QUALI INFORMAZIONI DEVE FORNIRE OGGI L'ANATOMO-PATOLOGO AL CLINICO? QUALI SOTTOGRUPPI SONO OGGI IDENTIFICABILI?

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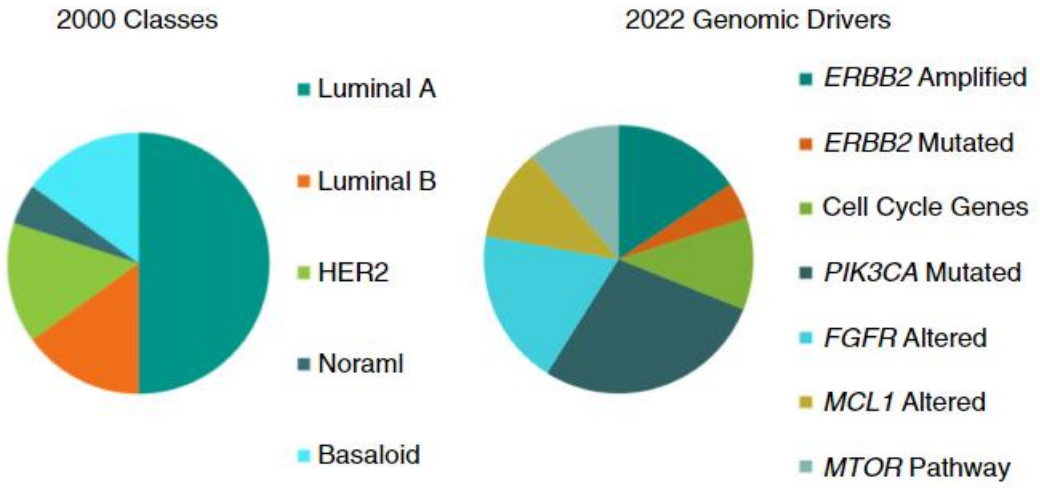
DISCLOSURES

Commercial Interest	Relationships
MSD, Novartis, AstraZeneca, Adicet Bio, Sermonix, Roche, Menarini, Gilead, Veracyte Inc.	Consulting/advisory role
MSD, Novartis, AstraZeneca, Daiichi Sankyo, GSK, Gilead, Roche, Leica Biosystems, Lilly	Speaker bureau
Novartis, Reply	Research grants
Roche	Travel grants

NOVEL AND EMERGING BIOMARKERS IN BREAST CANCER

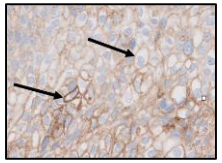
- **PD-L1 (CPS/IC)** → **IHC**
- HER2-low → IHC/ISH
- **PIK3CA** → **SEQ**
- **ESR1** → **SEQ**
- g/s/tBRCA → SEQ
- TROP-2 → tbd



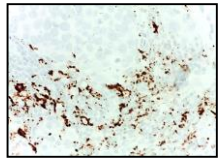
Fusco N, Malapelle U, Criscitiello C. Editorial: Diagnosis and Treatment of Breast Cancer in 2022: The Rise of Novel Molecular Biomarkers. Front Mol Biosci. 2023

Immune-related biomarkers in TNBC

PD-L1

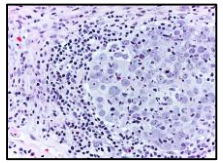


CPS



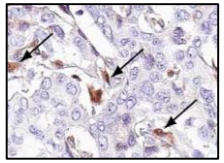
IC

TILs



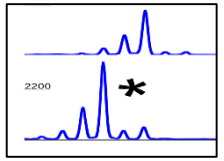
sTILs

MMR

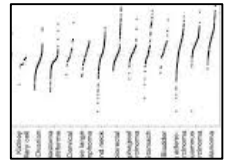


MLH1, MSH2
MSH6, PMS2

MSI



CGP



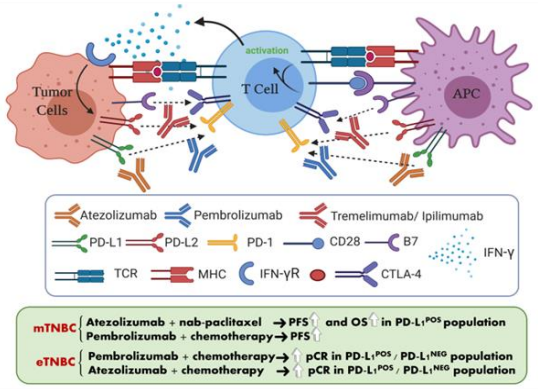
Histology-based

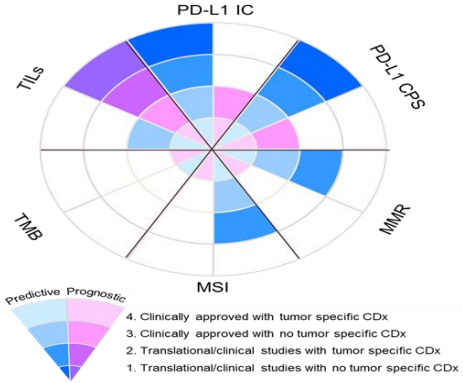
Sequencing-based

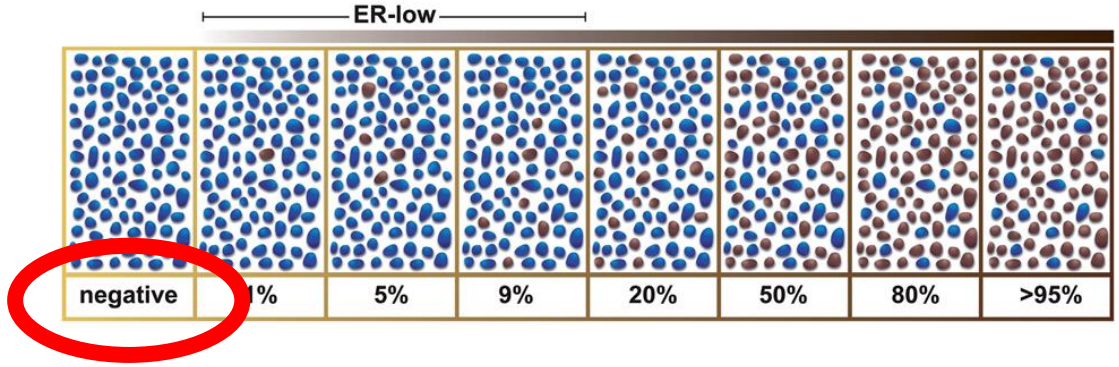
Immunotherapy in Breast Cancer Patients: A Focus on the Use of the Currently Available Biomarkers in Oncology

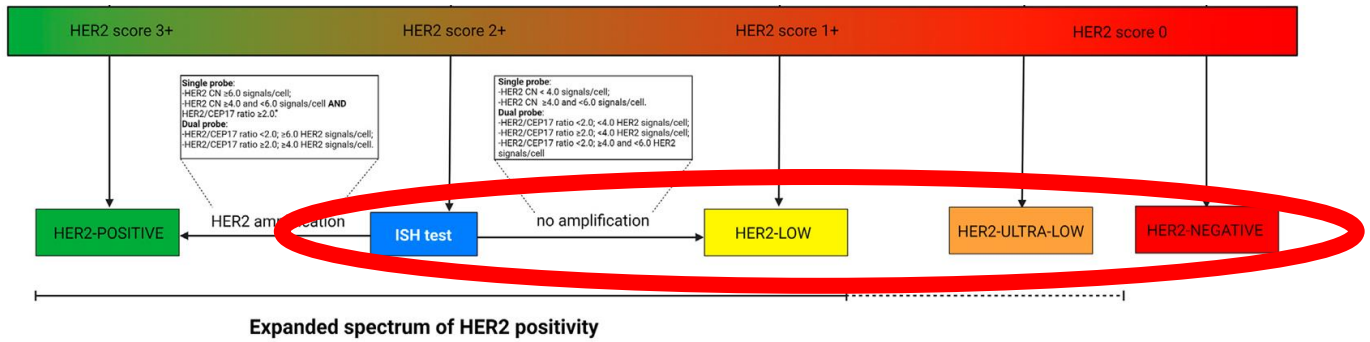
Author(s): Carmen Criscitiello, Elena Guerini-Rocco, Giulia Viale, Caterina Fumagalli, Elham Sajjadi, Konstantinos Venetis, Roberto Piciotti, Marco Invernizzi, Umberto Malapelle and Nicola Fusco*

Volume 22 , Issue 4 , 2022









ER-, PgR- **HER2-**

TNBC

Metastatic/locally advanced (biomarker-based) **PD-L1 CPS≥10**

Neoadjuvant (not biomarker-based) **PD-L1 IC≥1**

frontiers in Molecular Biosciences
REVIEW
published: 15 March 2022
doi: 10.3389/fmolb.2022.834651

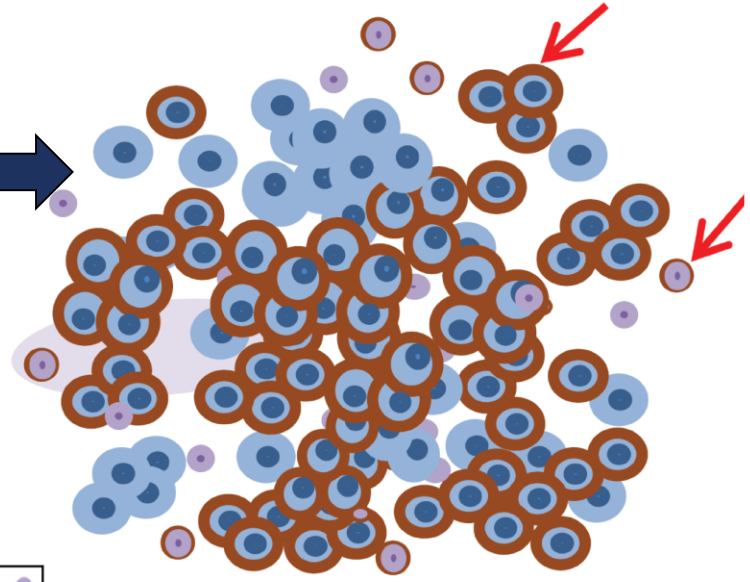
HER2 Low, Ultra-low, and Novel Complementary Biomarkers: Expanding the Spectrum of HER2 Positivity in Breast Cancer

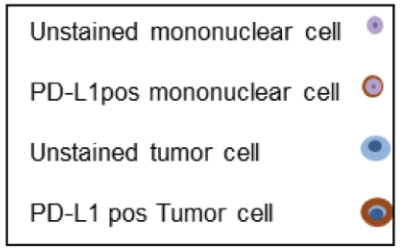
Konstantinos Venetis[1,2†], Edoardo Crimini[2,3†], Elham Sajjadi[1,2], Chiara Corti[2,3], Elena Guerini-Rocco[1,2], Giuseppe Viale[1,2], Giuseppe Curigliano[2,3], Carmen Criscitiello[2,3] and Nicola Fusco[1,2*]*

