)*

RARA



NUP98-LAMC3 (novel) fusion

t(9;11) (q34 ;p15)

319 fusion reads

NUP98 fusion partners

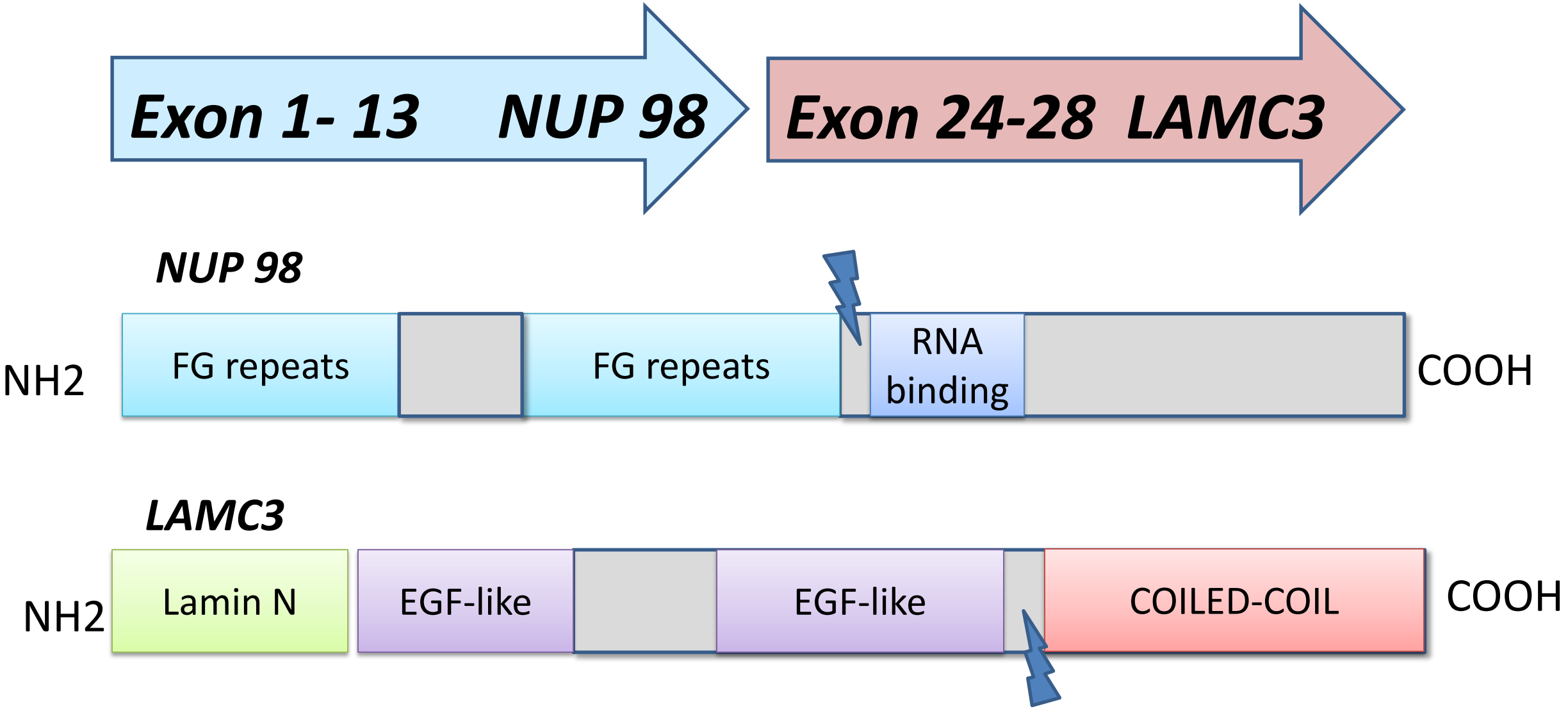
- ***Homeodomain proteins*** *(e.g. HOX proteins)*

