

Molecular Diagnostics 2024 *Symposium*

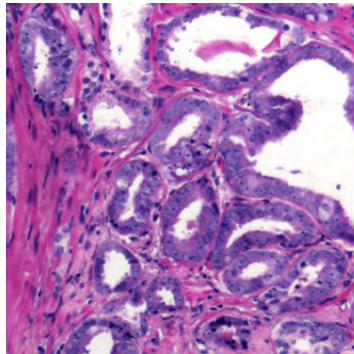
State of the Art and Real-World Molecular Testing Practices for Non-Small-Cell Lung Cancer



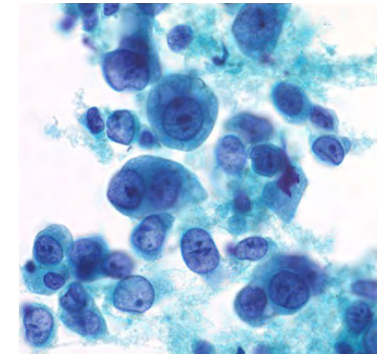
Obinna Chijioke
Institute of Medical Genetics and Pathology

Pathology

Histopathology



Cytopathology



Molecular Pathology

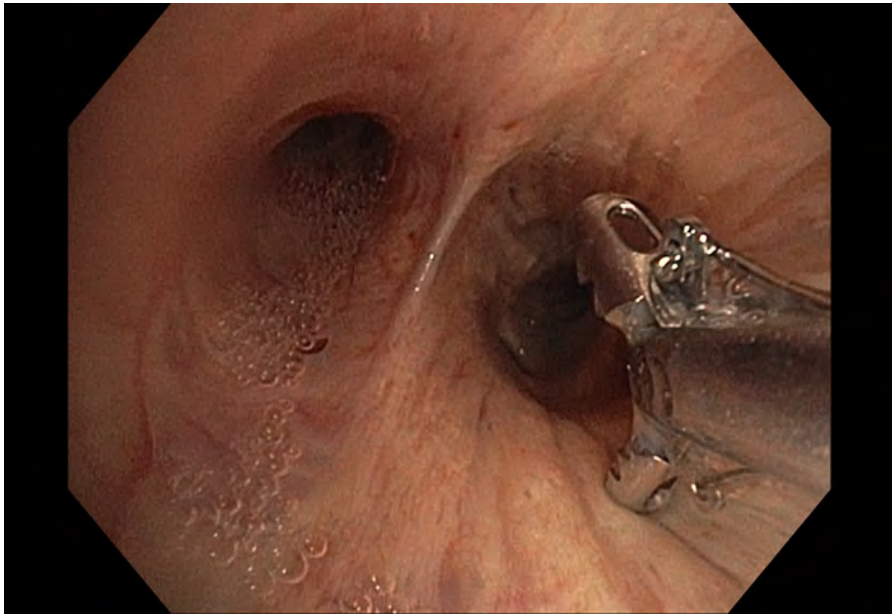


Autopsy

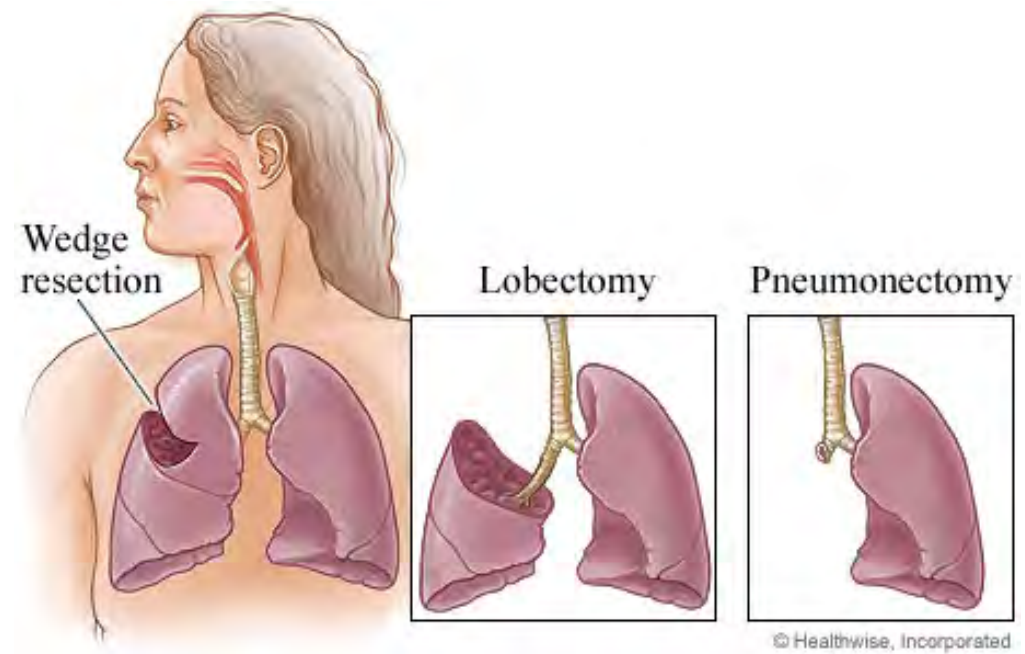


Histology

biopsy



surgery



Histology: sample processing



- formalin fixation
- paraffin embedding



- cutting: 5 μm
- staining (HE, ...)



Cytology

exfoliative cytology

fluids, surfaces (pleural effusion, urine, bronchus, uterine cervix ...)



fine needle aspiration

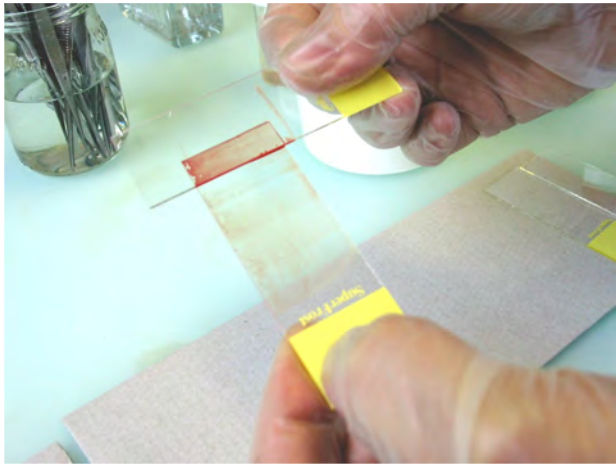
superficial or deep



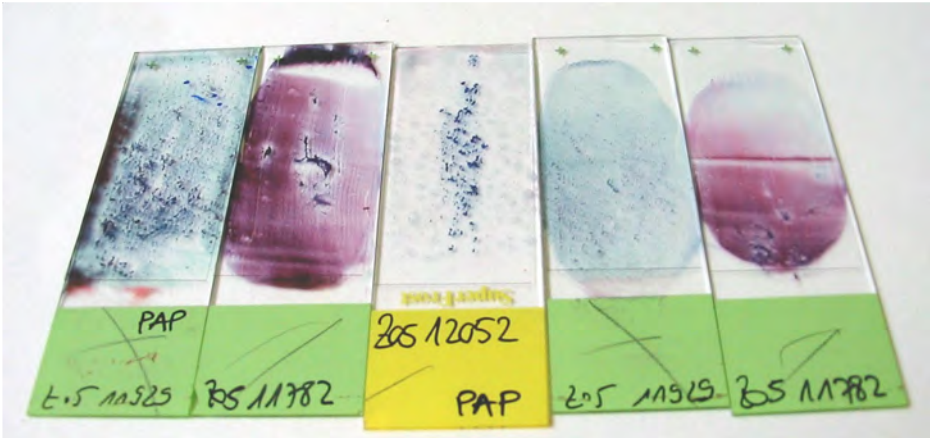
Cytology: effusions (exfoliative cytology)



Cytology: sample processing

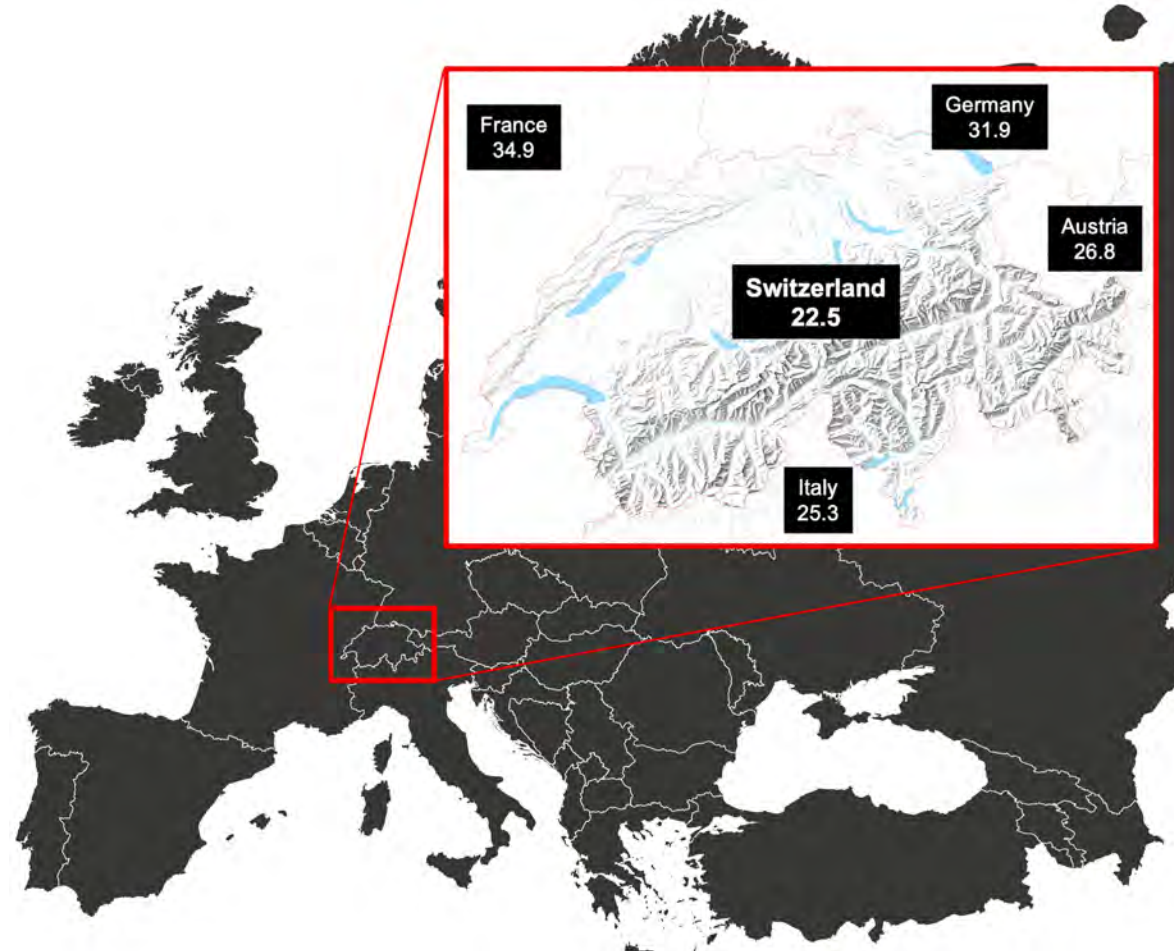


- alcohol fixation
- air drying



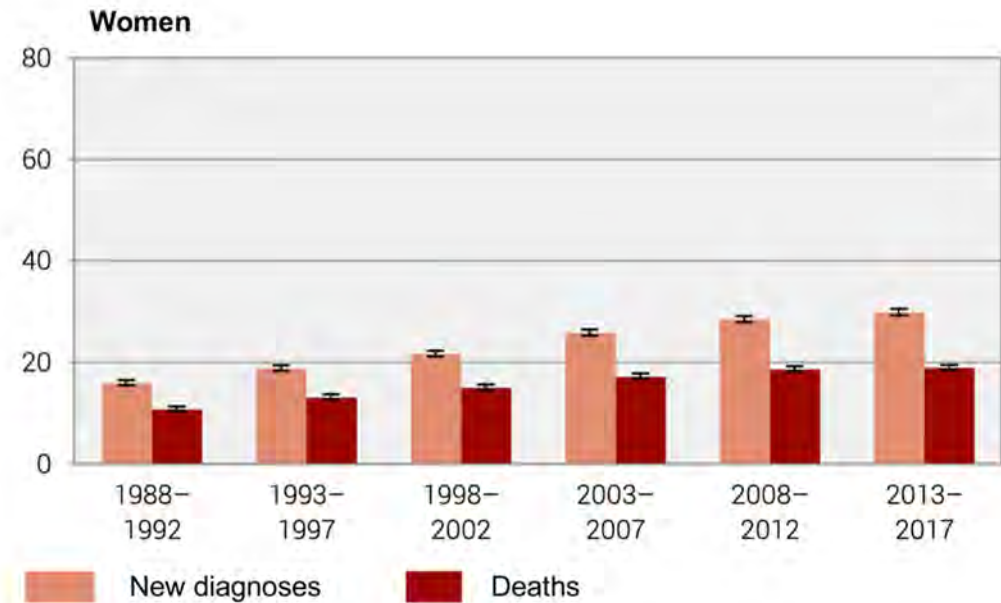
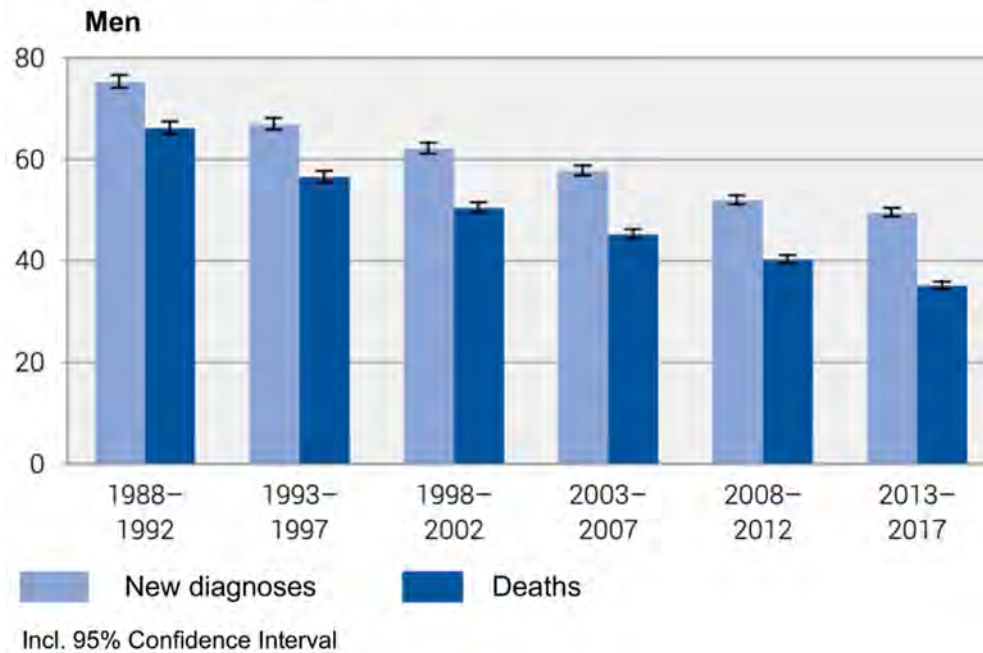
staining