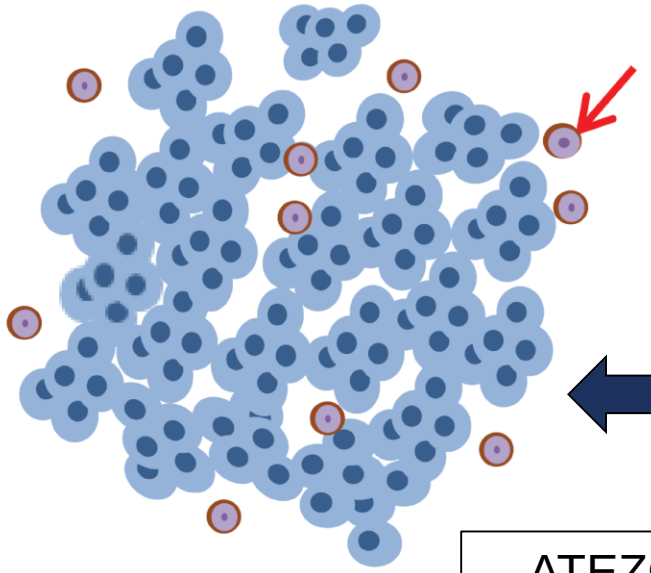
[1]Division of Pathology, IEO, European Institute of Oncology IRCCS, Milan, Italy, [2]Department of Oncology and Hemato-Oncology, University of Milan, Milan, Italy, [3]Division of Early Drug Development for Innovative Therapy, IEO, European Institute of Oncology, IRCCS, Milan, Italy

PD-L1 TEST IN mTNBC

PEMBROLIZUMAB







ATEZOLIZUMAB

CPS

$$CPS = \frac{\text{PD- L1 staining} \left[\begin{array}{l}\text{Tumor cells}\\ \text{Lymphocytes}\\ \text{Macrophages}\end{array}\right.}{\text{viable tumor cells}} 100$$

IC

$$IC = \frac{\text{PD- L1 staining} \left[\begin{array}{l}\text{Lymphocytes}\\ \text{Macrophages}\end{array}\right.}{\text{tumor area}} 100$$

Validated assays for CPS:
- Ventana SP263
- Dako 22-C3

TNBCs are scored and divided into:

CPS < 10 IC < 1

CPS ≥ 10 IC ≥ 1

Validated assays for IC:
- Ventana SP142

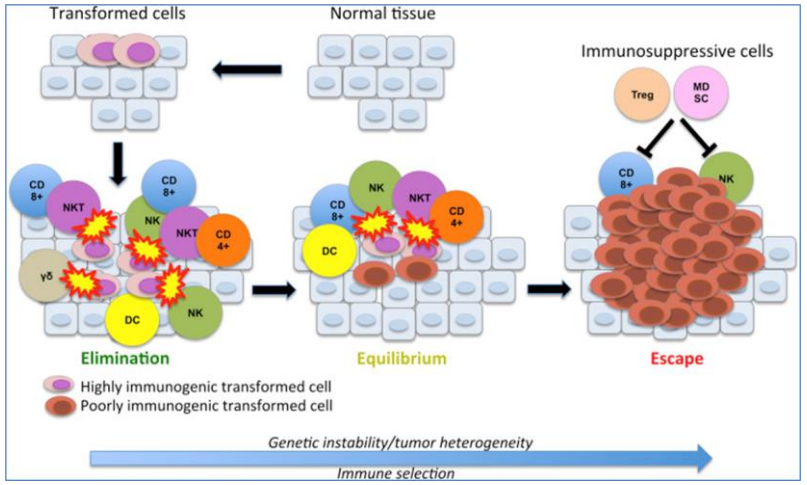
ecancermedicalscience

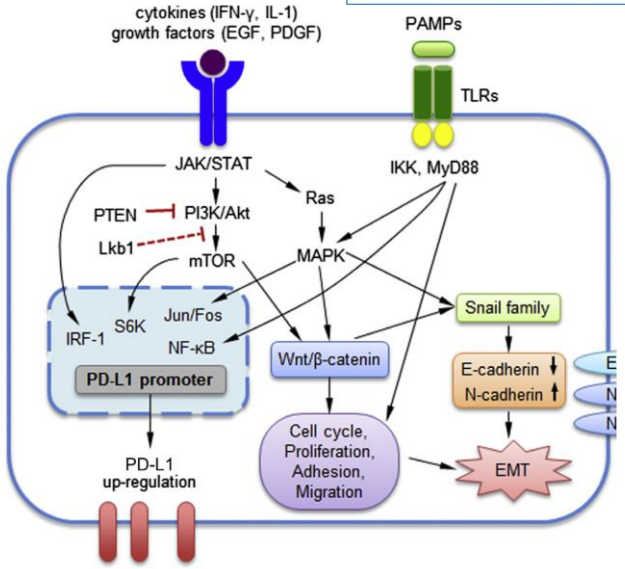
Biomarkers for precision immunotherapy in the metastatic setting: hope or reality?

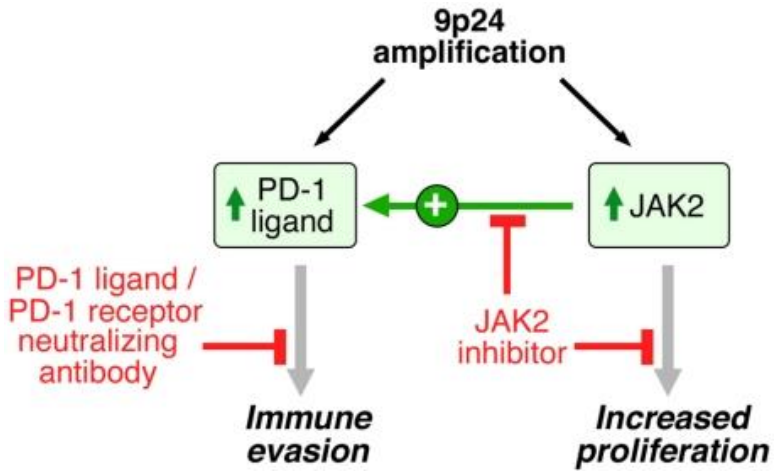
Elham Sajjadi[1,2], Konstantinos Venetis[1,2], Cristian Scatena[3] and Nicola Fusco[1,2]

PREDICTIVE ROLE OF PD-L1 EXPRESSION: PITFALLS

- **Assay variability**
  - Different antibodies
  - Different platforms
  - Different scoring systems
- **Biological variability**
  - Spatial variability
  - Temporal variability
  - Tumor or microenvironment
  - Constitutive vs. inflammation-induced

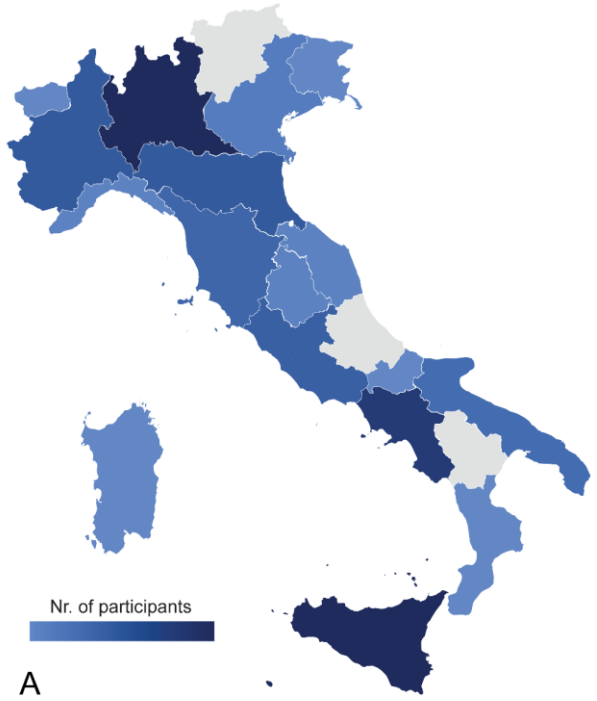






Marletta S, et al. Atlas of PD-L1 for Pathologists: Indications, Scores, Diagnostic Platforms and Reporting Systems. J Pers Med. 2022

PD-L1 CPS in mTNBC: A REAL-LIFE ITALIAN PORTRAIT



North-west	**31**
Valle d'Aosta	1
Piemonte	9
Lombardia	19
Liguria	2
North-east	**13**
Veneto	3
Friuli Venezia Giulia	1
Emilia Romagna	9
Center	**19**
Toscana	7
Umbria	2
Marche	2
Lazio	8
South and islands	**43**
Molise	1
Campania	15
Puglia	6
Calabria	1
Sardegna	1
Sicilia	19

