Approccio diagnostico per l'analisi delle alterazioni di PIK3CA, AKT, PTEN

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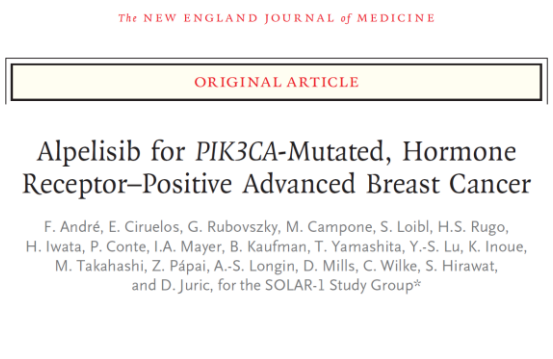
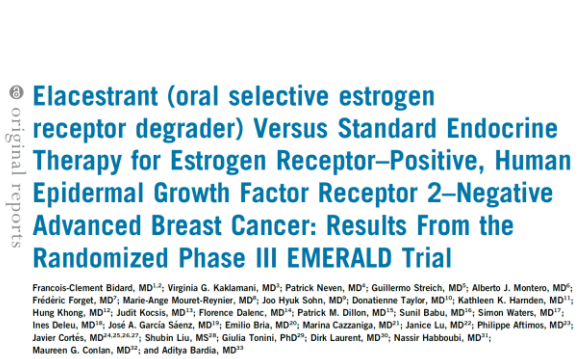
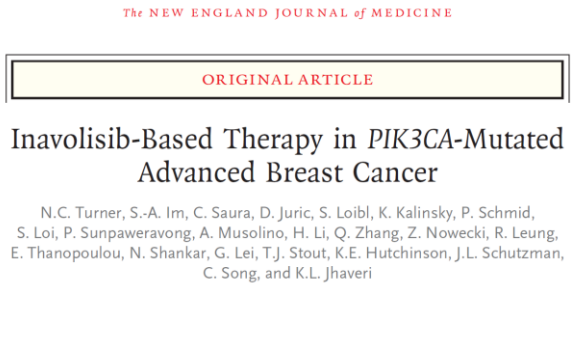
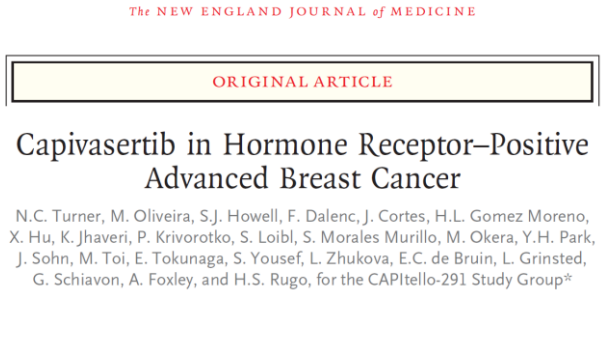
CLINICAL RATIONALE FOR MOLECULAR TESTING IN HR+/HER2- ADVANCED BREAST CANCER









Biomarker	Year	Drug	Target/class	Sample	Method /CDx
PIK3CA mutations	2019	Alpelisib	PIK3CA	Tissue (LB)	RT-PCR/NGS
PIK3CA mutations	Q1 2025 (EAP)	Inavolisib	PIK3CA	LB	NGS
PIK3CA/AKT1/PTEN alterations	2024 (EAP)	Capivasertib	AKT1	Tissue	NGS
ESR1 mutations	2024	Elacestrant	SERD	LB	ddPCR/NGS

EAP: expanded access program; LB: Lliquid Biopsy; ddPCR: digital droplet PCR

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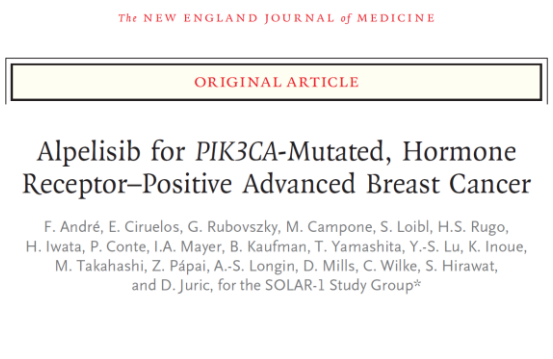

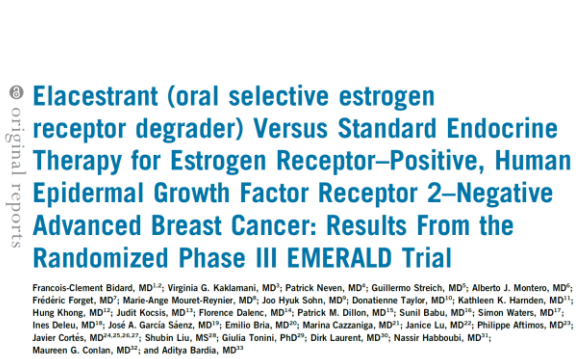

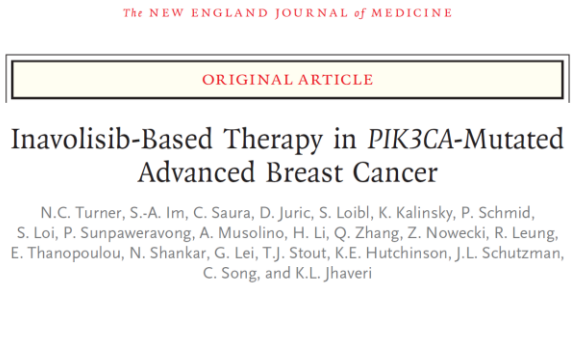

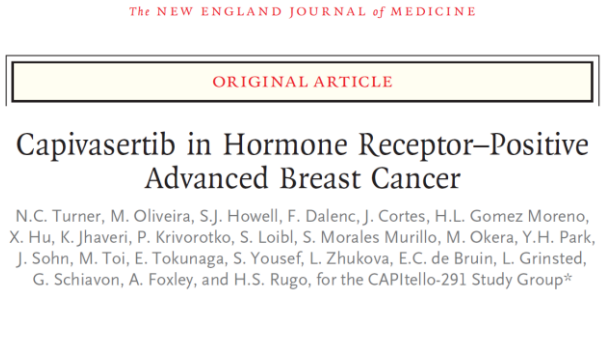

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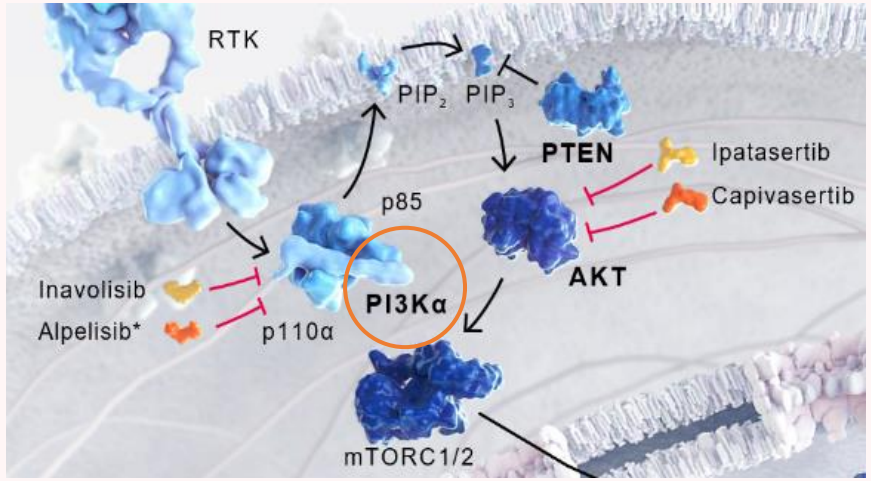
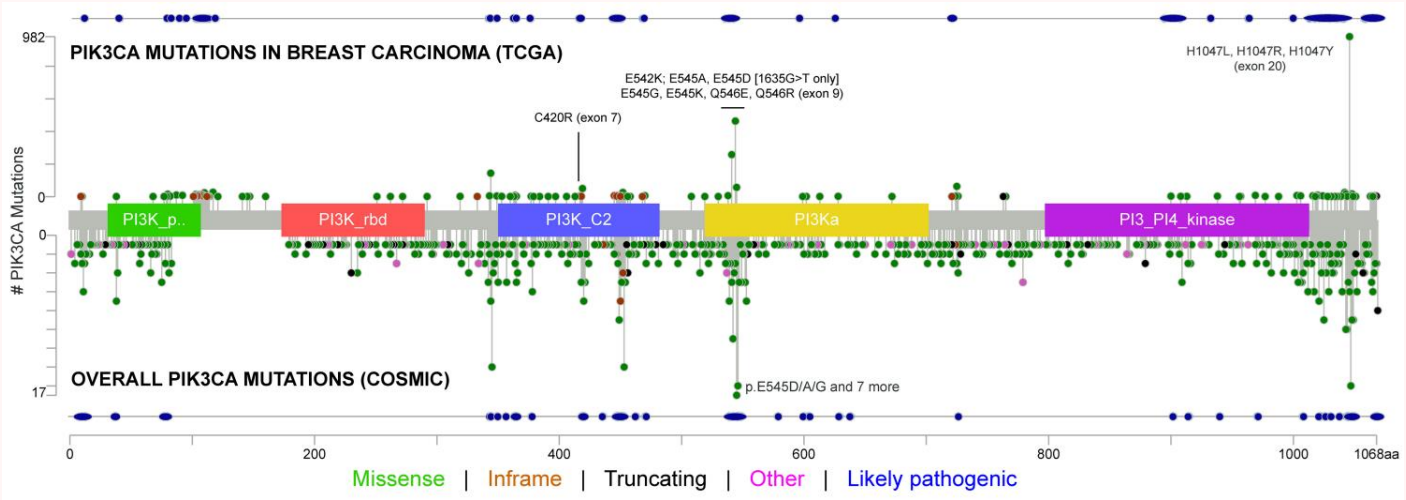
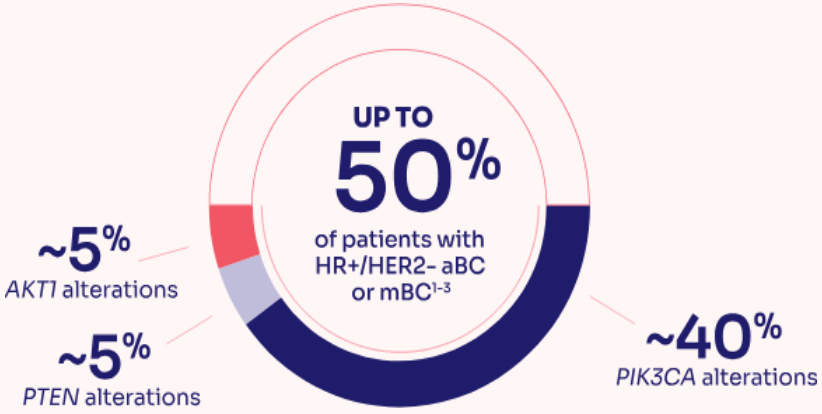
PI3K pathway increased relevance in HR+ Breast Cancer. Molecular information to guide treatment & improve patient outcomes







