Breast Cancer Session

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THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

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FOR CLINICAL TRIALS IN ONCOLOGY

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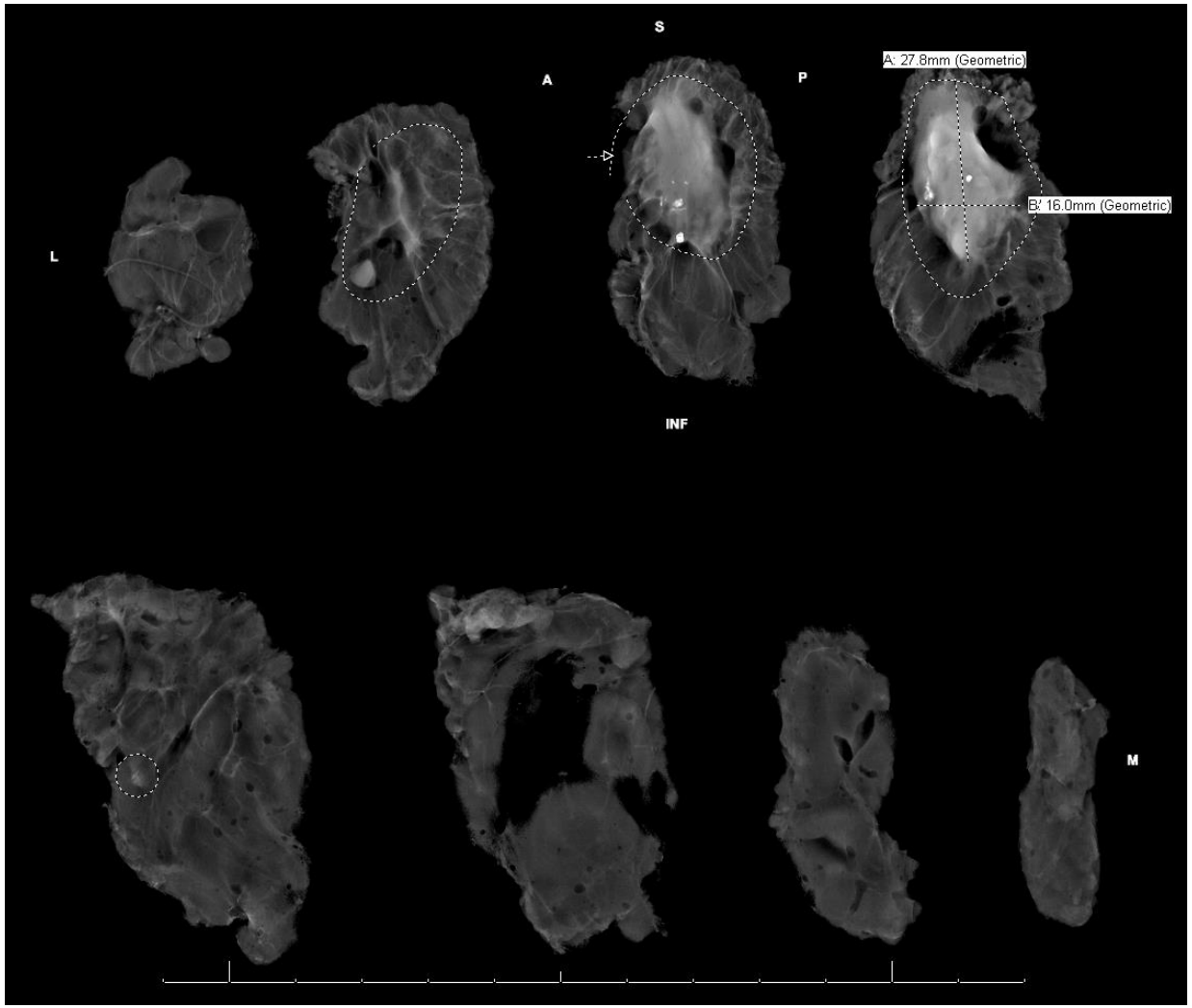
AbbVie	**Travel expenses to speak at meeting**
Luminex	**Travel expenses to speak at meeting**
Merck	**Travel expenses to speak at meeting**

How we assess a lumpectomy breast resection specimen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B: 16.0mm (Geometric)
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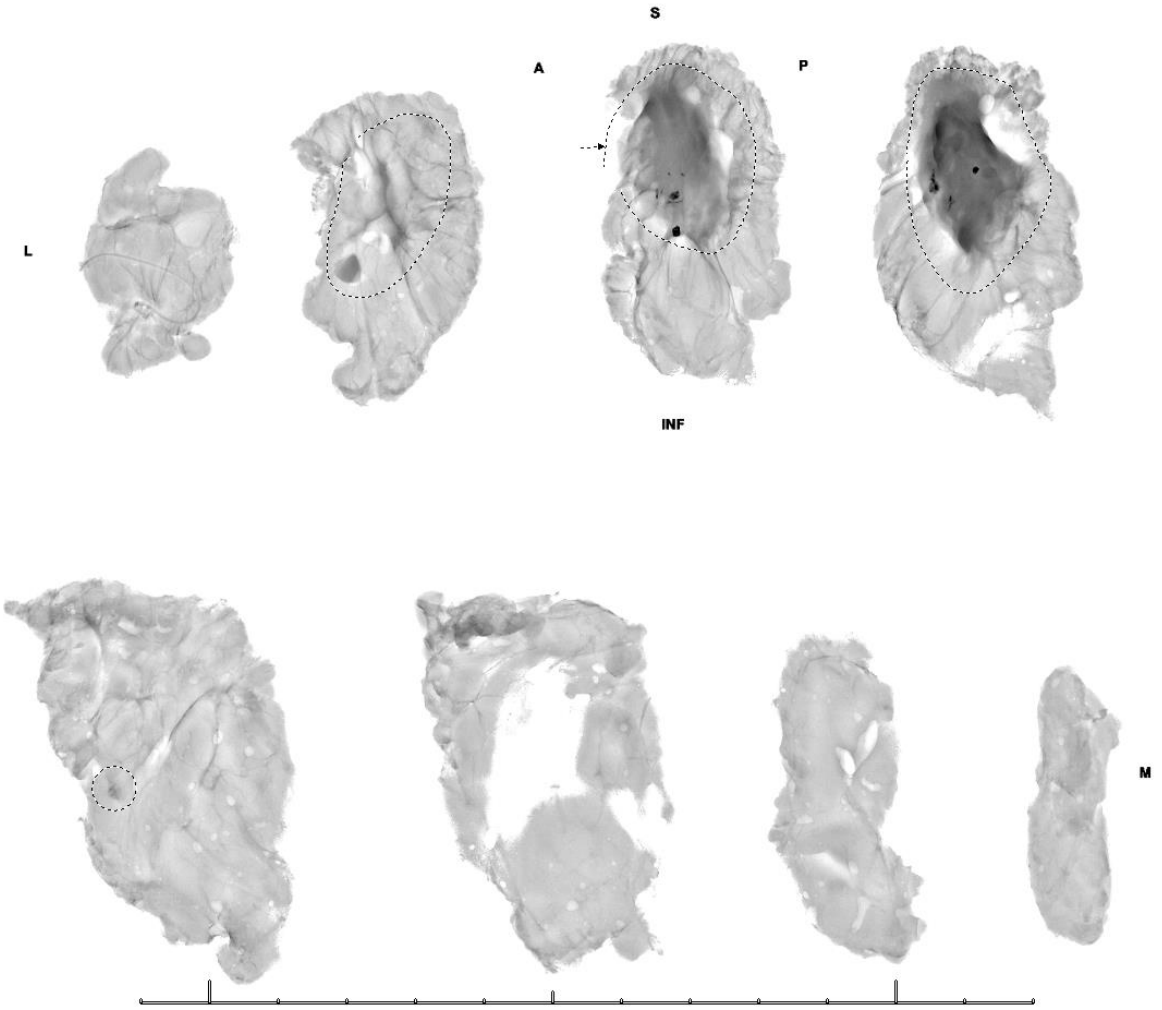
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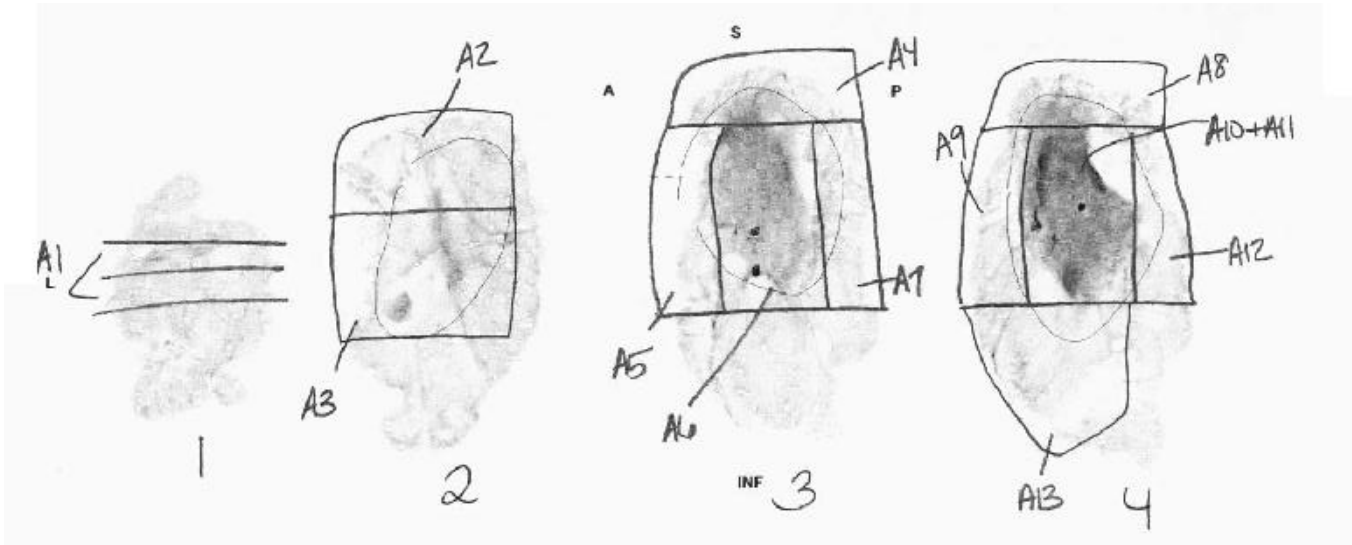
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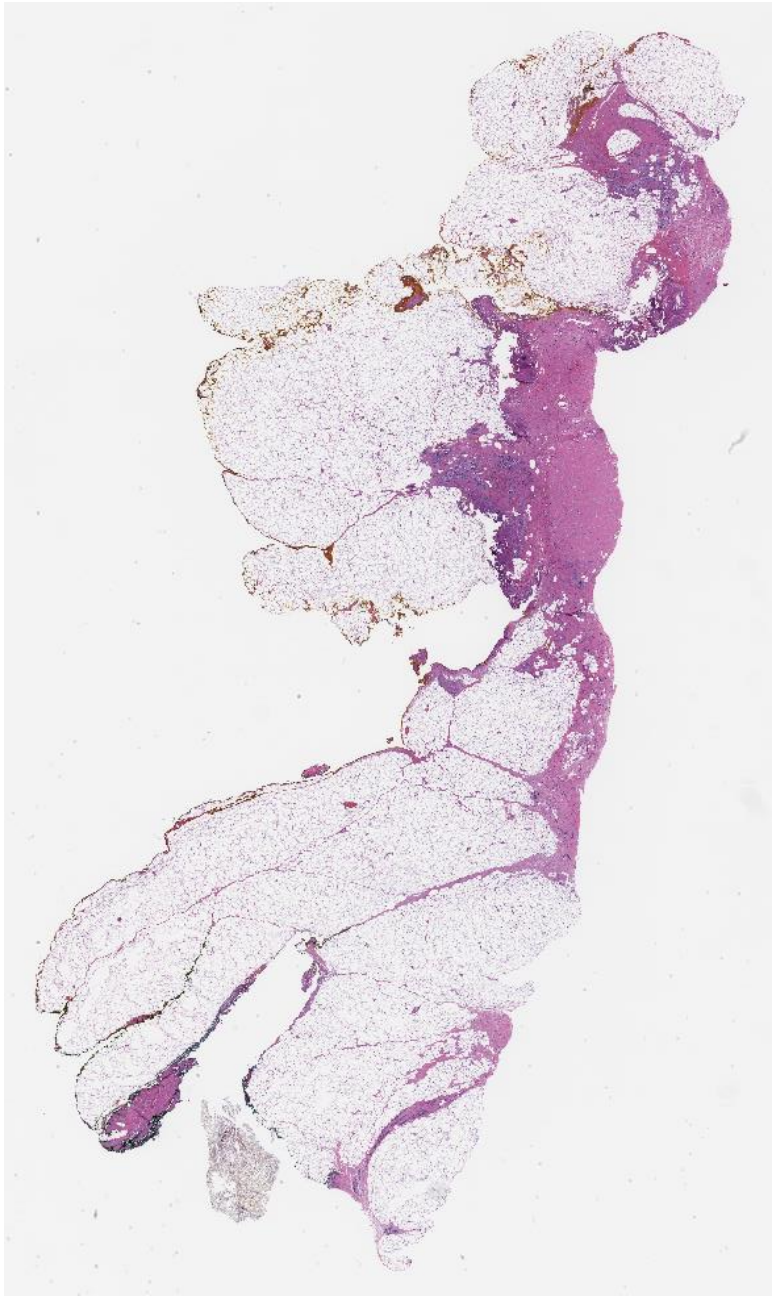

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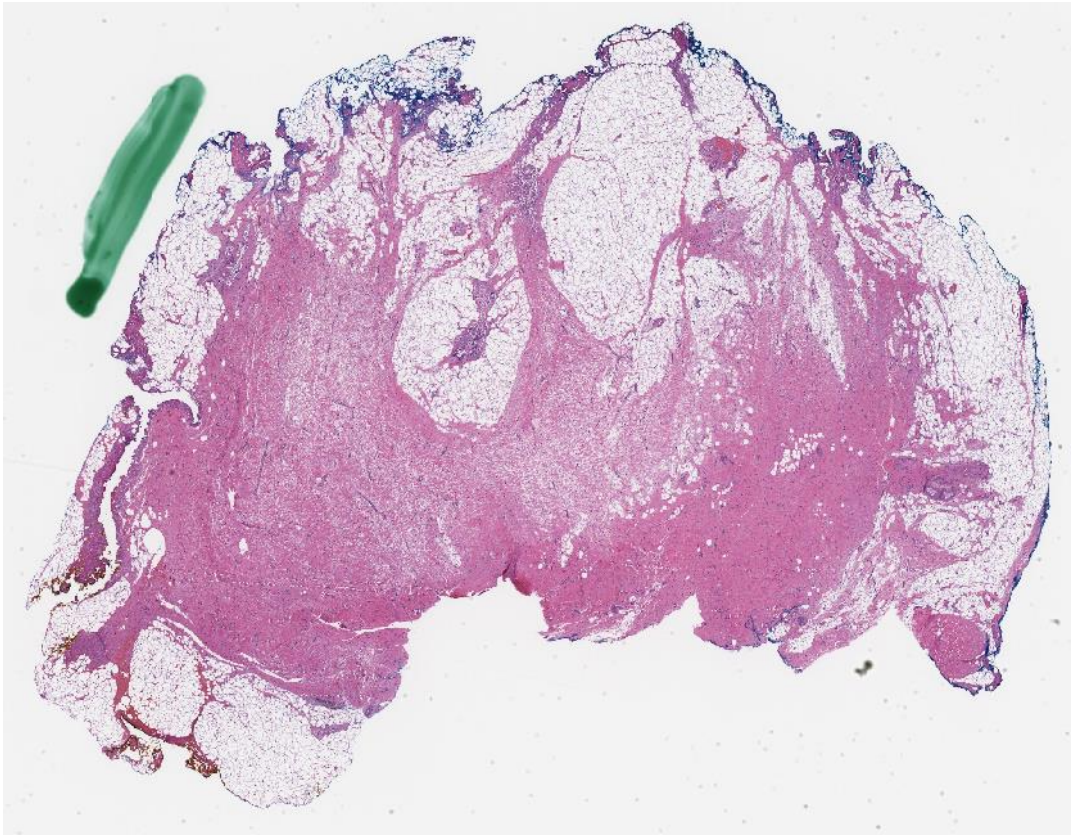

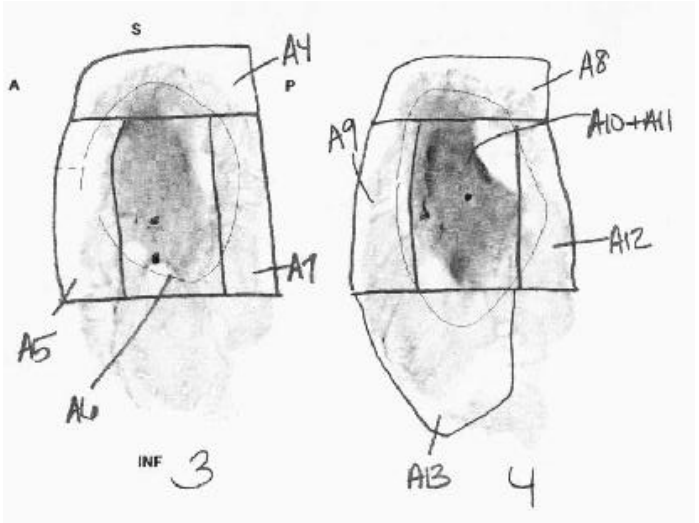

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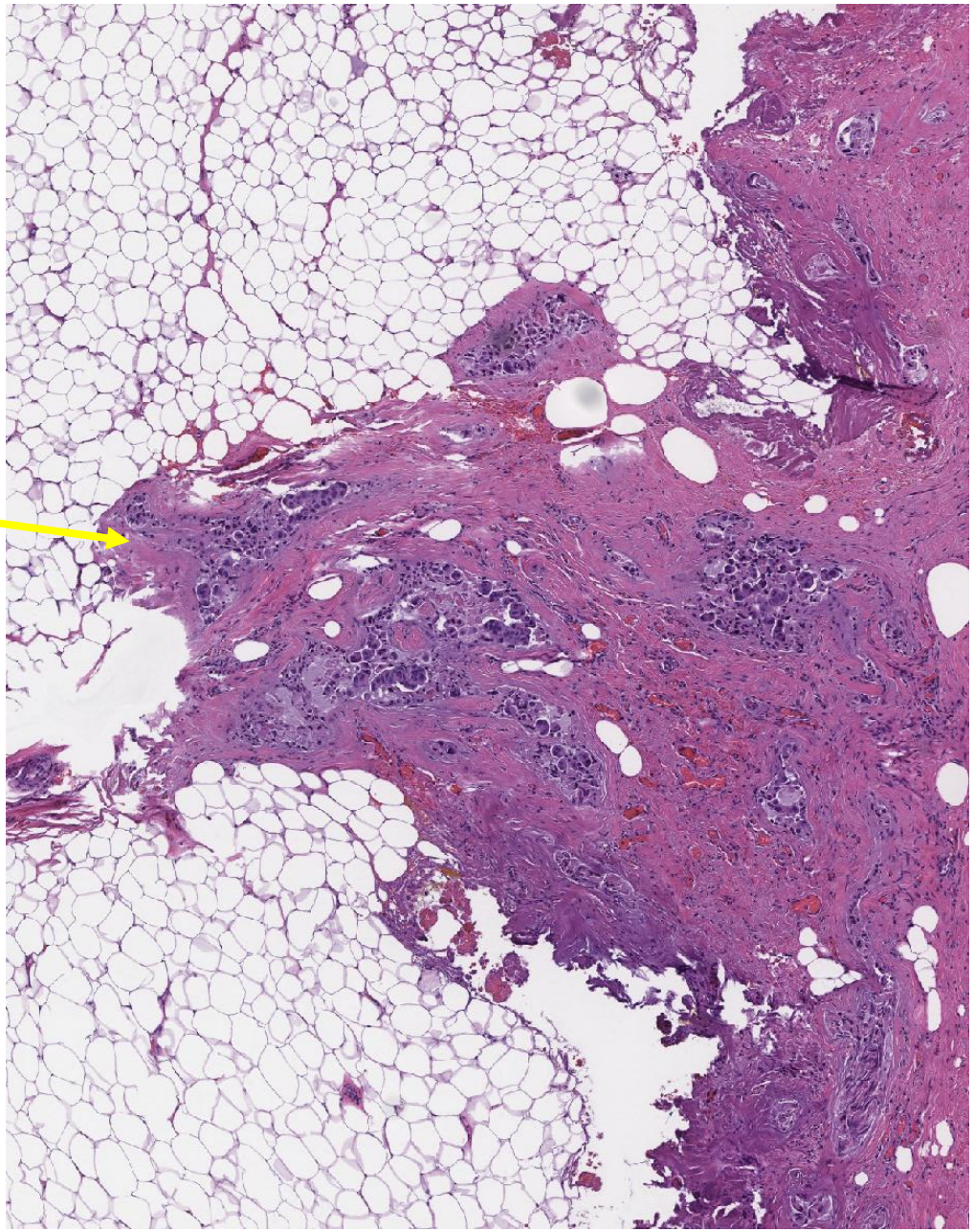

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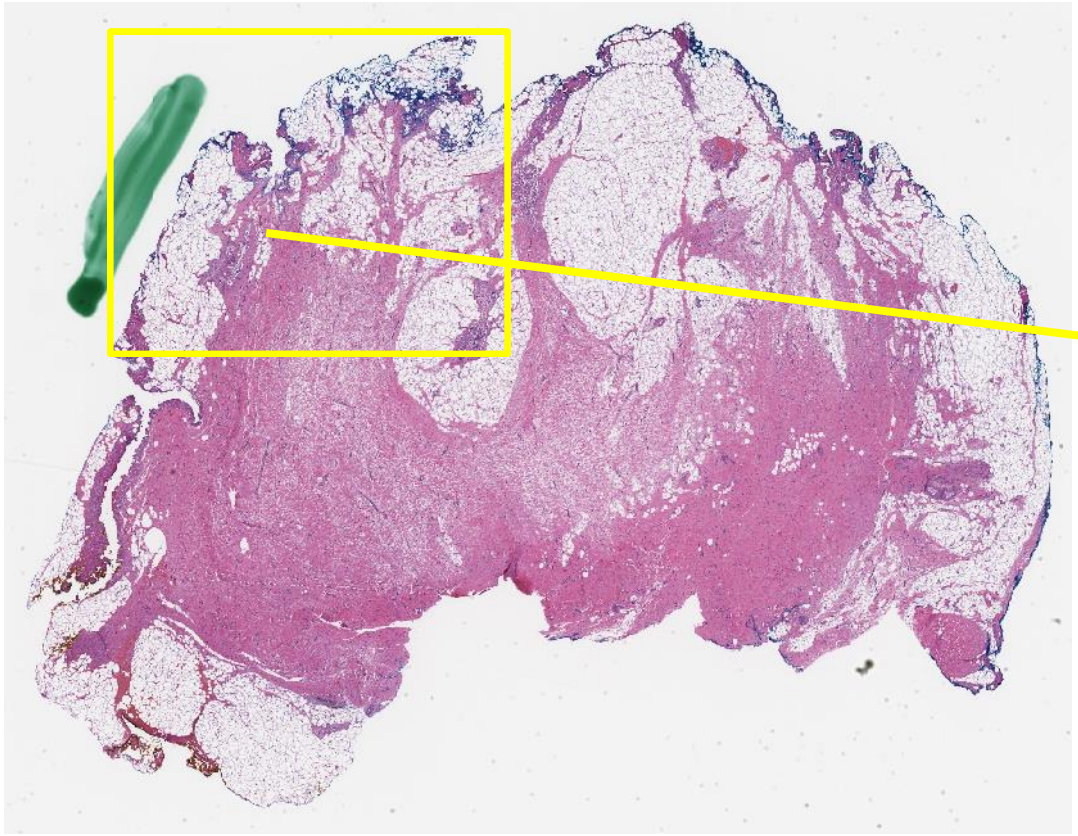
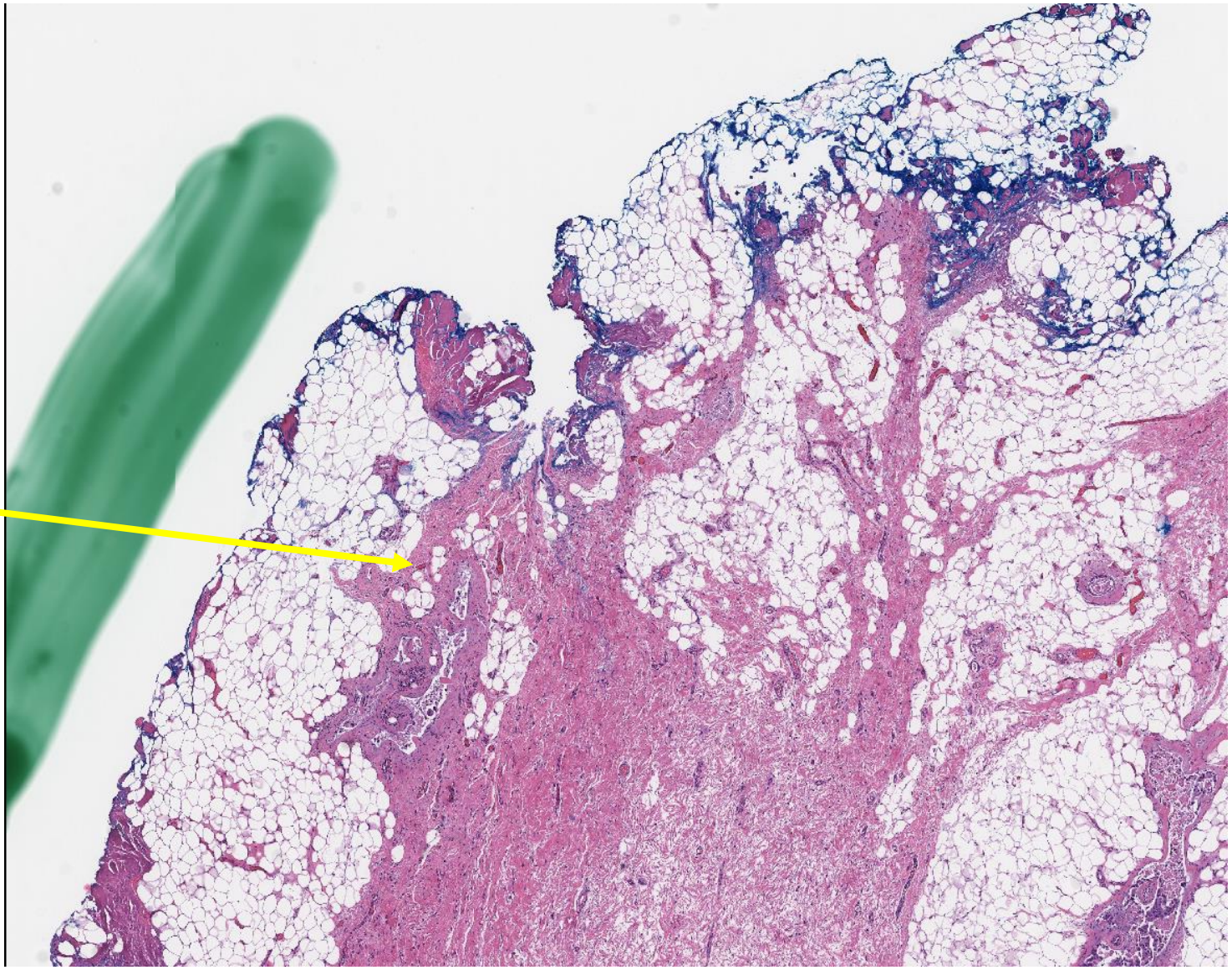




How we assess a mastectomy specimen for a non-palpable disease

Case: Pathologist's Preparatory Notes

49 year old with multifocal HER2+ left breast cancer (T2, N1)

Imaging

A) Tumor at 6 o'clock, 6 cm from nipple (biopsy + clip)
B) Tumor at 5 o'clock (anterior), 1 cm from nipple (biopsy + clip)
C) Intervening tumor 1.3 cm at 5 o'clock, 5 cm from nipple
D) Tumor 0.6 cm at 5 o'clock, 1.7 cm lateral to B).

Ultrasound showed one 2 cm axillary node with enlarged cortex

Pathology From Biopsy

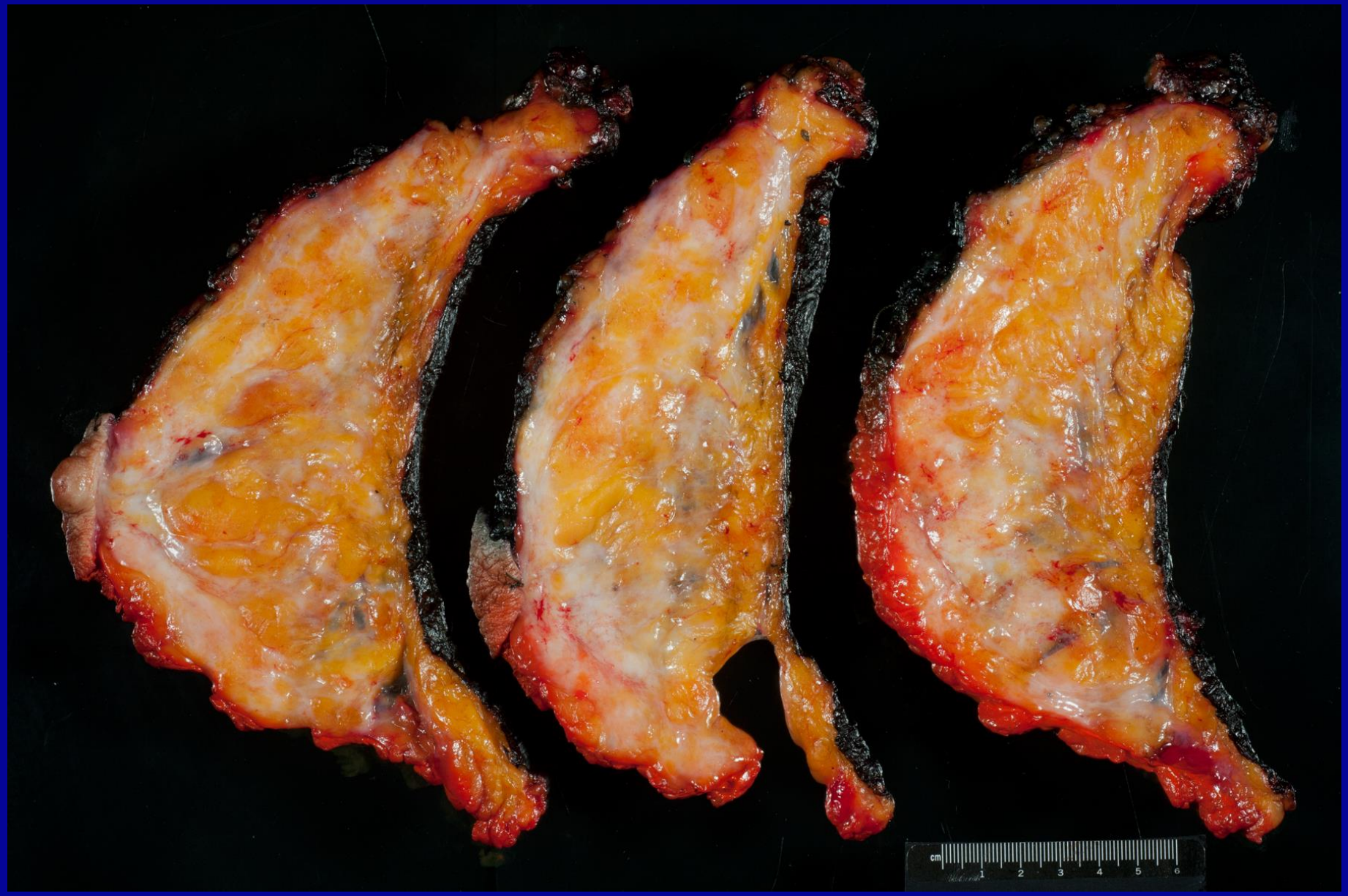
IDC grade 3, HR+ / HER2- in tumors A) and B)
Metastatic breast cancer in LN, HR+, HER2+

Treatment & Response

3-weekly FEC x 4 then weekly paclitaxel x 12 with concurrent trastuzumab throughout (FEC-H/T-H)