Critical Reviews in Oncology / Hematology 190 (2023) 104103

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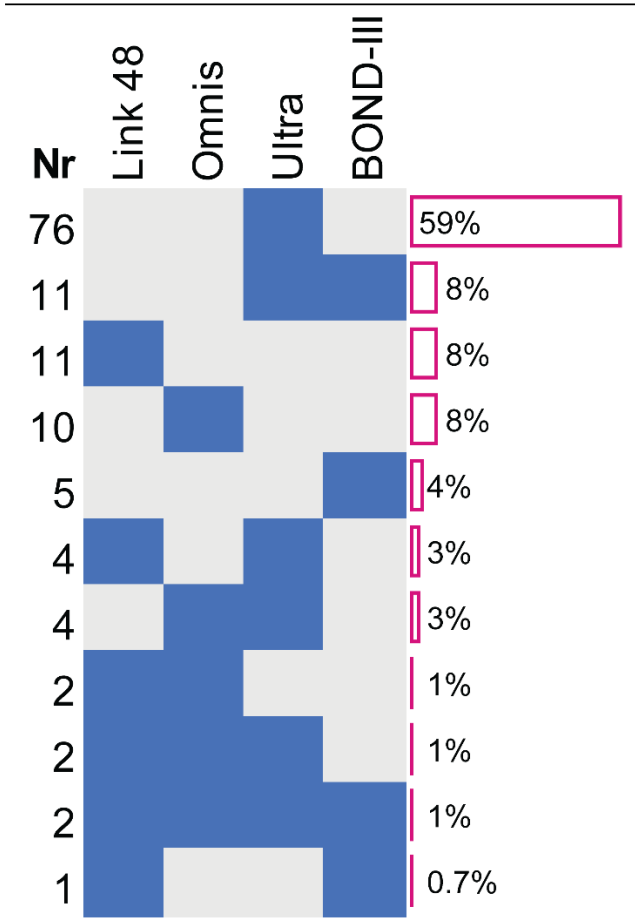
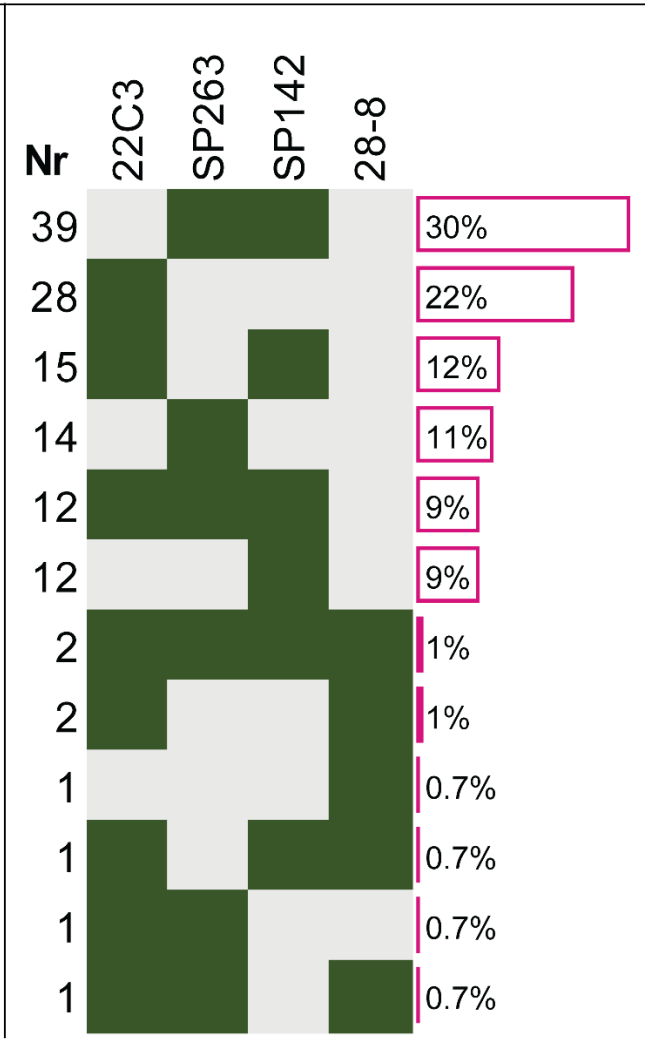
journal homepage: www.elsevier.com/locate/critrevonc

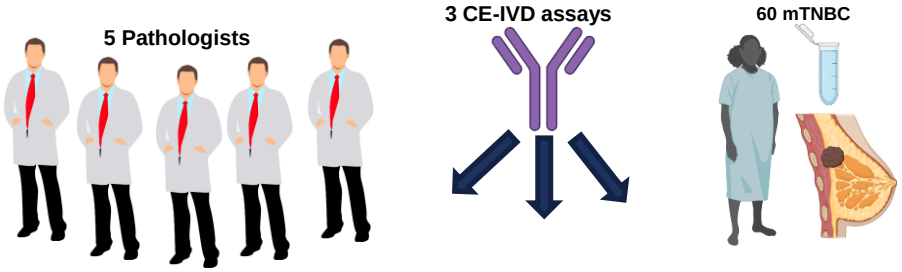
Advancing the PD-L1 CPS test in metastatic TNBC: Insights from pathologists and findings from a nationwide survey

Nicola Fusco [a,b,*], Mariia Ivanova [a], Chiara Frascarelli [a,b], Carmen Criscitiello [a,c], Bruna Cerbelli [d], Maria Gemma Pignataro [d], Angelina Pernazza [d], Elham Sajjadi [a,b], Konstantinos Venetis [a], Giulia Cursano [a], Fabio Pagni [e,f], Camillo Di Bella [f], Marina Accardo [g], Michelina Amato [h], Paolo Amico [i], Caterina Bartoli [j], Giuseppe Bogina [k], Laura Bortesi [k], Renzo Boldorini [l], Sara Bruno [m], Daniela Cabibi [n], Pietro Caruana [o], Emanuele Dainese [p], Elisa De Camilli [a], Vladimiro Dell'Anna [q], Loren Duda [r], Carmela Emmanuele [s], Giuseppe Nicolò Fanelli [t], Bethania Fernandes [u], Gerardo Ferrara [v], Letizia Gnetti [x], Alessandra Gurrera [w], Giorgia Leone [x], Raffaella Lucci [y], Cristina Mancini [z], Grazia Marangi [z], Mauro G. Mastropasqua [aa], Lorenzo Nibid [ab,ac], Sandra Orrù [ad], Maria Pastena [ae], Monica Peresi [af], Letizia Perracchio [ag], Angela Santoro [ah], Vania Vezzosi [ai], Claudia Zambelli [aj], Valeria Zuccalà [ak], Antonio Rizzo [x], Leopoldo Costarelli [b], Francesca Pietribiasi [al], Alfredo Santinelli [am], Cristian Scatena [t], Giuseppe Curigliano [b,c], Elena Guerini-Rocco [a,b], Maurizio Martini [an], Paolo Graziano [ao], Isabella Castellano [ap], Giulia d'Amati [d]

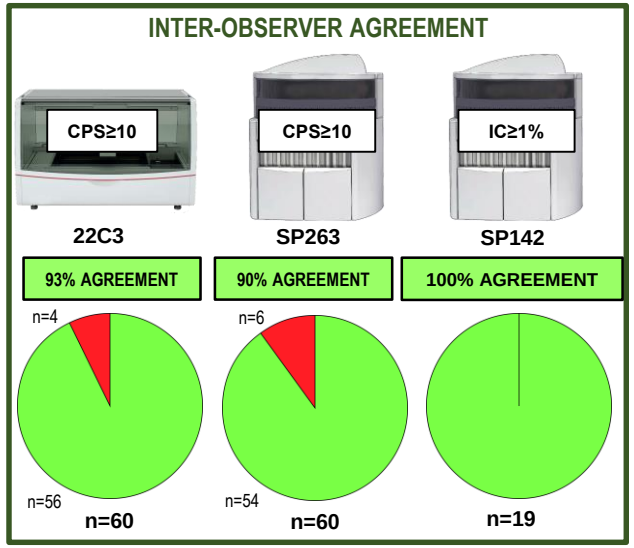


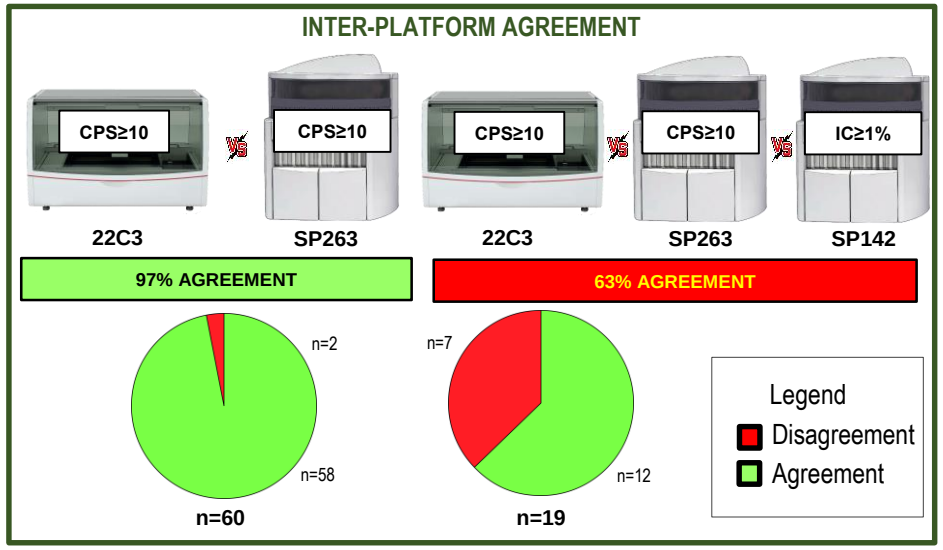


Conclusions (1): a reproducibility study



- In mTNBC, CPS can be reliably assessed either by 22C3 (which was used in the KEYNOTE studies) or SP263, providing the use of the dedicated platform (i.e. Dako and Ventana).
- CPS and IC are not interchangeable tests in mTNBC
- PD-L1 test in mTNBC is reproducible when assessed by specifically trained pathologists using CE-IVD assays, i.e. 22C3 and SP263 for CPS and SP142 for IC score.





Test (Assay)	ICC	Kappa
CPS (22C3)	0.939 (CI 0.913-0.96)	0.938 (CI 0.857-1.018)
CPS (SP263)	0.972 (CI 0.96-0.982)	0.972 (CI 0.890-1.052)
CPS (22C3 vs SP263)	0.909 (CI 0.874-0.938)	0.907 (CI 0.869-0.945)
IC (SP142)	1	1 (CI 0.725-1.274)
CPS (22C3 vs SP263) vs IC (SP142)	0.634 (CI 0.455-0.816)	0.619 (CI 0.537-0.700)

IEO European Institute of Oncology
UNIVERSITÀ DEGLI STUDI DI MILANO
SAPIENZA UNIVERSITÀ DI ROMA

PD-L1 testing in mTNBC: Interplatform and interobserver reproducibility of CE-IVD assays for CPS and IC scores

Nicola Fusco[1,2], Mariia Ivanova[1], Chiara Frascarelli[1,2], Bruna Cerbelli[3], Gemma Pignataro[3], Carmen Criscitiello[1,4], Konstantinos Venetis[1,2], Elham Sajjadi[1,2], Elena Guerini-Rocco[1,2], Paolo Graziano[5], Maurizio Martini[6], Giulia d'Amati[3]

[1]Division of Pathology, IEO, European Institute of Oncology IRCCS Milan, Italy, [2]Department of Oncology and Hemato-Oncology, University of Milan, Italy, [3]La Sapienza, University of Rome, Italy, [4] Division of Early Drug Development for Innovative Therapy, IEO, European Institute of Oncology, Milan, Italy, [5] Pathology Unit, Foundation IRCCS "Casa Sollievo della Sofferenza", San Giovanni Rotondo, Foggia, Italy, [6] Department of Human Pathology of the Adult and Developmental Age "Gaetano Barresi", University of Messina, Italy.

