A Pediatric Cancer Research Gene Panel

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Outline

- **Panel Content**
- Technical aspects of the Panel
- Performance Verification
- Research Case Study
- Conclusions

A tool to identify genetic defects in pediatric cancer research

- Developed with Ion Torrent and AmpliSeq technology*
- Tumor-specific gene fusions
- Over-expressed genes
- Amplified genes
- Known gene mutations, insertions, and deletions
- Gene mutations identified in the NCI MATCH program as candidate therapeutic targets

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Designed Specifically for Use in Pediatric Cancer Research

Hotspot (82)

ABL1	FGFR2	NT5C2
ABL2	FGFR3	PAX5
ALK	FLT3	PDGFRA
ACVR1	GATA2	PDGFRB
AKT1	GNA11	PIK3CA
ASXL1	GNAQ	PIK3R1
ASXL2	H3F3A	PPM1D
BRAF	HDAC9	PTPN11
CALR	HIST1H3B	RAF1
CBL	HRAS	RET
CCND3	IDH1	RHOA
CCR5	IDH2	SETBP1
CDK4	IL7R	SETD2
CIC	JAK1	SH2B3
CREBBP	JAK2	SH2D1A
CRLF2	JAK3	SMO
CSF1R	KDM4C	STAT3
CSF3R	KDR	STAT5B
CTNNB1	KIT	TERT
DAXX	KRAS	TPMT
DNMT3A	MAP2K1	USP7
EGFR	MAP2K2	ZMYM3
EP300	MET	
ERBB2	MPL	
ERBB3	MSH6	
ERBB4	MTOR	
ESR1	NCOR2	
EZH2	NOTCH1	
FASLG	NPM1	
FBXW7	NRAS	

CNV (24)

ALK
BRAF
CCND1
CDK4
CDK6
EGFR
ERBB2
ERBB3
FGFR1
FGFR2
FGFR3
FGFR4
GLI1
GLI2
IGF1R
KIT
KRAS
MDM2
MDM4
MET
MYC
MYCN
PDGFRA
PIK3CA

Full-gene CDS (44)

APC	PHF6
ARID1A	PRPS1
ARID1B	PSMB5
ATRX	PTCH1
CDKN2A	PTEN
CDKN2B	RB1
CEBPA	RUNX1
CHD7	SMARCA4
CRLF1	SMARCB1
DDX3X	SOCS2
DICER1	SUFU
EBF1	SUZ12
EED	TCF3
FAS	TET2
GATA1	TP53
GATA3	TSC1
GNA13	TSC2
ID3	WHSC1
IKZF1	WT1
KDM6A	XIAP
KMT2D	
MYOD1	
NF1	
NF2	

187 unique genes
3,110 amplicons in DNA assay
1,427 amplicons in RNA assay

Fusion & Expression (78)

ABL1	MECOM	RELA
ABL2	MET	RET
ALK	MKL1	ROS1
BCL11B	MLLT10	RUNX1
BCOR	MYB	SS18
BCR	MYH11	SSBP2
BRAF	MYH9	STAT6
CAMTA1	NCOA2	TAL1
CCND1	NOTCH1	TCF3
CIC	NOTCH2	TFE3
CREBBP	NPM1	TSLP
CRLF2	NR4A3	USP6
CSF1R	NTRK1	YAP1
ETV6	NTRK2	ZNF384
EWSR1	NTRK3	
FGFR1	NUP214	**Gene Expression**
FGFR2	NUP98	
FGFR3	NUTM1	BCL2
FLT3	PAX3	BCL6
FUS	PAX5	FGFR1
GLIS2	PAX7	FGFR4
JAK2	PDGFB	IGF1R
KAT6A	PDGFRA	MET
KMT2A	PDGFRB	MYCN
KMT2B	PLAG1	TOP2A
KMT2C	RAF1	
KMT2D	RANBP17	
MAML2	RARA	

All Major Pediatric Leukemia Translocations Are Represented

- **Acute lymphoblastic leukemia** — ETV6-RUNX1, E2A-PBX, BCR-ABL1, MLL-AF4, CDKN2A
- **Ph+ –like B-precursor ALL CD22delE12** — ABL1, ABL2, CSF1R, PDGFRB, EPOR, AK2, CRLF2, FLT3, KRAS,
- **Acute myelogenous leukemia** — FLT3, NPM1, KIT, IDH1, IDH2, DNMT3A, RAS, RUNX1, TET2, CEBPA
- **Acute promyelocytic leukemia** — PML-RARα

Pediatric Brain Tumors:
Comprehensive Coverage Across All Common Types

• AT/RT, cribiform neuro-epithelial tumor, Schwannoma	SMARCB1
• Medulloblastoma, WNT, RT (Rhabdoid Tumor)	SMARCA4
• Medulloblastoma	GLI2, MSH2, MSH6, MYCN, PMS2, PTCH1, SUFU
• Ependymoma	RELA
• Ependymoma, Meningioma	NF2
• Astrocytoma	FGFR1, HIST1H3B, MDM2, MLH1, NF PTPN11, TERT, TP53, QK1
• Glioblastoma	MDM4
• Glioma, Astrocytoma gr I-IV, Ependymoma Gr 3-4	PTEN
• Glioma, Astrocytoma I-IV, Oligoastrocytoma	H3F3A
• Pilocytic Astrocytoma	BRAF, FAM131B, NTRK2

Panel Identifies Key Gene Fusions in Pediatric Sarcomas

• Rhabdomyosarcoma (embryonal & alveolar)	PAX3/7-FOXO1
• Ewing sarcoma	EWS-FLI1/ERG
• Synovial cell sarcoma	SYT-SSX1/2/4
• Infantile(congenital) fibrosarcoma	ETV6 -NTRKC
• Desmoplastic small round cell tumor	EWS-WT1
• Alveolar soft part sarcoma	TFE3-ASPSCR1 (ASPL)
• Clear cell sarcoma (melanoma of soft parts)	EWS-ATF1, EWS-CREB1
• Inflammatory myofibroblastic tumor	ALK-TPM3/4, CLTC, ATIC
• Fibromyxoid sarcoma	FUS-CREBB3L2/1
• Dermatofibrosarcoma protuberans	COL1A-PDGFB
• Epithelioid sarcoma	SMARCB1
• Angiomatoid fibrous histiocytoma	EWS-CREB1
• Epithelioid hemangioendothelioma	WWTRC1-CAMTN
• Mesenchymal chondrosarcoma	HEY1-NCOA2
• Malignant peripheral nerve sheath tumor	NF1/NF2 mut
• Undifferentiated sarcoma	BCOR-CCNB3, CIC-DUX4
• Midline carcinoma	NUT-BRD4
• Low grade fibromyxoid sarcoma	FUS-CREB1L1 and FUS-CREB1L3

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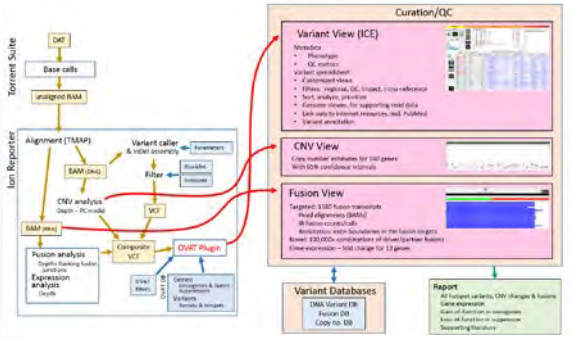
There are Three Main Parts of the workflow

Ampliseq Library Preparation → Ion Torrent Sequencing → Bio-Informatic Analysis

Ion Chef™ ICE℠

Ion™ S5 Sequencer™



Ion Reporter &

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There are Advantages to Each Component of the assay

- ***Library Preparation = Ampliseq™****
  - Interrogate DNA and RNA isolated from FFPE
  - Small input (≥ 20 ng RNA and DNA)
  - Ion Chef™ automated library prep and chip loading

- ***Sequencing = Ion Torrent™****
  - Fast turn-around time (2 hours)
  - Automated alignment (FASTQ to BAM) and variant calling (VCF)
  - Ion S5 sequencer

- ***Bio-Informatic Analysis***
  - Commercial Pipeline (Ion Torrent Suite™ & Ion Reporter™) *
  - Custom Scripts (ICE℠)

