Navigating Clonal Evolution in Metastatic Breast Cancer

Integrating longitudinal genomic profiling to counter multi-pathway resistance.

Cengiz Yakicier
AQUARIUS NPG Cancer Genomics Laboratory, Istanbul
Bahrain Oncology Center, King Hamad University Hospital, Manama/Bahrain

Breast Cancer Exists as Two Distinct Clinical Realities

Early-Stage Disease

Clinical Outcome: ~70–80% Curable.

Biological State: Static and manageable.

Strategy: Governed by distinct, stable molecular subtyping (HER2/ERBB2, HR+, BRCA mutations).

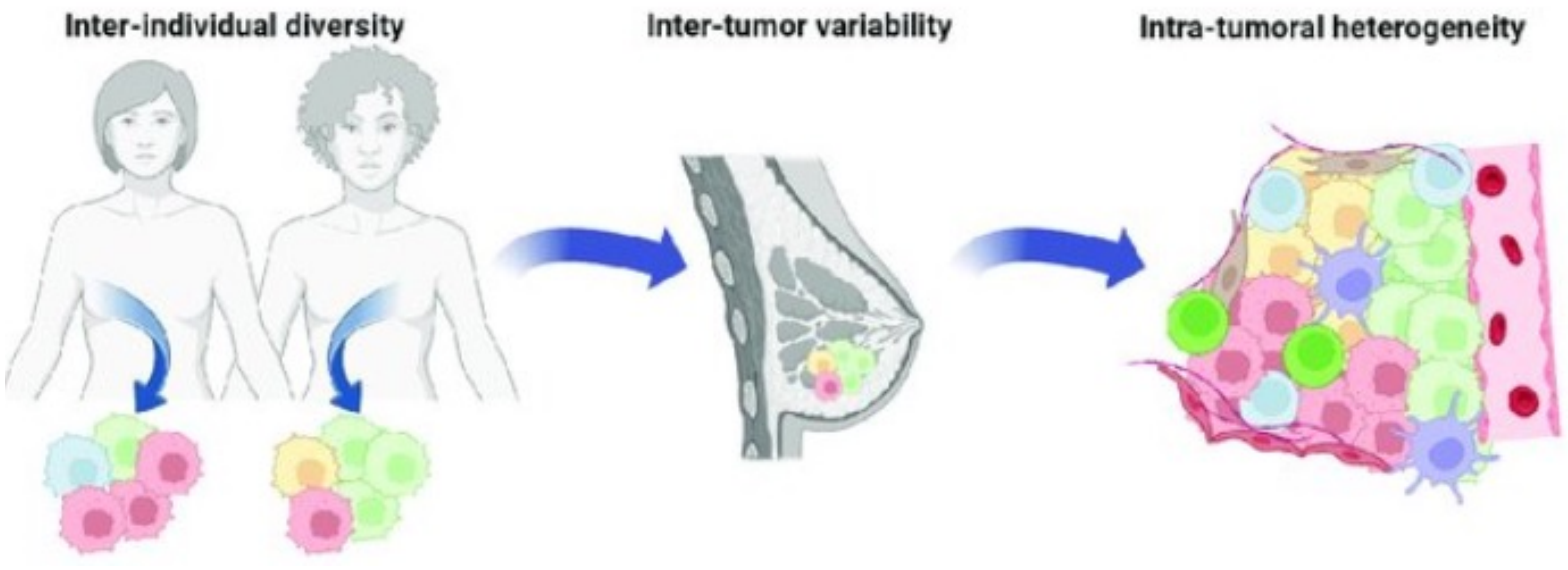
Metastatic Breast Cancer (mBC)

Clinical Outcome: Largely incurable with current therapies; leading cause of mortality.

Biological State: Highly dynamic and aggressively adaptive.

The Evasion Triad: Why does mBC evade cure?
Driven by Intratumoral Heterogeneity, Clonal Evolution, and Adaptive Resistance.

Understanding Breast Cancer Diversity



Azam & Sounni Cancers (Basel). 2022

Breast

Cohort not saved

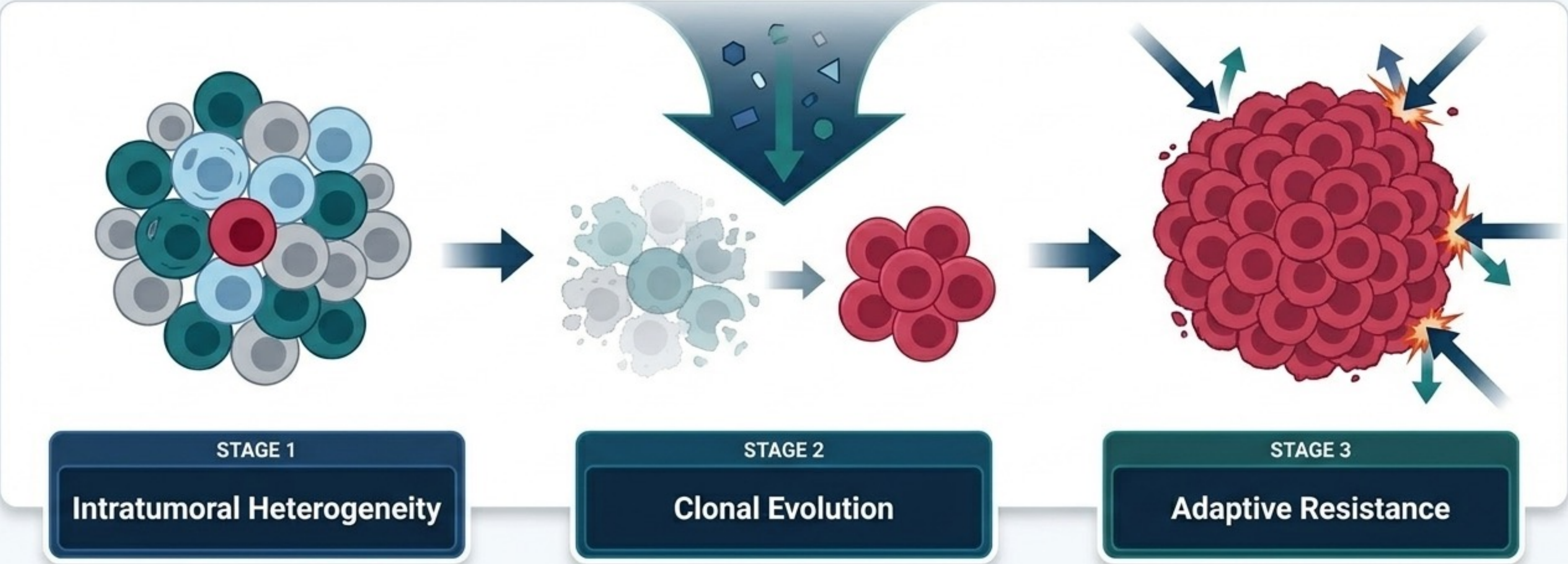
4.047 CASES

PRIMARY SITE: breast

TISSUE OR ORGAN OF ORIGIN: axillary tail of breast, breast, nos, central portion of br..., lower-inner quadran..., lower-outer quadran..., nipple, overlapping lesion of..., upper-inner quadran..., upper-outer quadran...

