



Multi Institutional Evaluation of a High Sensitive NGS Assay for Liquid Biopsy Mutation Detection in Lung Cancer

Dr. Claudia Vollbrecht
Charité Medical University Berlin
Institute of Pathology

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Conflicts of interest

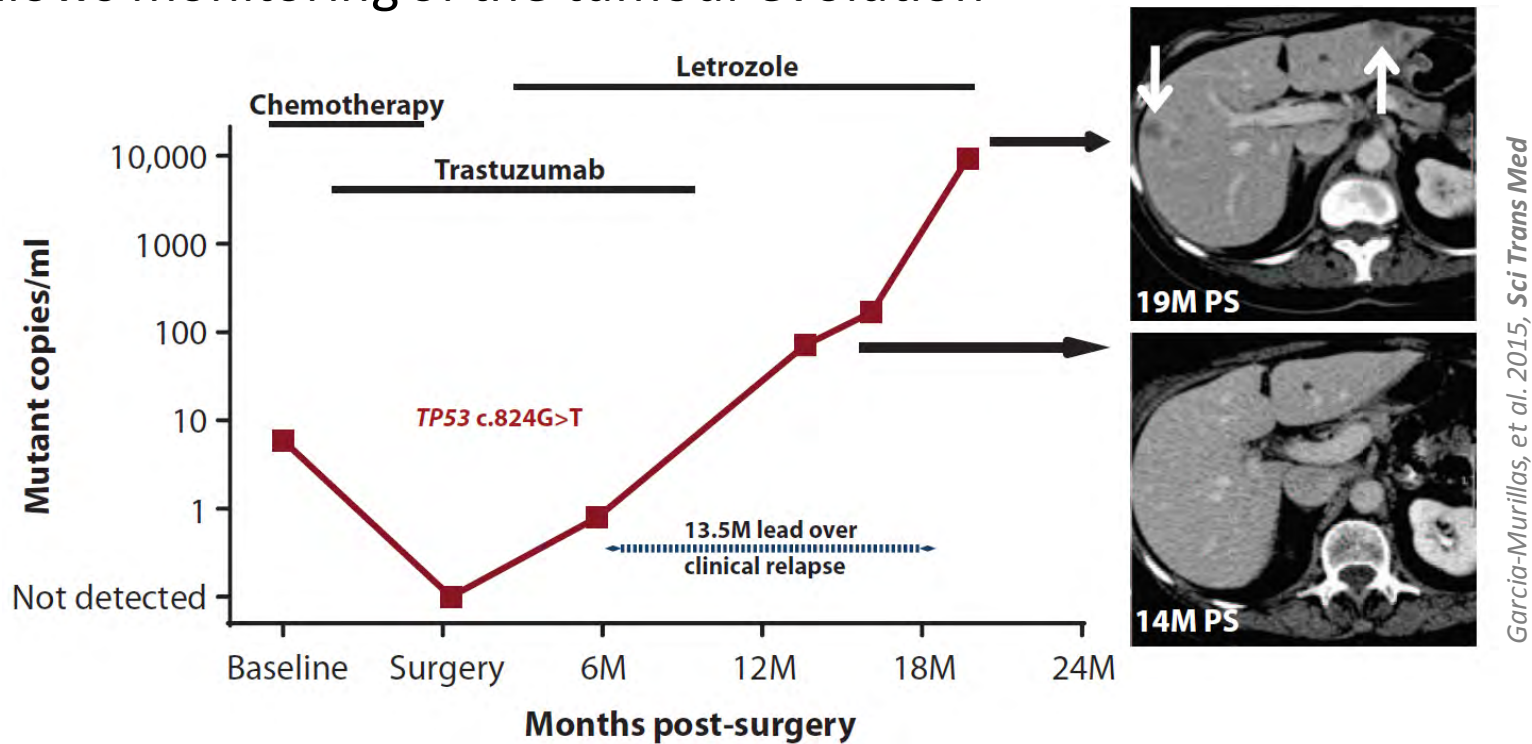
DISCLOSURE

I have the following potential conflict(s) of interest to report

- Congress fees paid by Thermo Fisher Scientific

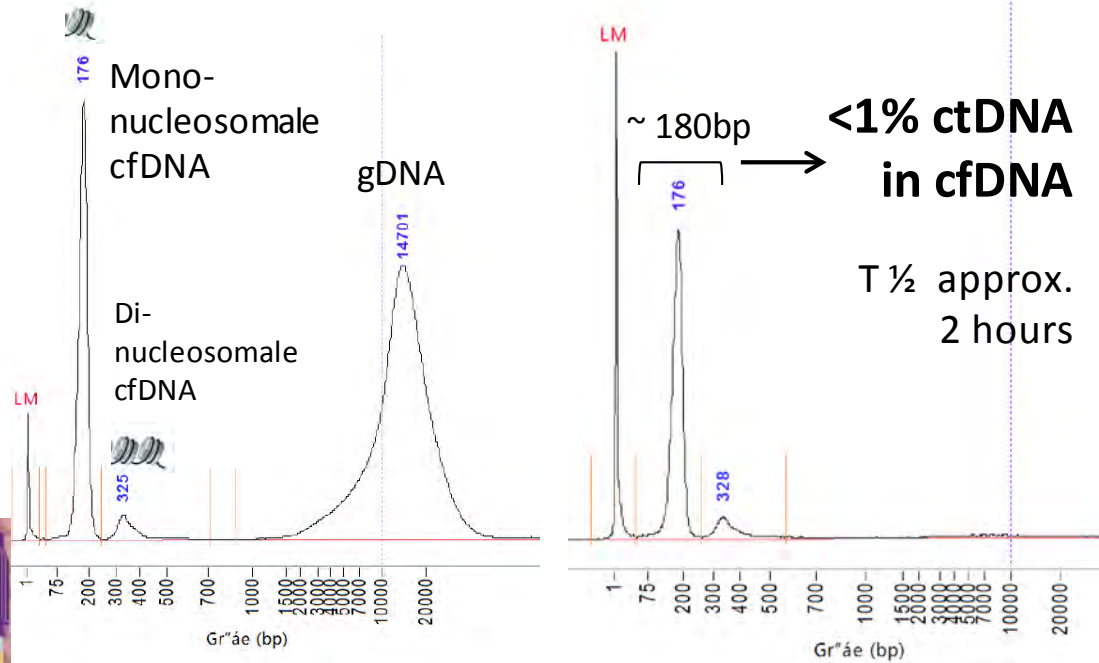
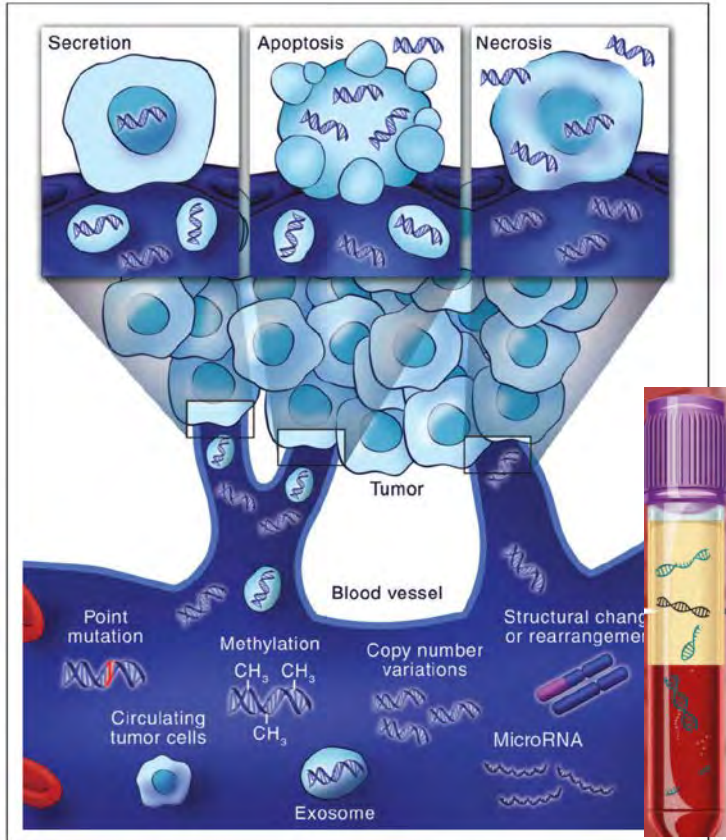
LIQUID BIOPSIES - A PROMISING TOOL FOR FUTURE

- Early, non-invasive detection of MRD & resistances with sensitive methods like parallel-sequencing
- Allows monitoring of the tumour evolution



LIQUID BIOPSY – CFDNA EXTRACTION

Liquid Biopsies: Genotyping Circulating Tumor DNA, Diaz LA, et al. 2014, J Clin Oncol



cfDNA Release Depends on:

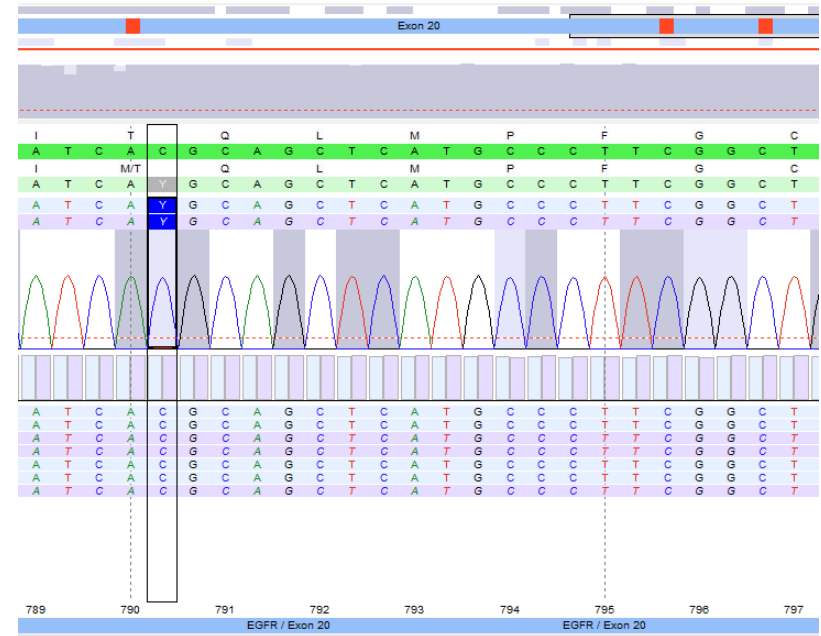
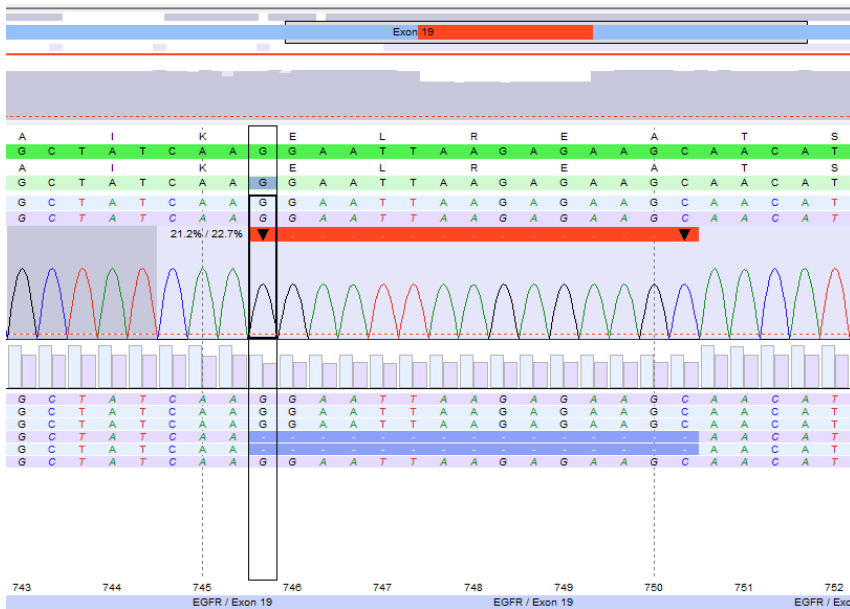
- Localization of tumor
- Tumors size
- Vascularity
- Therapy status