Pre-surgical MRI = complete response

For skin-sparing mastectomy and axillary node dissection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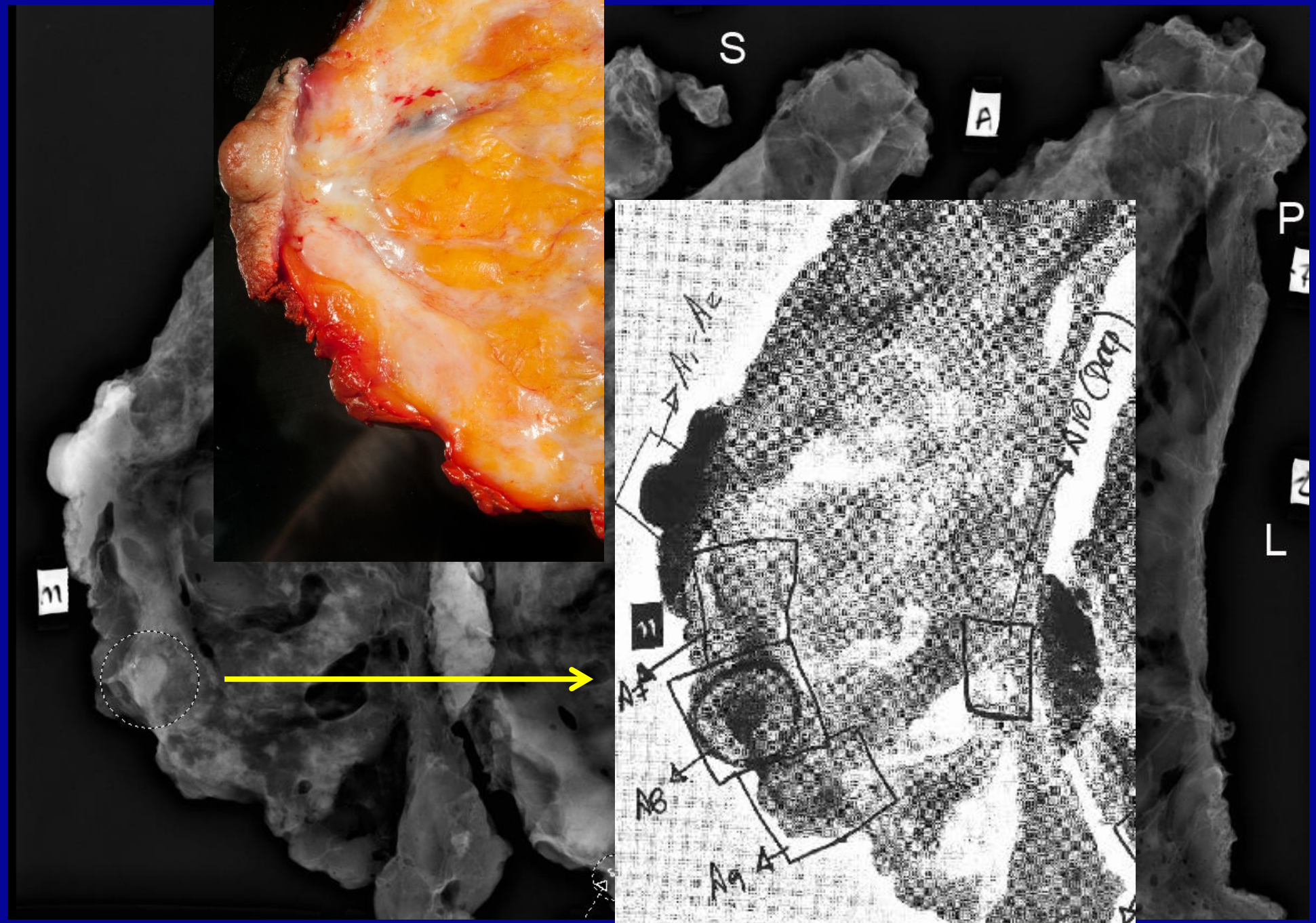
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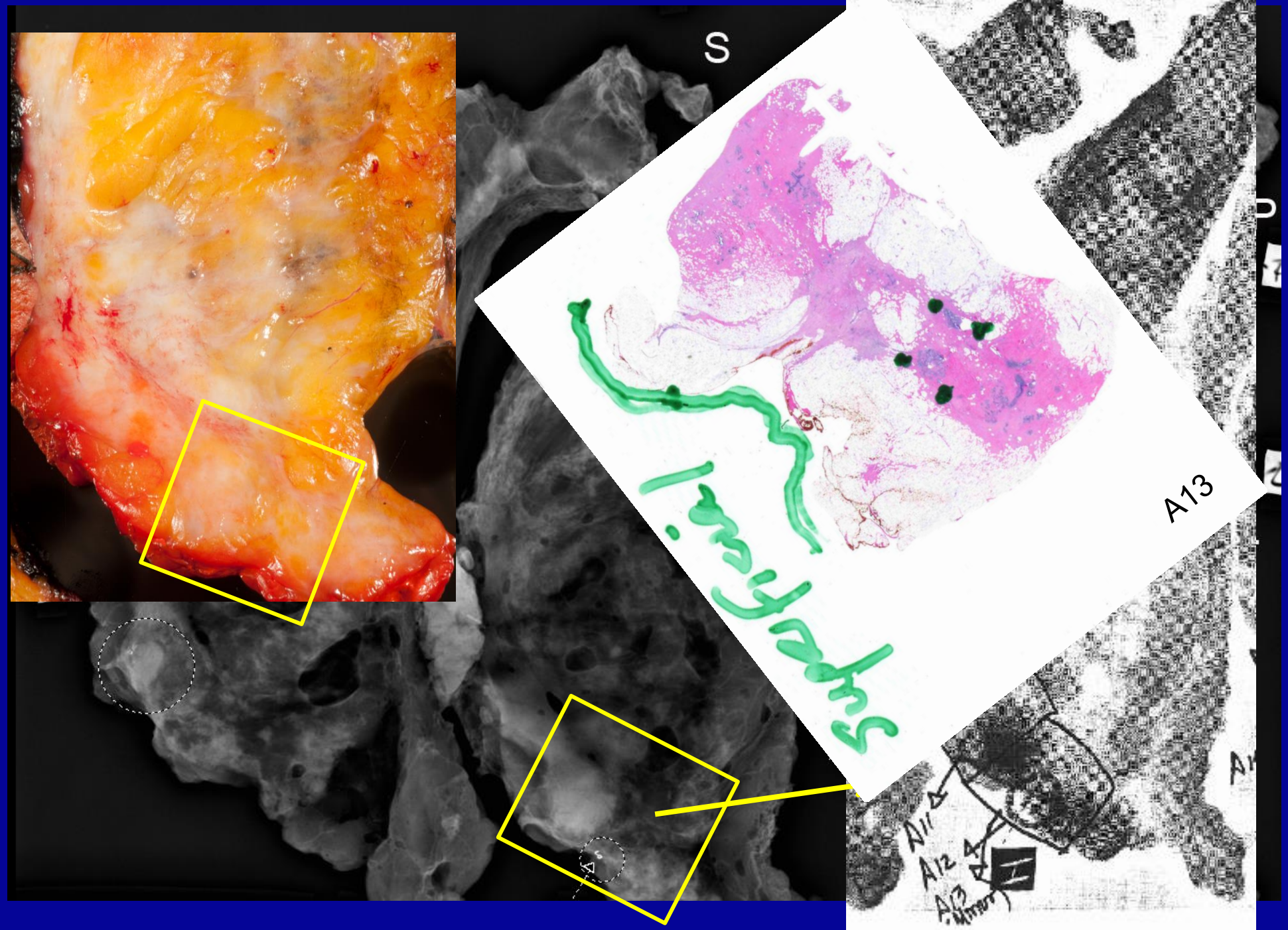
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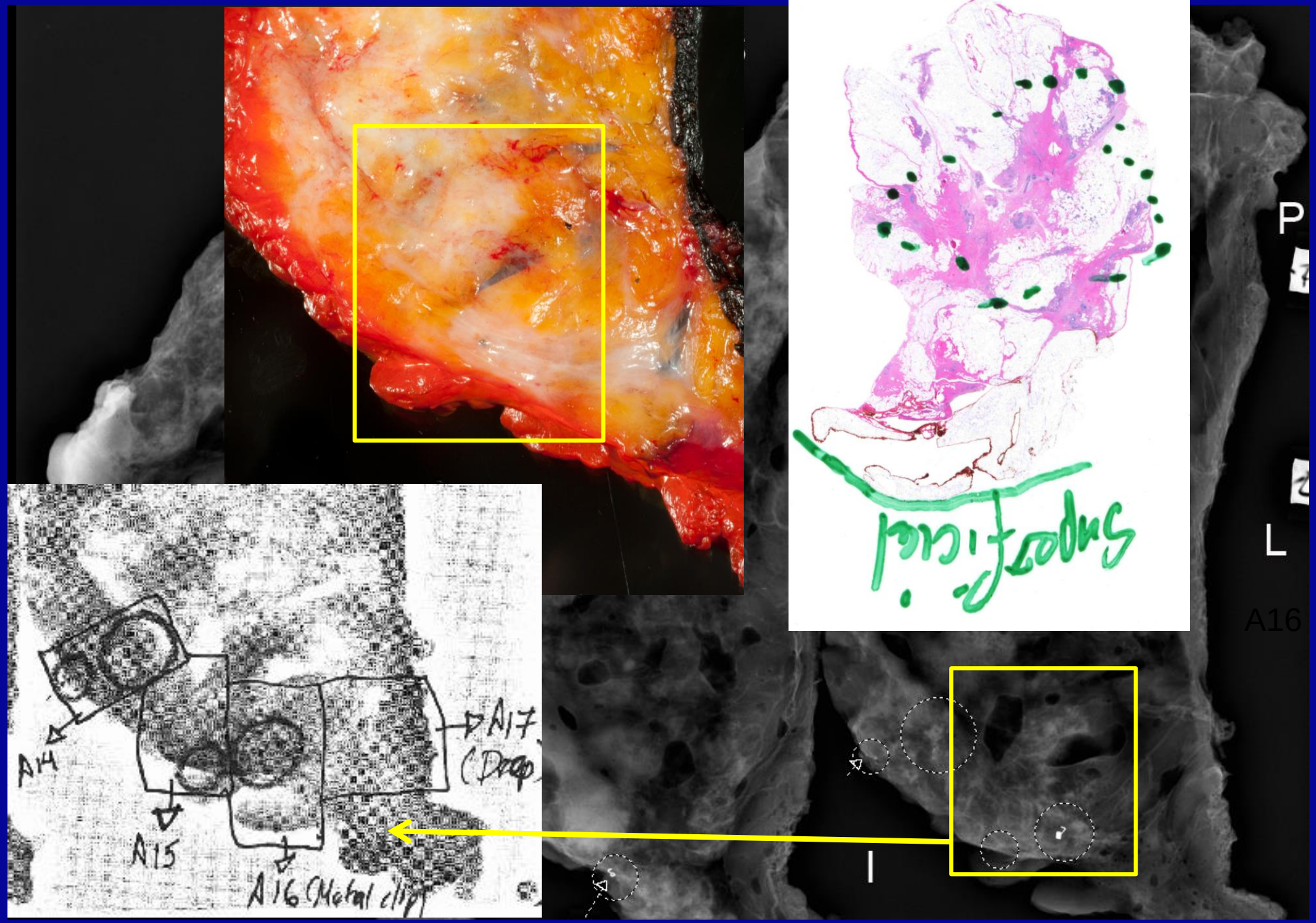
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A16 (Metal clip)
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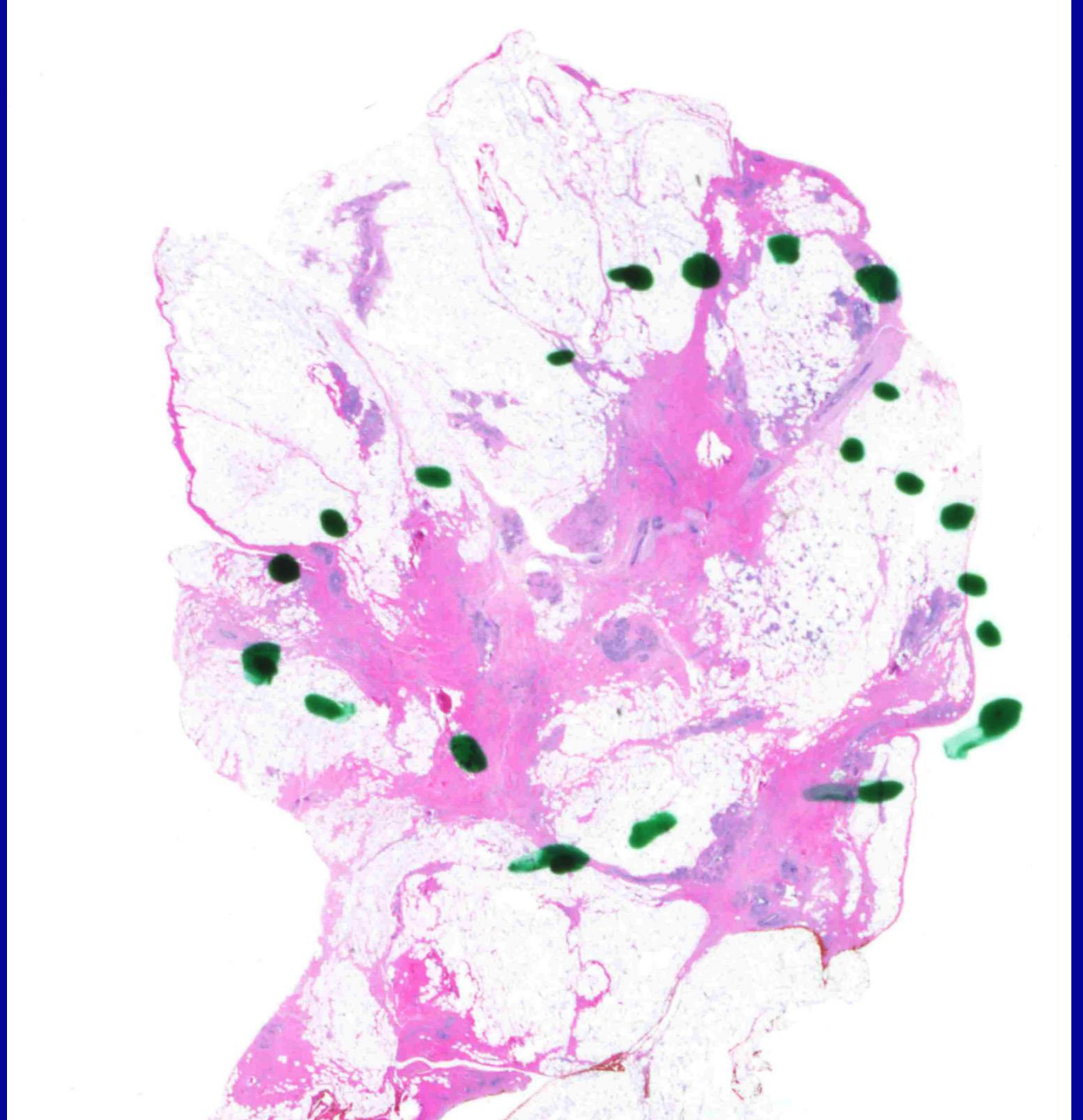

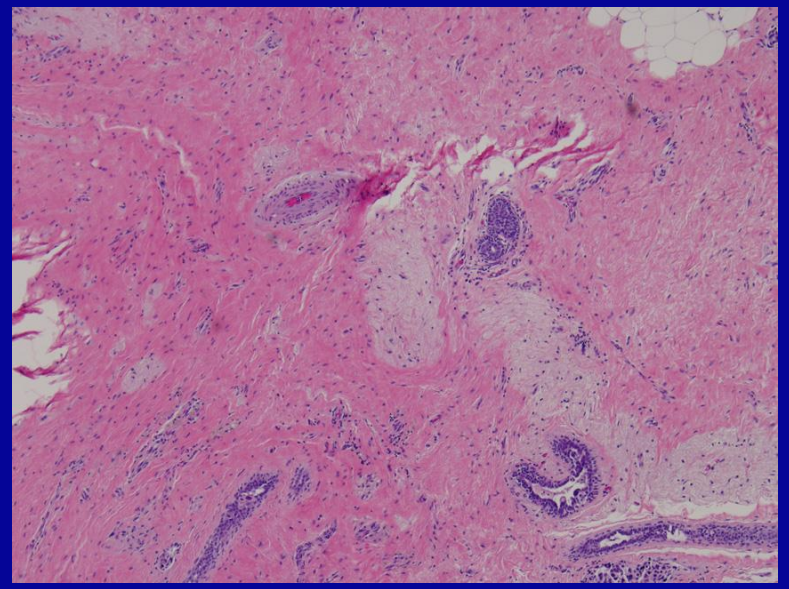

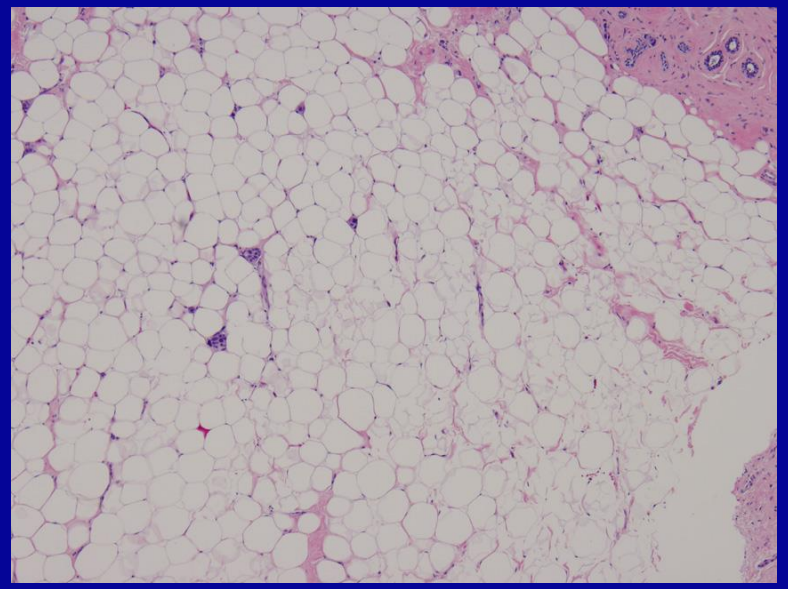

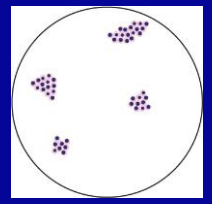

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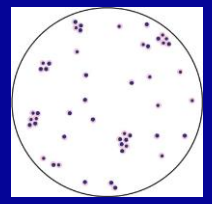

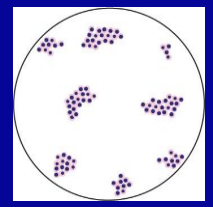

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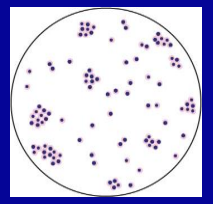
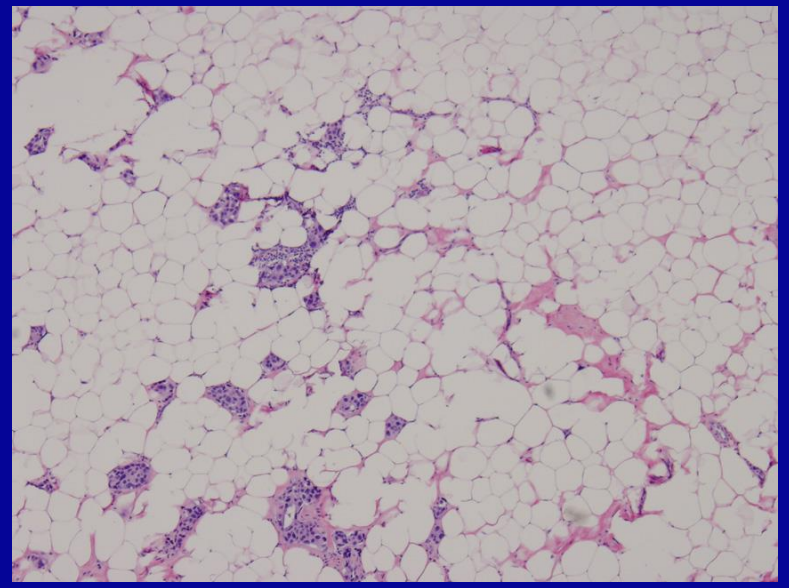
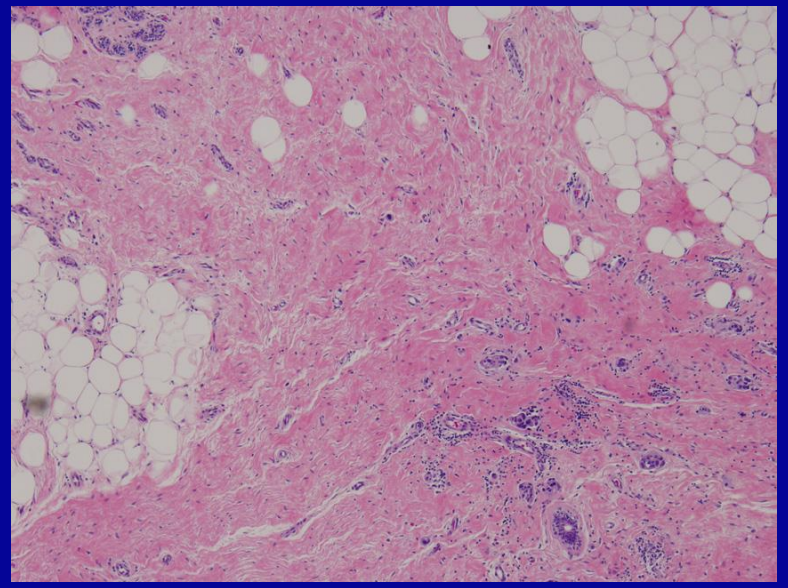
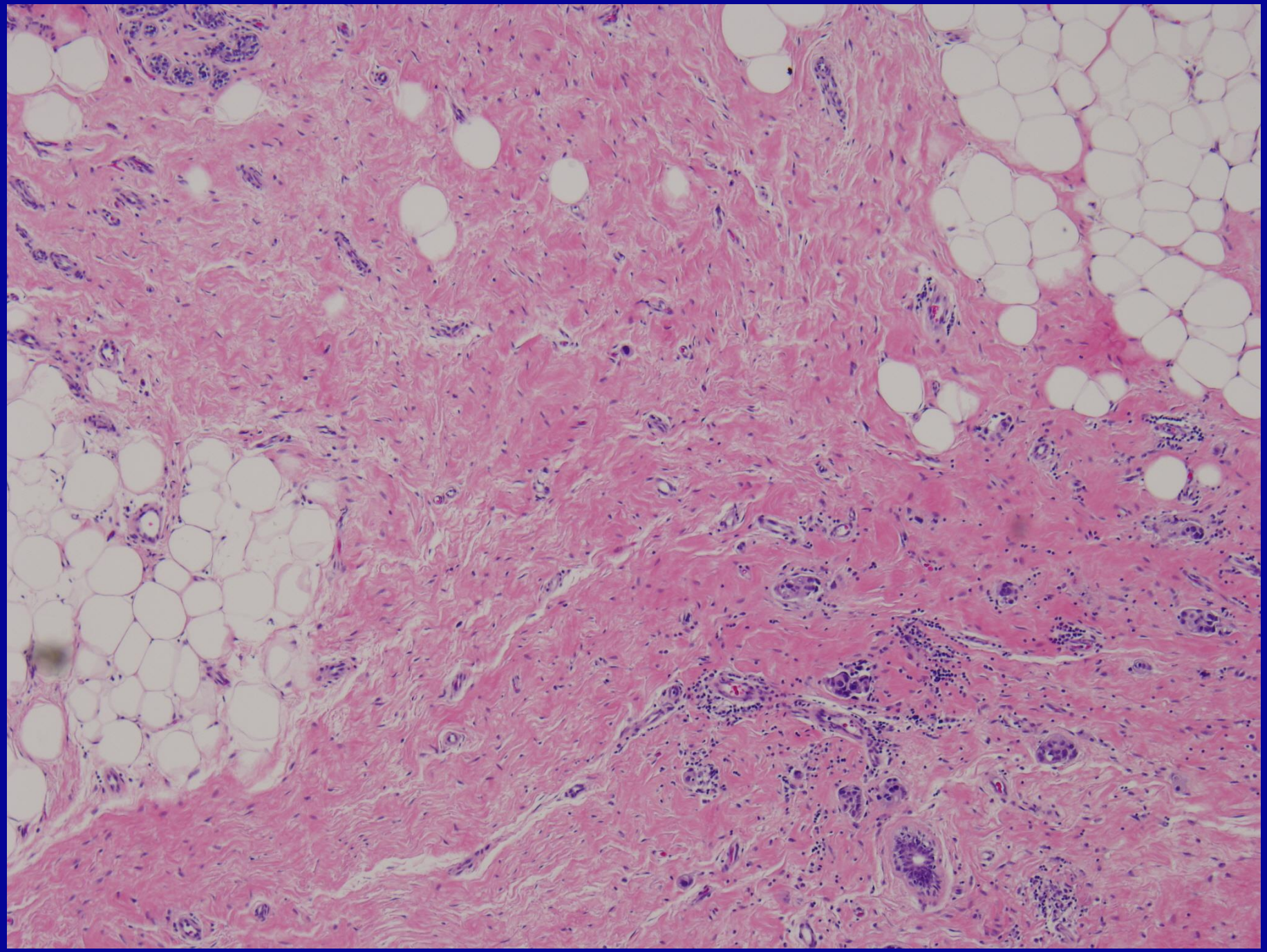
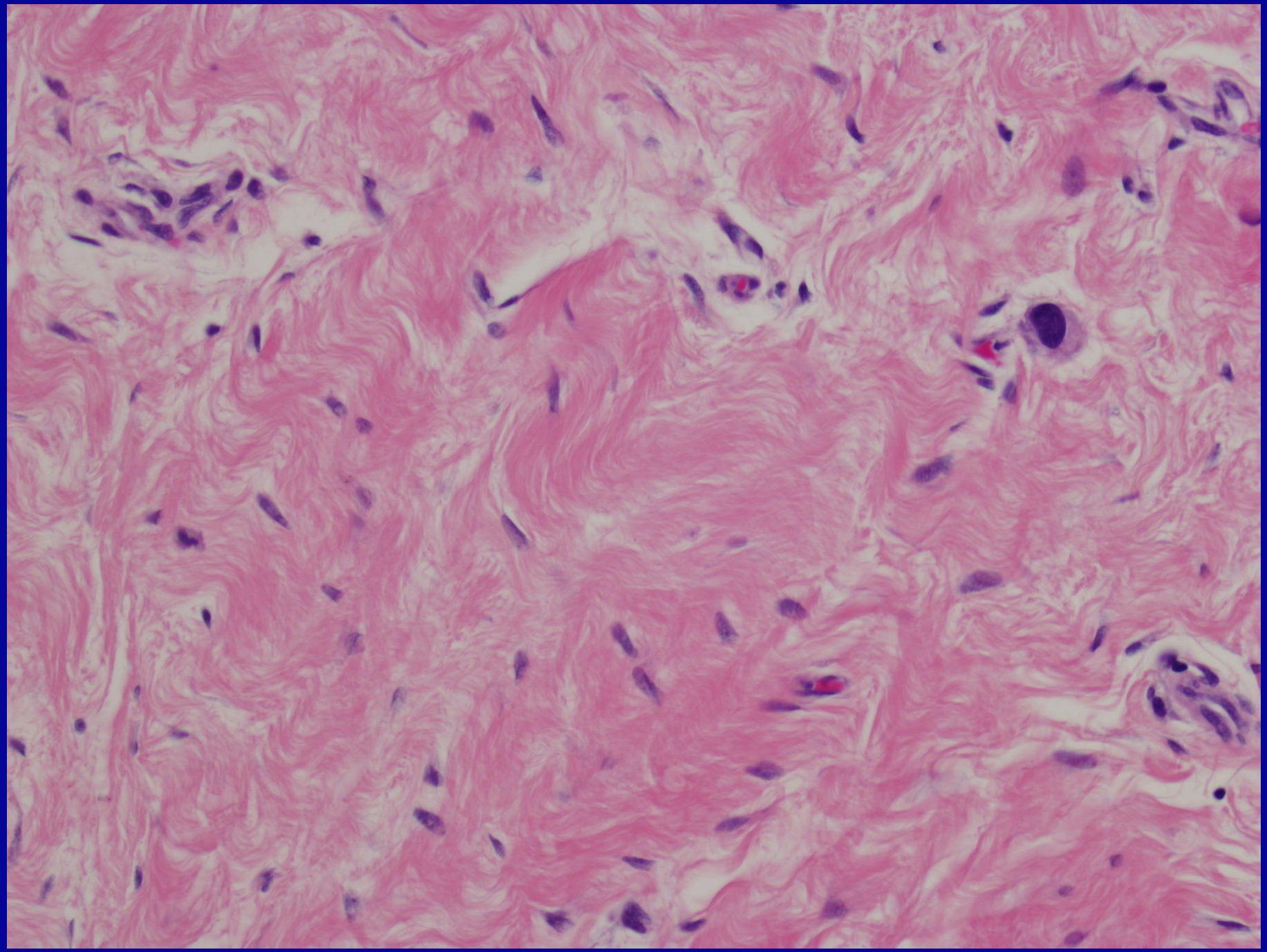
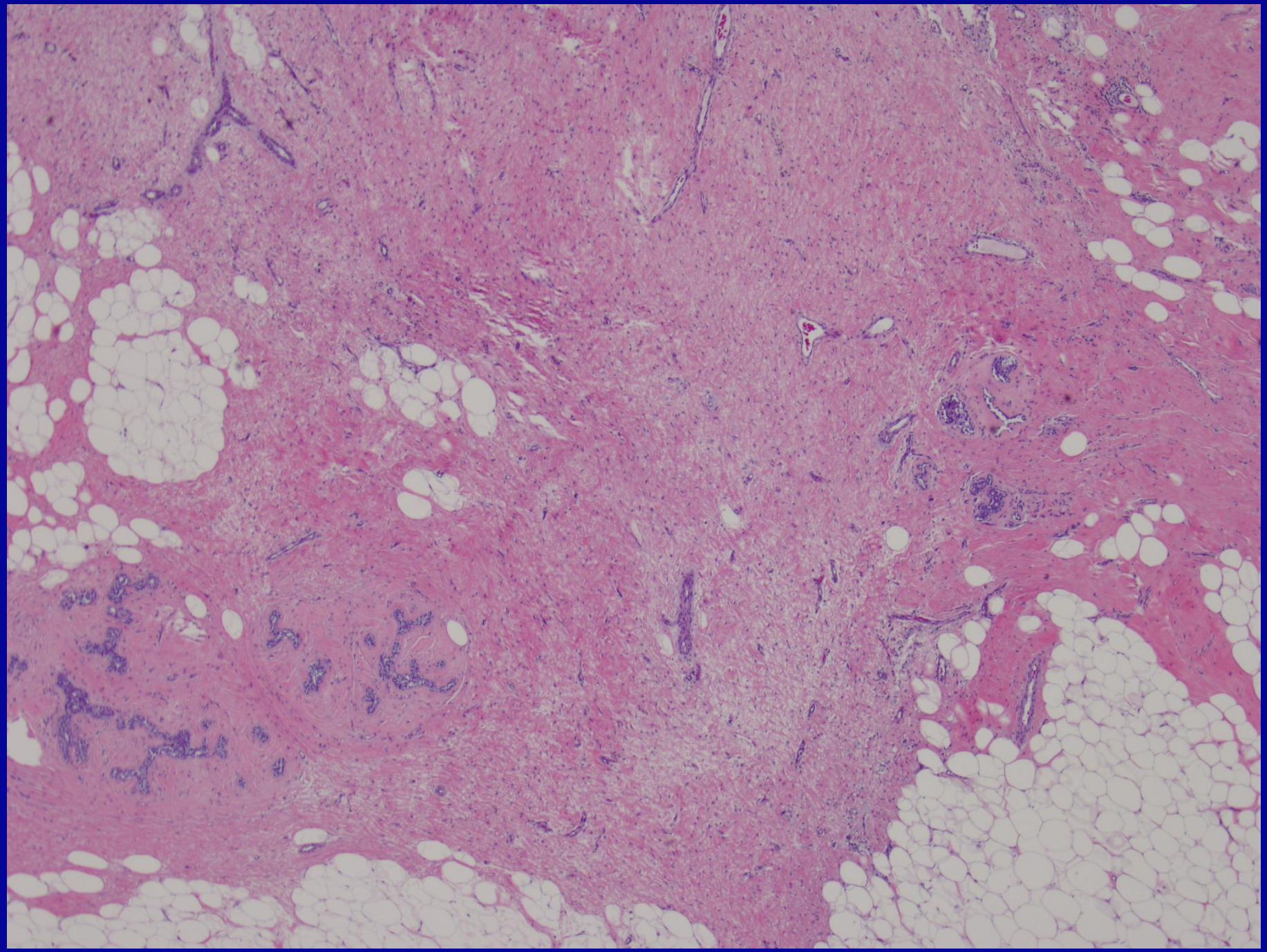


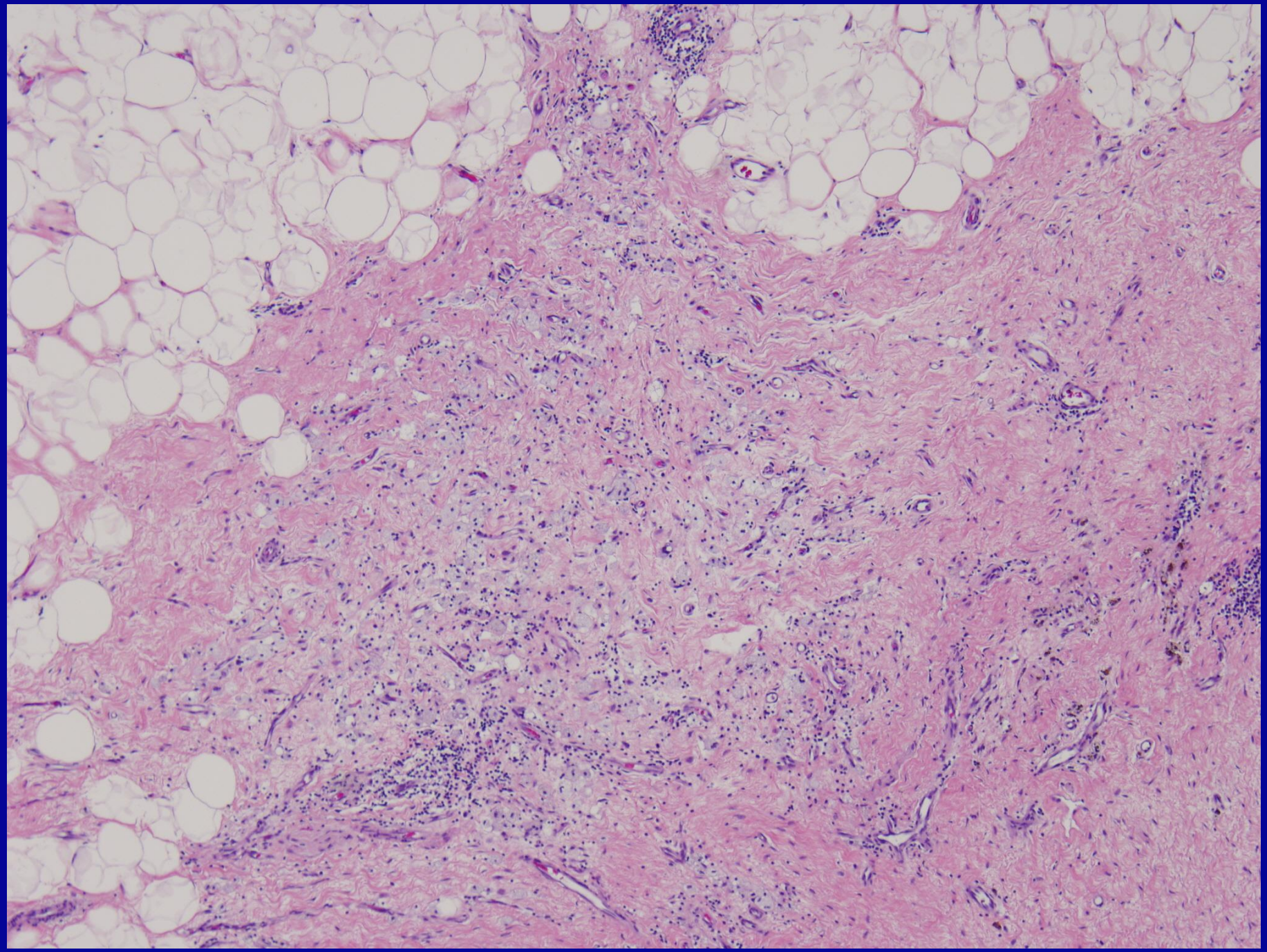


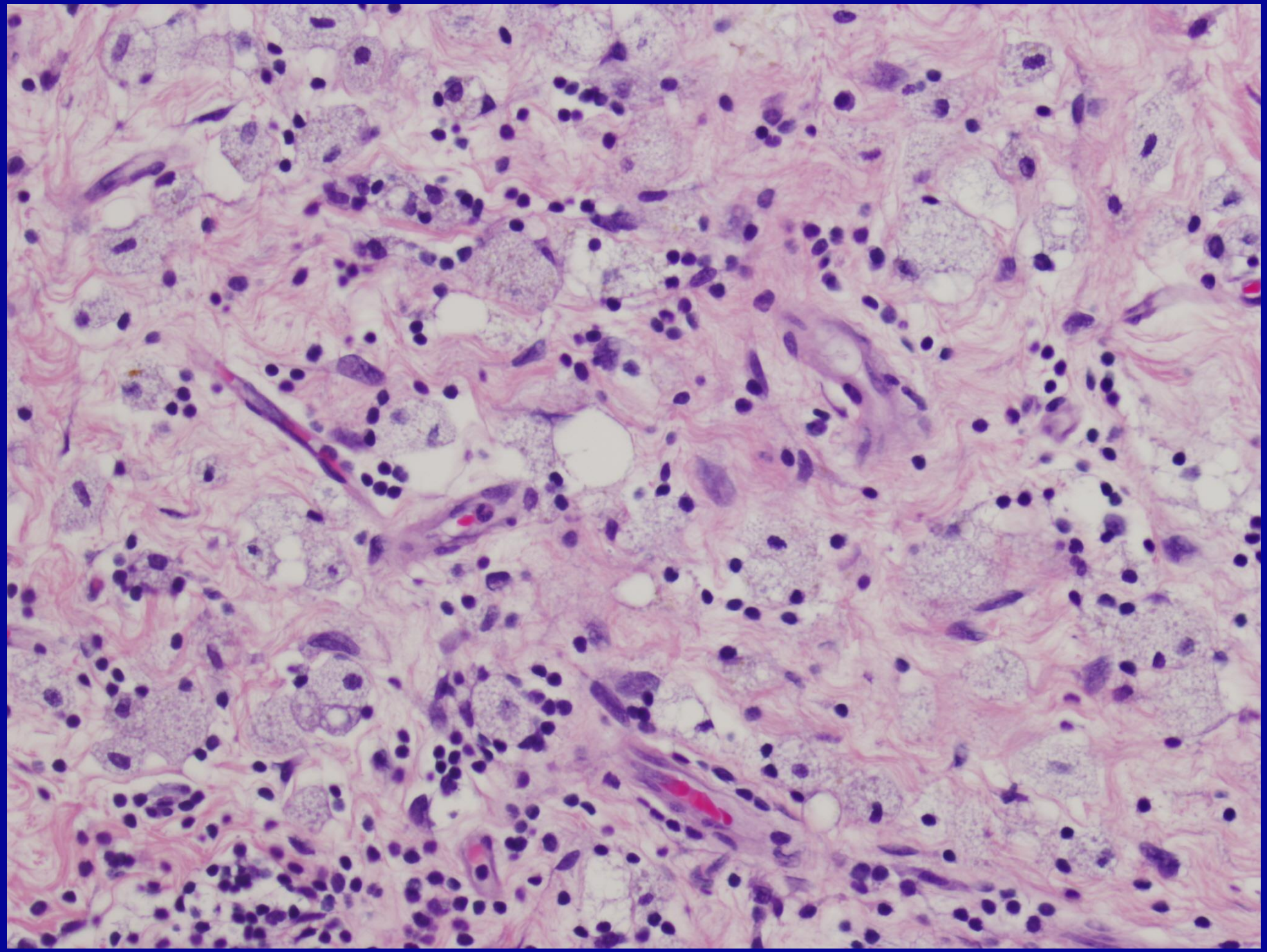












Resections: Before We Start

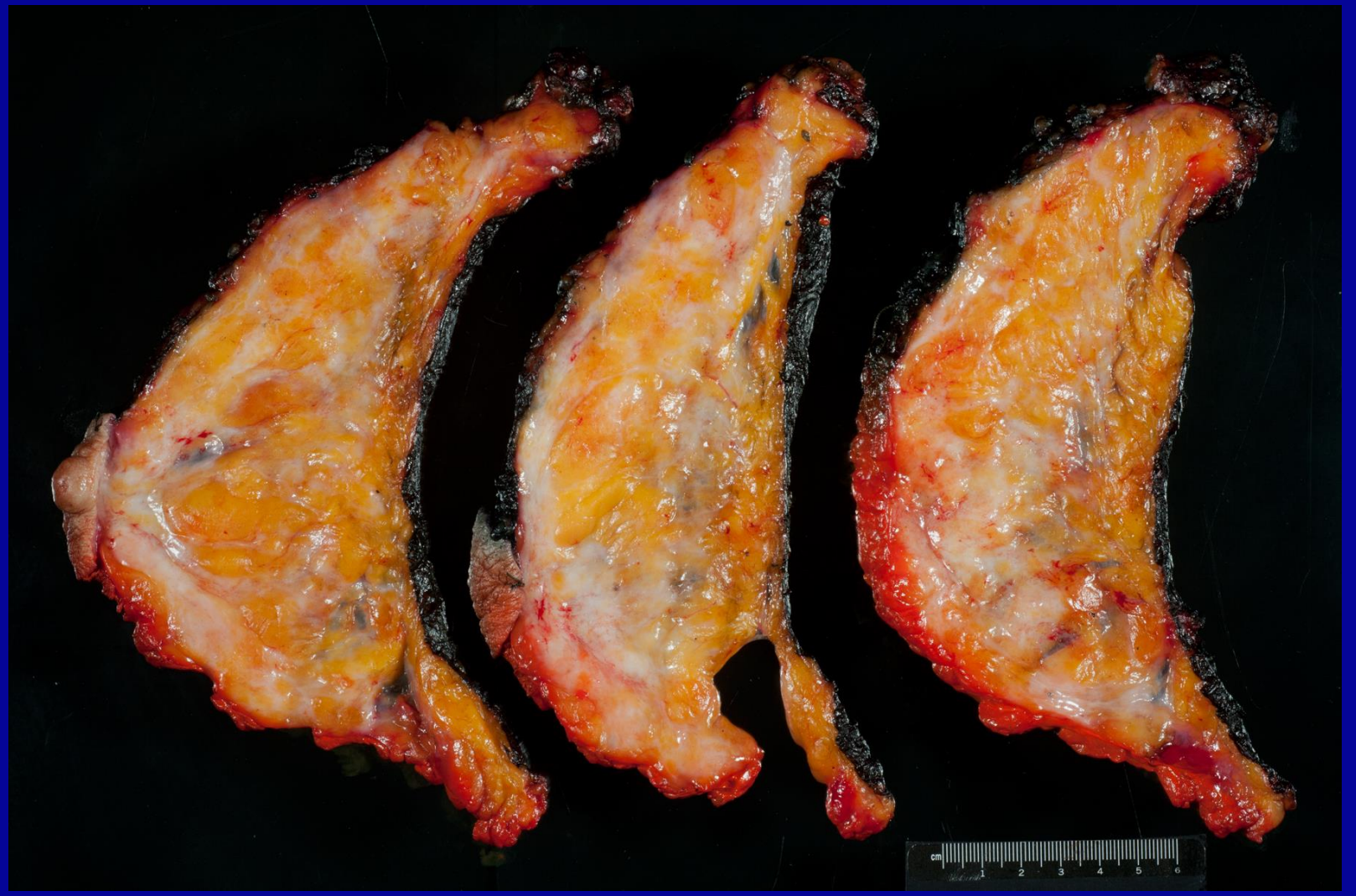
- Clinical summary
  - Pathology requisition form
  - Direct communication with pathologist
  - Electronic medical record
- Knowledge of the pre-surgical pathology findings
  - Report
  - Digital image

Localization and Extent

- Medical records
- Clips, coils, seeds, wires, tatoos, …
- Specimen radiography
- Sliced specimen radiography
- Section maps
- Digital measurement tools

