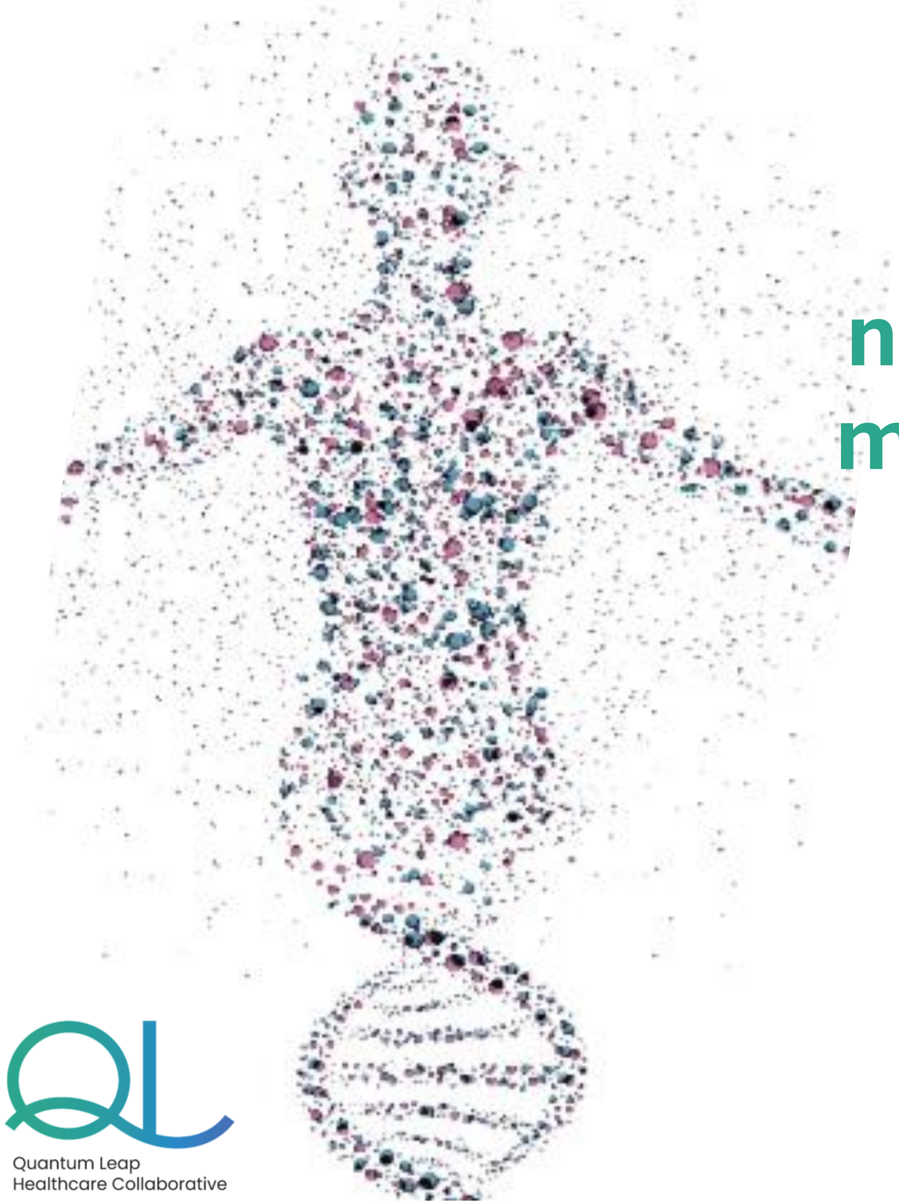


Improving risk stratification and prediction of response to neoadjuvant therapy (NAT) by serial monitoring of circulating tumor DNA (ctDNA) in high-risk early-stage breast cancer

Mark Jesus M. Magbanua PhD
I-SPY2 ctDNA working group

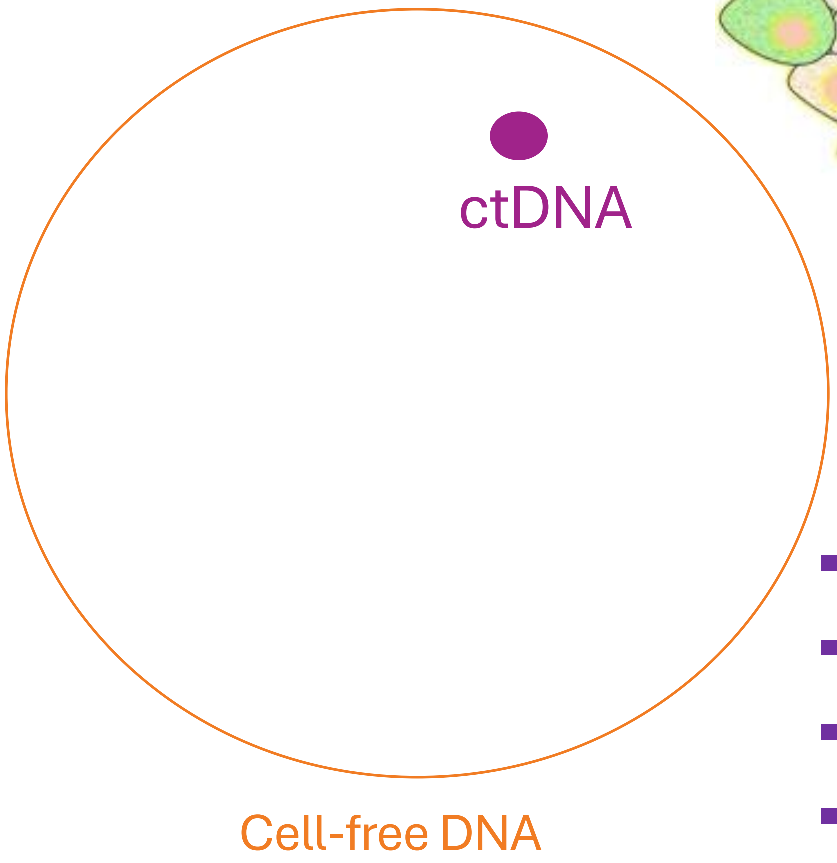
Special thanks to Nayelis Manon
(Poster)

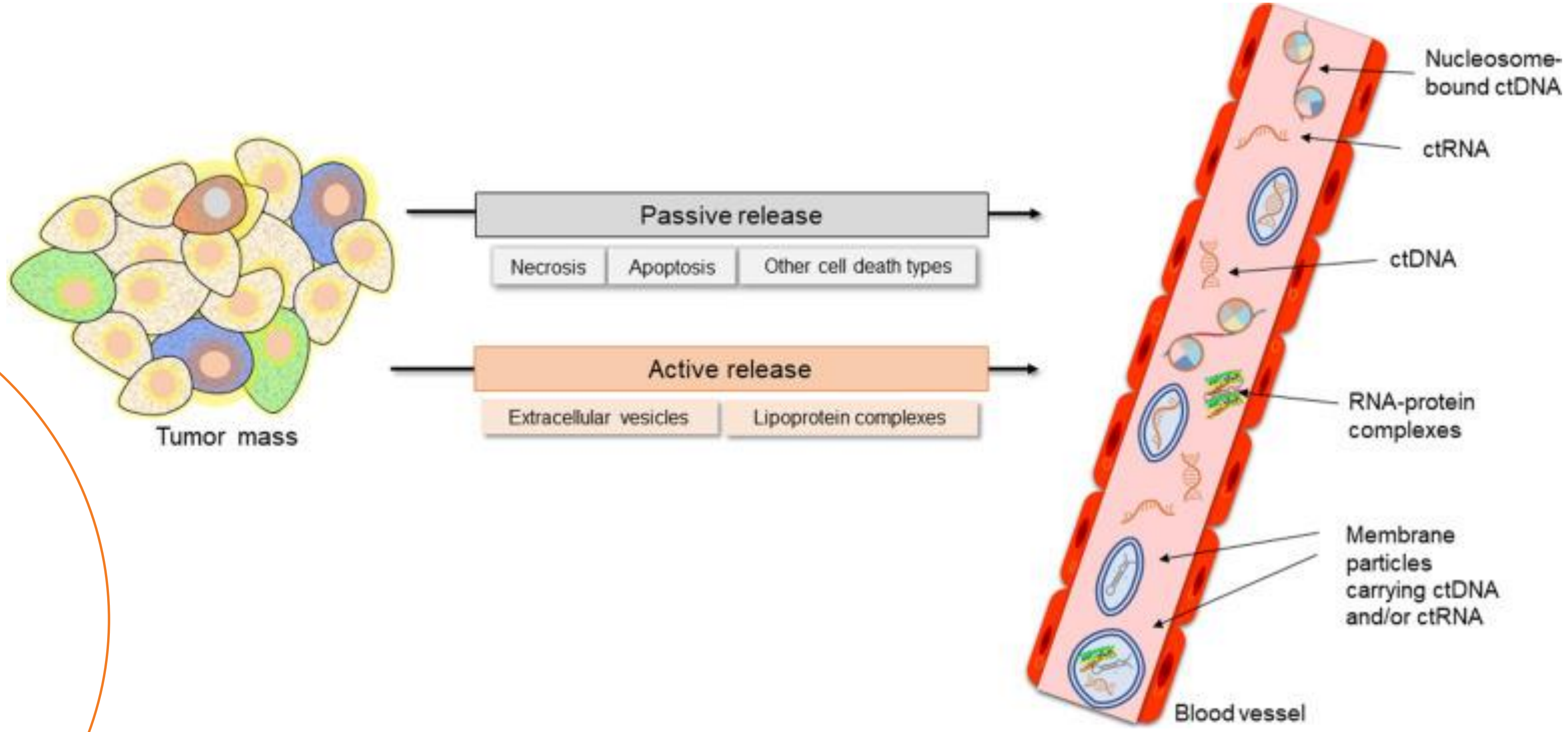
Quantum Leap
Healthcare Collaborative

Outline

I. ctDNA 101
II. ctDNA studies in the neoadjuvant I-SPY2 trial
III. Prognostic value of ctDNA in the neoadjuvant setting
IV. Predictive value of ctDNA in the neoadjuvant setting
V. Summary, Future Directions, and Clinical Implications

Circulating tumor DNA (ctDNA) is a subset of cell-free DNA shed from tumors

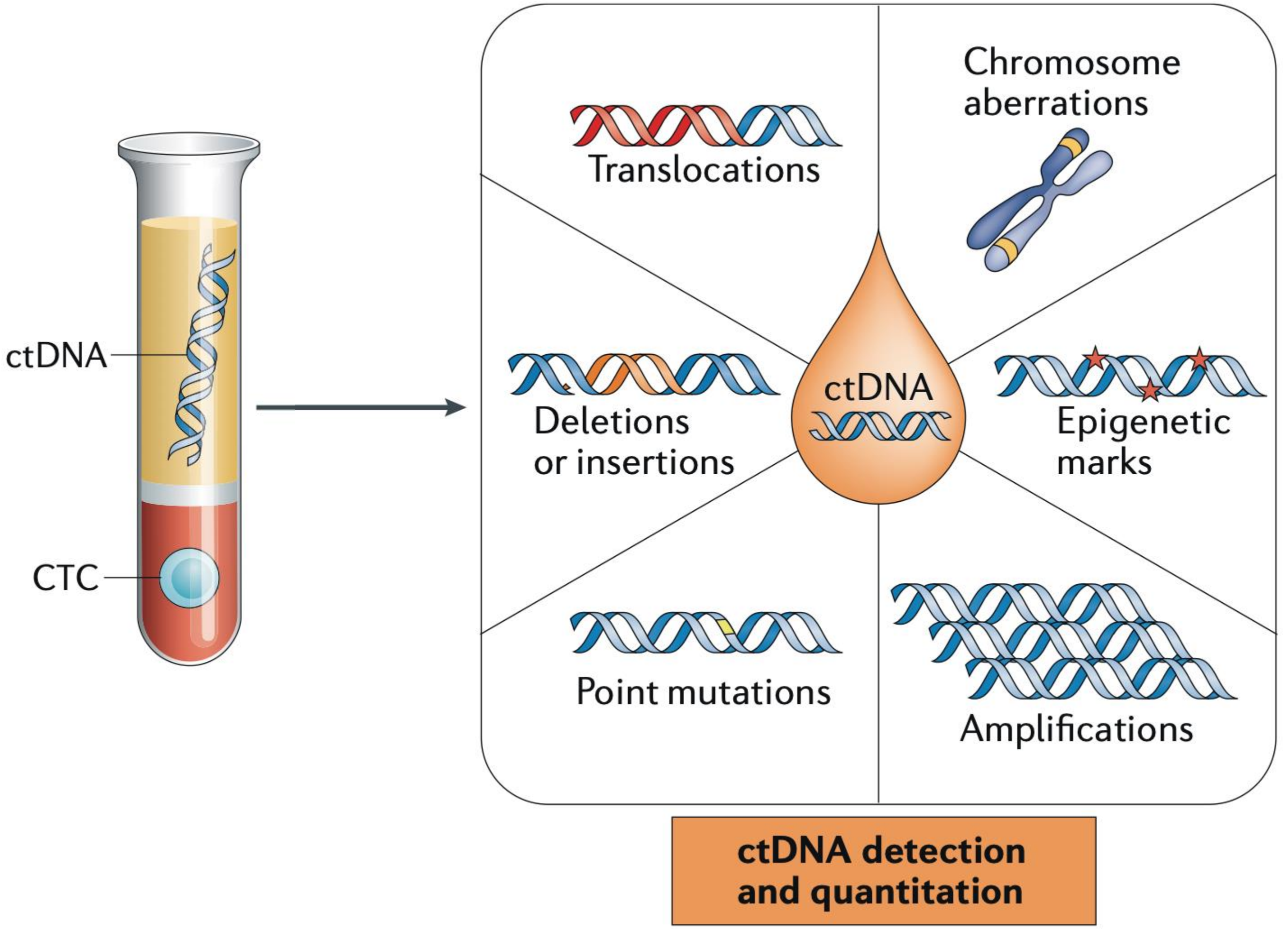

ctDNA
Cell-free DNA


Tumor mass
Passive release
Necrosis
Apoptosis
Other cell death types
Active release
Extracellular vesicles
Lipoprotein complexes
Nucleosome-bound ctDNA
ctRNA
ctDNA
RNA-protein complexes
Membrane particles carrying ctDNA and/or ctRNA
Blood vessel

- ctDNA is a subset of cell-free DNA shed exclusively by tumor cells.
- The total amount of DNA floating in the blood is called cell-free DNA.
- Most DNA (70%) in the blood comes from dying hematopoietic cells.
- The fraction of ctDNA depends on many factors, including tumor characteristics (e.g., subtype and size).

Stejskal P...Magbanua MJM. *Mol Cancer*. 2023;22(1):15.

ctDNA carries genetic information (e.g., mutations) found in the tumor of origin



ctDNA

CTC

Translocations

Chromosome aberrations

Deletions or insertions

ctDNA

Epigenetic marks

Point mutations

Amplifications

ctDNA detection and quantitation

Liquid biopsy and minimal residual disease — latest advances and implications for cure

Klaus Pantel[1] and Catherine Alix-Panabières[2]*

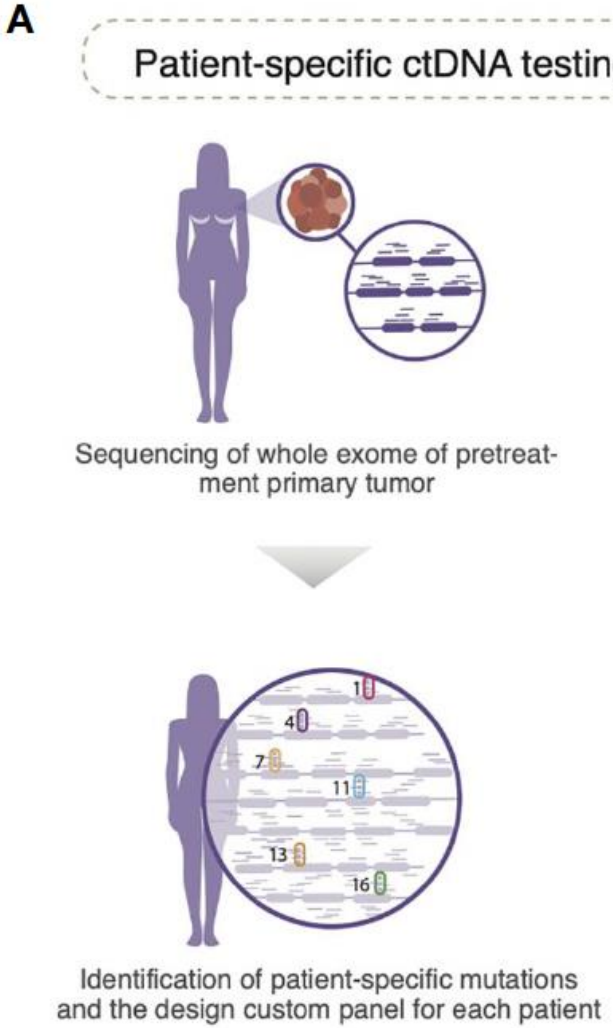
NATURE REVIEWS | CLINICAL ONCOLOGY

VOLUME 16 | JULY 2019 | **409**

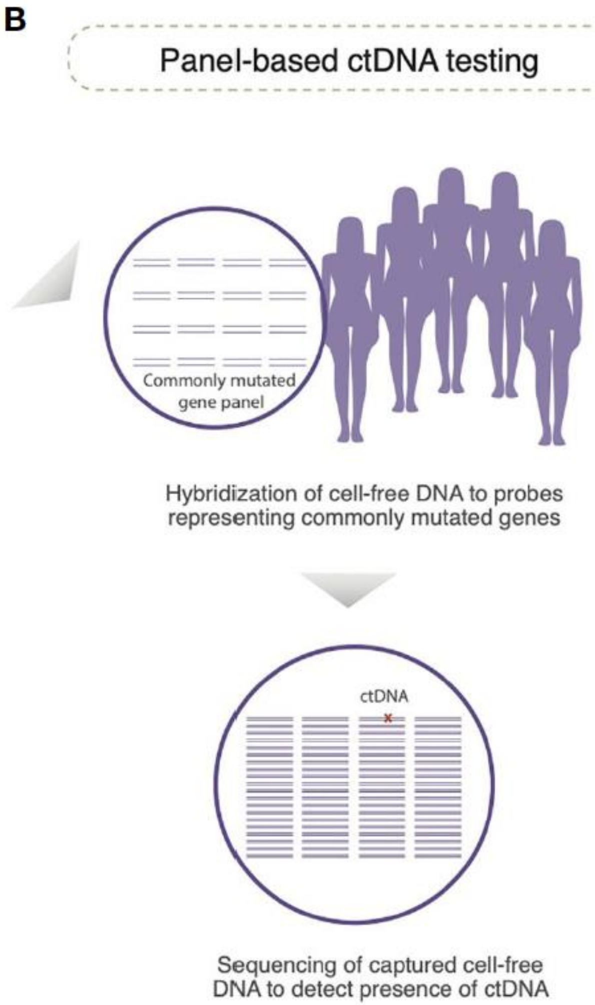
Pantel K et al. *Nat Rev Clin Oncol*. 2019;16(7):409-424.

Two major types of ctDNA detection platforms

Tumor-informed

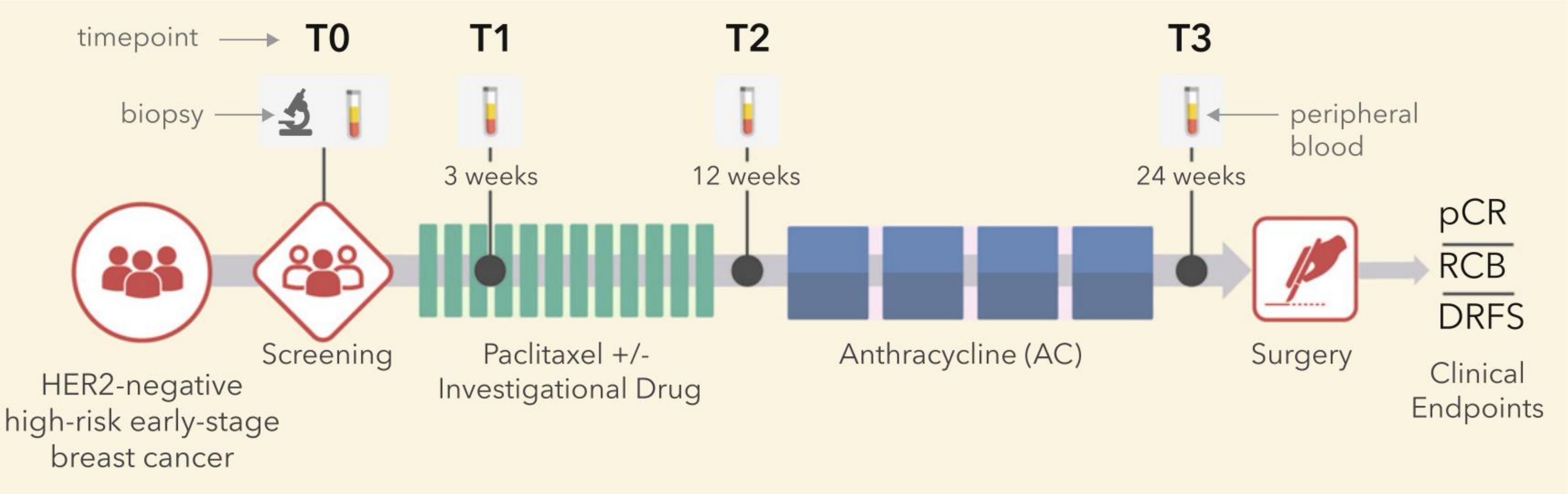

A
Patient-specific ctDNA testing
Sequencing of whole exome of pretreatment primary tumor
1
4
7
11
13
16
Identification of patient-specific mutations and the design custom panel for each patient

Tumor-agnostic


B
Panel-based ctDNA testing
Commonly mutated gene panel
Hybridization of cell-free DNA to probes representing commonly mutated genes
ctDNA
Sequencing of captured cell-free DNA to detect presence of ctDNA

Magbanua MJM et al. *Front Oncol*. 2022;12:802579.

ctDNA analysis in I-SPY2: Schema and Endpoints


timepoint
T0
T1
T2
T3
biopsy
peripheral blood
3 weeks
12 weeks
24 weeks
pCR
RCB
DRFS
HER2-negative high-risk early-stage breast cancer
Screening
Paclitaxel +/- Investigational Drug
Anthracycline (AC)
Surgery
Clinical Endpoints

Residual cancer burden (RCB) index – continuous measure of invasive cancer in the breast and regional lymph nodes after treatment

RCB-0 – pathologic complete response or absence of invasive cancer in the breast and regional lymph nodes

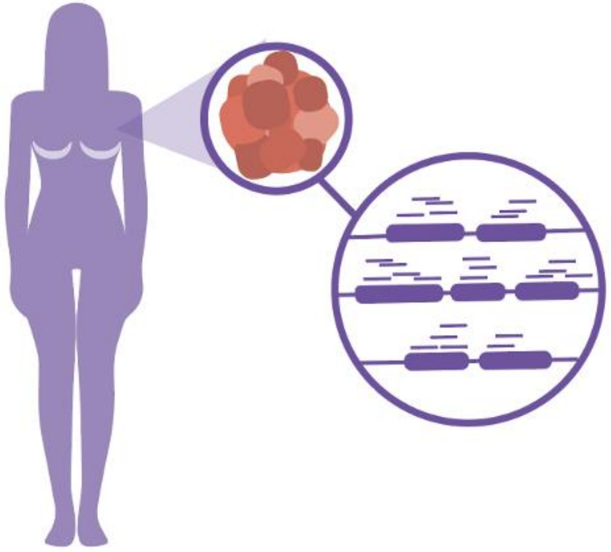
RCB-I – limited

RCB-II – moderate

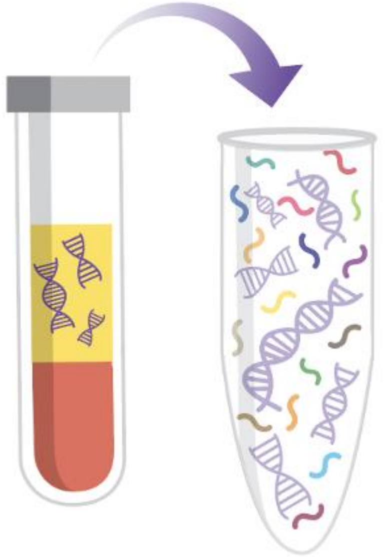
RCB-III – extensive

Distant recurrence-free survival (DRFS) – the time interval between patient consent to treatment and metastatic recurrence or death of any cause

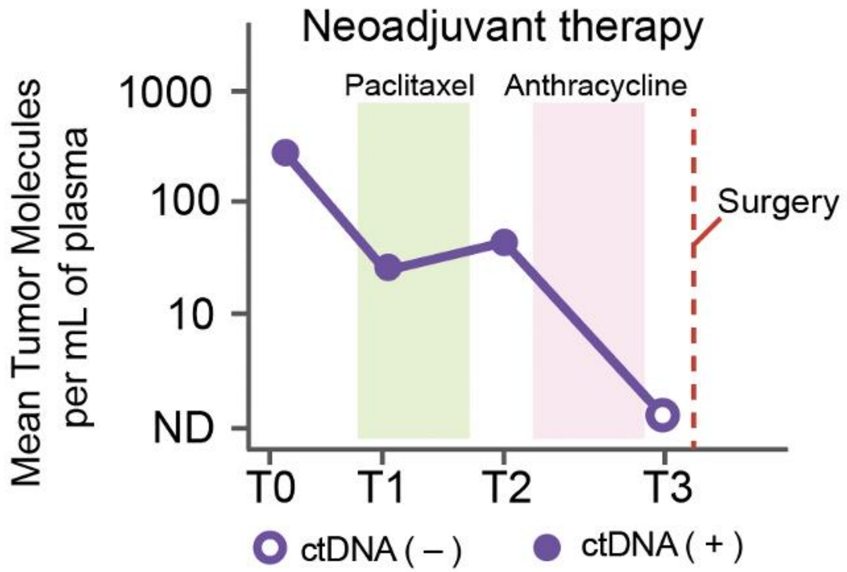
Tumor-informed patient-specific ctDNA testing



1 Sequence pretreatment tumor to identify clonal mutations



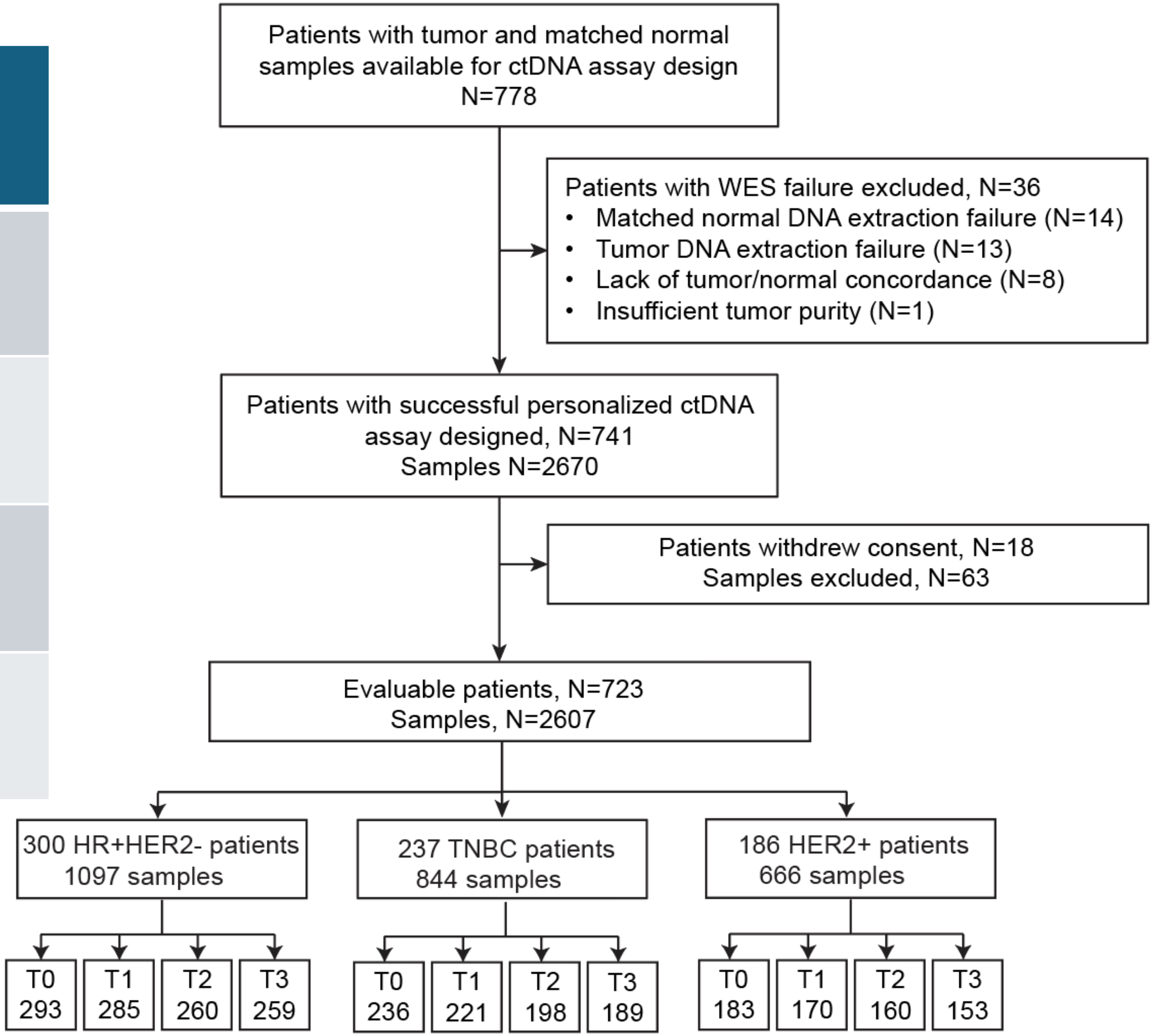
2 Custom design and manufacture of multiplex PCR assay for each patient targeting 16 clonal mutations



3 Use pCR assay and sequence amplicons to test for the presence of ctDNA in patient's blood

Biomarker cohort: Patients in I-SPY 2 with ctDNA

Subtype	No. of Patients	No. of Samples
HR+HER2-	300	1097
TNBC	237	844
HER2+	186	666
Total	723	2067



Patients with tumor and matched normal samples available for ctDNA assay design
N=778

Patients with WES failure excluded, N=36
- Matched normal DNA extraction failure (N=14)
- Tumor DNA extraction failure (N=13)
- Lack of tumor/normal concordance (N=8)
- Insufficient tumor purity (N=1)

Patients with successful personalized ctDNA assay designed, N=741
Samples N=2670

Patients withdrew consent, N=18
Samples excluded, N=63

Evaluable patients, N=723
Samples, N=2607

300 HR+HER2- patients
1097 samples

T0	T1	T2	T3
293	285	260	259

237 TNBC patients
844 samples

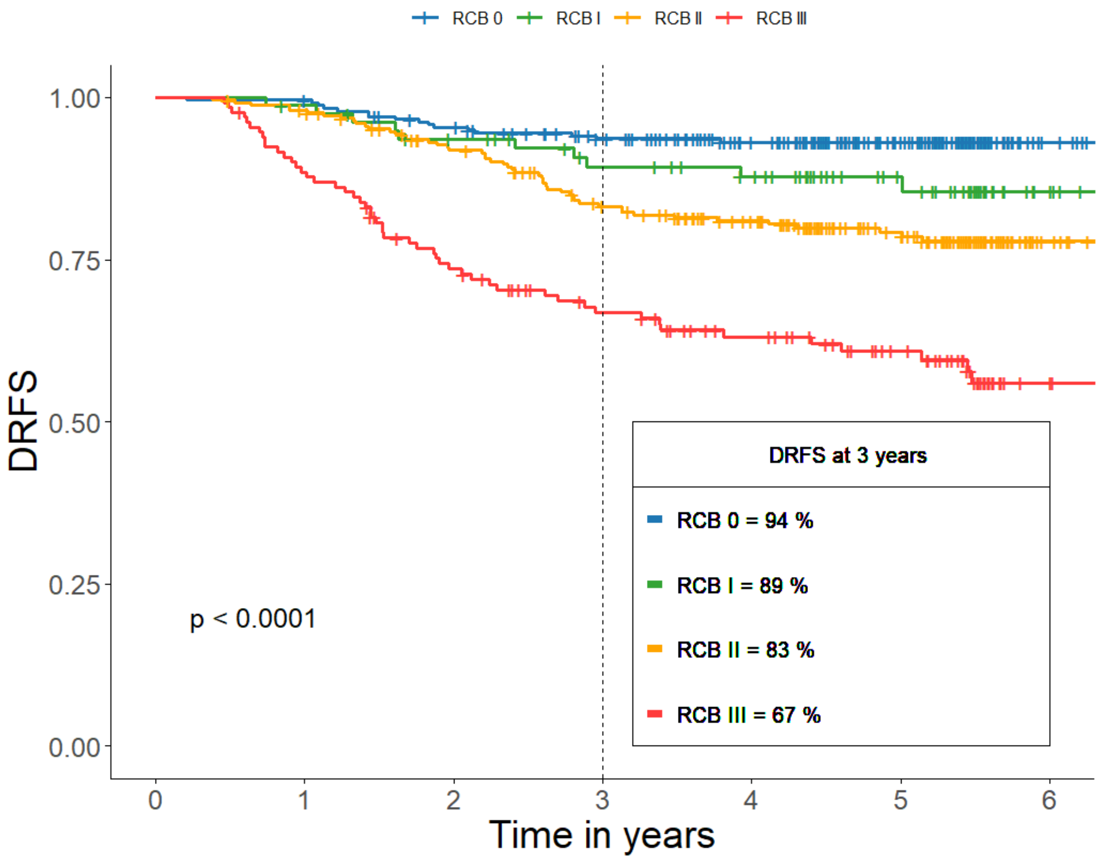
T0	T1	T2	T3
236	221	198	189

186 HER2+ patients
666 samples

T0	T1	T2	T3
183	170	160	153

Can ctDNA refine the risk stratification of patients using RCB?

Patients with ctDNA data



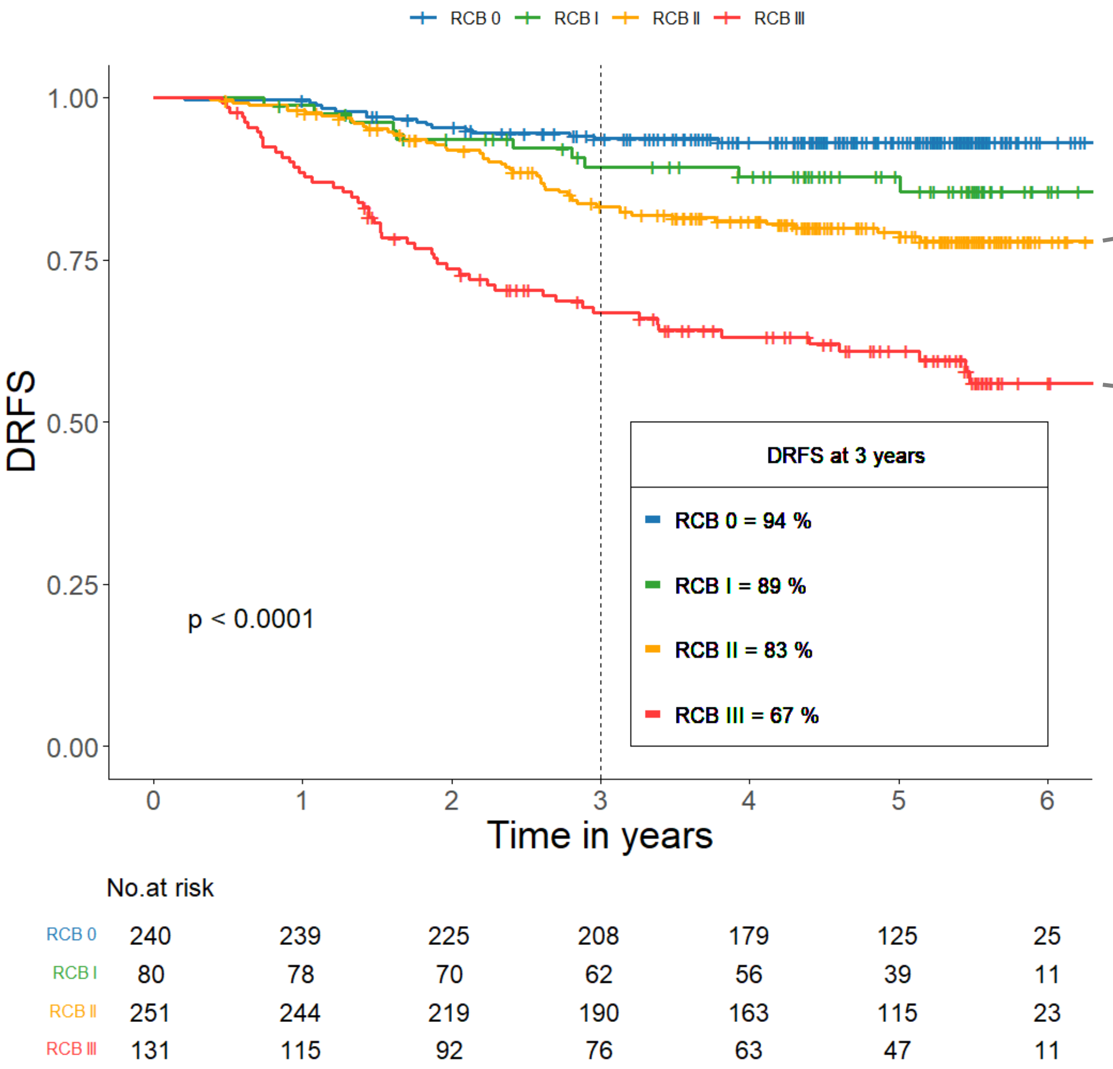
No.at risk

RCB 0	240	239	225	208	179	125	25
RCB I	80	78	70	62	56	39	11
RCB II	251	244	219	190	163	115	23
RCB III	131	115	92	76	63	47	11

- RCB-0/I is an excellent early surrogate of favorable survival (~90% 3-year DRFS rate)
- RCB-II is associated with a 17% metastatic recurrence rate at 3 years
- RCB-III is associated with a 33% metastatic recurrence rate at 3 years

Can ctDNA refine the risk stratification of patients with RCB-II and RCB-III?

Patients with ctDNA data



No.at risk

	0	1	2	3	4	5	6
RCB 0	240	239	225	208	179	125	25
RCB I	80	78	70	62	56	39	11
RCB II	251	244	219	190	163	115	23
RCB III	131	115	92	76	63	47	11

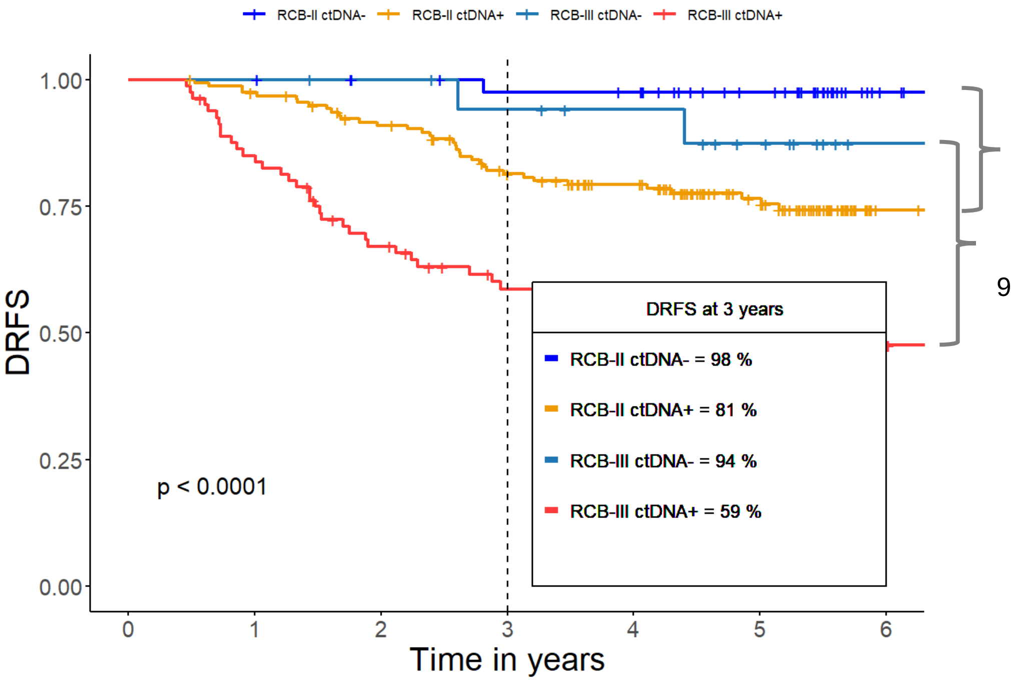
Sub-stratify based on:

ctDNA status/
ctDNA dynamics

ctDNA status/
ctDNA dynamics

ctDNA negativity at T0 and T3 is associated with favorable survival in patients with RCB-II and RCB-III

ctDNA status at pretreatment

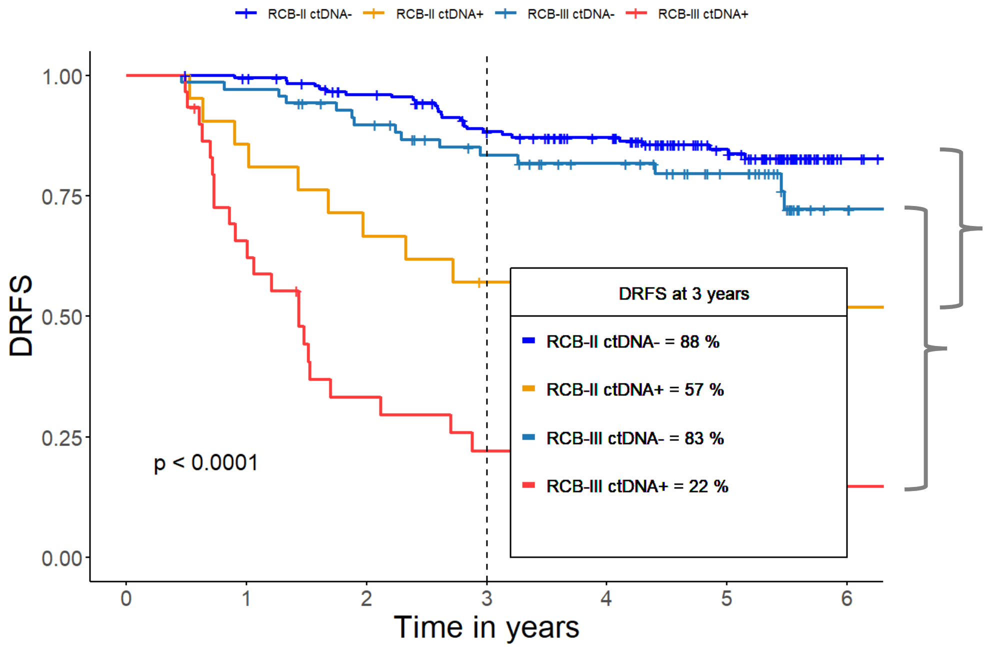


*HzR=0.10
95% CI 0.01-0.73
p=0.02

*HzR=0.28
95% CI 0.09-92
p=0.04

No.at risk							
RCB-II ctDNA-	45	45	42	40	39	27	4
RCB-II ctDNA+	159	153	138	117	98	70	13
RCB-III ctDNA-	19	19	18	16	14	10	3
RCB-III ctDNA+	81	68	51	40	32	25	7

ctDNA status at post-NAT

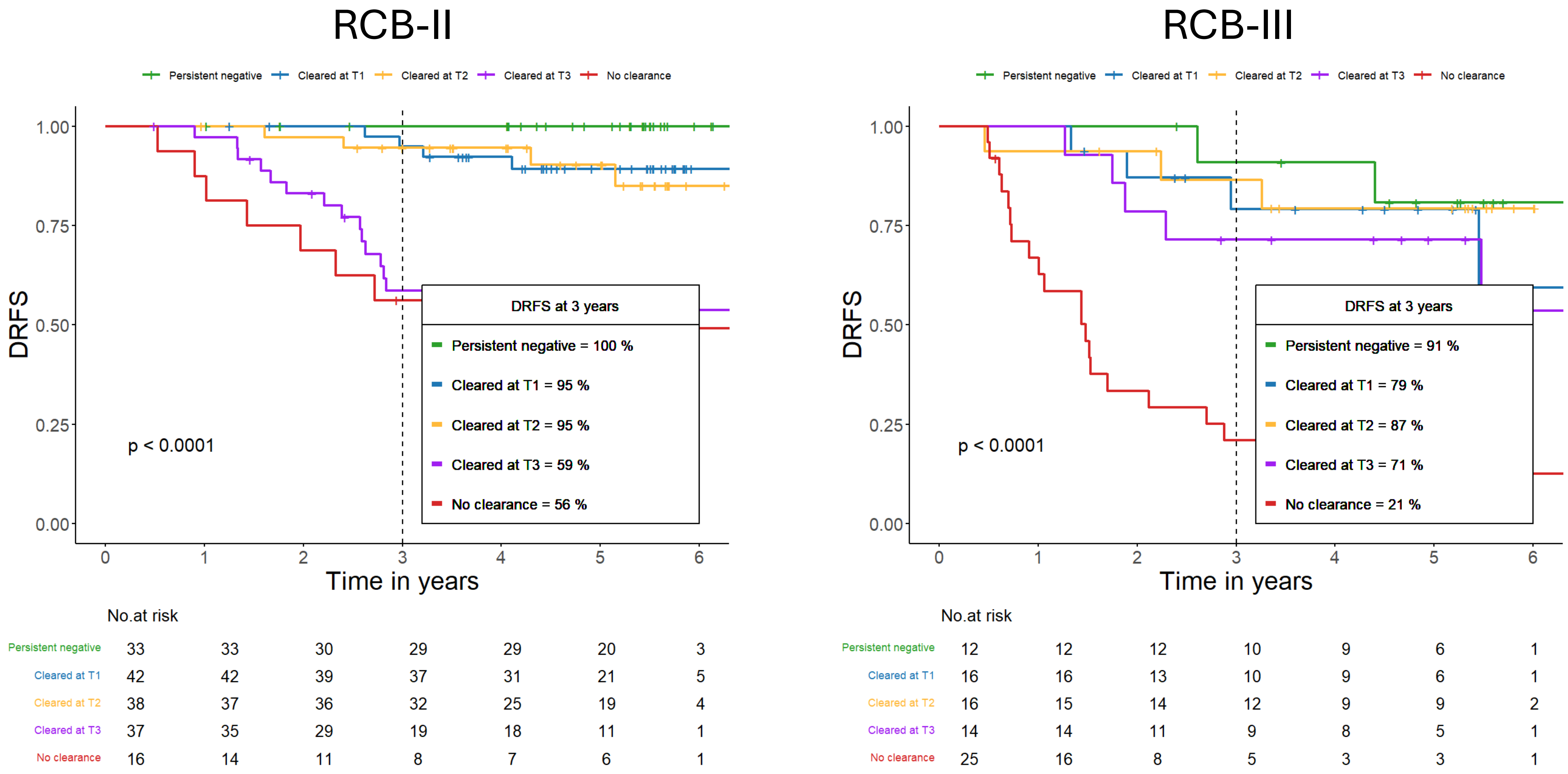


*HzR=0.29
95% CI 0.13-0.61
p=0.001

*HzR=0.14
95% CI 0.07-0.26
P<0.001

No.at risk							
RCB-II ctDNA-	183	180	166	146	127	89	16
RCB-II ctDNA+	21	18	14	11	10	8	1
RCB-III ctDNA-	70	68	60	50	42	31	9
RCB-III ctDNA+	30	19	9	6	4	4	1

Persistent ctDNA negativity and early ctDNA clearance are associated with favorable survival in patients with RCB-II and RCB-III



RCB-II

DRFS at 3 years

- Persistent negative = 100 %
- Cleared at T1 = 95 %
- Cleared at T2 = 95 %
- Cleared at T3 = 59 %
- No clearance = 56 %

No.at risk

	0	1	2	3	4	5	6
Persistent negative	33	33	30	29	29	20	3
Cleared at T1	42	42	39	37	31	21	5
Cleared at T2	38	37	36	32	25	19	4
Cleared at T3	37	35	29	19	18	11	1
No clearance	16	14	11	8	7	6	1

RCB-III

DRFS at 3 years

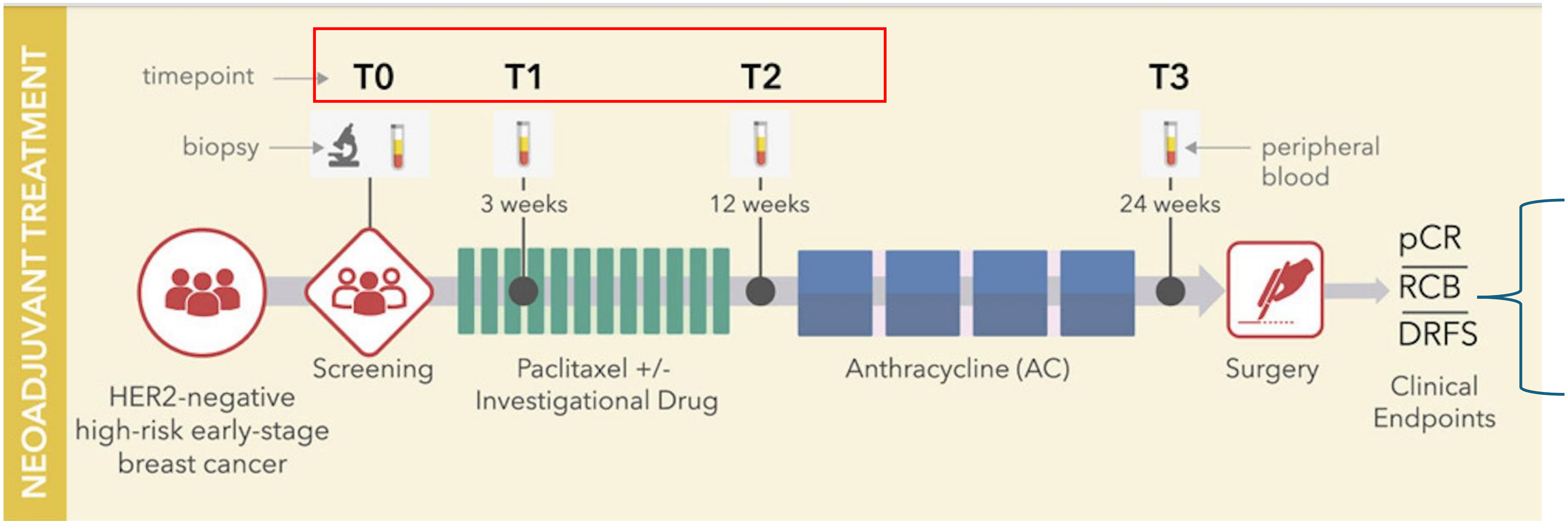
- Persistent negative = 91 %
- Cleared at T1 = 79 %
- Cleared at T2 = 87 %
- Cleared at T3 = 71 %
- No clearance = 21 %

No.at risk

	0	1	2	3	4	5	6
Persistent negative	12	12	12	10	9	6	1
Cleared at T1	16	16	13	10	9	6	1
Cleared at T2	16	15	14	12	9	9	2
Cleared at T3	14	14	11	9	8	5	1
No clearance	25	16	8	5	3	3	1

Can ctDNA improve the prediction of residual cancer burden (RCB) after NAT?