Lung cancer: incidence

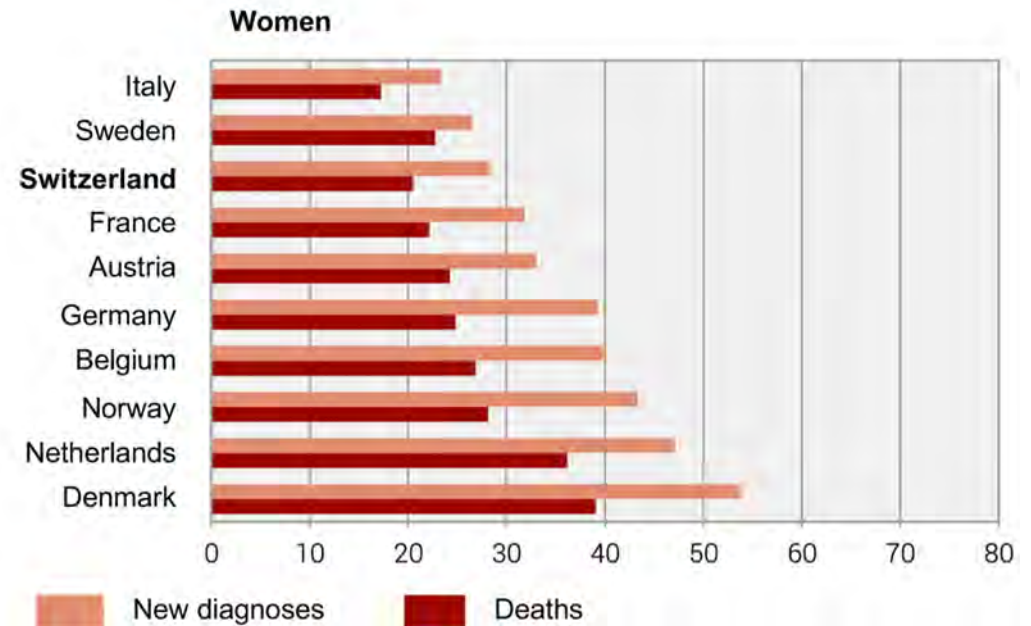
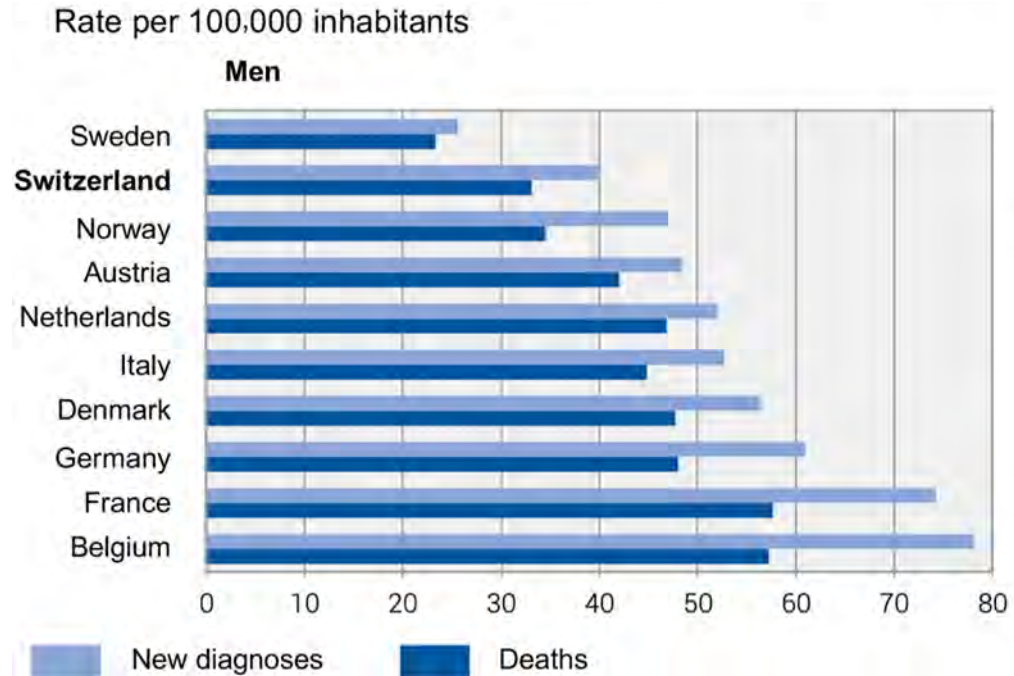


Lung cancer: incidence

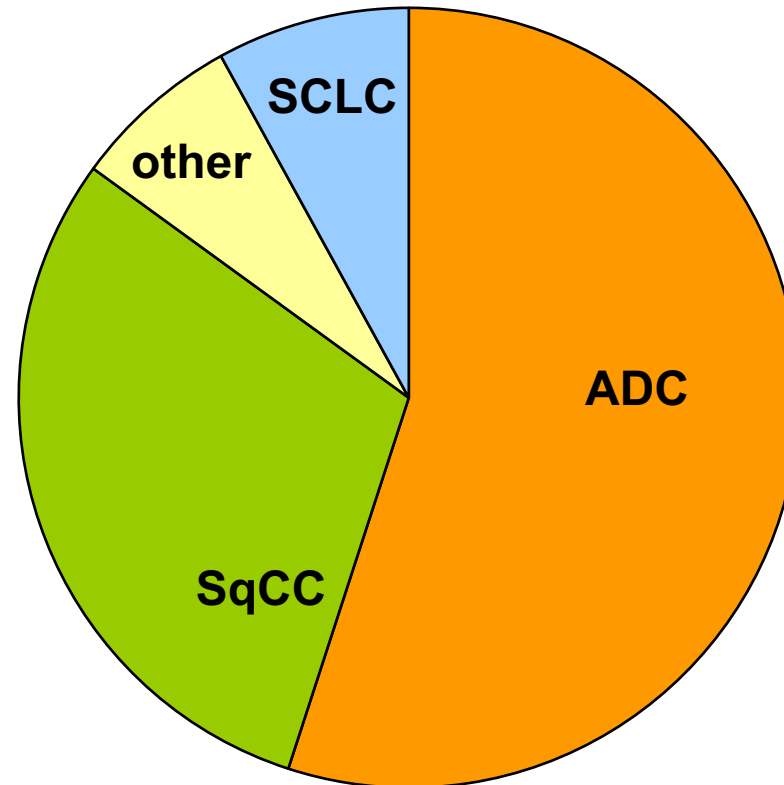
Rate per 100,000 inhabitants



Lung cancer: incidence



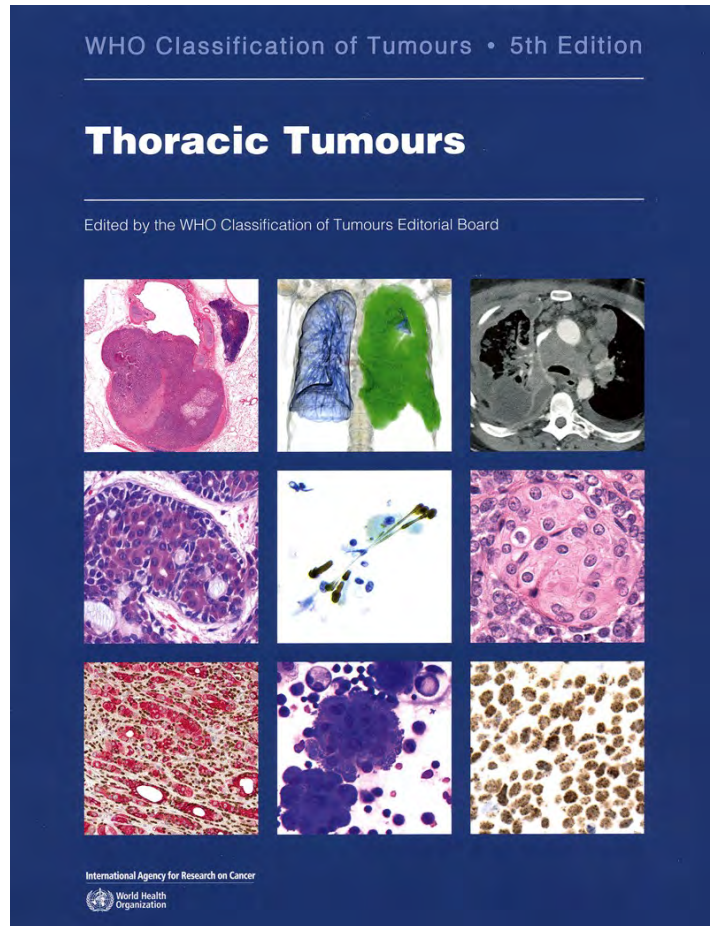
Lung cancer: subtypes



ADC: adenocarcinoma; **SqCC:** squamous cell cancer; **SCLC:** small cell lung cancer

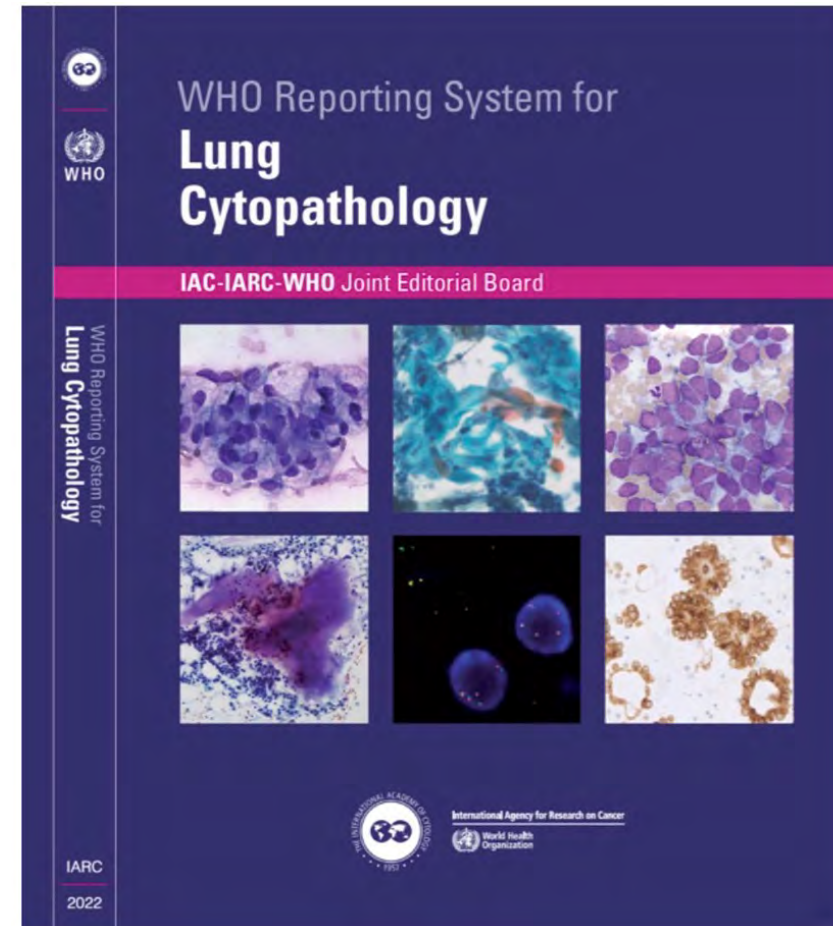
WHO Classification and Reporting System

the “blue book”



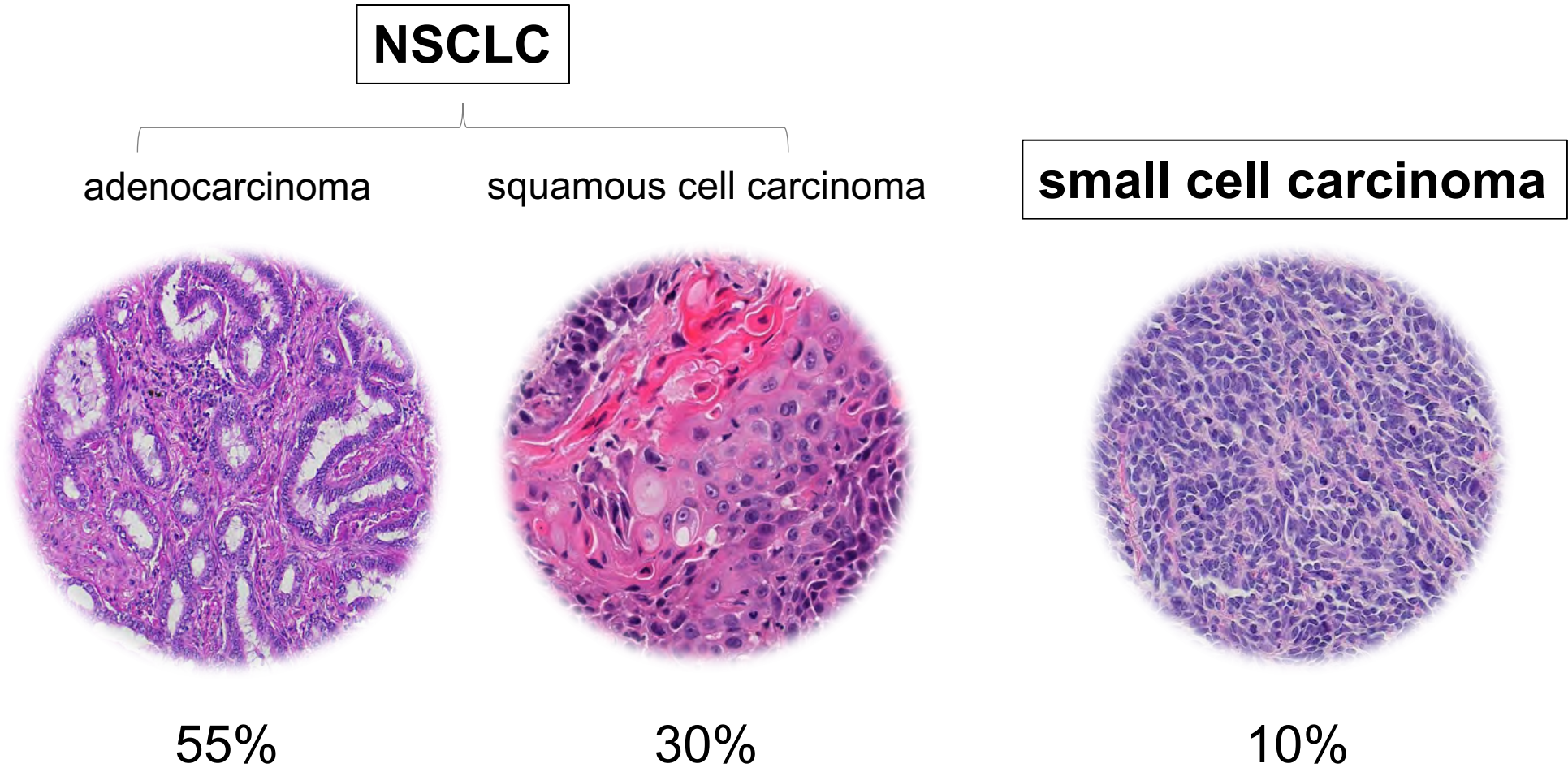
IARC/WHO, 2021

the “purple book”