cfDNA: cell-free DNA
 ctDNA: circulating tumor DNA

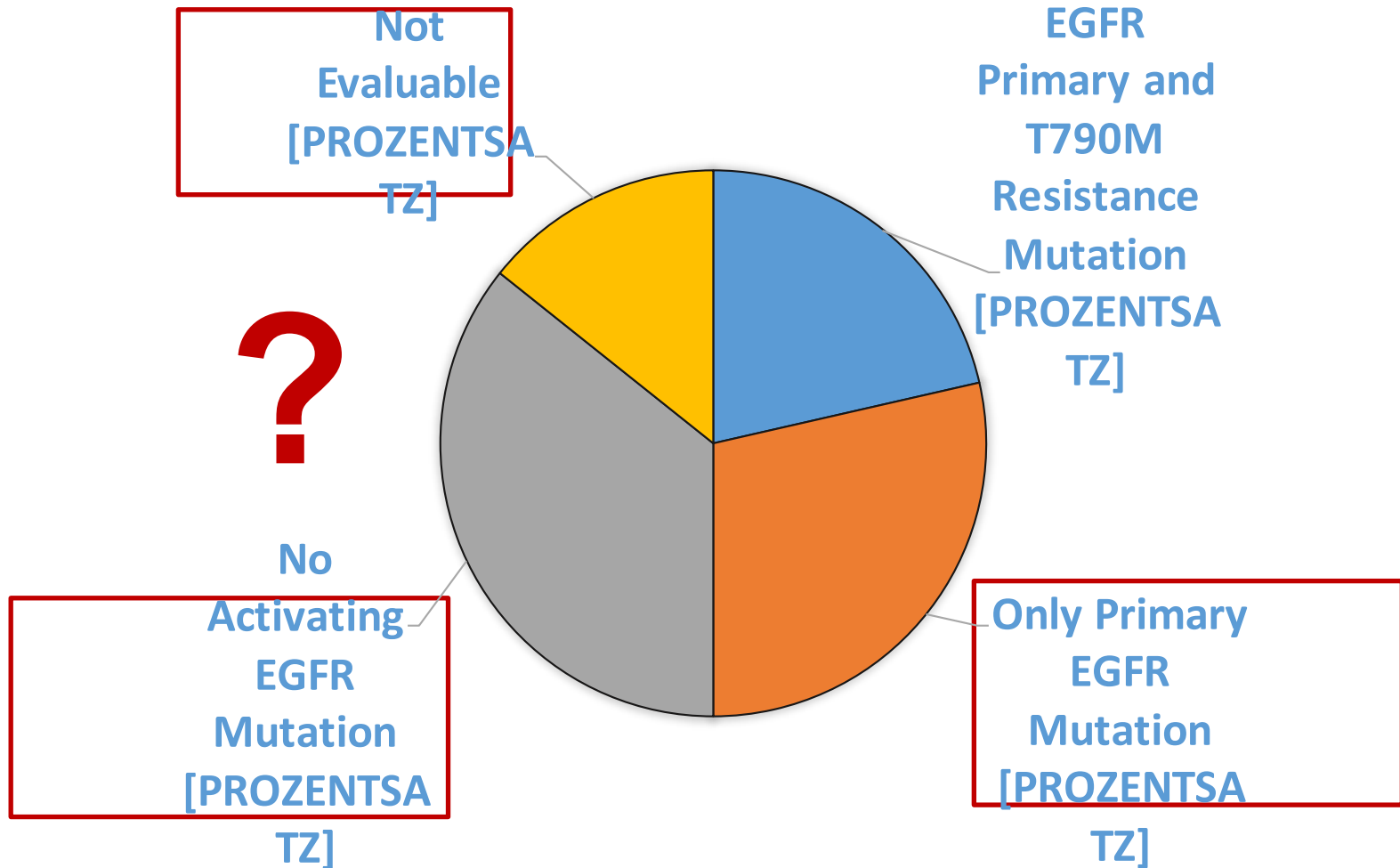
CTDNA DETECTION – PARALLEL SEQUENCING

Targeted Multiplex PCR Panel CLv2 (92 Amplicons)

- Primary mutation: *EGFR* p.E746_A750delELREA
- LB: *EGFR* p.E746_A750delELREA 22% AF + p.T790M 3% AF



CTDNA DETECTION – PARALLEL SEQUENCING



ONCONETWORK CONSORTIUM

Oncomine cfDNA Lung Assay Multicenter Study

Prof. Michael Hummel
Institute of Pathology Charité
Berlin, Germany

Prof. Harriet Feilotter
Dr. Paul Park
Queen's University, Ontario
Canada

Marjolijn J.L. Ligtenberg
Radboud University Nijmegen
Medical Centre, Netherlands

