

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

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"Saper leggere" uno studio clinico per migliorare la pratica clinica

Coordinatori Scientifici:
Stefania Gori
Giovanni L. Pappagallo

Verona, 28 - 29 Marzo 2025
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CLINICAL RATIONALE FOR MOLECULAR TESTING IN HR+/HER2- ADVANCED BREAST CANCER

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

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original reports

Elacestrant (oral selective estrogen receptor degrader) Versus Standard Endocrine Therapy for Estrogen Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: Results From the Randomized Phase III EMERALD Trial

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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: Liquid Biopsy; ddPCR: digital droplet PCR

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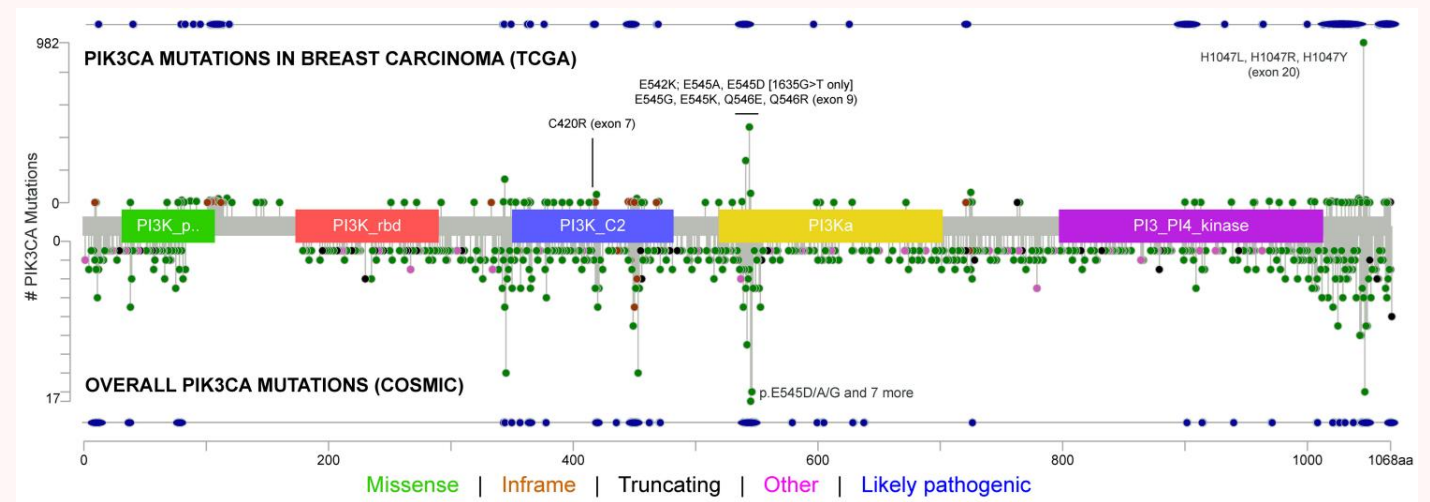
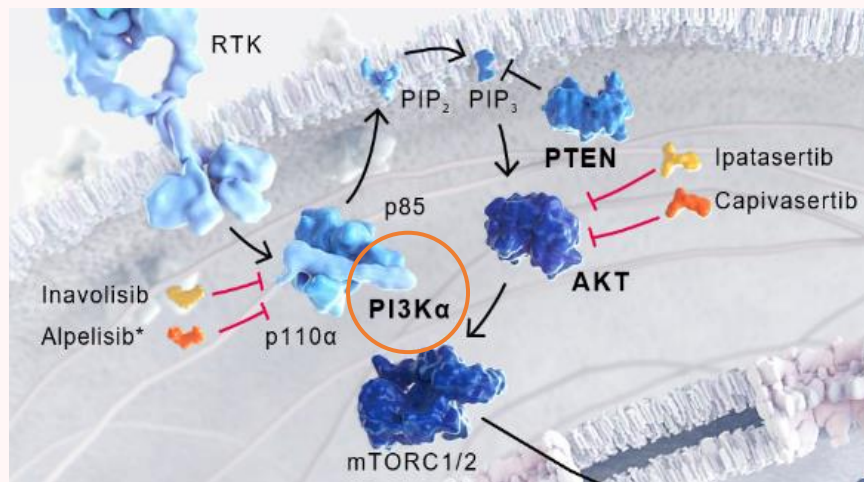
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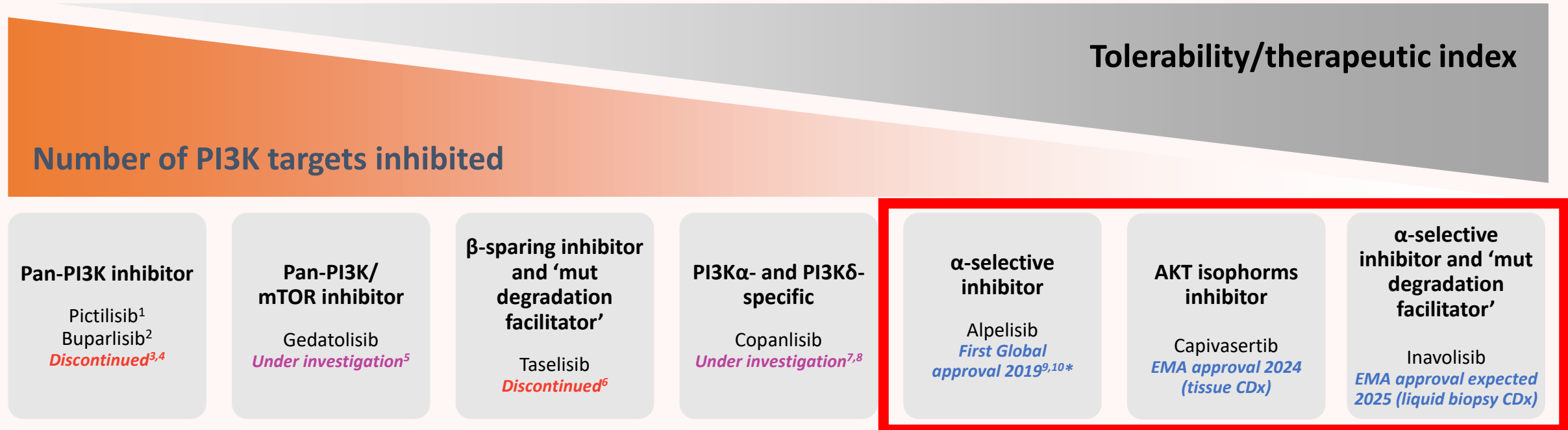
EAP: expanded access program; LB: Liquid Biopsy; ddPCR: digital droplet PCR

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 testing⁴⁻⁸

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



- **Achieving** an acceptable therapeutic index has been a challenge due to on-target toxicities
- **Specificity** toward p110 α 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>

