Improving Cancer Care With NGS Biomarker Testing

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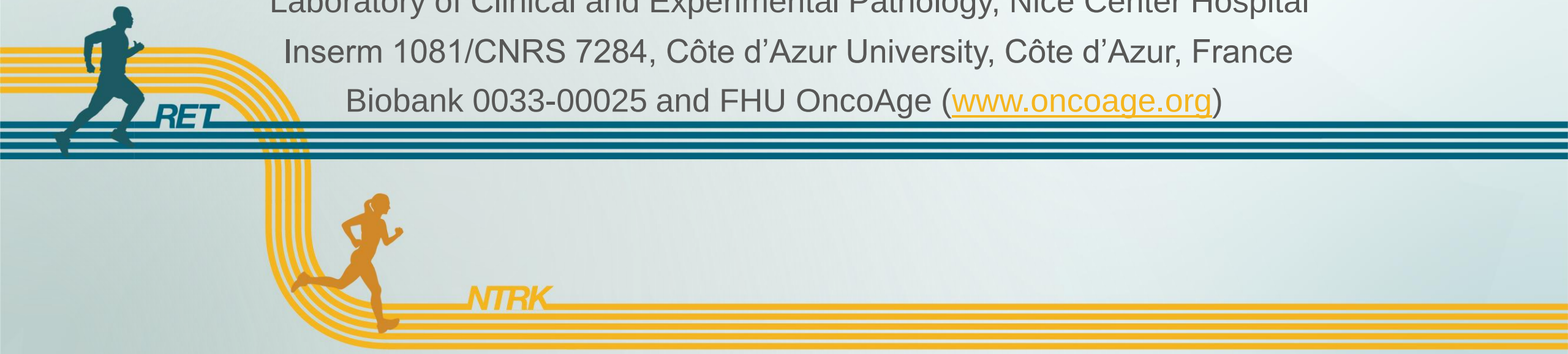
Biobank 0033-00025 and FHU OncoAge (www.oncoage.org)



Brief Comparison of the Advantages and Limitations of the Main Molecular Biology Approaches

Approach	Advantages	Limitations
RT-PCR	Short turnaround time Easy to use Cost-effective	Very limited possibility for multiplexing Low limit of detection Detection of well-known genomic alterations
Digital-PCR	Sensitive Cost-effective Short turnaround time	Detection of well-known genomic alterations Very limited possibility for multiplexing
Next Generation Sequencing (NGS)	Multiplexing from dozens to hundreds of genes at the same time Detection of variants at low allelic frequency Fits with current ESMO guidelines in NSCLC for genomic assessment	Need for bioinformatic support Cost (?) Accreditation process can be difficult

Targeted single gene sequencing

Explores one gene at a time

NGS using small panels
(up to 50 genes)

Explores different genes of interest at the same time

NGS using large panels
(up to 500 genes)

Explores numerous genes at the same time

Bottlenecks and limitations

- Turnaround time/urgent need
- Quality/quantity of the nucleic acids
- Panel size
- Cost/reimbursement
- Sensitivity for different genomic alterations detection
- Time needed for running single-gene tests sequentially

RET

NTRK

ThermoFisher SCIENTIFIC

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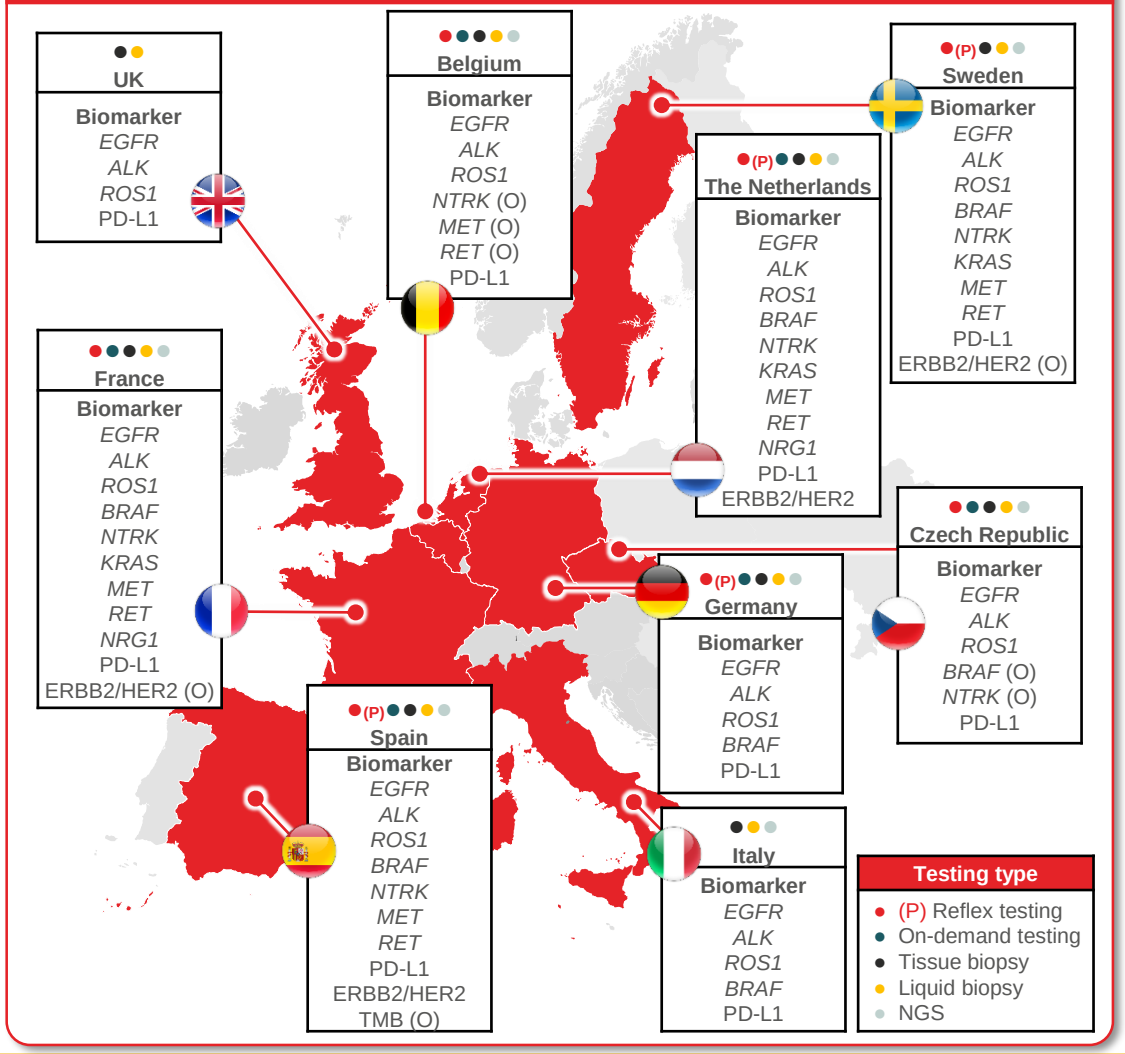
Biomarker testing for non-small cell lung cancer in Europe (2021)