Zoom 1,0

Three interlocking mechanisms drive therapeutic failure in metastatic disease



STAGE 1

Intratumoral Heterogeneity

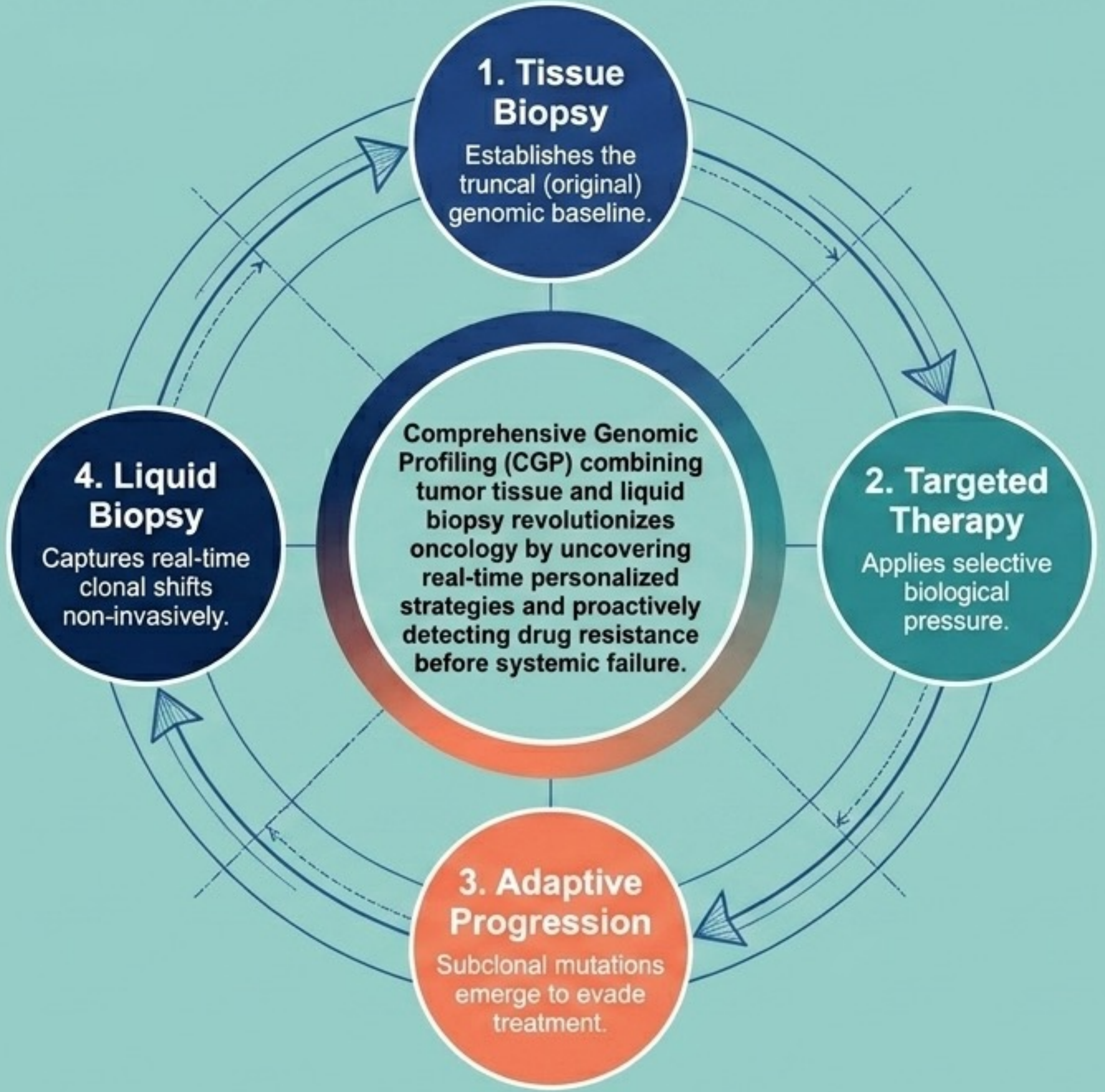
STAGE 2

Clonal Evolution

STAGE 3

Adaptive Resistance

Static Baseline Testing Cannot Track an Evolving Malignancy



1. Tissue Biopsy

Establishes the truncal (original) genomic baseline.

2. Targeted Therapy

Applies selective biological pressure.

3. Adaptive Progression

Subclonal mutations emerge to evade treatment.

4. Liquid Biopsy

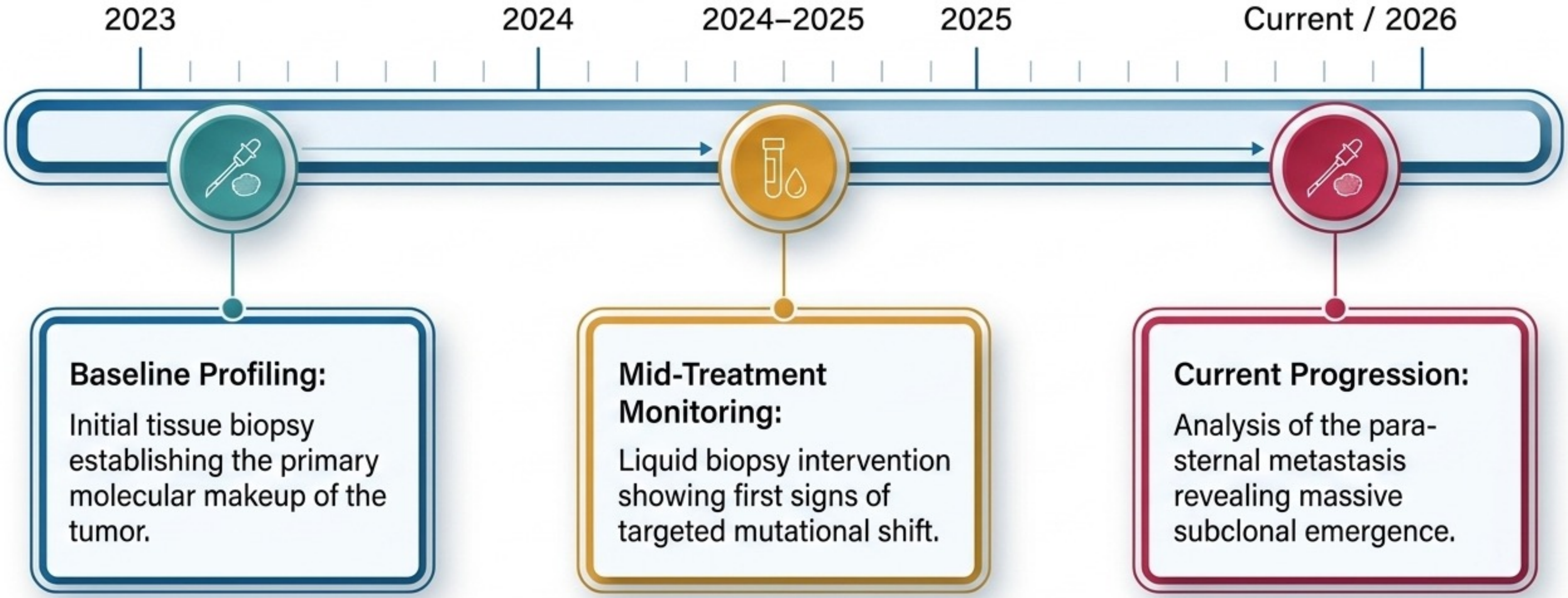
Captures real-time clonal shifts non-invasively.

Comprehensive Genomic Profiling (CGP) combining tumor tissue and liquid biopsy revolutionizes oncology by uncovering real-time personalized strategies and proactively detecting drug resistance before systemic failure.

Case

- Gender: Female
- Age: 58 yo
- Tumor Diagnosis: Breast Cancer
- Tumor Stage: Metastatic Breast Cancer
  - ER: %7 (+)
  - PR: (-)
  - HER-2:   (+++)
  - KI-67: %60

Longitudinal sampling provides a roadmap of the tumor’s evolutionary timeline



Baseline Profiling:

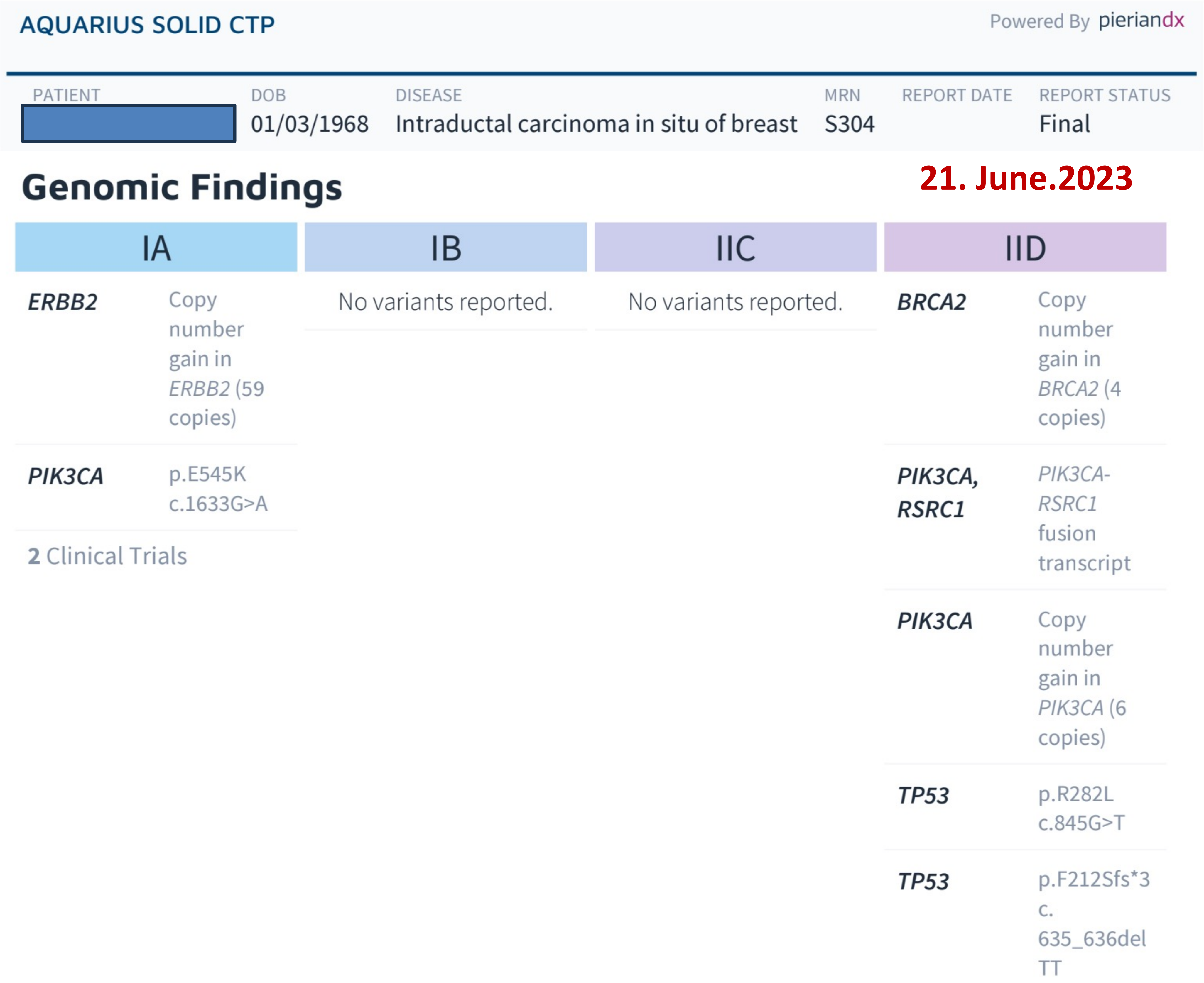
Initial tissue biopsy establishing the primary molecular makeup of the tumor.

