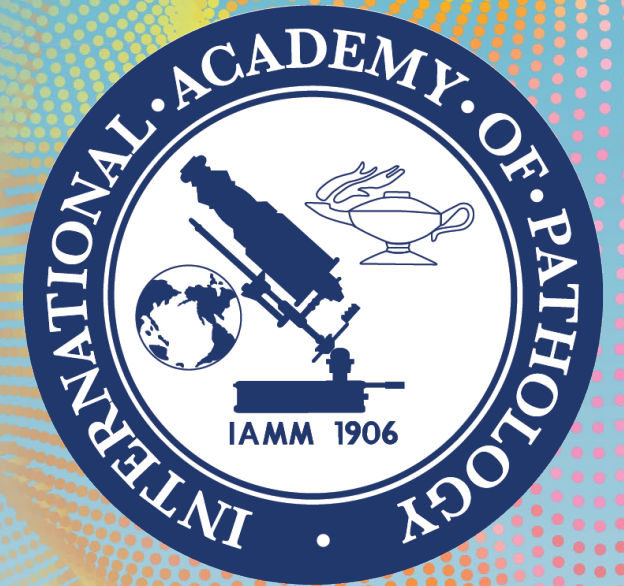


Changing landscape of NSCLC molecular testing in Australia

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 The 48th Annual Scientific Meeting *of the*


Australasian Division of the
International Academy of Pathology

Disclosure of Relevant Financial Relationships

Honoraria in past from AstraZeneca, Bayer, Illumina, Specialized Therapeutics, Servier, GSK, and Limbic for participations and presentations

Prior to Nov 2023

Category 6 - PATHOLOGY SERVICES

73337 

Group

P7 - Genetics

A test of tumour tissue from a patient with a new diagnosis of non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, requested by, or on behalf of, a specialist or consultant physician, if the test is:

- (a) to determine if requirements relating to epidermal growth factor receptor (EGFR) gene status for access to an immunotherapy listed under the Pharmaceutical Benefits Scheme (PBS) are fulfilled; and
- (b) not associated with a service to which item 73437 or 73438 applies

Fee: \$397.35 **Benefit:** 75% = \$298.05 85% = \$337.75

(See para [PN.7.15](#) of explanatory notes to this Category)

[← Previous - Item 73336](#)

[Next - Item 73338 →](#)

Prior to Nov 2023

- Limited reimbursement available which would hardly cover the testing cost
 - Most labs were performing single gene or limited NGS panel testing for DNA variants like *EGFR*, *KRAS*, *BRAF*
 - Testing for *MET* exon 14 skipping variant – non-existent
 - *ALK* and *ROS1* IHC was done separately on FFPE block (needed confirmatory FISH if IHC positive)
 - *RET*, *NTRK* and other fusions not routinely tested
- Sequential testing - tissue not utilized well and TAT was an issue

New MBS item (#73437) from Nov 2023

Category 6 - PATHOLOGY SERVICES

73437 ⓘ

Group

P7 - Genetics

A nucleic acid-based multi-gene panel test of tumour tissue from a patient with a new diagnosis of non-small cell lung cancer requested by, or on behalf of, a specialist or consultant physician, if the test is:

- (a) to detect variants in at least EGFR, BRAF, KRAS and MET exon 14 to determine access to specific therapies relevant to these variants listed on the Pharmaceutical Benefits Scheme (PBS); and
- (b) to detect the fusion status of at least ALK, ROS1, RET, NTRK1, NTRK2 and NTRK3; and
 - (i) to determine access to specific therapies relevant to these variants listed on the PBS; or
 - (ii) determine if the requirements relating to EGFR, ALK and ROS1 status for access immunotherapies listed on the PBS are fulfilled; and
- (c) not associated with a service to which item 73438, 73439, 73337, 73341, 73344, 73436 or 73351 applies

Fee: \$1,247.00 **Benefit:** 75% = \$935.25 85% = \$1,148.30

(See para [PN.7.15](#) of explanatory notes to this Category)

