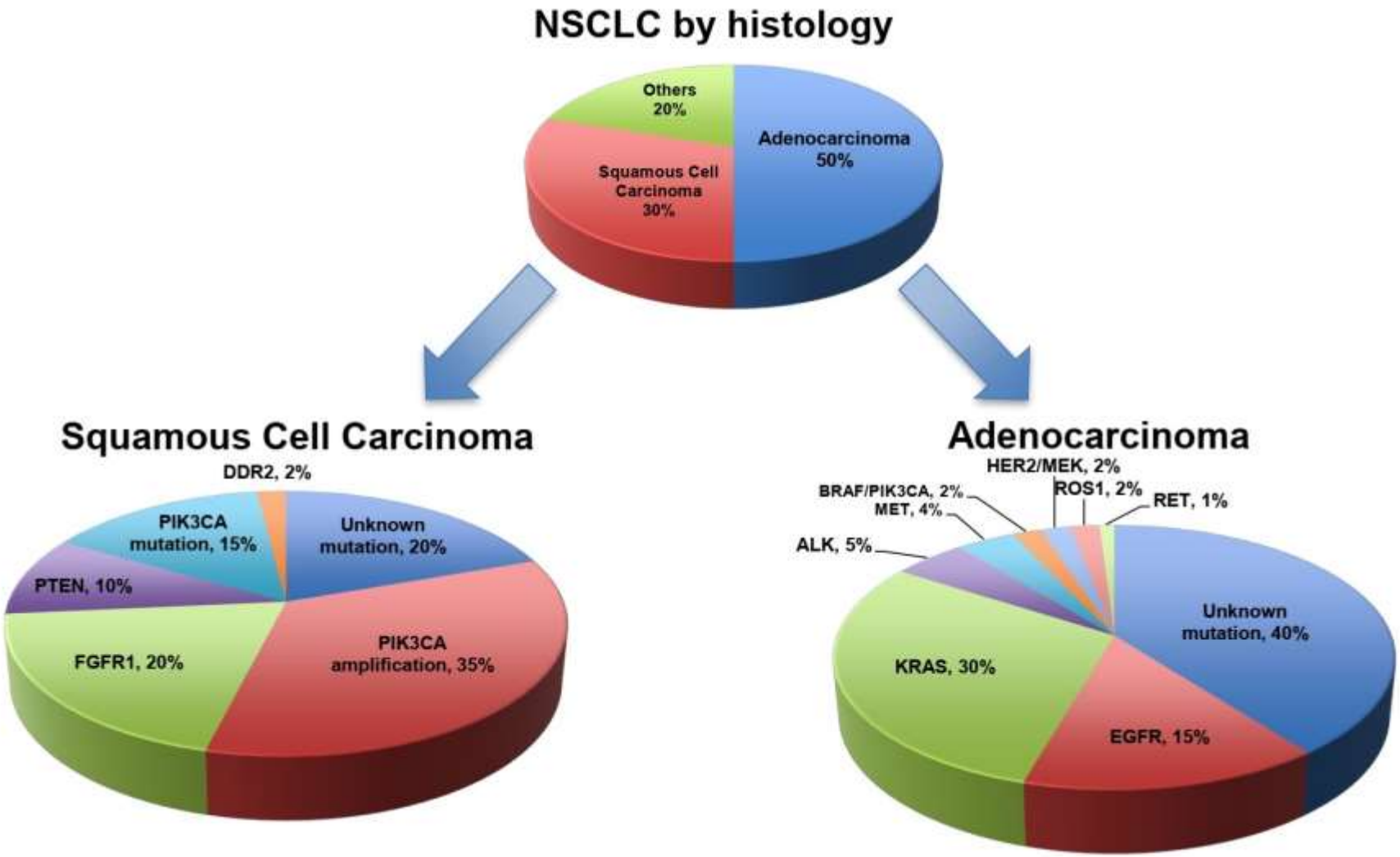
Targeted DNA +RNA NGS testing on NSCLC-- what we have learned so far?