Mid-Treatment Monitoring:

Liquid biopsy intervention showing first signs of targeted mutational shift.

Current Progression:

Analysis of the para-sternal metastasis revealing massive subclonal emergence.



AQUARIUS SOLID CTP

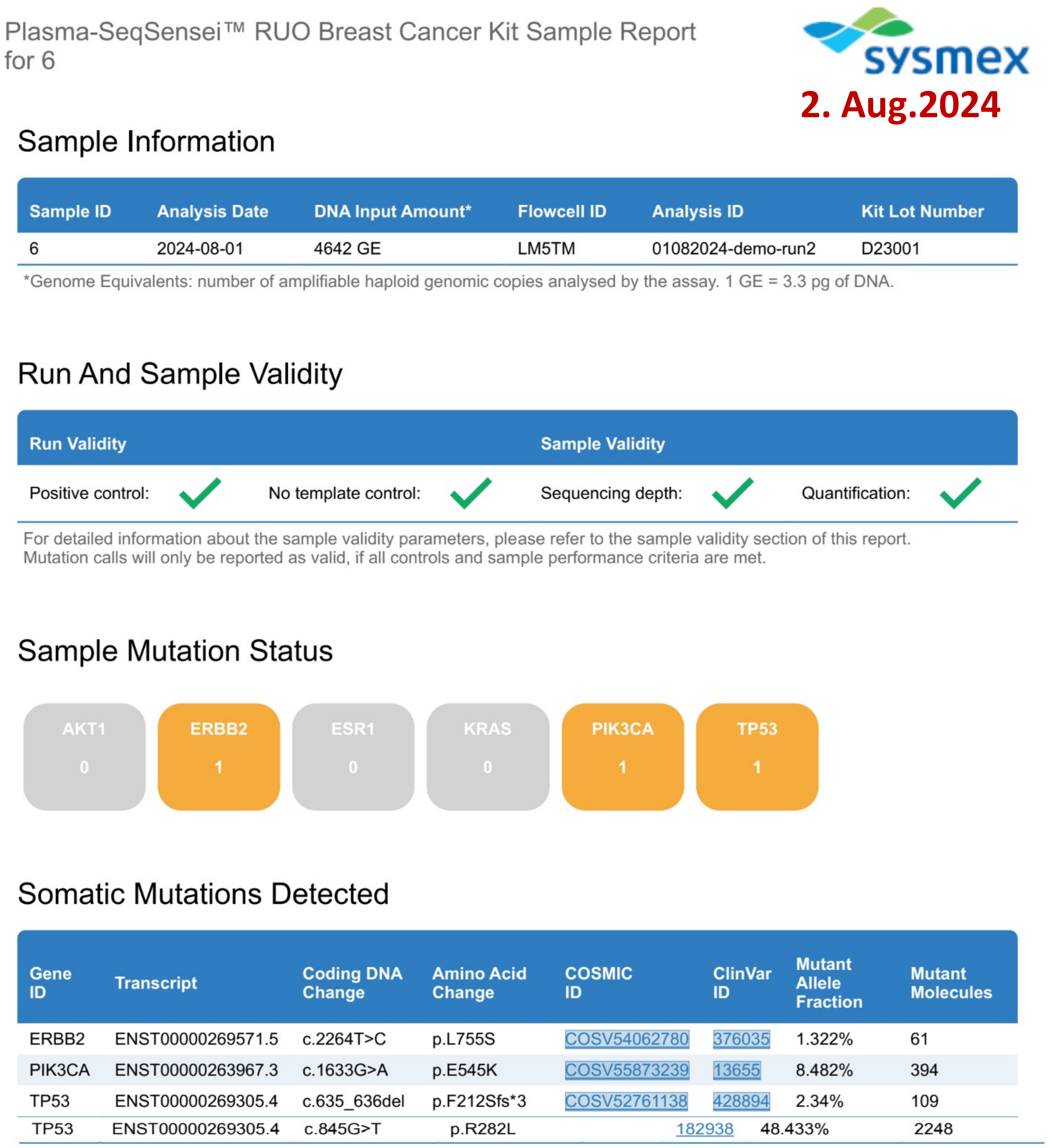
Powered By pieriandx

PATIENT	DOB	DISEASE	MRN	REPORT DATE	REPORT STATUS
[redacted]	01/03/1968	Intraductal carcinoma in situ of breast	S304		Final

Genomic Findings

21. June.2023

IA		IB	IIC	IID	
ERBB2	Copy number gain in *ERBB2* (59 copies)	No variants reported.	No variants reported.	***BRCA2***	Copy number gain in *BRCA2* (4 copies)
PIK3CA	p.E545K c.1633G>A			***PIK3CA, RSRC1***	*PIK3CA-RSRC1* fusion transcript
2 Clinical Trials				***PIK3CA***	Copy number gain in *PIK3CA* (6 copies)
				TP53	p.R282L c.845G>T
				TP53	p.F212Sfs*3 c. 635_636del TT



Plasma-SeqSensei™ RUO Breast Cancer Kit Sample Report for 6

sysmex

2. Aug.2024

Sample Information

Sample ID	Analysis Date	DNA Input Amount*	Flowcell ID	Analysis ID	Kit Lot Number
6	2024-08-01	4642 GE	LM5TM	01082024-demo-run2	D23001

*Genome Equivalents: number of amplifiable haploid genomic copies analysed by the assay. 1 GE = 3.3 pg of DNA.

Run And Sample Validity

Run Validity				Sample Validity			
Positive control:	✓	No template control:	✓	Sequencing depth:	✓	Quantification:	✓

