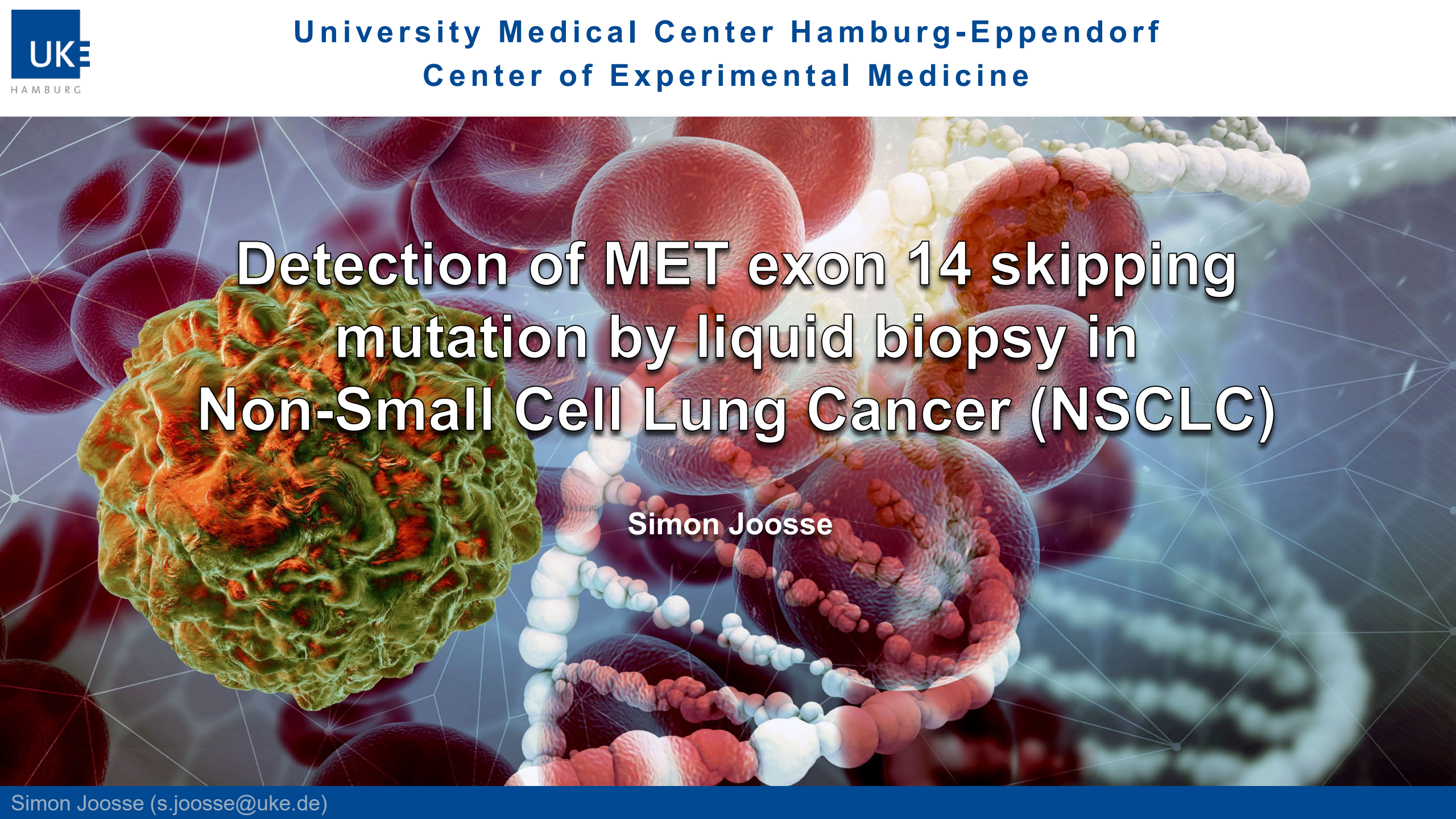
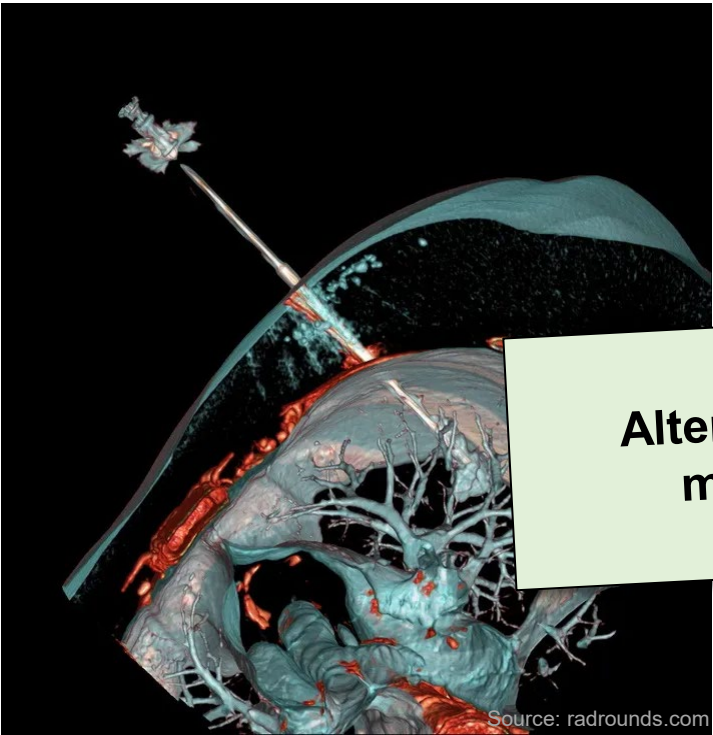




Detection of MET exon 14 skipping mutation by liquid biopsy in Non-Small Cell Lung Cancer (NSCLC)