A/ Prof Min Ru Qiu

SydPath, St Vincent's Hospital, Sydney

Disclosure of Relevant Financial Relationships

I have no commercial disclosure



https://www.quora.com/What-are-the-major-drug-targets-for-lung-cancer

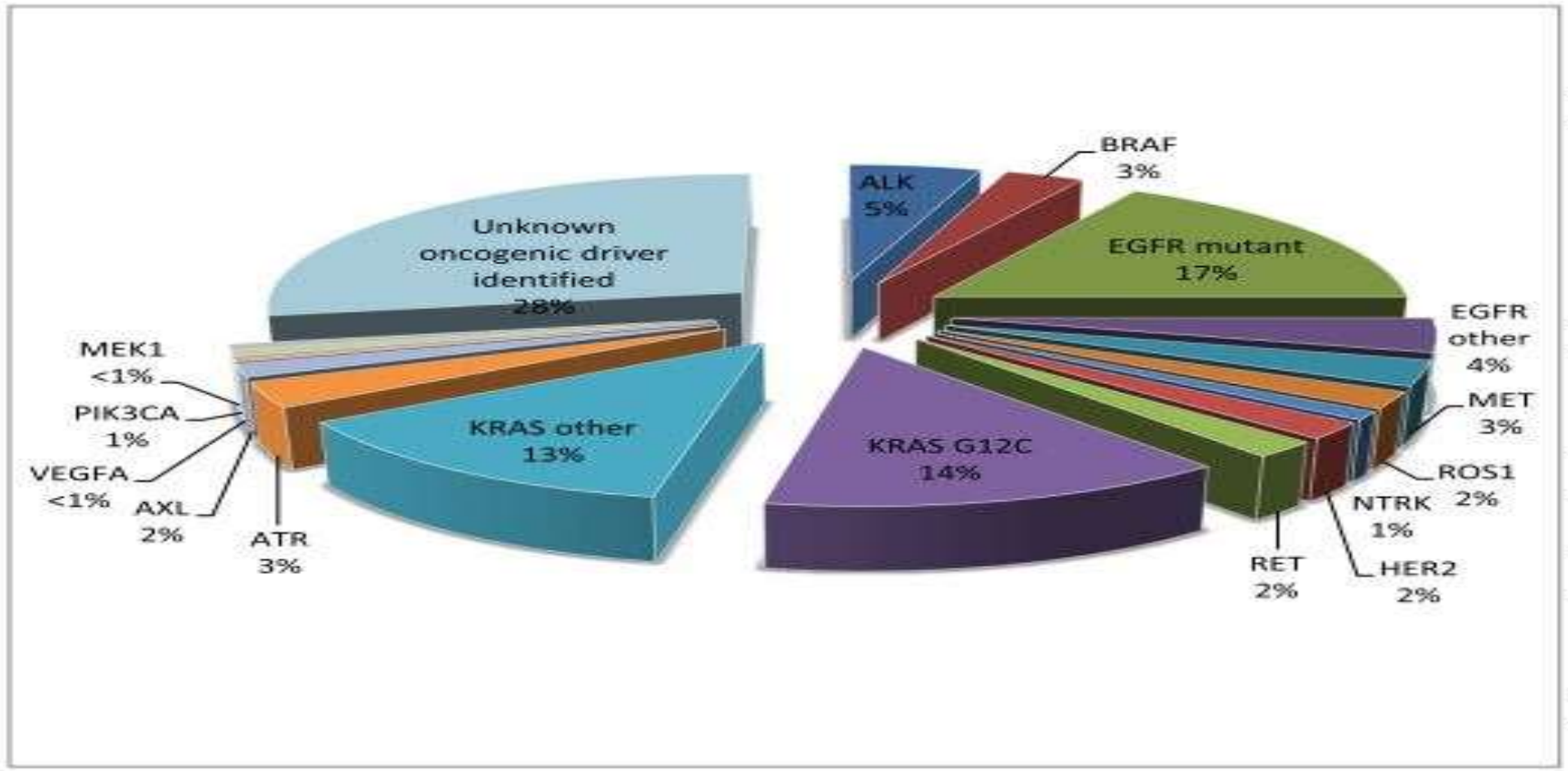


Fig. 1 Mutation frequency of lung adenocarcinoma and emerging drugs targeted these mutations. Frequency data is a combination from the AACR GENIE data and Lung Cancer Mutation Consortium [73, 74]

A

Predictive biomarkers	ESMO guidelines	NCCN guidelines	CAP/IASLC/AMP guidelines	ASCO guidelines	Pan-Asian guidelines
EGFR	●	●	●	●	●
ALK	●	●	●	●	●
ROS1	●	●	●	●	●
BRAF	●	●	●	●	●
PD-L1	●	●	●	●	●
NTRK	●	●	●	●	●

B

Emerging biomarkers	ESMO guidelines	NCCN guidelines	CAP/IASLC/AMP guidelines	ASCO guidelines	Pan-Asian guidelines
KRAS	●	●	●	●	●
MET	●	●	●	●	●
RET	●	●	●	●	●
ERBB2/HER2	●	●	●	●	●
TMB	●	●	●	●	●

● Testing recommended

● Expanded panel testing recommended

● Single gene or expanded panel testing recommended

● No guideline recommendations to date

https://doi.org/10.1016/j.lungcan.2021.02.035

The Evolution of Biomarker Testing for NSCLC in SydPath

2012-2020

PCR based EGFR testing + ALK IHC FISH + ROS1 IHC FISH+ PDL1 IHC

2020-2022(Nov)

DNA based NGS panel testing +ALK IHC FISH +ROS1 IHC FISH+ PDL1 IHC

2022(Nov)- Now

DNA+ RNA based NGS panel testing +ALK IHC +ROS1 IHC+ PDL1 IHC

Current Testing Panel and Platform Used in SydPath

Genexus System from Thermo Fisher

Oncomine Precision Assay (50 gene panel)

Gene list for Oncomine Precision Assay

Hotspot genes

AKT1	CHEK2	FGFR3	KIT	NTRK3
AKT2	CTNNB1	FGFR4	KRAS	PDGFRA
AKT3	EGFR	FLT3	MAP2K1	PIK3CA
ALK	ERBB2	GNA11	MAP2K2	PTEN
AR	ERBB3	GNAQ	MET	RAF1
ARAF	ERBB4	GNAS	MTOR	RET
BRAF	ESR1	HRAS	NRAS	ROS1
CDK4	FGFR1	IDH1	NTRK1	SMO
CDKN2A	FGFR2	IDH2	NTRK2	TP53