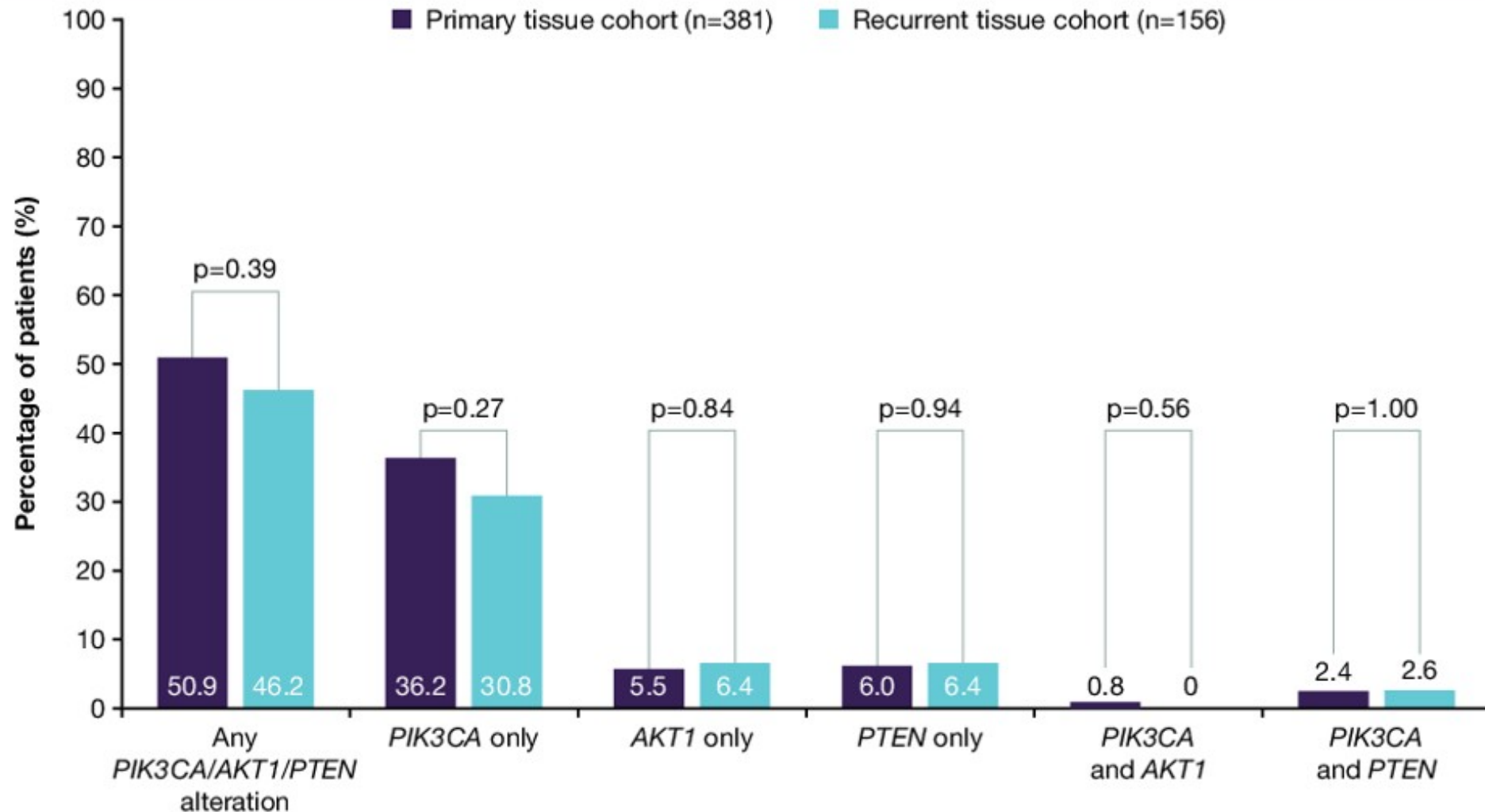
(accessed March 2024); 11. Dey A, et al. SABCS 2019 (Poster P3-11-23); 12. Roche. Data on file; 13. <https://clinicaltrials.gov/ct2/show/NCT05216432> (accessed March 2024);

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?

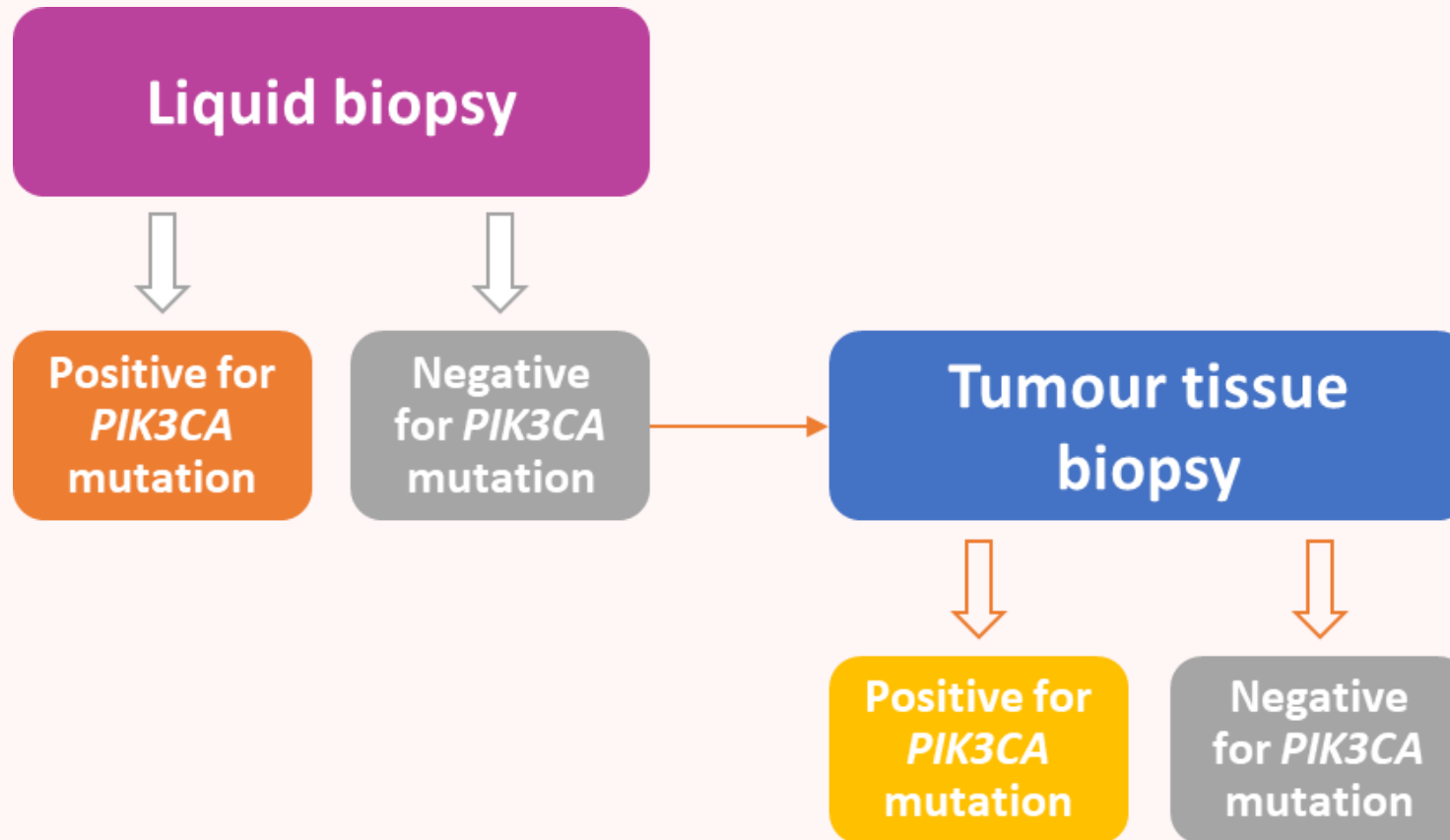
STABILITY OF PI3K PATHWAY ALTERATIONS IN PRIMARY AND RECURRENT BREAST CANCER TISSUES