Cecily P. Vaughn
ARUP- Institute for Clinical and
Experimental Pathology
Utah, USA

Dr. Henriette Kurth
VIOLLIER AG Basel ,
Switzerland

Prof. Orla Sheils
St James's Hospital Dublin,
Ireland

Prof. Kazuto Nishio
Faculty of Medicine, Kinki
University Osaka, Japan

Prof. Pierre Laurent Puig
Université Paris Descartes,
Paris, France

Prof. Aldo Scarpa
ARC-NET University of
Verona Italy

Prof. Ian Cree
UHCW
United Kingdom

Dr. Jose Costa
IPATIMUP, Medical Faculty of
Porto. Portugal

Dr. Nicola Normanno
Centro Ricerche Oncologiche
Mercogliano, Italy

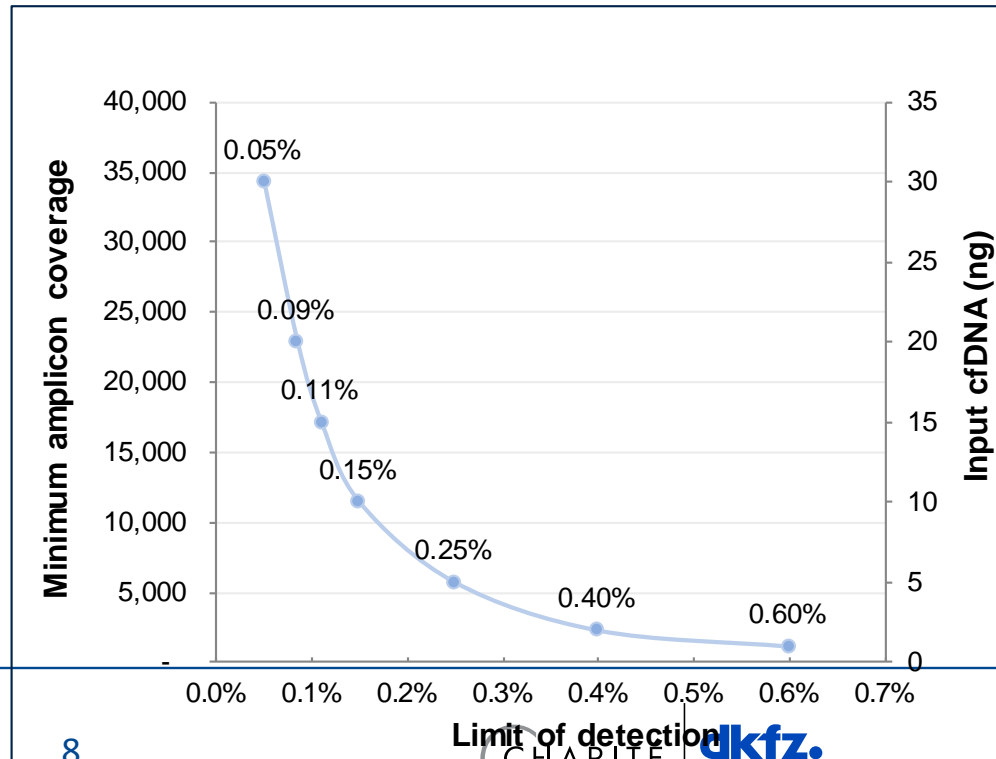
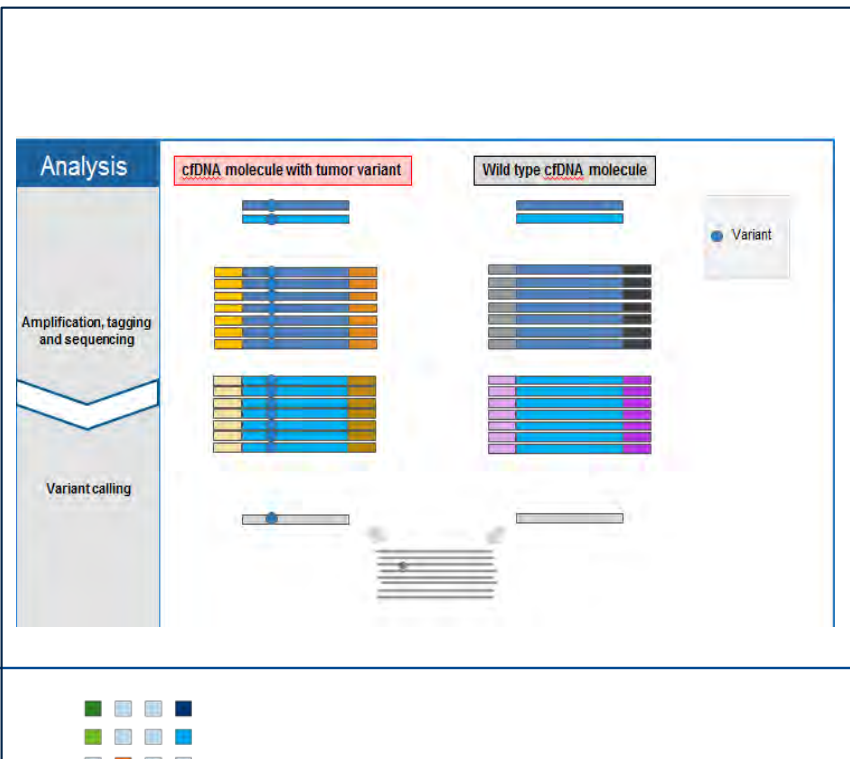