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Virtually Any Type of Specimen Can Be Profiled

- **Blood and bone marrow (purple top tube)**
- **Fresh/frozen tissue**
- **FFPE tissue (unstained slide, blocks, scrolls)**
  - **sample quality is assessed prior to library preparation**

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Performance Verification

Over 500 Samples Processed

- **503 samples have been run to date**
- **237 unique tumor samples**
- **Also measured panel against synthetic control material (Acrometrix, with known SNVs, InDels)**

Performance

- >5000X average coverage for DNA variants
- Average uniformity >95%
- Average mapped reads >2,000,000 for RNA fusions

DNA Features Detected

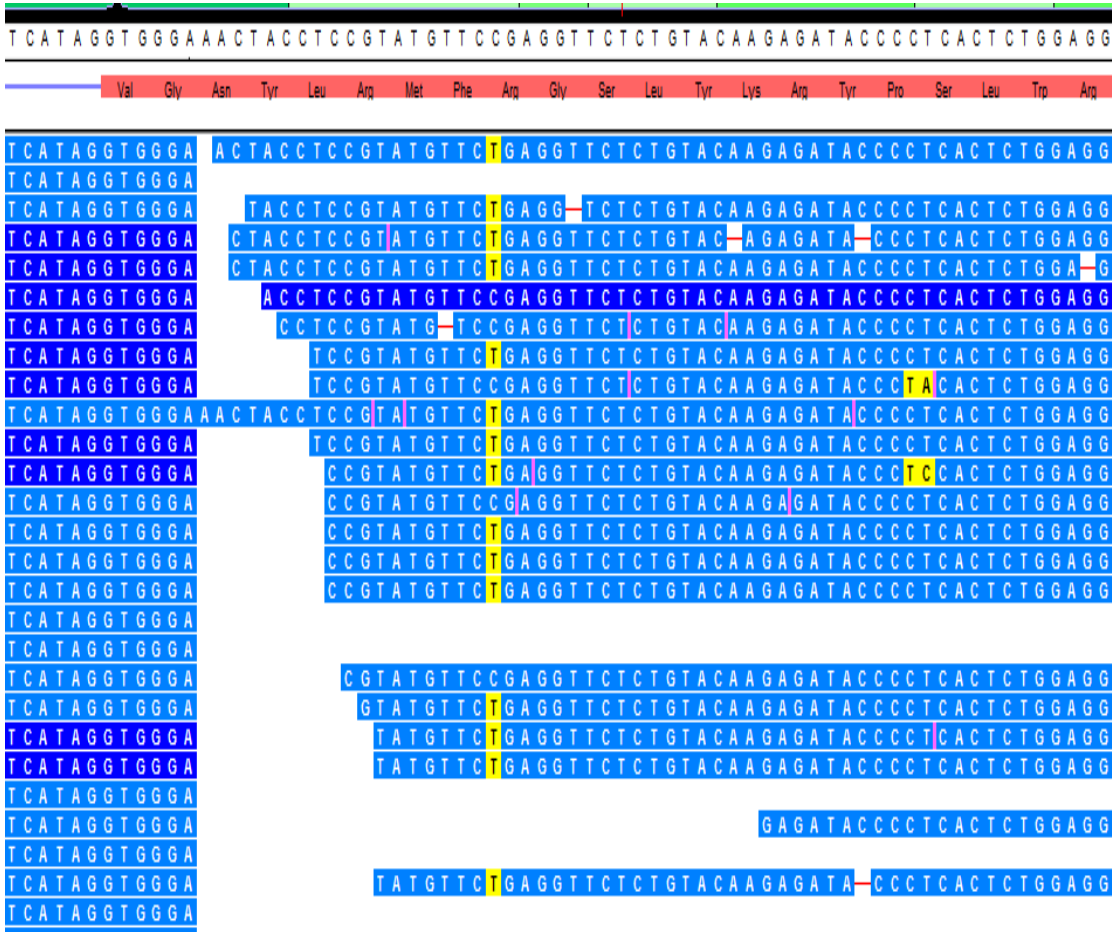
- SNV = single nucleotide variant
- InDel = insertion/deletion
- Gene Amplification: ≥ 6-fold

Verified technical performance:

- SNVs: 5% variant allele frequency
- InDels: 10% variant allele frequency

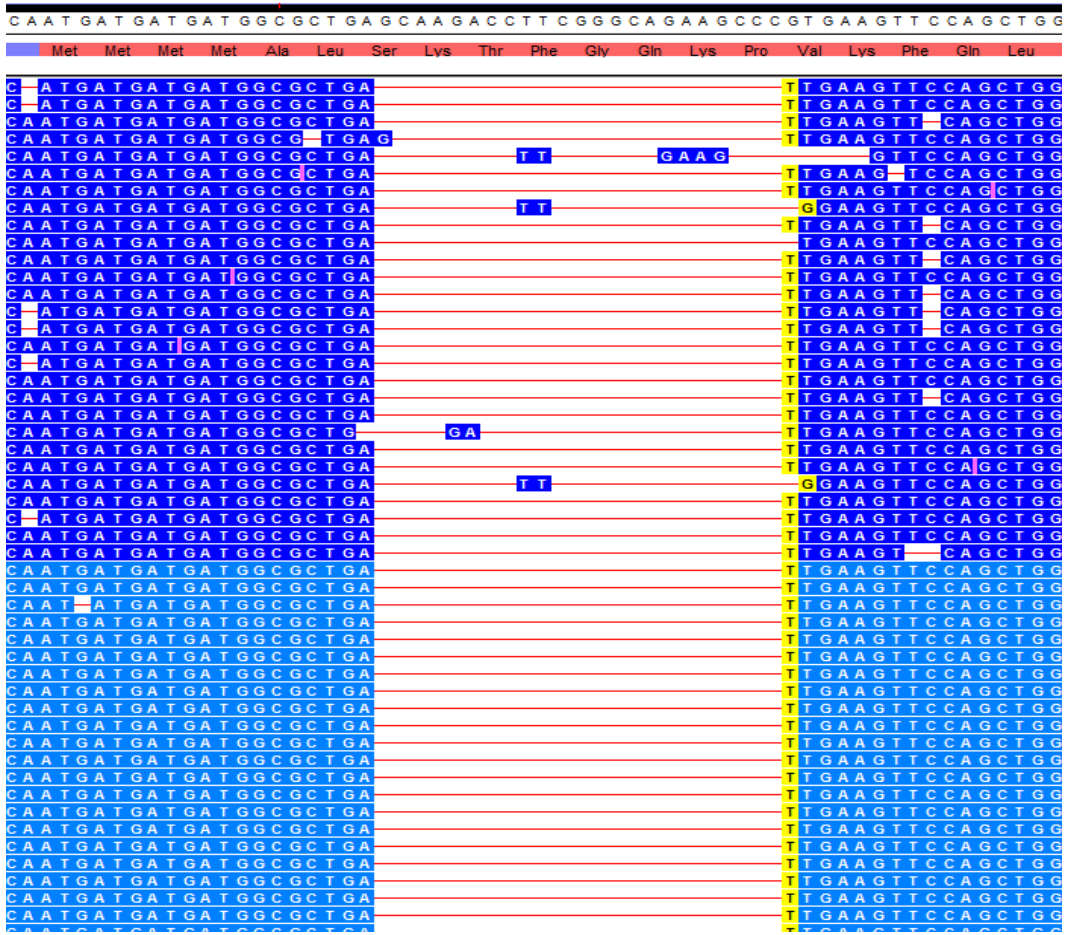
Assay is Sensitive and Specific

Single Nucleotide Variant: C to T



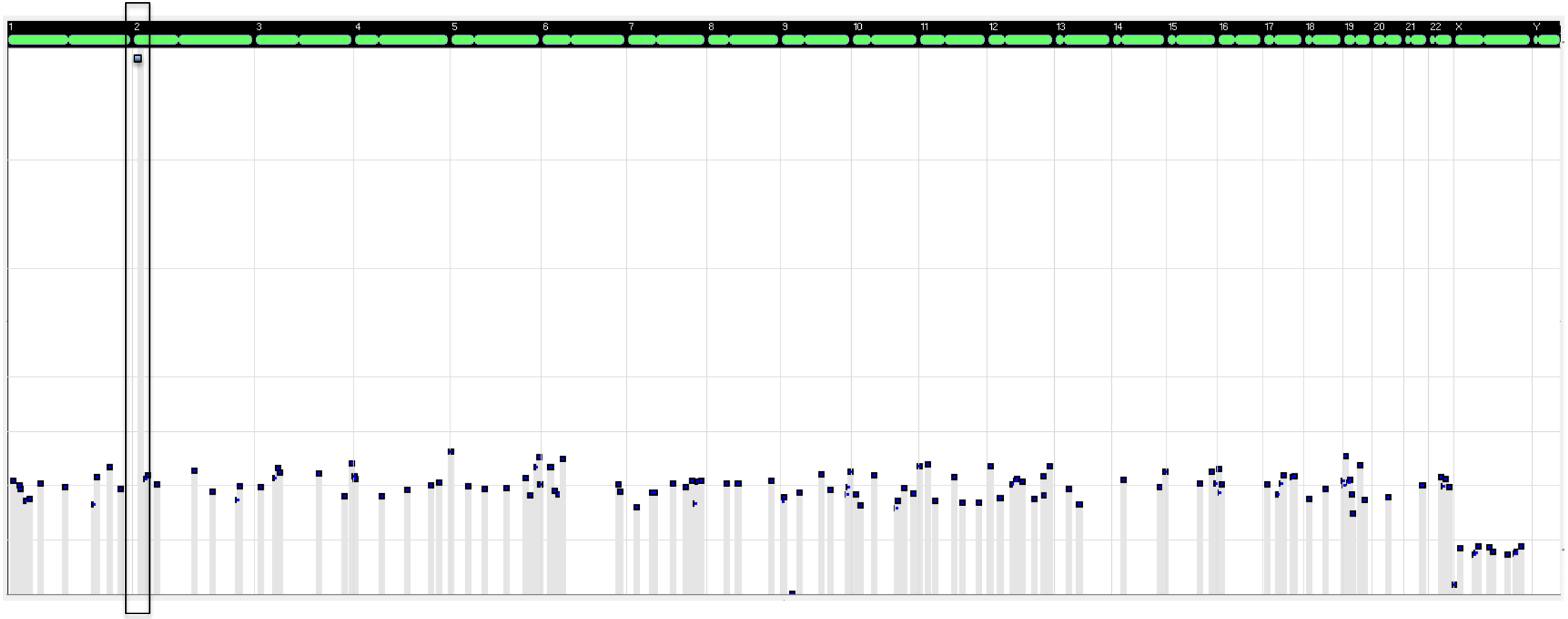
SMARCB1
c.118C>T, p.Arg40*

InDel: 24 base deletion



SMARCB1
c.20_43delGCAAGACCTTCGGGCAGAAGCCCGinsT
(p.Ser7Ilefs*56)

Detection of DNA Amplifications is Highly Specific



RNA Features Detected

- **Gene Fusions – annotated and unannotated (novel pairing)**

- **Gene Expression - # reads per gene, c/w average of 4 housekeeping genes**

n.b.: Gene Fusions:

- **78 parent fusions**
- **>1,400 variants**
- **Ability to detect *de novo* fusions from pairing of existing primer pairs**

A Diverse Range of Hematologic Fusions are Detected*

ATF7IP-JAK2	ETV6-NTRK3	P2RY8-CRLF2	RCSD1-ABL2
BCR-ABL1	ETV6-RUNX1	PAG1-ABL2	SSBP2-JAK2
BCR-JAK2	FIP1L1-PDGFRA	PAK5-JAK2	STIL-TAL1
CRLF2-P2RY8	FOXP1-ABL1	PML-RARA	TERF2-JAK2
EBF1-PDGFRB	MLL Rearrangement	RANBP2-ABL1	ZC3HAV1-ABL2
ETV6-ABL1	NUP214-ABL1	RBM15-MKL1	ZEB2-PDGFRB
ETV6-JAK2	NUP98-NSD1	RCSD1-ABL1	ZMIZ1-ABL1

Confirmed samples used for verification

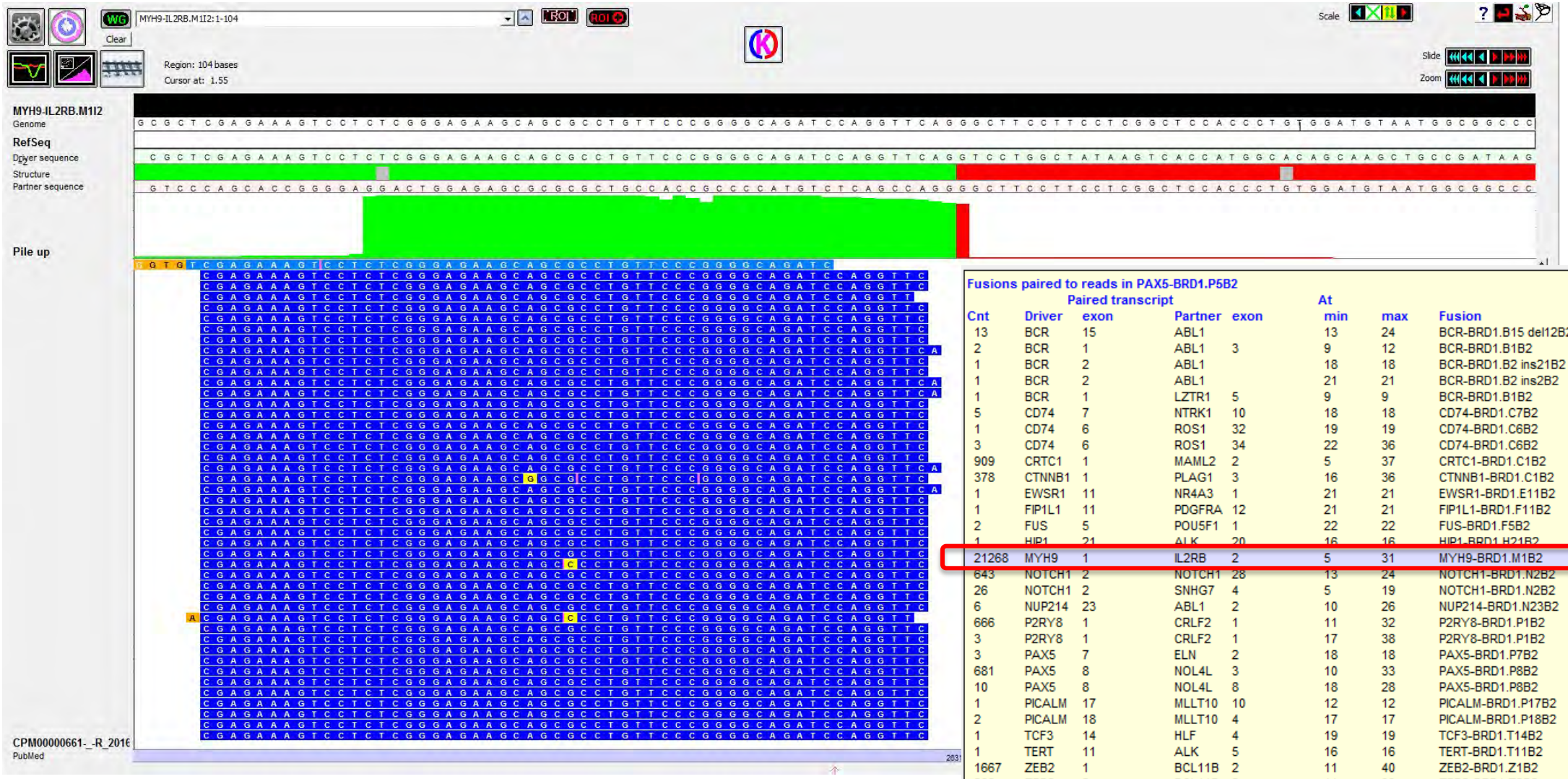
Key Solid Tumor Fusions were verified*

CCDC6-RET	Lung Adenocarcinoma
Cllorf95-RELA	Ependymoma
EML4-ALK	Lung Adenocarcinoma
ETV6-NTRK3	Congenital Mesoblastic Nephroma
EWSR1 Rearrangement	Ewing Sarcoma
FUS-CREB3L2	Fibromyxoid Sarcoma
GOPC-ROS1	Glioblastoma Multiforme
KIAA1549-BRAF	Pilocytic Astrocytoma
NPM1-ALK	Anaplastic Large Cell Lymphoma
PAX3-FOXO1	Alveolar Rhabdomyosarcoma
SS18-SSX1	Synovial Sarcoma