- **~40% of HR+/HER2– aBC patients have a mutation in the *PIK3CA* gene** and can have endocrine resistance and/or shorter mPFS
- **Hotspot regions in *PIK3CA*: ex 7, 9, 20** but also outside hot spots
- *PIK3CA* mutations can be detected in tissue (FFPE) or plasma samples
- *PIK3CA* mutations are **considered to be truncal**; samples from both primary and metastatic tumours can be used for testing[4-8]

1. Miricescu D, et al. Int J Mol Sci. 2020; 2. TCGA Nature. 2012; 3. Chung JH, et al. Ann Oncol. 2017; 4. Chang DY, *et al. Ther Clin Risk Manag* 2021; 5. O'Leary B, *et al. Cancer Discov* 2018; 6. Mroz EA and Rocco JW. *Cancer* 2017; 7. Miron A, et al. *Cancer Res* 2010; 8. Fusco N, et al. Front Oncol 2021

Leveraging past experiences to identify the ‘holy grail’ of PI3K inhibitors

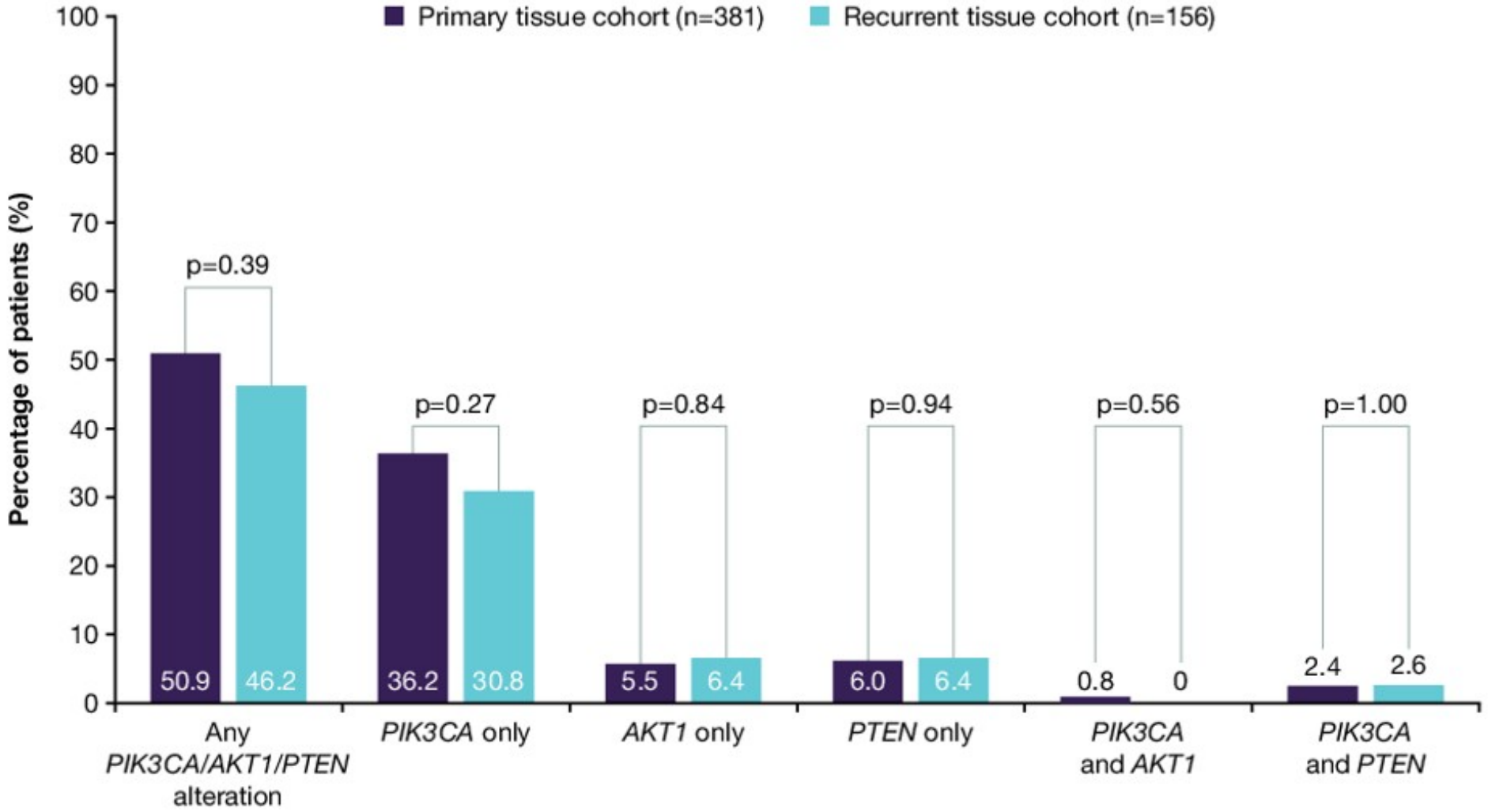
Pan-PI3K inhibitor	Pan-PI3K/ mTOR inhibitor	β-sparing inhibitor and ‘mut degradation facilitator’	PI3Kα- and PI3Kδ-specific	α-selective inhibitor	AKT isophorms inhibitor	α-selective inhibitor and ‘mut degradation facilitator’
Pictilisib[1] Buparlisib[2] *Discontinued*[3,4]	Gedatolisib *Under investigation*[5]	Taselisib *Discontinued*[6]	Copanlisib *Under investigation*[7,8]	Alpelisib *First Global approval 2019*[9,10]*	Capivasertib *EMA approval 2024 (tissue CDx)*	Inavolisib *EMA approval expected 2025 (liquid biopsy CDx)*

- **Achieving** an acceptable therapeutic index has been a challenge due to on-target toxicities
- **Specificity** toward p110α is paramount to enable a greater therapeutic index in a biomarker-defined population

Alpelisib is also approved in the EU.[17]

1. Krop IE, *et al. Lancet Oncol* 2016; 2. Di Leo A, *et al. Lancet Oncol* 2018; 3. https://ascopost.com/News/40583 (accessed March 2024);
4. https://www.cancernetwork.com/view/buparlisib-effective-toxic-advanced-breast-cancer-patients (accessed March 2024); 5. https://clinicaltrials.gov/ct2/show/NCT05501886 (accessed May 2023); 6. Jhaveri K, *et al. Clin Cancer Res* 2021; 7. https://clinicaltrials.gov/ct2/show/NCT03939897 (accessed March 2024);
8. https://www.hcp.aliqopa-us.com/mechanism-of-action (accessed March 2024); 9. PIQRAY PI 2024;
10. https://www.novartis.com/news/media-releases/fda-approves-novartis-piqray-first-and-only-treatment-specifically-patients-pik3ca-mutation-hrher2-advanced-breast-cancer (accessed March 2024); 11. Dey A, *et al*. SABCS 2019 (Poster P3-11-23); 12. Roche. Data on file; 13. https://clinicaltrials.gov/ct2/show/NCT05216432 (accessed March 2024);
14. Varkaris A, *et al.* AACR 2023 (Oral CT017); 15. https://clinicaltrials.gov/study/NCT05768139 (accessed March 2024); 16. https://clinicaltrials.gov/study/NCT05307705 (accessed March 2024); 17. PIQRAY SmPC 2024.