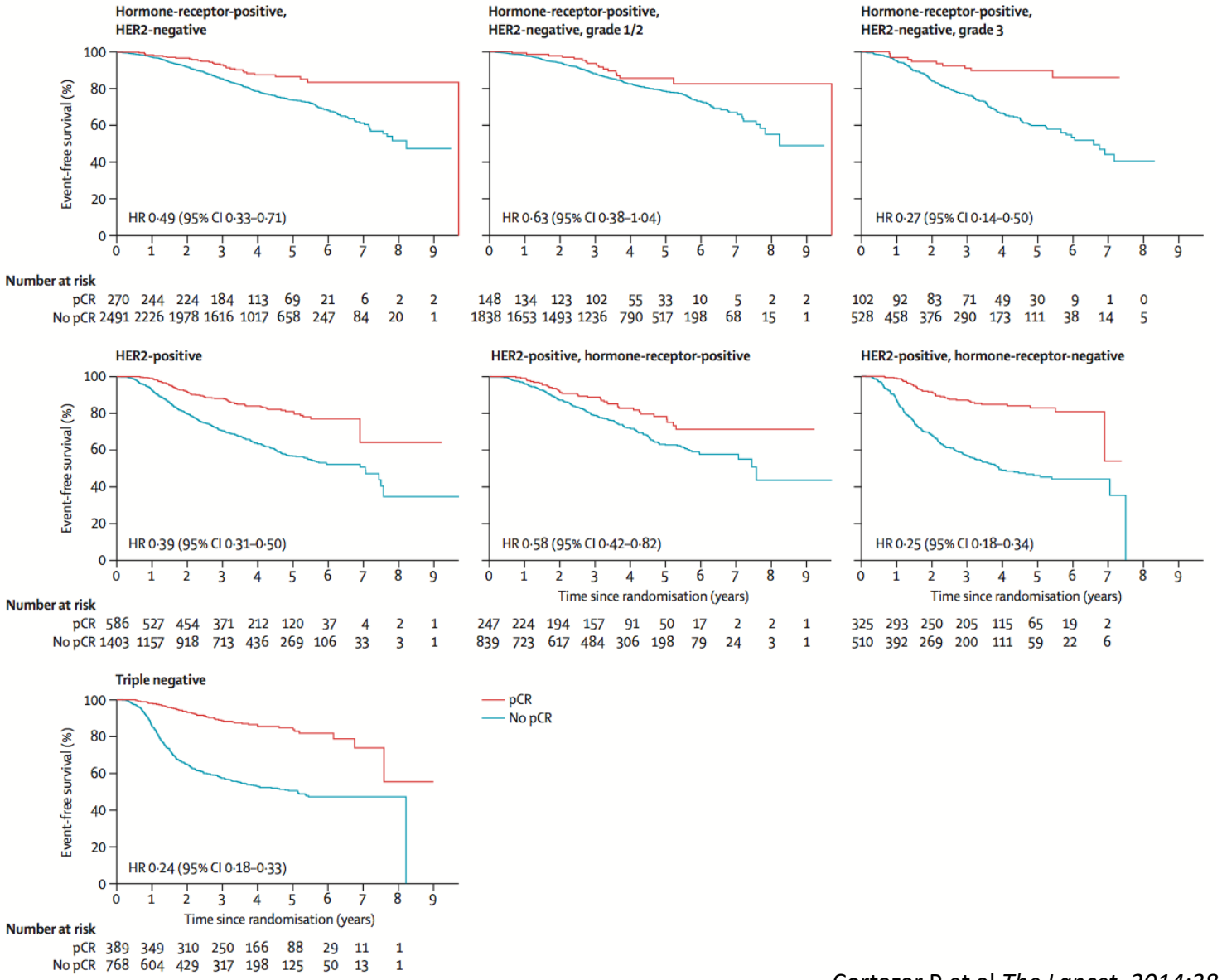
Response to Chemotherapy

The 3 Informative Slices Of The 13 Slices From The Mastectomy After Neoadjuvant Chemotherapy



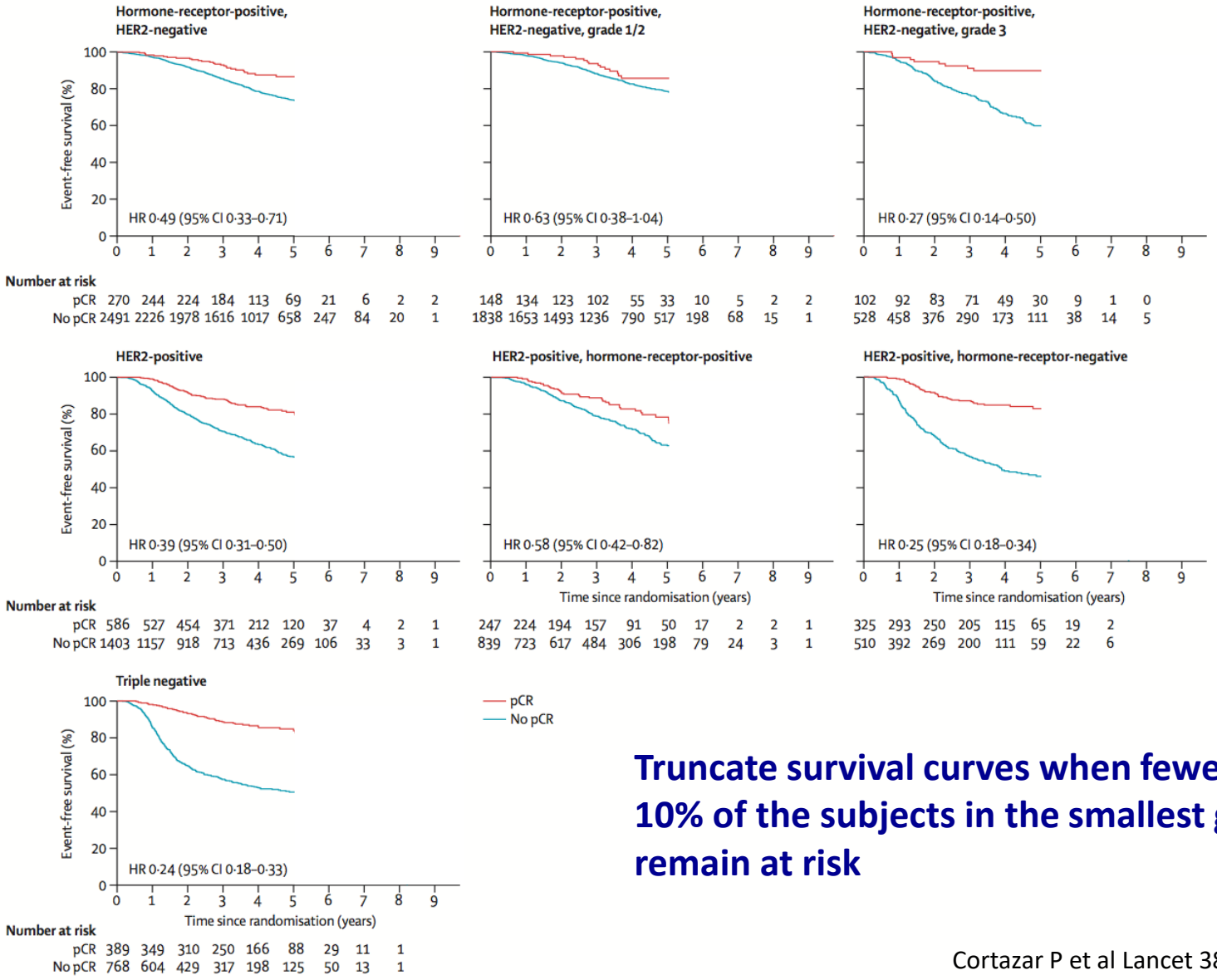
Can you imagine a primary endpoint for clinical trials that is defined by absence of disease, but relies on preferences of local sites to identify and sample the correct area within each resection specimen?

Pathologic Complete Response (pCR) indicates good prognosis



Cortazar P et al *The Lancet. 2014;384:164-72*

Pathologic Complete Response (pCR) indicates good prognosis



Truncate survival curves when fewer than 10% of the subjects in the smallest group remain at risk

Cortazar P et al Lancet 384:164-72, 2014

Pocock SJ, Clayton TC, Altman DG: Survival plots of time-to-event outcomes in clinical trials: good practice and pitfalls. Lancet 359:1686-9, 2002

LEFT BREAST, SKIN-SPARING MASTECTOMY:

RESIDUAL INVASIVE DUCTAL CARCINOMA WITH TREATMENT EFFECT.
INVASIVE CARCINOMA MEASURES 1.3 X 1.3 CM AND CONTAINS APPROXIMATELY 5% CANCER CELLULARITY BY AREA.
FOCAL LYMPHOVASCULAR INVASION IS PRESENT.
SCATTERED SMALL FOCI OF INTRADUCTAL CARCINOMA (DCIS) SURROUNDING INVASIVE CARCINOMA AND ADJACENT TO PRIOR BIOPSY SITES.
DCIS IS PRESENT 3 MM FROM INFERIOR SUPERFICIAL MARGIN.
INVASIVE CARCINOMA IS PRESENT AT LEAST 3 MM FROM INFERIOR SUPERFICIAL MARGIN.
MICROCALCIFICATIONS ASSOCIATED WITH DCIS AND BENIGN BREAST TISSUE.

LYMPH NODES, LEFT AXILLA, LEVELS I AND II, DISSECTION:

Fourteen lymph nodes, no carcinoma identified (0/14).

LEFT BREAST, NEW INFERIOR MARGIN, EXCISION:

No tumor present.

LEFT BREAST, NEW INFERIOR LATERAL MARGIN, EXCISION:

No tumor present.

COMMENTS: There was no residual invasive carcinoma at the sites of the clips. Pathologic AJCC Stage y-pT1c, y-pN0. Residual disease with pathologic findings c/w RCB-I.

Recommendations for standardized pathological characterization of residual disease for neoadjuvant clinical trials of breast cancer by the BIG-NABCG collaboration

V. Bossuyt[1*], E. Provenzano[2], W. F. Symmans[3], J. C. Boughey[4], C. Coles[5], G. Curigliano[6], J. M. Dixon[7], L. J. Esserman[8], G. Fastner[9], T. Kuehn[10], F. Peintinger[11,12], G. von Minckwitz[13], J. White[14], W. Yang[15], S. Badve[16], C. Denkert[17], G. MacGrogan[18], F. Penault-Llorca[19], G. Viale[20] & D. Cameron[21] of the Breast International Group-North American Breast Cancer Group (BIG-NABCG) collaboration

Annals of Oncology 00: 1–12, 2015 doi:10.1093/annonc/mdv161

Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group

Elena Provenzano[1], Veerle Bossuyt[2], Giuseppe Viale[3], David Cameron[4], Sunil Badve[5], Carsten Denkert[6], Gaëtan MacGrogan[7], Frédérique Penault-Llorca[8], Judy Boughey[9], Giuseppe Curigliano[10], J Michael Dixon[11], Laura Esserman[12], Gerd Fastner[13], Thorsten Kuehn[14], Florentia Peintinger[15,16], Gunter von Minckwitz[17], Julia White[18], Wei Yang[19] and W Fraser Symmans[20] on behalf of the Residual Disease Characterization Working Group of the Breast International Group-North American Breast Cancer Group (BIG-NABCG) collaboration

Modern Pathol 00: 1–17, 2015 doi:10.1038/modpathol.2015.74

Summary of Recommendations

Mandate of this working group committee was limited to recommendations for clinical trials

Provide the following information:

1. pCR (ypT0 ypN0 and ypT0/is ypN0) *versus* residual disease,
2. ypT and ypN Stage using the current AJCC/UICC staging system, &
3. Residual cancer burden (RCB)

A single standardized approach to macroscopic and microscopic pathologic examination makes it easy to reliably provide all 3 results!

Annals of Oncology 00: 1–12, 2015 doi:10.1093/annonc/mdv161

Modern Pathol 00: 1–17, 2015 doi:10.1038/modpathol.2015.74

Post-treatment yp-Stage (AJCC)

8th Edition AJCC (2017) Summary of Changes

Categories of *yp*-Stage are the same as for *p*-Stage

Primary Tumor: The Expert Panel clarified that ….

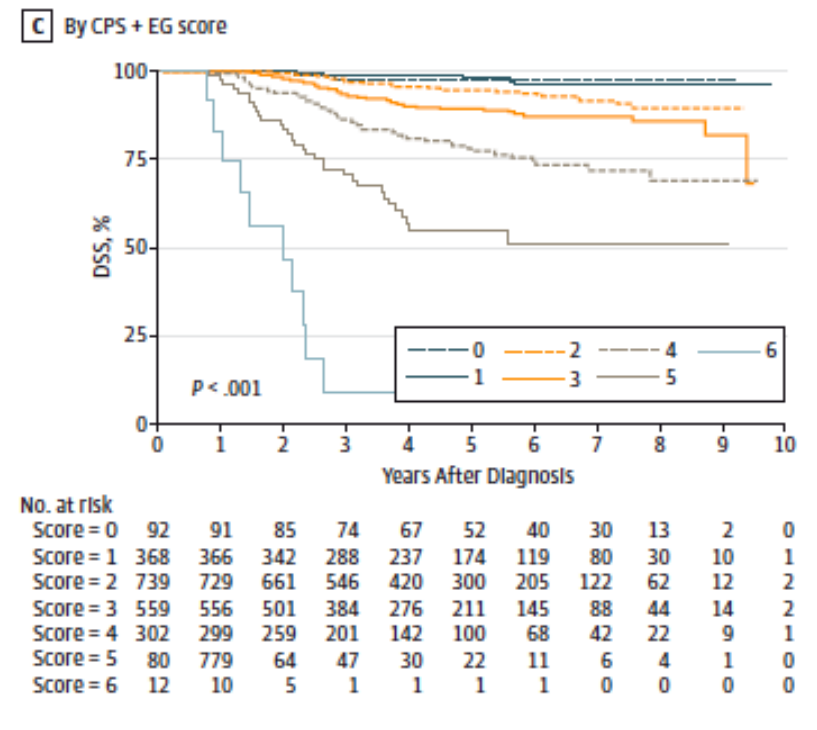
- Measurement for *yp*T is based on the largest focus of *continuous* residual invasive carcinoma
- Treatment-related fiborosis adjacent to residual invasive carcinoma is not included in the *yp*T measurement
- When multiple foci are present the (*m*) modifier is included

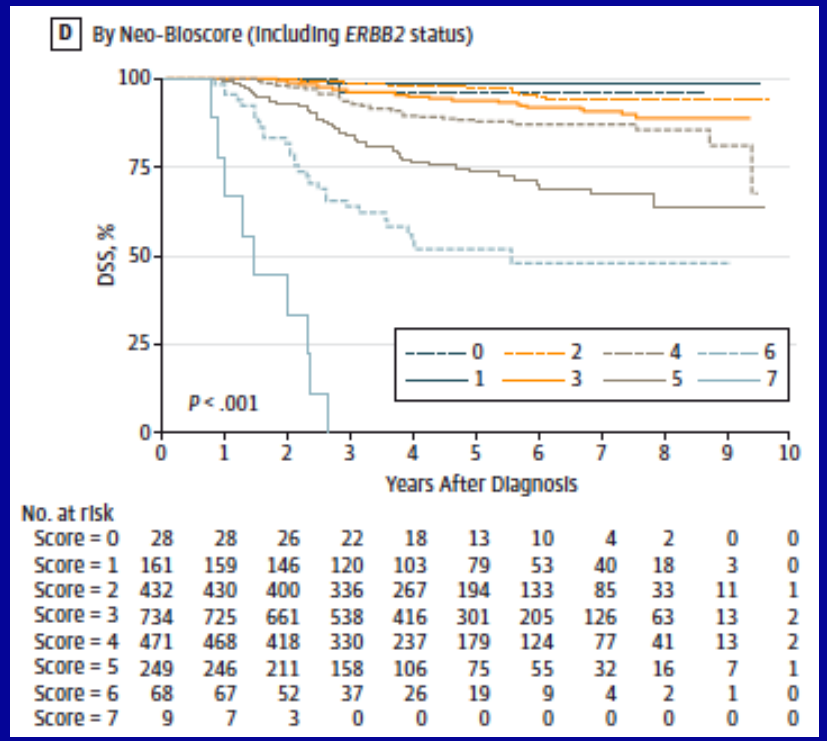
Nodal Metastases: The Expert Panel clarified that ….

- Measurement for *yp*N is based on the largest focus of *continuous* residual tumor
- Treatment-related fiborosis adjacent to residual invasive carcinoma is not included in the *yp*T measurement
- Isolated tumor cells *yp*N0*i* rules out pCR

NeoBioscore Modification of CPS-EG

Pre-Rx Stage (c)		Pre-Rx Pathobiology						Post-Rx Stage (yp)	
c Stage	=	ER (1%)	=	N Grade	=	HER2	=	yp Stage	=
I - IIA	0	Positive	0	1 - 2	0	Positive	0	0 - I	0
IIB - IIIA	1	Negative	1	3	1	Negative	1	IIA - IIIB	1
IIIB - IIIC	2							IIIC	2



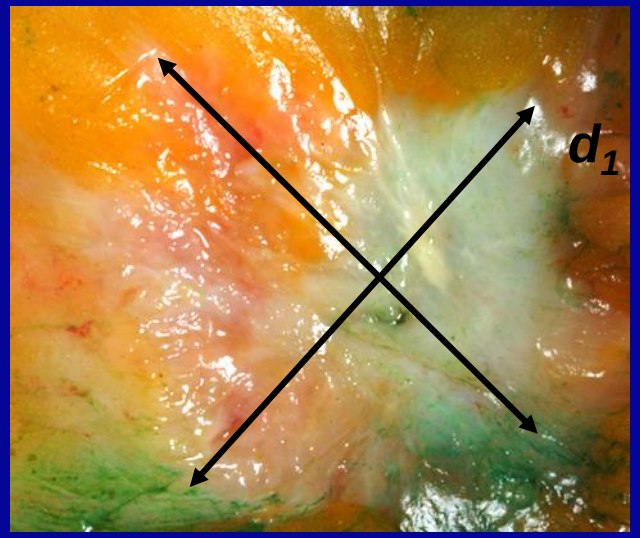


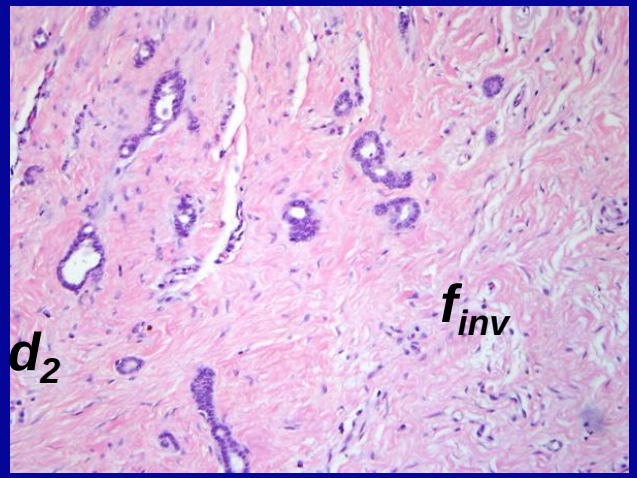
Mittendorf et al JAMA Oncol. 2016; doi: 10.1001/jamaoncol.2015.6478

Residual Cancer Burden (RCB)

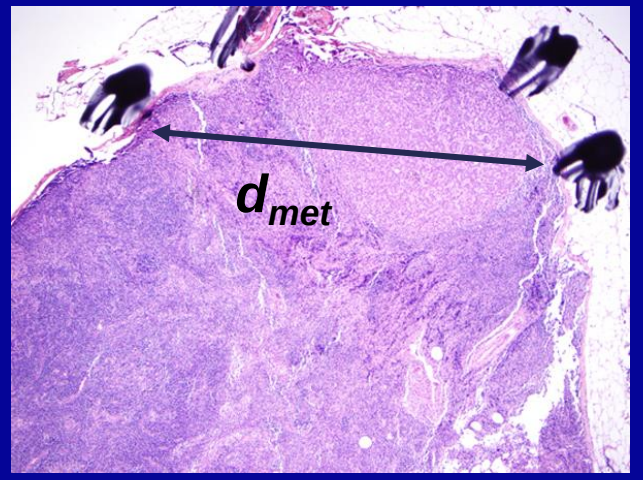
Residual Cancer Burden (RCB)

Primary Tumor Bed





Lymph Nodes



$d_{prim} = \sqrt{d_1 d_2}$

f_{inv} = % area with invasive CA

LN = Number of Positive Nodes

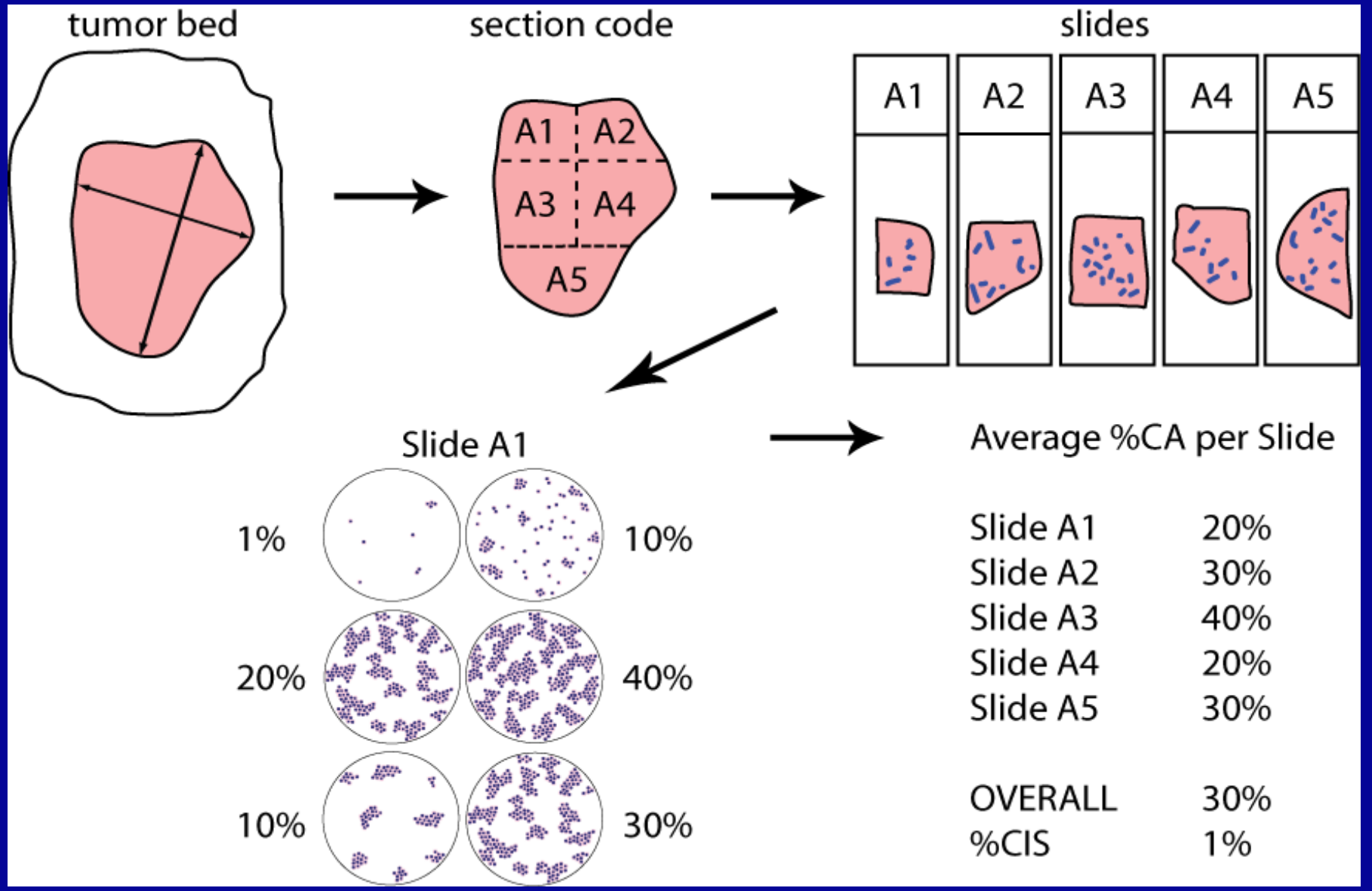
d_{met} = size largest metastasis

DRFS Following Neoadjuvant T/FAC Chemotherapy (N=241)

Variable	Hazard Ratio (95% CI)	P value
Primary tumor bed size (d_{prim})	1.24 (1.04-1.48)	0.02
Fraction of invasive cancer (f_{inv})	7.37 (2.16-25.1)	0.001
Number of positive lymph nodes (*LN*)	1.11 (1.04-1.19)	0.002
Size of largest metastasis (d_{met})	1.17 (0.99-1.38)	0.06

Symmans et al JCO 2007;25:4414-22

Pathologic Assessment Of The Primary Tumor Bed



See downloadable protocol and illustrations at www.mdanderson.org/breastcancer_RCB

Example: Pathologist's Preparatory Notes

52 year old with triple-negative right breast cancer (T2, N0)

Imaging

- Tumor in right breast, 11-12 o'clock position, 8 cm from nipple
- 2.7 cm mass + minute satellites, overall 3.4 cm greatest dimension
- Metal clip placed in tumor at time of diagnostic core biopsy
- Ultrasound of regional nodal basins did not show any abnormal LNs

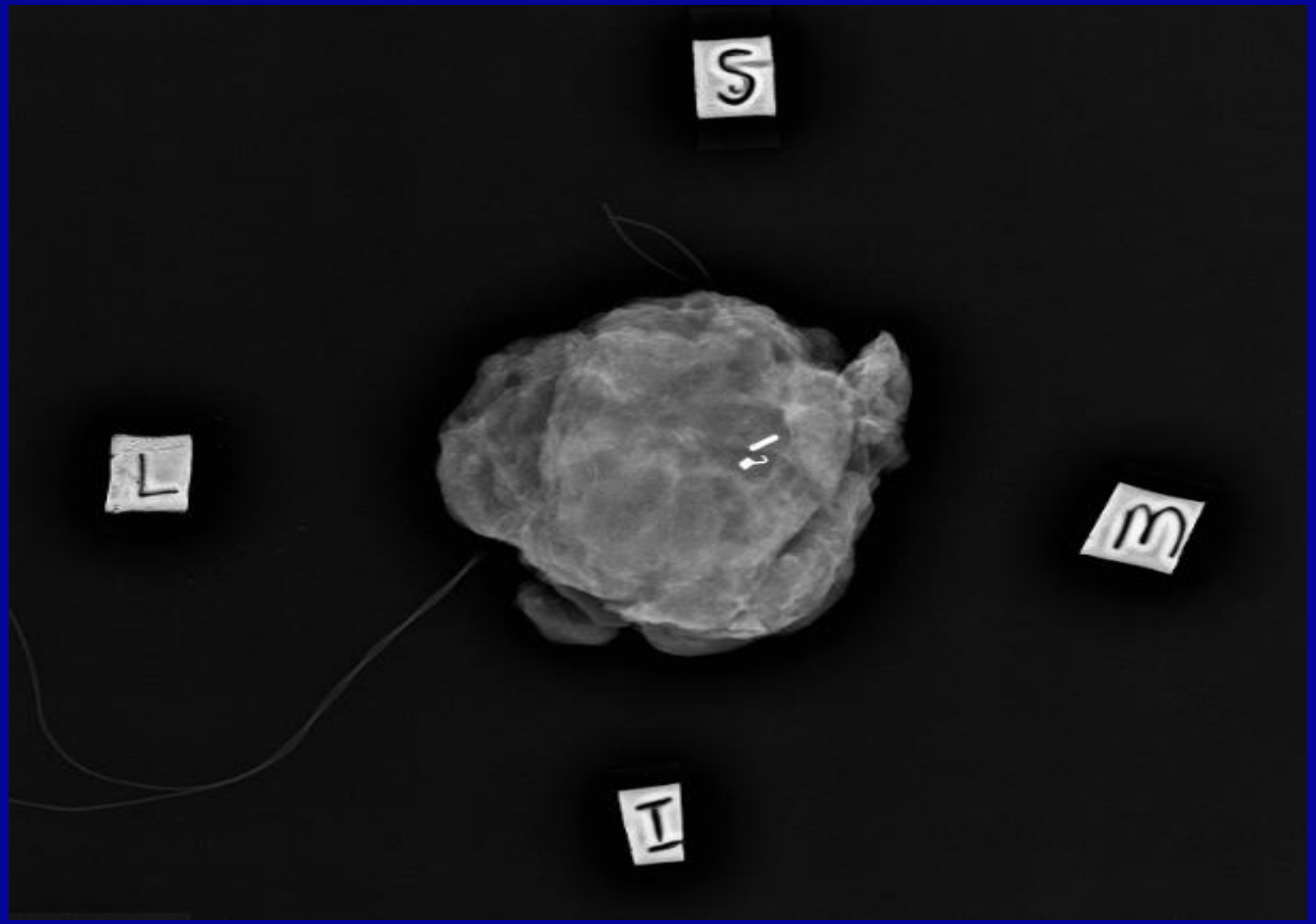
Pathology From Biopsy

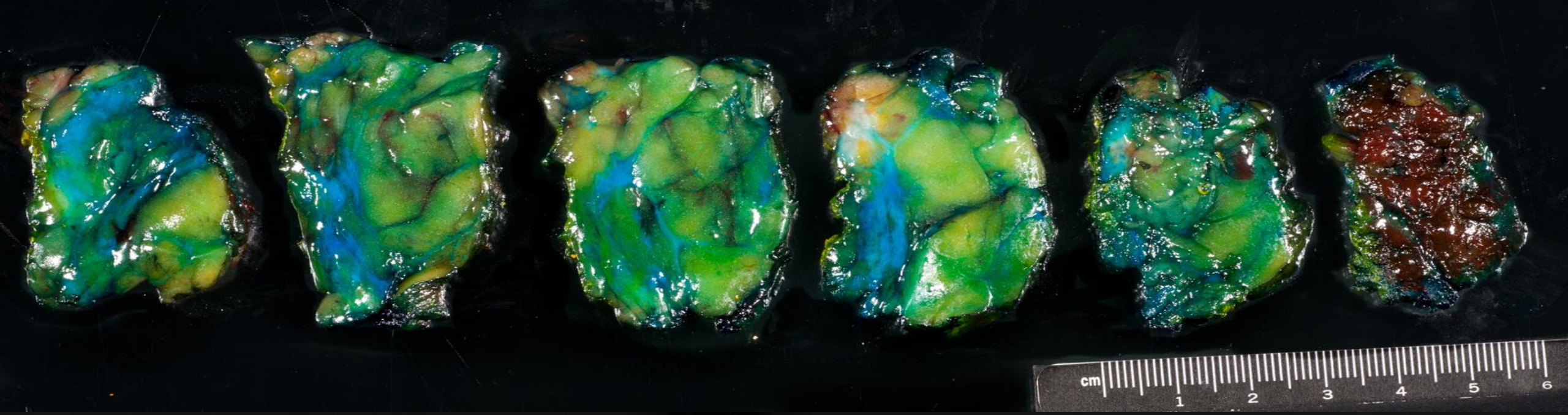
- IDC grade 3, HR- / HER2- (TNBC)

Treatment & Response

- Weekly paclitaxel x 12 then 3-weekly FAC x 4 (T/FAC)
- Residual architectural distortion, but no mass
- Radioactive seed placed in tumor on morning of surgery
- For segmental mastectomy and sentinel node biopsy procedure

The clip and the seed are in the specimen

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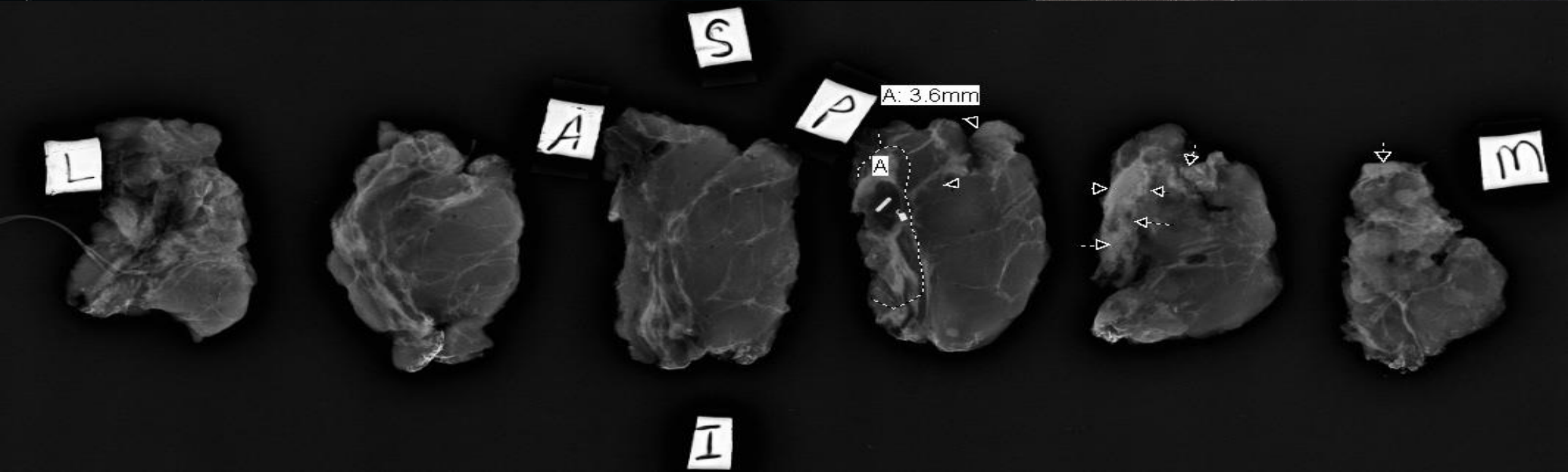
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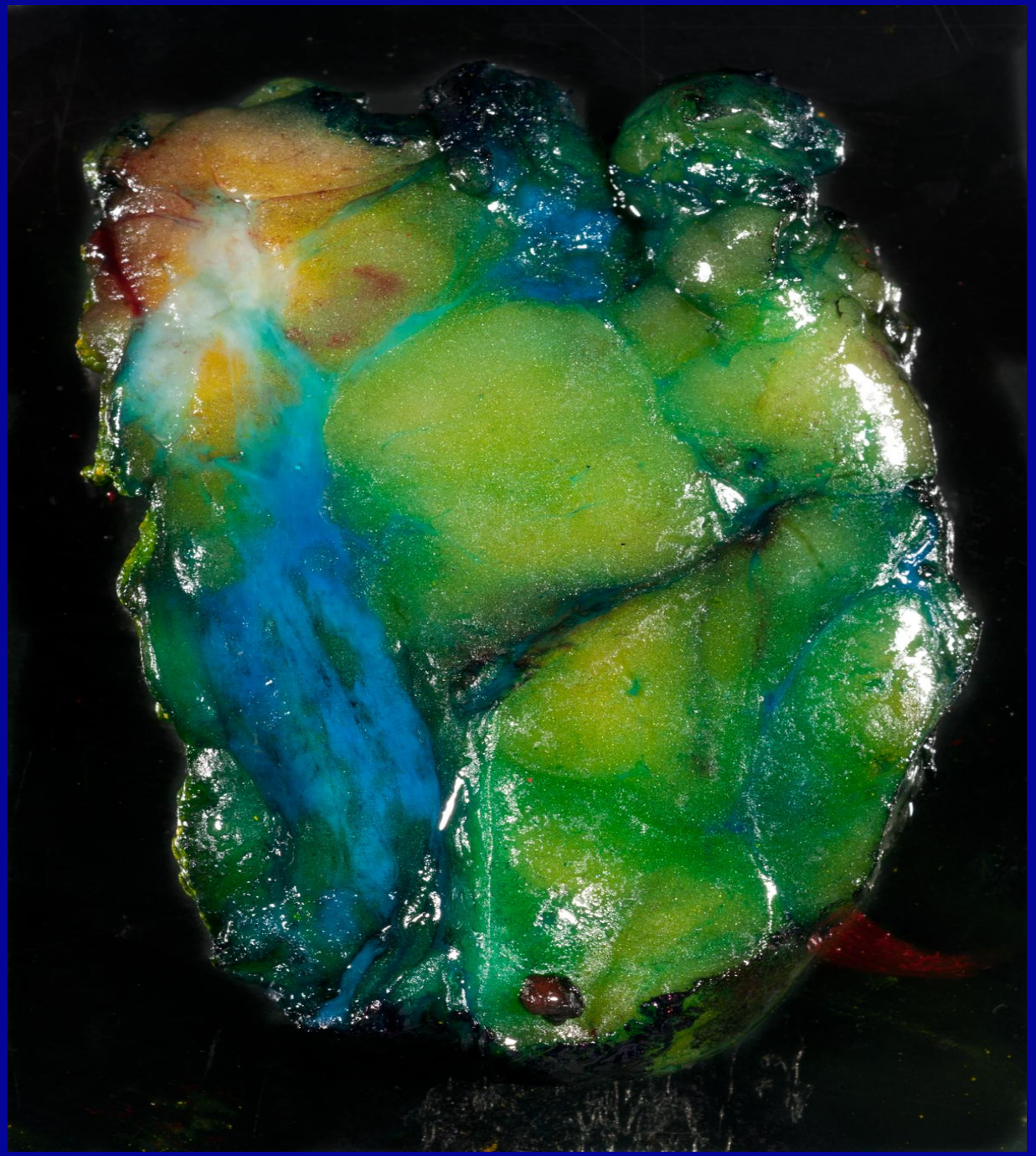