Simon Joosse

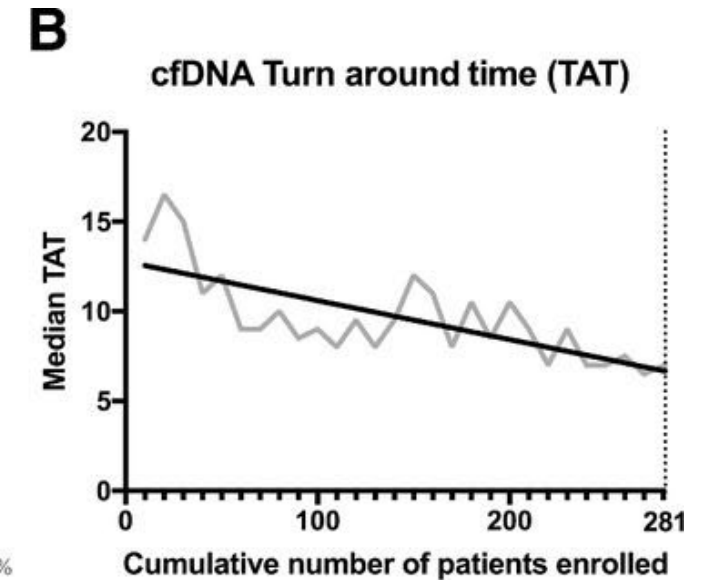
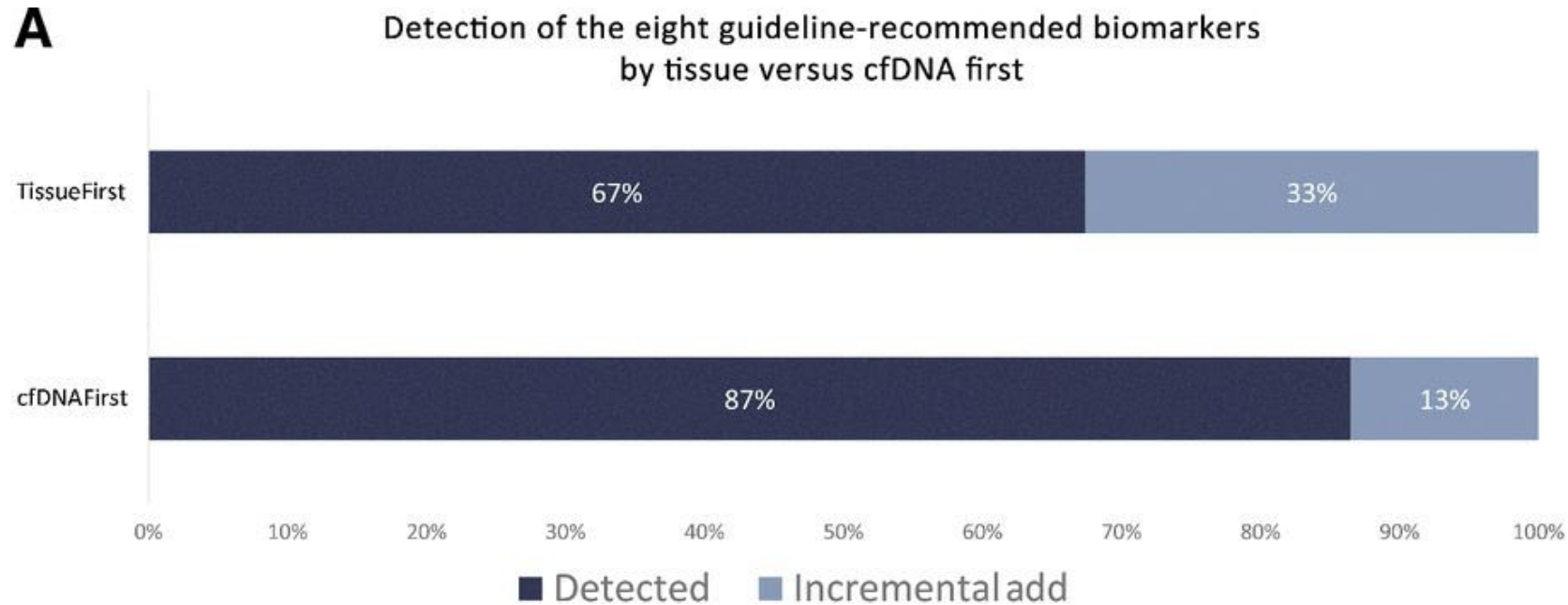
Comprehensive molecular genotyping of lung cancer