CNVs

ALK	FGFR3
AR	KRAS
CD274	MET
CDKN2A	PIK3CA
EGFR	PTEN
ERBB2	
ERBB3	
FGFR1	
FGFR2	

Genetic fusions

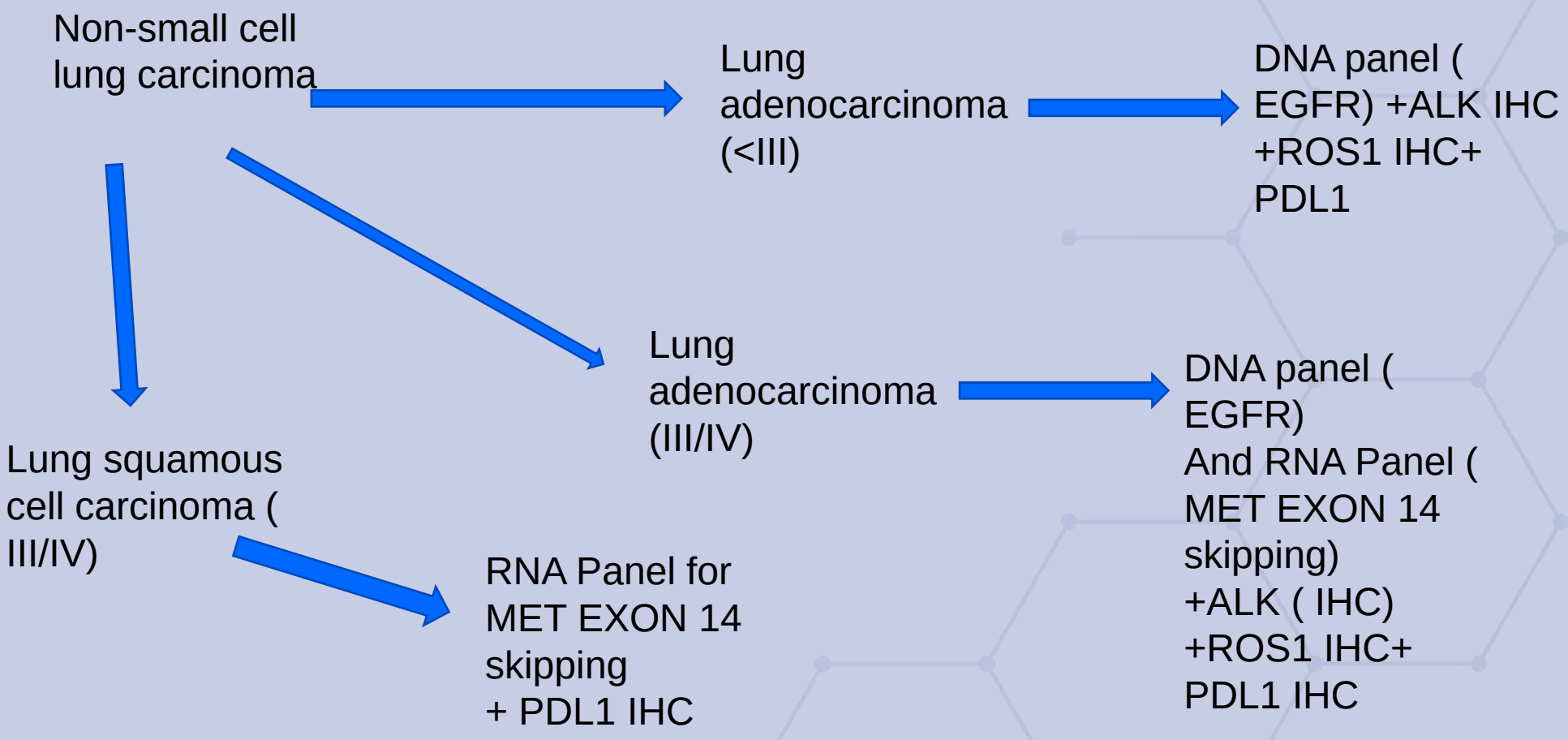
Inter-genetic fusions

ALK	MET	RET
BRAF	NRG1	ROS1
ESR1	NTRK1	RSPO2
FGFR1	NTRK2	RSPO3
FGFR2	NTRK3	
FGFR3	NUTM1	

Intra-genetic fusions

AR
EGFR
MET

Non-small Cell Lung Carcinoma Biomarker Testing Alogrithm



Total NSCLC Tested Between 1/11/2022 to 30/04/2023

Histology type	DNA panel only	DNA + RNA Panel	RNA panel only	Total
Adenocarcinoma	283	280	4	567
Squamous cell carcinoma	2	4	17	23
Total	285	284	21	**590**

Overview of Genetic Alterations in NSCLC

	DNA panel only	DNA + RNA Panel	RNA panel only
Genetic alterations	266	266	5
No genetic alteration	19	18	17

Targetable Genetic Alerations Detected by Using DNA + RNA Panel

	EGFR	KRAS	BRAF	ERBB 2	ALK	ROS1	RET	MET	NTRK 1	NTRK 2	NTRK 3	CDKN 2A
SNV/indel/insertion	133 (23.5%)	170 (30%)	25 (4.4%)	15	1(resistant)	0		17(3%)				
Fusion					20 (3.5%)		6 (1%)	11 (2%)		2 (0.4%)	2 (0.4%)	
CNV	20 (amplification) (3.5%)			6 (amplification) (1%)				11 (amplification) (2%)				26 (deletion) (4.5%)

Advantage by Using Targeted DNA+ RNA Panel Testing for NSCLC

1. Able to test all essential biomarkers including EGFR, ALK, ROS1 plus many new biomarkers including MET (both amplification and MET EXON 14 skipping), RET fusion, NTRK1, 2, 3 fusion, BRAF, KRAS, HER2 amplification and Exon20 insertion.
2. Able to test CDKN2A and TP53 gene alterations to help predict the prognosis.
3. Fast TAT. Our average TAT is 6 days from receiving the tumour sample to finalizing the reports.
4. Cost effective. We no longer perform ROS1 or ALK FISH if no fusion or gene expression imbalance detected, unless there is strong immunohistochemical staining.

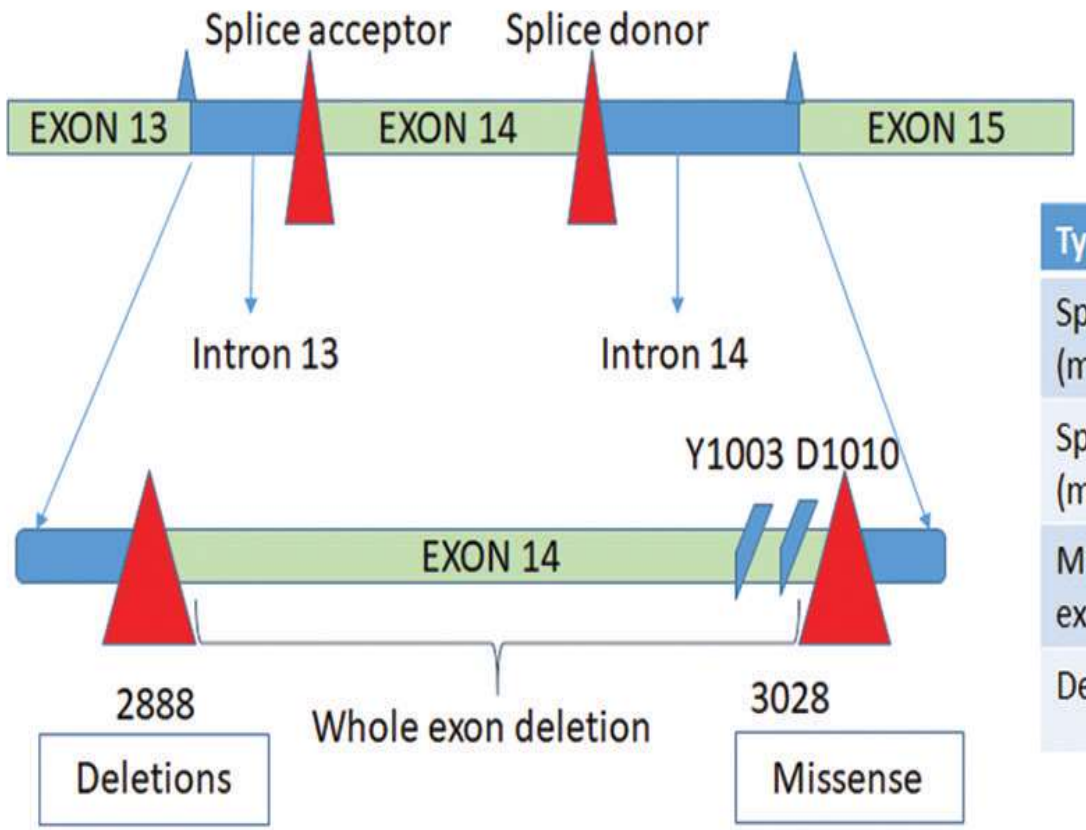
New Biomarkers and New Challenges

- MET EXON 14 Skipping
- Novel NTRK fusions
- Gene expression imbalance
- CDKN2A deletion

MET GENE

- The *MET* (mesenchymal epithelial transition factor) **proto-oncogene** maps to the 7q31 locus of chromosome 7 and encodes for a receptor tyrosine kinase (RTK) for *HGF*, also known as scatter factor.
- MET copy number gain/amplification and **exon 14 skipping mutation (*MET*ex14)**, is known to be one of the secondary mechanisms of resistance to EGFR tyrosine kinase inhibitors (TKIs).
- *MET*ex14 NSCLC can also be targeted using specific TKIs. The rapid approvals of newer MET-selective TKIs, such as **capmatinib and tepotinib,** mandate *MET* testing as a part of the first line molecular testing in NSCLC

MET EXON 14 Skipping



Type	Site	Frequency
Splice site alteration (mainly missense)	Splice Donor	42%
Splice site alteration (mainly deletions)	Splice Acceptor	11.2%
Missense , whole exon deletion	Exon 14	25.4%
Deletion	Polypyrimidine tract	21.5%