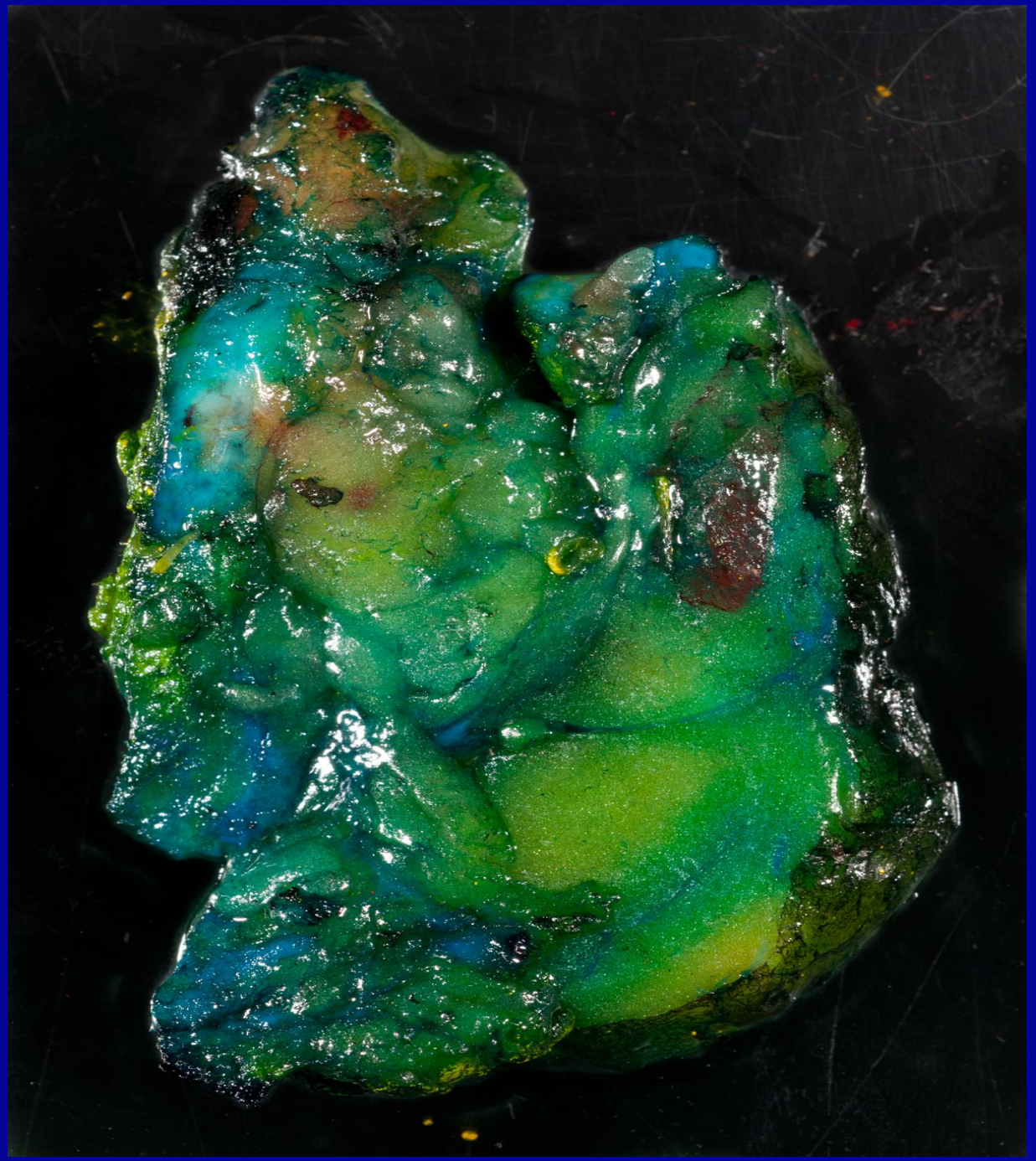

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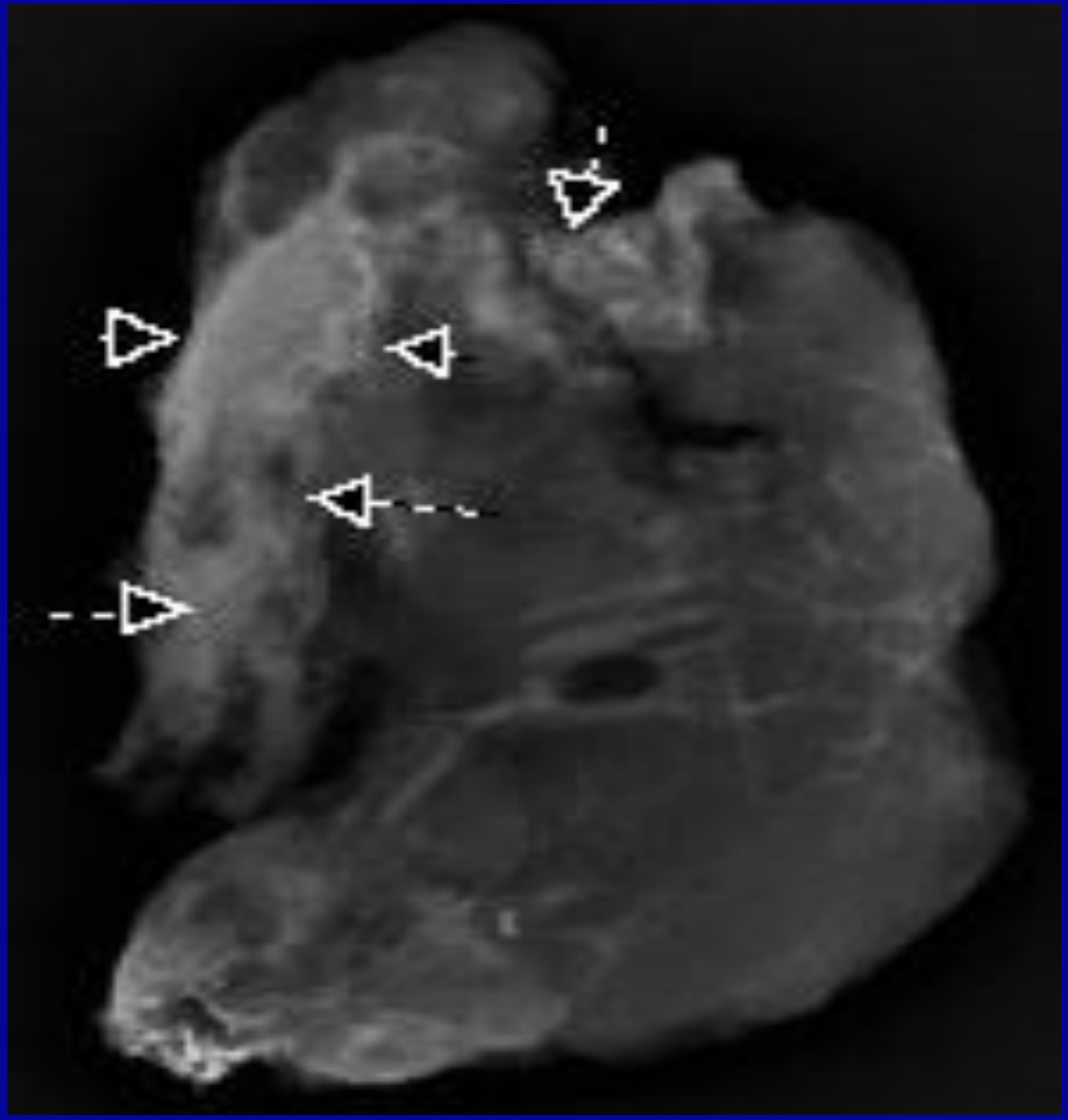

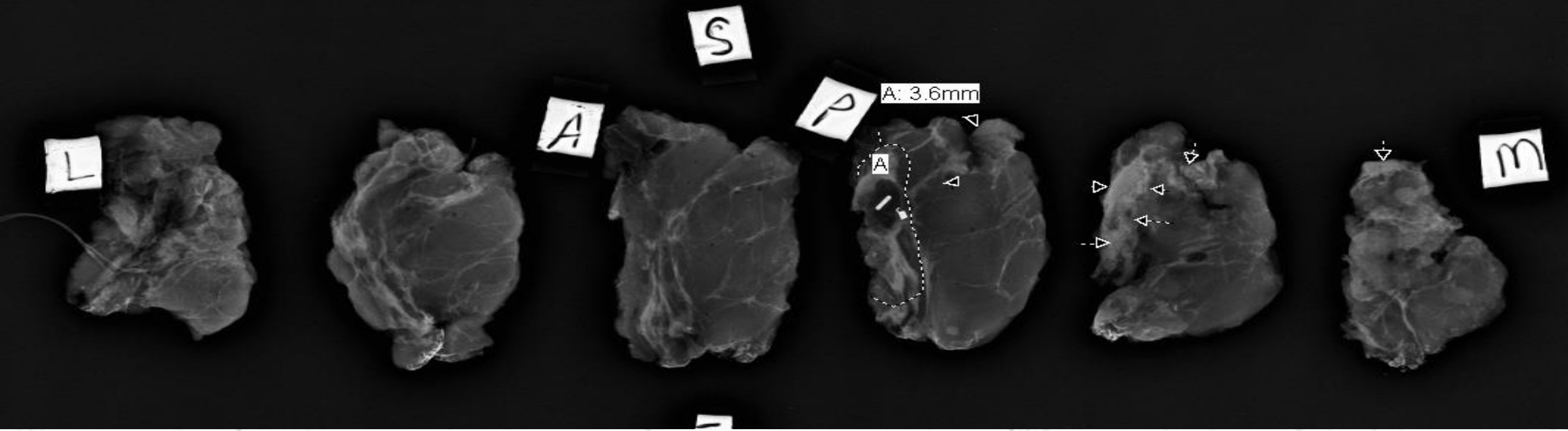
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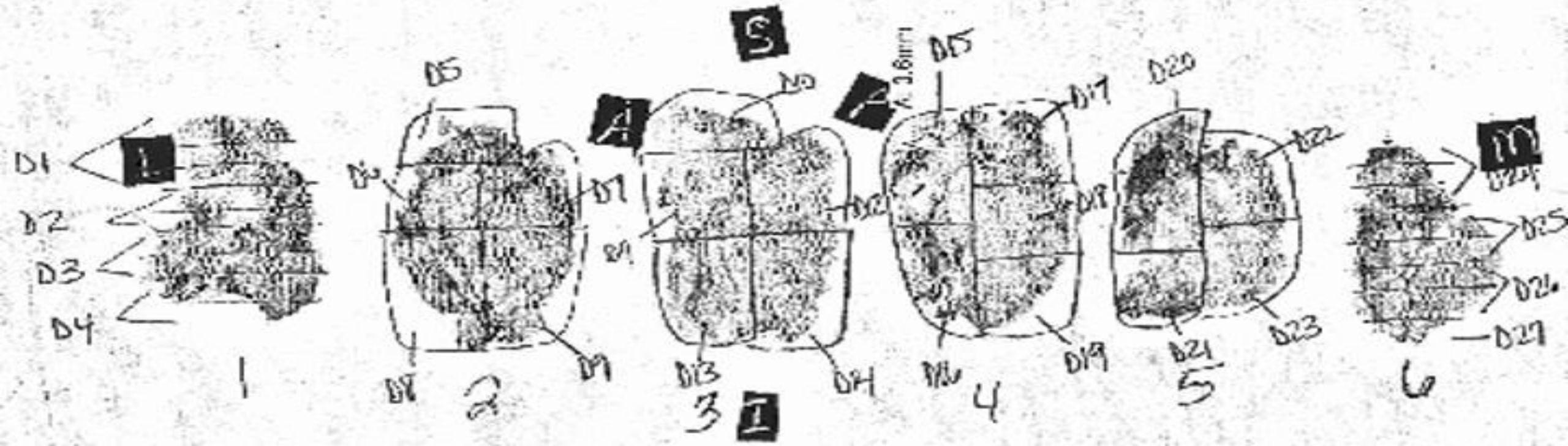
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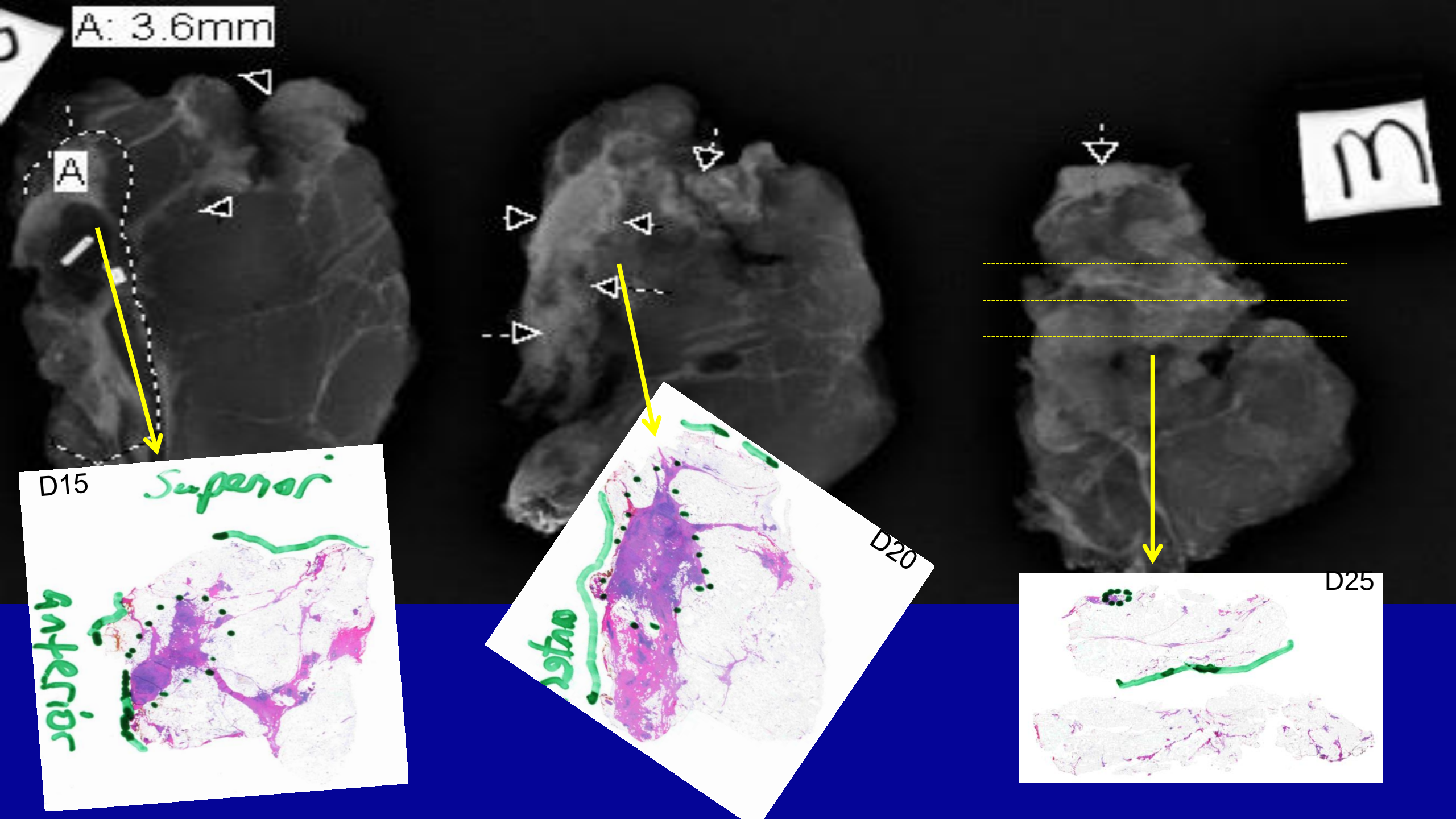
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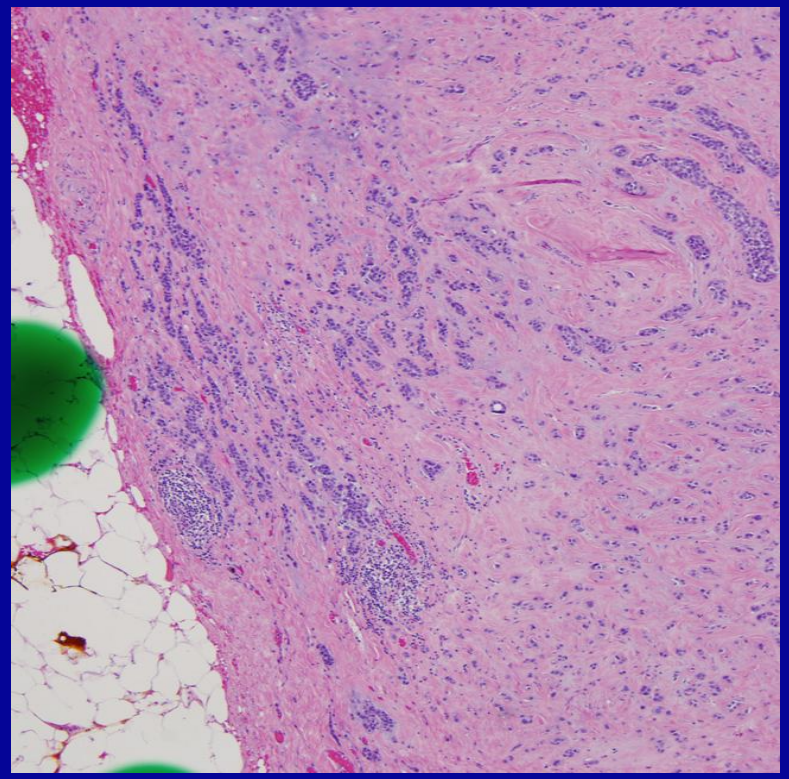

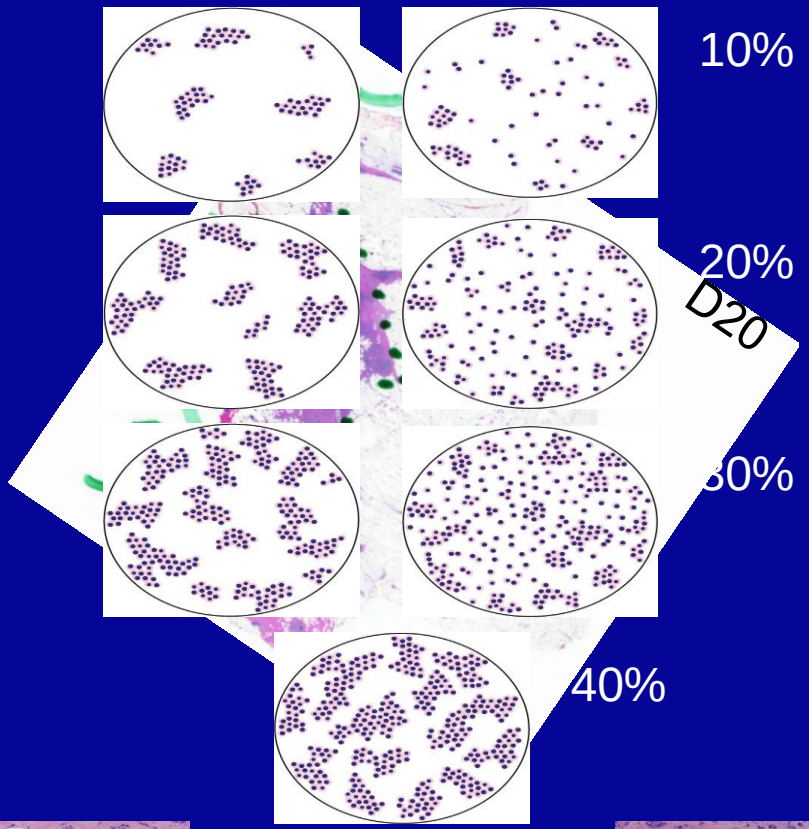
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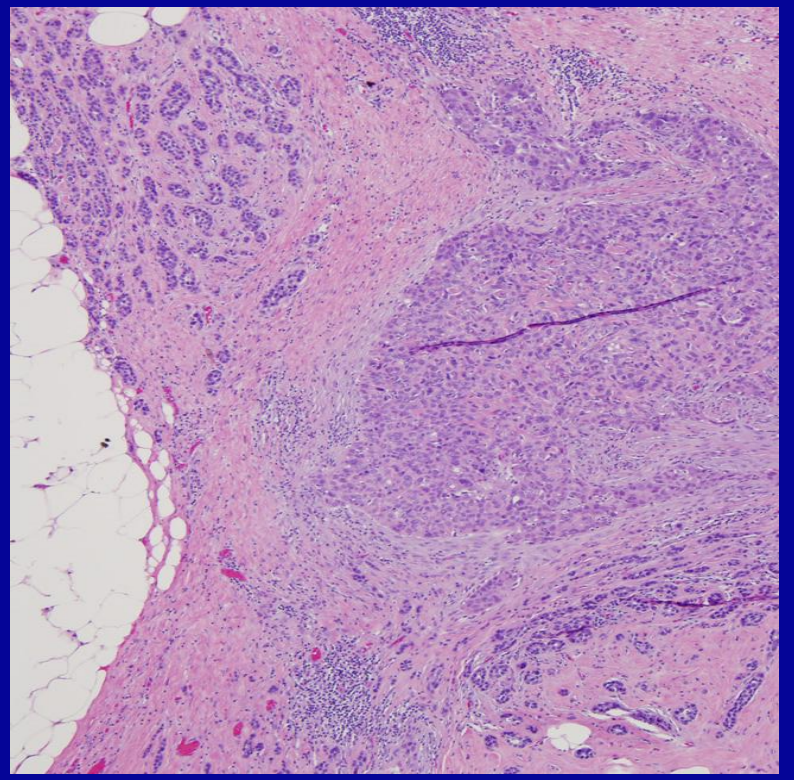
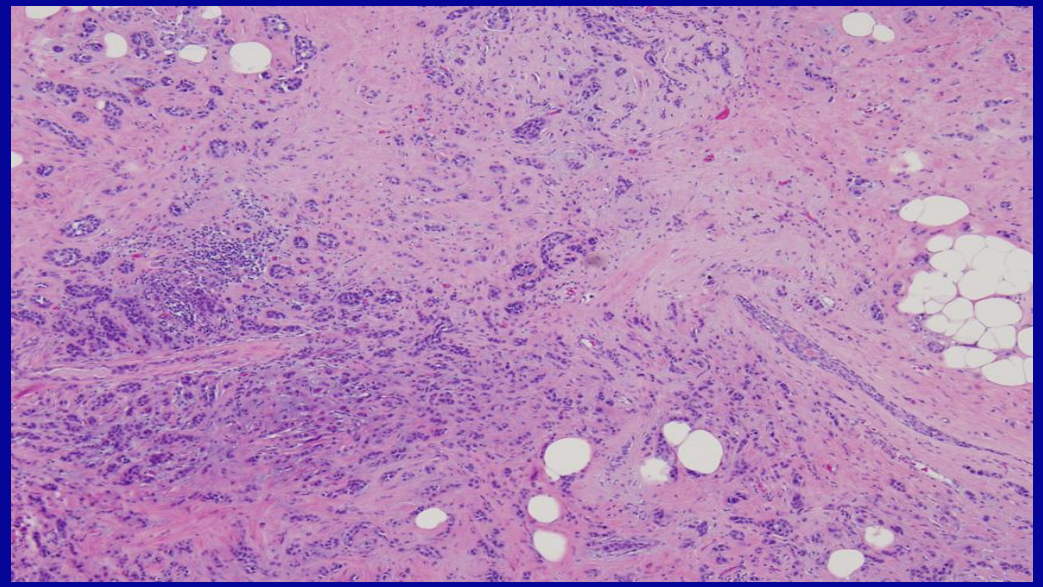
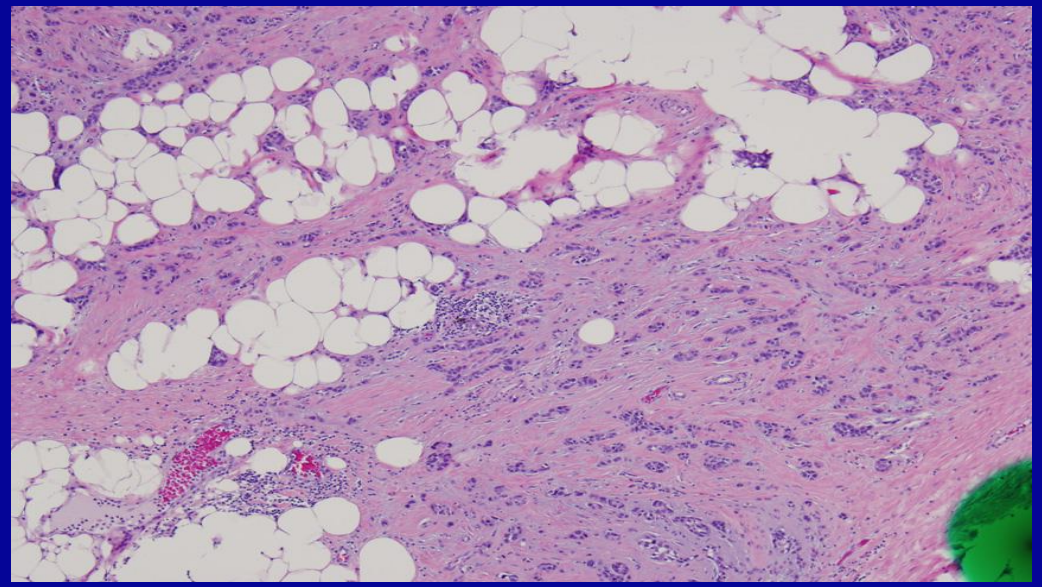
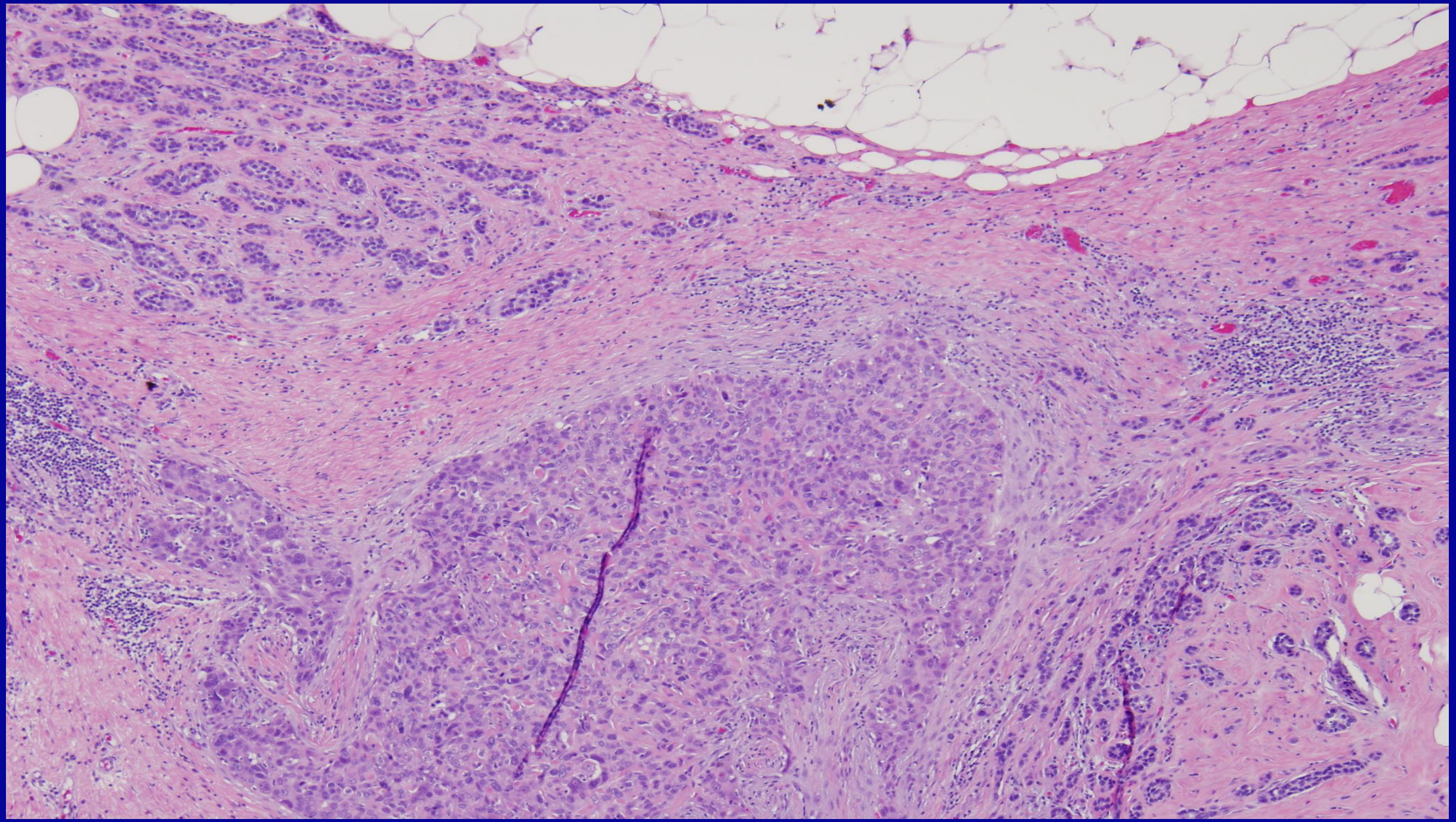
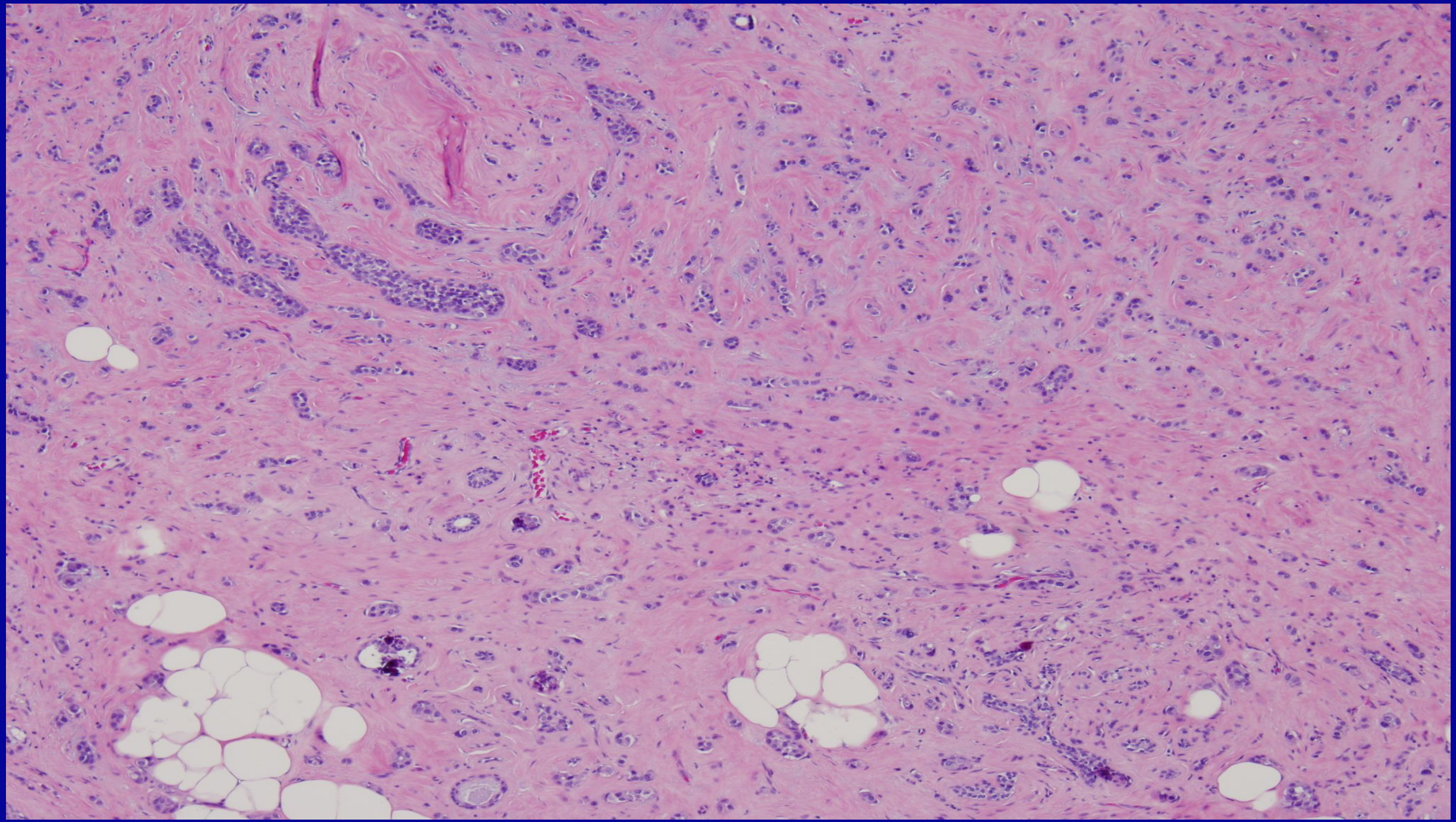










Residual Cancer Burden Calculator

*Values must be entered into all fields for the calculation results to be accurate.

(1) Primary Tumor Bed

Primary Tumor Bed Area:	14 (mm) X 7	(mm)
Overall Cancer Cellularity (as percentage of area):	30	(%)
Percentage of Cancer That Is *in situ* Disease:	0	(%)

(2) Lymph Nodes

Number of Positive Lymph Nodes:	0	
Diameter of Largest Metastasis:	0	(mm)

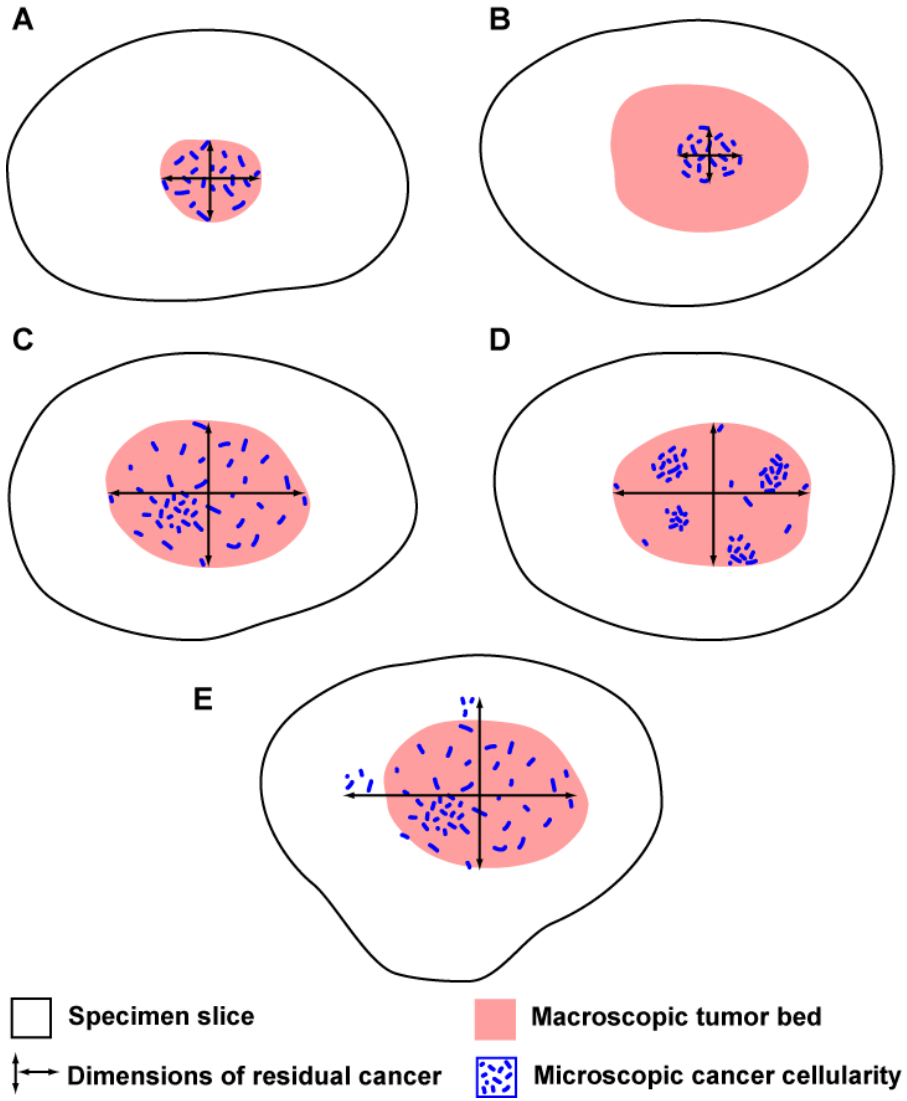
Reset Calculate

Residual Cancer Burden:	1.685
Residual Cancer Burden Class:	RCB–II

Google terms: residual cancer burden breast

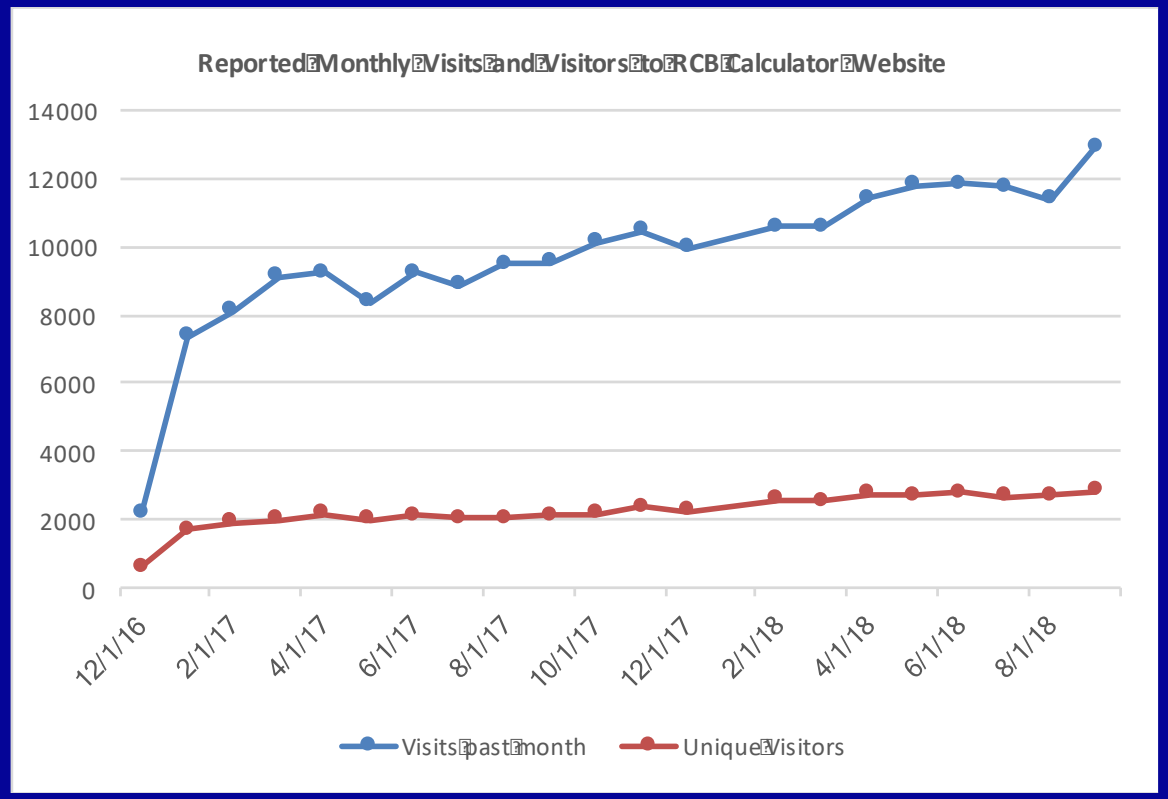
www.mdanderson.org/breastcancer_RCB

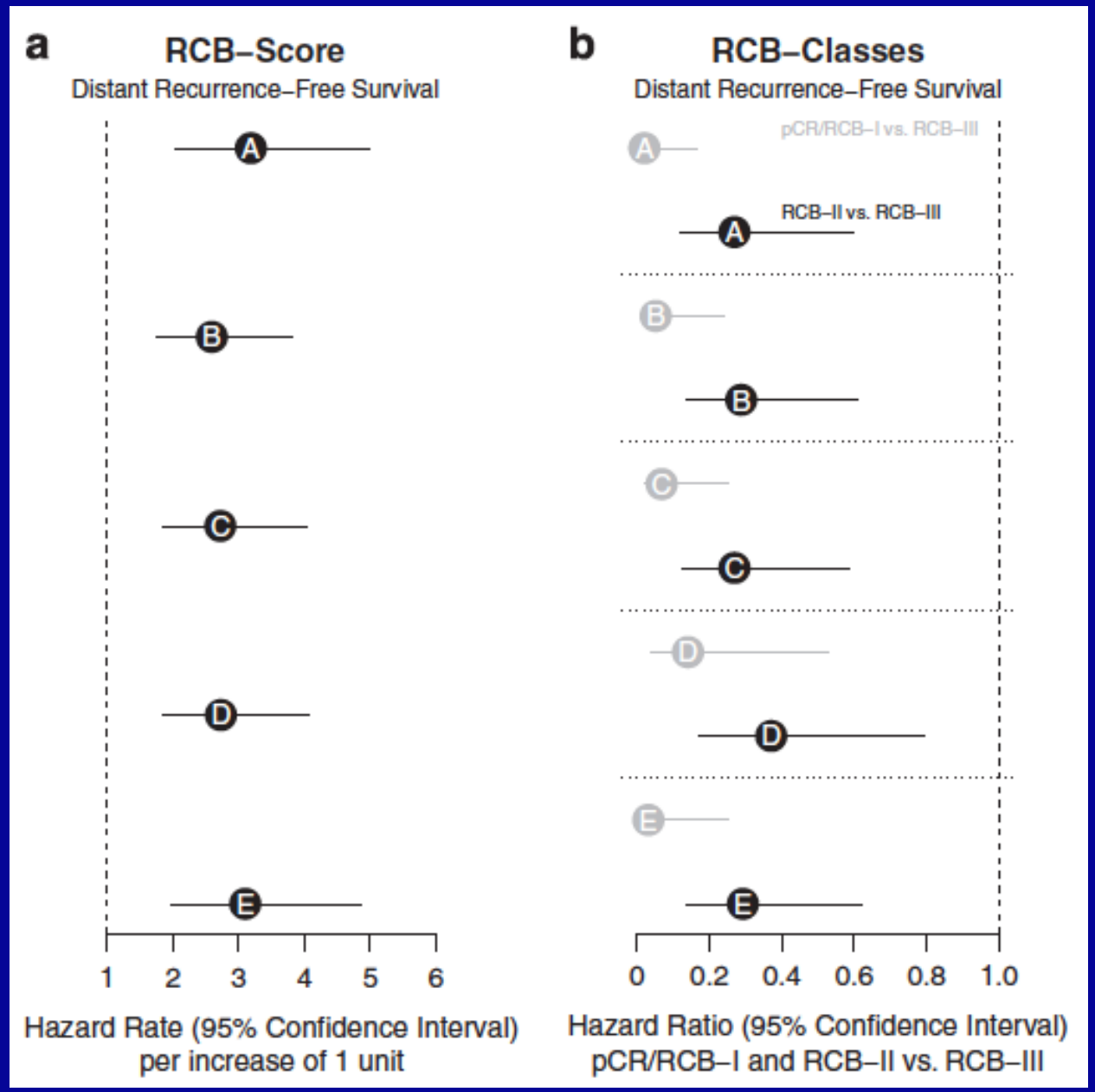
Measure Residual Invasive Primary Tumor Bed:
Macroscopic Findings Qualified By Microscopic Findings