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.

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 Gonzalez³

¹Precision Medicine and Biosamples, AstraZeneca, Cambridge, UK; ²Global Oncology Diagnostics, AstraZeneca, Cambridge, UK; ³Queen'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
<i>AKT1</i> (NM_001014431)	Short variant	Any short variant with protein effect E17K
<i>PIK3CA</i> (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
	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
<i>PTEN</i> (NM_000314)	Copy number alteration	Any homozygous deletion of one or more exons, regardless of transcript Any rearrangement that disrupts protein function, regardless of transcript
	Rearrangement	<ul style="list-style-type: none"> Intragenic events including duplications of only part of the gene, deletions, or inversions Translocations, deletions, or inversions where one breakpoint is in <i>PTEN</i> 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 <i>AKT1/PIK3CA/PTEN</i>	✓	✓	✓	✓	✓	✓	✓
Complex <i>PTEN</i> 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)

Fluorescence *in situ* hybridisation (FISH)

Polymerase chain reaction (PCR)

NGS

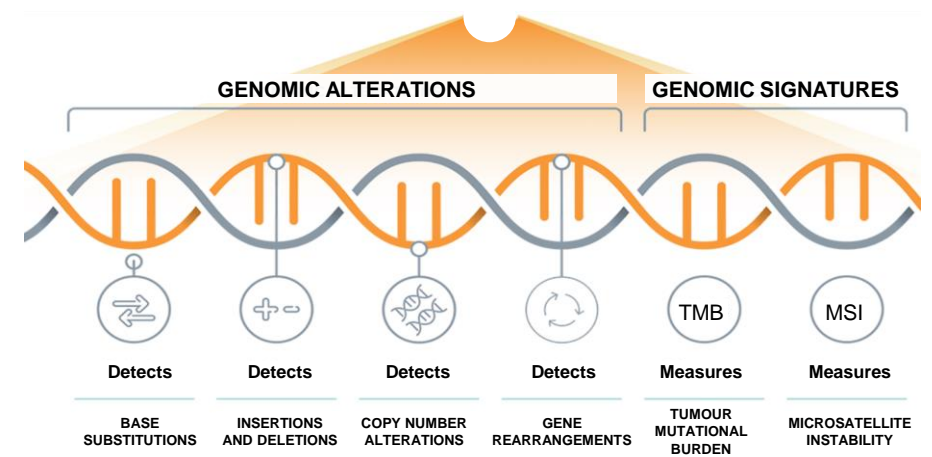
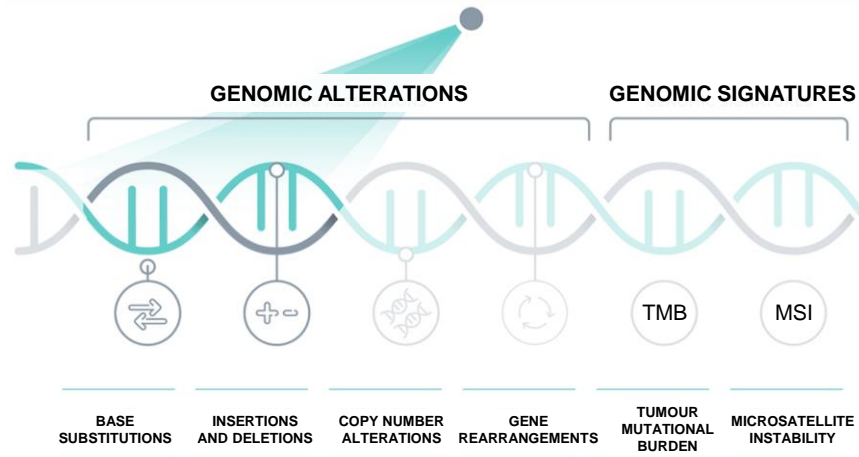
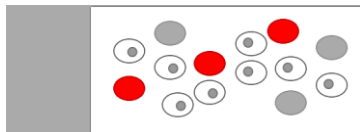
- Single biomarker analysis (protein expression)

