Biology and Genetics of Breast Cancer

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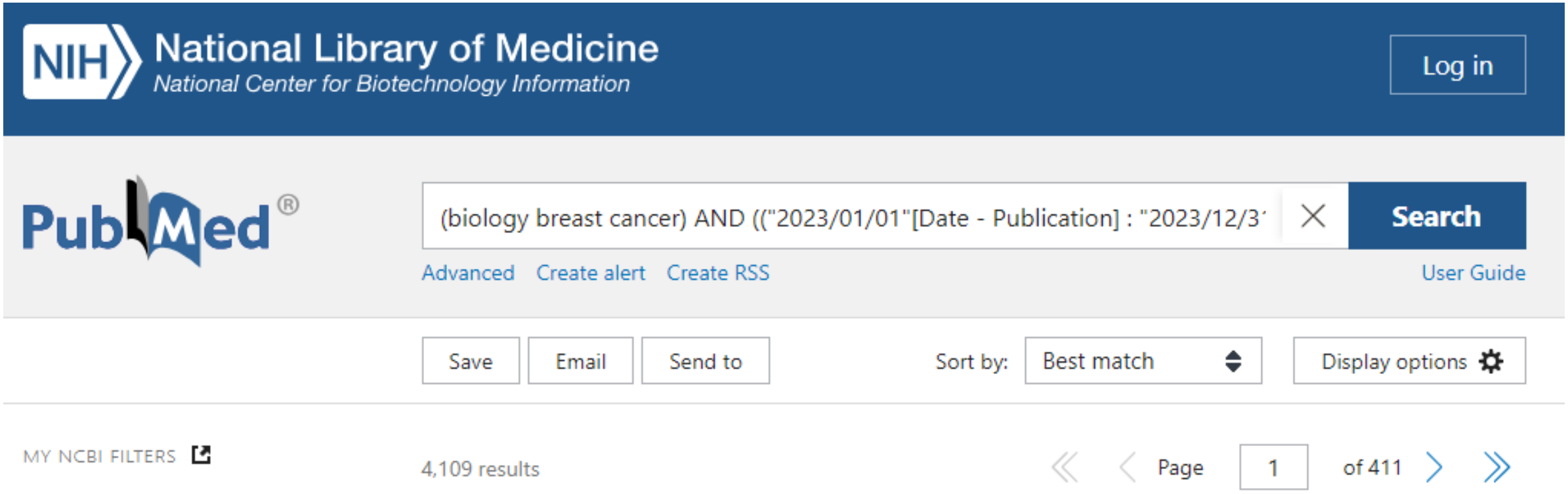
Medical Oncology and Clinical Genetics

Conflicts of interest

- My institution has received payments from the following pharmaceutical companies on my behalf :
  - Amgen
  - AstraZeneca
  - Daiichi Sankyo
  - Eli Lilly
  - Gilead
  - MSD
  - Mundi Pharma
  - Novartis
  - Pfizer
  - Pierre Fabre
  - Roche
  - Seagen
  - Teva

Size of the task

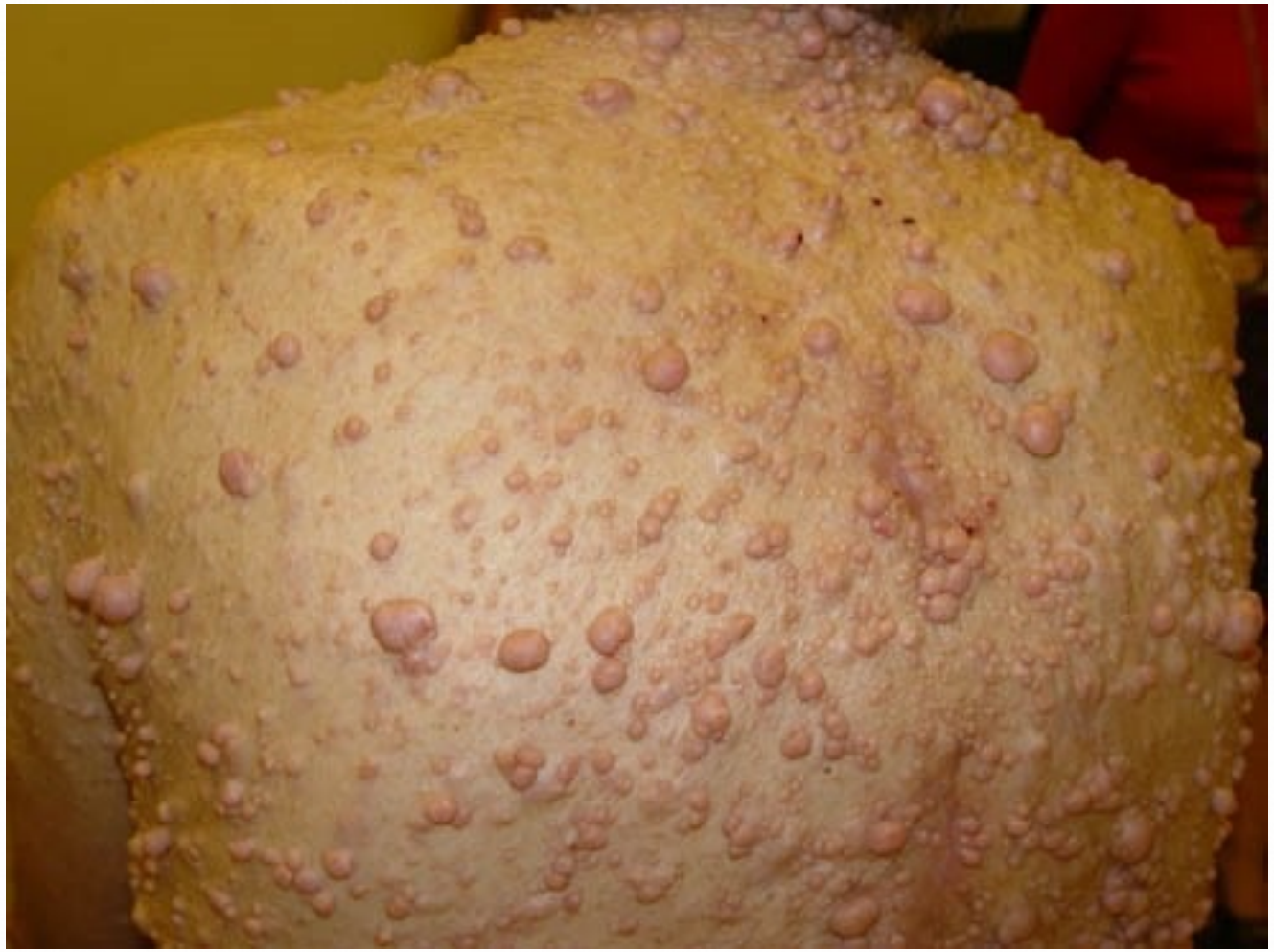


https://pubmed.ncbi.nlm.nih.gov/, accessed on 21 January 2024

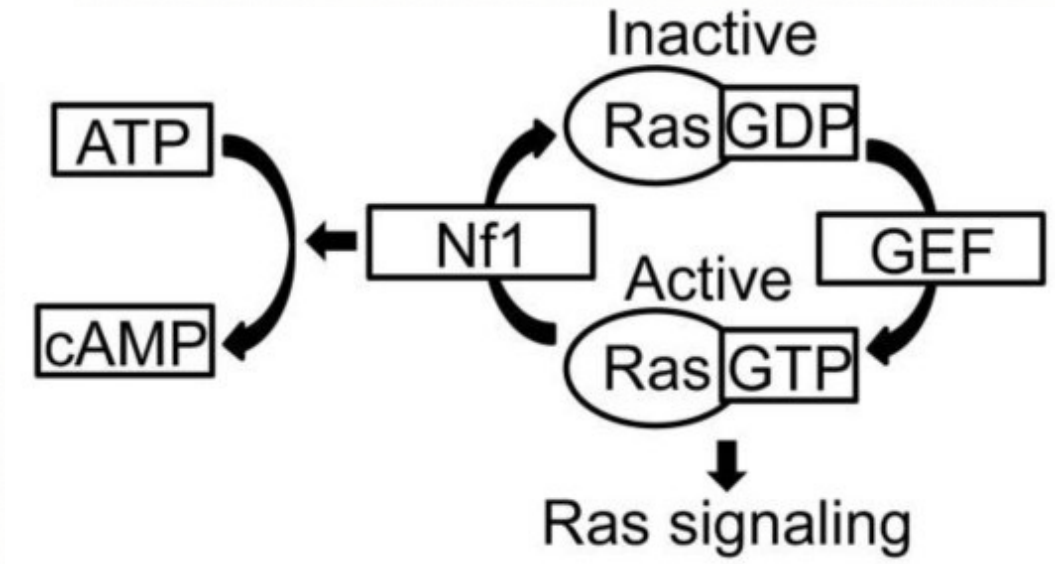
Topic n°1

Loss of *NF1* is associated with poor prognosis on endocrine therapy but might be synthetic lethal with CDK4/6 inhibition

The neurofibromin (NF1) tumor suppressor protein



NF1: a Ras GTPase activating protein



↓NF1 → ↑Ras activity → ↑MAPK activity

↑Ras activity → ↑PI3K activity

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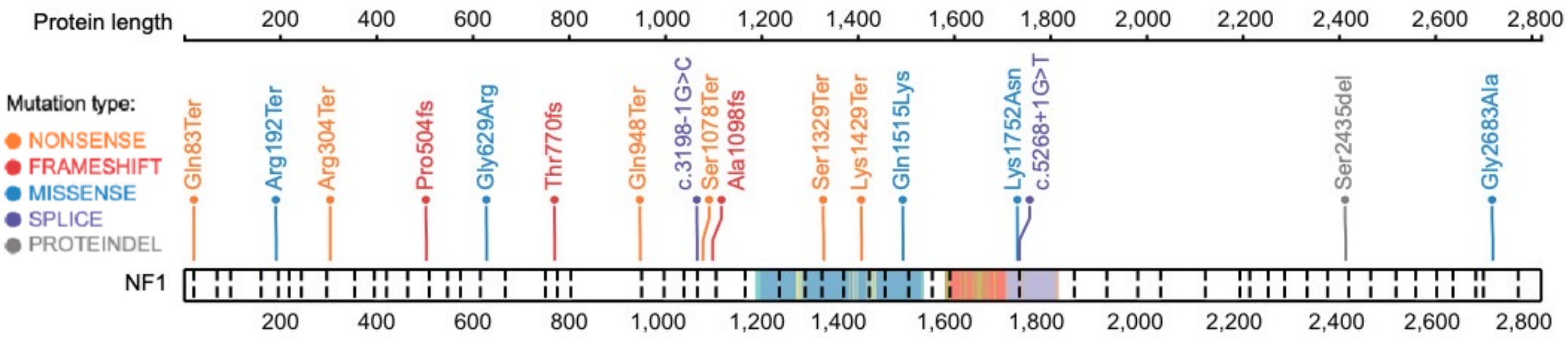
Goel et al, SABCS 2023, GS01-09

NF1 mutations in luminal breast cancer



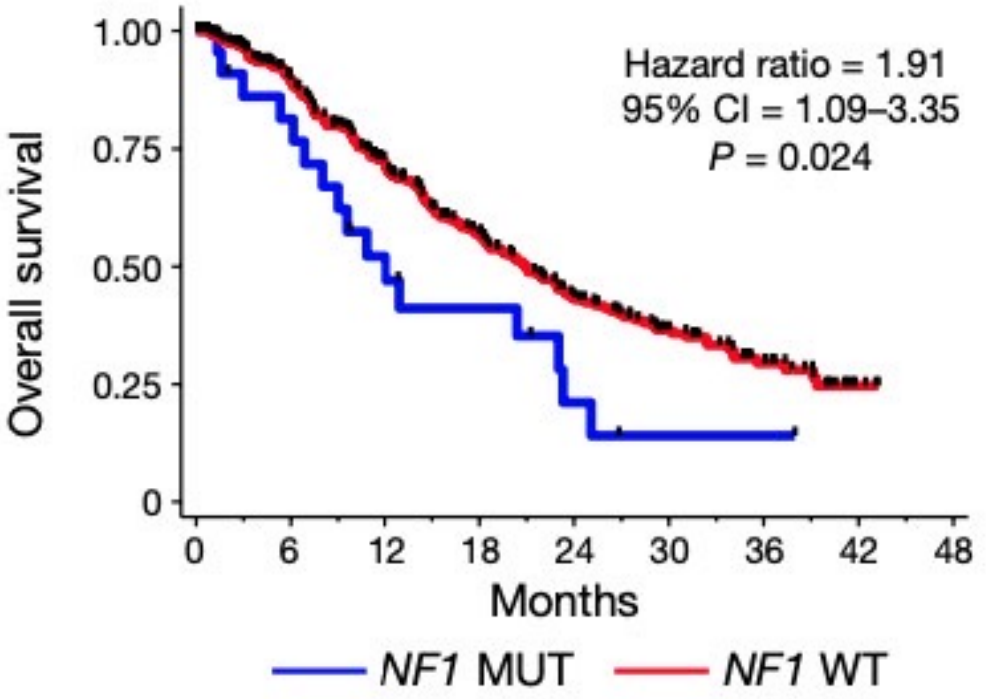
Often nonsense or frameshift mutations
Result in low/absent *NF1* mRNA and protein

Pearson *Clin Cancer Res* 2020

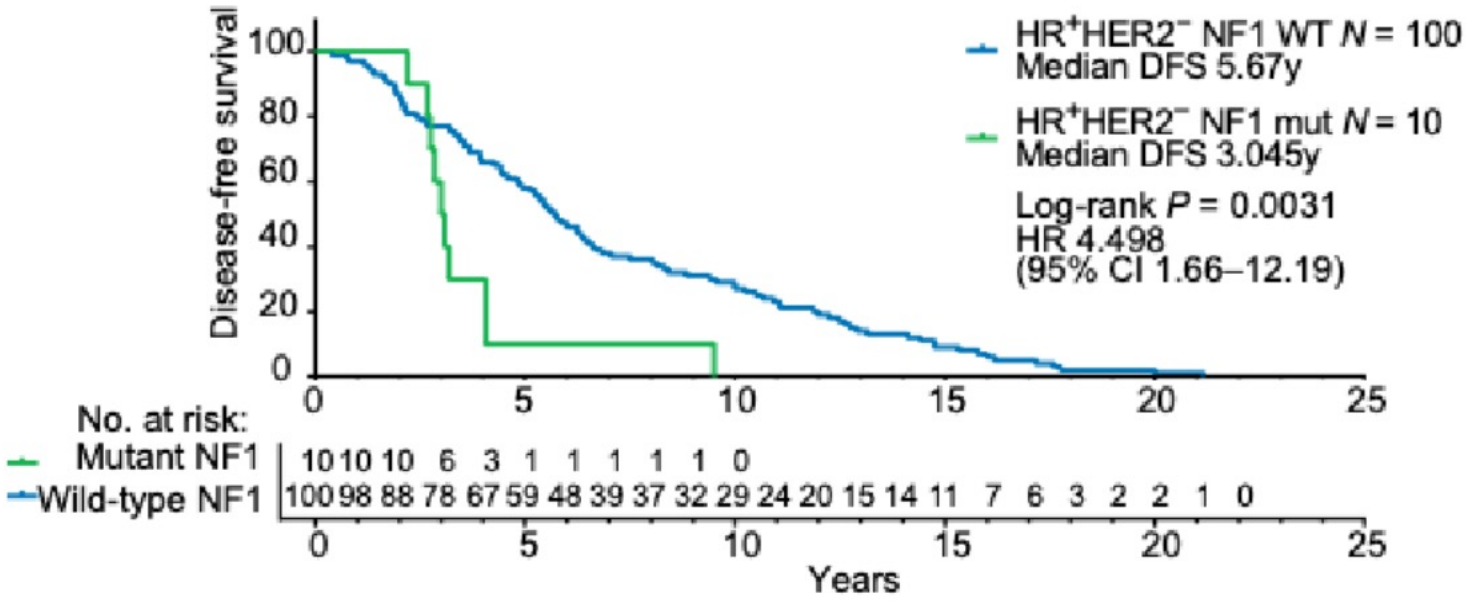
Goel et al, SABCS 2023, GS01-09

Association between *NF1* mutation and poor outcome on ET

Andre
Nature 2019

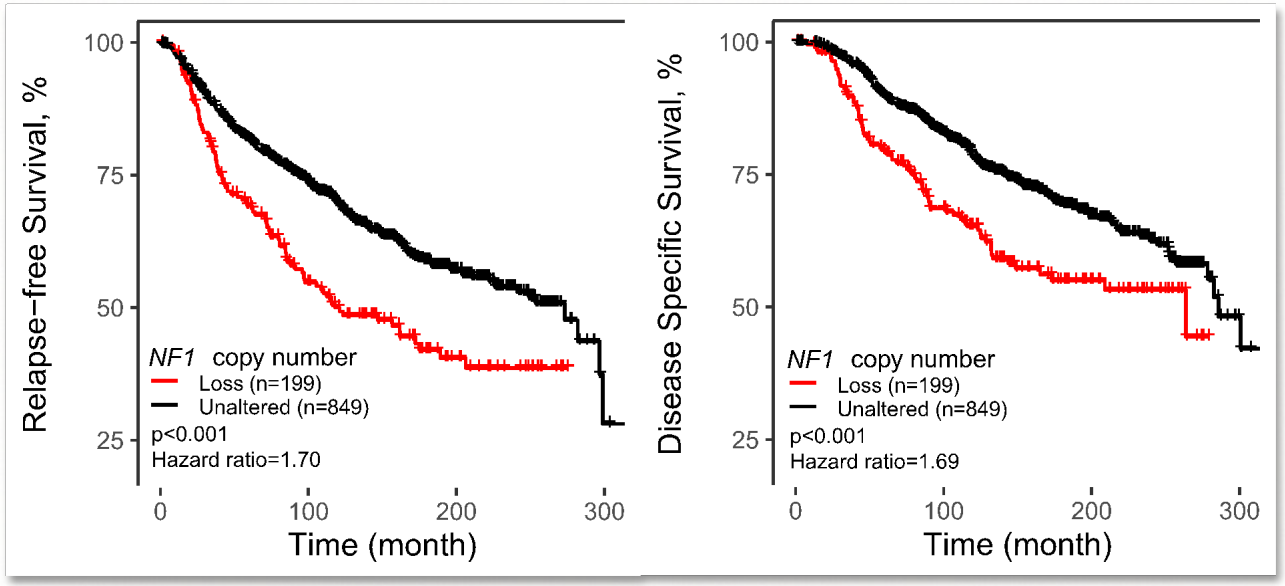


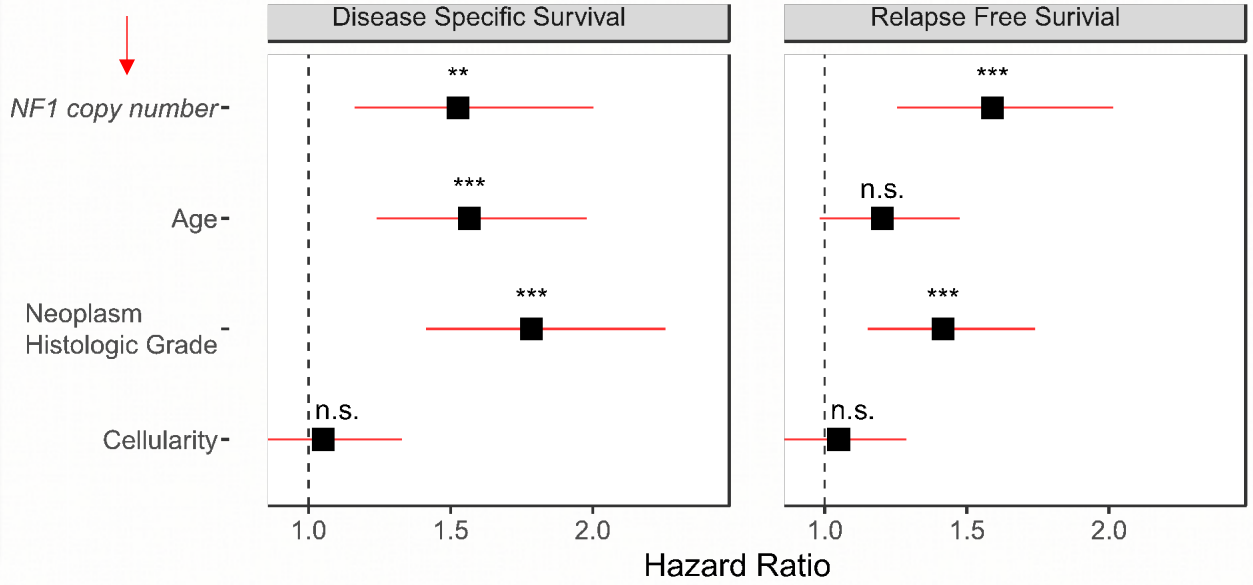
Pearson
Clin Cancer Res 2020



Goel et al, SABCS 2023, GS01-09

NF1 shallow deletion is present in 19% of ER+ primary breast cancer and independently associated with poor survival in ER+ primary breast cancer



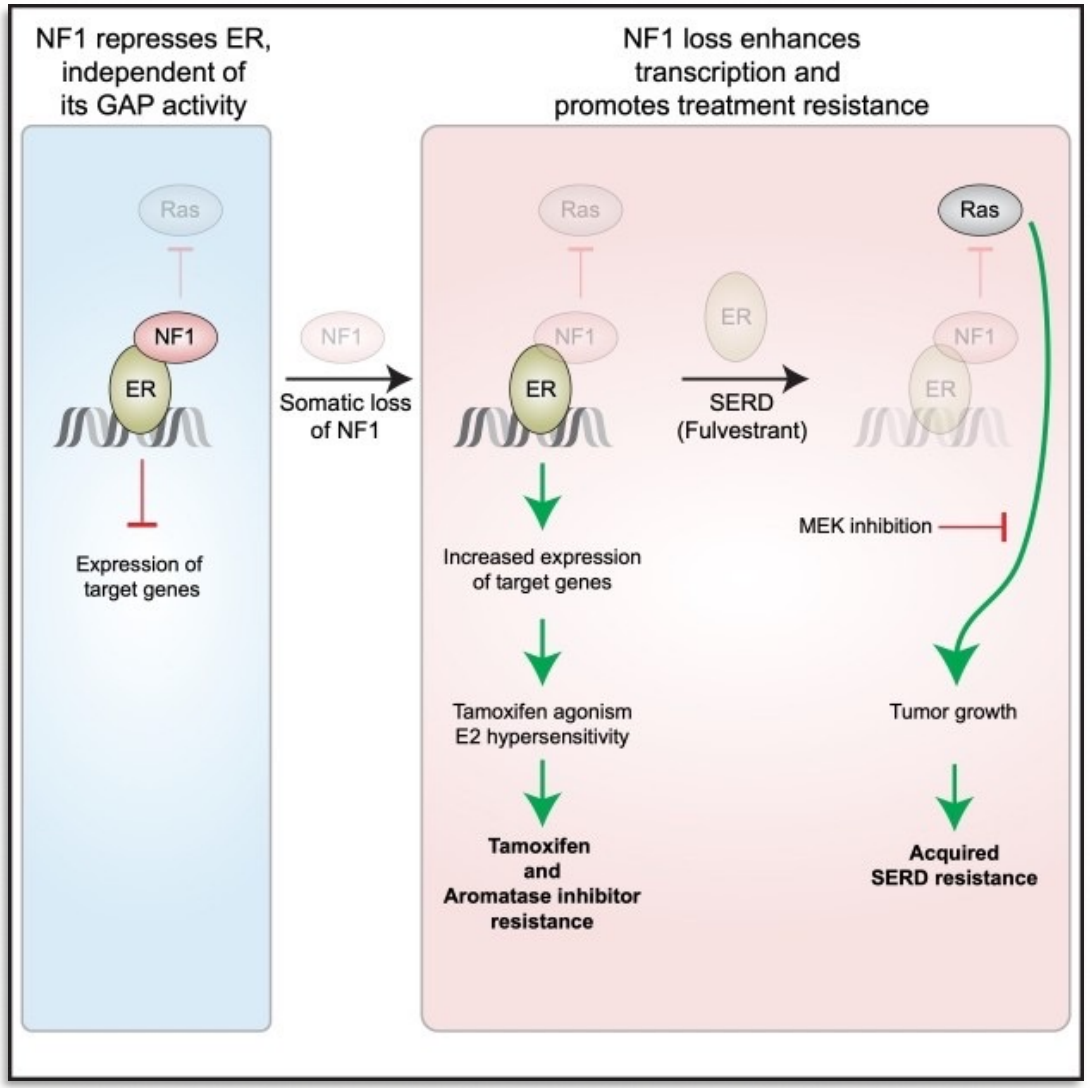


199/1,048 = 19% ($NF1^{low}$)

METABRIC analysis: Ze-Yi Zheng and Anran Chen et al, *in preparation*

Chang et al, SABCS 2023, GS01-08

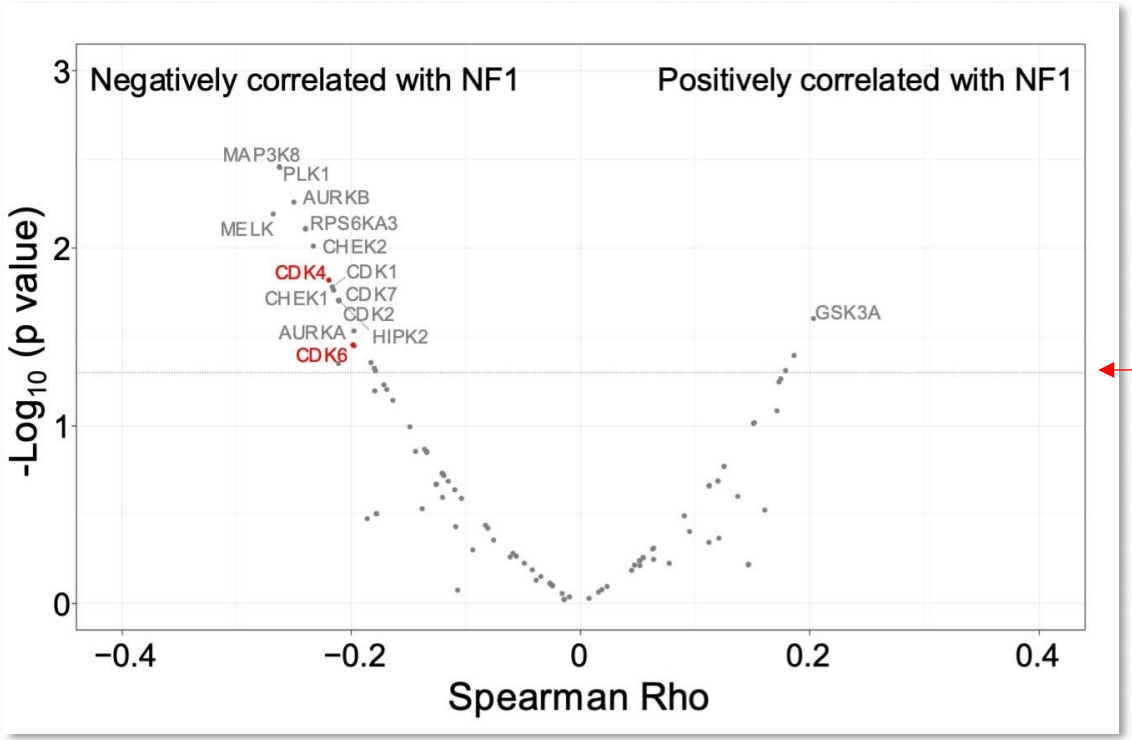
NF1 is also an ERα transcriptional co-repressor, *independent* of GAP activities (RAS repression)



(Zheng et al, 2020, Cancer Cell)

Elevated CDK4/6-RB pathway activity in NF1^{low} ER^{+} breast tumors

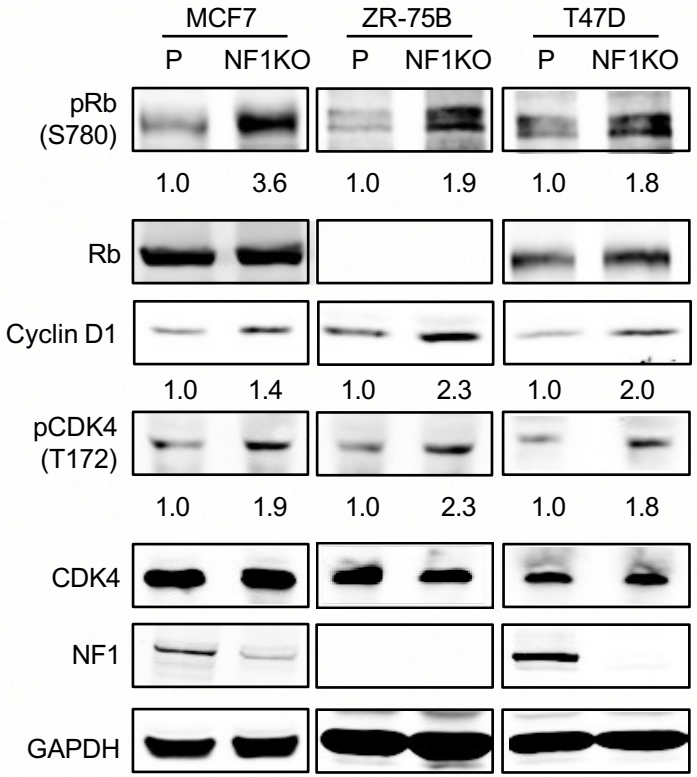
Protein kinase activity in breast tumors (CPTAC)



P=0.05

NF1↓ → pRb/Rb$_t$↑

CDK4 activation and Rb inactivation *in vitro* with NF1 loss

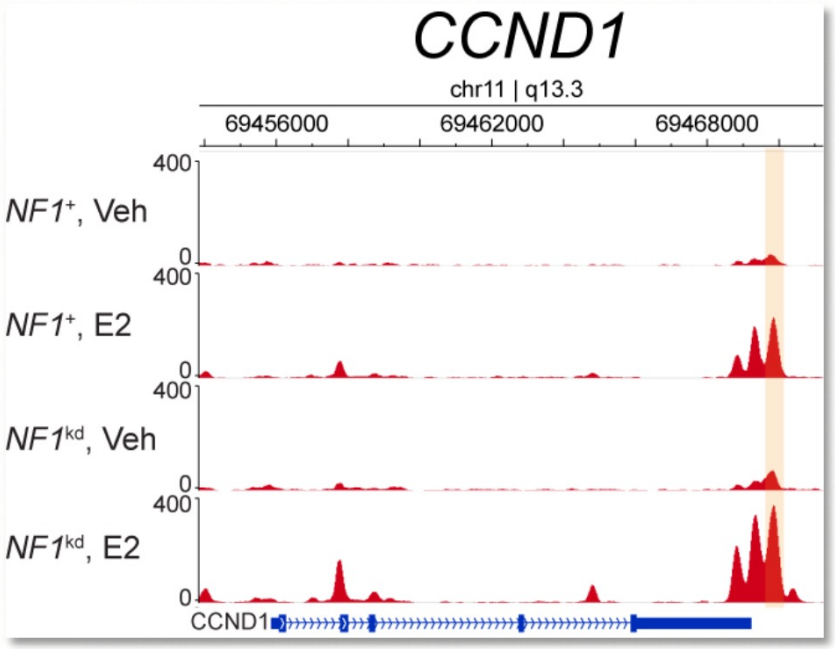


(Chen and Zheng et al, in preparation)

Chang et al, SABCS 2023, GS01-08

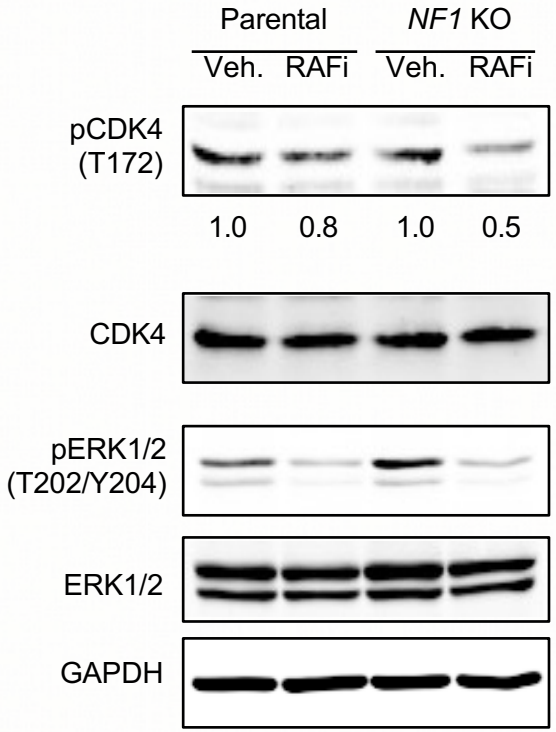
CDK4 is jointly regulated by ER and RAS signaling, and its activity is enhanced upon NF1 loss

ER recruitment to *CCND1* (ChIP-seq)



(MCF7)

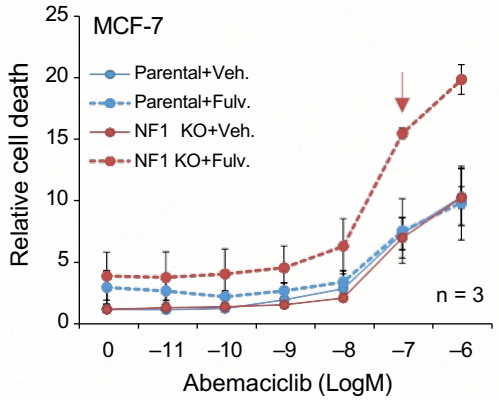
RAF-dependent T172 phosphorylation



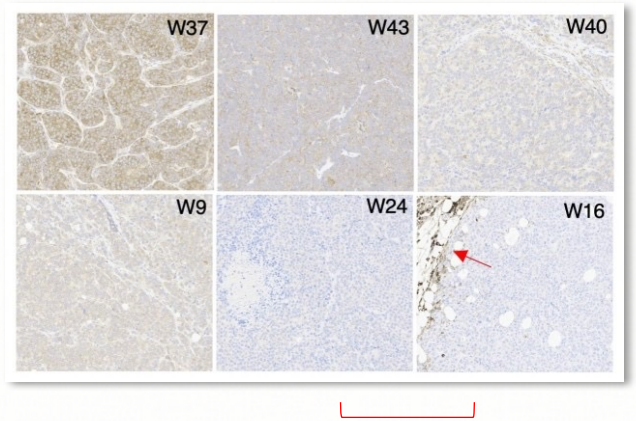
(MCF7)

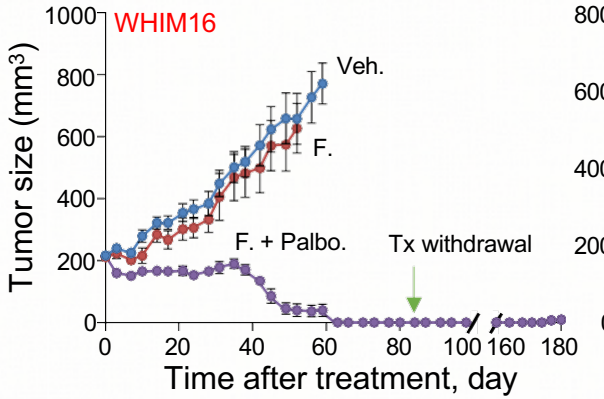
Chang et al, SABCS 2023, GS01-08

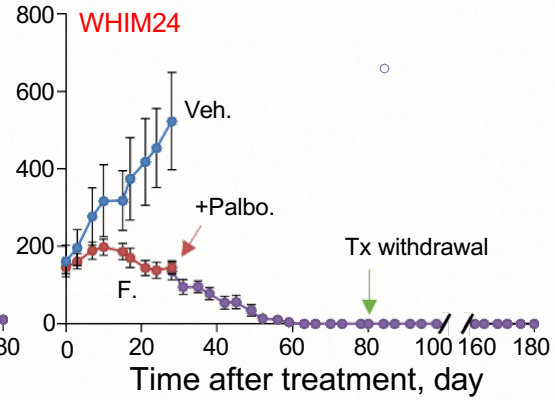
NF1^{low} ER^{+} tumors demonstrate deep responses to fulvestrant + palbociclib

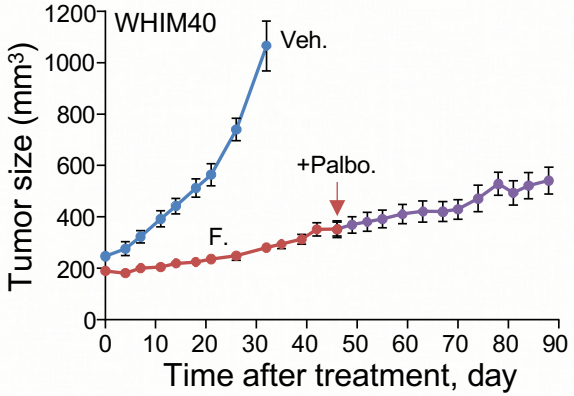


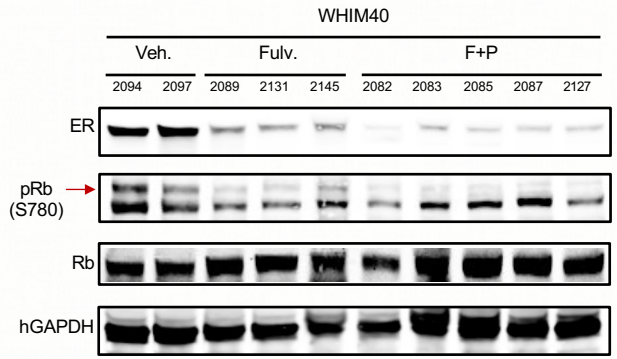
[Pearson et al 2020, Clin. Cancer Res.]