NSCLC 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

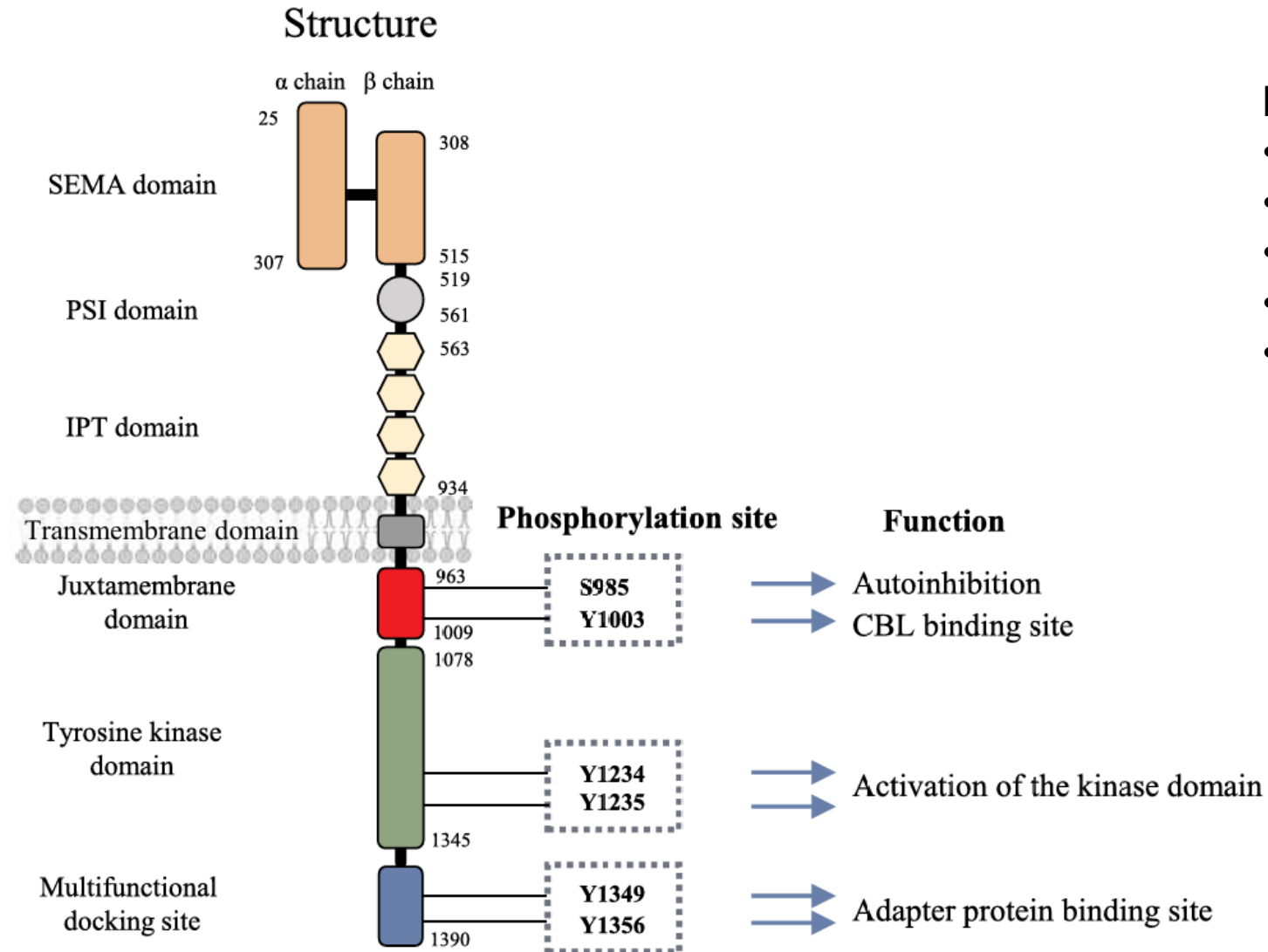
Lung cancer patients with insufficient quantity or quality of tissue DNA for comprehensive molecular genotyping		Reference
	58%	Aggarwal <i>et al</i> , <i>JAMA Oncol.</i> 2019
52/102	51%	Thompson <i>et al</i> , <i>Clin Cancer Res.</i> 2016
21/174	12%	Karlovich <i>et al</i> , <i>Clin Cancer Res.</i> 2016
601/2601	23%	Meric-Bernstam <i>et al</i> , <i>JCO.</i> 2015
735/1739	42%	Hellmann <i>et al</i> , <i>NEJM.</i> 2018
231/282	82%	Leighl <i>et al</i> , <i>Clin Cancer Res.</i> 2019

Lung cancer genotyping using cfDNA liquid biopsy



Leighl et al, *Clin Cancer Res.* 2019, García-Pardo et al, *JAMA netw Open.* 2023

MET: receptor tyrosine kinase for hepatocyte growth factor

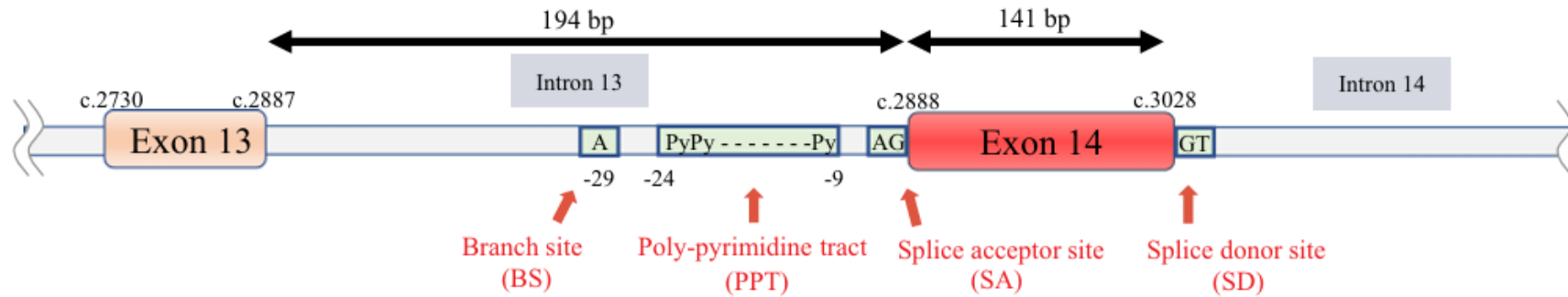


MET is involved in

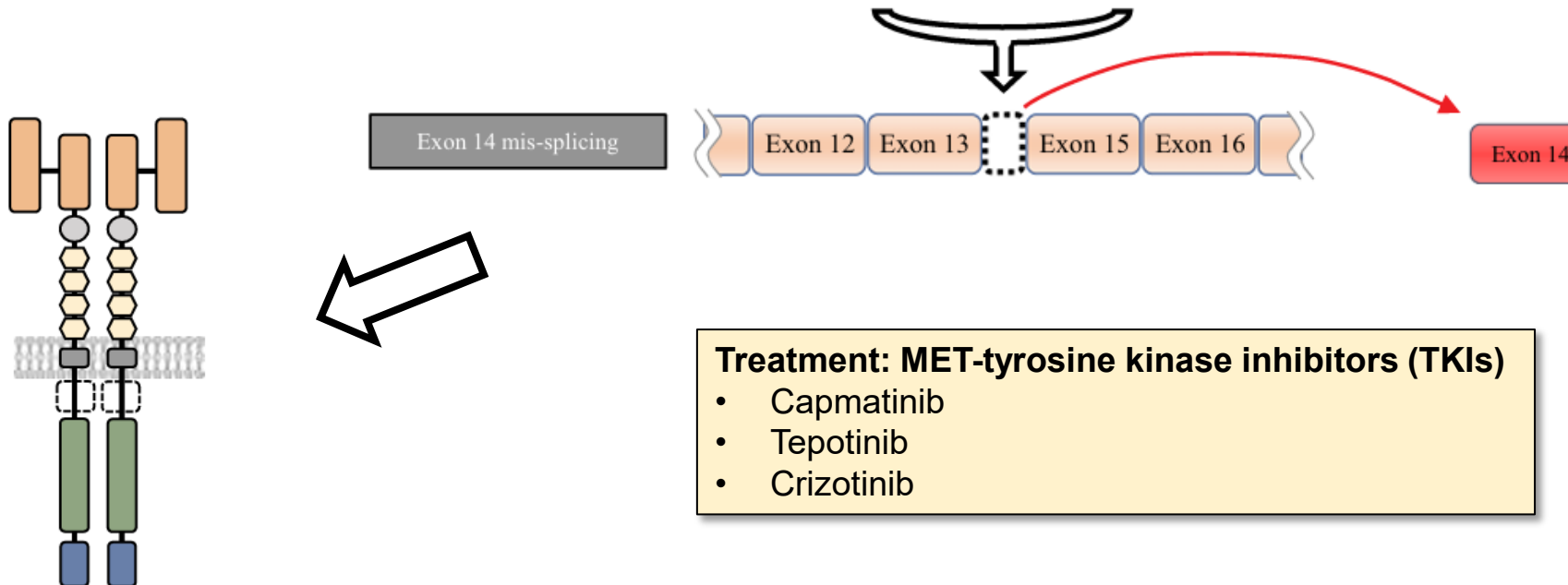
- Organogenesis
- Wound healing
- Cell motility
- Proliferation
- Embryogenesis

