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Interpretation Of c*MET* Amplification, EXON 14 Skipping Mutation In NSCLC FISH, IHC, NGS, qPCR

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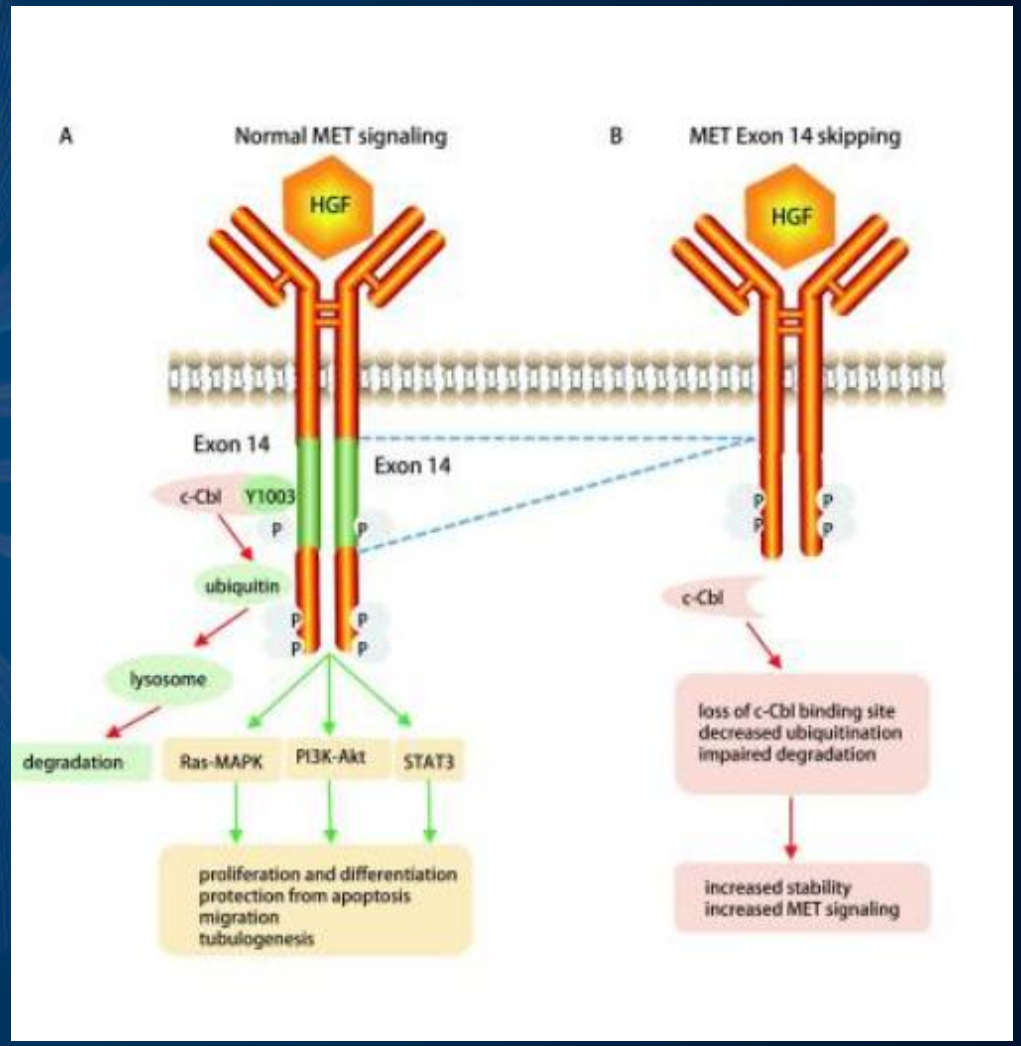
Backed by illumina

MET alterations in cancer

Major types

- *MET* exon 14 skipping mutation – 5%, MET overexpression
- *MET* amplification (1-5% denovo), MET overexpression
- *MET* fusion

MET overexpression as a mechanism of resistance to EGFR TKI can be monitored, however, 64% of them were only expression, while 5-22% of them had MET amplification



Experimental Hematology & Oncology **volume 10**,

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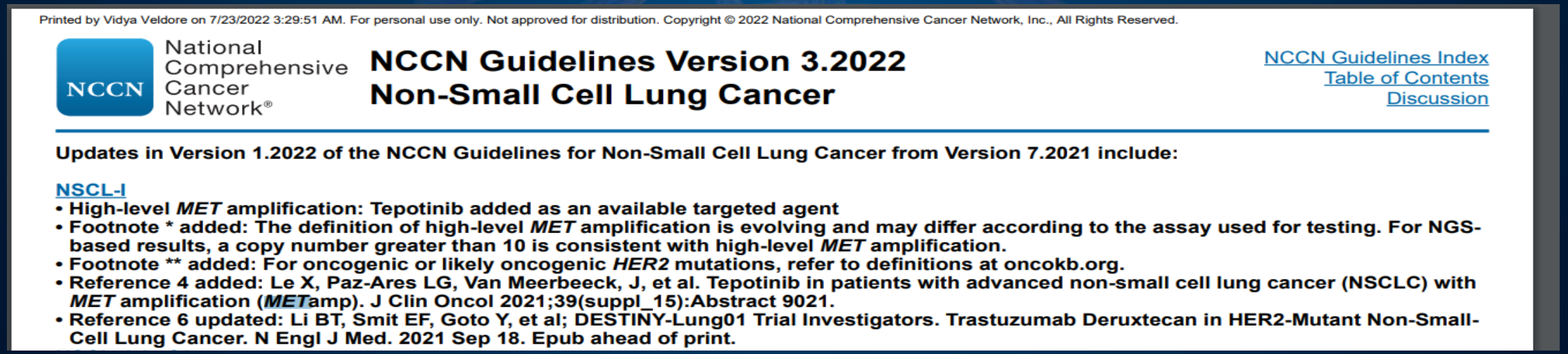
Detection of MET amplification at the DNA level