Confirmed samples used for verification

Novel Tumor Fusions were Discovered

MYH9-IL2RB transcript – reads partially aligned to MYH9 portion



Fusions paired to reads in PAX5-BRD1.P5B2

Cnt	Driver	exon	Partner	exon	Paired transcript At min	max	Fusion
13	BCR	15	ABL1		13	24	BCR-BRD1.B15 del12B2
2	BCR	1	ABL1	3	9	12	BCR-BRD1.B1B2
1	BCR	2	ABL1		18	18	BCR-BRD1.B2 ins21B2
1	BCR	2	ABL1		21	21	BCR-BRD1.B2 ins2B2
1	BCR	1	LZTR1	5	9	9	BCR-BRD1.B1B2
5	CD74	7	NTRK1	10	18	18	CD74-BRD1.C7B2
1	CD74	6	ROS1	32	19	19	CD74-BRD1.C6B2
3	CD74	6	ROS1	34	22	36	CD74-BRD1.C6B2
909	CRTC1	1	MAML2	2	5	37	CRTC1-BRD1.C1B2
378	CTNNB1	1	PLAG1	3	16	36	CTNNB1-BRD1.C1B2
1	EWSR1	11	NR4A3	1	21	21	EWSR1-BRD1.E11B2
1	FIP1L1	11	PDGFRA	12	21	21	FIP1L1-BRD1.F11B2
2	FUS	5	POU5F1	1	22	22	FUS-BRD1.F5B2
1	HIP1	21	ALK	20	16	16	HIP1-BRD1.H21B2
21268	MYH9	1	IL2RB	2	5	31	MYH9-BRD1.M1B2
643	NOTCH1	2	NOTCH1	28	13	24	NOTCH1-BRD1.N2B2
26	NOTCH1	2	SNHG7	4	5	19	NOTCH1-BRD1.N2B2
6	NUP214	23	ABL1	2	10	26	NUP214-BRD1.N23B2
666	P2RY8	1	CRLF2	1	11	32	P2RY8-BRD1.P1B2
3	P2RY8	1	CRLF2	1	17	38	P2RY8-BRD1.P1B2
3	PAX5	7	ELN	2	18	18	PAX5-BRD1.P7B2
681	PAX5	8	NOL4L	3	10	33	PAX5-BRD1.P8B2
10	PAX5	8	NOL4L	8	18	28	PAX5-BRD1.P8B2
1	PICALM	17	MLLT10	10	12	12	PICALM-BRD1.P17B2
2	PICALM	18	MLLT10	4	17	17	PICALM-BRD1.P18B2
1	TCF3	14	HLF	4	19	19	TCF3-BRD1.T14B2
1	TERT	11	ALK	5	16	16	TERT-BRD1.T11B2
1667	ZEB2	1	BCL11B	2	11	40	ZEB2-BRD1.Z1B2
286	ZEB2	2	BCL11B	2	11	33	ZEB2-BRD1.Z2B2

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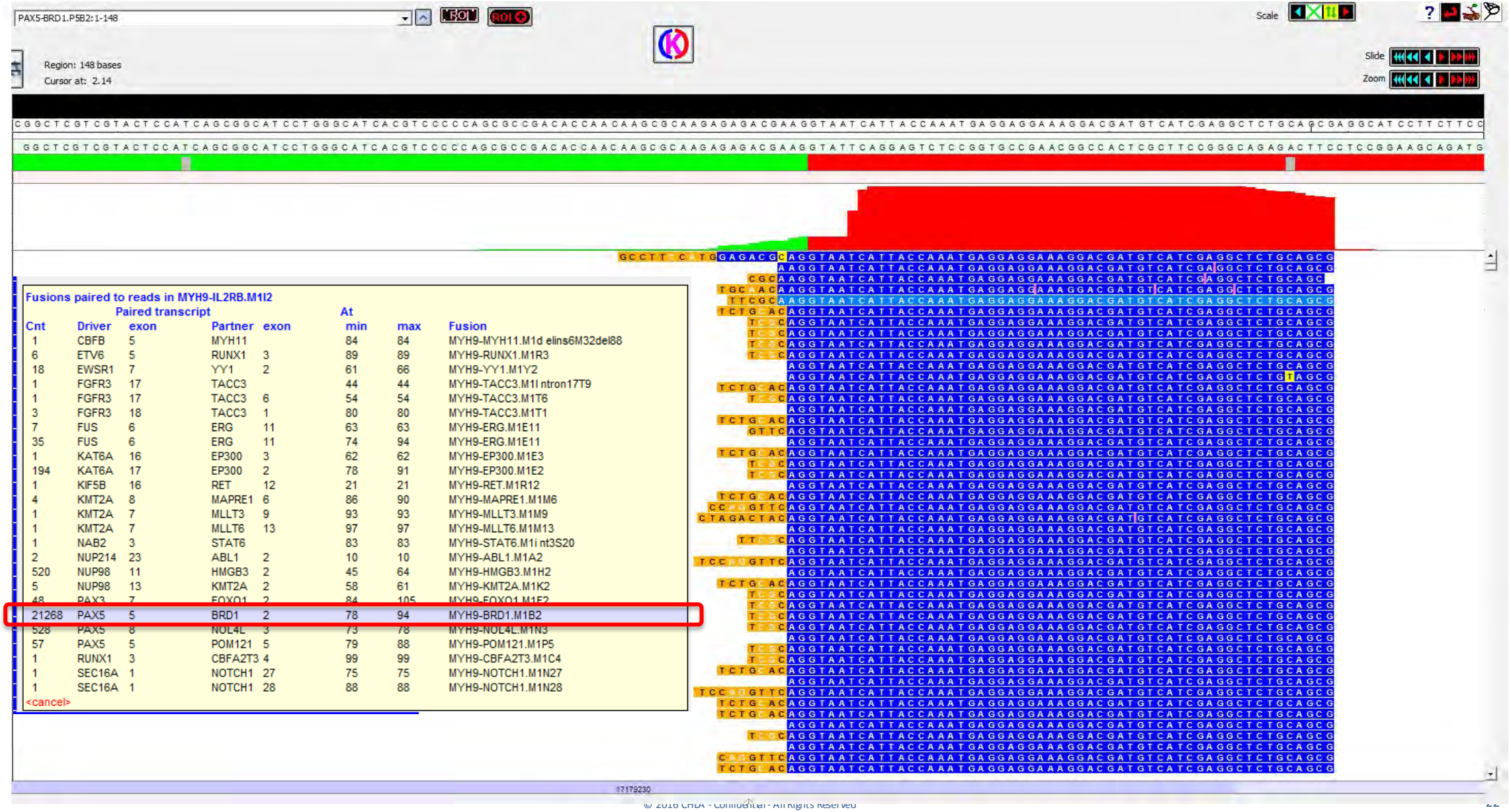
Other Half of Reads– Partially Aligned to BRD1 portion of PAX5-BRD1



