Detection of *MET* Exon 14 skipping mutation by MALDI-TOF based liquid biopsy in Non-Small Cell Lung Cancer (NSCLC)

UKE HAMBURG

EUROPEAN LIQUID BIOPSY SOCIETY

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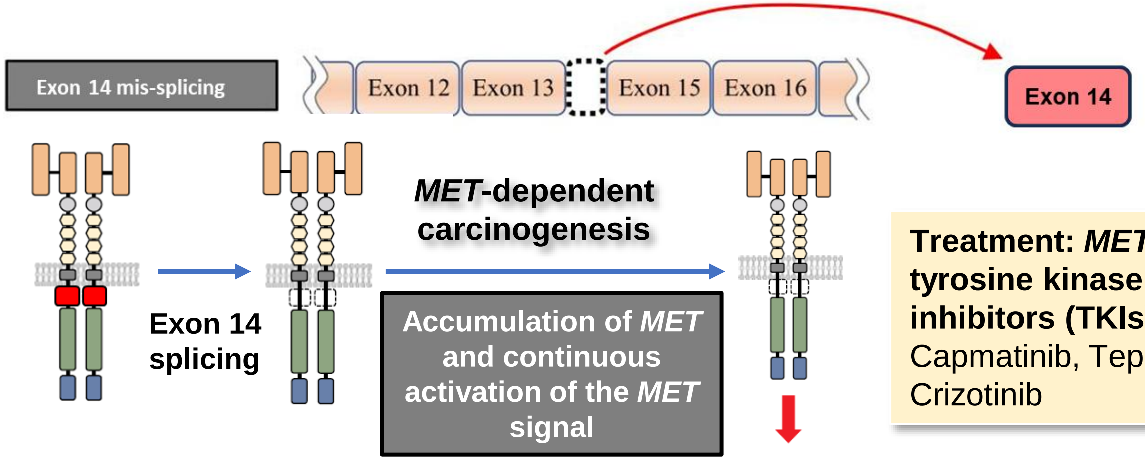
Background

***MET* is required for multiple cellular functions**
Organogenesis, Wound healing, Cell motility, Proliferation, Embryogenesis

How *MET* Exon 14 skipping (METex14) promotes carcinogenesis?



Accumulation of *MET* and continuous activation of the *MET* signal

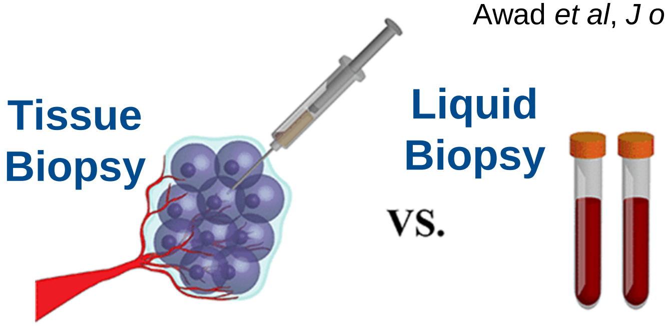
Treatment: *MET*-tyrosine kinase inhibitors (TKIs)
Capmatinib, Tepotinib, Crizotinib

Non-small cell lung cancer patients with comprehensive molecular genotyping (*EGFR*, *ALK*, *BRAF*, *ROS1*, *MET*, *RET*, and *NTRK*) have superior OS compared to those with incomplete or no testing (24.6 mos vs. 6.2 mos, p < 0.0001)
Aggarwal *et al*, *JCO Precis Oncol.* 2023

High rate of failure in NGS based comprehensive molecular genotyping due to insufficient quantity or quality of tissue DNA

Patient number	Failure rate	Reference
79/207	38%	Aggarwal *et al*, *JAMA Oncol*. 2019
52/102	51%	Thompson *et al*, *Clin Cancer Res*. 2016
21/174	12%	Karlovich *et al*, *Clin Cancer Res*. 2016
601/2601	23%	Meric-Bernstam *et al*, *JCO*. 2015
735/1739	42%	Hellmann *et al*, *NEJM*. 2018
231/282	82%	Leighl *et al*, *Clin Cancer Res*. 2019

Awad *et al*, *J of Clin. Onco*. 2016



Tissue Biopsy	Liquid Biopsy
Time-Intensive Procedure	Quick
Localized Sampling of Tissue	Comprehensive Tissue Profile
Not Easily Obtained	Easily Obtained
Some Pain/Risk	Minimal Pain/Risk
Invasive	Minimally Invasive

Liquid biopsy as a better alterative to tissue biopsy for METex14 RNA detection in NSCLC patients

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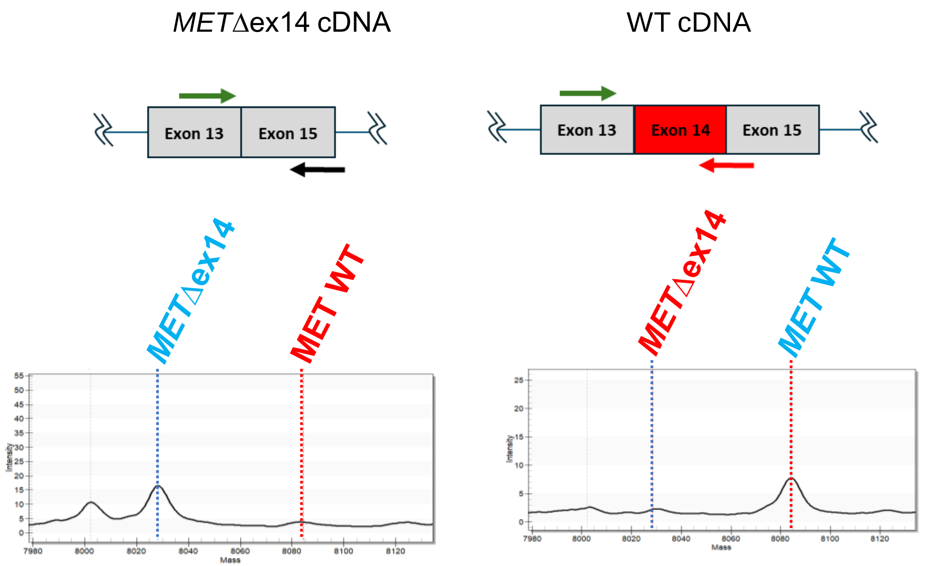
Material and Methods: Using MALDI-TOF and ddPCR to detect METex14 RNA

