Zukünftige therapierelevante Veränderungen per NGS in nur einem Tag

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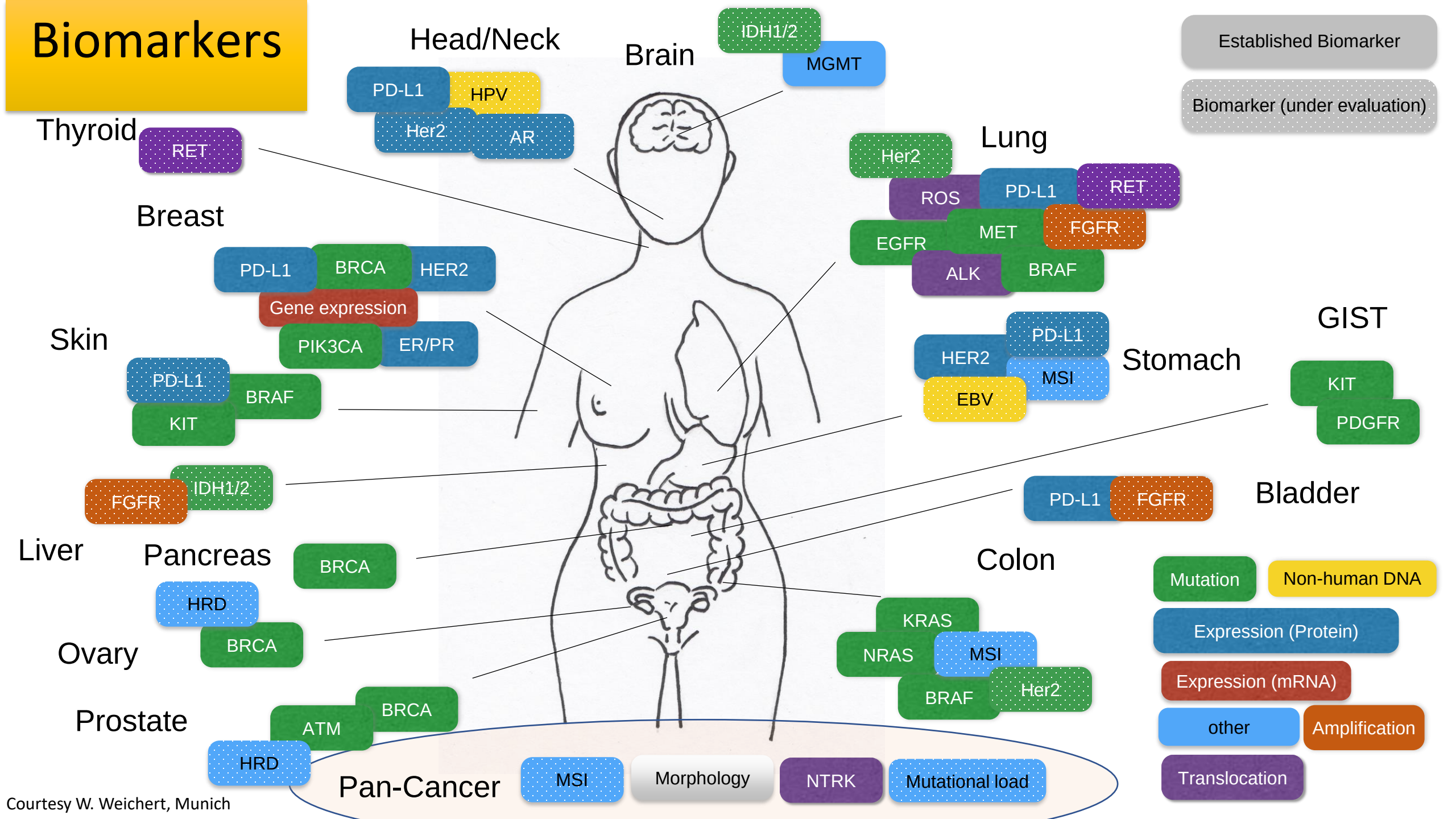
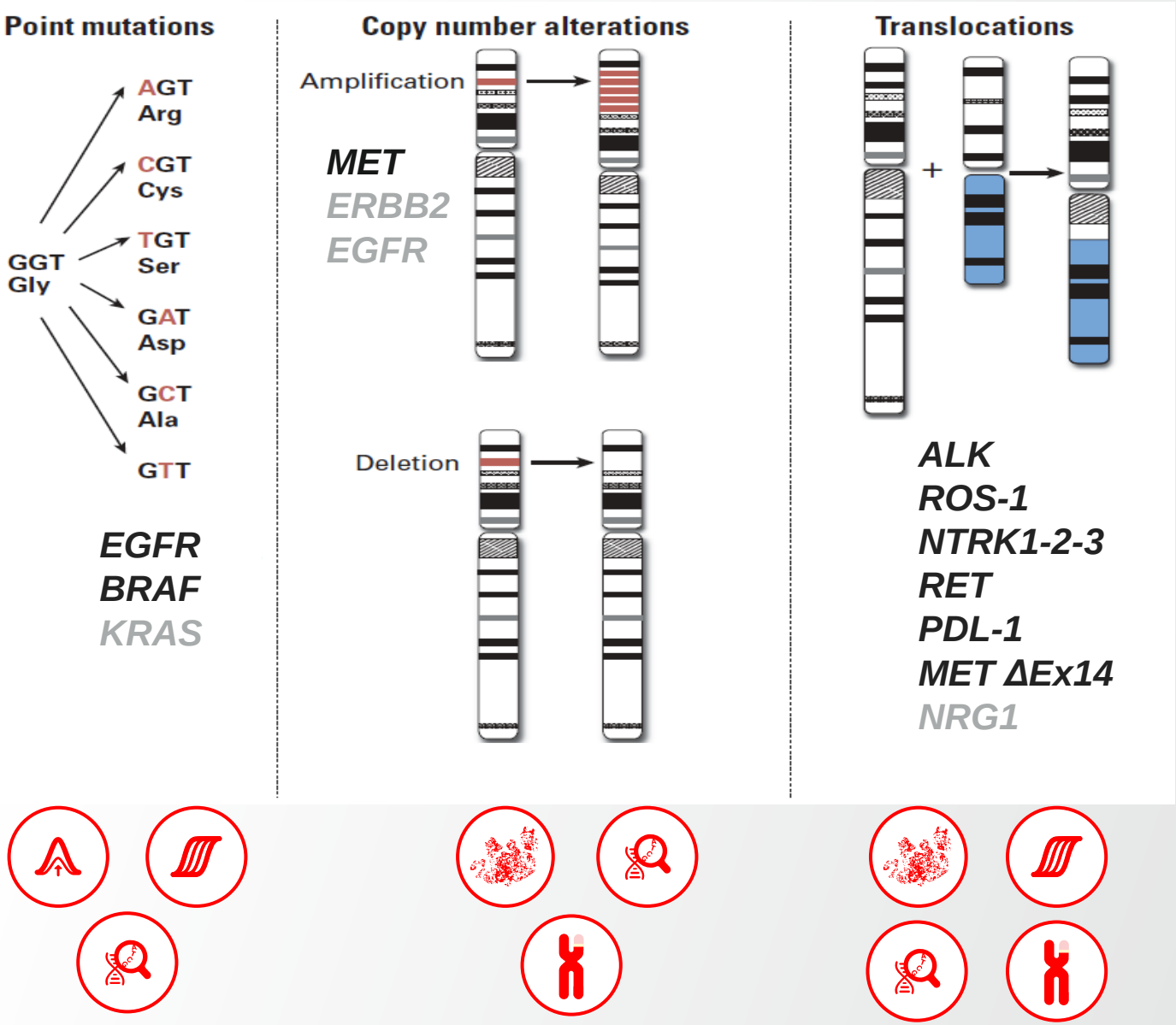
Biomarkers