[← Previous - Item 73436](#)

[Next - Item 73438 →](#)

SEQUENTIAL TESTING: RECOMMENDED REFLEX TESTS FOR NSCLC (2024)					
No. of tests	Test	Item No.	Fee	Benefit %	Cost
Single FISH	ALK FISH	73341	\$400.00	85%	\$340.00
Single FISH	ROS1 FISH	73344	\$400.00	85%	\$340.00
Single DNA	EGFR exons 18-21	73337	\$397.35	85%	\$337.75
Single DNA	EGFR T790M Resistance	73351	\$397.35	85%	\$337.75
Single DNA/RNA	MET exon 14 skipping alterations	73436	\$397.35	85%	\$337.75
Multigene DNA/RNA	EGFR, KRAS, BRAF, MET-exon 14, BRAF, KRAS, RET, NTRK 1/2/3 fusion status (NGS for biomarkers under 73337, 73341, 73344, 73436)	73437	\$1,247.00	85%	\$1,148.30
Multigene DNA	EGFR, BRAF, KRAS, MET-exon 14	73438	\$682.35	85%	\$583.65
Multigene RNA	ALK, ROS1, RET, NTRK 1/2/3 fusions, in absence of BRAF/KRAS/MET abnormalities	73439	\$682.35	85%	\$583.65

Aligns to currently recommended STANDARD OF CARE

MOLECULAR ABNORMALITY

- ✦ **EGFR – Tier 1**
- ✦ **ALK – Tier 1**
- ✦ **KRAS – Tier 1**
- ✦ **ROS1 – Tier 1**
- ✦ **BRAF – Tier 1**
- ✦ **NTRK1/2/3 – Tier 1**
- ✦ **MET ex 14 skip- Tier1**
- ✦ **RET – Tier 1**
- ✦ **ERBB2 (HER-2)- Tier 2A**

INDICATION

- Adenocarcinoma
- Large Cell
- NSCLC Not Otherwise Specified (NOS):
Advanced or Metastatic

TEST

- Preferable NGS, combined RNA/DNA multigene sequencing, best sensitivity and specificity.
- Targeted real-time PCR assays are used in some settings, although it is unlikely to detect fusions and all SNV.
- Single gene approach in combination with IHC for ALK, ROS-1, and then confirmation by FISH is also acceptable

TISSUE TYPE

- FFPE Biopsy
- Pleural or pericardial effusion
- Cytology material (FNA, EBUS, TBNA, etc)