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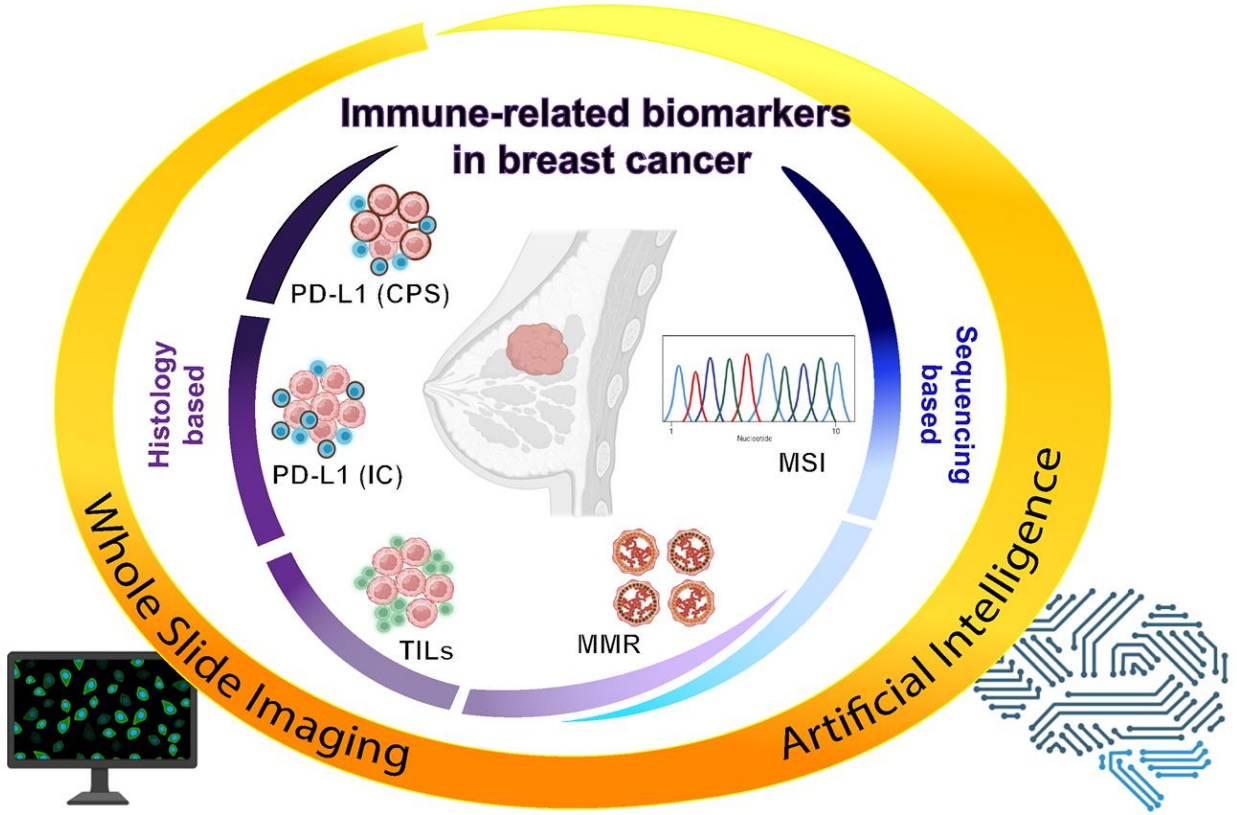
ESMO BREAST CANCER
BERLIN GERMANY 11-13 MAY 2023
Poster #22P

35th European Congress of Pathology
9 – 13 September 2023
Convention Centre Dublin, Ireland
European Society of Pathology

Conclusions (2): The role of Digital Pathology

- Harmonization efforts aim to standardize PD-L1 testing for better patient selection.
- TILs correlate with favorable outcomes in TNBC.
- MMR alterations are rare but predictive, indicating potential ICI response.
- AI plays a growing role in enhancing biomarker assessment and precision medicine.
- Standardized practices and validation are essential for successful AI application.



Journal of Personalized Medicine J. Pers. Med. 2023, 13, 1176. https://doi.org/10.3390/jpm13071176

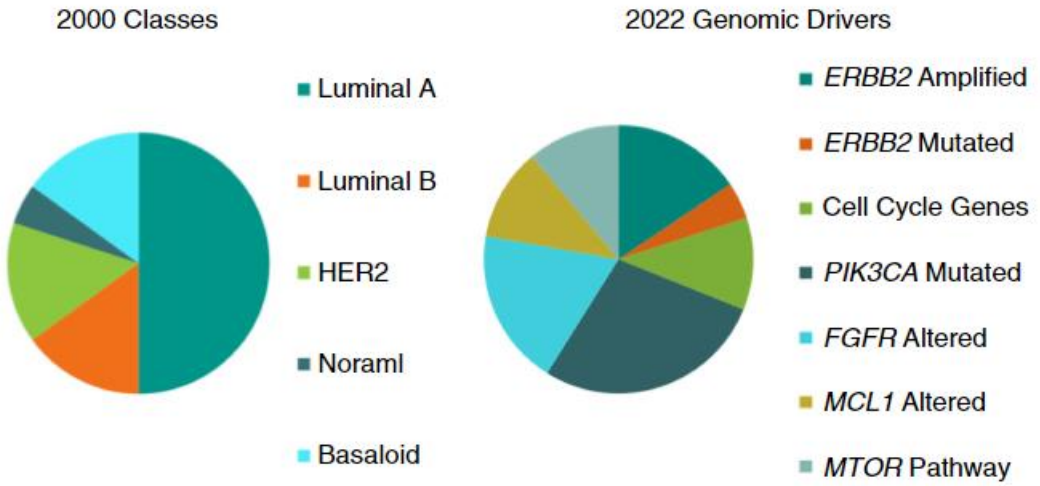
Review

Immune Biomarkers in Triple-Negative Breast Cancer: Improving the Predictivity of Current Testing Methods

Francesca Maria Porta [1], Elham Sajjadi [1,2], Konstantinos Venetis [1], Chiara Frascarelli [1,2], Giulia Cursano [1], Elena Guerini-Rocco [1,2], Nicola Fusco [1,2,*] and Mariia Ivanova [1]

NOVEL AND EMERGING BIOMARKERS IN BREAST CANCER

- PD-L1 (CPS/IC) → I-A
- HER2-low → I-A
- **PIK3CA → I-A**
- **ESR1 → II-A**
- TROP-2 → I-C



Fusco N, Malapelle U, Criscitiello C. Editorial: Diagnosis and Treatment of Breast Cancer in 2022: The Rise of Novel Molecular Biomarkers. Front Mol Biosci. 2023

PIK3CA mutations in breast cancer

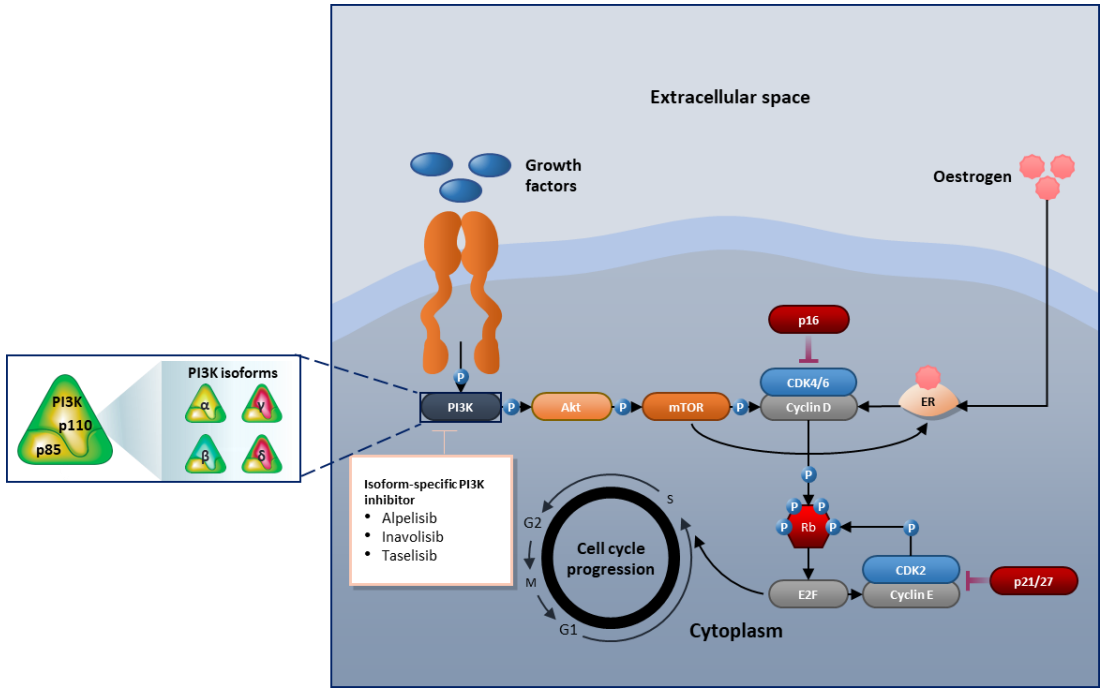
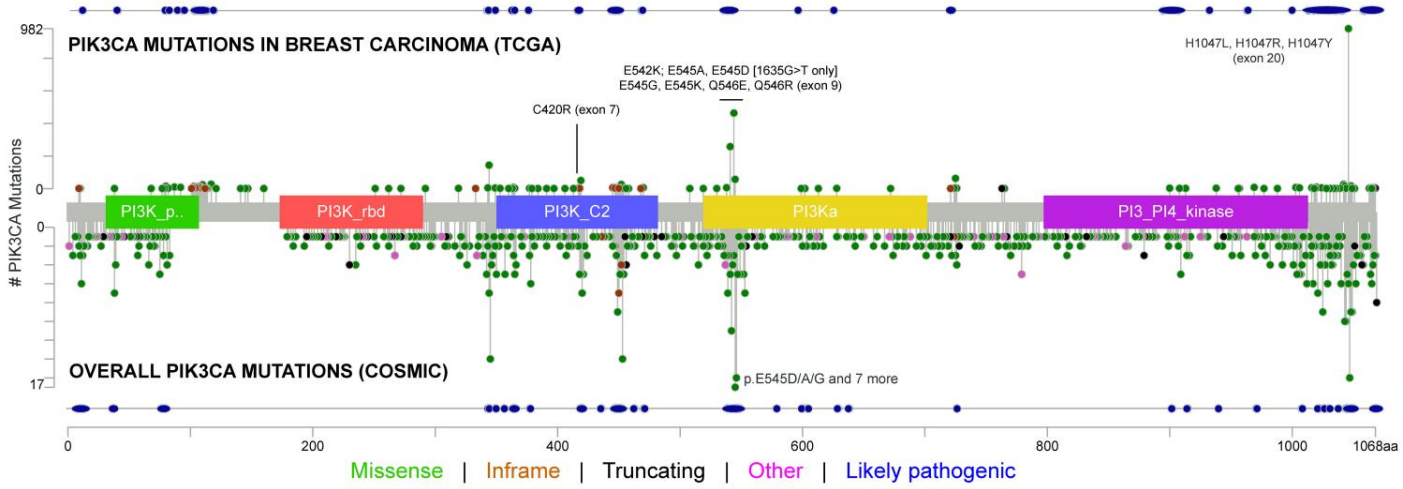
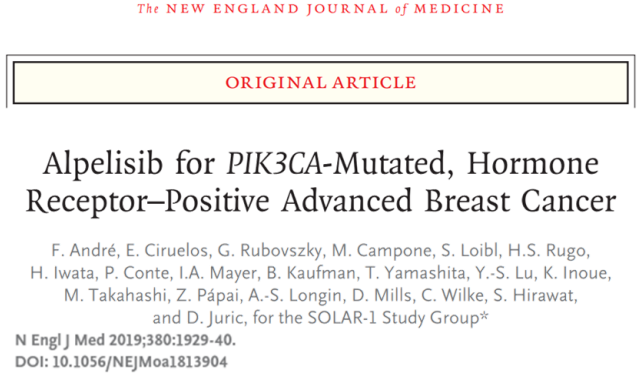


Image adapted from: Brufsky AM & Dickler MN. Oncologist. 2018; **23:**528 .
1.Hanah et al J. Med. Chem. 2022, 65, 16589–16621



- ~40% of HR+/HER2- aBC patients have a *PIK3CA* mutation, and can have endocrine resistance and/or shorter mPFS
- Hotspot regions: ex 7, 9, 20
- PIK3CA mutations can be detected in tissue (FFPE) or plasma samples.