Early ctDNA dynamics (T0-T2)


NEOADJUVANT TREATMENT
timepoint
T0
T1
T2
T3
biopsy
peripheral blood
3 weeks
12 weeks
24 weeks
HER2-negative high-risk early-stage breast cancer
Screening
Paclitaxel +/- Investigational Drug
Anthracycline (AC)
Surgery
pCR
RCB
DRFS
Clinical Endpoints

RCB index

RCB class (0/I vs. II/III)

RCB index – continuous measure of invasive cancer in the breast and regional lymph nodes after treatment

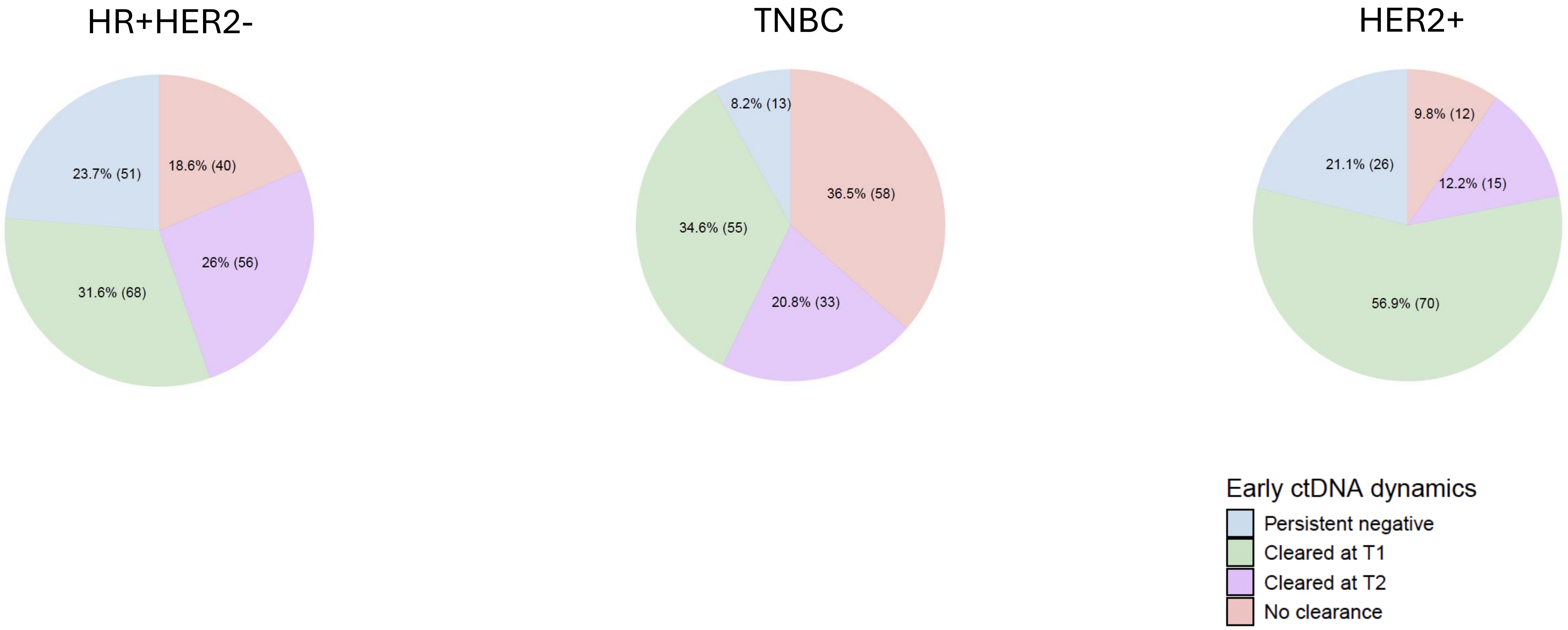
RCB-0 – pathologic complete response or absence of invasive cancer in the breast and regional lymph nodes

RCB-I – limited

RCB-II – moderate

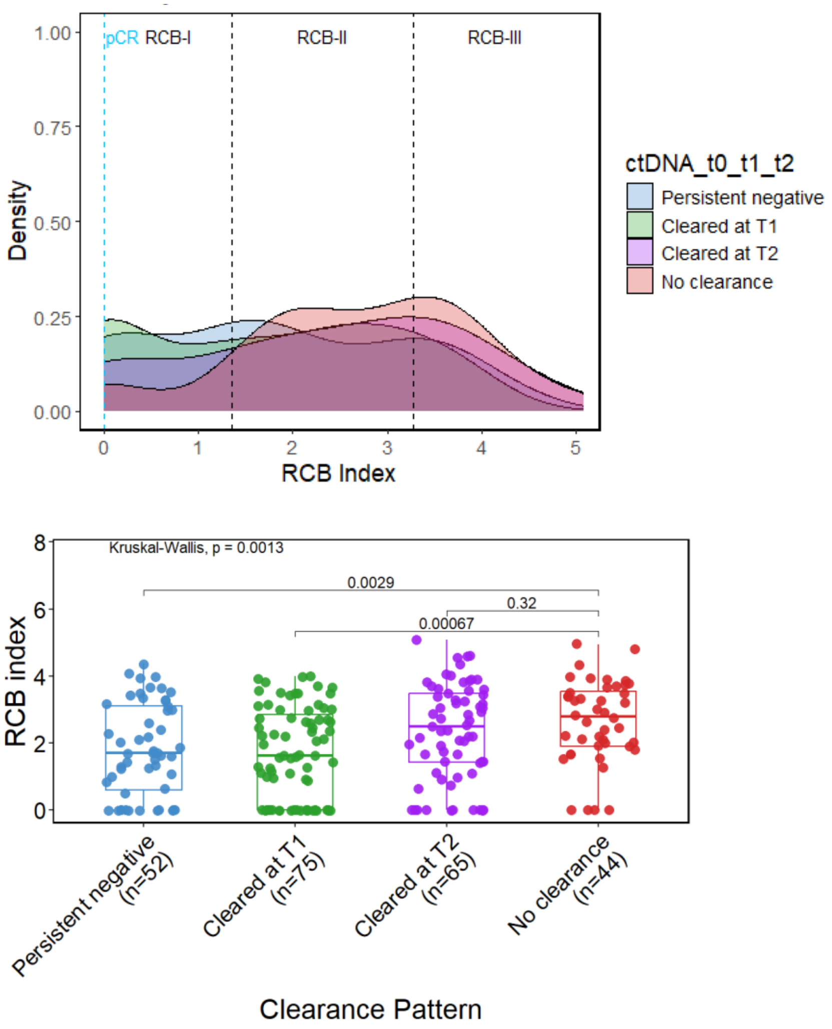
RCB-III – extensive

Early ctDNA clearance at week 3 is enriched in HER2+

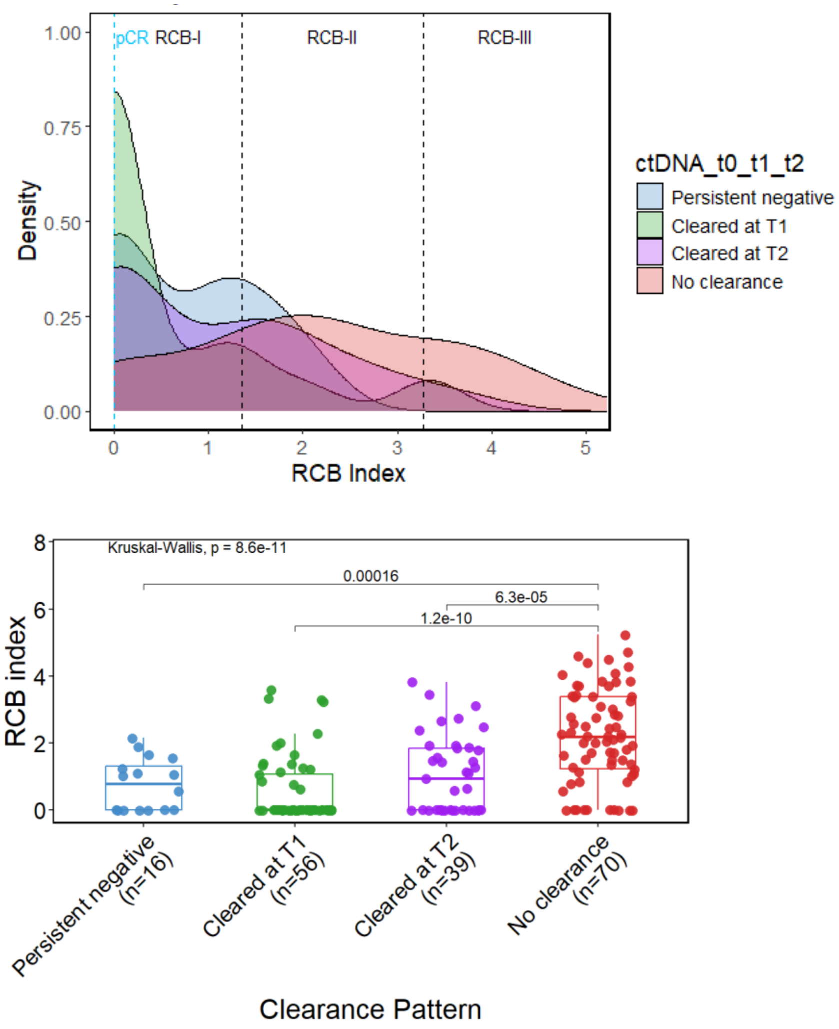


Early ctDNA clearance (week 3) skews the distributions towards lower RCB indices (favorable survival)

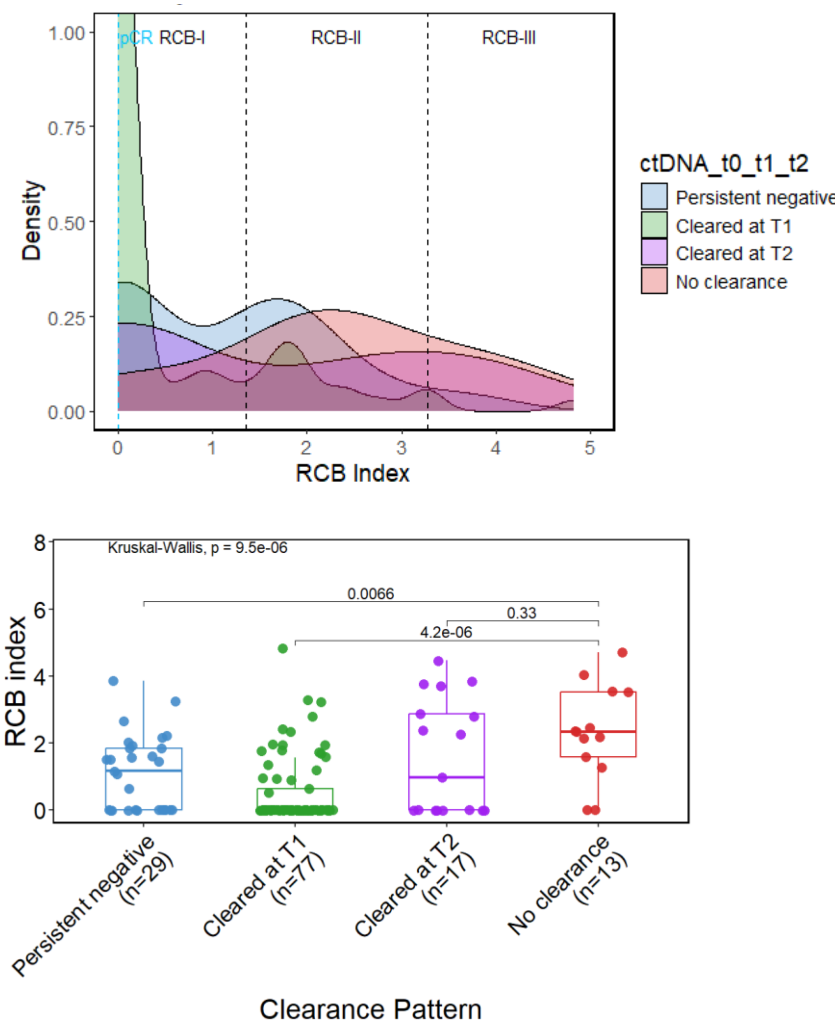
HR+HER2-



TNBC



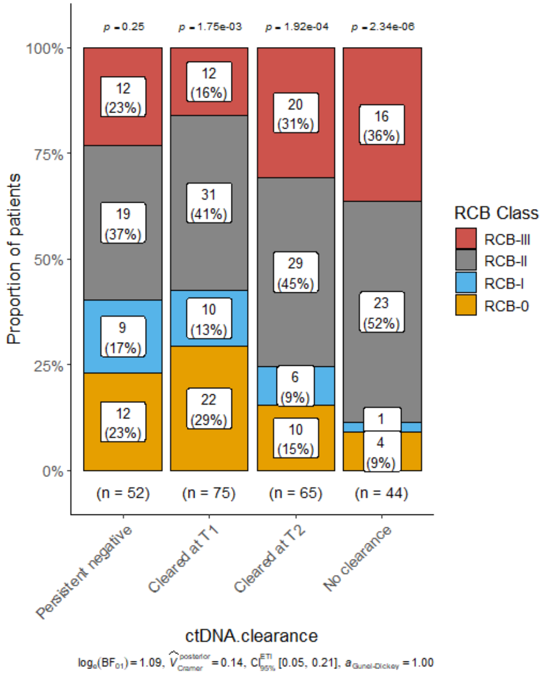
HER2+



ctDNA clearance is more predictive in TNBC and HER2+

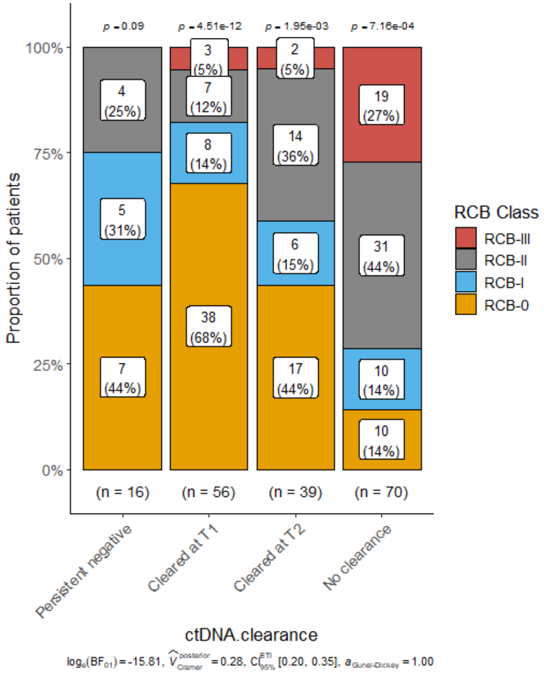
HR+HER2-

p=0.02



TNBC

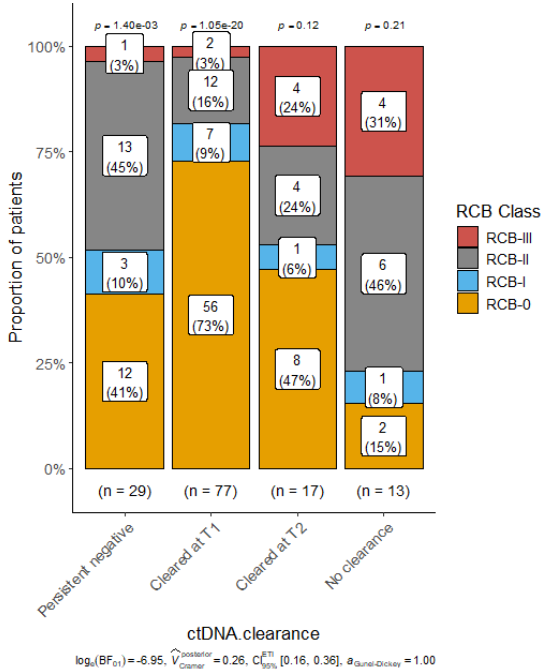
p<0.001



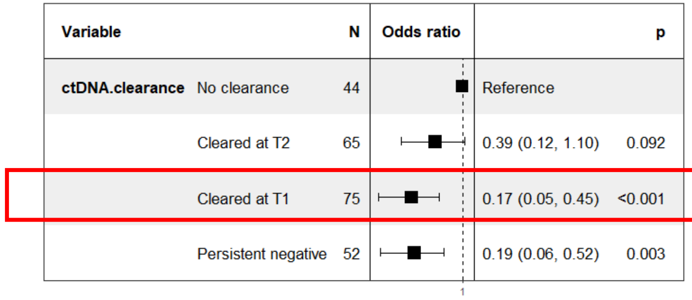
HER2+

p<0.001

136

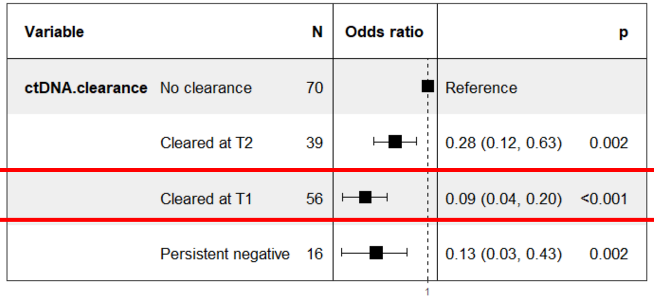


RCB class ~ clearance T0/T1/T2 in HR+HER2-



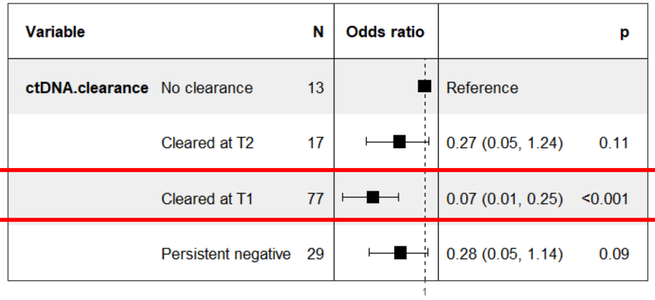
Variable		**N**	**Odds ratio**		**p**
ctDNA.clearance	No clearance	44		Reference	
	Cleared at T2	65		0.39 (0.12, 1.10)	0.092
	Cleared at T1	75		0.17 (0.05, 0.45)	<0.001
	Persistent negative	52		0.19 (0.06, 0.52)	0.003

RCB class ~ clearance T0/T1/T2 in TNBC



Variable		**N**	**Odds ratio**		**p**
ctDNA.clearance	No clearance	70		Reference	
	Cleared at T2	39		0.28 (0.12, 0.63)	0.002
	Cleared at T1	56		0.09 (0.04, 0.20)	<0.001
	Persistent negative	16		0.13 (0.03, 0.43)	0.002

RCB class ~ clearance T0/T1/T2 in HER2+



Variable		**N**	**Odds ratio**		**p**
ctDNA.clearance	No clearance	13		Reference	
	Cleared at T2	17		0.27 (0.05, 1.24)	0.11
	Cleared at T1	77		0.07 (0.01, 0.25)	<0.001
	Persistent negative	29		0.28 (0.05, 1.14)	0.09

RCB0/I ← 1 → RCBII/III

Summary

- ctDNA refined risk stratification in patients with RCB-II/III by identifying subgroups with improved survival
- Early ctDNA clearance markedly skewed the distribution towards lower RCB indices (favorable survival) in TNBC and HER2+
- The association between early ctDNA clearance and favorable response (RCB-0/I) is stronger in TNBC and HER2+ vs. HR+HER2-

Future directions

- Examine the correlation between ctDNA and response-predictive subtype (RPS, Wolf et al 2022)
- Elucidate the biology of NAT-resistant tumors while considering their ctDNA status and dynamics (Denise Wolf)
- Validate findings in a larger cohort (~1500 patients)
- Evaluate correlation between ctDNA and imaging (Wen Li)
- Examine the correlation between ctDNA vs. response according to the type of treatment received (e.g., immune checkpoint inhibitor, small molecule inhibitors, HER2-targeted)

Clinical Impact of ctDNA testing in I-SPY2.2

- In I-SPY2.2, ctDNA, in conjunction with imaging and pathology information, can inform treatment redirection
- Patients predicted to have a pCR/RCB-0 can receive surgery early to minimize toxicity (treatment de-escalation)
- Patients predicted to be a no pCR, or RCB-II/III can elect to change therapy to improve the chances of achieving a pCR (treatment escalation)
- ctDNA status/dynamics must be considered in treatment redirection in patients predicted to have RCB-II/III
- Not all NAT-resistant breast tumors are created equal!

pCR – pathologic complete response; RCB – residual cancer burden

Acknowledgements

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Director of Clinical Operations: M. Pitsiouni

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The steroid hormone receptor signature (SRS) in stage II/III TNBC correlates with highly actionable functional protein drug target activation and is associated with early recurrence

Julia D Wulfkuhle, PE Blas, HL Williams, C Mueller, I-SPY 2 Investigators, RI Gallagher, M Pierobon, JA O'Shaughnessy, EF Petricoin III

George Mason University; QuantumLeap Healthcare Collaborative; Baylor Scott and White Research Institute; Texas Oncology, Sarah Cannon Research Institute

RISE UP for Breast Cancer Conference
Nov.1, 2024

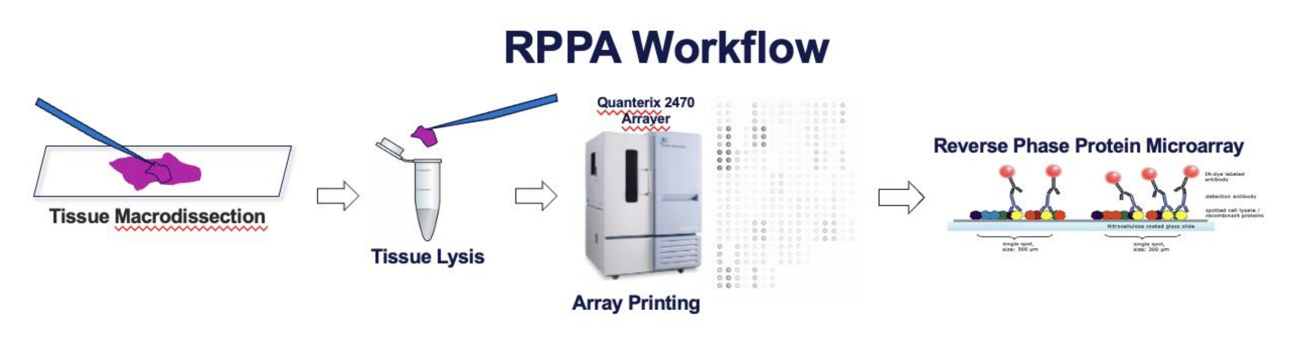
Conflict of Interest

JDW has ownership interest in Ignite Proteomics, Inc. and receives royalties from GMU licenses including protein and phosphoprotein biomarkers

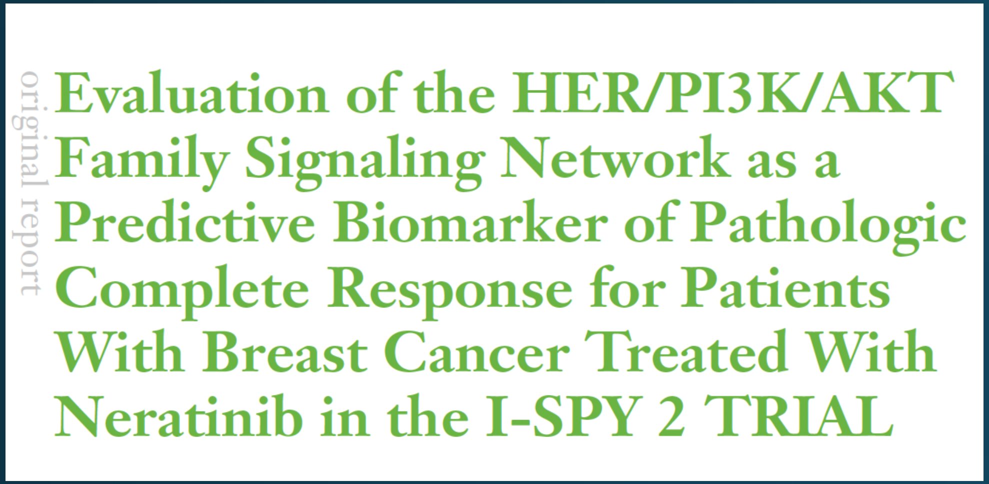
EFP is a consultant and Chair of the Science Advisory Board for Ignite Proteomics, Inc. and receives royalties from GMU licenses that cover protein and phosphoprotein biomarkers.

TNBC is a molecularly heterogeneous disease with poor prognosis

- There is a growing interest in more sensitive and quantitative measurement of biomarkers such as ER and HER2 in the ER neg and HER2 low/neg setting.
  - There are analytical limitations to IHC in quantitating ER and HER2 in low expressing (0-1+) tumors
  - There is a subset of TNBC that are ER and/or HER2 expressing/active (HARPS+) who could potentially benefit from ER or HER2-directed therapies.
- We used highly sensitive reverse phase protein array (RPPA) technology to quantitate expression of these therapeutic targets along with downstream signaling activation mapping in a pilot set of patient-matched TNBC primary (P) and axillary lymph node (LN) metastases obtained synchronously and in TNBC samples from the ISPY2 TRIAL.


RPPA Workflow
Tissue Macrodissection
Tissue Lysis
Quanterix 2470
Arrayer
Array Printing
Reverse Phase Protein Microarray
IR-dye labeled antibody
detection antibody
spotted cell lysate / recombinant proteins
Nitrocellulose coated glass slide
single spot, size: 300 µm
single spot, size: 300 µm

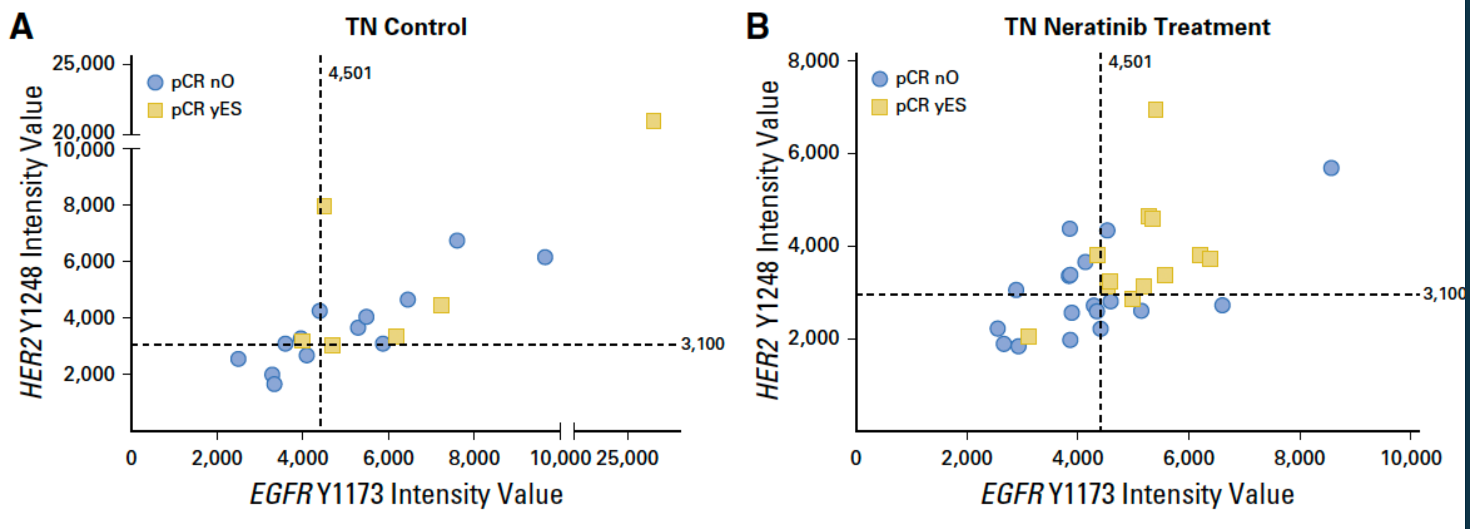
Elevated Estrogen Receptor Expression is Observed in HER2 Activation Response Predictive Signature (HARPS) -Positive TN Tumors Treated with Neratinib in I-SPY 2 TRIAL

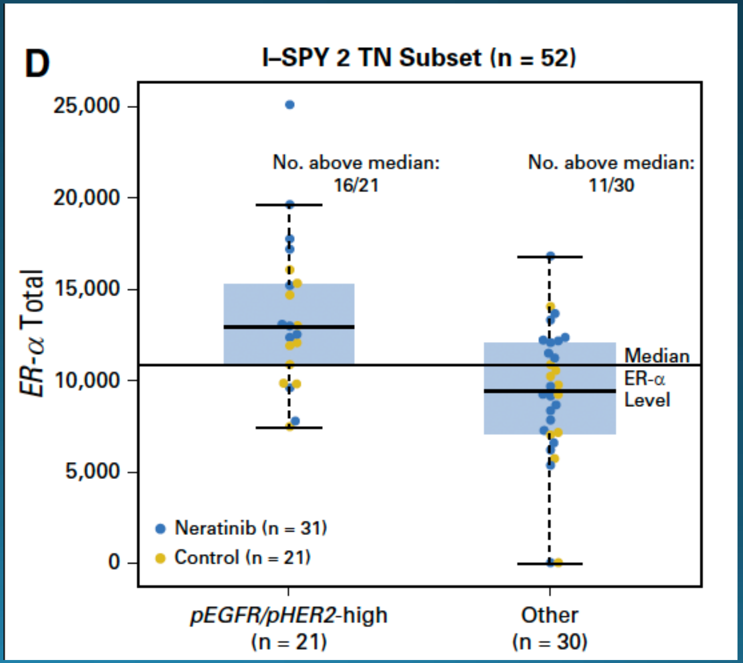


original report

Evaluation of the HER/PI3K/AKT Family Signaling Network as a Predictive Biomarker of Pathologic Complete Response for Patients With Breast Cancer Treated With Neratinib in the I-SPY 2 TRIAL

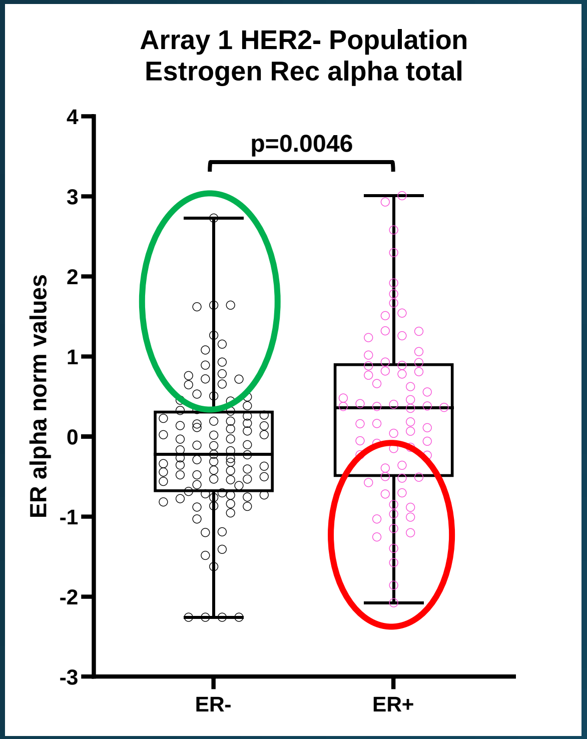
Wulfkuhle et al. JCO Precis Oncol. 2018 Aug 16;2:PO.18.00024. doi: 10.1200/PO.18.00024.

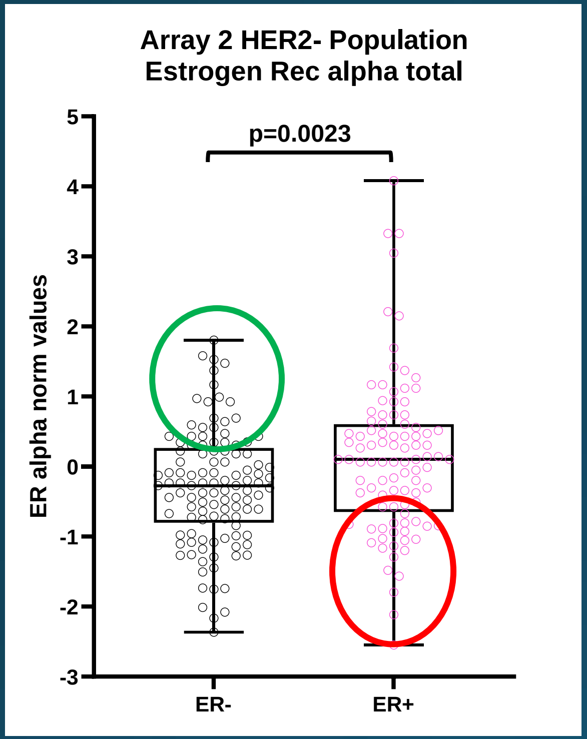


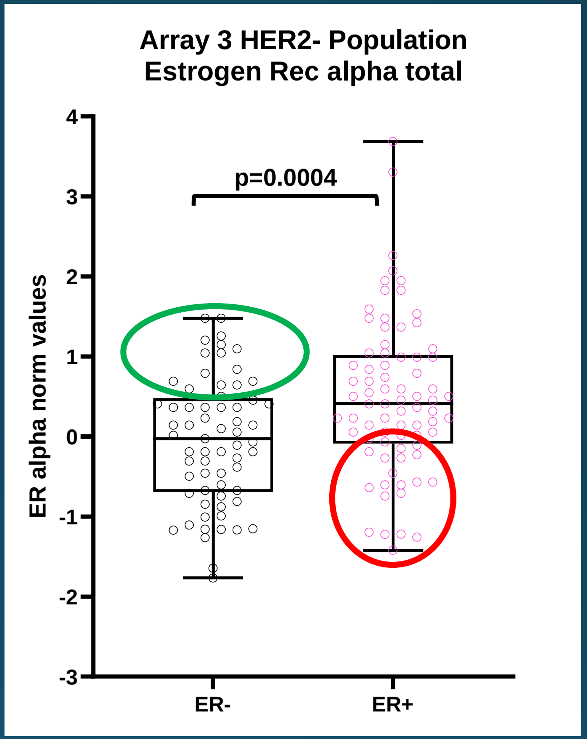


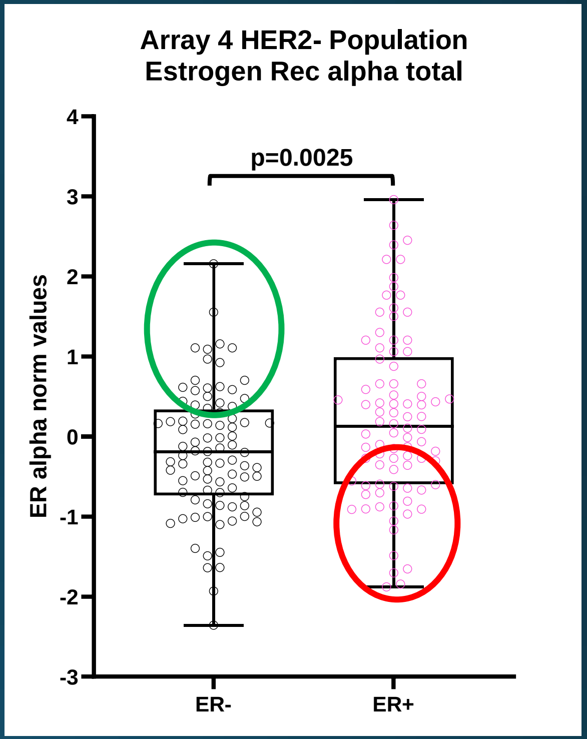
- A subset of TN tumors has activated EGFR and HER2
  - → HER2 Activation Response Predictive Signature (HARPS)
  - →associates with response to neratinib
- We also observed elevated expression of Estrogen Receptor α in HARPS+ tumors

Quantitative RPPA-based Estrogen Receptor α Expression in I-SPY2 HER2- Population


Array 1 HER2- Population
Estrogen Rec alpha total


Array 2 HER2- Population
Estrogen Rec alpha total

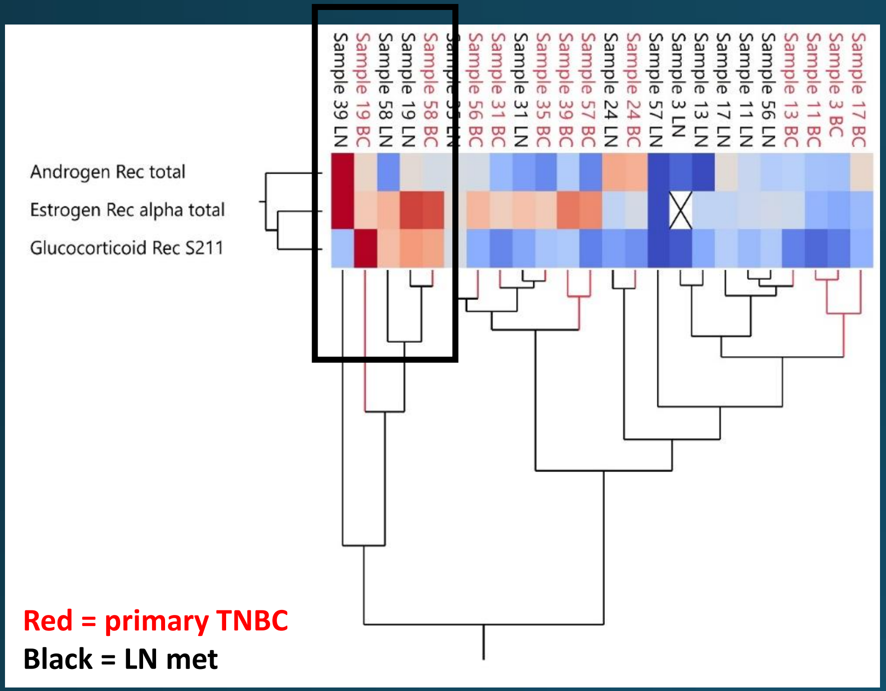

Array 3 HER2- Population
Estrogen Rec alpha total


Array 4 HER2- Population
Estrogen Rec alpha total

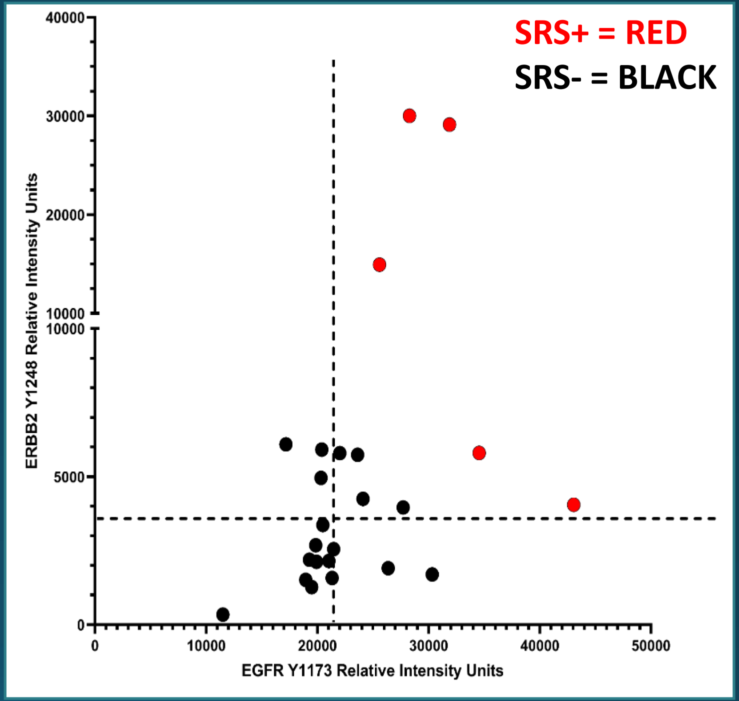
- While significantly different there are largely overlapping distributions between ER- and ER+ populations
- Low end of ER+ population (red circles) may act more like ER- population?
- High end of ER- population (green circles) may act more like ER+ population?
- Provided motivation to explore the role of ER and other steroid hormone receptors (AR and GR) in TNBC tumors