See downloadable protocol and illustrations at www.mdanderson.org/breastcancer_RCB

www.mdanderson.org/breastcancer_RCB

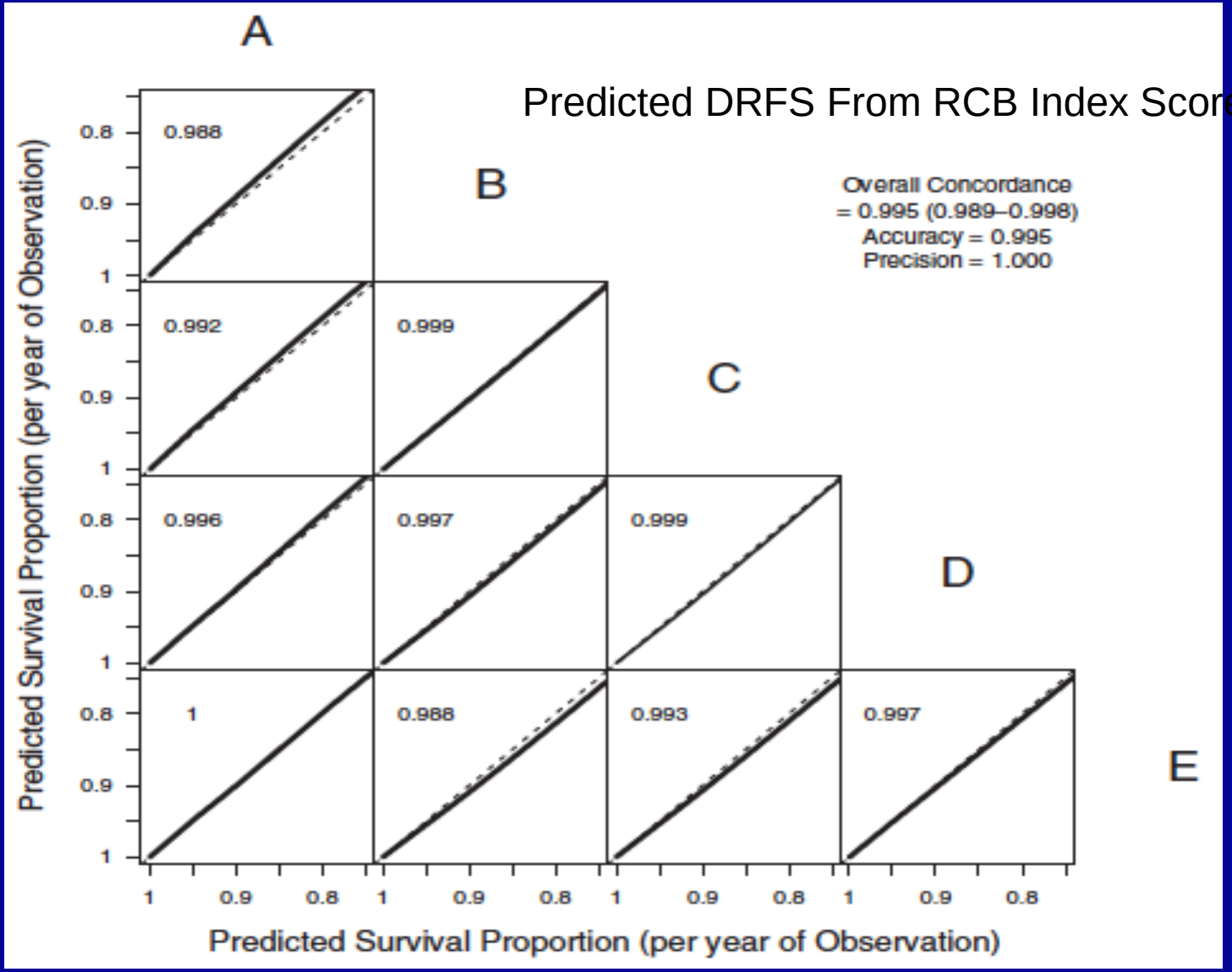




MDACC Website Tracking

Peintinger et al. Modern Pathology 2015 ;28:913-20

Inter-Pathologist Concordance of Predicted DRFS



Peintinger et al. Modern Pathology 2015 ;28:913-20

RCB Classes Within *yp*-Stage Categories (RFS)

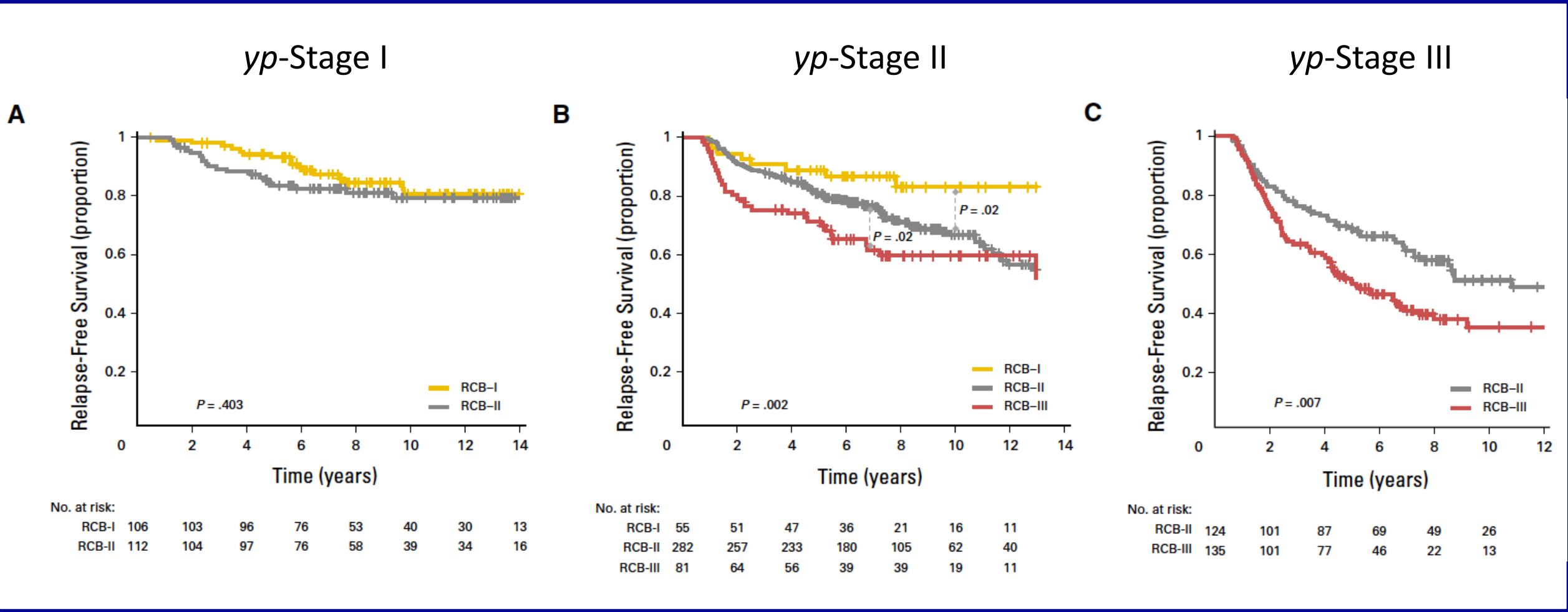
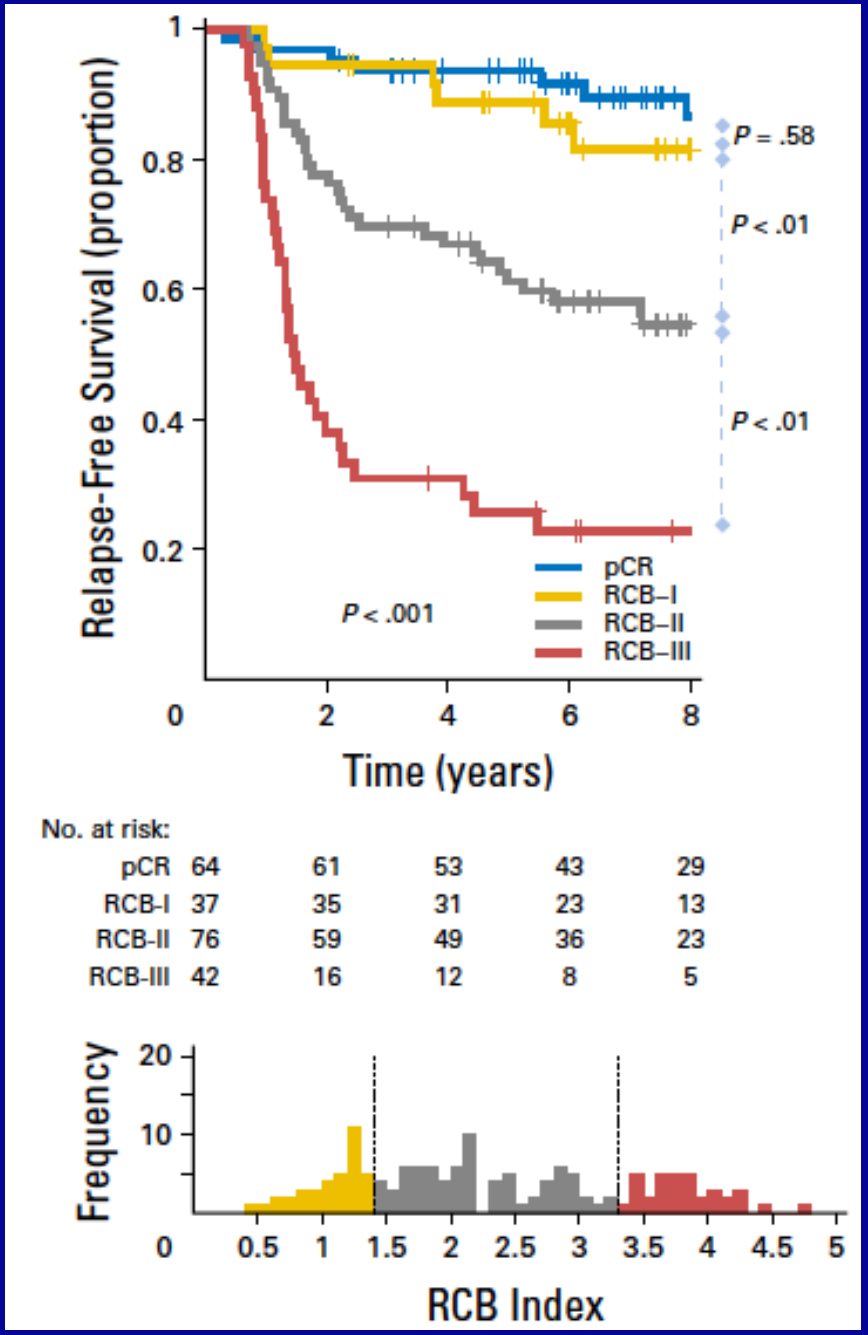
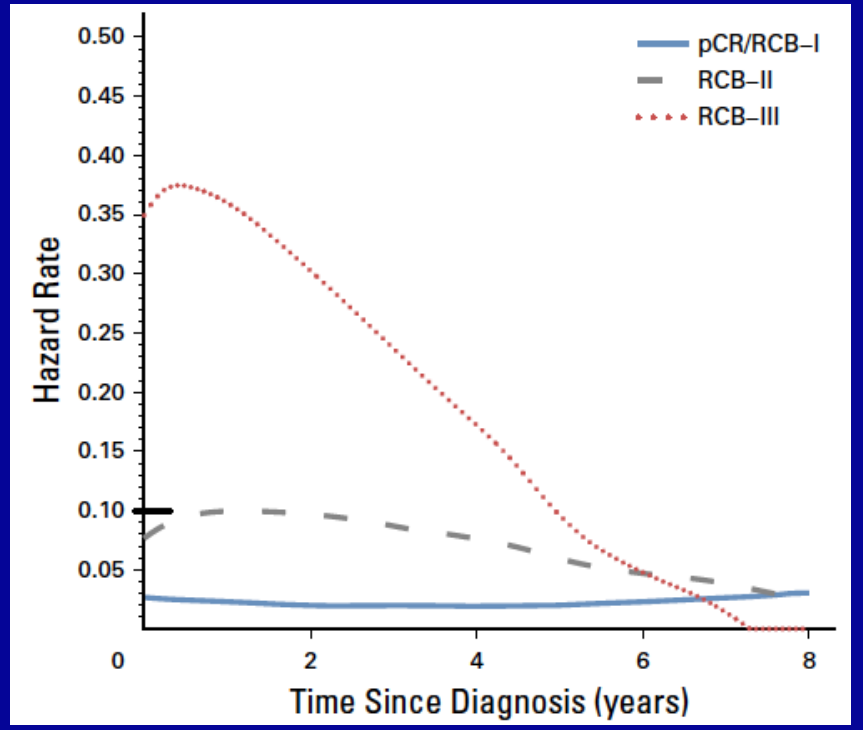


Figure 1. Symmans et al JCO 2017;35:1049-60

TNBC

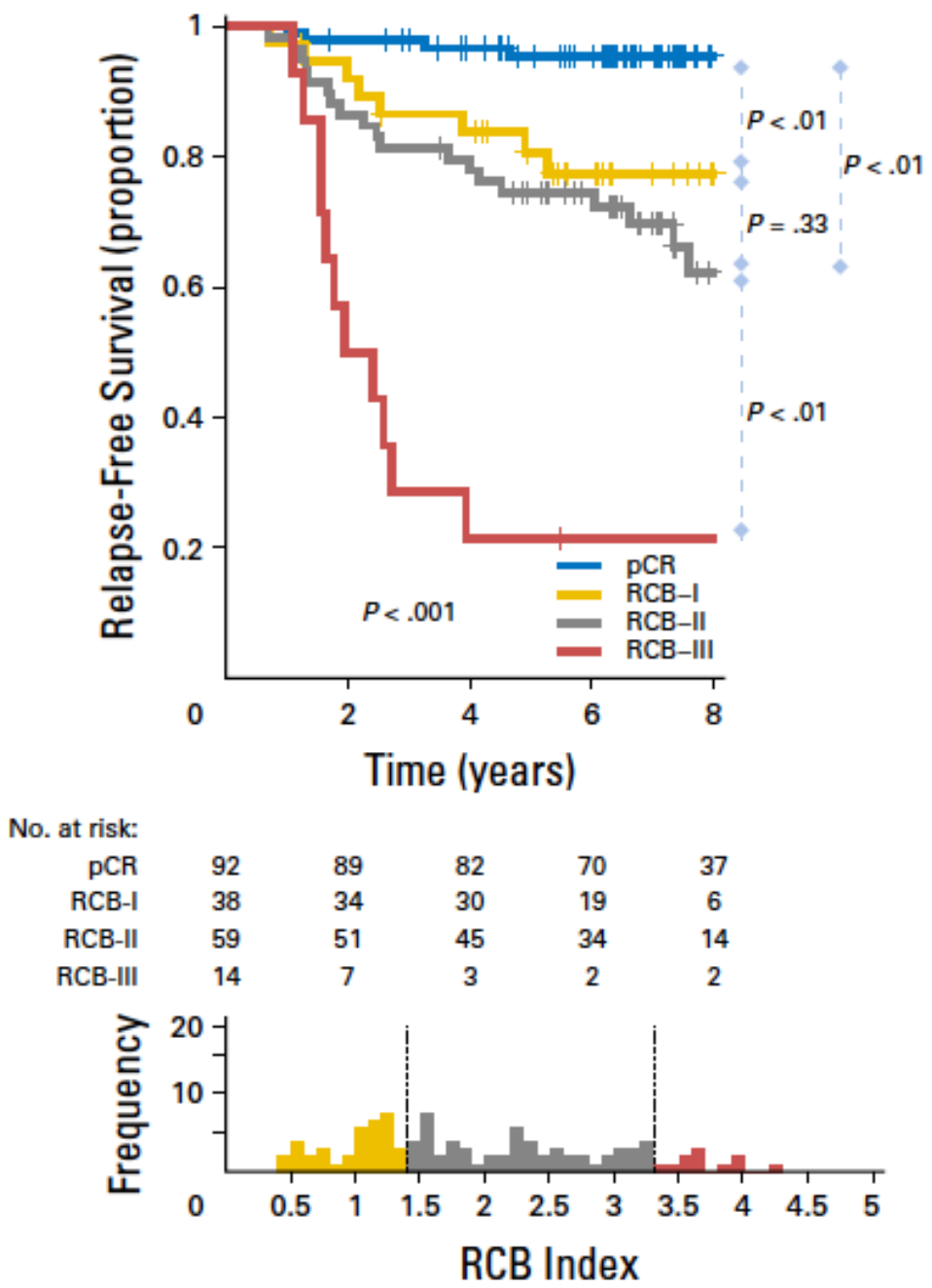


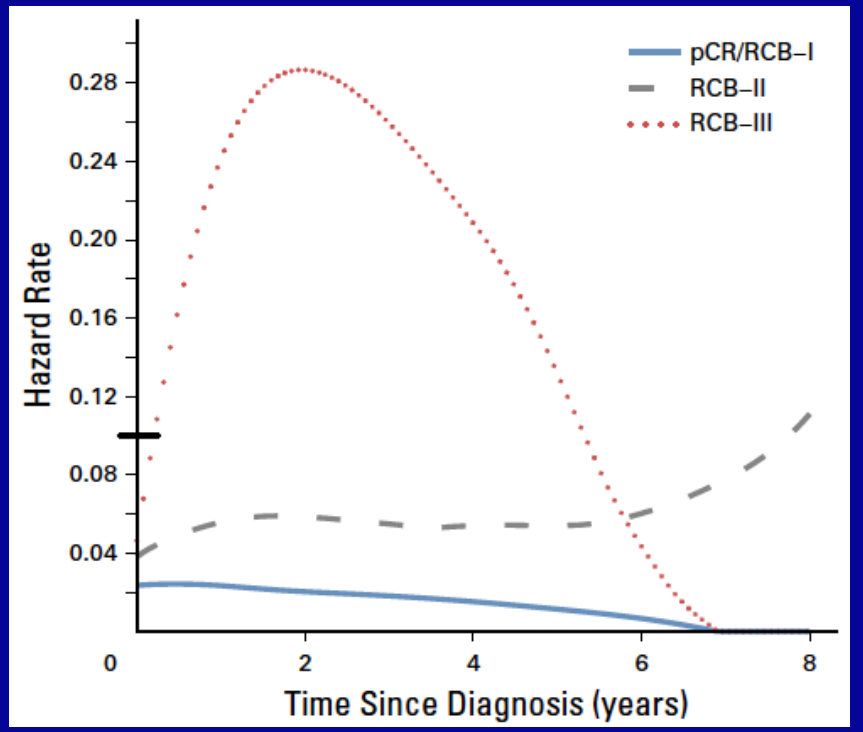


Multivariate Cox Regression				
TNBC Cases in MDACC RCB Study (n = 219)				
	HR	lower 95	upper 95	p =
Age	0.99	0.97	1.01	NS
Grade 3	0.96	0.54	1.69	NS
c-Stage III	1.19	0.73	1.96	NS
RCB index	1.89	1.56	2.29	<0.01

Figures 2B, 3B. Symmans et al JCO 2017;35:1049-60

HER2+ (H+T/FEC)

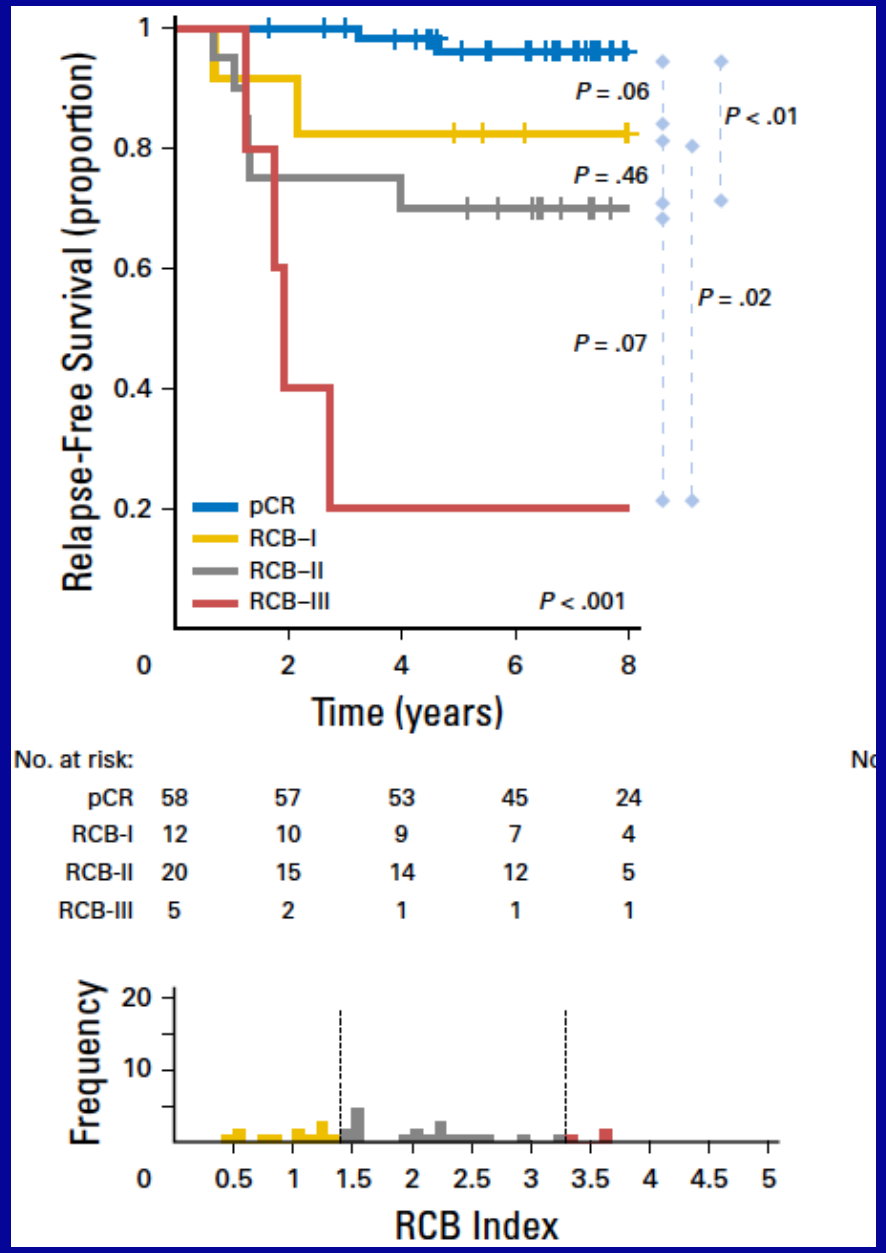




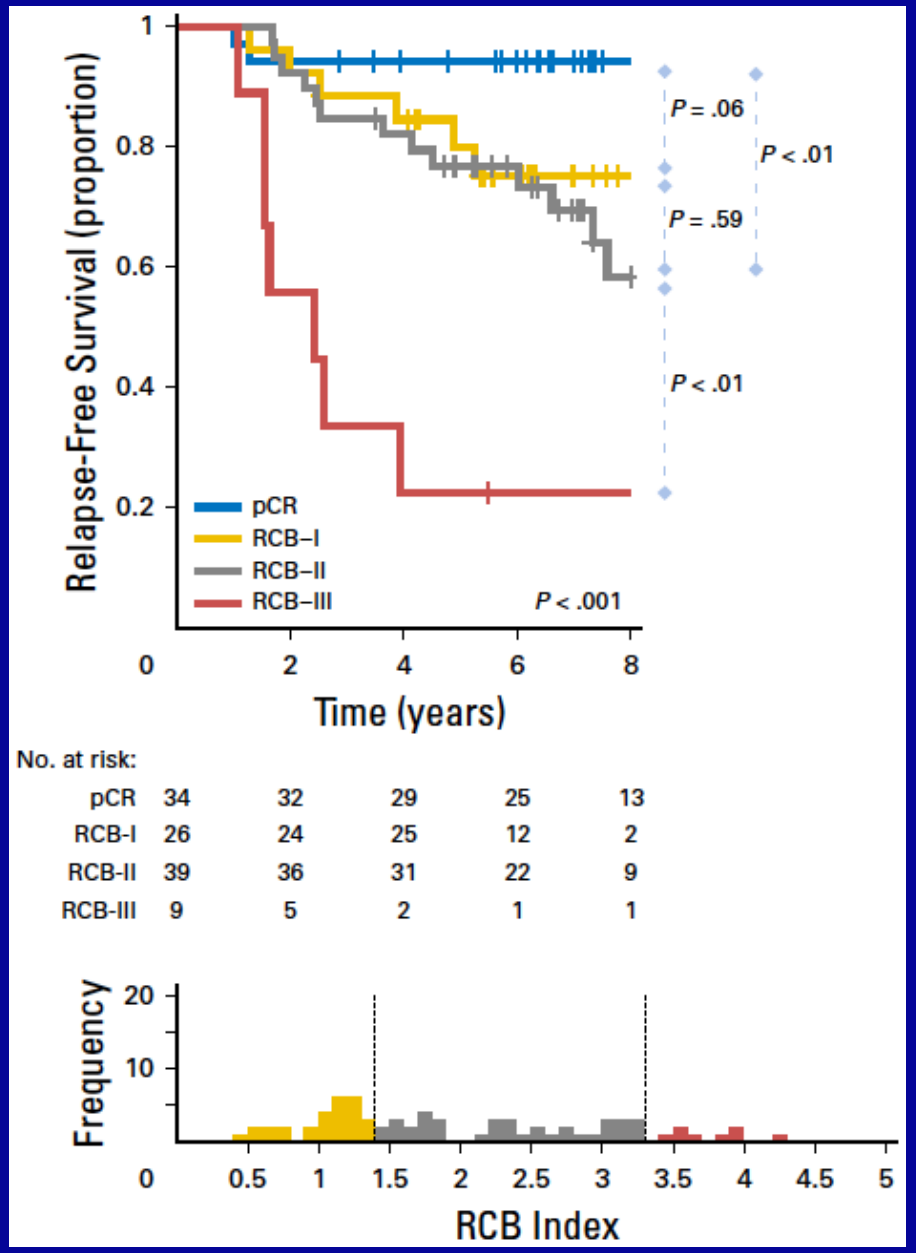
Multivariate Cox Regression				
HER2+ Cases in MDACC RCB Study (n = 203)				
	HR	lower 95	upper 95	p =
Age	1.02	0.99	1.06	NS
Grade 3	0.56	0.29	1.10	NS
c-Stage III	1.33	0.70	2.55	NS
Multifocal	1.48	0.72	3.07	NS
pCR	0.34	0.08	1.35	NS
RCB index	1.80	1.26	2.59	<0.01

Figures 2D, 3D. Symmans et al JCO 2017;35:1049-60

HER2+/HR- (H+T/FEC)

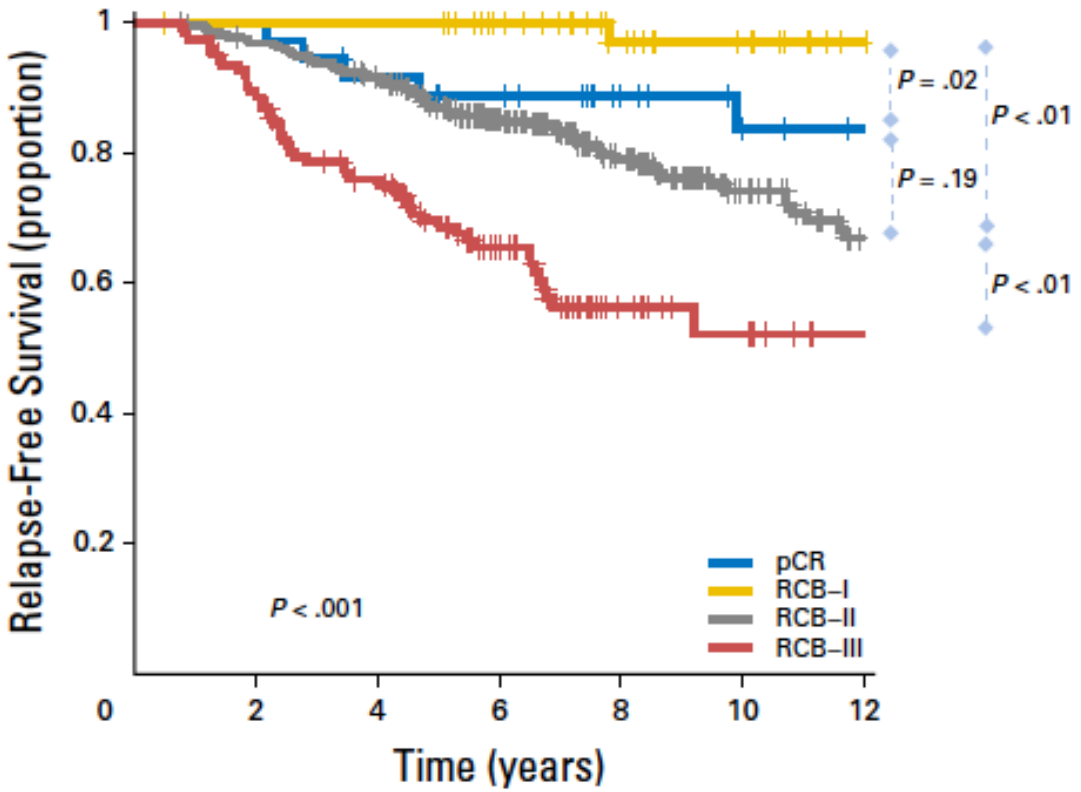


HER2+/HR+ (H+T/FEC)



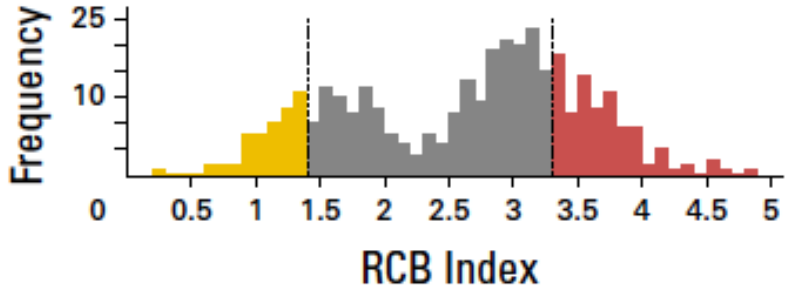
Figures 3E, 3F. Symmans et al JCO 2017;35:1049-60

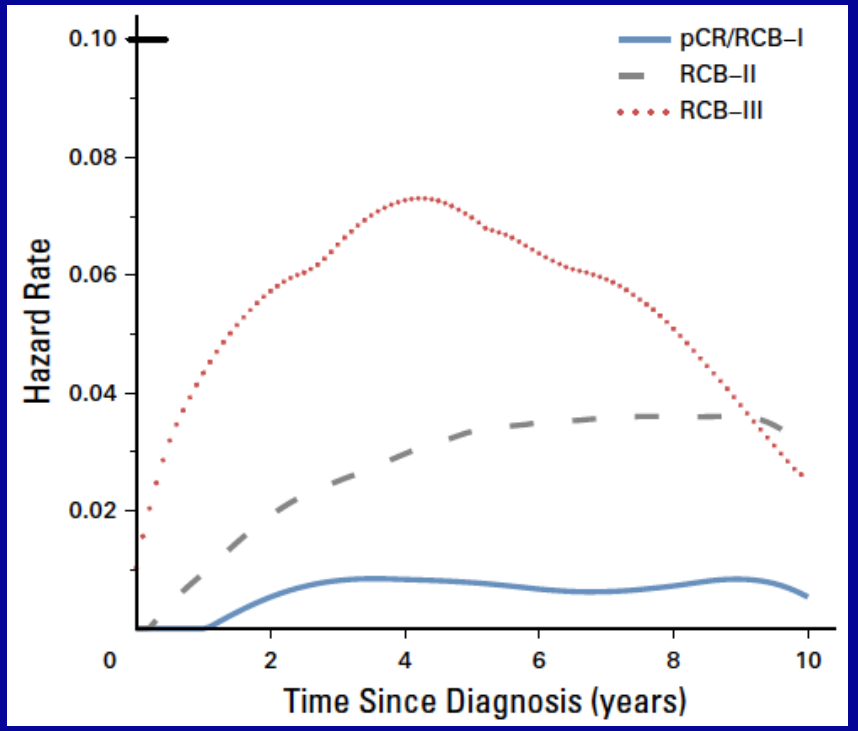
HR+/HER2-



No. at risk:

pCR	36	36	32	29	21	17	14
RCB-I	52	51	51	44	32	26	15
RCB-II	290	278	258	199	127	73	48
RCB-III	123	108	88	55	22	13	5

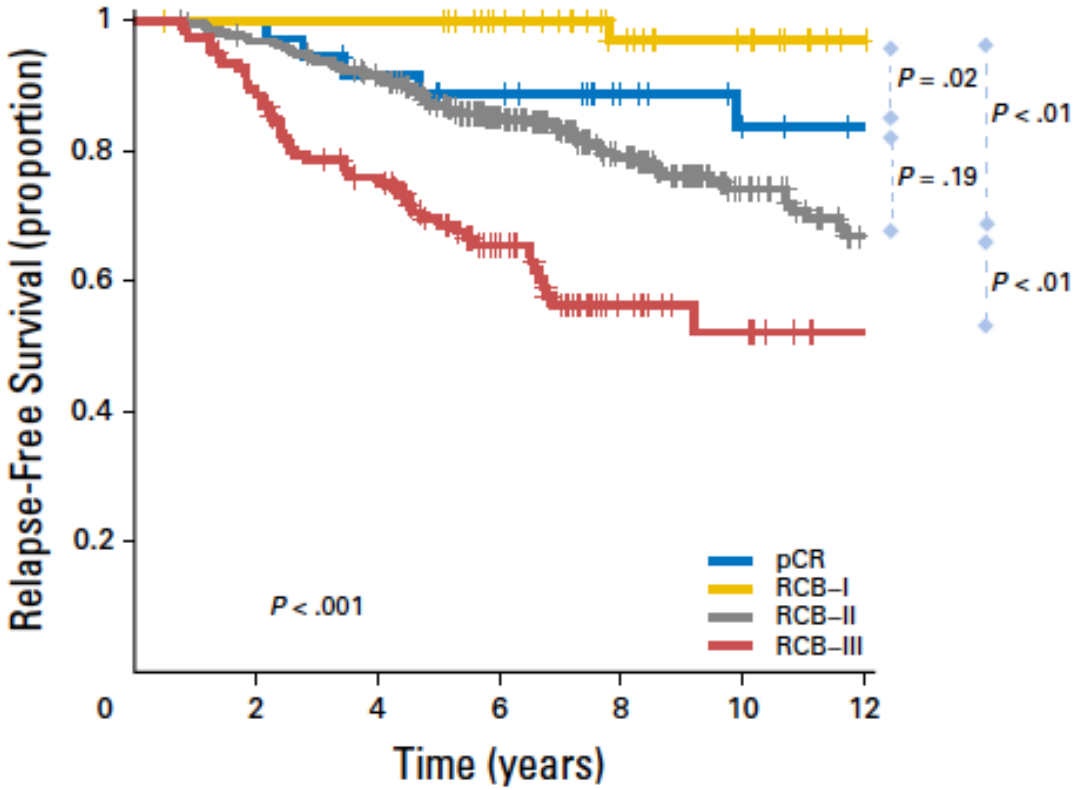




Multivariate Cox Regression				
HR+/HER2- Cases in MDACC RCB Study (n = 501)				
	HR	lower 95	upper 95	p =
Age	0.99	0.97	1.01	NS
Grade 3	1.23	0.82	1.84	NS
c-Stage III	2.53	1.73	3.71	<0.01
RCB index	1.95	1.57	2.41	<0.01

Figures 2B, 3B. Symmans et al JCO 2017;35:1049-60

HR+/HER2-



No. at risk:

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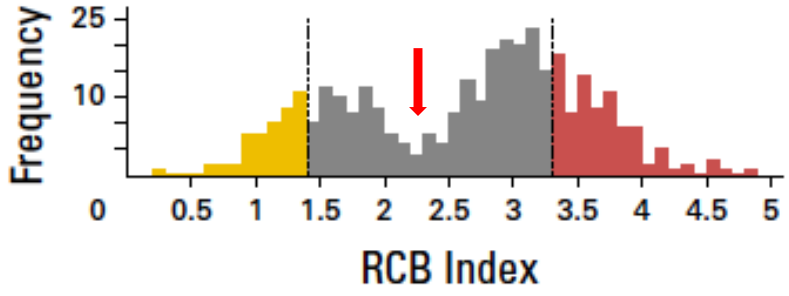
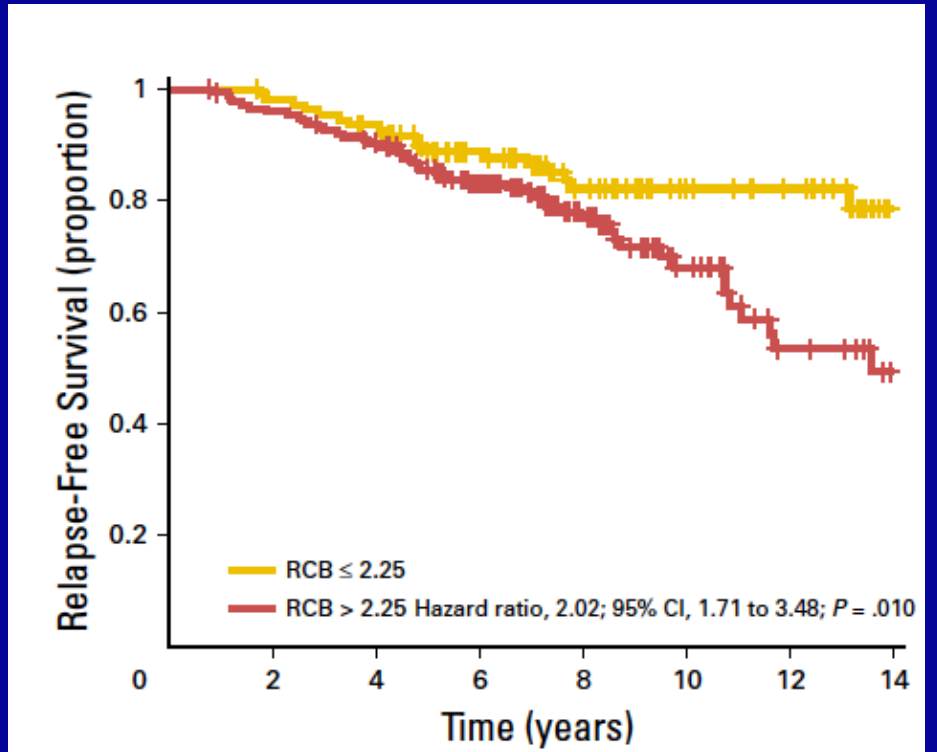