ONCONETWORK CONSORTIUM

OncoPrint Lung cfDNA Assay*

- 169 Hotspots, 35 Amplicons, 11 Genes
- Amplification-based Assay

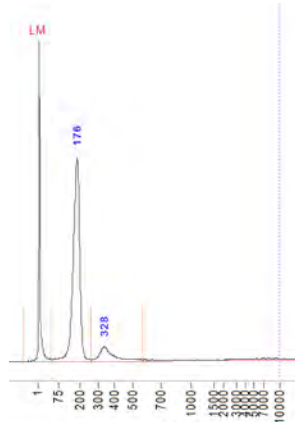
Genes	Hotspots
<i>ALK, BRAF, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, ROS1, TP53</i>	>169 hotspots including: <i>EGFR</i> : T790M, C797S, L848R, Exon 19 del <i>KRAS</i> : G12X, G13X, Q61X <i>BRAF</i> : V600E <i>ALK</i> : Exon 21-25 <i>PIK3CA</i> : E545K, H1047R, E542K



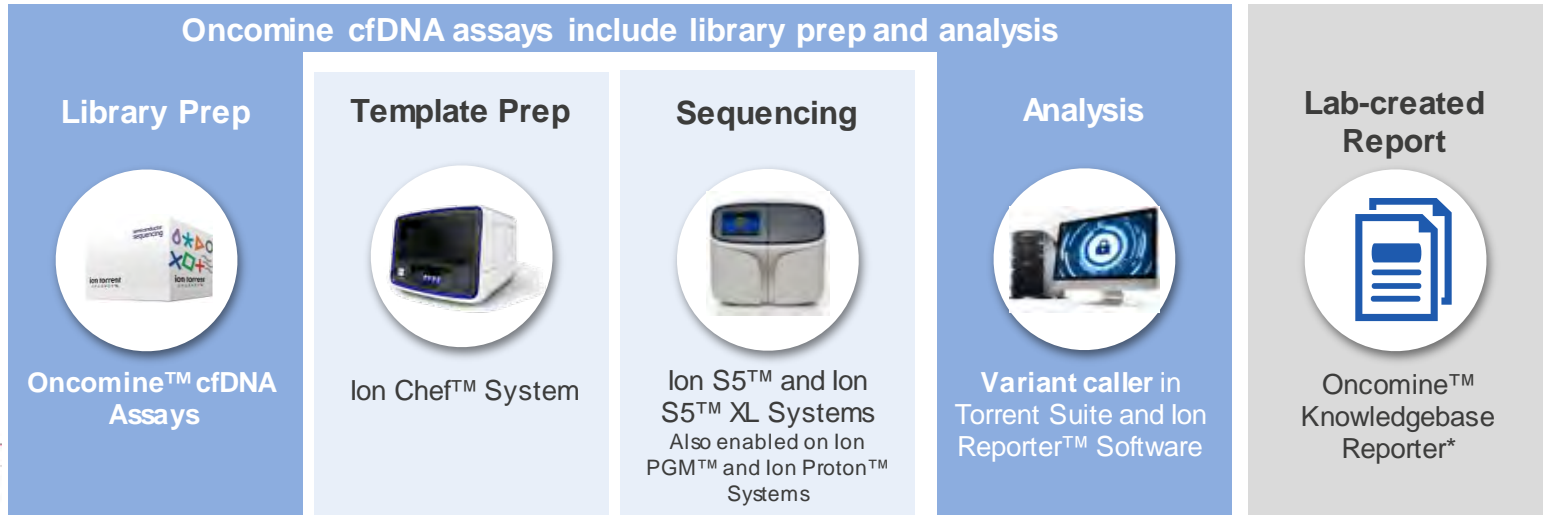
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OncoPrint cfDNA assays include library prep and analysis