Steroid Receptor Signature (SRS) Characterization in TNBC

- Matched pairs of primary TNBC and synchronous LN metastases



Unsupervised hierarchical clustering of steroid receptor analytes



SRS positive tumors are EGFR-HER2 coactivated

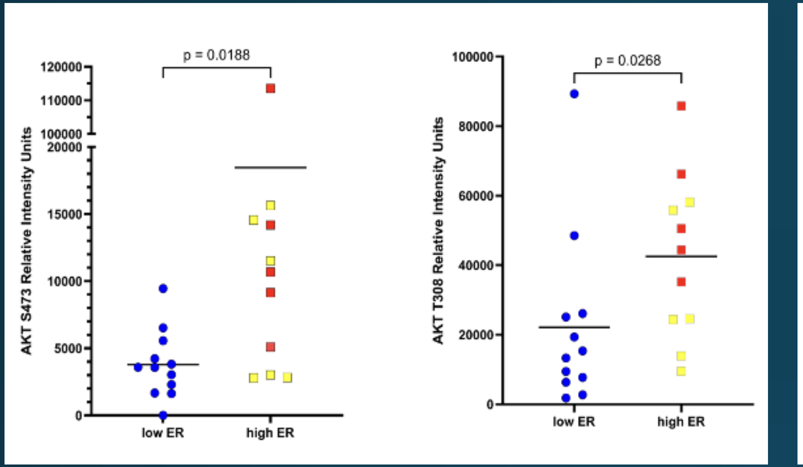
- **We observed that tumors with high relative ER alpha expression (top quartile) had co-incident high relative expression of AR and/or activated/phosphorylated GR (S211).**

- **We categorized these tumors with high ER/AR/GR as Steroid hormone Receptor Signature (SRS) POSITIVE.**

- **Thes SRS+ tumors were also found to be HARPS+ (high co-incident phosphoHER2 and phosphoEGFR)**

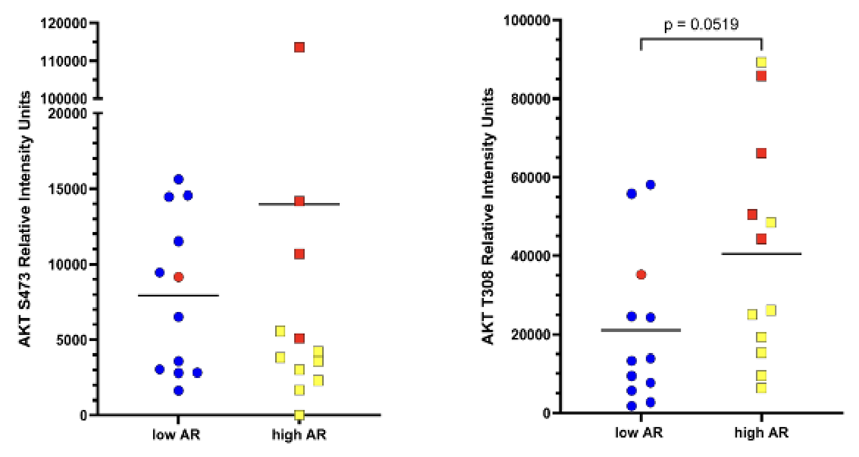
TNBC SRS+ Signature Correlates With AKT Signaling Activation

AKT S473 **AKT T308**



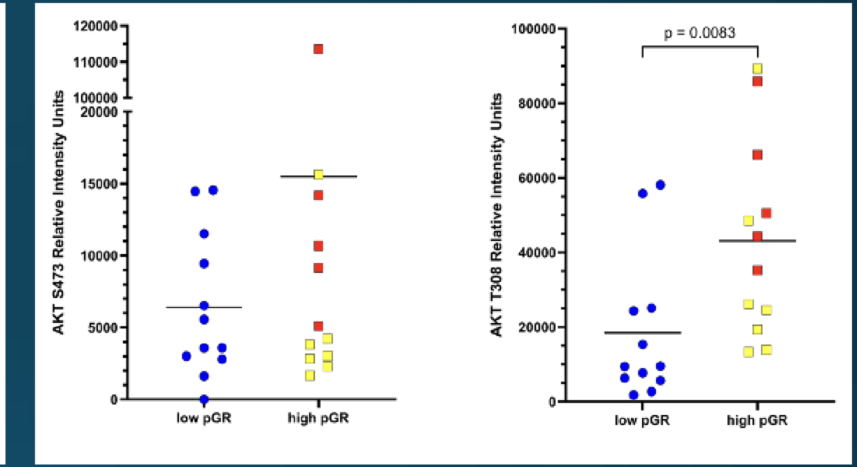
Estrogen Receptor α

AKT S473 **AKT T308**



Androgen Receptor

AKT S473 **AKT T308**

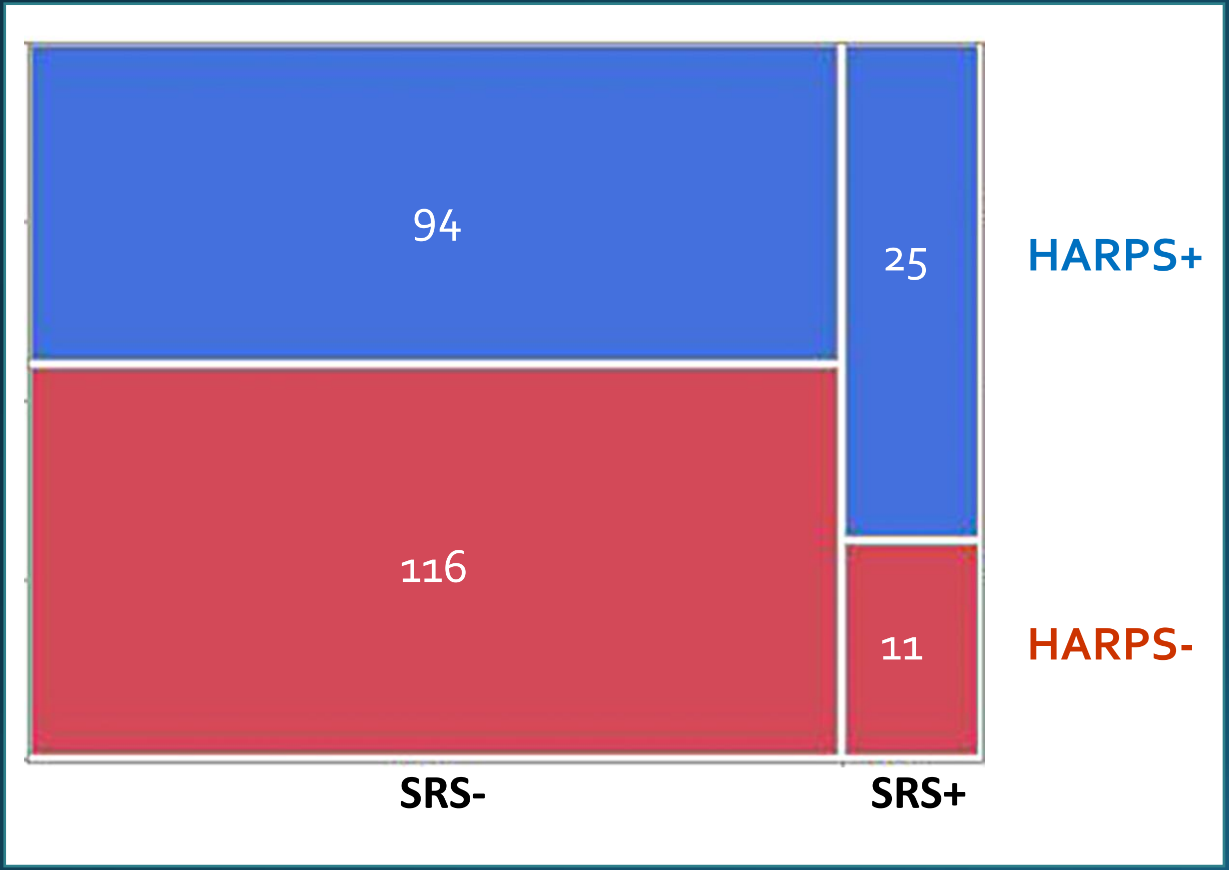


Glucocorticoid Receptor S211

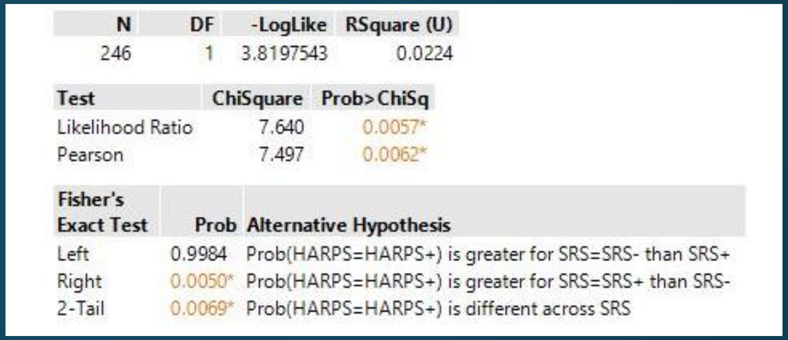
Scatter plots of phosphorylated AKT (S473) and AKT T308 (Right) quantitative levels in TNBCs that have low (below median in BLUE) or high (above median in TELLOW and RED) relative levels of ER, AR, and phospho GR (S211) (BOTTOM). In RED are the 5 SRS Biomarker + samples.

AKT pathway is systemically activated in SRS + TNBCs.

Examination of SRS in I-SPY2 TNBC Population



SRS defined by ER alpha, AR and pGR S211 all above median



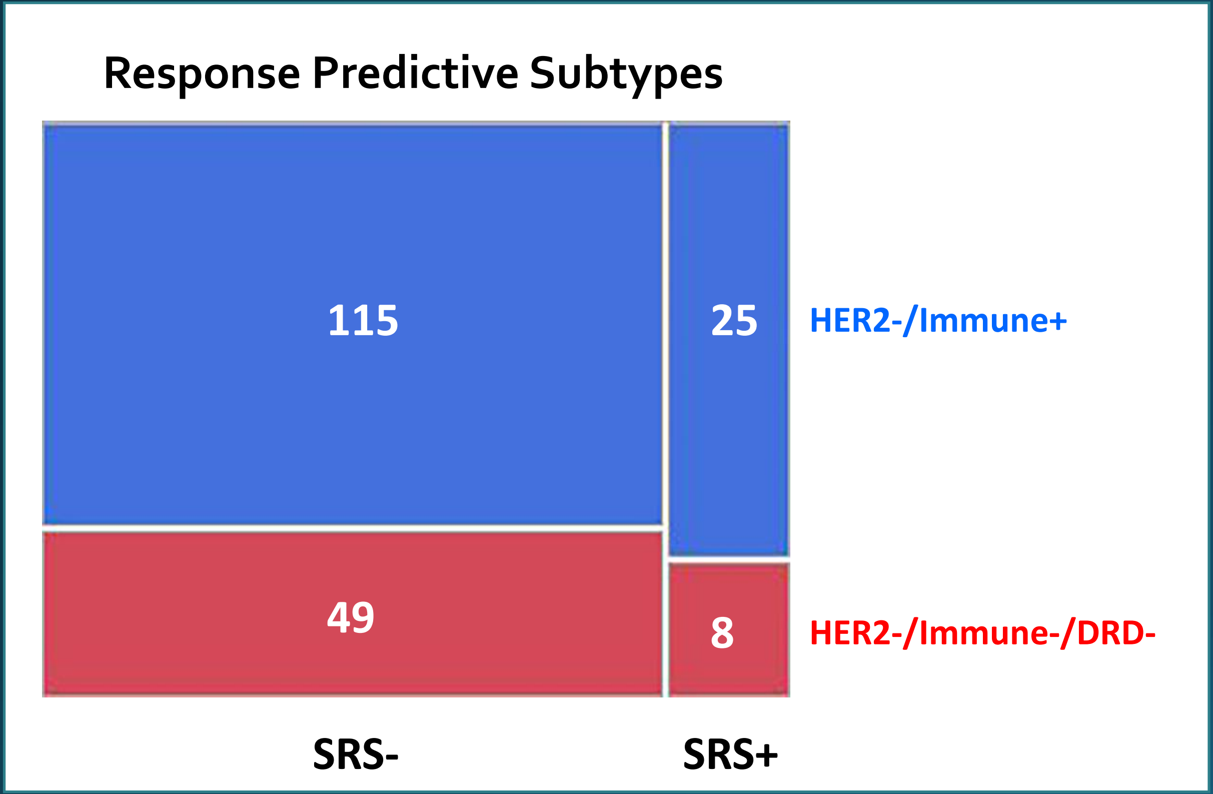
N	DF	-LogLike	RSquare (U)
246	1	3.8197543	0.0224

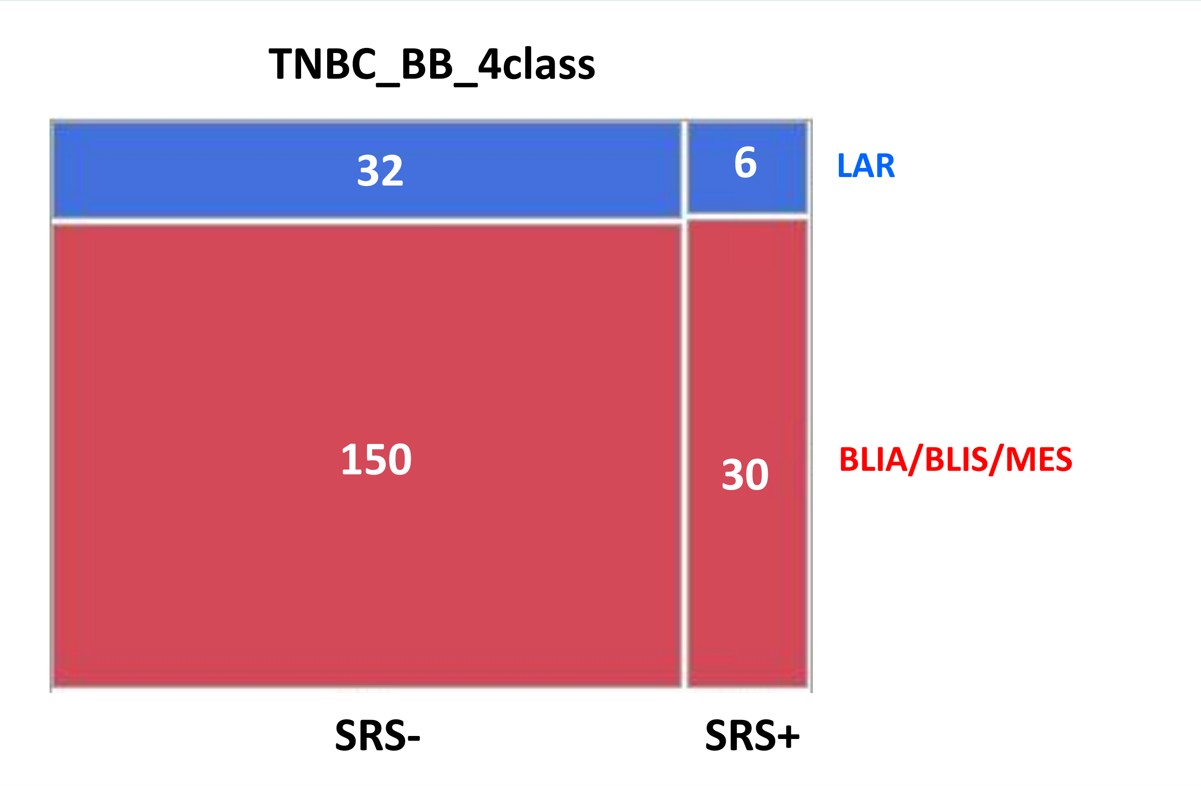
Test	ChiSquare	Prob>ChiSq
Likelihood Ratio	7.640	0.0057*
Pearson	7.497	0.0062*

Fisher's Exact Test	Prob	Alternative Hypothesis
Left	0.9984	Prob(HARPS=HARPS+) is greater for SRS=SRS- than SRS+
Right	0.0050*	Prob(HARPS=HARPS+) is greater for SRS=SRS+ than SRS-
2-Tail	0.0069*	Prob(HARPS=HARPS+) is different across SRS

HARPS positivity significantly associates with SRS positivity in TNBC population from I-SPY 2

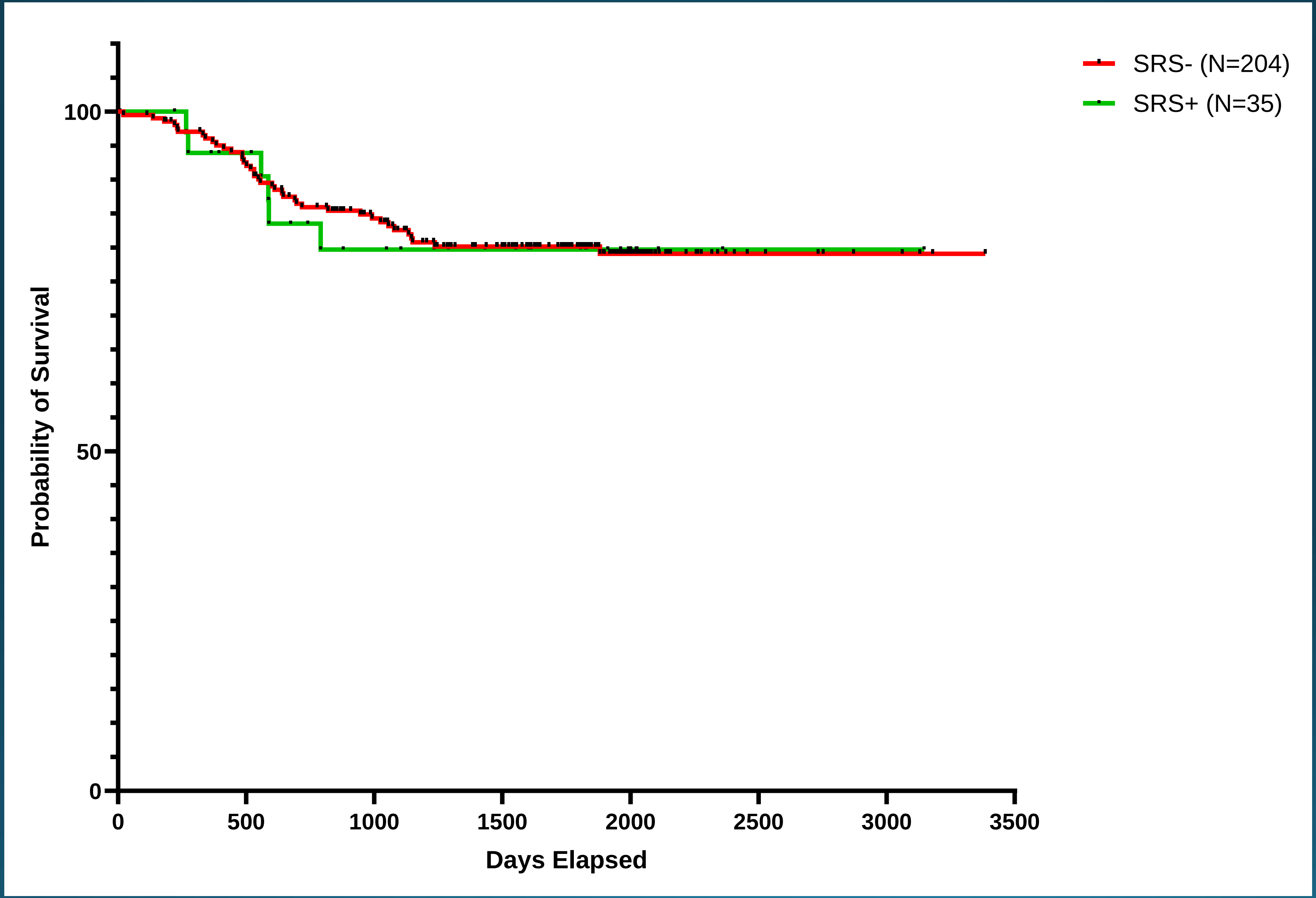
SRS Status Does Not Significantly Associate with Gene Expression Subtypes



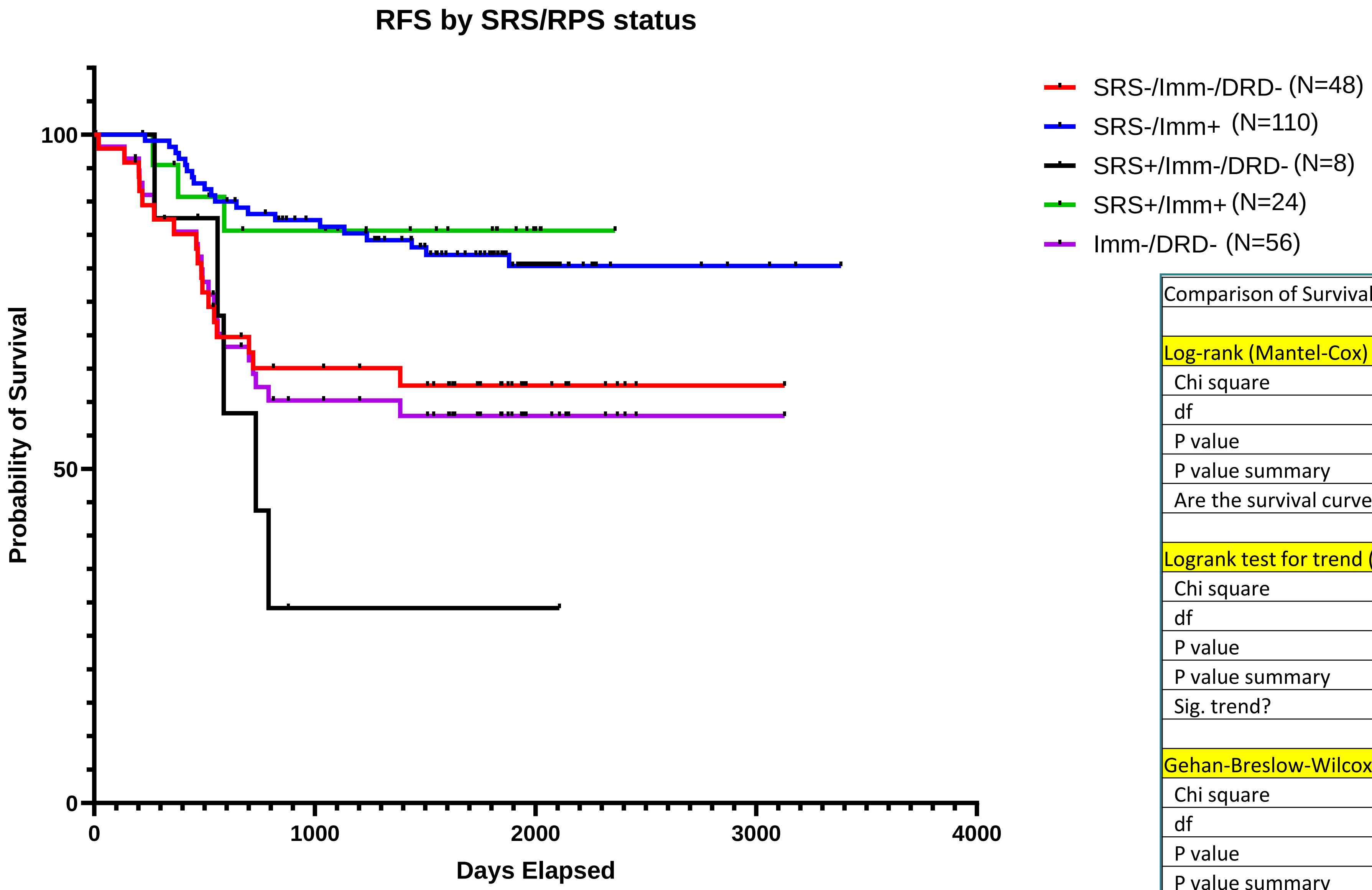


SRS positivity is not explained by RPS Subtypes or TNBC LAR subtype

SRS Status Does Not Associate with Overall Survival in I-SPY2 TN Population

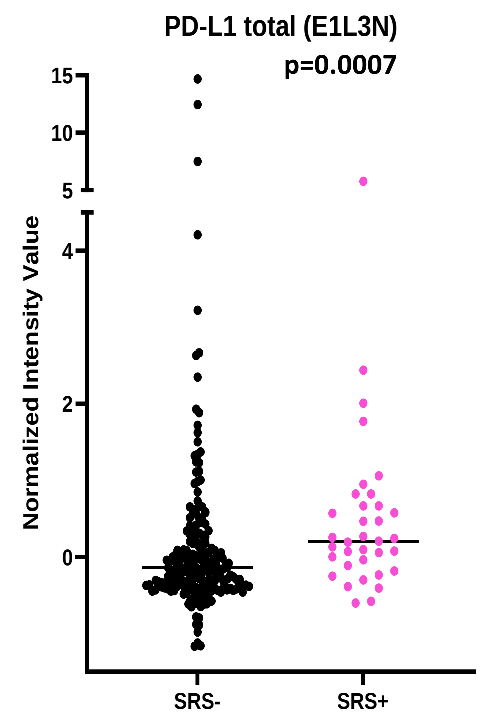


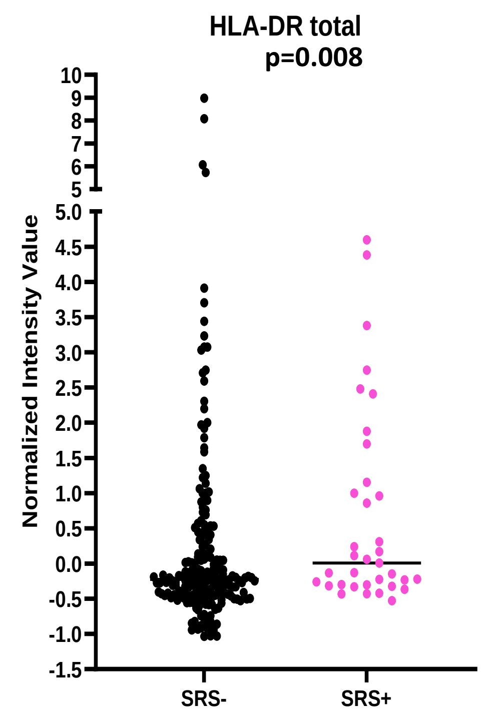
SRS+/Imm-DRD- TNBC Has Significantly Worse RFS

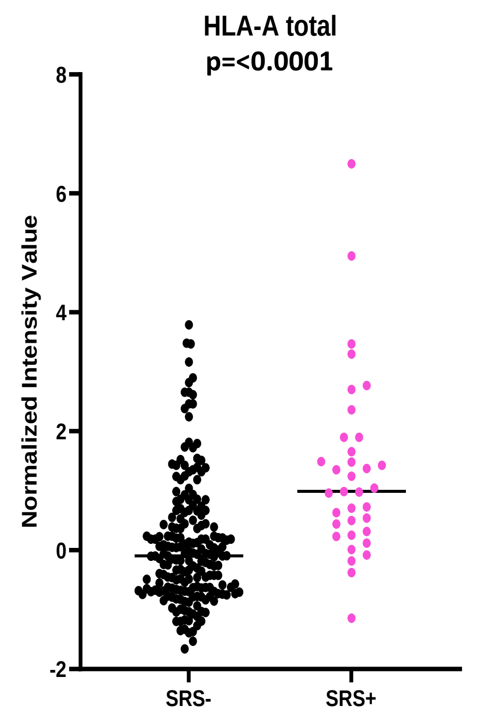


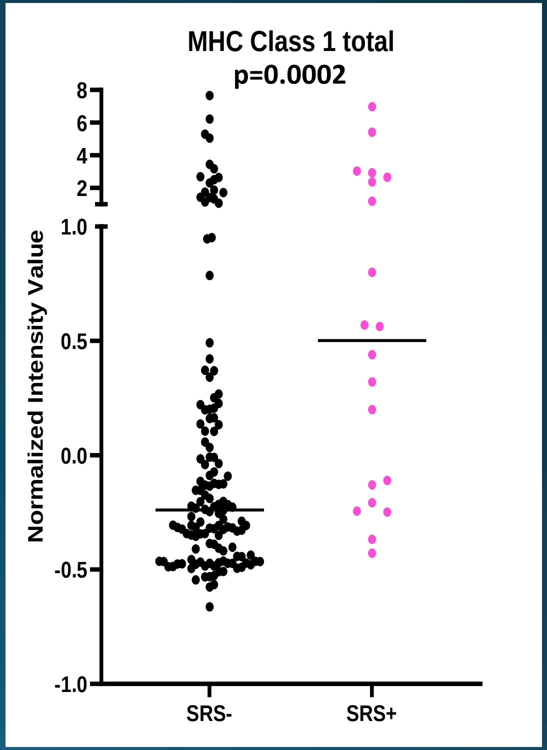
Comparison of Survival Curves	RFS by SRS/RPS + Imm-/DRD- pop
Log-rank (Mantel-Cox) test (recommended)	
Chi square	20.83
df	4
P value	0.0003
P value summary	***
Are the survival curves sig different?	Yes
Logrank test for trend (recommended)	
Chi square	2.199
df	1
P value	0.1381
P value summary	ns
Sig. trend?	No
Gehan-Breslow-Wilcoxon test	
Chi square	21.07
df	4
P value	0.0003
P value summary	***
Are the survival curves sig different?	Yes

Immune Checkpoint Drug Targets are elevated in SRS+ TNBC



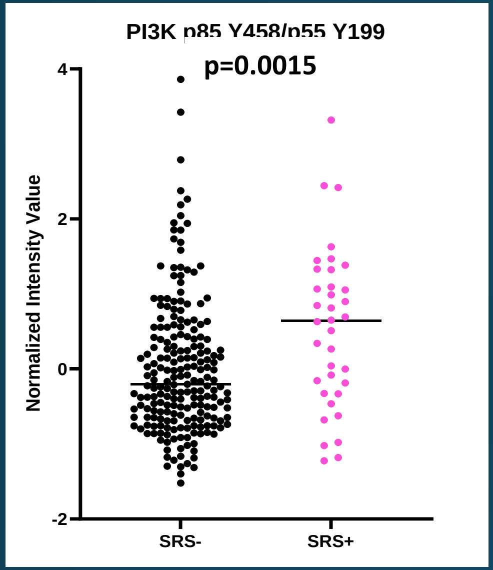


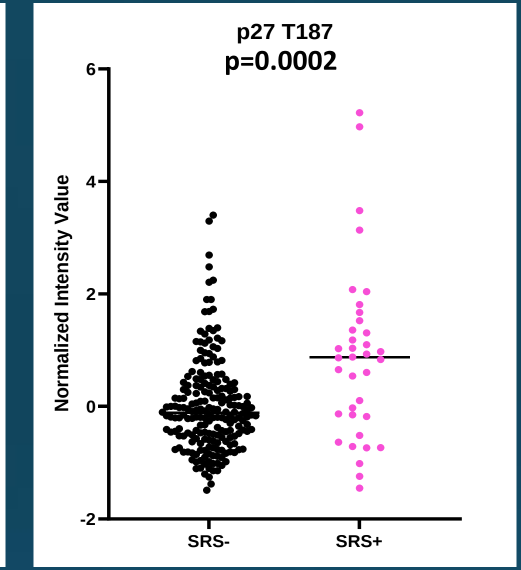


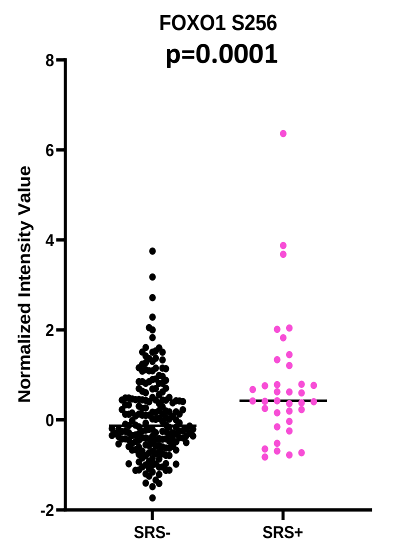


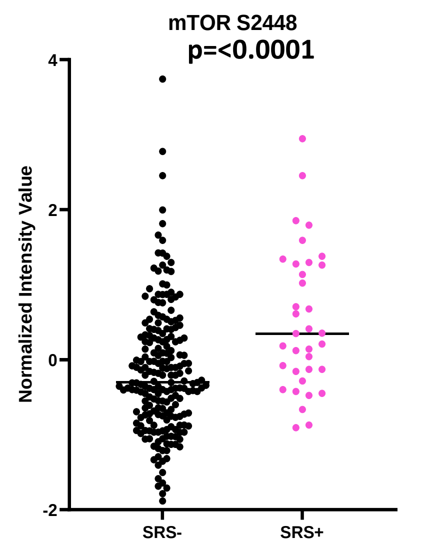
*All p=values are FDR corrected

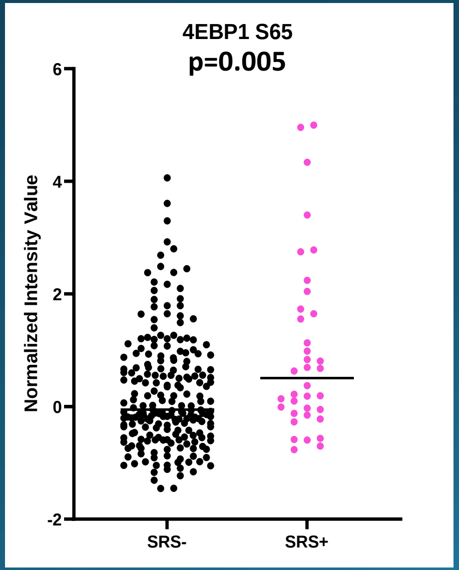
AKT-mTOR pathway is activated in SRS+ TNBC

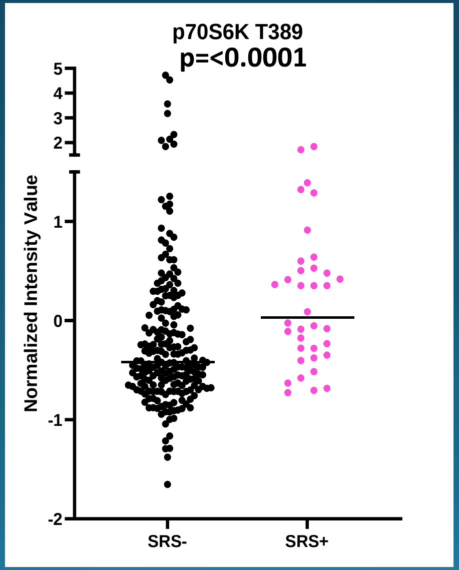


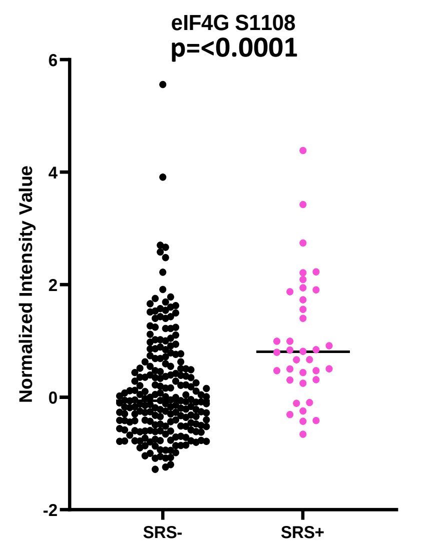






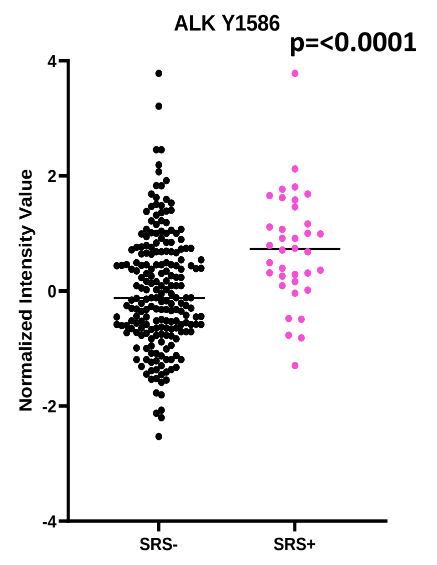


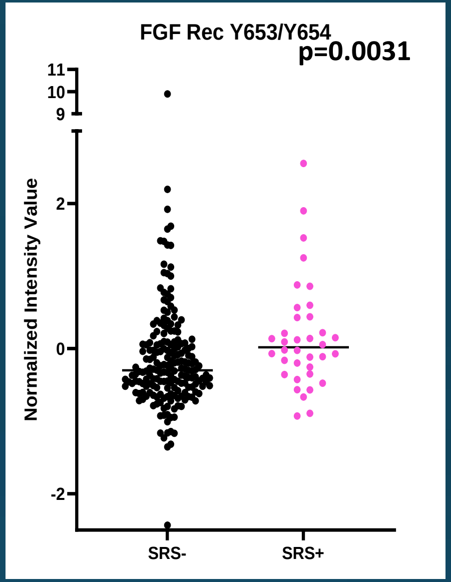


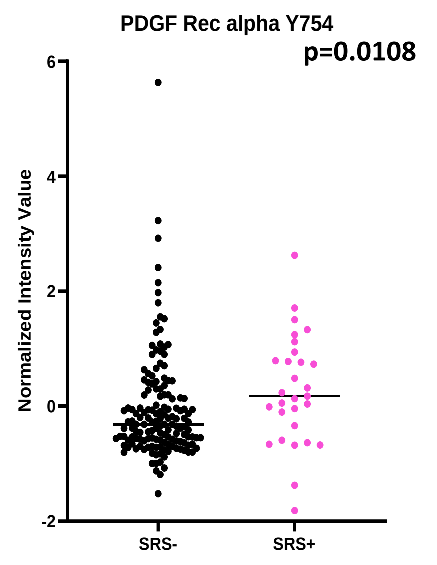


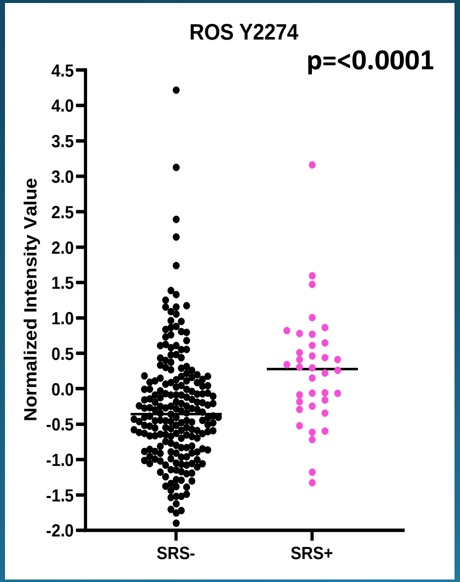
*All p=values are FDR corrected

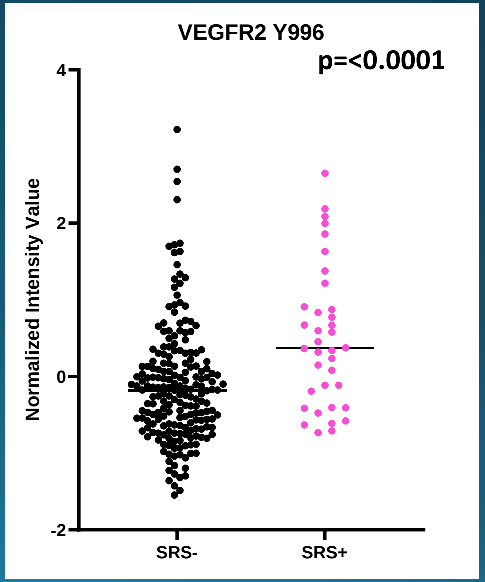
Receptor Tyrosine Kinases are Elevated in SRS+ TNBC





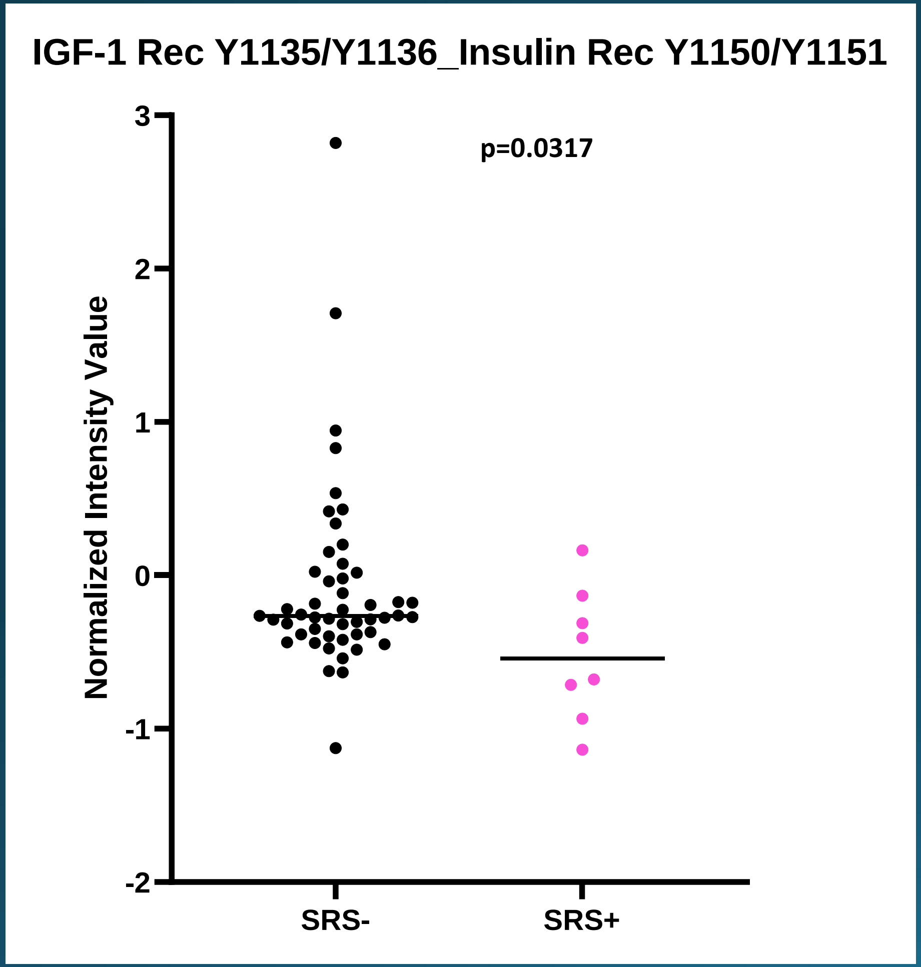


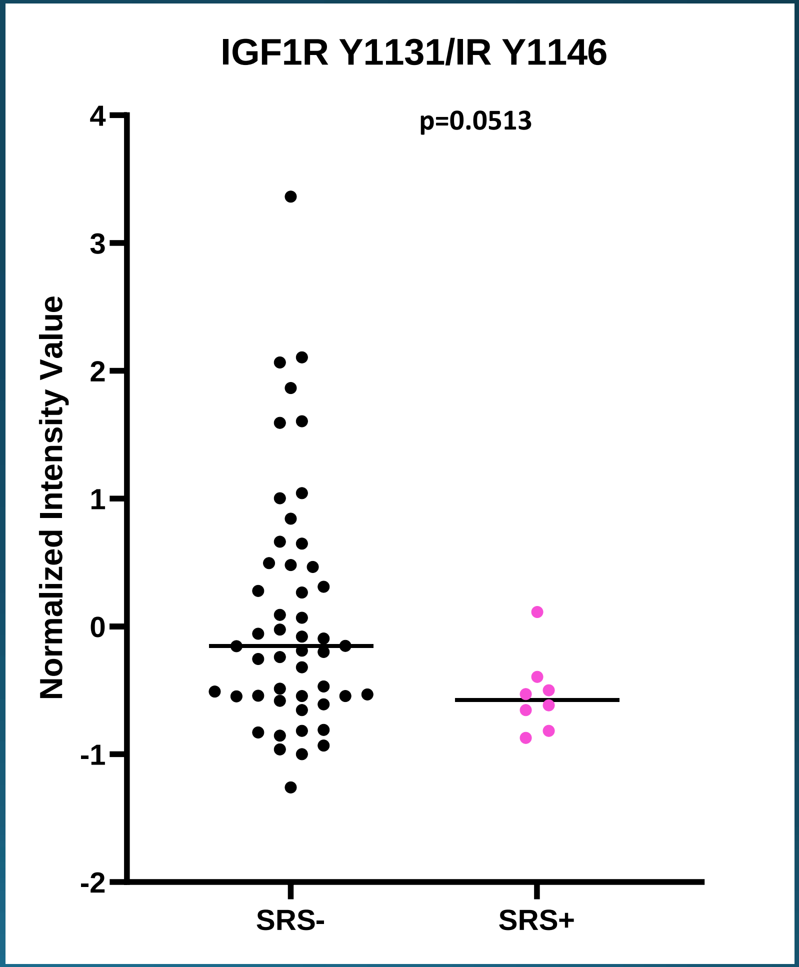




*All p=values are FDR corrected

IGFR/Insulin Receptor Activity Trends Higher in SRS- TNBC
Patient cohort: Recurrence <4y





*p-values uncorrected

SRS+ defined by:

↑
ER/AR/GR
RTK (ALK, HER2, EGFR, HER3,MET etc)
ROR
eNOS pathway
B-catenin pathway
AKT-mTOR pathway
RAS-RAF-ERK-p38 pathway
DDR pathway (p53, ATM, ATR, CHK, MDM2 etc)
I/O (JAK-STAT, PDL1, MHCI, MHCII, MSH,etc)
Autophagy

Found in:
all TNBC
TNBC with RCB II/III
TNBC with < 4 YR recurrence
TNBC who are dead/recur vs alive never recur

SRS- defined by:

↑ IGFR/IR pathway

Found in:
TNBC with < 4 YR recurrence
TNBC who are dead/recur vs alive never recur

Summary

- TNBC have a subpopulation of tumors with high relative (above median) ER expression that have co-incident high expression (above median) of either AR or GR or both.