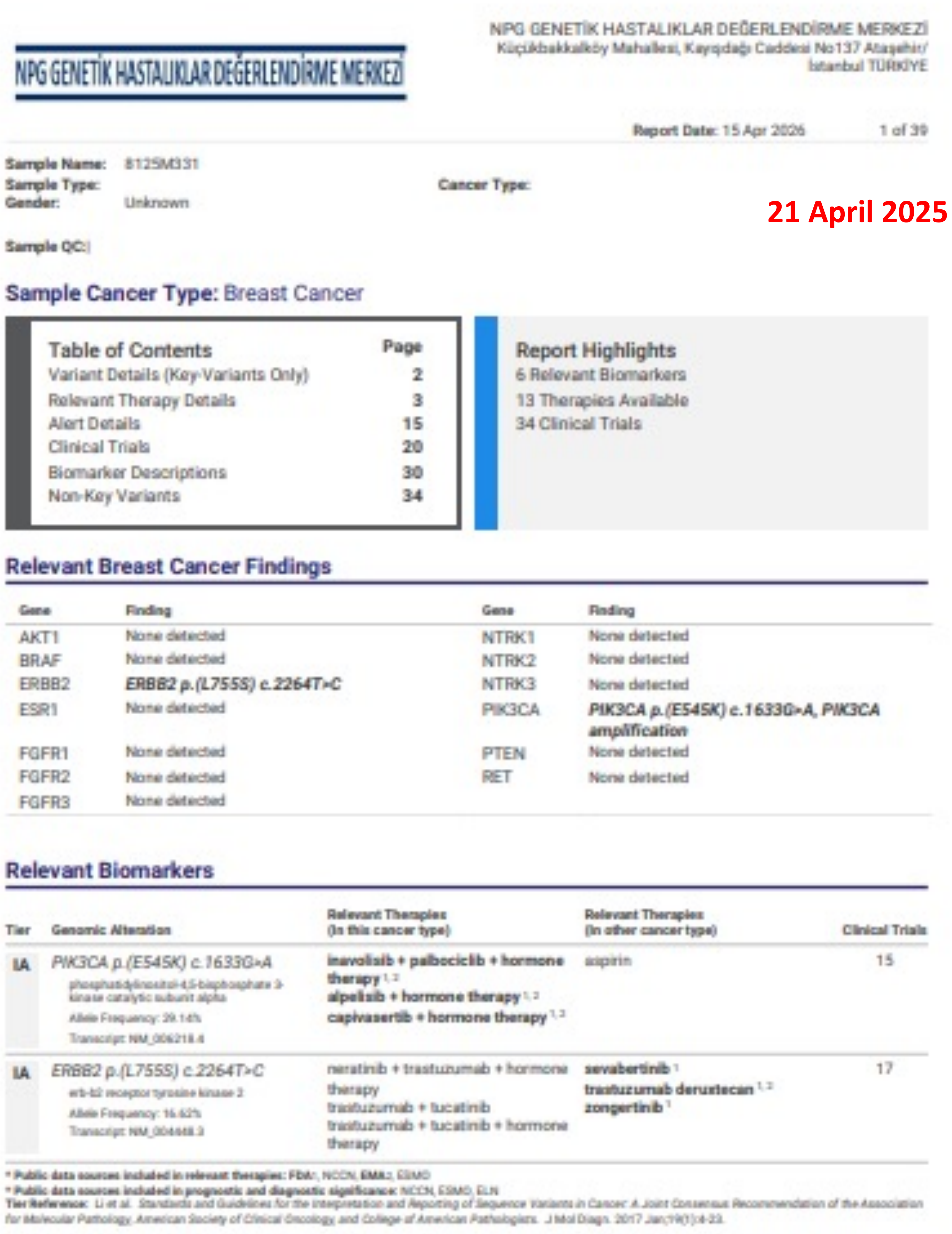
For detailed information about the sample validity parameters, please refer to the sample validity section of this report.
Mutation calls will only be reported as valid, if all controls and sample performance criteria are met.

Sample Mutation Status

AKT1	ERBB2	ESR1	KRAS	PIK3CA	TP53
0	1	0	0	1	1

Somatic Mutations Detected

Gene ID	Transcript	Coding DNA Change	Amino Acid Change	COSMIC ID	ClinVar ID	Mutant Allele Fraction	Mutant Molecules
ERBB2	ENST00000269571.5	c.2264T>C	p.L755S	COSV54062780	376035	1.322%	61
PIK3CA	ENST00000263967.3	c.1633G>A	p.E545K	COSV55873239	13655	8.482%	394
TP53	ENST00000269305.4	c.635_636del	p.F212Sfs*3	COSV52761138	428894	2.34%	109
TP53	ENST00000269305.4	c.845G>T	p.R282L		182938	48.433%	2248



NPG GENETİK HASTALIKLAR DEĞERLENDİRME MERKEZİ
Küçükbakkalköy Mahallesi, Kayışdağı Caddesi No137 Ataşehir/
İstanbul TÜRKİYE

Sample Name: 8125M331
Sample Type: **Cancer Type:**
Gender: Unknown

21 April 2025

Sample QC:

Sample Cancer Type: Breast Cancer

Report Highlights

6 Relevant Biomarkers
13 Therapies Available
34 Clinical Trials

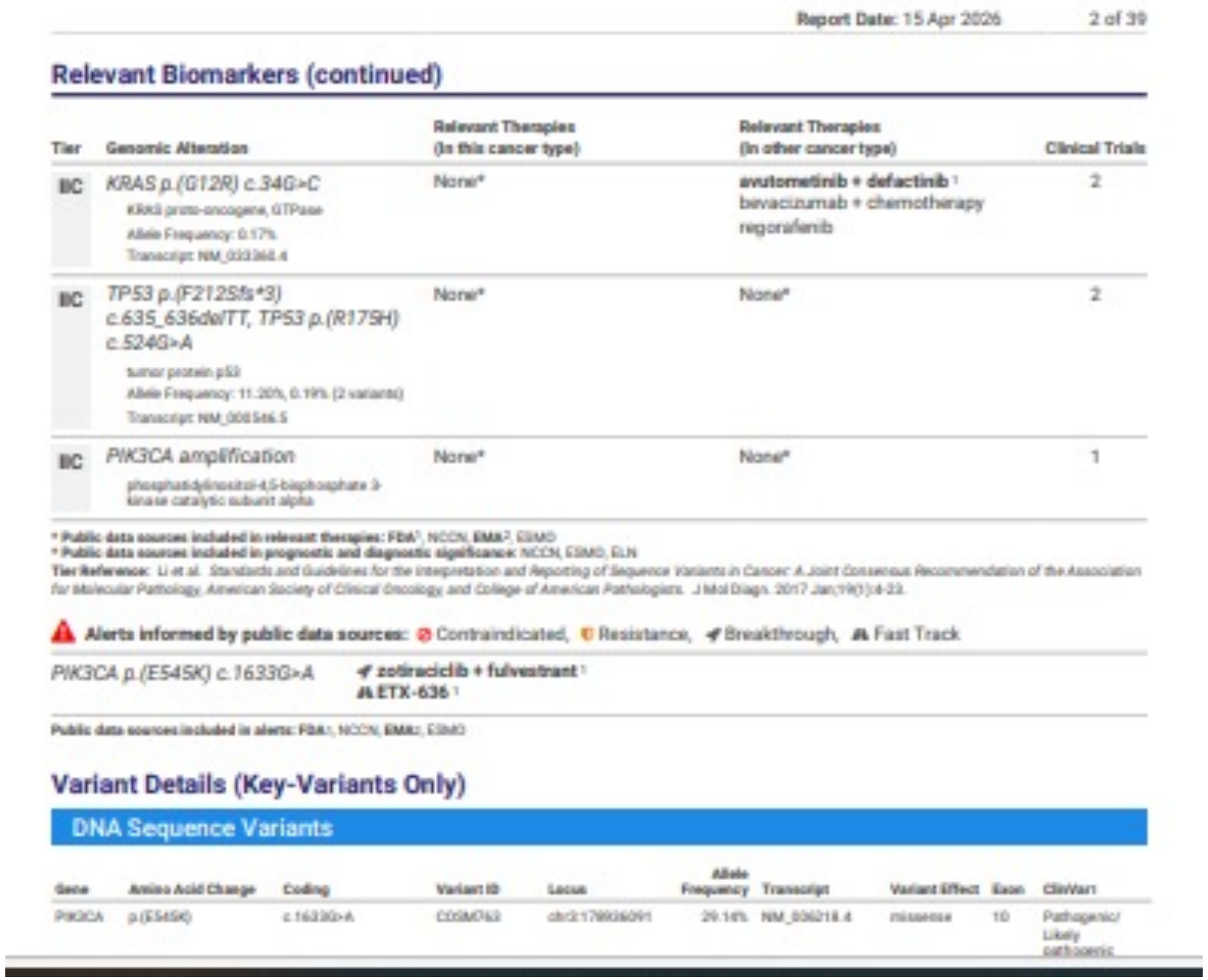
Relevant Breast Cancer Findings

Gene	Finding	Gene	Finding
AKT1	None detected	NTRK1	None detected
BRAF	None detected	NTRK2	None detected
ERBB2	***ERBB2 p.(L755S) c.2264T>C***	NTRK3	None detected
ESR1	None detected	PIK3CA	***PIK3CA p.(E545K) c.1633G>A, PIK3CA amplification***
FGFR1	None detected	PTEN	None detected
FGFR2	None detected	RET	None detected
FGFR3	None detected		

Relevant Biomarkers

Tier	Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
IA	*PIK3CA p.(E545K) c.1633G>A* phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha Allele Frequency: 29.14% Transcript: NM_006218.4	**inavolisib + palbociclib + hormone therapy** [1, 2] **alpelisib + hormone therapy** [1, 2] **capivasertib + hormone therapy** [1, 2]	aspirin	15
IA	*ERBB2 p.(L755S) c.2264T>C* erb-b2 receptor tyrosine kinase 2 Allele Frequency: 16.62% Transcript: NM_004448.3	neratinib + trastuzumab + hormone therapy trastuzumab + tucatinib trastuzumab + tucatinib + hormone therapy	**sevabertinib** [1] **trastuzumab deruxtecan** [1, 2] **zongertinib** [1]	17

* **Public data sources included in relevant therapies:** FDA[1], NCCN, EMA[2], ESMO
* **Public data sources included in prognostic and diagnostic significance:** NCCN, ESMO, ELN
Tier Reference: Li et al. *Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists.* J Mol Diagn. 2017 Jan;19(1):4-23.