Courtesy W. Weichert, Munich

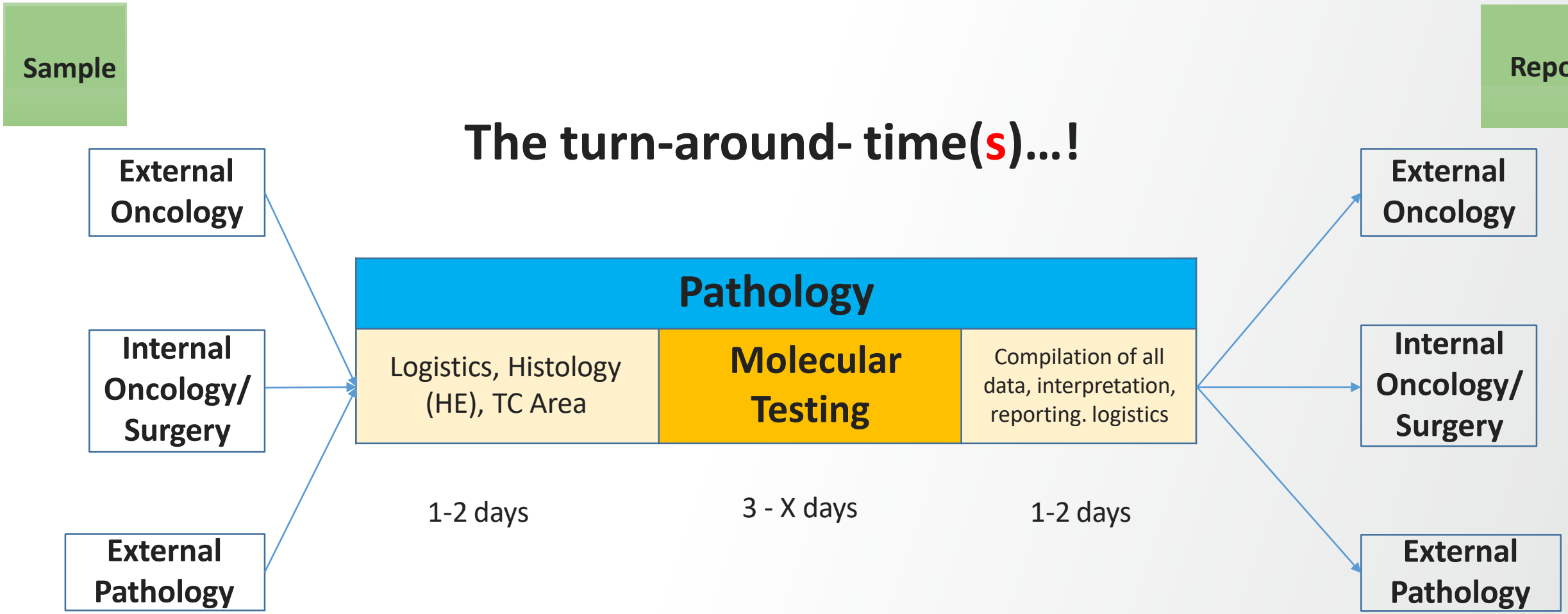
Major Genomic Alterations in NSCLS and How to Identify

- **CE-Sequencing**
  - LOD: 10-15%
  - TAT: 2 days
- **FISH**
  - LOD: 15%
  - TAT: 2days
- **IHC**
  - TAT: 1-2 days
- **qPCR**
  - LOD: 1%
  - TAT: 1 day
- **NGS**
  - LOD: 0.1%
  - TAT: 1 - 5 days