- This subpopulation is defined as SRS (Steroid Receptor Signature) positive and accounts for approx 30% of TNBC.

- SRS positivity is characterized by activation of AKT-mTOR pathway, RTK activation, HARPS positivity, increased autophagy, ROR and immune checkpoint protein expression.

- These expression/activation phenotypes of SRS+ are observed in the T0 of TNBC with RCB II/III, TNBC with < 4y recurrence and TNBC who are dead/recur vs alive never recur.

- SRS+/- does not appear to correlate with survival although SRS+/Imm-DRD- TNBC has significantly worse RFS then any other subtype analyzed to date.

- While a number of these results generalized across independent study sets, we will continue to explore the clinical and biochemical significance of SRS in TNBC tumors in expanded study sets.

- SRS+ may define a subset of TNBC who would be especially sensitive to treatment with HER2 TKIs, AR/GR inhibitors, AKT-mTOR inhibition, etc. and these therapeutics may effectively target tumors that are especially aggressive and have worse outcomes in the adjuvant setting.

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BSW/Texas Oncology/Sarah Cannon Research Inst

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MASONIC CANCER CENTER
UNIVERSITY OF MINNESOTA

Examining APOBEC3B in TCGA and ICGC Breast Cancer Datasets Reveals Altered Drug Metabolism Pathways

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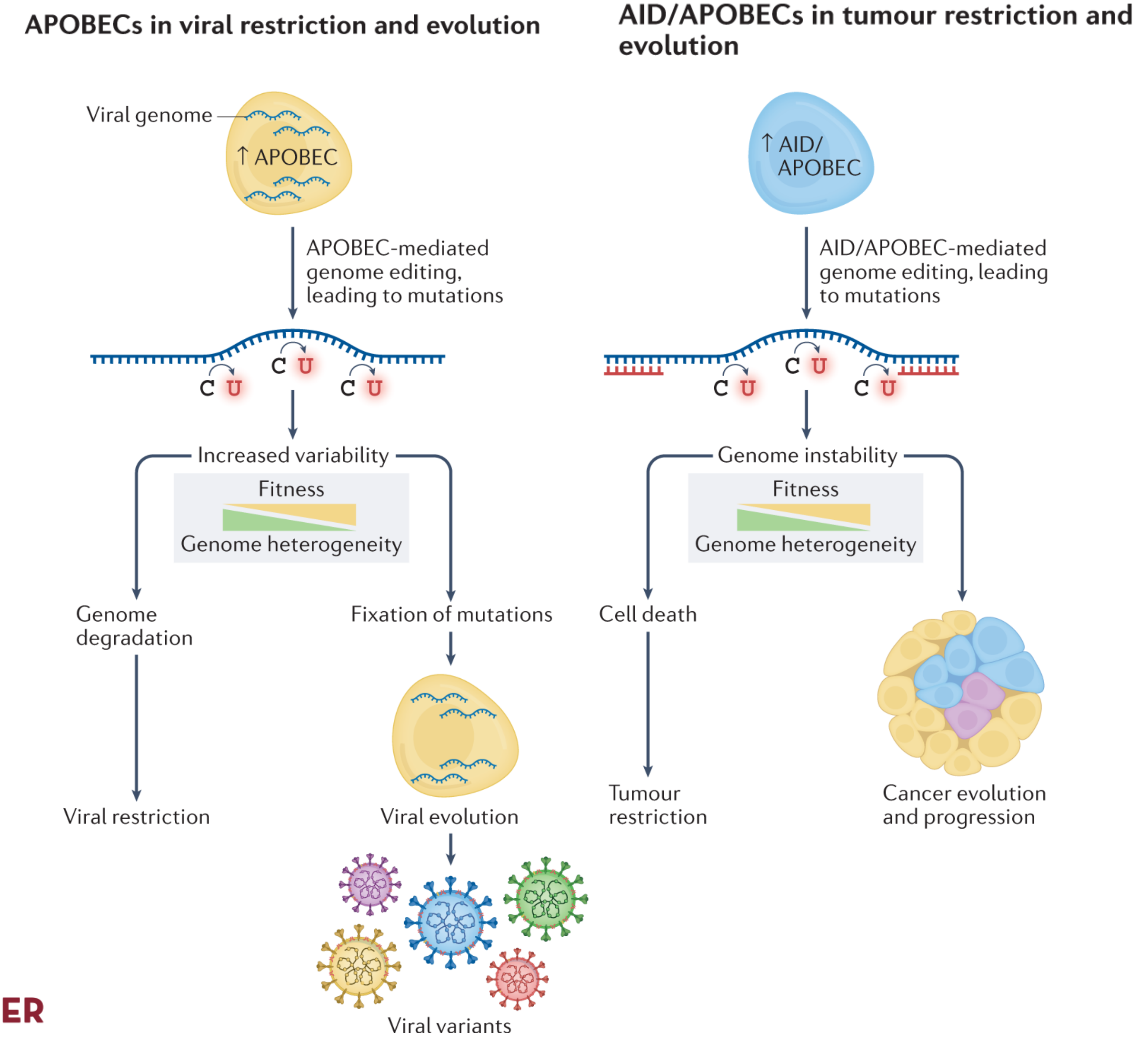
Pardo034@umn.edu
RISE-UP
November 1-3, 2024

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

Causes of mutations in cancer

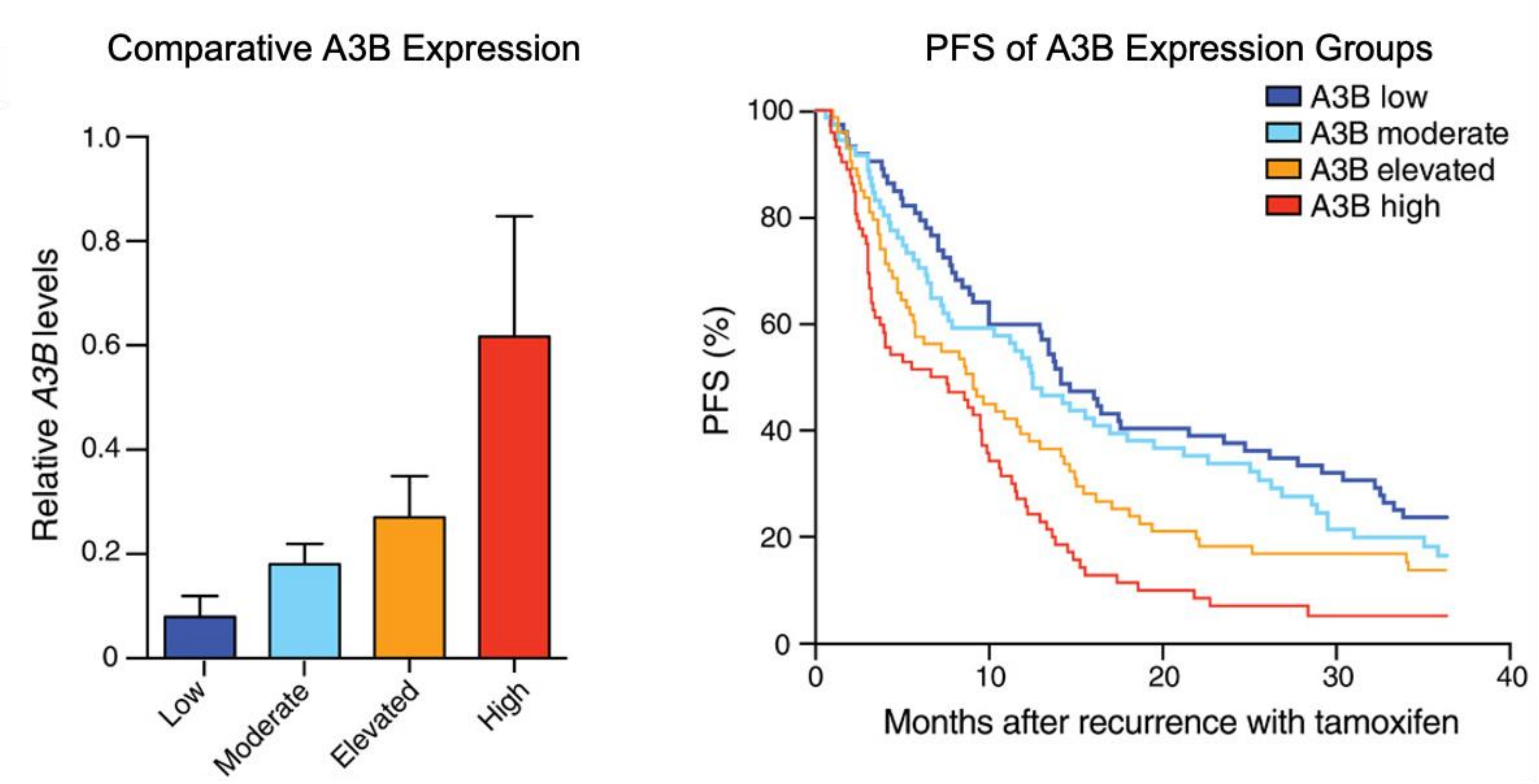
- Known causes:
  - Aging
  - Repair deficiencies (i.e., BRCA)
  - Viruses (i.e., HPV)
  - Endogenous Proteins (i.e., APOBECs)

Endogenously, APOBECs act as viral restriction enzymes


APOBECs in viral restriction and evolution
AID/APOBECs in tumour restriction and evolution
Viral genome
↑ APOBEC
APOBEC-mediated genome editing, leading to mutations
C U
C U
C U
Increased variability
Fitness
Genome heterogeneity
Genome degradation
Fixation of mutations
Viral restriction
Viral evolution
Viral variants
↑ AID/ APOBEC
AID/APOBEC-mediated genome editing, leading to mutations
C U
C U
C U
Genome instability
Fitness
Genome heterogeneity
Cell death
Tumour restriction
Cancer evolution and progression

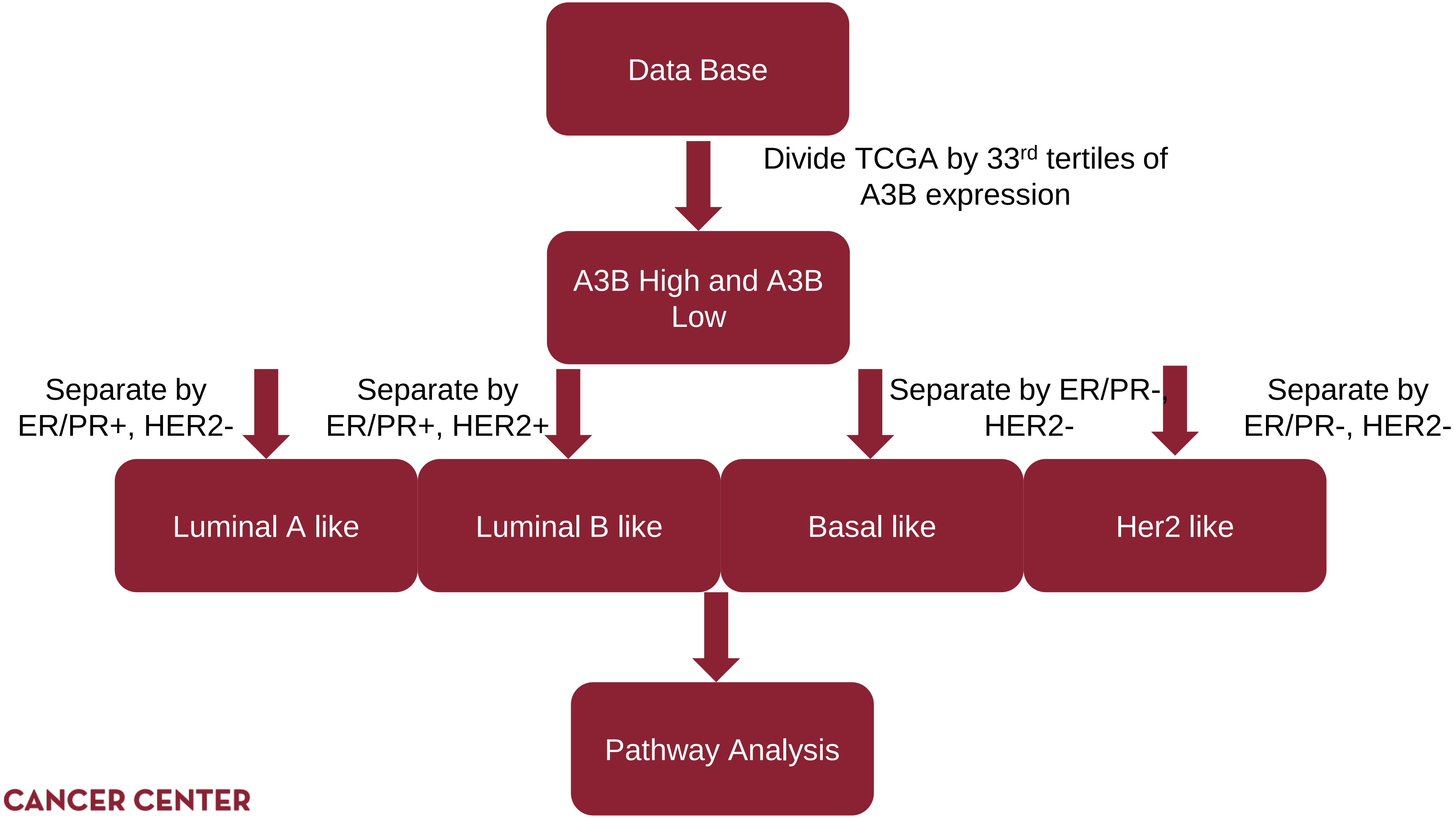
Pecori R, Di Giorgio S, Paulo Lorenzo J, et al. *Nat Rev Genet* 2022;23(8):505-518

APOBEC3B (A3B) level correlates with higher rates of recurrence in HR+ tumors



Law EK, Sieuwerts AM, LaPara K, et al. *Sci Adv* 2016;2(10):e1601737

TCGA Pathway Analysis



Data Base

Divide TCGA by 33rd tertiles of A3B expression

A3B High and A3B Low

Separate by ER/PR+, HER2-

Separate by ER/PR+, HER2+

Separate by ER/PR-, HER2-

Separate by ER/PR-, HER2-

Luminal A like

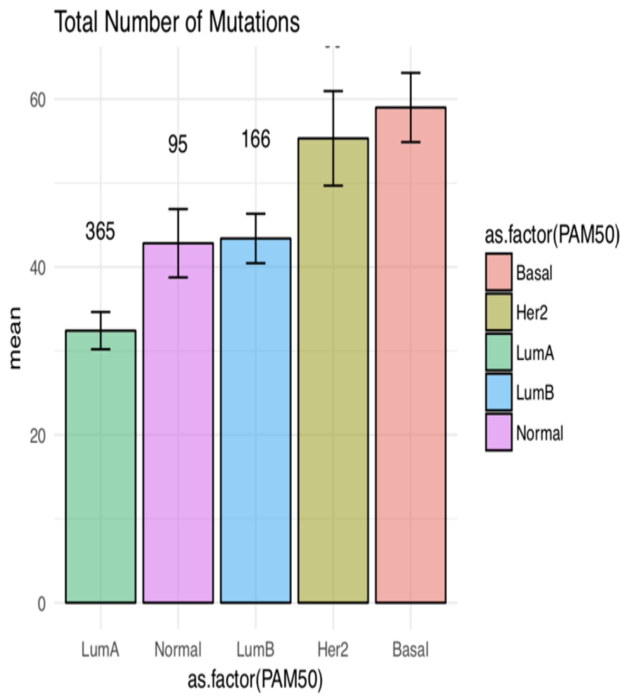
Luminal B like

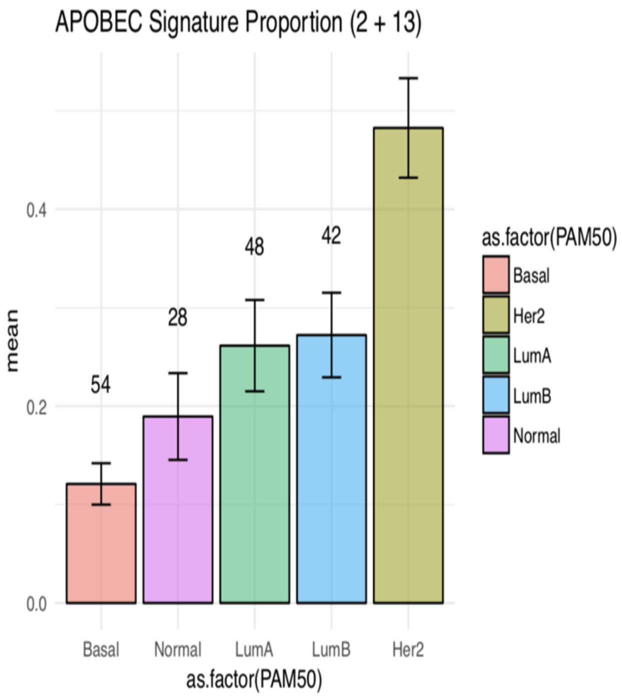
Basal like

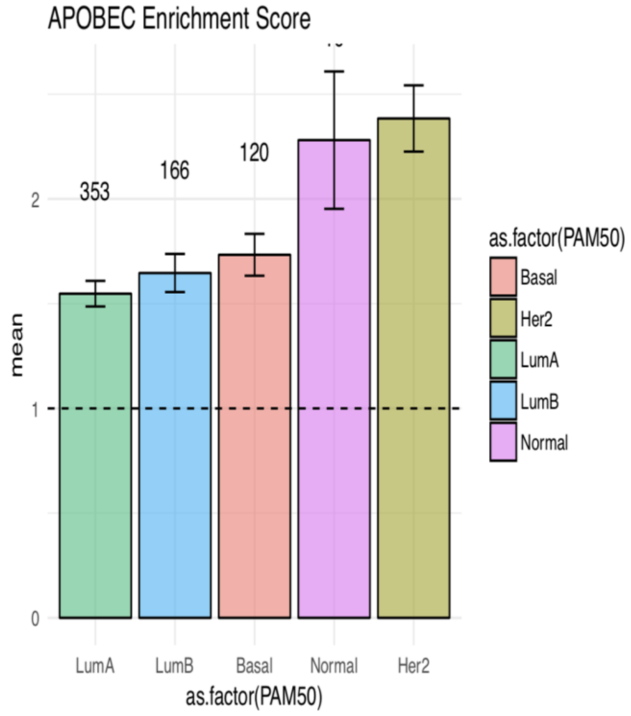
Her2 like

Pathway Analysis

APOBEC mutations across breast cancer subtypes

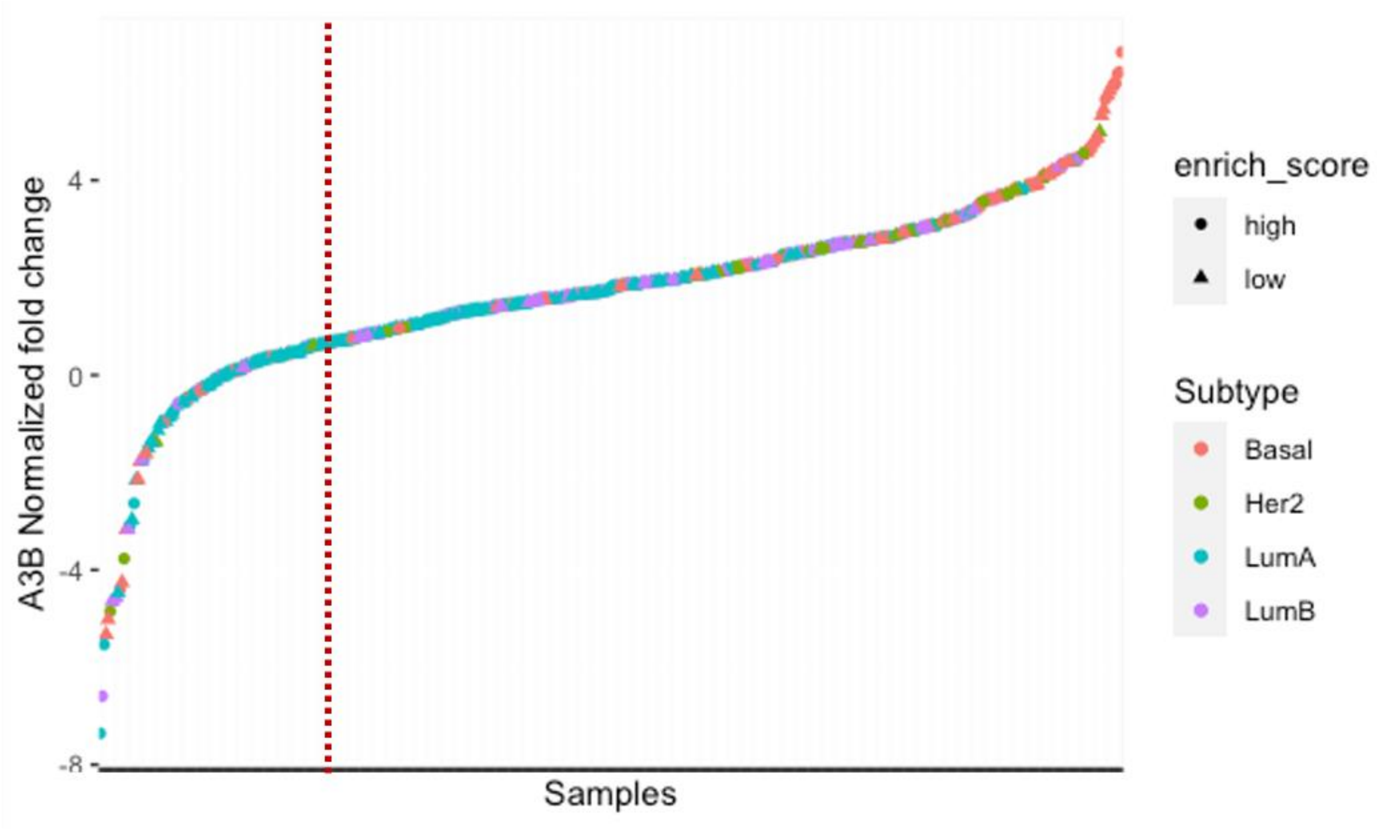


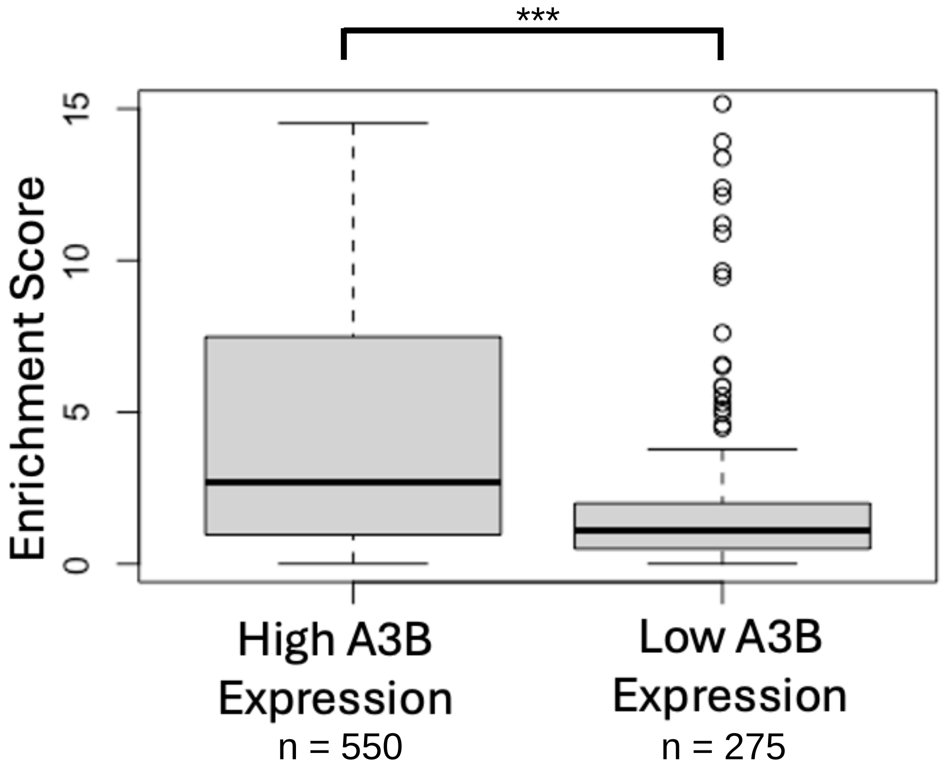




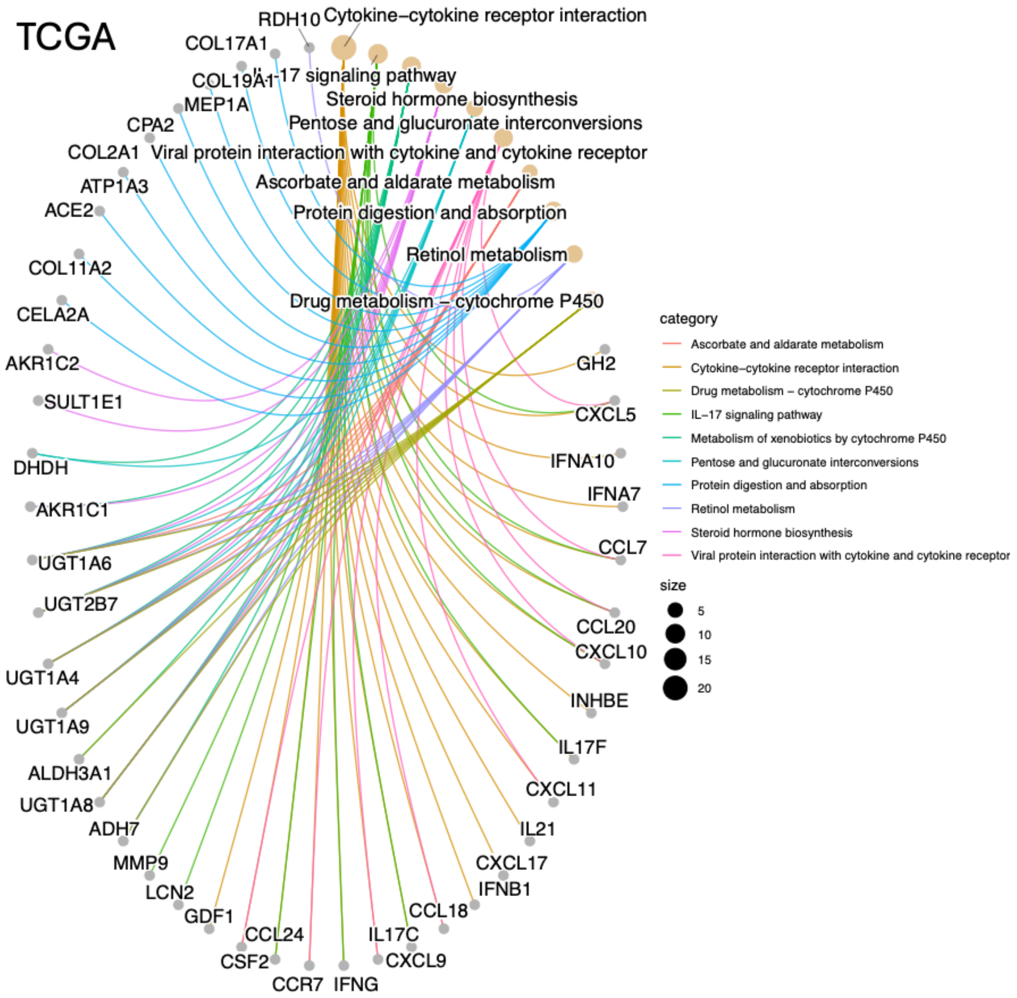
From TCGA

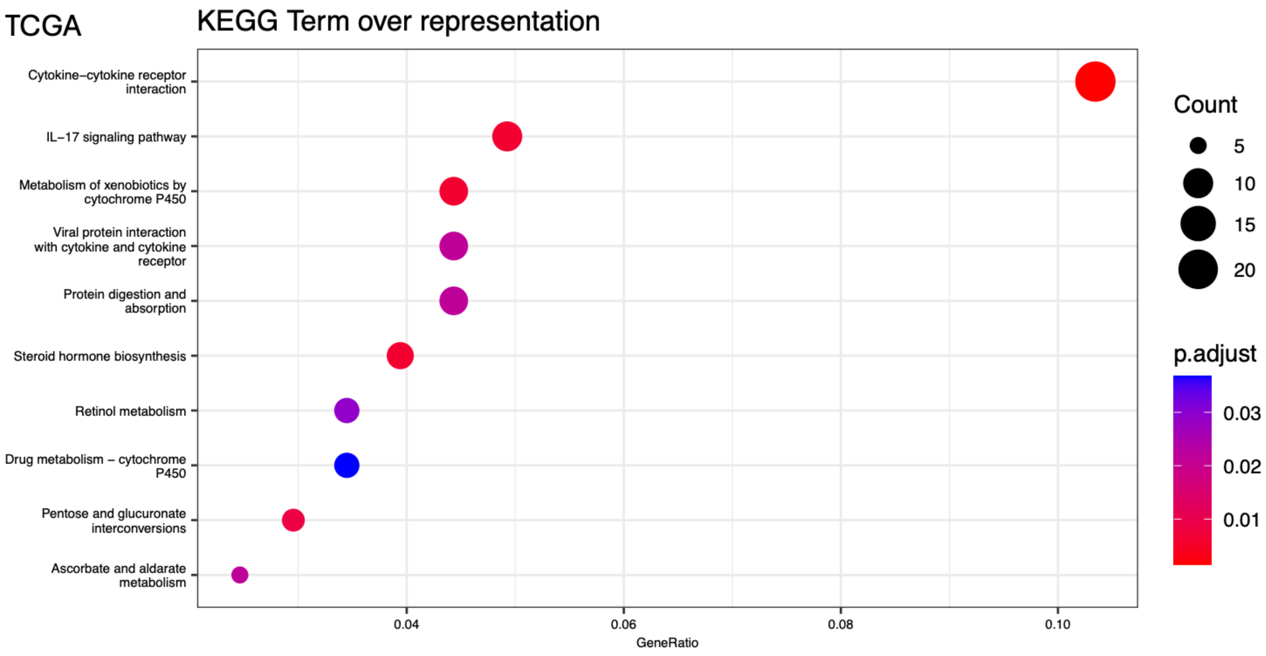
Tumors with higher levels of A3B expression have a higher enrichment score





TCGA pathway analysis shows several immune related pathways





A3B High vs Low

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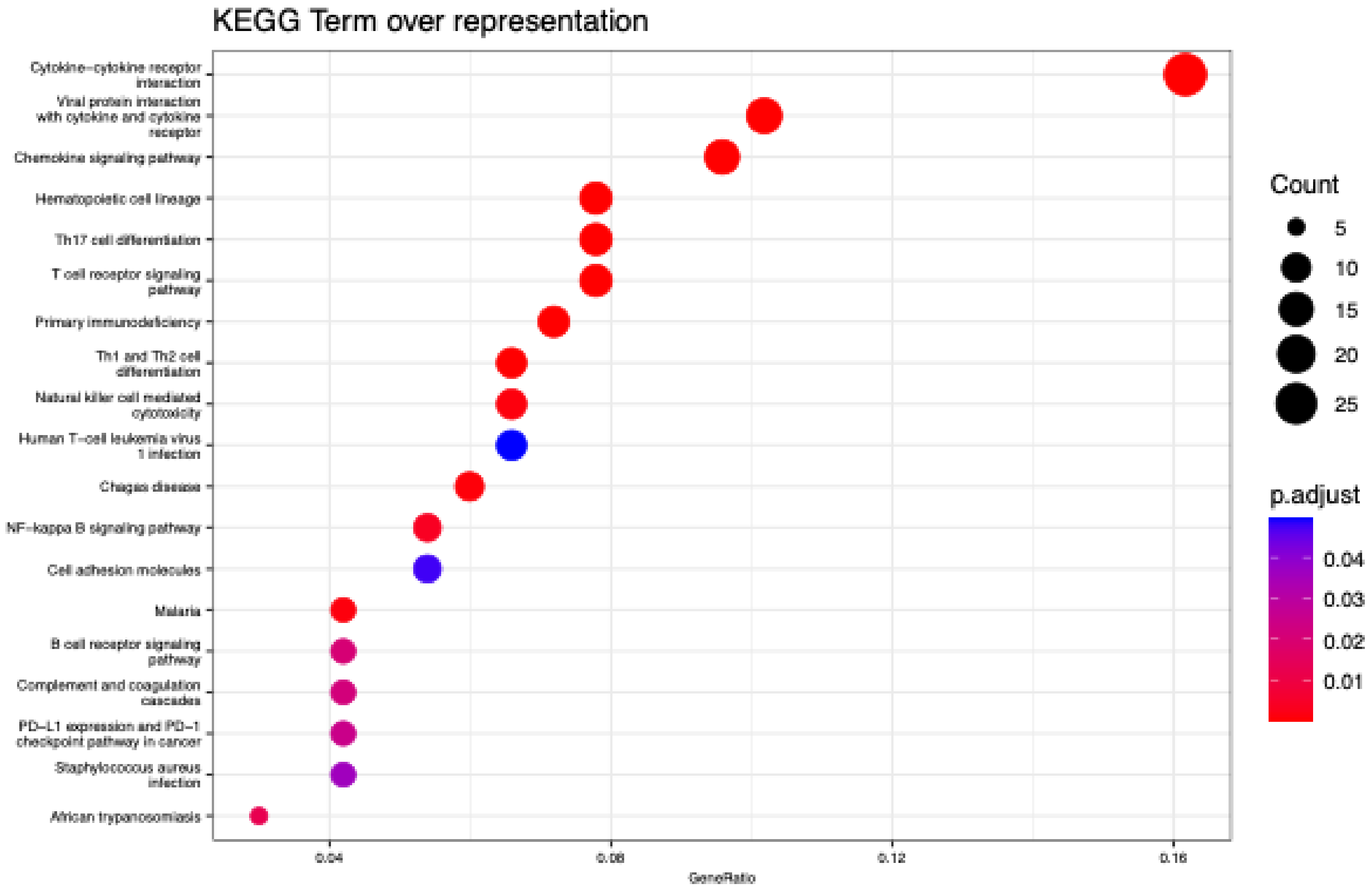
In ICGC, pathway analysis is dominated by immune mediated signaling



A3B High vs Low

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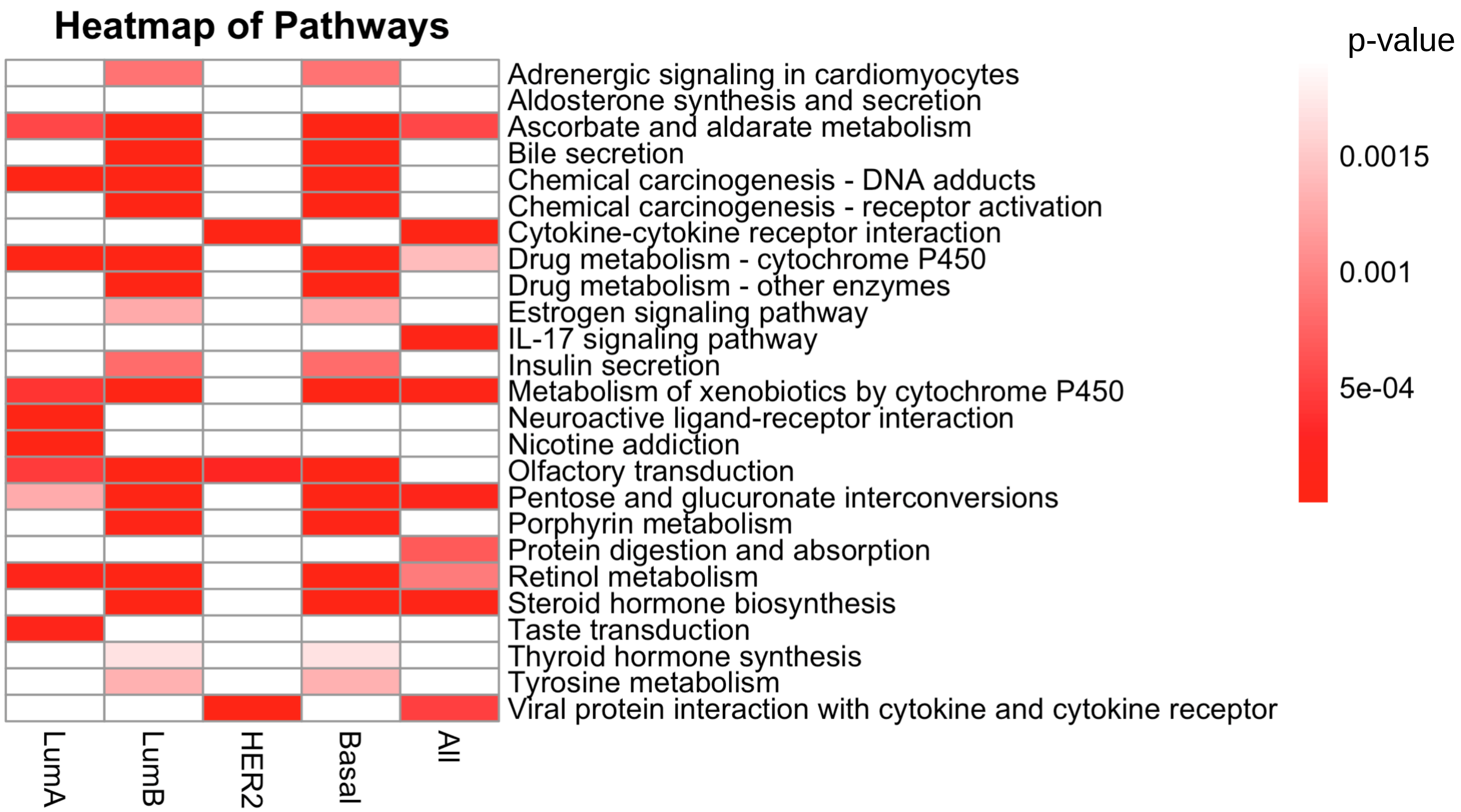
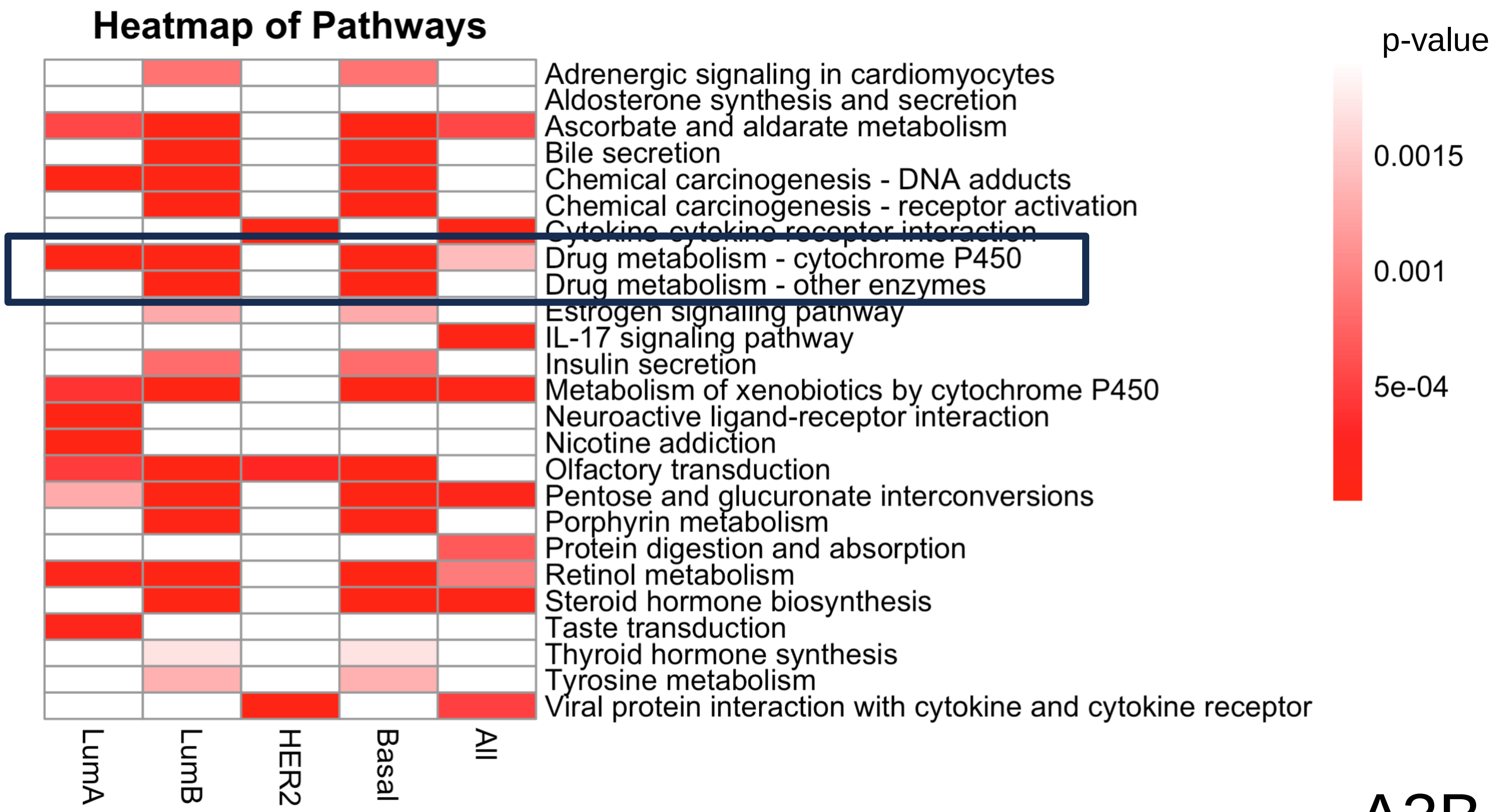
Pathways related to the P450 system are shared between most subtypes