Country-specific guidelines for advanced or recurrent NSCLC



Recommendations to guide selection of precision therapies

Predictive biomarkers	ESMO guidelines	NCCN guidelines	CAP/IASLC/ AMP guidelines	ASCO guidelines	Pan-Asian guidelines
EGFR	●	●	●	●	●
ALK	●	●	●	●	●
ROS1	●	●	●	●	●
BRAF	●	●	●	●	●
PD-L1	●	●	●	●	●
NTRK	●	●	●	●	●

Emerging biomarkers	ESMO guidelines	NCCN guidelines	CAP/IASLC/ AMP guidelines	ASCO guidelines	Pan-Asian guidelines
KRAS	●	●	●	●	●
MET	●	●	●	●	●
RET	●	●	●	●	●
ERBB2/HER2	●	●	●	●	●
TMB	●	●	●	●	●

● Testing recommended

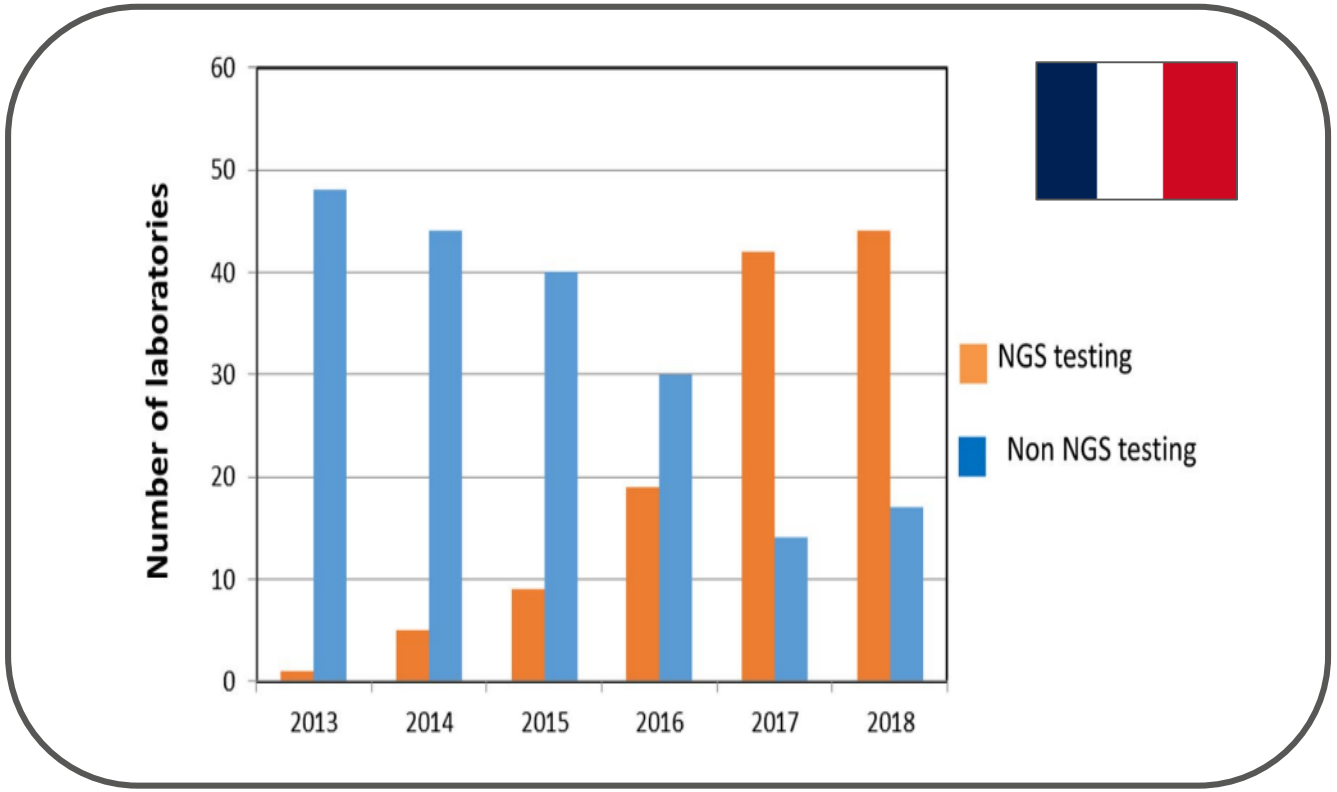
● Expanded panel testing recommended

● Single gene or expanded panel testing recommended

● No guideline recommendations to date

Kerr K, et al. *Lung Cancer* 2021;154:161–75.

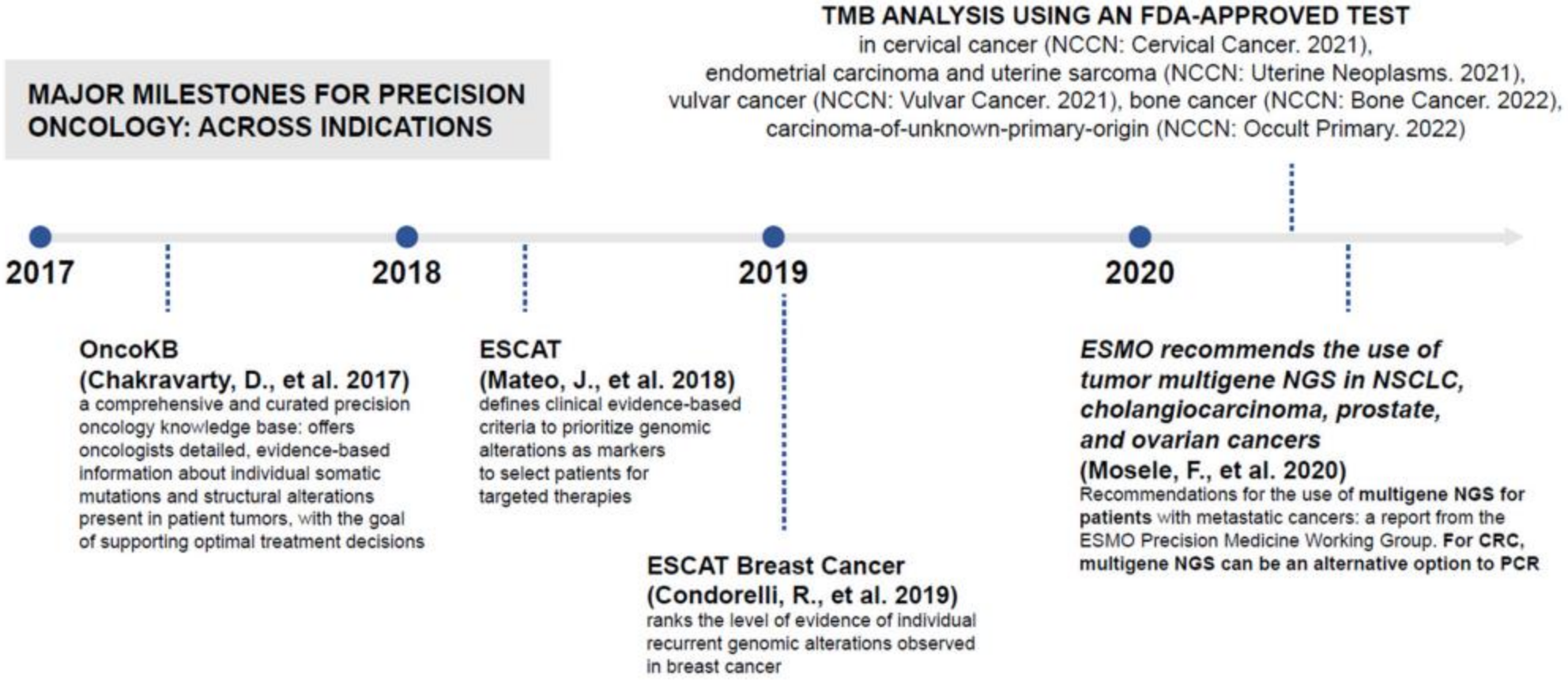
The Promising Increased Use of NGS During the Last Few Years[1,2]