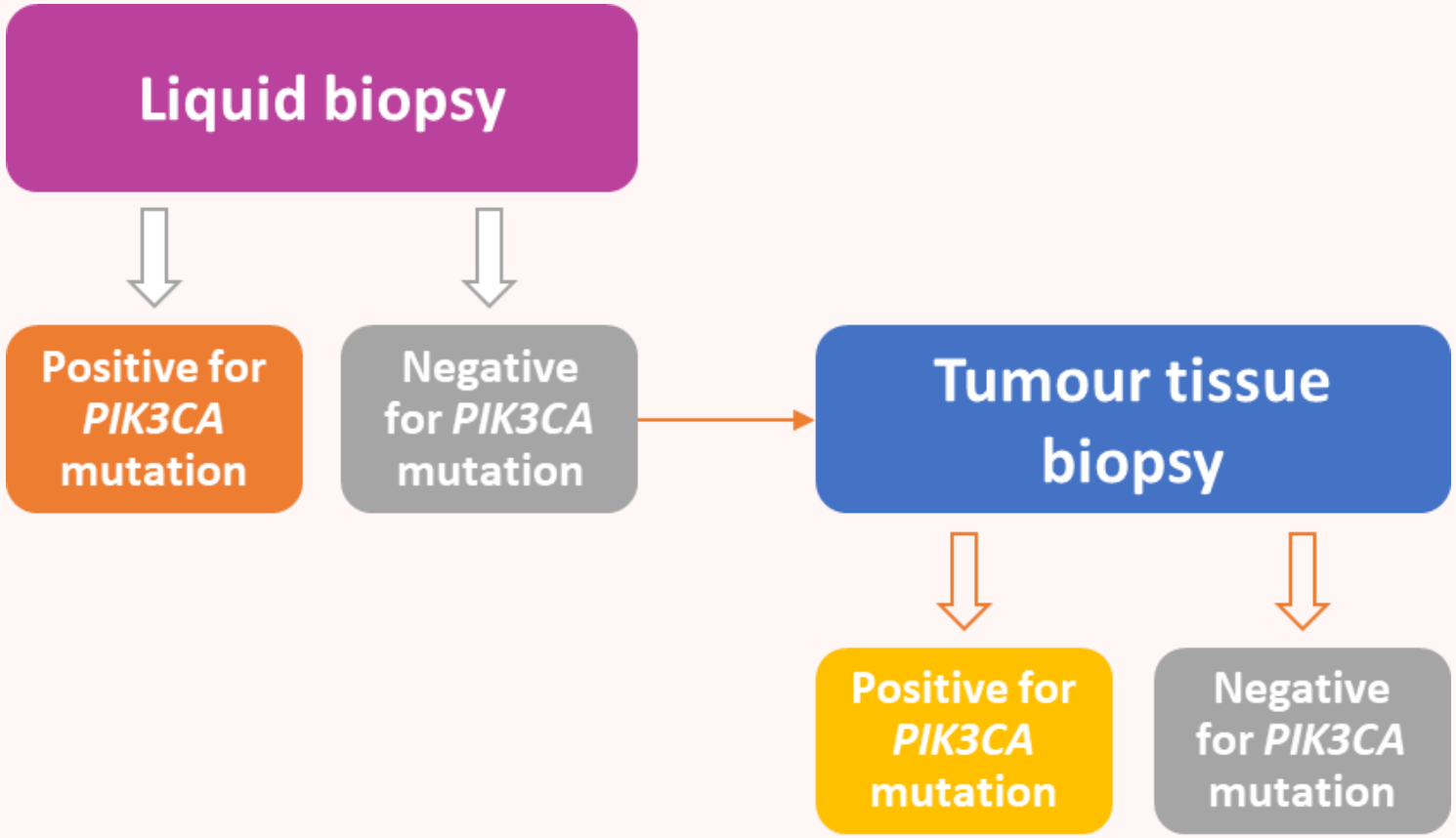
TISSUE OR LIQUID BIOPSY?
STABILITY OF PI3K PATHWAY ALTERATIONS IN PRIMARY AND RECURRENT BREAST CANCER TISSUES



Analysis using Chi-square or Fisher's exact test. No correction for multiplicity of analysis applied.

Cortes et al. SABCS 2024

Clinical guidelines recommend liquid or tumour tissue biopsy; if liquid biopsy is negative, tumour tissue testing is recommended



1. Henry LN, *et al. J Clin Oncol* 2022;
2. NCCN Breast Cancer Guidelines; Version 1, 2024;
3. Gennari A, *et al. Ann Oncol* 2021.

San Antonio Breast Cancer Symposium® – December 10–13, 2024
Poster number: P1-05-27

Analytical comparison of tissue-based next-generation sequencing assays for the detection of *PIK3CA*, *AKT1*, and *PTEN* tumor alterations in breast cancer

Xiaodun Li,*[1] Alexander Yarunin,[2] Benjamin Chaffey,[2] Manisha Maurya,[3] Peter Stewart,[3] Fionn Corr,[3] Efstratios Efstratiou,[3] Kirsty Trewellard,[3] & David Gonzalez[3]

[1]Precision Medicine and Biosamples, AstraZeneca, Cambridge, UK; [2]Global Oncology Diagnostics, AstraZeneca, Cambridge, UK; [3]Queen's University, Belfast, UK

*Presenting author

Table 1. Eligible *PIK3CA*, *AKT1*, and *PTEN* alterations for FoundationOne®CDx companion diagnostic

Gene (transcript)	Variant class	Biomarker rules defining biomarker positive status
AKT1 (NM_001014431)	Short variant	Any short variant with protein effect E17K
PIK3CA (NM_006218)	Short variant	Any of 19 short variants: R88Q, N345K, C420R, E542K, E545A, E545D, E545Q, E545K, E545G, Q546E, Q546K, Q546R, Q546P, M1043V, M1043I, H1047Y, H1047R, H1047L, and G1049R
PTEN (NM_000314)	Short variant	Any of 13 short variants: C124R, C124S, G129E, G129V, G129R, R130Q, R130G, R130L, R130P, C136R, C136Y, S170R, and R173C Any nonsense, frameshift, or splice site alteration
	Copy number alteration	Any homozygous deletion of one or more exons, regardless of transcript
	Rearrangement	Any rearrangement that disrupts protein function, regardless of transcript • Intragenic events including duplications of only part of the gene, deletions, or inversions • Translocations, deletions, or inversions where one breakpoint is in *PTEN* and the other breakpoint is in another gene or intergenic region

Table 2. Technical specifications of NGS assays

	FoundationOne® CDx used in CAPItello-291	AVENIO-CGP (reference)	TruSight Oncology 500	oncoReveal Core LBx	Oncomine Comprehensive Assay v3	AmoyDx HANDLE Classic	SOPHiA ExtHRS
Manufacturer	Foundation Medicine	Roche Diagnostics	Illumina	Pillar Biosciences	Thermo Fisher Scientific	Amoy Diagnostics	SOPHiA GENETICS
Input DNA source material	FFPE solid tissue	FFPE solid tissue	FFPE solid tissue	Liquid biopsy	FFPE solid tissue	FFPE solid tissue	FFPE solid tissue
No. of genes profiled	324	324	523	104	161	40	28
Target enrichment	Hybrid-capture	Hybrid-capture	Hybrid-capture	Amplicon-based	Amplicon-based	HANDLE system	Amplicon-based
Single nucleotide variants *AKT1*/*PIK3CA*/*PTEN*	✓	✓	✓	✓	✓	✓	✓
Complex *PTEN* alterations (copy number alterations/ rearrangements)	✓	✓	✓				✓
NGS sequencing platform	Illumina HighSeq 2500/4000	Illumina NextSeq 500/550	Illumina NextSeq 500/550	Illumina NextSeq 500/550	Ion Torrent	Illumina NextSeq 500/550	Illumina NextSeq 500/550