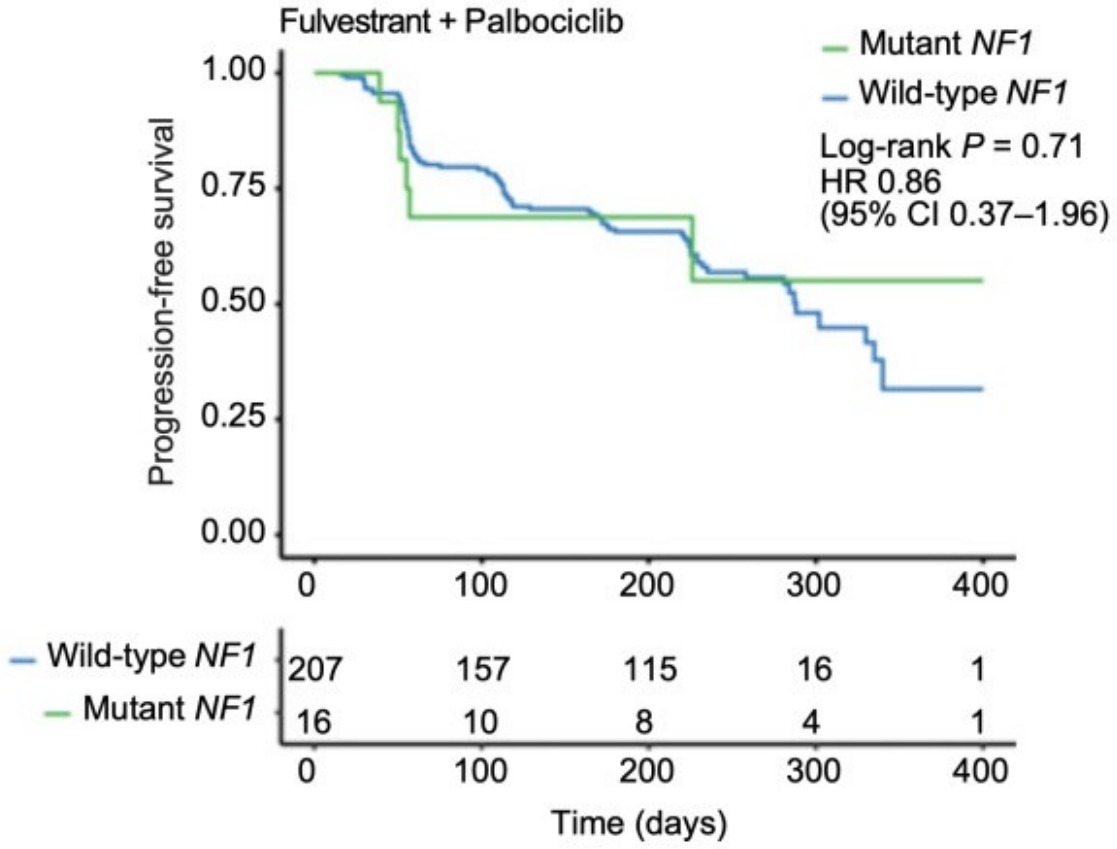




Chang et al, SABCS 2023, GS01-08

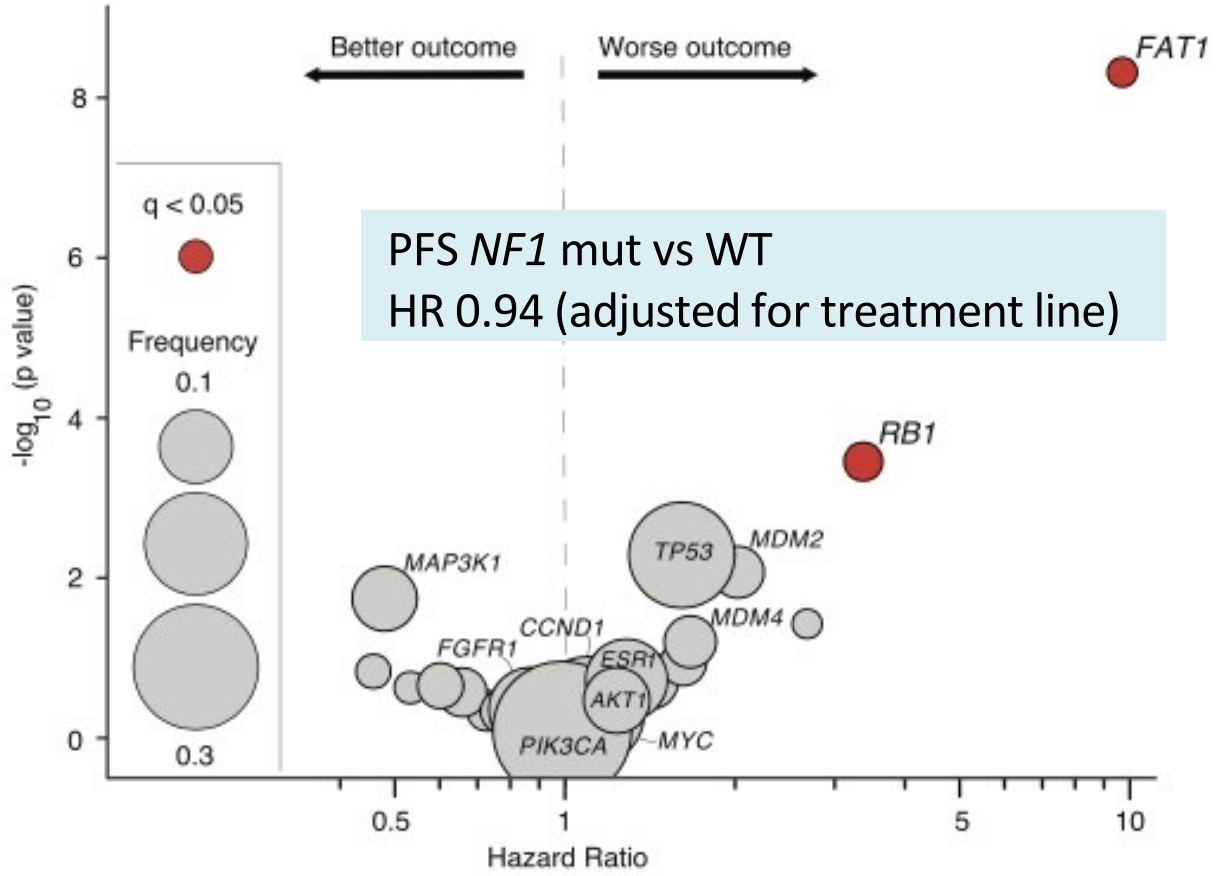
What does this mean in the era of CDK4/6i?

PALOMA-3 trial (palbo patients only)



Pearson *Clin Cancer Res* 2020

MSKCC cohort (all CDK4/6i-treated)



PFS *NF1* mut vs WT
HR 0.94 (adjusted for treatment line)

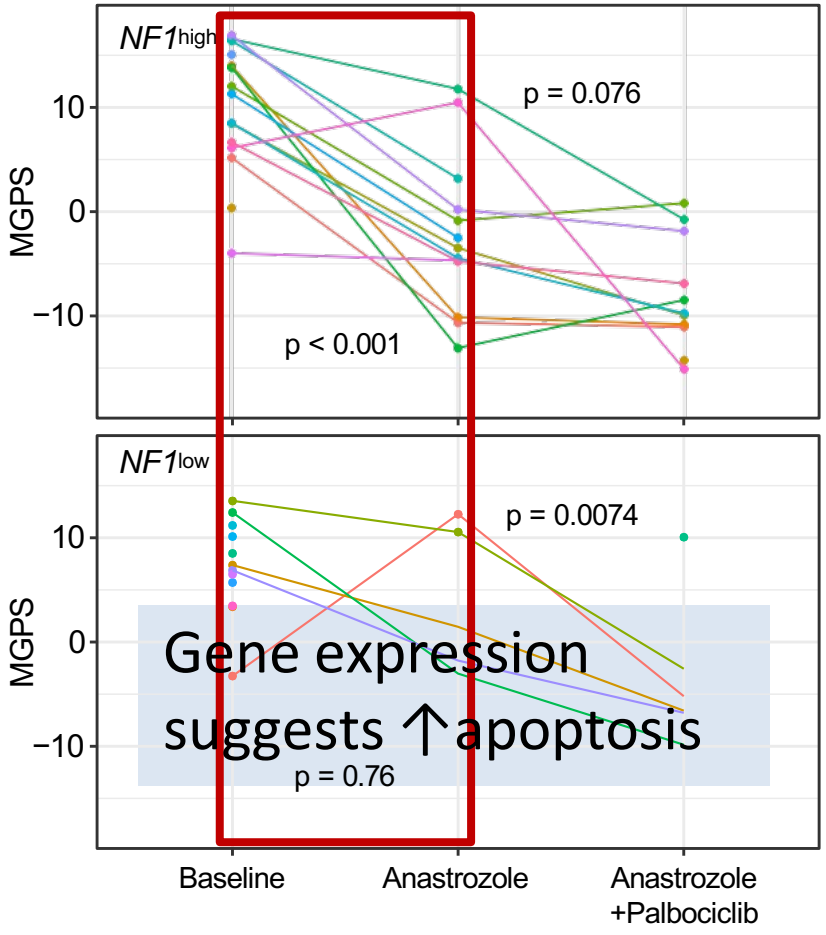
Li *Cancer Cell* 2018

Impact of *NF1* alterations on CDK4/6i response

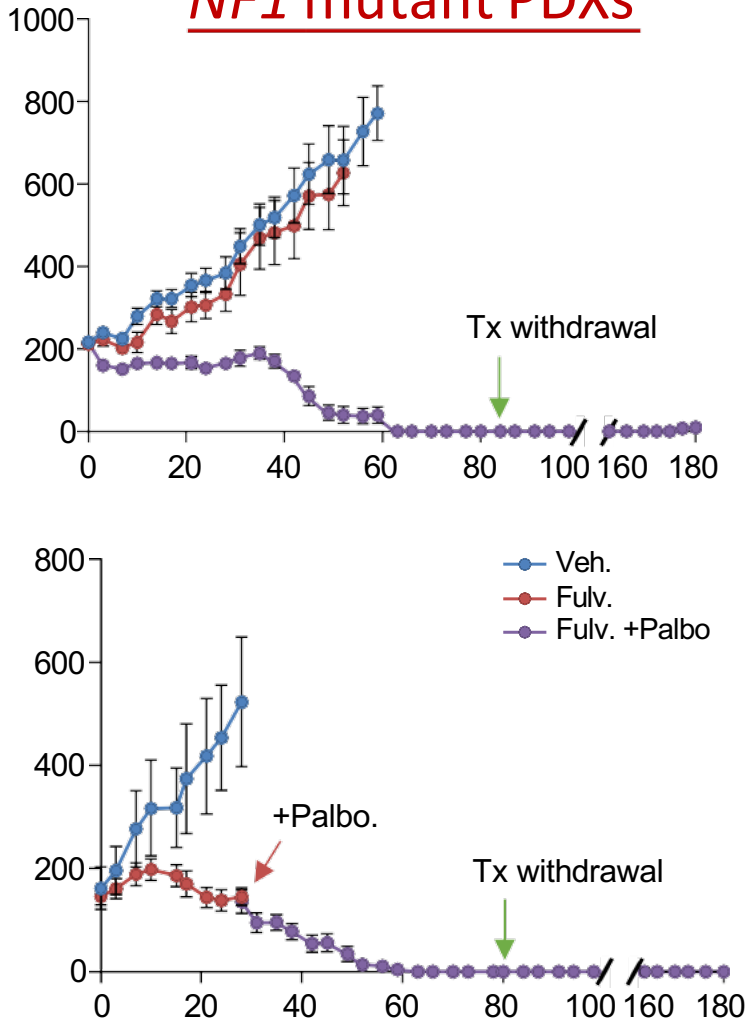
NeoPalAna

NF1 mRNA high

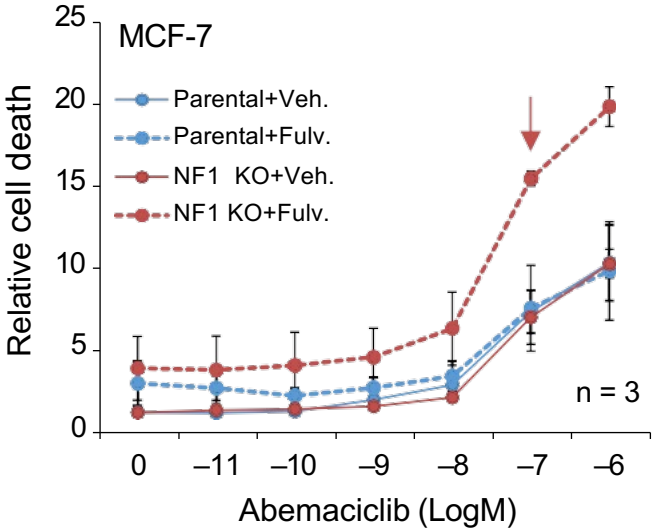
NF1 mRNA low



NF1 mutant PDXs

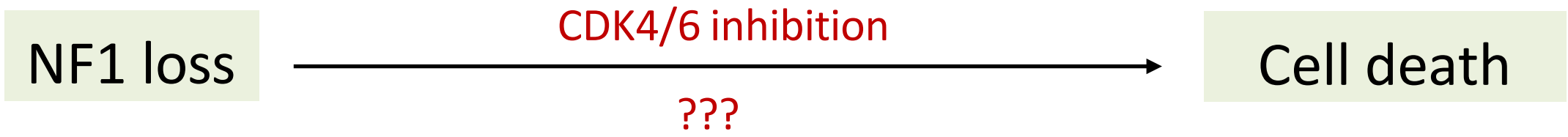


Cell death



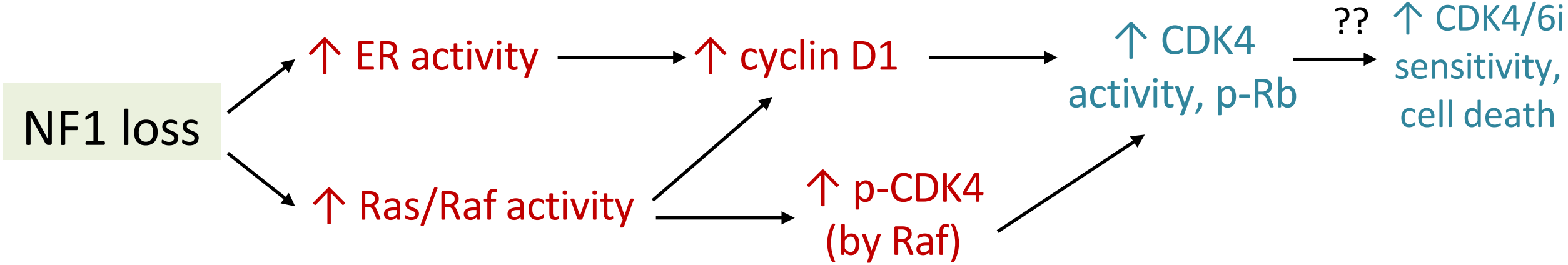
Goel et al, SABCS 2023, GS01-09<

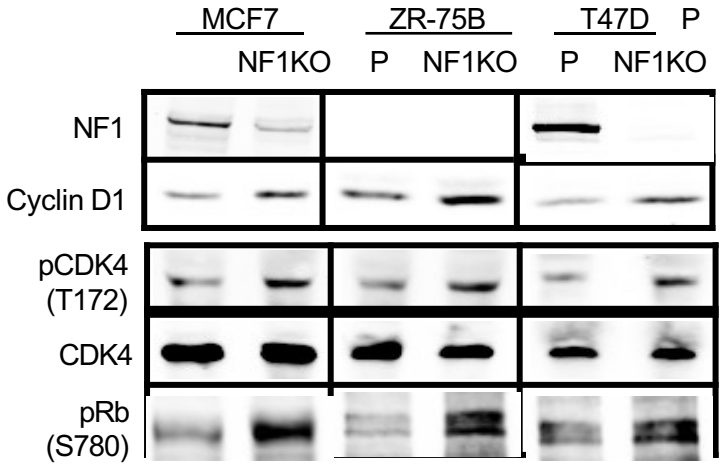
What is the mechanism?



Goel et al, SABCS 2023, GS01-09

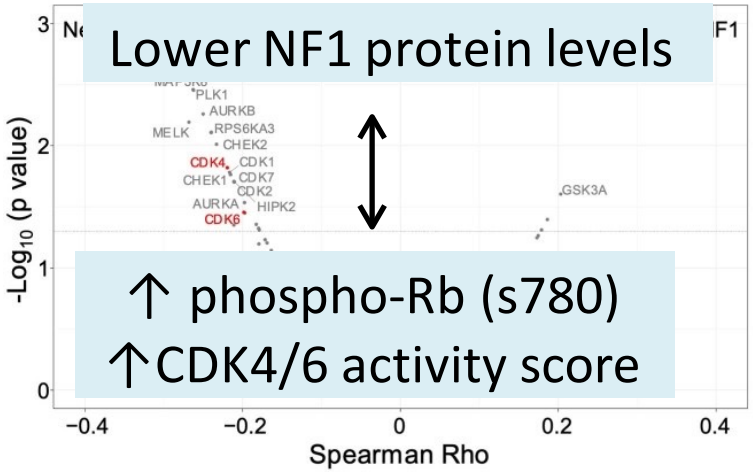
Proposed mechanism of CDK4/6i sensitivity in NF1-low cancers





Consistent with published work (Pearson *Clin Cancer Res* 2020)

CPTAC cohort (proteomics)



Goel et al, SABCS 2023, GS01-09

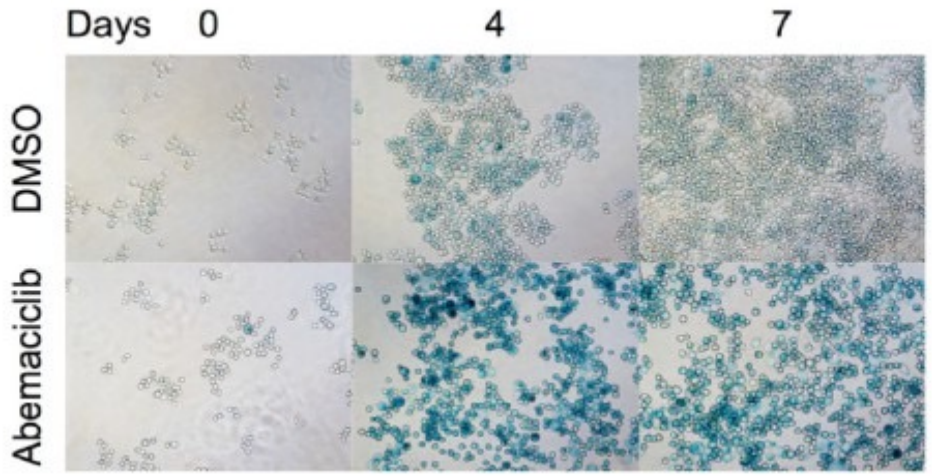
Implications of this study

1. “Functional loss” of NF1 might be more common in ER+ breast cancer than previously thought.
2. Confirmation that NF1 deficiency is associated with poor outcome on ET.
3. Loss of NF1 might be synthetic lethal with CDK4/6 inhibition, overcoming endocrine resistance in these tumors.

Goel et al, SABCS 2023, GS01-09

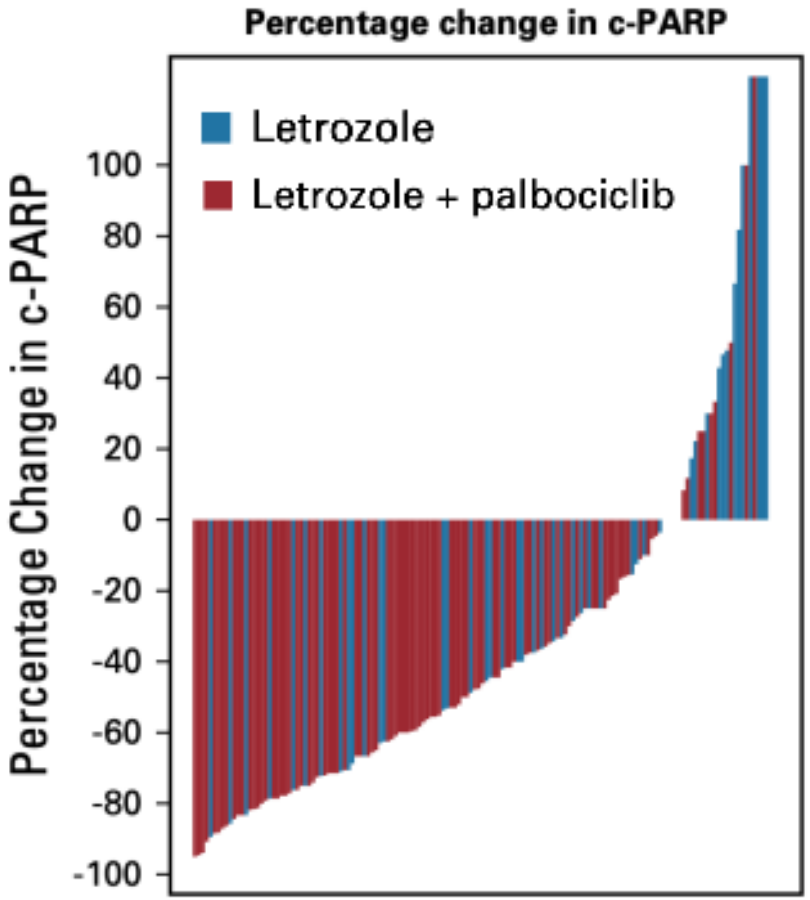
Outstanding biological questions: senescence vs apoptosis

CDK4/6 inhibitors induce “senescence”



AND

Senescence is an anti-apoptotic state



Goel *Nature* 2017
Watt *Nature Cancer* 2021
Johnston *J Clin Oncol* 2019

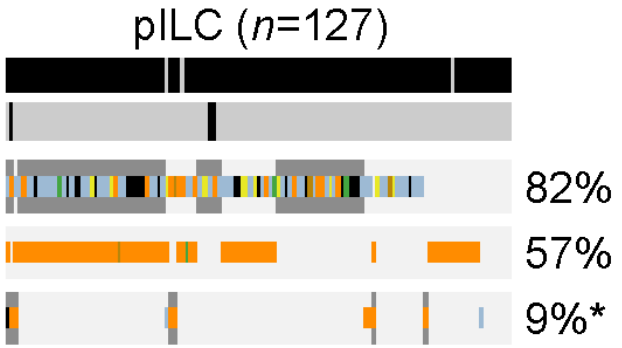
Topic n°2

INSTITUT ROI ALBERT II
Cliniques universitaires SAINT-LUC
UCLouvain BRUXELLES

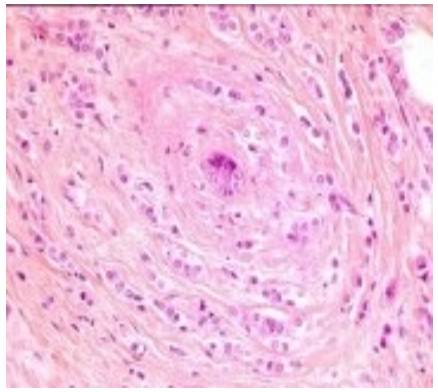
Artificial intelligence can unravel epigenetic and genetic mechanisms

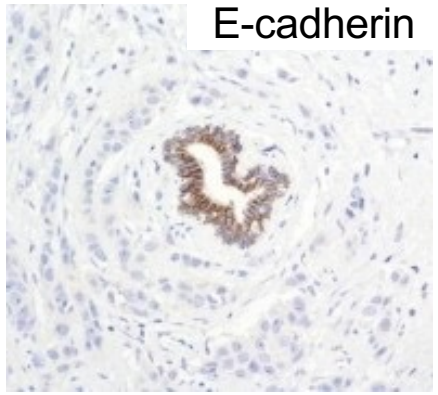
Invasive Lobular Carcinoma

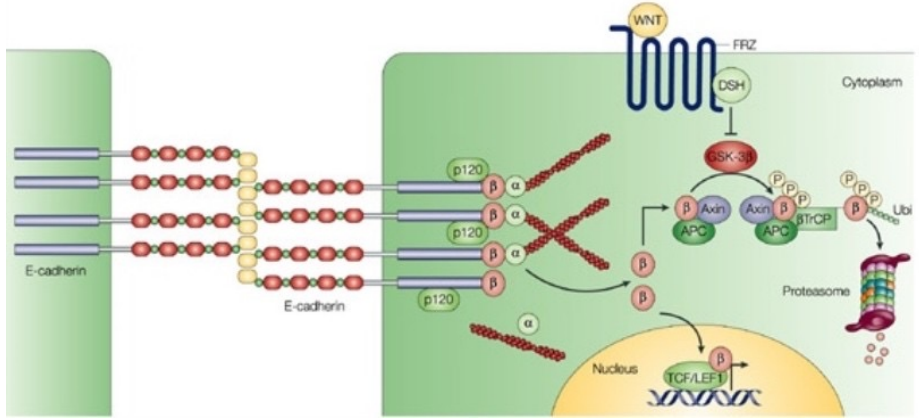
- Most frequent breast cancer special histologic subtype
- Distinctive phenotype
- *CDH1* bi-allelic inactivation



CDH1 – E-cadherin LOF mutations







Mechanisms:

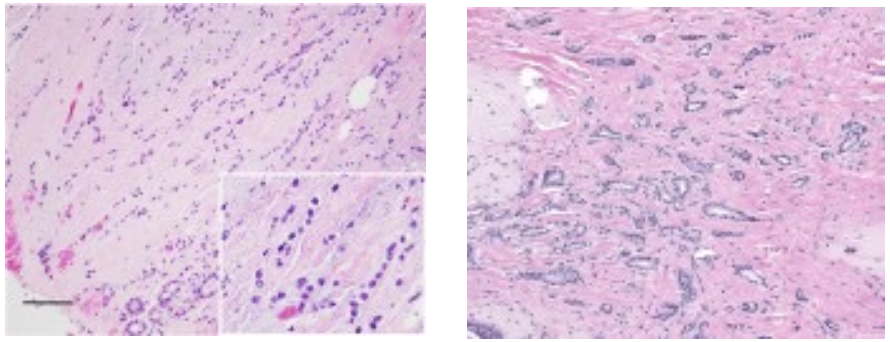
- Bi-allelic mutation
- Homozygous deletion
- Promoter methylation

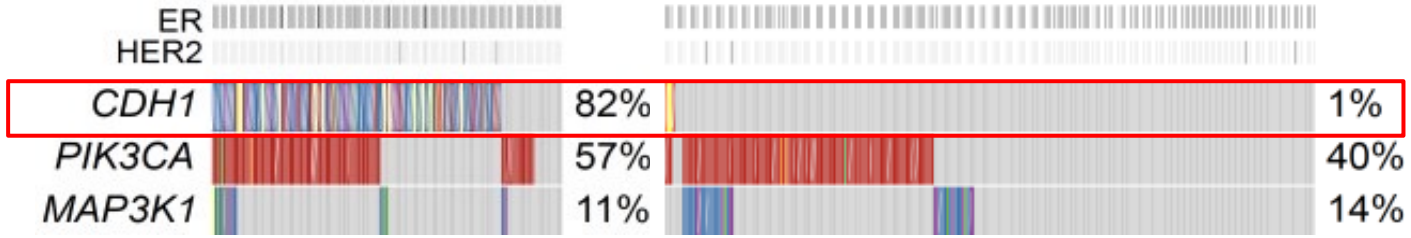
Ciriello et al, Cell 2015; Pareja et al, NPJ Breast Cancer 2020; Lee et al, Clin Cancer Res 2018

Pareja et al, SABCS 2023, GS03-04

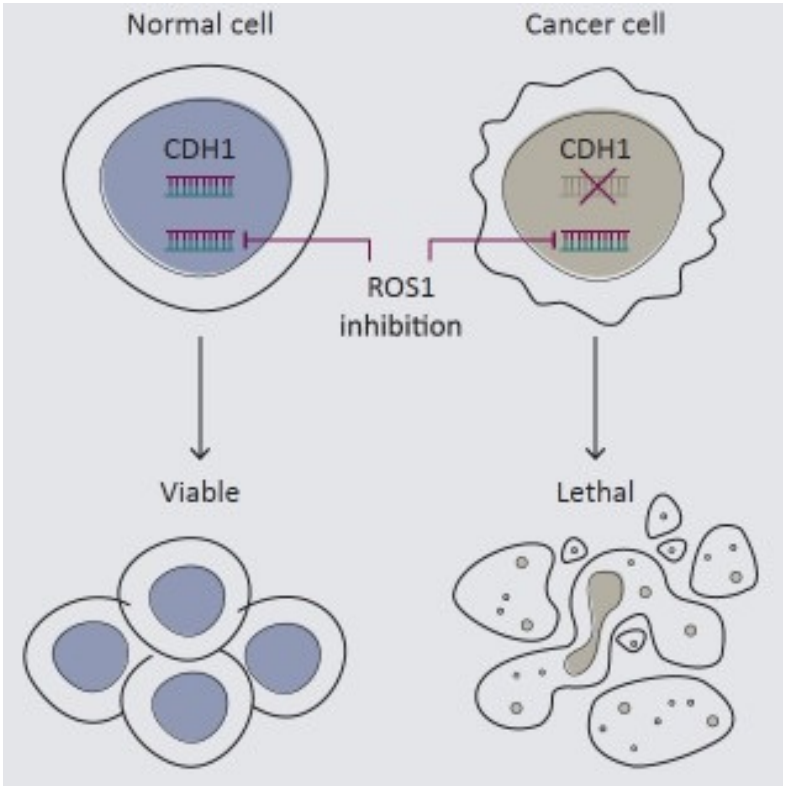
Invasive Lobular Carcinoma: Genotypic-Phenotypic Correlation

CDH1 biallelic mutations are pathognomonic for ILC





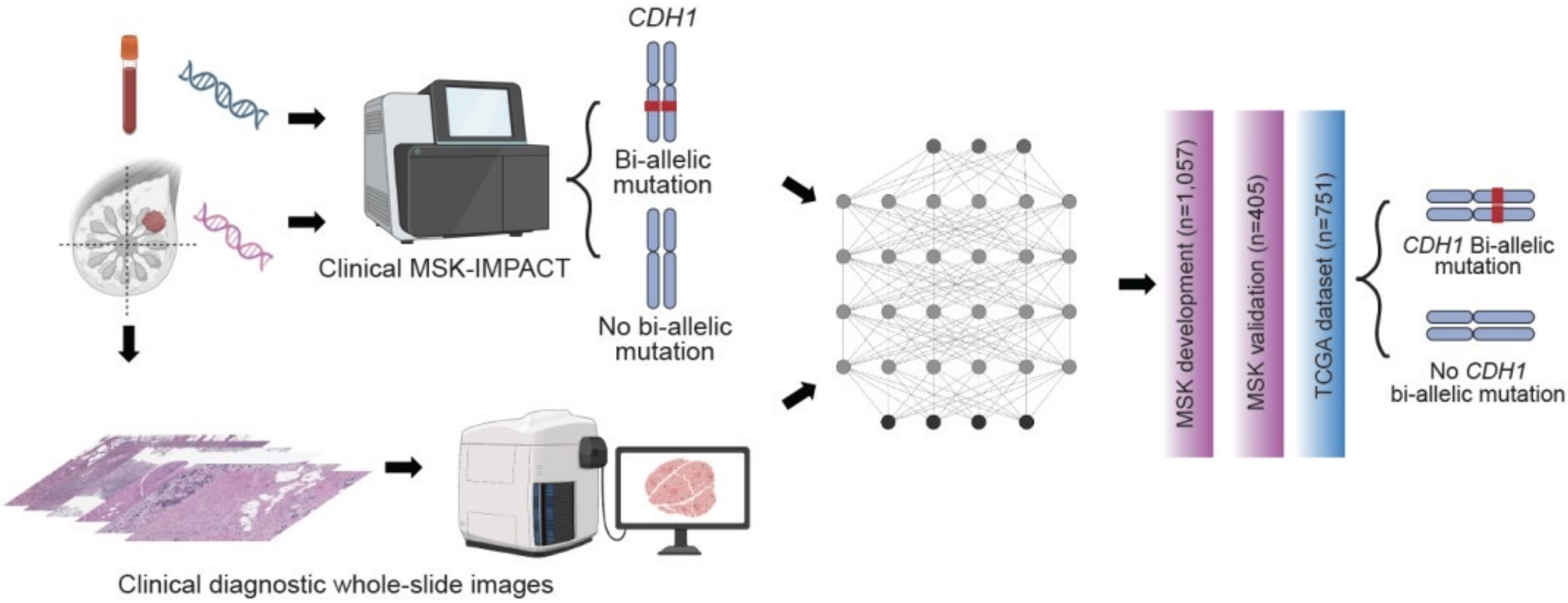
Unique genetic vulnerabilities



Pareja et al, NPJ Breast Cancer 2020; Van Baelen et al, Ann Oncol 2022

Van Baelen et al, Ann Oncol 2022

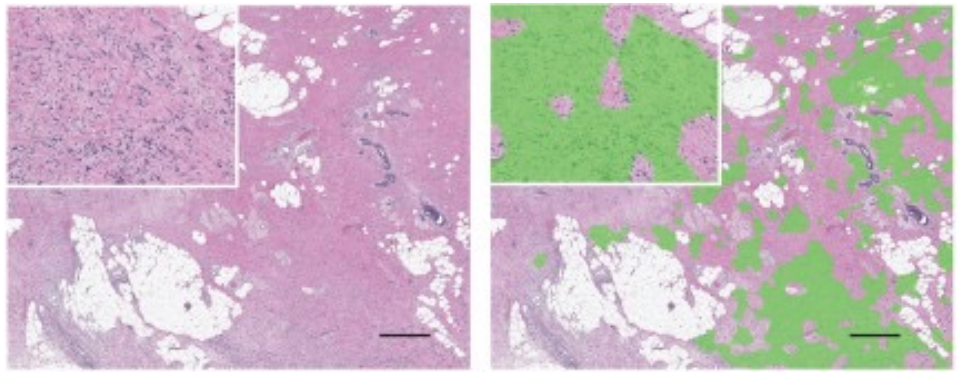
Genomics-Driven AI-System for Prediction of *CDH1* Bi-allelic Mutations