- Single biomarker analysis
- Copy number alterations

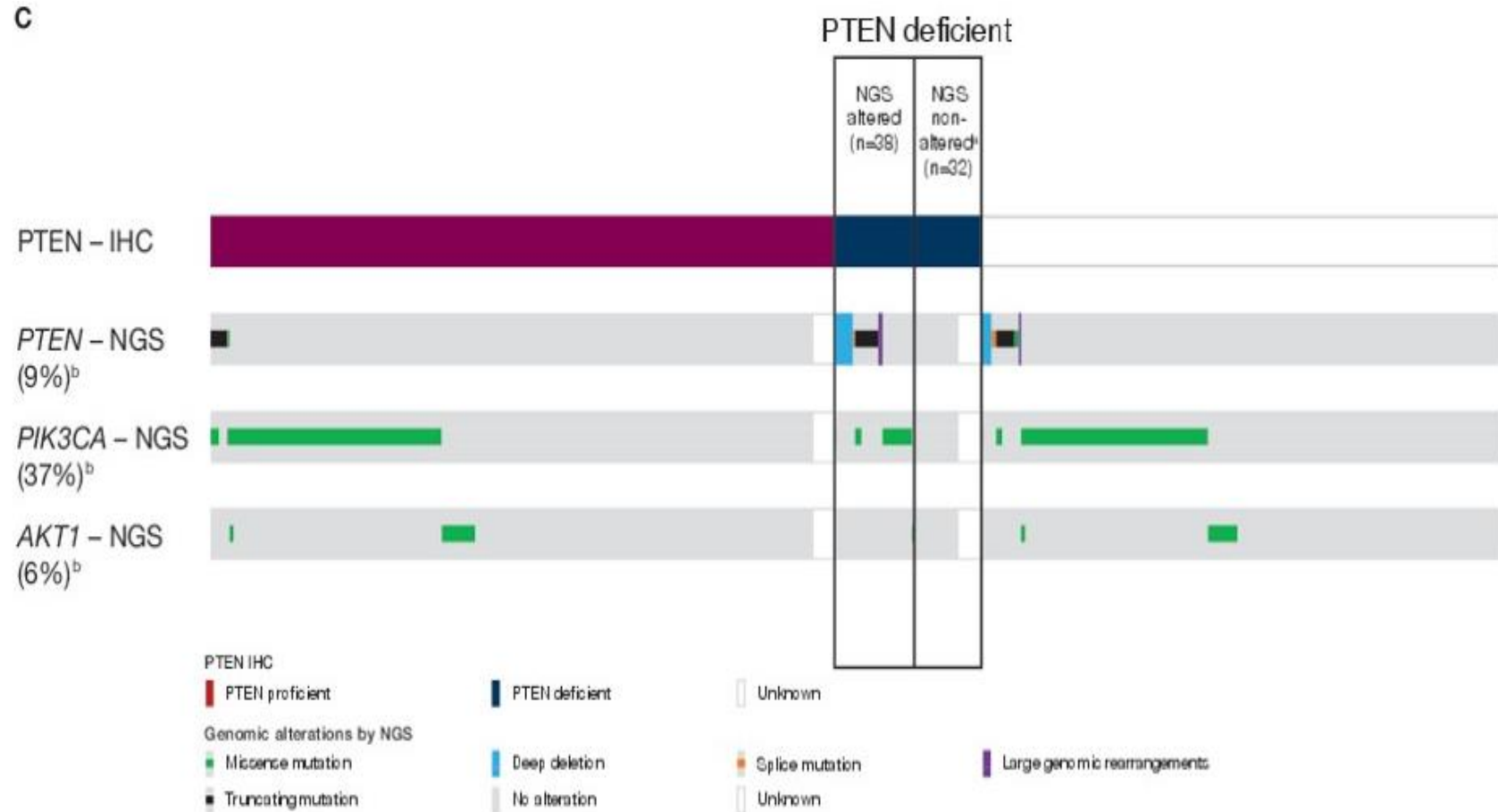
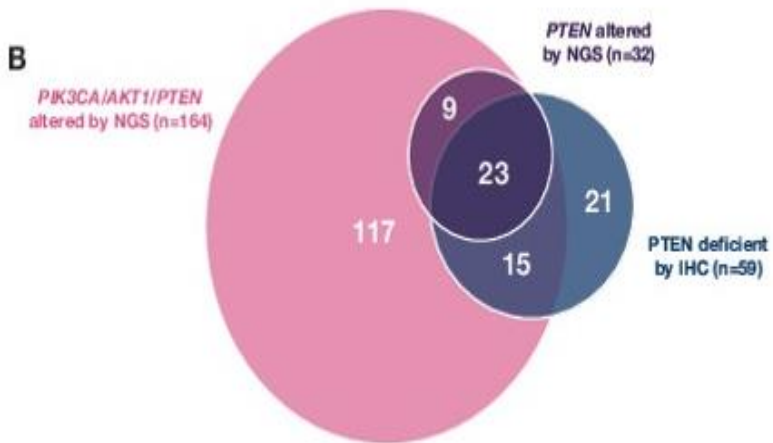
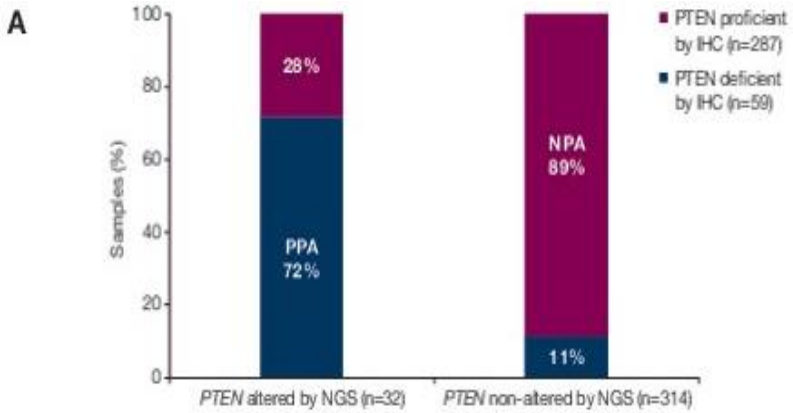
- Detects a predefined set of specific gene alterations that are associated with an effective targeted therapy
- Can **miss** indels, copy number alterations, rearrangements

- 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

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



PTEN IHC VS. NGS



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

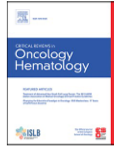


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Letter to the Editor

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

ARTICLE INFO

ABSTRACT

Keywords:

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

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*

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.

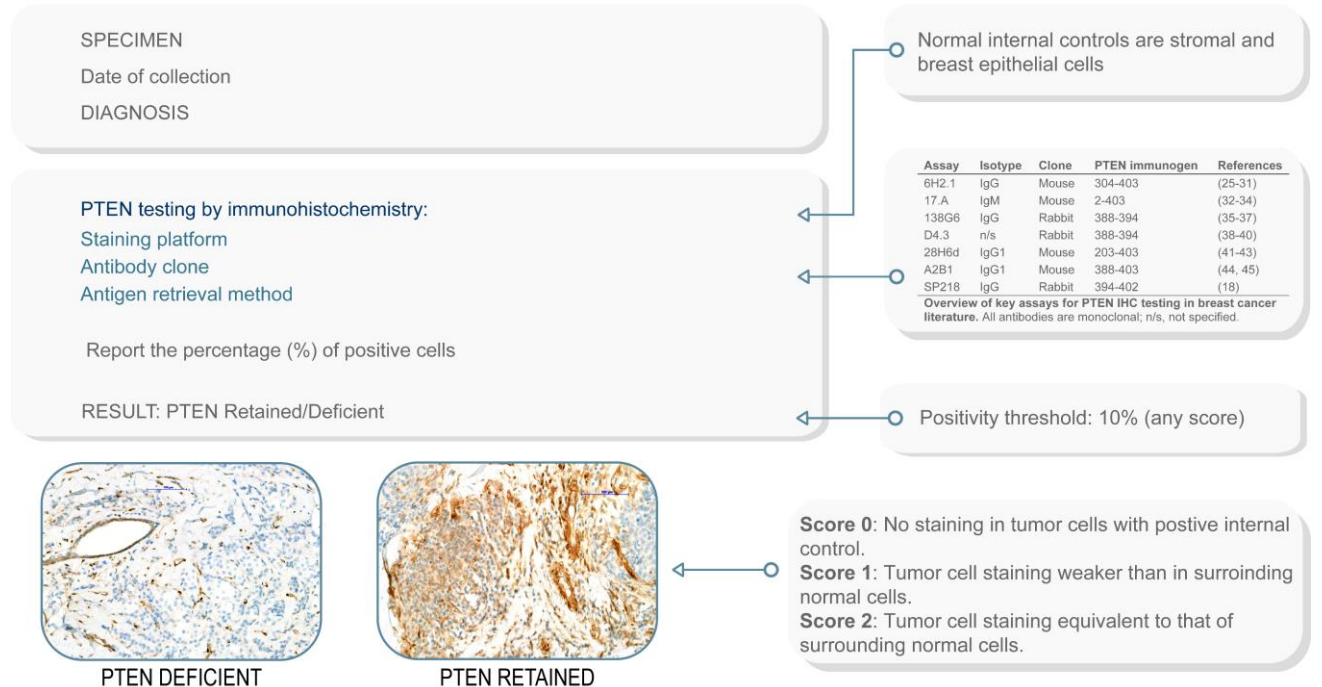
³ Department of Public Health, University Federico II of Naples, Naples, Italy.