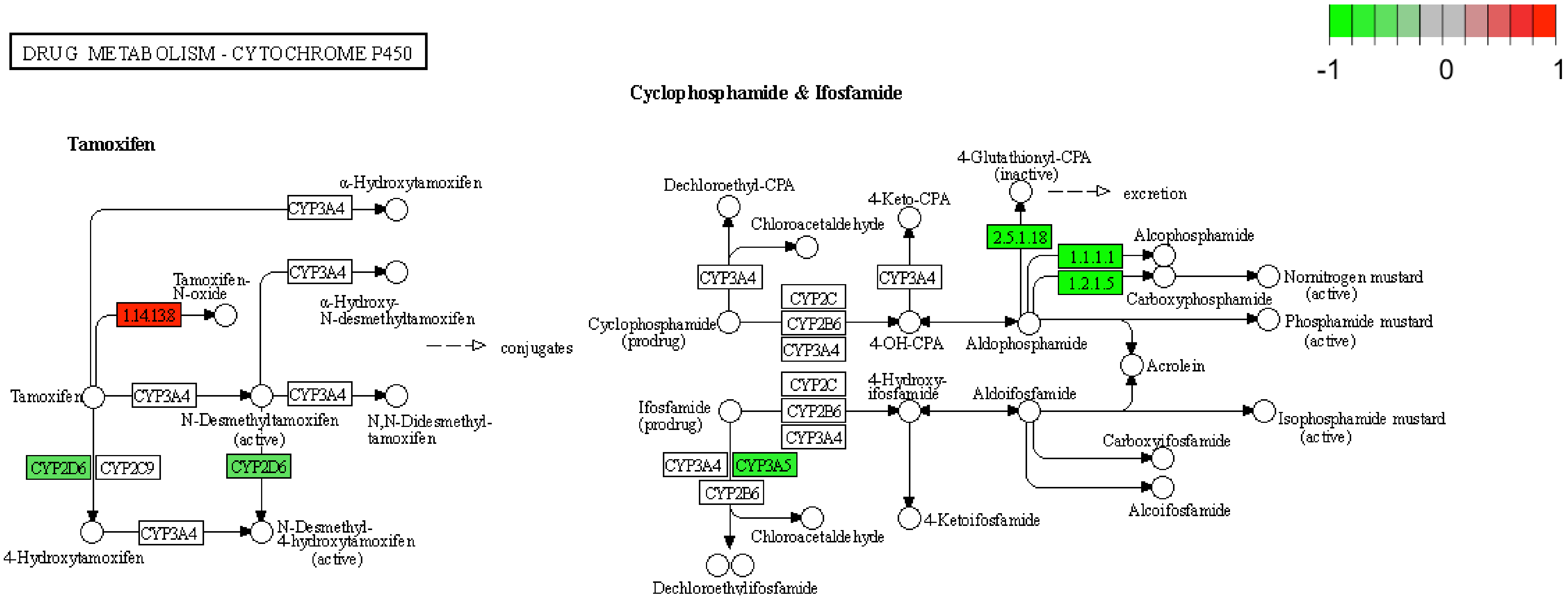
A3B High vs Low

Pathways related to the P450 system are shared between most subtypes



A3B High vs Low

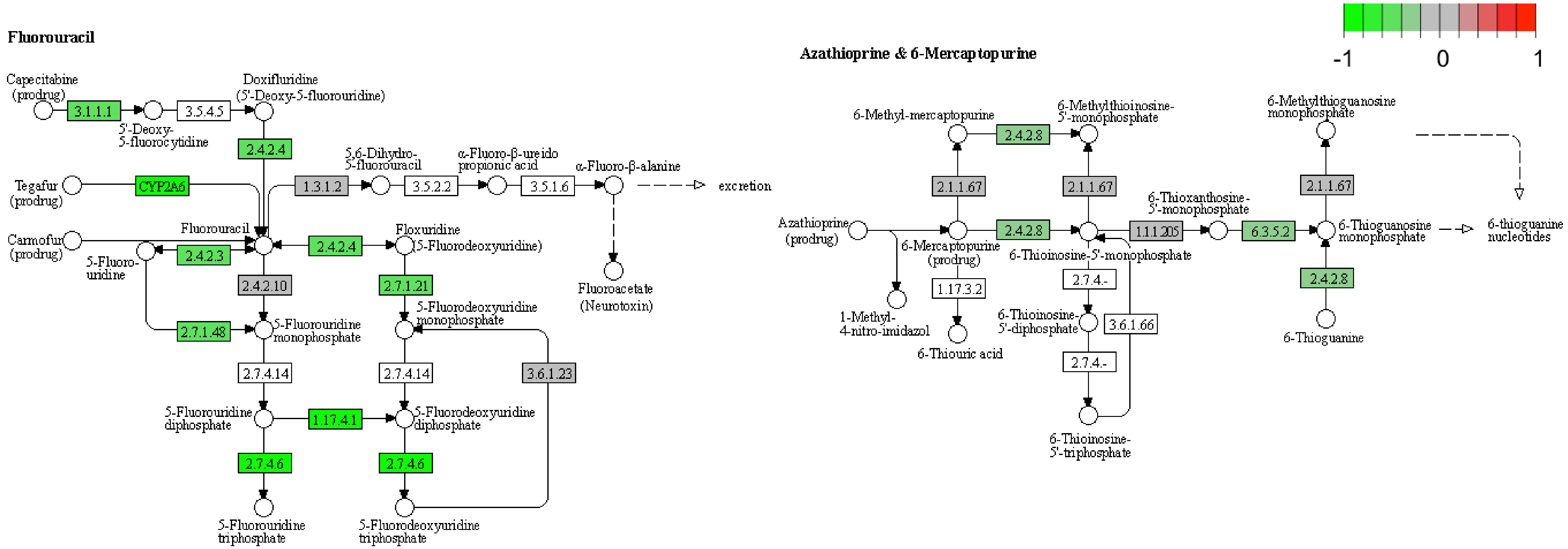
CYP2D6 downregulation is seen in most subtypes



DRUG METABOLISM - CYTOCHROME P450
-1
0
1
Cyclophosphamide & Ifosfamide
Tamoxifen
α-Hydroxytamoxifen
CYP3A4
CYP3A4
Tamoxifen-N-oxide
1.14.13.8
α-Hydroxy-N-desmethyltamoxifen
conjugates
Tamoxifen
CYP3A4
N-Desmethyltamoxifen (active)
CYP3A4
N,N-Didesmethyl-tamoxifen
CYP2D6
CYP2C9
CYP2D6
4-Hydroxytamoxifen
CYP3A4
N-Desmethyl-4-hydroxytamoxifen (active)
Dechloroethyl-CPA
Chloroacetaldehyde
CYP3A4
4-Keto-CPA
CYP3A4
4-Glutathionyl-CPA (inactive)
excretion
2.5.1.18
1.1.1.1
1.2.1.5
Alcophosphamide
Carboxyphosphamide
Nornitrogen mustard (active)
Cyclophosphamide (prodrug)
CYP2C
CYP2B6
CYP3A4
4-OH-CPA
Aldophosphamide
Phosphamide mustard (active)
Acrolein
Ifosfamide (prodrug)
CYP2C
CYP2B6
CYP3A4
4-Hydroxy-ifosfamide
Aldoifosfamide
Isophosphamide mustard (active)
CYP3A4
CYP3A5
CYP2B6
Carboxyifosfamide
Alcoifosfamide
Chloroacetaldehyde
4-Ketoifosfamide
Dechloroethylifosfamide

Data on KEGG graph
Rendered by Pathview

Enzymes related to pyrimidine metabolism are also shared between most subtypes


Fluorouracil
Capecitabine (prodrug)
3.1.1.1
5'-Deoxy-5-fluorocytidine
3.5.4.5
Doxifluridine (5'-Deoxy-5-fluorouridine)
2.4.2.4
Tegafur (prodrug)
CYP2A6
Carmofur (prodrug)
Fluorouracil
2.4.2.3
5-Fluoro-uridine
1.3.1.2
5,6-Dihydro-5-fluorouracil
3.5.2.2
α-Fluoro-β-ureido propionic acid
3.5.1.6
α-Fluoro-β-alanine
excretion
Fluoroacetate (Neurotoxin)
2.4.2.4
Floxuridine (5-Fluorodeoxyuridine)
2.4.2.10
2.7.1.21
2.7.1.48
5-Fluorouridine monophosphate
5-Fluorodeoxyuridine monophosphate
2.7.4.14
2.7.4.14
3.6.1.23
5-Fluorouridine diphosphate
1.17.4.1
5-Fluorodeoxyuridine diphosphate
2.7.4.6
2.7.4.6
5-Fluorouridine triphosphate
5-Fluorodeoxyuridine triphosphate
-1
0
1
Azathioprine & 6-Mercaptopurine
6-Methyl-mercaptopurine
2.4.2.8
6-Methylthioinosine-5'-monophosphate
6-Methylthioguanosine monophosphate
2.1.1.67
2.1.1.67
2.1.1.67
Azathioprine (prodrug)
6-Mercaptopurine (prodrug)
2.4.2.8
6-Thioinosine-5'-monophosphate
1.1.1.205
6-Thioxanthosine-5'-monophosphate
6.3.5.2
6-Thioguanosine monophosphate
6-thioguanine nucleotides
2.4.2.8
6-Thioguanine
1-Methyl-4-nitro-imidazol
1.17.3.2
6-Thiouric acid
2.7.4.-
6-Thioinosine-5'-diphosphate
3.6.1.66
2.7.4.-
6-Thioinosine-5'-triphosphate

Data on KEGG graph
Rendered by Pathview

MASONIC CANCER CENTER
UNIVERSITY OF MINNESOTA

Conclusions

- HER2-enriched and basal-like subtypes have among the highest expression and mutational pattern for APOBEC3B

- APOBEC3B expression can be a useful surrogate for mutations

- APOBEC3B high expressing tumor samples have altered drug metabolism pathways
  - Pathways related to tamoxifen metabolism are altered and may suggest a potential mechanism driving tamoxifen resistance.
  - High APOBEC3B expressing tumors may be more susceptible to other treatments like 5-Fluorouracil

Acknowledgements

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Douglas Yee M.D.
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Nicholas Berg

Harris Lab

Reuben Harris Ph.D.
William Brown Ph.D.

Sachdev Lab

Deepali Sachdev Ph.D.
Courtney Baar
Summer Gerhardt

Thesis Committee

Alpay Temiz Ph.D.
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David Largaespada Ph.D.
Stephanie Huang Ph.D.

MICaB

2022 Cohort
Ameeta Kelekar Ph.D.
Megan Ruf

Questions?

NCI Comprehensive Cancer Center
A Cancer Center Designated by the National Cancer Institute

MINNESOTA'S CANCER CENTER

MASONIC CANCER CENTER

MASONIC CANCER CENTER

UNIVERSITY OF MINNESOTA

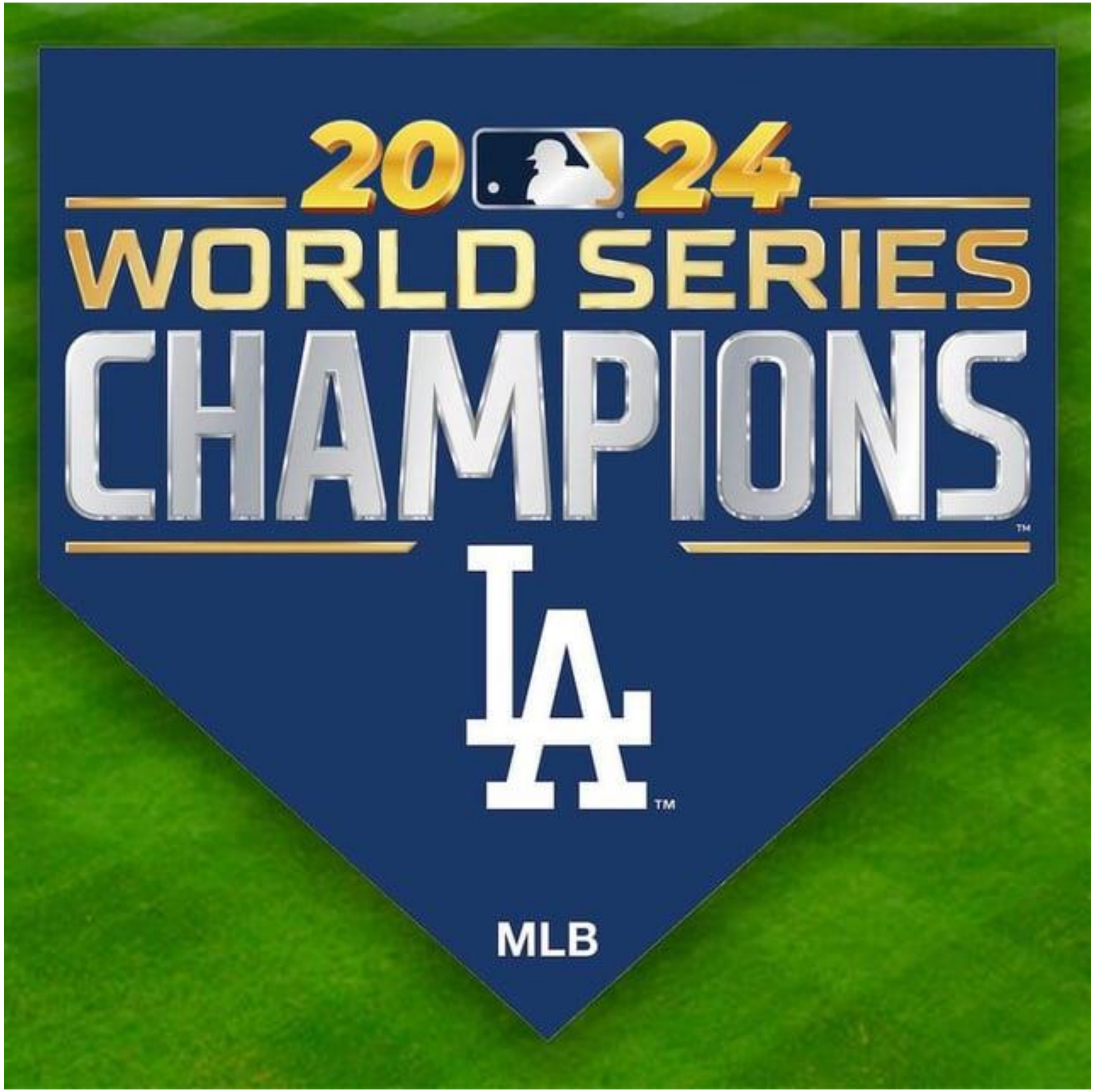
SPECIAL TIME EDITION
THE FUTURE OF MEDICINE
Amazing Breakthroughs
How Artificial Intelligence Will Help Patients
New Hope for Cancer, Heart Disease and Alzheimer's

VISION

Diagnostics 2030: What AI can (and can't) do for you.

As foretold by: Sandy Borowsky in 2024

And reported at the RISE UP for Breast Cancer meeting, November 2024


2024
WORLD SERIES
CHAMPIONS
LA
MLB

Wait... How did that get in there?

- Condolences Yankees fans…
- If you want to relive the highlights…
  - Like I do…. Use this QR code
- I have several other QR codes embedded in this talk, so this is a heads up to get your phones ready.

gettyimages
Credit: New York Daily News Archive

Disclosures:

- I will talk briefly about technology developed at UCDavis that is licensed to a start-up company “HISTOLIX” - I have founders’ shares.

Histolix

Crisis: Necessity is the mother of invention…

John F. Kennedy pointed out that the Chinese word for “crisis” has symbol components of “danger” and “opportunity.”[*]

危机	*Crisis*
危险	*Danger*
机会	*Opportunity, Chance, Odds*

---

[*] *This is not precisely accurate, of course, but some poetic license might be granted. For details see wikipedia:*

Crisis: Three Big Deals in Pathology Today 危机

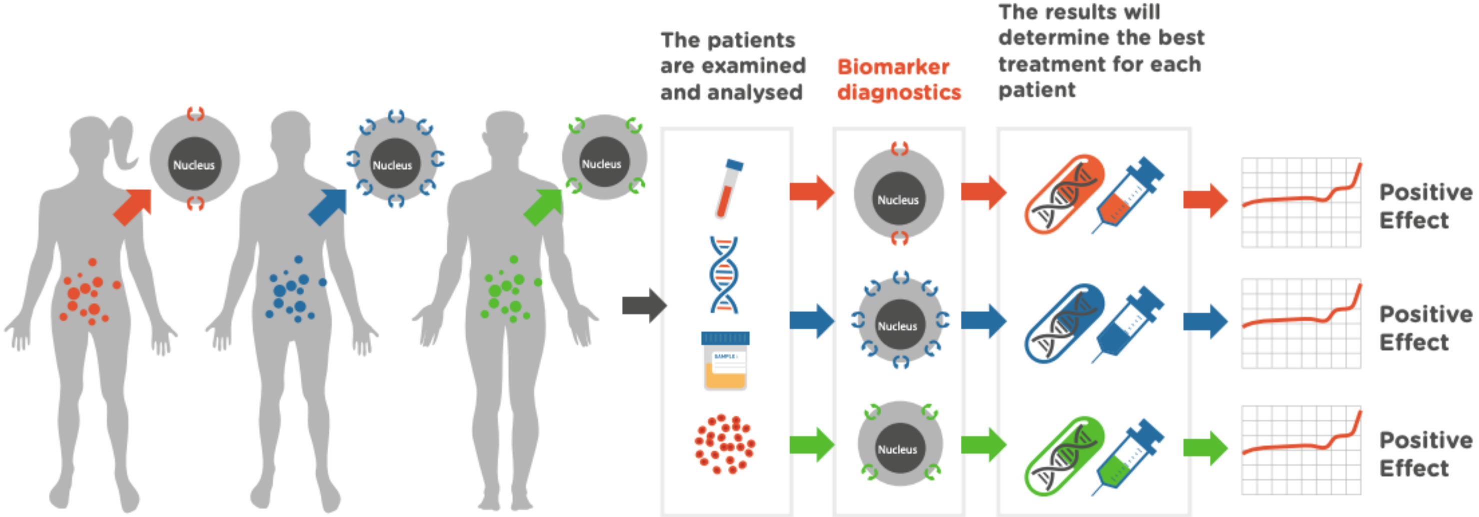
- Omics technologies (NGS, transcriptomes, metagenomics, metabolomics)
  - Investments in the technologies are initially outside the realm of any single hospital system… venture capital… industry investments… and their need to generate revenue drive the innovation…
- Blood-based testing (Combine imaging with ctDNA)
  - This testing, again developed primarily by industry could bypass pathology--- which would be unfortunate.
- **AI digital pathology image analysis**
  - **Could replace the cumbersome and slow and expensive to train carbon life forms… but ideally will be used to make us more efficient, accurate, and reproducible.**

What is precision medicine?

- Evidence-based measurable characteristics with implications for outcome and optimal treatment.

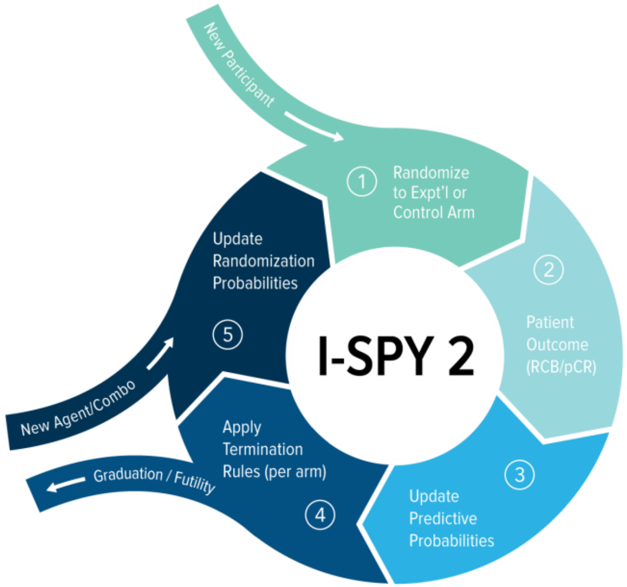
  Wait a second… that’s what we in pathology call: Diagnosis!

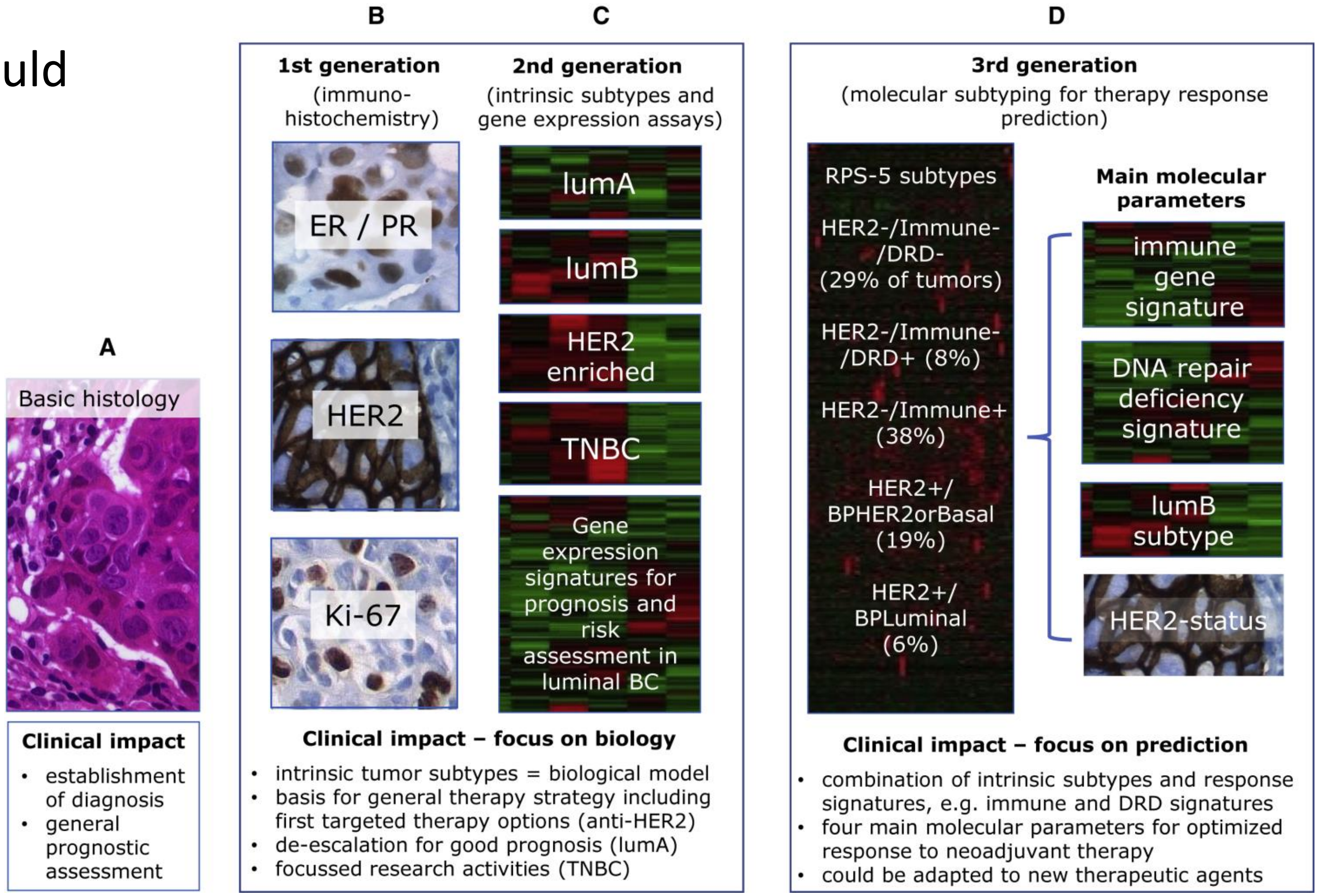
- *We are the integrators of ever-increasing data sources: DNA* sequencing, gene expression signatures, meta-genomics/microbiomes, data analytics.


Nucleus
Nucleus
Nucleus
The patients are examined and analysed
Biomarker diagnostics
The results will determine the best treatment for each patient
Nucleus
Nucleus
Nucleus
Positive Effect
Positive Effect
Positive Effect

Molecular Diagnostic Classification

- Rather than layering, this should be primary.
- Evolving knowledge, nimble criteria changes.


New Participant
1 Randomize to Expt'l or Control Arm
2 Patient Outcome (RCB/pCR)
3 Update Predictive Probabilities
4 Apply Termination Rules (per arm)
5 Update Randomization Probabilities
I-SPY 2
New Agent/Combo
Graduation / Futility


A
Basic histology
B
1st generation
(immuno-histochemistry)
ER / PR
HER2
Ki-67
C
2nd generation
(intrinsic subtypes and gene expression assays)
lumA
lumB
HER2 enriched
TNBC
Gene expression signatures for prognosis and risk assessment in luminal BC
D
3rd generation
(molecular subtyping for therapy response prediction)
RPS-5 subtypes
HER2-/Immune-/DRD- (29% of tumors)
HER2-/Immune-/DRD+ (8%)
HER2-/Immune+ (38%)
HER2+/BPHER2orBasal (19%)
HER2+/BPLuminal (6%)
Main molecular parameters
immune gene signature
DNA repair deficiency signature
lumB subtype
HER2-status

Clinical impact

- establishment of diagnosis
- general prognostic assessment

Clinical impact – focus on biology

- intrinsic tumor subtypes = biological model
- basis for general therapy strategy including first targeted therapy options (anti-HER2)
- de-escalation for good prognosis (lumA)
- focussed research activities (TNBC)

Clinical impact – focus on prediction

- combination of intrinsic subtypes and response signatures, e.g. immune and DRD signatures
- four main molecular parameters for optimized response to neoadjuvant therapy
- could be adapted to new therapeutic agents

From: Cancer Cell Volume 40, Issue 6, 13 June 2022, Pages 592-594

So… we all need to be Molecular Pathologists.

- Incorporating the molecular with the morphology to provide complete diagnosis.
- Understand the testing methods, especially the limitations/pitfalls

VISION

I think we will see molecular methods for tissue analysis take the place of much of our current IHC. The technology allows simultaneous quantitative and spatially localized assessment of thousands of molecules simultaneously. The pathologist's role will be to sort out which are informative for diagnosis, prognosis, and precision therapy response prediction.

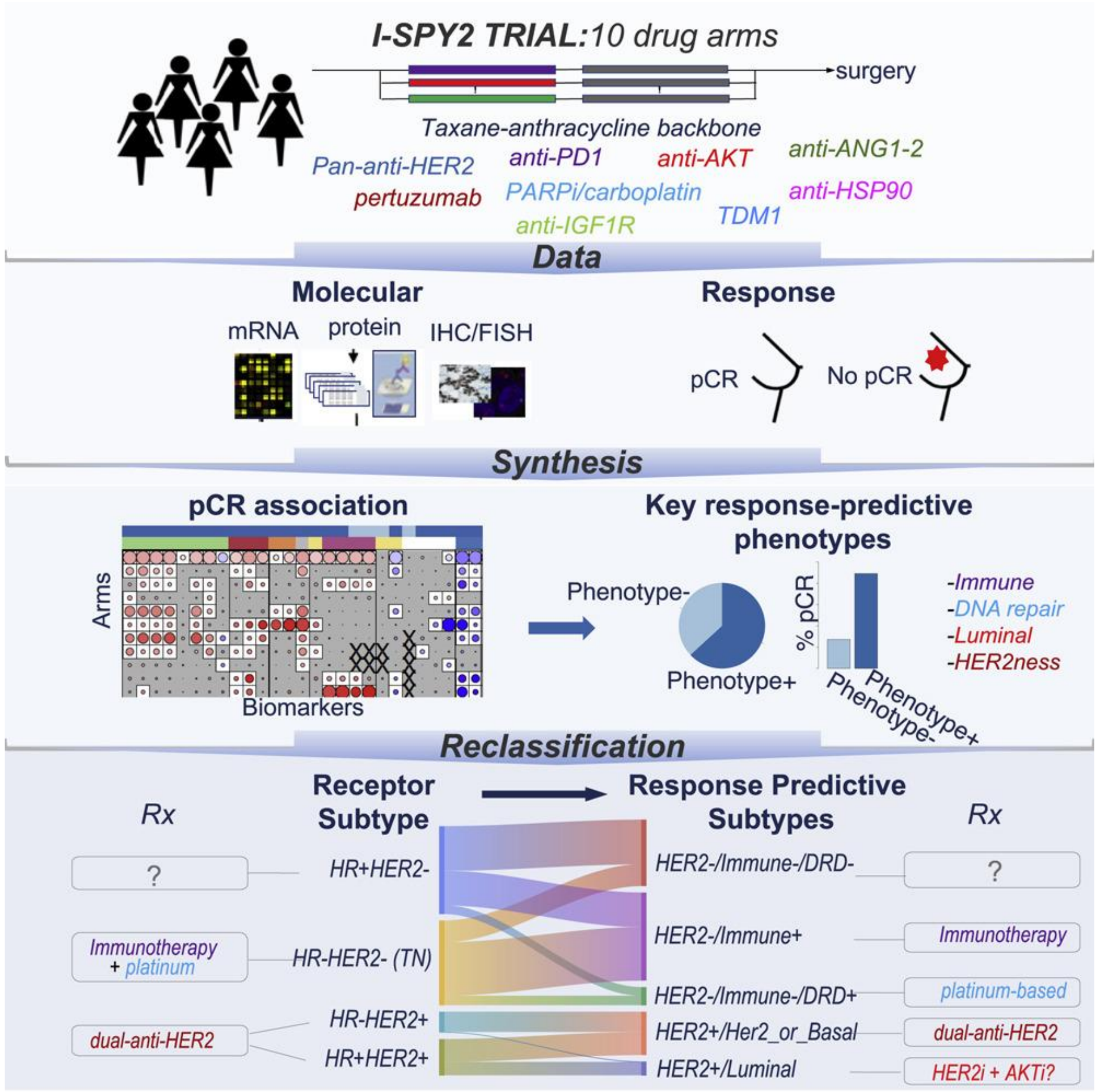
What to do about it…

TO DO LIST

1. Keep learning…
2. Bring technology in-house
3. Control the reporting and context of sendout test results
4. Be involved in the development and validation of the next generation of tests

Biomarker Discovery

- Drives more impactful clinical trials. (Not driven by a single drug application).
- Can be reported *ahead* of clinical adoption. (Pathology can drive understanding).


I-SPY2 TRIAL:10 drug arms
surgery
Taxane-anthracycline backbone
Pan-anti-HER2
anti-PD1
anti-AKT
anti-ANG1-2
pertuzumab
PARPi/carboplatin
anti-HSP90
anti-IGF1R
TDM1
Data
Molecular
Response
mRNA
protein
IHC/FISH
pCR
No pCR
Synthesis
pCR association
Arms
Biomarkers
Key response-predictive phenotypes
Phenotype-
Phenotype+
% pCR
Phenotype-
Phenotype+
-Immune
-DNA repair
-Luminal
-HER2ness
Reclassification
Rx
Receptor Subtype
Response Predictive Subtypes
Rx
?
HR+HER2-
HER2-/Immune-/DRD-
?
Immunotherapy + platinum
HR-HER2- (TN)
HER2-/Immune+
Immunotherapy
HER2-/Immune-/DRD+
platinum-based
dual-anti-HER2
HR-HER2+
HER2+/Her2_or_Basal
dual-anti-HER2
HR+HER2+
HER2+/Luminal
HER2i + AKTi?

Digital Pathology and AI Image Analysis

VISION

With the right tools applied by pathologists, our histopathologic diagnoses will be faster (maybe 10x), more objective/quantitative, and reproducible.

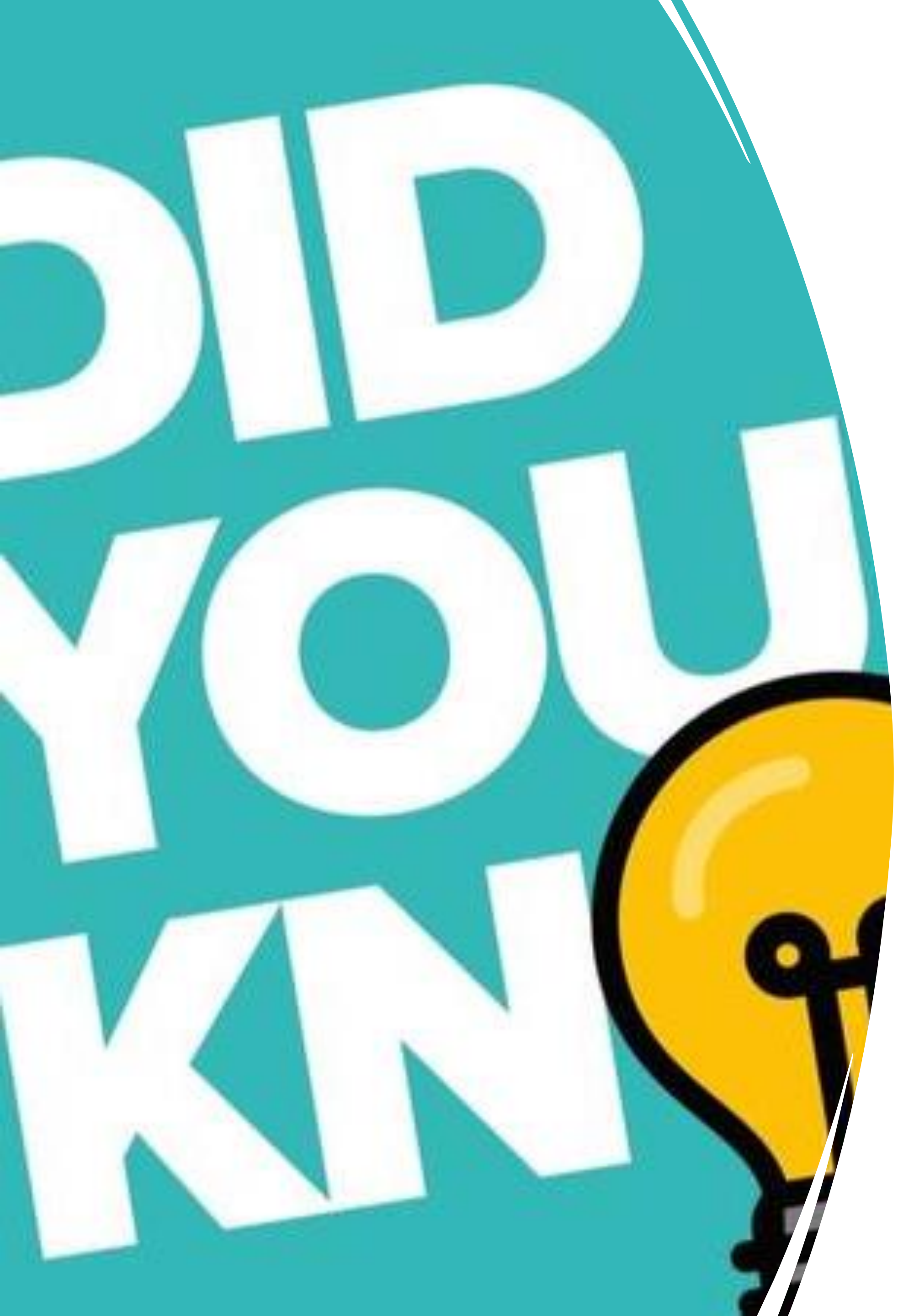
With the right data, we can catalog better classifiers and response predictors that are fast and easy to apply.

Developers who think this can be done without pathologists… well… they are blind.

What to do about it…

TO DO LIST

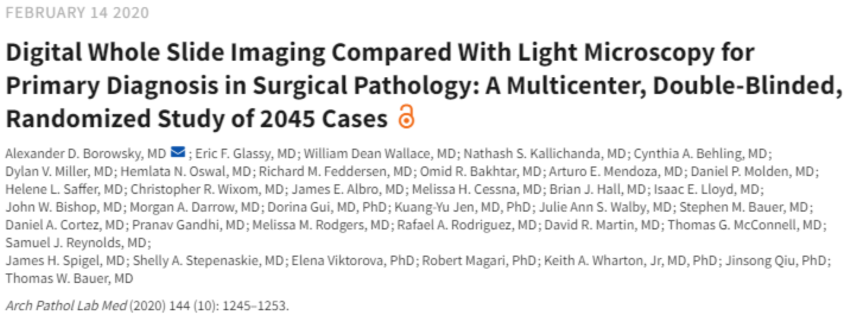
1. Develop direct digital capture methods.
2. Tools run in the background to speed up histology evaluation and ensure quality
3. Tools for report generation.
4. Learning engines for improved classification.
5. Pathologists are the primary users.



These tools require digital pathology images:

- UC Davis Borowsky/Cardiff was the first site to own/use an Aperio ImageScope digital slide scanner circa 1999.
- UC Davis SOM was the first to use WSIs in the medical school curriculum.
- UC Davis led the multi-site validation study to provide data for FDA approval of digital pathology for primary diagnosis.

What to do about it…



FEBRUARY 14 2020

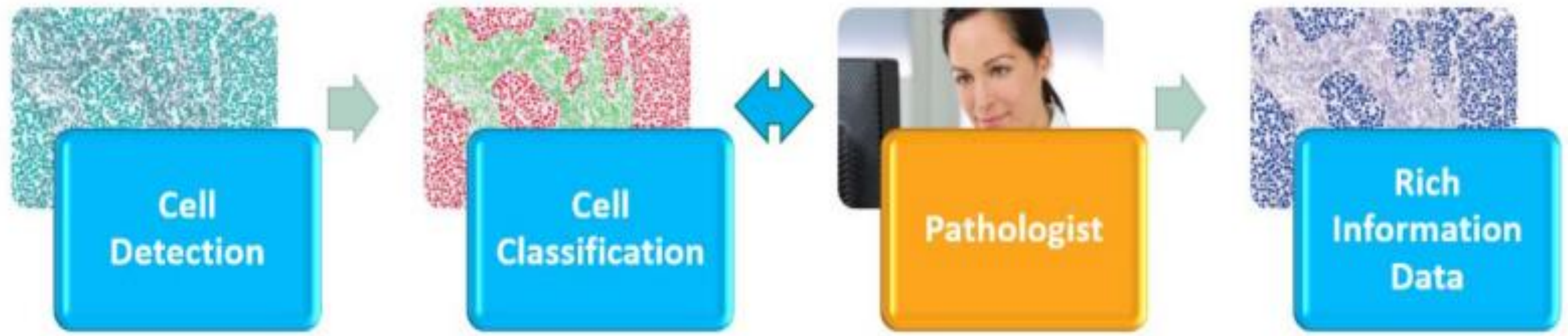
Digital Whole Slide Imaging Compared With Light Microscopy for Primary Diagnosis in Surgical Pathology: A Multicenter, Double-Blinded, Randomized Study of 2045 Cases

Alexander D. Borowsky, MD ✉; Eric F. Glassy, MD; William Dean Wallace, MD; Nathash S. Kallichanda, MD; Cynthia A. Behling, MD; Dylan V. Miller, MD; Hemlata N. Oswal, MD; Richard M. Feddersen, MD; Omid R. Bakhtar, MD; Arturo E. Mendoza, MD; Daniel P. Molden, MD; Helene L. Saffer, MD; Christopher R. Wixom, MD; James E. Albro, MD; Melissa H. Cessna, MD; Brian J. Hall, MD; Isaac E. Lloyd, MD; John W. Bishop, MD; Morgan A. Darrow, MD; Dorina Gui, MD, PhD; Kuang-Yu Jen, MD, PhD; Julie Ann S. Walby, MD; Stephen M. Bauer, MD; Daniel A. Cortez, MD; Pranav Gandhi, MD; Melissa M. Rodgers, MD; Rafael A. Rodriguez, MD; David R. Martin, MD; Thomas G. McConnell, MD; Samuel J. Reynolds, MD;
James H. Spigel, MD; Shelly A. Stepenaskie, MD; Elena Viktorova, PhD; Robert Magari, PhD; Keith A. Wharton, Jr, MD, PhD; Jinsong Qiu, PhD; Thomas W. Bauer, MD

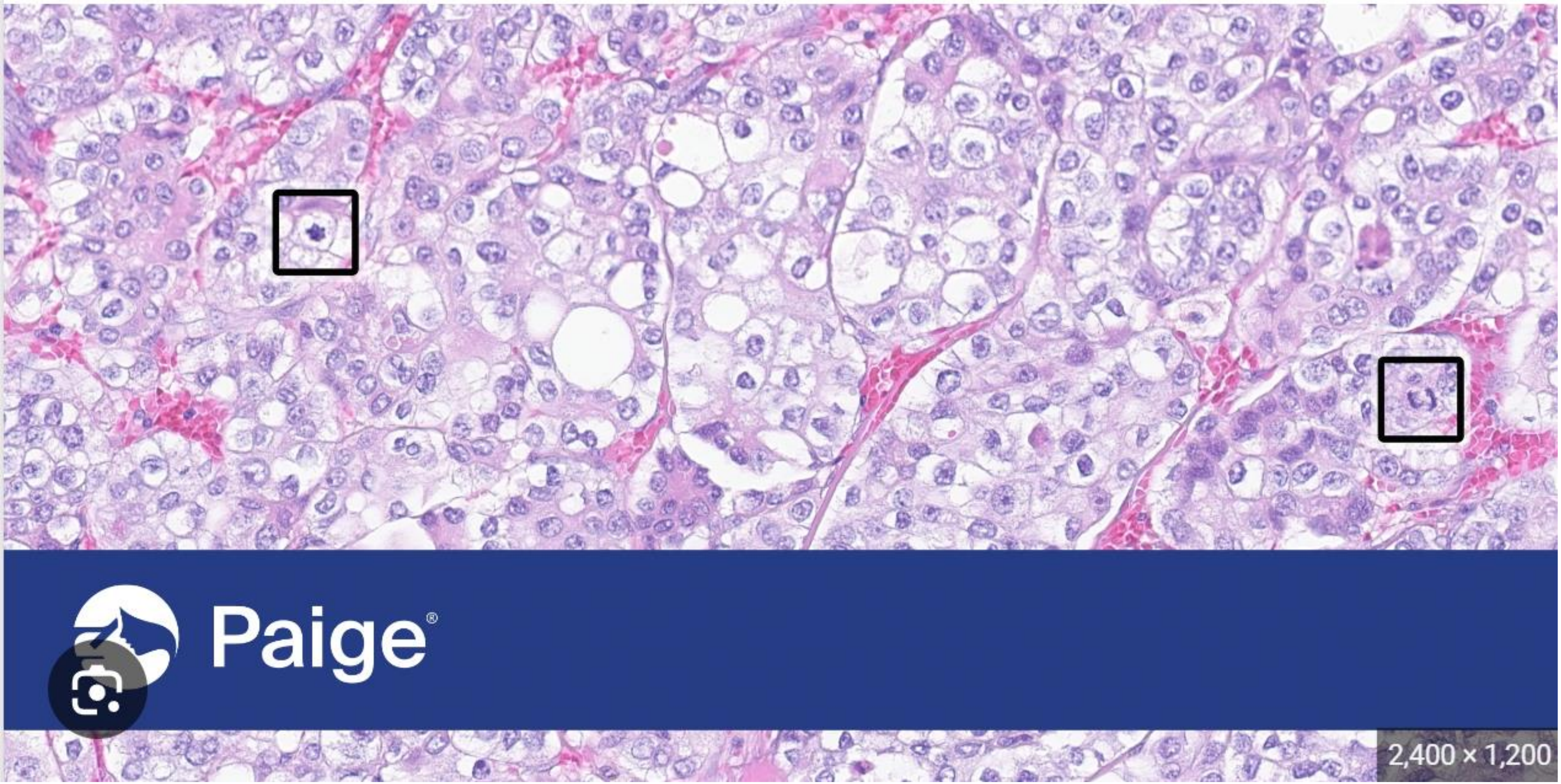
Arch Pathol Lab Med (2020) 144 (10): 1245–1253.

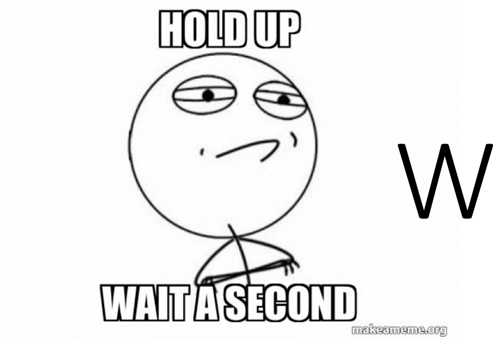
The Plan for AI: Insist on the tools that help.

- Work with ‘em not against ‘em
- Insist on the tools we need, not what they imagine
- Incorporate “big” data analysis, multiple IHCs, DNA/RNA sequence


Cell Detection
Cell Classification
Pathologist
Rich Information Data

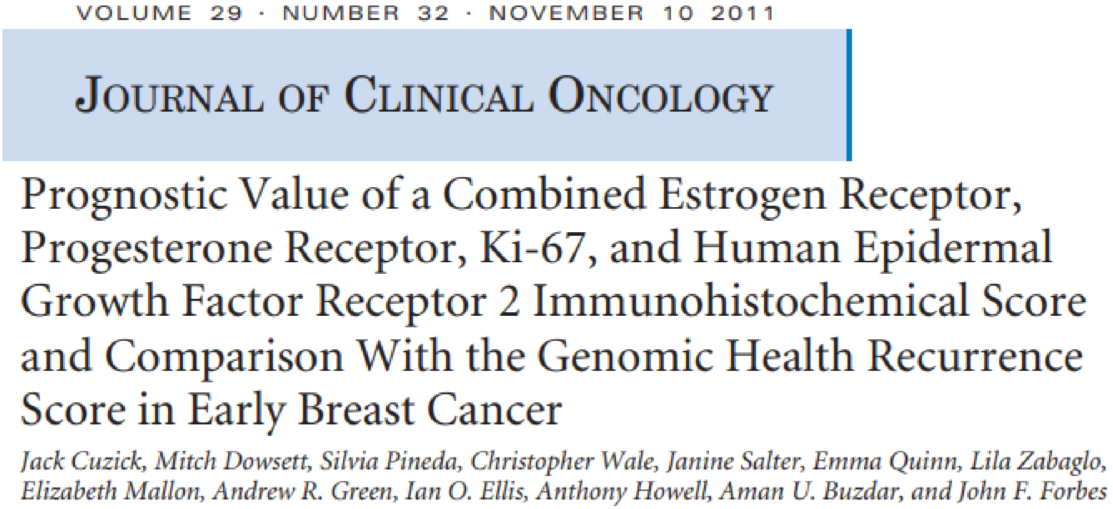
Helpful things: Counting mitoses.


Paige®
2,400 × 1,200



Why is counting mitoses important?

- Proliferative rate is the primary driver of the OncotypeDX score.
    - *Oncotype is expensive and proven to be replacable.*
    - *Example: IHC4   (below)*
- *High inter-observer variability.*
- *Time consuming/tedious*.