Hofman P, et al. Cancer Cytopathol 2020;128:601–10

1. Lin HM, et al. *JTO Clin Res Rep* 2022;3:100285; 2. Hofman P, et al. *Cancer Cytopathol* 2020;128:601–10.

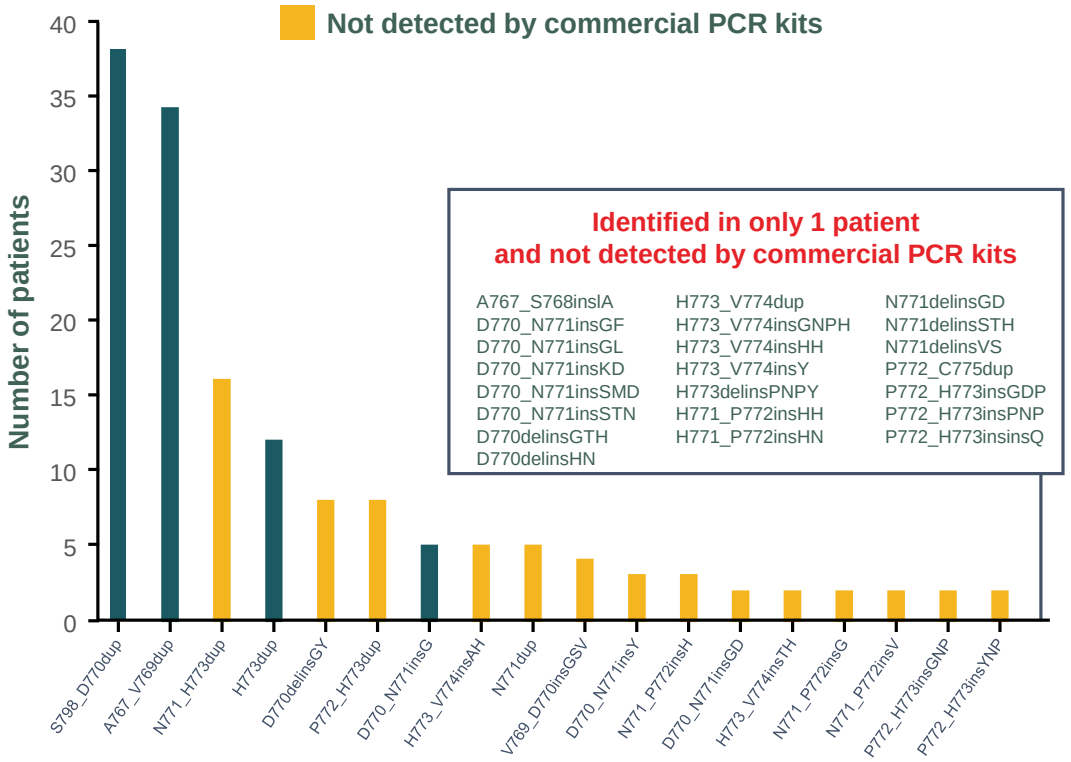
Evolution of Support for Genomic Testing Within Recommendations



Horgan D, et al. *J Pers Med* 2022;12:72.

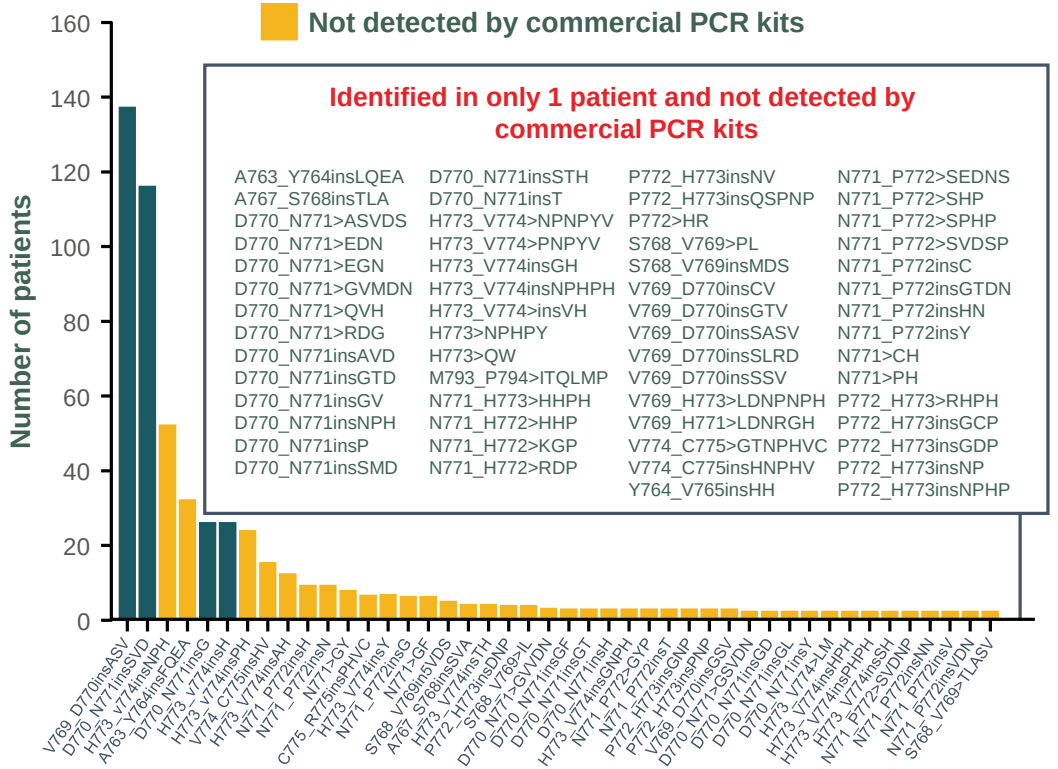
PCR vs NGS for Detecting EGFR Exon20ins

PCR would be expected to **miss 49.1% of exon20ins** cases identified by NGS (PCR = 89; NGS = 175)



GENIE PCR detection results

PCR would be expected to **miss 51.4% of exon20ins** cases identified by NGS (PCR = 305; NGS = 627)