MET amplification is **a potential resistance pattern of EGFR-TKIs in NSCLC, accounting for 50–60% of the first- and second-generation EGFR-TKIs acquired resistance**
Noro R, Seike M, Zou F, et al. MET FISH-positive status predicts short progression-free survival and overall survival after gefitinib treatment in lung adenocarcinoma with EGFR mutation. BMC Cancer. 2015;

Positivity for mean MET/cell was defined as: low: ≥5 to <6 copies, intermediate: ≥ 6 to <7 copies, or high: ≥7 copies. Ratio MET/CEP7 positivity was defined as: **low: ≥1.8 to ≤2.2, intermediate: >2.2 to < 5, or high: ≥5**. J Thorac Oncol. 2016 Aug; 11(8): 1293–1304.



NCCN National Comprehensive Cancer Network®

NCCN Guidelines Version 3.2022
Non-Small Cell Lung Cancer

NCCN Guidelines Index
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Discussion

Updates in Version 1.2022 of the NCCN Guidelines for Non-Small Cell Lung Cancer from Version 7.2021 include:

NSCL-I

- **High-level *MET* amplification: Tepotinib added as an available targeted agent**
- **Footnote * added: The definition of high-level *MET* amplification is evolving and may differ according to the assay used for testing. For NGS-based results, a copy number greater than 10 is consistent with high-level *MET* amplification.**
- **Footnote ** added: For oncogenic or likely oncogenic *HER2* mutations, refer to definitions at oncokb.org.**
- **Reference 4 added: Le X, Paz-Ares LG, Van Meerbeeck, J, et al. Tepotinib in patients with advanced non-small cell lung cancer (NSCLC) with *MET* amplification (*MET*amp). J Clin Oncol 2021;39(suppl_15):Abstract 9021.**
- **Reference 6 updated: Li BT, Smit EF, Goto Y, et al; DESTINY-Lung01 Trial Investigators. Trastuzumab Deruxtecan in HER2-Mutant Non-Small-Cell Lung Cancer. N Engl J Med. 2021 Sep 18. Epub ahead of print.**

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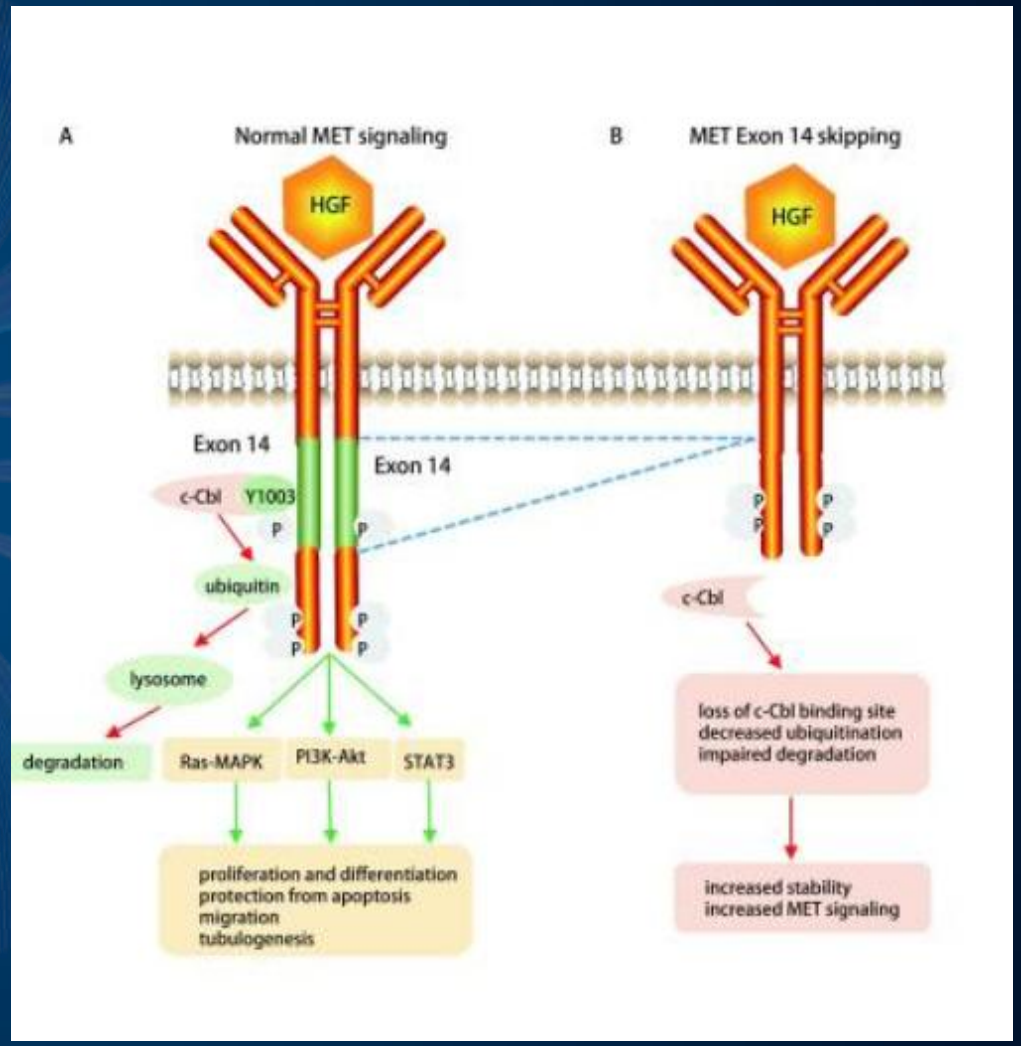
MET Exon 14 Skipping- Structure and Biology