cfDNA Input



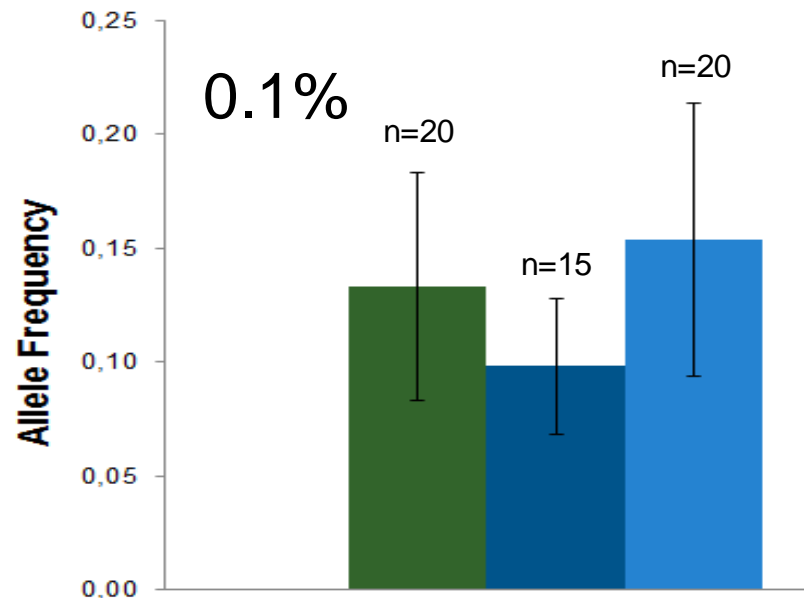
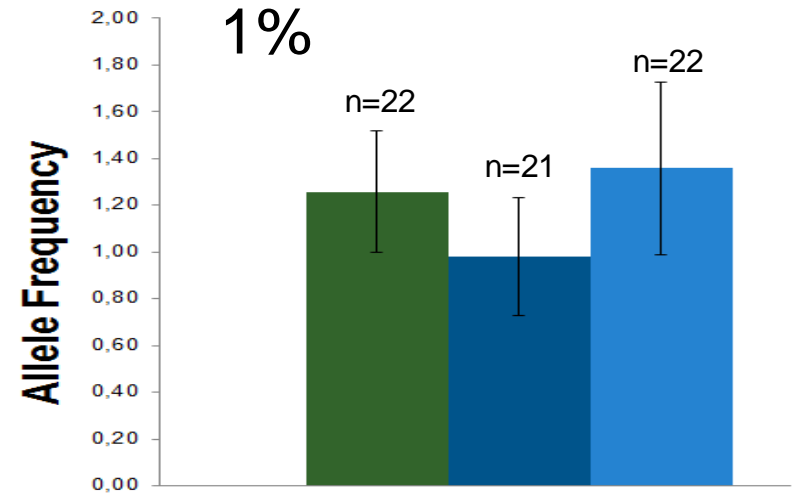
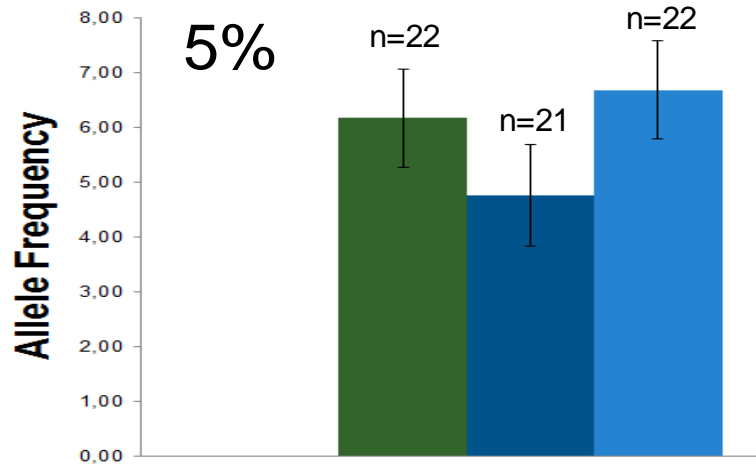
Refined variant data

Sample	EGFR E746_A750 deIELREA	EGFR L858R	EGFR T790M	EGFR V769_D770 insASV	KRAS G12D	NRAS A59T	NRAS Q61K	PIK3CA E545K
0.1% HDX	0.06	0.17	0.06	0.10	0.22	0.17	0.15	0.10
1% HDX	0.72	1.07	0.75	0.74	1.14	1.15	1.15	2.29
5% HDX	4.52	4.86	6.32	3.97	6.34	6.11	6.94	5.29
100% WT	0	0	0	0	0	0	0	0