VOLUME 29 · NUMBER 32 · NOVEMBER 10 2011

JOURNAL OF CLINICAL ONCOLOGY

Prognostic Value of a Combined Estrogen Receptor, Progesterone Receptor, Ki-67, and Human Epidermal Growth Factor Receptor 2 Immunohistochemical Score and Comparison With the Genomic Health Recurrence Score in Early Breast Cancer

Jack Cuzick, Mitch Dowsett, Silvia Pineda, Christopher Wale, Janine Salter, Emma Quinn, Lila Zabaglo, Elizabeth Mallon, Andrew R. Green, Ian O. Ellis, Anthony Howell, Aman U. Buzdar, and John F. Forbes

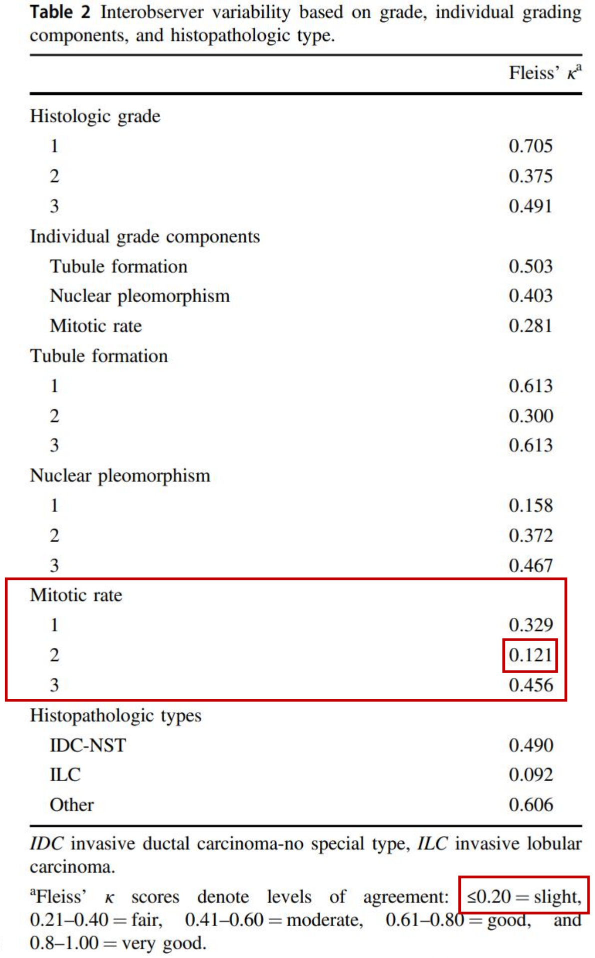


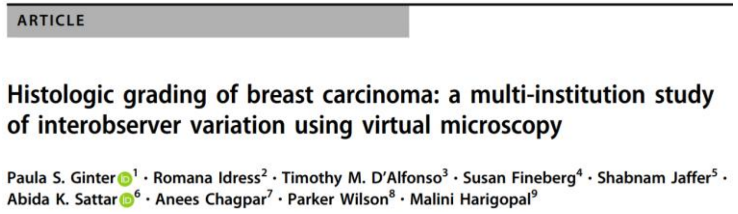
Table 2 Interobserver variability based on grade, individual grading components, and histopathologic type.

	Fleiss' κ^a
Histologic grade	
1	0.705
2	0.375
3	0.491
Individual grade components	
Tubule formation	0.503
Nuclear pleomorphism	0.403
Mitotic rate	0.281
Tubule formation	
1	0.613
2	0.300
3	0.613
Nuclear pleomorphism	
1	0.158
2	0.372
3	0.467
Mitotic rate	
1	0.329
2	0.121
3	0.456
Histopathologic types	
IDC-NST	0.490
ILC	0.092
Other	0.606

IDC invasive ductal carcinoma-no special type, *ILC* invasive lobular carcinoma.

[a] Fleiss' κ scores denote levels of agreement: ≤0.20 = slight, 0.21–0.40 = fair, 0.41–0.60 = moderate, 0.61–0.80 = good, and 0.8–1.00 = very good.

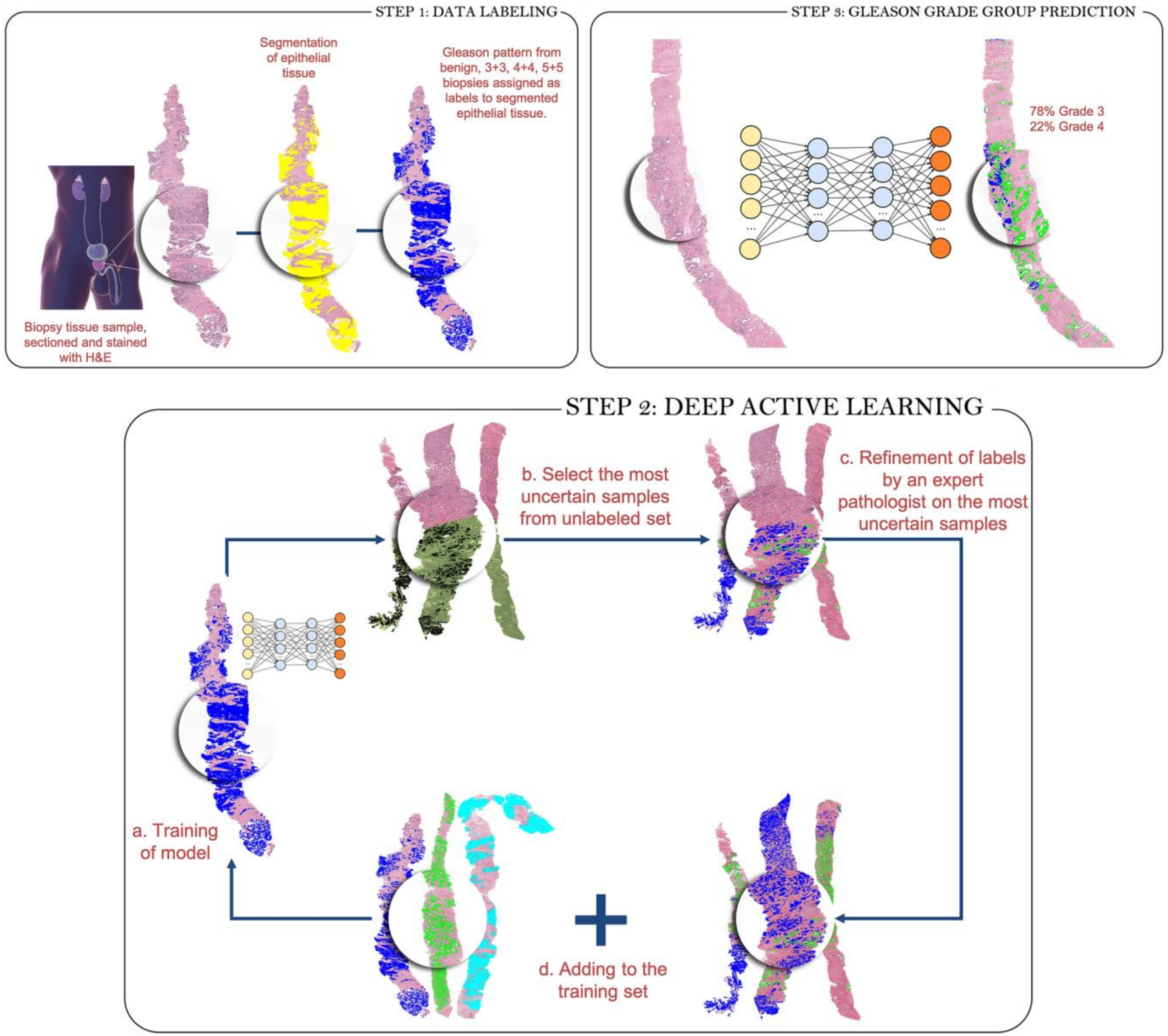
Modern Pathology (2021) 34:701–709
https://doi.org/10.1038/s41379-020-00698-2


ARTICLE

Histologic grading of breast carcinoma: a multi-institution study of interobserver variation using virtual microscopy

Paula S. Ginter[1] · Romana Idress[2] · Timothy M. D'Alfonso[3] · Susan Fineberg[4] · Shabnam Jaffer[5] · Abida K. Sattar[6] · Anees Chagpar[7] · Parker Wilson[8] · Malini Harigopal[9]

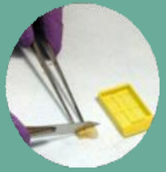
Helpful things: Quantitative grading


STEP 1: DATA LABELING
Segmentation of epithelial tissue
Gleason pattern from benign, 3+3, 4+4, 5+5 biopsies assigned as labels to segmented epithelial tissue.
Biopsy tissue sample, sectioned and stained with H&E
STEP 3: GLEASON GRADE GROUP PREDICTION
78% Grade 3
22% Grade 4
STEP 2: DEEP ACTIVE LEARNING
b. Select the most uncertain samples from unlabeled set
c. Refinement of labels by an expert pathologist on the most uncertain samples
a. Training of model
d. Adding to the training set

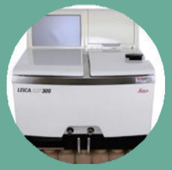
Scientific Reports **volume 12**, Article number: 3383 (2022)

Traditional WSI

8 hours to days


Grossing

2 to 3 minutes


Processing*

4-12 hours

Wax embedding

10 minutes

Sectioning

10 minutes

Air drying

1-2 hours

Staining, cover slipping

45 minutes

Digital pathology conversion

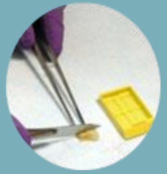
AI Molecular Assessment . . ,

Histolix Workflow

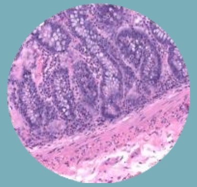
UC Davis IP startup company

Histolix

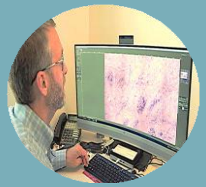
5 Minutes


Grossing & Staining

2 to 3 minutes

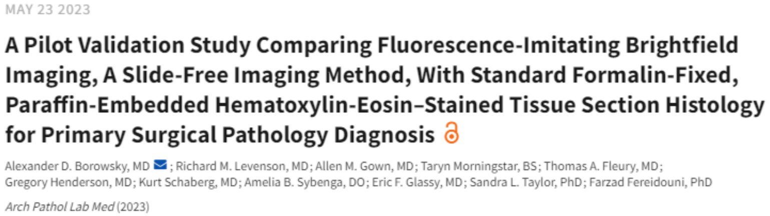

Direct to Digital Image processing

2 minutes


Digital Image to pathologist with AI additional assessment

What to do about it...

R01 CA277527; R33 CA278544- IMAT; U01 CA269191


MAY 23 2023

A Pilot Validation Study Comparing Fluorescence-Imitating Brightfield Imaging, A Slide-Free Imaging Method, With Standard Formalin-Fixed, Paraffin-Embedded Hematoxylin-Eosin–Stained Tissue Section Histology for Primary Surgical Pathology Diagnosis

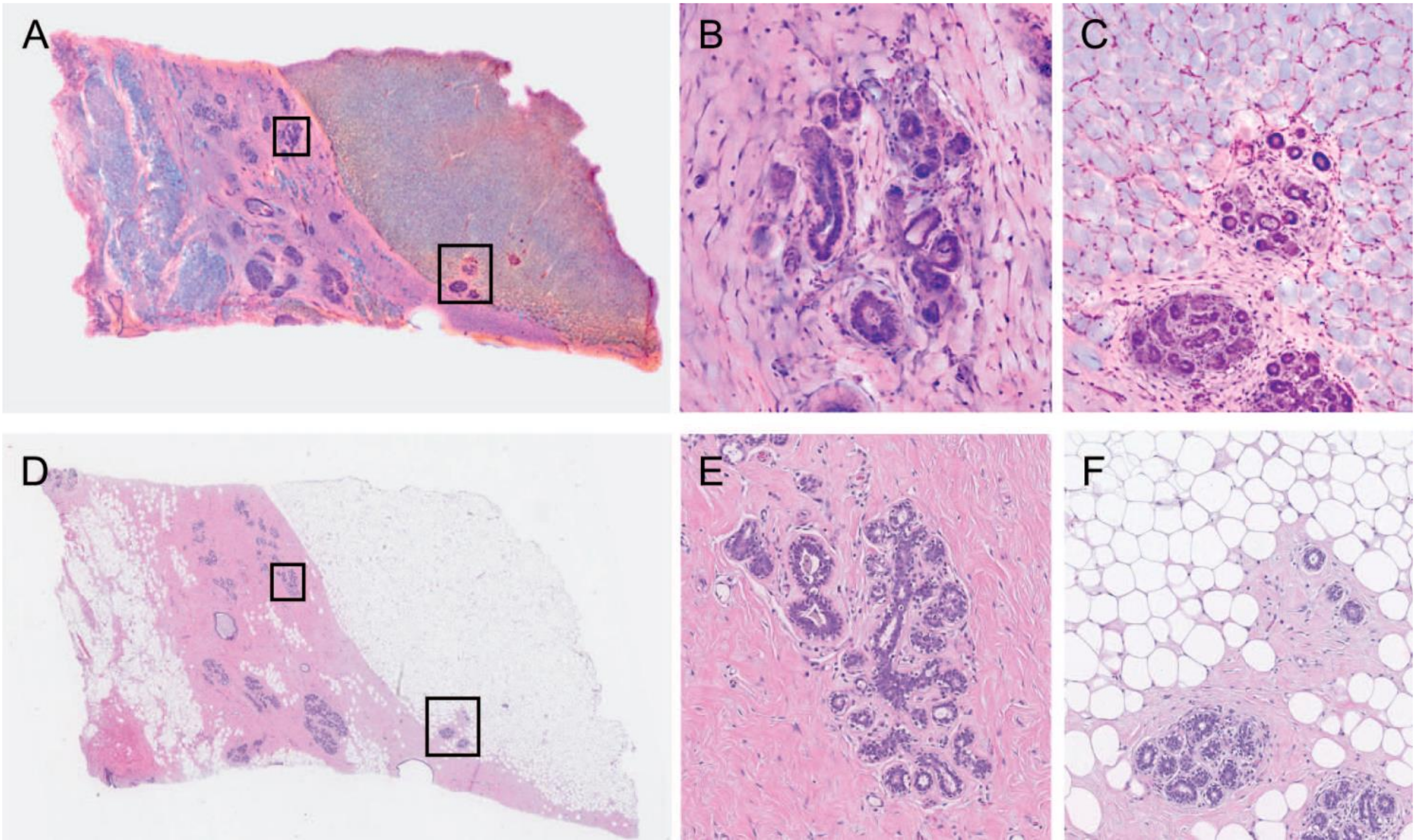
Alexander D. Borowsky, MD; Richard M. Levenson, MD; Allen M. Gown, MD; Taryn Morningstar, BS; Thomas A. Fleury, MD; Gregory Henderson, MD; Kurt Schaberg, MD; Amelia B. Sybenga, DO; Eric F. Glassy, MD; Sandra L. Taylor, PhD; Farzad Fereidouni, PhD

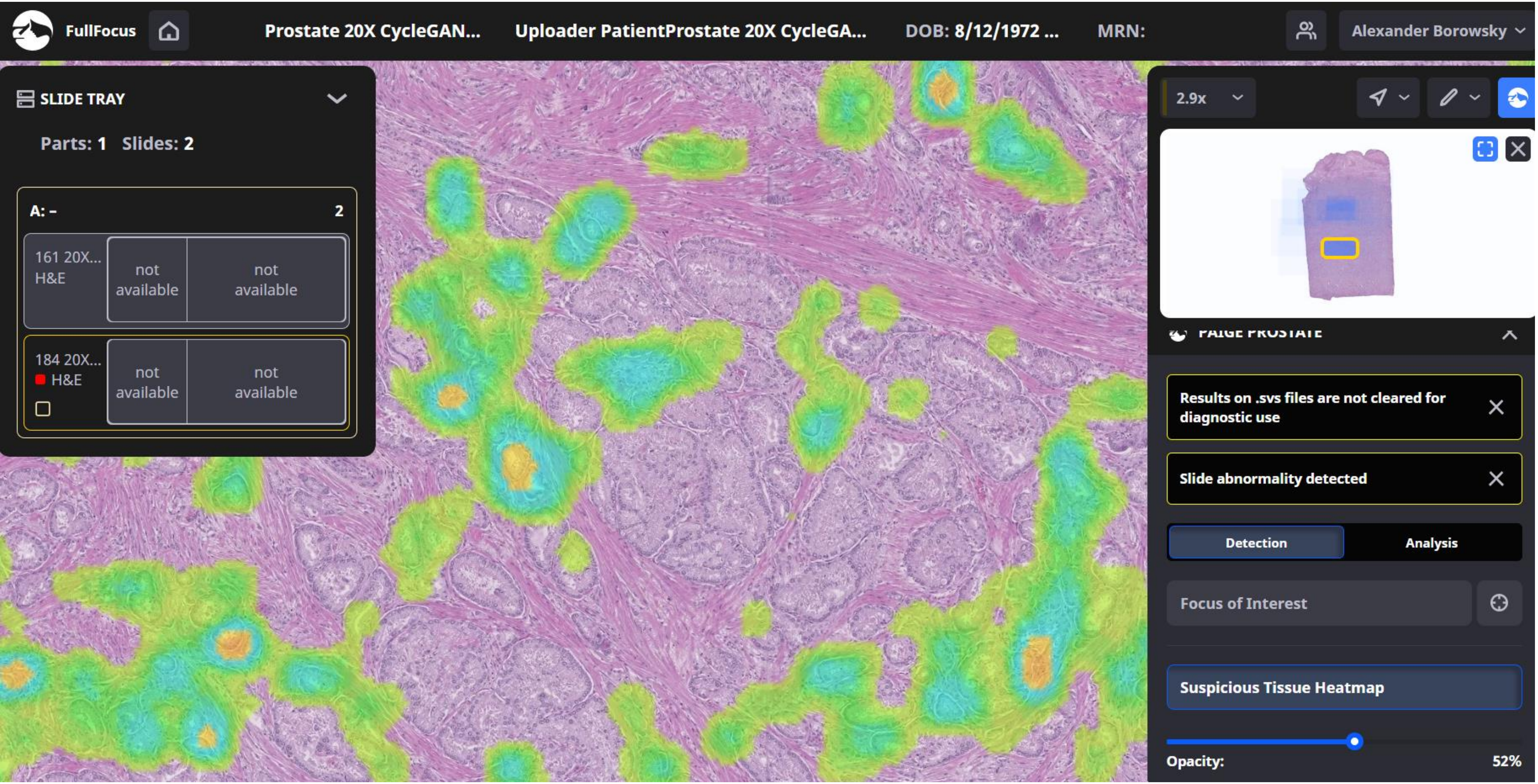
Arch Pathol Lab Med (2023)

A Pilot Validation Study Comparing Fluorescence-Imitating Brightfield Imaging, A Slide-Free Imaging Method, With Standard Formalin-Fixed, Paraffin-Embedded Hematoxylin-Eosin-Stained Tissue Section Histology for Primary Surgical Pathology Diagnosis

Alexander D. Borowsky, MD; Richard M. Levenson, MD; Allen M. Gown, MD; Taryn Morningstar, BS; Thomas A. Fleury, MD; Gregory Henderson, MD; Kurt Schaberg, MD; Amelia B. Sybenga, DO; Eric F. Glassy, MD; Sandra L. Taylor, PhD; Farzad Fereidouni, PhD

Arch Pathol Lab Med. 2024 Mar 1;148(3):345-352



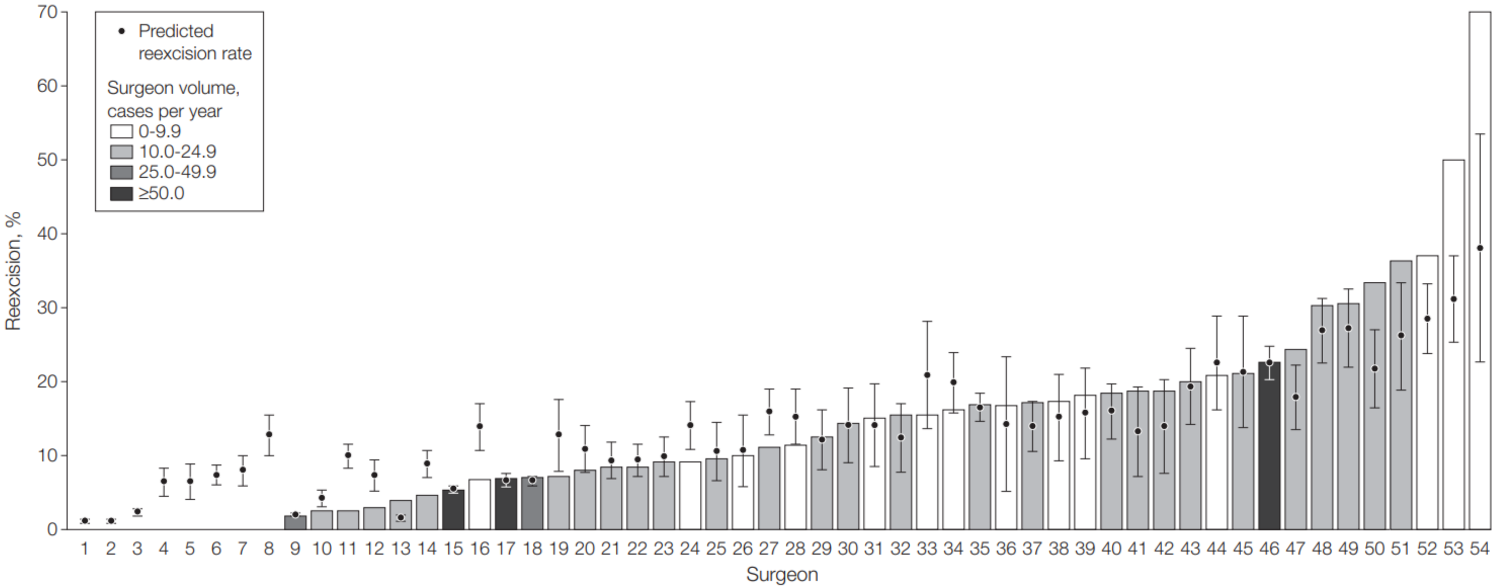

FullFocus
Prostate 20X CycleGAN...
Uploader PatientProstate 20X CycleGA...
DOB: 8/12/1972 ...
MRN:
Alexander Borowsky
SLIDE TRAY
Parts: 1 Slides: 2
A: –
2
161 20X...
H&E
not available
not available
184 20X...
🟥 H&E
☐
not available
not available
2.9x
PAIGE PROSTATE
Detection
Analysis
Focus of Interest
Suspicious Tissue Heatmap
Opacity:
52%

Results on .svs files are not cleared for diagnostic use

Slide abnormality detected

Variability in Reexcision Following Breast Conservation Surgery
McCahill, et al
JAMA. 2012;307(5):467-475.

Figure. Observed Reexcision Rates for Each of 54 Surgeons With at Least 10 Patients in the Study



Predicted reexcision rates, based on the random effects logistic regression model controlling for clinical covariates, are plotted as a circle above the encrypted surgeon identifiers along the horizontal axis. Error bars indicate 95% CIs. Surgeon-level predicted values were computed by averaging the patient-level predicted probabilities for all patients treated by that surgeon. Bars are shaded to indicate categories of annual surgeon volume (average cases per year, see "Methods" section). Surgeons 1 through 8 had zero observed reexcisions, thus there is no bar associated with these surgeons. These surgeons had average annual volumes of 0 to 9.9 cases per year, with the exception of surgeons 2 and 5 who had average volumes of 10.0 to 24.9 cases per year.

474 JAMA, February 1, 2012—Vol 307, No. 5

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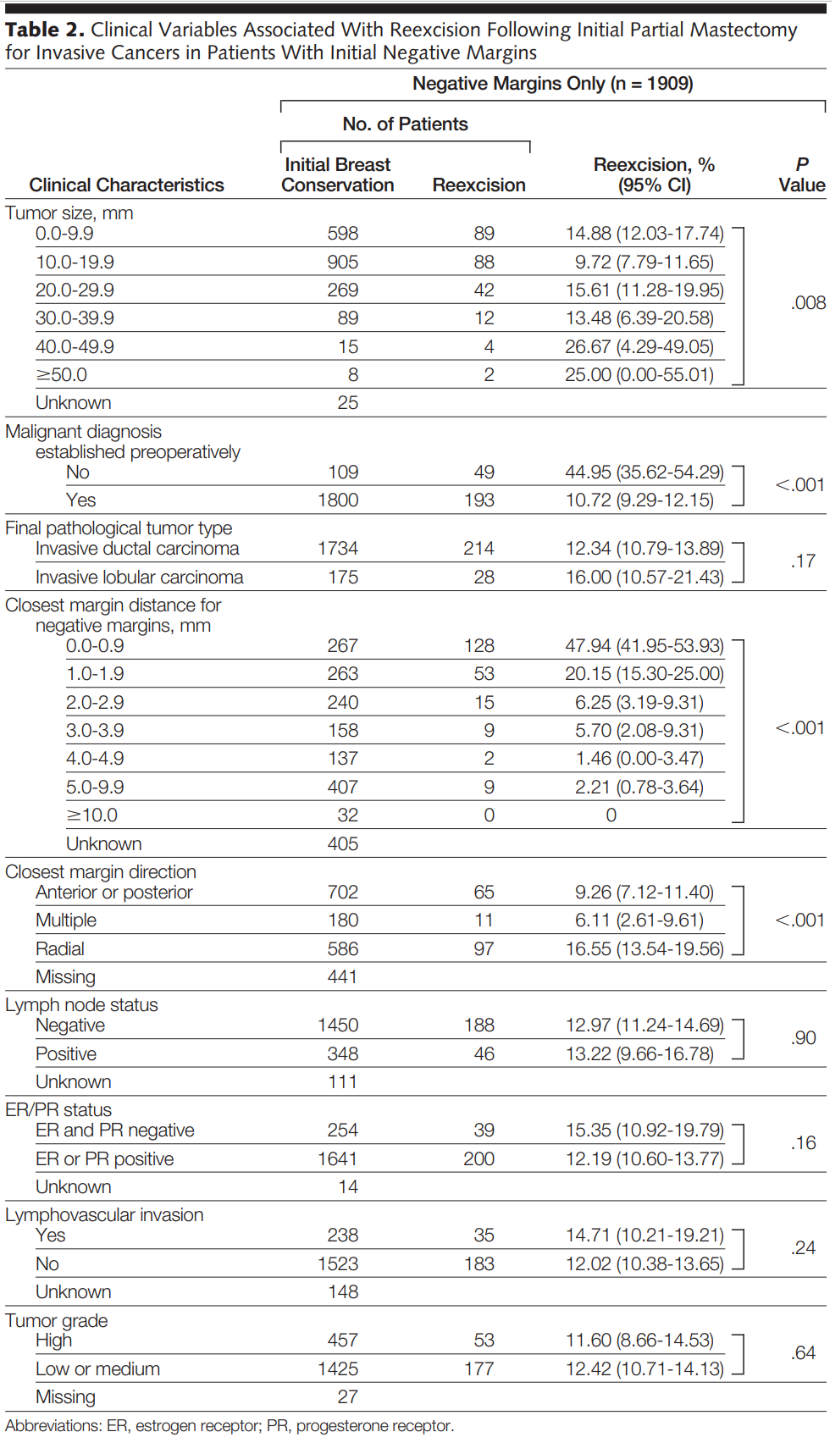


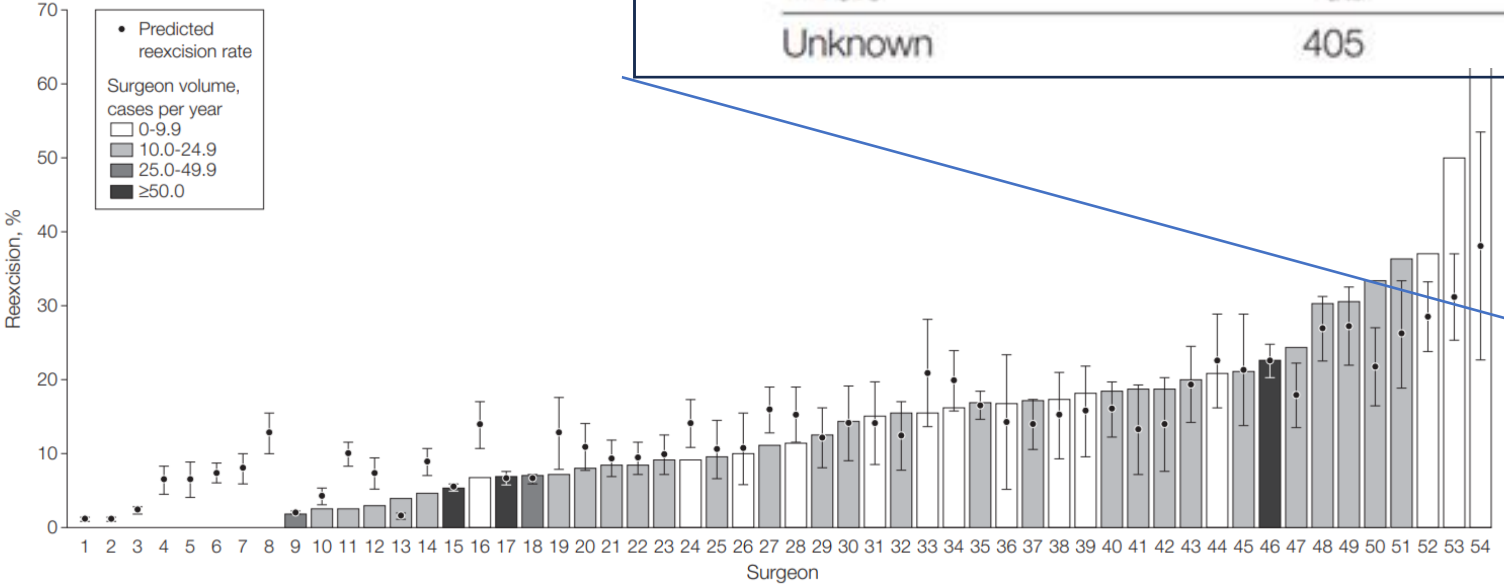
Table 2. Clinical Variables Associated With Reexcision Following Initial Partial Mastectomy for Invasive Cancers in Patients With Initial Negative Margins

	Negative Margins Only (n = 1909)			
	No. of Patients			
Clinical Characteristics	Initial Breast Conservation	Reexcision	Reexcision, % (95% CI)	P Value
Tumor size, mm				
0.0-9.9	598	89	14.88 (12.03-17.74)	.008
10.0-19.9	905	88	9.72 (7.79-11.65)	
20.0-29.9	269	42	15.61 (11.28-19.95)	
30.0-39.9	89	12	13.48 (6.39-20.58)	
40.0-49.9	15	4	26.67 (4.29-49.05)	
≥50.0	8	2	25.00 (0.00-55.01)	
Unknown	25			
Malignant diagnosis established preoperatively				
No	109	49	44.95 (35.62-54.29)	<.001
Yes	1800	193	10.72 (9.29-12.15)	
Final pathological tumor type				
Invasive ductal carcinoma	1734	214	12.34 (10.79-13.89)	.17
Invasive lobular carcinoma	175	28	16.00 (10.57-21.43)	
Closest margin distance for negative margins, mm				
0.0-0.9	267	128	47.94 (41.95-53.93)	<.001
1.0-1.9	263	53	20.15 (15.30-25.00)	
2.0-2.9	240	15	6.25 (3.19-9.31)	
3.0-3.9	158	9	5.70 (2.08-9.31)	
4.0-4.9	137	2	1.46 (0.00-3.47)	
5.0-9.9	407	9	2.21 (0.78-3.64)	
≥10.0	32	0	0	
Unknown	405			
Closest margin direction				
Anterior or posterior	702	65	9.26 (7.12-11.40)	<.001
Multiple	180	11	6.11 (2.61-9.61)	
Radial	586	97	16.55 (13.54-19.56)	
Missing	441			
Lymph node status				
Negative	1450	188	12.97 (11.24-14.69)	.90
Positive	348	46	13.22 (9.66-16.78)	
Unknown	111			
ER/PR status				
ER and PR negative	254	39	15.35 (10.92-19.79)	.16
ER or PR positive	1641	200	12.19 (10.60-13.77)	
Unknown	14			
Lymphovascular invasion				
Yes	238	35	14.71 (10.21-19.21)	.24
No	1523	183	12.02 (10.38-13.65)	
Unknown	148			
Tumor grade				
High	457	53	11.60 (8.66-14.53)	.64
Low or medium	1425	177	12.42 (10.71-14.13)	
Missing	27			

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.

Variability in Reexcision
Surgery
McCahill, et al
JAMA. 2012;307(5):467

Figure. Observed Reexcision Rates for Each of 54 Surgeon



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≥10.0	32	0	0	
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Should Intraoperative Frozen Section Evaluation of Breast Lumpectomy Margins Become Routine Practice?

Stuart J. Schnitt, MD, Monica Morrow, MD

American Journal of Clinical Pathology, Volume 138, Issue 5, November 2012, Pages 635–638.

“Positive margins (ie, invasive carcinoma or DCIS at an inked tissue edge) have consistently been associated with a higher risk of local recurrence than negative margins.[2] Therefore, obtaining negative margins is the primary goal of breast-conserving surgery. Unfortunately, there is far from universal agreement as to what constitutes an adequate negative margin.”

“Ultimately, patient outcomes will be optimized by considering the full spectrum of factors that predict tumor burden and impact on local recurrence. Much of this information, however, is not available at the time of initial surgical resection of the primary tumor.”

Other problems with frozen sections:

- Tissue destructive.
  - Possible that a true positive margin is lost in processing to result in a false negative margin (irretrievable on permanents)
- Artifacts can make interpretation difficult.
  - Alteration of architectural features makes benign lesions such as sclerosing adenosis more closely mimic invasive carcinoma
  - Artifactual clefting / spaces around tumor cells simulates lymphovascular invasion
- Digital imaging requires an extra step.

Fereidouni and Borowsky 1R01 CA277527-01, “GigaFIBI: rapid, large-format histology-resolution imaging for intraoperative assessment of breast lumpectomy margins.”

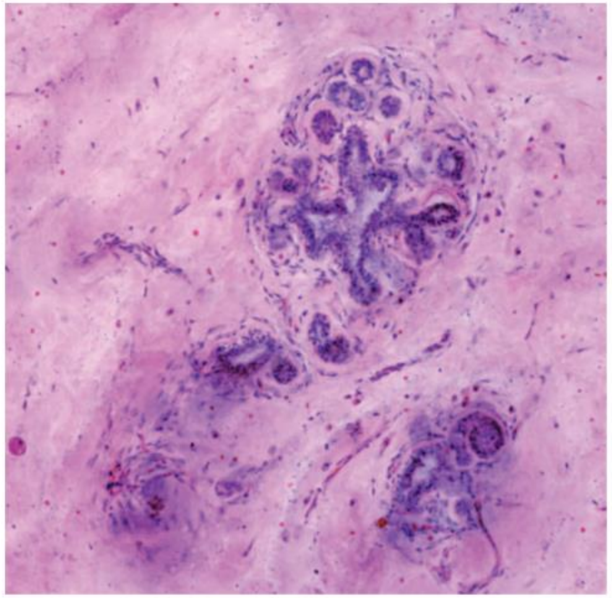
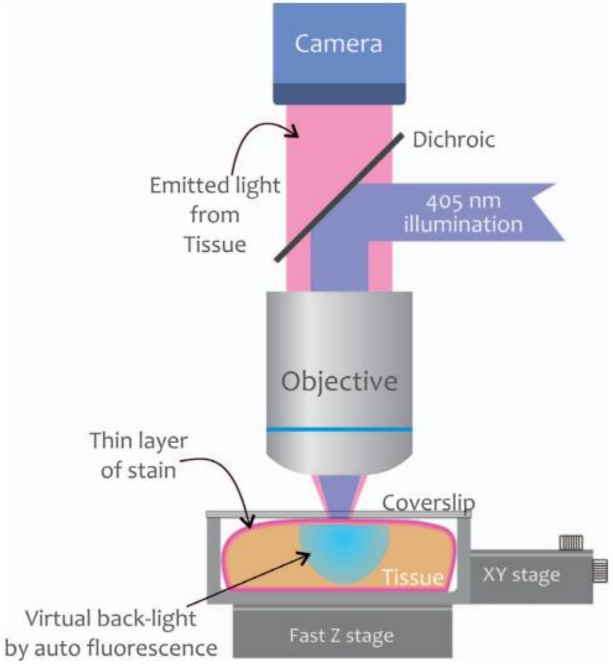


Figure 1. *Fluorescence-imitating brightfield imaging image of breast lobules, ducts, stroma, and microvasculature (original magnification ×80).*


Camera
Dichroic
Emitted light from Tissue
405 nm illumination
Objective
Thin layer of stain
Coverslip
Tissue
XY stage
Virtual back-light by auto fluorescence
Fast Z stage

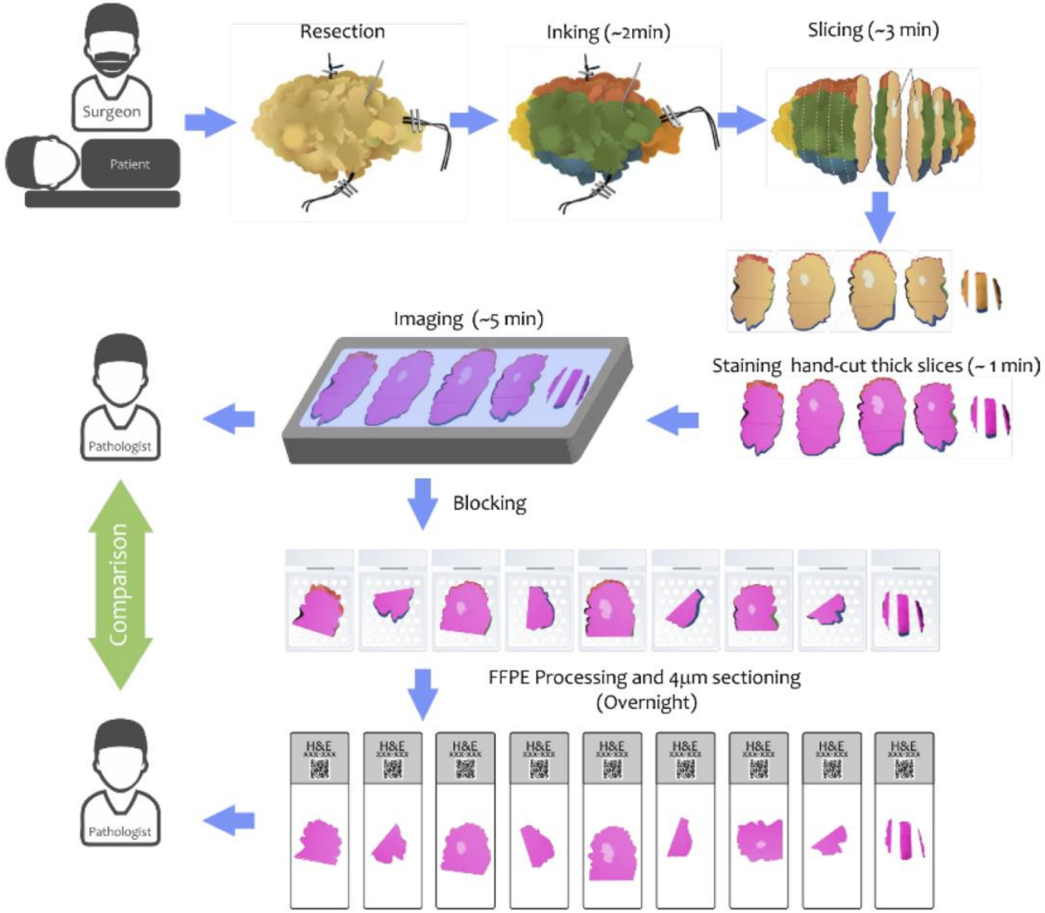
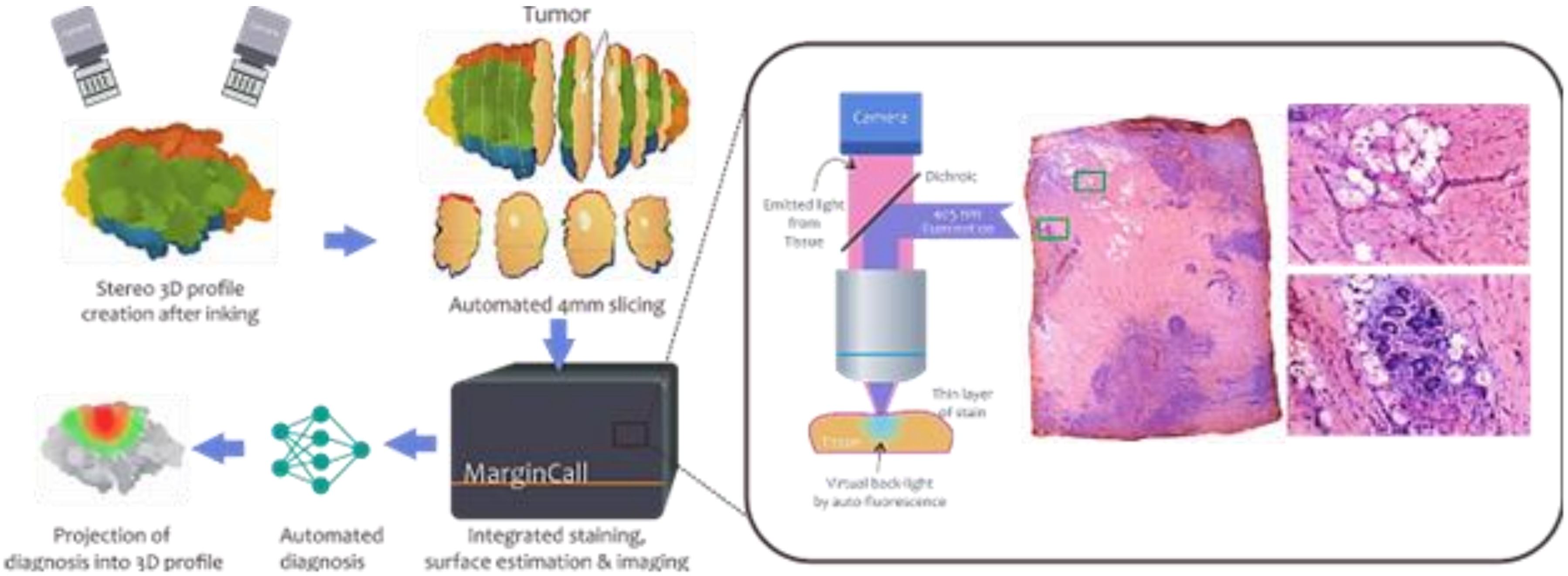

Surgeon
Patient
Resection
Inking (~2min)
Slicing (~3 min)
Imaging (~5 min)
Staining hand-cut thick slices (~1 min)
Pathologist
Comparison
Blocking
FFPE Processing and 4μm sectioning (Overnight)
H&E
Pathologist

Figure 9. The proposed workflow of GigaFIBI. Surgical resection of breast specimen by surgeon is marked with sutures to identify the orientation;

ARPA-H: Precision Surgical Interventions program

"…to classify margins as positive or negative within 15 minutes *without a pathologist.*" Fereidouni, Borowsky and Madabhushi (Emory).