PIK3CA: RATIONALE FOR CLINICAL TESTING 2024?

Compound / Generic Name	Trade Name	Combination	Indication	Phase 1 2 3 F	Expected Filing
RG6114 Inavolisib		plus palbociclib plus fulvestrant	1L metastatic ER-positive and HER2-negative breast cancer (1L HR+ mBC)	■ ■ ■ □	2024

Description/ Summary:

Inavolisib (RG6114, GDC-0077) is a small molecule PI3 kinase (PI3K) inhibitor. Dysregulation of PI3K signaling is implicated in a broad range of human cancers, and activating mutations in the PI3K alpha-isoform gene (PIK3CA) are common oncogenic drivers. The PI3K/Akt/mTOR pathway regulates cell growth and survival.

Managed By:

Roche Late Stage Product Development

Inavolisib is a PI3Kα-specific inhibitor that also promotes degradation of mutant p110α. It has demonstrated encouraging preliminary antitumor activity in pts with PIK3CA-mutated HR+ BC as a monotherapy, and in combo with other anticancer agents

https://www.roche.com/solutions/pipeline/#81ad1b25-f415-4714-9450-45c188614658

ctDNA IN MBC

- As in early breast cancer, the quantity of ctDNA correlates with poor survival
- serial measurement of ctDNA has the potential to monitor and predict treatment response.
- PADA-1 trial (NCT03079011) is currently investigating the utility of serial ESR1 ctDNA measurements in HER2-negative MBC patients treated with palbociclib and AI
- mutation status of specific genes
  - HER2
  - **ESR1**
  - TP53
  - **PIK3CA**

Tumor type	Recommendation	FDA-approved assays
NSCLC[1,2]	Testing of plasma ctDNA should be performed by a clinically validated, NGS platform for genotyping of all guideline-recommended and treatable oncogenic drivers in patients with newly diagnosed NSCLC, as a plasma first approach: • *ALK* rearrangements • *ROS1* rearrangements • *BRAF* alterations • *MET* alterations • *RET* rearrangements • *ERBB2* (formerly *HER2*) alterations	Roche cobas *EGFR* Mutation Test v2 Guardant360 CDx (*EGFR* and *KRAS*)[3] FoundationOne Liquid CDx (*EGFR*, *ALK*, and *MET*)[a]
Advanced HR-positive, *ERBB2*-negative breast cancer[4]	Assessment of *PIK3CA* alterations in patients under consideration for treatment with alpelisib or fulvestrant	FoundationOne Liquid CDx[a]

ESTABLISHED PREDICTIVE BIOMARKER

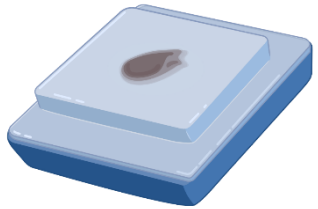
JAMA Oncol, yesterday, ahead of print

Practical Considerations for the Use of Circulating Tumor DNA in the Treatment of Patients With Cancer
A Narrative Review

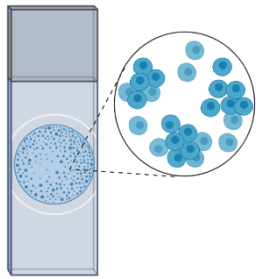
Matthew G. Krebs, MD, PhD; Umberto Malapelle, PhD; Fabrice André, MD, PhD; Luis Paz-Ares, MD, PhD; Martin Schuler, MD; David M. Thomas, PhD; Gilad Vainer, MD, PhD; Takayuki Yoshino, MD, PhD; Christian Rolfo, MD, PhD, MBA

PIK3CA mutational analysis

Which sample?

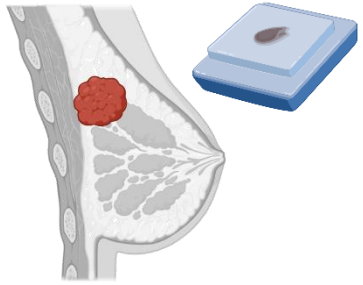


Histological specimen

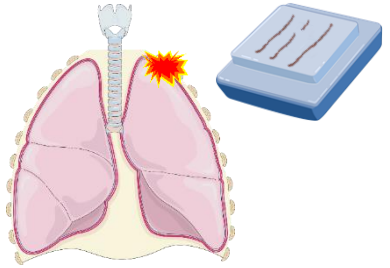


Cytological specimen

Liquid biopsy

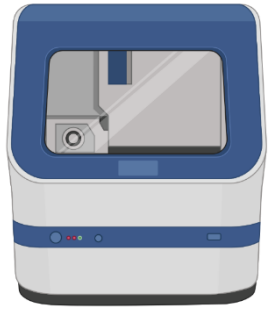


Primary tumor

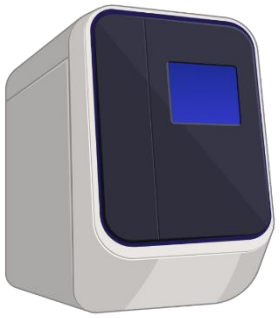


Metastatic sample

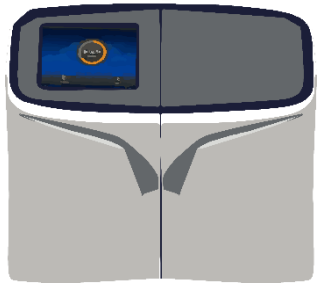
Which method?



Direct sequencing



Real Time PCR



Next-Generation Sequencing

Pros and cons of the *PIK3CA* molecular testing methods

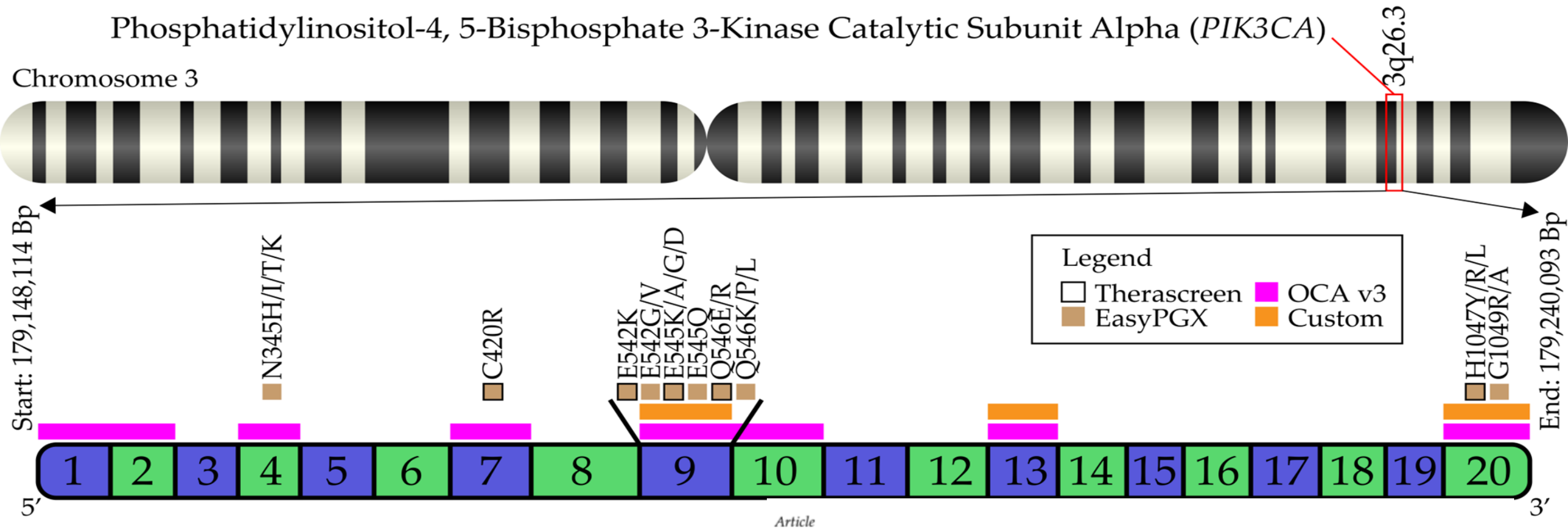
Real Time PCR

PROS	CONS
• Cost-effective • Short turnaround time • Widely available • High sensitivity • Wide choice of panels	• High amount of material required • Affected by low tumor cell content • Variable reference range • No allele frequency • Affected by the pre-analytical phase

Next-Generation Sequencing

PROS	CONS
• Higher sequencing depth for increased sensitivity (down to 1%) • Multi-target panels • Low input of nucleic acid needed • Wide choice of panels	• Expensive • Long turnaround time • Not widely available • Affected by the pre-analytical phase • Dedicated personnel required