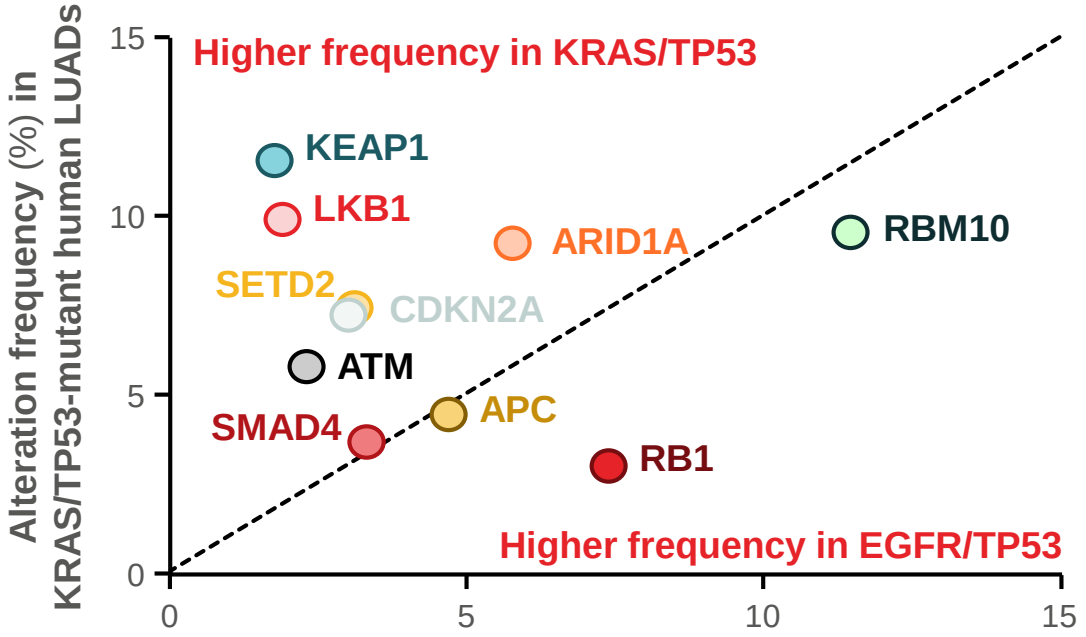


FoundationInsights® PCR detection results

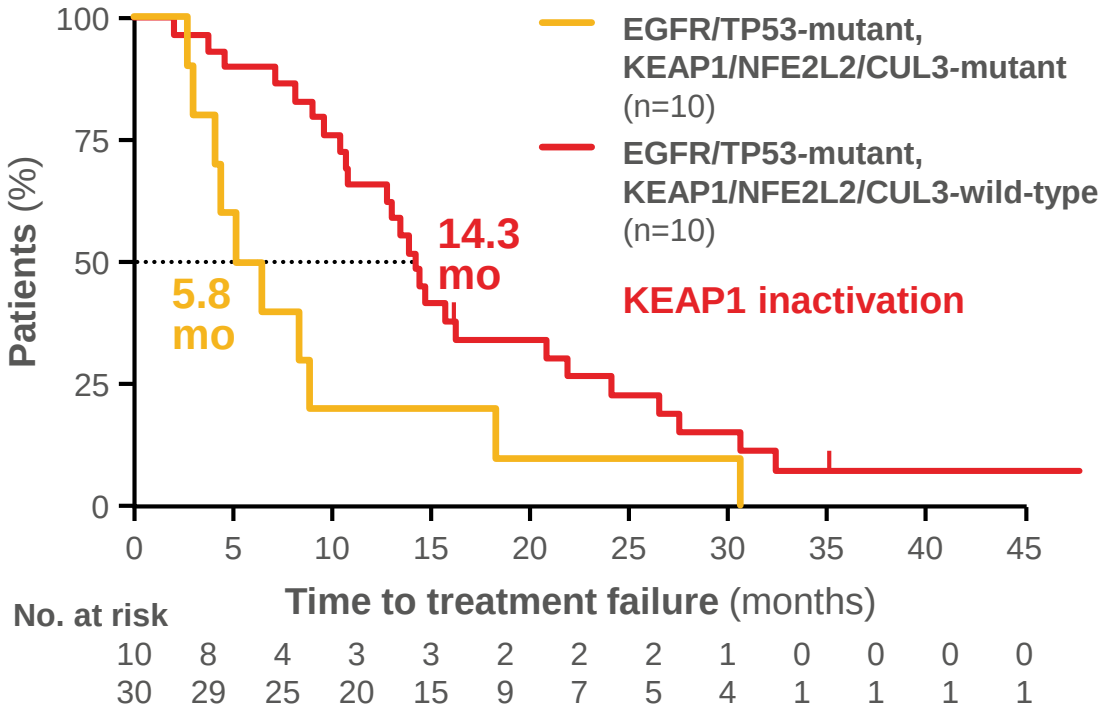
Bauml J, et al. Presented at WCLC 2020: FP07.12.

Co-Occurring Gene Alterations and Their Effect on Therapeutic Response

Frequency of tumor suppressor gene alterations that co-occur with EGFR or KRAS and TP53 mutations in lung adenocarcinoma

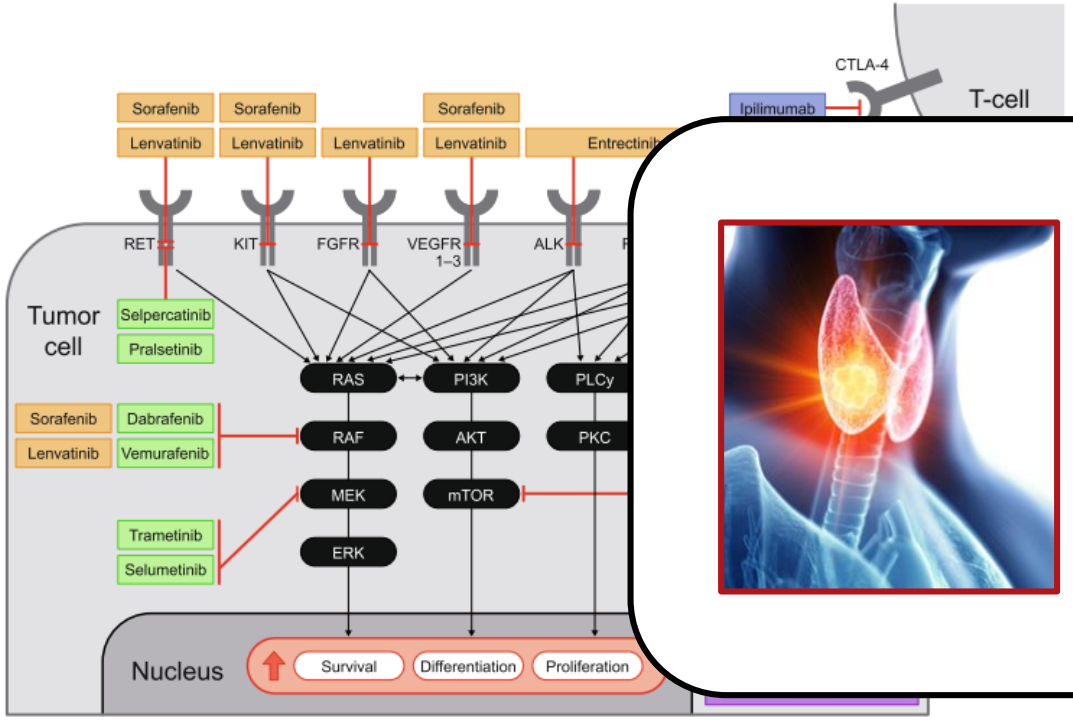


KEAP1 inactivation correlates with reduced response to TKIs in EGFR-driven lung adenocarcinomas