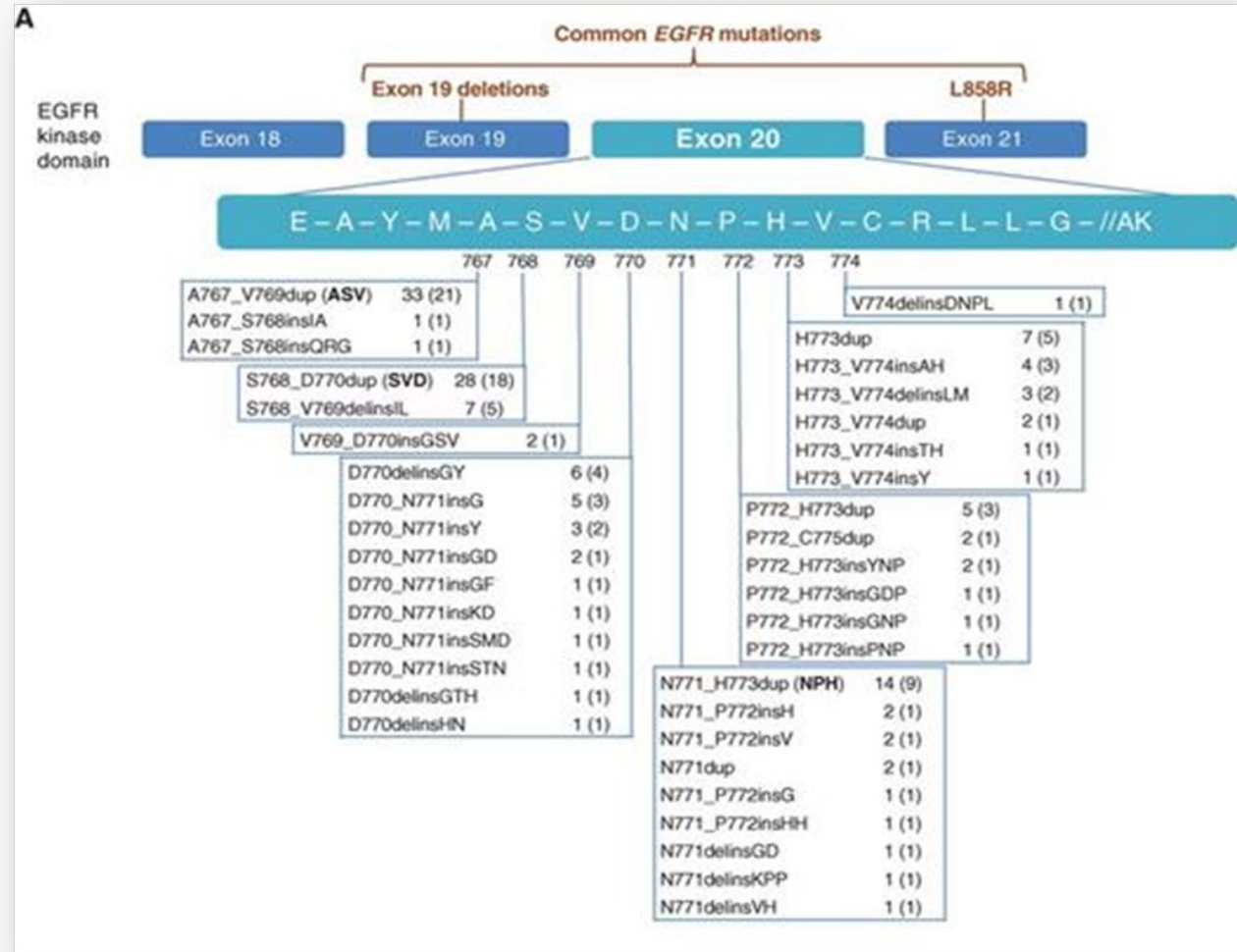
- Preferable for testing to be conducted as part of broad molecular profiling.
- NCCN NSCLC guideline panel strongly advises broader molecular profiling, able to identifying rare driver mutations for which effective drugs may already be available
- Tiered approaches based on low prevalence of co-occurring biomarkers are acceptable

Modified from : Benhur E. PCPA workshop, Melb, Feb 2024

Paradigm shift for NSCLC molecular testing (and treatment)

- Many genomic labs across Australia now perform NGS panels for NSCLC
- Both DNA variants and fusions can now be performed simultaneously, in one test
- Judicious use of tissue
- Rapid turnaround times possible (within 5 days)
- Better alignment with Anatomical Pathology results

EGFR exon 20 variants – heterogenous group of insertions/indels



Cancer Discov. 2021 Jul;11(7):1672-1687

Testing of co-occurring mutations is important in NSCLC (a study on 133 *EGFR* mutant cases)



[Cancers \(Basel\)](#). 2019 Mar; 11(3): 341.

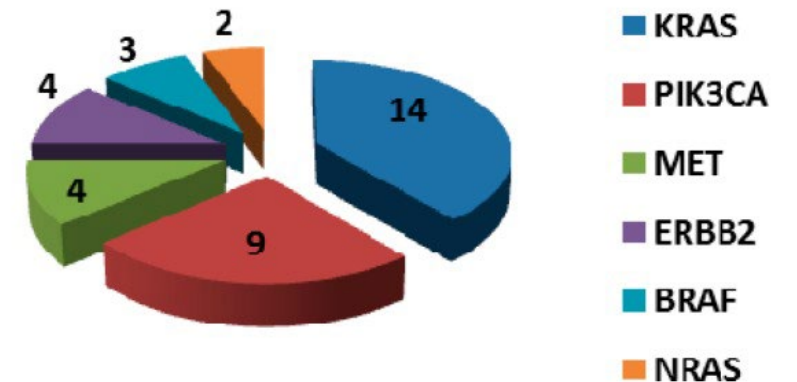
Published online 2019 Mar 10. doi: [10.3390/cancers11030341](https://doi.org/10.3390/cancers11030341)