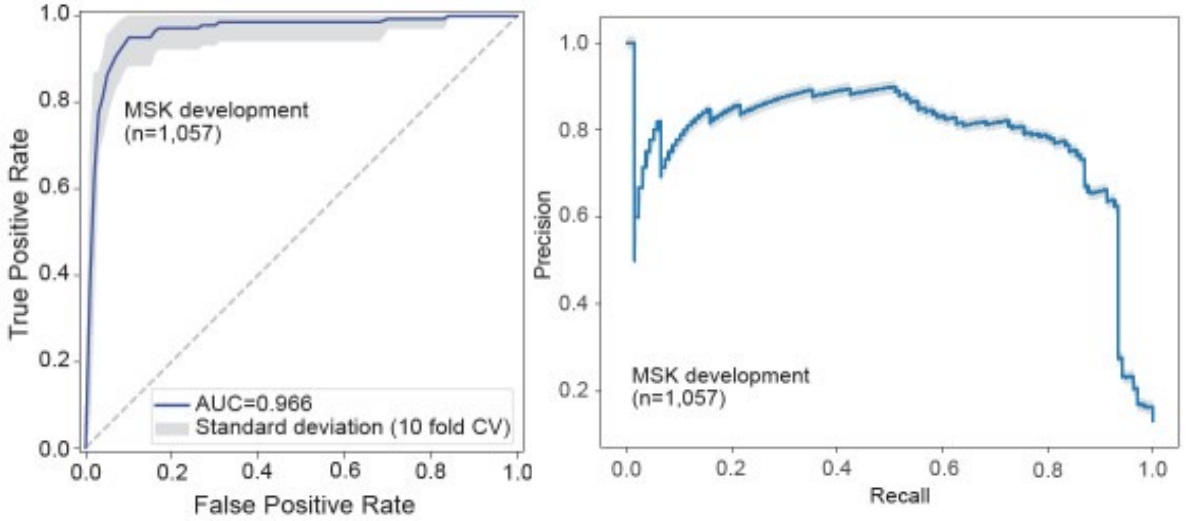


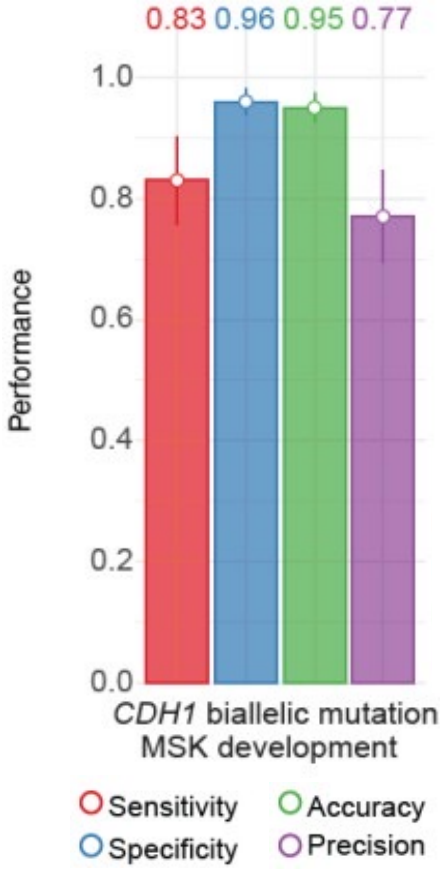
Pareja, Reis-Filho et al, Unpublished

Pareja et al, SABCS 2023, GS03-04

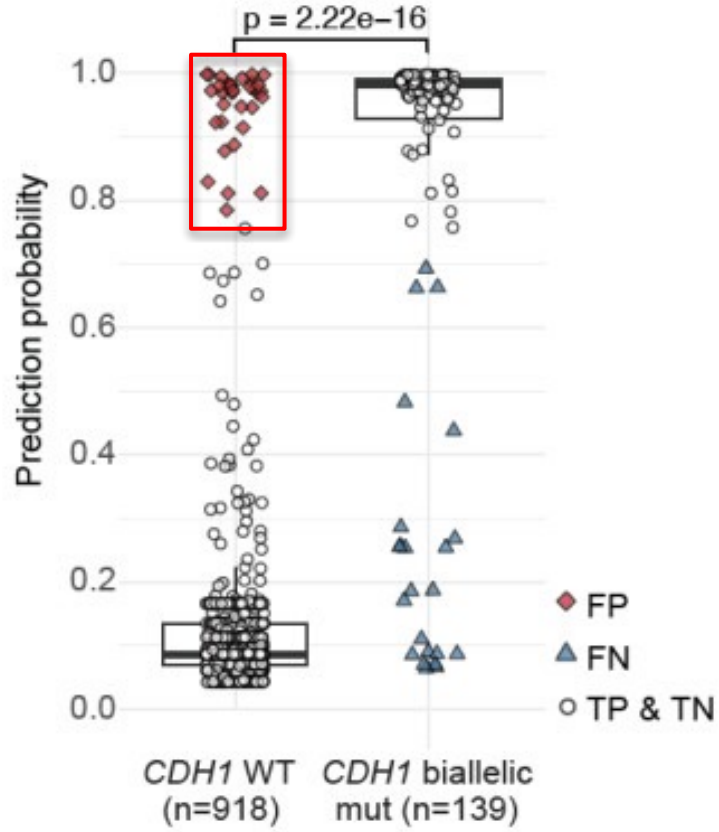
Prediction of *CDH1* Bi-allelic Mutations by AI-Model





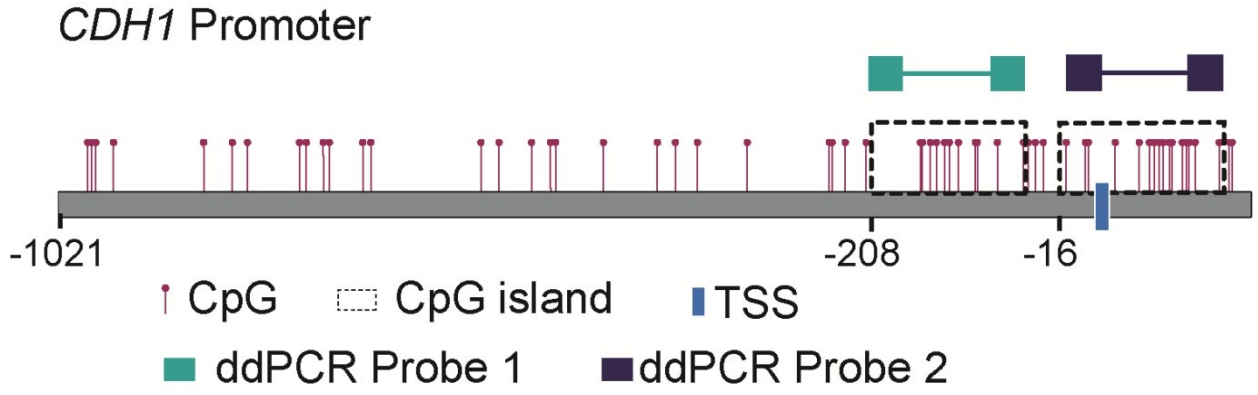


False Positives (n=34)



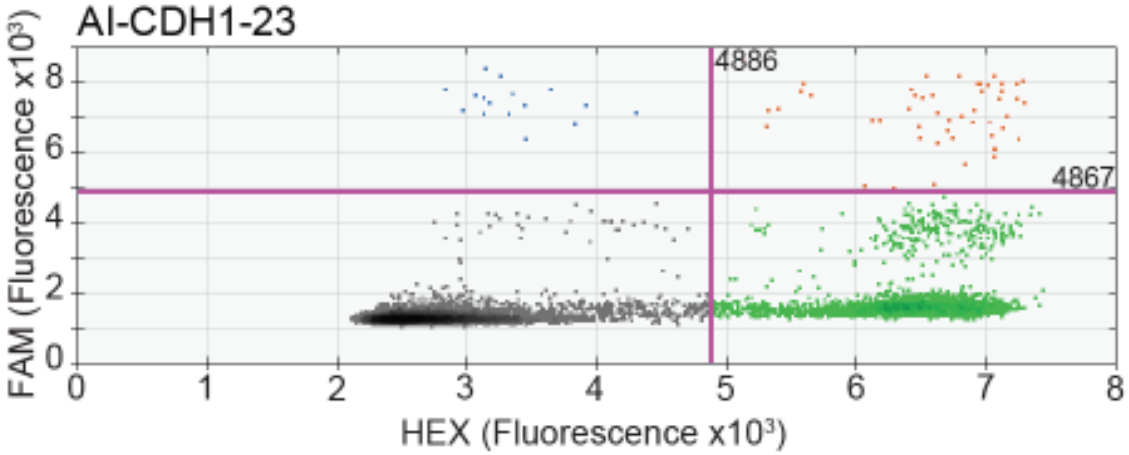
What is the molecular underpinning of this subset cases?

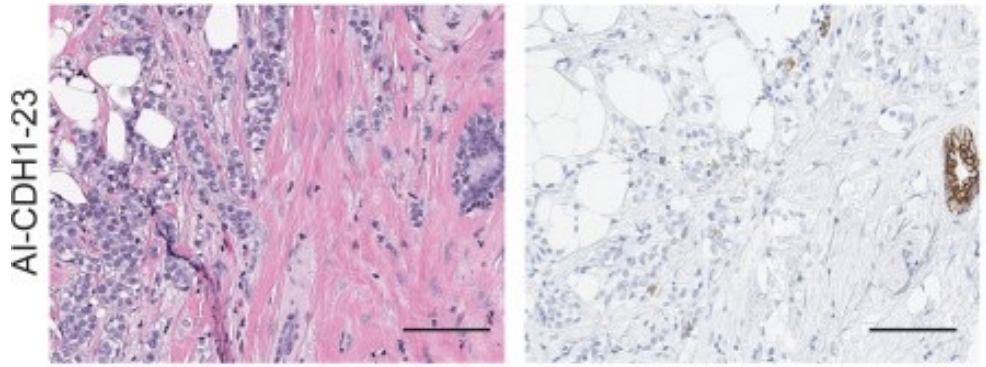
Alternative Mechanisms of *CDH1* Inactivation: Epigenetic Silencing



***CDH1* promoter methylation:**

18/28 (64%) of cases interrogated

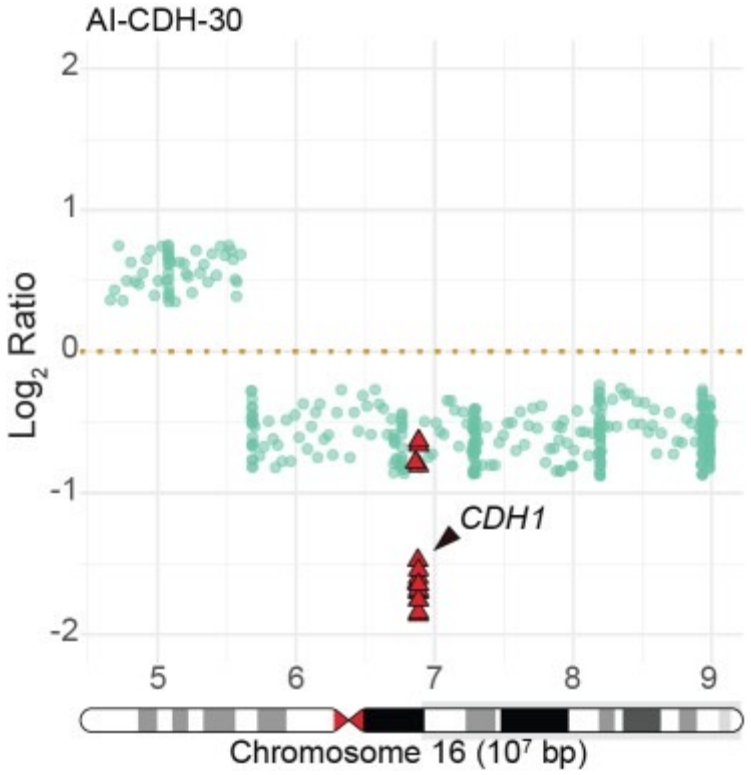




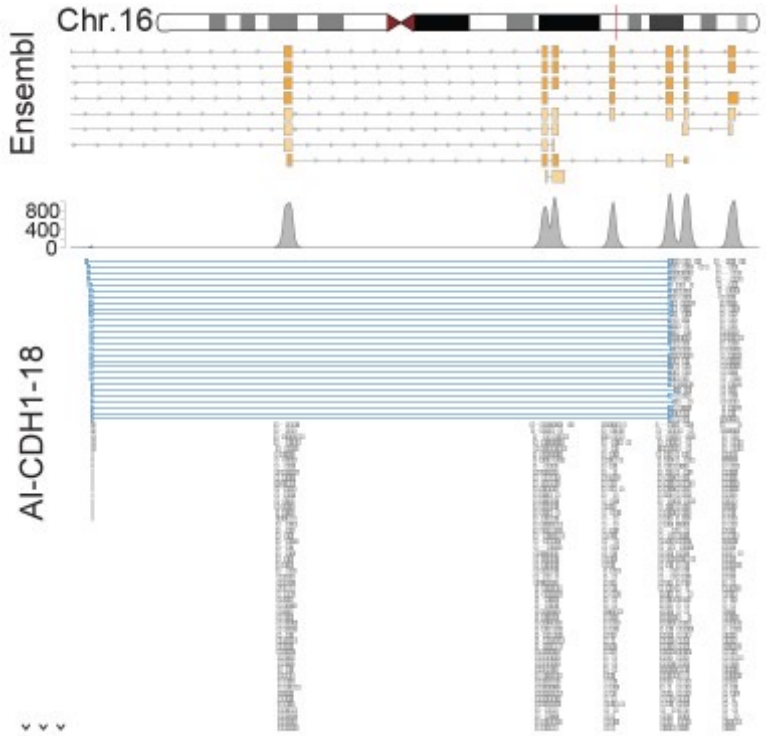
CDH1 promoter methylation is prevalent in ILCs lacking CDH1 pathogenic mutations

Alternative *CDH1* Inactivating Mechanisms: Coding and Non-Coding Genetic Alterations

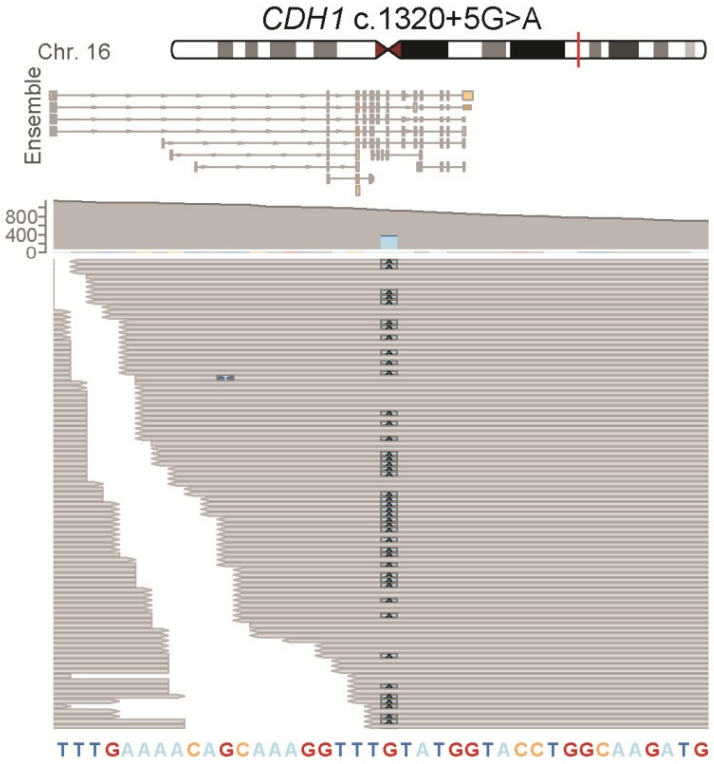
Homozygous Deletion



Intragenic Deletion

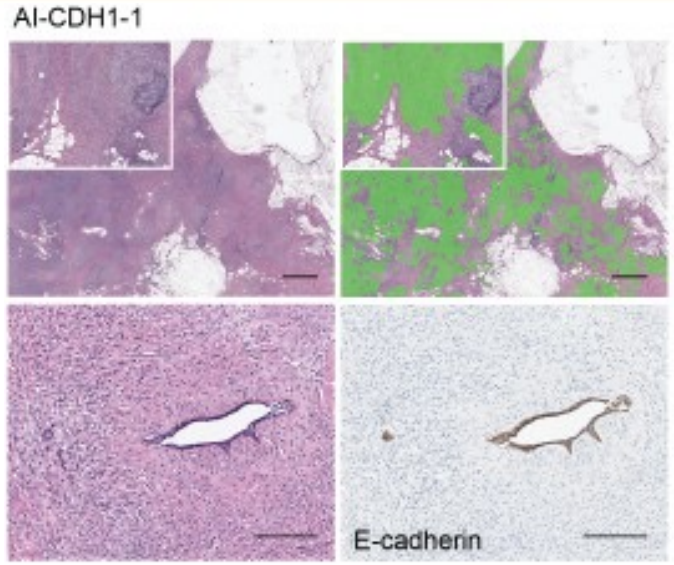


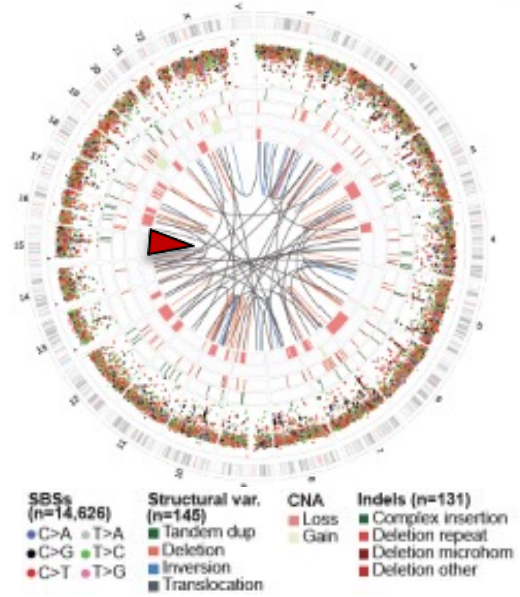
Non-coding alterations

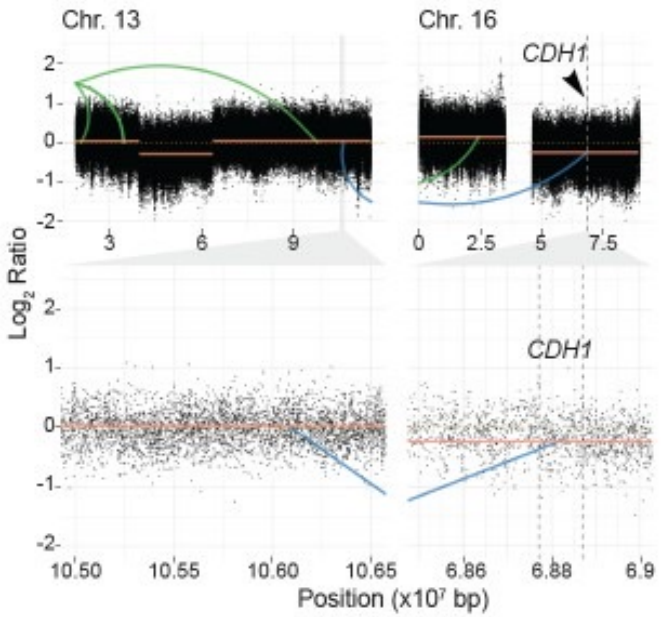


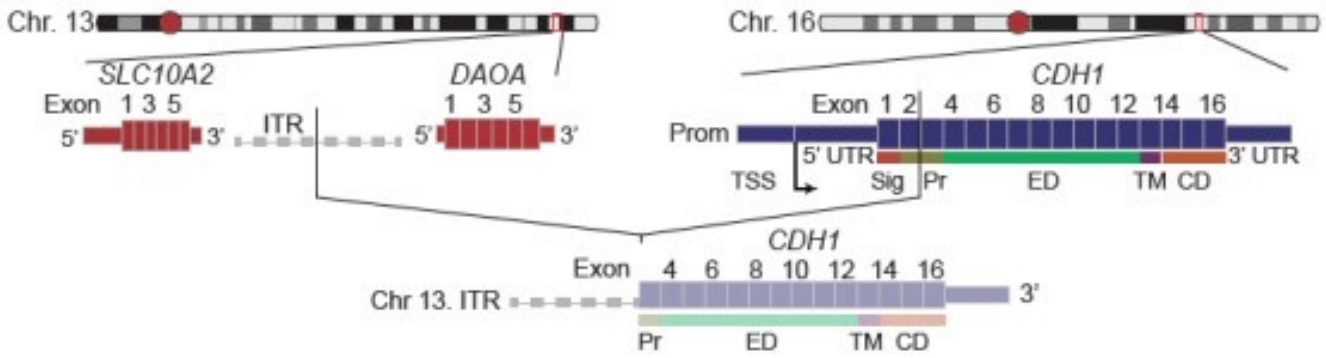
ILC is a convergent phenotype underpinned by various molecular mechanisms

Alternative *CDH1* Inactivating Mechanisms: Novel *CDH1* Deleterious Fusion gene





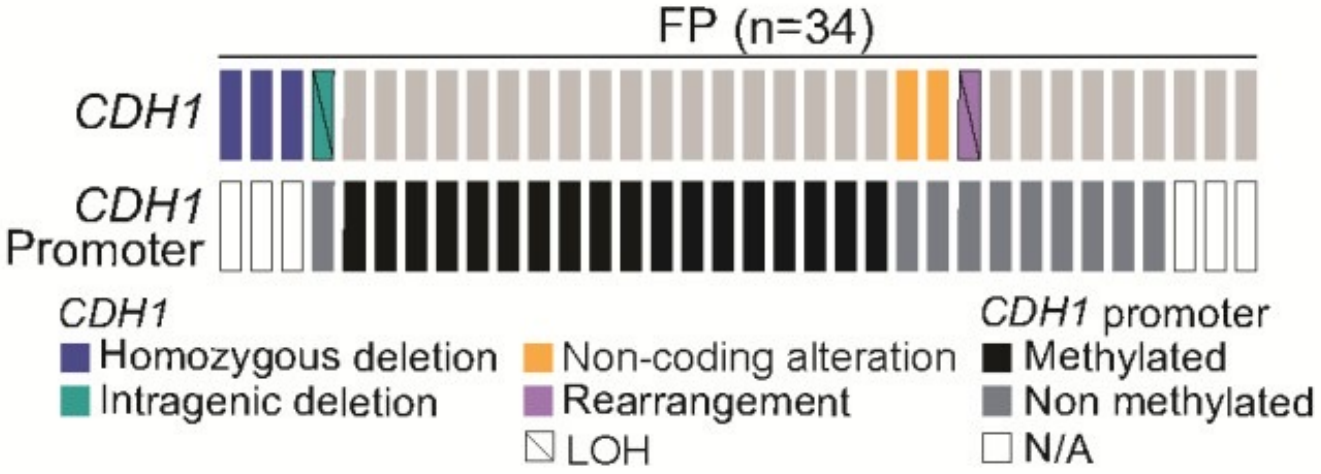




AI model detects CDH1 inactivation regardless of mechanism

CDH1 inactivating Molecular Mechanisms unveiled by the Integration of AI and Genomics

ILCs lacking *CDH1* biallelic mutations identified by a genomics-driven AI-model



CDH1 genetic/ epigenetic inactivation: 74% (25/34)

CDH1 inactivation:

- Promoter methylation
- Homozygous deletions
- Intragenic deletion
- Novel deleterious fusion
- Non-coding alterations

Application of AI-tools trained to detect a genetic alteration could decipher novel biology

Pareja et al, SABCS 2023, GS03-04

Conclusions

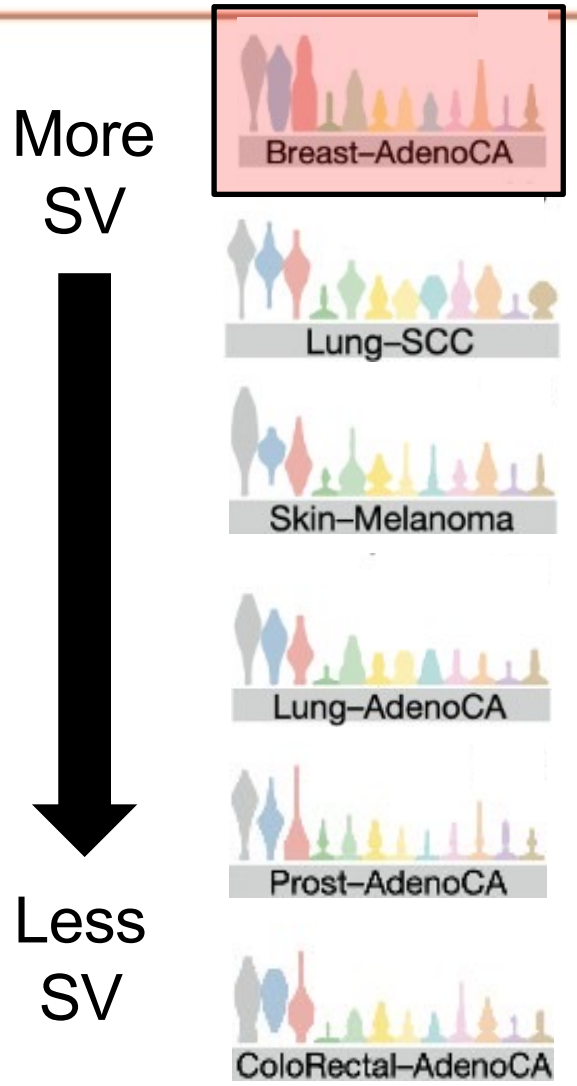
- By applying an AI-system trained to detect a genetic alteration (i.e. *CDH1* bi-allelic mutations), we identified alternative epigenetic and genetic *CDH1* inactivating mechanisms in a histologic entity (ILC).

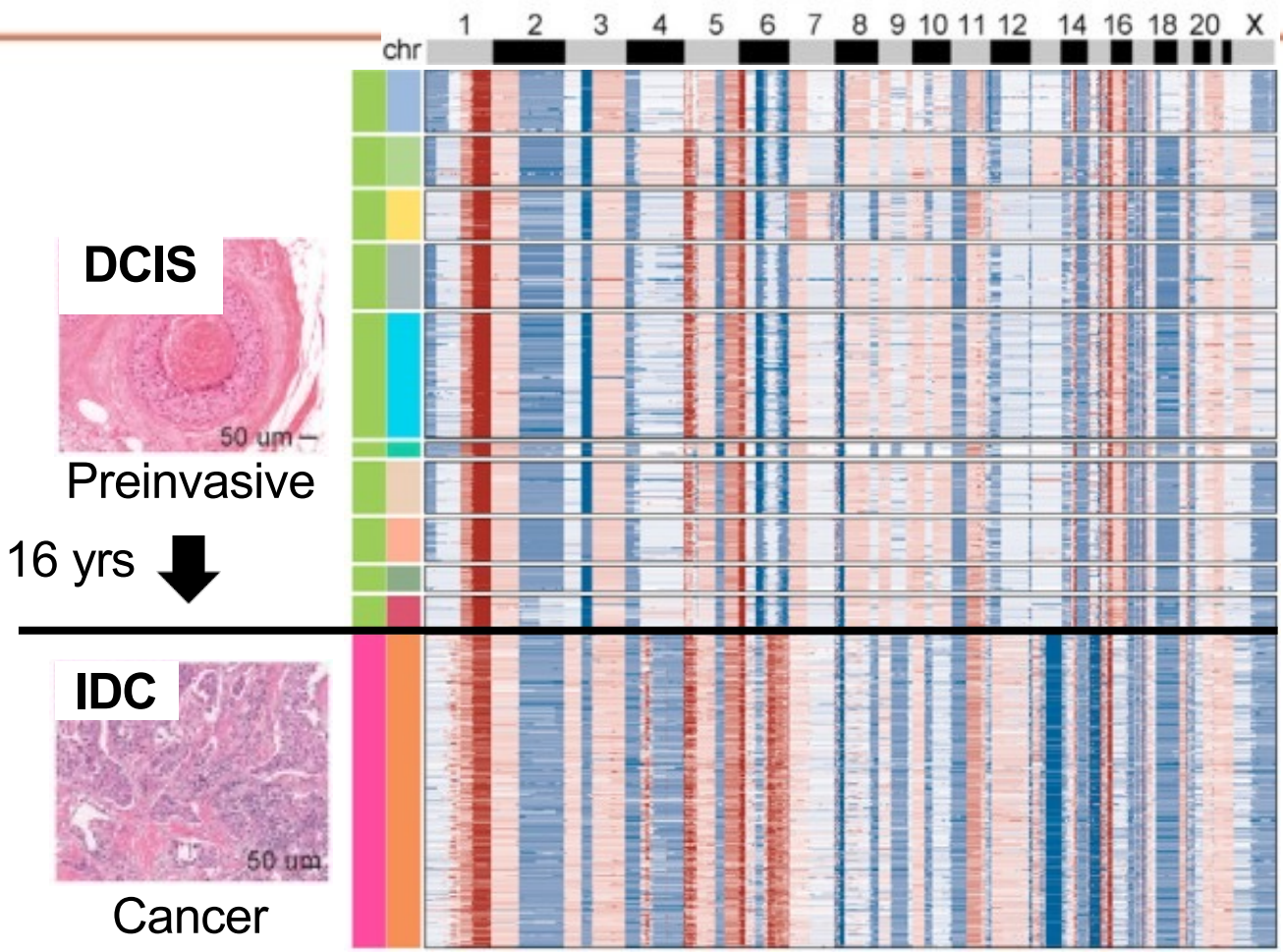
- Molecular mechanisms converging on the same phenotype can be unveiled by the integration of AI and genomics, highlighting the robustness of this approach for the discovery of novel biology.

Topic n°3

Fusion RNAs are frequent in breast cancer and may be new therapeutic targets

Structural variation (SV) in Breast Cancer



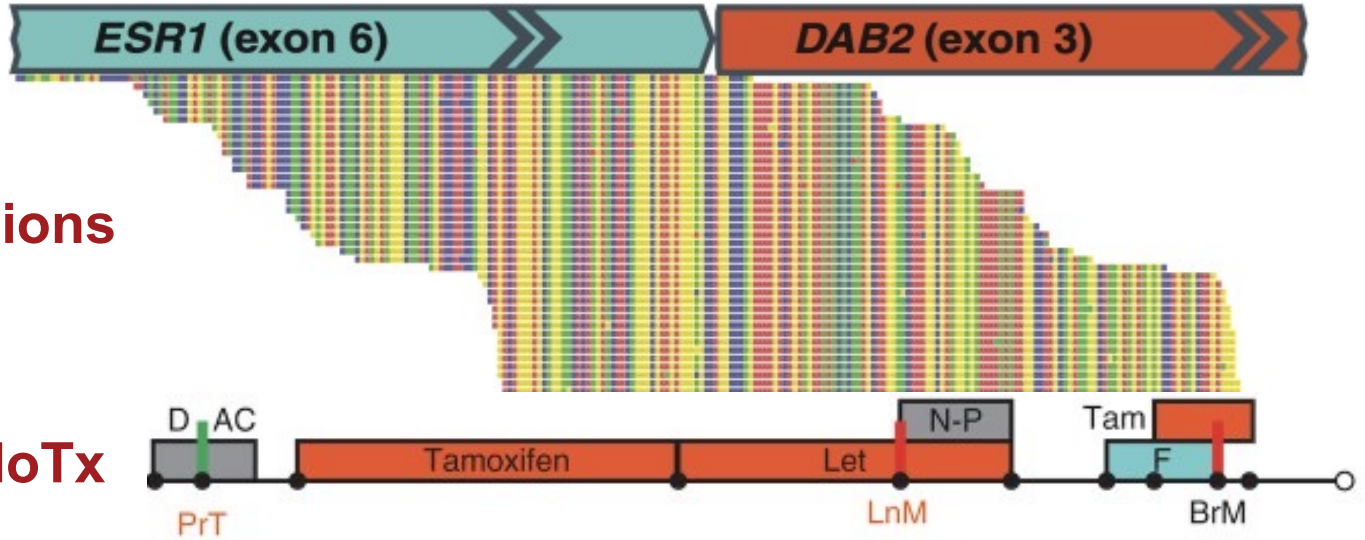


Somatic SVs are common and can be some of the most foundational events in BrCa

Nature. 2020 Feb;578(7793):112-121

Cell. 2023 Aug 31;186(18):3968-3982.e15

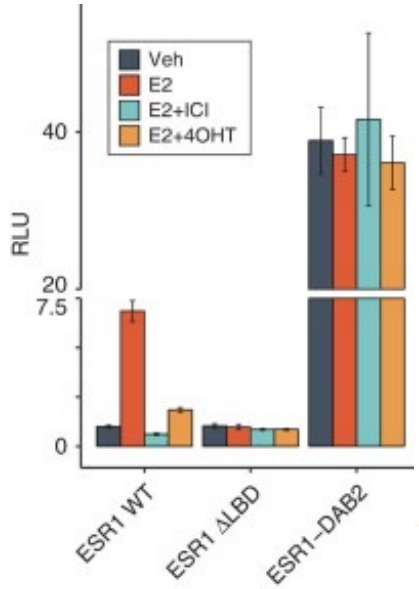
SV in BrCa can produce clinically meaningful fusion RNAs

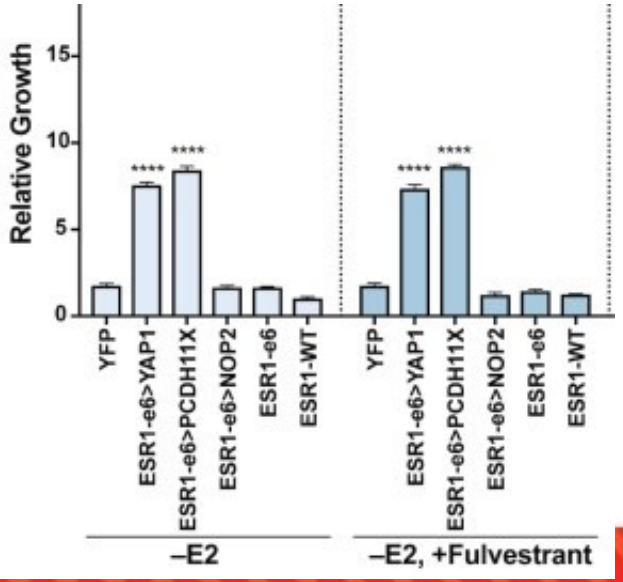


ER Fusions

s/p EndoTx

Only in recurrence





Confer estrogen independent activation and EndoTx resistance

Ann Oncol. 2018 Apr 1;29(4):872-880
Cell Rep. 2018 Aug 7;24(6):1434-144

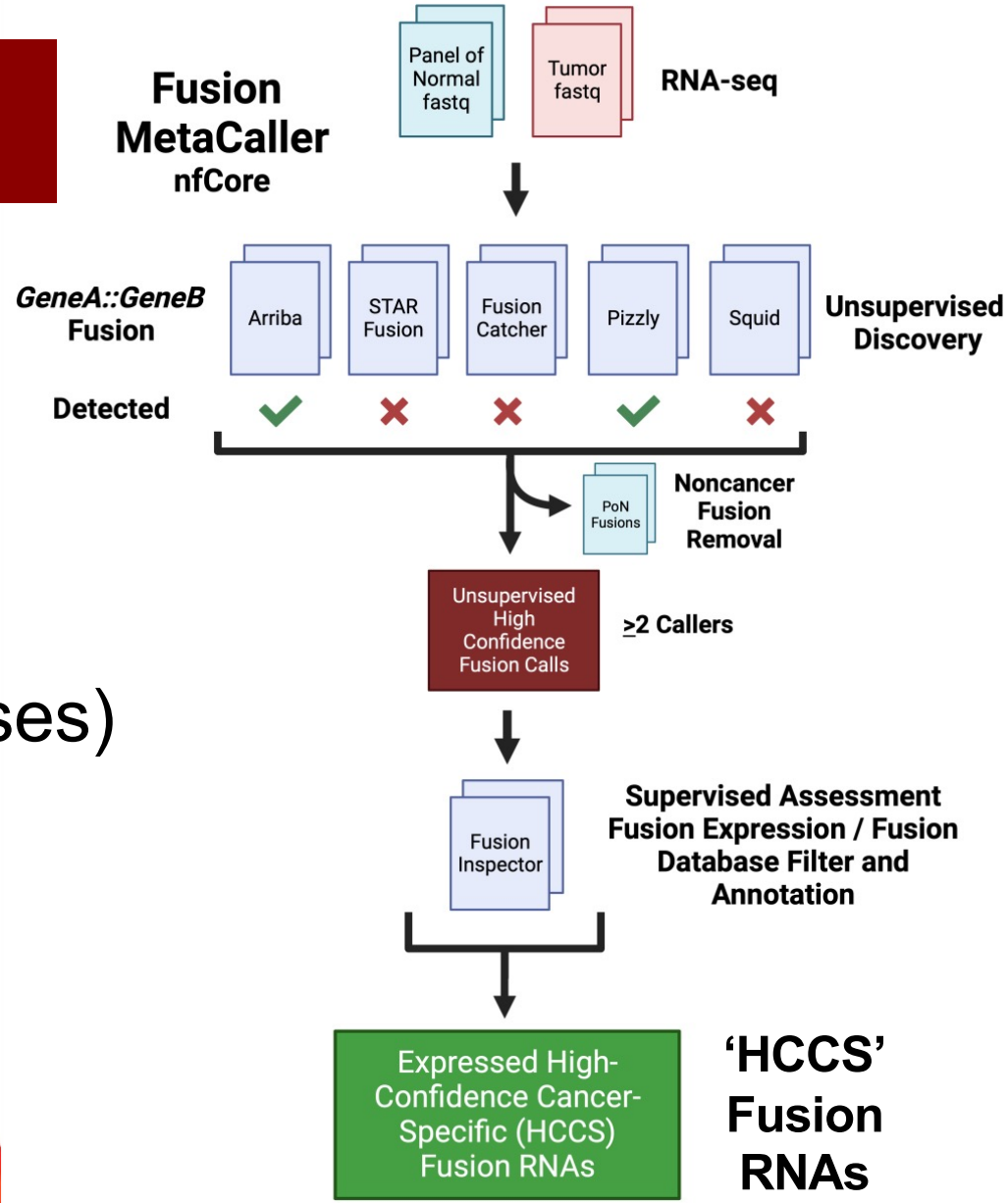
Fusion RNA Discovery in MBC

To better define the landscape of targetable fusion RNAs in treatment refractory BrCa