PIK3CA MUTATIONS IN BREAST CANCERS: TESTING STRATEGIES

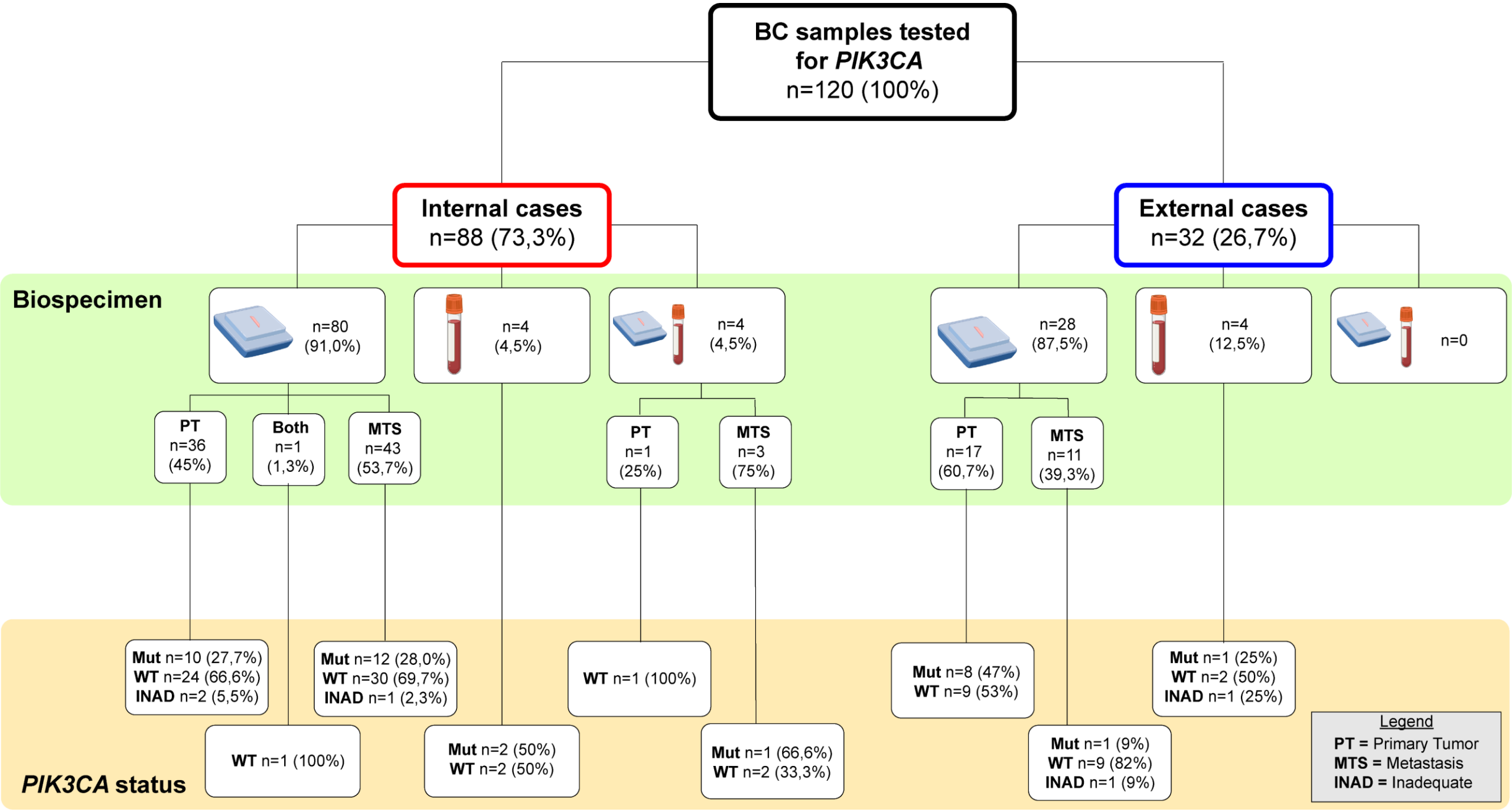


Article

Analytical Performance of Next-Generation Sequencing and RT-PCR on Formalin-Fixed Paraffin-Embedded Tumor Tissues for *PIK3CA* Testing in HR+/HER2− Breast Cancer

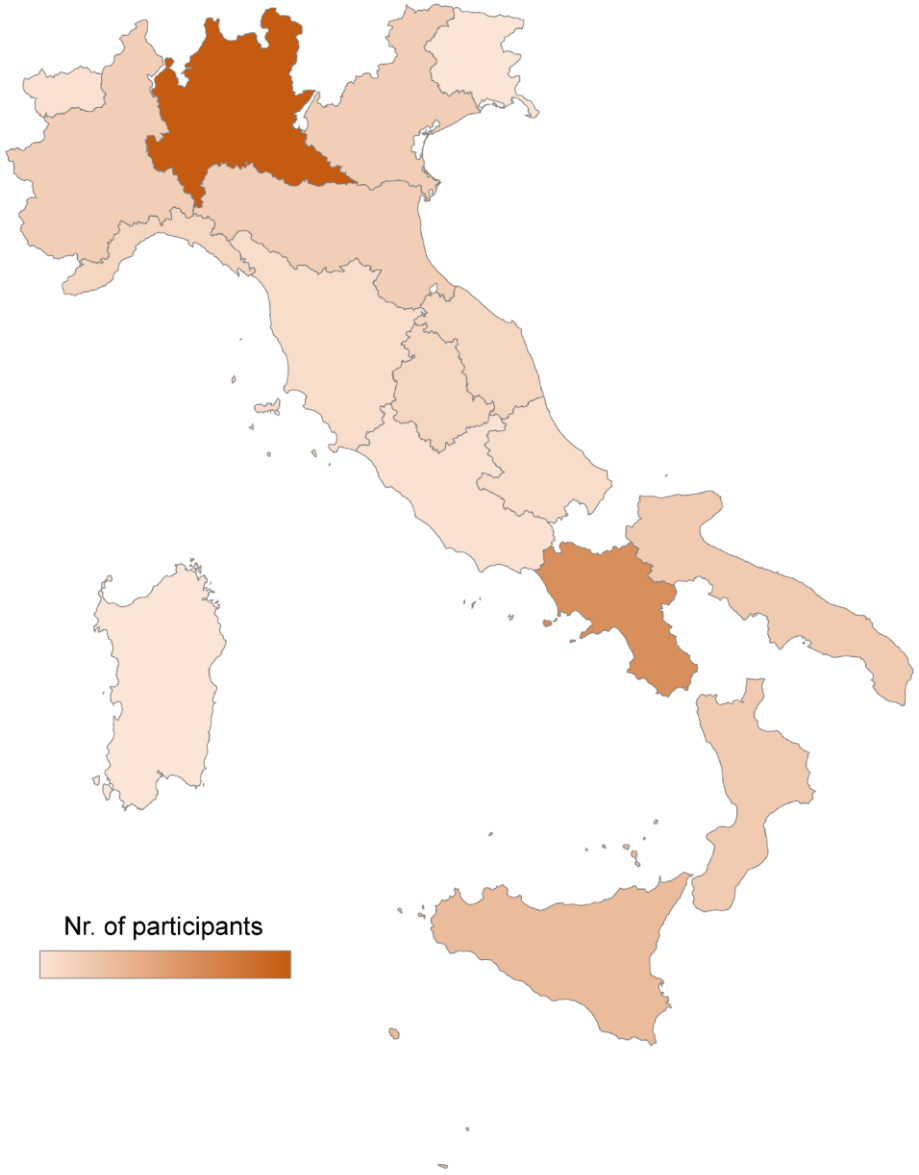
Konstantinos Venetis [1,2,†], Francesco Pepe [3,†], Elisabetta Munzone [4], Elham Sajjadi [1,2], Gianluca Russo [3], Pasquale Pisapia [3], Mariia Ivanova [1], Giuseppina Bonizzi [1], Davide Vacirca [1], Alessandra Rappa [1], Alberto Ranghiero [1], Sergio Vincenzo Taormina [1], Giuseppe Viale [1,2], Giancarlo Troncone [3], Massimo Barberis [1], Elena Guerini-Rocco [1,2], Umberto Malapelle [3,*,‡] and Nicola Fusco [1,2,*,‡]

Cells. 2022 Nov 9;11(22):3545. doi: 10.3390/cells11223545.



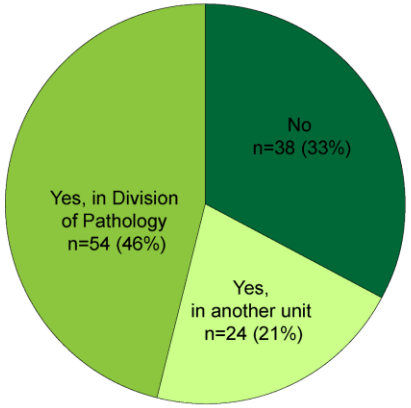
Pepe et al. Unpublished 2023

Landscape of *PIK3CA* mutation testing in Italy – Nationwide survey

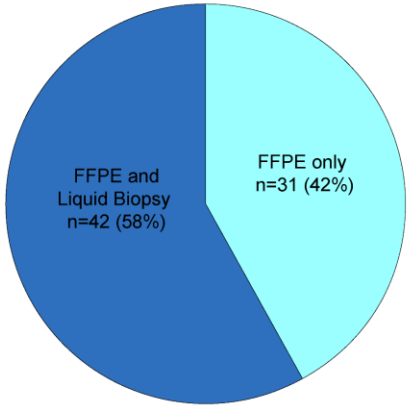


North-west	**52**
Valle d'Aosta	2
Piemonte	7
Lombardia	38
Liguria	5
North-east	**15**
Veneto	7
Friuli Venezia Giulia	1
Emilia Romagna	7
Center	**18**
Toscana	3
Umbria	5
Marche	5
Lazio	2
Abruzzo	3
South and islands	**53**
Campania	24
Puglia	8
Calabria	8
Sardegna	1
Sicilia	12

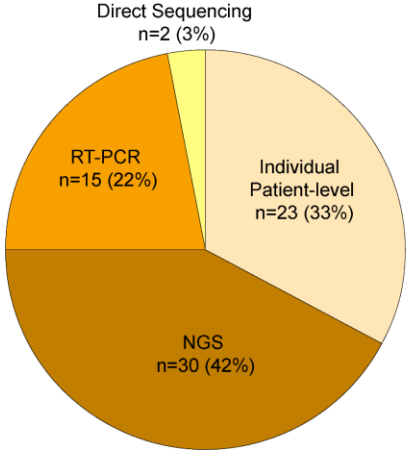
Q1: Do you have the possibility to carry out the test in your center?
n=116 answers



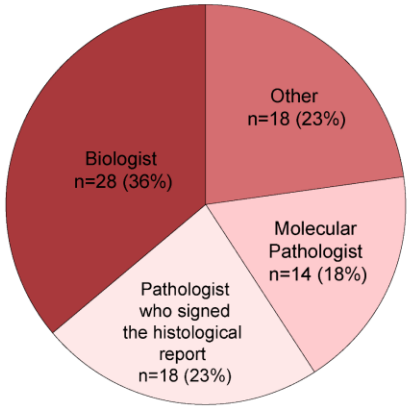
Q2: On which biomaterial can you conduct the analysis?
n=73 answers



Q3: Which technology is adopted for this test?
n=70 answers



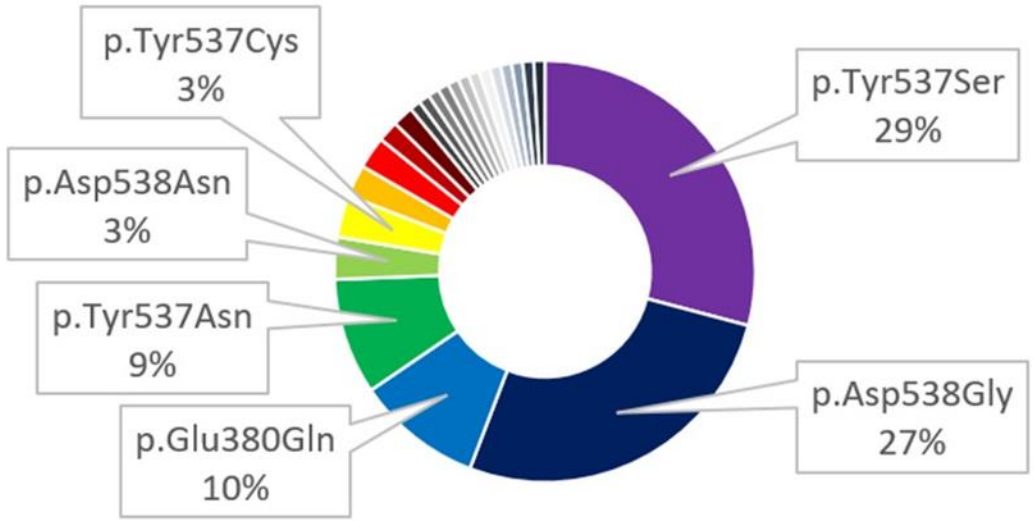
Q4: Which professionals sign the clinical report of this molecular test for breast cancer in your center ?
n=78 answers