PMCID: PMC6468673

PMID: [30857358](https://pubmed.ncbi.nlm.nih.gov/30857358/)

The Presence of Concomitant Mutations Affects the Activity of EGFR Tyrosine Kinase Inhibitors in EGFR-Mutant Non-Small Cell Lung Cancer (NSCLC) Patients

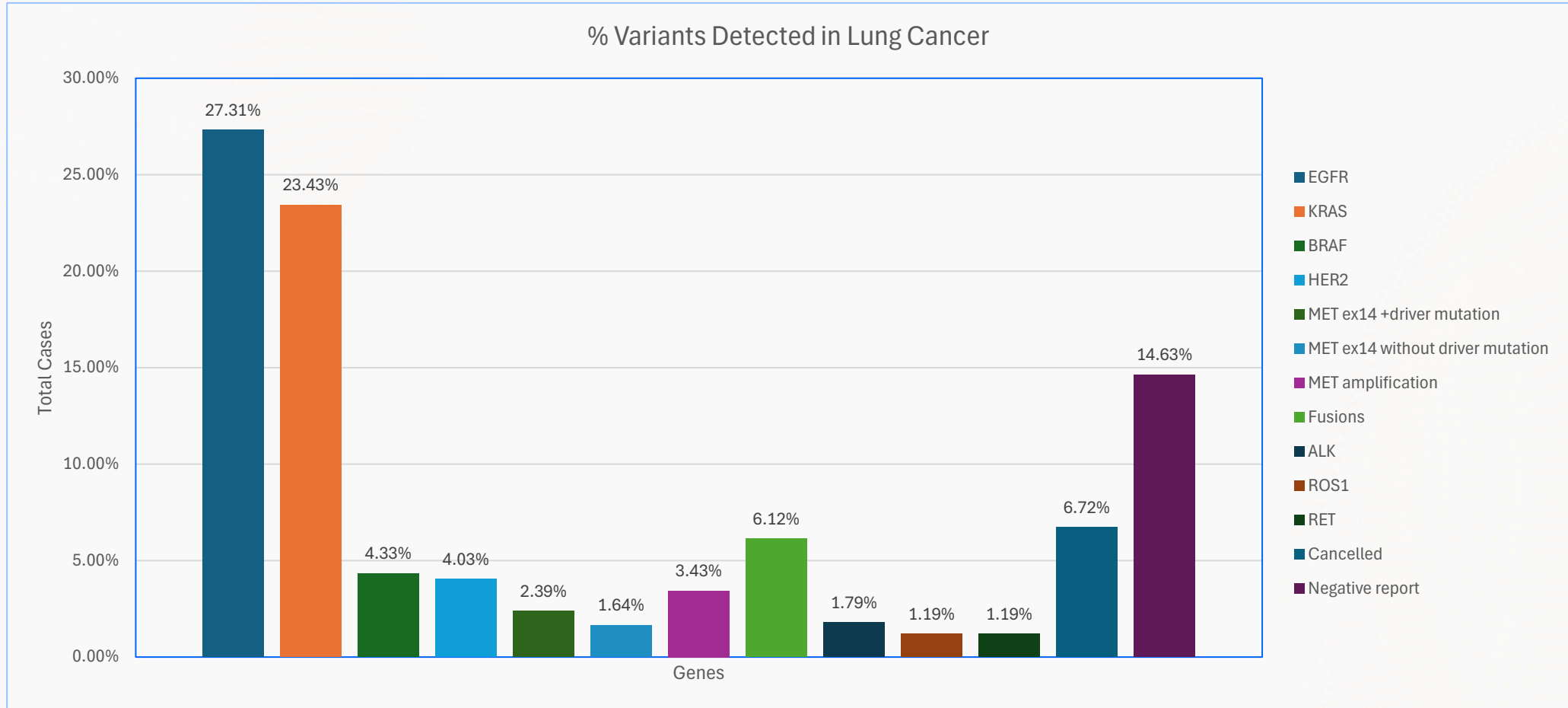
[Anna Maria Rachiglio](#)^{1,†}, [Francesca Fenizia](#)^{1,†}, [Maria Carmela Piccirillo](#)^{2,†}, [Domenico Galetta](#)³, [Lucio Crinò](#)⁴, [Bruno Vincenzi](#)⁵, [Emiddio Barletta](#)⁶, [Carmine Pinto](#)⁷, [Francesco Ferrau](#)⁸, [Matilde Lambiase](#)¹, [Agnese Montanino](#)⁹, [Cristin Roma](#)¹, [Vienna Ludovini](#)¹⁰, [Elisabetta Sara Montagna](#)³, [Antonella De Luca](#)¹, [Gaetano Rocco](#)^{11,‡}, [Gerardo Botti](#)¹², [Francesco Perrone](#)², [Alessandro Morabito](#)⁹ and [Nicola Normanno](#)^{1,*}