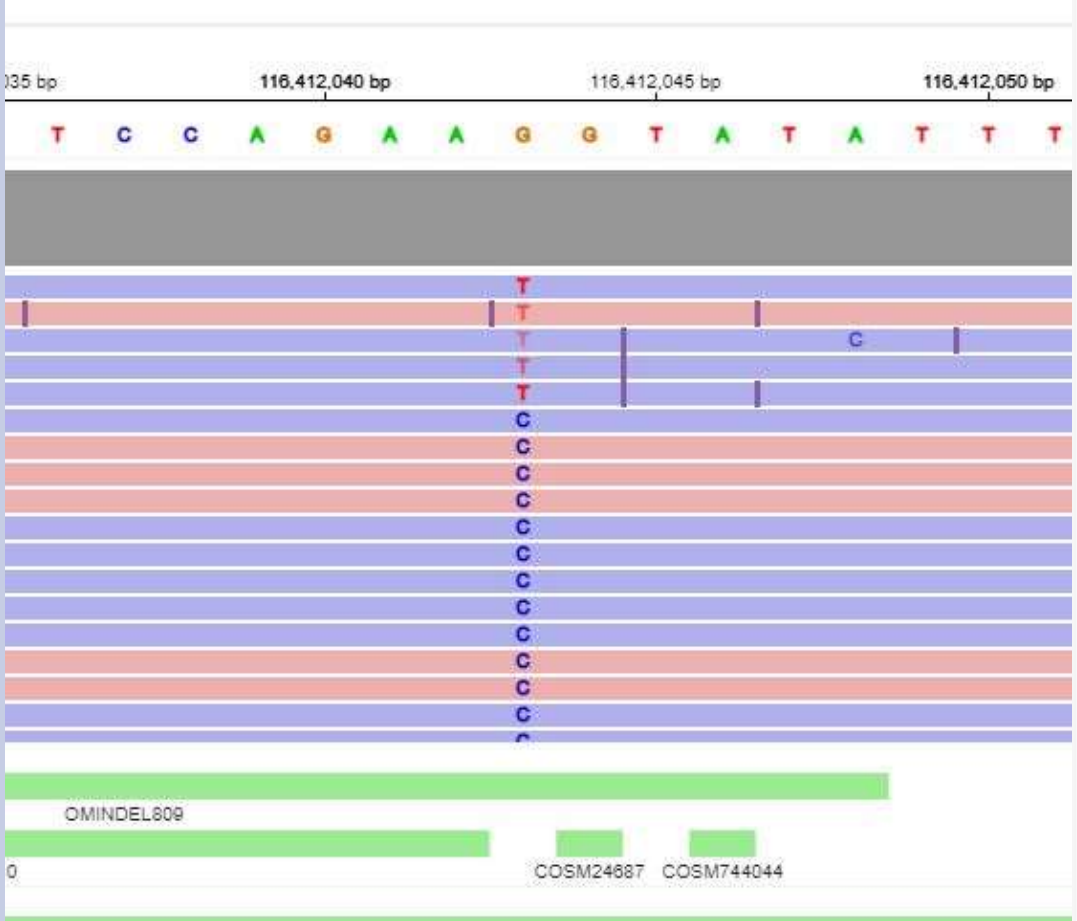
DETECTION OF MET EXON 14 Skipping

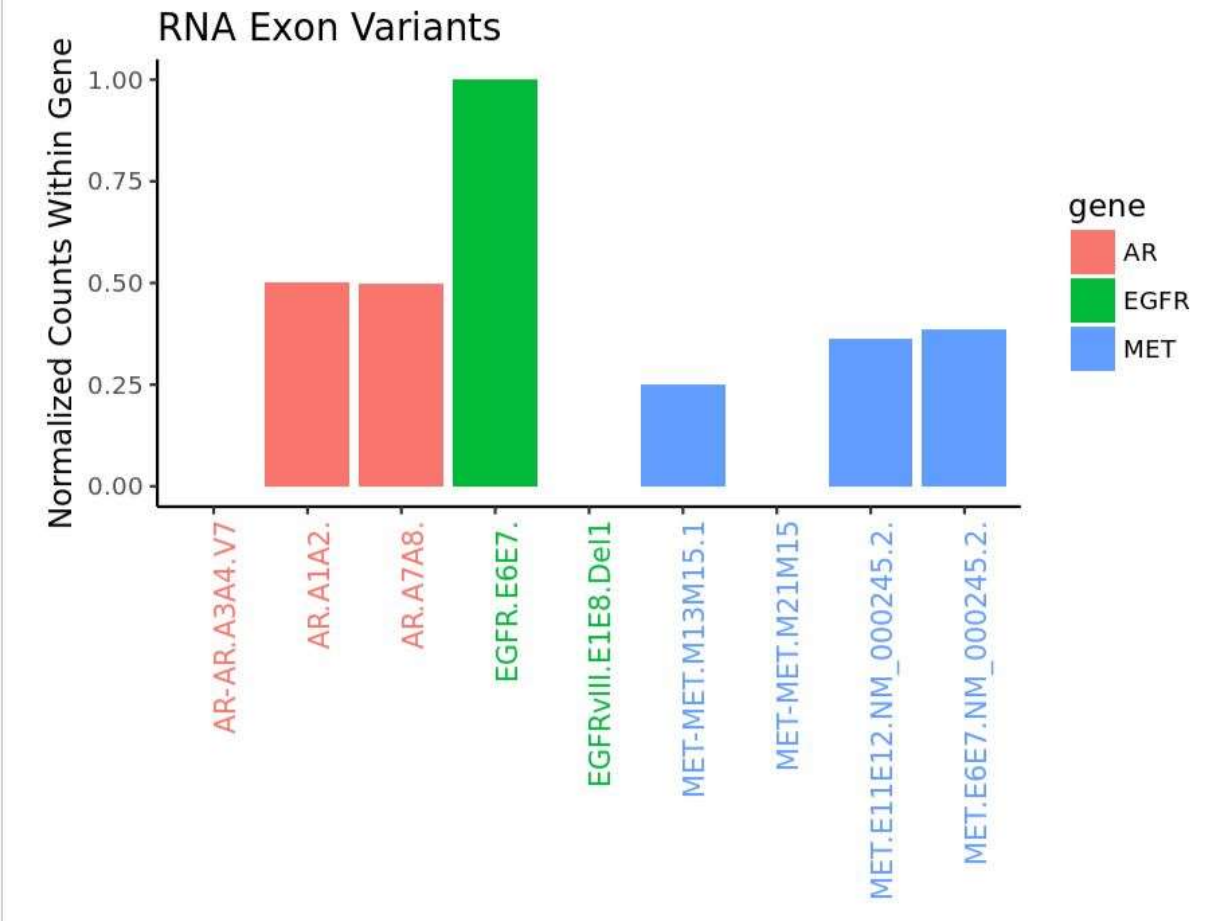
Table 1: Various assays available for the detection of *MET exon* 14 skipping mutation