MBC RNA-seq Cohorts

DFCI (n = 276 samples, 252 cases)
MichiganCSER (n = 190 samples, 171 cases)

466 MBC specimens



Exploratory:
AURORA Retrospective Autopsy Cases
Breast cancer lines, UPitt PDOs/PDXs

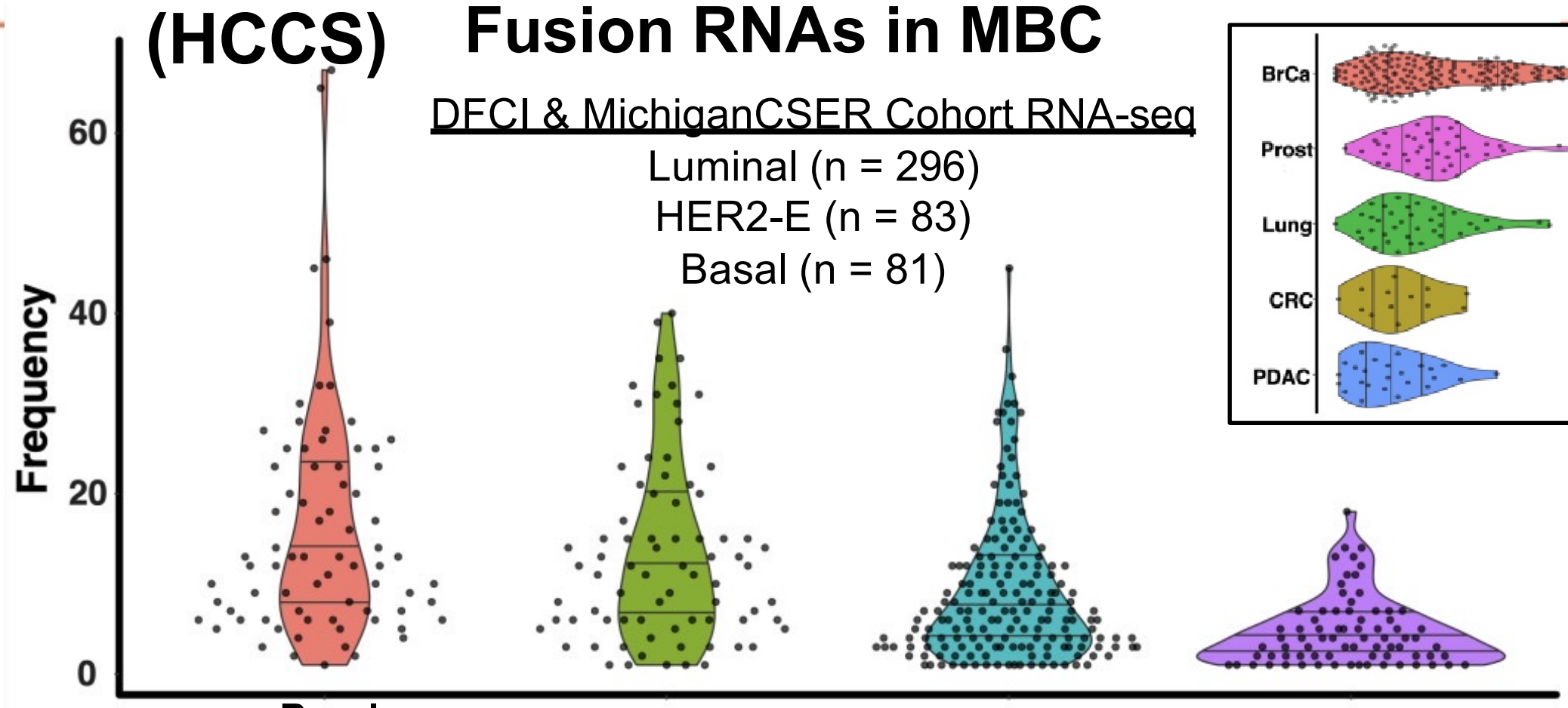
High-Confidence Cancer-Specific Fusion RNAs in MBC (HCCS)

DFCI & MichiganCSER Cohort RNA-seq

Luminal (n = 296)

HER2-E (n = 83)

Basal (n = 81)



Median HCCS Fusion RNAs / sample	Basal	HER2-E	LumB	LumA
	13	12	7	4

65% involve cancer-related gene* | 18% cases w/ in-frame kinase fusion

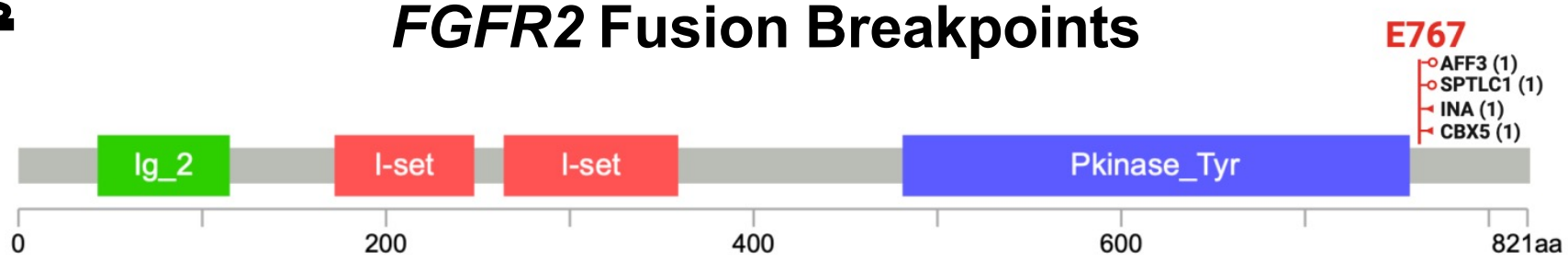
*OncoKB

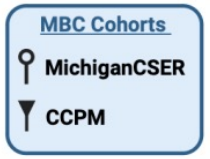
Kinase and Loss-of-Function Fusions in MBC

In-Frame Kinase Fusions

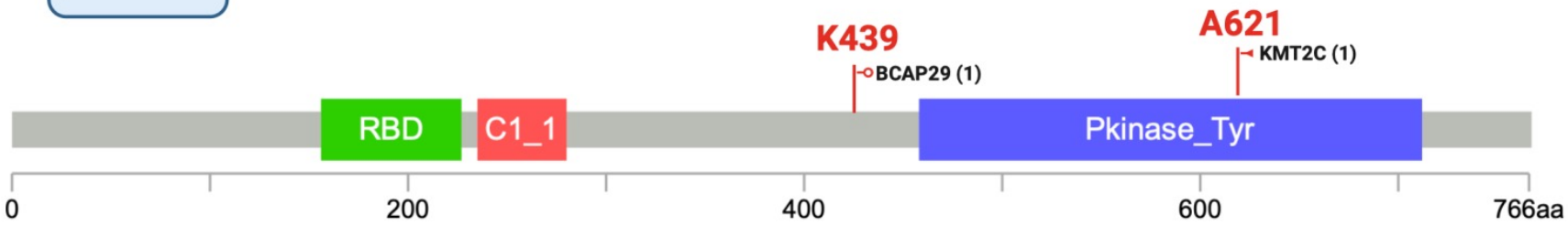
5' Gene	3' Gene	PAM50
FGFR2	AFF3	LumA
FGFR2	SPTLC1	LumB
FGFR2	INA	LumB
FGFR2	CBX5	LumB
BCAP29	BRAF	LumA
KMT2C	BRAF	LumB
CAMK2G	ADK	Basal
CUL3	ADK	LumB
WDR17	ADK	LumB
MED13	TLK2	LumB
PPFIA1	TLK2	LumB
TANC2	TLK2	LumB
ACER3	PRKCA	Basal
BCAS3	PRKCA	LumB
FMN1	PRKCA	Her2

FGFR2 Fusion Breakpoints





BRAF Fusion Breakpoints



Recurrent Loss of Function Fusions

NF1, *MSI2*, *USP32*, *PTEN*, and *CDH1*

Priedigkeit et al, SABCS 2023, GS03-09

Protein domain Images from OncoKB

Recurrent Fusion RNA Partners in MBC

Higher Frequency

Lower Frequency

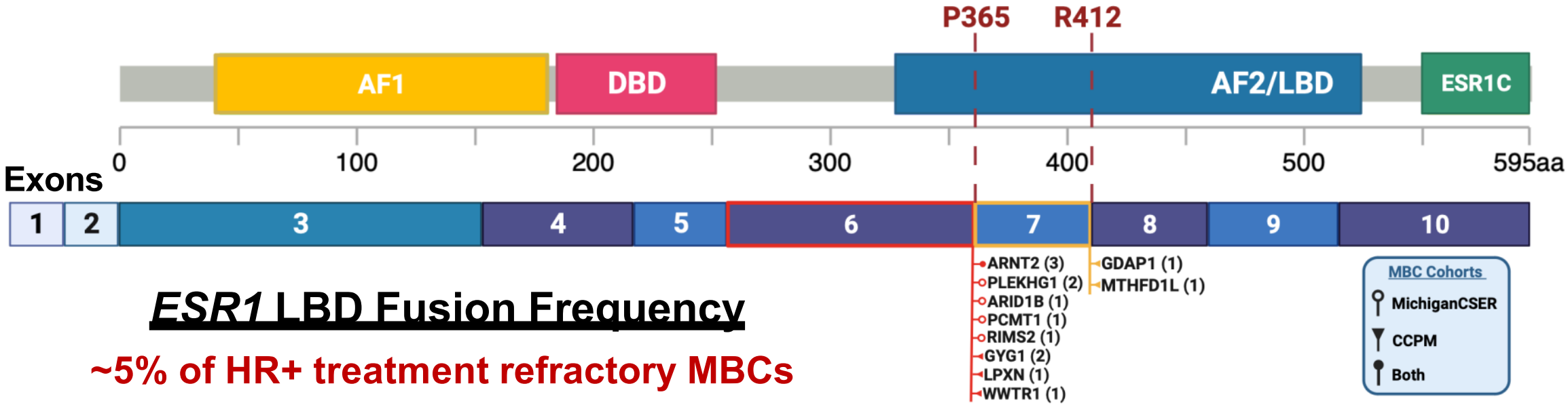
	Basal	HER2-E	LumA	LumB	Normal
MSI2	2	4	2	7	0
ESR1	0	1	0	14	0
NF1	3	6	2	2	0
YAF2	4	1	1	3	0
SEPTIN9	5	2	0	2	0
NSD3	0	3	1	5	0
GAB2	1	1	1	3	3
LASP1	2	5	0	1	0
RYBP	2	1	1	3	0
MYH9	4	0	0	3	0
KMT2C	1	2	2	2	0
FGFR1	0	3	0	4	0
ERBB2	0	4	0	3	0
SNX29	1	2	0	3	0
NOTCH3	2	1	0	3	0
IKZF3	0	6	0	0	0
BRD4	4	0	0	1	1
AGO2	2	1	0	2	0
FANCC	2	0	0	2	0
CDH1	2	0	0	2	0
Total N =	**81**	**83**	**97**	**199**	**6**

Most recurrent subtype enriched fusion RNA were those involving *ESR1*

Significantly enriched in LumB classified MBCs (padj < 0.01)

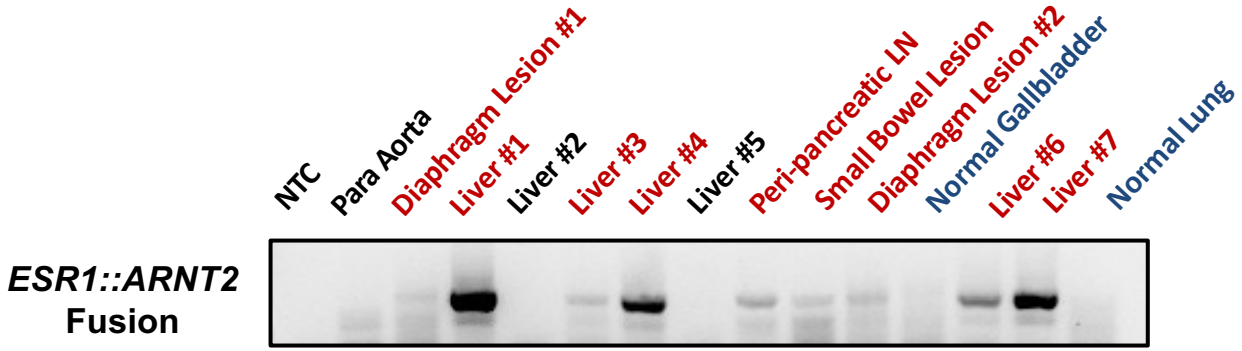
***Limited to OncoKB genes**

ESR1 Fusions in HR+ MBC



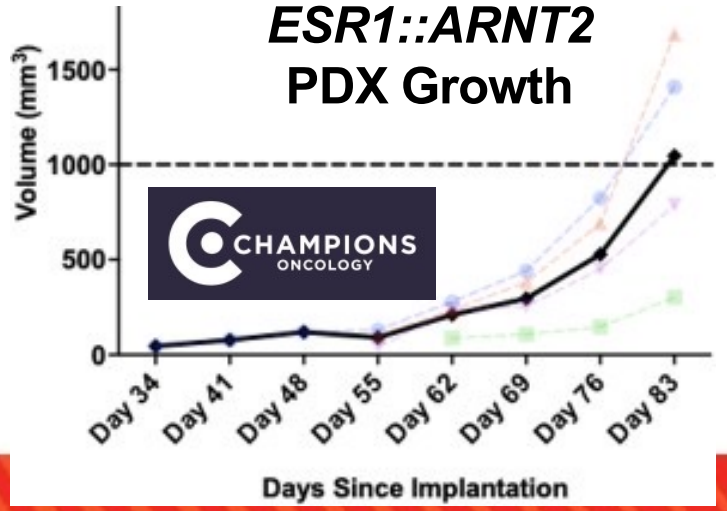
ESR1 LBD Fusion Frequency

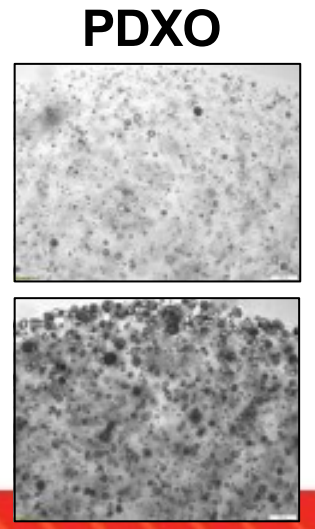
~5% of HR+ treatment refractory MBCs



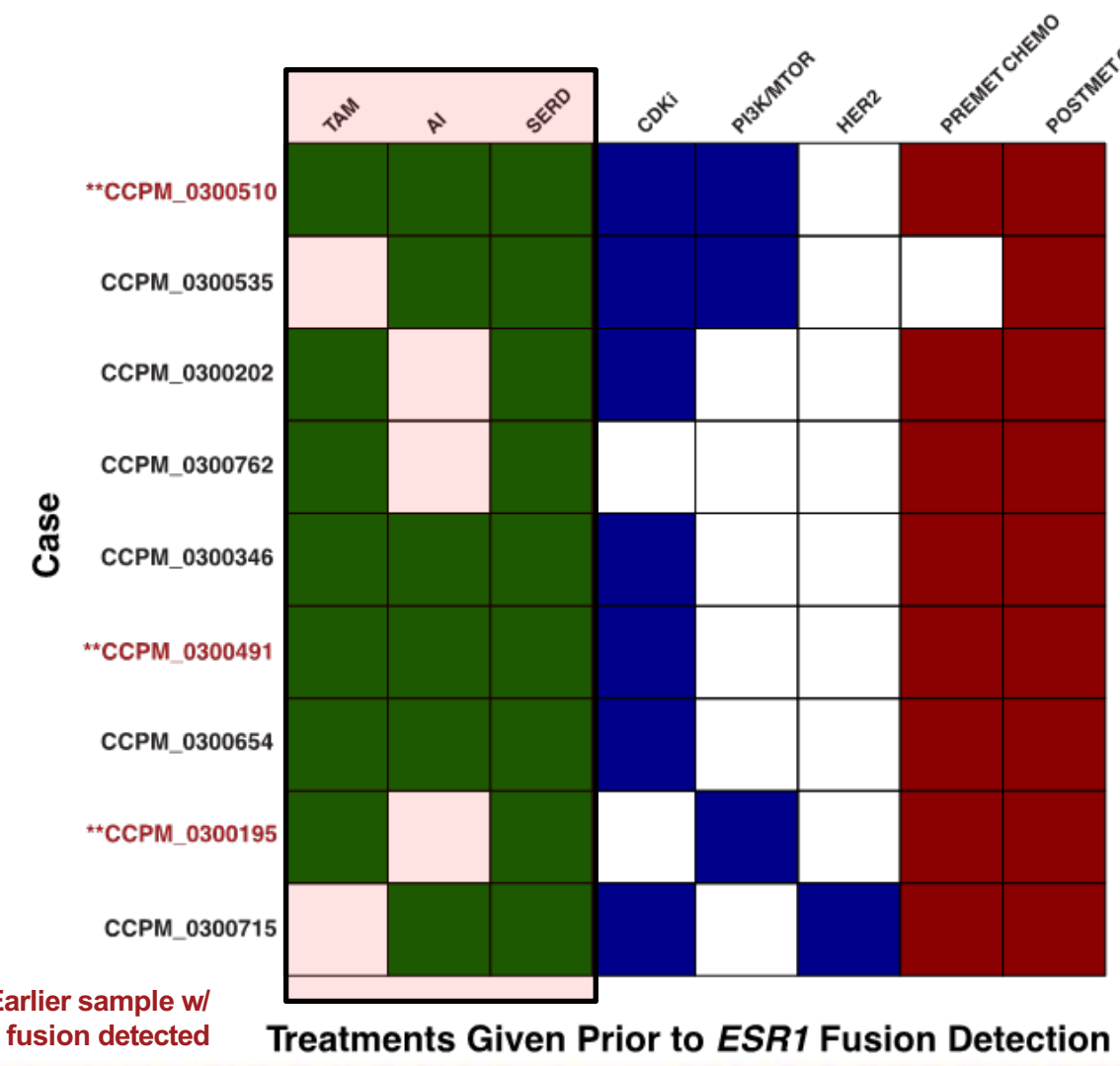
***ESR1* fusions can be expressed in most metastatic sites**

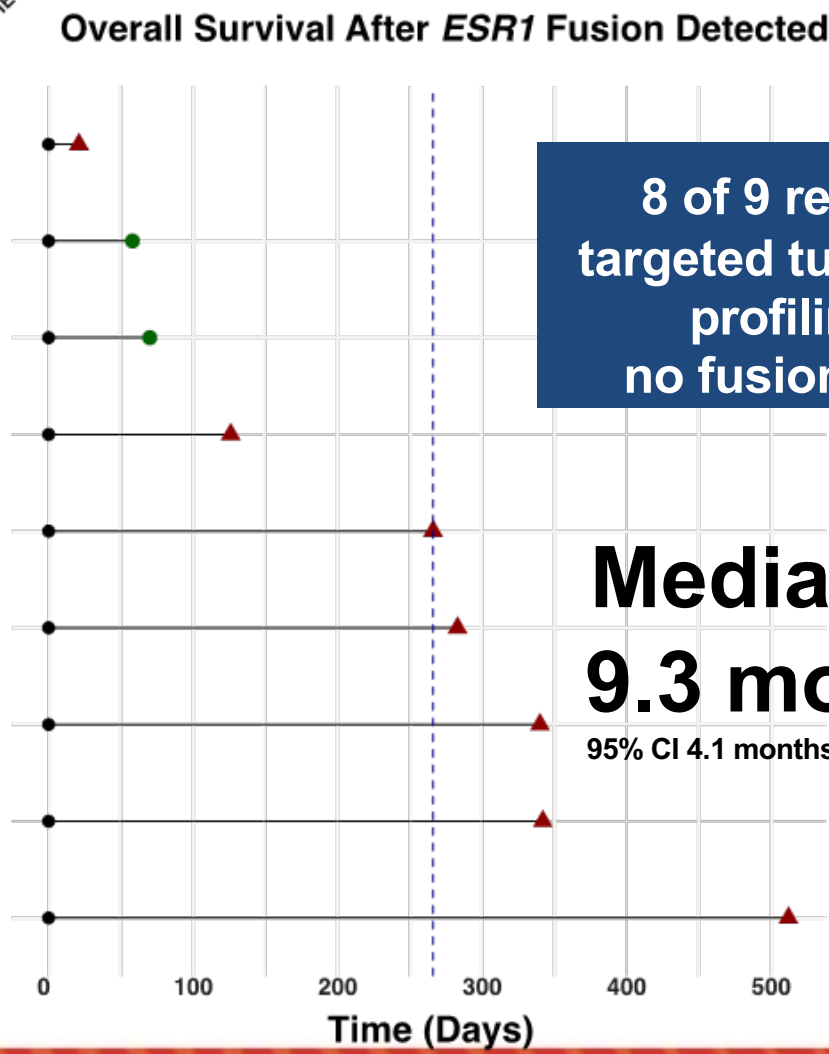
U. Pittsburgh
Lee/Oesterreich





Tx Exposures & Outcomes of *ESR1* Fusion Positive MBC



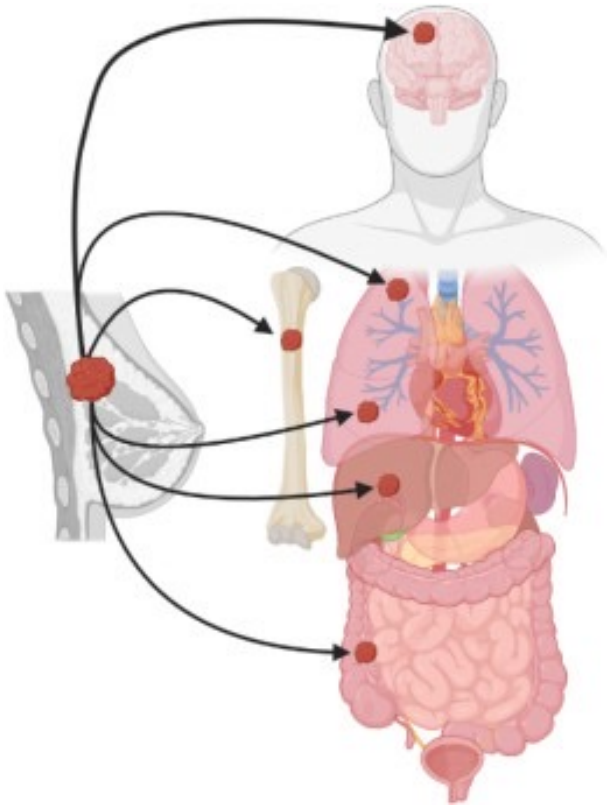


8 of 9 received targeted tumor DNA profiling— no fusion called

Median OS 9.3 months

95% CI 4.1 months – not reached

Fusion RNAs can be widely expressed in an individual patient across multiple metastatic sites



Sample	Tumor Site	Gene A	Gene B	Protein
AUR_01_11_01	**Breast**	GTF3C1	AKAP10	in-frame
AUR_01_11_03	**Skin**	GTF3C1	AKAP10	in-frame
AUR_01_11_04	**Thyroid**	GTF3C1	AKAP10	in-frame
AUR_01_11_05	**Diaphragm**	GTF3C1	AKAP10	in-frame
AUR_01_11_07	**Stomach**	GTF3C1	AKAP10	in-frame
AUR_01_11_08	**Rectum**	GTF3C1	AKAP10	in-frame
AUR_01_11_09	**Soft tissue**	GTF3C1	AKAP10	in-frame
AUR_01_11_11	**Liver**	GTF3C1	AKAP10	in-frame
AUR_01_11_12	**Peritoneum**	GTF3C1	AKAP10	in-frame
AUR_01_11_01	**Breast**	METTL9	GSG1L	in-frame
AUR_01_11_03	**Skin**	METTL9	GSG1L	in-frame
AUR_01_11_04	**Thyroid**	METTL9	GSG1L	in-frame
AUR_01_11_05	**Diaphragm**	METTL9	GSG1L	in-frame
AUR_01_11_07	**Stomach**	METTL9	GSG1L	in-frame
AUR_01_11_08	**Rectum**	METTL9	GSG1L	in-frame
AUR_01_11_09	**Soft tissue**	METTL9	GSG1L	in-frame
AUR_01_11_11	**Liver**	METTL9	GSG1L	in-frame
AUR_01_11_12	**Peritoneum**	METTL9	GSG1L	in-frame

Highly expressed, cancer-specific, in-frame fusions can be found in all cancer tissue tested from a single patient that has undergone many lines of therapy

***Not present in matched normal tissue**

Priedigkeit et al, SABCS 2023, GS03-09

Summary

- **Patient-specific, highly expressed fusion RNAs are present in over one-third of MBCs**
- Low-frequency, recurrent gain/LOF fusion RNAs **likely drive therapy resistance in MBC—some potentially druggable with FDA-approved small molecules (FGFR family, BRAF fusions)** and likely missed with current testing standards
- ***5' ESR1* fusions** are some of the most recurrent fusion RNAs in MBC with a frequency of **~5% in HR+ disease**—acquired in the treatment-refractory setting
- **Fusion RNAs can be widely expressed in multiple metastatic sites of an individual patient—**possibly serving as more homogenous targets
- **Breakthrough advances in gene therapy are here**—and we postulate exploiting cancer-specific, SV nucleotide breakpoints may be a compelling therapeutic approach—currently testing various technologies.

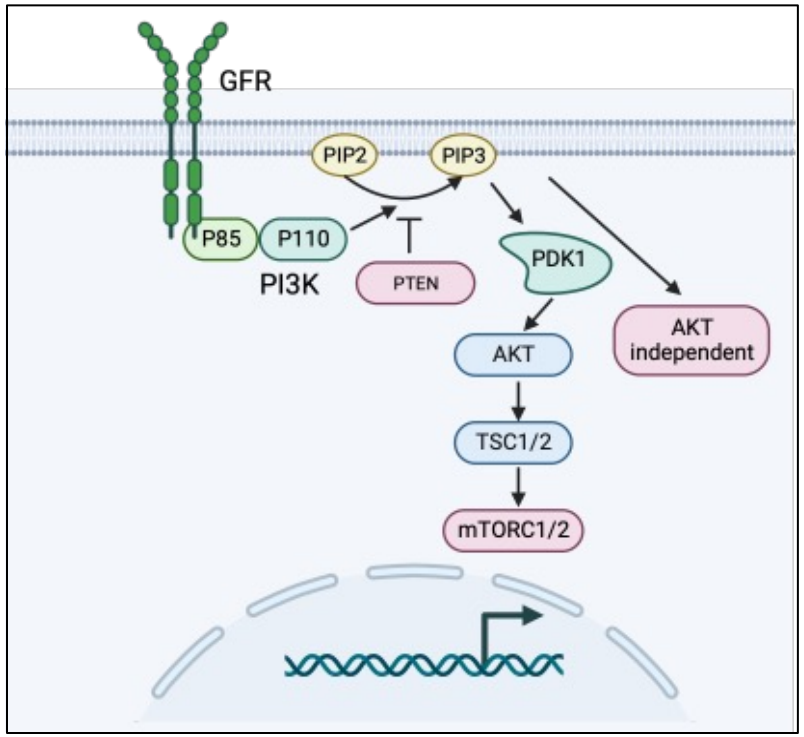
Priedigkeit et al, SABCS 2023, GS03-09

Topic n°4

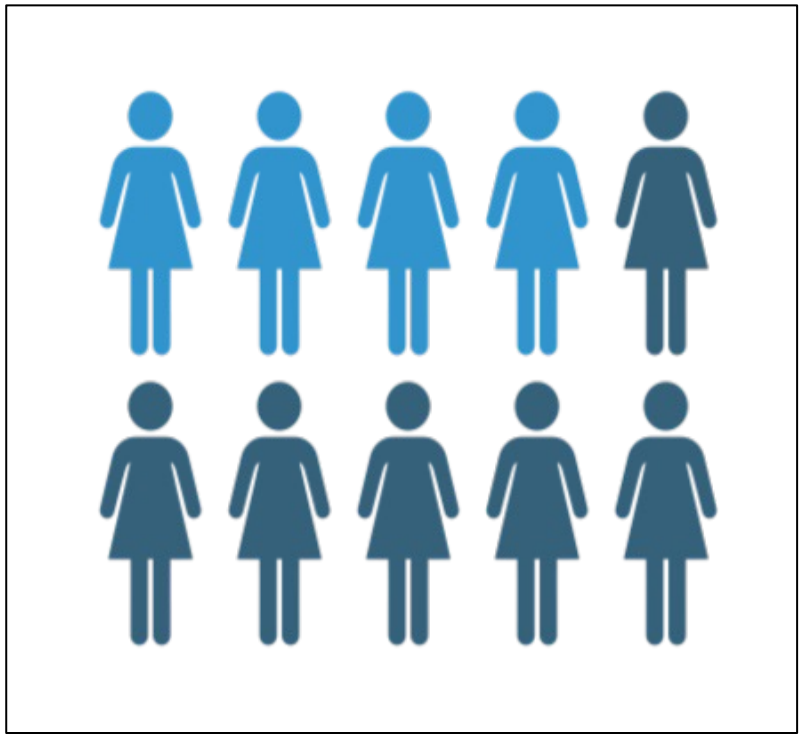
Secondary *PIK3CA* fusions can be targeted by allosteric PI3K-alpha inhibitors

PIK3CA mutations are the most common actionable alterations in HR+/HER2- MBC

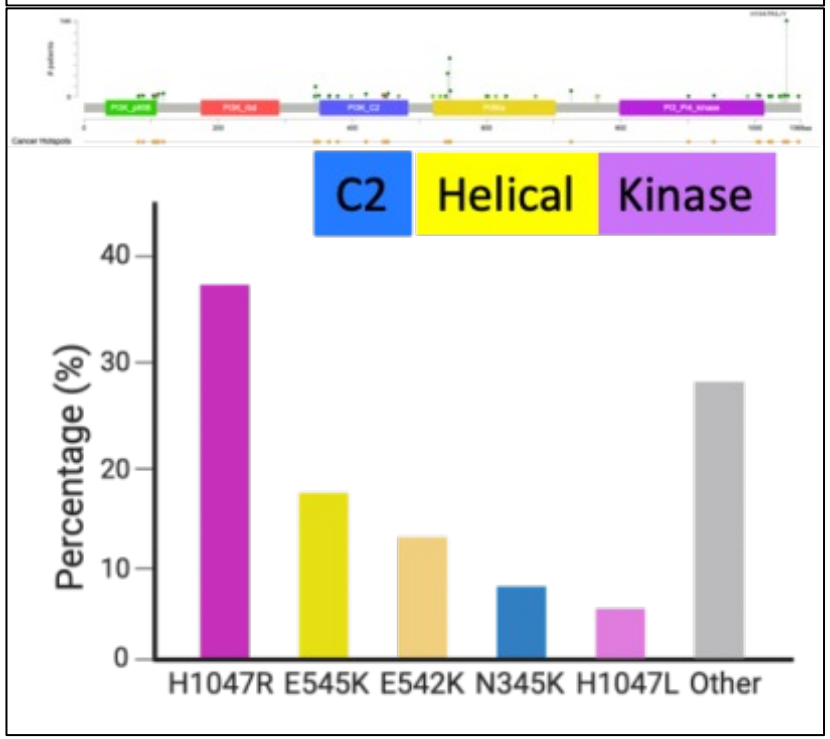
PI3K/Akt/mTOR Signaling



Mutations detected in 40% of advanced HR+ BC



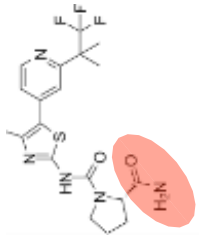
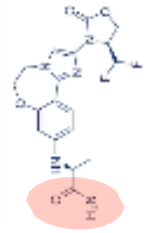
Non-random distribution with hot spots located in the helical and kinase domains

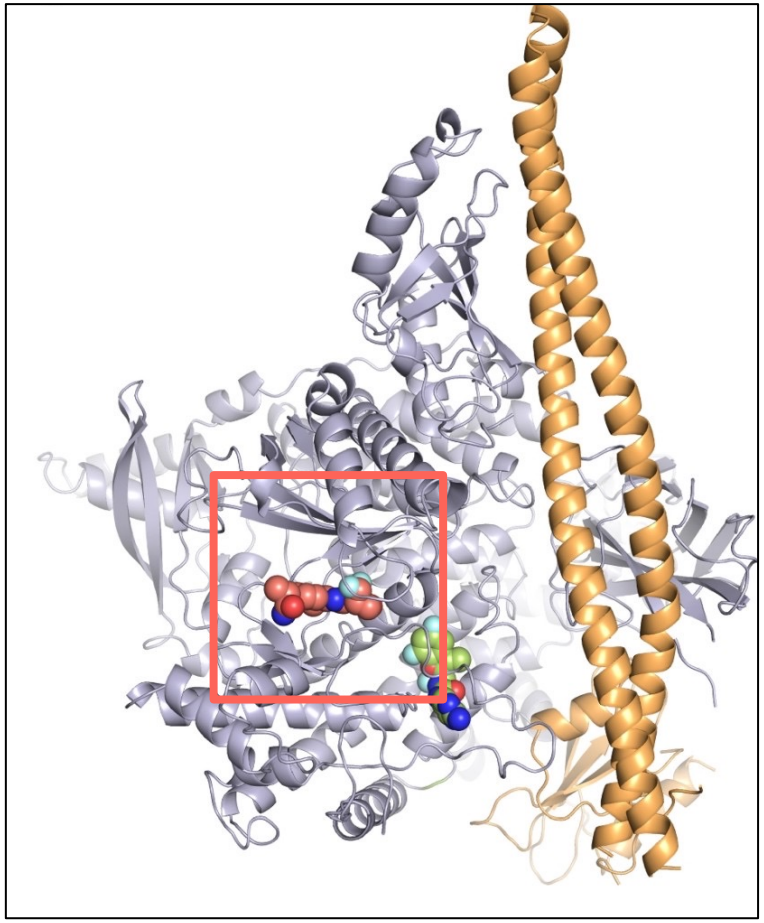


Varkaris et al, SABCS 2023, GS03-10

TCGA, PanCancer Atlas
Marinez et al. BCR. 2020

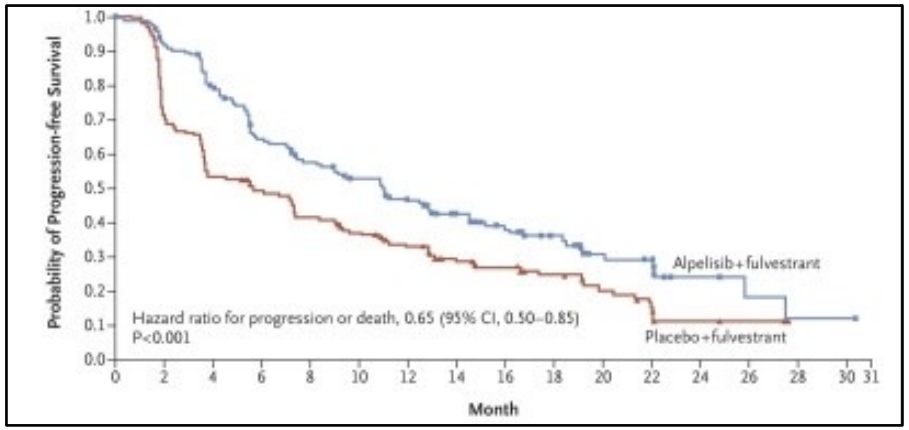
Orthosteric PI3K alpha inhibitors in HR+/HER2- PIK3CA-mutated MBC