Tumor
Stereo 3D profile creation after inking
Automated 4mm slicing
MarginCall
Integrated staining, surface estimation & imaging
Automated diagnosis
Projection of diagnosis into 3D profile
Camera
Dichroic
Emitted light from Tissue
405 nm Illumination
Thin layer of stain
Tissue
Virtual back light by auto fluorescence

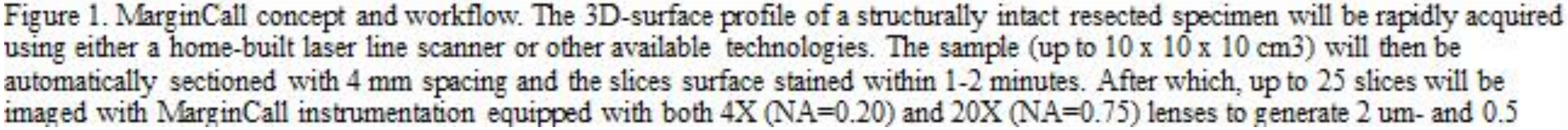
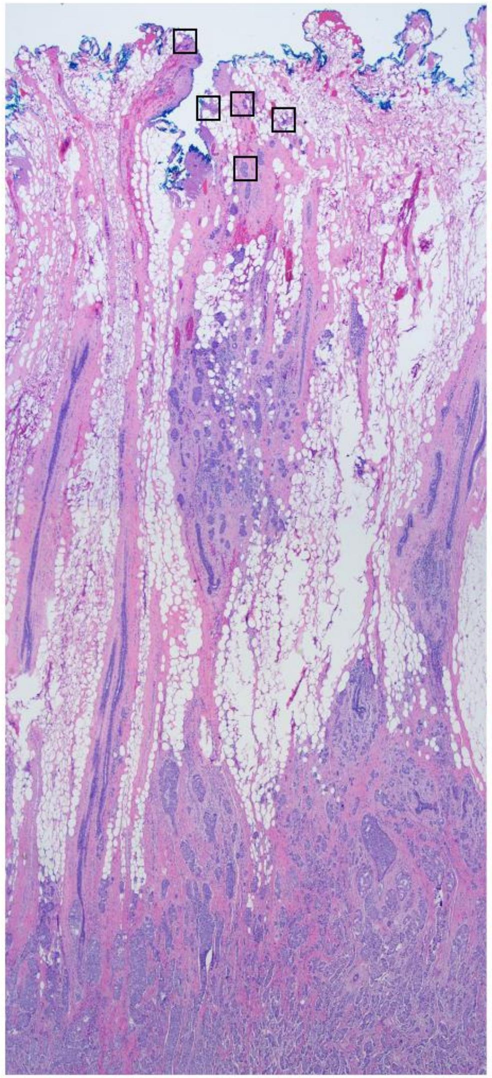
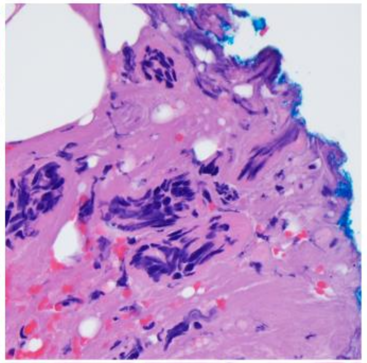
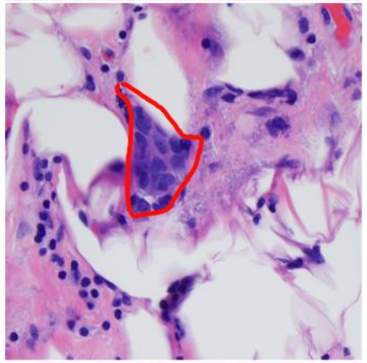


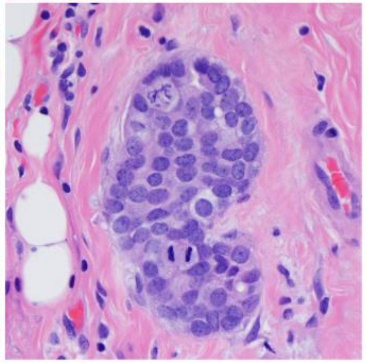
Figure 1. MarginCall concept and workflow. The 3D-surface profile of a structurally intact resected specimen will be rapidly acquired using either a home-built laser line scanner or other available technologies. The sample (up to 10 x 10 x 10 cm3) will then be automatically sectioned with 4 mm spacing and the slices surface stained within 1-2 minutes. After which, up to 25 slices will be imaged with MarginCall instrumentation equipped with both 4X (NA=0.20) and 20X (NA=0.75) lenses to generate 2 um- and 0.5

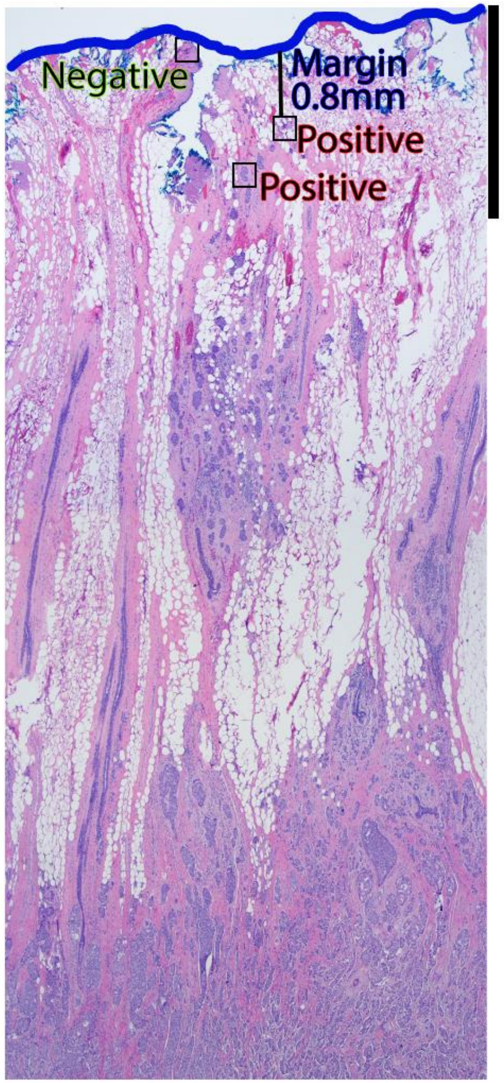
Teaching AI to work like a pathologist….


Low Mag


High Mag Negative


High Mag Positive


High Mag Cancer Cell Reference

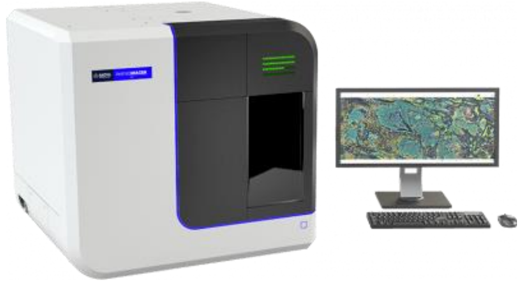

Negative
Margin 0.8mm
Positive
Positive
High Mag Scan Area

Final Margin Analysis

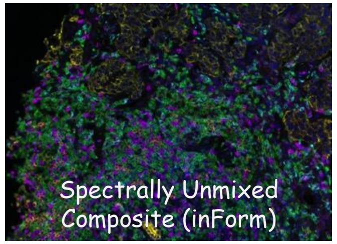
Borowsky Resource Core Laboratories

- ***Center for Genomic Pathology Laboratory***: Core laboratory, spin off from MBP providing advanced histology, immunohistochemistry, multiplex*IF*, image analysis including ML/AI methods, advanced experimental techniques, 3d culture etc.

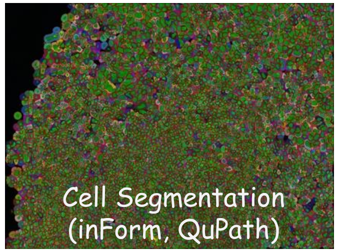
Autostainer: Leica Bond Rx
Opal fluorophores



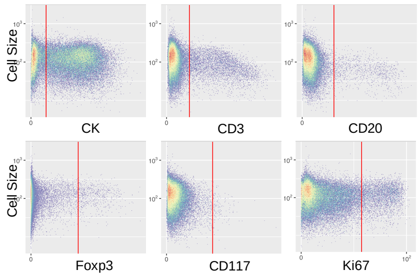
Polaris Imaging Platform



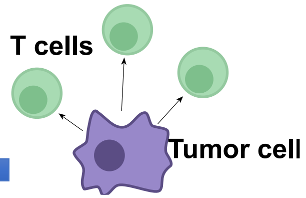
Spectrally Unmixed Composite (inForm)



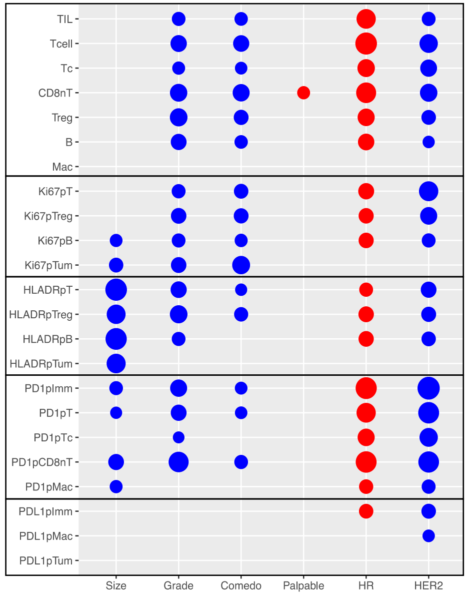
Cell Segmentation (inForm, QuPath)



Phenotyping/Gating
(flowDensity R package)



Spatial Analyses
(spatstat R package)



Spot matrix illustrating associations
with high-risk clinical features

Serves over 50 Investigator Teams across SOM, SVM, others including extramural. Roughly $1,000,000 annual business.

We all need to also become ***Immunologists***

- Medicine is still at the very start of harnessing/enhancing the immune system to treat and prevent disease.
- PHENOTYPERs needed!

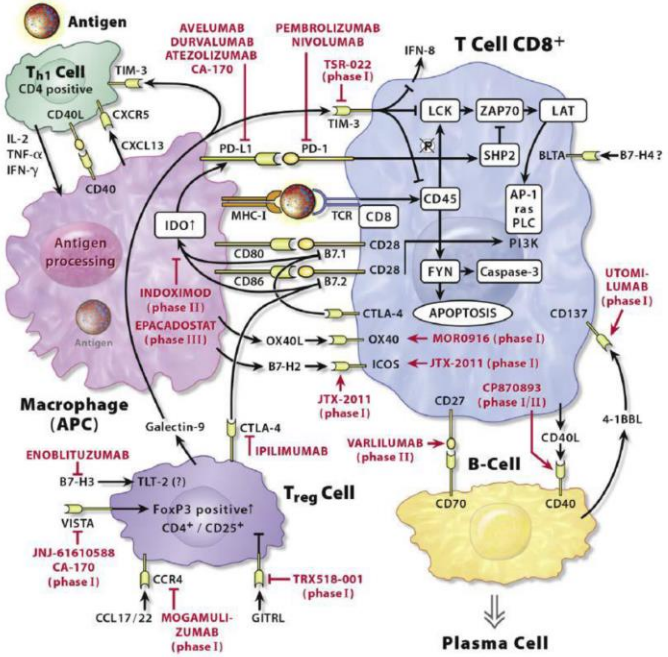

Antigen
AVELUMAB
DURVALUMAB
ATEZOLIZUMAB
CA-170
PEMBROLIZUMAB
NIVOLUMAB
IFN-8
T Cell CD8+
T$_{h1}$ Cell
CD4 positive
TIM-3
TSR-022
(phase I)
TIM-3
CD40L
CXCR5
LCK
ZAP70
LAT
IL-2
TNF-α
IFN-γ
CXCL13
PD-L1
PD-1
SHP2
BLTA
B7-H4 ?
CD40
CD45
AP-1
ras
PLC
MHC-I
TCR
CD8
IDO↑
Antigen
processing
CD80
B7.1
CD28
PI3K
CD86
B7.2
CD28
FYN
Caspase-3
UTOMI-
LUMAB
(phase I)
INDOXIMOD
(phase II)
EPACADOSTAT
(phase III)
CTLA-4
APOPTOSIS
CD137
Antigen
OX40L
OX40
MOR0916 (phase I)
B7-H2
ICOS
JTX-2011 (phase I)
JTX-2011
(phase I)
CD27
CP870893
(phase I/II)
Macrophage
(APC)
Galectin-9
CTLA-4
IPILIMUMAB
4-1BBL
CD40L
VARLILUMAB
(phase II)
B-Cell
ENOBLITUZUMAB
B7-H3
TLT-2 (?)
T$_{reg}$ Cell
CD70
CD40
FoxP3 positive↑
CD4+ / CD25+
VISTA
JNJ-61610588
CA-170
(phase I)
CCR4
TRX518-001
(phase I)
CCL17/22
MOGAMULI-
ZUMAB
(phase I)
GITRL
Plasma Cell

Figure1. Drugs, approved and in development to control immune checkpoint molecules (pathways and targets in black with all drugs in RED TYPE).*Adapted from: Second-And Third-Generation Drugs for Immuno-Oncology Treatment, Dempkeet al., Eur. J. Cancer (2017)*

It is Time for TIME (Tumor Immune Microenvironment) in Experimental and Diagnostic Pathology

Immunology for Cancer and for Health

- In addition to the Tumor Immune MicroEnvironment and precision cancer immuno-oncology…
  - Immune monitoring for health and disease prevention
  - The immune system as the effectors of microbiome changes
  - GMP for cell-based therapies… apheresis centers and Car-T cells.
- Experimental models for immune interactions
  - Building a better mouse
  - 3D microfluidics

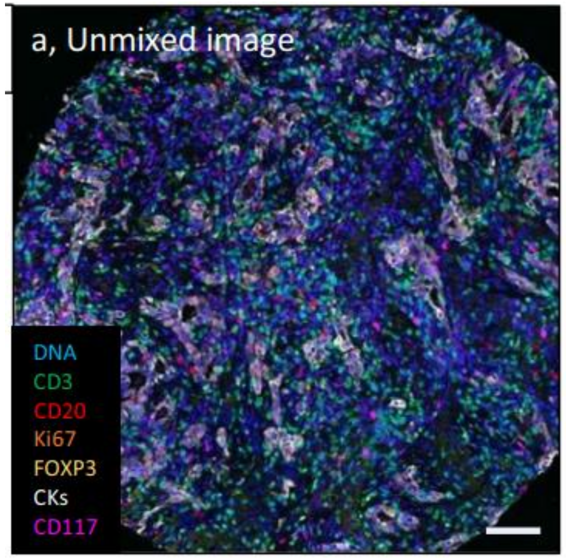
VISION

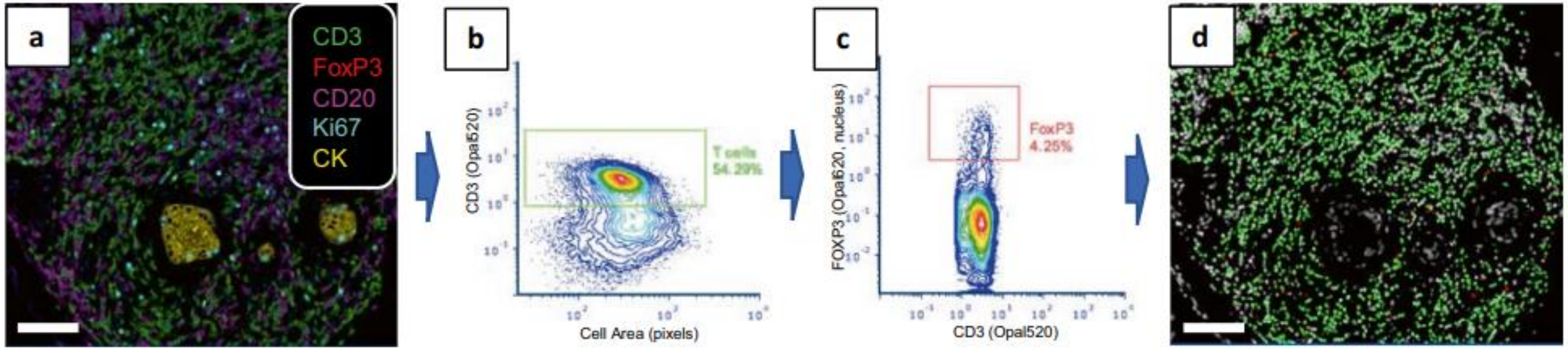
I believe we are only at the tip of the iceberg in harnessing and augmenting the immune system for a wide array of disease treatments… and more importantly disease prevention.

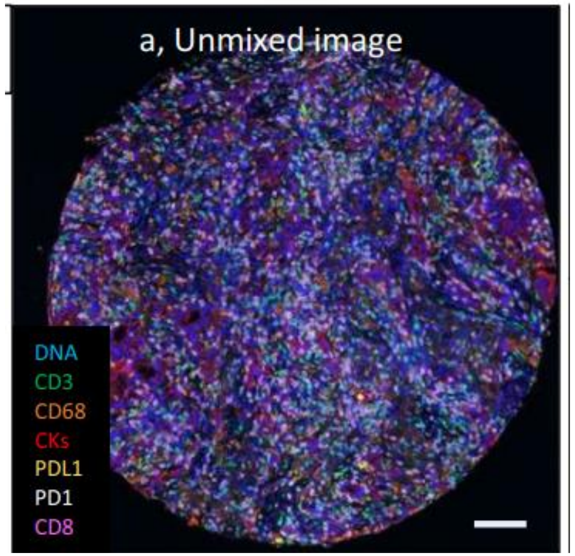
What to do about it…

TO DO LIST

1. Keep learning… and teaching immunology
2. Bring technology in-house
3. Validate these immune system biomarkers
4. Develop experimental pathology models and methods







Characterizing the Tumor Immune Microenvironment with Tyramide-Based Multiplex Immunofluorescence

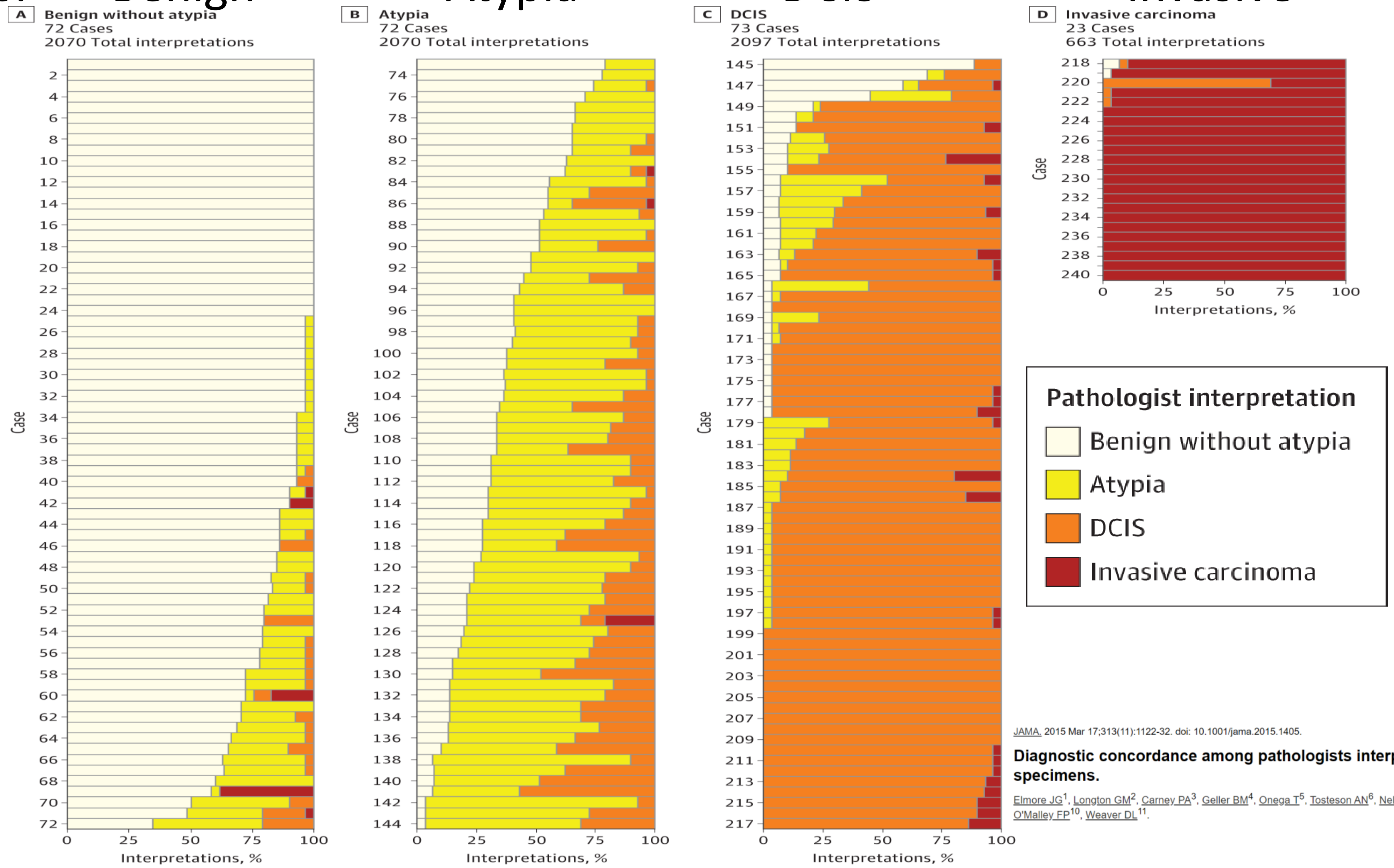
Hidetoshi Mori[1] · Jennifer Bolen[2] · Louis Schuetter[1] · Pierre Massion[3] · Clifford C. Hoyt[4] · Scott VandenBerg[2] · Laura Esserman[5,6] · Alexander D. Borowsky[1,7] · Michael J. Campbell[5]

Table1. Staining conditions for multiplex IHC for IP1 and IP2

Staining cycle	Marker	Clone	Company	Product	Antibody dilution	Fluorophore	Fluoro-phore dilution
IP1							
1	FOXP3	SP97	Spring	M3972	1:25	Opal620	1:250
2	CKs	AE1/AE3	DAKO	M3515	1:200	Opal650	1:200
3	Ki67	30-9	Ventana	790-4286	RTU	Opal690	1:100
4	CD20	L26	Ventana	790-2531	RTU	Opal540	1:250
5	CD3	2GV6	Ventana	790-4341	RTU	Opal520	1:100
6	CD117	c-kit	DAKO	A4502	1:100	Opal570	1:300
7			Perkin Elmer	FP1490		DAPI	RTU
IP2							
1	PDL1	E1L3N	CST	13648e	1:100	Opal620	1:100
2	PD1	EPR4877	Abcam	ab137132	1:100	Opal650	1:200
3	CD8	4B11	Leica	CD8-4B11-L-CE	1:100	Opal690	1:100
4	CKs	AE1/AE3	DAKO	M3515	1:200	Opal570	1:300
5	CD68	PG-M1	DAKO	M0876	1:100	Opal540	1:250
6	CD3	2GV6	Ventana	790-4341	RTU	Opal520	1:100
7			Perkin Elmer	FP1490		DAPI	RTU

Inter-observer diagnostic variability

Experts
Answers: Benign Atypia DCIS Invasive



JAMA. 2015 Mar 17;313(11):1122-32. doi: 10.1001/jama.2015.1405.

Diagnostic concordance among pathologists interpreting breast biopsy specimens.

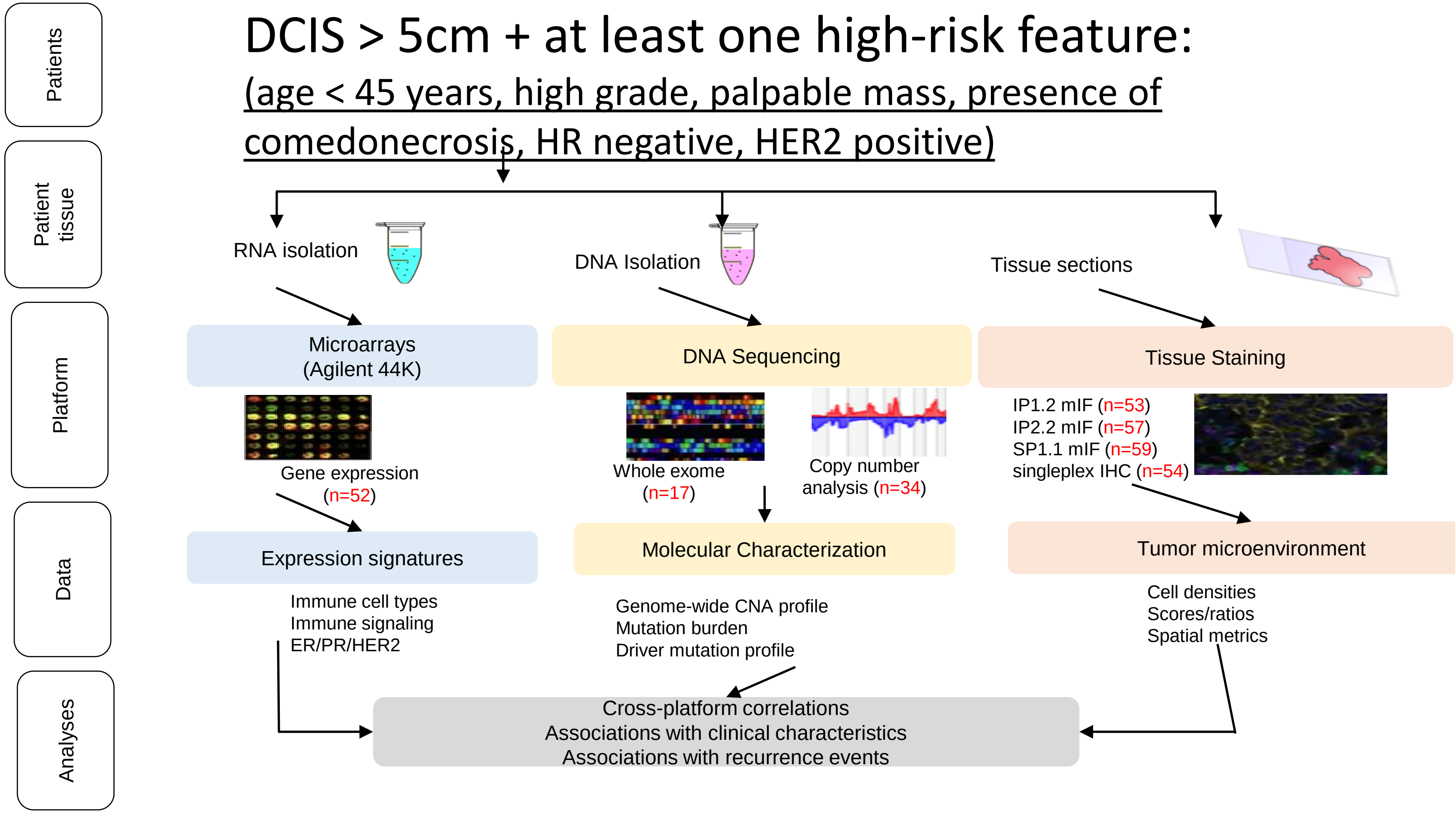
Elmore JG[1], Longton GM[2], Carney PA[3], Geller BM[4], Onega T[5], Tosteson AN[6], Nelson HD[7], Pepe MS[2], Allison KH[8], Schnitt SJ[9], O'Malley FP[10], Weaver DL[11].

DEFENSE Study Cohort (n=63)

*DCIS: **E**laboration of **F**actors from **E**nlarged lesions that **N**evertheless **S**tay **E**ncapsulated*

DCIS > 5cm + at least one high-risk feature:

(age < 45 years, high grade, palpable mass, presence of comedonecrosis, HR negative, HER2 positive)



RNA isolation

DNA Isolation

Tissue sections

Microarrays (Agilent 44K)

DNA Sequencing

Tissue Staining

Gene expression (n=52)

Whole exome (n=17)

Copy number analysis (n=34)

IP1.2 mIF (n=53)
IP2.2 mIF (n=57)
SP1.1 mIF (n=59)
singleplex IHC (n=54)

Expression signatures

Molecular Characterization

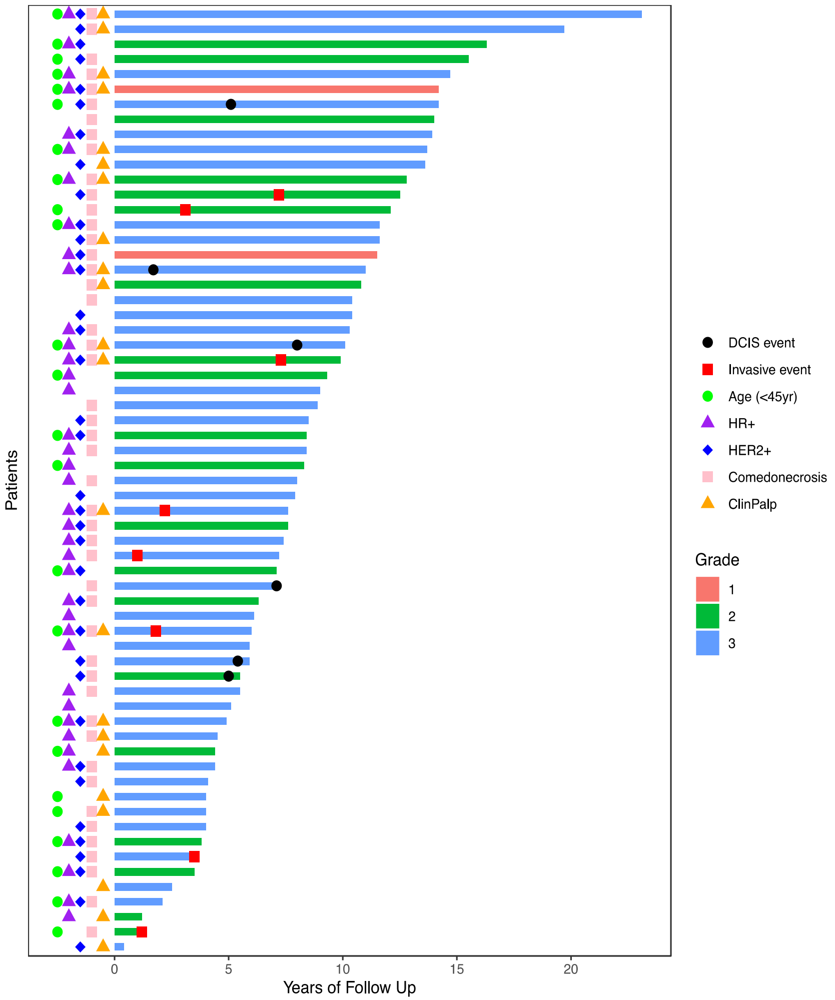
Tumor microenvironment

Immune cell types
Immune signaling
ER/PR/HER2

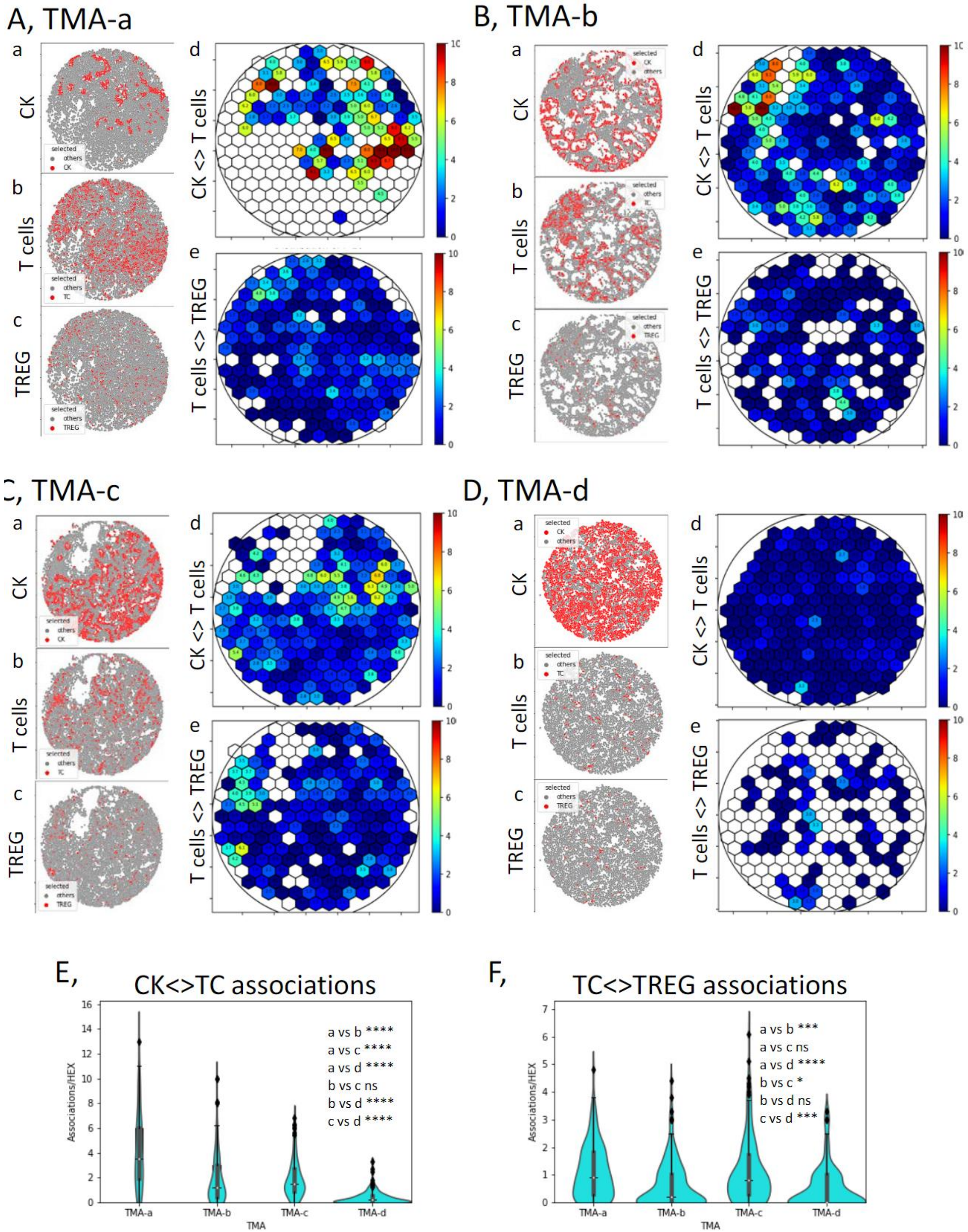
Genome-wide CNA profile
Mutation burden
Driver mutation profile

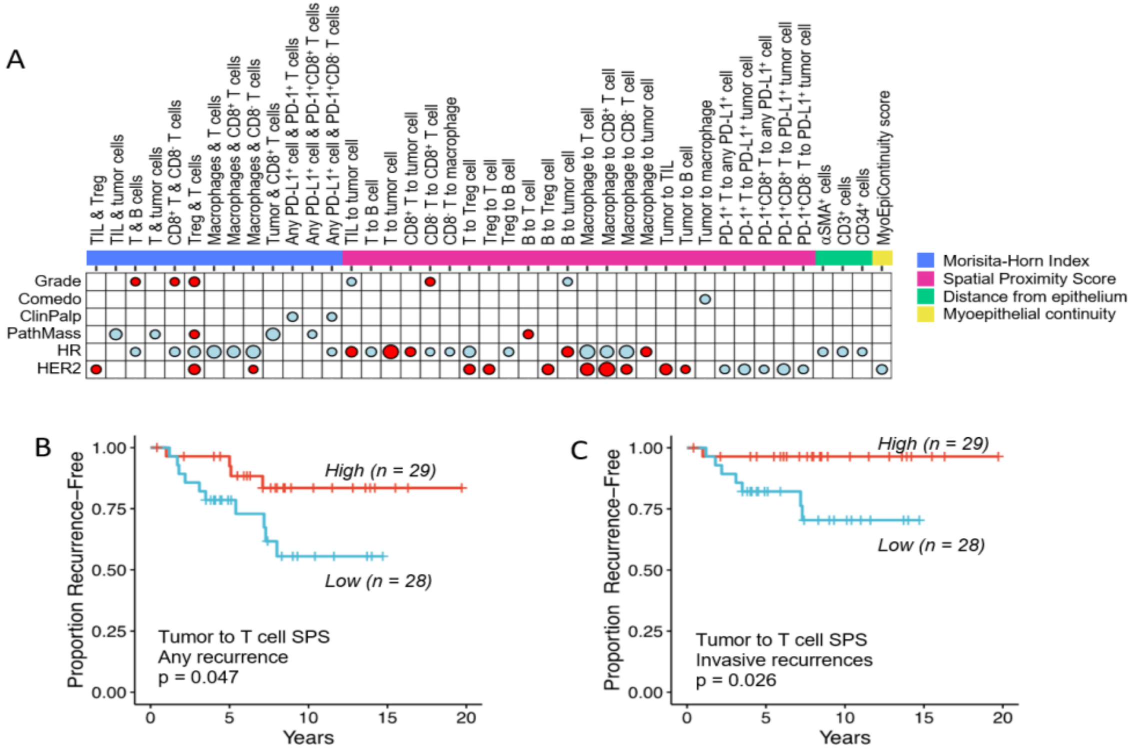
Cell densities
Scores/ratios
Spatial metrics

Cross-platform correlations
Associations with clinical characteristics
Associations with recurrence events



Will the *Real* Pre-cancers please reveal yourself…





Cell-Cell proximities

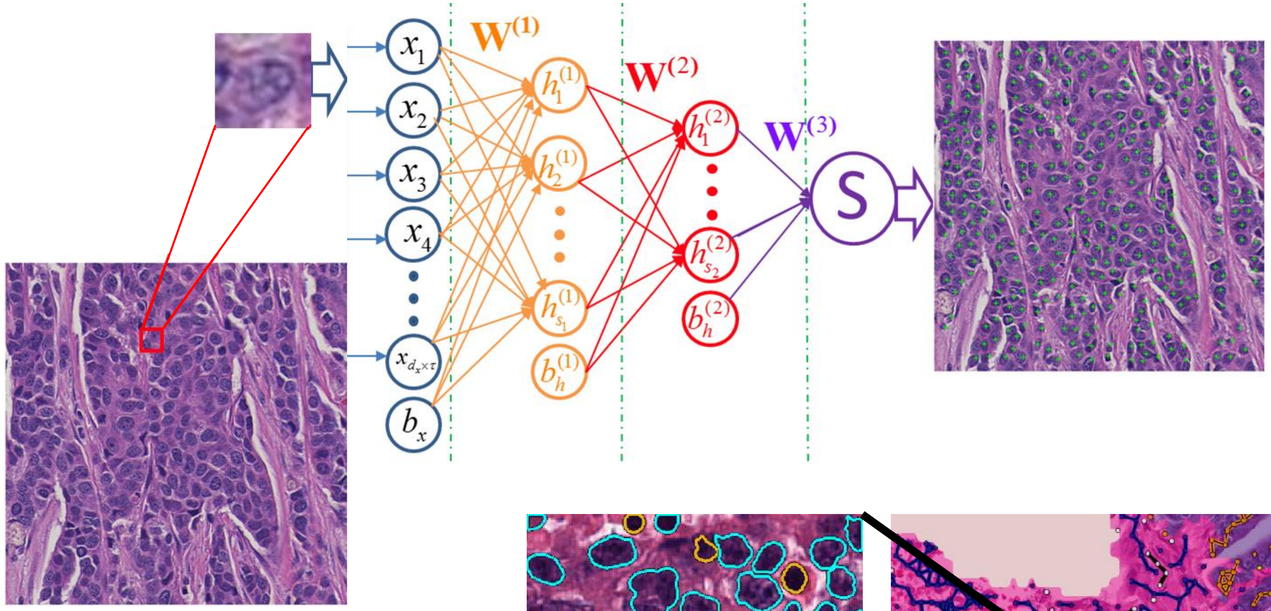
DEFENSE Summary

Cold Spring Harbor Laboratory bioRχiv THE PREPRINT SERVER FOR BIOLOGY

Conclusions/Hypotheses:

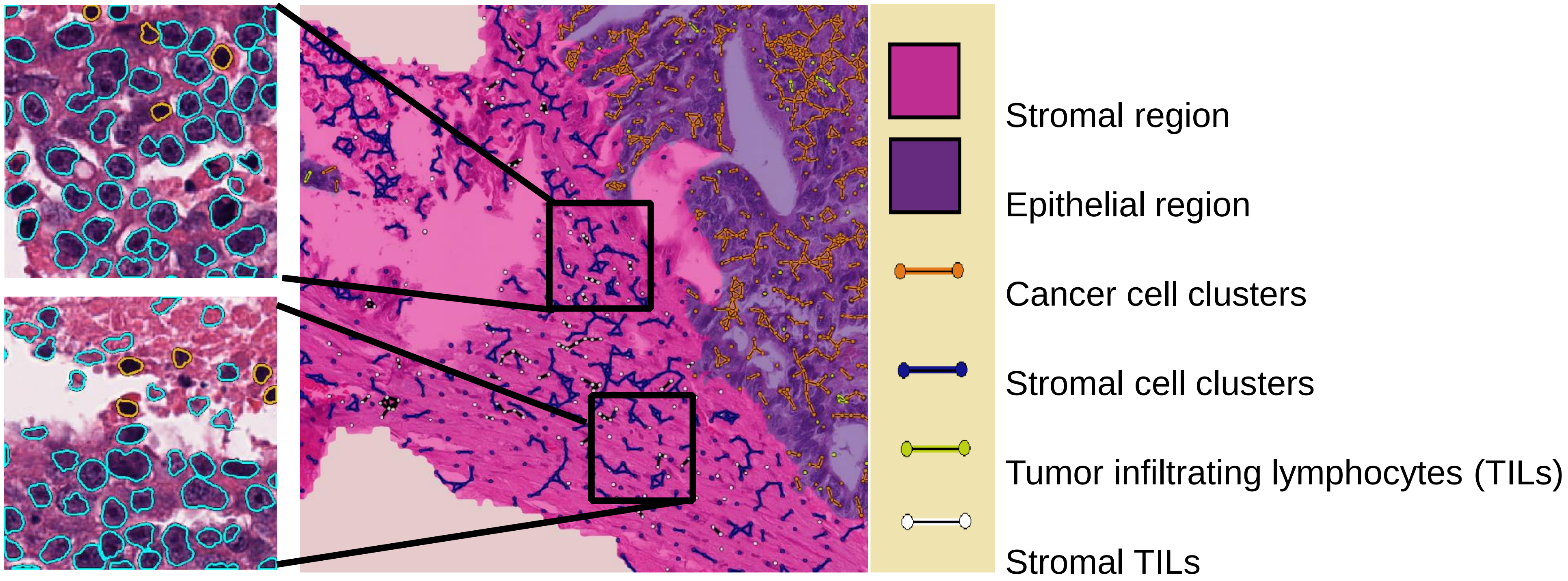
1. Myoepithelial cells may limit host immune recognition of the DCIS cells harboring neoantigens.
2. HER2 amplification/overexpression itself may be one of these neoantigens *(high prevalence in our cohort, but also high prevalence in all DCIS compared to invasive carcinomas.)*
3. DCIS can be stratified based upon features of the tumor immune microenvironment
4. Intrinsic epithelial subtypes include propensity for high neo-antigenicity.