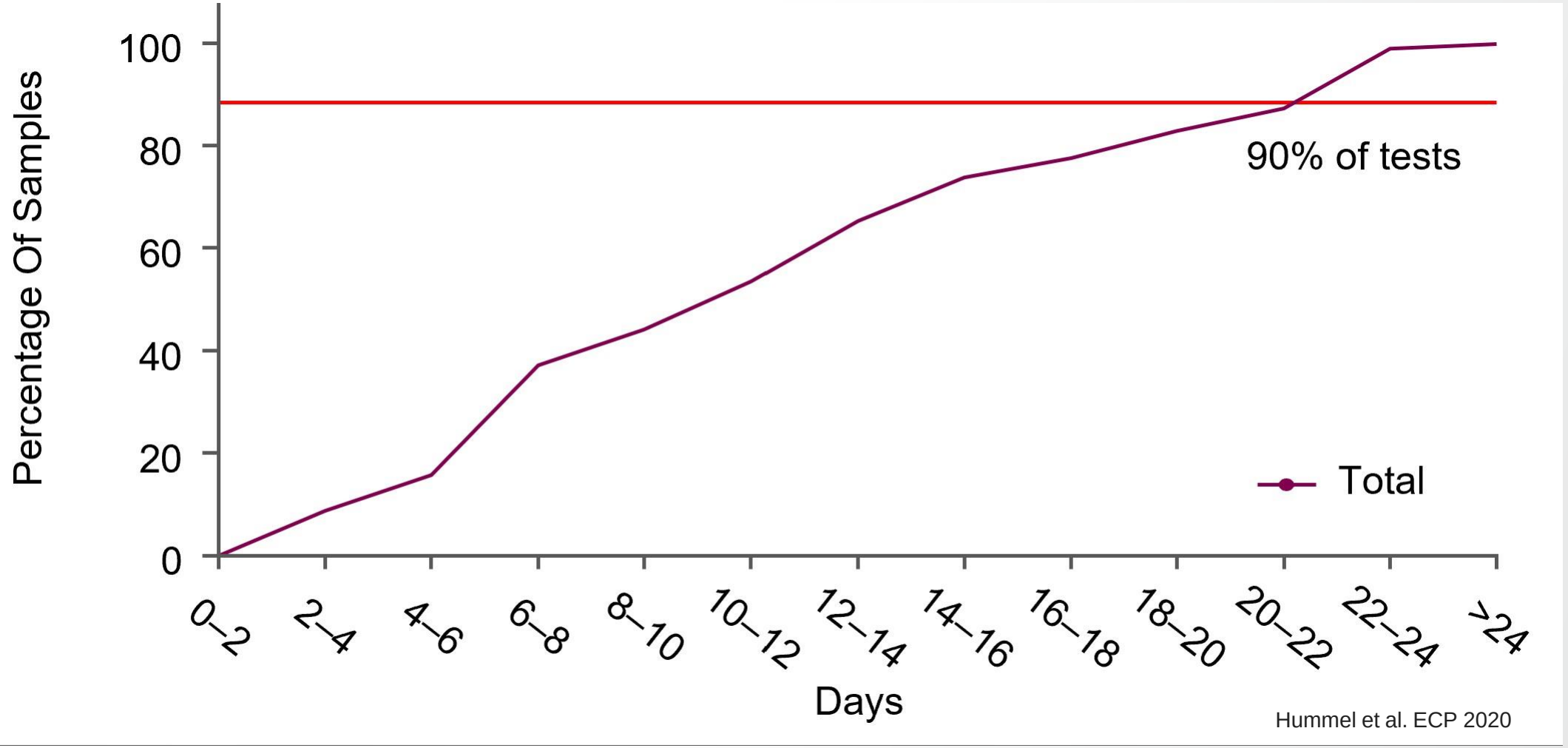


Modified from: McConaill et al (2010) - *J Clin Oncol*

The entire sample workflow – including molecular pathology



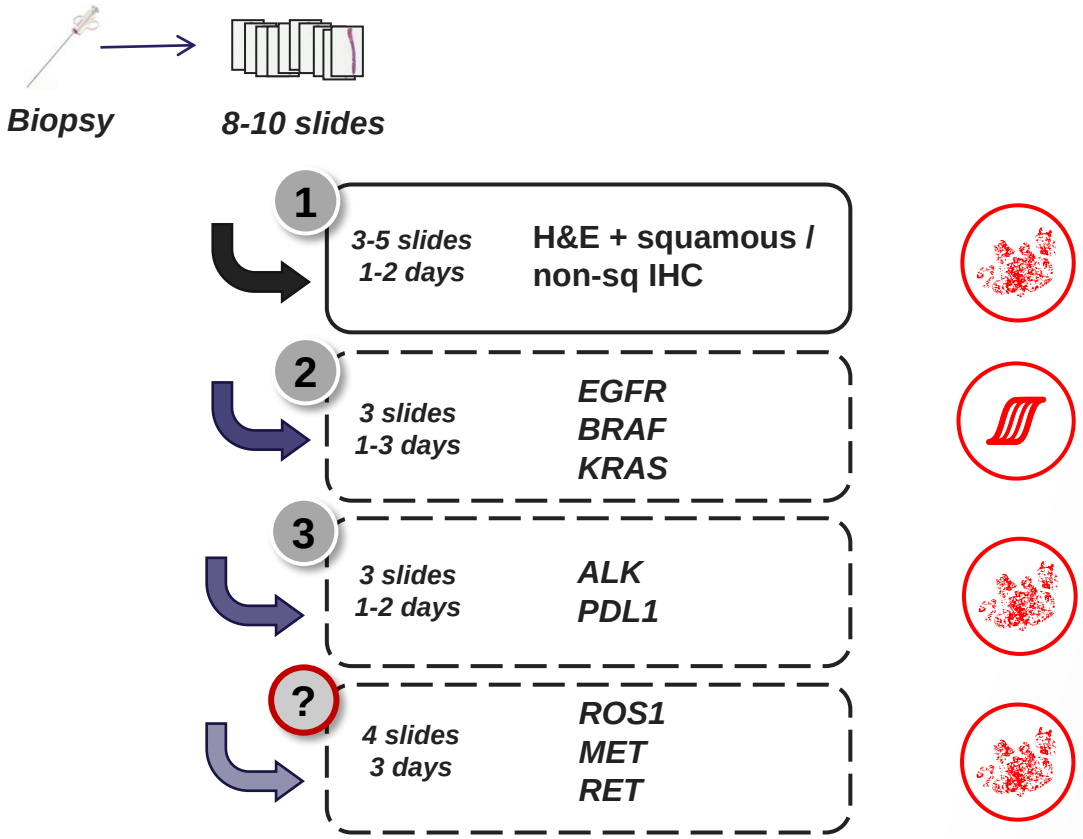
Testing for EGFR mutations: 16 centers in 4 EU countries