- ***Non homeodomain proteins***

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

  -- *histone "reading" or "writing" domain*

Case 7


Exon 1- 13 NUP 98
Exon 24-28 LAMC3
NUP 98
NH2
FG repeats
FG repeats
RNA binding
COOH
LAMC3
NH2
Lamin N
EGF-like
EGF-like
COILED-COIL
COOH

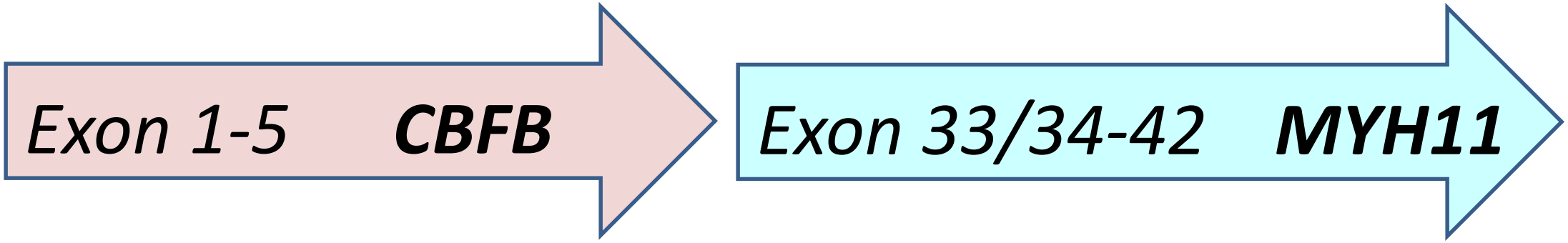
Case 8

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



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)

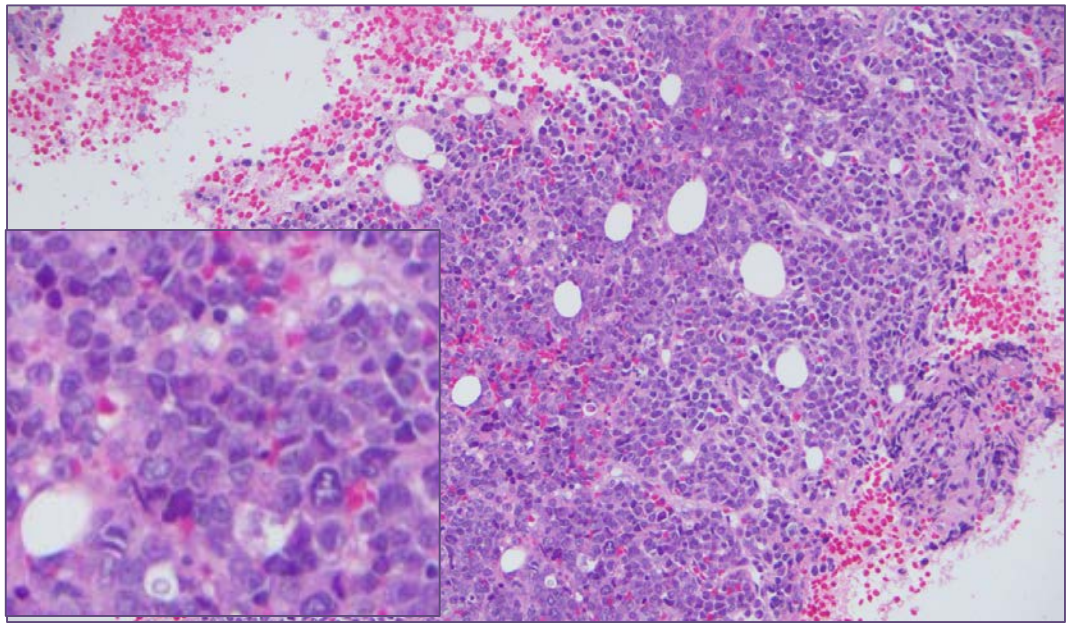
-- good prognosis with no need of BMT (case 6)

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

Case 9

ZMYND8 exon 22--RELA exon 3 fusion

Fusion of *ZMYND8* and *RELA* Genes in Acute Erythroid Leukemia

Ioannis Panagopoulos, Francesca Micci, Jim Thorsen, Lisbeth Haugom, Jochen Buechner, Gitte Kerndrup, Anne Tierens, Bernward Zeller, Sverre Heim