Relevant Biomarkers (continued)

Tier	Genomic Alteration	Relevant Therapies (In this cancer type)	Relevant Therapies (In other cancer type)	Clinical Trials
IIC	*KRAS p.(G12R) c.34G>C* KRAS proto-oncogene, GTPase Allele Frequency: 0.17% Transcript: NM_033360.4	None*	**avutometinib + defactinib** [1] bevacizumab + chemotherapy regorafenib	2
IIC	*TP53 p.(F212Sfs*3) c.635_636delTT, TP53 p.(R175H) c.524G>A* tumor protein p53 Allele Frequency: 11.20%, 0.19% (2 variants) Transcript: NM_000546.5	None*	None*	2
IIC	*PIK3CA amplification* phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	None*	None*	1

* **Public data sources included in relevant therapies:** FDA[1], NCCN, EMA[2], ESMO
* **Public data sources included in prognostic and diagnostic significance:** NCCN, ESMO, ELN
Tier Reference: Li et al. *Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists.* J Mol Diagn. 2017 Jan;19(1):4-23.

⚠ **Alerts informed by public data sources:** ⊘ Contraindicated, ◐ Resistance, ✈ Breakthrough, ⏩ Fast Track

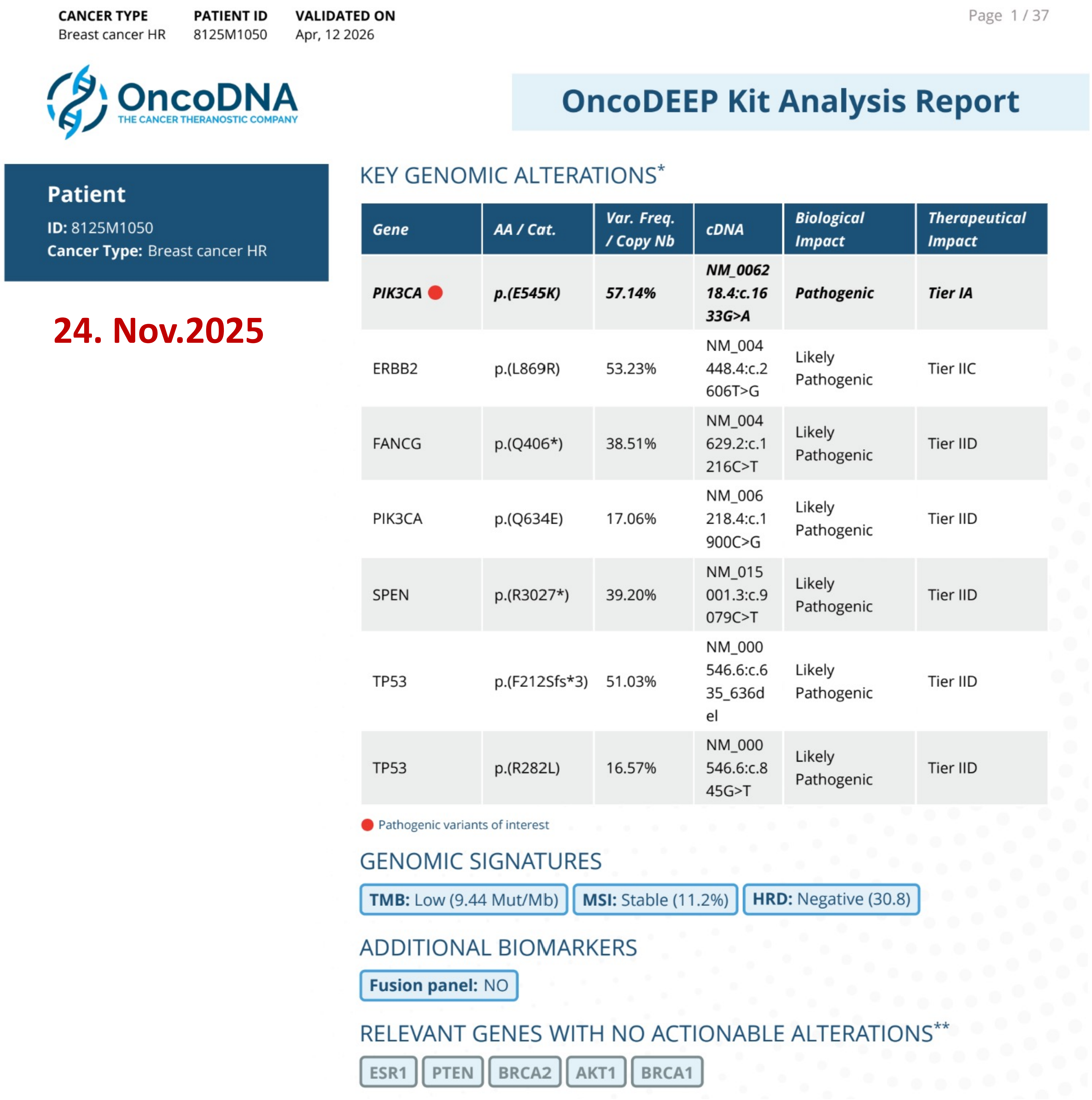
PIK3CA p.(E545K) c.1633G>A	✈ **zotiraciclib + fulvestrant** [1] ⏩ **ETX-636** [1]

Public data sources included in alerts: FDA[1], NCCN, EMA[2], ESMO

Variant Details (Key-Variants Only)

DNA Sequence Variants

Gene	Amino Acid Change	Coding	Variant ID	Locus	Allele Frequency	Transcript	Variant Effect	Exon	ClinVar
PIK3CA	p.(E545K)	c.1633G>A	COSM763	chr3:178936091	29.14%	NM_006218.4	missense	10	Pathogenic/ Likely pathogenic



CANCER TYPE Breast cancer HR | **PATIENT ID** 8125M1050 | **VALIDATED ON** Apr, 12 2026

Page 1 / 37

OncoDNA
THE CANCER THERANOSTIC COMPANY

OncoDEEP Kit Analysis Report

Patient

ID: 8125M1050
Cancer Type: Breast cancer HR

24. Nov.2025

KEY GENOMIC ALTERATIONS*

Gene	AA / Cat.	Var. Freq. / Copy Nb	cDNA	Biological Impact	Therapeutical Impact
PIK3CA ●	***p.(E545K)***	***57.14%***	***NM_006218.4:c.1633G>A***	***Pathogenic***	***Tier IA***
ERBB2	p.(L869R)	53.23%	NM_004448.4:c.2606T>G	Likely Pathogenic	Tier IIC
FANCG	p.(Q406*)	38.51%	NM_004629.2:c.1216C>T	Likely Pathogenic	Tier IID
PIK3CA	p.(Q634E)	17.06%	NM_006218.4:c.1900C>G	Likely Pathogenic	Tier IID
SPEN	p.(R3027*)	39.20%	NM_015001.3:c.9079C>T	Likely Pathogenic	Tier IID
TP53	p.(F212Sfs*3)	51.03%	NM_000546.6:c.635_636del	Likely Pathogenic	Tier IID
TP53	p.(R282L)	16.57%	NM_000546.6:c.845G>T	Likely Pathogenic	Tier IID

● Pathogenic variants of interest

GENOMIC SIGNATURES

TMB: Low (9.44 Mut/Mb) | **MSI:** Stable (11.2%) | **HRD:** Negative (30.8)