Hummel et al. ECP 2020

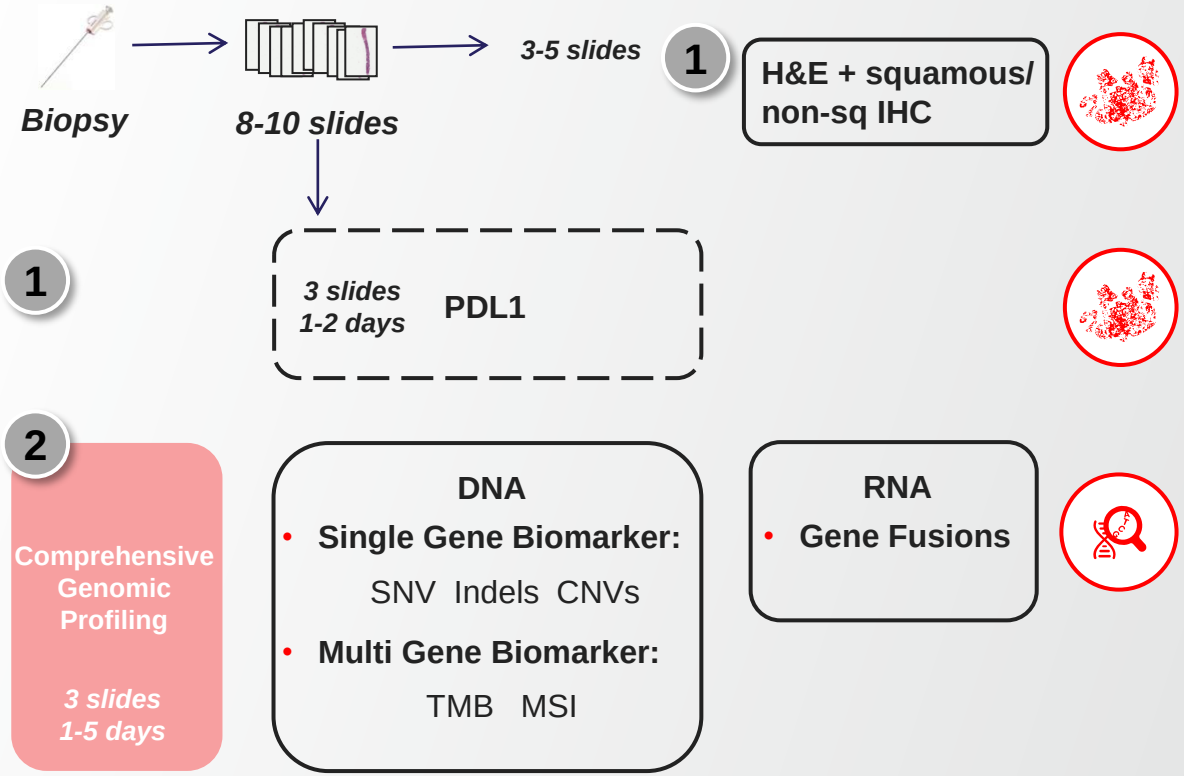
Multi-Test or Multi-Gene: Which Approach to choose?

Sequential Single Gene assays



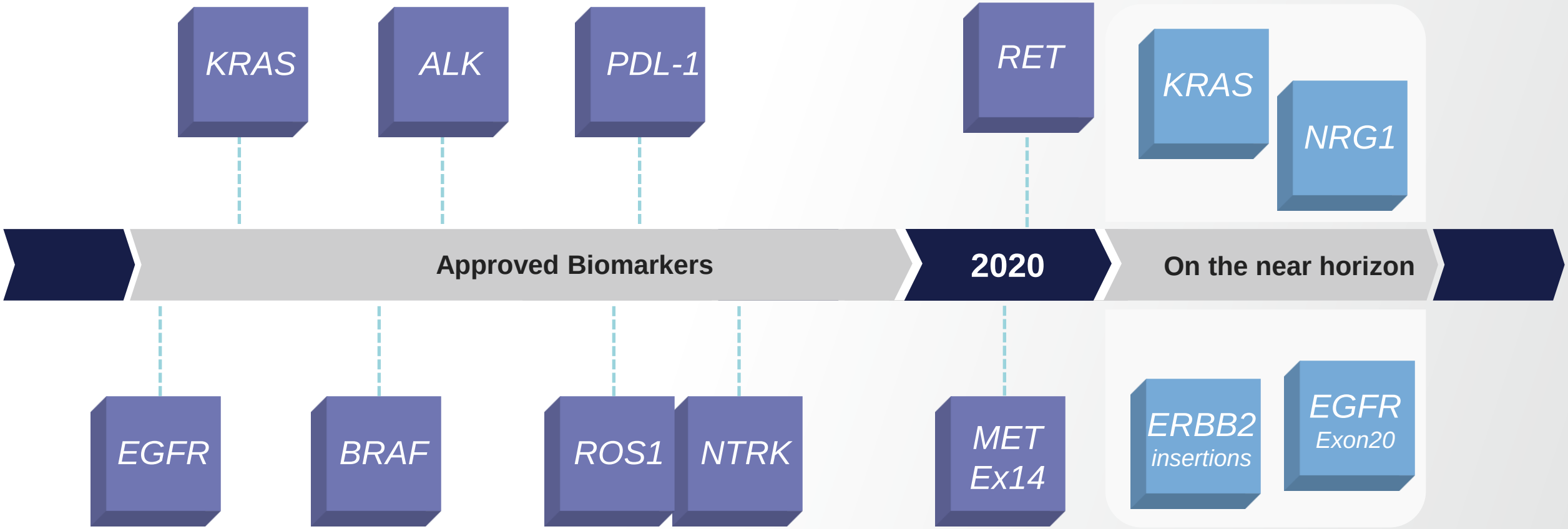
- Tissue often not sufficient for all assays
- Altogether associated with longer TAT

Multiplex assay through NGS panels



- Tissue not the Issue
- Shorter TAT

NSCLC Predictive Biomarkers Landscape



Tissue preservation has become a major challenge

The Ideal Testing Method

Fast

Hands Free

Cost Saving

Tissue Saving

Precision medicine: fast TAT and ease of use

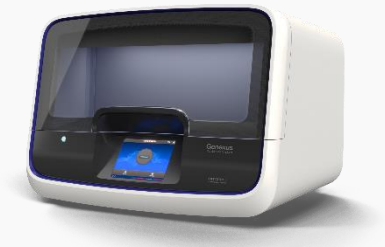
Genexus System - Specimen-to-Report NGS Workflow

- FFPE
- Tissue
- Bone marrow
- Whole blood
- PBL
- Urine
- Saliva

Genexus Software

Nucleic acid purification and quantitation*	Library preparation to variant interpretation	Report*

Ion Torrent™ Genexus™
Purification System (Available 2020)