activation of RAS/RAF/MEK/ERK
and PI3K/AKT/mTOR pathways

MET exon 14 skipping (*MET* Δ ex14)



More than 500 different mutations (point, indel, insertion) reported in 3% of NSCLC

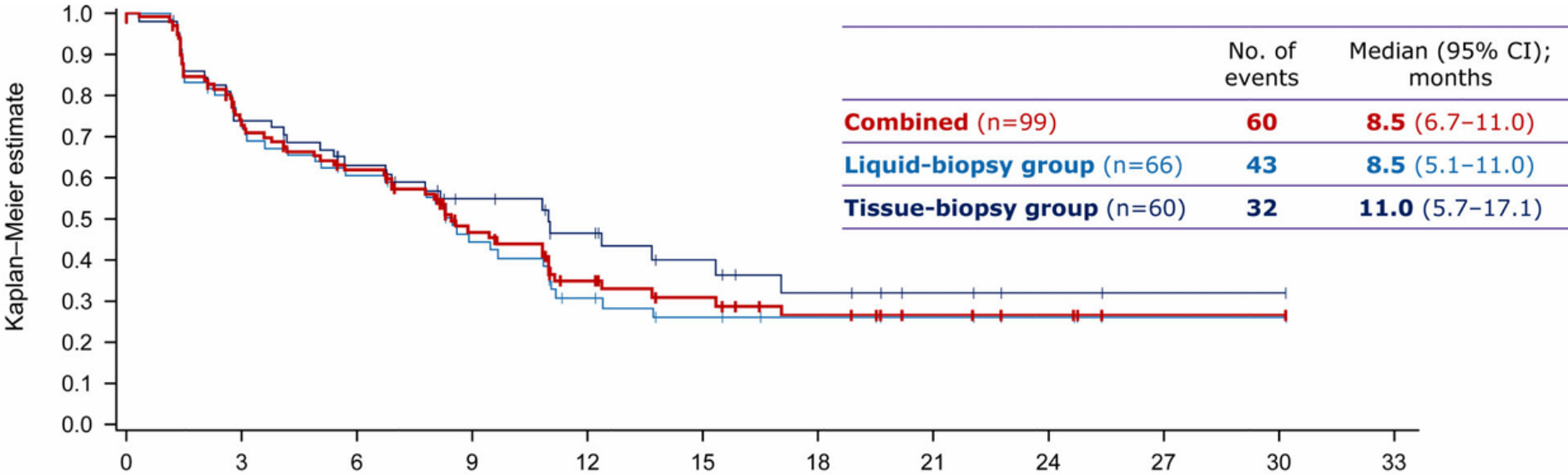


Treatment: *MET*-tyrosine kinase inhibitors (TKIs)

- Capmatinib
- Tepotinib
- Crizotinib

MET exon 14 skipping (METΔex14)