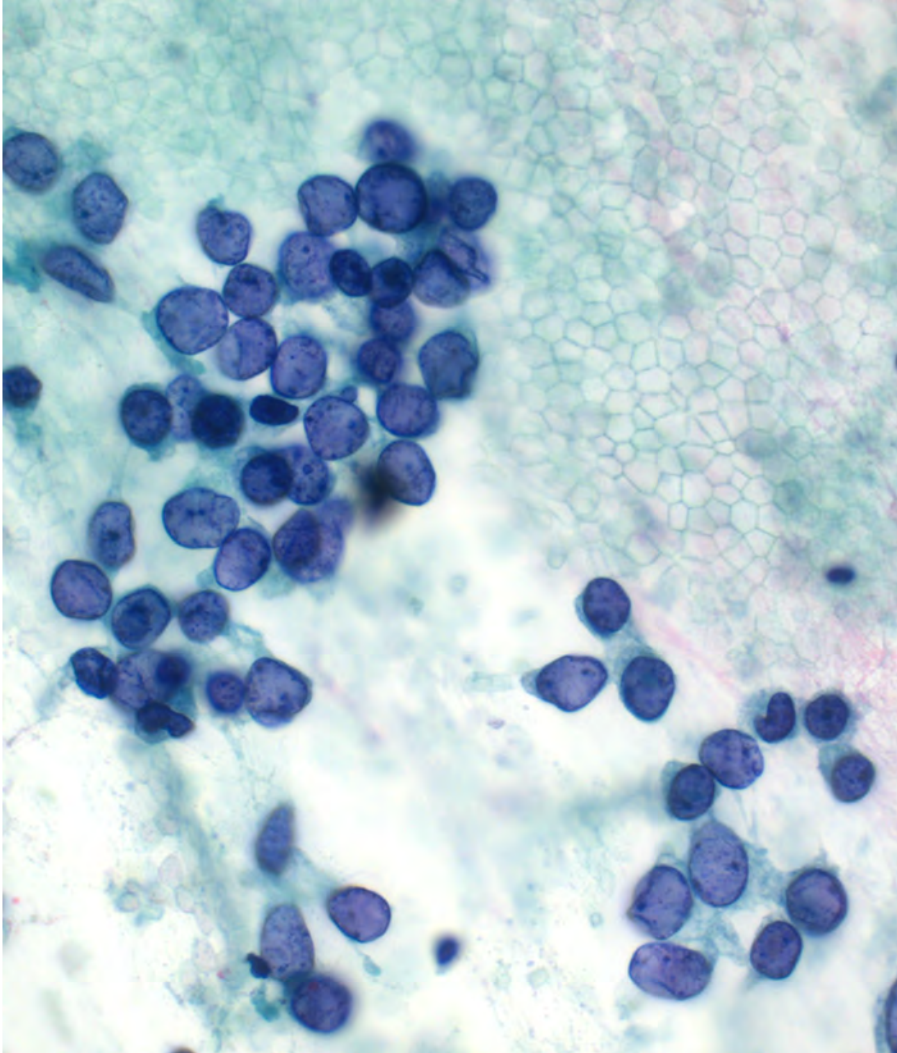
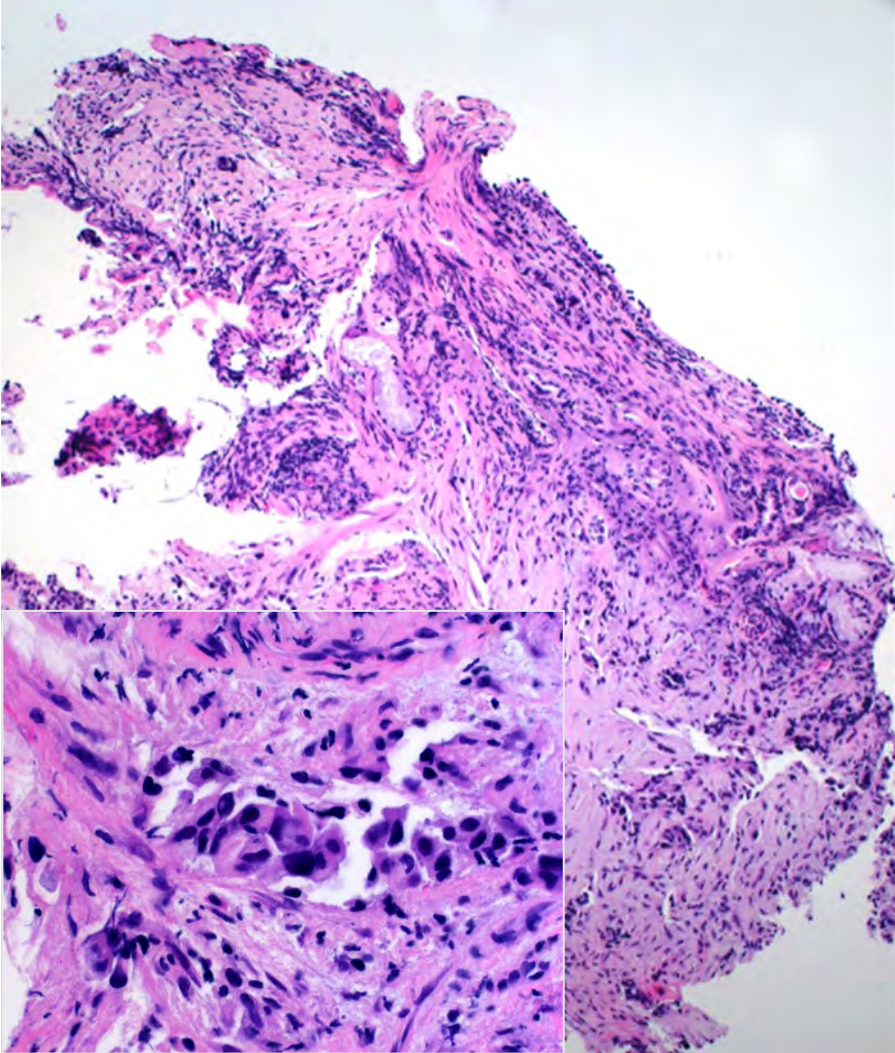
IAC – IARC/WHO, 2022

Lung cancer: subtypes



NSCLC: non-small cell lung cancer

NSCLC: subtypes

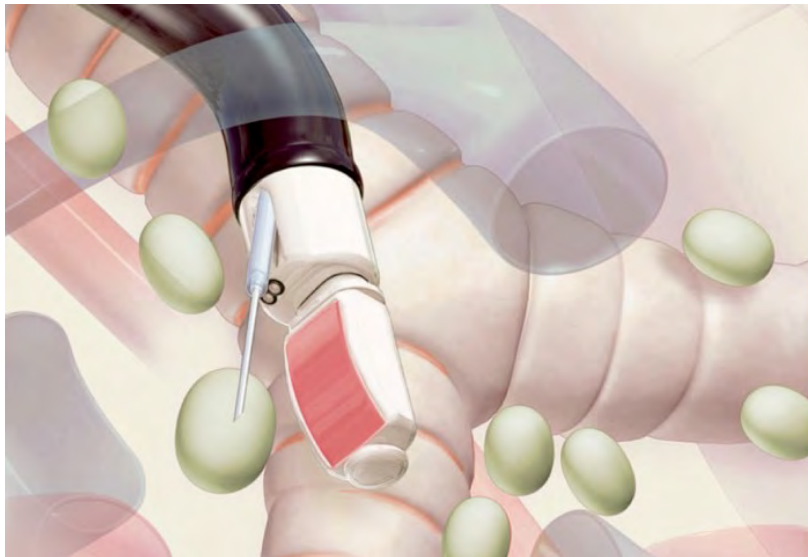


NSCLC: stage

70% of NSCLC are in an **advanced**, inoperable stage:

- diagnosis and predictive markers on small samples
- 40% diagnosed by cytology

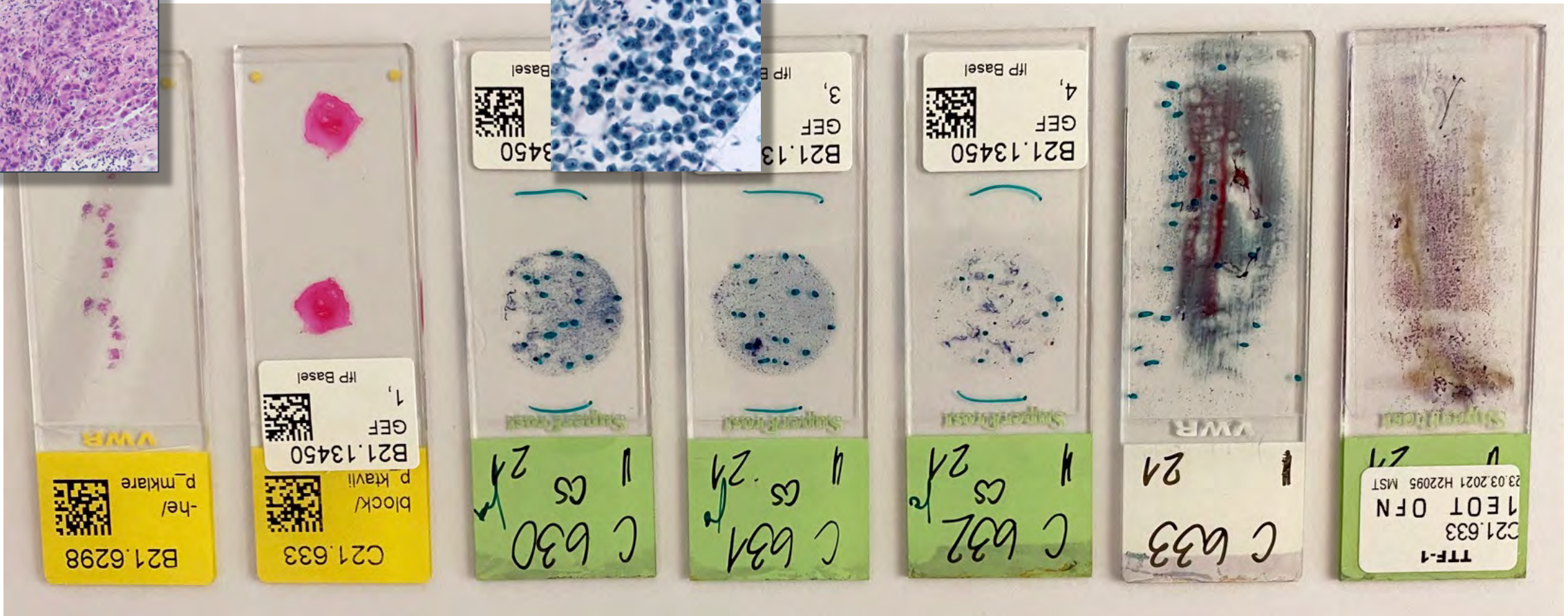
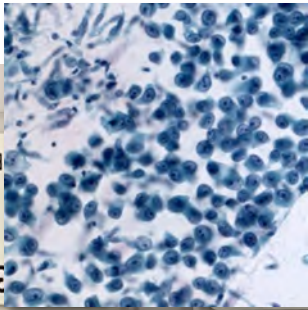
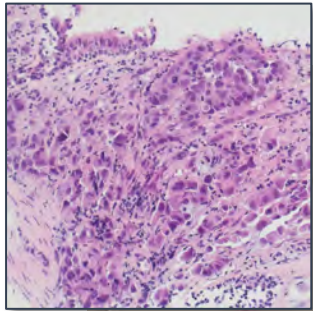
endobronchial ultrasound-guided
TBNA



electromagnetic navigation
bronchoscopy



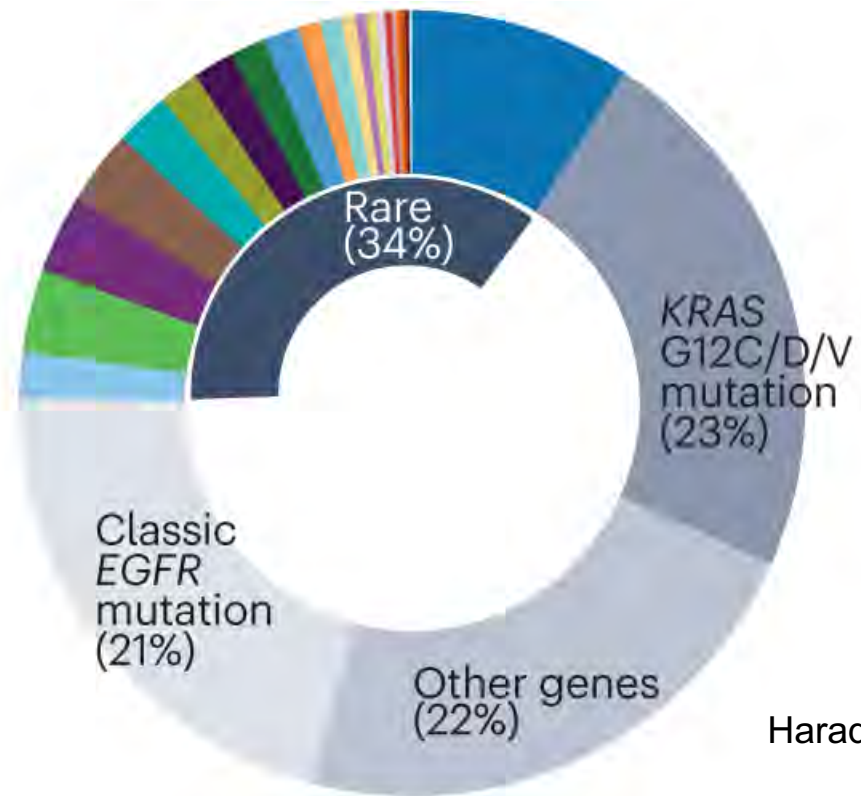
NSCLC: the material we get



every case is different

NSCLC: predictive biomarkers

- all NSCLC: **PD-L1** by IHC
- non-squamous NSCLC: testing for **targetable oncogenic drivers**



Methods for biomarker analysis

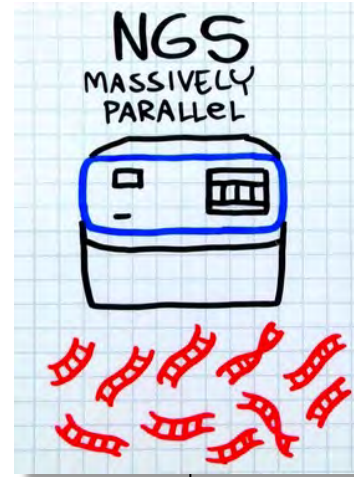
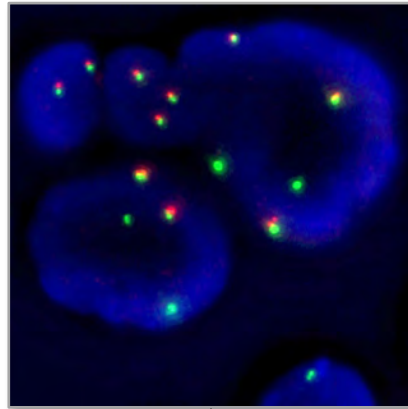
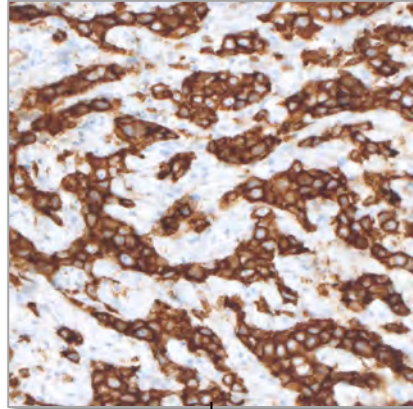
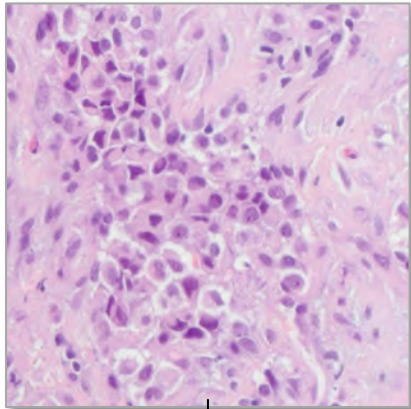
histological
Subtype

ALK/ROS1/NTRK
MET/PD-L1

ALK/ROS1/RET/
NTRK/MET

- predictive mutations
- rearrangements
- “mutational burden”

- resistance mutations
- monitoring



histology/
cytology

IHC

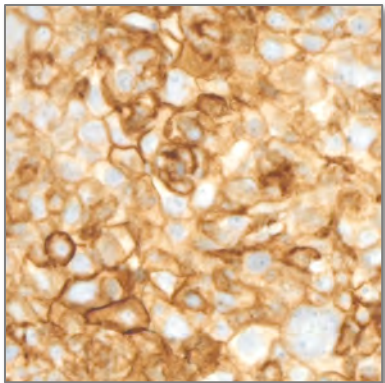
FISH

NGS
(DNA/RNA)

liquid biopsies
(ctDNA)