MALDI-TOF mass spectrometry

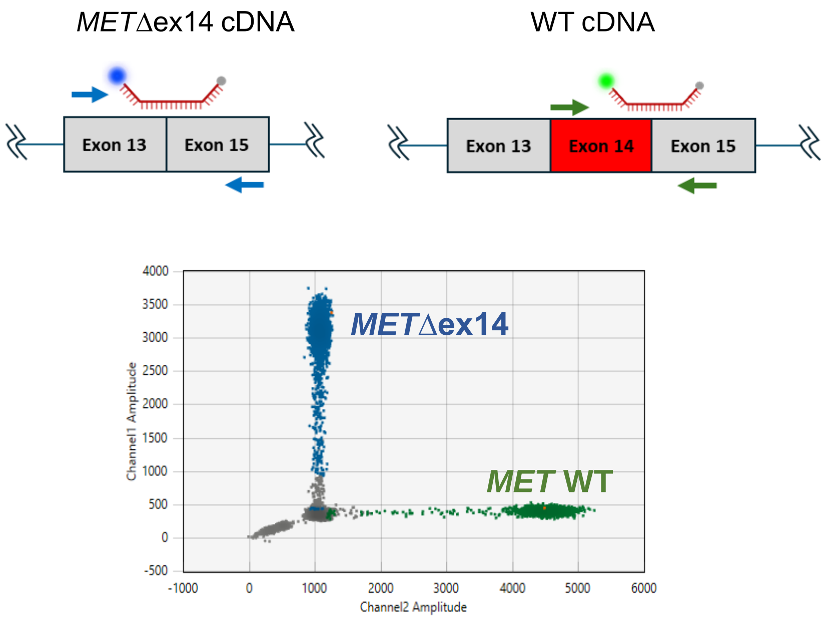


***MET*Δex14 RNA/cDNA**

***MET* WT RNA/cDNA**

Mass of PCR fragments determine time of flight

Droplet digital PCR



Target sequence gives fluorescent signal

- Proof-of-principle study using MALDI-TOF
- Quantification of mutant copies using ddPCR
- Materials used: H596 cell line bearing *MET* Exon 14 skipping mutation, healthy donor blood.

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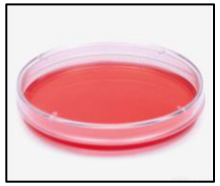
Results

H596: cell line with *MET*Δex14 genomic mutation

H596 supernatant: filtered culture media from 48 hour cultured H596 cells at 70% confluency

Sera Care METΔex14 RNA: Synthetic commercial naked RNA with *MET*Δex14



Exp. #1: Healthy donor blood incubated with **H596 live cells, supernatant,** and **Sera Care**

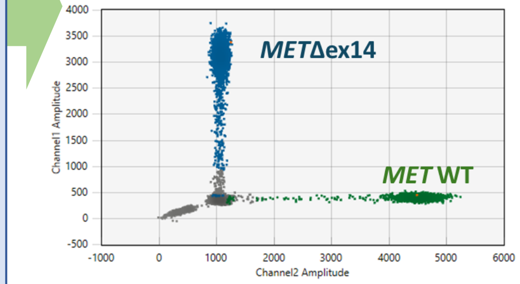
Exp. #2: Healthy donor blood incubated with **H596 supernatant volumes ranging from 0-4ml**

Incubation at RT, cfRNA extraction, cDNA synthesis, analysis with MALDI-TOF and ddPCR

2 hours incubation at RT

cfRNA isolation & cDNA synthesis

Agena FusionNTRK Panel on MassARRAY



ddPCR

Exp. #1 outcome

	MET Exon 14 status (MALDI-TOF)	*MET* Exon 14 status (ddPCR copies)
H596 cells	NOT DETECTED	NOT DETECTED
Sera Care *MET*Δex14 RNA	NOT DETECTED	NOT DETECTED
Cell supernatant (4ml)	DETECTED	101.5

Exp. #2 outcome

Supernatant volume (ml)	*MET* Exon 14 status (MALDI-TOF)	*MET* Exon 14 status (ddPCR copies)
4	DETECTED	88.1
2	DETECTED	42.4
1	DETECTED	12.7
0.5	DETECTED	5.8
0.25	DETECTED	2.5
0	NOT DETECTED	0

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Conclusion - Discussion

- cfRNA can be a good analyte for splice variants
- However, naked RNA is not suitable for liquid biopsy validation
- Protected RNA (protein bound, EVs) from cell culture are suitable for validation

Measurement	Quantitative	Qualitative
Number of targets	Few	Many (*e.g.*, *NTRK1-3, ALK, RET, ROS1, MET*)

Looking for patient material
Collaboration partners?

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