Assay	Target	Sample quantity	Number of genes	Detection limit	Sensitivity/Specificity
RT-PCR	RNA	10 ng	1	5%	100/97.4
Sanger	DNA	10 ng	1	25%	61.5/100
NGS-Tumor					
FoundationOne CDx	DNA	50-100 ng	324	2-5%	Not computed
TruSight Oncology	DNA/RNA	40 ng	523	5%	Not computed
NGS-Liquid based					
Guardant360	cfDNA	5-30 ng	73	0.1%	Not computed
FoundationOne Liquid	cfDNA	17 ml blood sample	70	0.5%	Not computed

Acronyms: RT-PCR: reverse transcription polymerase chain reaction, ng: nanogram, NGS: next-generation sequencing, DNA: deoxyribonucleic acid, RNA: ribonucleic acid, cf: cell-free

DETECTION of MET EXON 14 Skipping by ONCOMINE PRECISION ASSAY

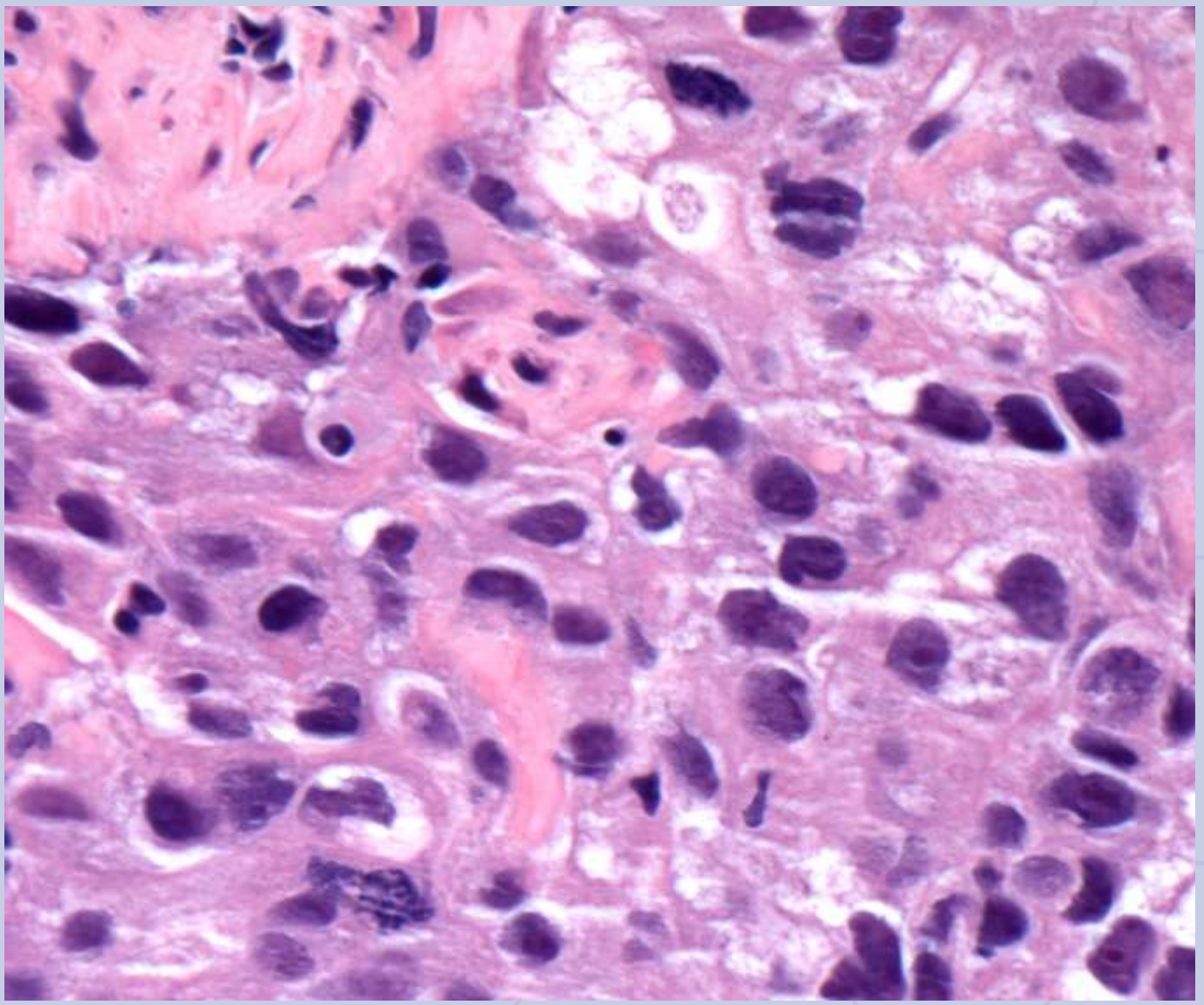




DETECTION OF MET EXON 14 SKIPPING

MET EXON 14 Skipping	Detected by DNA only	Detected by both DNA and RNA	Detected by RNA only
28	9	18	1 (4%)