NGS OR PCR? CLINICALLY RELEVANT *PIK3CA* PATHWAY ALTERATIONS MAY BE DETECTED USING DIFFERENT TECHNIQUES

Single biomarker and/or multi-gene hotspot NGS panel tests

Hybrid capture-based NGS/CGP

Immunohistochemistry (IHC)

- ***Single biomarker analysis (protein expression)***

Fluorescence *in situ* hybridisation (FISH)

- ***Single biomarker analysis***
- ***Copy number alterations***

Polymerase chain reaction (PCR)

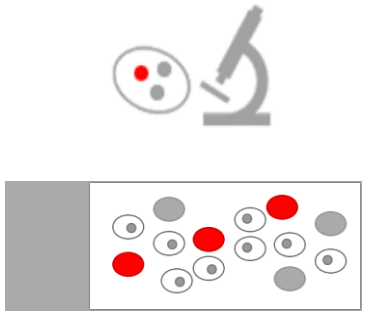
NGS

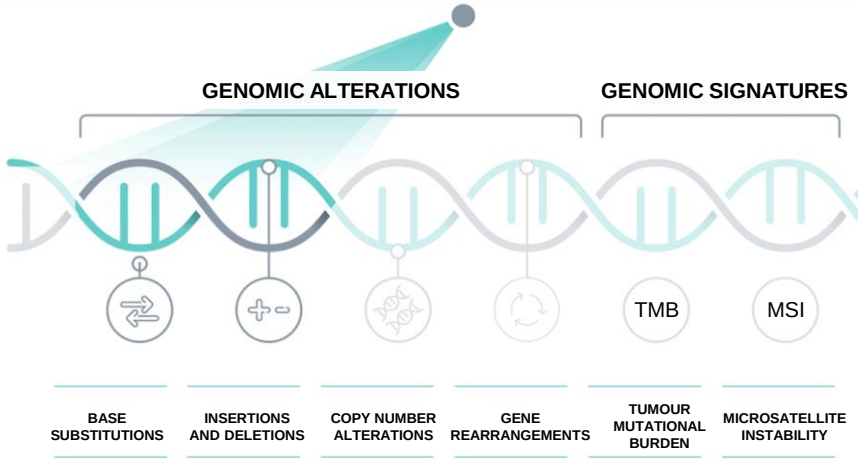
Conventional methods for identifying targetable alterations in cancer, including IHC and FISH, identify single protein or nucleic acid biomarker

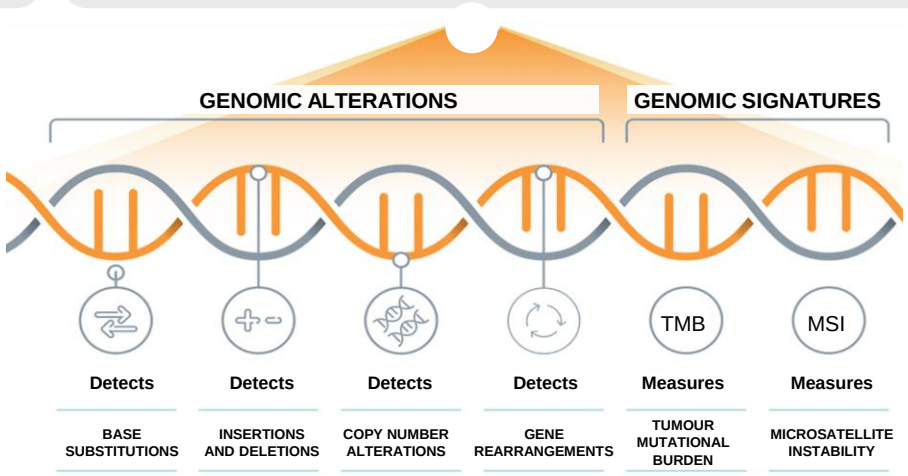
Not suitable for *PIK3CA and AKT1* mut but suitable for *PTEN* loss

- Detects a predefined set of specific gene alterations that are associated with an effective targeted therapy
- Can **miss** indels, copy number alterations, rearrangements

- Can target the whole coding sequence of a gene and is able to detect all four main classes of gene alterations
- Also allows for the characterisation of both **known and novel variants** for discovery-related applications