- MET proto-oncogene is a receptor tyrosine kinase, which activates MAPK, PI3K/AKT, SRC, and STAT pathways to promote cell proliferation, invasion, and angiogenesis

- Exon 14 encodes a juxtamembrane domain loss of this region results in increased oncogenic signaling**.**

- Exon 14 is skipping results in impairment of CBL-mediated MET protein degradation

- MET exon 14 skipping mutation (METΔex14) is **present about 3% of non-small cell lung cancers (NSCLCs)**. NSCLC patients with METΔex14 are characterized by an average age of over 70 years at diagnosis, a smoking history and a higher frequency in pleomorphic carcinoma and adenosquamous cell carcinoma than in adenocarcinoma



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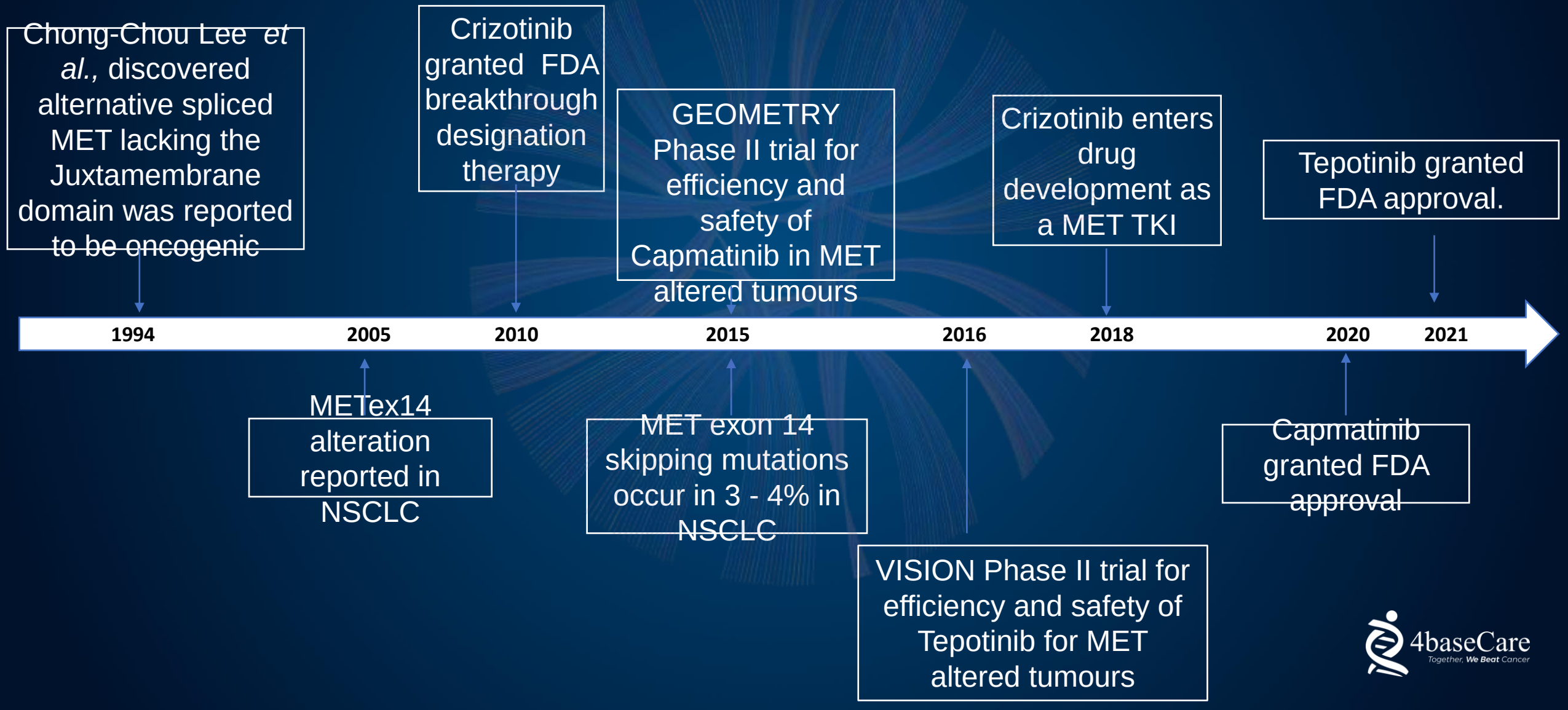
Discovery of Exon 14 Skipping:



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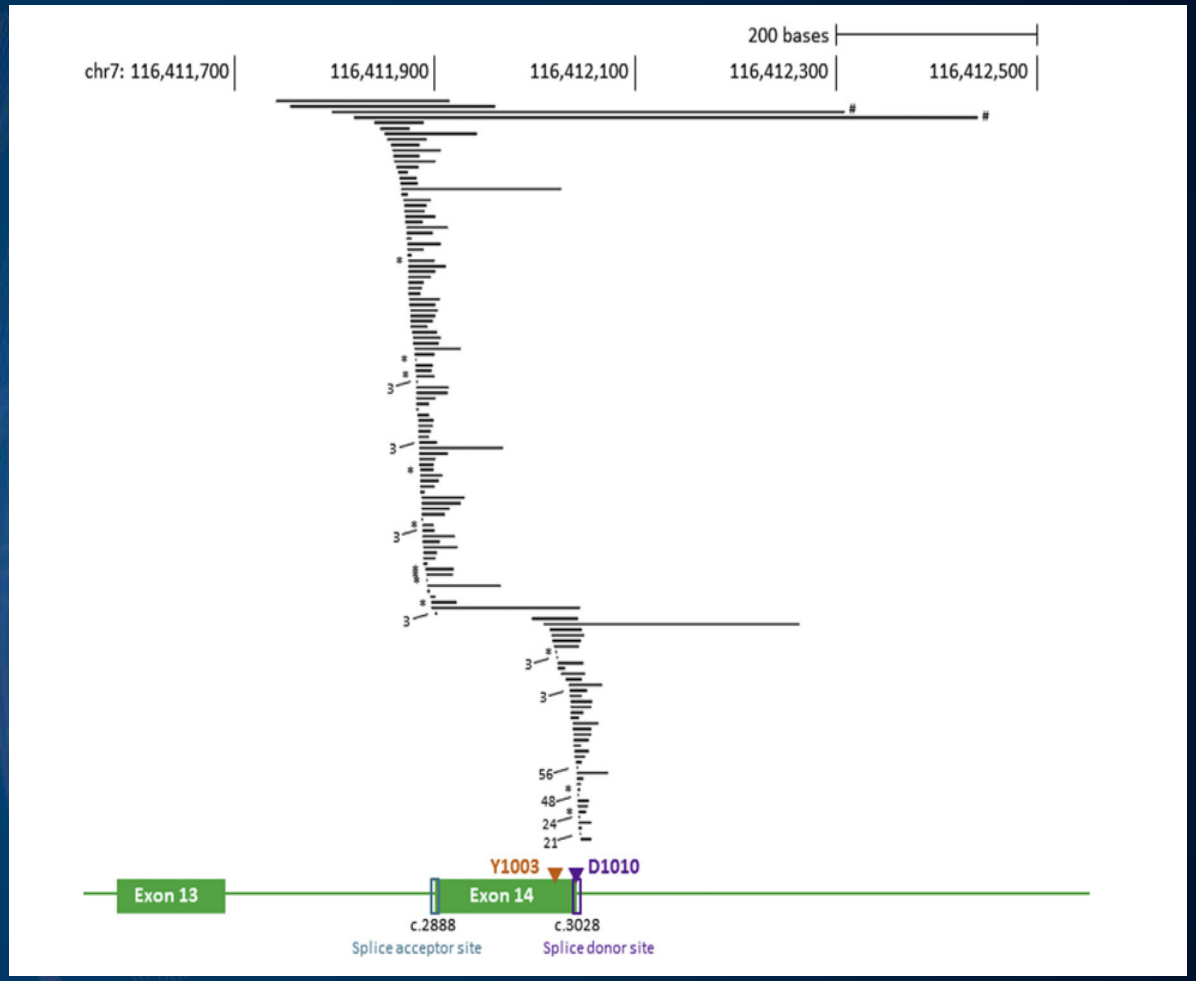
MET Exon 14 Skipping Mutations: Reasons to remember for the assay design

In a study conducted by Schrock AB et al., METex14 alterations was analyzed by hybrid capture-based approach in 298 cases where 165 different variants were predicted to affect MET exon 14.

These genomic alterations were manually inspected to identify those that affected the MET exon 14 splicing or delete the exon completely as described by Garrett M Frampton et al., where FoundationOne DNA assay was used.

Patient Data	Values					
	All	AdenoCA	NOS	SqCC	AdenoSqCC	Sarcomatoid
Total Cases	11,205	7140	1659	1206	98	104
METex14 alterations, n (%)[b]						
Base substitution splice donor	149 (49.1)	103 (50)	24 (49)	15 (60)	3 (38)	3 (38)
Indel splice acceptor	100 (32.9)	71 (35)	18 (37)	5 (20)	4 (50)	1 (12)
Indel splice donor	42 (13.8)	28 (14)	4 (8)	4 (16)	1 (12)	4 (50)
Base substitution splice acceptor	4 (1.3)	3 (1)	0	1 (4)	0	0
Base substitution noncoding adjacent splice acceptor	4 (1.3)	2 (1)	2 (4)	0	0	0
Indel noncoding adjacent splice acceptor	3 (1.0)	2 (1)	1 (2)	0	0	0
Whole exon 14 deletion	2 (0.7)	1 (0.5)	1 (2)	0	0	0



Schematic of all the genomic positions of (MET) exon 14 skipping alterations (165 variants detected)

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Is IHC validated for Exon14 skipping mutation?