DETECTION OF MET EXON 14 SKIPPING



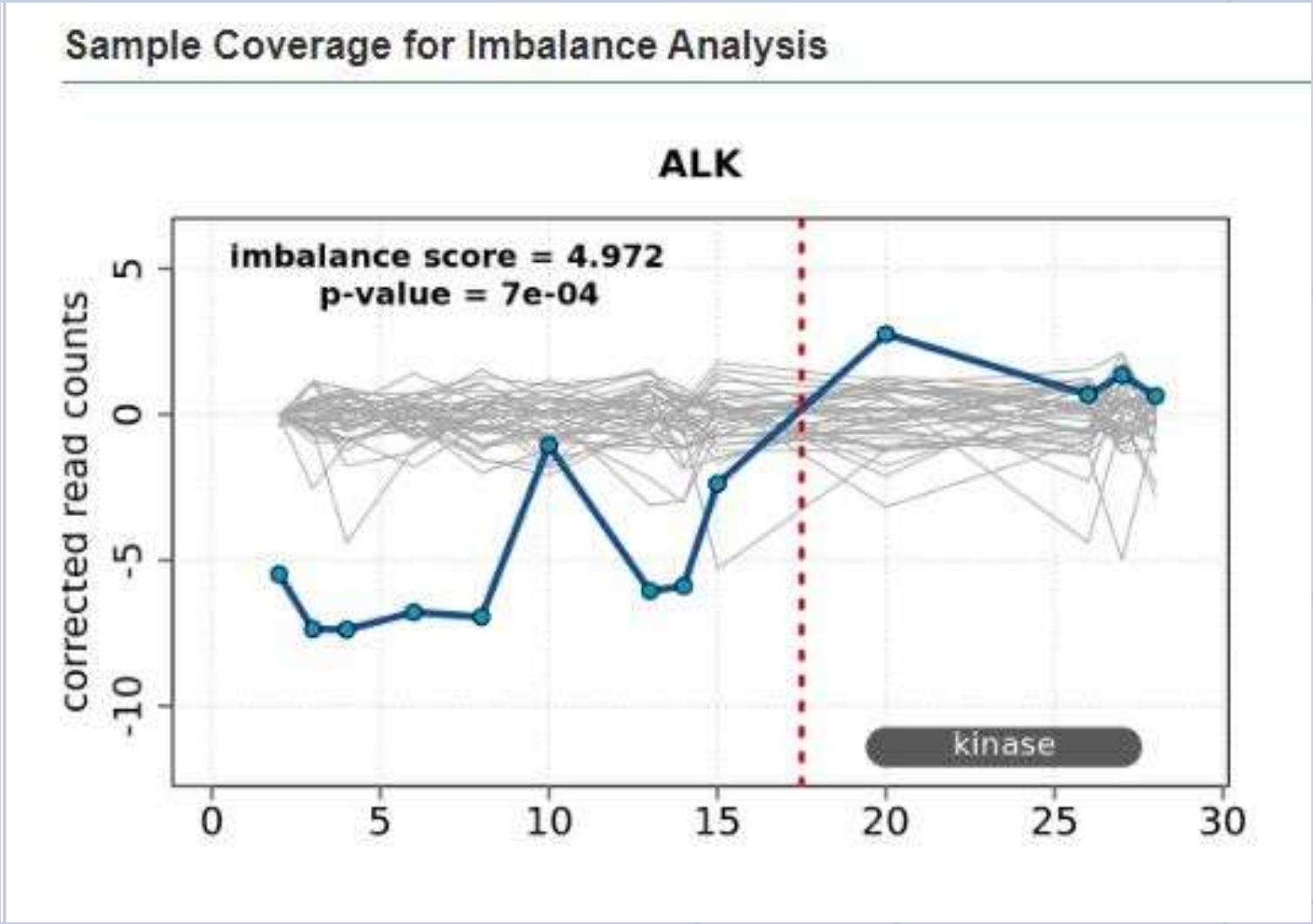
CHALLENGES DURING RNA BASED TESTING

- **Higher quality of tumour sample**
- **Higher tumour cellularity**
- **Curation and annotation of the results: Novel genetic fusions and gene expression imbalance only without fusion detected**

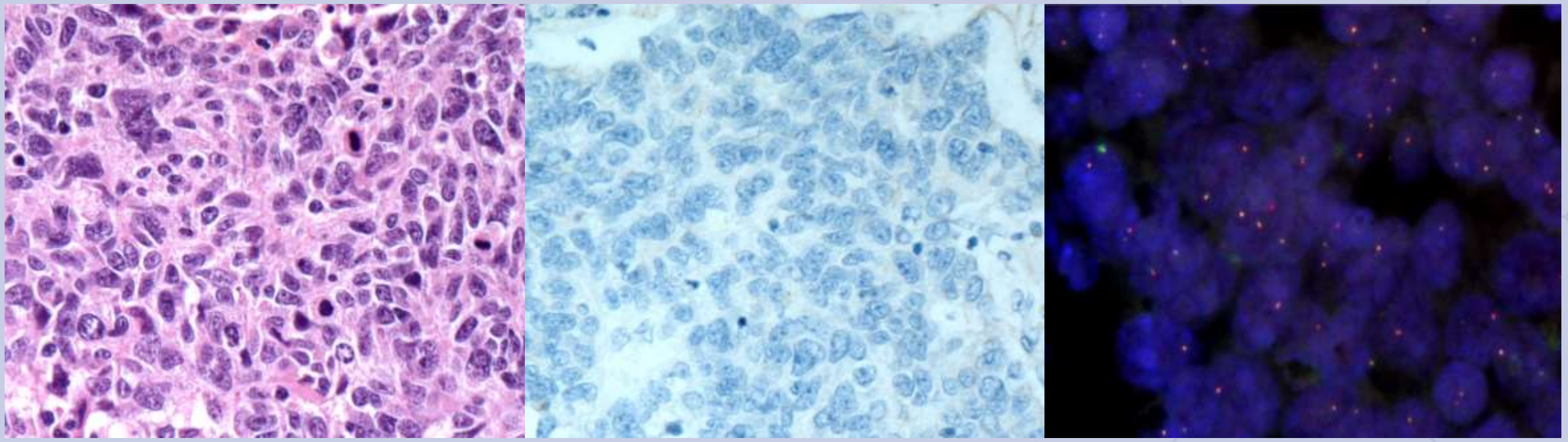
GENE EXPRESSION IMBALANCE

Total cases been tested with RNA based NGS testing	ALK expression imbalance only	NTRK3 expression imbalance only	Total cases with gene expression imbalance
305	9	2	11 (3.6%)

GENE EXPRESSION IMBALANCE



GENE EXPRESSION IMBALANCE



NTRK Fusion

- NTRK (neurotrophic tyrosine receptor kinase) composed of NTRK1, NTRK2, NTRK3
- They are receptor tyrosine kinases expressed in human neuronal tissue.
- NTRK gene fusions are oncogenic drivers of various adult and many paediatric tumours.
- Testing methods: IHC, FISH, RT-PCR, RNA based NGS testing, NTRK fusion can be targeted by TRK inhibitors, such as larotrectinib (FDA approved).

Penault-Llorca F, et al. J Clin Pathol 2019;**72**:460–467. doi:10.1136/jclinpath-2018-205679

Table 1 *NTRK* gene fusions identified in adult and paediatric cancers by relative frequency of *NTRK* gene fusions