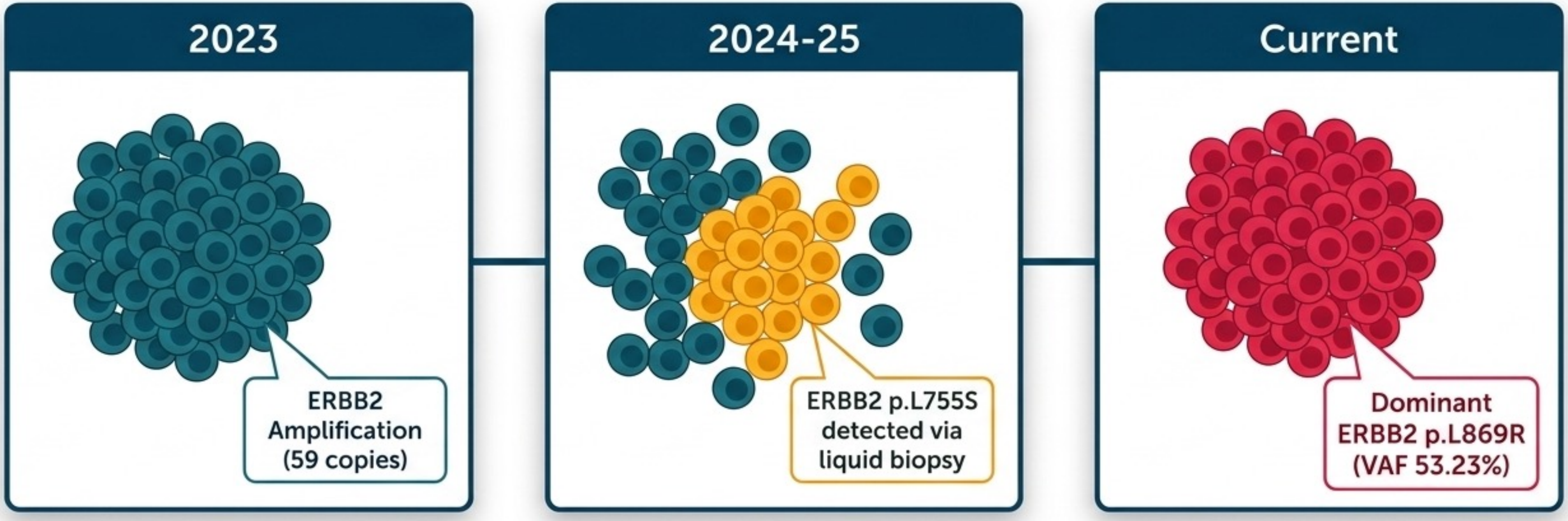
ADDITIONAL BIOMARKERS

Fusion panel: NO

RELEVANT GENES WITH NO ACTIONABLE ALTERATIONS**

ESR1 | PTEN | BRCA2 | AKT1 | BRCA1

Continuous monitoring reveals a definitive clonal sweep replacing initial ERBB2 amplification


2023

ERBB2 Amplification (59 copies)

2024-25

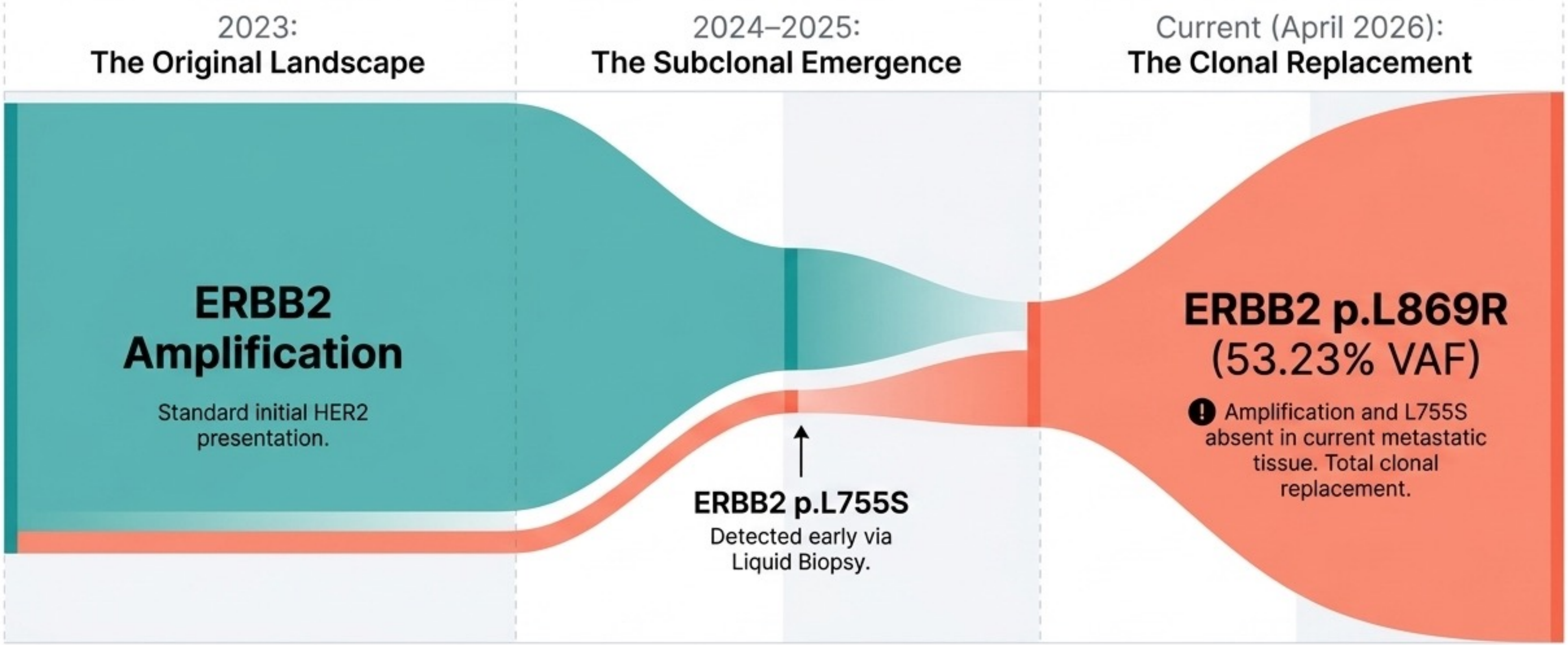
ERBB2 p.L755S detected via liquid biopsy

Current

Dominant ERBB2 p.L869R (VAF 53.23%)

Definitive Clonal Replacement

The clonal sweep: A shifting genomic landscape under selective pressure



2023:
The Original Landscape

2024–2025:
The Subclonal Emergence

Current (April 2026):
The Clonal Replacement

ERBB2 Amplification

Standard initial HER2 presentation.

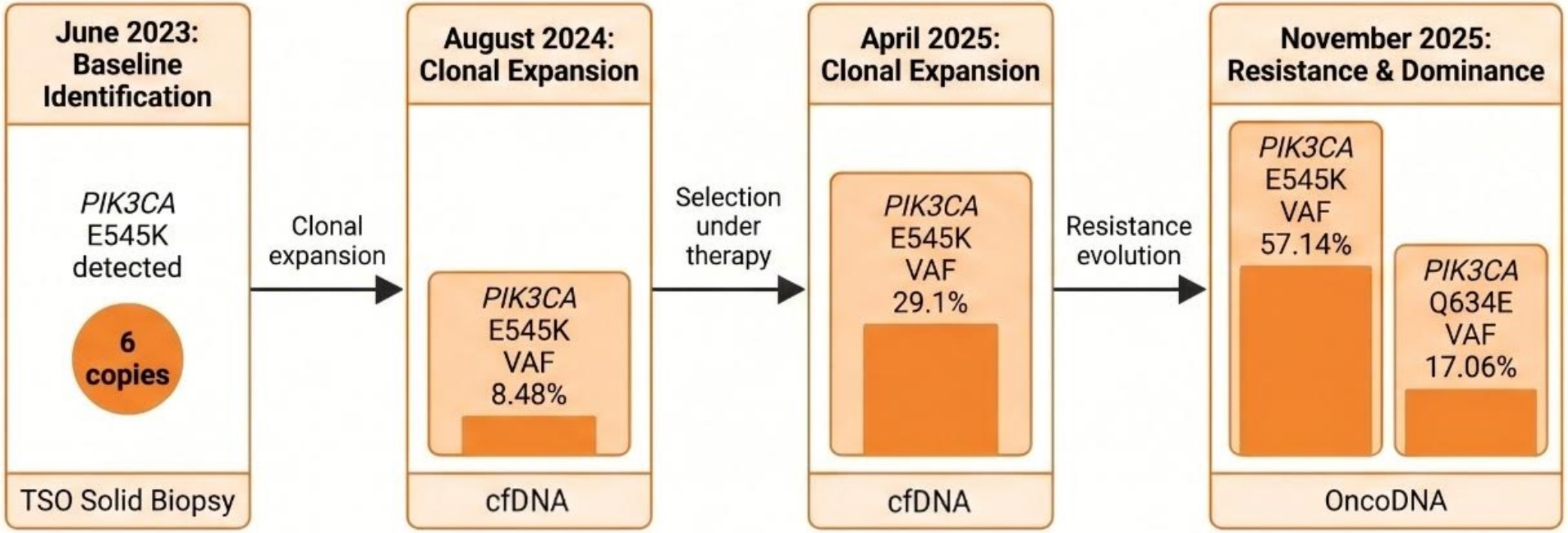
ERBB2 p.L755S

Detected early via Liquid Biopsy.

ERBB2 p.L869R (53.23% VAF)

❗ Amplification and L755S absent in current metastatic tissue. Total clonal replacement.

Longitudinal Evolution of *PIK3CA*-Driven Resistance in Breast Cancer



June 2023: Baseline Identification

PIK3CA E545K detected

6 copies

TSO Solid Biopsy

Clonal expansion

August 2024: Clonal Expansion

PIK3CA E545K VAF 8.48%

cfDNA

Selection under therapy

April 2025: Clonal Expansion

PIK3CA E545K VAF 29.1%

cfDNA

Resistance evolution

November 2025: Resistance & Dominance

PIK3CA E545K VAF 57.14%

PIK3CA Q634E VAF 17.06%

OncoDNA

Clinical Insights & Resistance Conclusion

- **Evidence of Clonal Dominance**
  The persistent and dramatic increase in *PIK3CA* E545K VAF E545K signifies selective advantage.
- **Emergence of Secondary Mutation**
  The new Q634E mutation indicates further branching and complexity in resistance.
- **Conclusion: PIK3CA-Driven Evolution**
  Data confirms PIK3CA as the primary driver of therapeutic resistance evolution.