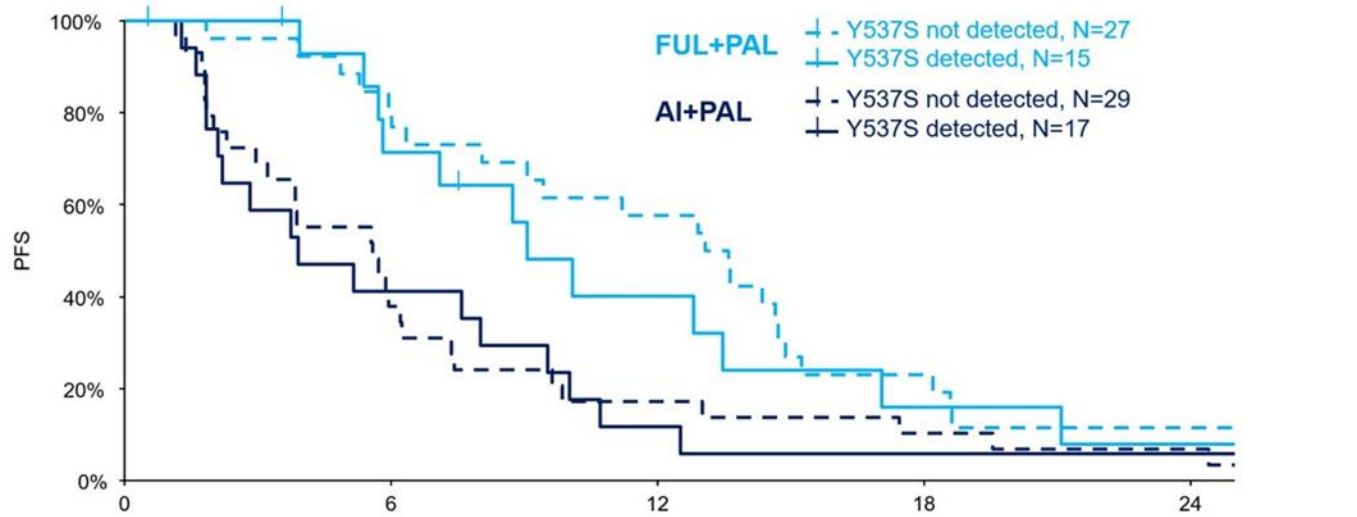


Pepe et al. Unpublished 2023

Mutation features & dynamics did not significantly predict switch benefit

- No difference by <u>which</u> *ESR1*mut





ESR1$_{mut}$ & PADA-1 design

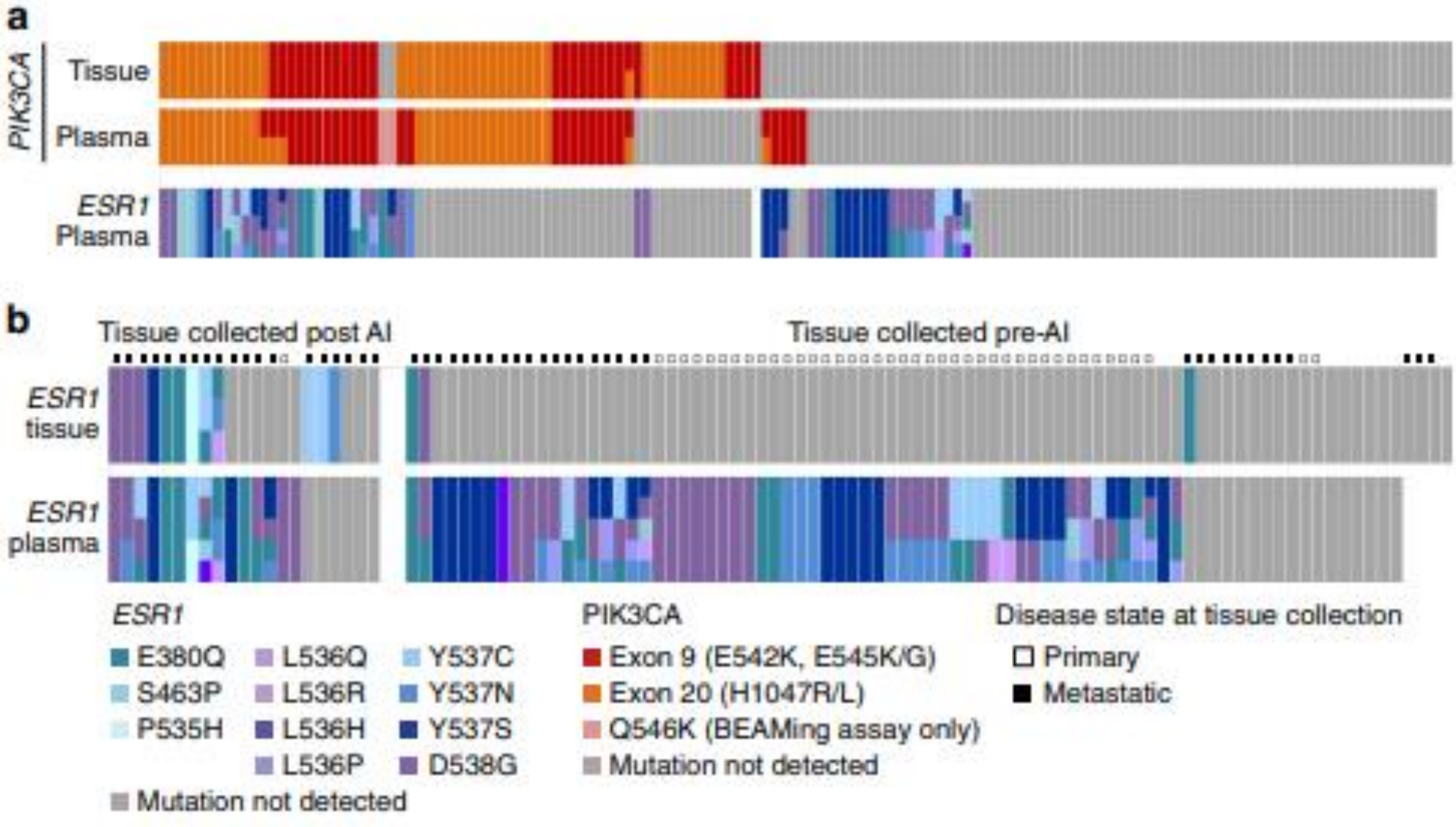
2023 ASCO ANNUAL MEETING #ASCO23

***ESR1* mutations**

- are acquired during aromatase inhibitors (AI) therapy in ~40% of ER+ HER2- mBC pts and drive resistance
- can be detected by ctDNA analysis in blood (*bESR1*$_{mut}$)
- retain partial sensitivity to fulvestrant (FUL), a selective estrogen receptor dégrader (SERD)

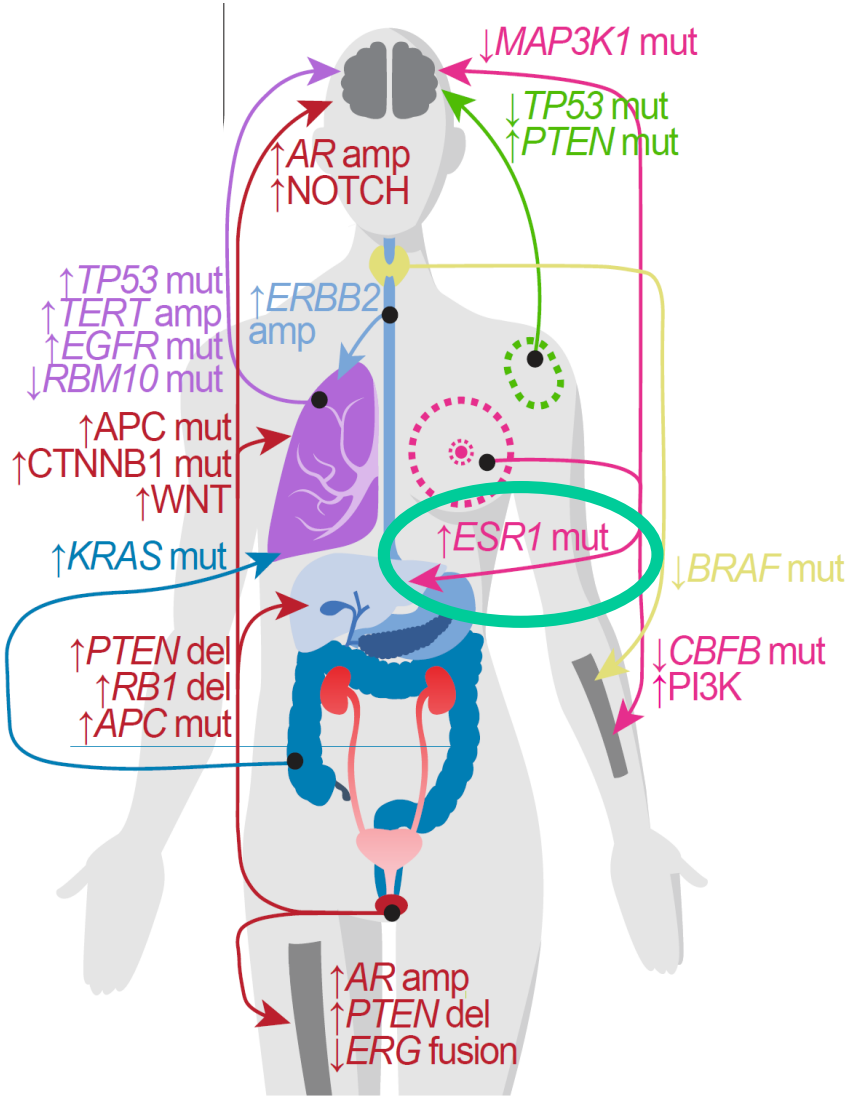
PADA-1

- Strategy: **targeting rising *bESR1*$_{mut}$ when they become detectable** under AI+Palbociclib (PAL) [1]