Fusions paired to reads in MYH9-IL2RB.M1I2

Cnt	Driver	exon	Partner	exon	Paired transcript At min	max	Fusion
1	CBFB	5	MYH11		84	84	MYH9-MYH11.M1d elins6M32del88
6	ETV6	5	RUNX1	3	89	89	MYH9-RUNX1.M1R3
18	EWSR1	7	YY1	2	61	66	MYH9-YY1.M1Y2
1	FGFR3	17	TACC3		44	44	MYH9-TACC3.M1I ntron17T9
1	FGFR3	17	TACC3	6	54	54	MYH9-TACC3.M1T6
3	FGFR3	18	TACC3	1	80	80	MYH9-TACC3.M1T1
7	FUS	6	ERG	11	63	63	MYH9-ERG.M1E11
35	FUS	6	ERG	11	74	94	MYH9-ERG.M1E11
1	KAT6A	16	EP300	3	62	62	MYH9-EP300.M1E3
194	KAT6A	17	EP300	2	78	91	MYH9-EP300.M1E2
1	KIF5B	16	RET	12	21	21	MYH9-RET.M1R12
4	KMT2A	8	MAPRE1	6	86	90	MYH9-MAPRE1.M1M6
1	KMT2A	7	MLLT3	9	93	93	MYH9-MLLT3.M1M9
1	KMT2A	7	MLLT6	13	97	97	MYH9-MLLT6.M1M13
1	NAB2	3	STAT6		83	83	MYH9-STAT6.M1i nt3S20
2	NUP214	23	ABL1	2	10	10	MYH9-ABL1.M1A2
520	NUP98	11	HMGB3	2	45	64	MYH9-HMGB3.M1H2
5	NUP98	13	KMT2A	2	58	61	MYH9-KMT2A.M1K2
48	PAX3	7	FOXO1	2	84	105	MYH9-FOXO1.M1F2
21268	PAX5	5	BRD1	2	78	94	MYH9-BRD1.M1B2
528	PAX5	8	NOL4L	3	73	78	MYH9-NOL4L.M1N3
57	PAX5	5	POM121	5	79	88	MYH9-POM121.M1P5
1	RUNX1	3	CBFA2T3	4	99	99	MYH9-CBFA2T3.M1C4
1	SEC16A	1	NOTCH1	27	75	75	MYH9-NOTCH1.M1N27
1	SEC16A	1	NOTCH1	28	88	88	MYH9-NOTCH1.M1N28

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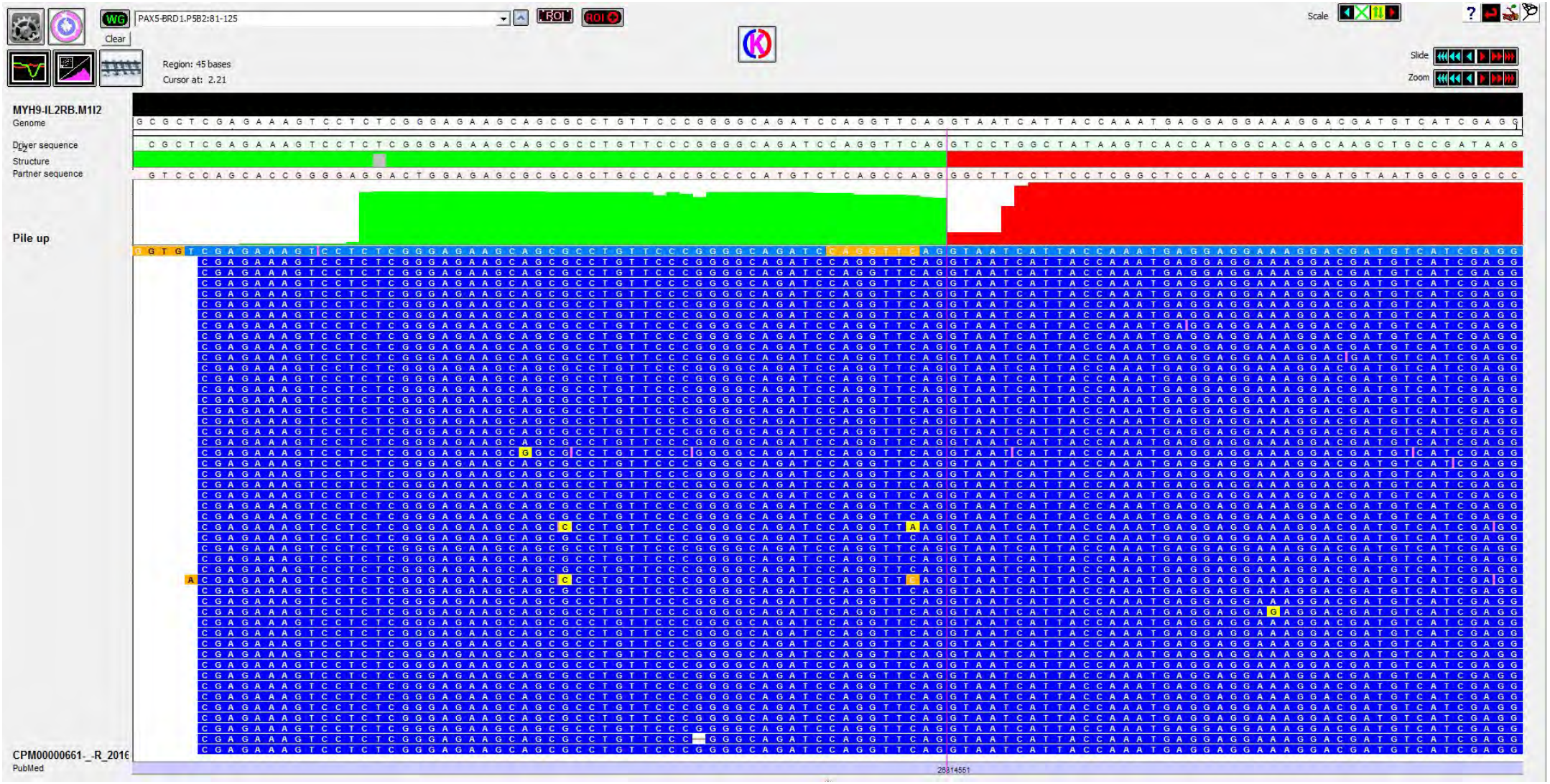
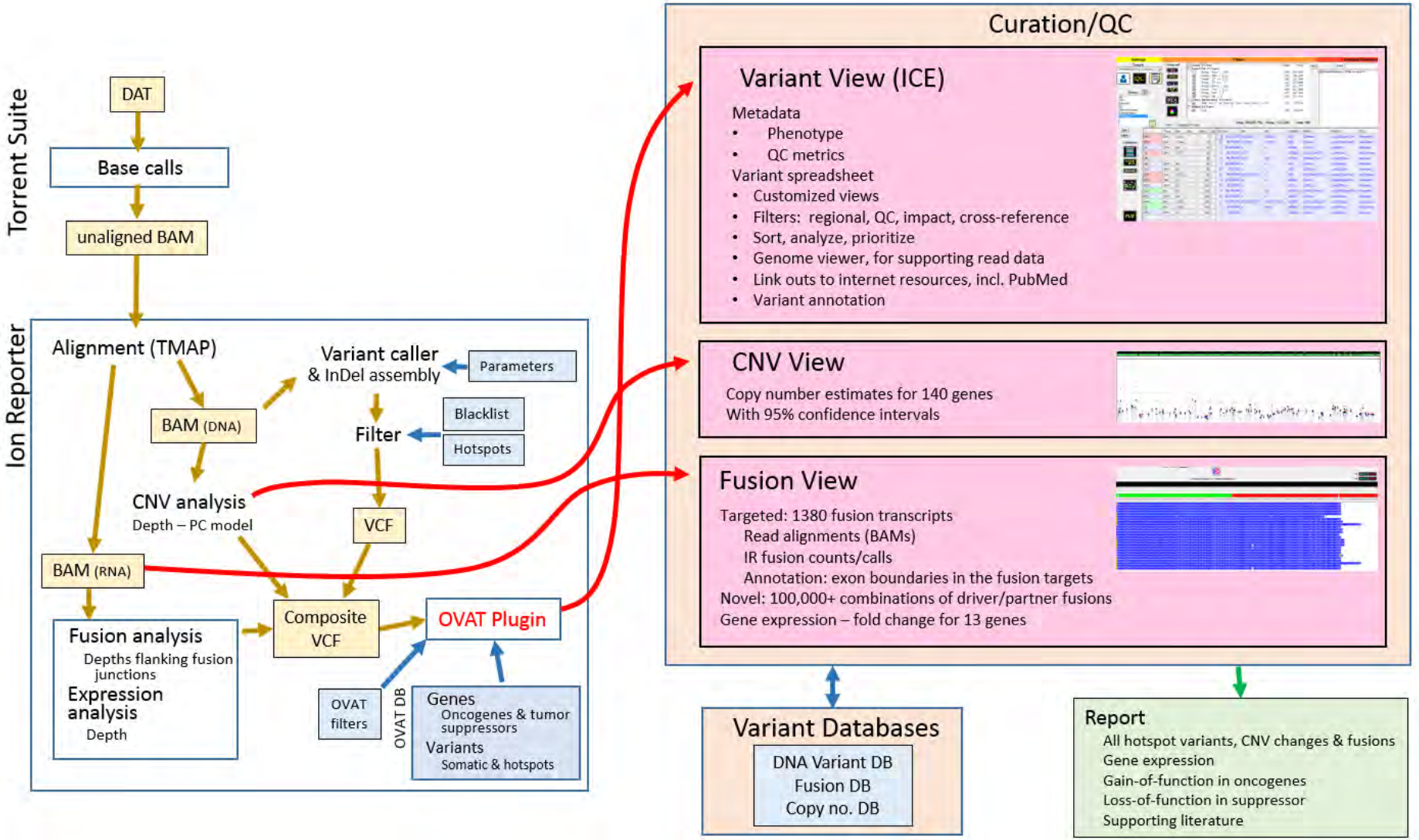
Stitching these together – full reads aligned to MYH9-BRD1 transcript