NSCLC: predictive biomarkers - IHC

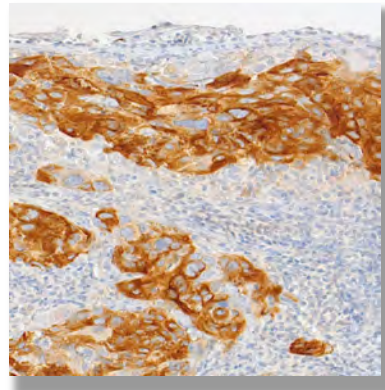
unrelated to
genetic alteration



PD-L1

all NSCLC

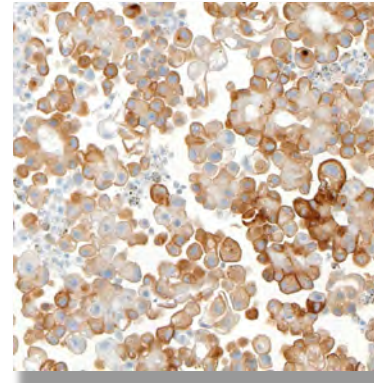
surrogate marker of
genetic alteration



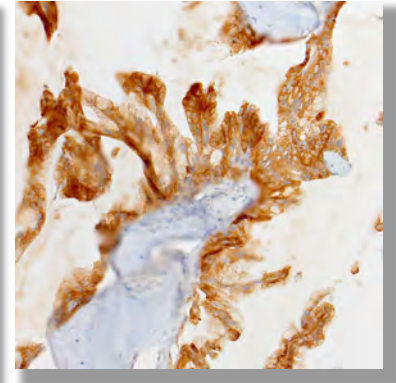
ALK

non-squamous NSCLC

confirmation needed



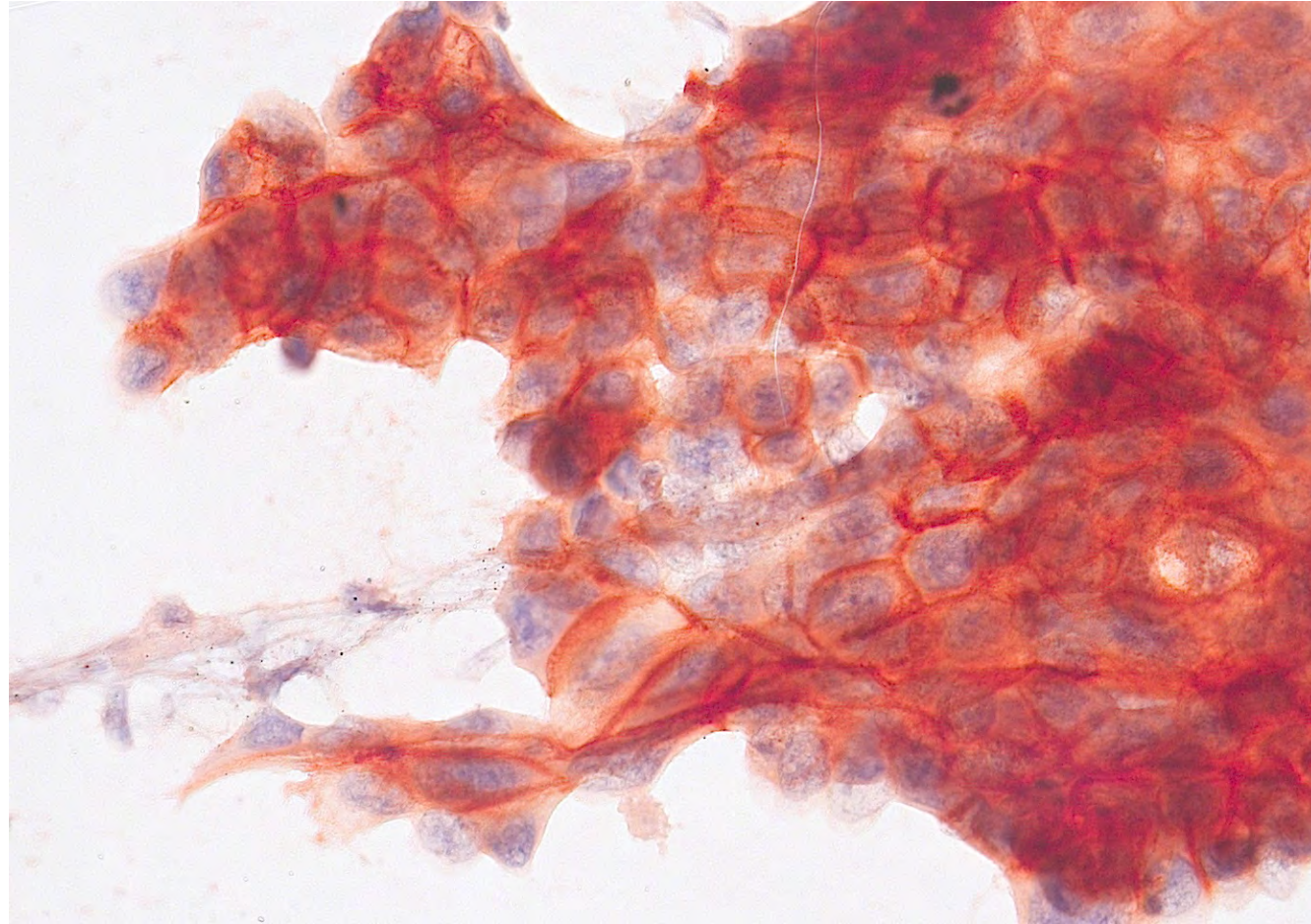
ROS1



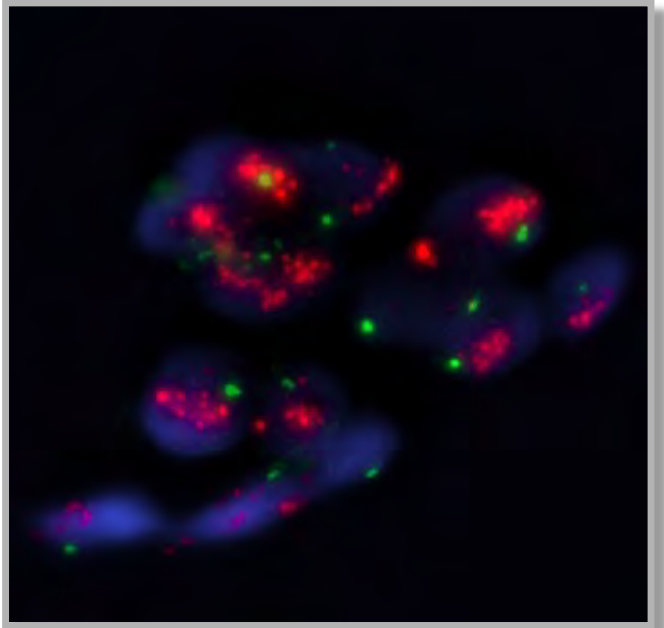
NTRK



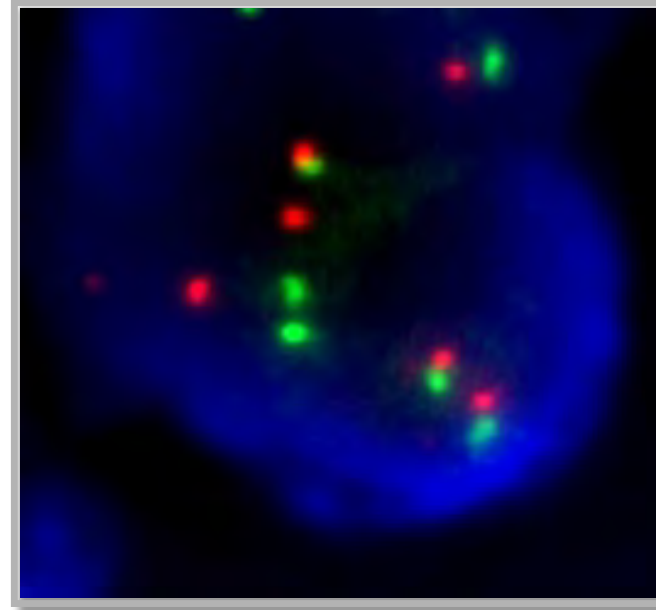
NSCLC: predictive biomarkers - PD-L1



NSCLC: predictive biomarkers - FISH

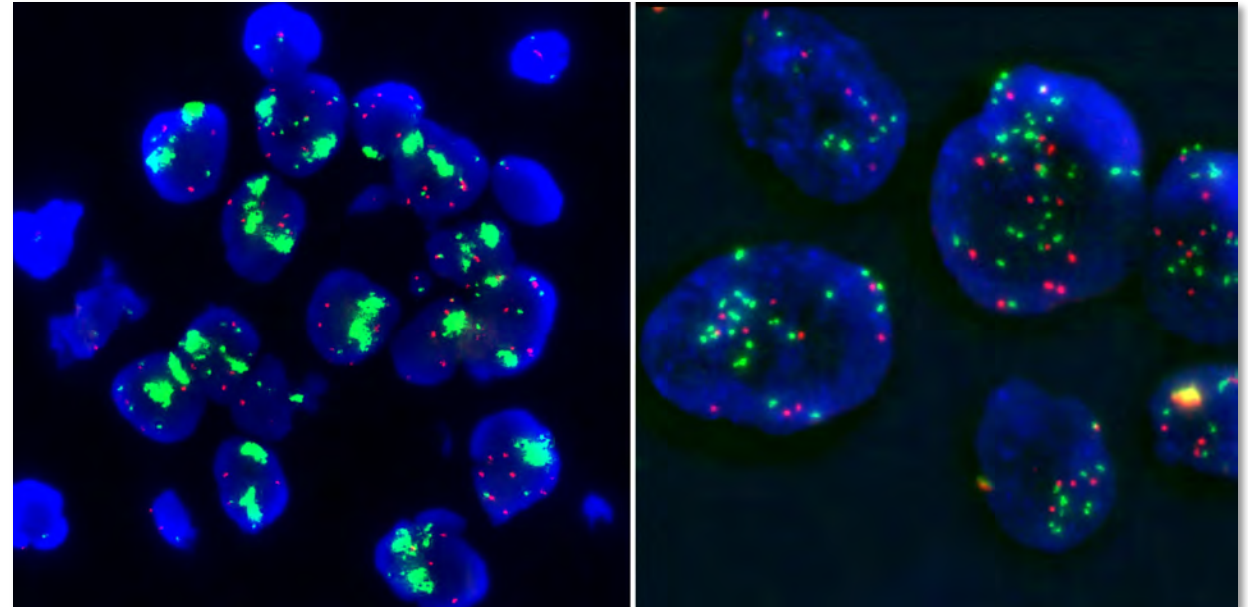
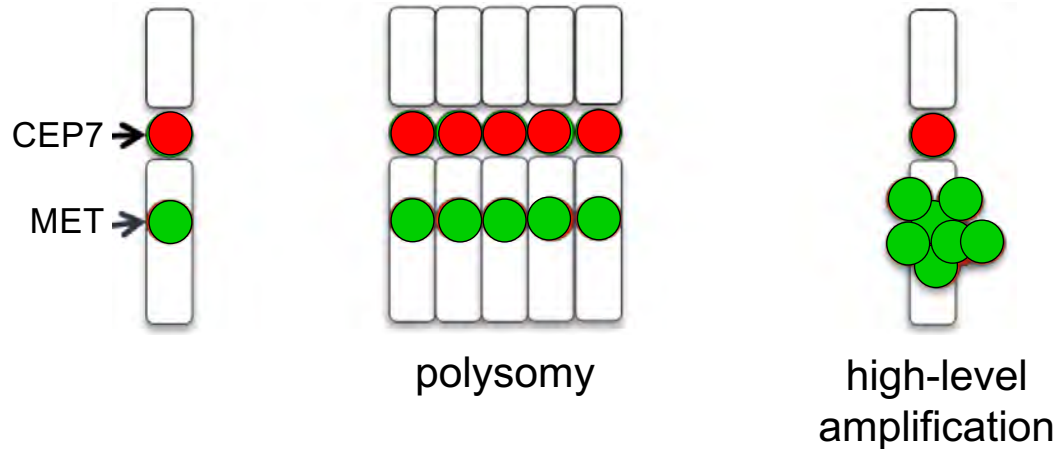


amplifications
(MET, HER2)



rearrangements
(ALK/ROS1/RET/NTRK1-3)

NSCLC: MET amplification



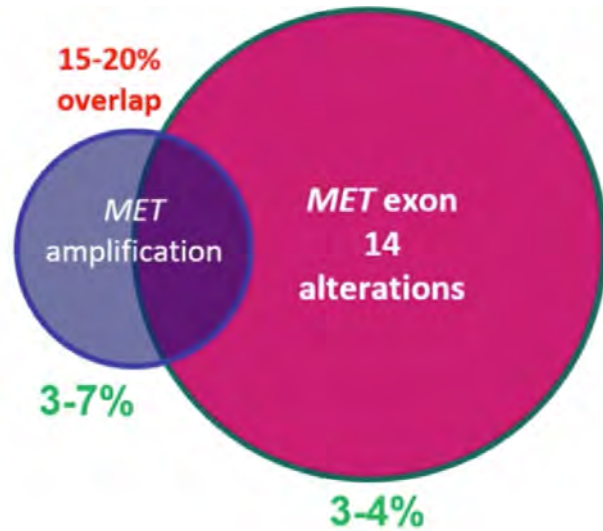
MET/CEP7: >5
(high-level)

MET/CEP7: 2.8
(intermediate level)

best response to MET inhibitors in NSCLC with **high-level amplification** (GCN ≥ 10 , MET/CEN7 ratio ≥ 5.0) **determined by FISH**

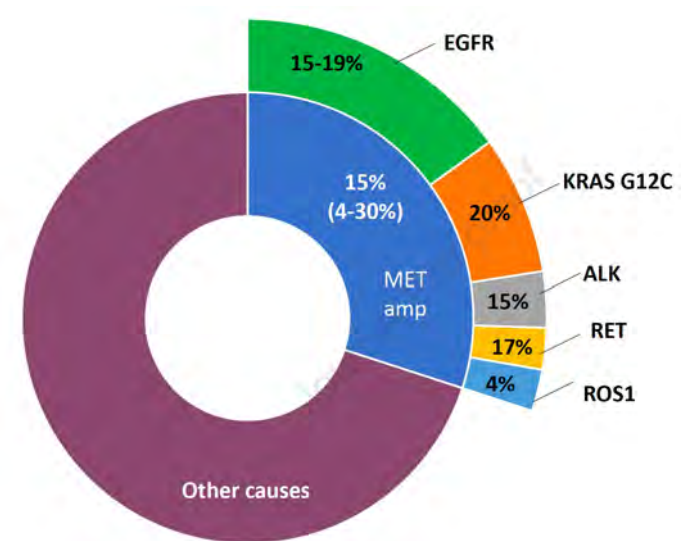
NSCLC: MET amplification

as a primary driver



courtesy Laetitia Mauti

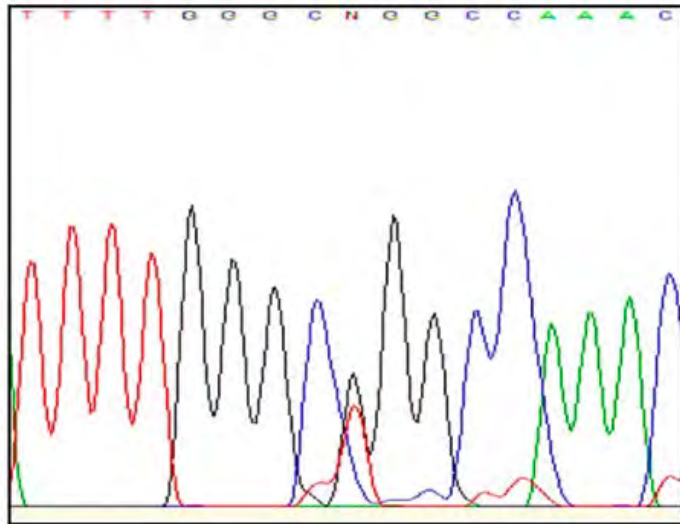
as mechanism of acquired resistance



Comoglio et al., Nat Rev Cancer 2018; Remon et al., J Thorac Oncol 2022

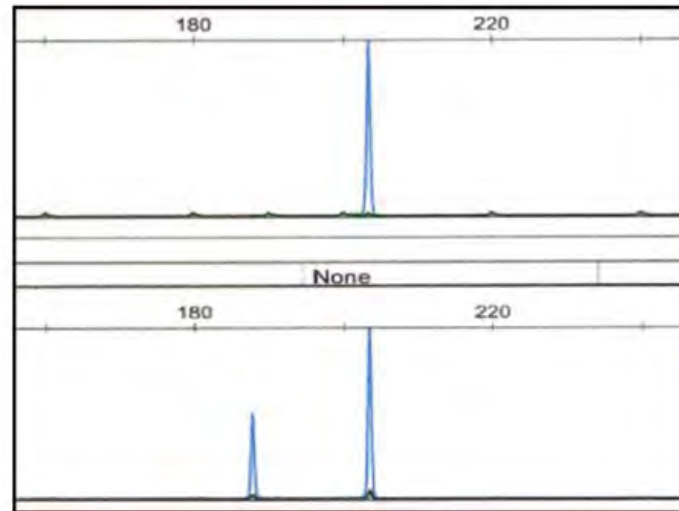
Methods for variant detection

CAP/IASLC/AMP recommended assays



Sanger sequencing

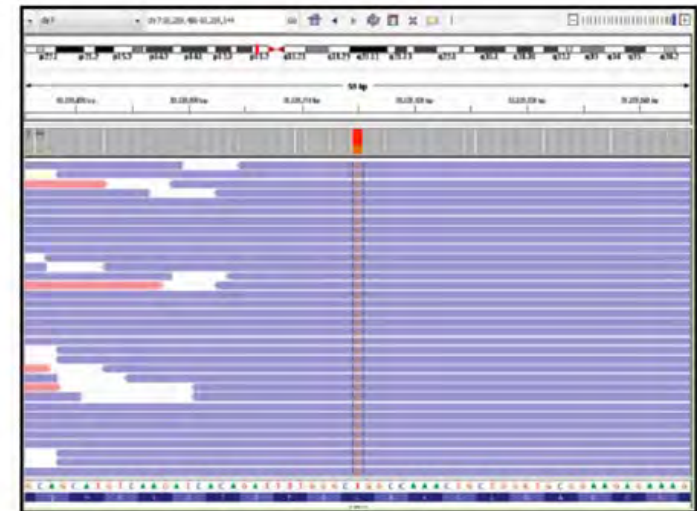
lower sensitivity
selected genes



PCR-based methods

e.g. Cobas®, Idylla™

selected genes



Targeted next generation sequencing (NGS)

many genes (broad testing)

Multiplex PCR



- single genes (e.g. EGFR)
- convenient
- fast TAT (1-2 days)
- tissue consumption
- not comprehensive