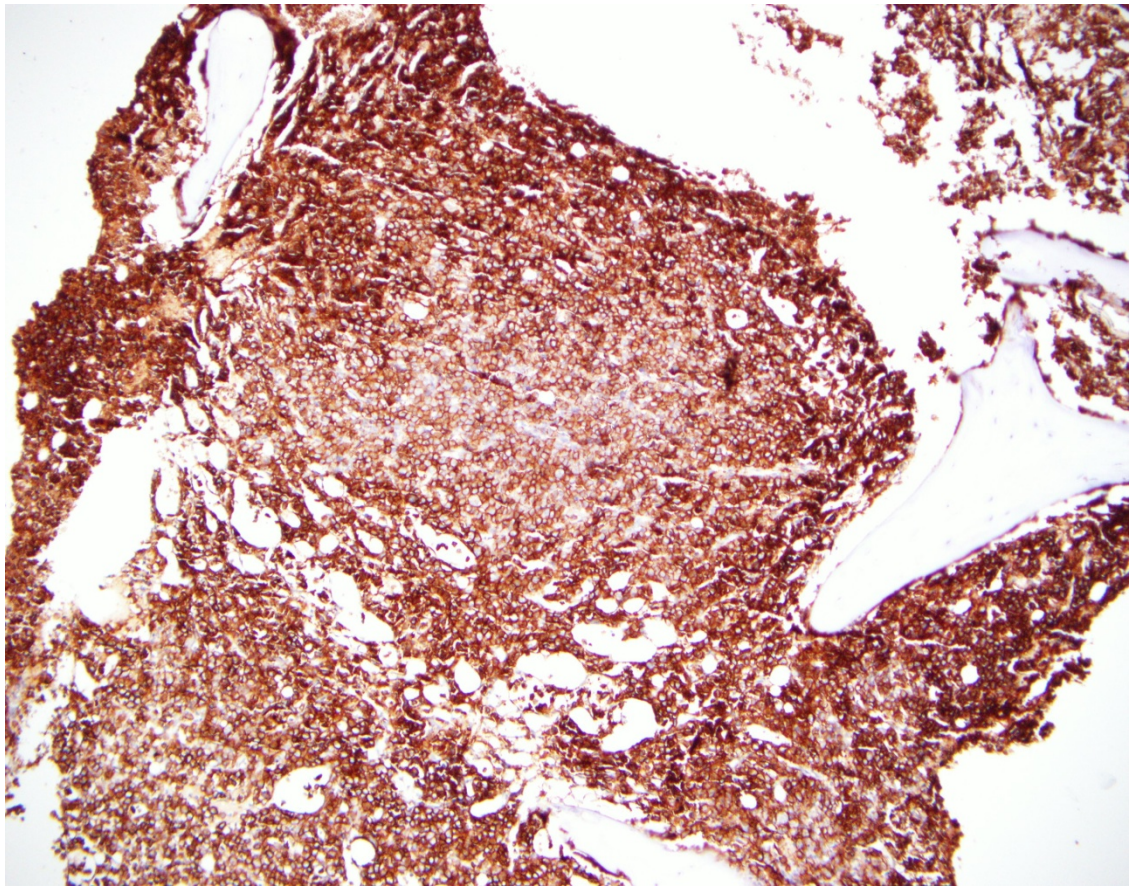
Published: May 7, 2013 • http://dx.doi.org/10.1371/journal.pone.0063663

Abstract

Acute erythroid leukemia was diagnosed in a 4-month-old boy. Cytogenetic analysis of bone marrow (BM) cells showed a t(11;20)(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 der(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-PHD 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 leukemogenically by influencing several cellular processes including the NF-kappa-B 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)