⁴ Pathology Unit, Department of Medical Sciences, City of Health and Science University Hospital, University of Turin, Turin, Italy.

* These authors contributed equally to this work

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Simplified report for *PTEN* test by immunohistochemistry in breast cancer

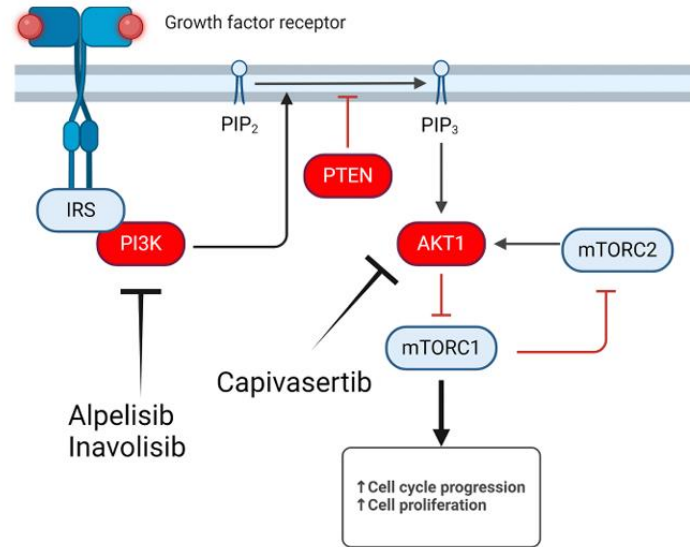
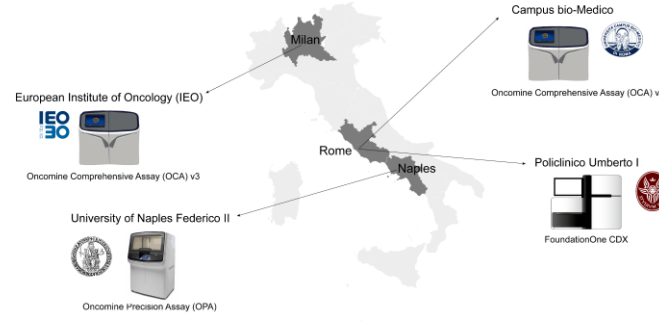


NGS vs PCR and TISSUE + LB integration

Tissue Biopsy



- Invasive
- Longer time
- Low sensitivity
- High cost of sample isolations
- Clinically validated
- Provides histological evaluation
- Organ penetration required
- Not capable of accessing tumor evolution
- No real time monitoring of drug response
- Repeated surgeries not feasible
- Does not reveal tumor heterogeneity



Legend

- Missense mutations
- Deletion
- Fusion

Lymph node

- T140K (1)
- E380Q (1)

Contralateral breast

- Y537S (2)
- D538G (1)

Lung

- Y537S (1)
- D538G (1)

Liver

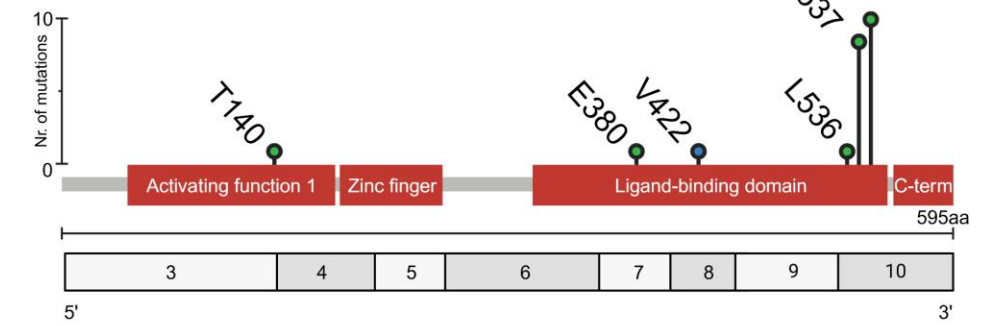
- V422del (1)
- L536P (1)
- Y537C (1)
- Y537N (2)
- Y537S (3)
- D538G (5)