All 8 mutant hotspots were detected at 0.1%, no false positives

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Repeatability & Reproducibility

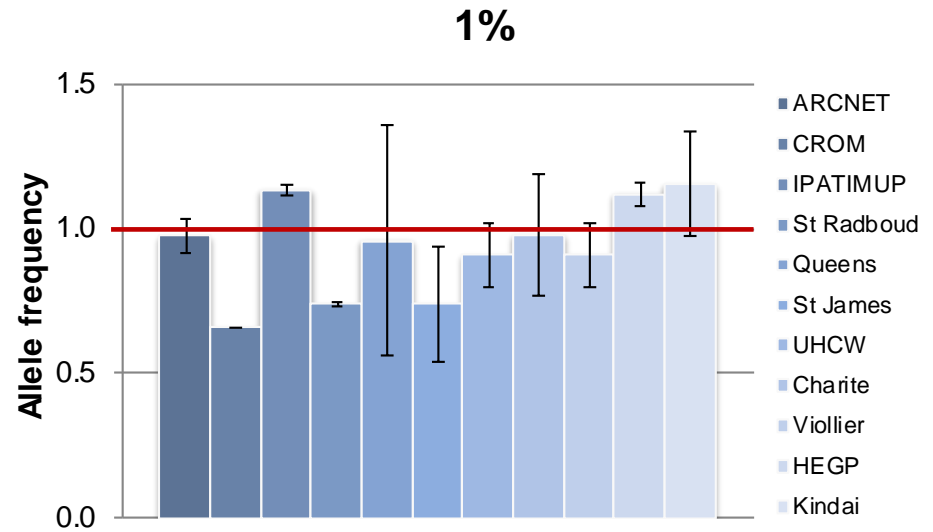
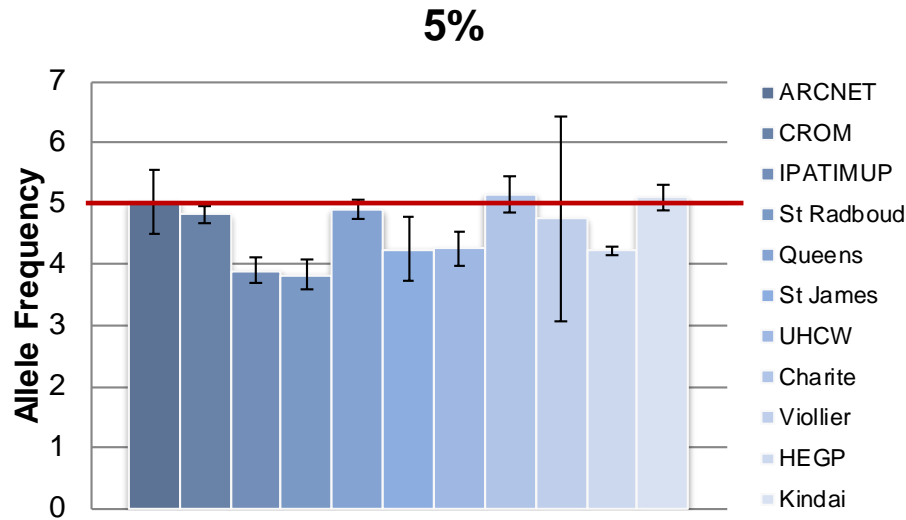


KRAS p.G12D

EGFR p.E746_A750del ELREA

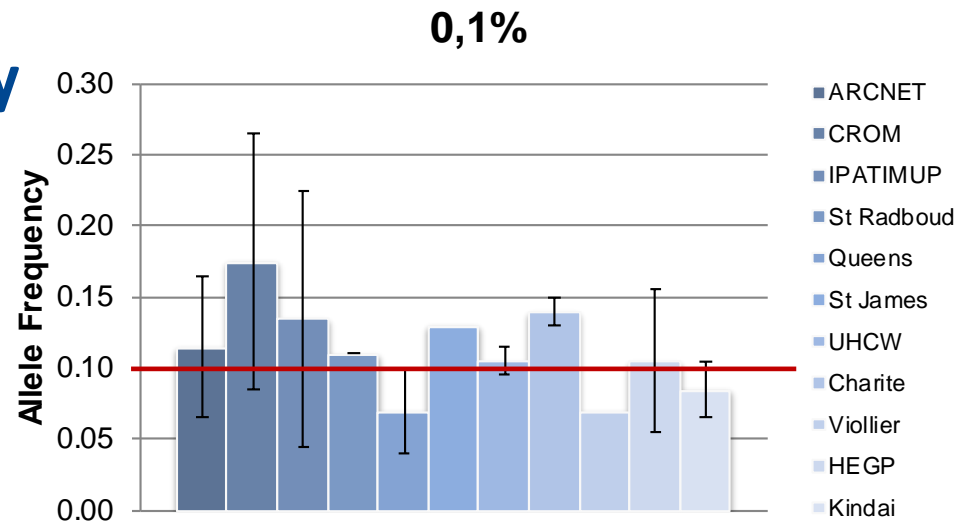
PIK3CA p.E545K

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Repeatability & Reproducibility