Performance Improved with ICE (Integrated Curation Environment)



ICE Performance Specifications*

SNVs

Acrometrix test sample; >5% VAF)

SNV

Thermo Fisher Variant Caller	Absent	Present
No Call	213,510	0
Call	9	303

Sensitivity: 100%
Specificity: >99%

InDels

Acrometrix test sample; >10% VAF)

InDel

ICE InDel Variant Caller	Absent	Present
No Call	213,803	0
Call	0	19

Sensitivity: 100%
Specificity: 100%

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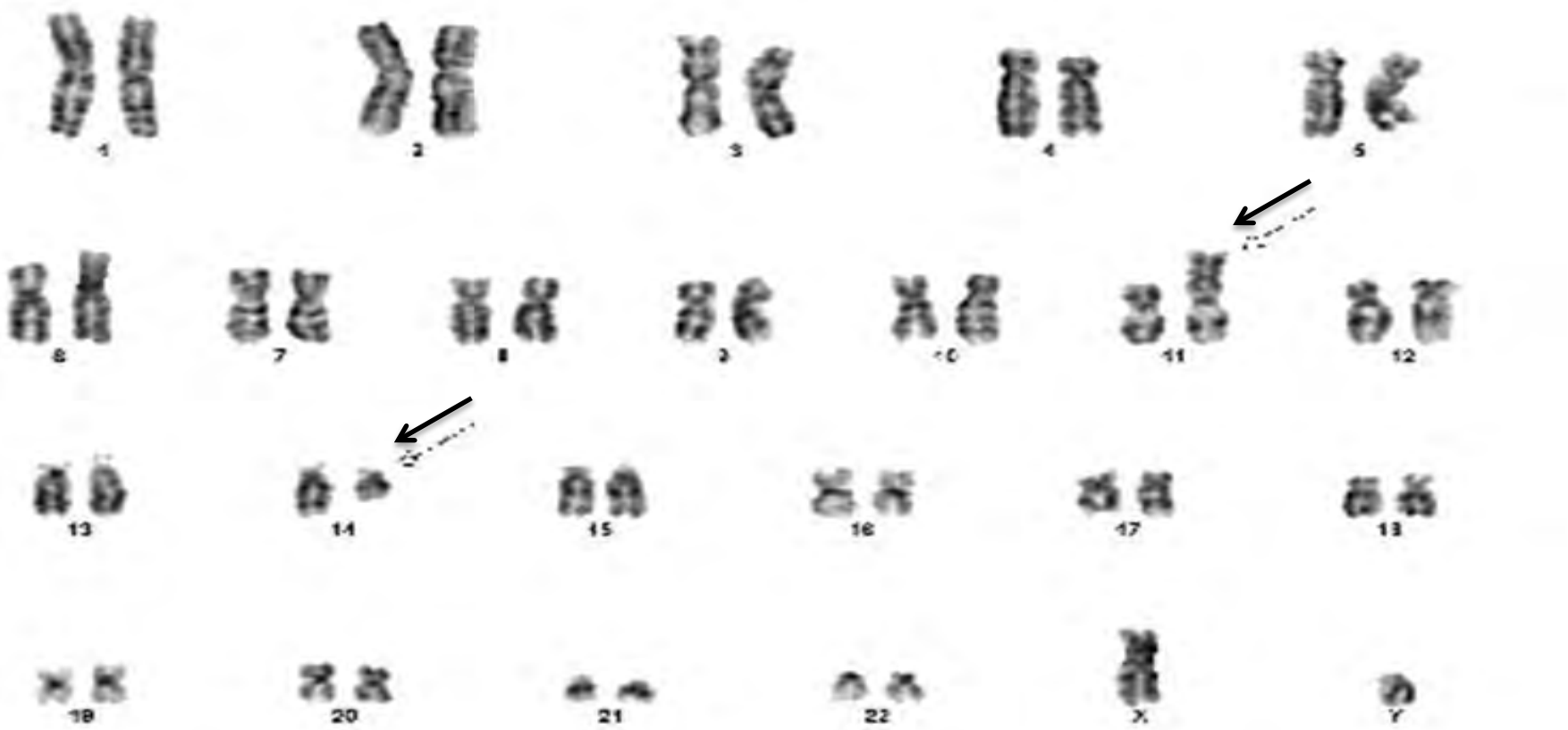
Clinical Research Case Study #1: A Pediatric Cancer Sample with T-ALL

Cytogenetics: *LMO2-TCRA* fusion 46,XY,t(11;14)(p13;q11.2)[7]/46,XY[1]

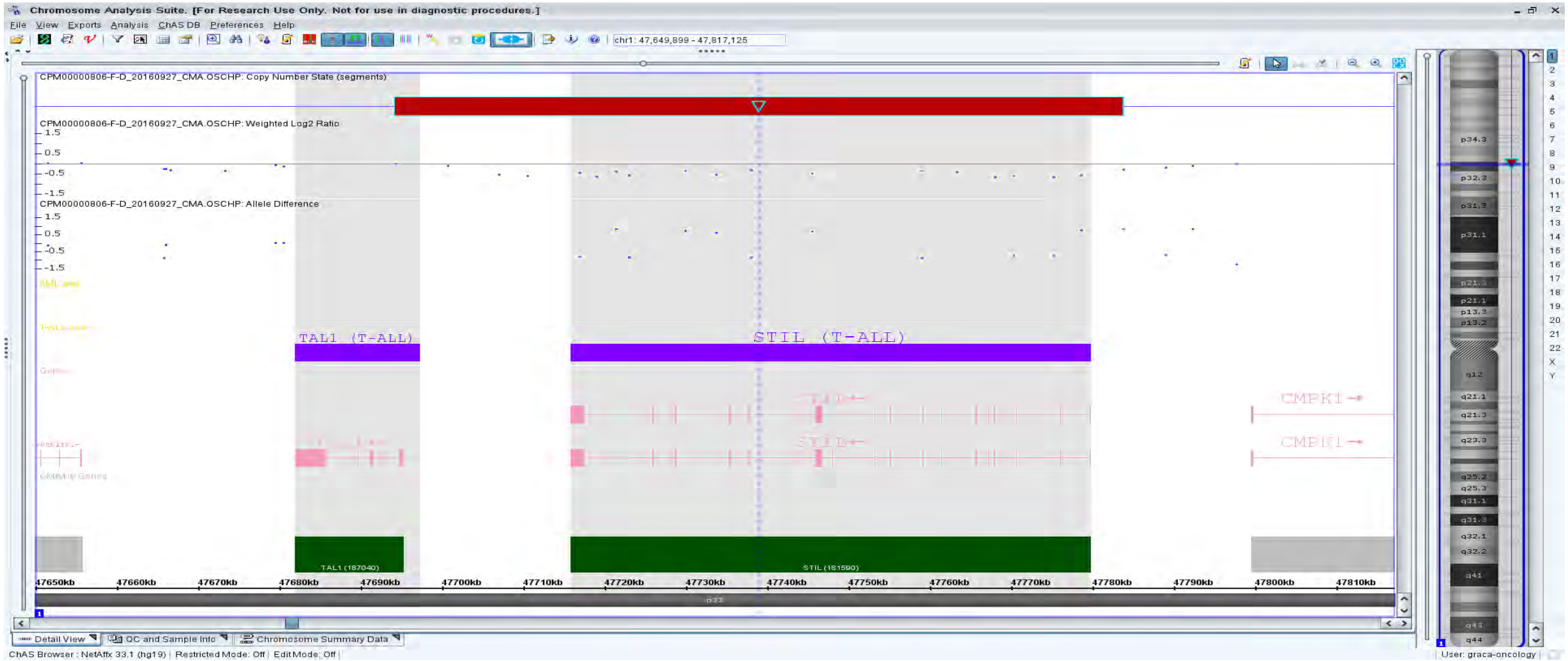


***LMO2-TCRA* fusion seen in 5-10% of pediatric T-ALL**

Results provided by Sammy Wu, CHLA cytogenetics

Chromosomal Microarray Results:

~80 kb Deletion in 1p33, Fusing 5' Portion of *STIL* to 3' Portion of *TAL1*



NGS Result: Two Dominant Fusions demonstrated :

STIL-TAL1 & FIP1L1-PDGFRA

- Two dominant fusions seen in the data
- The *PDGFRA* fusion can potentially be relevant for further clinical research

FUSION	FIP1L1(11) - PDGFRA(12)	158	Present	
FUSION	STIL(1) - TAL1(2)	124658	Present	Type2
FUSION	MET(13) - MET(15)	199	Present	
FUSION	STIL(1) - TAL1(2)	10225	Present	
FUSION	MET(17) - MET(20)	1367	Present	
FUSION	FIP1L1(13) - PDGFRA(12)	6689	Present	
FUSION	FIP1L1(13) - PDGFRA(12)	46	Present	

PDGFRA Hotspots Covered on the panel

Mutation
p.N659K
p.T674I
p.D842V
p.D848K

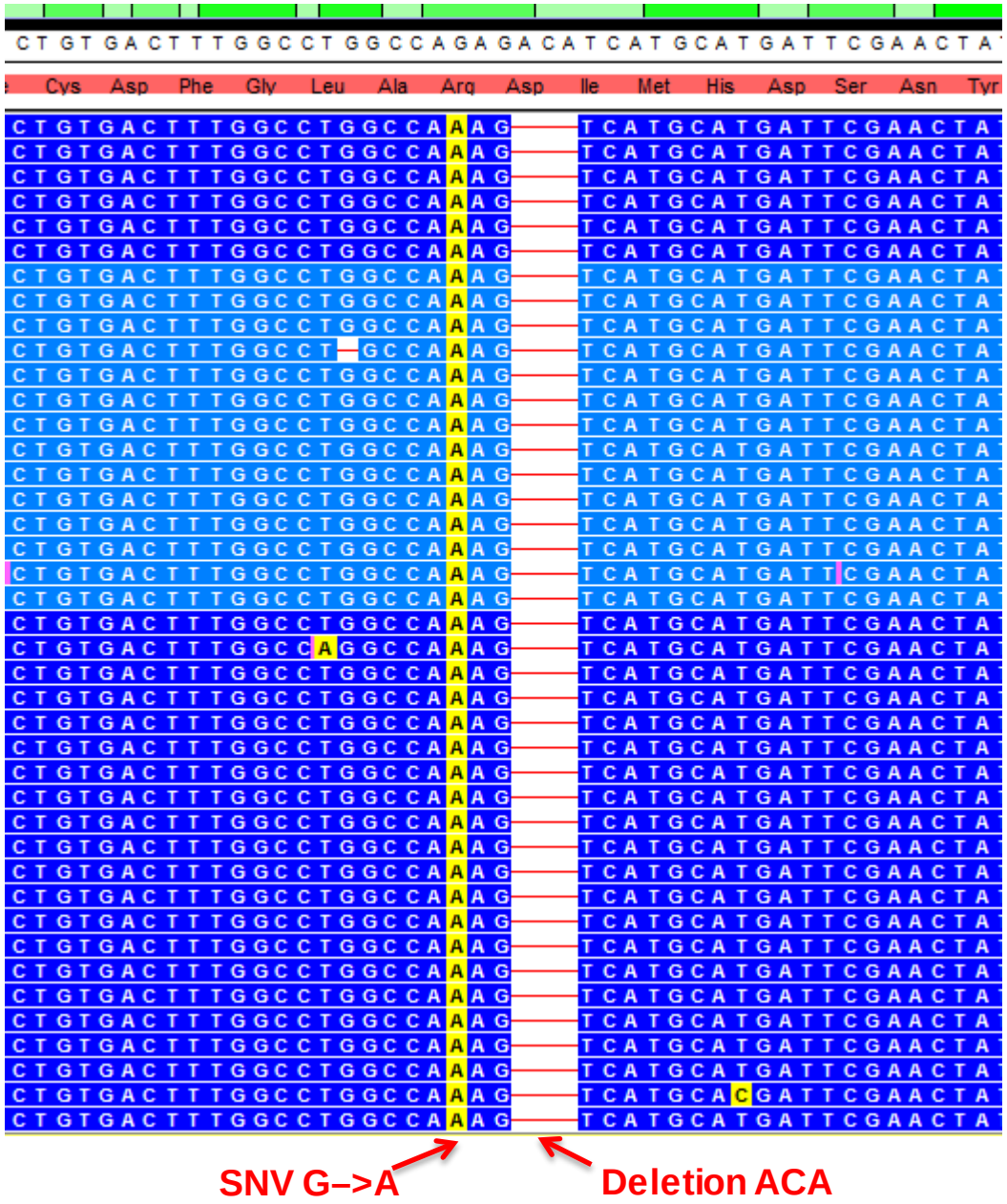
Detected *PDGFRA* Variant (D842V) [Deletion & Insertion]

NM_006206 (*PDGFRA):*
c.2522_2527delinsAAG
(p.Arg841_Ile843delinsLysVal)

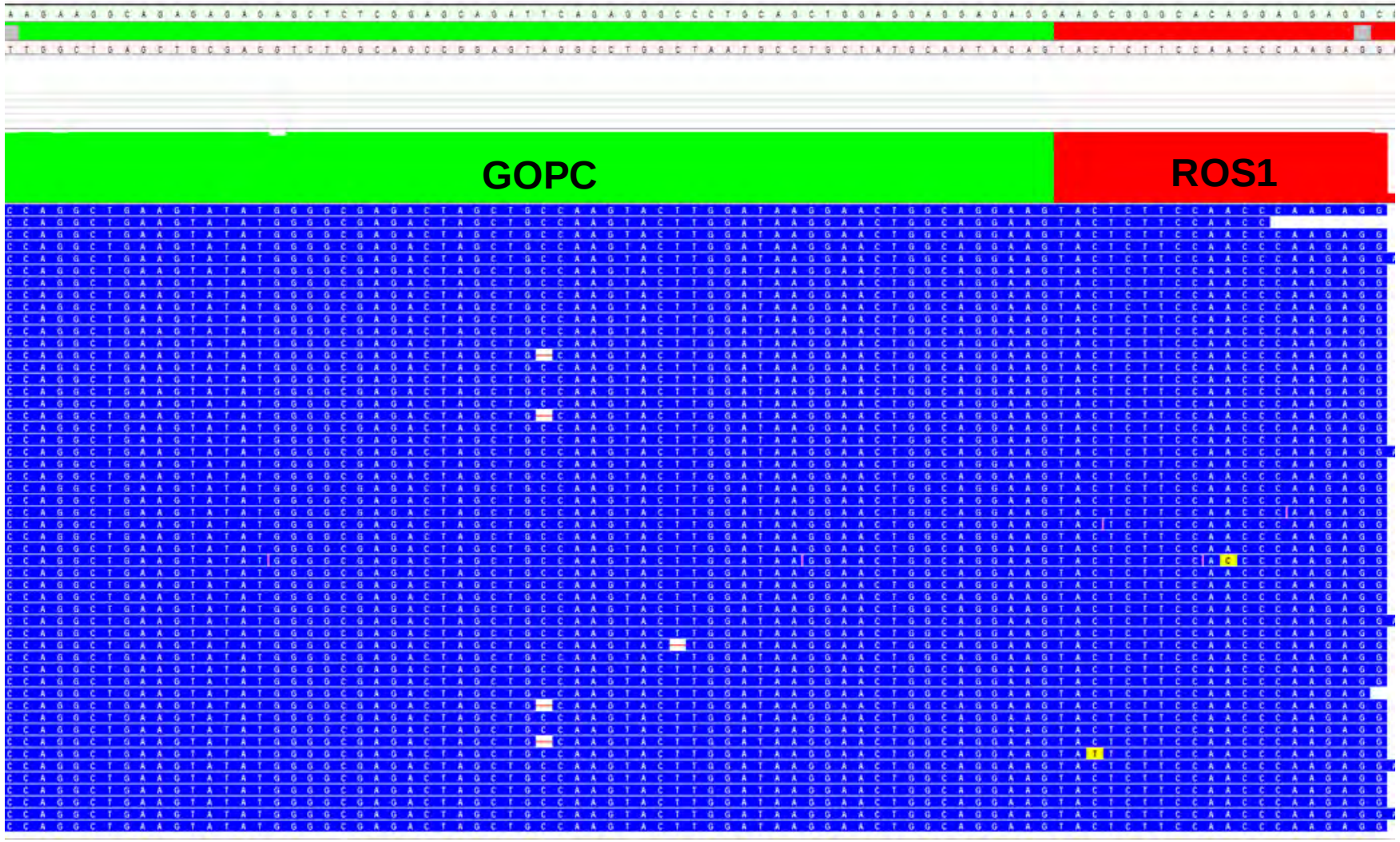
Present at roughly 14.22 % variant allele frequency