Patient Dossier

Patient 8125M1050 demonstrates the challenge of **multi-line resistance following early progression**

Demographics

Gender: Female

DOB: 01/03/1968

MRN: S304

ID: 8125M1050

Clinical History

- **Initial Diagnosis**: Intraductal carcinoma in situ of breast.
- **Progression**: Metastatic disease demonstrating adaptive resistance to multiple lines of standard endocrine and targeted therapies.

Sampling Parameters

Current Sample: Para-sternal metastatic tissue

Cellularity: 70% tumor cellularity

Analysis: Comprehensive 638-gene panel supported by longitudinal liquid biopsy and historical tissue profiling.

The Current Genomic Snapshot Reveals Extreme Instability and Critical Biomarkers

Genomic Signatures

TMB
Borderline High (9.44 Muts/Mb)
Low (<10 Muts/Mb)

MSI
Stable (11.2%)
Unstable (>10%)

HRD
Negative (30.8)
Positive (>42)

Critical Tier I/II Alterations

●	**PIK3CA**: p.E545K (VAF 57.14%)	●	**PIK3CA**: p.E2BBK (VAF 140.77%)
●	**PIK3CA**: p.Q634E (VAF 17.06%)		
●	**ERBB2**: p.L869R (VAF 53.23%)		
●	**TP53**: **p.F212Sfs*3** (51.03%) & **p.R282L** (16.57%)		

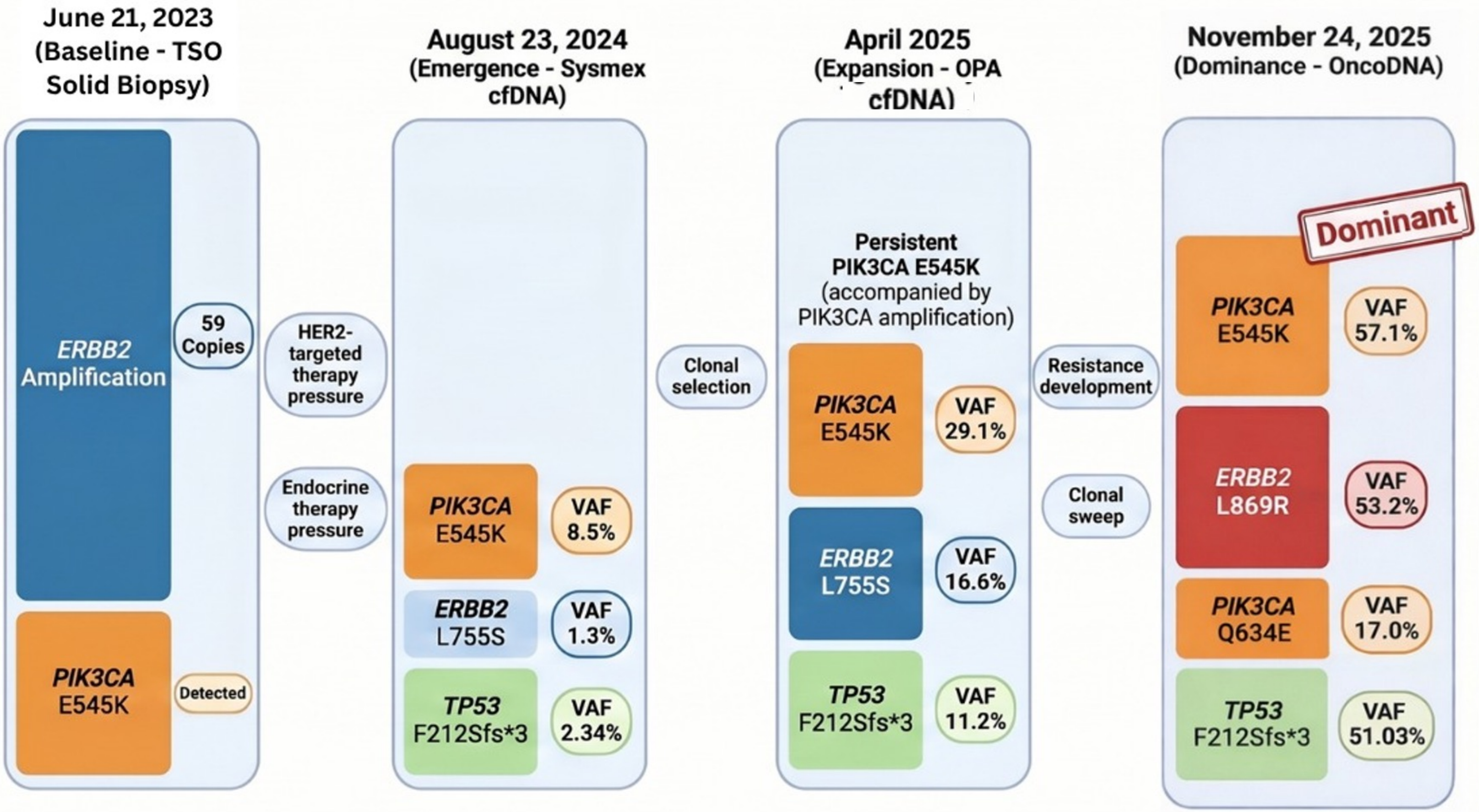
The Instability Footprint (LOH)

⚠

Extensive Loss of Heterozygosity (LOH) across 17 critical genes.

Impacted HRR Genes (1 Copy Only): ATM, ATR, BAP1, BRCA1, CDKN2A/B, CHEK1, FANCE, FBXW7, MLH1, MTAP, NF1, RAD51, RAD51D, RB1, BIRC3, TP53.

Clonal Evolution and Resistance Mechanisms in Metastatic Breast Cancer: A Longitudinal Genomic Study



June 21, 2023
(Baseline - TSO Solid Biopsy)

ERBB2 Amplification — 59 Copies

PIK3CA E545K — Detected

HER2-targeted therapy pressure

Endocrine therapy pressure

August 23, 2024
(Emergence - Sysmex cfDNA)

PIK3CA E545K — VAF 8.5%

ERBB2 L755S — VAF 1.3%

TP53 F212Sfs*3 — VAF 2.34%

Clonal selection

April 2025
(Expansion - OPA cfDNA)

Persistent PIK3CA E545K
(accompanied by PIK3CA amplification)

PIK3CA E545K — VAF 29.1%

ERBB2 L755S — VAF 16.6%

TP53 F212Sfs*3 — VAF 11.2%

Resistance development

Clonal sweep

November 24, 2025
(Dominance - OncoDNA)

Dominant

PIK3CA E545K — VAF 57.1%

ERBB2 L869R — VAF 53.2%

PIK3CA Q634E — VAF 17.0%

TP53 F212Sfs*3 — VAF 51.03%

Therapeutic Implications

Shift in HER2 Targeting Strategy: The evolution from amplification to specific kinase domain mutations (L755S, L869R) requires a change in HER2-directed agents.