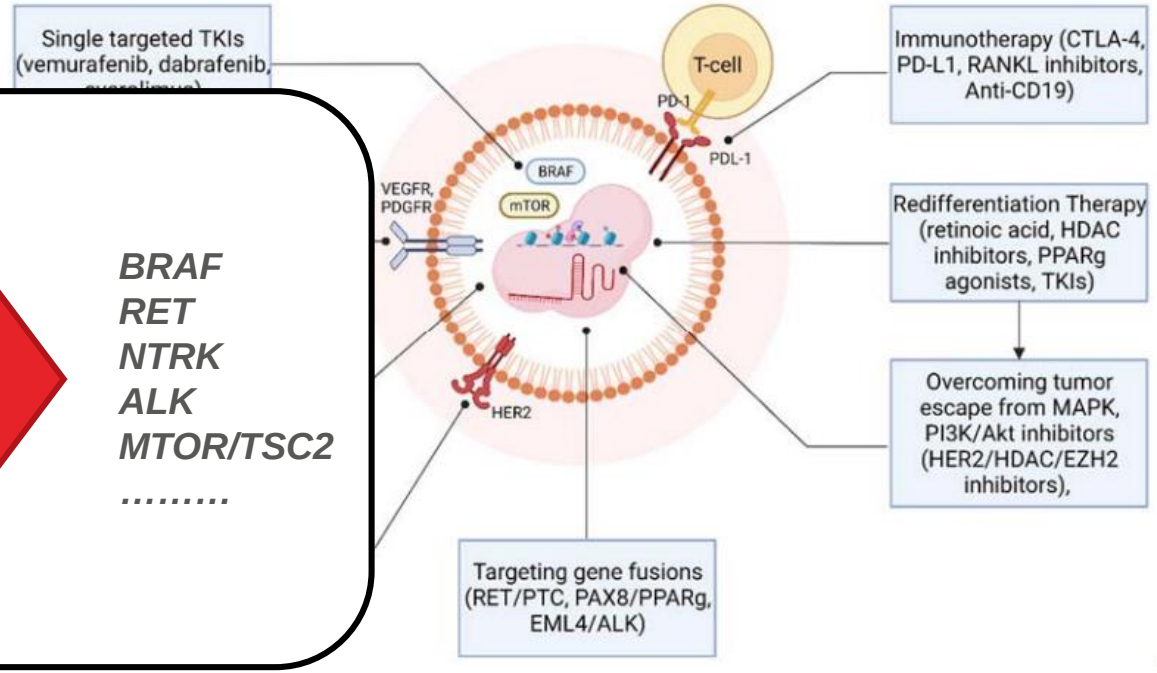
Foggetti G, et al. *Cancer Discov* 2021;11:1736–53.

Potential therapeutic strategies targeting cellular aberrations in thyroid cancer[1]



BRAF
RET
NTRK
ALK
MTOR/TSC2
……

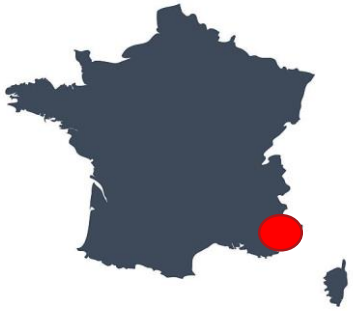
Potential therapeutic approaches in RAIR-DTC alternative or after 1L MKI failure[2]



1. Capdevila J, et al. *Cancer Treat Rev* 2022;106:102380; 2. Silaghi H, et al. *Int J Mol Sci* 2022;23:3470.

First scenario ← NGS analysis → Second scenario

GUARDANT



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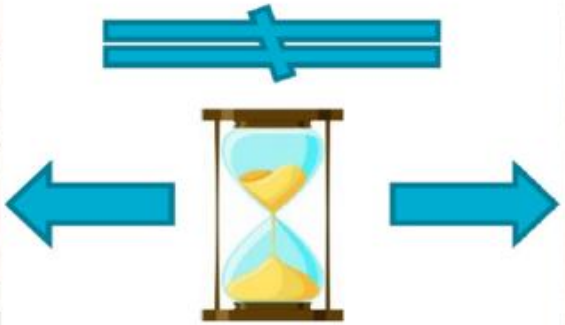
“In-House” NGS Set Up

Mandatory to check

1. Cost/reimbursement
2. Turnaround time for getting the results
3. Team expertise
4. Bioinformatic analyses
5. Accreditation (ISO 15189 for ex)
6. CE-IVD testing (?) / next IVDR

NGS
(S5 sequencer, Thermo Fisher)



Real-time PCR
(Idylla, Biocartis)

NGS
(Genexus sequencer, Thermo Fisher)

Immunohistochemistry
(Ventana, Roche Diagnostics)

LPCE Platform - ISO 15819

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From Tissue Biopsy to Treatment Decision Making

1

2

Clinical pathology

3

Cancer diagnosis

4

Molecular pathology

5

Genomic alterations assessment

6

7

24 h

24/48 h

30 h

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BAYER

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ODxET Evaluation at Clinical Lab Sites Across Europe

Genexus Purification System

Genexus Integrated Sequencer

Country	PI Name	KOL Site	Short Name
Italy	Edoardo Pescarmona	Istituti Fisioterapici Ospitalieri	Rome
Spain	Lara Navarro	Consorcio Hospital General de Valencia	Valencia

Genexus Integrated Sequencer

Country	PI Name	KOL Site	Short Name
France	Paul Hofman	Pasteur Hospital, University of Nice Sophia Antipolis	Nice
Italy	Nicola Normanno	CROM-Fondazione Pascale	Naples
Portugal	Jose Carlos Machado	Ipatimup	Porto
Switzerland	Philp Jermann	Universitätsspital Basel	Basel

Hofman P. Removing barriers to routine NGS testing – Results of multicentric evaluation of performance of the Oncomine Dx Express test. *The Pathologist* 2022. Available at https://thepathologist.com/webinar/connecting-patients-everywhere-to-precision-oncology (Accessed August 2022).