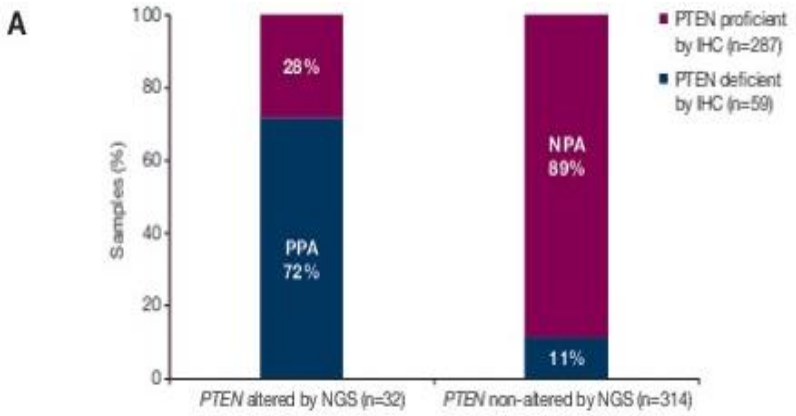


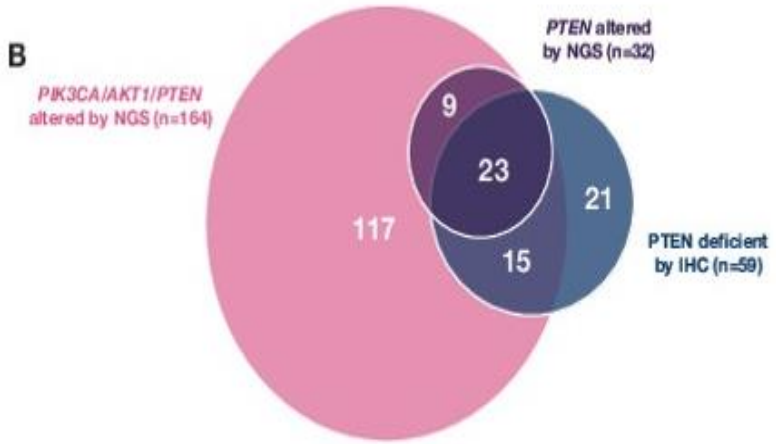


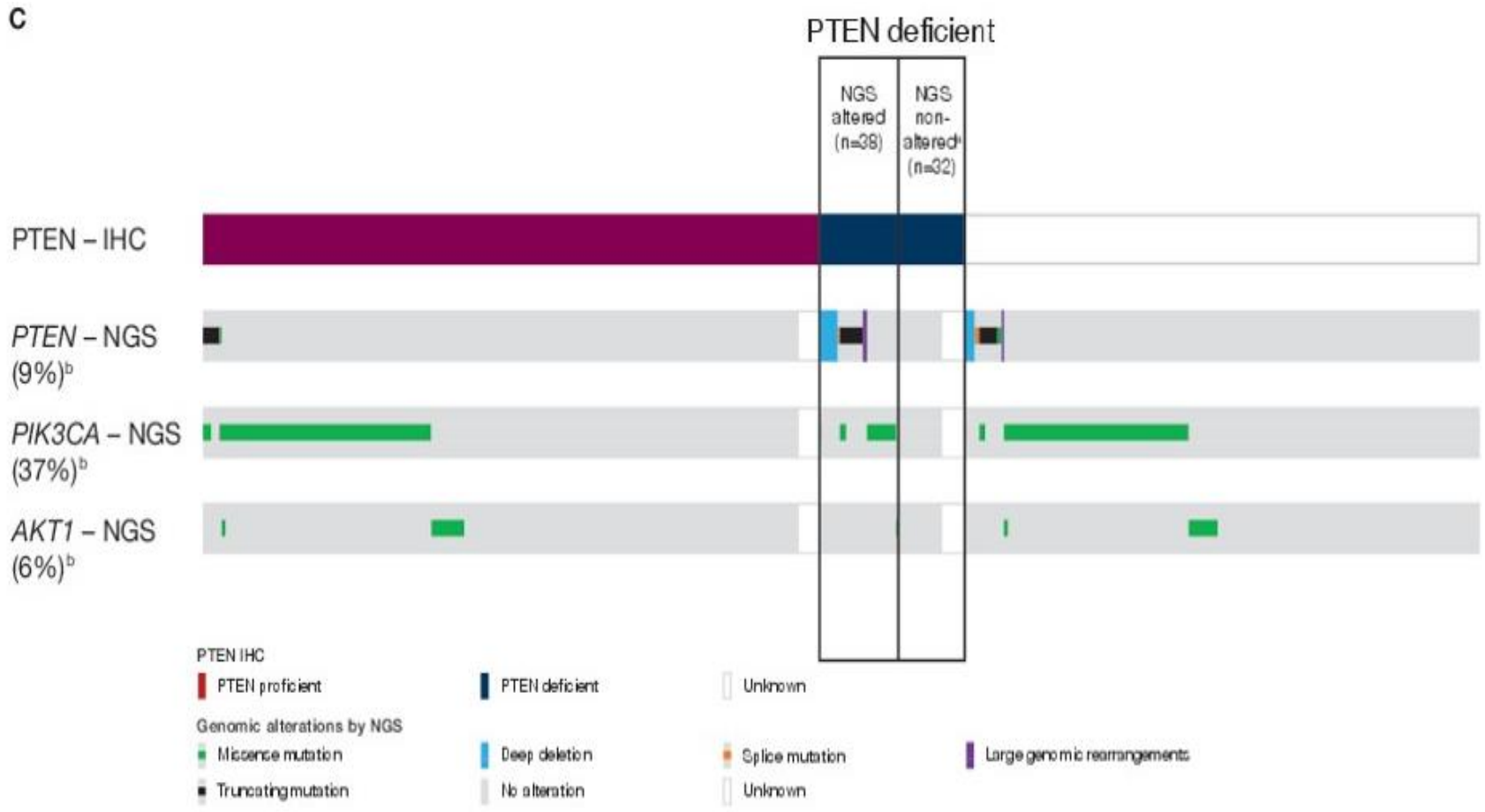


1. Dong L, *et al. Current Genomics* 2015; 2. Gray PN, *et al. Cancers* 2015;
3. Frampton GM, *et al. Nat Biotechnol* 2013; 4. Roche. Data on file.

PTEN IHC VS. NGS







[a]The *PIK3CA/AKT1/PTEN*-non-altered group includes patients with confirmed *PIK3CA/AKT1/PTEN*-non-altered and unknown results; [b]Out of 594 tumor tissue samples tested by NGS using the FoundationOne®CDx assay.

Jhaveri et al. SABCS 2024

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Letter to the Editor

Next-generation sequencing for *PTEN* testing in HR+/HER2− metastatic breast cancer

ARTICLE INFO

Keywords:
Hormone receptor-positive breast cancer
PIK3CA pathway
PTEN alterations
Next-generation sequencing (NGS)
Precision oncology

ABSTRACT

Molecular alterations in the Phosphoinositide 3-kinase (PI3K) pathway are key drivers of tumorigenesis and progression in hormone receptor-positive, HER2-negative (HR+/HER2 −) metastatic breast cancer (MBC). These genomic changes are actionable through targeted therapeutic agents. In particular, access to these therapies depends on accurate molecular testing of *PIK3CA*, *AKT1*, and *PTEN*. Next-generation sequencing (NGS) has emerged as a transformative diagnostic tool, offering a comprehensive analysis of PI3K pathway alterations while concurrently evaluating other actionable markers, such as *ESR1* and *BRCA*. Acknowledging its clinical importance, the European Society for Medical Oncology (ESMO) recommends NGS of tumor or plasma samples as the standard of care for patients with HR+ /HER2 − MBC. Although resource-intensive, NGS represents a significant advancement in MBC diagnostics, ensuring that therapeutic decisions are informed by a detailed and multidimensional molecular profile. This review highlights the capabilities of NGS for PI3K pathway testing in HR+ / HER2 − MBC, with a particular focus on the spectrum of *PTEN* alterations.

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NGS offers a comprehensive analysis of PI3K pathway alterations while concurrently evaluating other actionable markers, such as ESR1 and BRCA

Manuscript under review

Immunohistochemistry for PTEN testing in HR+/HER2- metastatic breast cancer

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[2] Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy.

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Simplified report for PTEN test by immunohistochemistry in breast cancer

SPECIMEN
Date of collection
DIAGNOSIS

PTEN testing by immunohistochemistry:
Staining platform
Antibody clone
Antigen retrieval method

Report the percentage (%) of positive cells

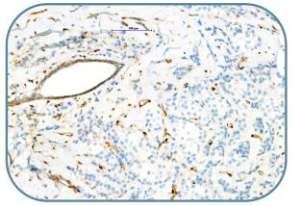
RESULT: PTEN Retained/Deficient

Normal internal controls are stromal and breast epithelial cells

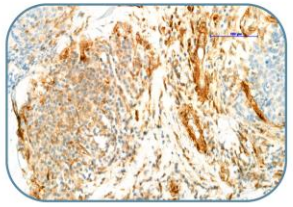
Assay	Isotype	Clone	PTEN immunogen	References
6H2.1	IgG	Mouse	304-403	(25-31)
17.A	IgM	Mouse	2-403	(32-34)
138G6	IgG	Rabbit	388-394	(35-37)
D4.3	n/s	Rabbit	388-394	(38-40)
28H6d	IgG1	Mouse	203-403	(41-43)
A2B1	IgG1	Mouse	388-403	(44, 45)
SP218	IgG	Rabbit	394-402	(18)

Overview of key assays for PTEN IHC testing in breast cancer literature. All antibodies are monoclonal; n/s, not specified.

Positivity threshold: 10% (any score)



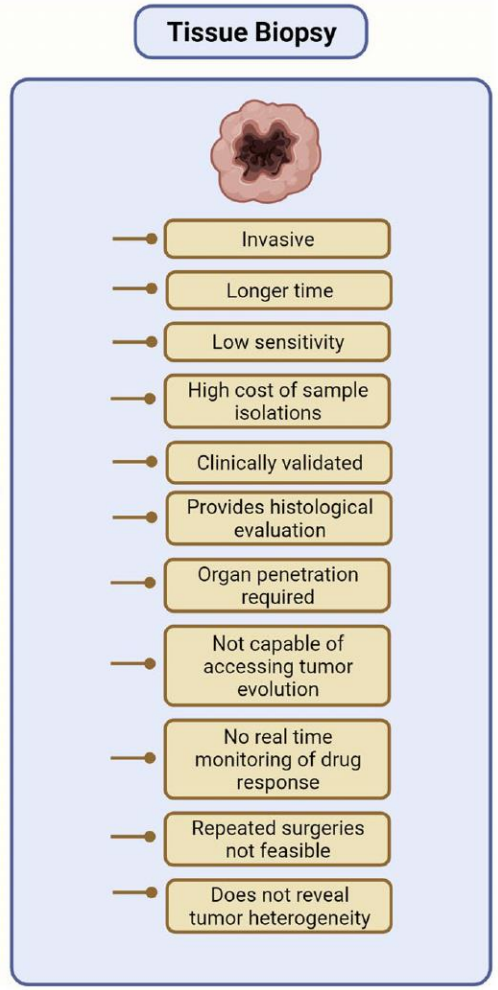
PTEN DEFICIENT



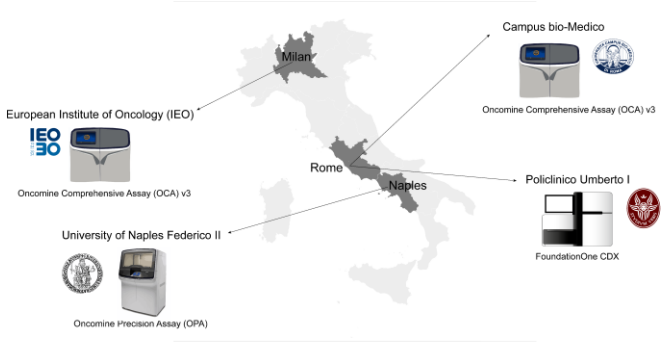
PTEN RETAINED

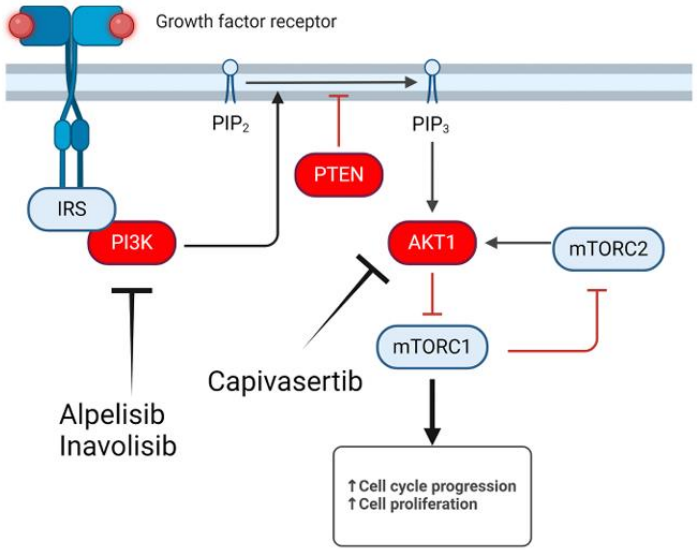
Score 0: No staining in tumor cells with postive internal control.
Score 1: Tumor cell staining weaker than in surroinding normal cells.
Score 2: Tumor cell staining equivalent to that of surrounding normal cells.