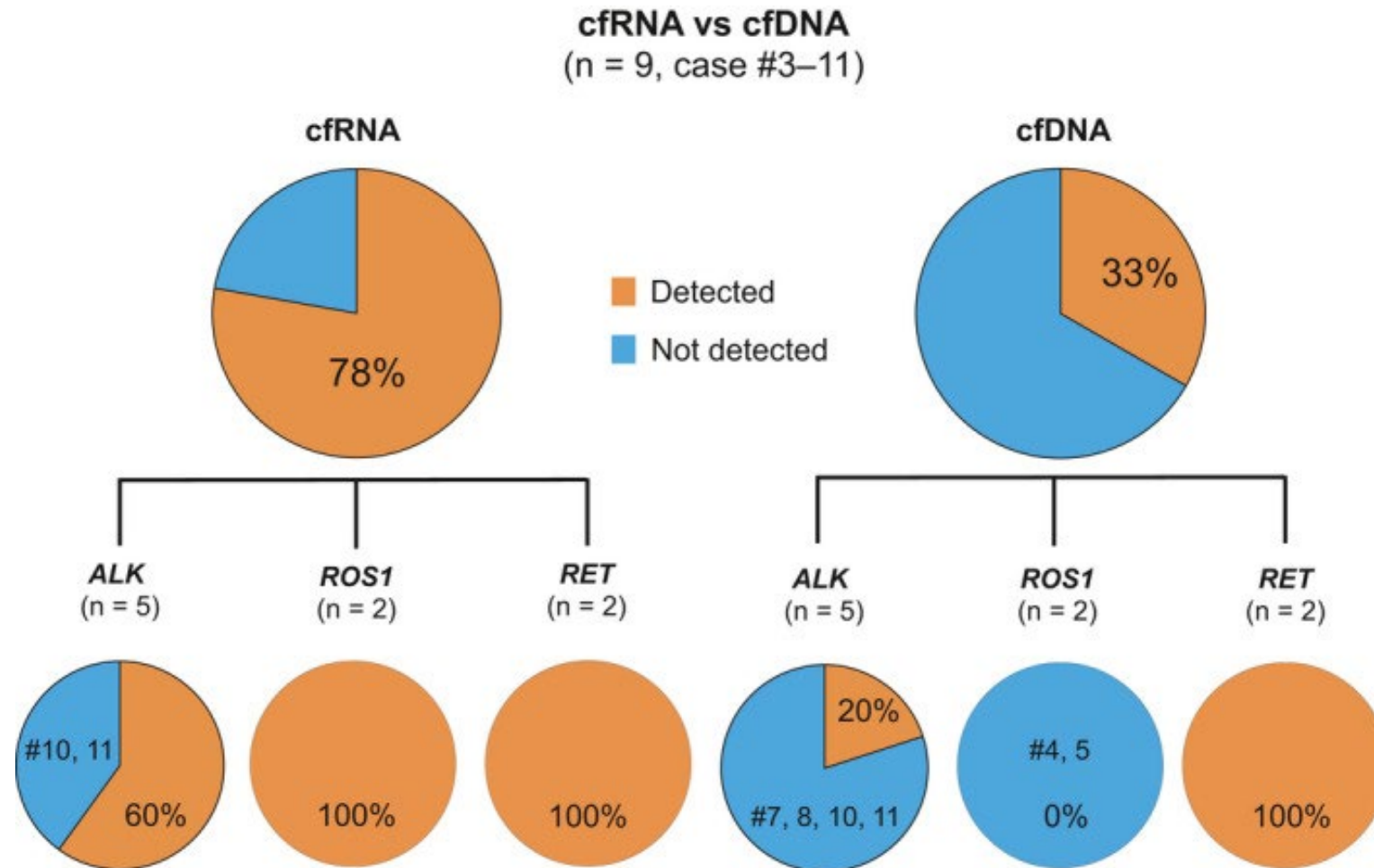
VISION study: 169/6708 patients with METΔex14 treated with Tepotinib



NGS panel
(Guardant360)

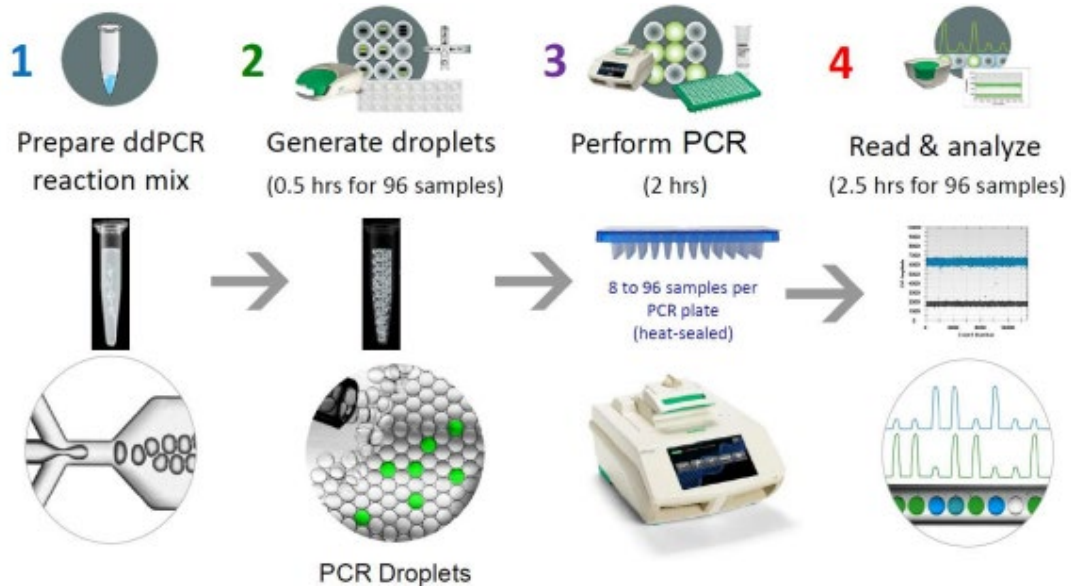
Number of patients at risk:		Time (months)											
Combined	99	67	53	33	20	15	10	6	4	1	1	0	
Liquid-biopsy group	66	44	36	23	14	10	8	6	4	1	1	0	
Tissue-biopsy group	60	42	32	22	16	11	7	4	2	1	1	0	

Fusion detection: cfRNA vs cfDNA



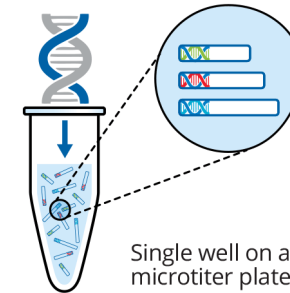
cfRNA detection

Droplet digital PCR



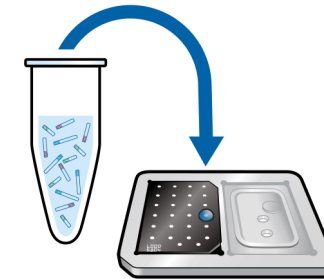
Target sequence gives fluorescent signal

MALDI-TOF mass spectrometry



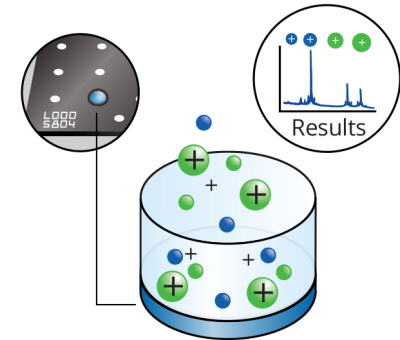
Endpoint PCR

Amplify and extend up to 40 target-specific DNA fragments in a single reaction.



Transfer Analyte

Transfer a small amount of sample to a single pad on the SpectroCHIP® Array.