Successful Detection of Expected Variants Across Sites

- Consistent results between internal and external testing of FFPE samples

DNA variants:

Sample	Cancer Type	Variant Type	Expected Variant (Pre-characterized)	Unit of Measurement	TFS R&D	Basel	Naples	Valencia	Rome	Nice
					Expected	Observed	Observed	Observed	Observed	Observed
1	Lung	Deletion	*EGFR exon 19 del*	Allele Frequency	**30.5%**	31.4%	‡	29.8%	32.3%	34.6%
2	Lung	Insertion	*EGFR exon 20 ins*	Allele Frequency	**41.4%**	34.0%	36.9%	35.7%	36.7%	32.2%
5	Bladder	CNV	*ERBB2 CNV*	Copy Number	**35.3**	37.5	36.9	36.3	36.0	37.7
6	Small Intestine	SNV	*BRAF V600E*	Allele Frequency	**53.3%**	53.1%	51.5%	53.0%	53.3%	51.2%

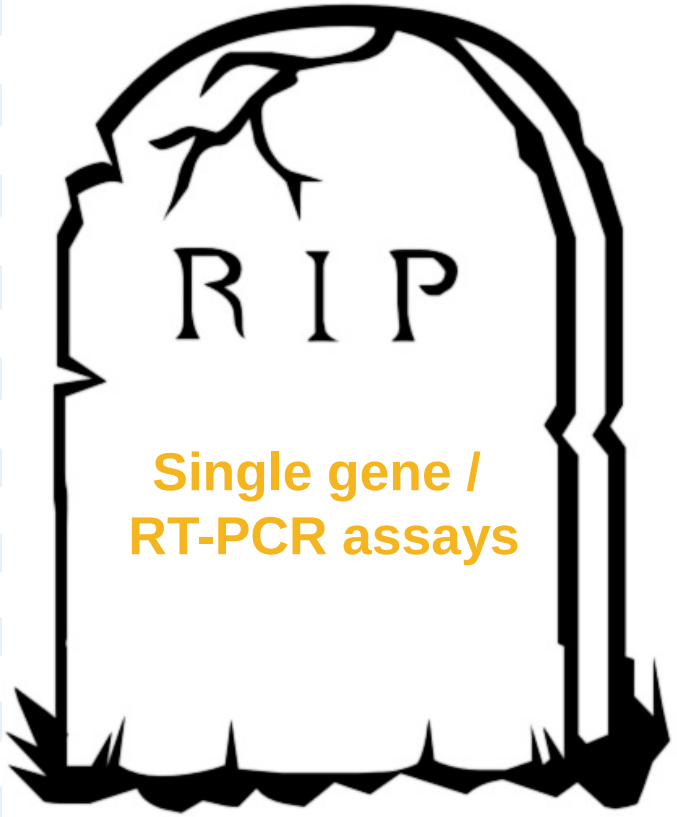
RNA variants:

Sample	Cancer Type	Variant Type	Expected Variant (Pre-characterized)	Unit of Measurement	TFS R&D	Basel	Porto	Naples	Valencia	Rome
					Expected	Observed	Observed	Observed	Observed	Observed
3	Lung	Splice Variant	*MET Exon 14 Skip*	# of Molecules	**1787**	1872	515	1955	1889	1974
4	Lung	Fusion	*KIF5B-RET*	# of Molecules	**110**	124	134	47	143	84

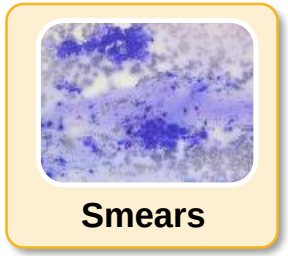
Note: Small number of additional variants have been detected in some samples which are also consistently detected across the sites with some variance in the measurement detection level. ‡ DNA sample failure.

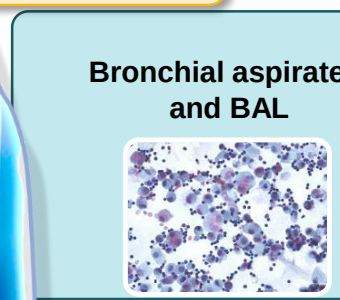
Hofman P. Removing barriers to routine NGS testing – Results of multicentric evaluation of performance of the Oncomine Dx Express test. *The Pathologist* 2022. Available at https://thepathologist.com/webinar/connecting-patients-everywhere-to-precision-oncology (Accessed August 2022).

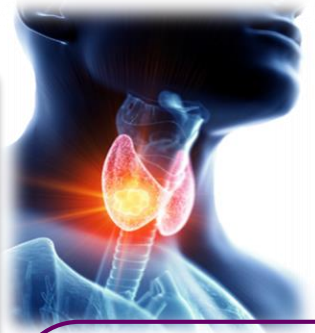
	SCENARIOS		
	Current Practice	In development	Future Horizons
EGFR 18,19,21	✓	✓	✓
BRAF	✓	✓	✓
PD-L1	✓	✓	✓
ALK			✓
ROS-1			✓
PANTRK			✓
RET			✓
MET exon 14 skip			✓
MET amplification			✓
EGFR ex 20			✓
KRAS			✓
HER2			✓
NRG1			✓
HRAS			✓
NRAS			✓
AKT			✓
TMB/CGP/HRD			?
STK11			✓
KEAP1			✓
TP53			✓
JAK2/3			✓
BRCA 1,2			✓
FGFR			✓

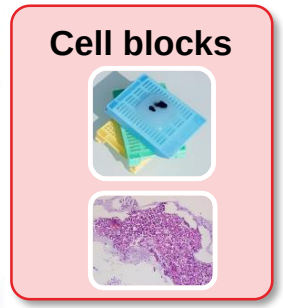


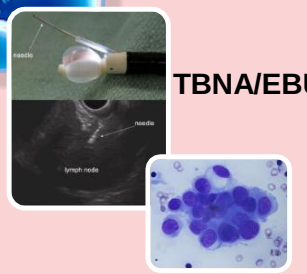
Examples of Bioresources for NGS at Diagnosis or at Progression