Bone

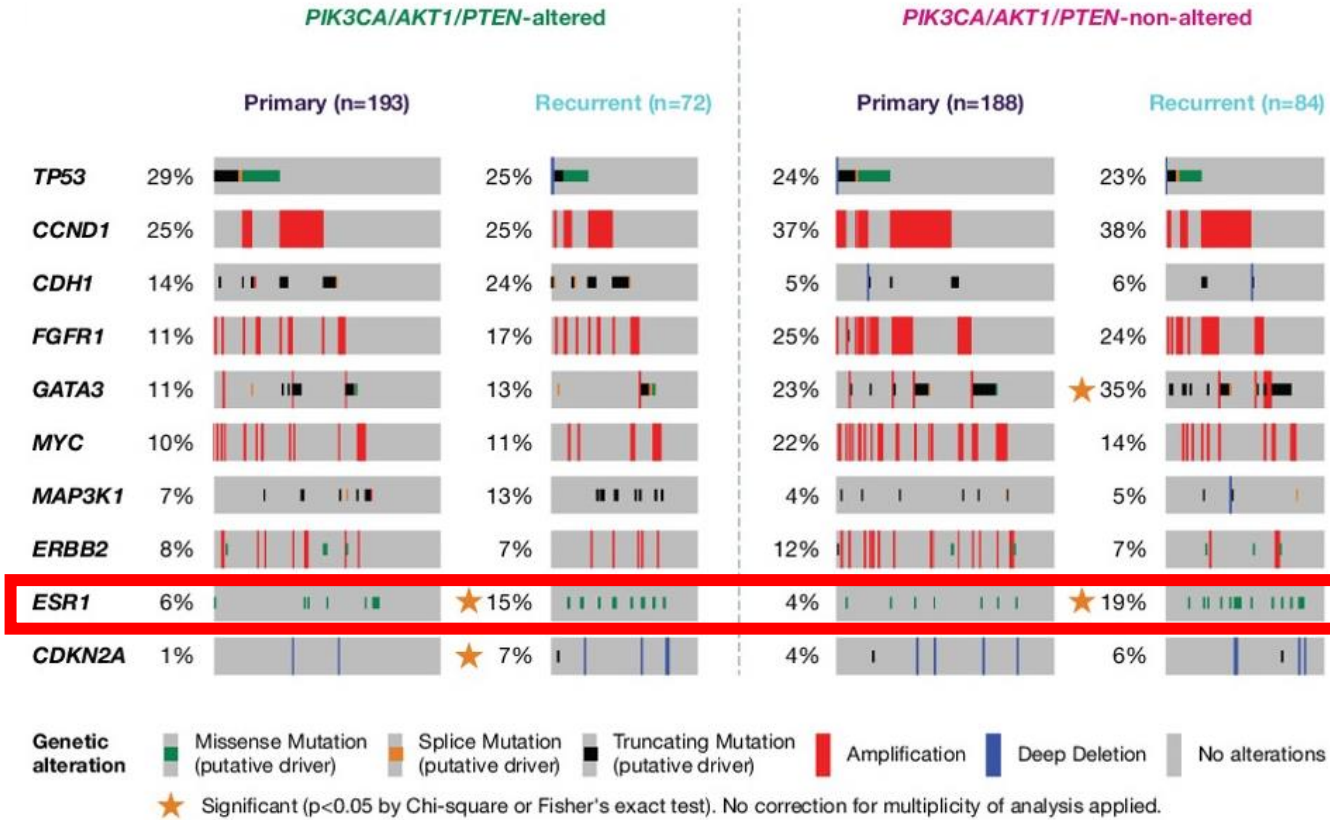
- ESR1-CCDC170 (1)
- D538G (1)

Other sites

- D538G (2)
- CNV (1)



CO-MUTATIONS

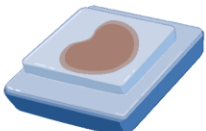










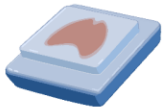


































	ESR1mut (n=24)	ESR1wt (n=14)	Total (n=38)
PIK3CA mutations, n (%)	8 (33.3)	8 (57.1)	16 (42.1)
p.V344M, c.1030G>A (E5)	1 (12.5)	0	1 (6.3)
p.E542K, c.1624G>A (E10)	0	2 (25.0)	2 (12.6)
p.E545K, c.1633G>A (E10)	2 (25.0)	1 (12.5)	3 (18.8)
p.E545Q, c.1633G>C (E10)	1 (12.5)	0	1 (6.3)
p.H556Y, c.1666C>T (E11)	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 *ESR1*mut group.

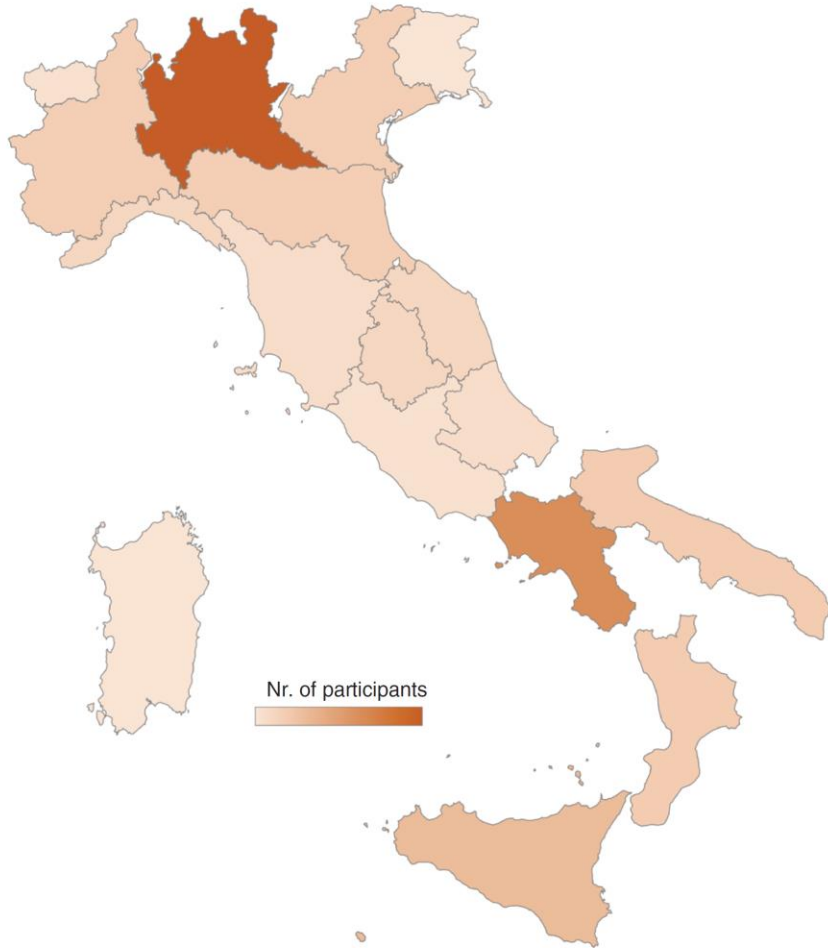
GENOMIC TESTING: WHAT, WHEN, HOW AND WHERE TO TEST?

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 decalcified bone metastasis)	ESR1				
	Primary tumor 		 If recent sample	 /  if old sample (more than 5 years or decalcified bone metastasis)	ESR1				
					PIK3CA				
					PIK3CA pathway				
	Liquid Biopsy 	ctDNA 			ESR1				
					PIK3CA				
PIK3CA pathway									

PIK3CA mutation testing 2022

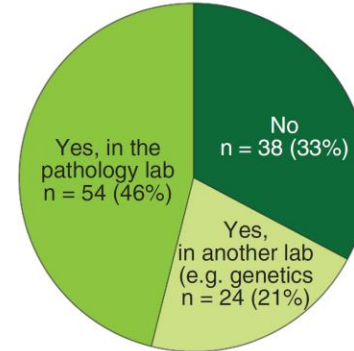
A



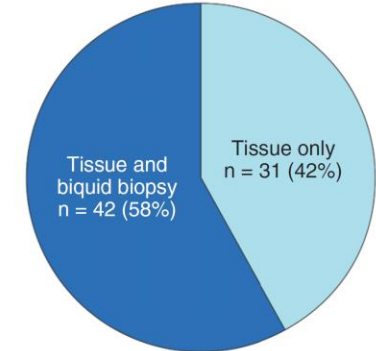
North-west	52
Valle d'Aosta	2
Piemonte	7
Lombardia	38
Liguria	5
North-east	15
Veneto	7
Friuli Venezia Giulia	1
Emilia Romagna	7
Center	18
Toscana	3
Umbria	5
Marche	5
Lazio	2
Abruzzo	3
South and islands	53
Campania	24
Puglia	8
Calabria	8
Sardegna	1
Sicilia	12