Detection and Analysis

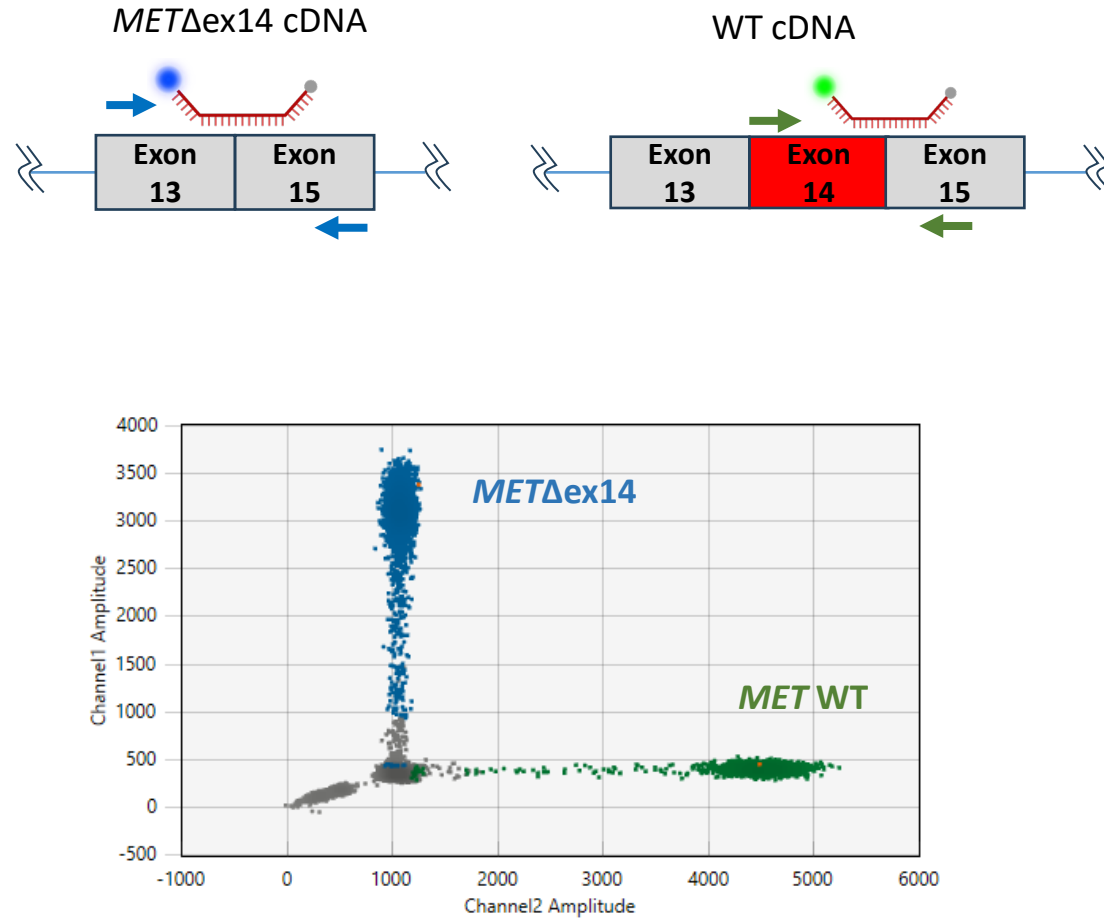
Multiple tests can be run on a single SpectroCHIP Array. Hundreds of mutations can be tested per sample.

* Use multiple reactions for >40 targets if required.

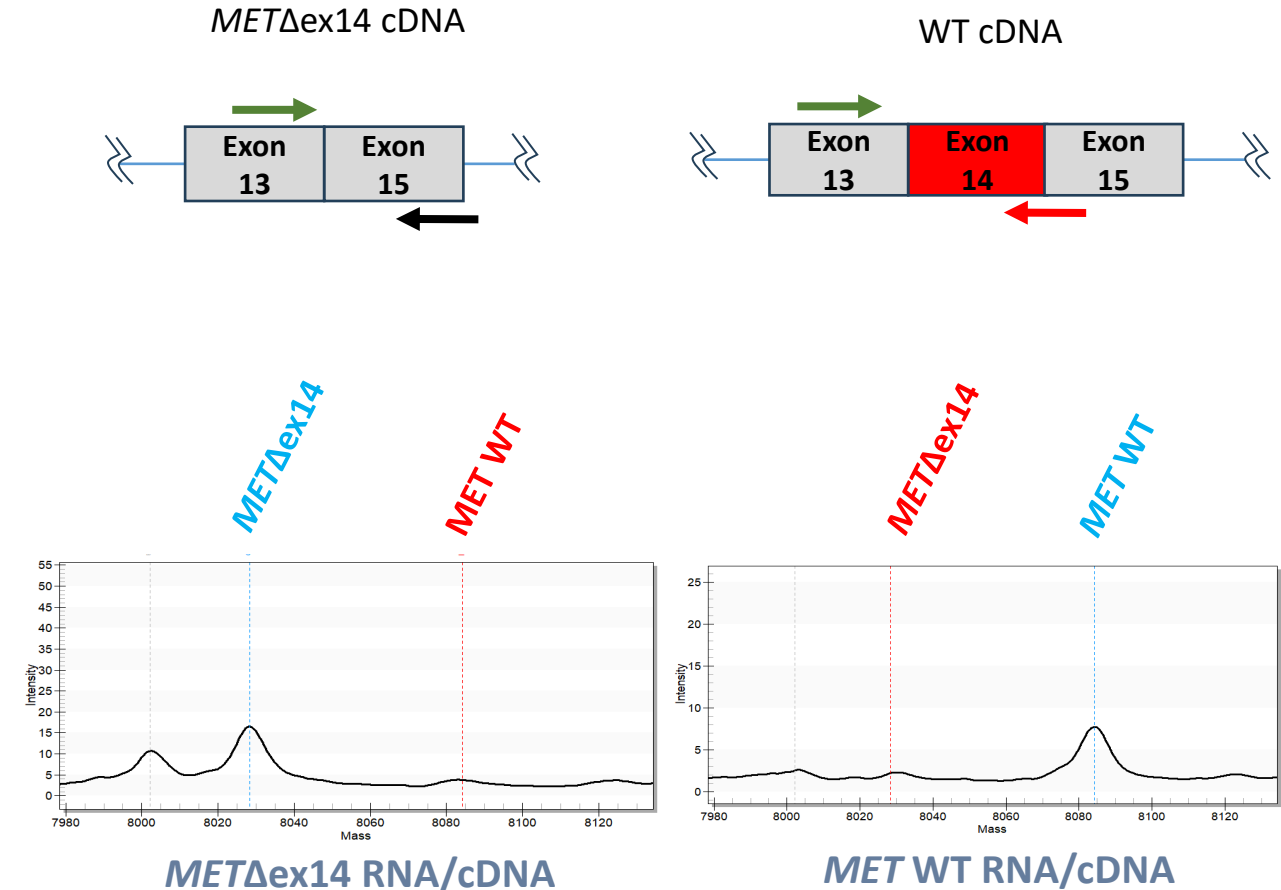
Mass of PCR fragments determine time of flight