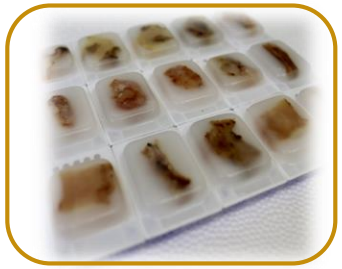




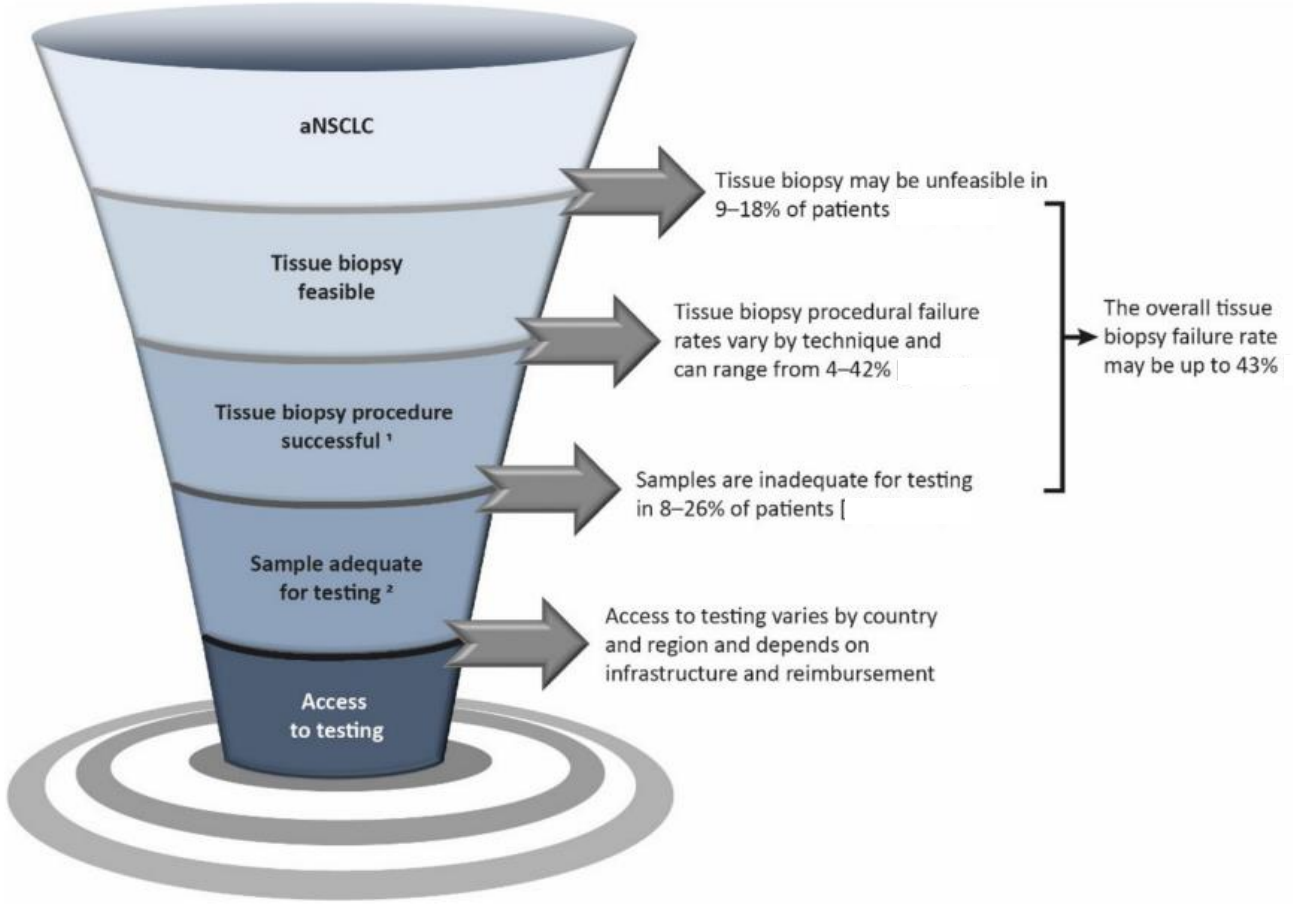








Reasons Why Patients Miss Out on Biomarker Testing from Tissue Biopsy

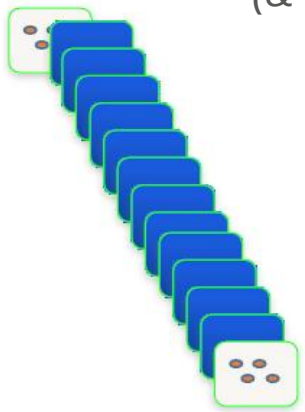


Malapelle U, et al. *J Mol Pathol* 2021;2:255–73.

More and more diagnostic (TTF1, P40, chromogranin, CD56, BRG1, NUT…)
and predictive biomarkers (**PD-L1**, *ALK*, *ROS1*, *NTRK*, *RET*, *VE1 BRAF*, *FGFR*?...)

(& MET IHC & DLL3 IHC are coming back)

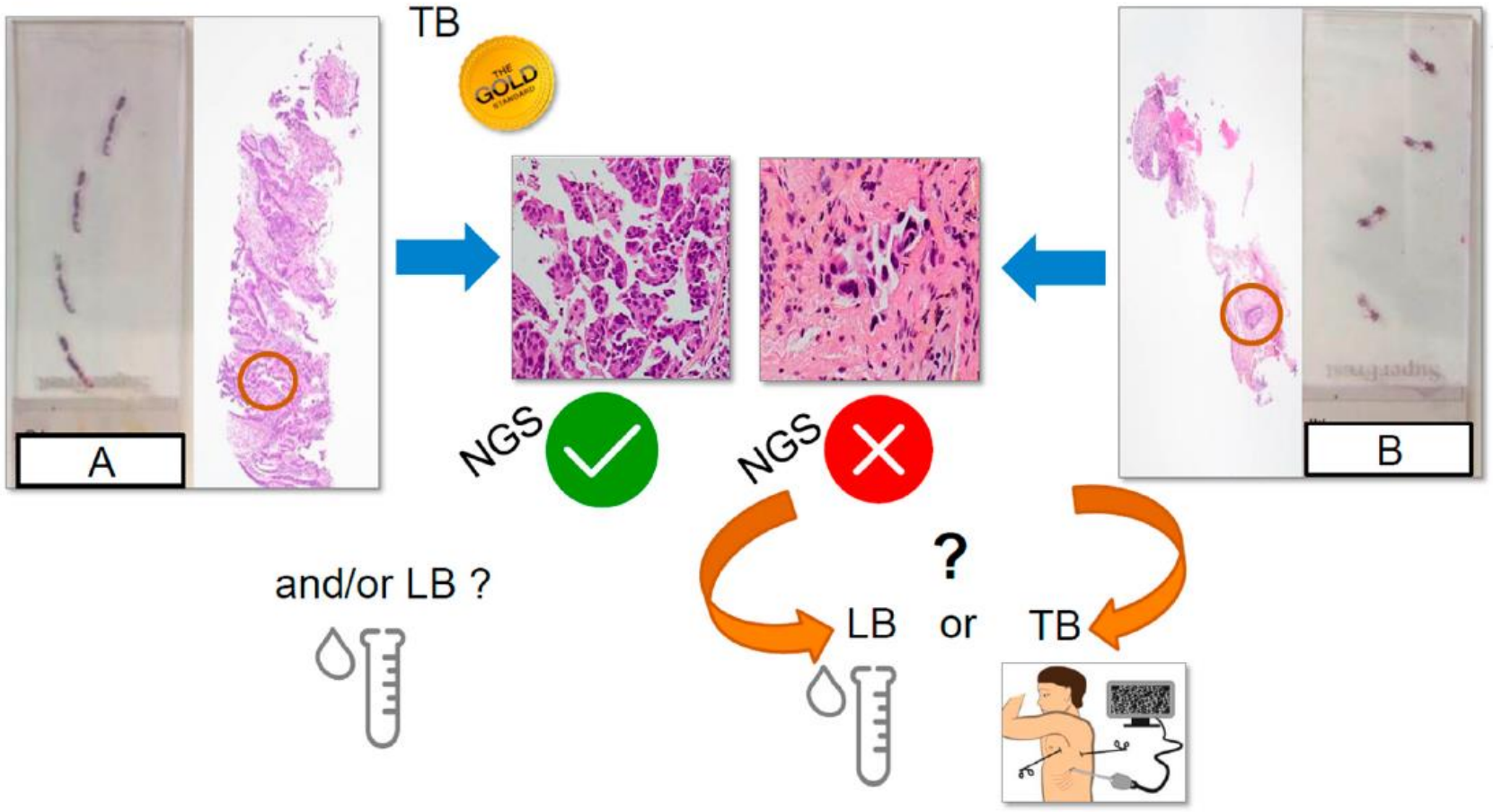
…with less and less tissue



Tissue exhaustion

Toward multiplex analyses?