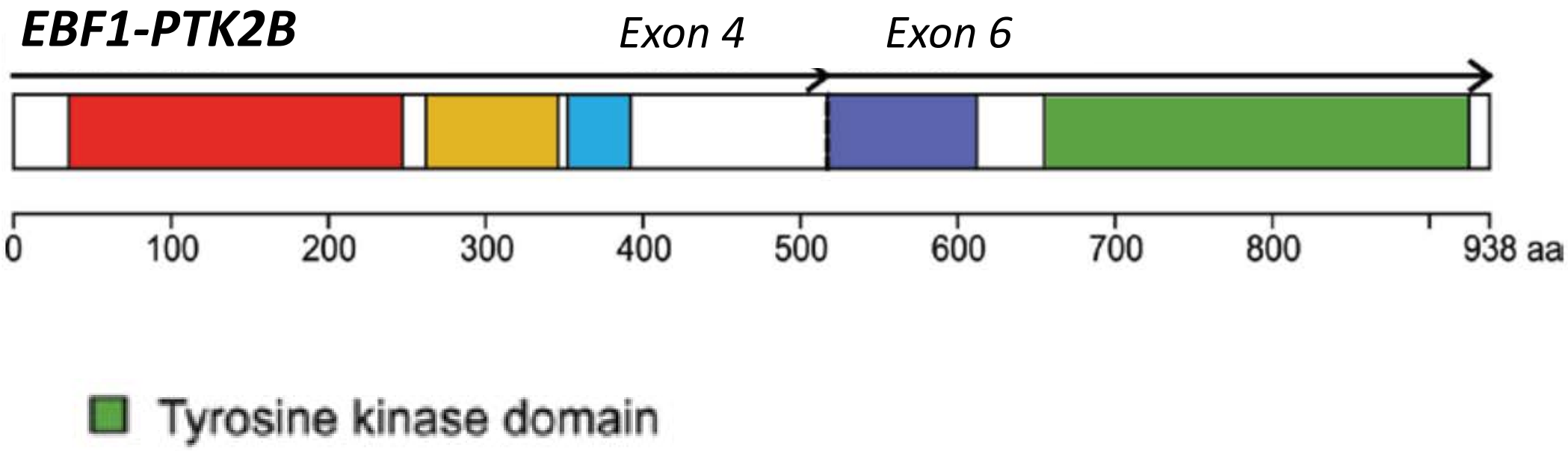
16 yo boy with precursor B- acute lymphoblastic leukemia

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



90 fusion reads

Case 1

Table 1. Kinase Fusions Identified in Ph-like Acute Lymphoblastic Leukemia.

Kinase Gene	Tyrosine Kinase Inhibitor	Fusion Partners	Patients	5′ Genes
		number		
ABL1	Dasatinib	6	14	*ETV6*,[11] *NUP214*,[11] *RCSD1*,[11] *RANBP2*,[11] *SNX2*,[19] *ZMIZ1*[20]
ABL2	Dasatinib	3	7	*PAG1*,* *RCSD1*,* *ZC3HAV1**
CSF1R	Dasatinib	1	4	*SSBP2**
PDGFRB	Dasatinib	4	11	*EBF1*,[11-13] *SSBP2*,* *TNIP1*,* *ZEB2**
CRLF2	JAK2 inhibitor	2	30	*IGH*,[21] *P2RY8*[22]
JAK2	JAK2 inhibitor	10	19	*ATF7IP*,* *BCR*,[11] *EBF1*,* *ETV6*,[23] *PAX5*,[11] *PPFIBP1*,* *SSBP2*,[24] *STRN3*,[11] *TERF2*,* *TPR**
EPOR	JAK2 inhibitor	2	9	*IGH*,[11] *IGK**
DGKH	Unknown	1	1	*ZFAND3**
IL2RB	JAK1 inhibitor, JAK3 inhibitor, or both	1	1	*MYH9**
NTRK3	Crizotinib	1	1	*ETV6*[25-27]†
PTK2B	FAK inhibitor	2	1	*KDM6A*,* *STAG2**
TSLP	JAK2 inhibitor	1	1	*IQGAP2**
TYK2	TYK2 inhibitor	1	1	*MYB**

* 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]

Roberts KG et al. N Engl J Med 2014;371:1005-1015.

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