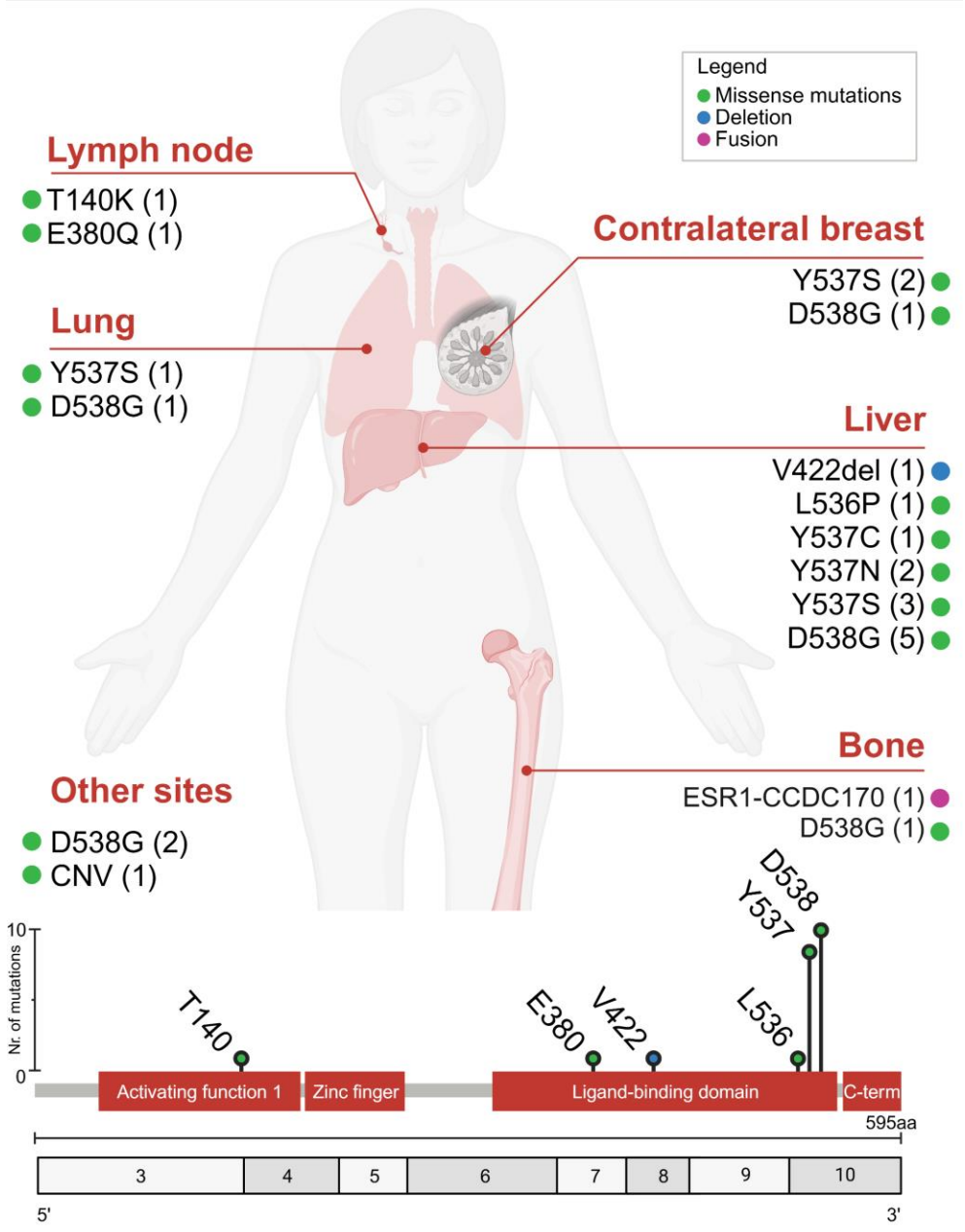
NGS vs PCR and TISSUE + LB integration



Kavan, S. et al., Cancer and Metastasis Reviews, 2022

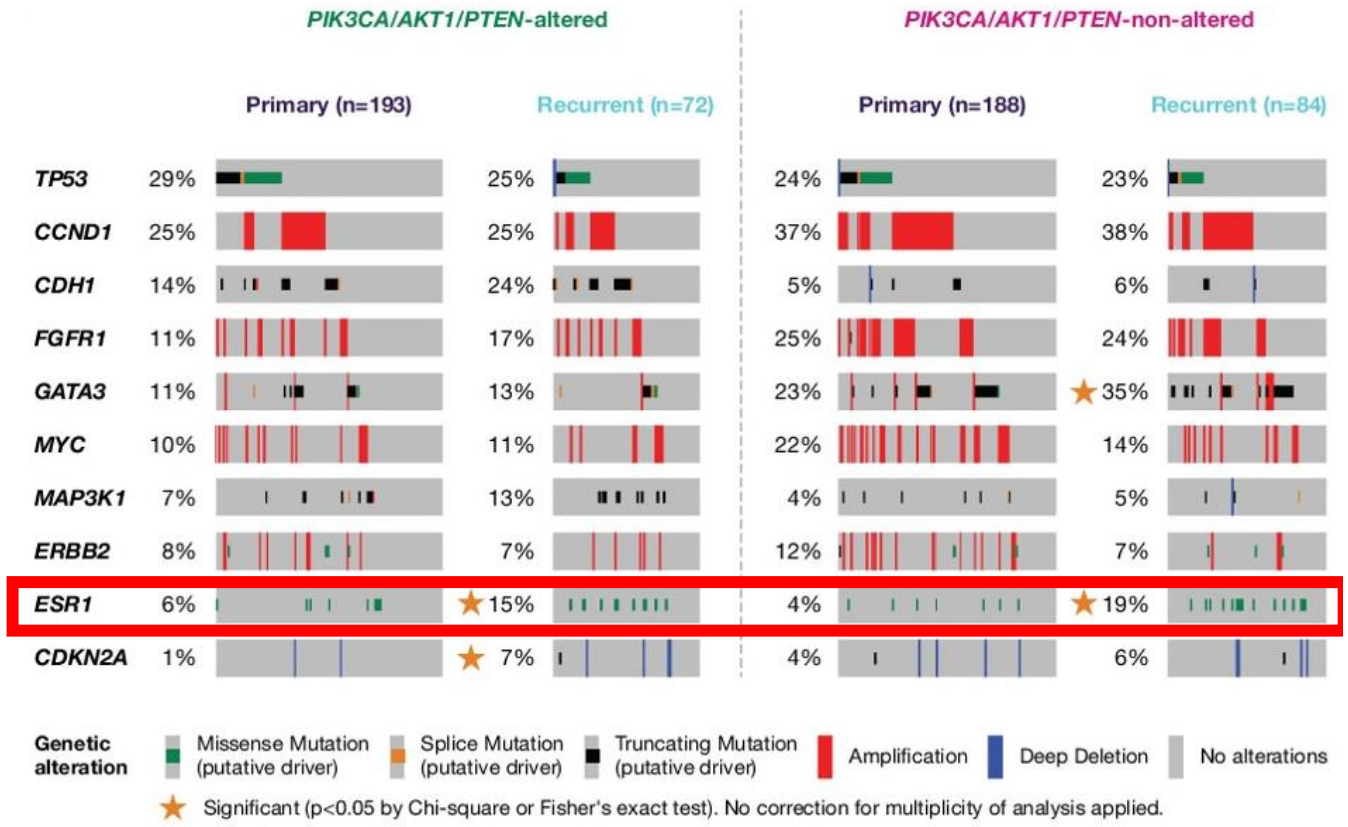






Venetis K. et al., Breast Cancer Research, 2025

CO-MUTATIONS

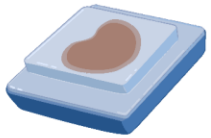


	ESR1mut (n=24)	ESR1wt (n=14)	Total (n=38)
PIK3CA mutations, n (%)	8 (33.3)	8 (57.1)	16 (42.1)
p.V344M, c.1030G>A (E5)	1 (12.5)	0	1 (6.3)
p.E542K, c.1624G>A (E10)	0	2 (25.0)	2 (12.6)
p.E545K, c.1633G>A (E10)	2 (25.0)	1 (12.5)	3 (18.8)
p.E545Q, c.1633G>C (E10)	1 (12.5)	0	1 (6.3)
p.H556Y, c.1666C>T (E11)	0	1 (12.5)	1 (6.3)
p.E726K, c.2176G>A (E14)	1 (12.5)	0	1 (6.3)
p.H1047R, c.3140A>G (E21)	2 (25.0)	3 (37.5)	5 (31.3)
p.H1047L, c.3140A>T (E21)	1 (12.5)	1 (12.5)	2 (12.6)

Table 4. Frequency and type of *PIK3CA* mutations according to *ESR1* status. A total of n=8 *PIK3CA* mutations was detected in n=6 *PIK3CA*-mutant cases in the ESR1mut group.