	Fusion partner		
Tumour	***NTRK1***	***NTRK2***	***NTRK3***
Adult cancers			
High frequency (>80%)			
Mammary analogue secretory carcinomas			*ETV6*[11]
Secretory breast carcinoma			*ETV6*[12]
Intermediate frequency (5%–25%)			
Papillary thyroid cancer	*TFG*,[13] *SSBP2*,[9] *SQSTM1*,[9] *TPR*,[7] *PPL*[7]		*ETV6*,[9 43] *RBPMS*[9]
Low frequency (<5%)			
Appendiceal cancer	*LMNA*[18]		
Glioma/glioblastoma	*ARHGEF2*,[19] *BCAN*,[20 21] *CHTOP*,[19] *NFASC*[20]	*BCR*,[18] *AFAP1*,[9] *SQSTM1*[9]	*AFAP1*,[18] *ZNF710*,[18] *EML4*[18]
Astrocytoma		*QK1*,[7] *NACC2*[7]	
Gastrointestinal stromal tumour			*ETV6*[15]
Head and neck cancer		*PAN3*[9]	*LYN*[9]
Lung cancer	*CD74*,[7] *GRPAP1*,[23] *IRF2BP2*,[18] *MPRIP*,[7] *P2RY8*,[18] *SQSTM1*,[24] *TPM3*[18]	*TRIM24*[9]	
Sarcoma	*TPM3*,[9] *LMNA*[18]		*TPM4*[10]
Breast cancer	*CGN*,[25] *GATAD2B*,[25] *LMNA*,[25] *MDM4*,[25] *PEAR1*,[25] *TPM3*,[10 25]		*ETV6*[25]
Acute lymphoblastic leukaemia, acute myeloid leukaemia, histiocytosis, multiple myeloma, dendritic cell neoplasms			*ETV6*[26]
Uterine sarcoma	*LMNA*,[27] *TPM3*,[27] *TPR*[27]		*RBPMS*[27]
Cholangiocarcinoma	*LMNA*,[10] *RABGAP1L*[28]		
Pancreatic cancer	*CTRC*[10]		
Melanoma	*DDR2*,[29] *GON4L*,[29] *TRIM63*[29]	*TRAF2*[29]	*ETV6*[9]
Colorectal cancer	*LMNA*,[10] *TPM3*,[10] *SCYL3*[30]		*ETV6*[18]
Paediatric cancers			
High frequency (>80%)			
Secretory breast carcinoma			*ETV6*[12]
Infantile fibrosarcoma and other mesenchymal tumours	*SQSTM1*,[31] *TPM3*,[41] *LMNA*[41]		*EML4*,[32 41] *ETV6*[34 63]
Cellular and mixed congenital mesoblastic nephroma	*TPR*,[40] *LMNA*[40]		EML4,[32 40] ETV6[33 40]
Intermediate frequency (5%–25%)			
Papillary thyroid cancer	*TPR*,[35] *IRF2BP2*,[10] *TPM3*[14]		*ETV6*[35]
Spitz tumours	*TP53*,[16] *LMNA*[16]		*ETV6*,[17] *MYH9*,[17] *MYO5A*[17]
Paediatric high-grade gliomas	*TPM3*[36]	*AGBL4*,[36] *VCL*[36]	*ETV6*,[36] *BTB1*[36]
Low frequency (<5%)			
Ganglioglioma		*TLE*[38]	
Astrocytoma		*NACC2*,[37] *QK1*[37]	

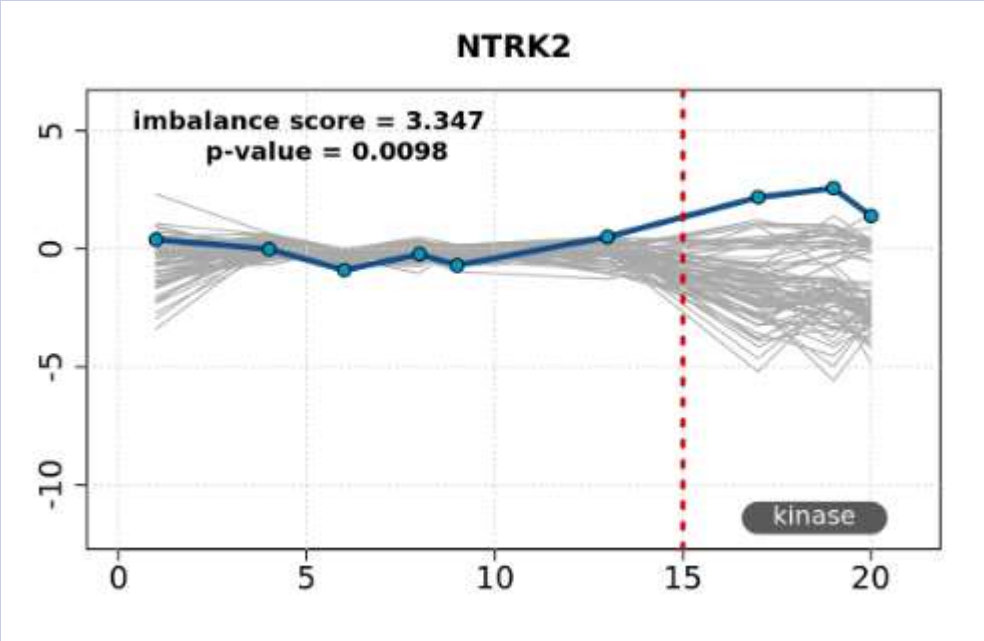
Penault-Llorca F, et al. J Clin Pathol 2019;**72**:460–467. doi:10.1136/jclinpath-2018-205679

NOVEL NTRK FUSION

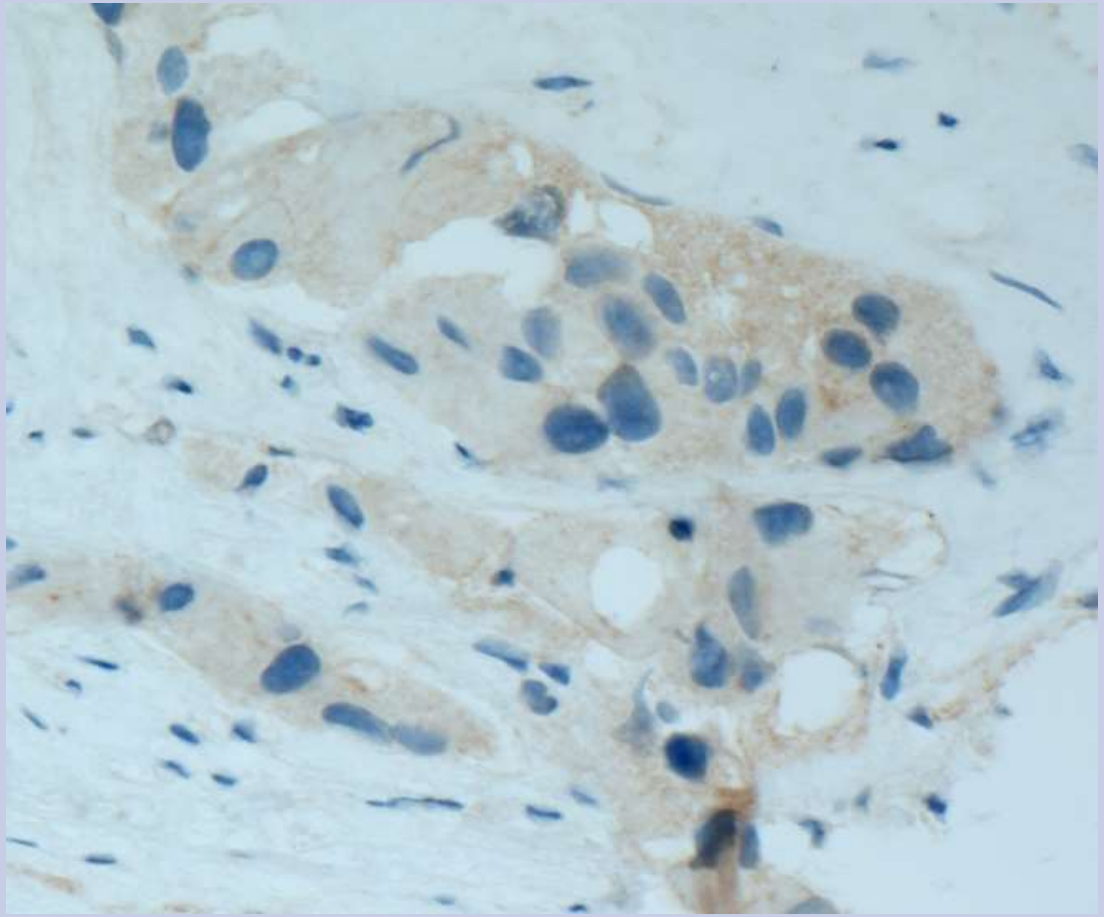
- 4 cases (1.3%) with NTRK fusion in a total of 305 cases tested with RNA panel
- 2 of the four cases are NTRK2 fusion with novel patterns (**TPR-NTRK2 FUSION** and **STRN3-NTRK2 FUSION**).