NO

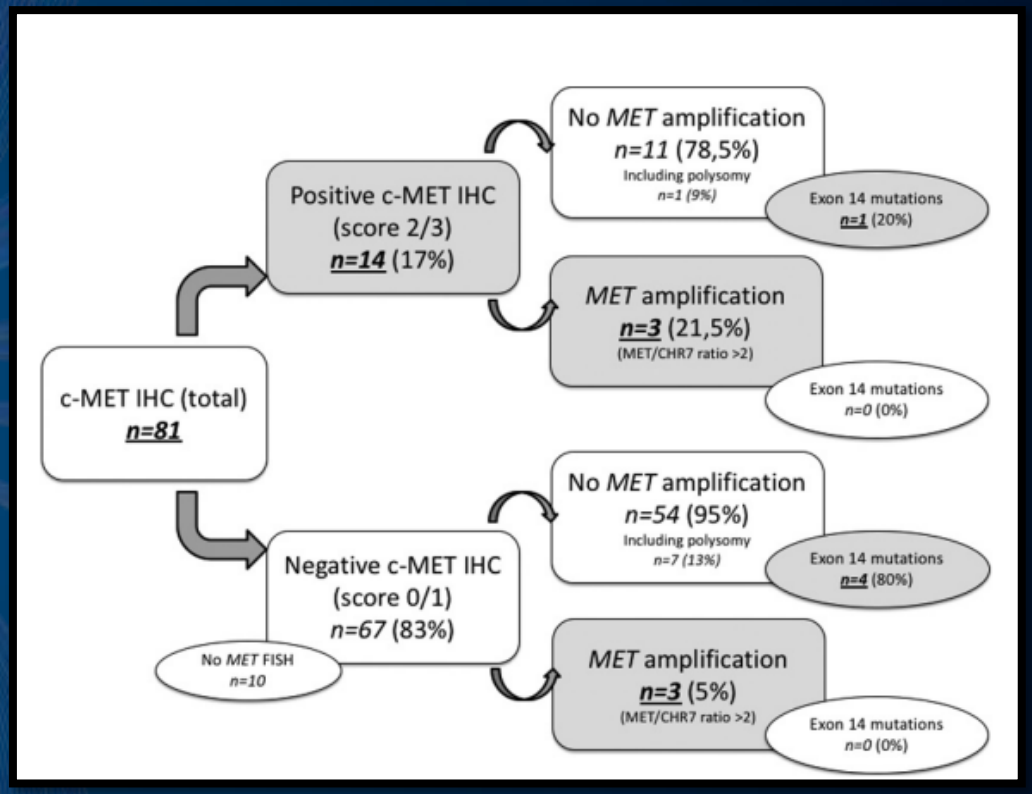
IHC –not an ideal assay for METex14 skipping:

- IHC is only able to detect MET overexpression, which may occur due to not only METex14 alterations but also increased gene copy number and gene amplification

- Several studies have shown MET IHC overexpression poorly predicts for the presence of METex14 alterations.

- As depicted in the figure MET amplification or MET exon 14 mutations has sensitivity (50% and 20%, respectively) and correlation (r ¼ 0.27) proved poor.

- In a study conducted by Robin Guo *et al.,* 2019 nearly all MET IHC–positive cases were negative for MET amplification or METex14 mutations



Xavier Mignard et al., 2018

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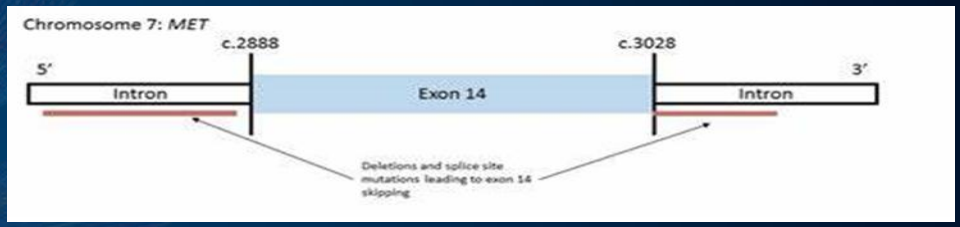
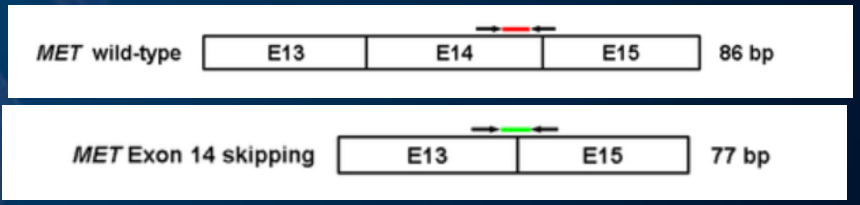
What is the most effective approach for detection of Exon14 skipping ?
- qPCR (RNA)

- Principle-In this method, RNA is first transcribed into complementary DNA (cDNA) by reverse transcriptase from total RNA or messenger RNA (mRNA). The cDNA is then used as the template for the qPCR reaction.



- Considering sensitivity(>90%) and specificity, qPCR may be the most reasonable single gene testing for detecting METex14



Disadvantages-

- The quality of nucleic acids is often seriously deteriorated after the routine FFPE tissue processing; hence, there is a significant chance of failure in RNA-based detection of MET exon 14 skipping mutations .
- This target-specificity of this is a disadvantage in the case of MET skipping, which can be originated by a variety of mutations on genomic DNA