B

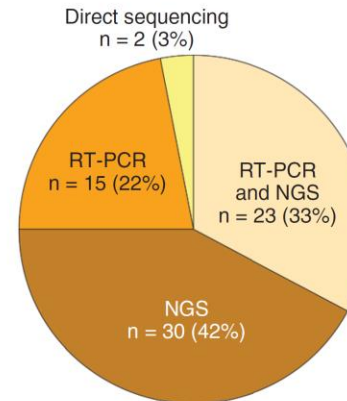
Q1: Is your center capable of conducting the PIK3CA test?
n = 116 answers



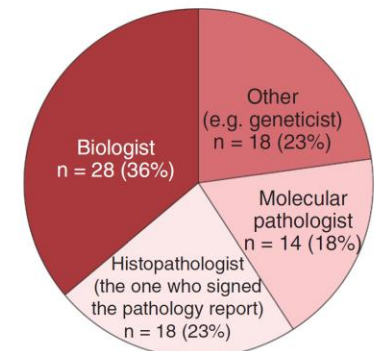
Q2: What are the acceptable biomaterials in your laboratory?
n = 73 answers



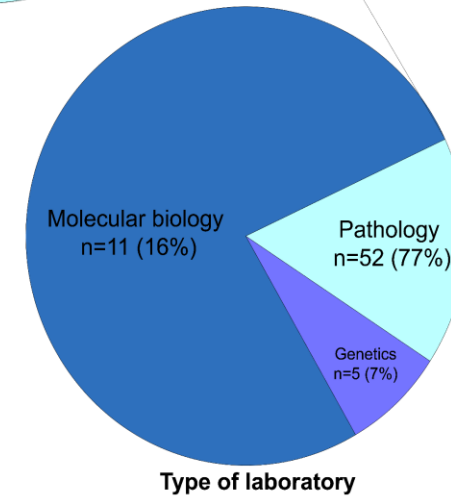
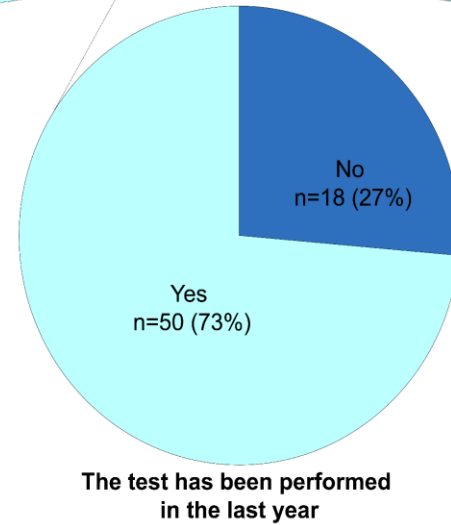
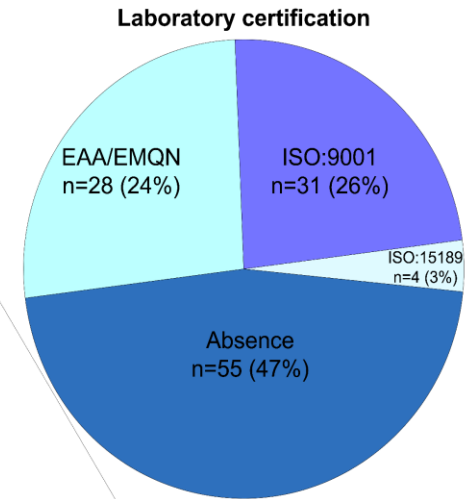
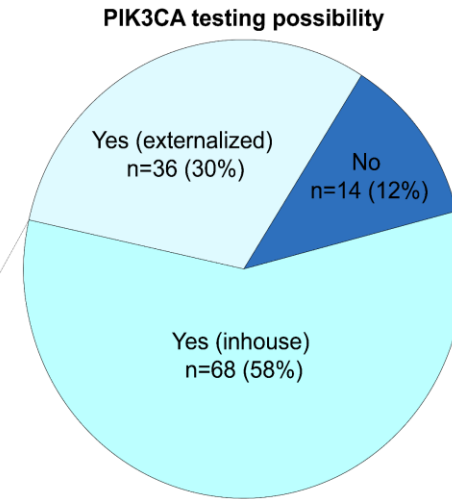
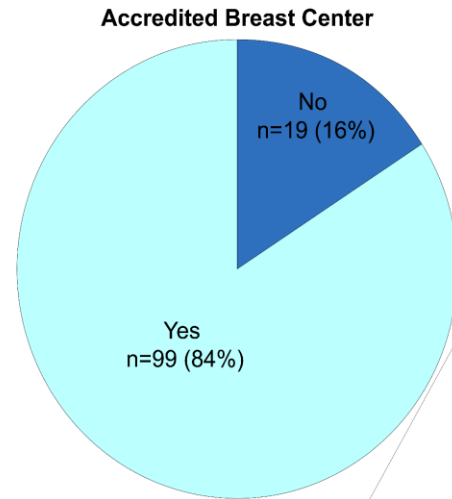
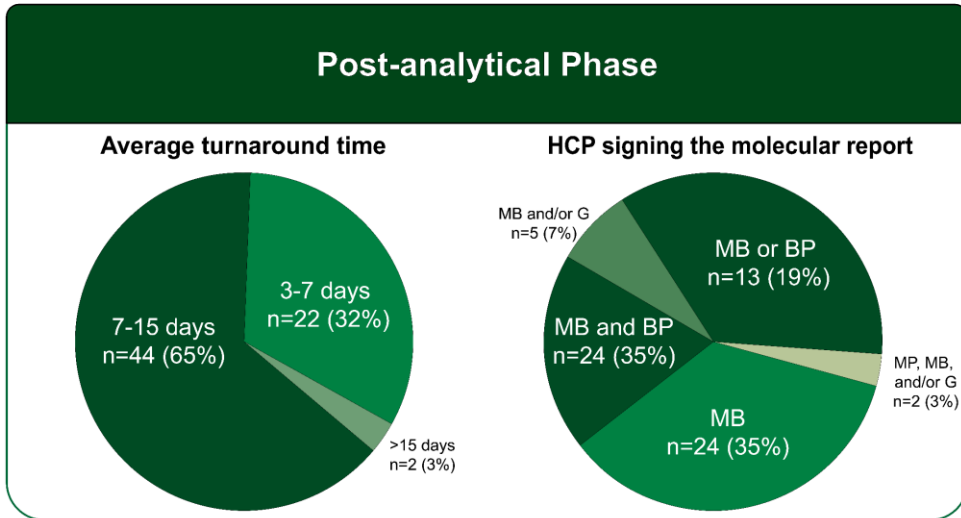
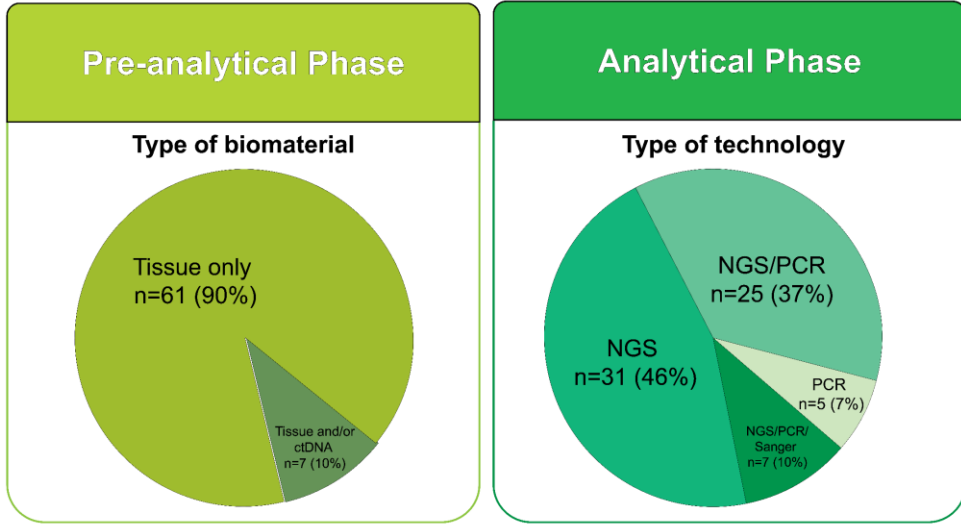
Q3: What technology options are available for this test at your center?
n = 70 answers