2 hour turnaround time

12 FFPE (DNA and RNA)

6 Plasma

Ion Torrent
Integrated Sequencer (A

Ion Torrent™ GX5™ Chip:
12–15M reads/lane

14 hours for a s

(approx. 24 to 30 h

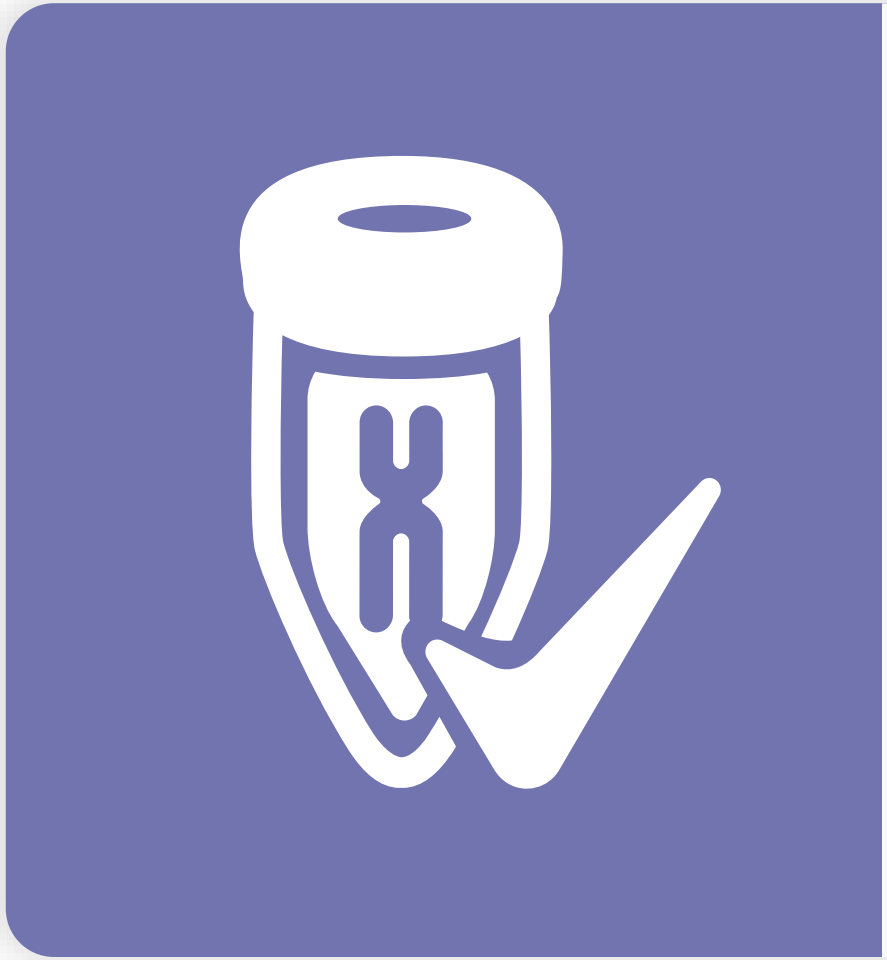
Up to 32 Sam

The Oncomine Precision Assay: Broad coverage of genomic alterations

The Oncomine Precision Assay content is carefully curated to include all relevant targets and targets of emerging importance in precision oncology and clinical research..

- 50 genes and 2,769 unique variants
- Mutations (45), CNVs (14), and fusion variants (19),
- Pan cancer span with NSCLC focus
- 218 potential resistance mutations across 22 genes

The Oncomine Precision Assay: Improved detection of fusion trancripts



Generally, there are **two** key features for optimal fusion detection:

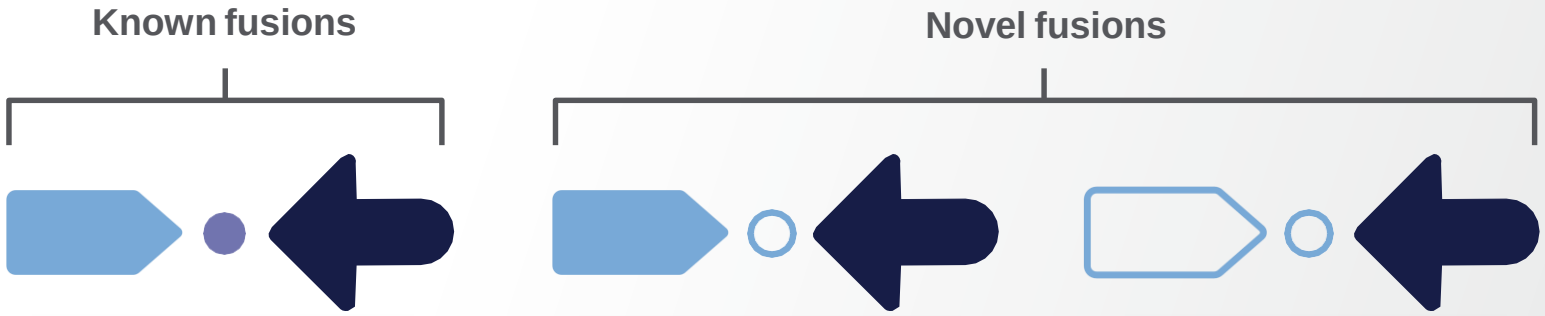
1. Performance of fusion detection with low input samples / low level transcripts
2. Ability to detect novel fusions for driver genes (e.g. *NTRK* and *FGFR*)

Many similar technologies emphasize #2 above but ignore #1.

With FusionSync™ detection, **BOTH #1 and #2**

Can be addressed

The Oncomine Precision Assay: Strategy for fusion transcript detection



Method	*Targeted*	*Non-targeted*	*Exon* tiling imbalance*
Driver	✓ Known	✓ Known	✓ Known
Partner	✓ Known	✓ Known	○ Unknown
Breakpoint	✓ Known	○ Unknown	○ Unknown

** Available for ALK, FGFR1, FGFR2, FGFR3, NTRK1, NTRK2, NTRK3, and RET fusion drivers*

Multi-centre study for evaluation of Genexus and Oncomine Precision Assay (OPA)

- The following sites contributed to this study: Porto, Basel, Charité Berlin, and Naples
- Each external customer site selected own banked samples pre-characterized by other assays and/or technologies.
- Since samples were unique to each site, reproducibility across sites was not assessed; however where possible concordance to previous results was completed.
- Results in this presentation will be structured by sample type (FFPE vs. plasma) and by variant type; mutations (SNV + INDEL), copy number variation, and fusions

FFPE Control Performance

Horizon Structural Multiplex Reference Standard (FFPE)