Alpelisib (BYL-719)	
Inavolisib (GDC-0077)	

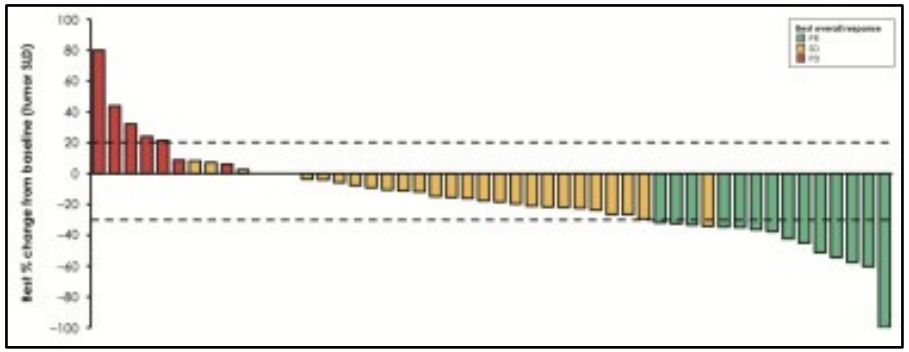


2.9Å X-ray structure of PI3Kα (p110 purple, p85 orange) with GDC-0077 (red spheres)

Alpelisib phase III data:



Inavolisib phase I/II data:

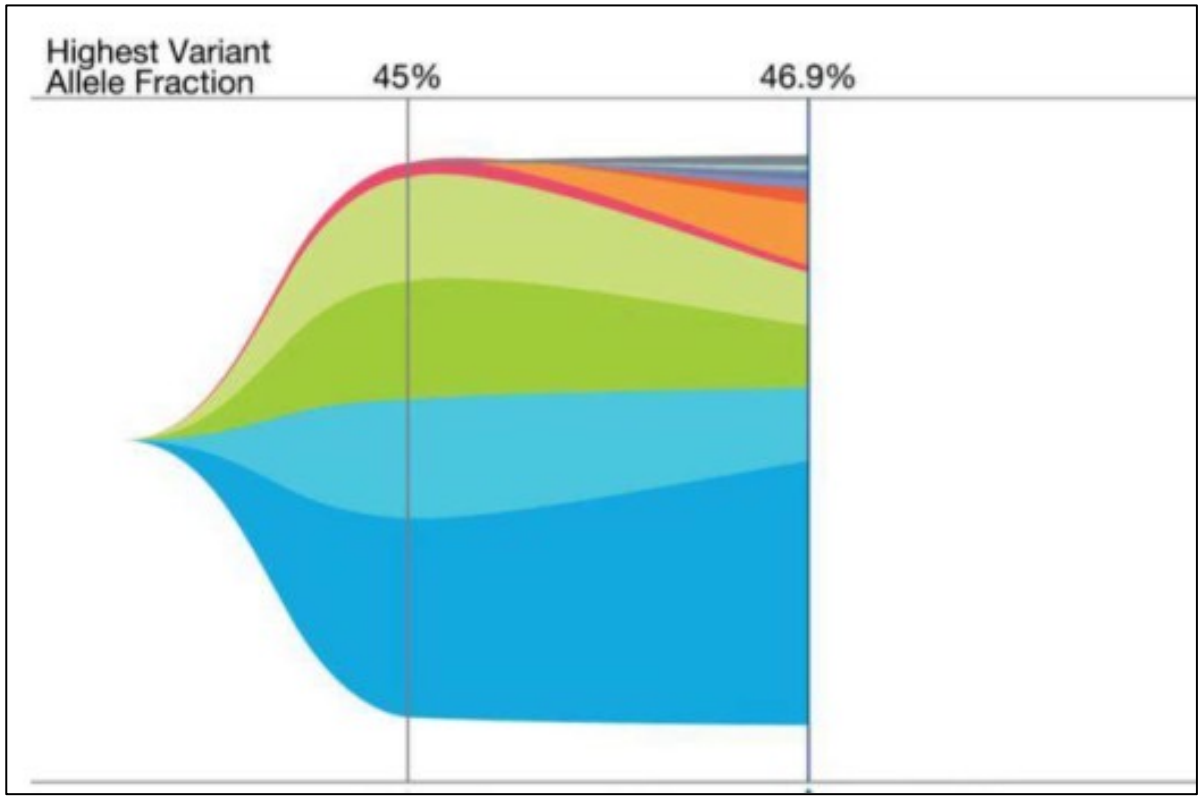


Varkaris et al, SABCS 2023, GS03-10

Andre et al. NEJM. 2019. Juric et al. JCO 2018. Juric et al. SABCS. 2021, Buckbinder et al. Cancer Discovery. 2023

Index Patient

75 yo female diagnosed with HR+/HER2 low PIK3CA mutant MBC enrolled on clinical trial with inavolisib plus fulvestrant. She was treated on trial for approximately 30 months. At the end of treatment visit she underwent testing with ctDNA analysis that demonstrated three acquired PIK3CA mutations beyond the primary pre-treatment PIK3CA mutation and AKT1 mutations.



Summary of Detected Somatic Alterations, Immunotherapy Biomarkers & Associated Treatment Options

KEY ✓ Approved in indication ~ Approved in other indication ✗ Lack of response

Detected Alteration(s) / Biomarker(s)	Associated FDA-approved therapies	Clinical trial availability (see page 6)	% cfDNA or Amplification
ESR1 Y537N	✗ Anastrozole, Exemestane, Letrozole	Yes	6.3%
ESR1 D538G	✗ Anastrozole, Exemestane, Letrozole	Yes	0.5%
PIK3CA H1047R	✓ Alpelisib	Yes	9.5%
ATM Copy Number Loss	~ Olaparib	Yes	DETECTED
CHEK2 Copy Number Loss	~ Olaparib	Yes	DETECTED
AKT1 E17K	None	Yes	1.3%
AKT1 L52R	None	Yes	0.7%
AKT1 Q79K	None	Yes	0.2%
GATA3 c.1051-5_1052del (Splice Site Indel)	None	No	7.6%
CDH1 S337fs	None	No	0.2%

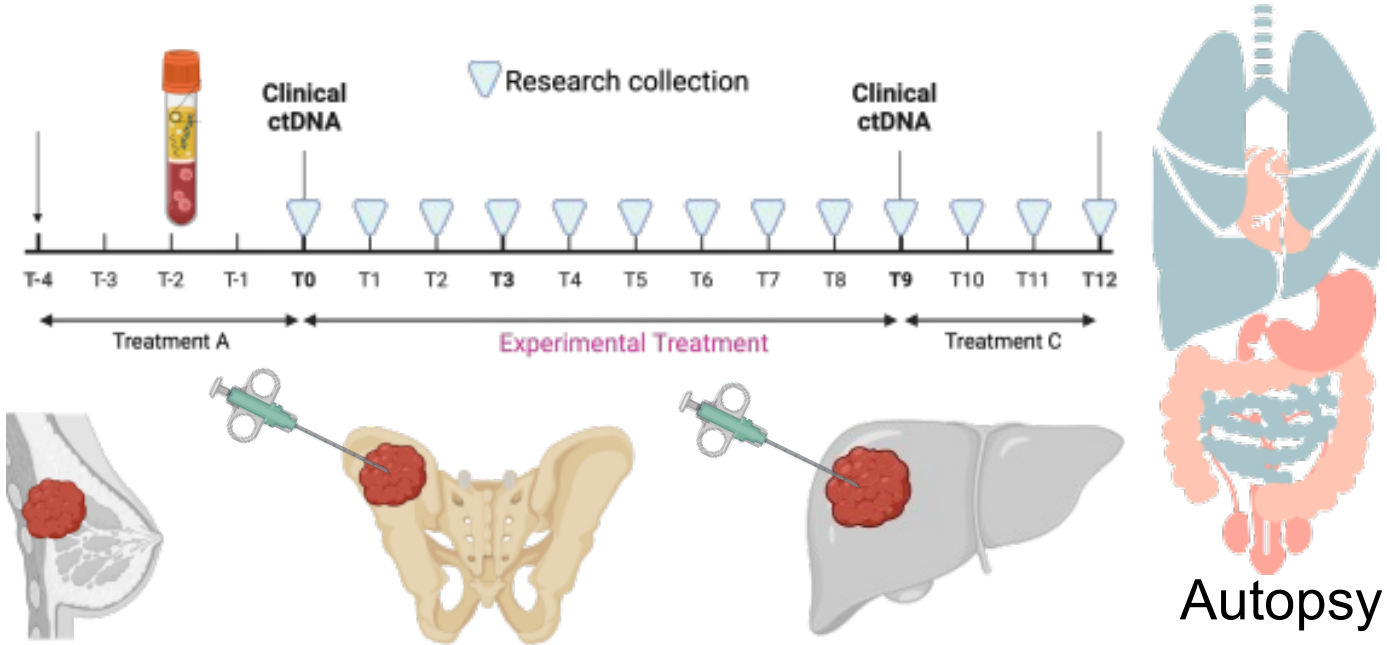
Variants of Uncertain Clinical Significance
CDK12 Splice Site SNV (46.9%), *PIK3CA* N107I (7.7%), *PIK3CA* Q859K (0.5%), *ATM* L2866I (0.2%), *PIK3CA* W780R (0.2%), *PIK3CA* Q859H (0.1%)
The functional consequences and/or clinical significance of alterations are unknown. Relevance of therapies targeting these alterations is uncertain.

Study overview

Hypothesis: H1: Acquired resistance to orthosteric PIK3CA inhibitors is mediated through frequent activating alterations within PI3K pathway. H2: These alterations may be amendable to specific therapeutic interventions with next generation PI3K/Akt inhibitors

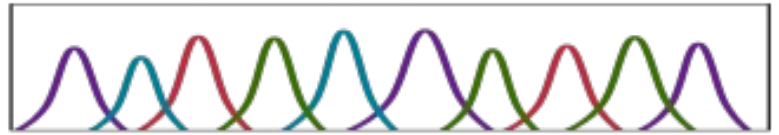
Aim: Characterize the clinical landscape of resistance to orthosteric PIK3CA inhibitors.

Clinical Research samples



Methodology

WES



Engineered Cell lines

Express genomic alterations found in clinical samples

Patients:

Serial ctDNA analysis

Autopsy samples analysis

Eligibility Criteria	
1	Histologically confirmed diagnosis of HR+/HER2- MBC
2	Documented baseline PIK3CA mutation(s) in blood or tumor per local assessment
3	Treatment with PI3K-alpha inhibitor (alpelisib and inavolisib) for >50 days.
4	Pretreatment and posttreatment evaluation with ctDNA analysis or autopsy

32 Patients

1 overlapping

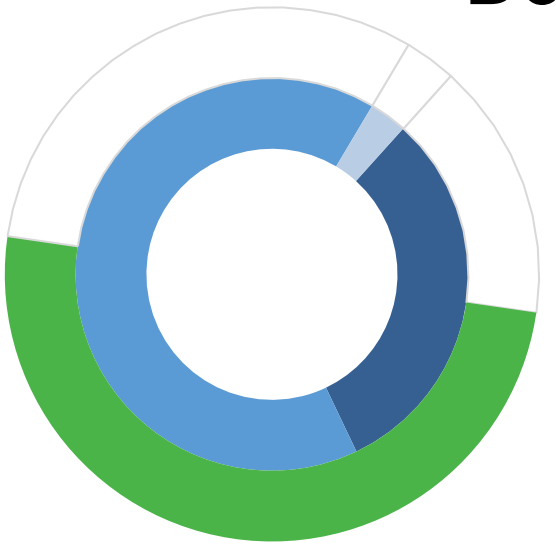
8 Patients

Within PI3K pathway alterations	
1	PIK3CA
2	PTEN
3	AKT
Other: mTOR, FGFR1/2, EGFR, HER2, RAS	

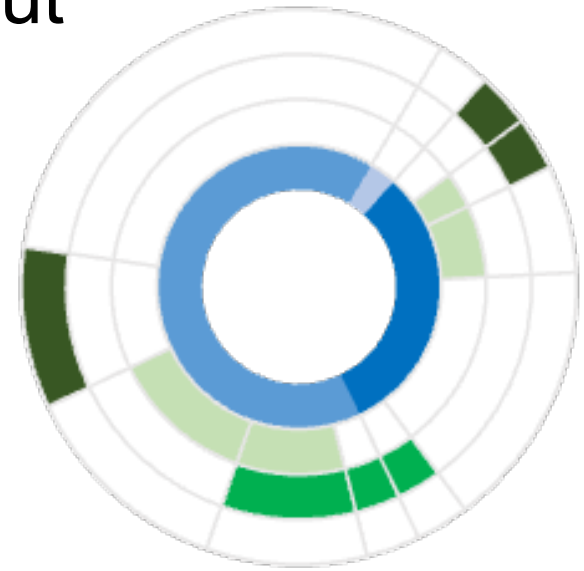
Varkaris et al, SABCS 2023, GS03-10

50% of patients acquired additional on-target and within pathway alterations beyond baseline PIK3CA mutations

Baseline PIK3CA Kinase/Helical Domain Mut

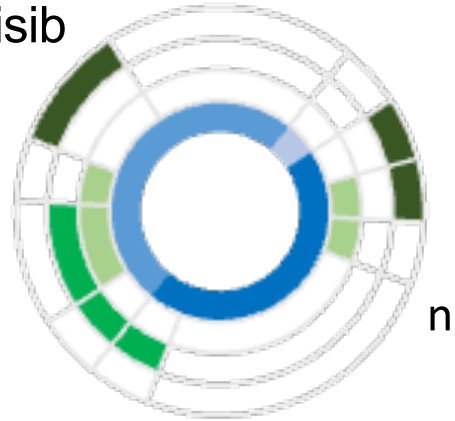


Total Acquired PI3K Pathway Alterations 50%



PIK3CA, PTEN, AKT1
28% 15% 15%

Inavolisib



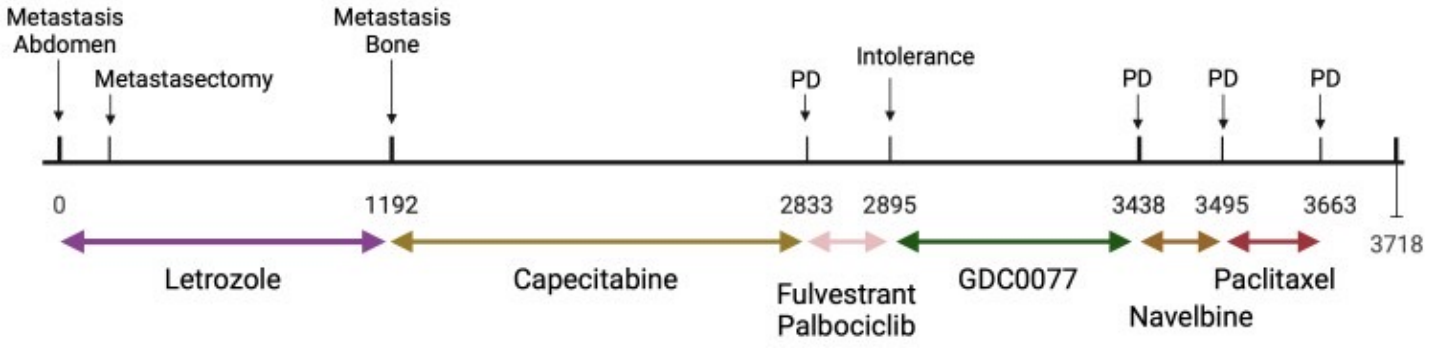
n:20

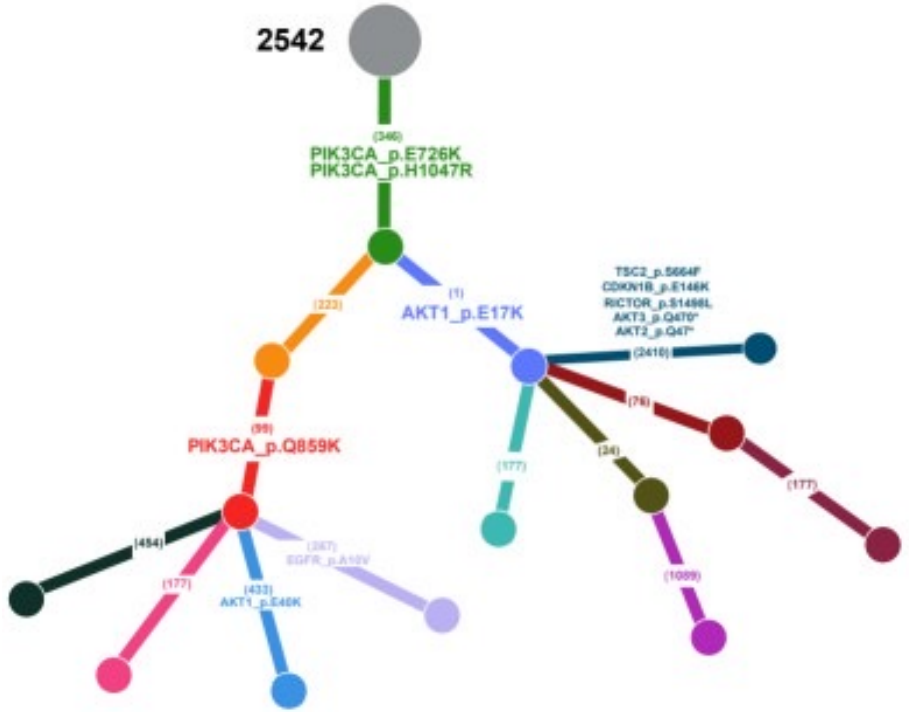
Alpelisib

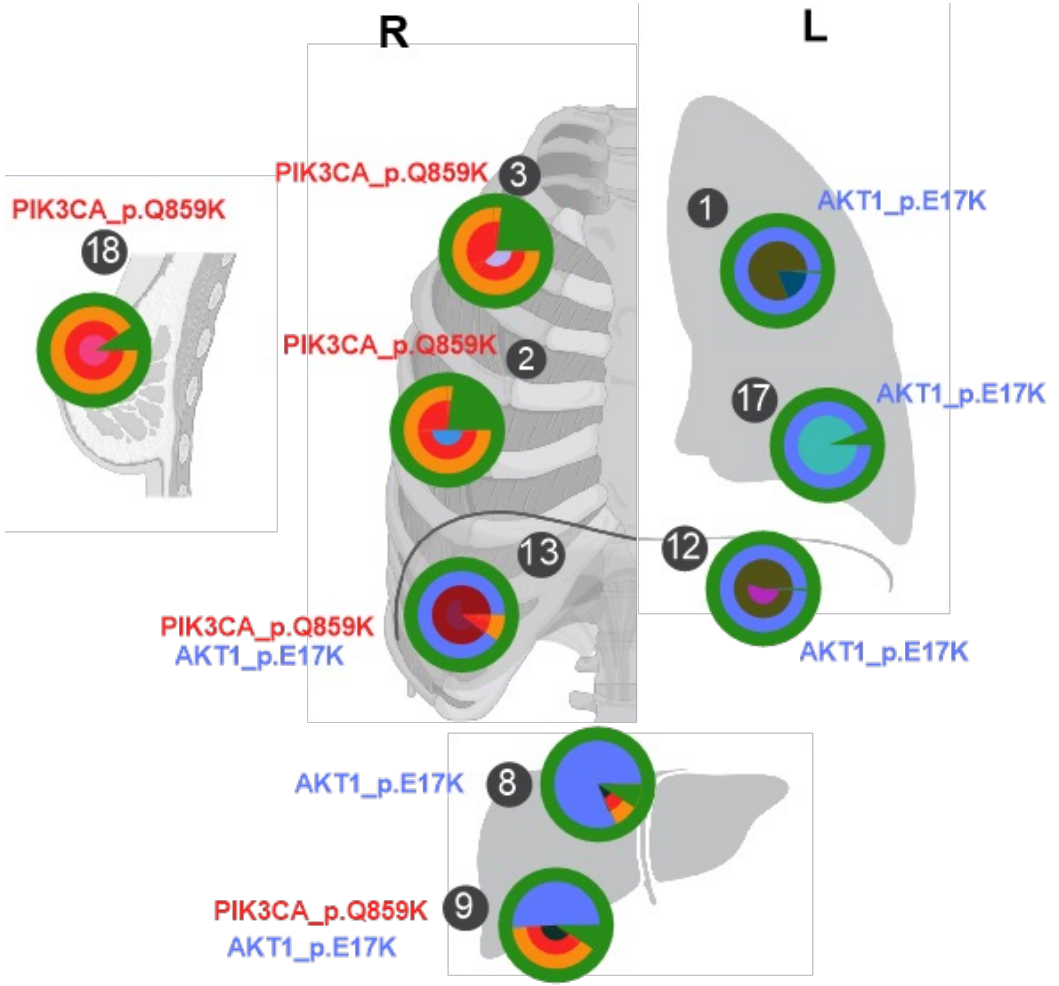
n:12

Varkaris et al, SABCS 2023, GS03-10

Rapid autopsy reveals intra- and inter-lesional heterogeneity of secondary PIK3CA and AKT1 mutations



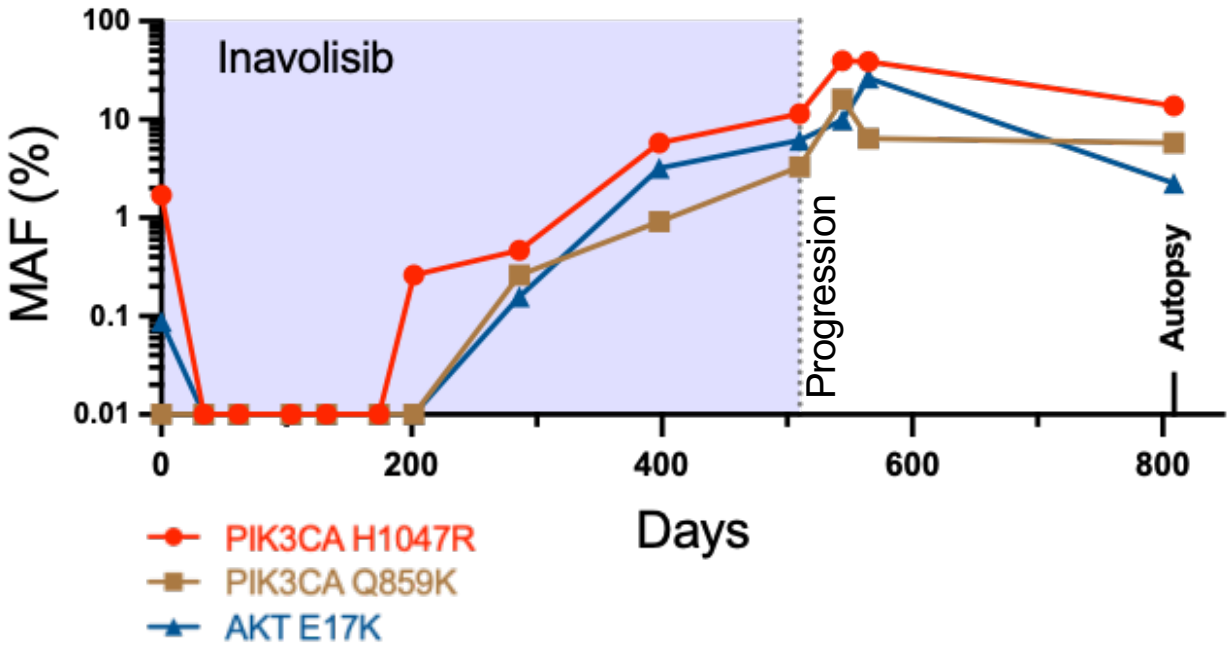




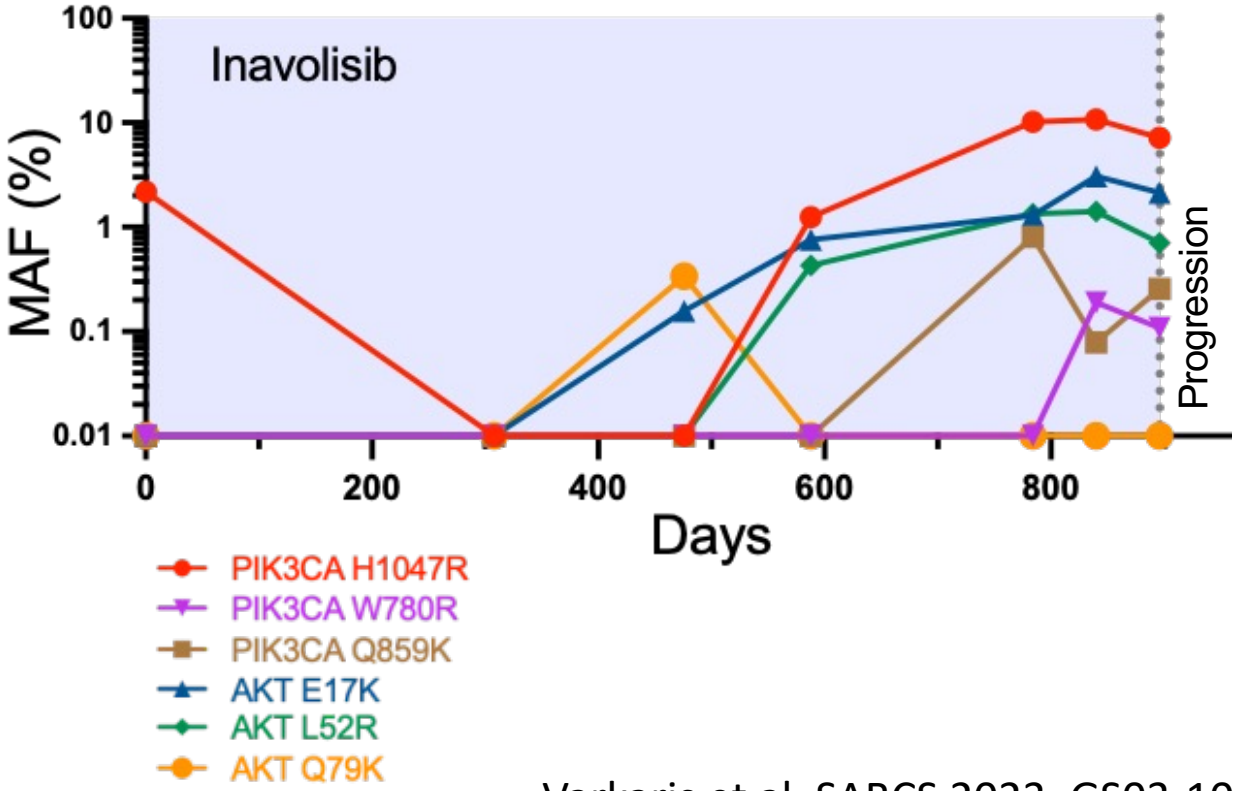
Serial ctDNA analysis reveals clonal dynamics of resistance drivers preceding radiographic progression

ddPCR

Patient A

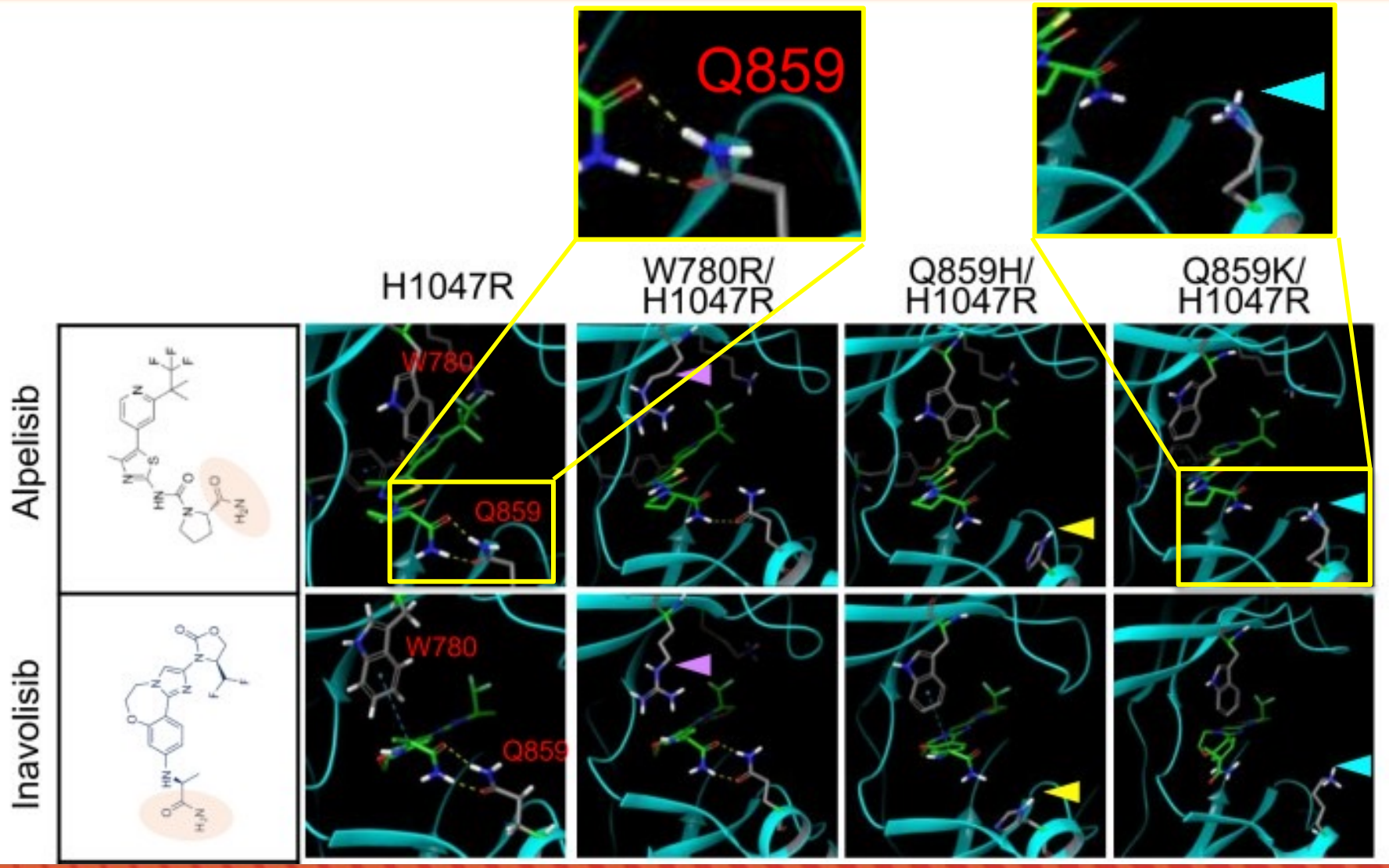


Patient B



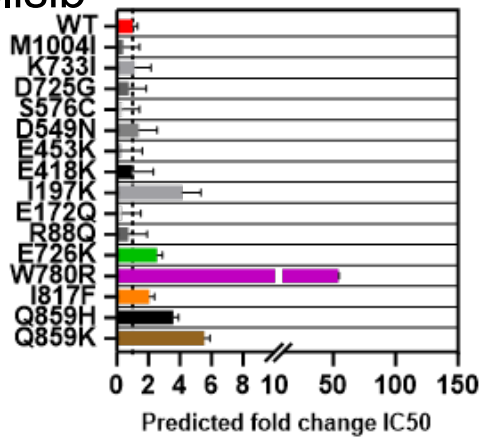
Varkaris et al, SABCS 2023, GS03-10

Structural modeling and FEP of acquired PIK3CA mutations predicts decreased affinity for alpelisib and inavolisib

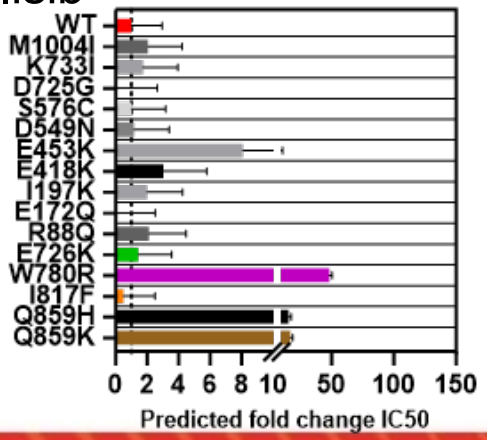


FEP Simulation

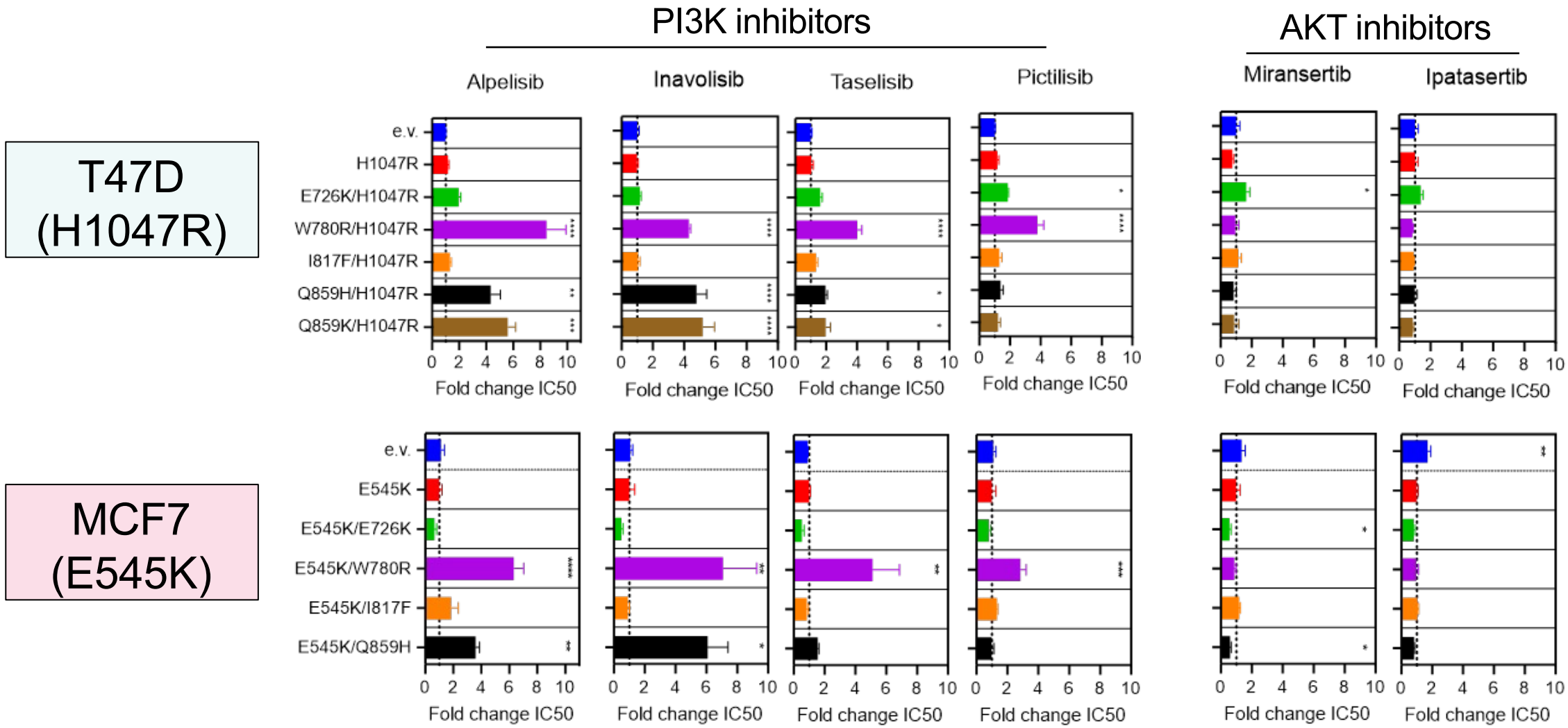
Alpelisib



Inavolisib

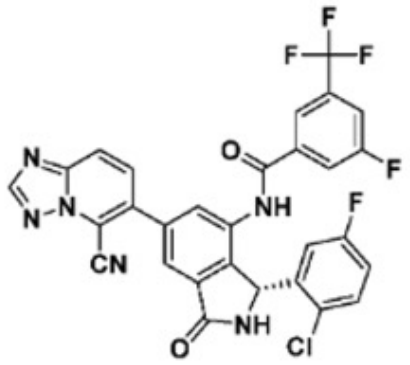


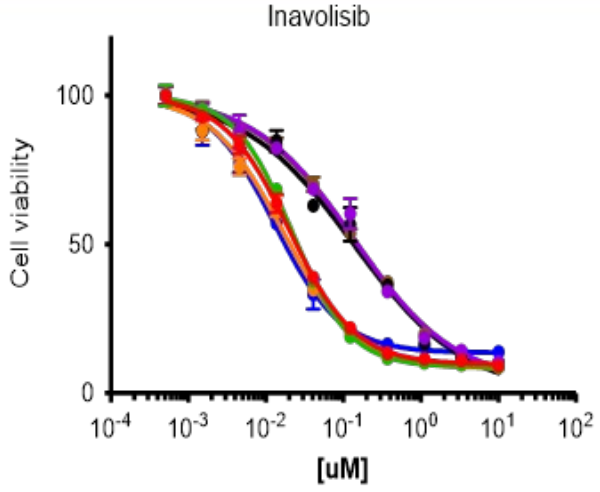
In-vitro functional analysis of acquired PIK3CA mutations confirms their role in resistance