There is a lot more to *EGFR* TKI resistance than just *EGFR* T790/C797S

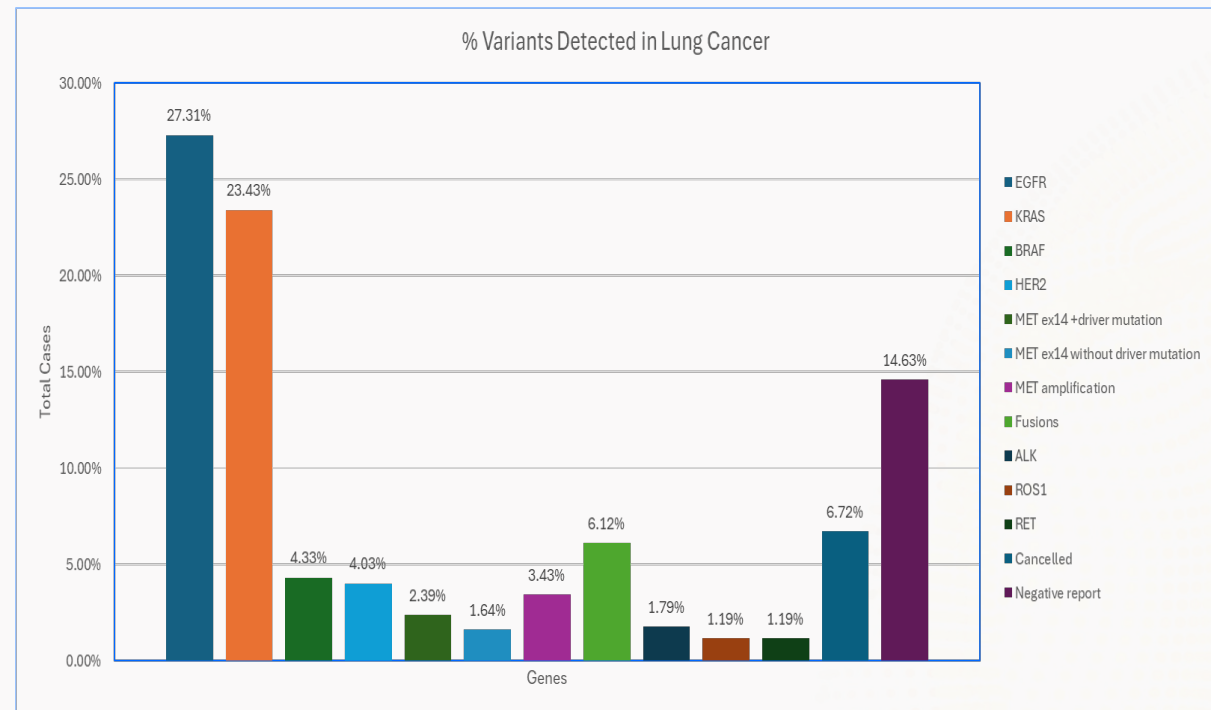
- Analysis of genomic DNA by Next Generation Sequencing (NGS) revealed the presence of hotspot mutations in genes other than the *EGFR*, including *KRAS*, *NRAS*, *BRAF*, *ERBB2*, *PIK3CA*, or *MET*, in 29/133 cases (21.8%)
- A p.T790M mutation was found in 9/133 tumour samples (6.8%).
- The progression free survival (PFS) of patients without other mutations was 11.3 months vs. 7 months in patients with other mutations
- In addition, patients with higher VAF of concomitant mutations showed further decrease in PFS