- EGFR p.T790M at 5%, 1% and 0.1% allele frequency



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Sensitivity & Specificity

Lab	Sensitivity	Specificity	NPV	PPV
ARCNET	89,58%	99,53%	99,61%	87,76%
CROM	95,83%	100,00%	99,84%	100,00%
IPATIMUP	95,83%	100,00%	99,84%	100,00%
Radboud	89,58%	99,84%	99,61%	95,56%
Queens	97,92%	99,92%	99,92%	97,92%
St James	95,83%	100,00%	99,84%	100,00%
UHCW	95,83%	99,76%	99,84%	93,88%
Charite	95,83%	99,76%	99,84%	93,88%
Viollier*	97,50%	99,82%	99,91%	95,12%
HEGP	93,75%	99,69%	99,76%	91,84%
Kindai*	95,83%	99,69%	99,84%	92,00%
Total	94,81%	99,82%	99,80%	95,93%

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Sensitivity & Specificity at 0.1% Allele Frequency

Lab	Sensitivity	Specificity	NPV	PPV
ARCNET	68,75%	100,00%	98,43%	100,00%
CROM	87,50%	100,00%	99,37%	100,00%
IPATIMUP	87,50%	100,00%	99,37%	100,00%
Radboud	68,75%	99,68%	98,43%	91,67%
Queens	93,75%	100,00%	99,68%	100,00%
St James	87,50%	100,00%	99,37%	100,00%
UHCW	87,50%	100,00%	99,37%	100,00%
Charité	87,50%	100,00%	99,37%	100,00%
Viollier	87,50%	100,00%	99,37%	100,00%
HEGP	81,25%	99,36%	99,05%	86,67%
Kindai	87,50%	99,68%	99,37%	93,33%
Total	83,93%	99,88%	99,19%	99,13%

ONCONETWORK CONSORTIUM

Sensitivity & Specificity

Allele Frequency	Sensitivity	Specificity
0.1% - 5%	94.81%	99.82%
0.1%	83.93%	99.88%

Data collected from 11 laboratories:

UHCW, CROM, ARCNET, IPATIMUP, Radboud University, Queen's University,
St James Hospital, Violler, HEGP, Kinday University, Charité Hospital

CONCLUSION

- The Oncomine Lung cfDNA Assay* enables detection of primary driver and resistance mutations to a level of 0.1%
- OncoNetwork Consortia evaluated the assay in a repeatability and reproducibility multicenter study using Horizon cfDNA Reference Set
- Results from 11 laboratories demonstrated more than **94% sensitivity** and **98% specificity**



*For Research use only

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ACKNOWLEDGEMENTS

OncoNetwork Consortium

Cecily P. Vaughn

ARUP- Institute for Clinical and Experimental Pathology, Utah, USA

Prof. Orla Sheils

St James's Hospital Dublin, Ireland

Prof. Pierre Laurent Puig

Université Paris Descartes, Paris, France

Prof. Ian Cree

UHCW, United Kingdom

Dr. Jose Costa

IPATIMUP, Medical Faculty of Porto, Portugal

Dr. Nicola Normanno

Centro Ricerche Oncologiche Mercogliano, Italy

Prof. Aldo Scarpa

ARC-NET University of Verona, Italy

Prof. Kazuto Nishio

Faculty of Medicine, Kinki University Osaka, Japan

Prof. Harriet Feilotter & Dr. Paul Park

Queen's University, Ontario, Canada

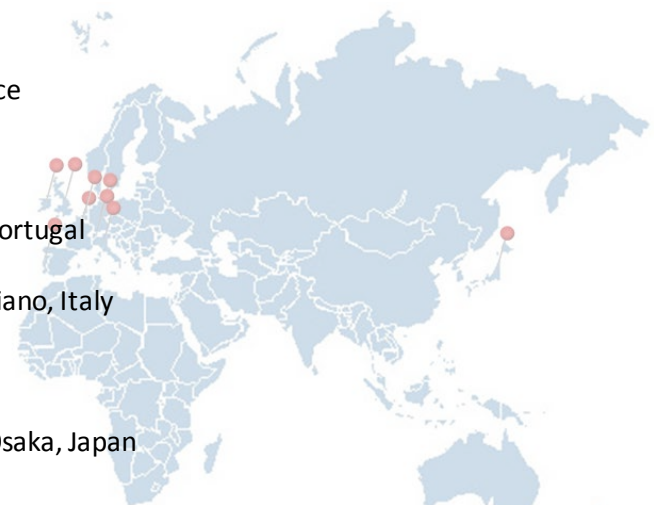
Marjolijn J.L. Ligtenberg, Radboud University Nijmegen, Medical Centre, Netherlands

Dr. Henriette Kurth

VIOLLIER AG Basel, Switzerland

Prof. Michael Hummel

Institute of Pathology Charité Berlin, Germany



Molpath Team AG Hummel

Thermo Fisher Scientific

Rosella Petraroli & Christopher Allen



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