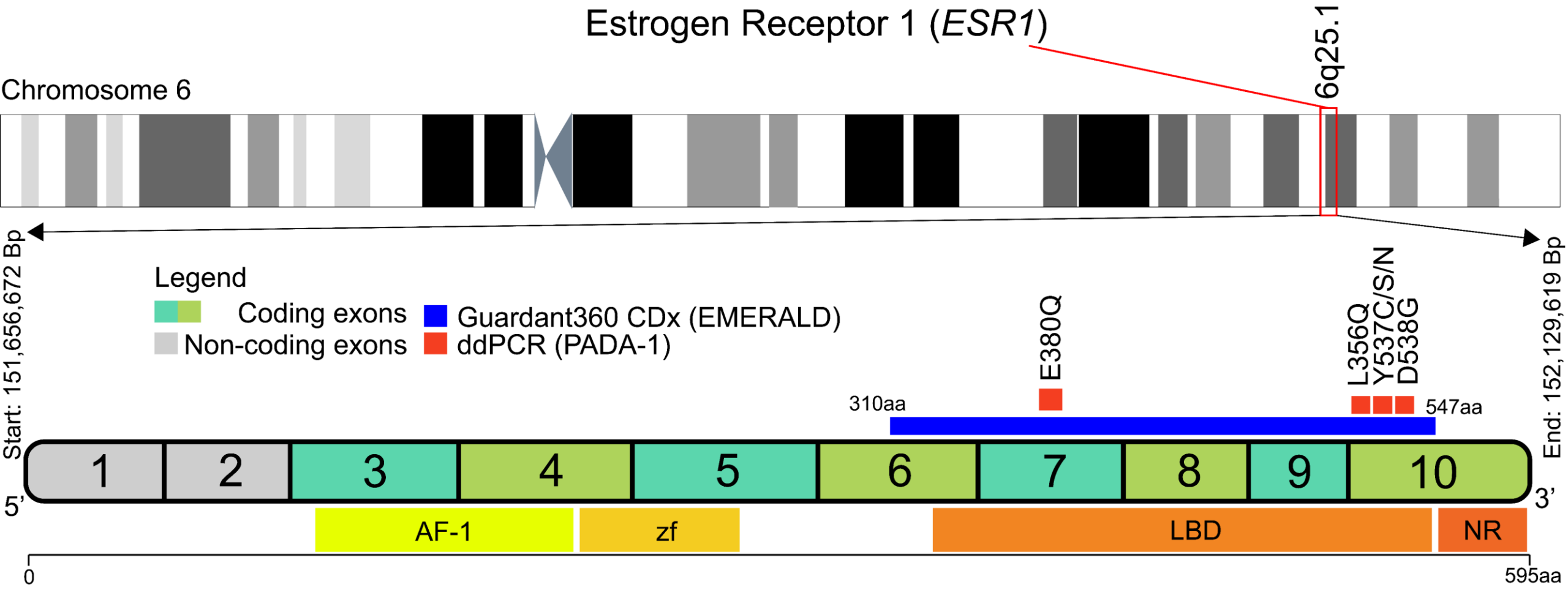


Spoerke JM, Gendreau S, Walter K, Qiu J, Wilson TR, Savage H, Aimi J, Derynck MK, Chen M, Chan IT, Amler LC, Hampton GM, Johnston S, Krop I, Schmid P, Lackner MR. Heterogeneity and clinical significance of ESR1 mutations in ER-positive metastatic breast cancer patients receiving fulvestrant. Nat Commun. 2016;7:11579.

ESR1 mutations associated with increased metastatic spread and liver metastasis



Nguyen B Cell 2022



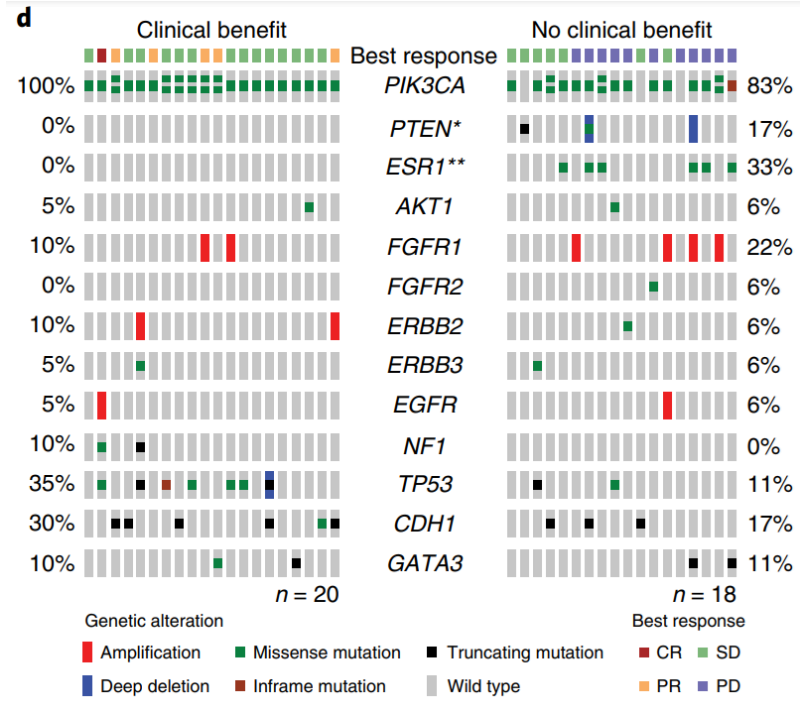
Venetis et al. Cancer Treat Rev 2023 (accepted)

ctDNA for the detection of *PIK3CA* mutations in breast cancer

ARTICLES
https://doi.org/10.1038/s43018-020-0047-1
nature cancer
Check for updates

Alterations in *PTEN* and *ESR1* promote clinical resistance to alpelisib plus aromatase inhibitors

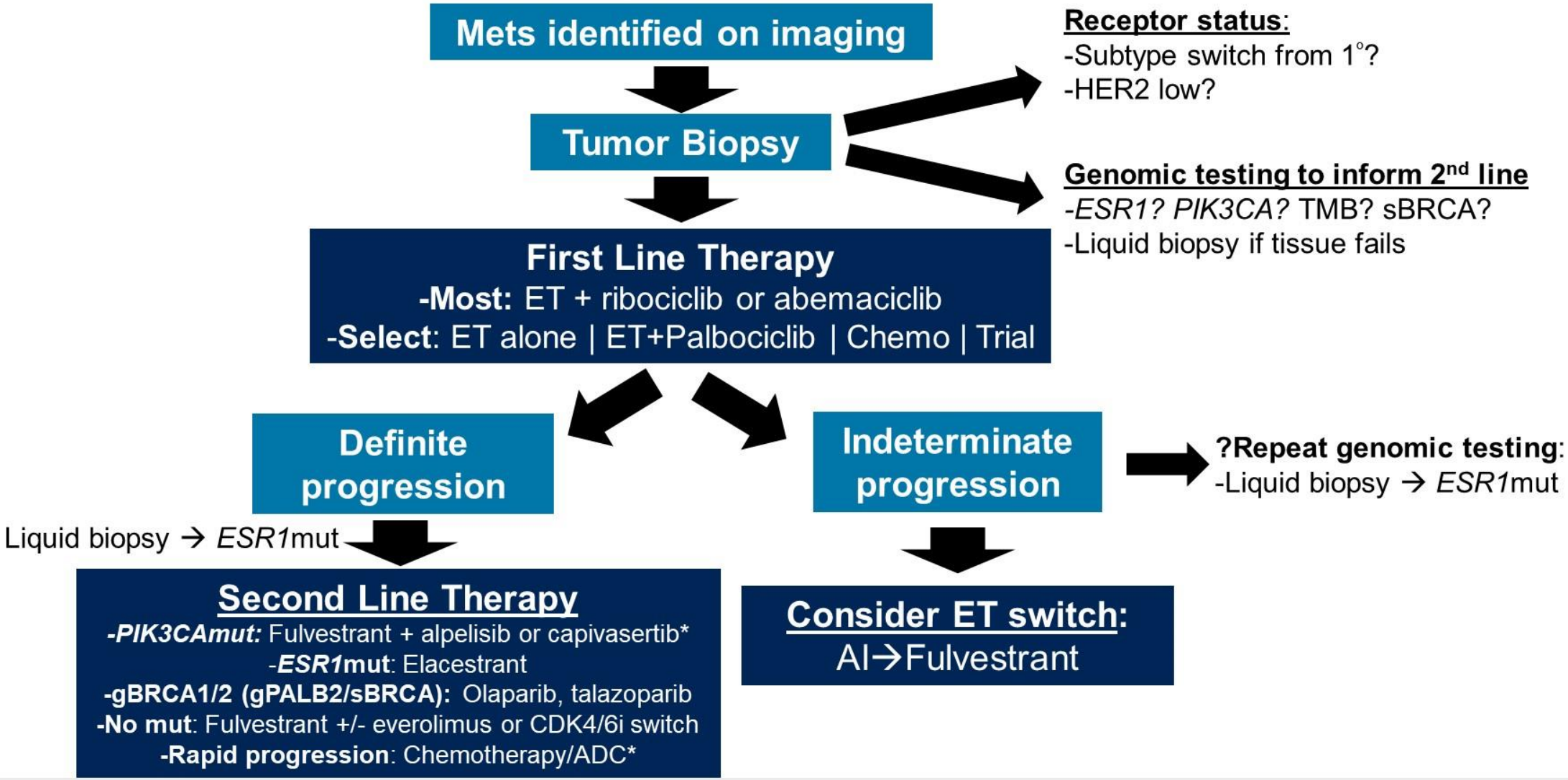
Pedram Razavi[1,2,3]✉, Maura N. Dickler[1,11], Payal D. Shah[4], Weiyi Toy[2], David N. Brown[5], Helen H. Won[6], Bob T. Li[1], Ronglai Shen[7], Neil Vasan[1,3], Shanu Modi[1,3], Komal Jhaveri[1,3], Betty Ann Caravella[8,3], Sujata Patil[3,7], Pier Selenica[5], Stephen Zamora[1], Aimee M. Cowan[1], Elizabeth Comen[1,3], Andy Singh[9], Anne Covey[8], Michael F. Berger[2,3,5,6], Clifford A. Hudis[1,3,10], Larry Norton[1,3], Rebecca J. Nagy[9], Justin I. Odegaard[9], Richard B. Lanman[9], David B. Solit[1,2,3,6], Mark E. Robson[1,3], Mario E. Lacouture[1,3], Edi Brogi[3,5], Jorge S. Reis-Filho[2,5], Mary Ellen Moynahan[1,3], Maurizio Scaltriti[2,3,5] and Sarat Chandarlapaty[1,2,3]✉



- Uncover mechanisms of resistance doing a longitudinal analysis of tumor and plasma circulating tumor DNA (ctDNA) among such PIK3CA-mutant, HR+ metastatic breast cancer patients from a phase I/II trial combining alpelisib with an aromatase inhibitor

Razavi, P. *et al.*, *Nat Cancer.* 2020

Approach to newly diagnosed HR+/HER2- MBC



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Thank you!

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