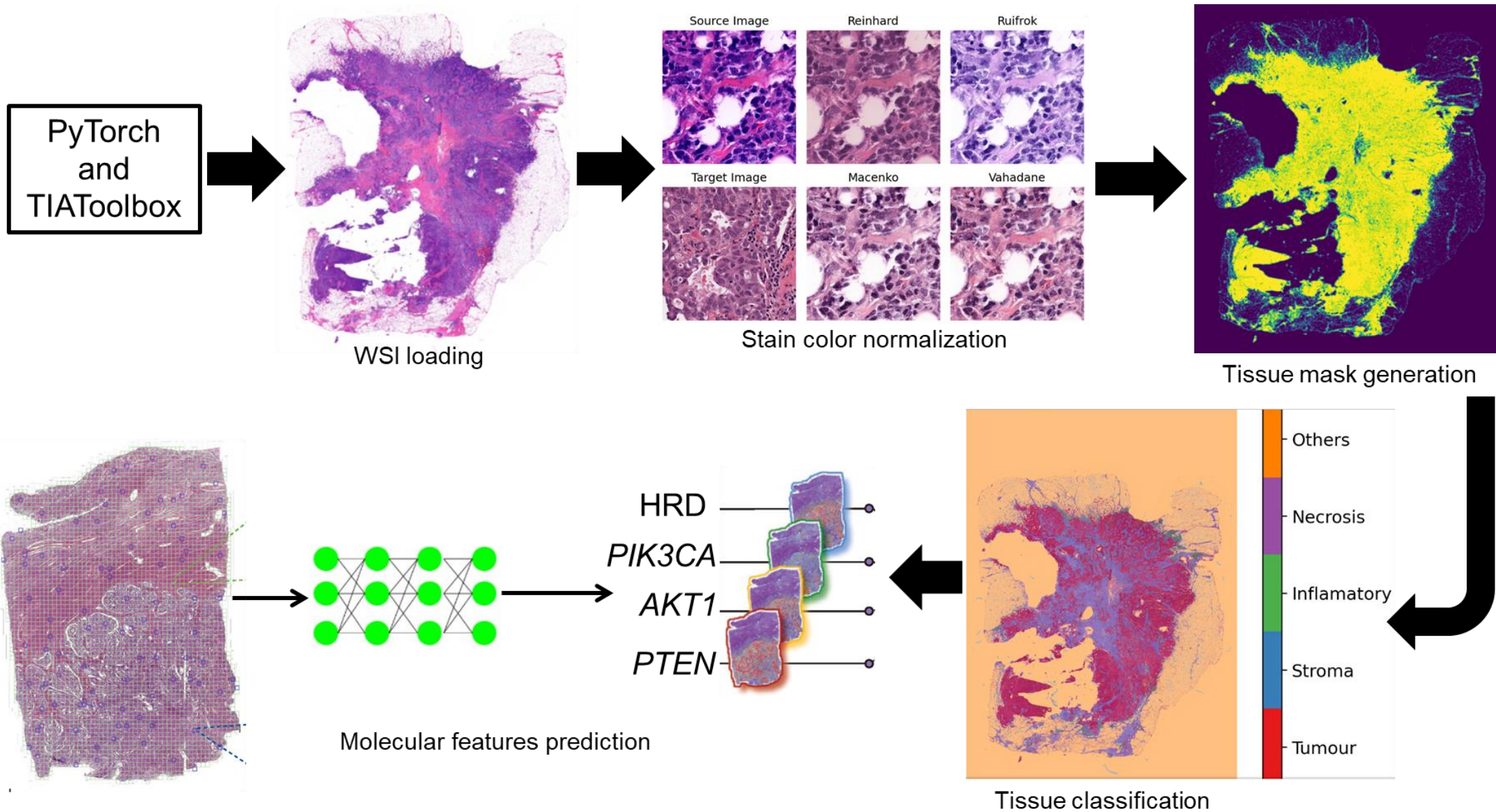
Q4: Which healthcare provider is responsible for signing the PIK3CA clinical report?
n = 78 answers



PIK3CA mutation testing 2025 (n=118 Centers)



PIPELINE FOR THE PREDICTION OF COMPLEX MOLECULAR BIOMARKERS IN BREAST CANCER



Thank you

Pathology team

Giuseppe Viale
Luca Bottiglieri
Silvestro Carinelli
Alberto Concardi
Anna Maria Croce
Elisa De Camilli
Marianna D'Ercole
Clementina Di Tonno
Mariacristina Ghioni
Elena Guerini Rocco
Mariia Ivanova
Daniela Lepanto
Renato Lobrano
Mariano Lombardi
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Biobank team

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Maria Capra
Cristina Cassi
Camilla Rosella Musico
Luca Leoni
Mariachiara Bonazzola



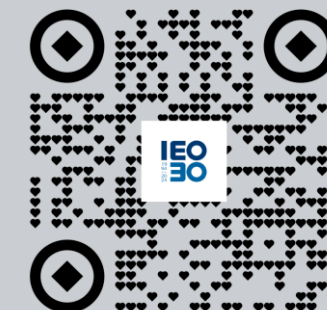
Dr. Clementina Di Tonno (Cytology), Dr. Giovanni Mazzarol (Histology), Prof. Elena Guerini Rocco (Molecular Pathology)



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