Variant Name	Berlin
AKT1 p.E17K	ND
EGFR p.E746_A750del	2.40%
EGFR p.A767_V769dup	2.40%
GNA11 p.Q209L	4.70%
PIK3CA p.E545K	5.60%
MET Amplification	2.07

Seraseq® Fusion RNA Mix v4

Variant Name	Berlin
ERBB2 Amplification	4.57
FGFR3 Amplification	2.33

Seraseq® FFPE NTRK Fusion RNA Reference Material

Variant Name	Berlin
AFAP1(14) - NTRK2(12)	●
BTBD1(4) - NTRK3(14)	●
ETV6(4) - NTRK3(14)	●
ETV6(4) - NTRK3(15)	●
ETV6(5) - NTRK3(14)	●
ETV6(5) - NTRK3(15)	●
IRF2BP2(1) - NTRK1(10)	●
LMNA(11) - NTRK1(11)	●
NACC2(4) - NTRK2(13)	●
PAN3(1) - NTRK2(17)	●
QKI(6) - NTRK2(16)	●
SQSTM1(5) - NTRK1(10)	●
TFG(5) - NTRK1(10)	●
TPM3(7) - NTRK1(10)	●
TRIM24(12) - NTRK2(15)	●

Seraseq® Fusion RNA Mix v4

Variant Name	Berlin
CCDC6(1) - RET(12)	●
CD74(6) - ROS1(34)	●
EGFR(1) - EGFR(8)	●
EML4(13) - ALK(20)	●
ETV6(5) - NTRK3(15)	●
FGFR3(17) - BAIAP2L1(2)	●
FGFR3(17) - TACC3(11)	●
KIF5B(24) - RET(11)	●
LMNA(2) - NTRK1(11)	●
MET(13) - MET(15)	●
NCOA4(7) - RET(12)	●
SLC34A2(4) - ROS1(34)	●
SLC45A3(1) - BRAF(8)	●
TFG(5) - NTRK1(10)	●
TPM3(7) - NTRK1(10)	●

Plasma Control Performance

CNV Fusion Plasma Control

Variant Name	Berlin
EGFR Amplification	1.6
MET Amplification*	1.27
TRMT61B(1) - ALK(9)	
EML4(6) - ALK(20)	
EML4(6) - ALK(20)	
SLC34A2(4) - ROS1(32)	
SLC34A2(4) - ROS1(34)	
MET(13) - MET(15)	
CCDC6(1) - RET(12)	

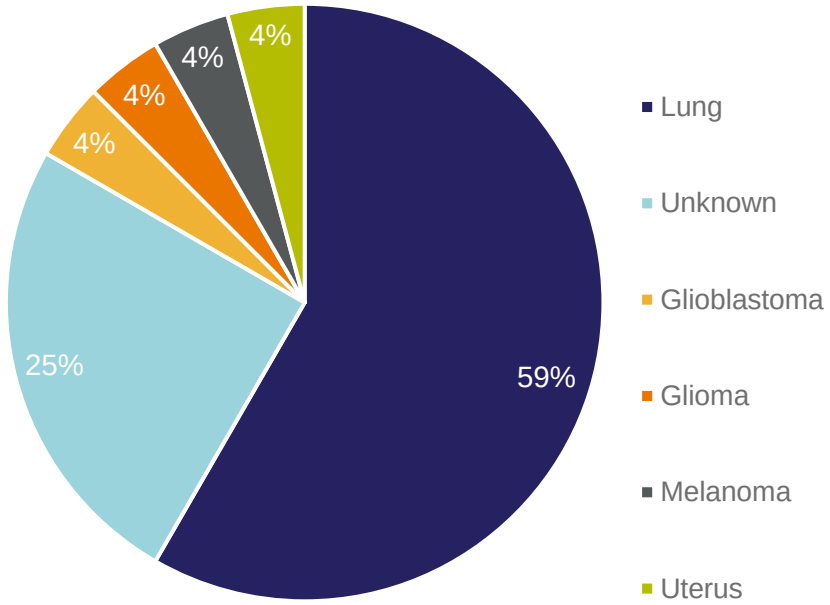
Horizon 1% Multiplex I cfDNA Reference Standard

Variant Name	Berlin
NRAS p.Q61K	1.19%
NRAS p.A59T	1.29%
PIK3CA p.E545K	1.61%
EGFR p.E746_A750del	0.91%
EGFR p.A767_V769dup	1.14%
EGFR p.T790M	0.69%
EGFR p.L858R	0.58%
KRAS p.G12D	1.18%

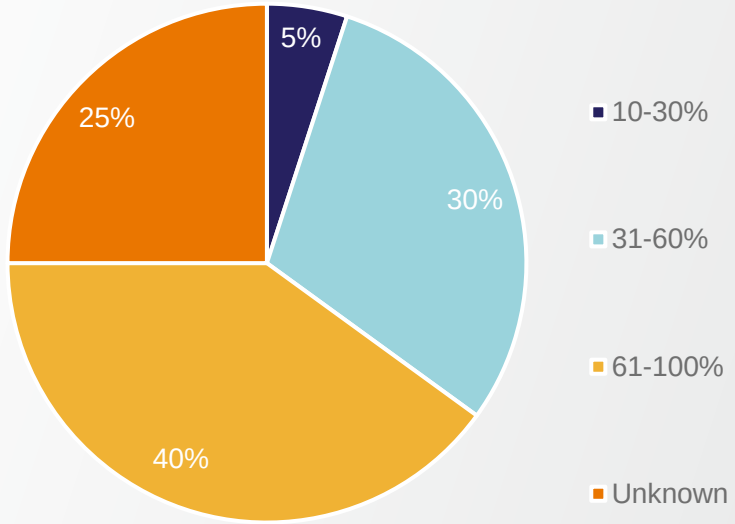
- MET Amplification in CNV Fusion Plasma Control is close to LOD therefore expected to be detected in 50%