Last 700 NSCLC cases tested at LSGA



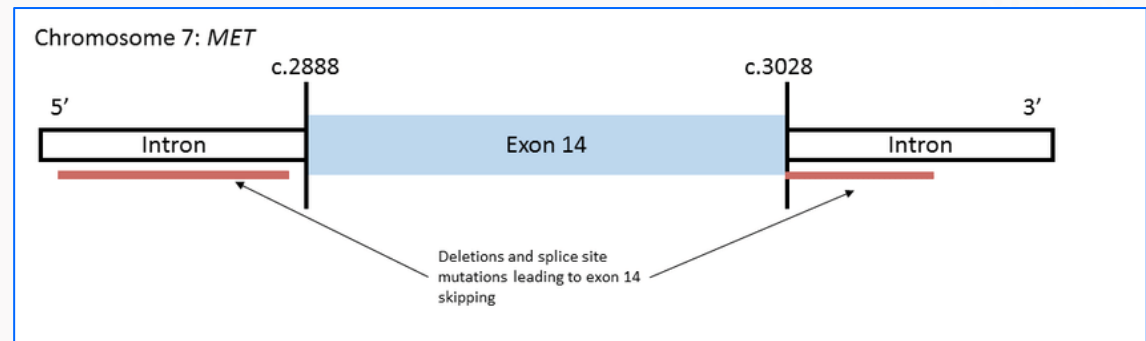
MET exon 14 skipping and *MET* amplification

- Tepotinib available for these targets
- LSGA data for last 700 cases - total of 7.5% cases showed *MET* exon 14 skipping variants (4%) or *MET* amplification (>10 copies of *MET*)
- This is more than the combined case numbers for *ALK*, *ROS1* and *RET* fusion positive cases (6.12%)
- Small number of SCC showed *MET* ex14 skipping as well



All *MET* exon 14 skipping variants cannot be detected by DNA only NGS panel

- Only 16 out of total 27 *MET* exon 14 skipping variants were picked up by the NGS DNA component
- All 27 cases showed splice variants on the RNA component of the assay
- 11 cases were detected only by the RNA component and missed by the DNA component
- DNA panels cannot detect all *MET* exon 14 variants