A Major Issue in Thoracic Pathology



Reflex testing pathway

TAT ↘

Physican, radiologist or surgeon obtains specimen

↓

Pathologist makes diagnosis, evaluates specimen, investigates and controls comprehensive profiling

TAT ↗

Bespoke testing pathway

Physician, radiologist or surgeon obtains specimen

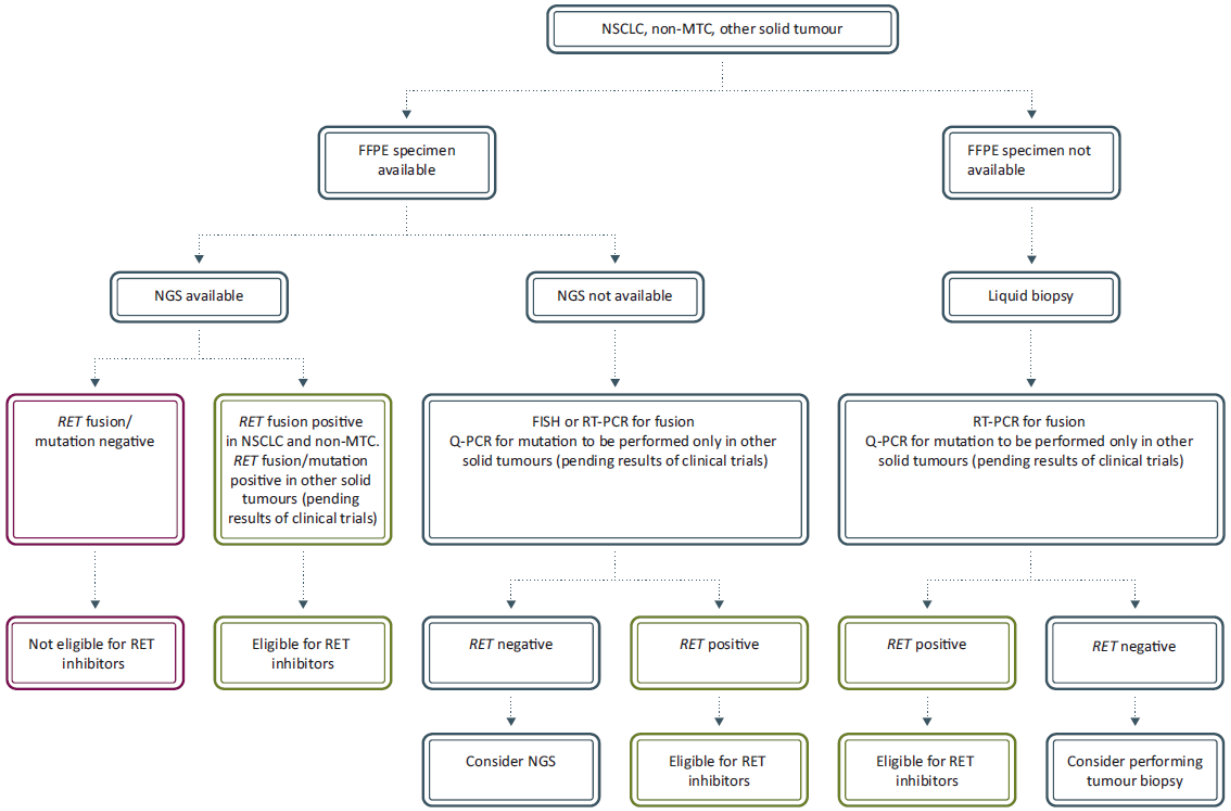
↓

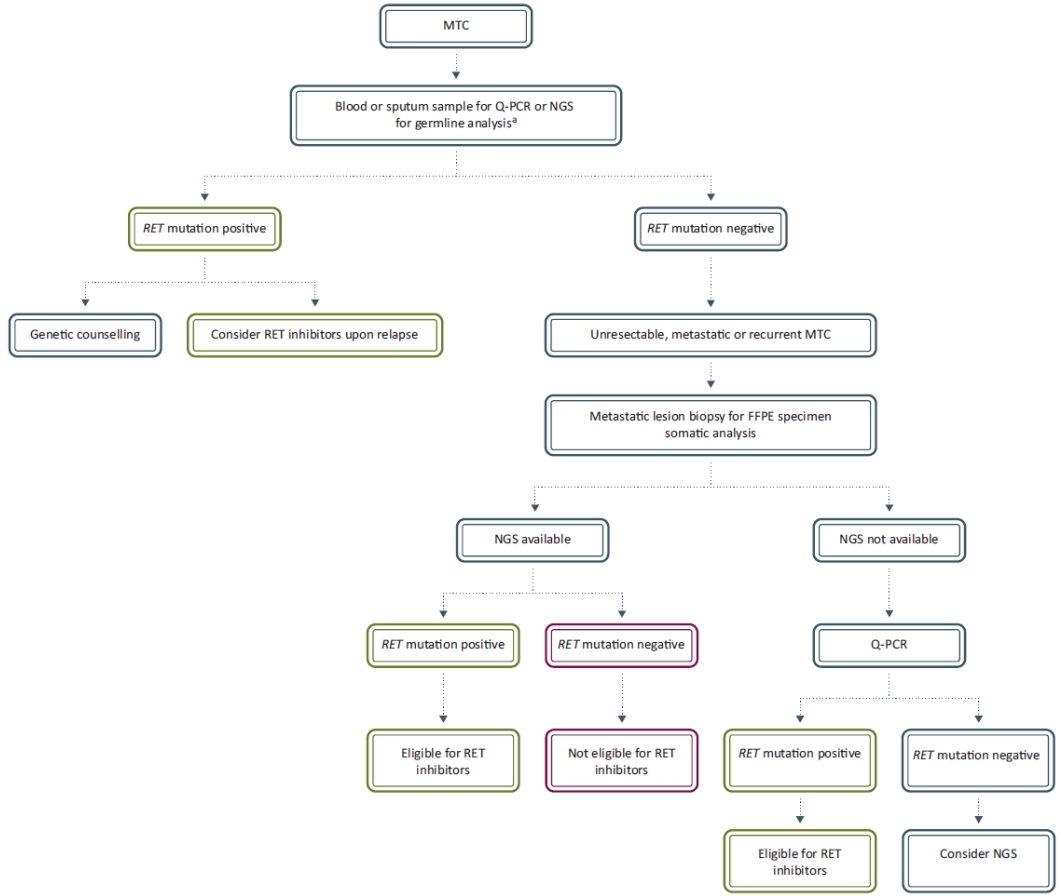
Pathologist makes diagnosis

↓

Oncologist makes treatment decision, considers further profiling and asks the pathologist to perform molecular profiling

ESMO recommended *RET* testing algorithms





Belli C, et al. *Ann Oncol* 2021;32:337–50.

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