NGS

TAT:
3-5 days



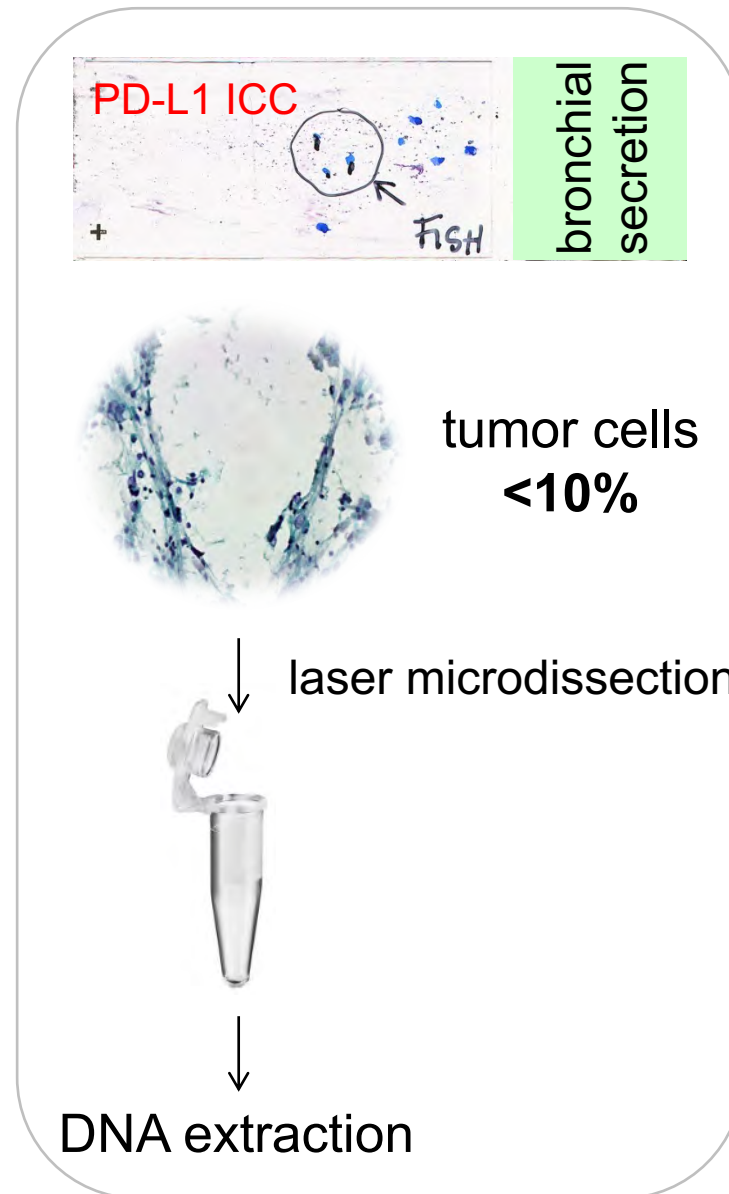
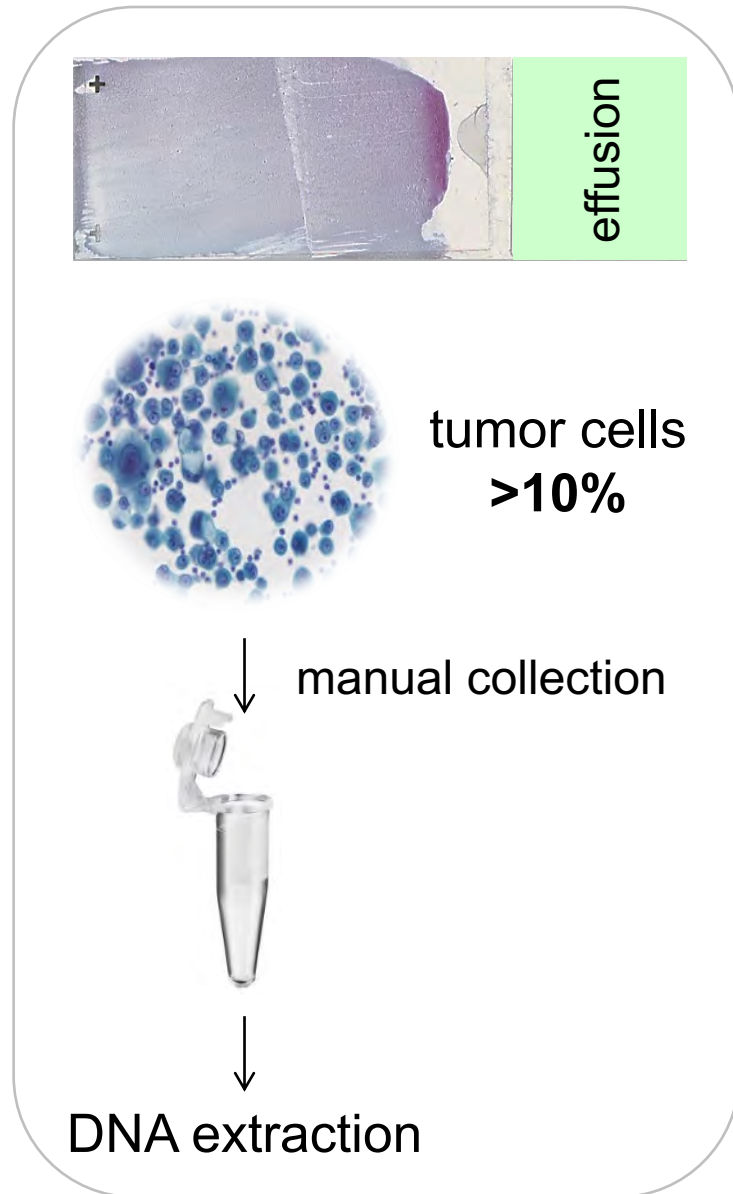
Ion Torrent Genexus

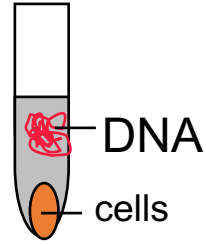


Illumina MiSeq

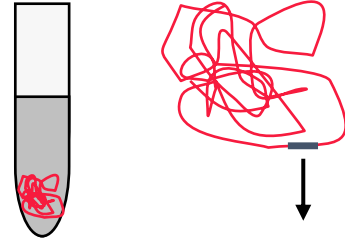
- comprehensive
- 10 - >500 genes at a time (gene panels)
- DNA & RNA (mutations and fusions)
- 10% - 20% tumor cell content