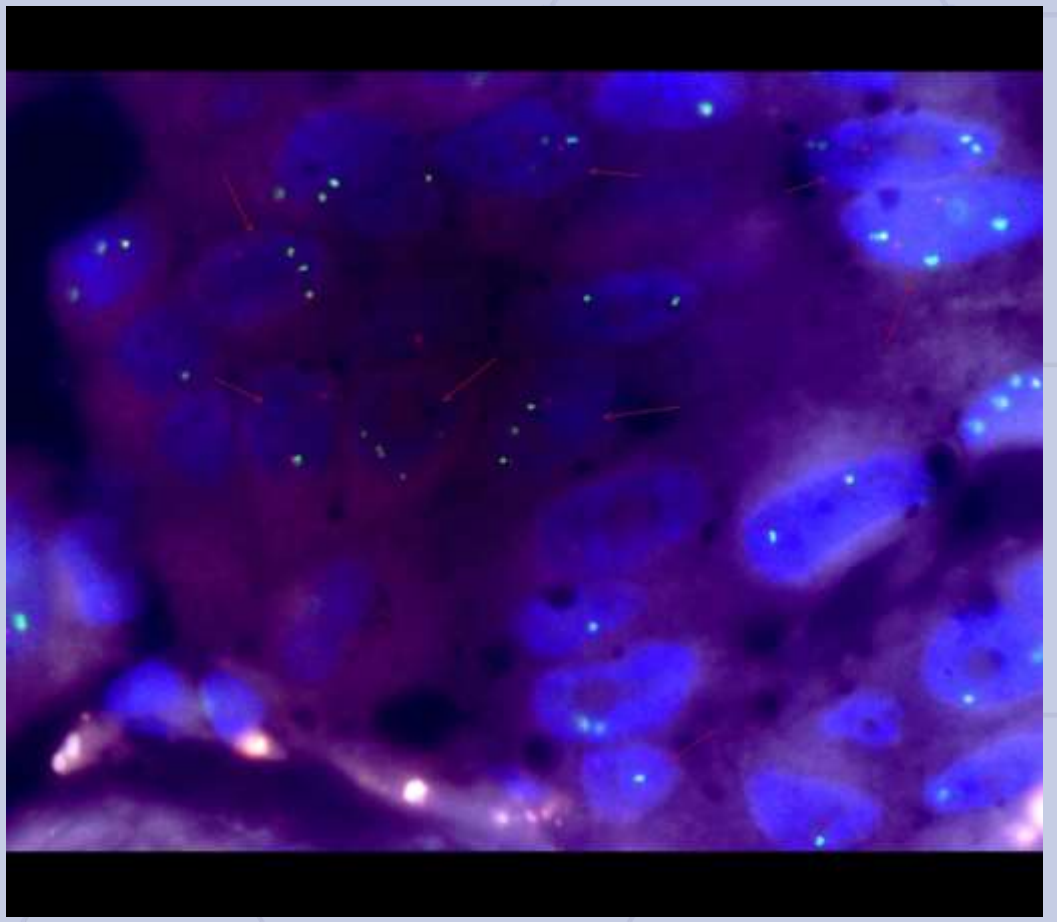
NOVEL TPR-NTRK2 FUSION

Variant ID	Key Variant	Locus	Oncomine Variant Class	Oncomine Gene Class	Genes (Exons)	Read Counts
NTRK2	Yes	chr9:87359940	ExpressionImbalance	Gain-of-Function	NTRK2	0
TPR-NTRK2.T21N15.Non-Targeted	No	chr1:186319355 - chr9:87475955			TPR(21) - NTRK2(15)	544
TPR-NTRK2.T21N16.Non-Targeted	No	chr1:186319355 - chr9:87482158			TPR(21) - NTRK2(16)	455



NOVEL TPR-NTRK2 FUSION





CDKN2A DELECTION

- CDKN2A stands for "Cyclin-Dependent Kinase Inhibitor 2A.
- It is located on chromosome 9.
- It is known to be an important tumor suppressor gene
- This gene is frequently mutated or deleted in a wide variety of tumors.
- CDKN2A loss-of-function predicts immunotherapy resistance in non-small cell lung cancer

DETECTION OF CDKN2A DELETION

- The best and simple testing method for CDKN2A loss is FISH.
- Since this gene is included in Oncomine Precision Panel, it is important to report this genetic alteration accurately.
- Our lab has used a criteria of CNV ratio (or fold-change) <0.5 as regarded homozygous deletion of CDKN2A (adapted from Gutiontov, S.I., et al. CDKN2A loss-of-function predicts immunotherapy resistance in non-small cell lung cancer. Sci Rep 11, 20059 (2021). https://doi.org/10.1038/s41598-021-99524-1)
- Confirmation study by immunohistochemical study with MTAP antibody or FISH

SUMMARY

- Targeted DNA +RNA Panel NGS testing is the most cost effective and fastest overall TAT for NSCLC patient's management.
- However, there are changlingers when reporting CNV and working on RNA based panel.
- It is important to using other testing methods such as IHC, FISH, RT-PCR to verifying any borderline result, novel gene fusion, or gene expression imbalance only.

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MET EXON 14 Skipping

The exon 14 of the *MET* gene encodes for 47 amino acids, which is the key region responsible for the prevention of over-signaling of the MET receptor. Alterations in the intronic regions around exon 14, that is, intron 13 and intron 14, or within exon 14 itself or whole exon deletion of exon 14 result in disruption of the transcription process of the *MET* gene, resulting in unabated *MET* signaling and thus carcinogenesis. Several types of changes including missense alterations, deletions, splice site changes, and whole exon deletions may result in the skipping of exon 14 of the *MET* gene.