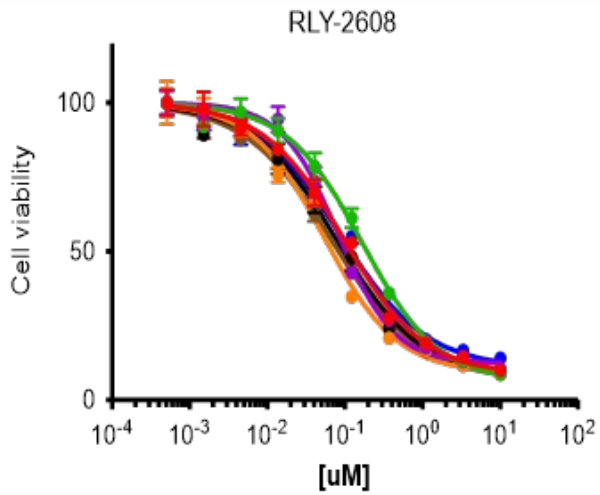


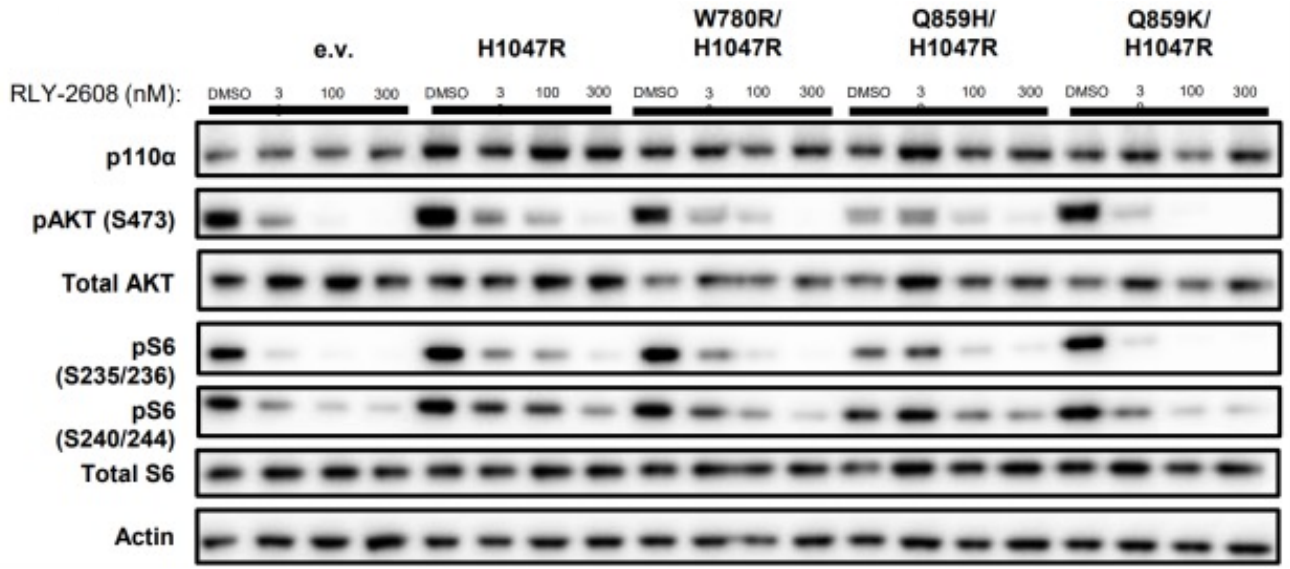
Allosteric mutant selective PIK3CA inhibitors overcome resistance due to acquired PIK3CA mutations

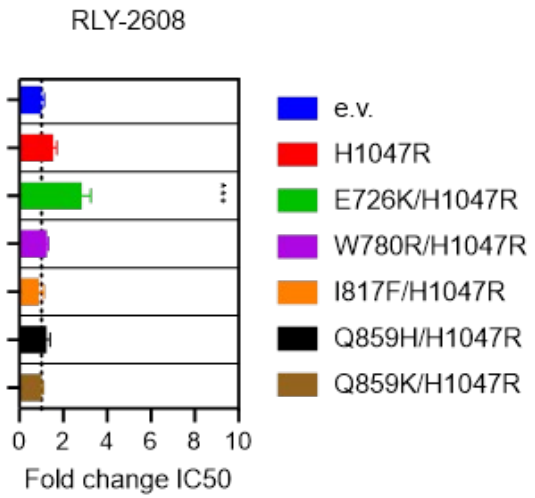
RLY-2608:
Pan-mutant selective allosteric PIK3CA inhibitor











Varkaris et al. Cancer Discovery 2023

Conclusions

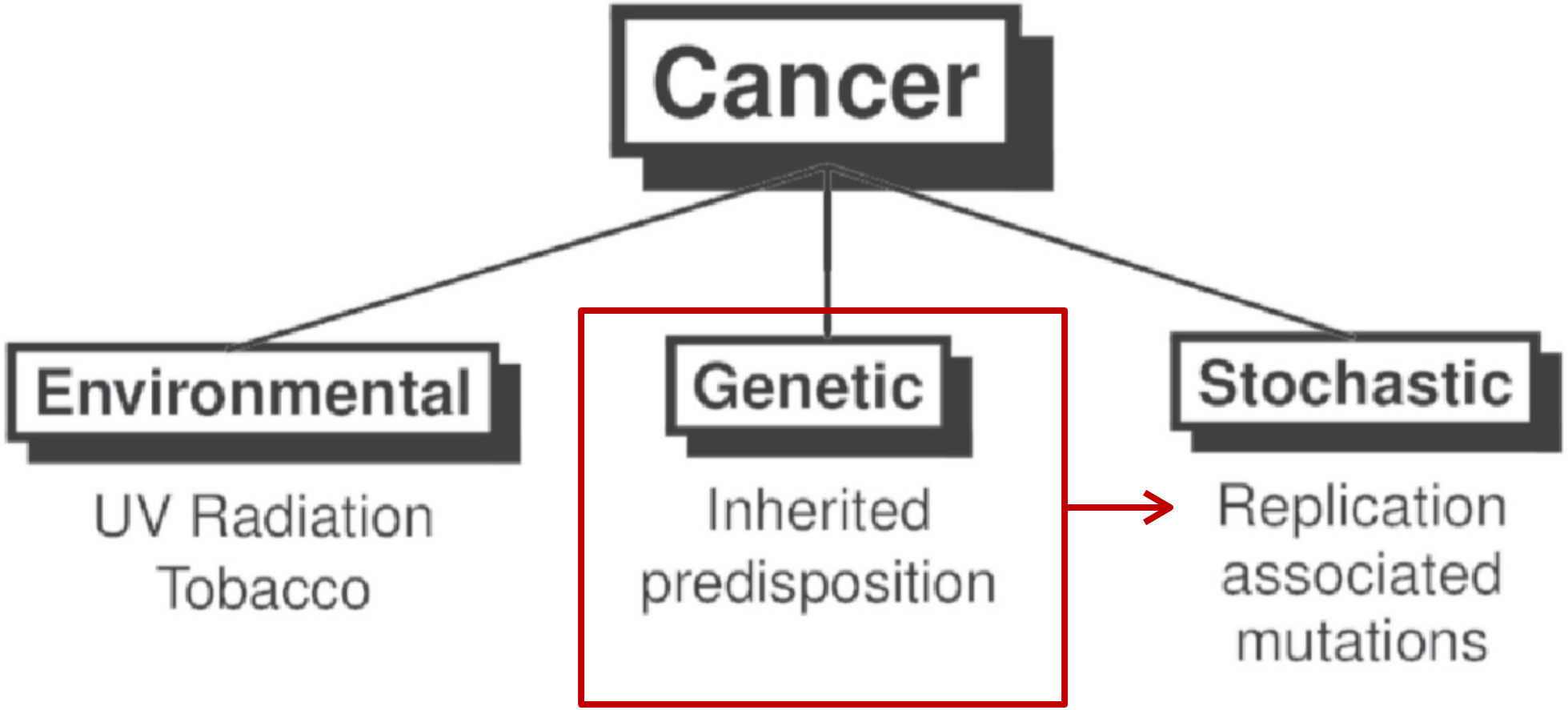
- Reactivation of PI3K signaling represents a dominant mode of acquired resistance to alpelisib and inavolisib, present in nearly half of patients and involving acquired PTEN loss, activating AKT1 mutations and secondary PIK3CA mutations.
- Acquired secondary PIK3CA mutations drive resistance by altering affinity of alpelisib and inavolisib for PI3K-alpha.
- Novel allosteric PI3K inhibitors and AKT inhibitors can overcome resistance driven by these acquired PIK3CA alterations.

Varkaris et al, SABCS 2023, GS03-10

Topic n°5

The germline influences somatic evolution and molecular subtypes via immunoediting

Does inherited variation bound the likelihood of specific stochastic mutations?



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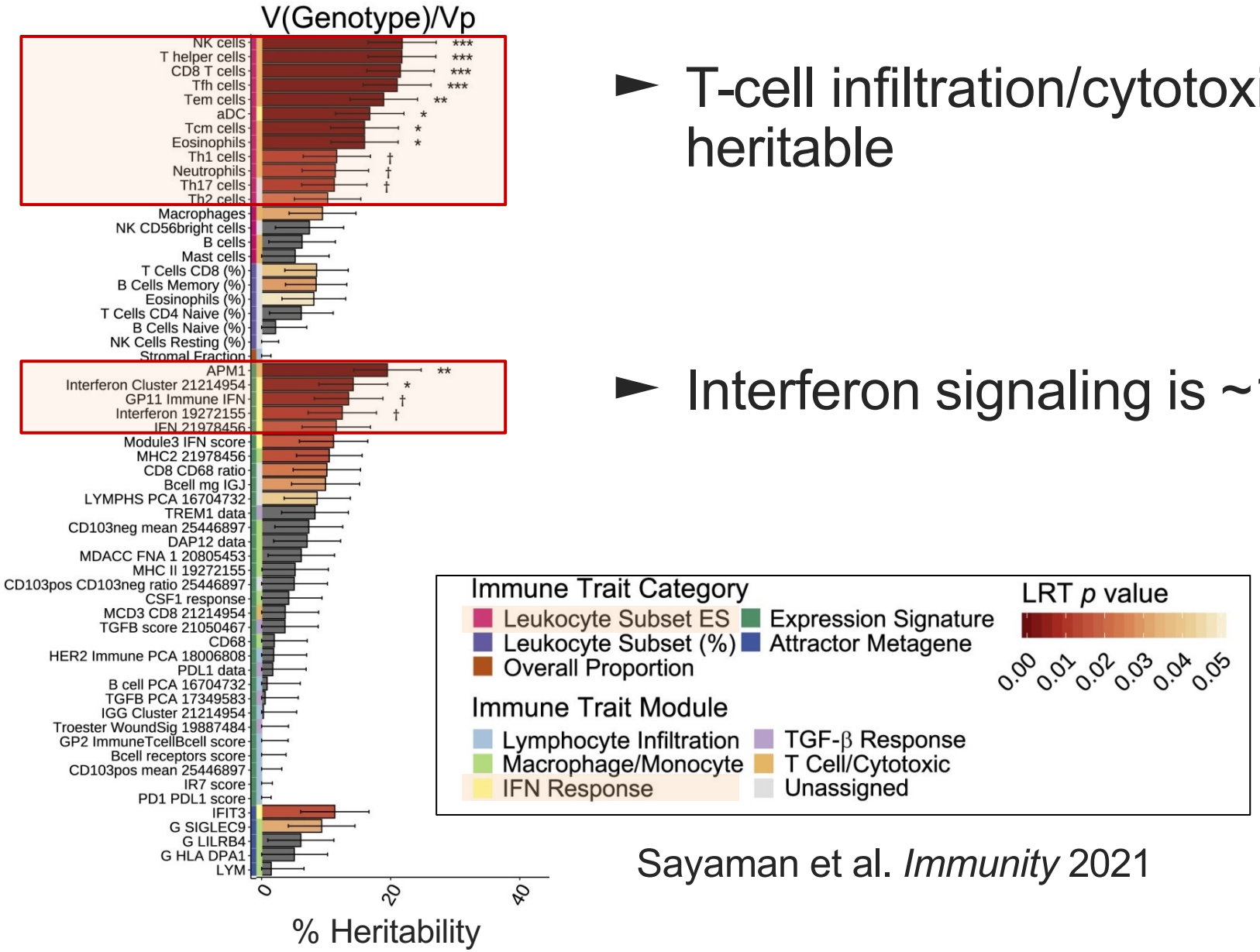
Curtis et al, SABCS 2023, GS03-11

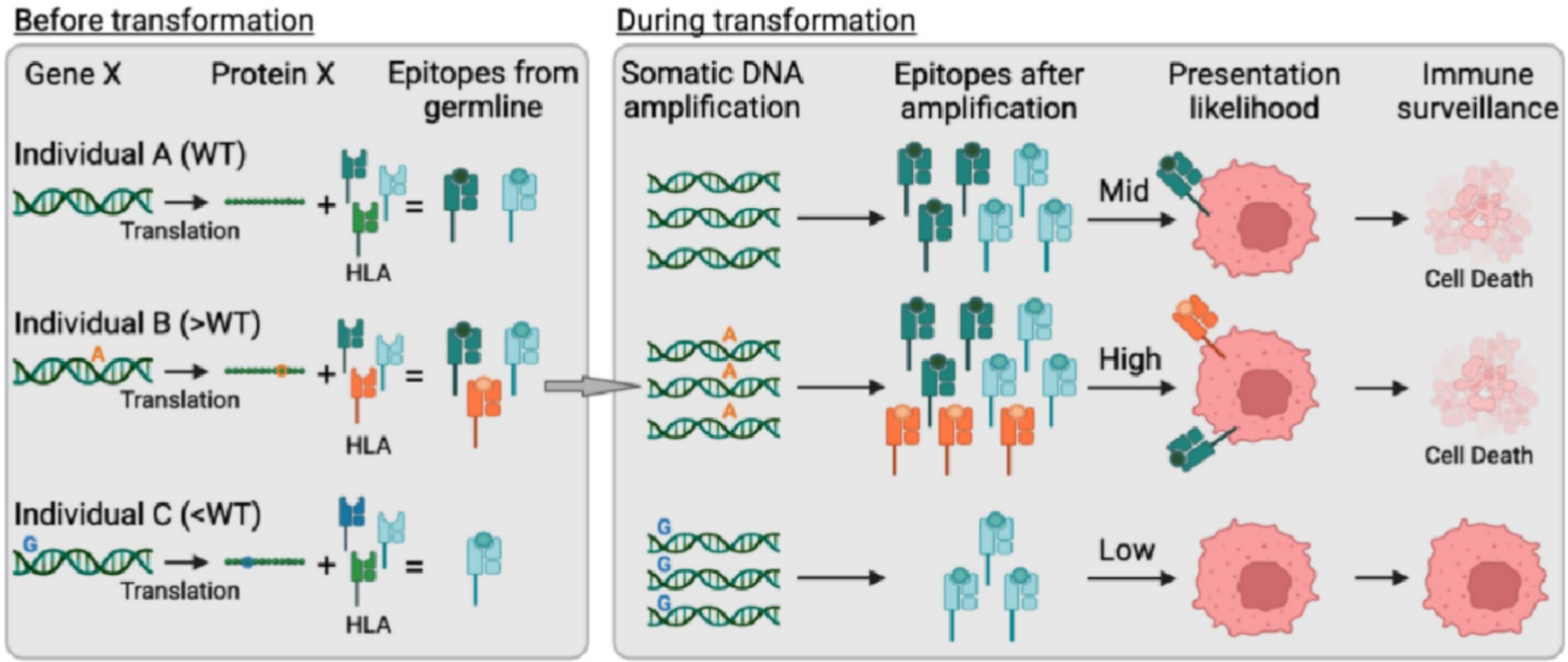
The germline genome modulates immune responses



- T-cell infiltration/cytotoxicity is ~15-20% heritable
- Interferon signaling is ~15% heritable

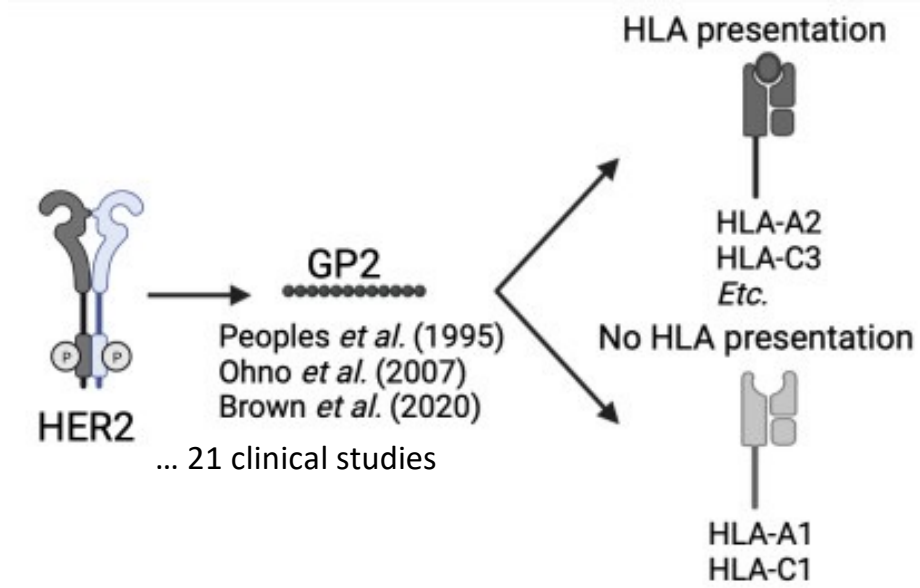
Sayaman et al. *Immunity* 2021

Hypothesis: Germline-derived epitope burden in oncogenes selects against oncogene amplification

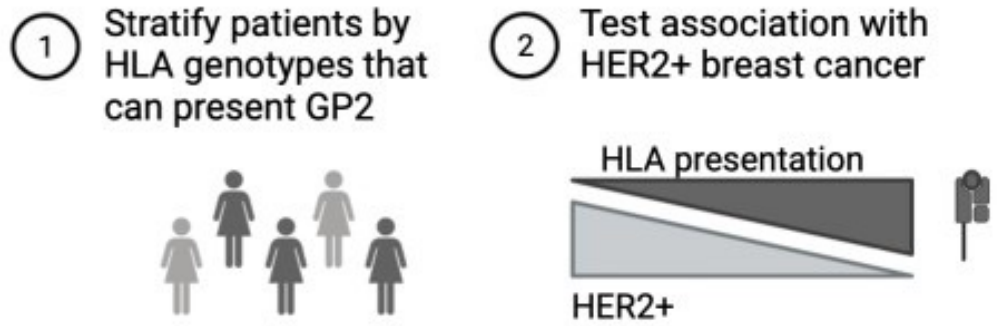


Curtis et al, SABCS 2023, GS03-11

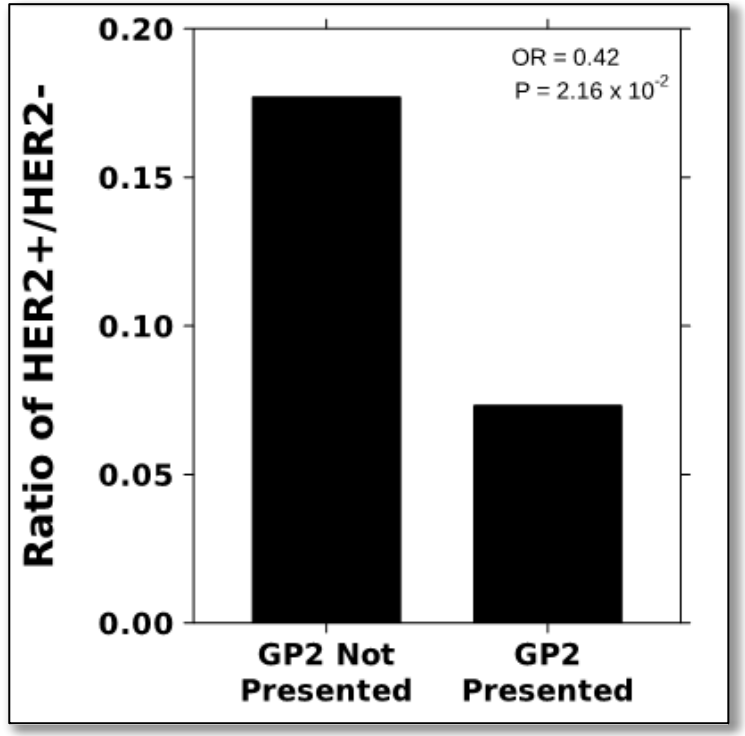
Proof of concept: HER2-derived GP2 peptide



HLA alleles that can bind GP2 GP2 “presented”

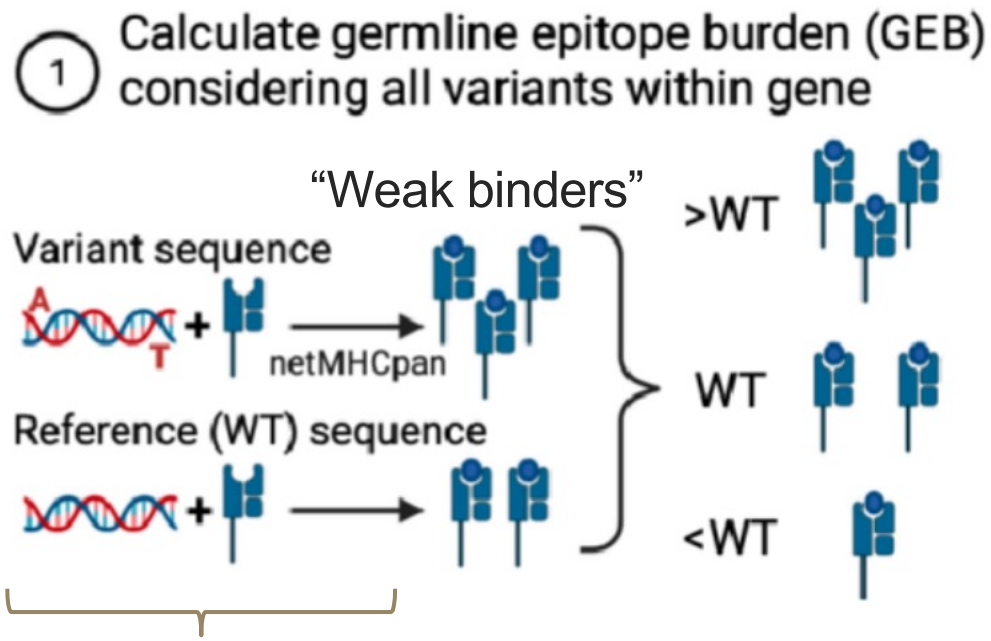


TCGA



- GP2: 9 amino acid, non-mutated immunogenic peptide (others E75, AE37, HLA-A2^{+} and HLA-A3^{+})
- Does ability to “present” GP2 associate with Her2 subtype?
- Yes, individuals with high burden of germline-derived GP2 are less likely to develop HER2+ disease

Predicting germline epitope burden (GEB)



Blood/non-malignant sample

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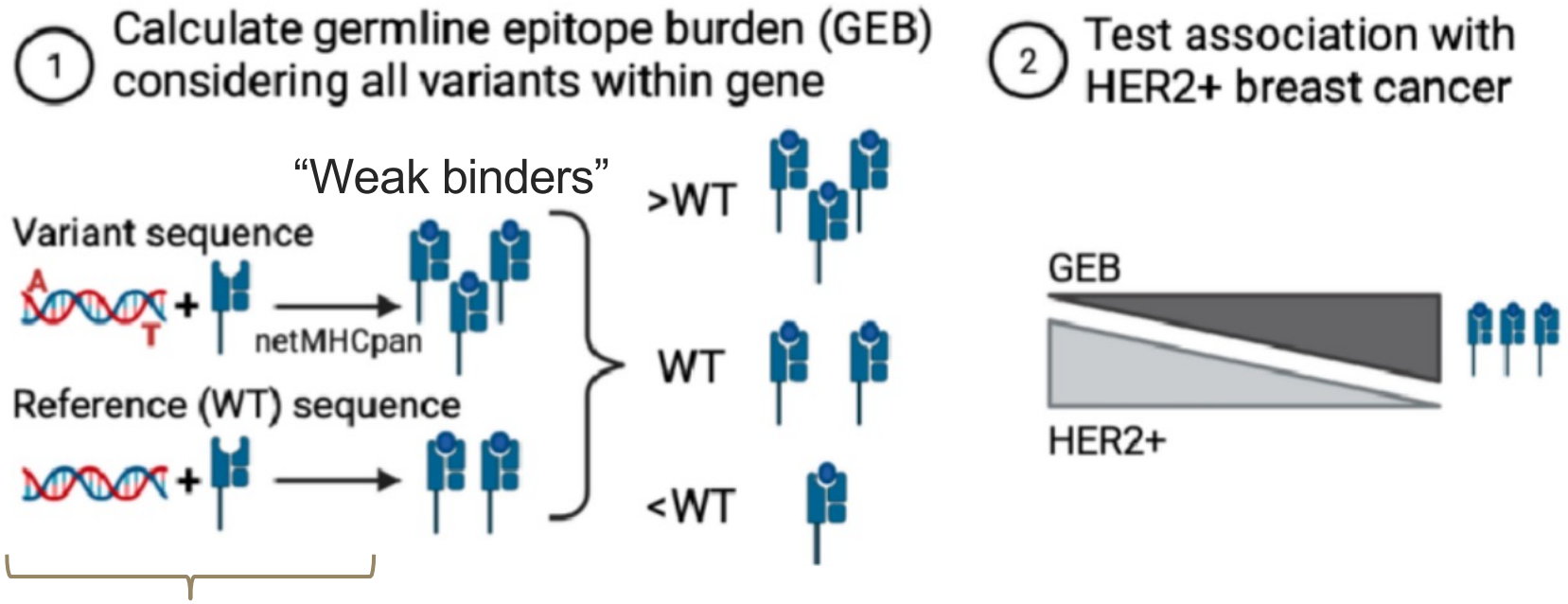
Predicting germline epitope burden (GEB)



Blood/non-malignant sample

Considered individuals of European descent
Corrected for six genetic principal components & somatic mutation burden

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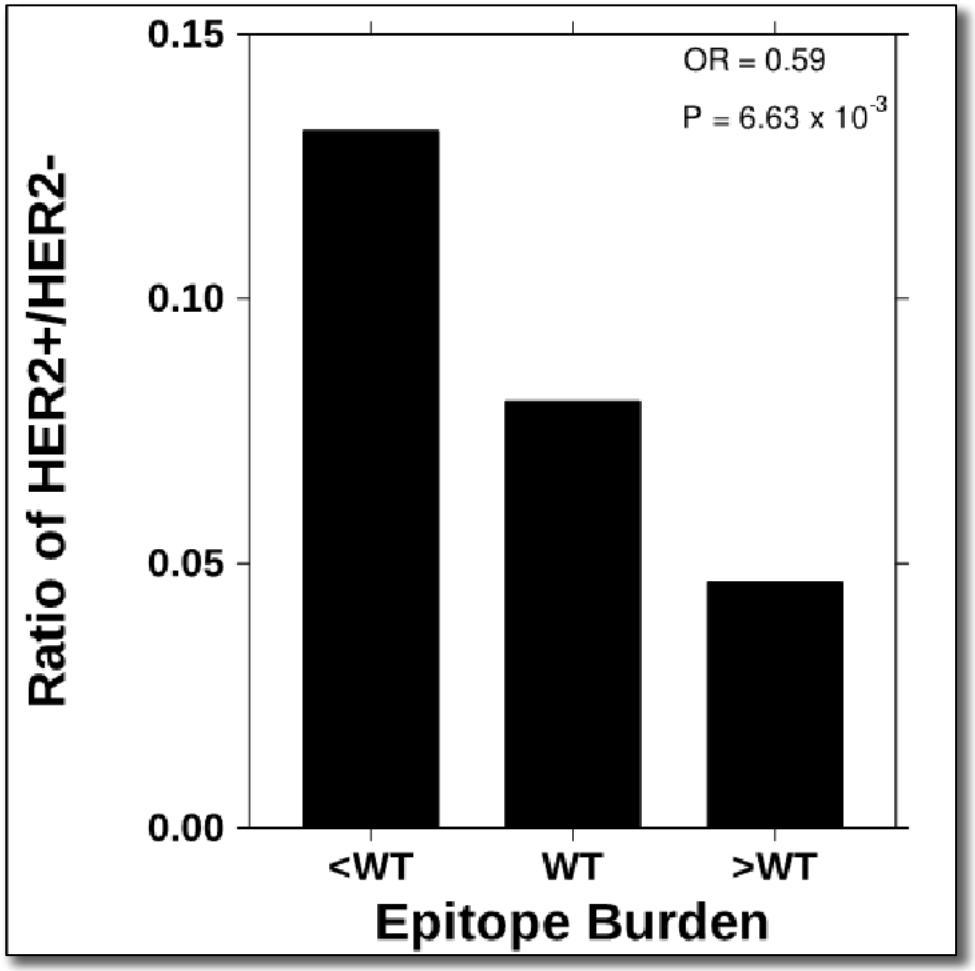
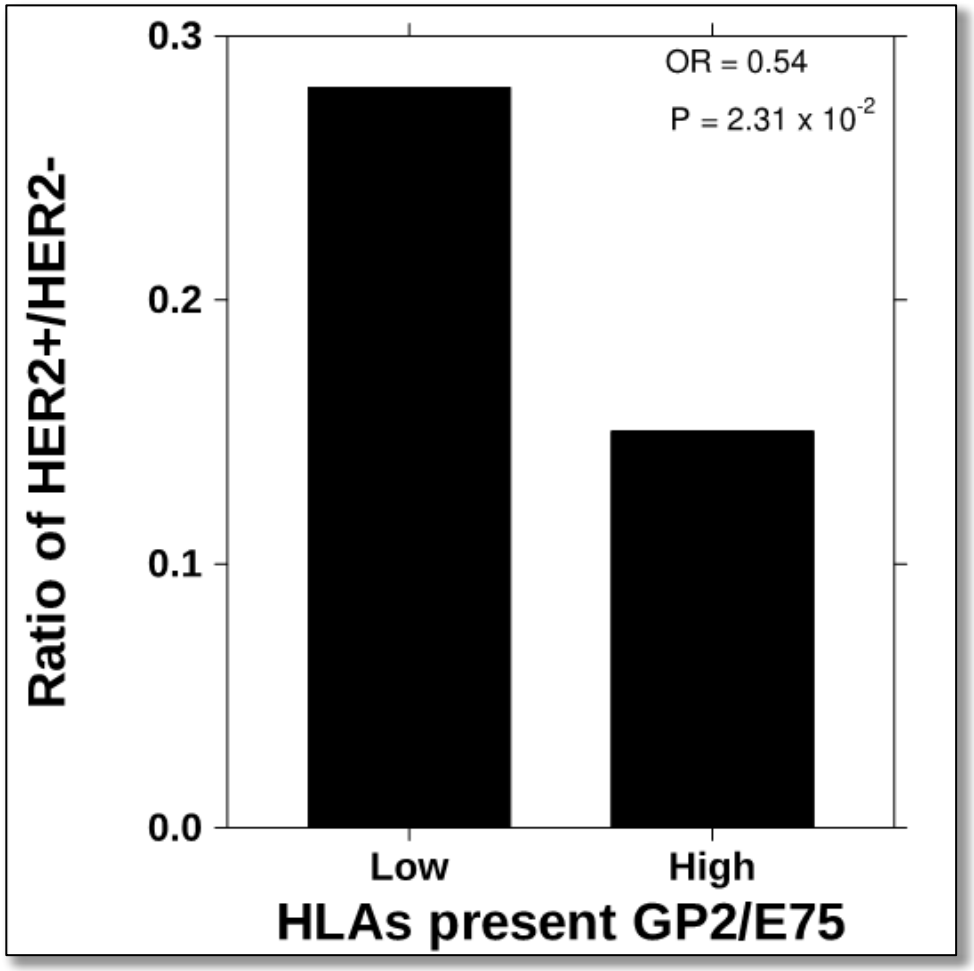
Germline epitope burden (GEB) in ERBB2 is negatively associated with HER2+ breast cancer

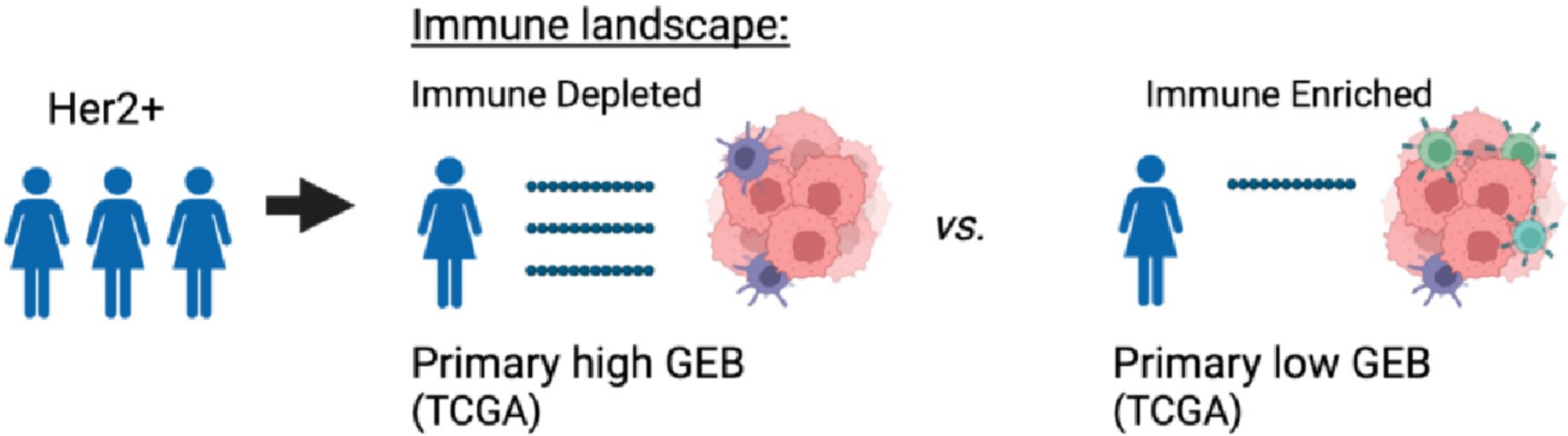
TCGA



TCGA



Why do tumors with high germline epitope burden (GEB) still acquire somatic amplification?