NSCLC: material - cytologic smears





DNA
extraction



PCR
amplification

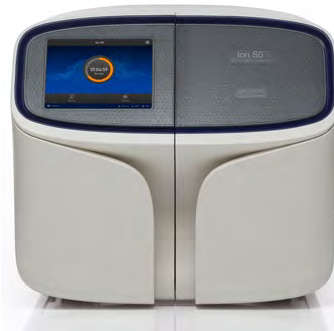


sequencing

data
generation

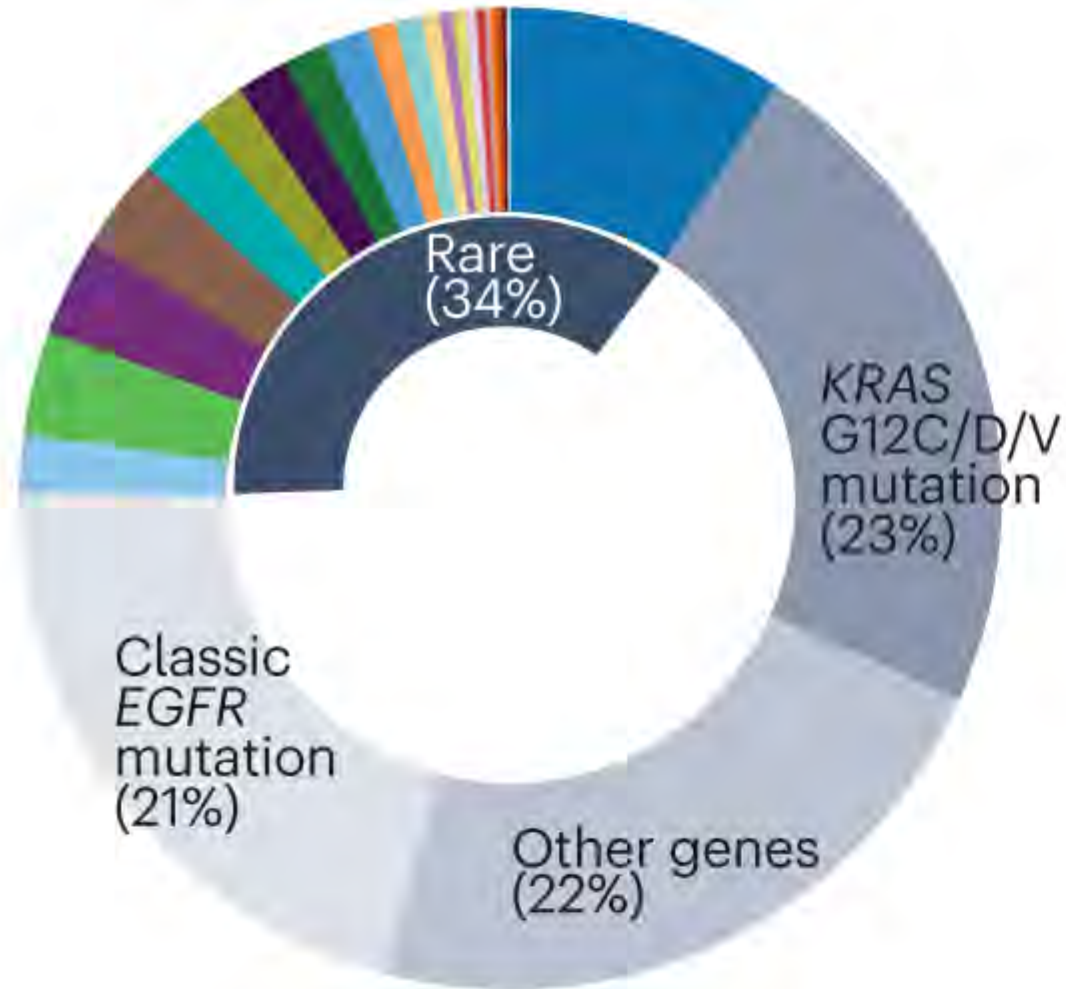


data analysis
and reporting

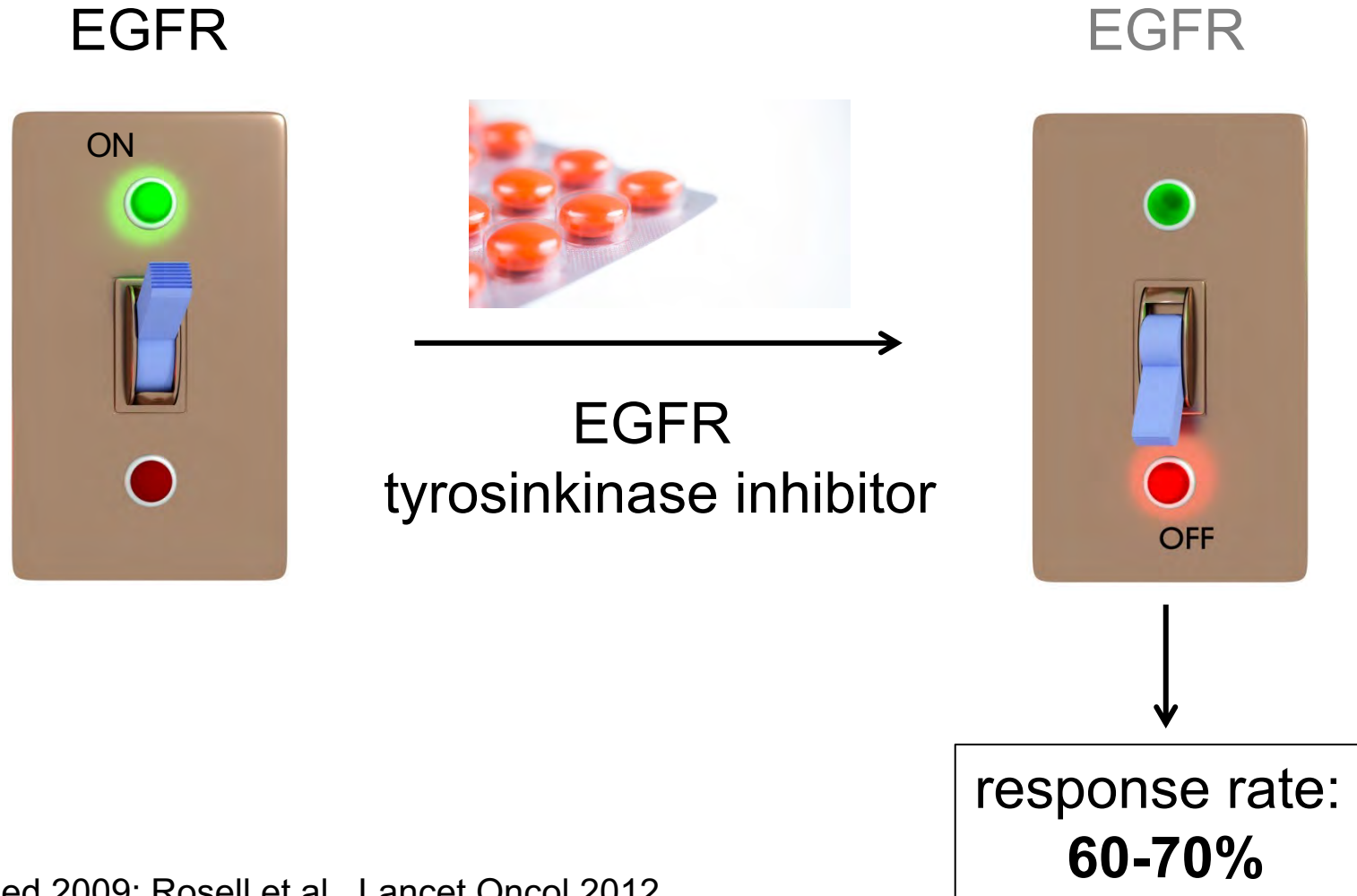


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ACGGATCAATGTAATGAACCGTGGGGATGACACCC
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ACGGATCAATGTAATGAACCGTGGGGATGACACCC
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TGACACCCCTCGCATCTGGC
TGACACCCCTCGCATCTGGCAGC
TGACACCCCTCGCATCTGGCAGCC
TGACACCCCTCGCATCTGGCAGCCA
TGACACCCCTCGCATCTGGCAGCCAGTC
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TGACACCCCTCGCATCTGGCAGCCAGTCATGGA
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GGCAGCCAGTCATGGA
GCAGCCAGTCATGGA
CAGTCATGGA
```

NSCLC: predictive biomarkers - EGFR

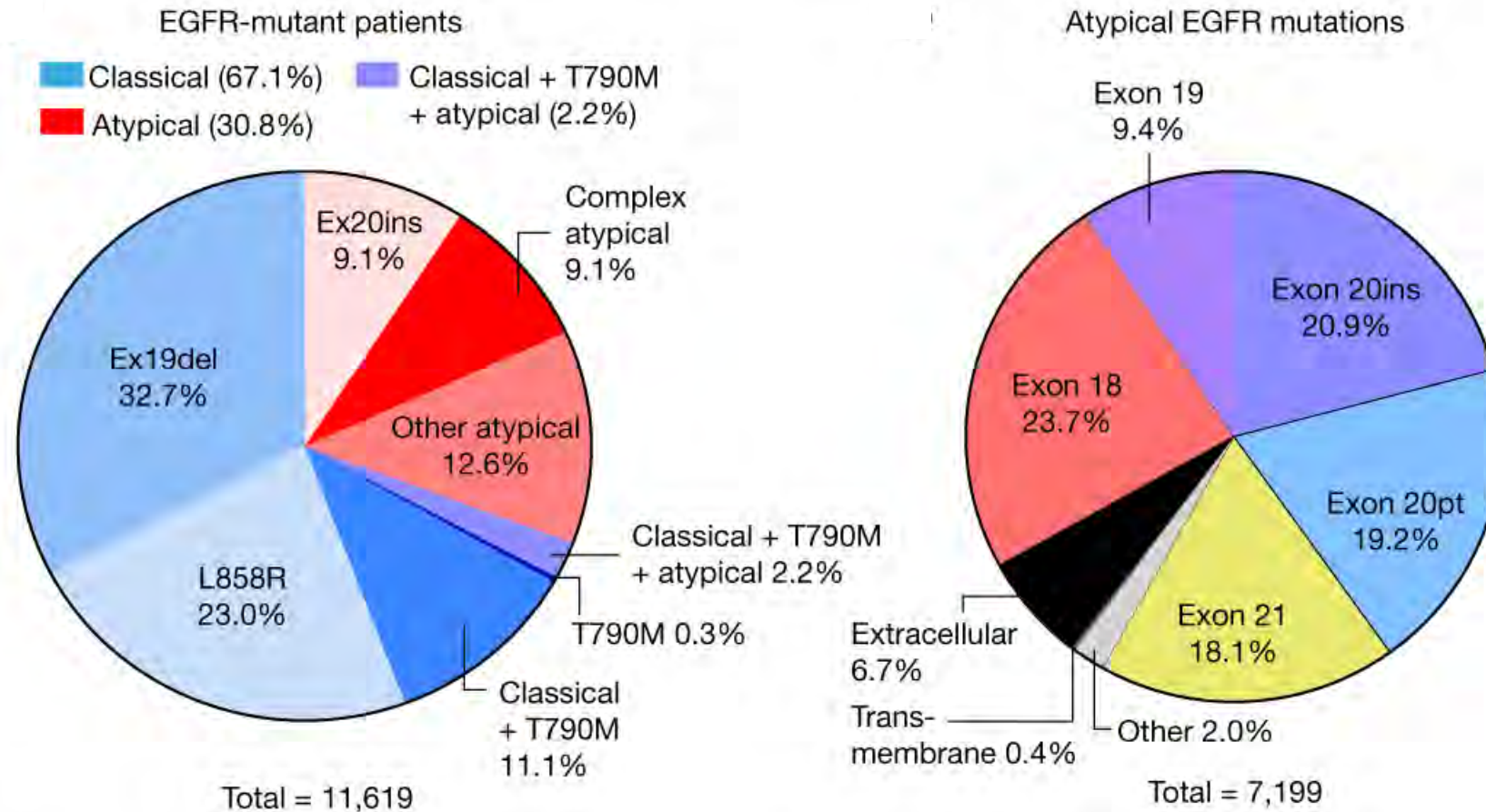


NSCLC: predictive biomarkers - EGFR

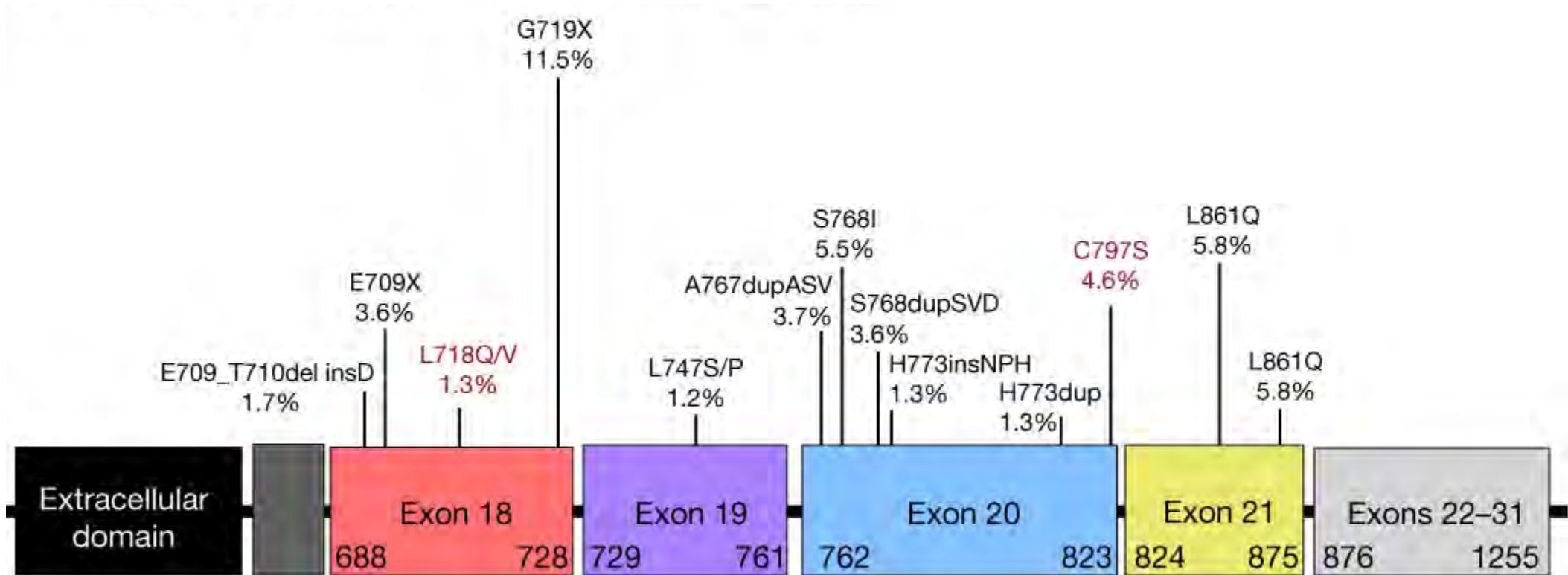


Mok et al., N Engl J Med 2009; Rosell et al., Lancet Oncol 2012

NSCLC: predictive biomarkers - EGFR



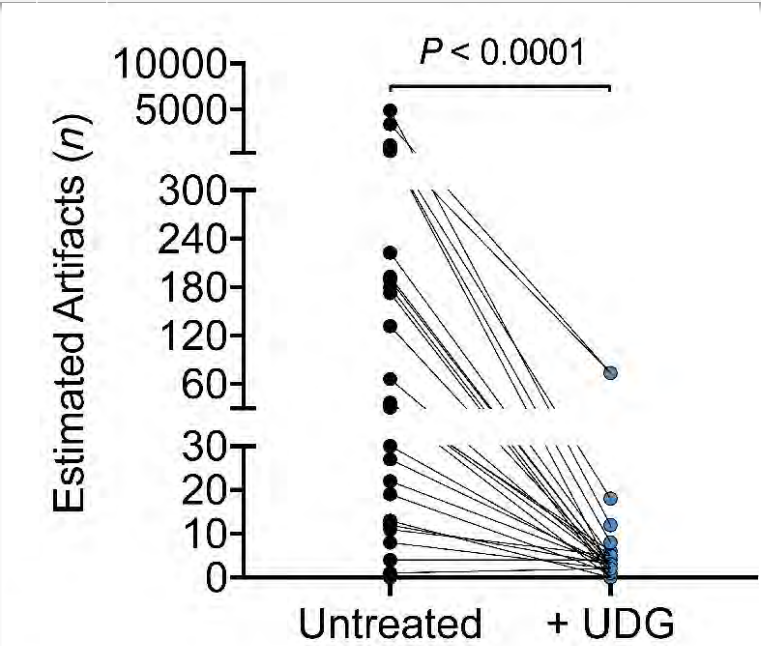
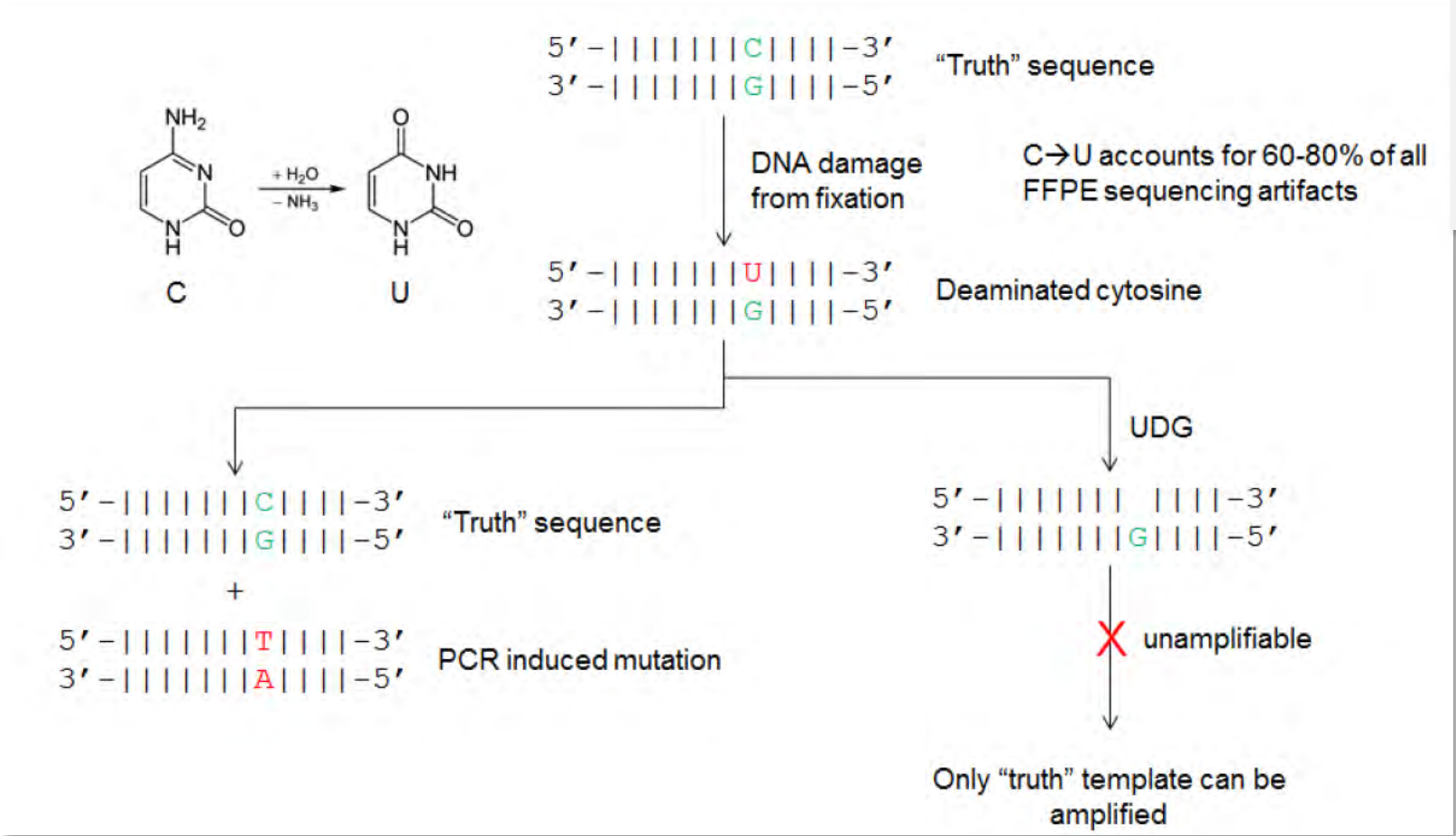
NSCLC: predictive biomarkers - EGFR