cfRNA detection

Droplet digital PCR



MALDI-TOF mass spectrometry



cfRNA based liquid biopsy assay: RESULTS



***MET*Δex14 RNA**
SeraCare control



blood from
healthy donors



PAXgene

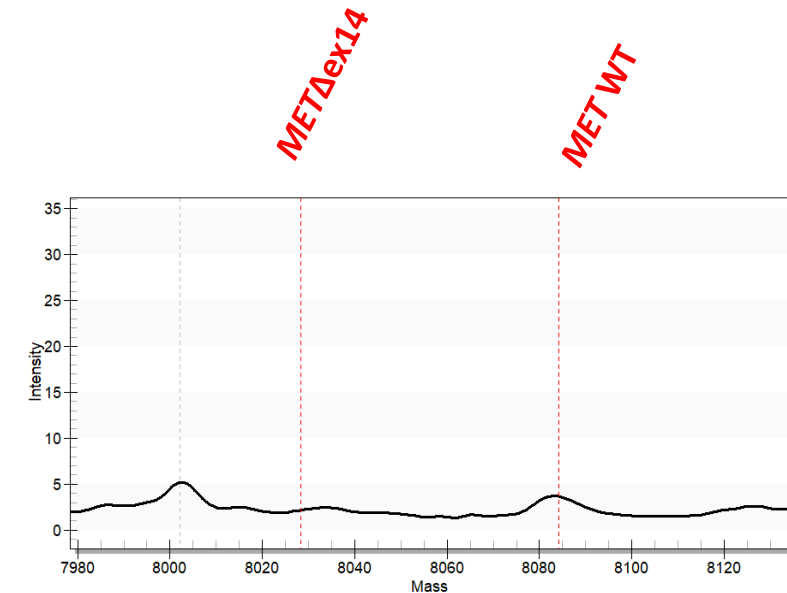


cfRNA extraction
&
cDNA synthesis



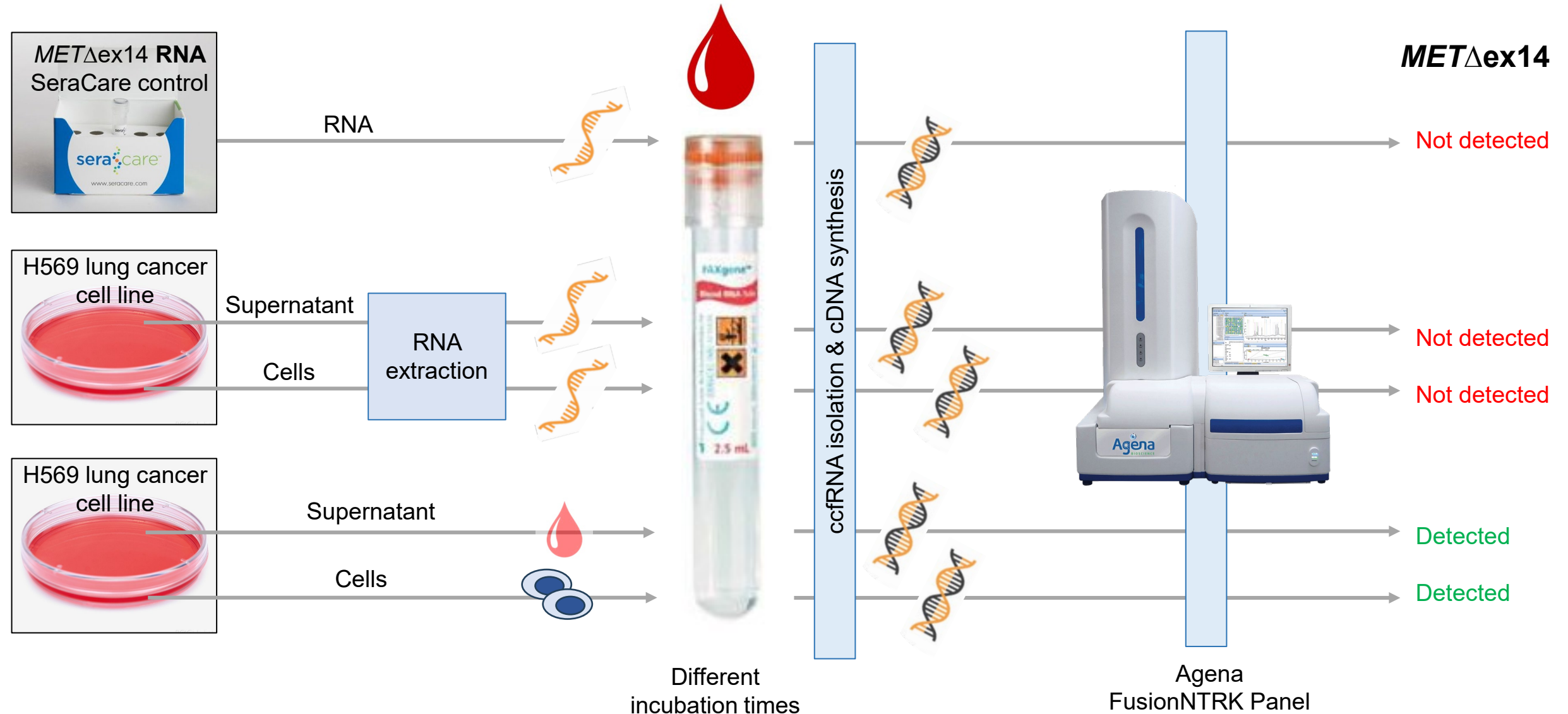
Agena
FusionNTRK Panel

*MET*Δex14 not detected

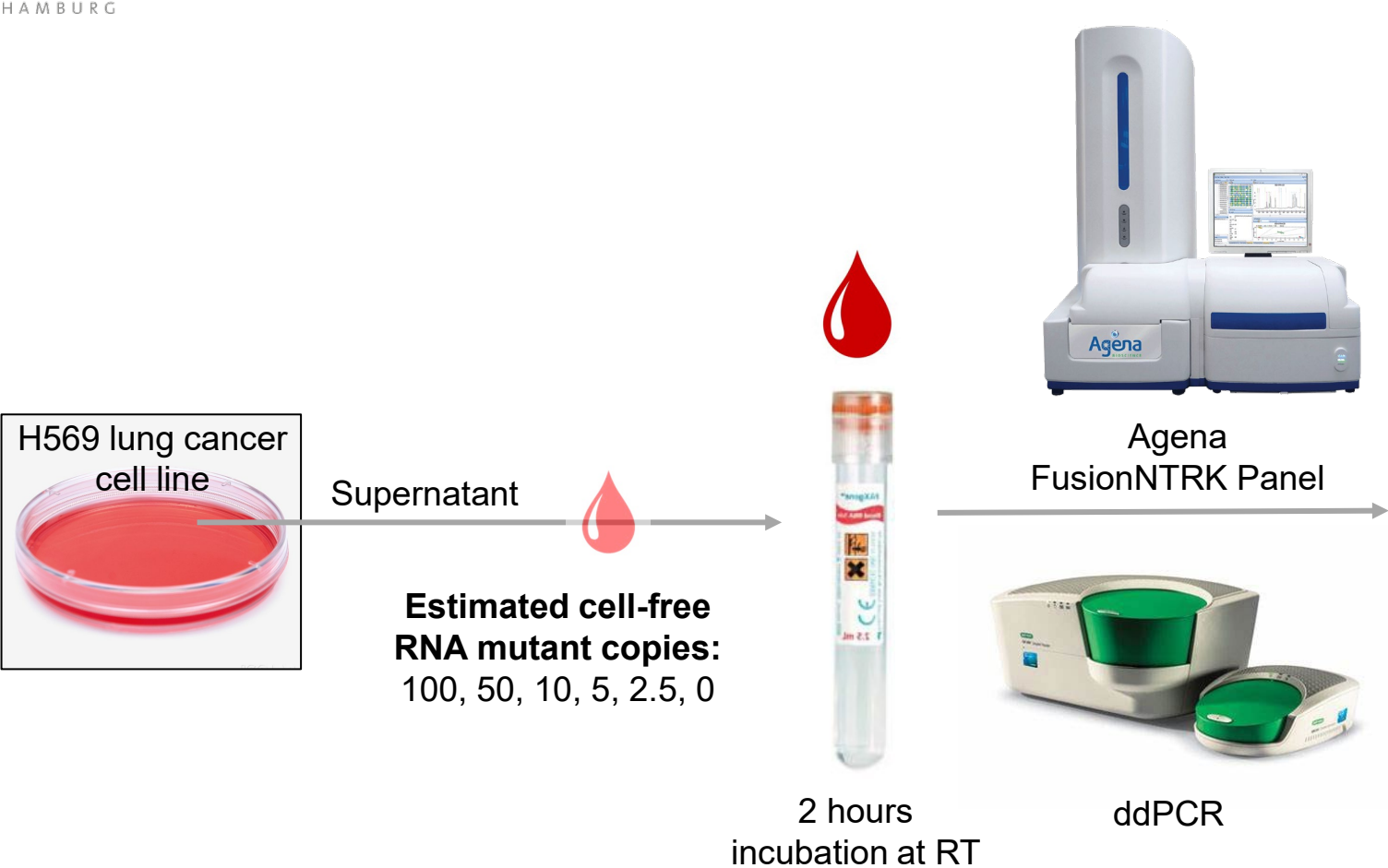


Different incubation times

cfRNA based liquid biopsy assay: RESULTS



cfRNA based liquid biopsy assay: RESULTS



Sample (RNA copies)	QC	MET Exon 14 status	ddPCR
100	✓	DETECTED	88.1
	✓	DETECTED	
	✓	DETECTED	
50	✓	DETECTED	42.4
	✓	DETECTED	
	✓	DETECTED	
10	✓	DETECTED	12.7
	✓	DETECTED	
5	✓	DETECTED	5.8
	✓	DETECTED	
2.5	✓	DETECTED	2.5
	✓	DETECTED	
	✓	DETECTED	
0	✓	INDETERMINATE	0

CONCLUSION



- 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**
- **Number of targets**

Quantitative
Few



Qualitative
Many (e.g, *NTRK1-3*, *ALK*, *RET*, *ROS1*, *MET*)

Looking for patient material
Collaboration partners?

Joosse Laboratory

Bidisha Paul
Luis Enrique Cortes Hernandez
Annabelle Lobermeyer
Peiyao Wang
Sandra Lenz
Christina Apostolopoulou
Anja Holz
Jenna Sophie Schoellhorn
Evelyn Duesterhoeft

Department of Tumor Biology (UKE)

Klaus Pantel
Sabine Riethdorf
Harriet Wikman
Stefan Werner

Oncology (UKE)

Benjamin Schmidt
Piet Sonnemann

Agena

Alexander Sartori
Darryl Irwin

University of Southern Denmark

Sara Wen

Funding received from



Federal Ministry
of Education
and Research



Hubertus Wald Tumorzentrum
Universitäres Cancer Center Hamburg

Ein Kompetenznetzwerk des UKE



Universitätsklinikum
Hamburg-Eppendorf