This variant was NOT DETECTED in the previous lymph node sample

DOD 12/30/2016



Clinical Research Case Study #2:
A Glioblastoma sample
- GOPC-ROS1 Fusion in 283317 Reads



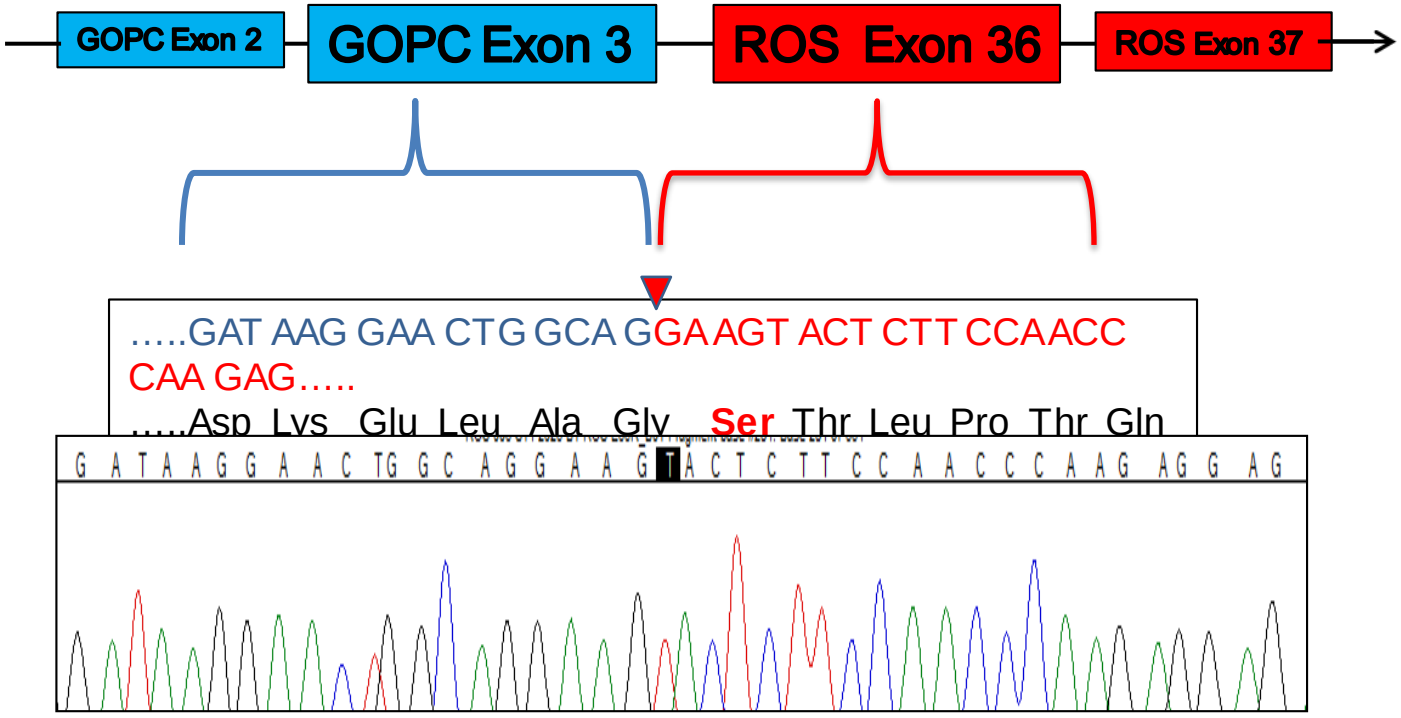
GOPC-ROS1 Fusion relevance

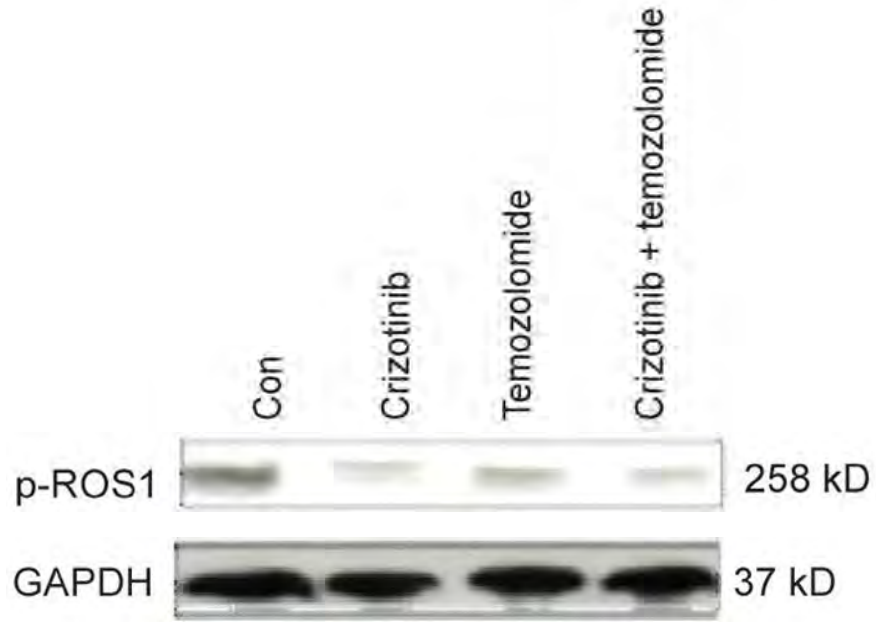
Synergistic Effects of Crizotinib and Temozolomide in Experimental FIG-ROS1 Fusion-Positive Glioblastoma

Libertas Academica

Arabinda Das[1], Ron Ron Cheng[1], Megan L.T. Hilbert[1], Yaenette N. Dixon-Moh[1], Michele Decandio[1], William Alex Vandergrift III[1], Naren L. Banik[1,2], Scott M. Lindhorst[1], David Cachia[1], Abhay K. Varma[1], Sunil J. Patel[1] and Pierre Giglio[1,3]

[1]Department of Neurosurgery, Medical University of South Carolina, Charleston, SC, USA. [2]Ralph H. Johnson VA Medical Center, Charleston, SC, USA. [3]Department of Neurological Surgery, The Ohio State University Wexner Medical Center, Columbus, OH, USA.





Cancer Growth Metastasis. 2015; 8:51-60.

Conclusions

- The assay is designed specifically for use in pediatric cancer research
  - Designed using AmpliSeq and Ion Torrent Technology*
  - Content developed in collaboration with CHLA & COG pediatric oncologists
  - 49 of 51 targets identified by COG TAP committee are included
- The same 52 genes designated as candidate therapeutic targets in Adult Oncomine Focus for NCI MATCH program are present in our panel as well
- Nearly 200 hotspot and full length genes already identified in pediatric cancer are also included
- Nearly 200 relevant gene fusions are included (> 1,500 combinatorial variants)
- Custom bioinformatics pipeline, ICE (Integtrated Curation Environment), enabling best in class precision, sensitivity, and specificity

*For Research Use Only. Not for use in diagnostic procedures

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Dennis Maglinte
Gig Ostrow
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