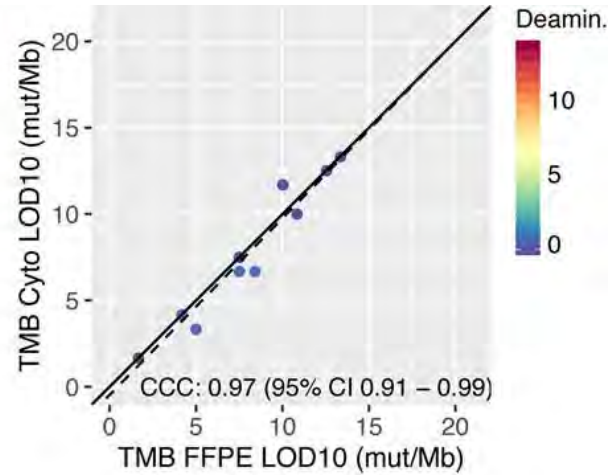
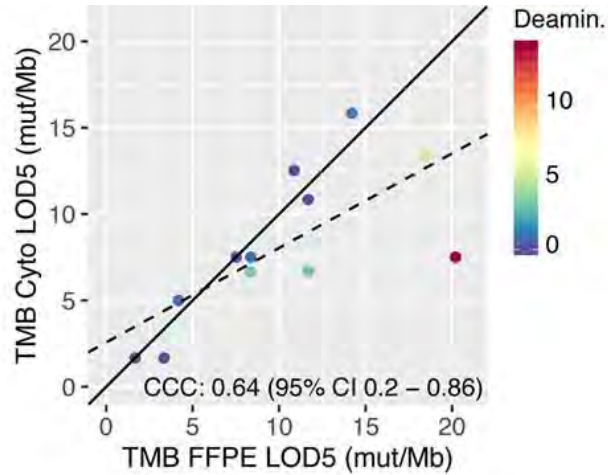
sequencing artifacts

C:G>T:A most common FFPE sequencing artifact

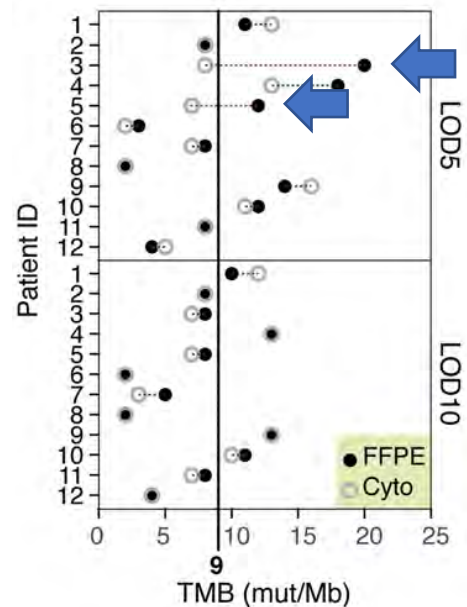
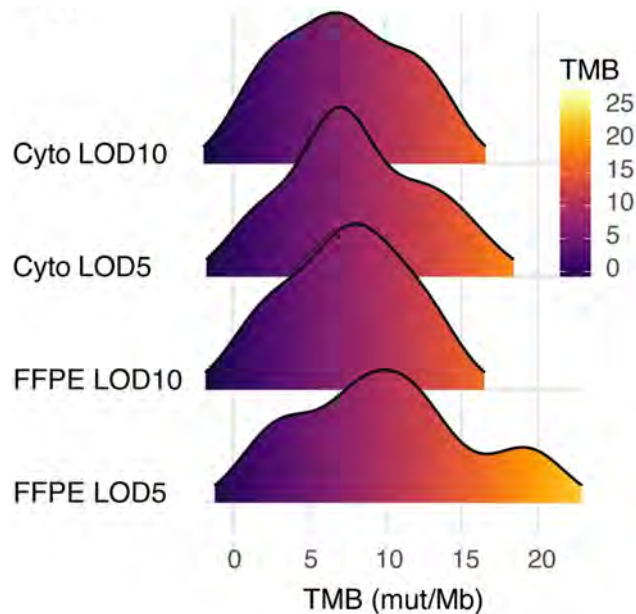
in vitro removal of uracil prior to PCR amplification of FFPE DNA by **uracil DNA glycosylase (UDG)**



NSCLC: predictive biomarkers - TMB



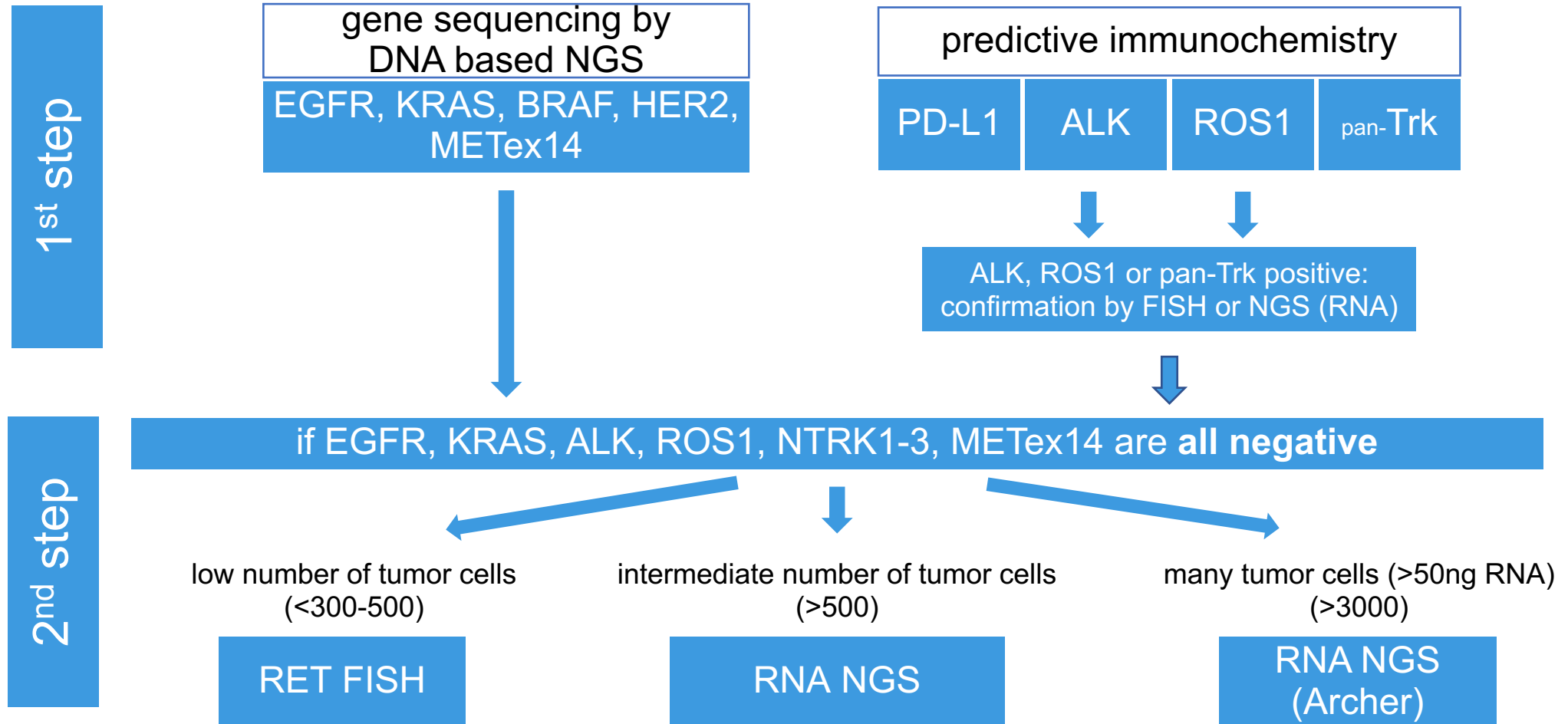
- excellent concordance for TMB (mut/Mb) between biopsy and cytology at higher VAF threshold



- FFPE sequencing artifacts might not only lead to false positive calls, but also to artificial inflation of TMB

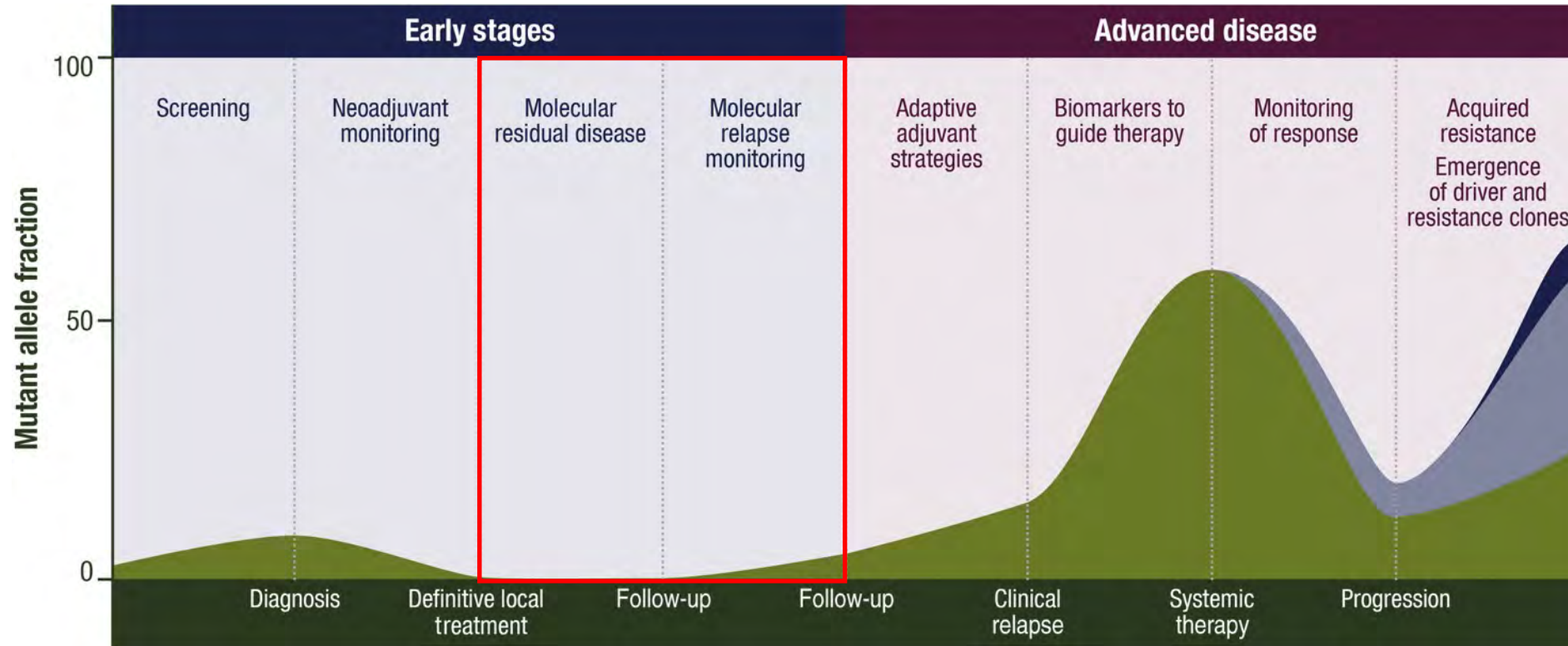
NSCLC: Basel predictive biomarker testing algorithm

stage II non-squamous NSCLC (squamous NSCLC if ≤ 50 years or never smoker)

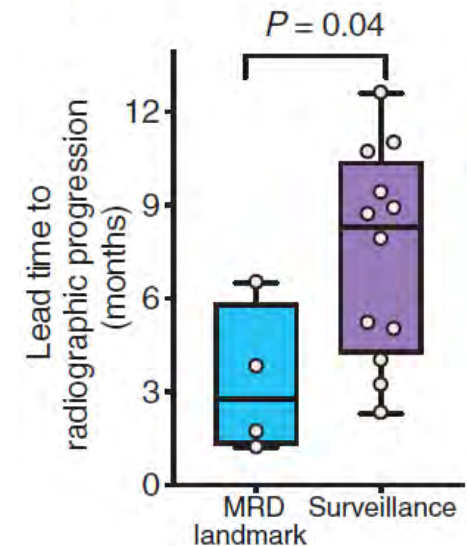
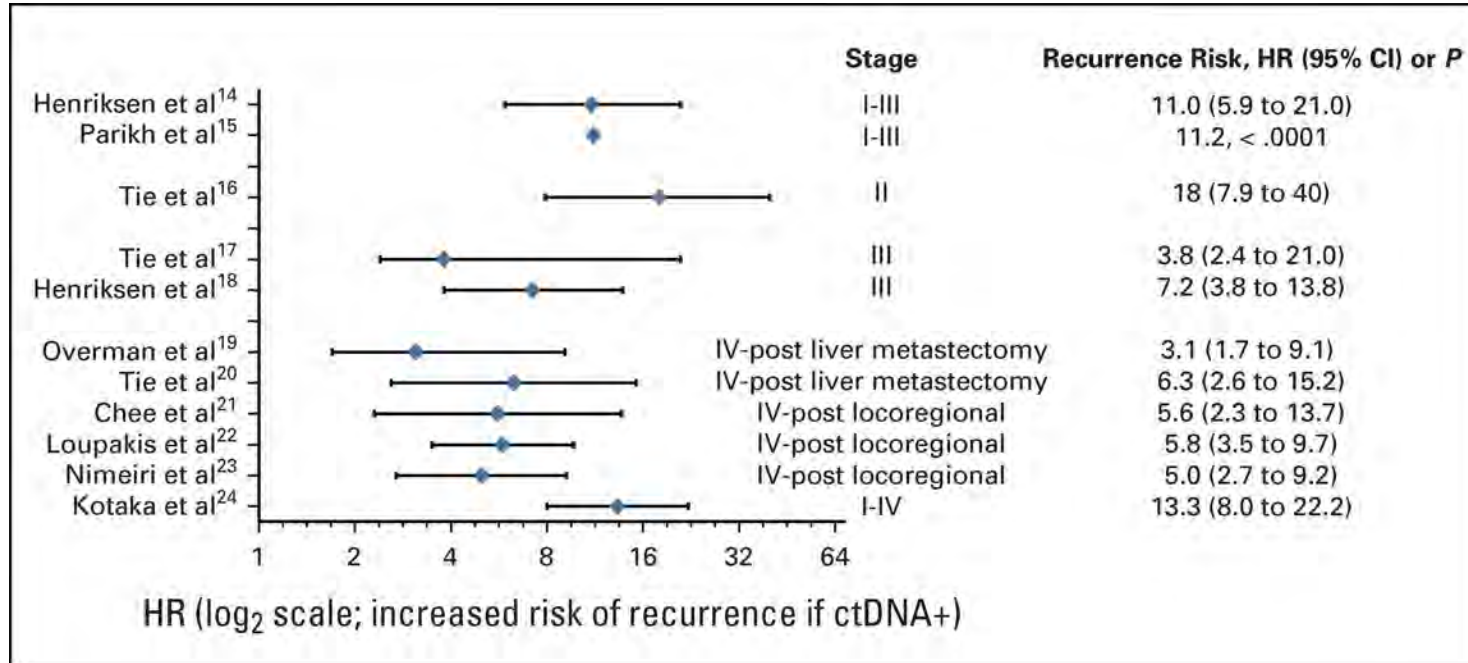


Liquid biopsy & MRD

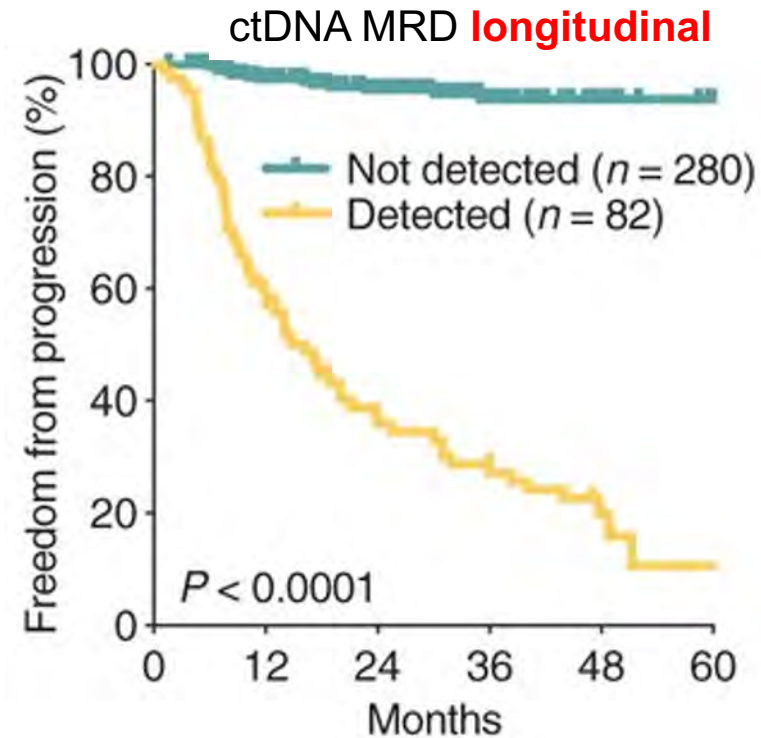
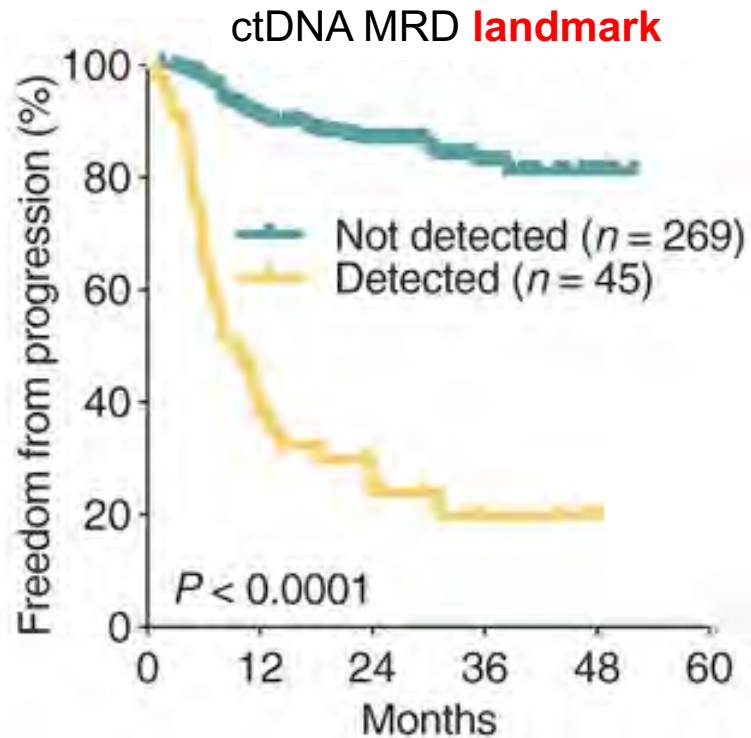
Minimal residual disease (MRD)



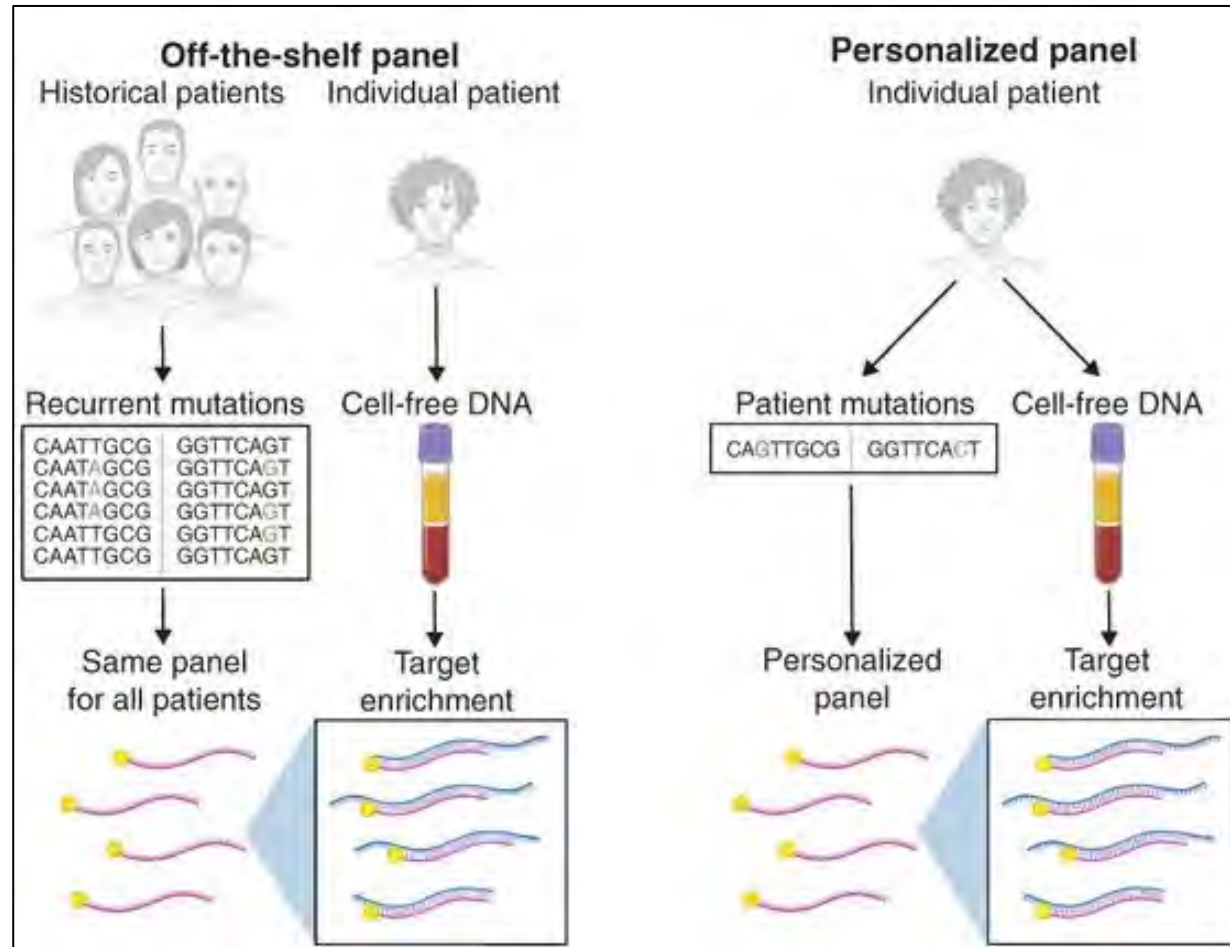
Recurrence risk & lead time



Landmark vs longitudinal analysis

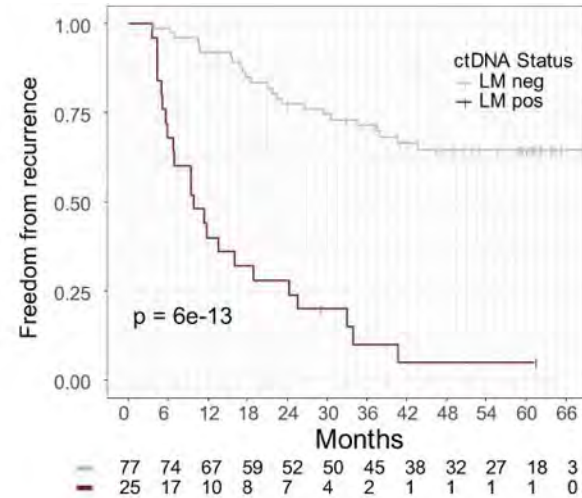
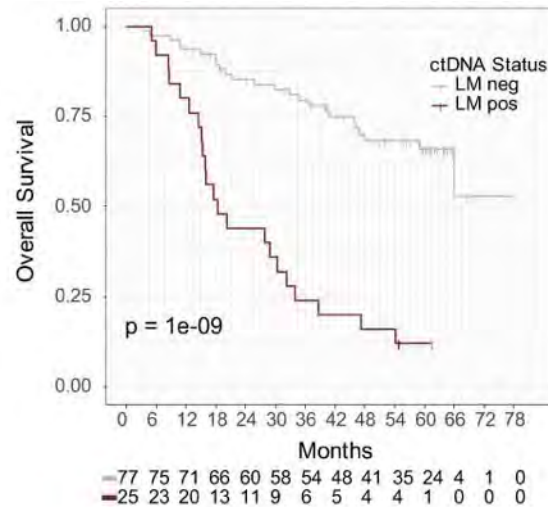


Fixed vs personalized panel