Early pressure to develop an immunosuppressive phenotype, leads to more aggressive disease

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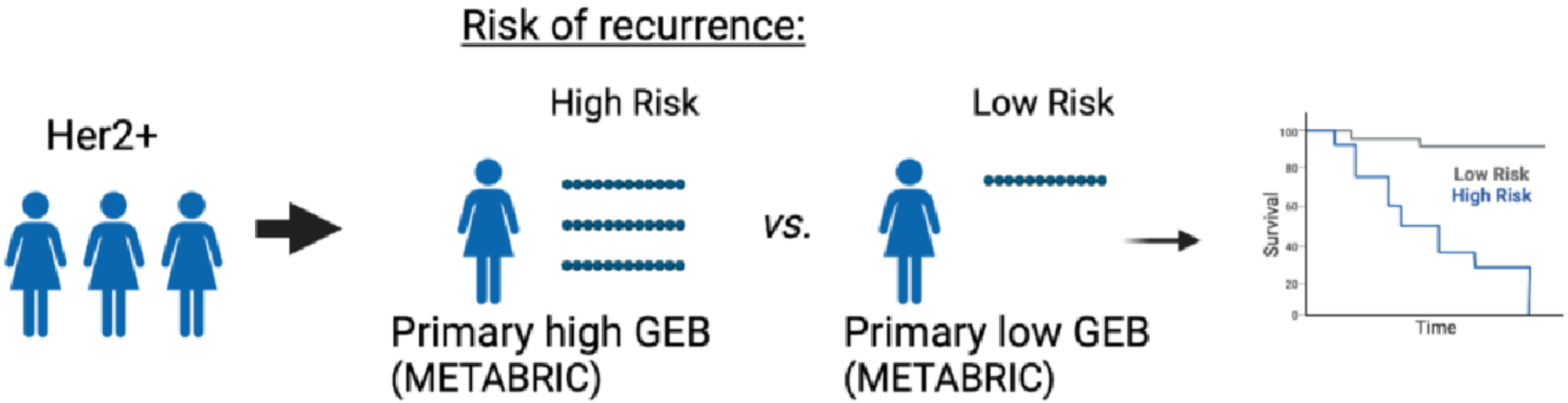
Is germline epitope burden (GEB) prognostic?



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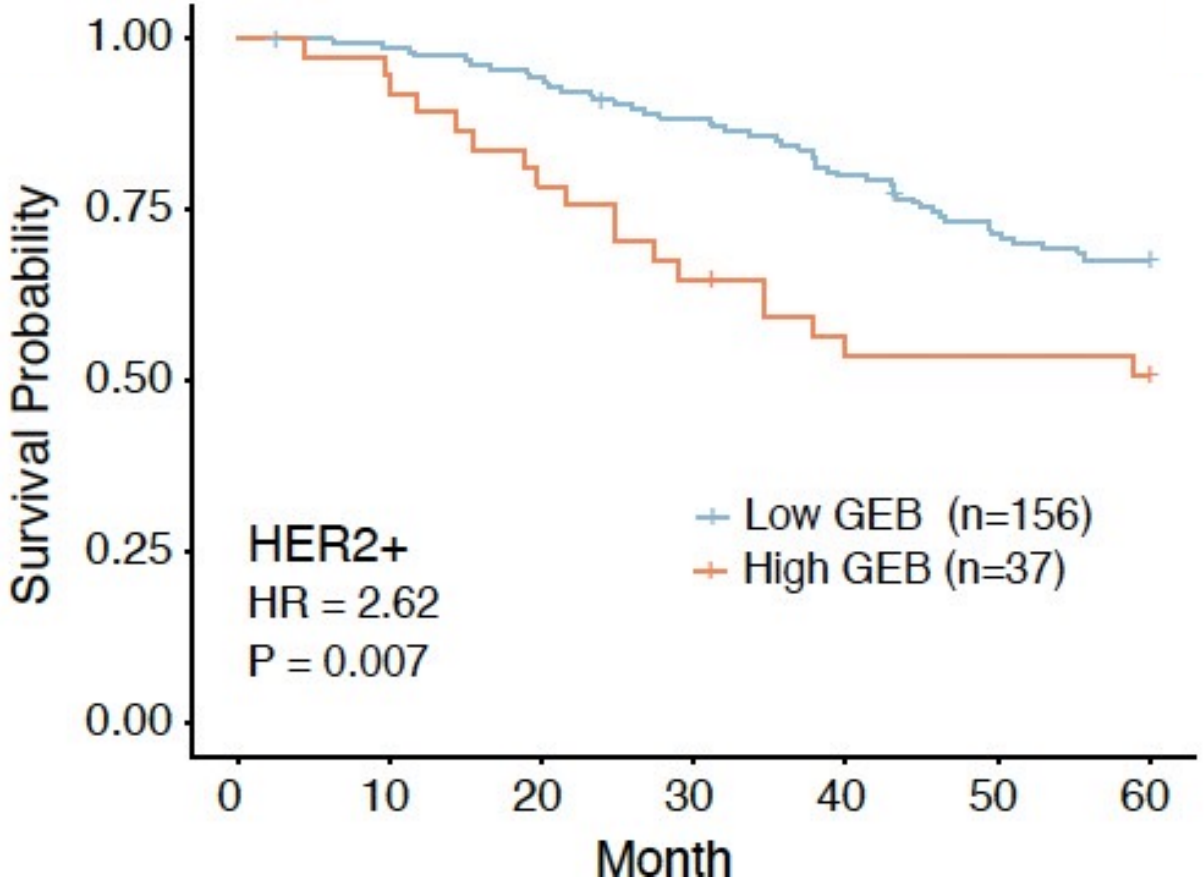
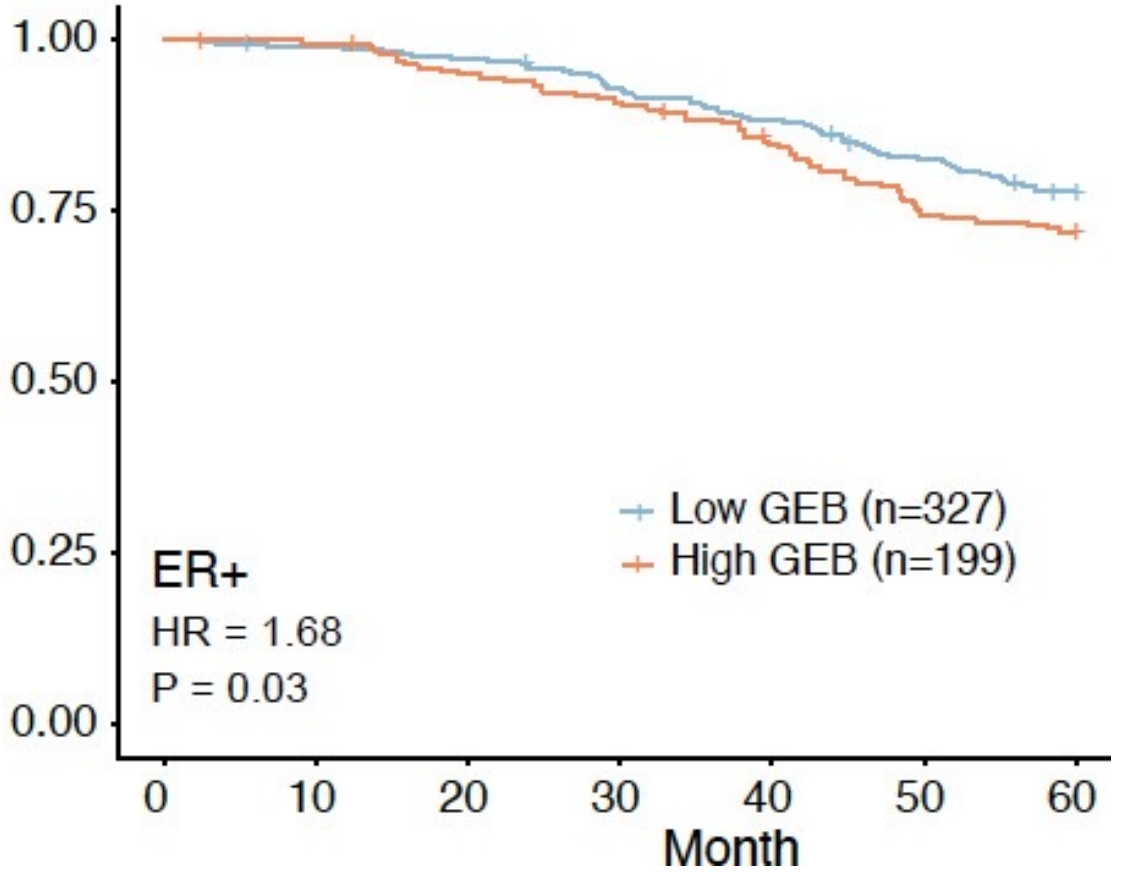
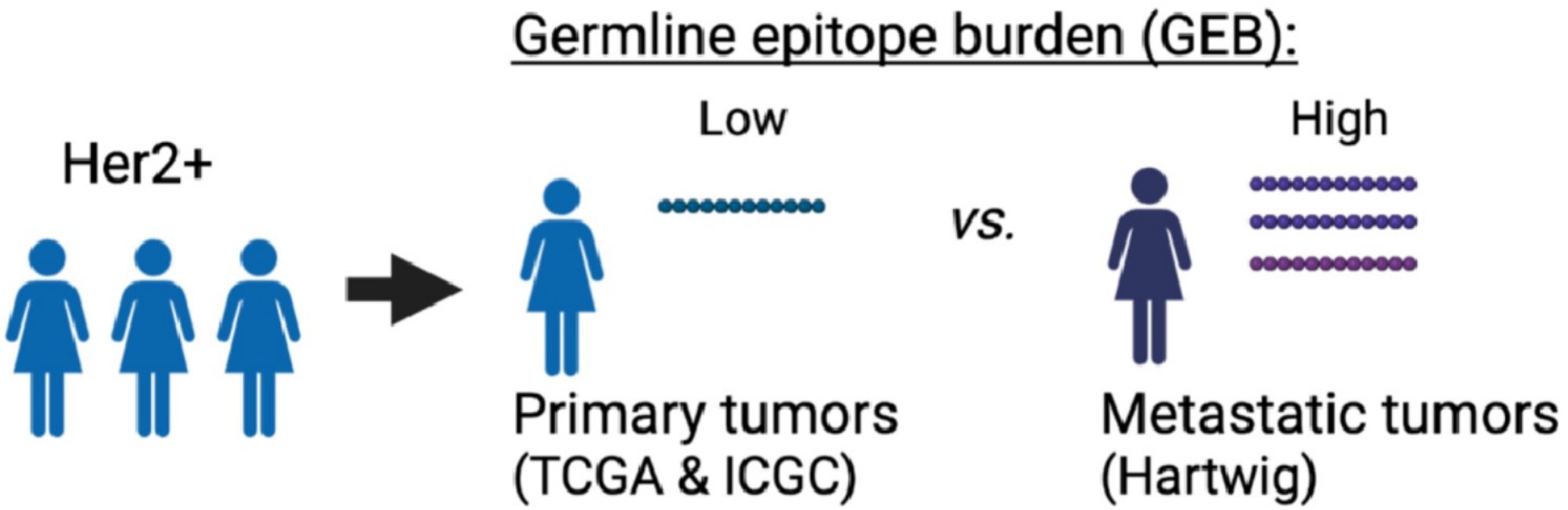
Tumors that overcome a high germline epitope burden (GEB) are more aggressive





Do metastatic tumors have higher germline epitope burden (GEB) than primary tumors?



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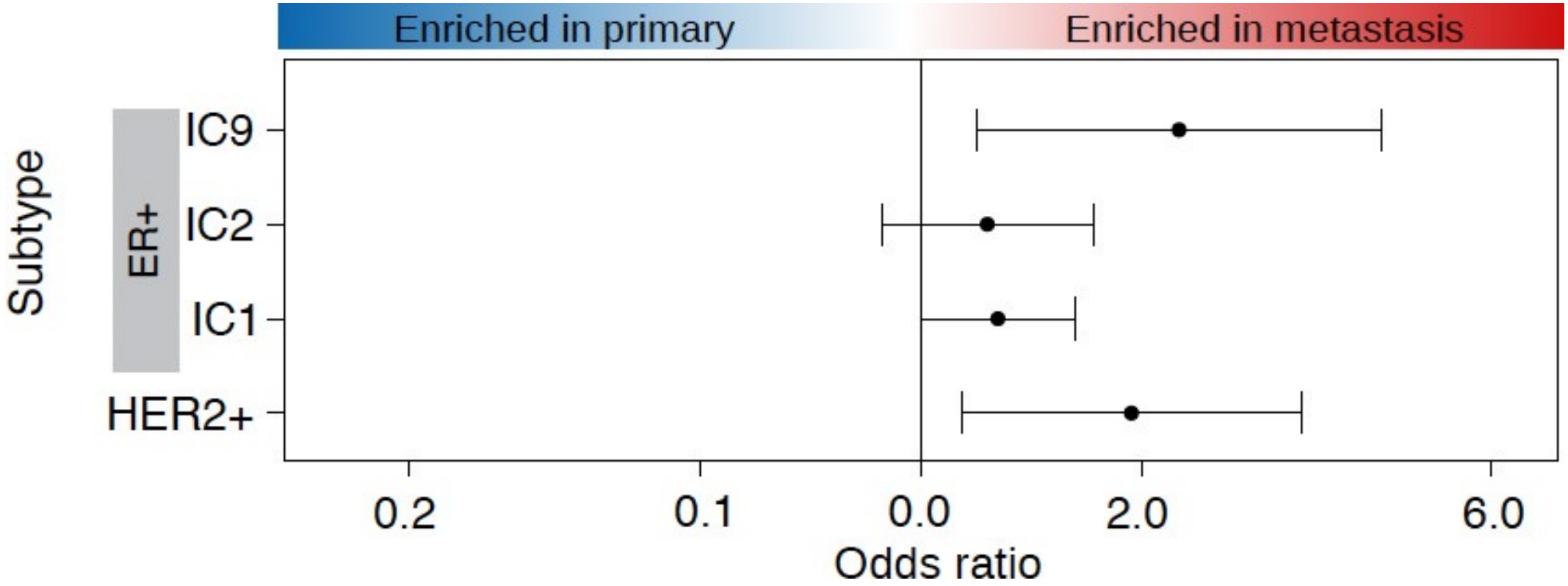
Metastatic tumors have higher germline epitope burden (GEB) than primary tumors



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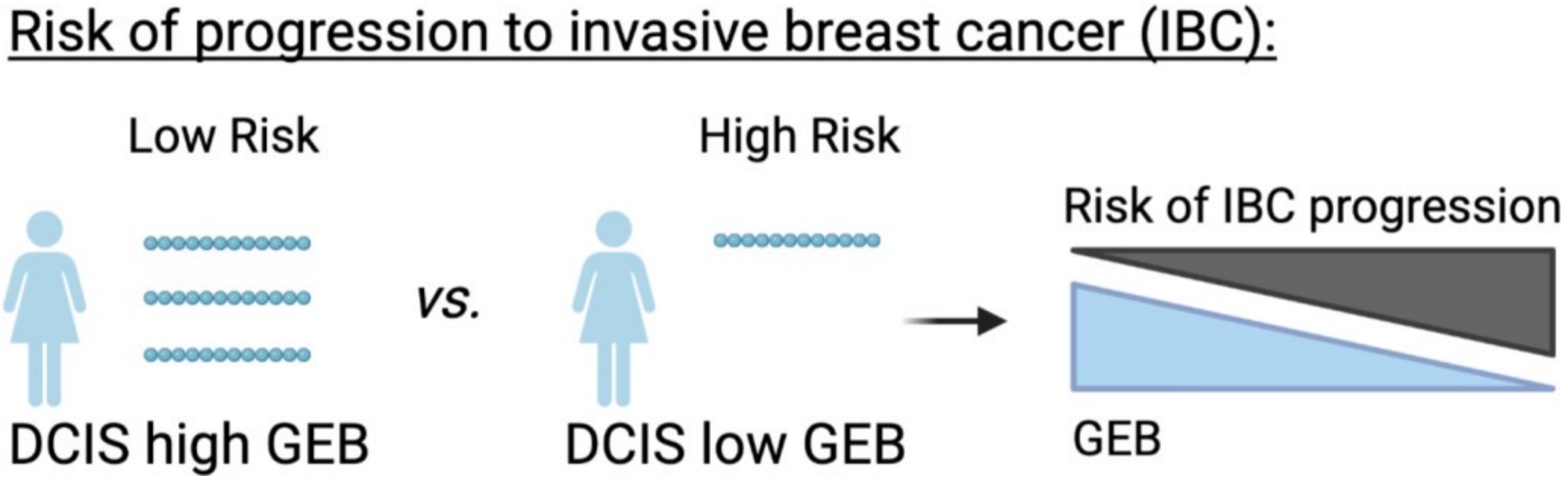
Is germline epitope burden (GEB) associated with progression from DCIS to invasive breast cancer (IBC)?



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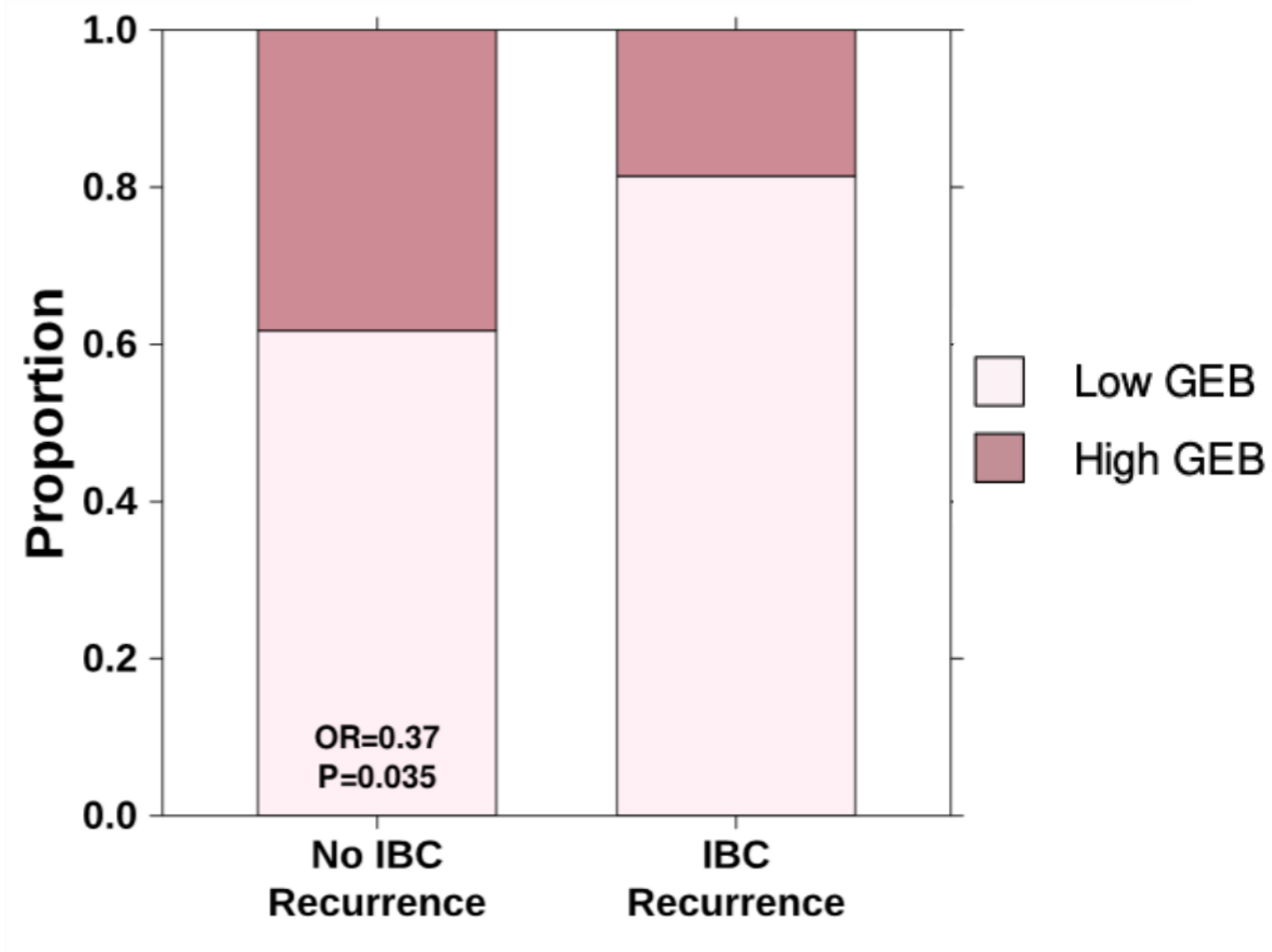
Low germline epitope burden (GEB) is associated with progression to invasion



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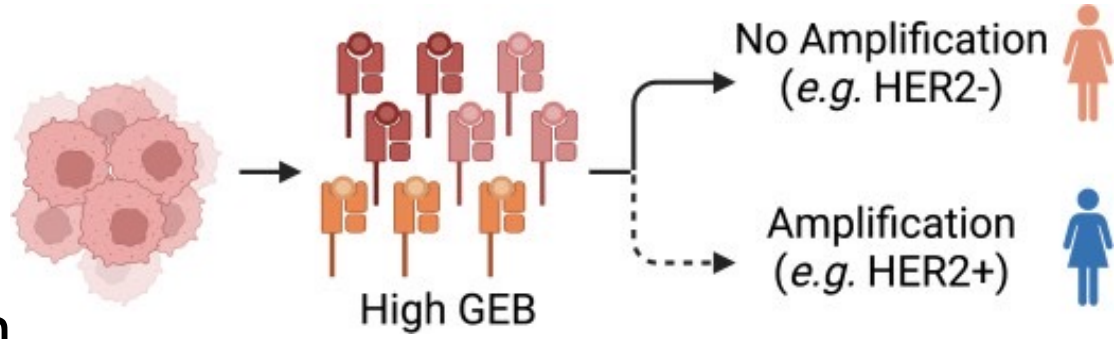
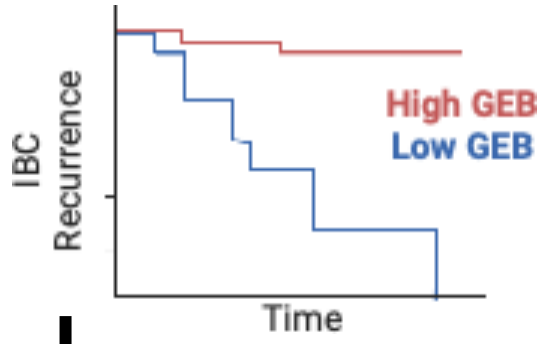
Summary

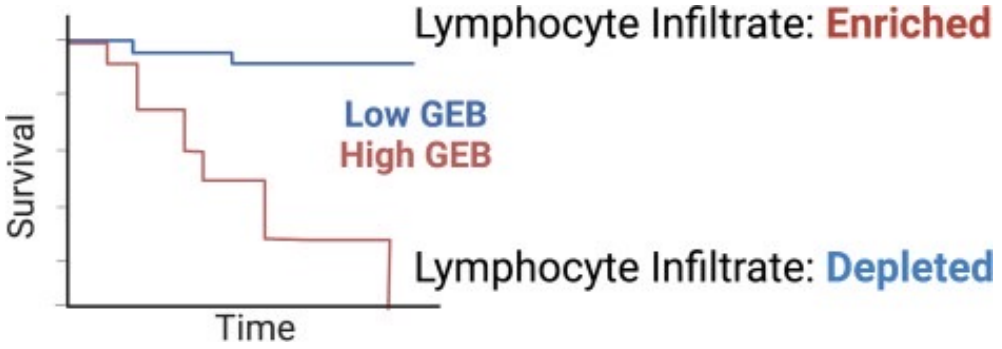


- The germline influences somatic evolution and molecular subtypes via immunoediting
- High GEB is associated with reduced likelihood of addiction to that oncogene
- Pressure to develop immune evasive phenotypes early leads to aggressive disease
  - In DCIS, high GEB protects against IBC
  - In IBC, high GEB portends worse prognosis
- Our findings uncover a broad source of underappreciated immunogenic antigens

Pre-cancerous stage (DCIS)



Invasive breast cancer (IBC)



Topic n°6

INSTITUT ROI ALBERT II
Cliniques universitaires SAINT-LUC
UCLouvain BRUXELLES

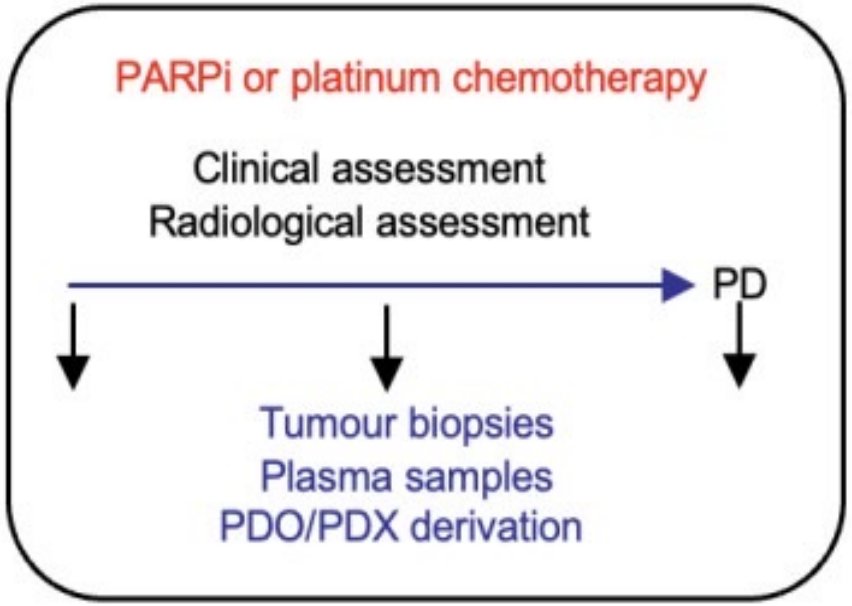
Biomarkers of PARPi resistance

Study outline

Real world data***

PARPi/Pt resistant
Metastatic breast cancer ER+ HER2- or TNBC

BRCA1m n=23 *BRCA2m* n=23 *PALB2m* n=1



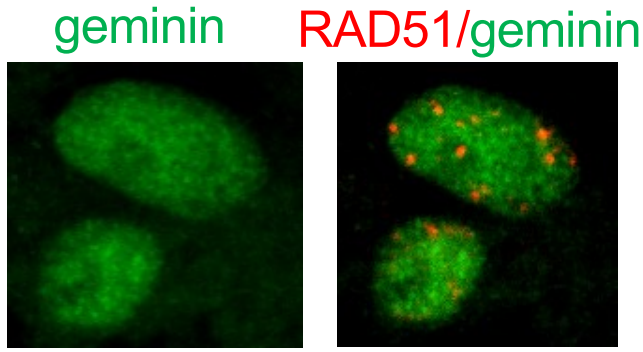
Predictive Biomarkers

1. Presence of a germline mutation in *BRCA1* or *BRCA2*
2. HRD* mutational signature analysis + HRD score

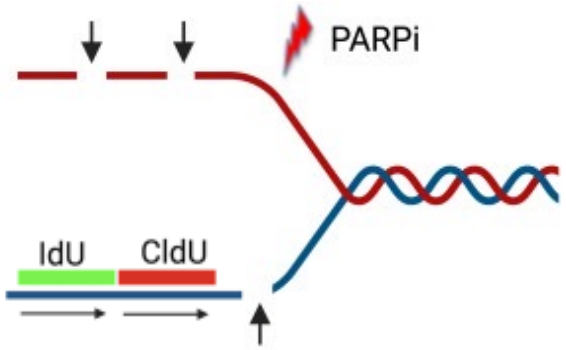
Limitations!

Functional analyses of HRR** proficiency- ?biomarkers of PARPi resistance

RAD51 nuclear foci analysis



DNA replication fork dynamics and stability



*HRD - homologous recombination deficiency - **HRR – homologous recombination repair

***Presented at AACR 2023: Poster 6094 - Longitudinal analysis of PARP inhibitor and platinum resistance in *BRCA1/2m* breast cancer using liquid biopsy Harvey-Jones et al.

Functional RAD51 restoration is associated with PARPi resistance

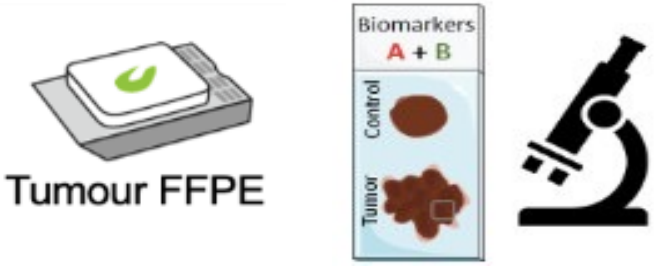
Restoration of HRR is a preclinically validated mechanism of resistance

Mechanisms of HRR restoration include:

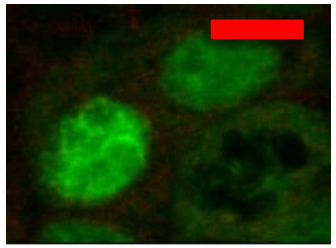
- Secondary reversion mutations in *BRCA1/2*
- Defects in the 53BP1-Shieldin complex
- BRCA hypomorph expression

Lead to restoration of RAD51 nuclear foci

RAD51 assay

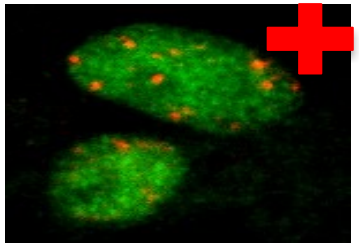


geminin



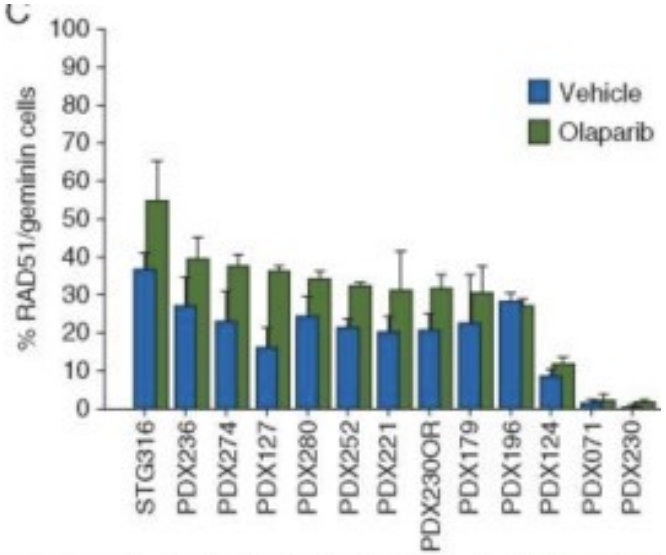
RAD51-Low HRD

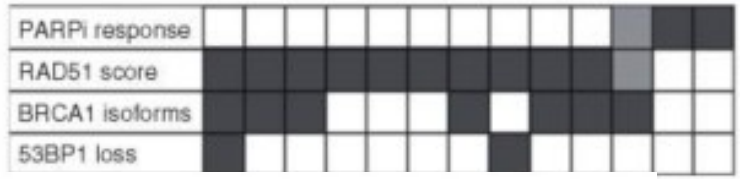
RAD51/geminin

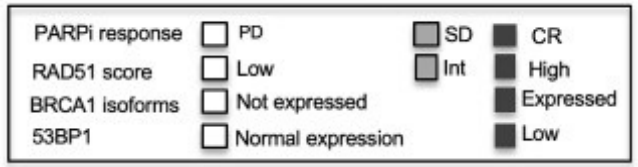


RAD51-high HRP

Evidence in PDX

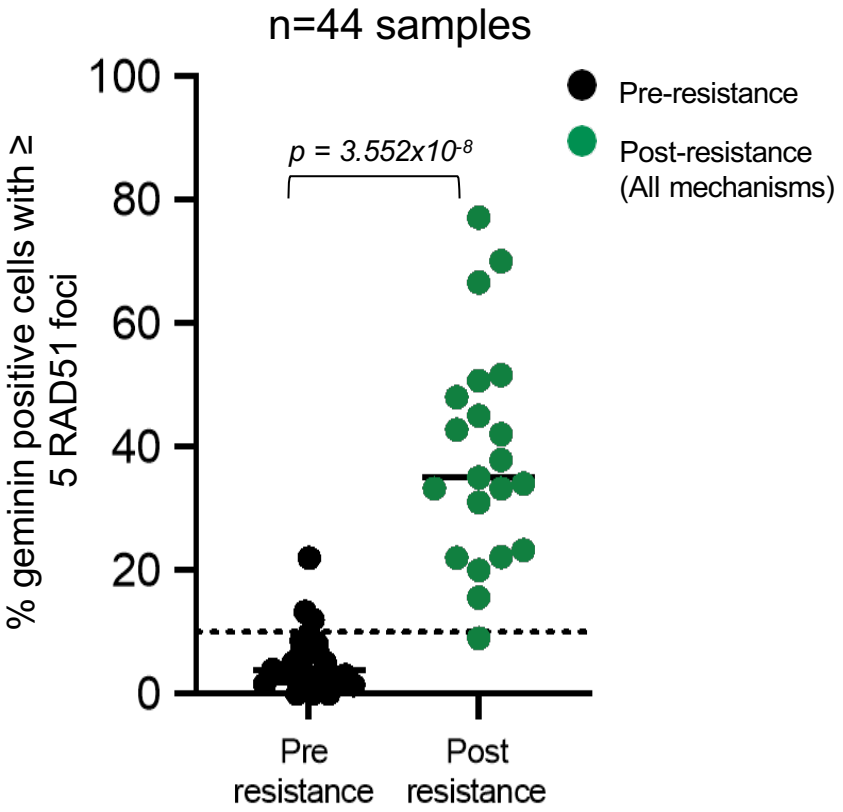






Cruz et al 2018; Castroviejo-Bermejo et al 2018; Pellegrino et al, 2022

Restoration of RAD51 function after PARPi resistance



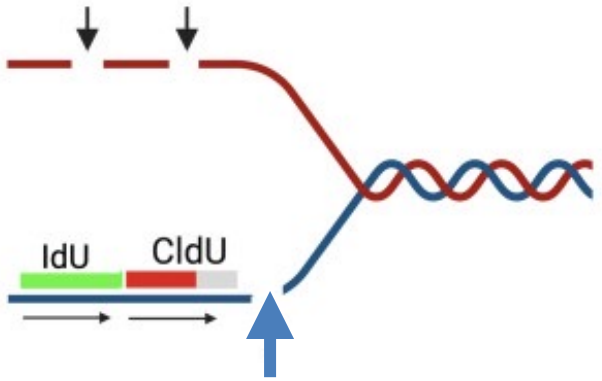
- RAD51 function analysed in geminin +ve cells
- 44 samples (29 patients)
  - 23 samples pre resistance
  - 21 samples post resistance
- Paired samples in 12 patients

High frequency of RAD51 restoration in real world practice

Harvey-Jones et al, SABCS 2023, RF01-05

Higher replication fork stability to HU induced SSBs can be seen with resistance to olaparib in breast cancer PDOs

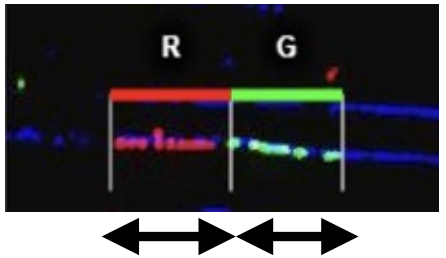
IdU and CldU are used as sequential labels and detected by fluorescently labelled antibodies



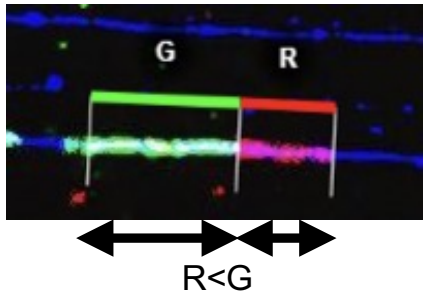
Hydroxyurea (HU) induces replication fork stalling

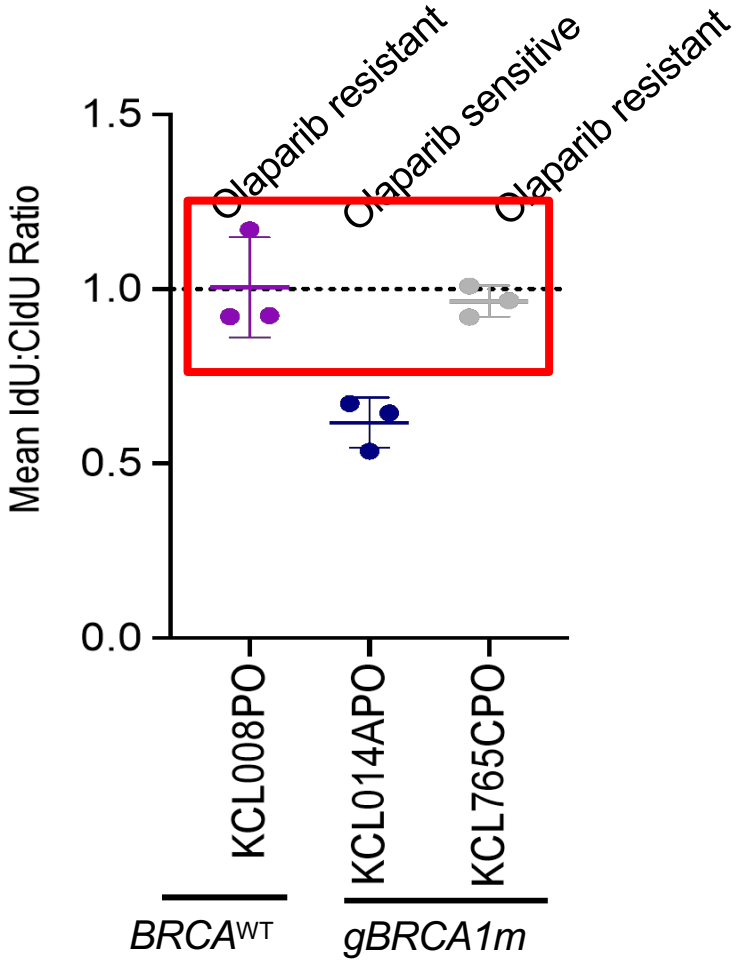
Intrinsic fork stability gives a read out of replication fork protection -> predictive of response to treatment

STABLE
Ratio ~1



UNSTABLE
Ratio <1





Summary

- HRR proficiency assessed by RAD51 foci is a potential dynamic biomarker of HRD-targeted therapy resistance
- Restoration of HRR upon resistance to HRD-targeted treatment results in change in RAD51 foci, but HRD scars do not reverse upon resistance
- Replication fork dynamics and stability measured by DNA fibre analysis can be measured in relation to therapy interventions in breast cancer PDOs
- This could be explored as a biomarker of PARPi resistance

Topic n°7

Does the *BRCA1/2* mutation status have an impact on OS?