DEEP PHENOTYPING: Digital Pathology


$W^{(1)}$
$W^{(2)}$
$W^{(3)}$
x_1
x_2
x_3
x_4
$x_{d_x \times \tau}$
b_x
$h_1^{(1)}$
$h_2^{(1)}$
$h_{s_1}^{(1)}$
$b_h^{(1)}$
$h_1^{(2)}$
$h_{s_2}^{(2)}$
$b_h^{(2)}$
S

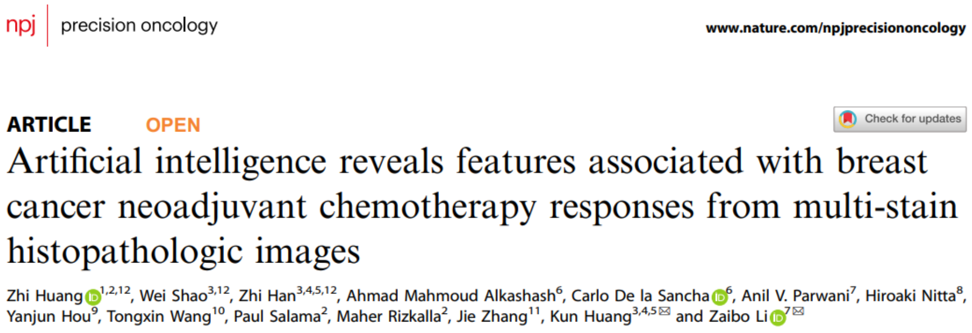
Deep learning-based mapping of cells, tissues, and structures on histopathology images

Mining the TIME and histologic primitives for AI-powered interpretable biomarkers


Stromal region
Epithelial region
Cancer cell clusters
Stromal cell clusters
Tumor infiltrating lymphocytes (TILs)
Stromal TILs

Dimensionality Reduction

- Can predictive immune infiltrate patterns be determined with fewer markers using AI?



npj | precision oncology

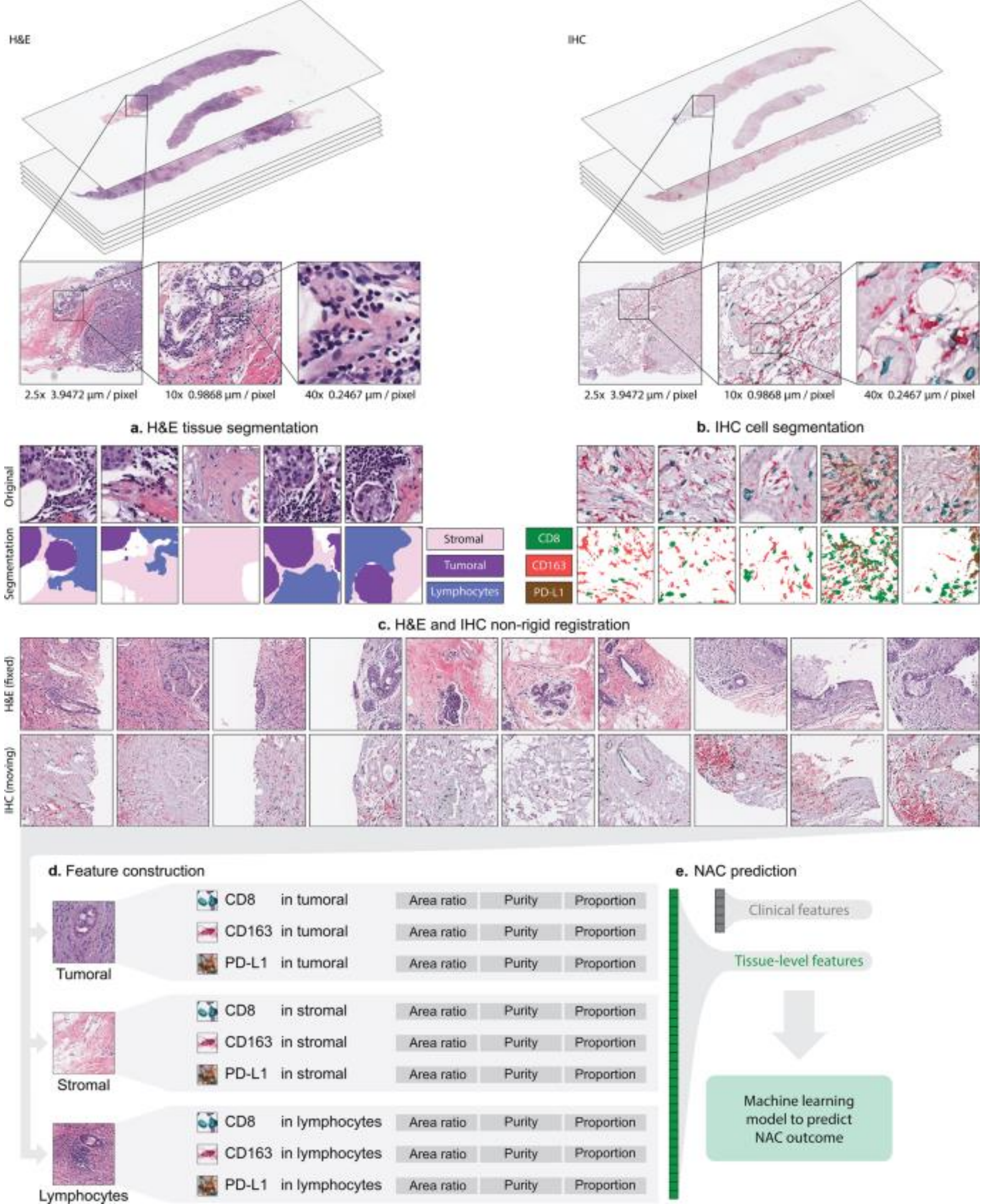
www.nature.com/npjprecisiononcology

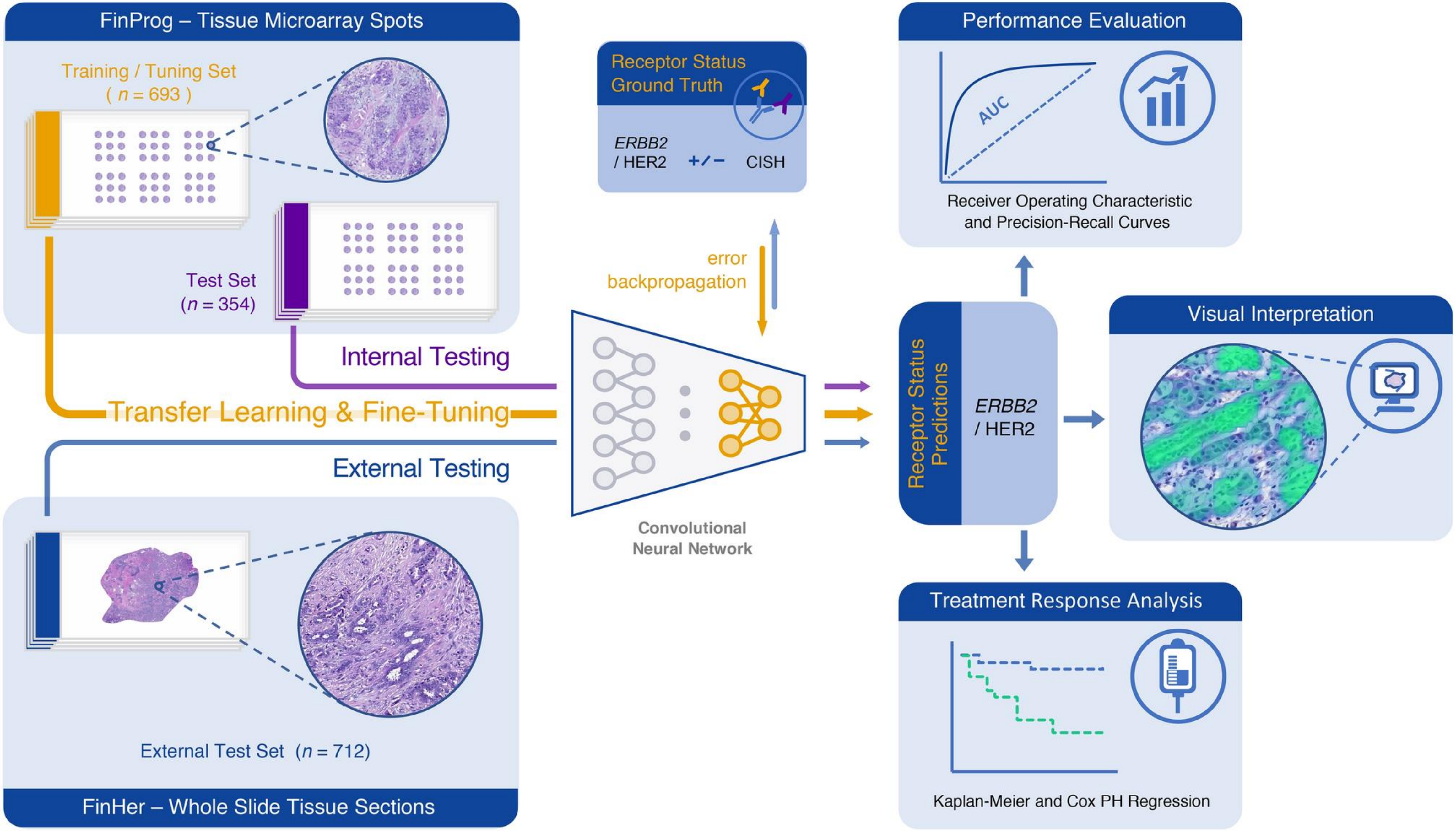
ARTICLE OPEN

Check for updates

Artificial intelligence reveals features associated with breast cancer neoadjuvant chemotherapy responses from multi-stain histopathologic images

Zhi Huang[1,2,12], Wei Shao[3,12], Zhi Han[3,4,5,12], Ahmad Mahmoud Alkashash[6], Carlo De la Sancha[6], Anil V. Parwani[7], Hiroaki Nitta[8], Yanjun Hou[9], Tongxin Wang[10], Paul Salama[2], Maher Rizkalla[2], Jie Zhang[11], Kun Huang[3,4,5]✉ and Zaibo Li[7]✉


H&E
IHC
2.5x 3.9472 μm / pixel
10x 0.9868 μm / pixel
40x 0.2467 μm / pixel
2.5x 3.9472 μm / pixel
10x 0.9868 μm / pixel
40x 0.2467 μm / pixel
a. H&E tissue segmentation
b. IHC cell segmentation
Original
Segmentation
Stromal
Tumoral
Lymphocytes
CD8
CD163
PD-L1
c. H&E and IHC non-rigid registration
H&E (fixed)
IHC (moving)
d. Feature construction
Tumoral
Stromal
Lymphocytes
CD8 in tumoral
CD163 in tumoral
PD-L1 in tumoral
CD8 in stromal
CD163 in stromal
PD-L1 in stromal
CD8 in lymphocytes
CD163 in lymphocytes
PD-L1 in lymphocytes
Area ratio
Purity
Proportion
e. NAC prediction
Clinical features
Tissue-level features
Machine learning model to predict NAC outcome



FinProg – Tissue Microarray Spots

Training / Tuning Set
(*n* = 693)

Test Set
(*n* = 354)

Internal Testing

Transfer Learning & Fine-Tuning

External Testing

External Test Set (*n* = 712)

FinHer – Whole Slide Tissue Sections

Receptor Status Ground Truth

ERBB2 / HER2 +/− CISH

error backpropagation

Convolutional Neural Network

Receptor Status Predictions

ERBB2 / HER2

Performance Evaluation

AUC

Receiver Operating Characteristic and Precision-Recall Curves

Visual Interpretation

Treatment Response Analysis

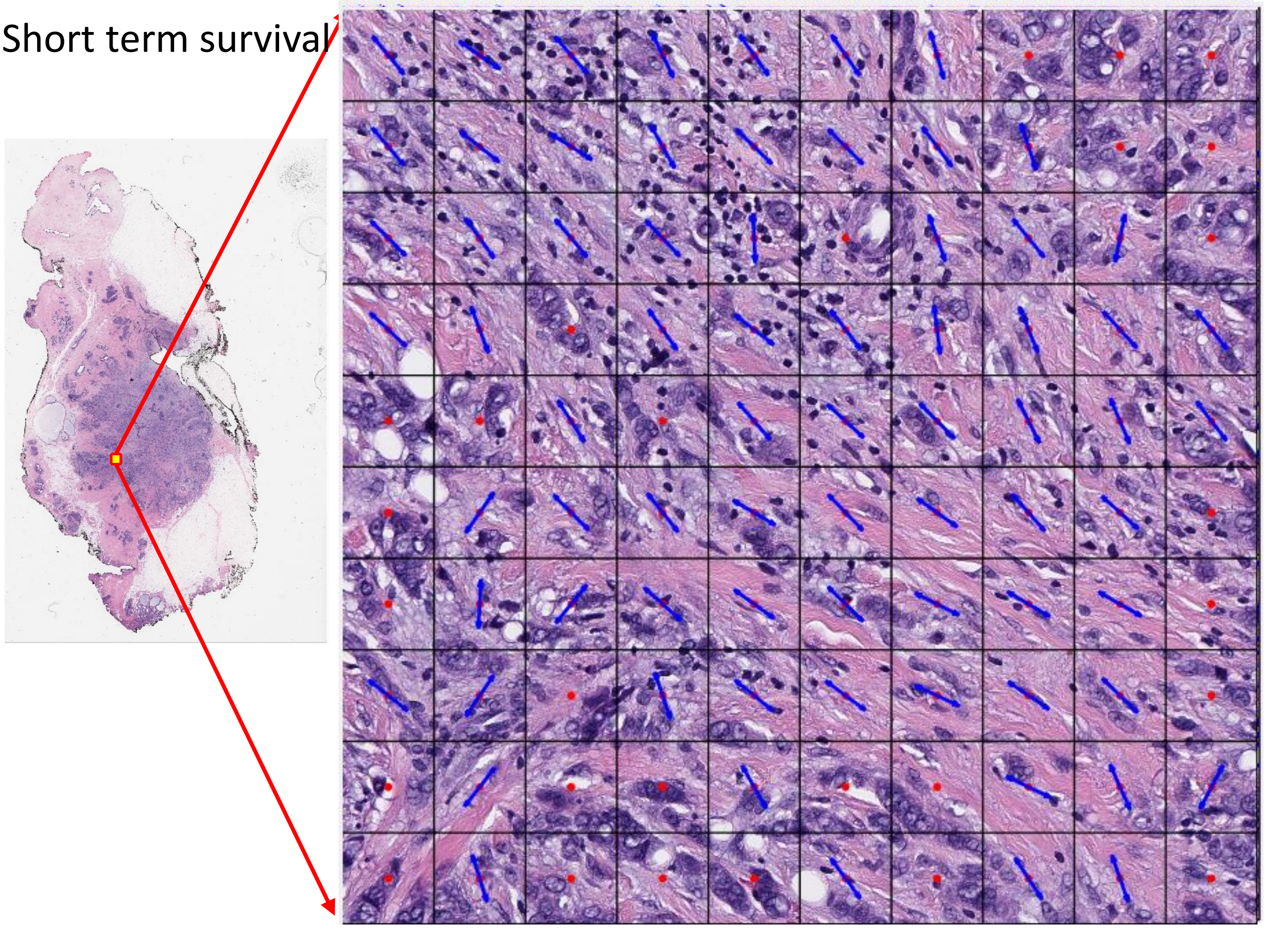
Kaplan-Meier and Cox PH Regression

https://www.nature.com/articles/s41598-021-83102-6

Collagen fiber detection
↓
Epithelium segmentation
↓
Collagen vectors in tumor-associated stroma
↓
Collagen fiber orientation disorder calculation

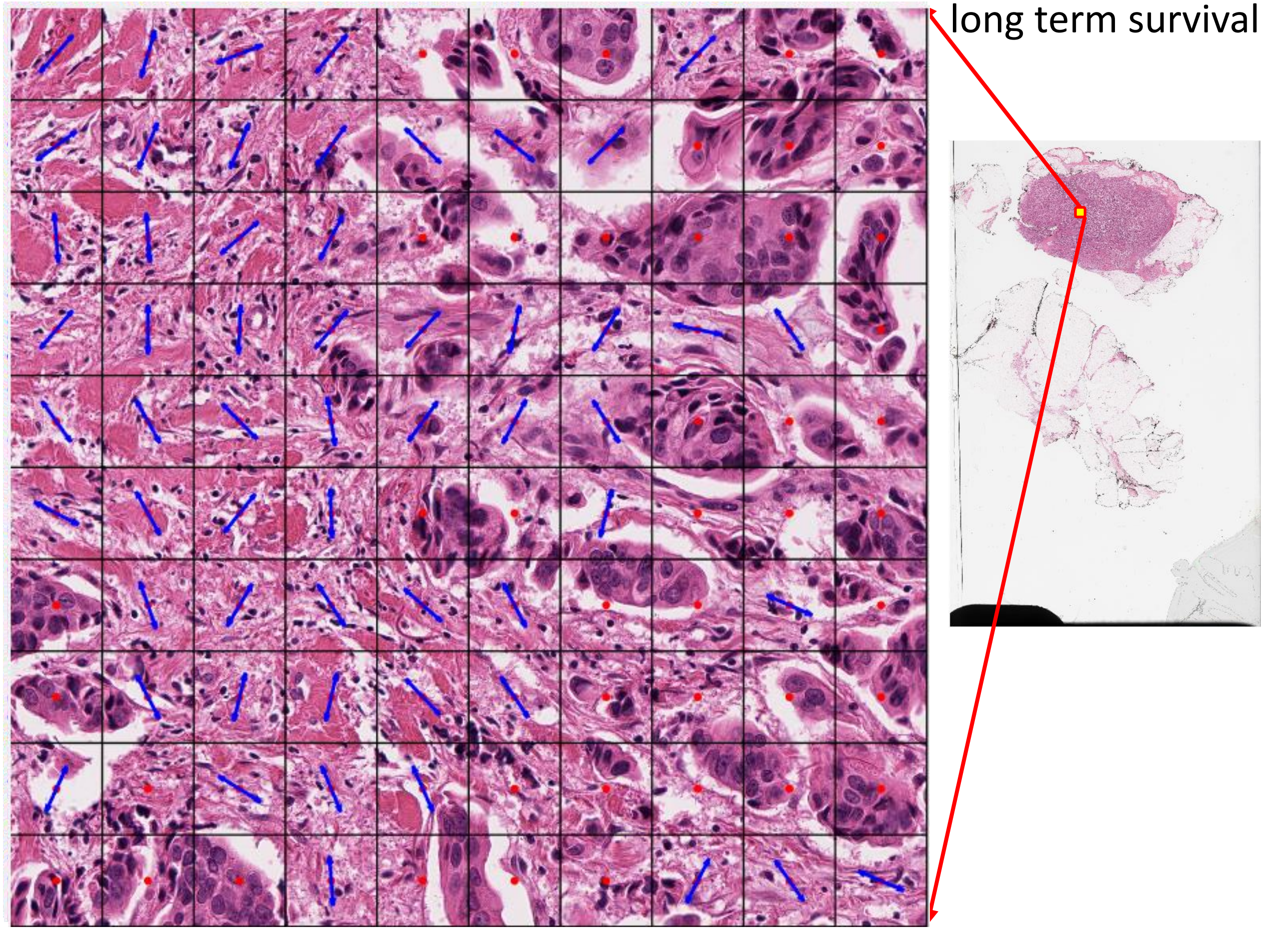
Low degree of disorder

Short term survival



High degree of disorder

long term survival



Li et al, npj Breast Cancer, 2021

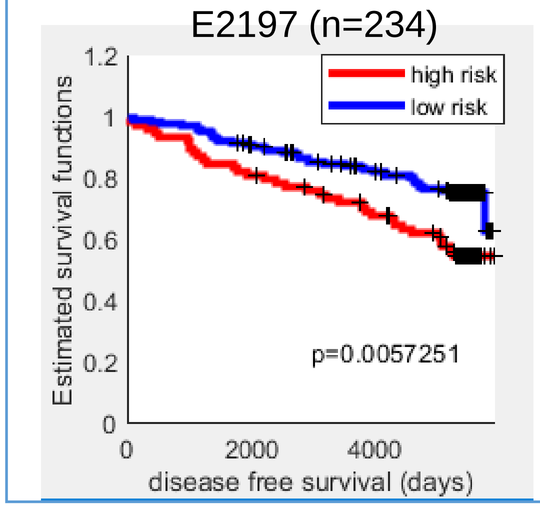
Disorder of collagen fiber orientation associated with risk of recurrence in ER+ breast cancers in ECOG-ACRIN E2197 & TCGA

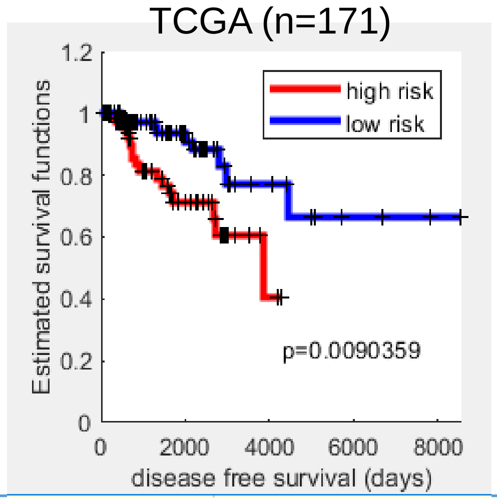
Unmet Clinical Need

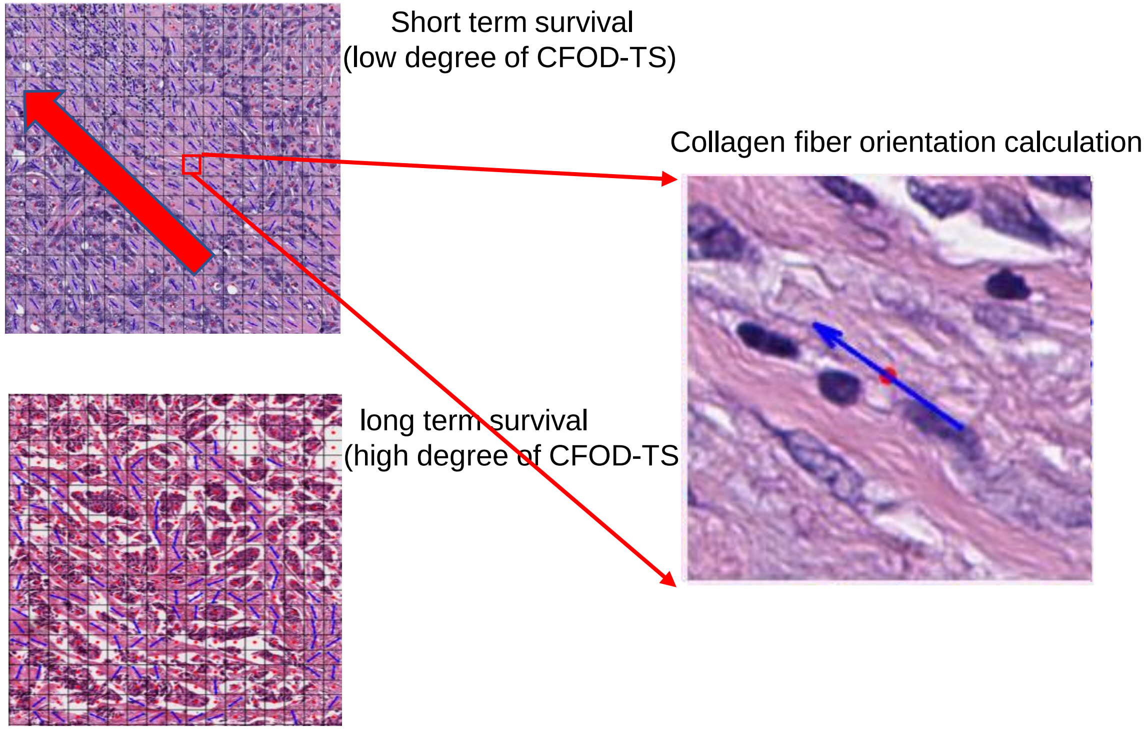
- Early stage ER+ breast cancer (BC) is the most common type of breast cancer in the United States
- Predicting the likelihood of recurrence for patients helps physicians plan more tailored treatment strategy to improve survival rate.

Results:

- Collagen Fiber Orientation Disorder in Tumor associated Stroma (CFOD-TS) was independently prognostic for ER+ BCs in E2197 and TCGA.







Short term survival
(low degree of CFOD-TS)

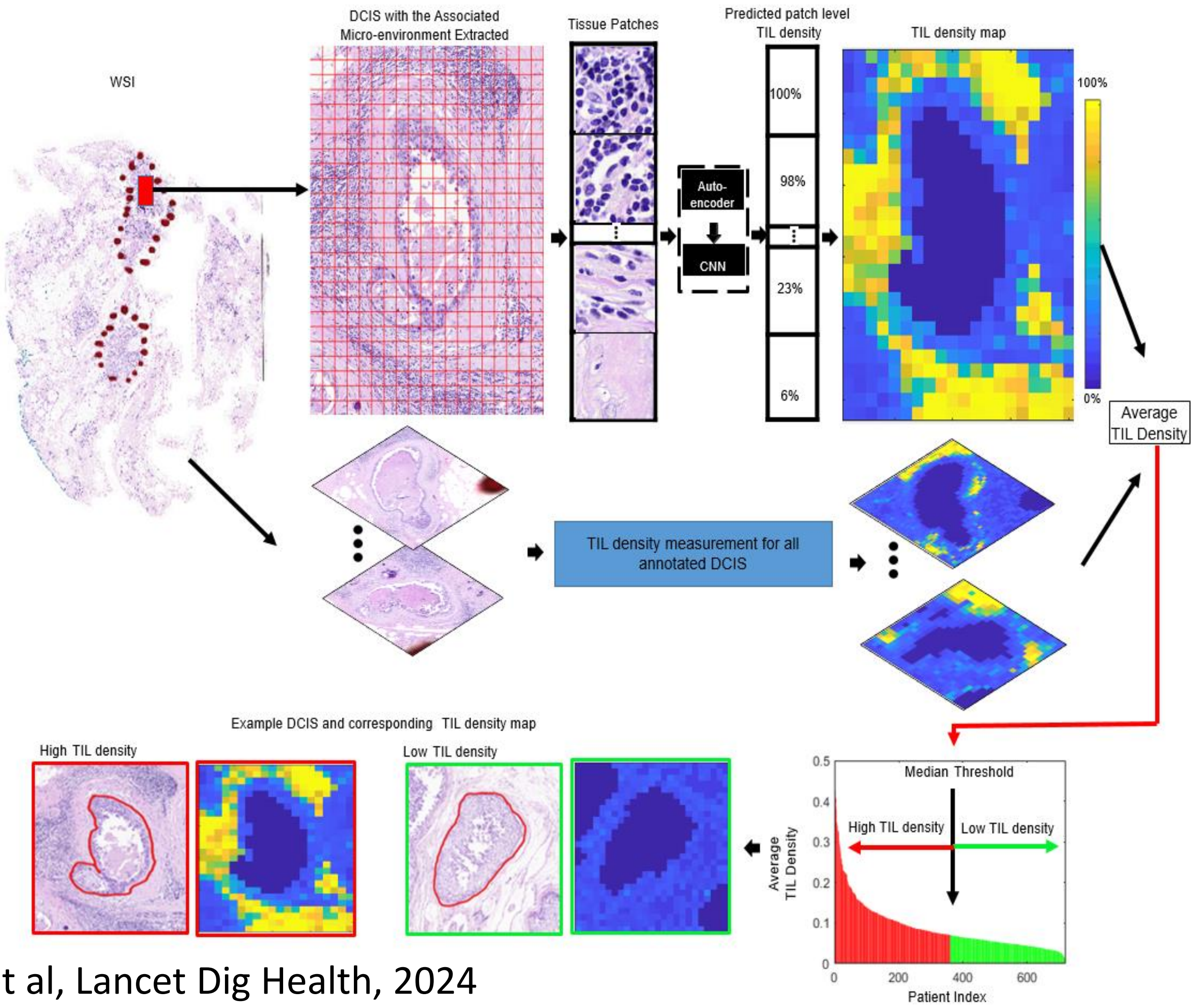
Collagen fiber orientation calculation

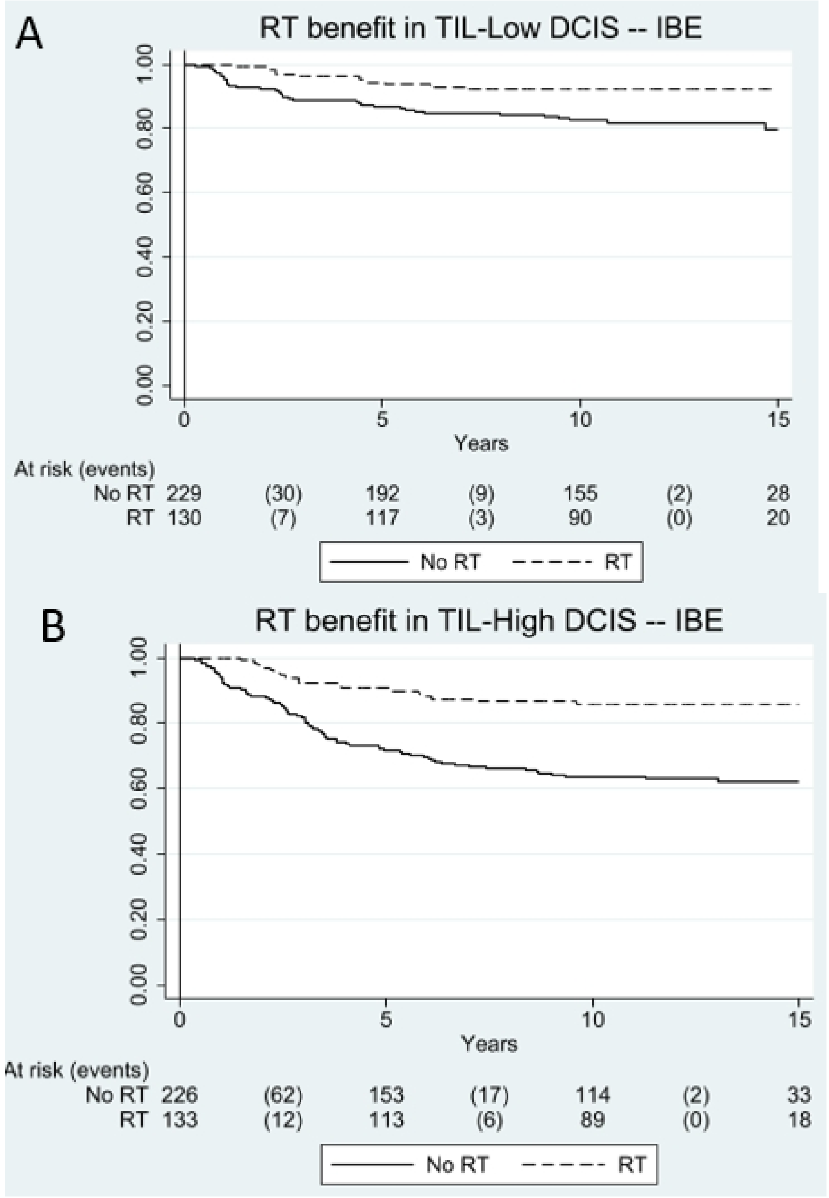
long term survival
(high degree of CFOD-TS

Take away:

Over-expression of CFOD-TS independently associated with lower likelihood of recurrence and could potentially serve as a prognostic marker of outcome for ER+ invasive breast cancer.

Computer extracted features of immune architecture from H&E Whole slide images are associated with disease-free survival and benefit of radiotherapy in Ductal Carcinoma in situ (DCIS)


WSI
DCIS with the Associated Micro-environment Extracted
Tissue Patches
Auto-encoder
CNN
Predicted patch level TIL density
100%
98%
23%
6%
TIL density map
100%
0%
Average TIL Density
TIL density measurement for all annotated DCIS
Example DCIS and corresponding TIL density map
High TIL density
Low TIL density
Median Threshold
High TIL density
Low TIL density
Average TIL Density
0.5
0.4
0.3
0.2
0.1
0
0
200
400
600
Patient Index



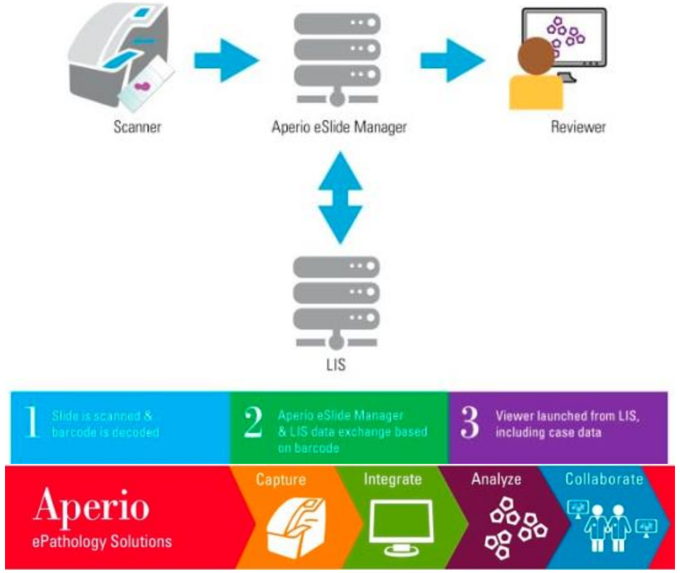
Li et al, Lancet Dig Health, 2024

Take Aways

- **AI is not magic –** Need to be thoughtful and intentional in developing algorithms.
- **Computational Analytics with routine imaging** and data could help address questions in precision medicine, specifically prognosis and predicting response to therapy
- **Low cost** computational diagnostics, need to be intentional in addressing **equity.**
- **Global impact**, especially **low and middle income** countries.
- Multi-scale disease associations, looking to establish the morphologic and molecular basis of the imaging features. Need an intuitive basis to drive clinical adoption

So we all need to be Bio-informaticists.

- Maintain and curate the databases.
- Choose and validate the software tools.


Scanner
Aperio eSlide Manager
Reviewer
LIS

1 Slide is scanned & barcode is decoded

2 Aperio eSlide Manager & LIS data exchange based on barcode

3 Viewer launched from LIS, including case data

Aperio
ePathology Solutions
Capture
Integrate
Analyze
Collaborate

OneSource

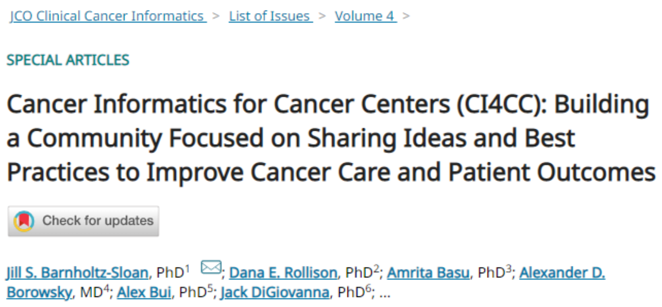
INNOVATIVE PRACTICES AWARDS
WINNER
Bio-IT World 2022

smart in media
the pathologists' company

medloop

REDCap
Research Electronic Data Capture

ENABLE MEDICINE



JCO Clinical Cancer Informatics > List of Issues > Volume 4 >

SPECIAL ARTICLES

Cancer Informatics for Cancer Centers (CI4CC): Building a Community Focused on Sharing Ideas and Best Practices to Improve Cancer Care and Patient Outcomes

Check for updates

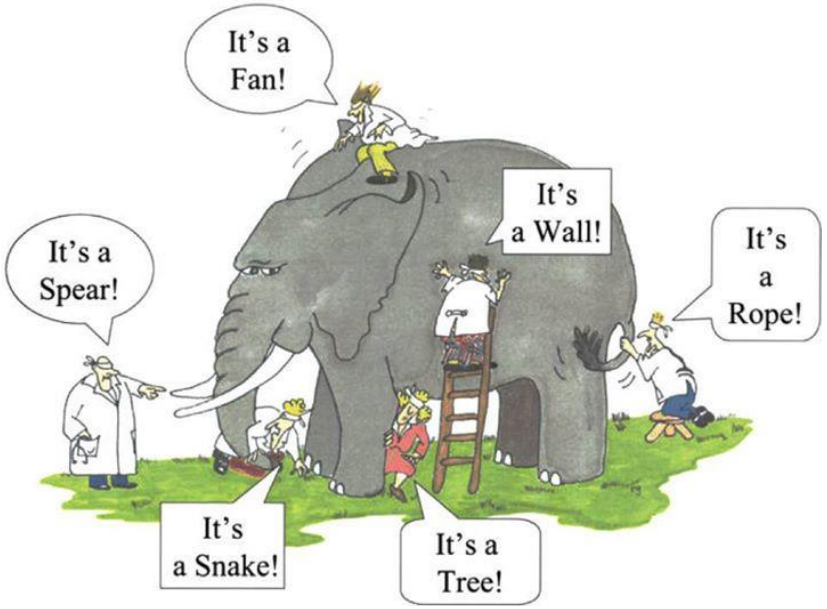
Jill S. Barnholtz-Sloan, PhD[1]; Dana E. Rollison, PhD[2]; Amrita Basu, PhD[3]; Alexander D. Borowsky, MD[4]; Alex Bui, PhD[5]; Jack DiGiovanna, PhD[6]; ...

The importance of teamwork:

Just when you think you've got it all figured out… everything turns out to be far more complex than you ever realized… (I say this a lot, but maybe someone said it first?)

The Blind Men and the Elephant. *Hindu parable c.1000BC.*

"We choose to go to the Moon in this decade and do the other things, not because they are easy, but because they are hard; because that goal will serve to organize and measure the best of our energies and skills, because that challenge is one that we are willing to accept, one we are unwilling to postpone, and one we intend to win, and the others, too."


It's a Fan!
It's a Wall!
It's a Spear!
It's a Rope!
It's a Snake!
It's a Tree!

Acknowledgements (Incomplete list)

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Avi Spira (J&J)

I-SPY The I-SPY Trials

AKOYA BIOSCIENCES®

Leica BIOSYSTEMS

Center for Genomic Pathology CGP
A non-profit dedicated to improvements and validation of experimental pathology and clinical diagnostics.

QL HC Quantum Leap Healthcare Collaborative

CDMRP

NIH NATIONAL CANCER INSTITUTE
Consortium for Molecular and Cellular Characterization of Screen-Detected Lesions

NCI CANCER MOONSHOT
Human Tumor Atlas Network
A program of the National Cancer Institute of the National Institutes of Health

Bristol-Myers Squibb

CENTER FOR IMMUNOLOGY & INFECTIOUS DISEASES

RISE UP for
Breast Cancer

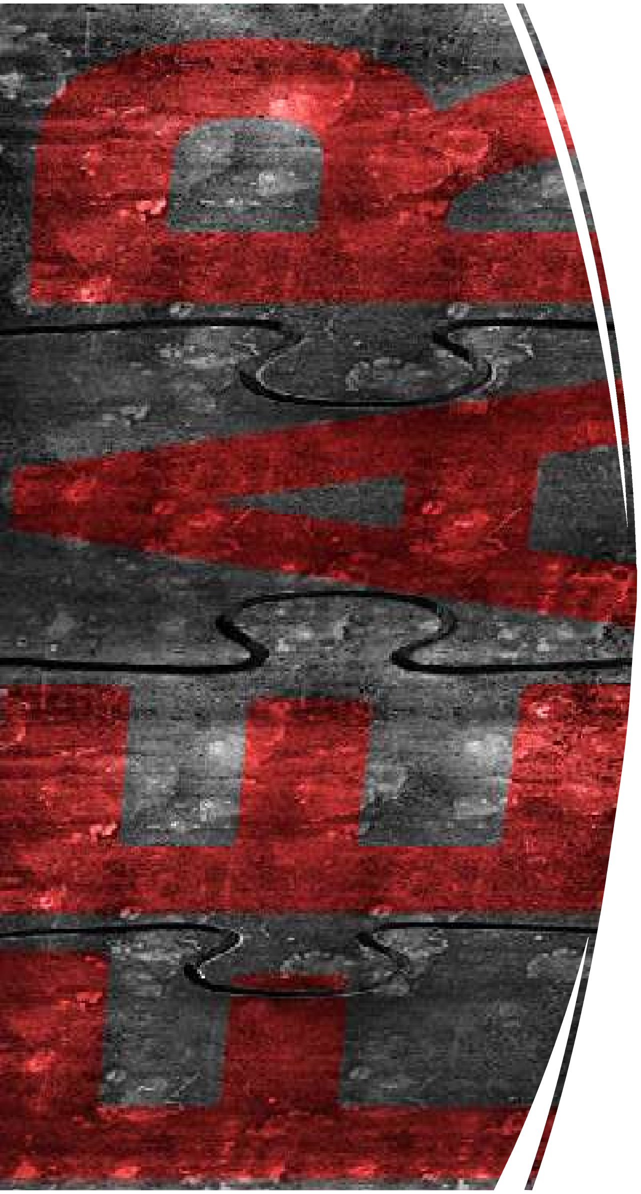
Tumor classification in optimizing therapy & outcomes

No Subtype Left Behind

Patient Perspective

Amy L Delson, AIA
UCSF BSAC
November 1, 2024

Apples & oranges of different colors - Cezanne



"Fear of cancer recurrence is considered one of the most pervasive and burdensome sources of distress for patients"
(KCCure)

Solving the puzzle of improving outcomes and QoL with better tumor classification?

- **What kind of cancer do I have?** Precision Dx Integration (Sandy Borowsky)
- **What is the right drug for me?** RPS Guided Therapy (Denise Wolf)
- **Is my tumor responding to treatment or recurring?** ctDNA trajectory (Mark Magbanua)
- **How can we stop it?** Targeting novel resistance pathways
  - CARM1 (Tam Binh Bui)
  - APOBEC3B (Temiz Pardo)
  - SRS: (Julia Wulfhulke)

Precision Dx: Integration

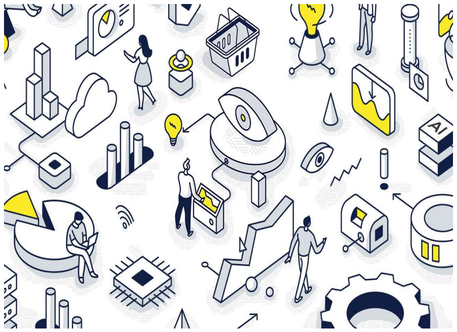
Recipe for Optimal Dx to Rx

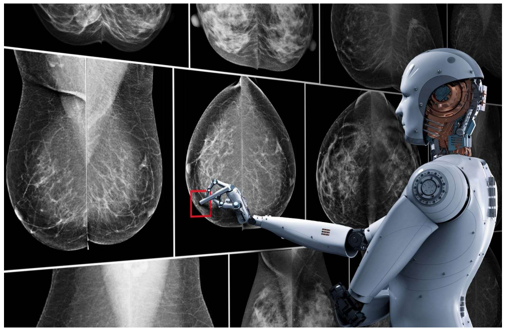
Digital Image Analysis + Incorporate:

+ AI (pathomics)
  - Faster with objective/consistent results
  - Can provide spatial organization & structure
+ Omics of all sorts
+ Liquid Biopsy
+ Immune Landscape (TIME)
+ TBD

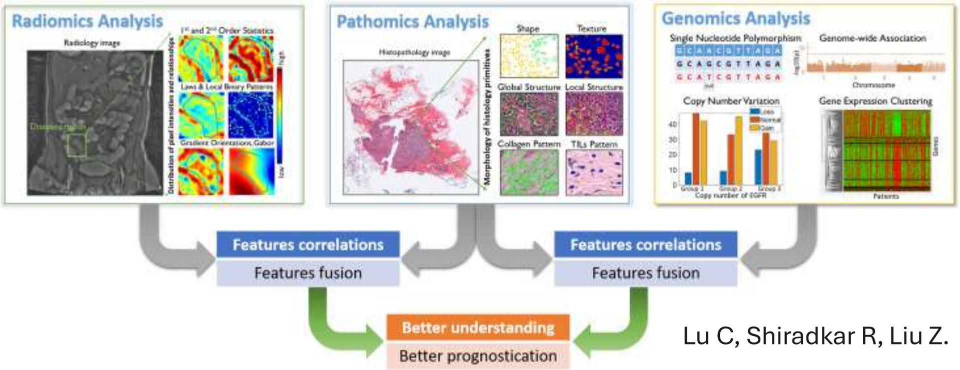
Multi-modal data integration, but how to sort out what is actionable/ meaningful?

(Sandy Borowsky)





Chick Digital


Radiomics Analysis
Pathomics Analysis
Genomics Analysis
Features correlations
Features fusion
Features correlations
Features fusion
Better understanding
Better prognostication

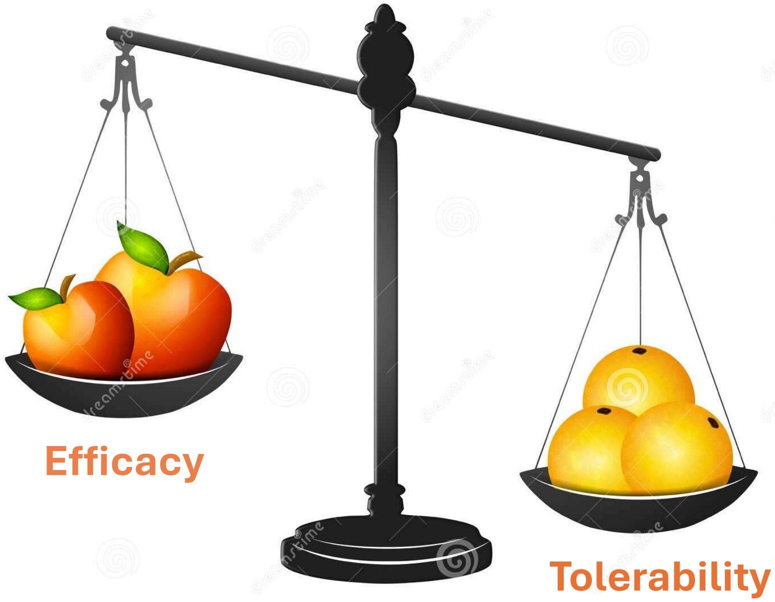
Lu C, Shiradkar R, Liu Z.

What is the right drug for me?

Molecular subtyping (RPS = tumor molecular signature + receptor) so far **improves pCR rates but not for all patients.**
Optimize RPS and identify resistance targets.

- How fine-grained can you get without losing statistical significance?
- **Add the history of response to predict response to “next block”?**

RPS+response_to_BlockA ---> assignment probabilities
Block B (Denise Wolf)



Efficacy

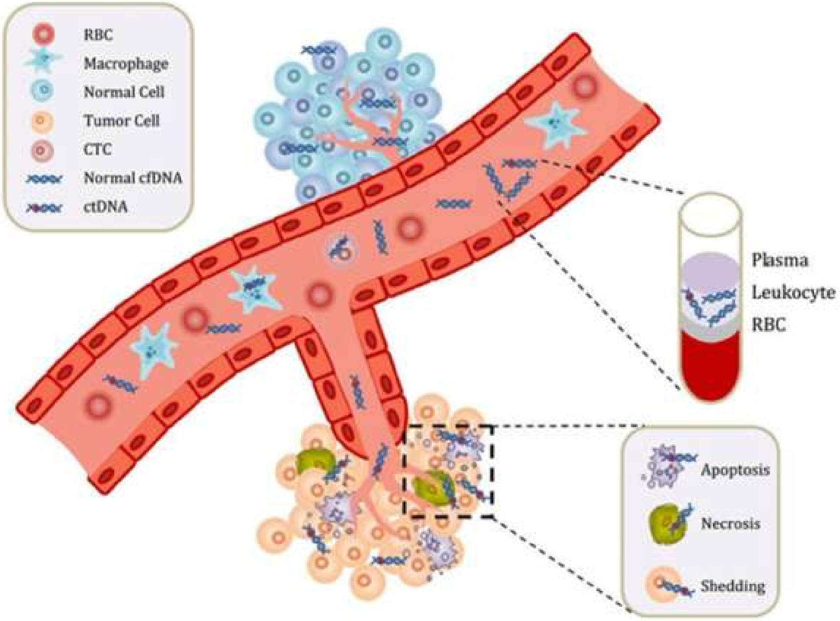
Tolerability

Integrate with QoL

ctDNA: Is my tumor responding to treatment? Will/has my cancer come back? (Mark Magbanua)

- Can discover before it shows up in imaging.
- ctDNA Trajectory: T_0, T_1, T_2, T_3 …. Clearance or not
- Prognostic/Predictive: resistance, local or distant recurrence
- Tumor-informed (\$\$\$ f(#mutations) & **tumor-agnostic** (when no tumor available)

“There are major gaps in understanding of the clinical implications and actionability of ctDNA in the early (nonmetastatic) setting, and there is no clear guidance on what to do with a positive ctDNA result if there is no clinically evident recurrence. Would regular testing intensify fear of recurrence and associated anxiety and affect overall health-related quality of life?” Yara Abdou MD


RBC
Macrophage
Normal Cell
Tumor Cell
CTC
Normal cfDNA
ctDNA
Plasma
Leukocyte
RBC
Apoptosis
Necrosis
Shedding

Dr. John Strickler – Cancer Connect

How can we stop resistance driving progression?

- New pathways in non-response biology: CARM1i (Tam Binh Bui)
- Avoid drug resistance: APOBEC3B (Temiz Pardo)
- Target steroid receptor signature (SRS) in TNBC (Julia Wulfhulke)

Monotherapy is not enough. How many targets must we hit to achieve a durable response and overcome tumor recurrence???

The power of synthetic lethality to overcome intrinsic drug resistance using drug combinations: "..such resistance to combination therapies may become less common when these combinations are ***used earlier in disease progression when tumor heterogeneity is lower****." Nature Reviews Drug Discovery*

Hitting all the right targets?