RCB-II Only



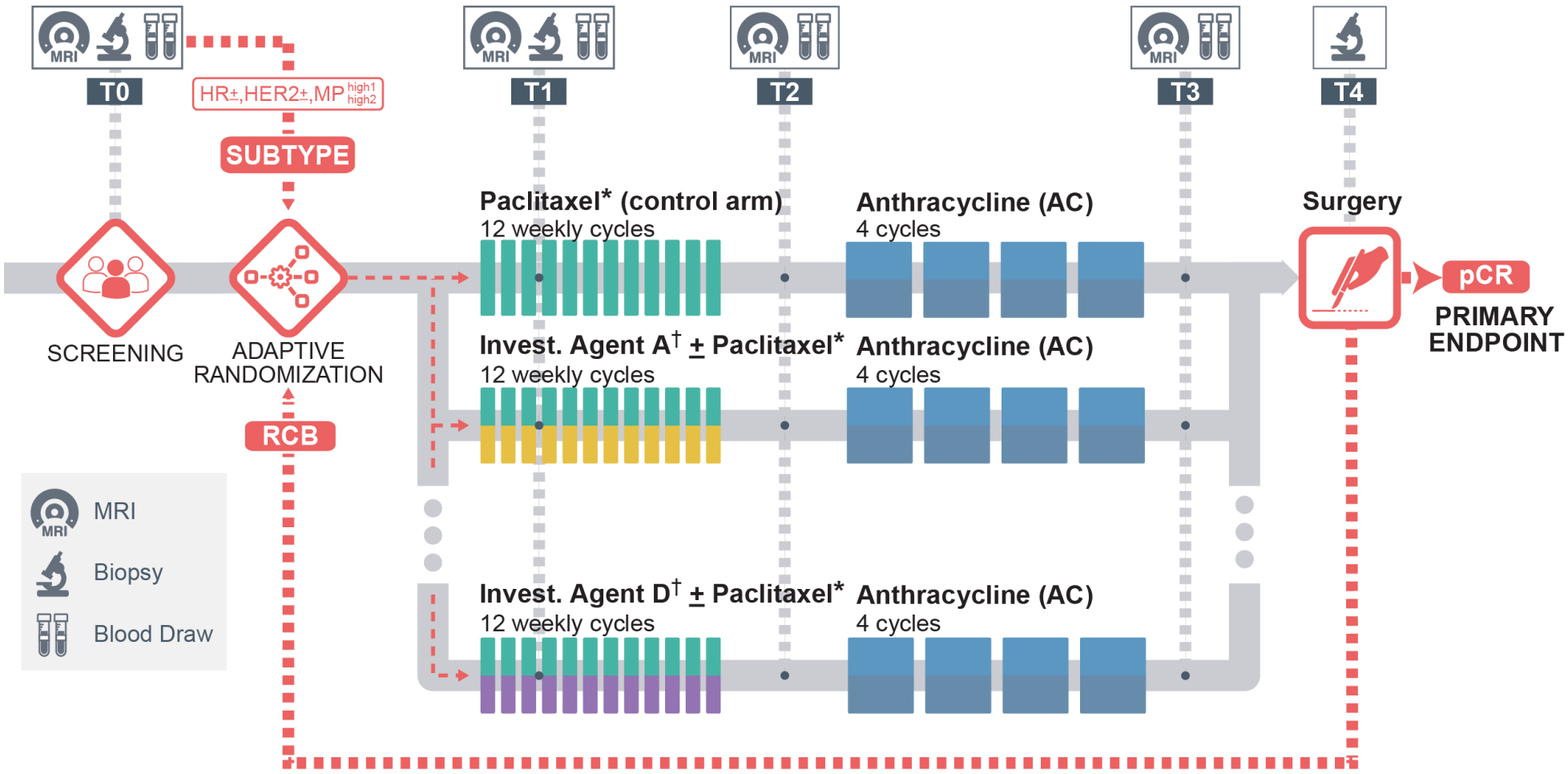
No. at risk:

RCB ≤ 2.25	111	108	101	79	55	37	30
RCB > 2.25	179	170	170	120	72	36	18

Supplemental Figure A6. JCO 2017;35:DOI: 10.1200/JCO.2015.63.1010

Independent Validation in Prospective Clinical Trials

I-SPY2 Trial: Adaptive Randomization

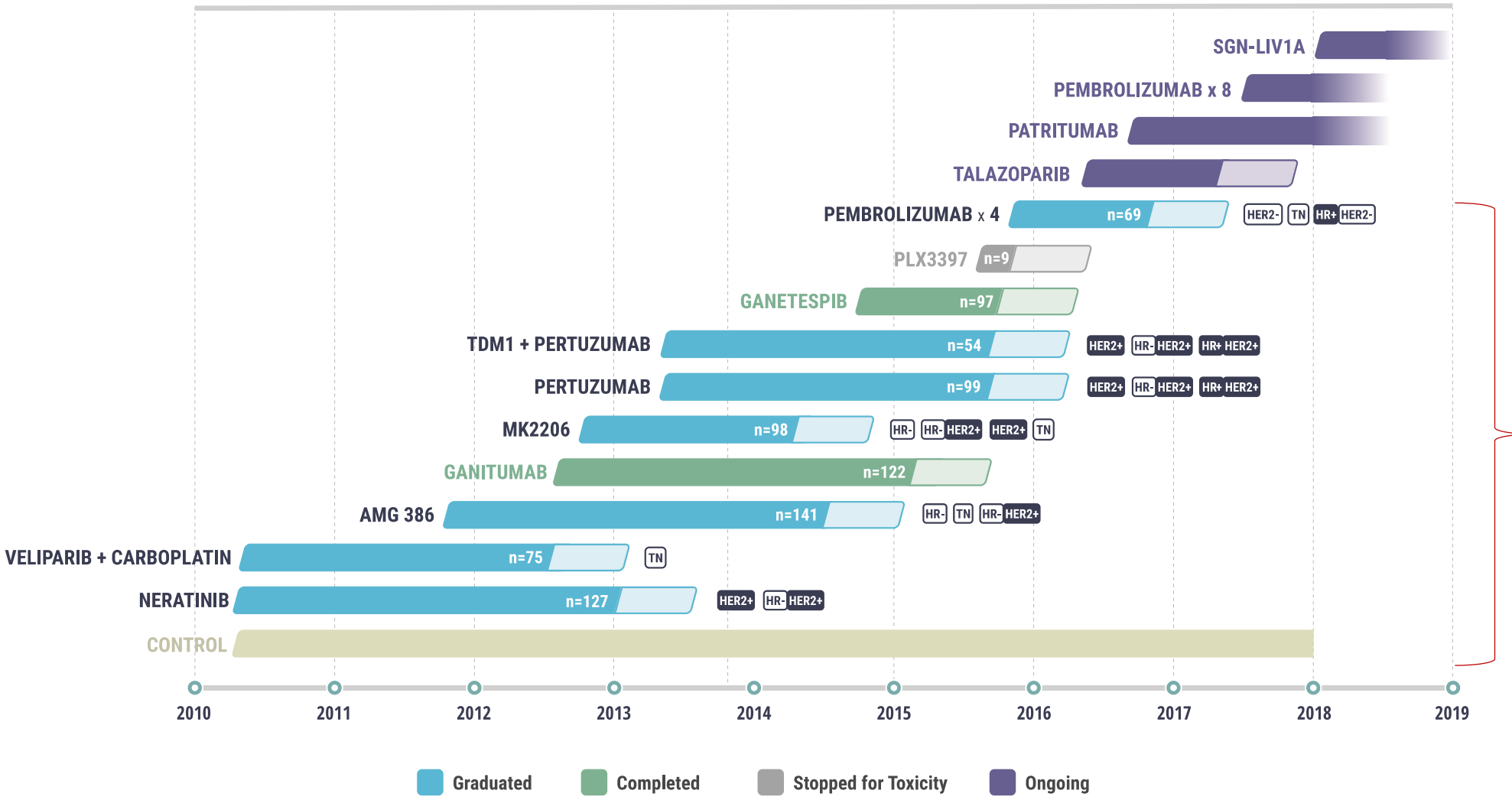


*Patients who are HER2+ may also receive tastuzumab (Herceptin)

†An investigational combination of one or more agents may be used to replace all or some of the standard therapy

Yee D. et al. San Antonio Breast Cancer Symposium, Dec 5-9, 2017

I-SPY2 Trial: Agent Timeline

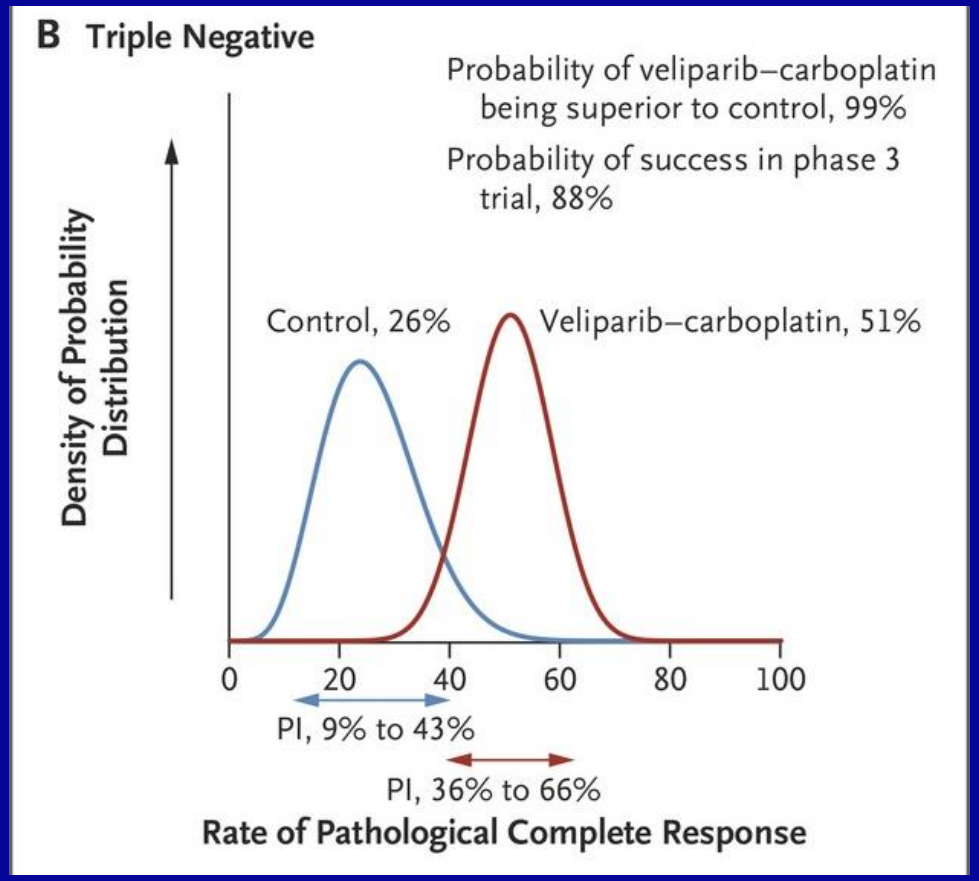


11 Agent Combinations included in current analysis, including control

Yee D. et al. San Antonio Breast Cancer Symposium, Dec 5-9, 2017

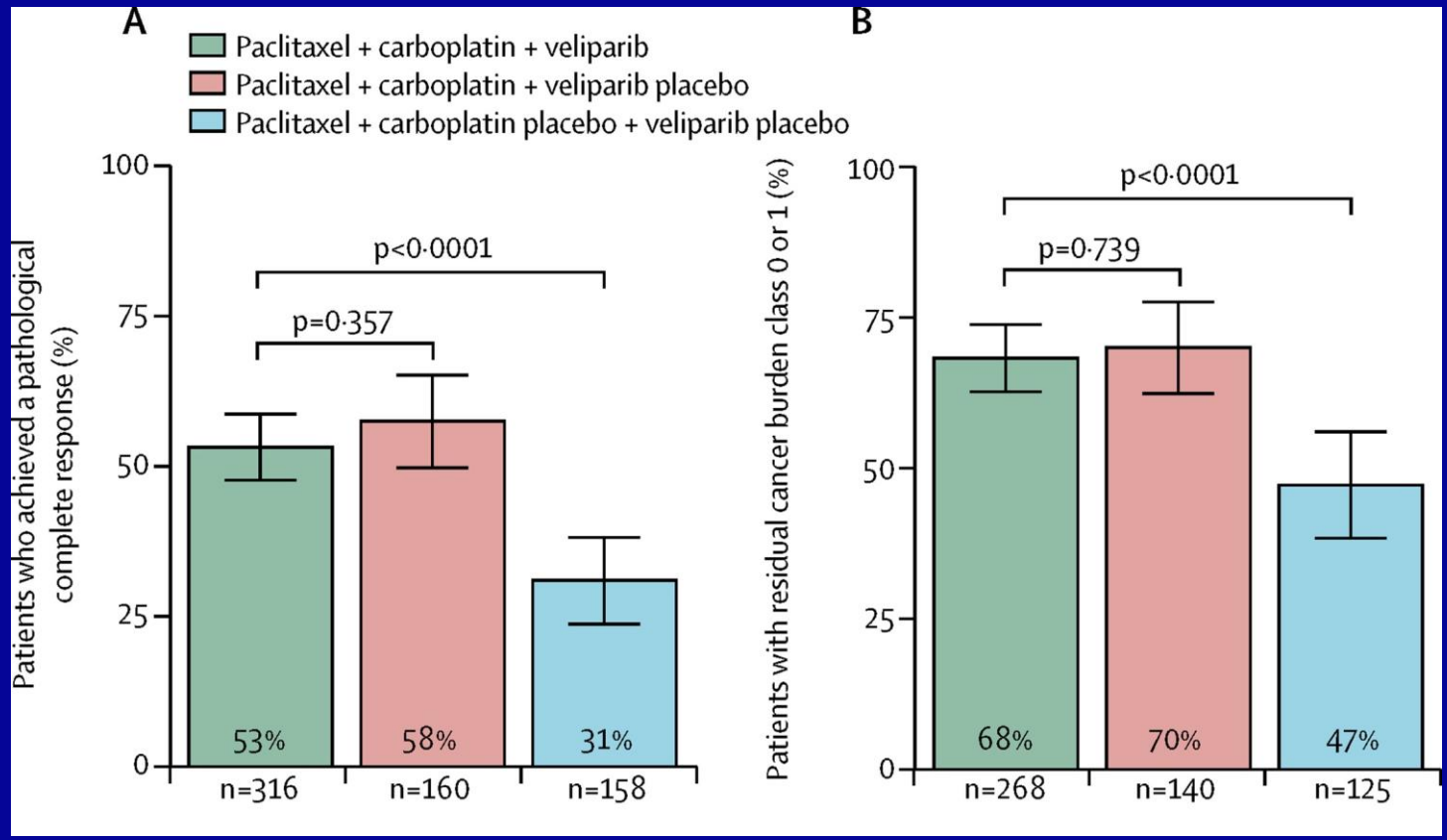
Implementation In Randomized Clinical Trials

I-SPY2 : Adaptive Randomization Phase 2. Addition of Veliparib and Carboplatin to Weekly Paclitaxel



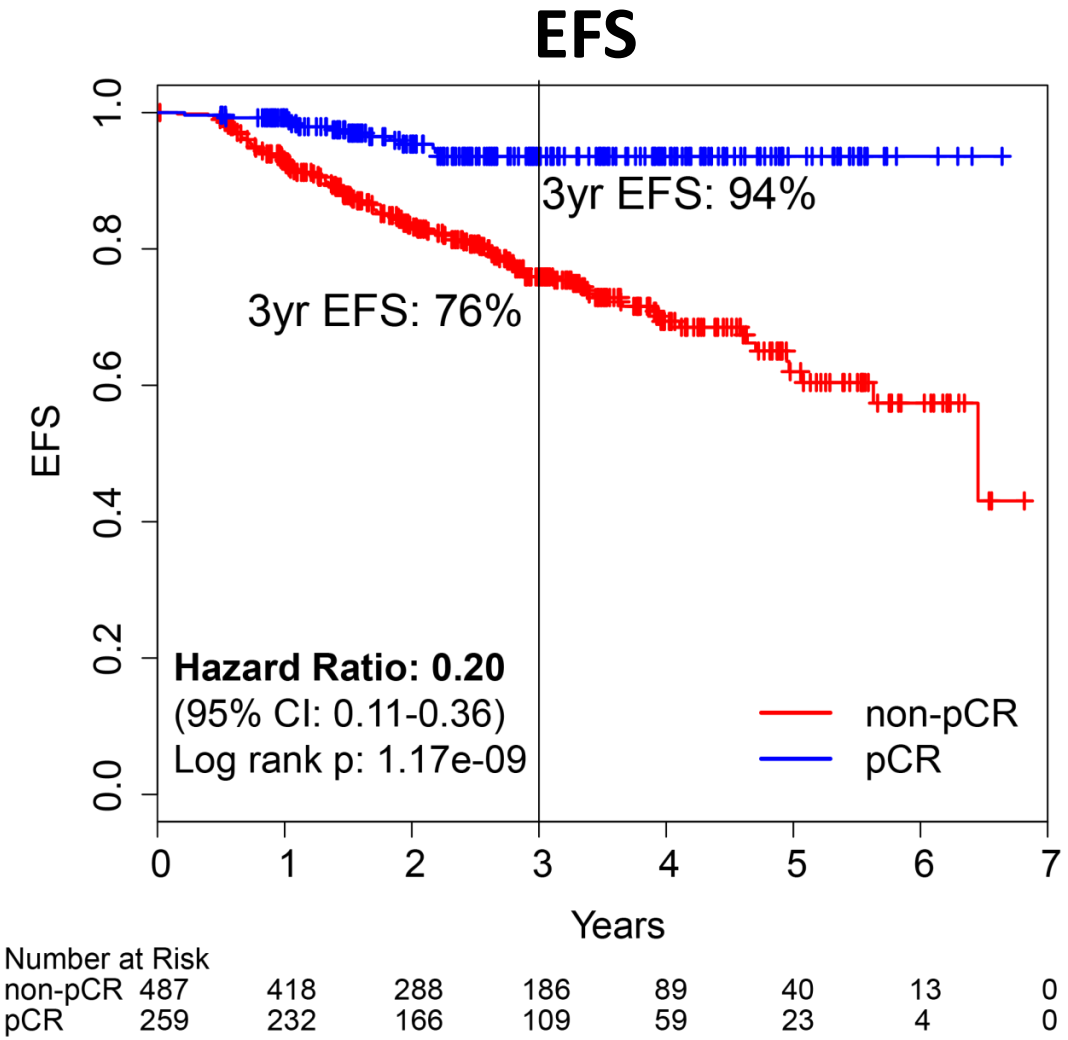
Rugo H, et al. NEJM 2016;375:23-34

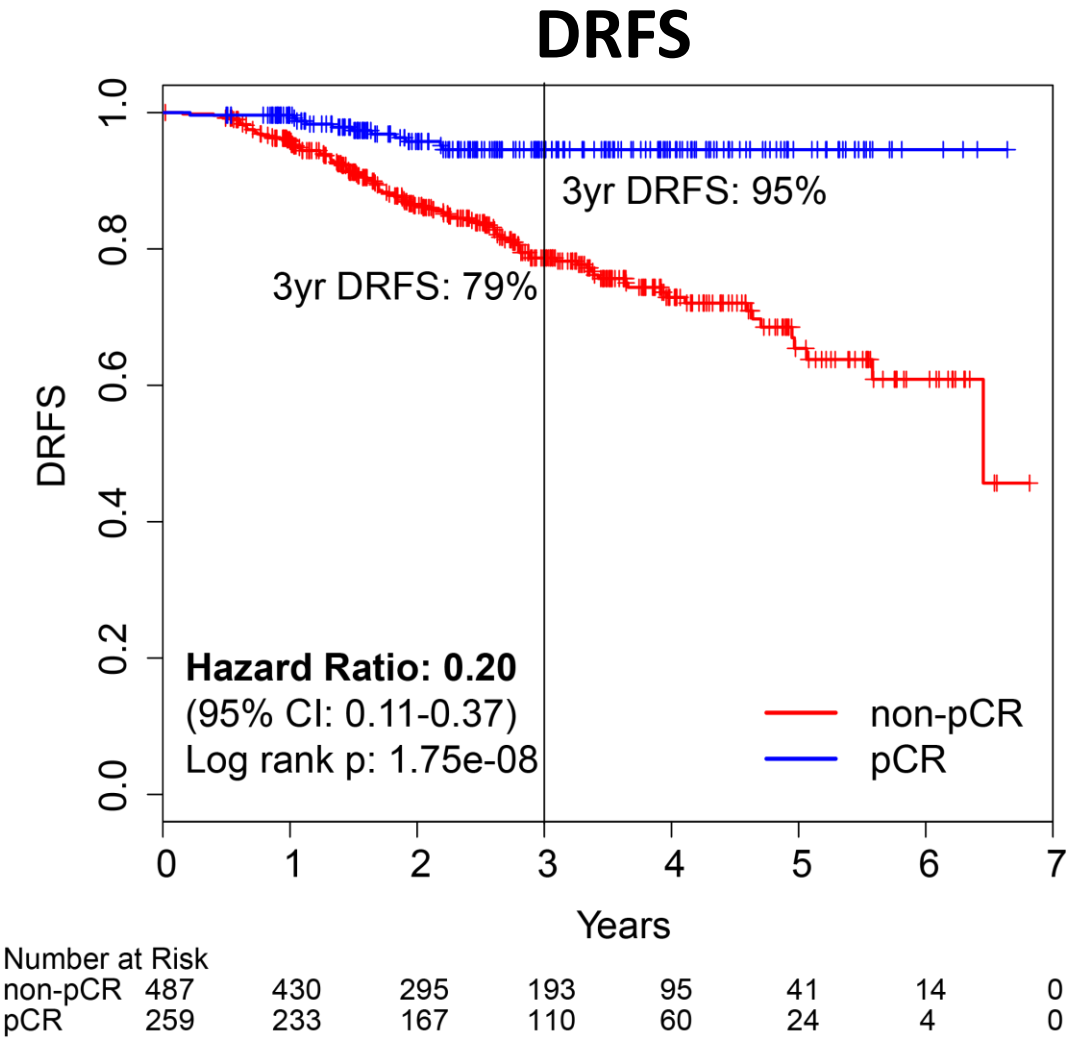
Brightness Trial (AFT8): Randomized Phase 3. Addition of Veliparib and Carboplatin, or Carboplatin to Weekly Paclitaxel



Loibl S, et al. Lancet Oncol 2018;19:497-509

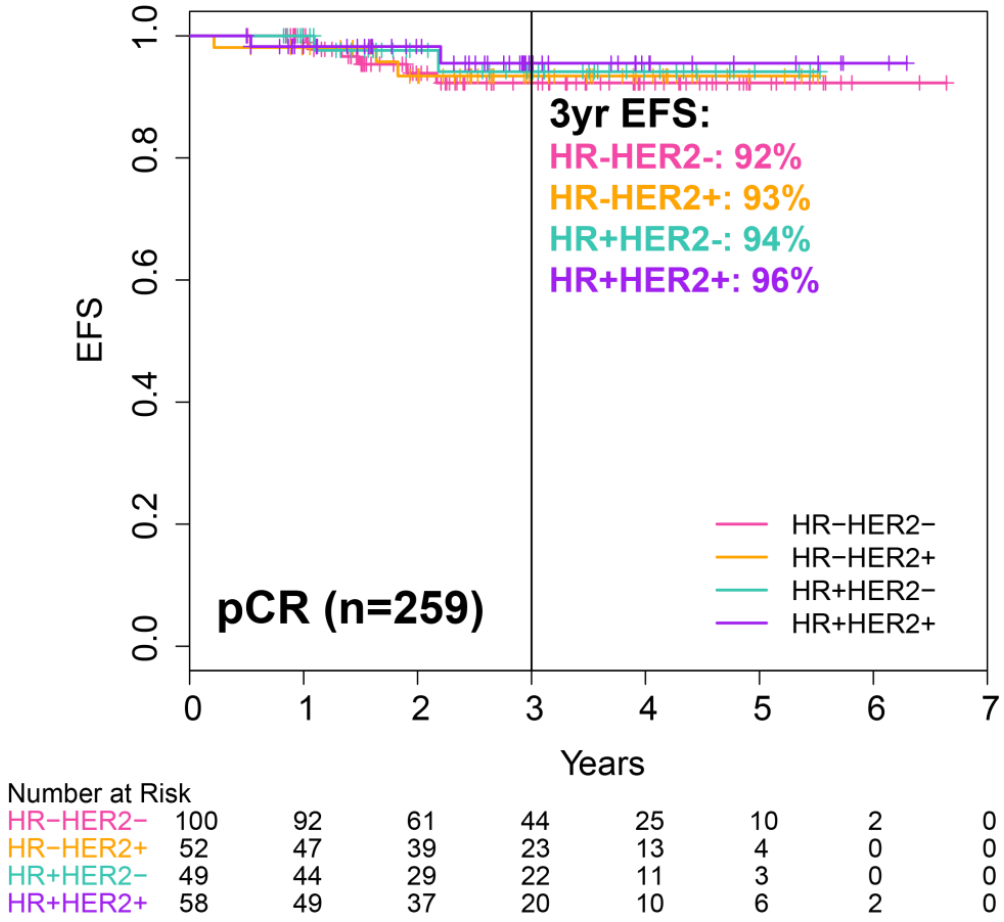
pCR is a highly significant predictor of EFS and DRFS





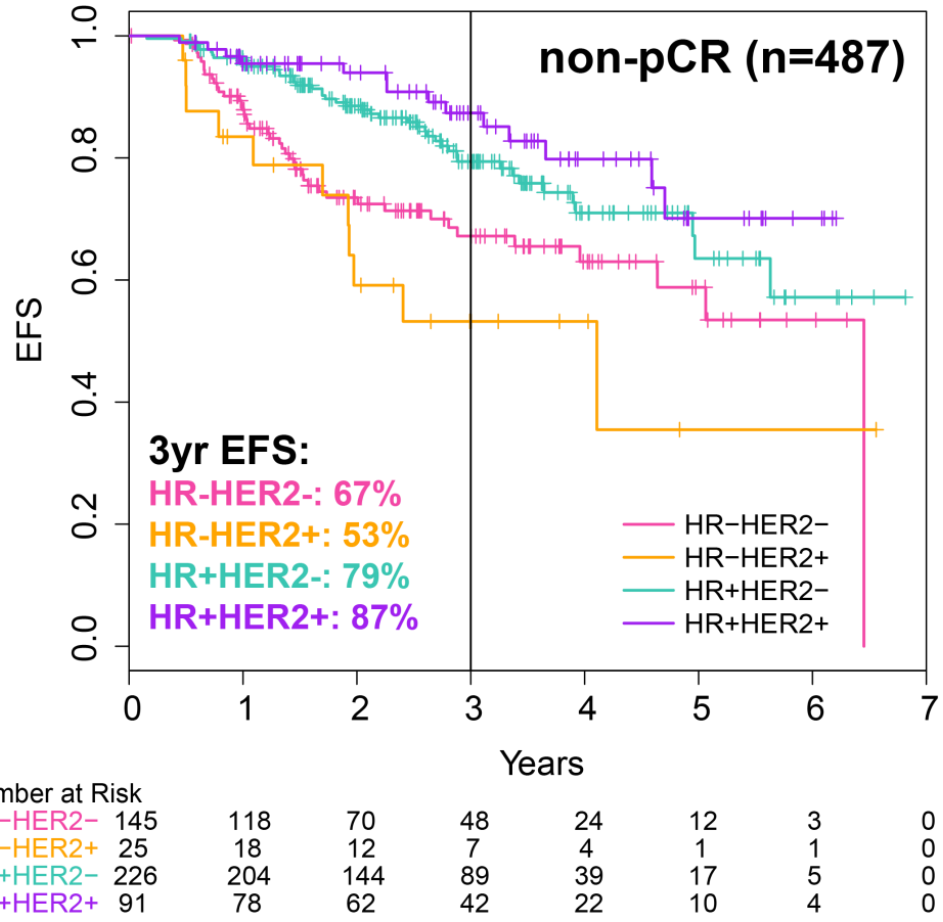
Yee D. et al. San Antonio Breast Cancer Symposium, Dec 5-9, 2017

EFS by pCR & non-pCR, by Subtype



Number at Risk

HR−HER2−	100	92	61	44	25	10	2	0
HR−HER2+	52	47	39	23	13	4	0	0
HR+HER2−	49	44	29	22	11	3	0	0
HR+HER2+	58	49	37	20	10	6	2	0

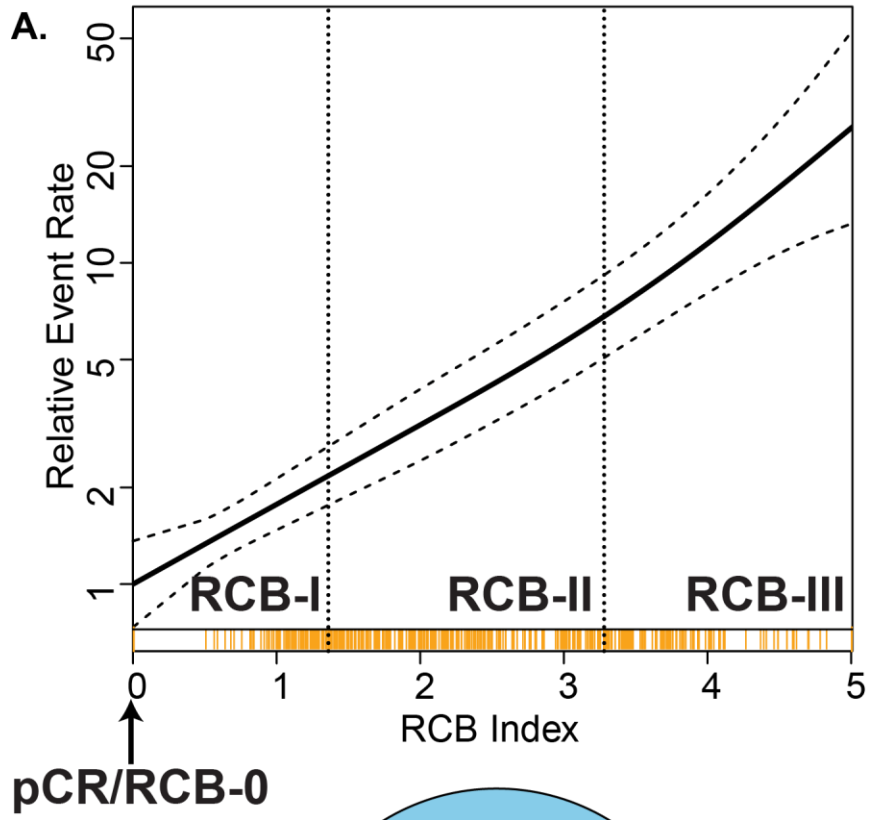


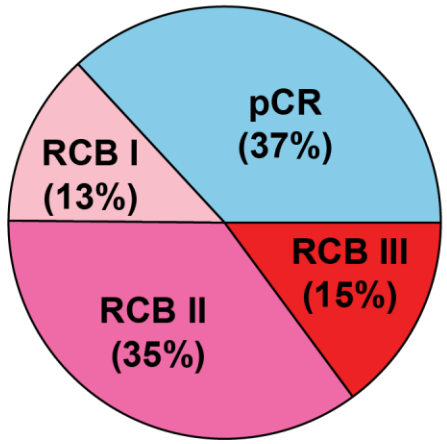
Number at Risk

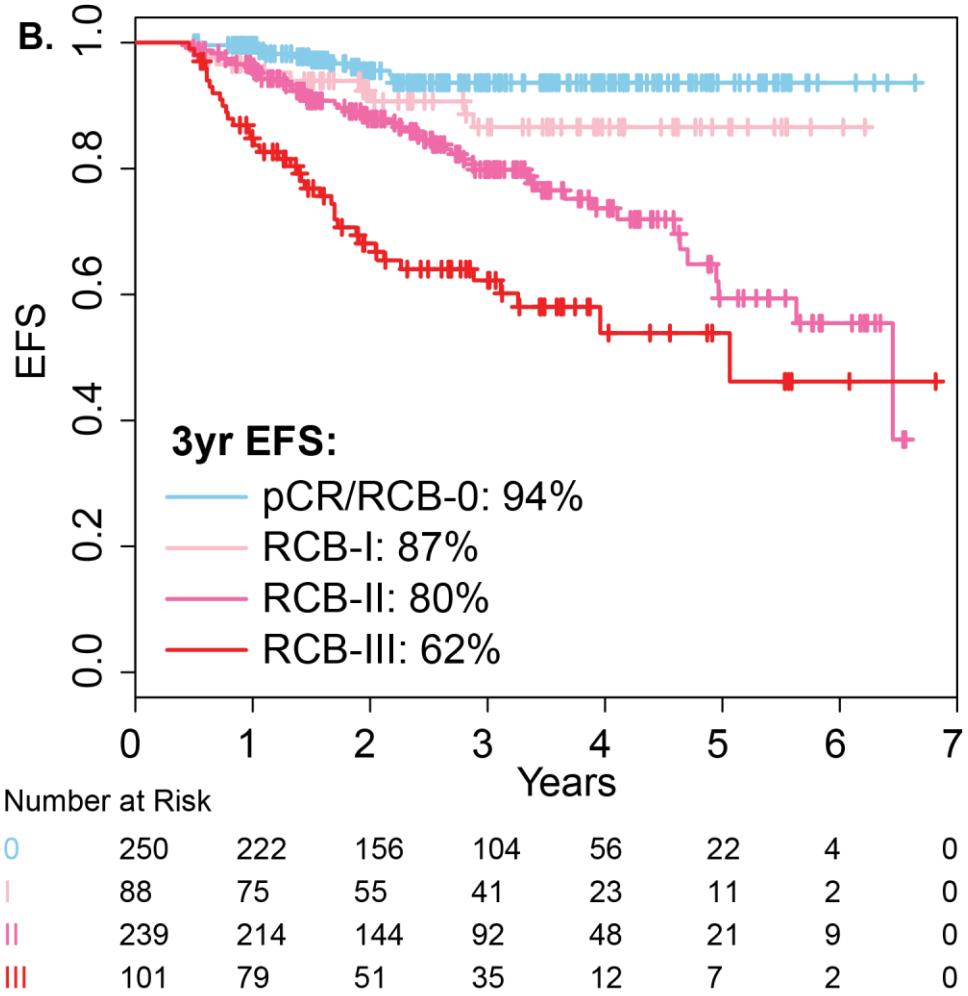
HR−HER2−	145	118	70	48	24	12	3	0
HR−HER2+	25	18	12	7	4	1	1	0
HR+HER2−	226	204	144	89	39	17	5	0
HR+HER2+	91	78	62	42	22	10	4	0

Yee D. et al. San Antonio Breast Cancer Symposium, Dec 5-9, 2017

Residual Cancer Burden in I-SPY2 (Site Pathologists' Reporting)

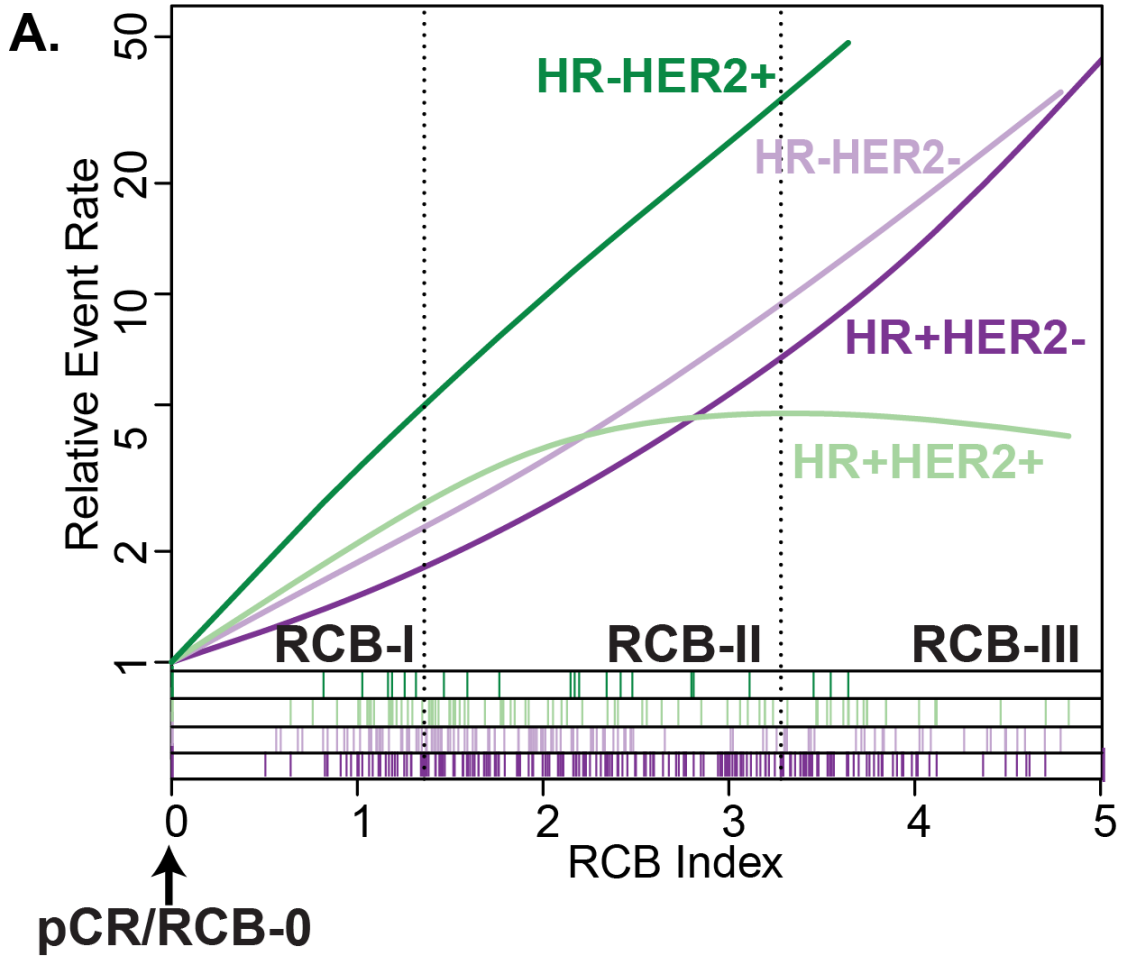






Symmans WF. et al. ASCO, 2018, abstract 520

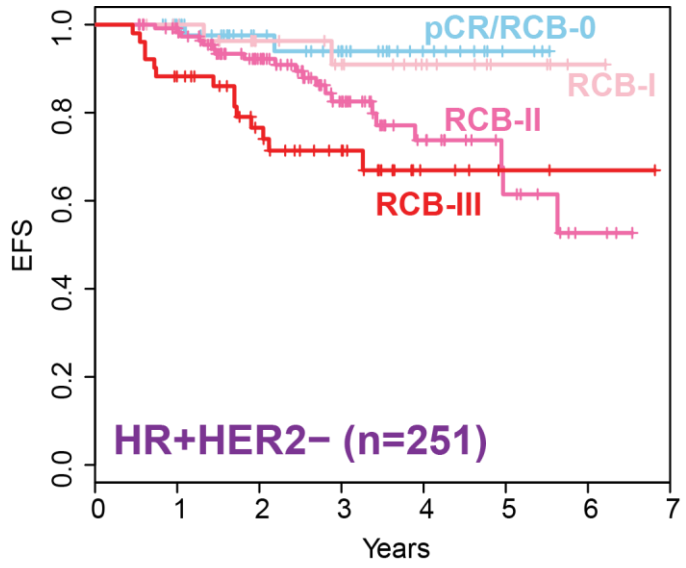
Relative Risk According to RCB Index Within Subtypes

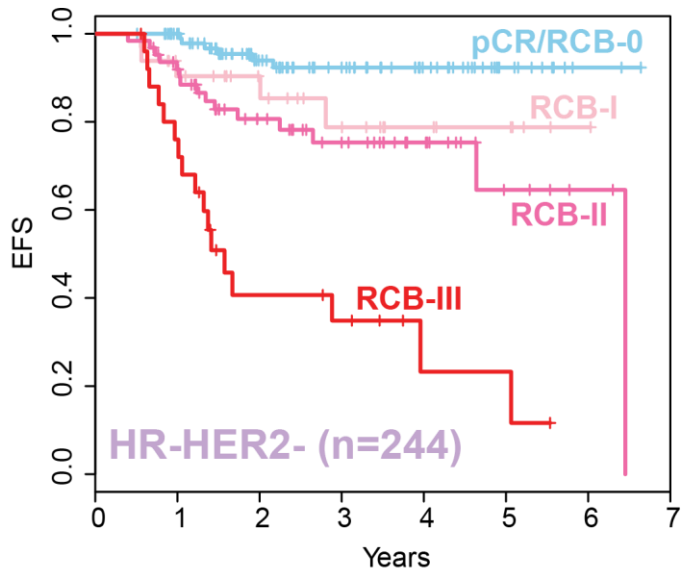


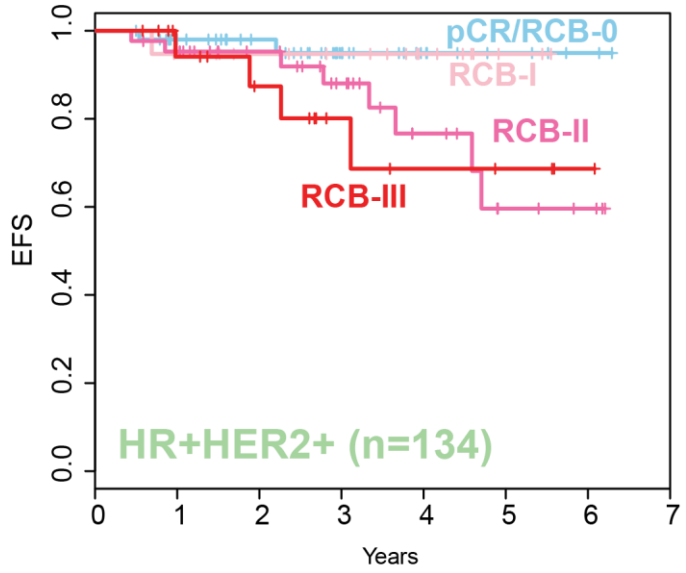
Relative risk of relapse within 3 years in breast cancer subtypes, according to RCB index.