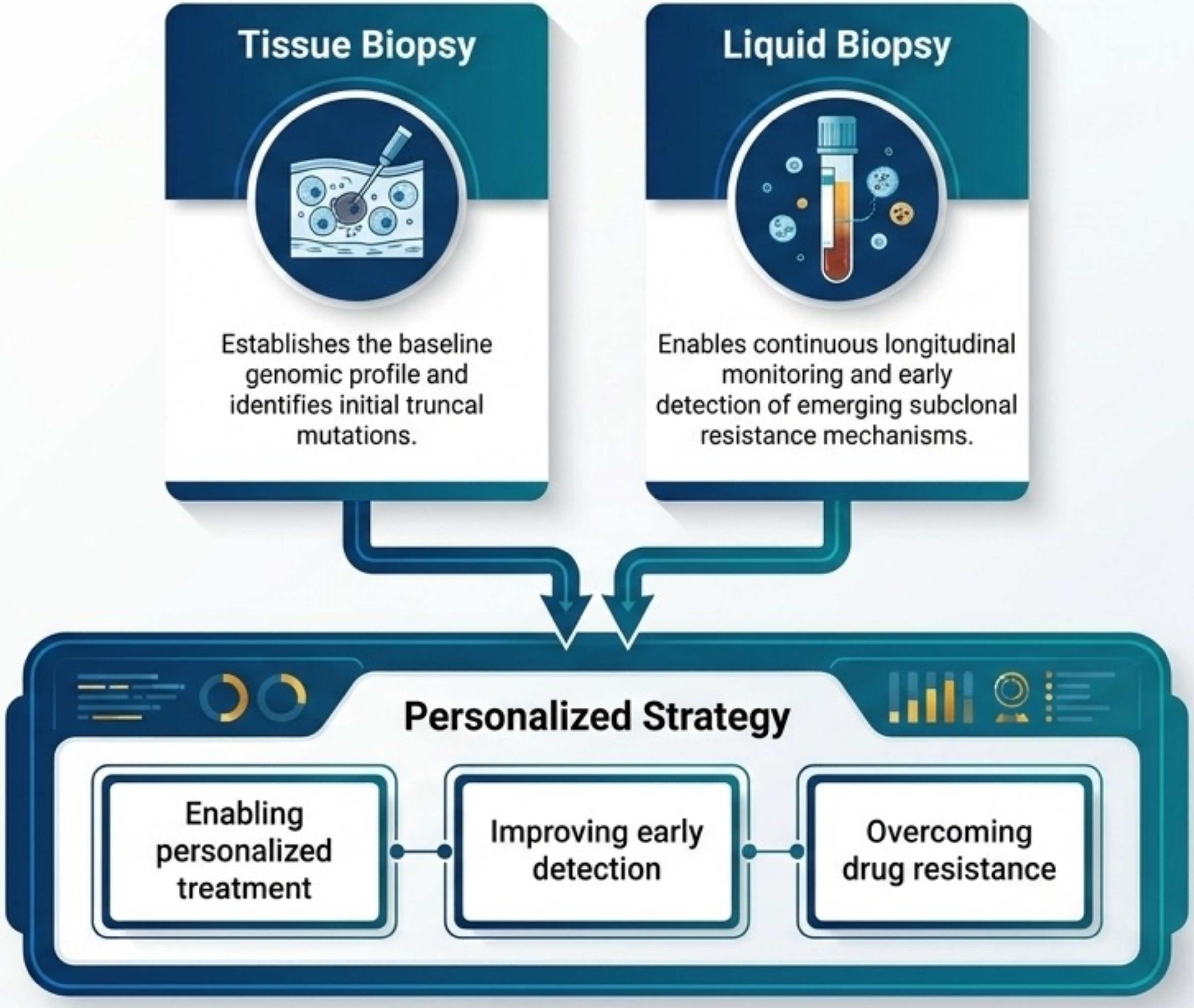
PI3K Pathway Inhibition Relevance: The high VAF and persistence of PIK3CA E545K emphasize the continued need for PI3K-alpha inhibitors.

Need for Combination Therapy: Dual resistance drivers suggest that single-agent therapy is insufficient, necessitating combination regimens to address multiple pathways.

Mapping genomic findings to a personalized multi-targeted strategy

Emergent Alteration	Clinical Evidence	Targeted Therapy	Rationale
ERBB2 p.L869R	SUMMIT Trial (PMID: 37597578)	Neratinib + Trastuzumab + Fulvestrant (NCCN Cat 2A)	Targets the mutated HER2 kinase domain directly.
PIK3CA mutations	SOLAR-1 Trial (PMID: 31094691)	Alpelisib or Inavolisib	Suppresses the parallel PI3K resistance pathway.
Extensive LOH (17 genes)	Functional HRR Deficiency	PARP Inhibitors	Exploits structural DNA repair vulnerabilities.
Borderline TMB (9.44)	TAPUR Study	Pembrolizumab	Potential secondary immunotherapy pathway.

Comprehensive genomic profiling fundamentally shifts how we detect and counter drug resistance



Tissue Biopsy

Establishes the baseline genomic profile and identifies initial truncal mutations.

Liquid Biopsy

Enables continuous longitudinal monitoring and early detection of emerging subclonal resistance mechanisms.

Personalized Strategy

- Enabling personalized treatment
- Improving early detection
- Overcoming drug resistance

Longitudinal tracking provides three pillars for actionable therapeutic decision-making

Therapy Switching

Identifying the precise moment a current therapy fails at the molecular level (e.g., detecting an ***ESR1* mutation** to trigger a switch to oral SERDs).

Combination Strategies

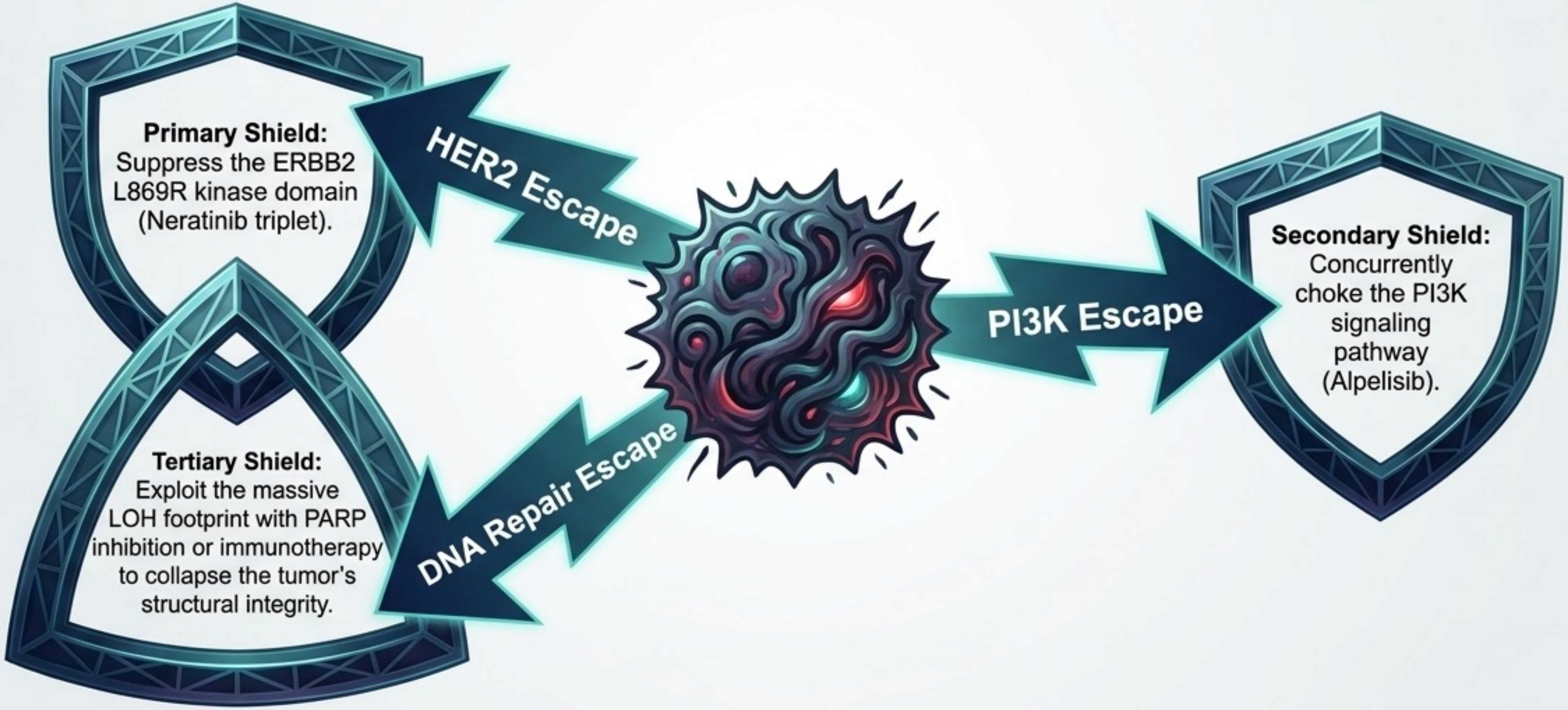
Designing multi-pronged clinical regimens to suppress parallel biological pathways simultaneously and cut off escape routes.

Clinical Trial Matching

Utilizing **newly emergent, actionable subclonal alterations** to continuously qualify patients for advanced targeted trials.

Synthesis: A Multi-Pronged Blockade is Required to Prevent Parallel Escape

The Biological Reality: Treating only the HER2 mutation will force the tumor to rely entirely on its **PI3K or HRR** deficiencies to survive. Single-agent therapy is guaranteed to fail.



Primary Shield:
Suppress the ERBB2 L869R kinase domain (Neratinib triplet).

Secondary Shield:
Concurrently choke the PI3K signaling pathway (Alpelisib).

Tertiary Shield:
Exploit the massive LOH footprint with PARP inhibition or immunotherapy to collapse the tumor's structural integrity.

Repeated genomic assessment is essential to outpace complex, adaptive resistance

The detection of a **complete clonal sweep**—replacing an initial *ERBB2* amplification with a **dominant L869R** mutation—**fundamentally altered the therapeutic strategy** for this patient.

Supporting Conclusion:

A static, single-point-in-time biopsy is insufficient for metastatic breast cancer. Only **continuous** profiling enables the multi-pronged targeting of both the HER2 kinase domain and the PI3K pathway necessary to manage dynamic resistance.

Teşekkür Ederim