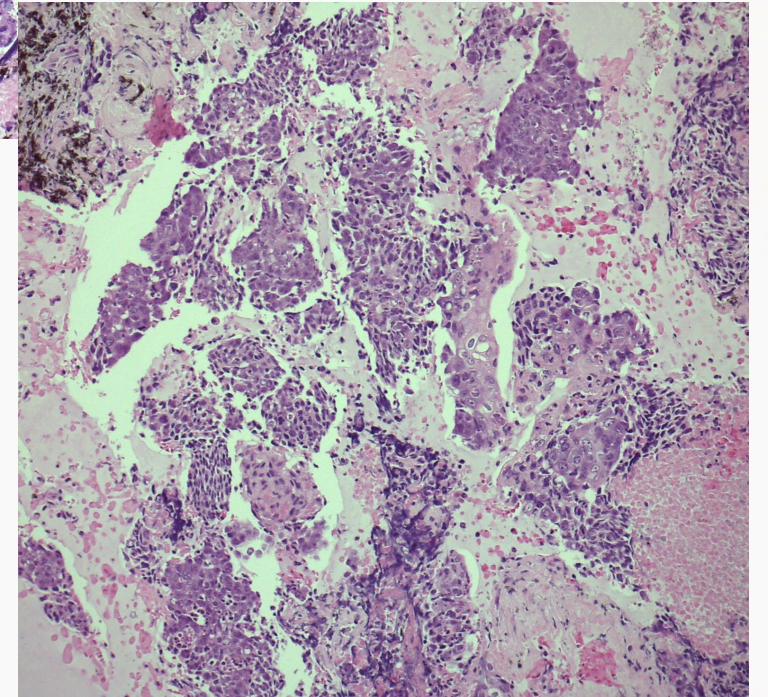
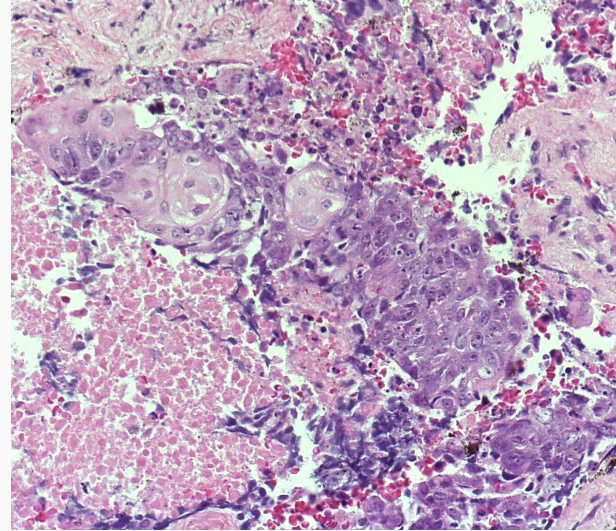


Clinically relevant fusions (apart from *ALK/ROS1/RET/NTRK*)

- The spectrum of clinically relevant fusions in NSCLC being detected is increasing
- Further, more novel fusion partners to *ALK, ROS1, RET, NTRK* are being detected (limitations with IHC, FISH, PCR tests)
- Following fusions detected at LSGA (apart from *ALK, ROS1, RET, NTRK*) –
 - *FGFR3::TACC3*
 - *ATP1B1::NRG1*
 - *CD74::NRG1*
 - *SLC3A2::NRG1*
 - *FGFR2::TACC2*
 - *BRD4::NUTM1*

Case Example

- 68-year-old female, non –smoker, suspicious lung mass
- EBUS performed
- TBNA, Station 7 LN
- Histology = NSCLC favouring poorly diff. SCC
 - Pan cytokeratin = Diffuse +ve
 - P40 = Diffuse +ve
 - TTF-1 = +ve in majority but not all tumour cells
 - Synaptophysin/chromogranin = -ve
 - Ki-67 = 60-70%



Molecular studies

- NGS performed using a multigene DNA+Fusion panel
- *BRD4::NUTM1* fusion detected
- Wild type for all other variants
 - No *EGFR*, *KRAS*, *BRAF*, *HER2*, *MET* ex 14
 - No *MET* amplification
 - No fusions in *ALK*, *ROS1*, *RET*, *NTRK1/2/3*

NUT carcinoma

- Extremely aggressive malignancy of thoracic/mediastinum
- Characteristic translocation *BRD4::NUTM1* (75% cases)
- *NUTM1* fused with *BRD3*, *NSD3*, *ZNF532*, *ZNF592* in rest
- NUT IHC available but not widely used
- Characteristic poorly diff. morphology as above (SCC like, mixed with small cell or undifferentiated)
- IHC - varied staining for p63/p40 and TTF-1, chromo, synapto

Thank you

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