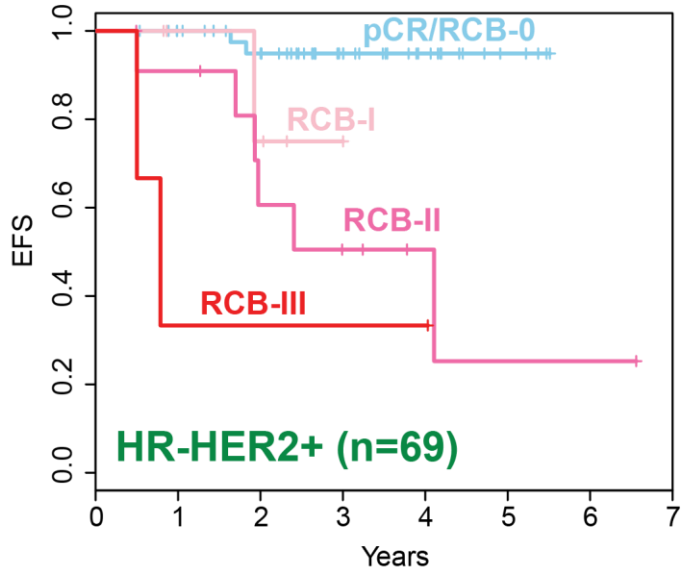
Symmans WF. et al. ASCO, 2018, abstract 520

Prognosis According to RCB Index Within Subtypes









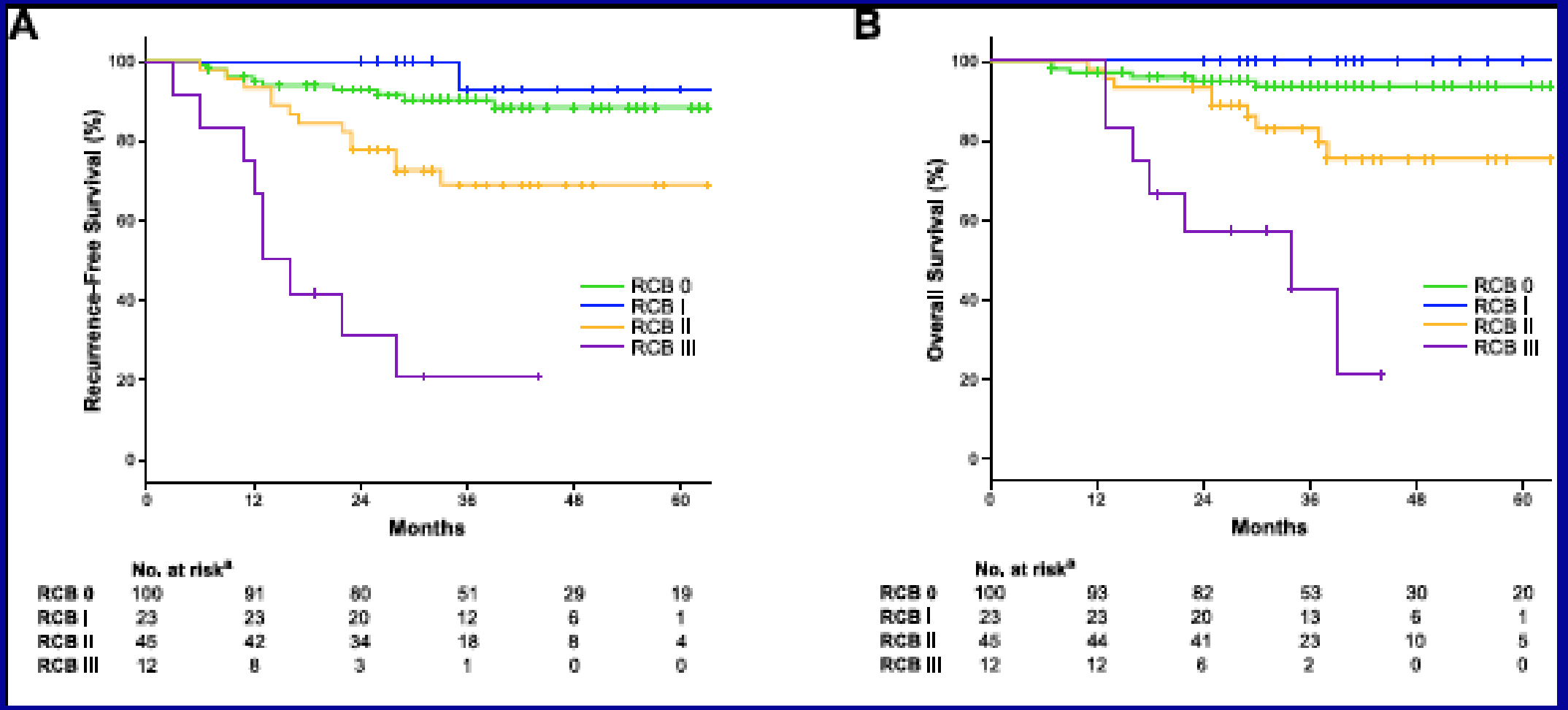
I-SPY2 TRIAL

Symmans WF. et al. ASCO, 2018, abstract 520

RCB in TNBC

Treated with Carboplatin and Docetaxel (6 cycles)

Recurrence Free Survival

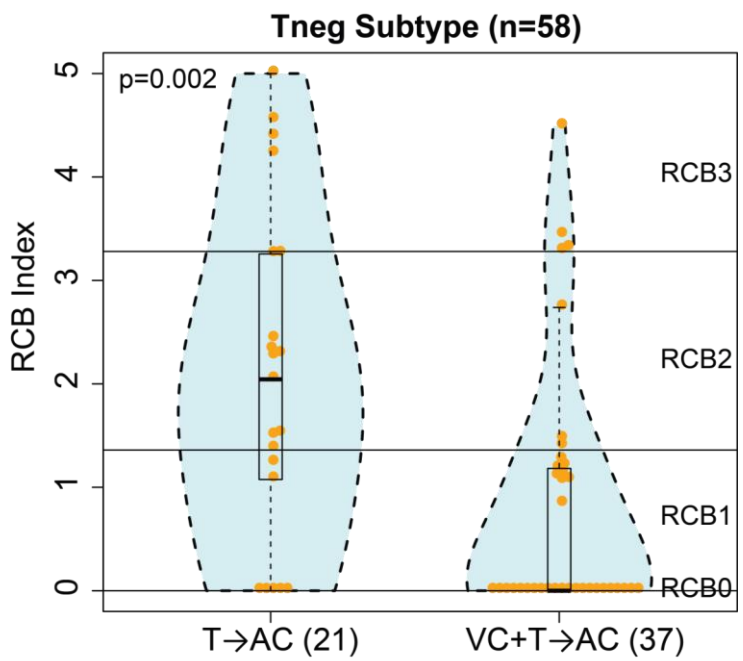


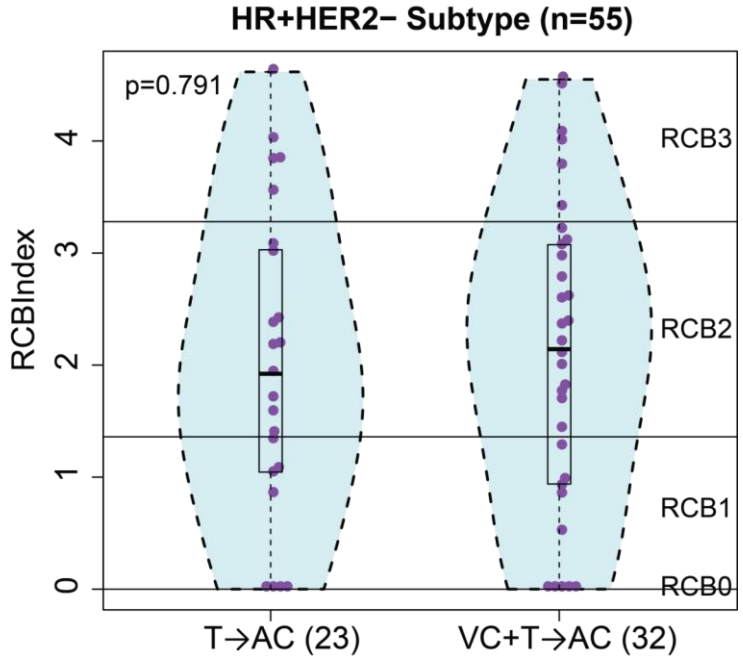
Overall Survival

Sharma et al. Clin Cancer Res 2018; epub 2018-08-01

Comparison In A Phase II Randomized Trial

I-SPY2 Trial: Addition of Veliparib and Carboplatin to Weekly Paclitaxel





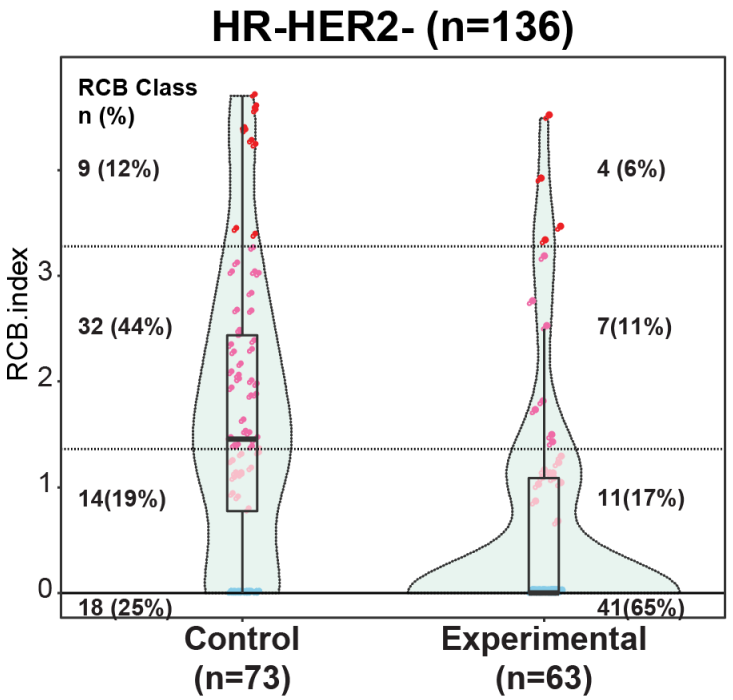
Response Endpoint

pCR	OR = 4.56, p = 0.013	OR = 0.88, p = 1
pCR/RCB-I	OR = 8.19, p = 0.0005	OR = 0.85, p = 1

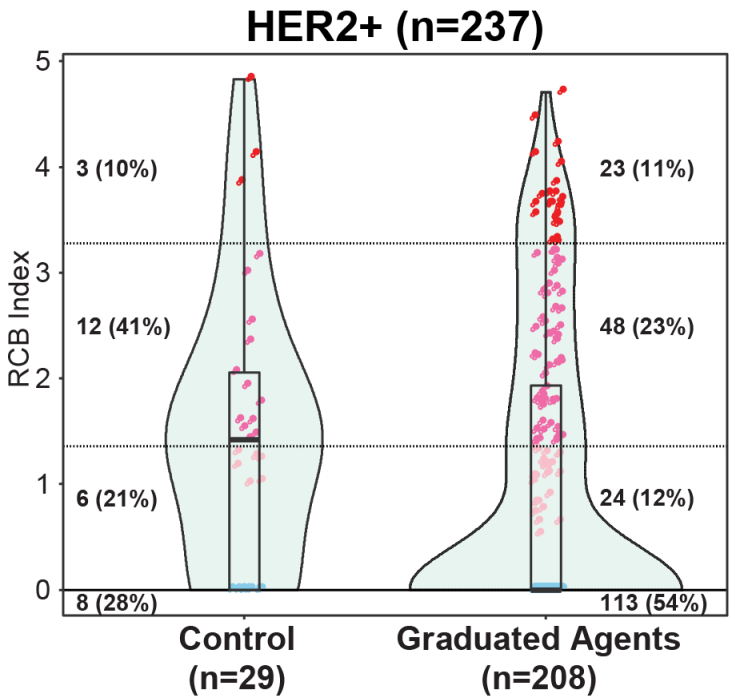
Liu MC, et al. SABCS, 2015, abstract P3-07-49

At A Glance: Responses In The Whole Trial

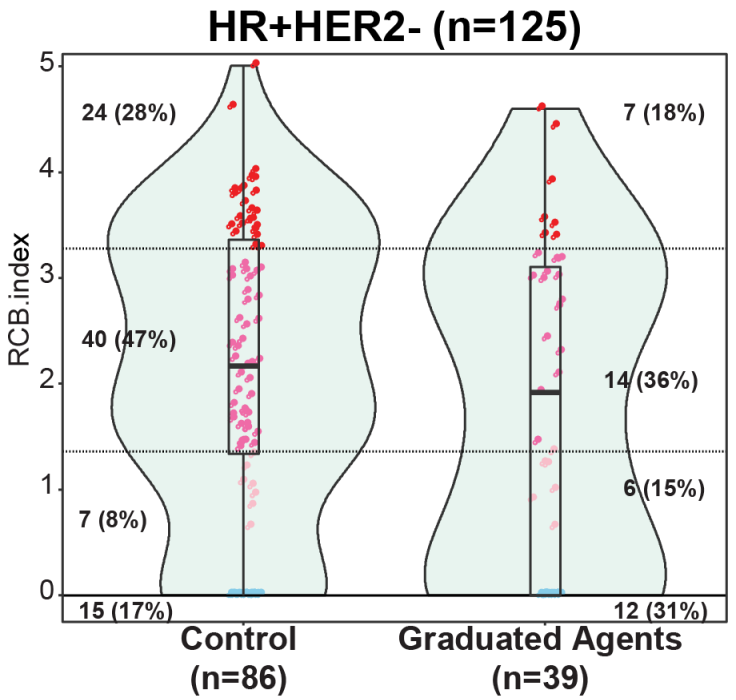
Comparison of RCB index distribution between graduated treatments and control treatment



Odds Ratio for achieving pCR:
5.61 (2.55-12.83)



Odds Ratio for achieving pCR:
3.11 (1.25-8.50)

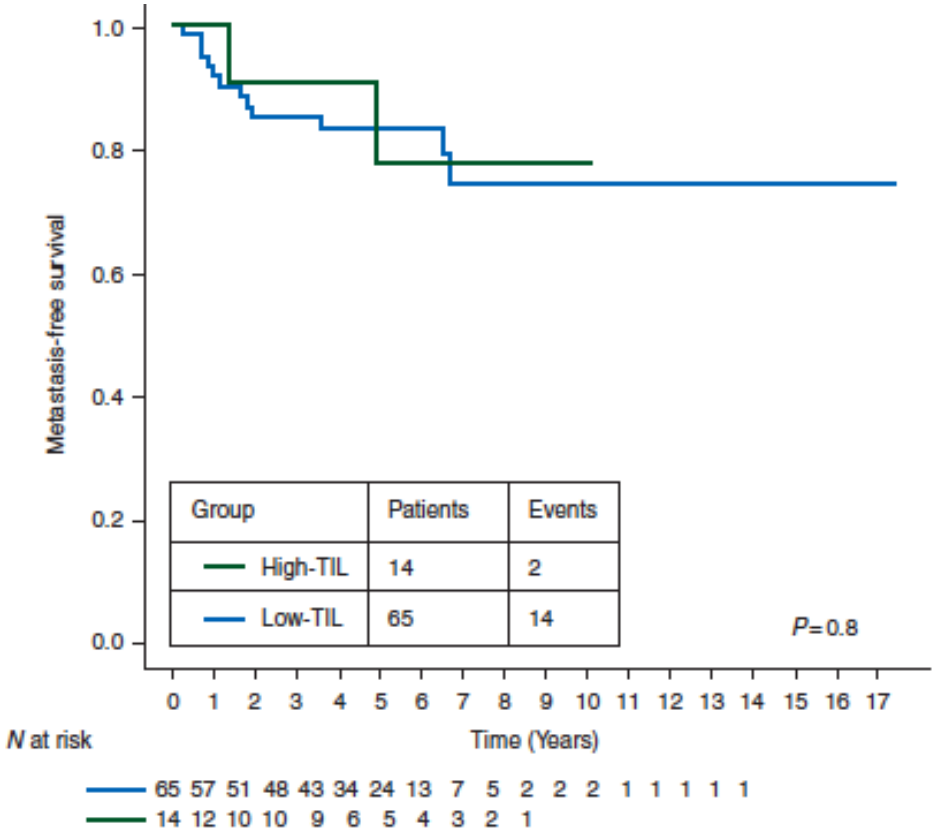


Odds Ratio for achieving pCR:
2.09 (0.78-5.51)

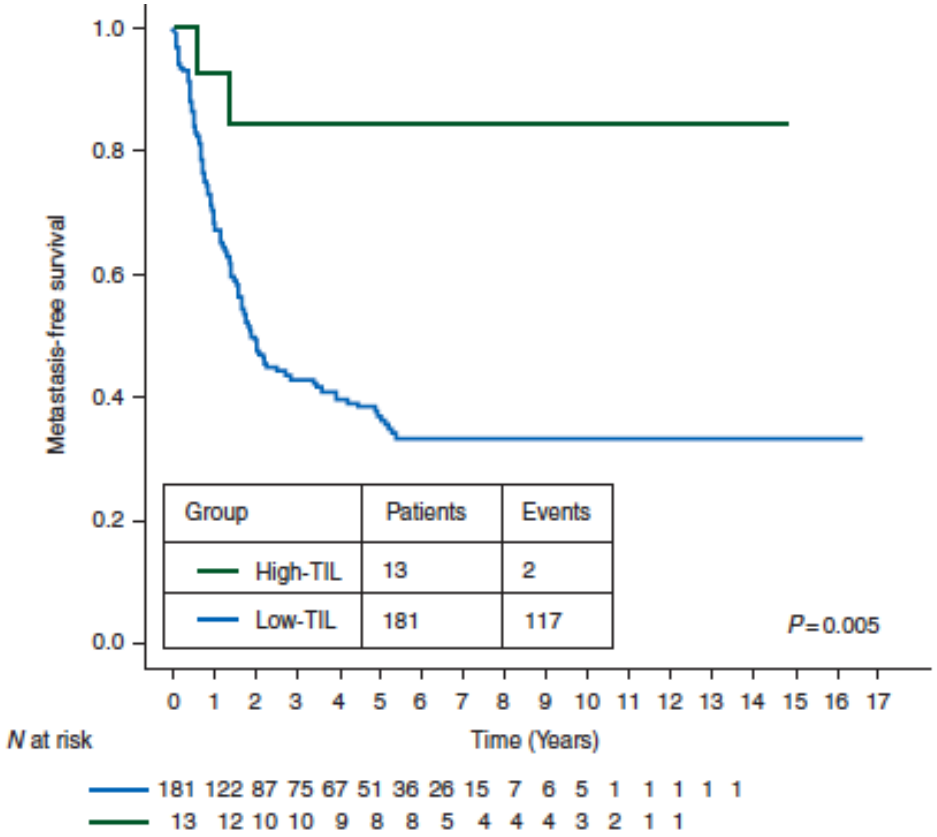
Symmans WF. et al. ASCO, 2018, abstract 520

TILs in the Specimen After Neoadjuvant Chemotherapy

ypT1 and ypN0

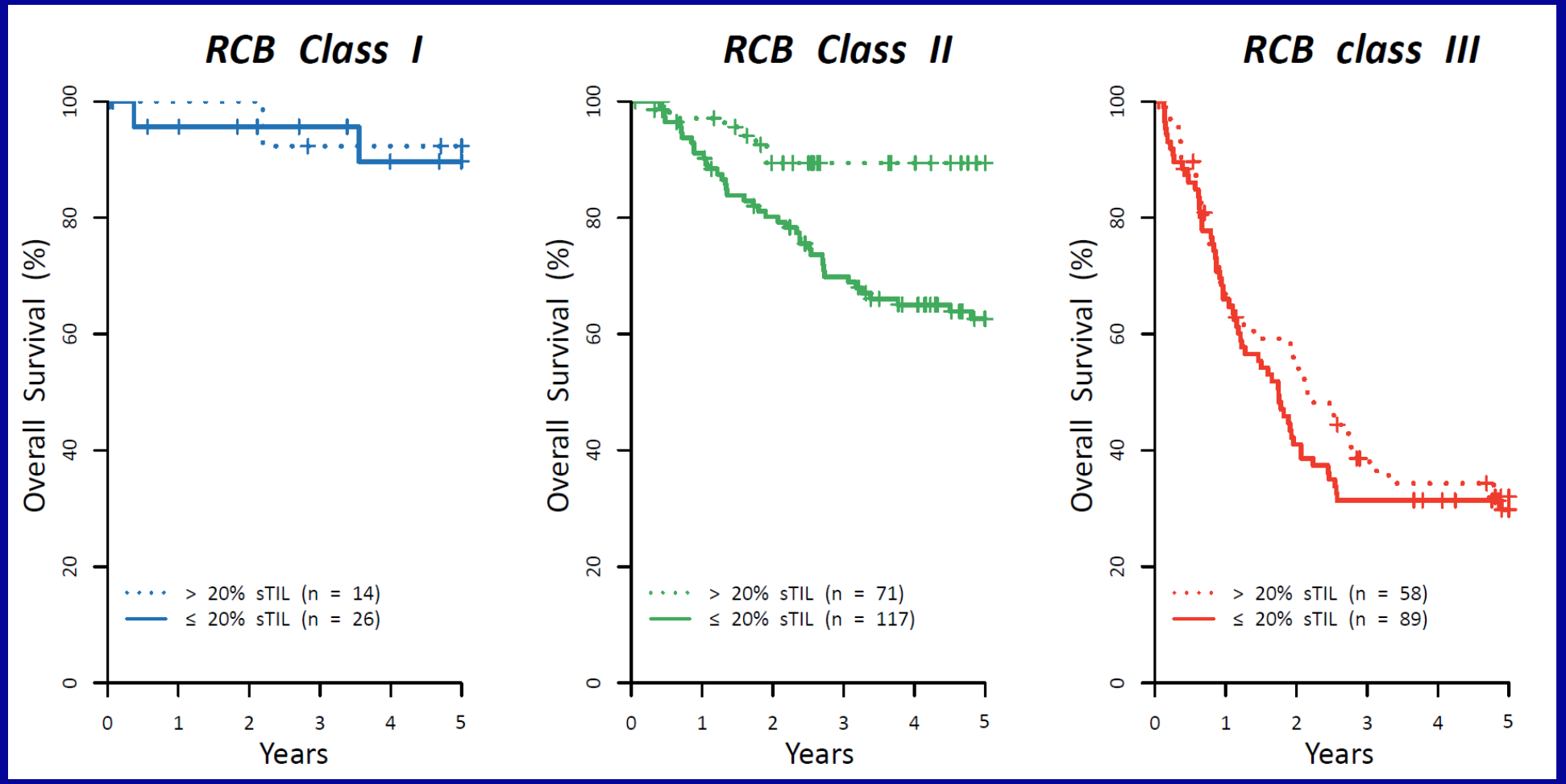


yp >T1 and/or ypN >0



Dieci, et al. *Ann Oncol* 2014; 25: 611–18

TILs in Residual TNBC



Luen et al. ASCO abstract. 2018

Recommendations for standardized pathological characterization of residual disease for neoadjuvant clinical trials of breast cancer by the BIG-NABCG collaboration

V. Bossuyt[1*], E. Provenzano[2], W. F. Symmans[3], J. C. Boughey[4], C. Coles[5], G. Curigliano[6], J. M. Dixon[7], L. J. Esserman[8], G. Fastner[9], T. Kuehn[10], F. Peintinger[11,12], G. von Minckwitz[13], J. White[14], W. Yang[15], S. Badve[16], C. Denkert[17], G. MacGrogan[18], F. Penault-Llorca[19], G. Viale[20] & D. Cameron[21] of the Breast International Group-North American Breast Cancer Group (BIG-NABCG) collaboration

Standardization of pathologic evaluation and reporting of postneoadjuvant specimens in clinical trials of breast cancer: recommendations from an international working group

Elena Provenzano[1], Veerle Bossuyt[2], Giuseppe Viale[3], David Cameron[4], Sunil Badve[5], Carsten Denkert[6], Gaëtan MacGrogan[7], Frédérique Penault-Llorca[8], Judy Boughey[9], Giuseppe Curigliano[10], J Michael Dixon[11], Laura Esserman[12], Gerd Fastner[13], Thorsten Kuehn[14], Florentia Peintinger[15,16], Gunter von Minckwitz[17], Julia White[18], Wei Yang[19] and W Fraser Symmans[20] on behalf of the Residual Disease Characterization Working Group of the Breast International Group-North American Breast Cancer Group (BIG-NABCG) collaboration

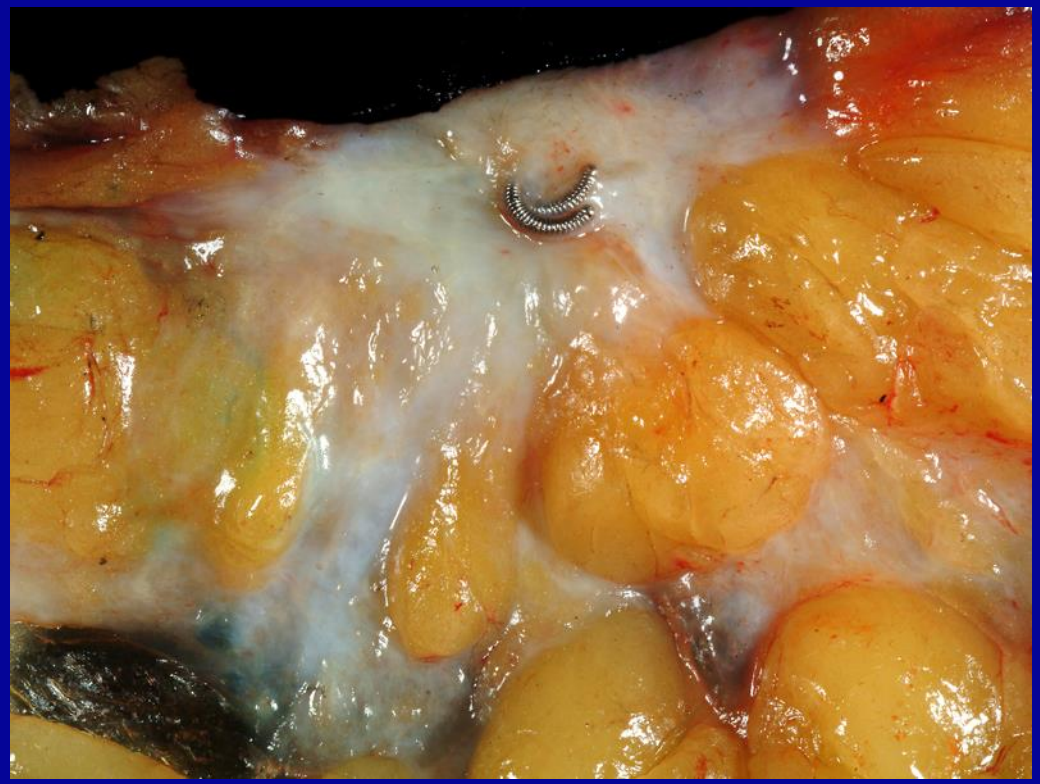
Recommendation

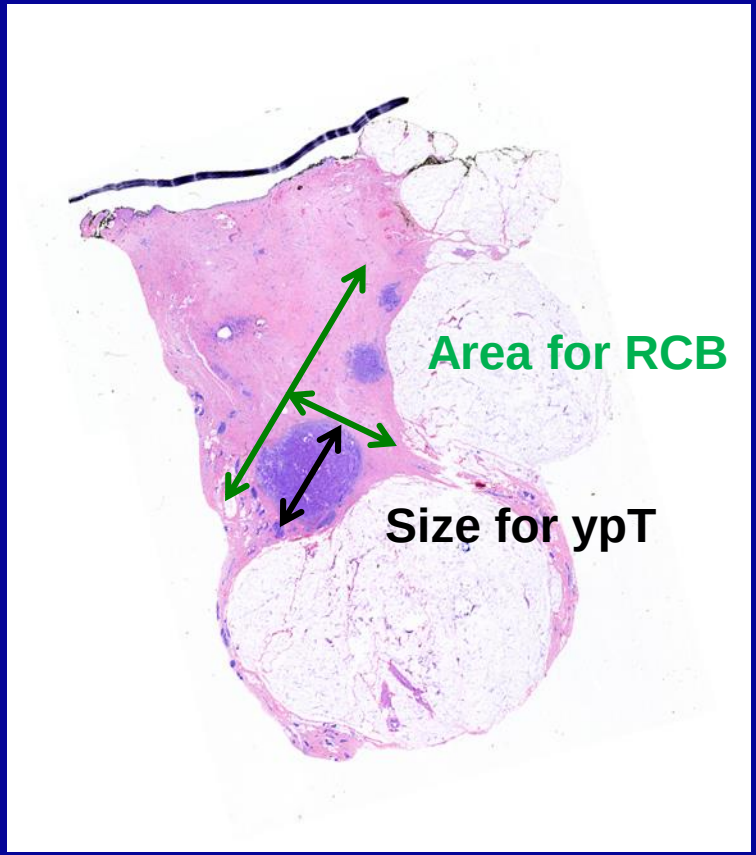
Provide the following information:

1. **pCR (ypT0 ypN0 and ypT0/is ypN0) *versus* residual disease,**
2. **ypT and ypN Stage using the current AJCC/UICC staging system,**
3. **Residual cancer burden (RCB)**

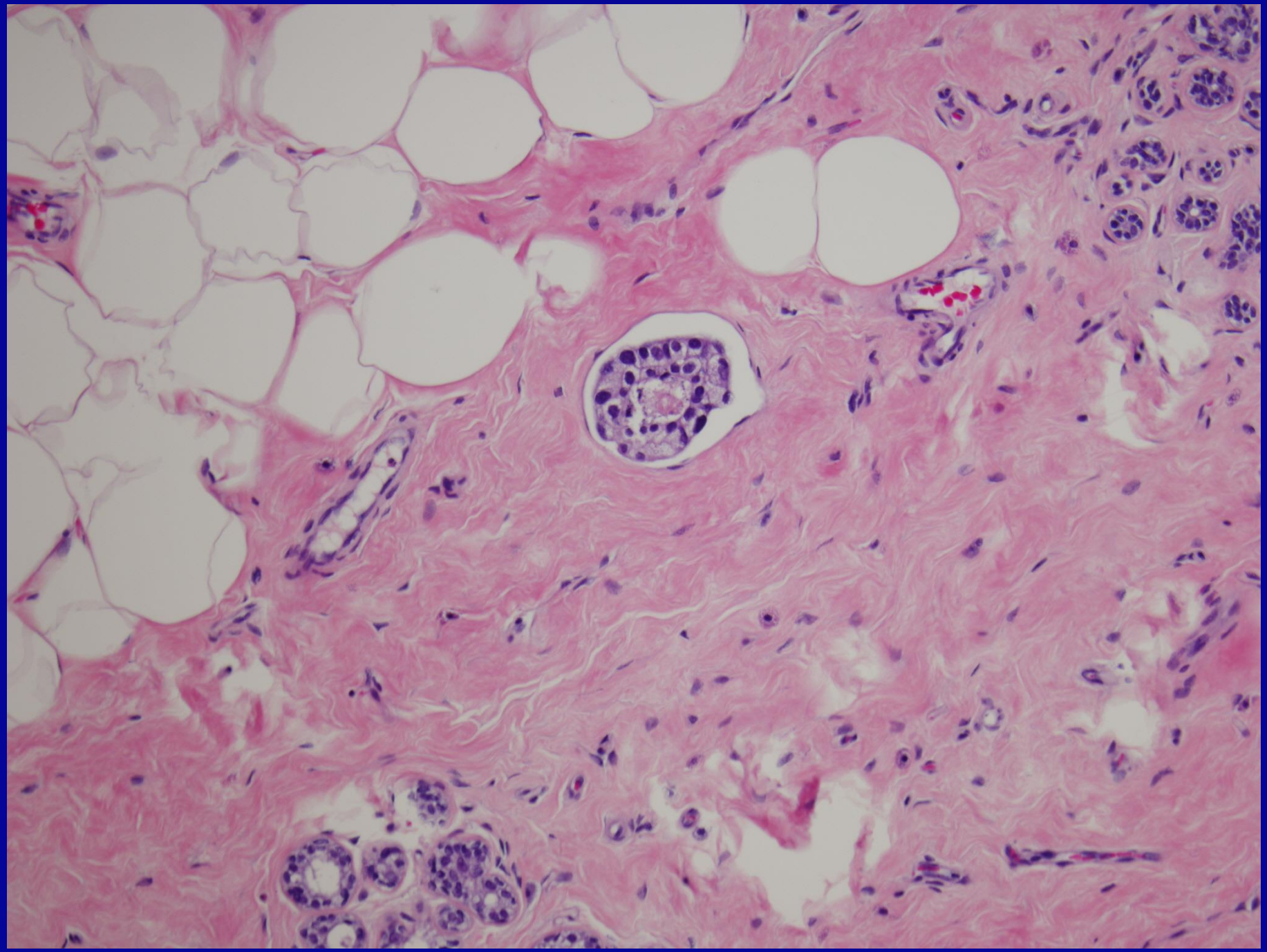
A single standardized approach to macroscopic and microscopic pathologic examination makes it easy to reliably provide all 3 results!