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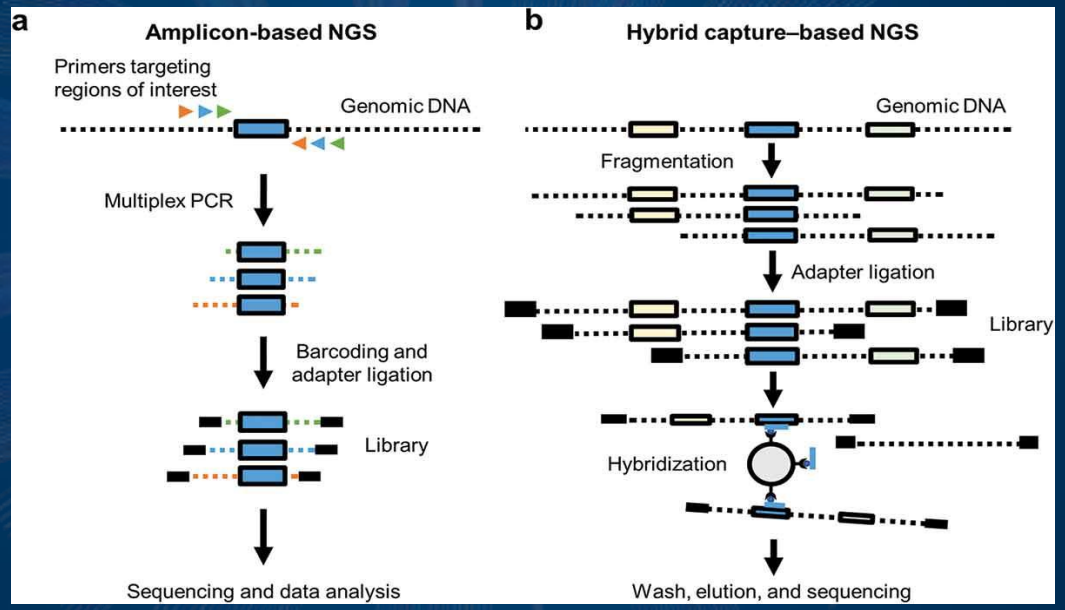
Detection of Exon 14skipping mutations at the DNA level:

Amplicon based Approach

- This methods utilize PCR primers that are designed to amplify targeted regions of interest in the genome
- Diversity in alterations leading leading to MET exon 14 skipping can lead to allele dropout and provide false negatives.
- Poor sequencing quality at the ends of amplicons may lead to miscalling of variant



Hybrid capture based approach

- These platforms are constructed using long biotinylated oligonucleotides
- The hybridization probes more tolerant to the presence of mismatches in the binding site, hence no allele dropout

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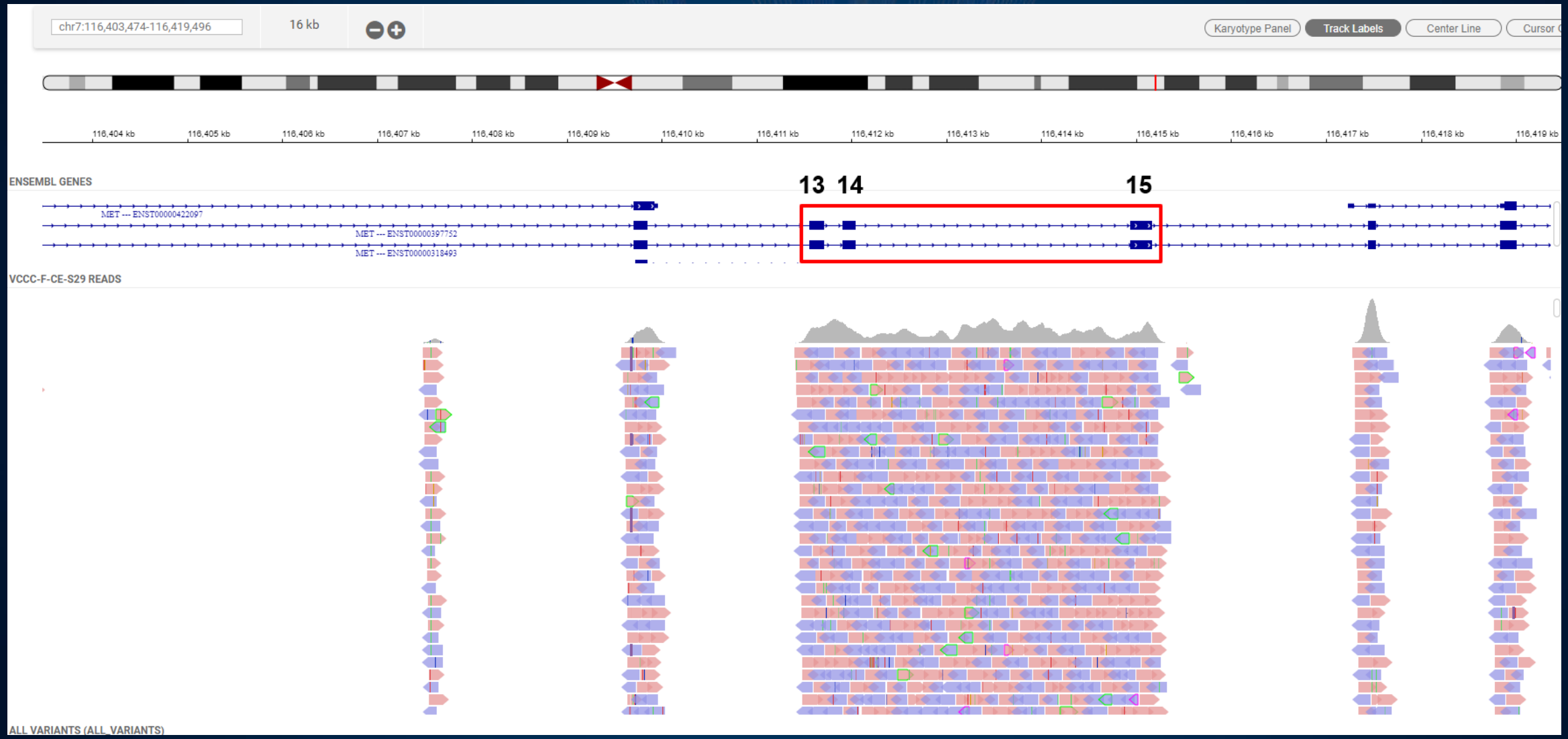
DNA VS RNA Sequencing:

NGS	RNA BASED	DNA BASED
Sensitivity	100% in mRNA based RT PCR	Technology-wise 100% with tumor content greater than 20%.
Detection principle	Fusion of ex 13 and 15 transcripts	Alterations around splice donor and acceptor sites of ex 14, as well as intronic regions
Limitations	Predefined assay, only documented mutations could be detected if it is an amplicon based PCR	Often it is a hybrid capture based approach, there is adequate coverage all through the scope of Exon14 skipping event
Limitations	Difficulty in obtaining high quality RNA as it is prone to degradation, for any technology	Panel design needs to be carefully checked, before recommendation
	Chances of false positivity if RNA analysis data is not carefully filtered based on Quality criteria	If the tumor content is less, then the chances of detecting would be compromised: >20% is the recommendation (more than 150 viable tumor cells/hpf)

DNA Sequence of MET ex 14 with flanking introns (13 & 15): √ Hybrid capture based approach (TARGT IndieGene Solid and Liquid Biopsy Panel)



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Different NGS assays for detection of Exon14 skipping:

- First assay by **Blidner** et al : for chimeric RNAs from gene fusions and exon skipping events in NSCLC

- **Oncomine Focus Assay**(OFA):combination of DNA and RNA sequencing.
  -DNA coverage detects mutations in the splice donor site (3' end) but not the splice acceptor site (5' end)

- **FoundationOne CDx** : FDA approved to identify mutations that lead to MET exon 14 skipping in advanced non-small cell lung cancer (NSCLC) (DNA based)

- **SNaPshot**: the SNaPshot-NGS panel has provided targeted next-generation DNA sequencing of specific exons in MET; this allowed us to identify MET exon 14 skipping events that result from specific SNVs.
  - this method does not identify all the genomic events that can lead to exon 14 skipping

- **TARGT IndieGene Solid and Liquid Biopsy** panel has been tailored to identify MET exon 14 skipping events although the exons and introns, thus allowing broad capture of this event regardless of the specific genomic change that produced

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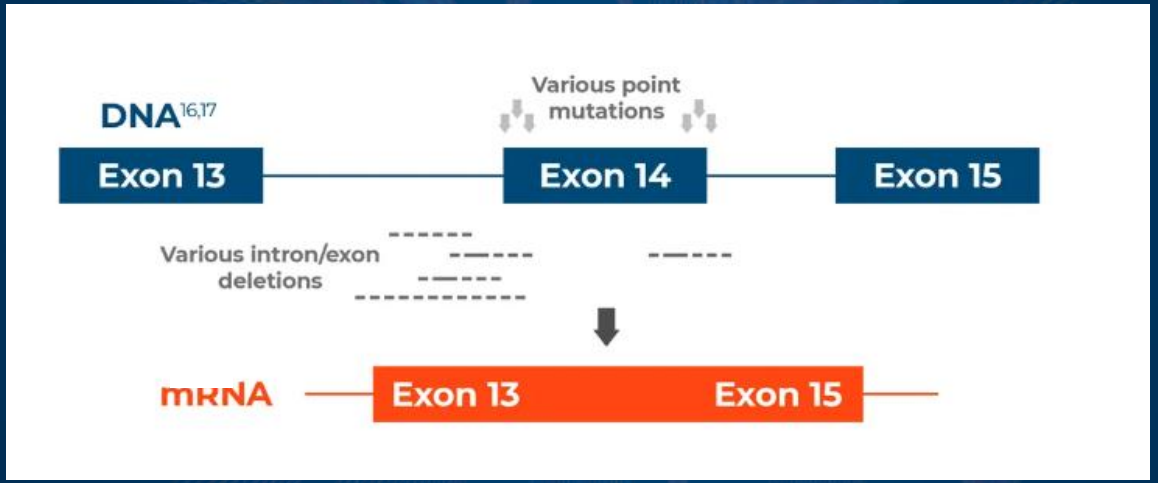
Detection of Exon 14 skipping mutations at the RNA level:

- Studies have found that RNA-based methods detect *MET*ex14 events at a higher rate than DNA-based approaches
- RNA based sequencing detects the product of exon 14 skipping mutations which is “fusion” of exon 13 to 15



MET **(mesenchymal-epithelial transition) *exon 14 (METex14)* skipping variants: MET is a receptor tyrosine kinase. A mutation that results in loss of *exon 14* can occur in NSCLC. Loss of *METex14* leads to dysregulation and inappropriate signaling.**

- **The presence of *METex14* skipping mutation is associated with responsiveness to oral MET TKIs.**
- **A broad range of molecular alterations lead to *METex14* skipping.**
- **Testing Methodologies: NGS-based testing is the primary method for detection of *METex14* skipping events; RNA-based NGS may have improved detection. IHC is not a method for detection of *METex14* skipping.**

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Journal of Thoracic Oncology

IASLC INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER

TUMOUR BIOLOGY AND PATHOLOGY | VOLUME 16, ISSUE 4, SUPPLEMENT , S701, APRIL 01, 2021

5P False positive errors in RNA based next generation sequencing of exon 14 skipping mutations in NSCLC