Venetis K, et al. Unpublished data 2025

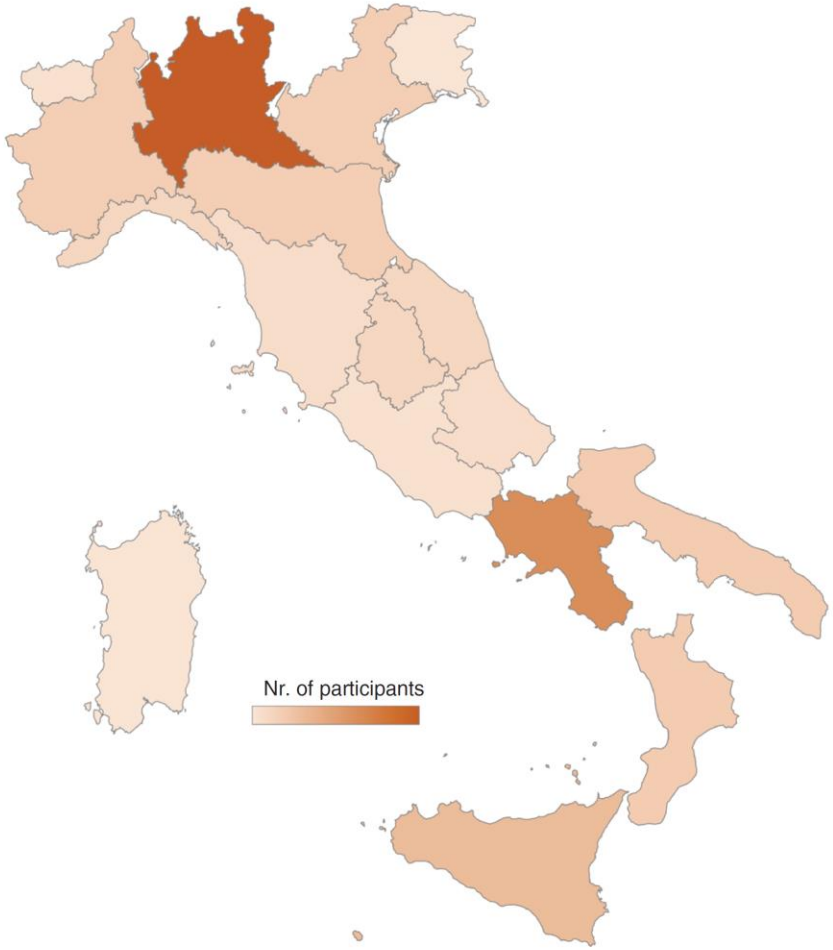
GENOMIC TESTING: WHAT, WHEN, HOW AND WHERE TO TEST? TIPS FOR ONCOLOGISTS

Sample type		DNA quantity	DNA quality		Biomarkers	RT-PCR	dPCR	Target NGS	CGP
Tissue (FFPE) 	Metastatic site 	→ / ↓	↑ If recent sample	→ / ↓ if old sample (more than 5 years or de-calcified bone metastasis)	ESR1				
					PIK3CA				
					PIK3CA pathway				
	Primary tumor 	↑	↑ If recent sample	→ / ↓ if old sample (more than 5 years or de-calcified bone metastasis)	ESR1				
					PIK3CA				
					PIK3CA pathway				
Liquid Biopsy 	ctDNA 	↓	↑		ESR1				
					PIK3CA				
					PIK3CA pathway				

De Angelis et al. 2025, in preparation

PIK3CA mutation testing 2022

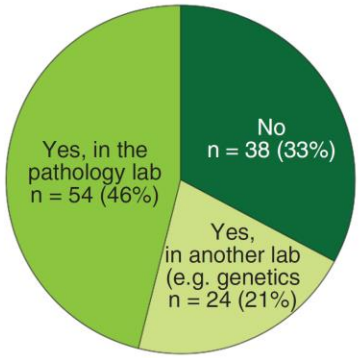
Ⓐ



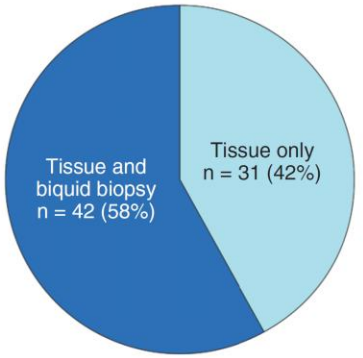
North-west	**52**
Valle d'Aosta	2
Piemonte	7
Lombardia	38
Liguria	5
North-east	**15**
Veneto	7
Friuli Venezia Giulia	1
Emilia Romagna	7
Center	**18**
Toscana	3
Umbria	5
Marche	5
Lazio	2
Abruzzo	3
South and islands	**53**
Campania	24
Puglia	8
Calabria	8
Sardegna	1
Sicilia	12

Ⓑ

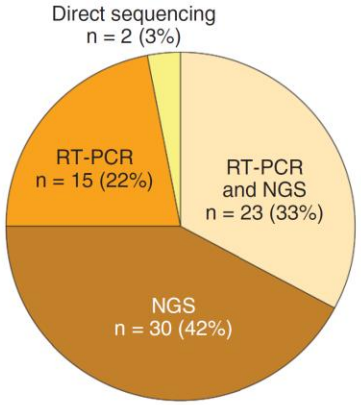
Q1: Is your center capable of conducting the *PIK3CA* test?
n = 116 answers



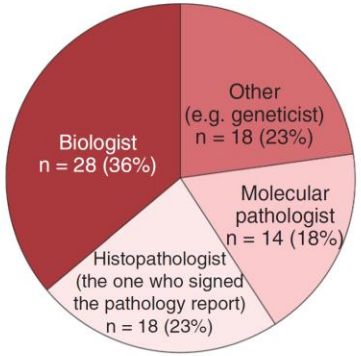
Q2: What are the acceptable biomaterials in your laboratory?
n = 73 answers



Q3: What technology options are available for this test at your center?
n = 70 answers



Q4: Which healthcare provider is responsible for signing the *PIK3CA* clinical report?
n = 78 answers