FFPE Samples Used for Detection of Fusions

23 FFPE samples of various cancer types with range of tumor content was tested for fusions



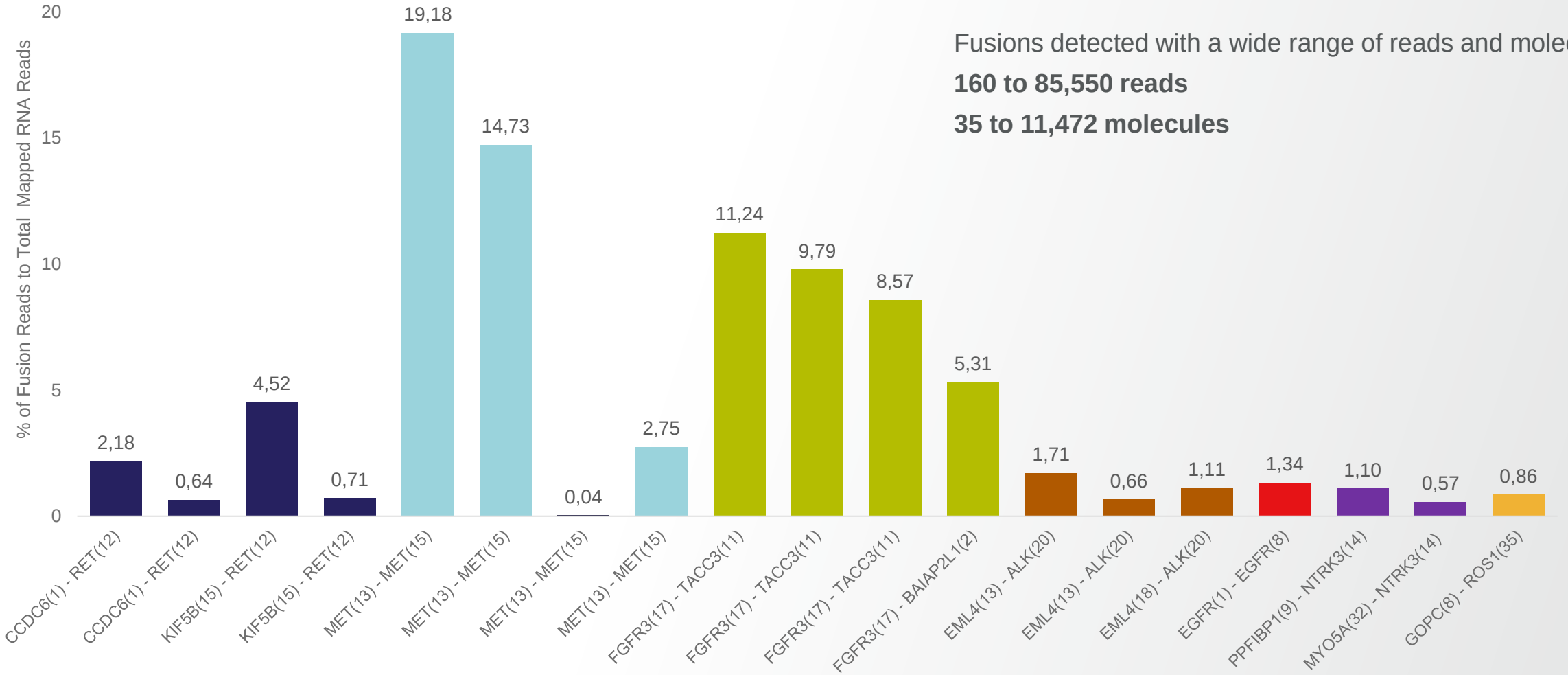
Distribution of tumor type



Distribution of tumor content

Targeted Fusion Detection Across Varying Isoforms and Driver Genes

Fusion isoforms detected with a wide range of relative transcript level (fusion reads/total RNA reads)



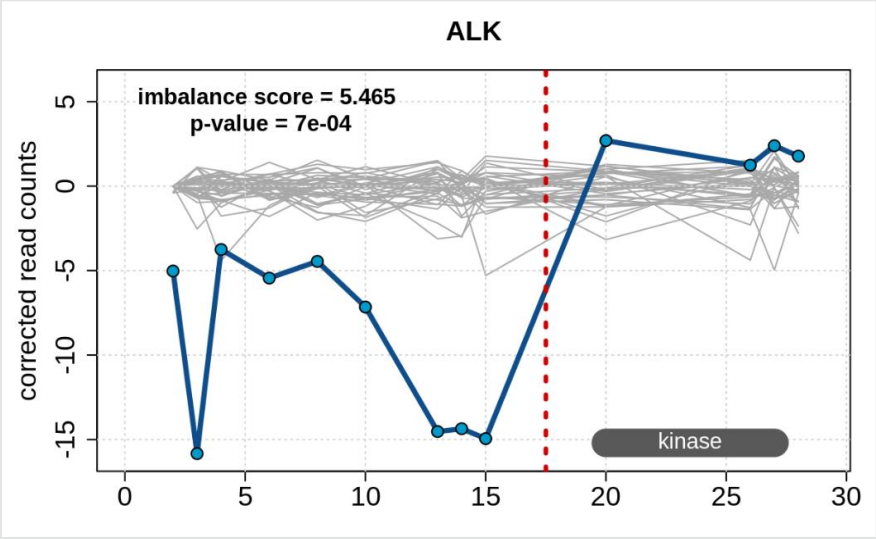
Fusions detected with a wide range of reads and molecules:

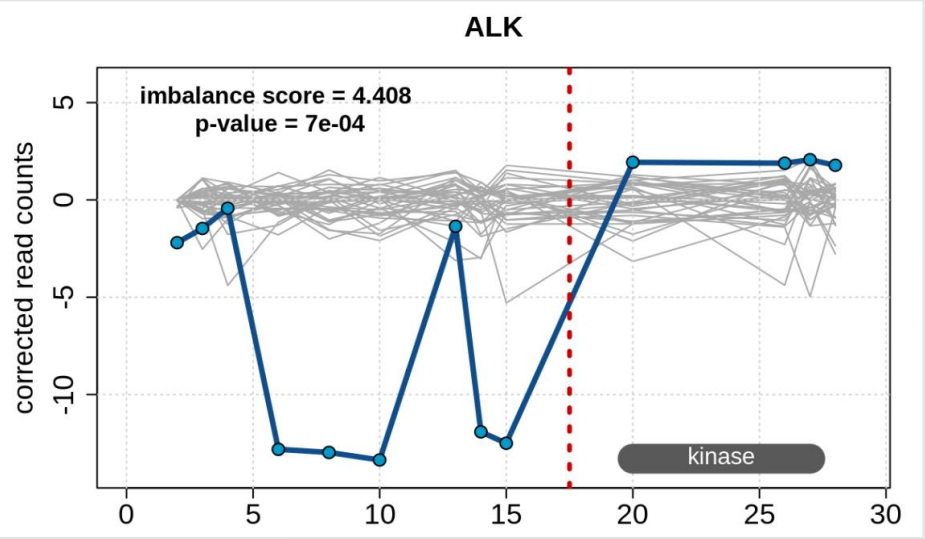
160 to 85,550 reads

35 to 11,472 molecules

Novel Fusion Detection in FFPE Samples without known isoform

Detection of two novel ALK fusions



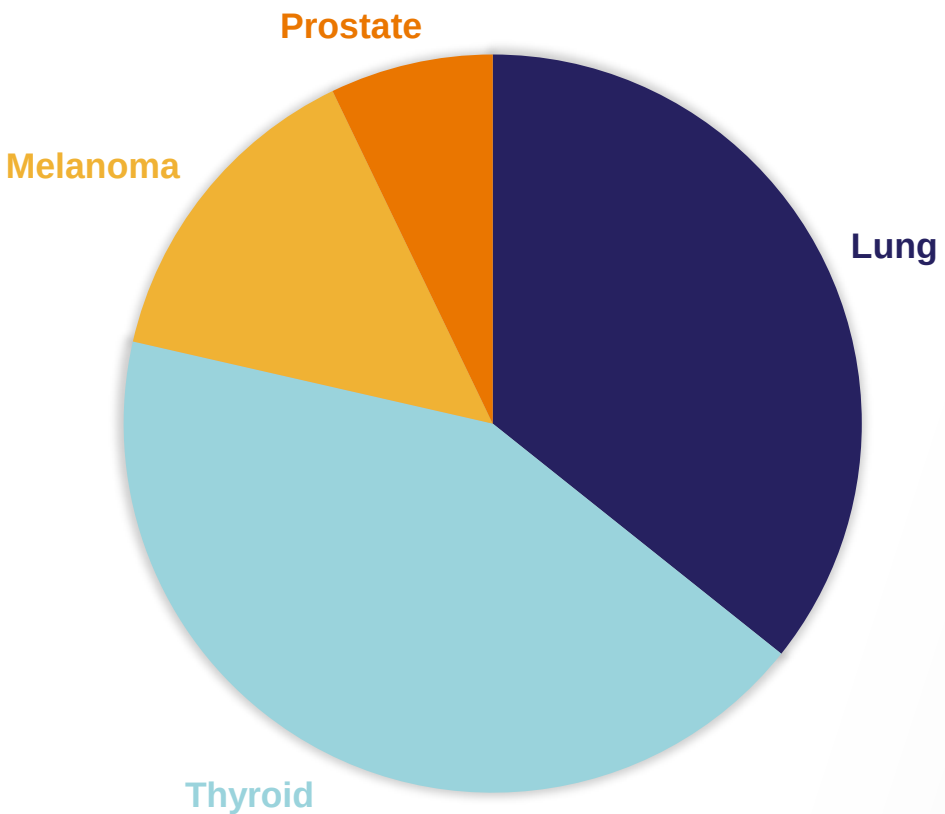


Testing using OPA/Genexus also suggested the presence of a novel ALK fusion (passing imbalance score and p-value). Both samples have a predicted breakpoint outside of the kinase domain, suggesting a potential activating fusion transcript.

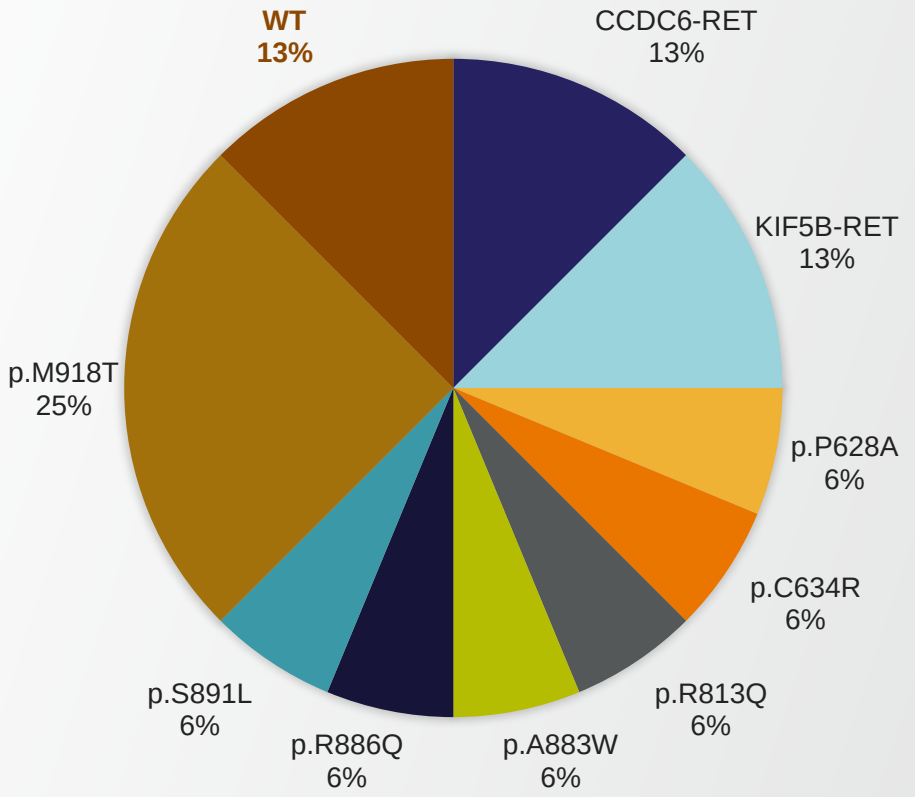
What's Next? RET fusion and mutation study with Genexus

16 FFPE samples of various cancer types with range of tumor content will be tested for RET fusions and RET mutations

Distribution of tumor type



Distribution of mutation



Conclusions

- Molecular diagnostics is becoming progressively demanding
- Flexible adaption to the lab needs is very important
- Increasing complexity requires solutions to minimized the workload
- Genexus is a potential future solution combining all steps of diagnostic NGS
- The resulting data are very robust and reliable across different sites
- Implementation of high-volume gene panels on Genexus

Acknowledgment

The colleagues of the other VTS sites: Basel, Naples and Porto

The colleagues of Thermo Fisher Scientific

The molecular pathology team at Charité Berlin, especially Burkhard Hirsch an Karsten Kleo