Introduction and Methods

- Overall survival of germline pathogenic variant (PV) carriers after breast cancer diagnosis has not been adequately investigated in population-based studies

- The CARRIERS Study[1] is a population-based case-control study:

16,797 women with loco-regional invasive breast cancer within the CARRIERS Study who underwent surgery

Inclusion:
-At least 1 year of follow-up
Exclusion:
- DCIS at initial diagnosis

Comparison:
- PV carriers: 5 genes[2]
- Non-carriers: Negative for 12 known breast cancer predisposition genes[3]

- Time-to-event analysis comparing OS between carriers in each gene vs. non-carriers
- Multivariable Cox proportional hazard regression analysis adjusting for:
  - Age and menopausal status at diagnosis, Race/ethnicity, ER status of the tumor, Type of surgery, Use of radiation, chemotherapy, and endocrine agents, and Prophylactic oophorectomy
  - Censored at second primary cancer (except for non-melanoma skin cancer)
- Subset OS analysis by ER status and Race/ethnicity adjusting for relevant covariates

[1]*Hu C.. Yadav S.. Couch FJ et. al. N Engl J Med 2021;384:440-451;*
[2]*ATM, BRCA1, BRCA2, CHEK2* and *PALB2;*
[3]*ATM, BARD1, BRCA1, BRCA2, BRIP1,CDH1,CHEK2, PALB2, PTEN, RAD51C, RAD51D* and *TP53;*

Results – Baseline Characteristics

	N=16,797 (%)
Median age at diagnosis	61 years
Race/ethnicity:	
Non-Hispanic White	10,392 (65%)
Black	2,364 (14%)
Post-menopausal status*	10,571 (63%)
ER-positive breast cancer*	12,780 (76%)
Ductal histology*	11,569 (69%)

Median follow-up duration = 10 years

Gene	Total (n)	Deaths (n)
Non-carriers	15,906	4,694
ATM	142 (0.8%)	33
BRCA1	206 (1.2%)	38
BRCA2	260 (1.5%)	46
CHEK2	167 (1.0%)	31
PALB2	116 (0.7%)	36
Total		**4,878**

Yadav et al, SABCS 2023, PS10-02

Results

Gene	Overall		ER-positive		ER-negative	
	HR (95%CI)	p-value	HR (95%CI)	p-value	HR (95%CI)	p-value
No PV	Reference		Reference		Reference	
ATM	1.09 (0.78 – 1.55)	0.59	1.17 (0.79 – 1.73)	0.44	0.72 (0.27 – 1.93)	0.52
BRCA1	1.22 (0.88 – 1.69)	0.22	1.53 (0.93 – 2.51)	0.09	1.00 (0.63 – 1.59)	0.99
BRCA2	1.09 (0.81 – 1.47)	0.54	1.51 (1.06 – 2.16)	0.02	0.63 (0.36 – 1.12)	0.12
CHEK2	1.01 (0.71 – 1.44)	0.95	1.11 (0.76 – 1.63)	0.58	0.65 (0.20 – 2.03)	0.45
PALB2	1.36 (0.98 – 1.89)	0.06	1.28 (0.83 – 1.97)	0.26	1.53 (0.86 – 2.71)	0.15

Conclusions:

- *BRCA1* and *BRCA2* PV carriers with ER+ breast cancer may have worse OS compared to non- carriers
- *CHEK2* and *ATM* carriers have similar OS as non-carriers

Yadav et al, SABCS 2023, PS10-02

Discussion

- Very large dataset; previously not reported information
- Worse OS in ER+ BRCA2 mutation carriers : CDK4/6 inhibitors have different benefit in BRCA PV carriers?
- Relevance for today? PARPi use
- Stage and grade at diagnosis not included in the analysis
- Screening detected cancers and outcome?

Topic n°8

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Pregnancy after breast cancer in *BRCA1/2* mutation carriers

Background

- A substantial proportion of young women with newly diagnosed breast cancer are interested in future fertility[1]
- More than 12% of young women with breast cancer carry a germline pathogenic variant in the *BRCA1* or *BRCA2* genes[2]
- Additional challenges should be considered in the reproductive counseling of *BRCA* carriers:
  - The psychological fear of transmitting the pathogenic variant to their offspring[3]
  - The possible negative impact of deficient BRCA function on ovarian reserve and fertility potential[4]
  - The indication to undergo risk-reducing bilateral salpingo-oophorectomy at a young age[5]
- While several studies have demonstrated the safety of conceiving following breast cancer diagnosis and treatment, the evidence in *BRCA* carriers is very limited[6]

1. Ruddy KJ et al, *J Clin Oncol* 2014;32(11):1151-6
2. Copson ER et al, *Lancet Oncol* 2018;19(2):169-80
3. Fine E et al, *JCO Oncol Pract* 2022;18(3):165-8
4. Turan V et al, *J Clin Oncol* 2021;39(18):2016-24
5. Sessa C et al, *Ann Oncol* 2023;34(1):33-47
6. Lambertini M et al, *J Clin Oncol* 2021;39(29):3293-305

Study Design and Participants

- International, multicenter, hospital-based, retrospective cohort study

Key inclusion criteria

- Stage I - III invasive breast cancer
- Diagnosis between January 2000 and December 2020
- Age ≤ 40 years at diagnosis
- Known germline likely pathogenic or pathogenic variants in *BRCA1* and/or *BRCA2* genes

Key exclusion criteria

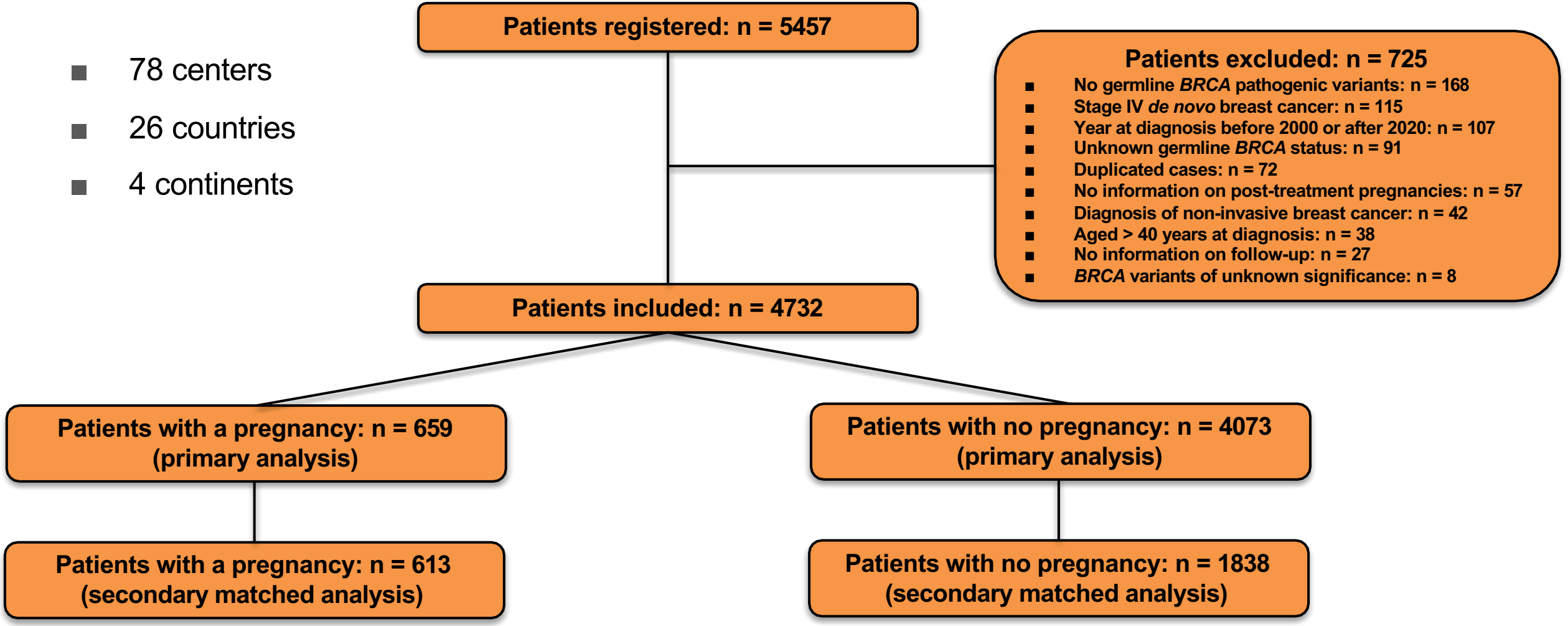
- Stage IV *de novo* breast cancer
- Lack of data on follow-up or post-treatment pregnancies
- History of ovarian cancer or other malignancies without prior breast cancer
- *BRCA* VUS or *BRCA* healthy carriers

ClinicalTrials.gov Identifier: NCT03673306

Lambertini et al, SABCS 2023, GS02-13

Participant Flow

- 78 centers
- 26 countries
- 4 continents



Median follow-up: 7.8 years (IQR 4.5 – 12.6 years) Lambertini et al, SABCS 2023, GS02-13

Participant and Treatment Characteristics

Key participant characteristics at breast cancer diagnosis

	Patients with a pregnancy n = 659, N (%)	Patients with no pregnancy n = 4073, N (%)
Region:		
Southern Europe	303 (46.0)	1777 (43.6)
Asia	130 (19.7)	650 (16.0)
Northern Europe	110 (16.7)	599 (14.7)
North America	59 (9.0)	460 (11.3)
Eastern Europe	22 (3.3)	282 (6.9)
Australia/Oceania	26 (3.9)	167 (4.1)
Latin/South America	9 (1.4)	138 (3.4)
Year at diagnosis:		
2000 – 2004	106 (16.1)	498 (12.2)
2005 – 2008	141 (21.4)	647 (15.9)
2009 – 2012	170 (25.8)	835 (20.5)
2013 – 2016	159 (24.1)	999 (24.5)
2017 – 2020	83 (12.6)	1094 (26.9)
Age at diagnosis, median (IQR) years	30 (28 – 33)	35 (32 – 38)
Specific *BRCA* gene		
BRCA1	483 (73.3)	2550 (62.6)
BRCA2	170 (25.8)	1493 (36.7)
BRCA1 and *BRCA2*	3 (0.5)	23 (0.6)
BRCA, unknown if 1 or 2	3 (0.5)	7 (0.2)
Tumor size:		
T1 (≤ 2 cm)	282 (44.8)	1529 (39.5)
T2 (>2 – ≤ 5 cm)	270 (42.9)	1780 (46.0)
T3 (> 5 cm) - T4	77 (12.2)	562 (14.5)
Unknown	30	202
Nodal status:		
N0	399 (62.5)	2035 (52.1)
N1	180 (28.2)	1376 (35.2)
N2 – N3	59 (9.3)	497 (12.7)
Unknown	21	165
Hormone receptor status:		
ER and/or PR positive	216 (33.3)	1910 (47.7)
ER and PR negative	432 (66.7)	2097 (52.3)
Unknown	11	66
HER2 status:		
HER2 negative	589 (94.2)	3562 (92.2)
HER2 positive	36 (5.8)	303 (7.8)
Unknown	34	208

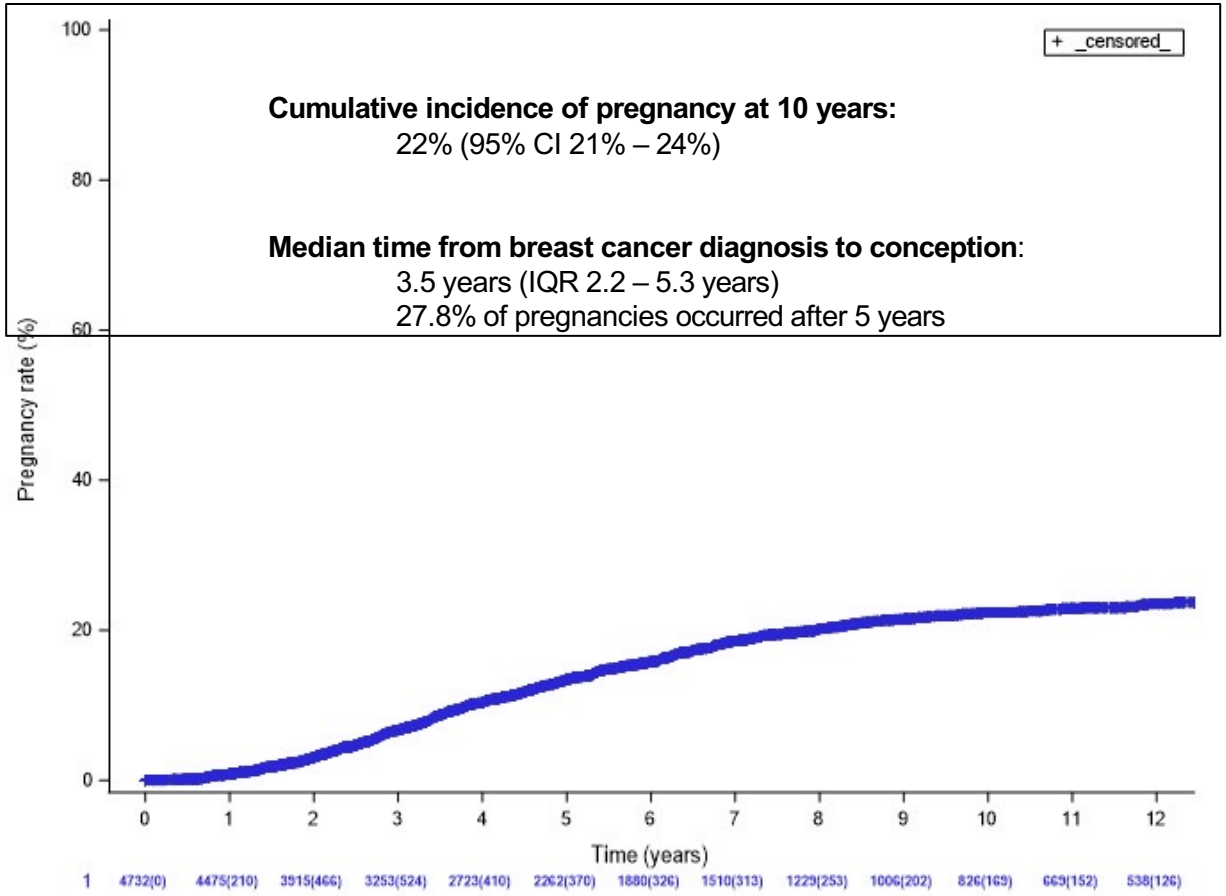
Treatment patterns

	Patients with a pregnancy n = 659, N (%)	Patients with no pregnancy n = 4073, N (%)
Breast surgery:		
None	2 (0.3)	13 (0.3)
Breast-conserving surgery	315 (48.8)	1511 (37.9)
Mastectomy	329 (50.9)	2465 (61.8)
Unknown	13	84
Received chemotherapy:		
No	47 (7.1)	334 (8.2)
Yes	611 (92.7)	3780 (91.0)
Unknown	1	31
Type of chemotherapy:		
Anthracycline- and taxane-based	414 (69.2)	2637 (73.8)
Anthracycline-based	143 (23.9)	655 (18.3)
Taxane-based	19 (3.2)	169 (4.7)
Other	22 (3.7)	110 (3.1)
Unknown	13	137
Received endocrine therapy:		
No	18 (8.3)	93 (4.9)
Yes	197 (91.6)	1790 (93.7)
Unknown	1	27
Type of endocrine therapy:		
Tamoxifen alone	64 (32.7)	638 (36.0)
Tamoxifen + LHRHa	81 (41.3)	469 (26.5)
LHRHa alone	7 (3.6)	36 (2.0)
AI ± LHRHa	21 (10.7)	334 (18.8)
Tamoxifen and AI (± LHRHa)	19 (9.7)	274 (15.5)
Other	4 (2.0)	22 (1.2)
Unknown	1	17
Duration of endocrine therapy, median (IQR) months	48 (24 – 60)	60 (28 – 60)
Unknown	40	467
Risk-reducing salpingo-oophorectomy:		
No	379 (57.6)	1844 (46.0)
Yes	279 (42.4)	2164 (54.0)
Unknown	1	65

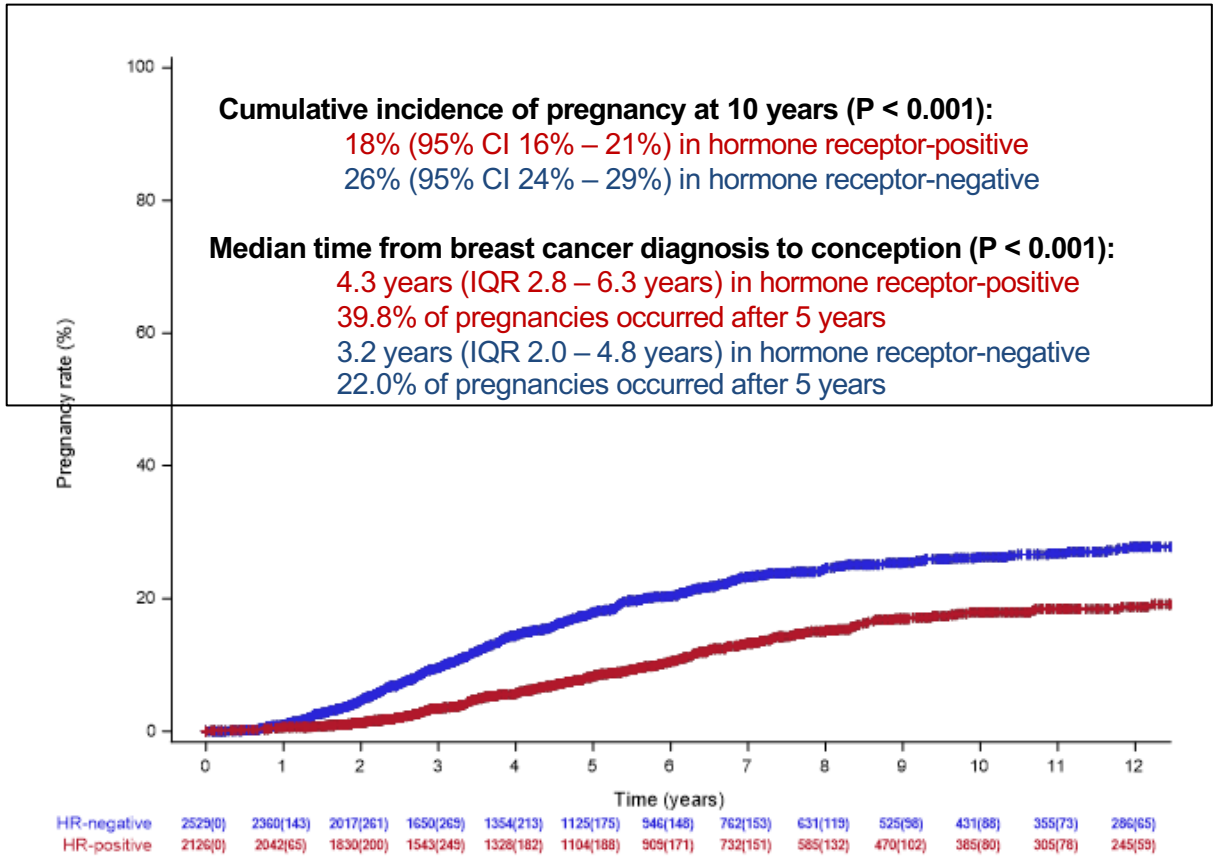
Lambertini et al, SABCS 2023, GS02-13

Study Results – Cumulative Incidence of Pregnancy

Overall cohort



According to hormone receptor status



Lambertini et al, SABCS 2023, GS02-13

Study Results – Reproductive Outcomes

	Patients with a pregnancy n = 659, N (%)
Age at pregnancy, median (IQR) years	34.7 (31.8-37.3)
Type of conception	
Spontaneous pregnancy	461 (79.2)
Use of assisted reproductive technology	121 (20.8)
Unknown	77
Pregnancy outcome	
Delivered a child	517 (79.7)
Ongoing pregnancy	24 (3.7)
Miscarriage	63 (9.7)
Induced abortion	45 (6.9)
Unknown	10
Number of live births at the first pregnancy after breast cancer	
1	463 (89.6)
2	54 (10.4)
Timing of delivery	
At term (≥ 37 weeks)	406 (91.0)
Preterm (< 37 weeks)	40 (9.0)
Unknown	71
Pregnancy complications	
None	365 (86.3)
Pregnancy complications	27 (6.4)
Delivery complications	22 (5.2)
Congenital abnormalities	4 (0.9)
Fetal complications	3 (0.6)
Other complications	2 (0.5)
Unknown	94
Breastfeeding	
No	270 (67.0)
Yes	133 (33.0)
Unknown	114
Duration of breastfeeding, median (IQR), months	5 (2 – 6)
Unknown duration of breastfeeding	50

Study Results – Disease-free Survival

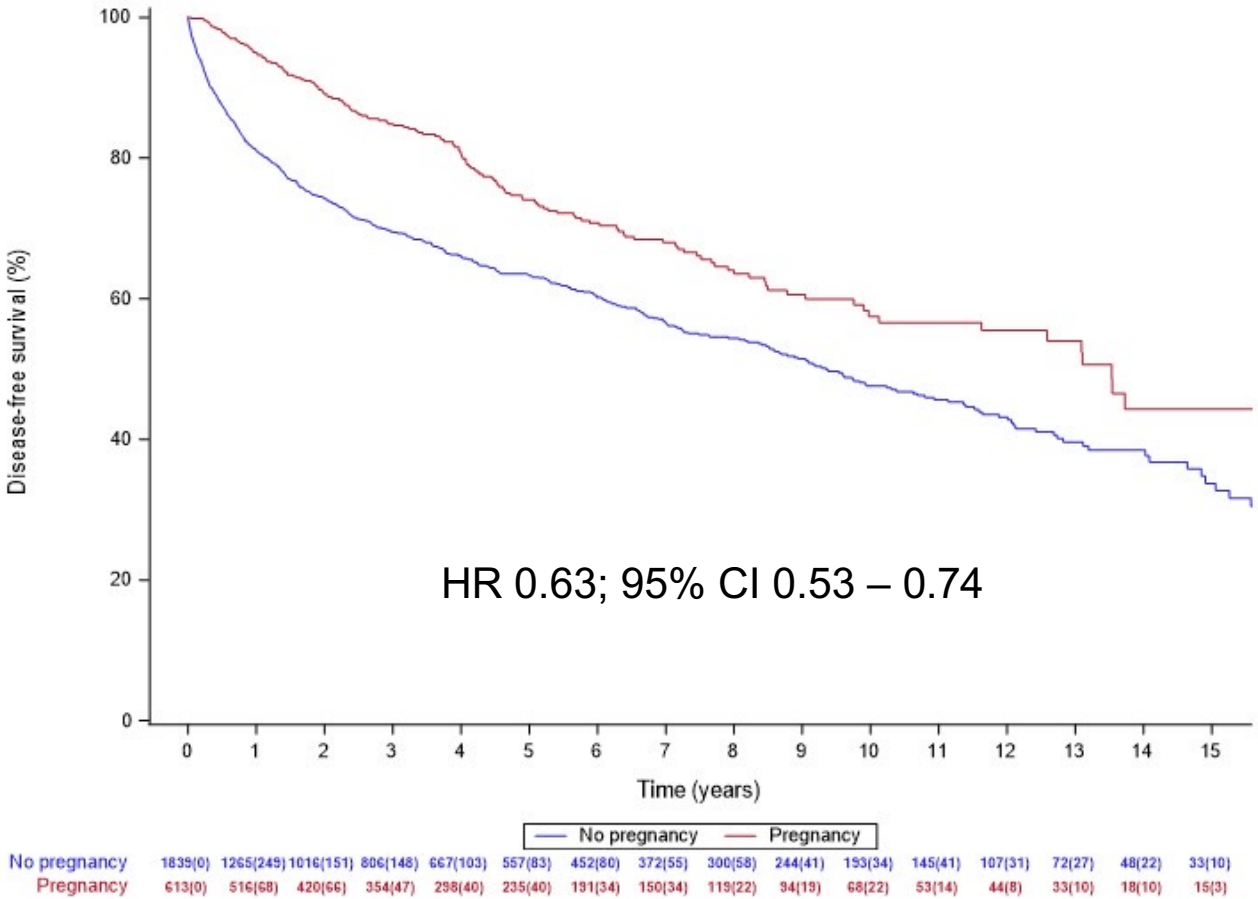
Primary analysis – Extended Cox model with occurrence of pregnancy as a time-varying covariate

Unadjusted HR 0.97; 95% CI 0.82 – 1.15
Adjusted HR* 0.99; 95% CI 0.81 – 1.20

Subgroup analyses	Multivariate HR* (95% CI)	P value for interaction
Specific *BRCA* gene		
BRCA1	0.80 (0.63 – 1.01)	0.007
BRCA2	1.55 (1.12 – 2.16)	
BRCA1 and *BRCA2*	4.49 (0.28 – 72.17)	
BRCA, unknown if 1 or 2	Not evaluable	
Hormone receptor status:		
ER and/or PR positive	1.30 (0.95 – 1.76)	0.009
ER and PR negative	0.76 (0.60 – 0.95)	
Unknown	0.28 (0.04 – 2.21)	
HER2 status:		
HER2 negative	0.61 (0.22 – 1.71)	0.08
HER2 positive	1.07 (0.87 – 1.31)	
Unknown	0.42 (0.17 – 1.02)	
Received chemotherapy:		
No	0.77 (0.39 – 1.52)	0.47
Yes	1.00 (0.82 – 1.23)	
Unknown	0.77 (0.39 – 1.52)	
Received endocrine therapy:		
No	0.85 (0.67 – 1.08)	0.01
Yes	1.55 (1.08 – 2.21)	
Unknown	0.13 (0.01 – 2.95)	

*Adjusted for: region, age, nodal status, hormone receptor status and type of breast surgery

Secondary matched analysis



Study Results – Secondary Survival Outcomes

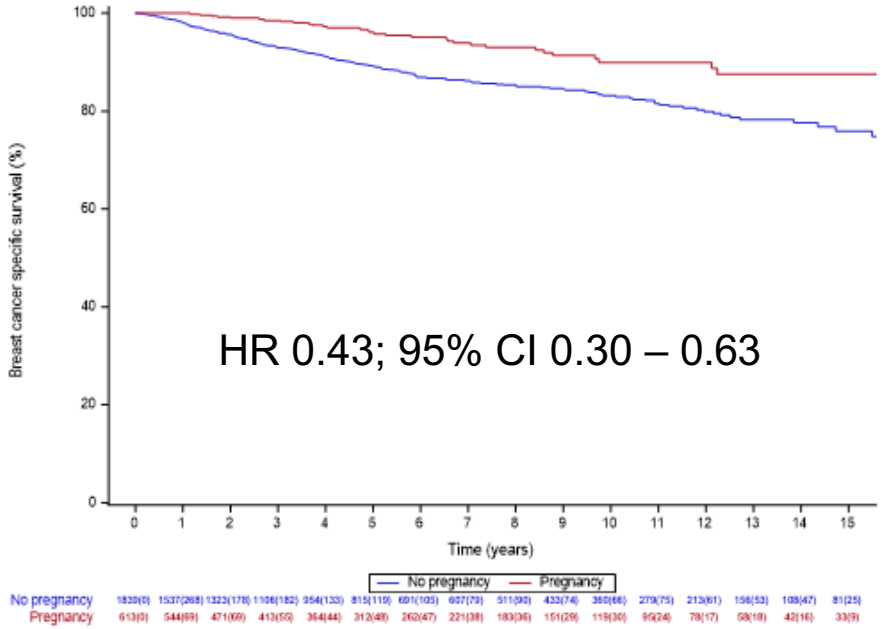
Breast cancer-specific survival

Extended Cox model:

Unadjusted HR 0.53; 95% CI 0.37 – 0.74

Adjusted HR* 0.60; 95% CI 0.40 – 0.88

Secondary matched analysis:



HR 0.43; 95% CI 0.30 – 0.63

*Adjusted for: region, age, nodal status, hormone receptor status and type of breast surgery

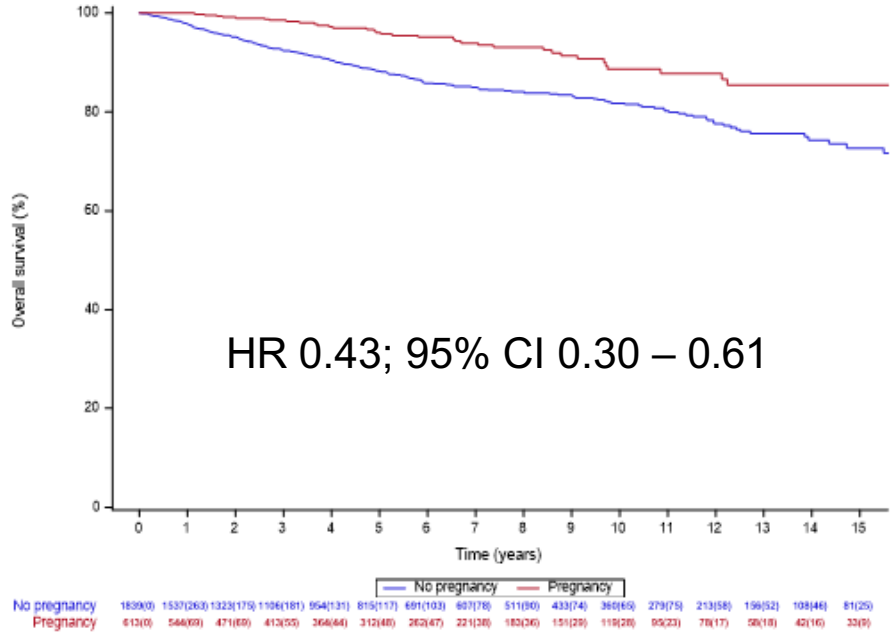
Overall survival

Extended Cox model:

Unadjusted HR 0.52; 95% CI 0.38 – 0.72

Adjusted HR* 0.58; 95% CI 0.40 – 0.85

Secondary matched analysis:



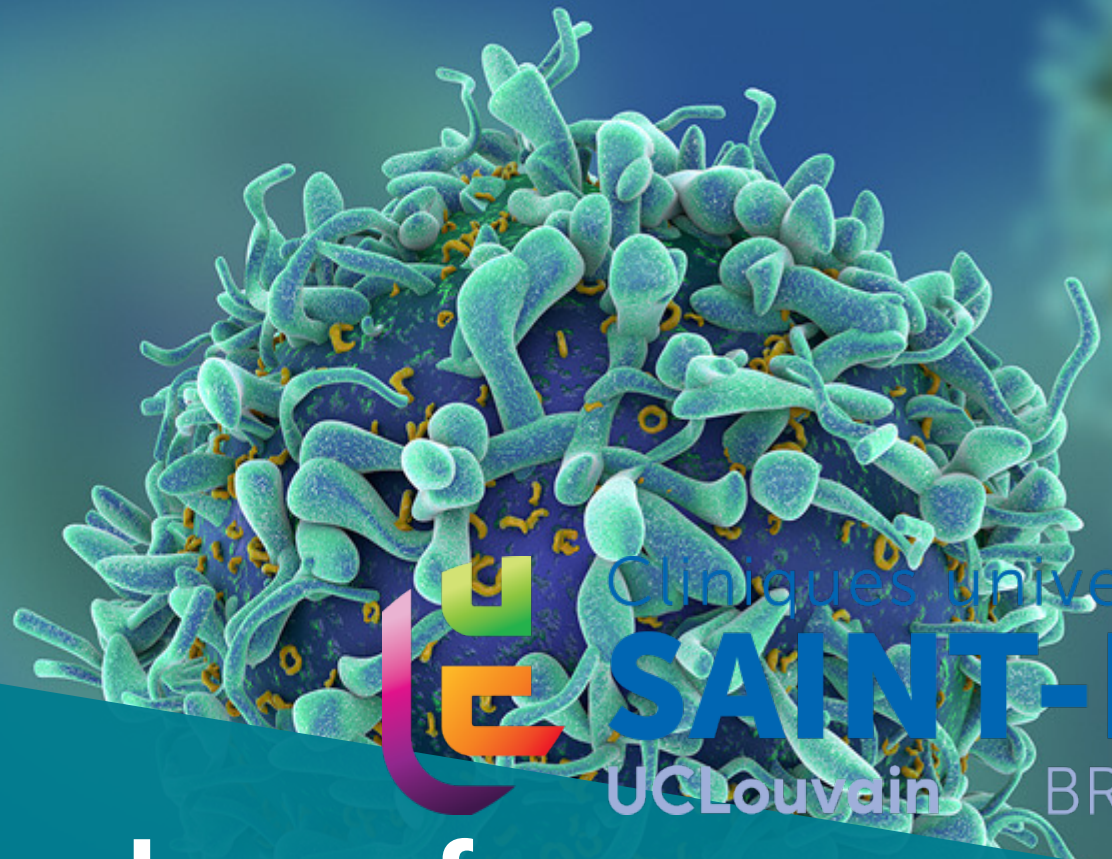
HR 0.43; 95% CI 0.30 – 0.61

Conclusions

- This global study including 4732 young *BRCA* carriers from 78 centers worldwide provides reassuring evidence for the oncofertility counseling of young *BRCA* carriers interested in conceiving following diagnosis and treatment for breast cancer
- More than one out of five (22%) young *BRCA* carriers became pregnant within 10 years after a breast cancer diagnosis
- The rate of pregnancy, fetal and obstetric complications was low and in line with the expectations in a population of women with similar age and no history of breast cancer
- No detrimental prognostic effect of pregnancy after breast cancer was observed, particularly in *BRCA1* carriers

- **Conceiving after proper treatment and follow-up for breast cancer should not be contraindicated in young *BRCA* carriers**

Lambertini et al, SABCS 2023, GS02-13



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Thank you for your attention