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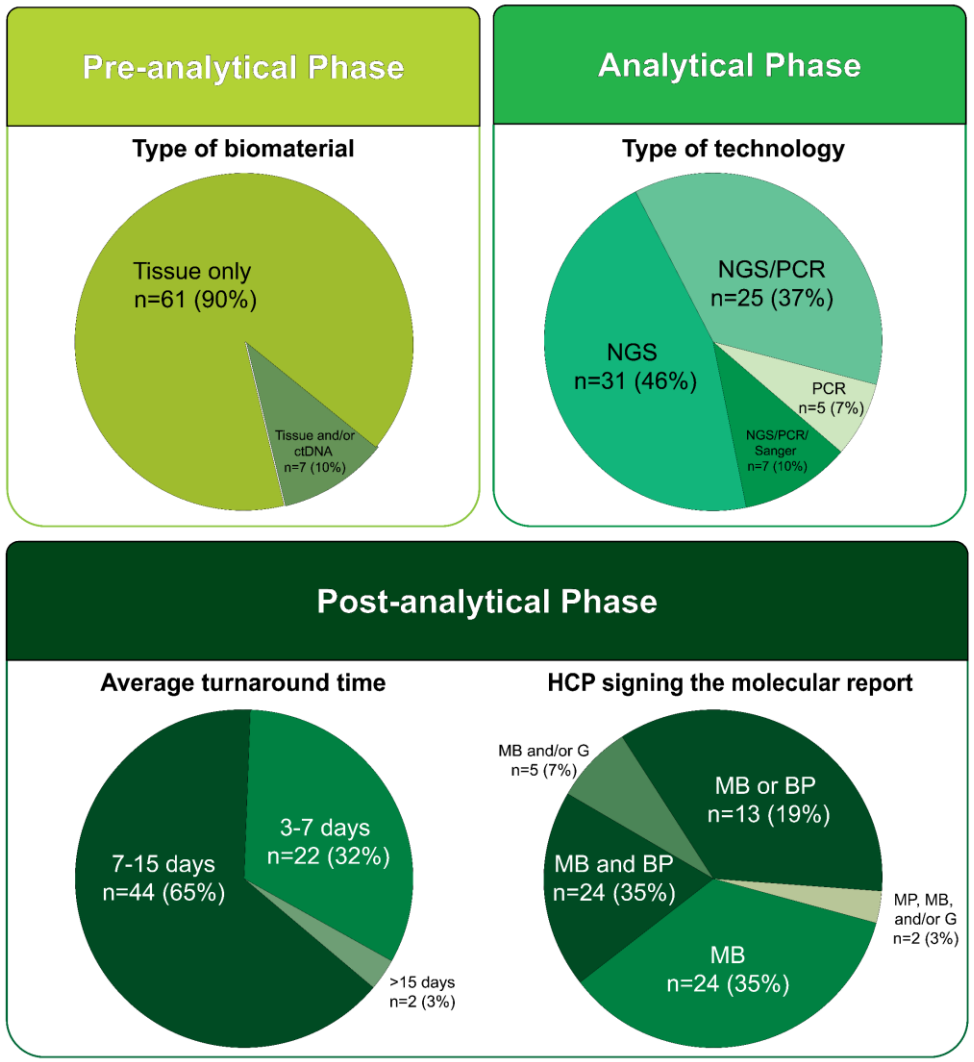
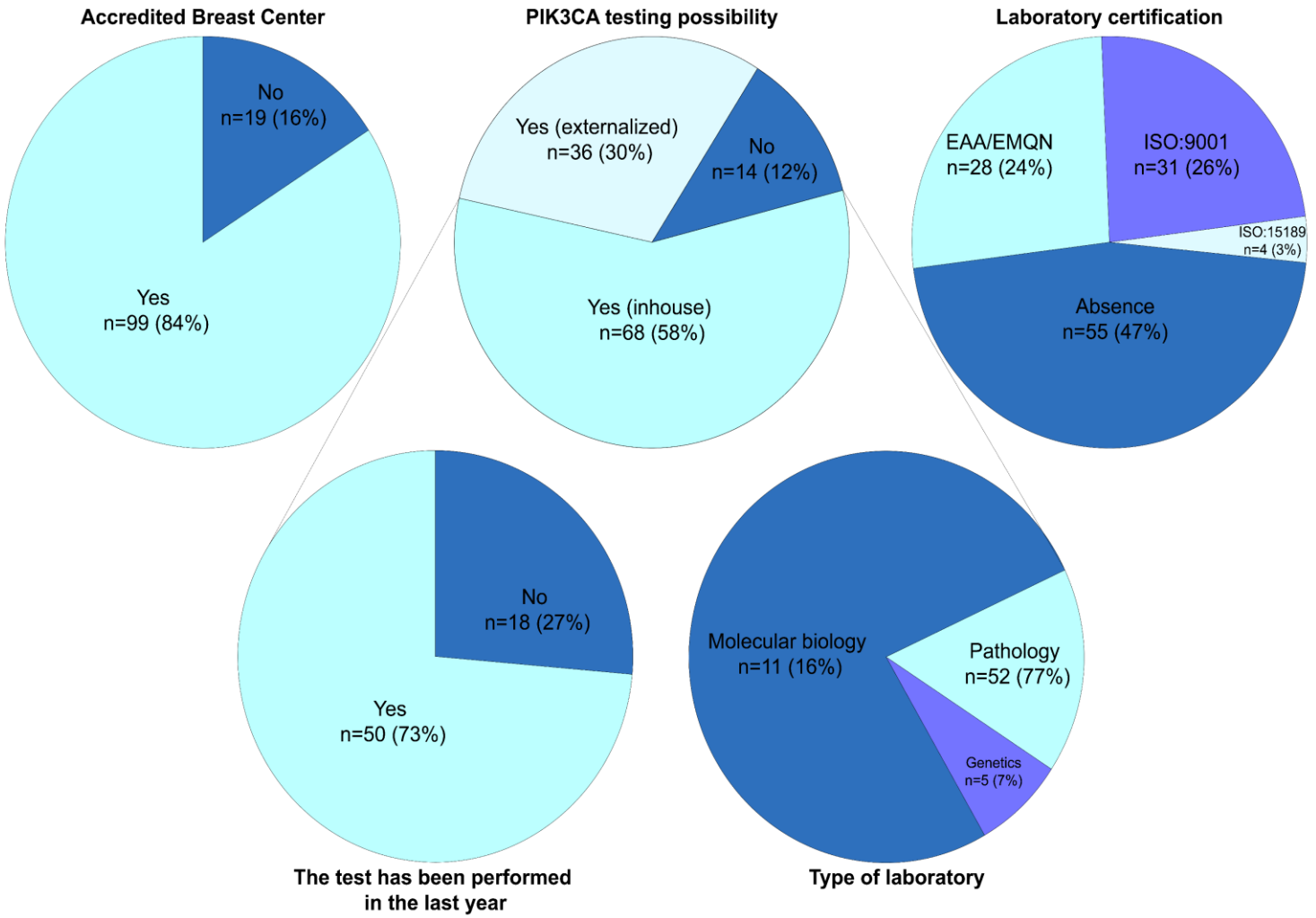
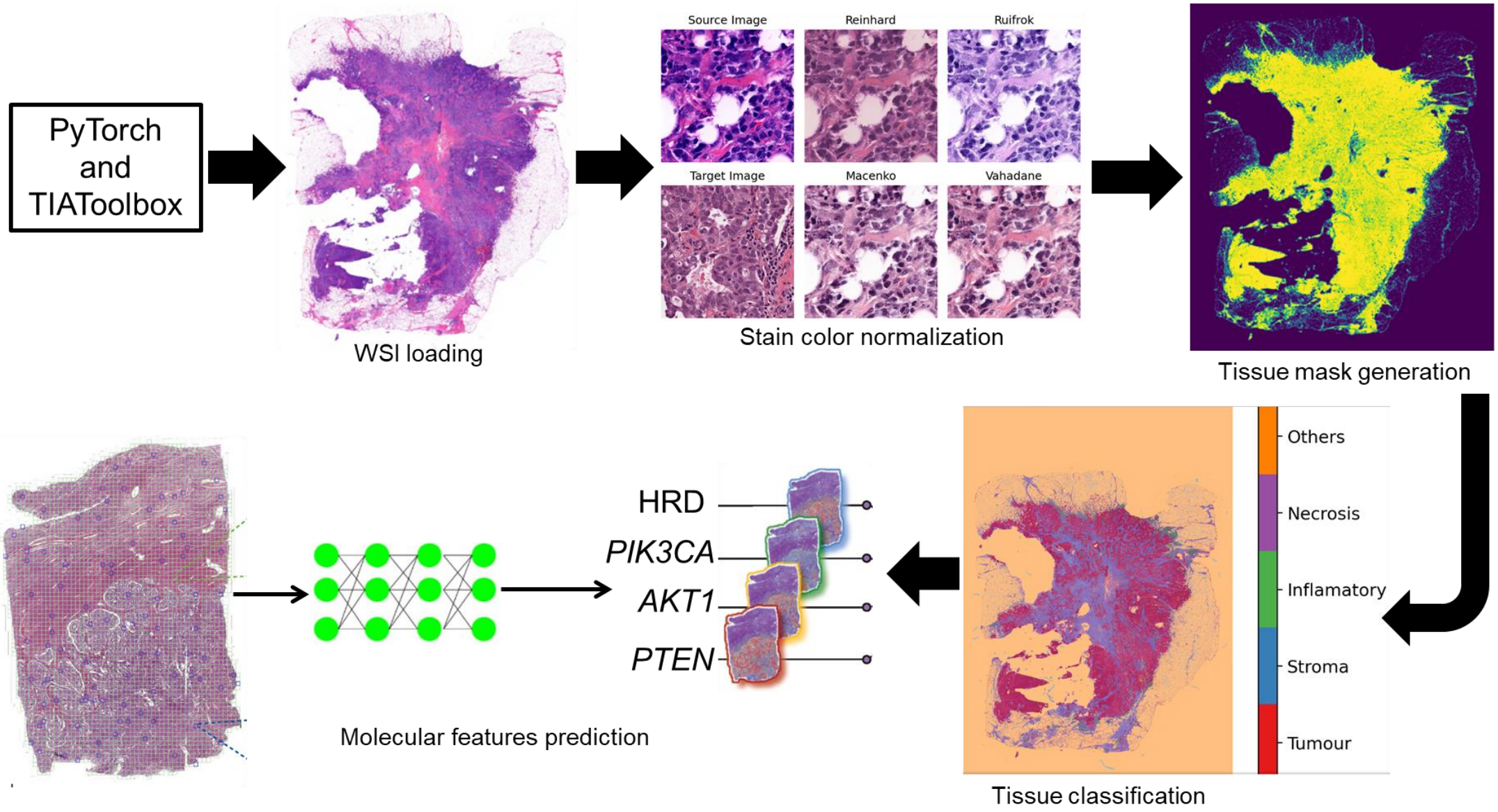
PIK3CA mutation testing 2025 (n=118 Centers)





Mane E, et al. In press 2025

PIPELINE FOR THE PREDICTION OF COMPLEX MOLECULAR BIOMARKERS IN BREAST CANCER



Frascarelli et al., Comput Struct Biotechnol J . 2024

Thank you

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Scan me!

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