M. Suryavanshi • S. Mattoo • U. Batra • S. Sharma • D. Kumar • A. Mehta

Open Archive • DOI: https://doi.org/10.1016/S1556-0864(21)01847-5

Article Info

Related Articles

Background: Exon 14 skipping mutations are found in approximately 3% of patients with NSCLC. Robust approaches for detection of MET exon 14 skipping events are crucial for treatment. About one-third of the mutations occur between exons 13 and 14 at acceptor site of exon 14, and two-thirds occur between exons 14 and 15 at donor site. In addition, MET exon 14 skipping can result from large deletions that can span not only all of exon 14 but large portions of the intronic sequence. This mutation can be detected by sequencing of DNA or RNA or both. DNA based approaches alone are not able to detect more than 60% of these mutations. RNA based sequencing detects the product of exon 14 skipping mutations which is "fusion" of exon 13 to 15 regardless of the underlying genomic event. Most studies have favoured a RNA based approach.

Methods: During the period from August 2017 to January 2021, a total of 231 samples of NSCL were assessed by routine clinical application of the Thermofischer Ion Torrent™ Oncomine™ Focus 52 gene Assay. Sequencing data were processed with the Ion Torrent Suite software. This assay detects MET mutations at 3′ end splice donor site by DNA sequencing and RNA sequencing for exon 13 and exon 15 fusion for exon 14 skipping mutation. All positive cases on RNA sequencing were reanalysed by PCR and Sanger sequencing for confirmation.

Results: Exon 13 and exon 15 fusion by RNA was detected in 20 cases. Read counts ranged from 143 to 7980. Two cases had additional MET amplification, one case had EGFR deletion 19,one case had CTNNB1 p. Ser37Phe and KRAS p.Gly12Asp, one case had RET KIF5B Fusion (read count 458), one case had EGFR amplification and in remaining cases exon 13 and exon 15 fusion was the sole abnormality. Only 6 out of 20 cases detected by NGS were confirmed by Sanger sequencing. All cases above the read count of 1607 were detected by sanger sequencing. All true positive cases had exon 3 and exon 15 fusion as the sole abnormality. Cases with MET amplification were also negative on sanger sequencing.

Conclusions: RNA based exon 13 and exon 15 fusion for detection of exon 14 skipping mutations can have false positive calls by Ion torrent-based sequencing and should be confirmed by alternate methods.

Legal entity responsible for the study: The authors.

Funding: Rajiv Gandhi Cancer Institute and Research Center, Delhi, India.

Capmatinib in *MET* Exon 14–Mutated or *MET*-Amplified Non–Small-Cell Lung Cancer

Jürgen Wolf, M.D., Takashi Seto, M.D., Ji-Youn Han, M.D., Ph.D., Noemi Reguart, M.D., Ph.D., Edward B. Garon, M.D., Harry J.M. Groen, M.D., Ph.D., Daniel S.W. Tan, M.D., Ph.D., Toyoaki Hida, M.D., Ph.D., Maja de Jonge, M.D., Ph.D., Sergey V. Orlov, M.D., Egbert F. Smit, M.D., Ph.D., Pierre-Jean Souquet, M.D., et al., for the GEOMETRY mono-1 Investigators*

September 3, 2020
N Engl J Med 2020; 383:944-957
DOI: 10.1056/NEJMoa2002787

48 References

Abstract

BACKGROUND

Among patients with non–small-cell lung cancer (NSCLC), *MET* exon 14 skipping mutations occur in 3 to 4% and *MET* amplifications occur in 1 to 6%. Capmatinib, a selective inhibitor of the MET receptor, has shown activity in cancer models with various types of MET activation.

CONCLUSIONS

Capmatinib showed substantial antitumor activity in patients with advanced NSCLC with a MET exon 14 skipping mutation, particularly in those not treated previously. The efficacy in MET-amplified advanced NSCLC was higher in tumors with a high gene copy number than in those with a low gene copy number. Low-grade peripheral edema and nausea were the main toxic effects. (Funded by Novartis Pharmaceuticals; GEOMETRY mono-1 ClinicalTrials.gov number, NCT02414139.)

Related Articles

ORIGINAL ARTICLE SEP 3, 2020

Tepotinib in Non–Small-Cell Lung Cancer v 14 Skipping Mutations

P.K. Paik and Others

Take home message: When selecting the most appropriate assay for broad molecular testing, carefully considering each assay's limitations is important

- Detection of METex14 in patients with NSCLC can be challenging due to the complex alterations leading to the loss of exon 14
- Both RNA-based and DNA-based approaches have been used to detect *MET*ex14 in NSCLC, there is Indian data evidence that, RNA based approaches have false positive rate of 5%.
- Many amplicon-based NGS panels are not properly optimized to detect METex14 (detection rate 0.3%), due to inadequate coverage and technical limitations.
- Wide range of *MET*ex14 events occurring within and surrounding exon 14 can be detected using a DNA-based hybrid capture NGS approach
- The use of broad molecular testing in patients with NSCLC is crucial for matching their tumors with the appropriate targeted therapy with regard to MET gene alterations

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Take Home Message

One needs to take a careful call for each case, based on factors: Tissue availability, FFPE RNA quality, Tumor Cell percentage, Availability of good technology – the choice of test and technology might change for one patient to another – Guidelines or Recommendations are there, however for each patient, the physician would take a call based on multiple factors as mentioned above