Prognostic accuracy

NSCLC (TRACERx): tumor-informed, personalized assay (up to 200 variants per patient)



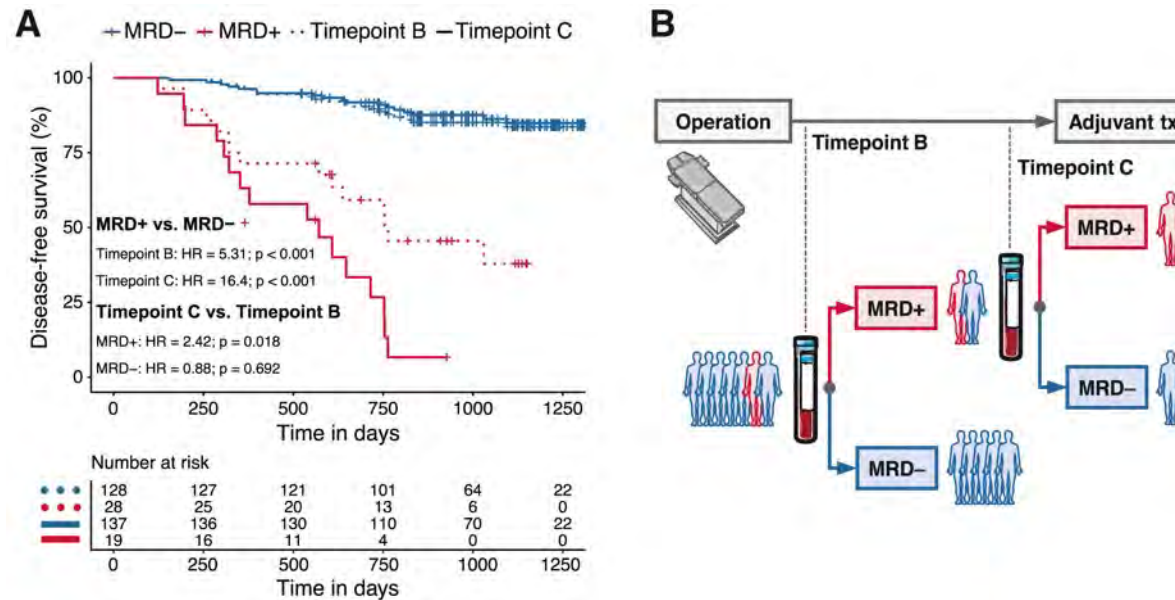
cfDNA input: 23 ng, LOD estimate: 0.008%

ctDNA level: 0.08% (range, 0.002–2.41%, n = 25)

lead time: 119 days

Prognostic accuracy

NSCLC (PROPHET): tumor-informed, personalized assay (50 patient-specific variants)



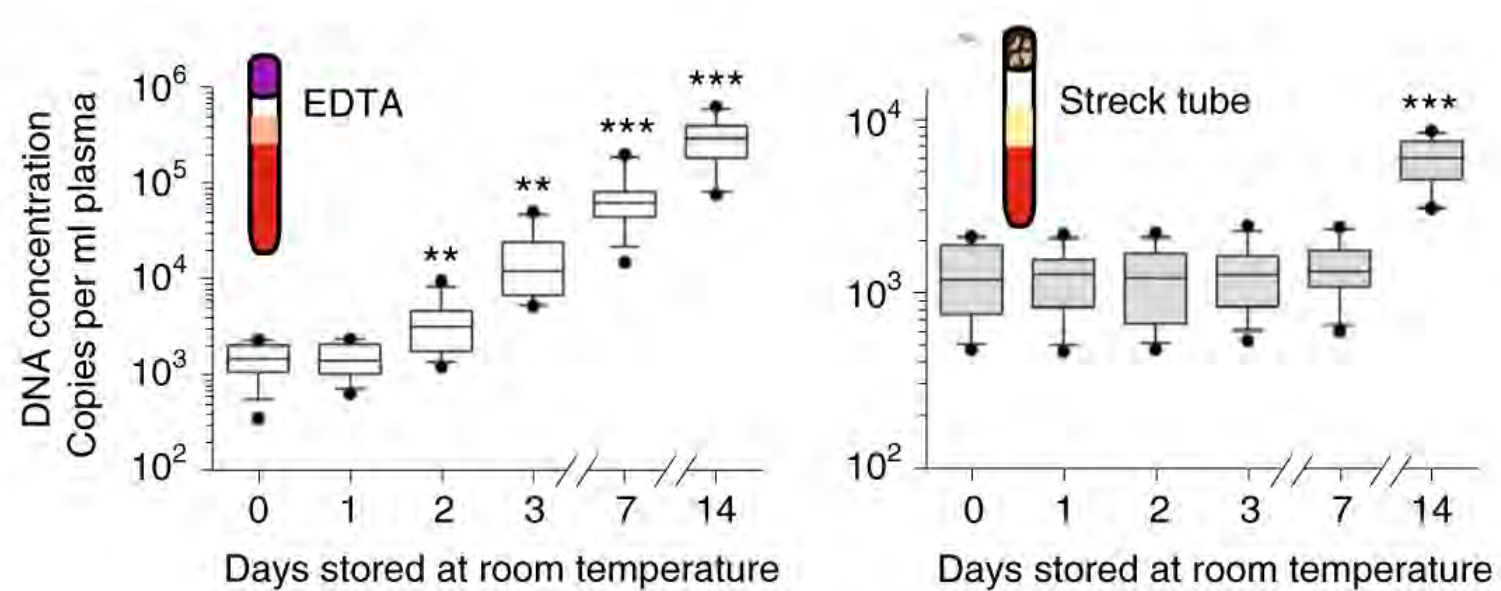
cfDNA input: 20 ng, LOD: 0.004%

ctDNA level: 0.01%

lead time: 299 days

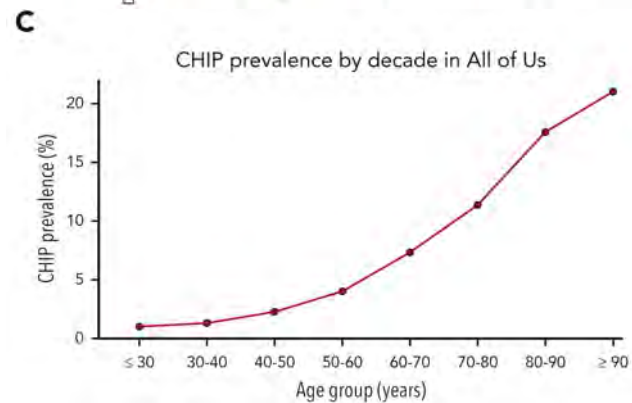
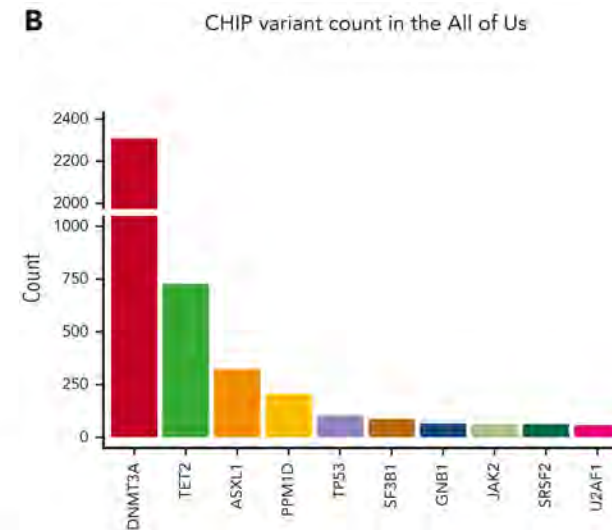
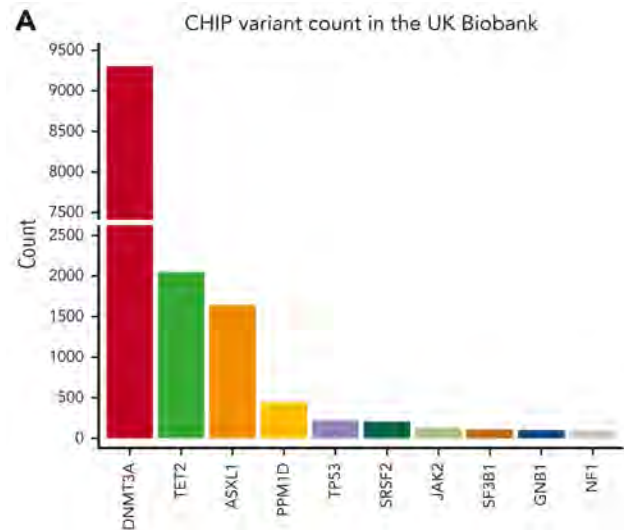
Assay sensitivity

Limitations: contaminants from leukocyte DNA (false positives)



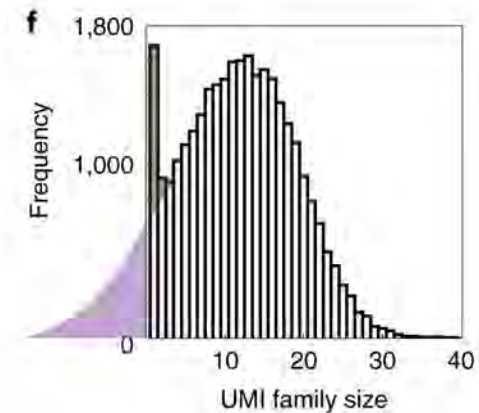
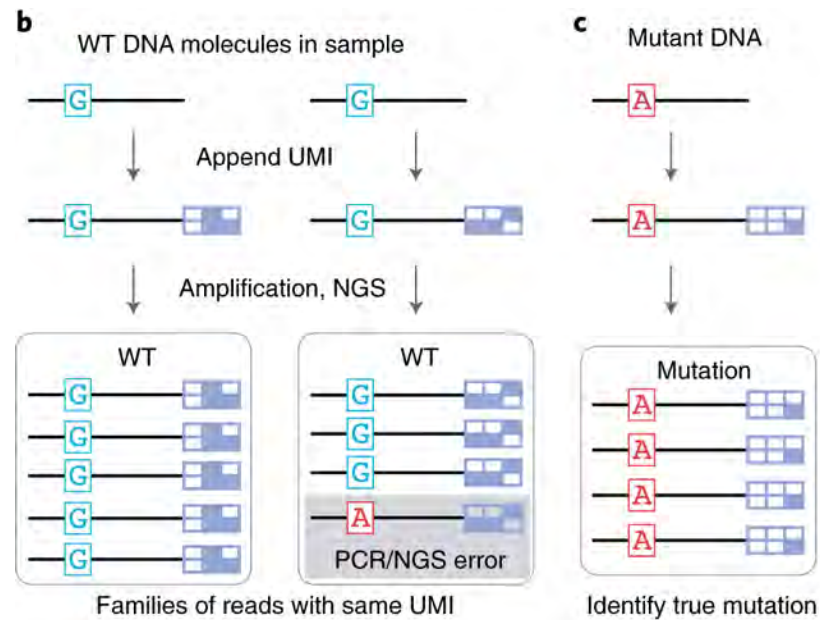
Assay sensitivity

Limitations: CHIP (false positives)

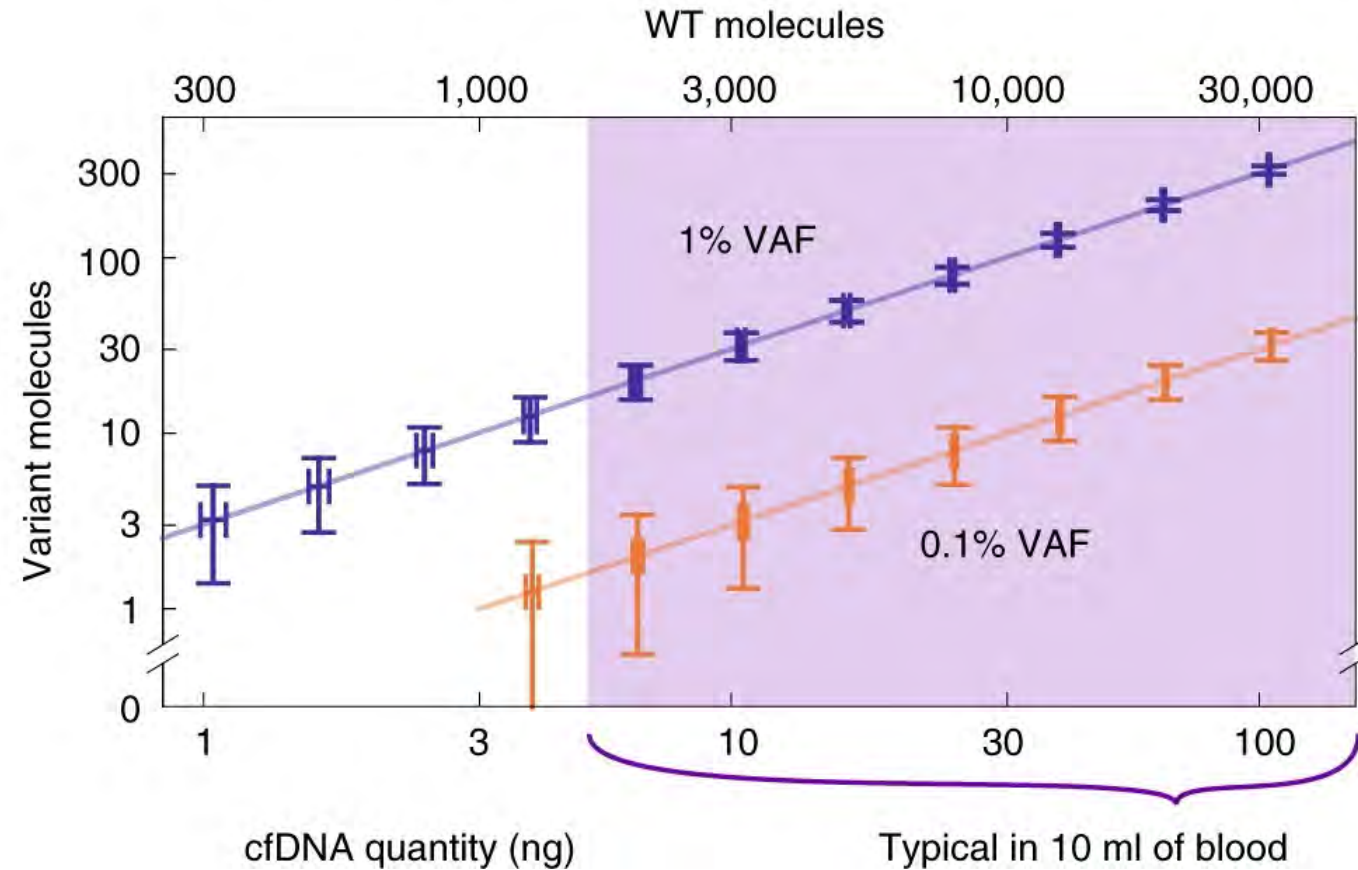


Assay sensitivity

Limitations: technical – PCR & NGS errors (false positives)



Assay sensitivity



Lukas Bubendorf
Spasenija Savic
Tatjana Vlajnic
Lab Teams

Matthias Matter
Ilaria Alborelli
Ivana Bratic Hench
Valeria Perrina

The Power of Diagnostics

Pathology Basel

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