Defining The Size Of The Residual Invasive Cancer





Same principle for measuring metastatic cancer in lymph nodes



*yp*Stage and RCB Are Different Systems

Main differences for interpreting residual disease in the breast

1. **Primary tumor dimensions:**
   - **longest continuous invasive cancer is measured to interpret *yp*T for Stage**
   - **overall extent of invasive cancer (including gaps) is used to measure tumor area for RCB**

2. **Lymphovascular invasion (LVI):**
   - **not measured for *yp*T (so can be *yp*T0 if only LVI) but cannot be called pCR**
   - **Interpreted as residual invasive cancer for RCB**

3. **Cellularity of residual invasive cancer:**
   - **not considered for Stage**
   - **AVERAGE CELLULARITY across the residual AREA of invasive disease for RCB**

*yp*Stage and RCB Are Different Systems

Main differences for interpreting residual disease in the regional lymph nodes

1. **Dimension of nodal metastases:**
   - **longest continuous metastatic deposit is interpreted for *yp*N**
   - **overall length of largest metastasis (inclusive of gaps) used for RCB and this would include extranodal extension**

2. **Isolated tumor cells < 0.2 mm (ITC):**
   - ***yp*N0(i+), but cannot be called pCR**
   - **interpreted as metastatic cancer for RCB, and measurement used if largest metastasis**

Excerpt From Our CAP Synoptic Report …

Lymph Nodes
- **Total number of lymph nodes examined (sentinel and non-sentinel):** 28
- **Number of sentinel lymph nodes examined:** 0
- **Lymph node involvement**
  - **Number of lymph nodes with macrometastases:** 1
  - **Number of lymph nodes with micrometastases:** 0
  - **Number of lymph nodes with isolated tumor cells:** 0
  - **Extranodal extension:** 0

RCB Index Input Variables
- **Dimensions of residual primary tumor area:** 100 mm X 80 mm
- **Percent cancer cellularity of residual tumor area:** 0.01%
- **Percent of residual cancer that is in situ:** 100%
- **Total number of lymph nodes with residual tumor:** 1
- **Size of largest metastasis:** 4.5 mm
- **RCB index computed value (optional):** 1.29
- **RCB class (optional):** RCB-I

Pathologic staging (pTNM)
- **Primary tumor (invasive carcinoma) (ypT):** ypT0is
- **Regional lymph nodes (ypN):** ypN1
  - **Additional modifiers:**
- **Distant metastasis (ypM):** ypMX

Online Educational Resources

Google Search Term: “RCB Breast” or “Residual Cancer Burden Breast”

Educational Videos – Macroscopic and Microscopic Evaluation

https://www.mdanderson.org/for-physicians/clinical-tools-resources/clinical-calculators/residual-cancer-burden.html

Protocol for Pathologists – Detailed SOP Document

https://www.mdanderson.org/education-and-research/resources-for-professionals/clinical-tools-and-resources/clinical-calculators/calculators-rcb-pathology-protocol2.pdf

Calibration of Percent Cellularity by Area – Computer Generated Examples

https://www.mdanderson.org/education-and-research/resources-for-professionals/clinical-tools-and-resources/clinical-calculators/calculators-cellularity-guide.pdf

Multigene Assays Foe Breast Cancer

Survival Risk of Breast Cancer in the Adjuvant Setting

The probability that micrometastatic disease ever existed, survived the entirety of treatments, and was able to reawaken and flourish

Original burden of cancer prior to treatment

Biology of the cancer – natural history prognosis

Sensitivity to chemotherapy

- e.g. extent of residual cancer after neoadjuvant treatment

Sensitivity to radiation therapy

Sensitivity to adjuvant endocrine therapy

- Duration, type, bisphosphonates, adherence

Constitutional health that promotes dormancy

- Immunity, metabolism, prevention agents, other

Survival Risk of Breast Cancer in the Adjuvant Setting

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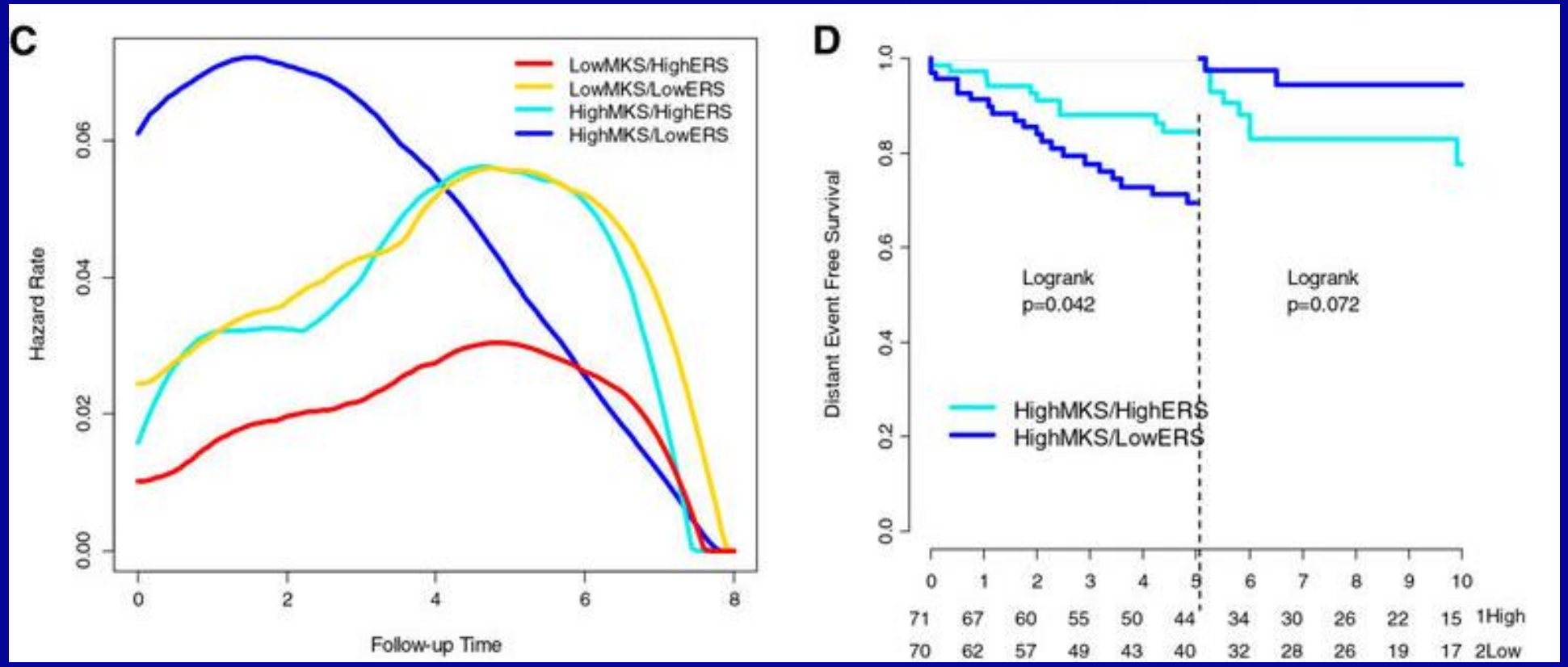
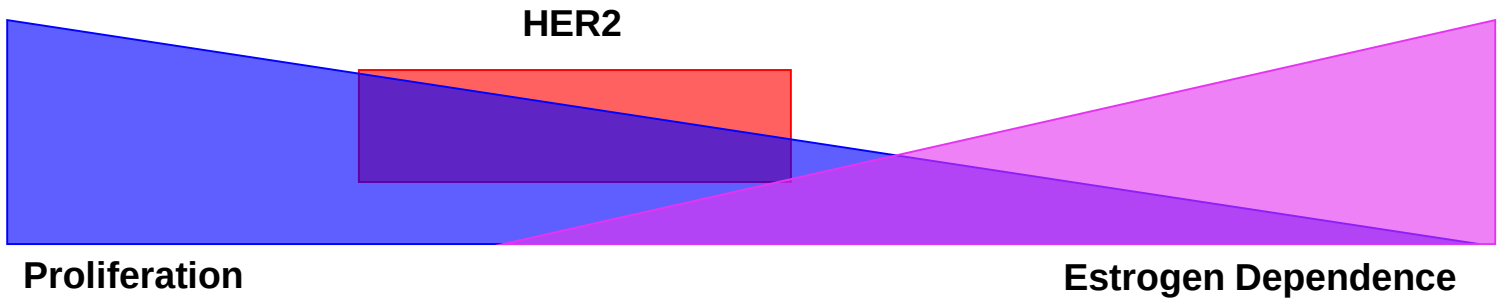
Time-Dependent Risk Related to Proliferation and to Endocrine Activity in HR+/HER2- Breast Cancers



Bianchini et al. Br Cancer Res 2013;15:R86



DIFFERENTIATION ⟶

Standard markers are generally sufficient for classification.

Multi-gene expression panels have favorable analytical characteristics that can:

- improve prognostic / theranostic performance.
- be useful when standard markers are indeterminate.

Different multi-gene expression panels have similar prognostic performance.

Have New Tests Met Clinical Need?

Therapeutic Decision (implied action)	HR+ / HER2-				HER2+				TNBC			
AJCC Stage	I	II	III	IV	I	II	III	IV	I	II	III	IV
Natural History Ultra-low Risk (no Rx)	✓ 1	-	-	-	-	-	-	-	?	-	-	-
Targeted Rx Alone Ultra-low Residual Risk (no CT)	✓ 5	?	-	-	-	-	-	-	-	-	-	-
CT + Targeted Rx Lower Residual Risk (standard of care)	✓ 5	-	-	-	✓ 1	✓ 1	✓ 1	✓ 1	-	-	-	-
CT + Targeted Rx High residual risk (clinical trial)	-	-	-	-	-	-	-	-	-	-	-	-
Susceptibility (prophylactic)	✓ 1				✓ 1				✓ 1			

Expression

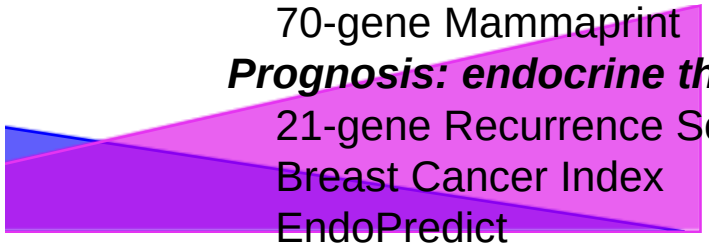
HER2 IHC/FISH

Germ line genetics

Modified from ASCO 2013 Annual Meeting: ASCO/ESMO Presidents' Symposium

PHENOTYPE

HR+/HER2-



Classification

PAM50 subtypes (& others)

Prognosis: natural history

70-gene Mammaprint

Prognosis: endocrine therapy

21-gene Recurrence Score

Breast Cancer Index

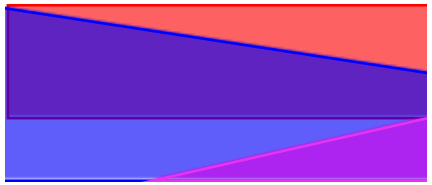
EndoPredict

Prosigna ROR score

Receptor Activity

SET index

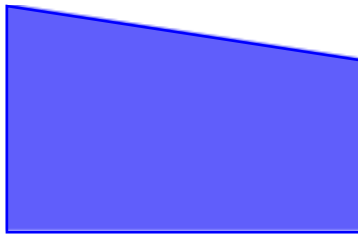
HER2+



Classification

HER2-like subtype of PAM50

TNBC



Classification

Basal-like subtype of PAM50

Vanderbilt subtypes

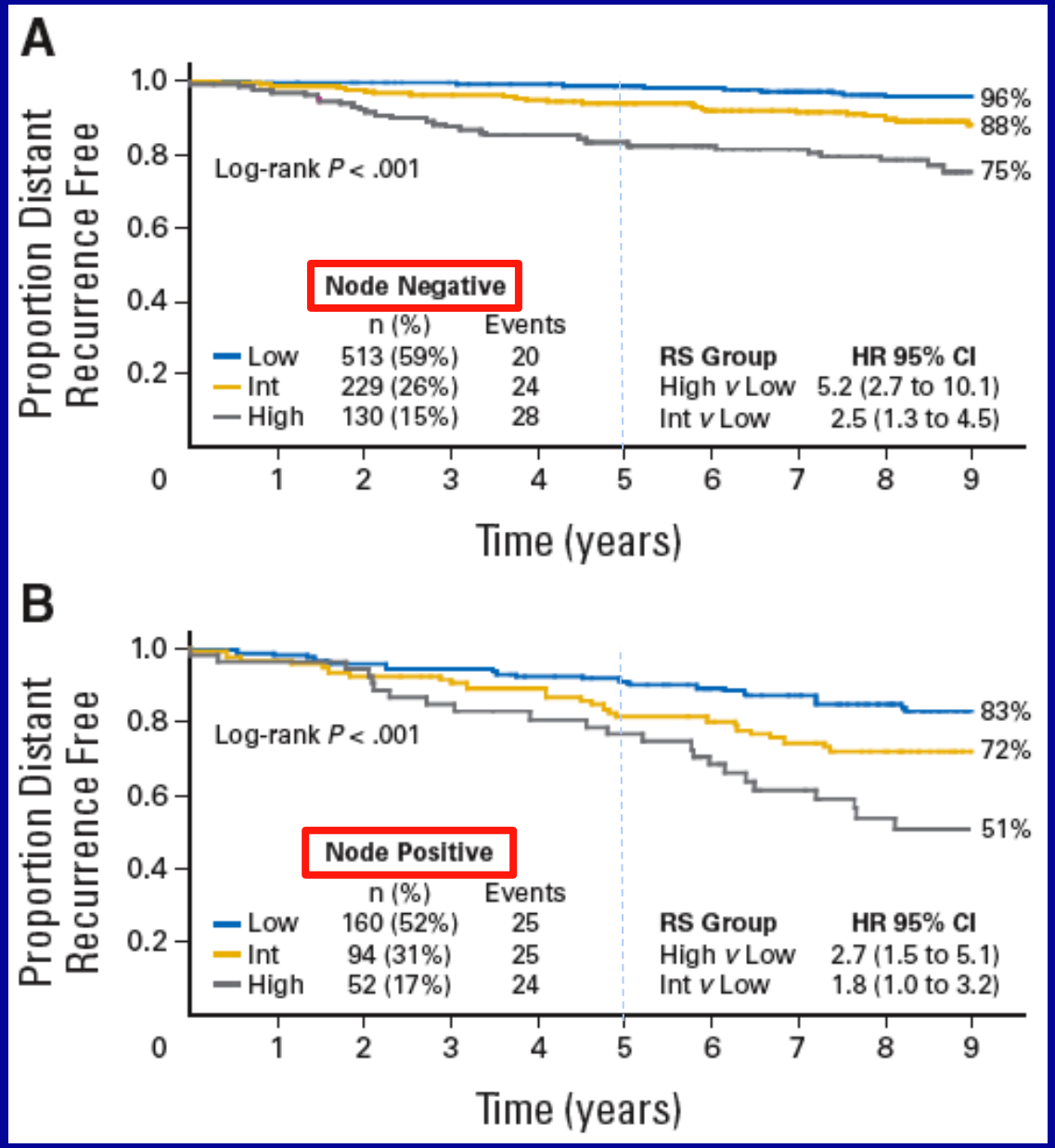
Prognosis: chemotherapy

Prognostic signature (immune)

Response & Prognosis

MDACC algorithm, Myriad HRD assay

21-Gene Recurrence Score: Tamoxifen or Anastrozole



Postmenopausal women

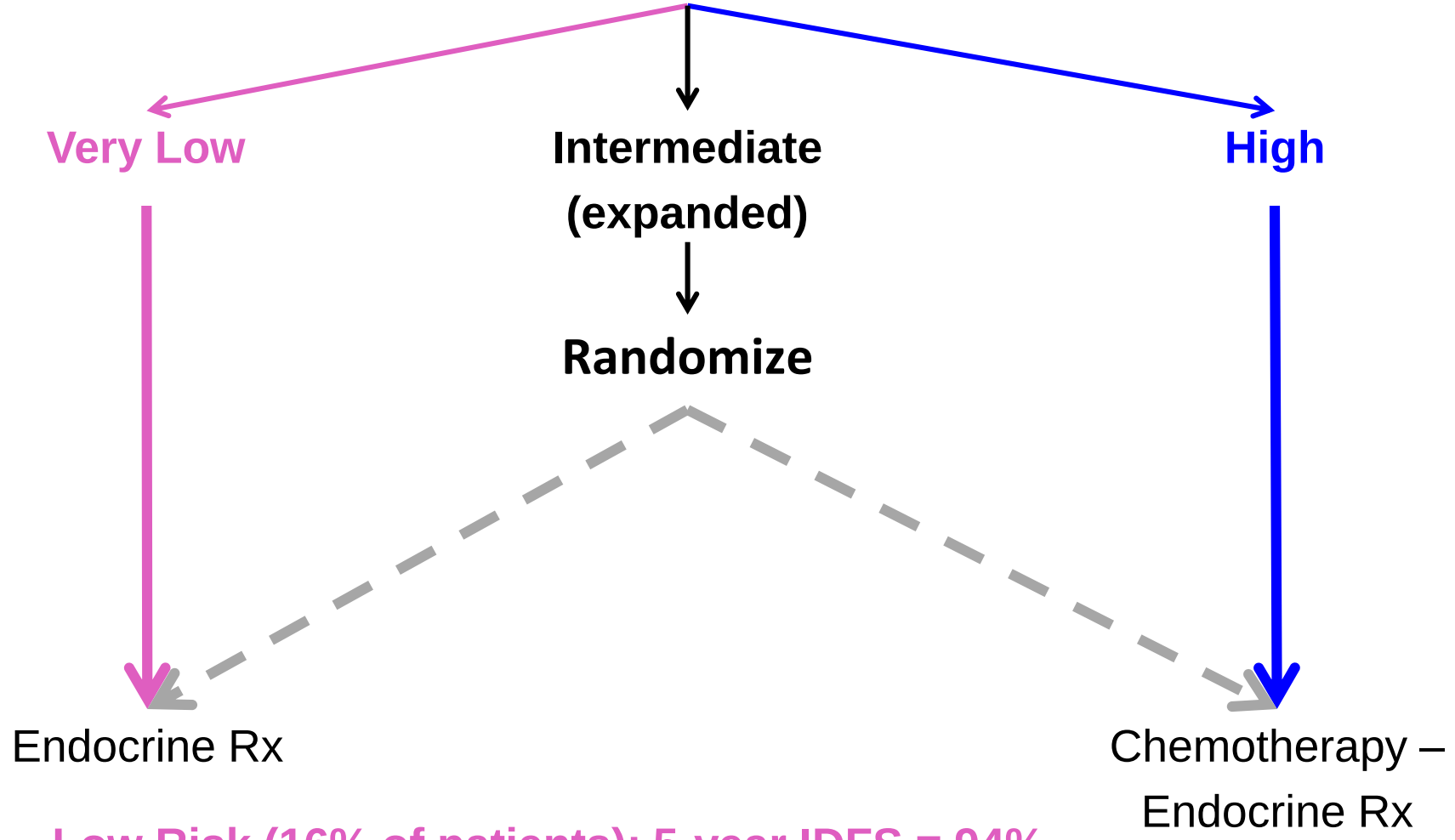
Nodal Status Matters

Hazard ratio for RS group was adjusted for tumor size, grade, age, and treatment

Dowsett et al JCO 2010;28:1829-34

TAILORx Trial

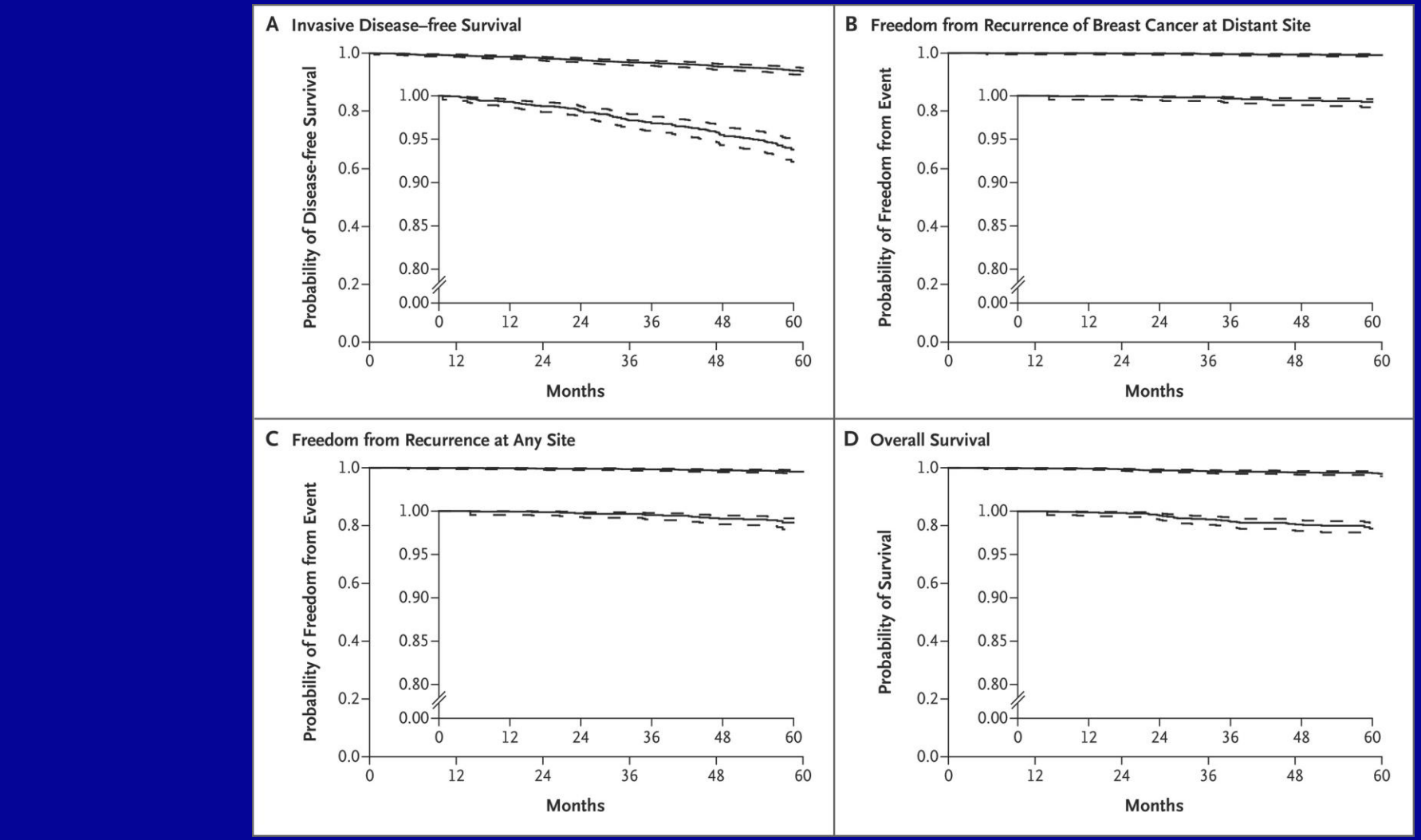
Recurrence Score in ER+, LN-



Low Risk (16% of patients): 5-year IDFS = 94%

Sparano et al NEJM 2015;373:2005-14.

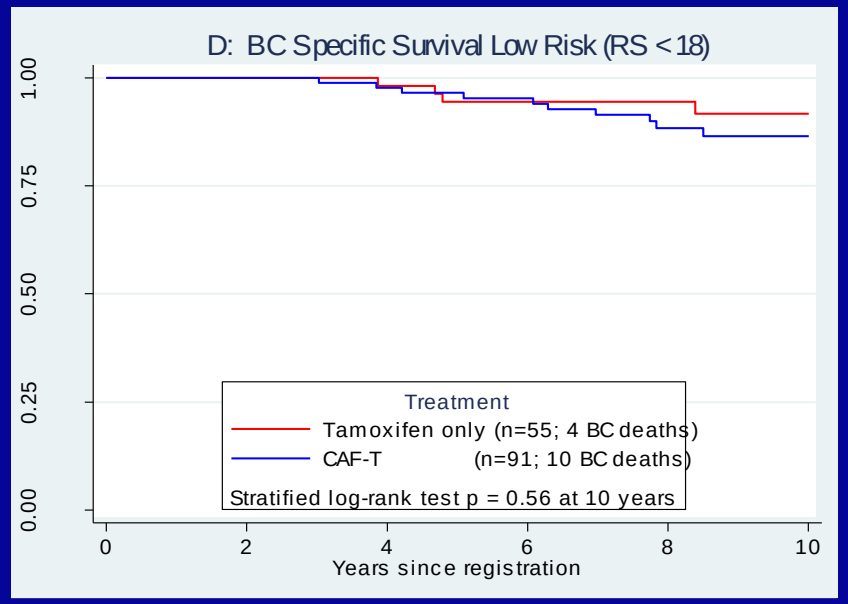
Kaplan–Meier Estimates in the Analyses of Invasive Disease–free Survival, Freedom from Recurrence of Breast Cancer at a Distant Site, Freedom from Recurrence at Any Site, and Overall Survival.

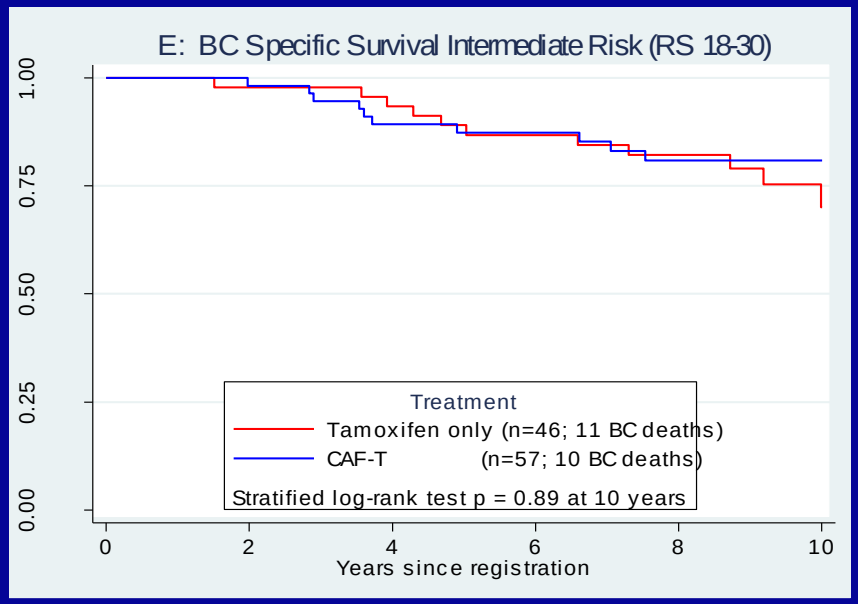


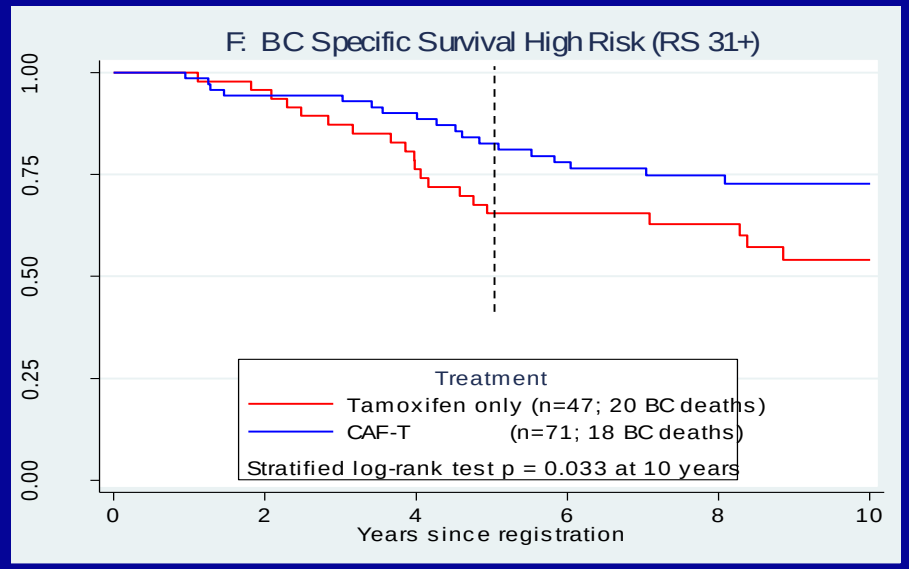
Sparano JA et al. N Engl J Med 2015;373:2005-2014

The NEW ENGLAND JOURNAL of MEDICINE

Sequential Chemo-endocrine Therapy, Node-positive: SWOG-8814 Results for Recurrence Score







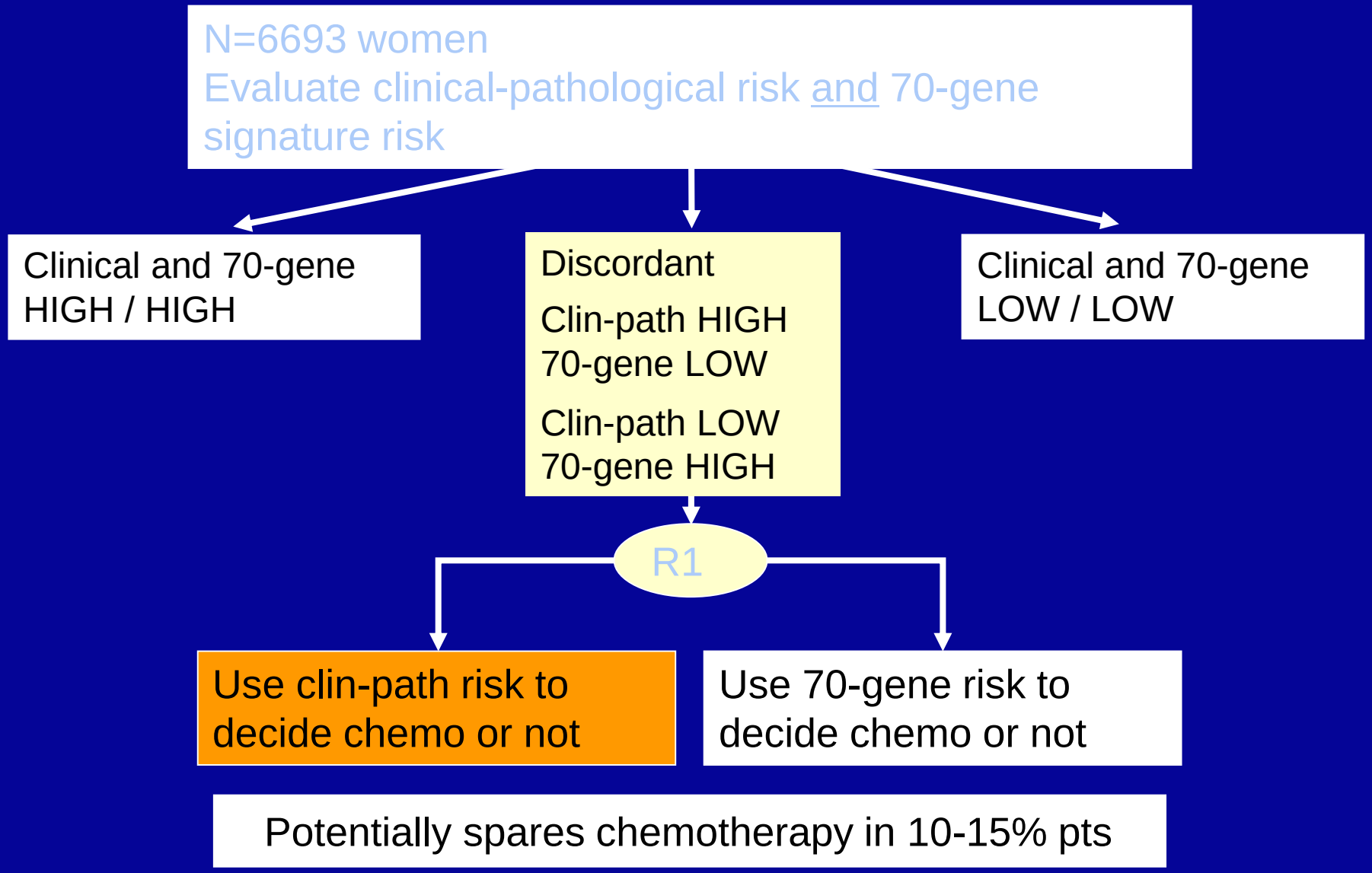
Interaction p = 0.021

GHI unable to identify reliable multi-gene panel for chemoprediction using RNAseq from FFPE samples

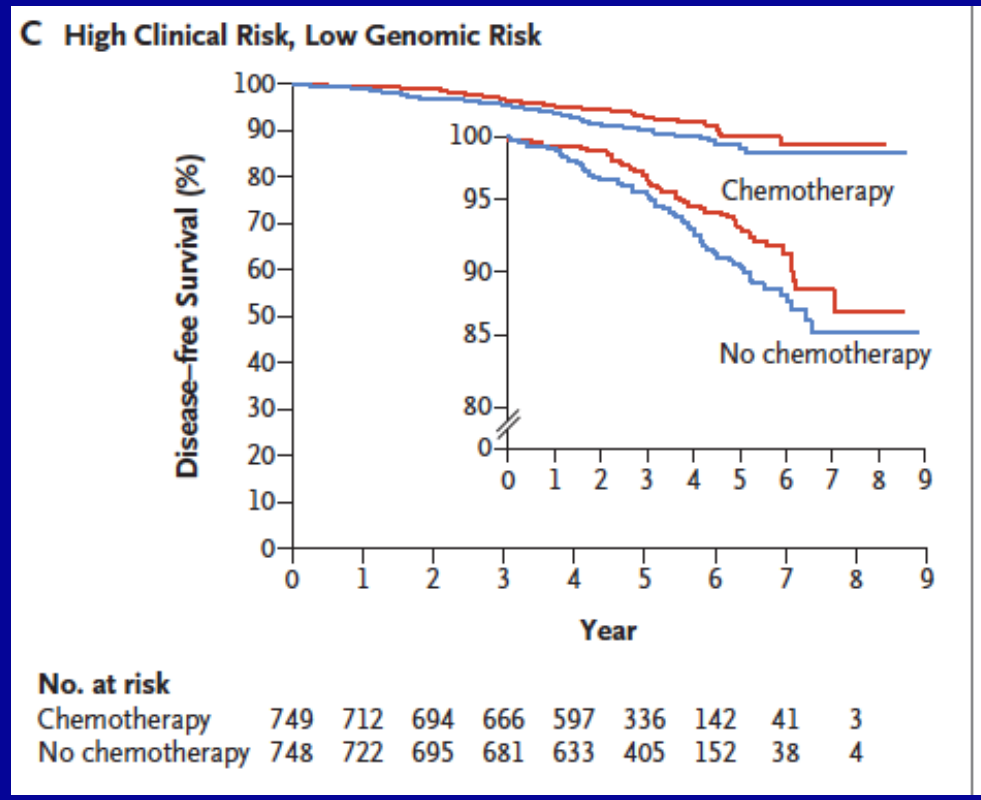
Albain et al SABCS December, 2015

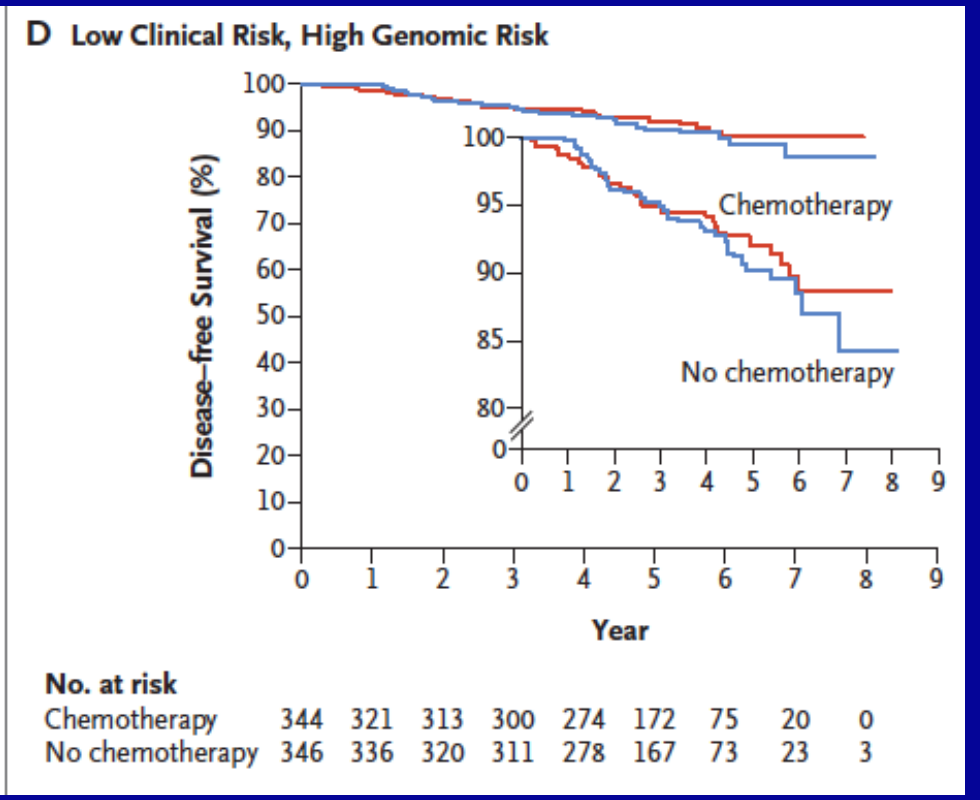
Albain et al. Lancet Oncol 2010, 11:55-65

EORTC-BIG MINDACT TRIAL



MindACT Trial: 70-gene Mammaprint Assay

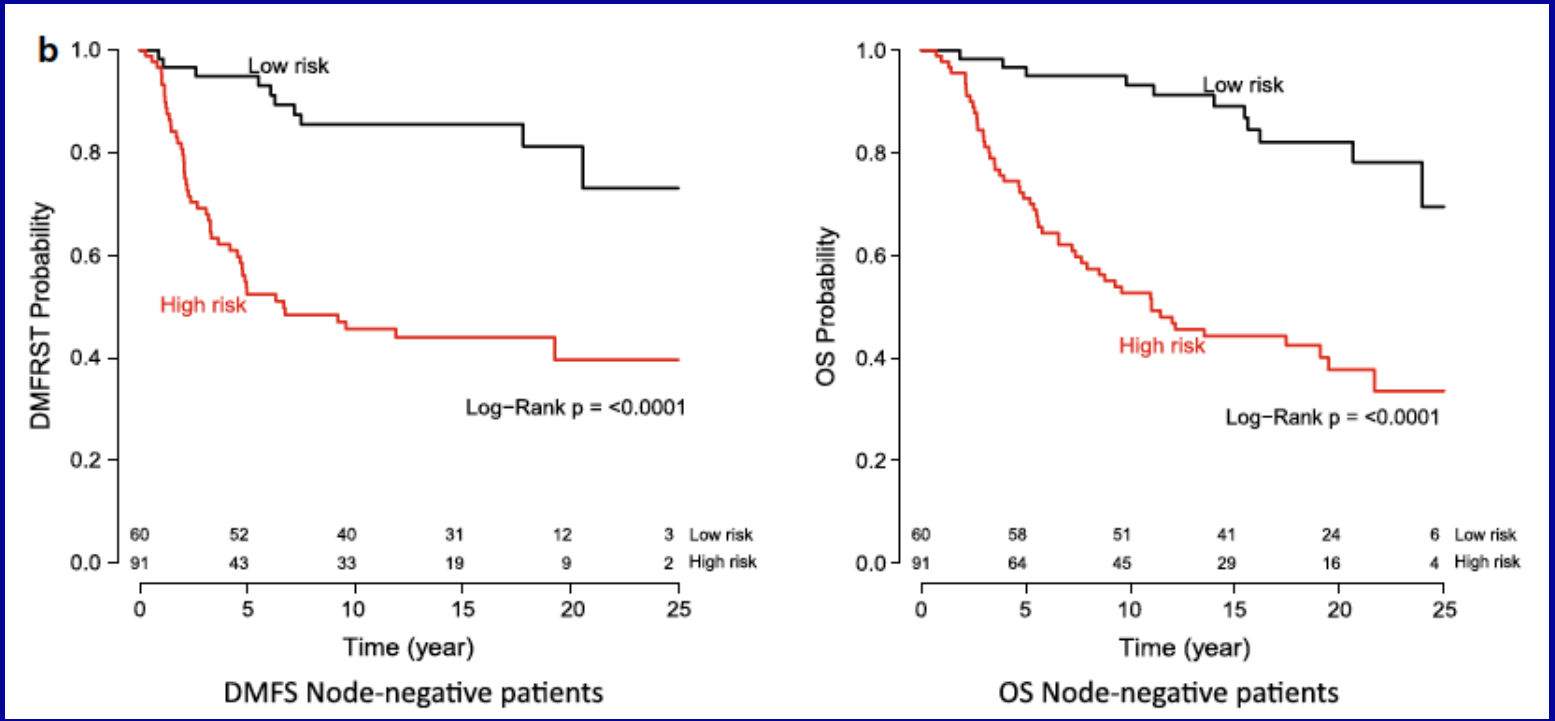




Cardoso et al. NEJM 2016; 375(8):717-729

70-Gene Mammaprint Assay
Very Long Term

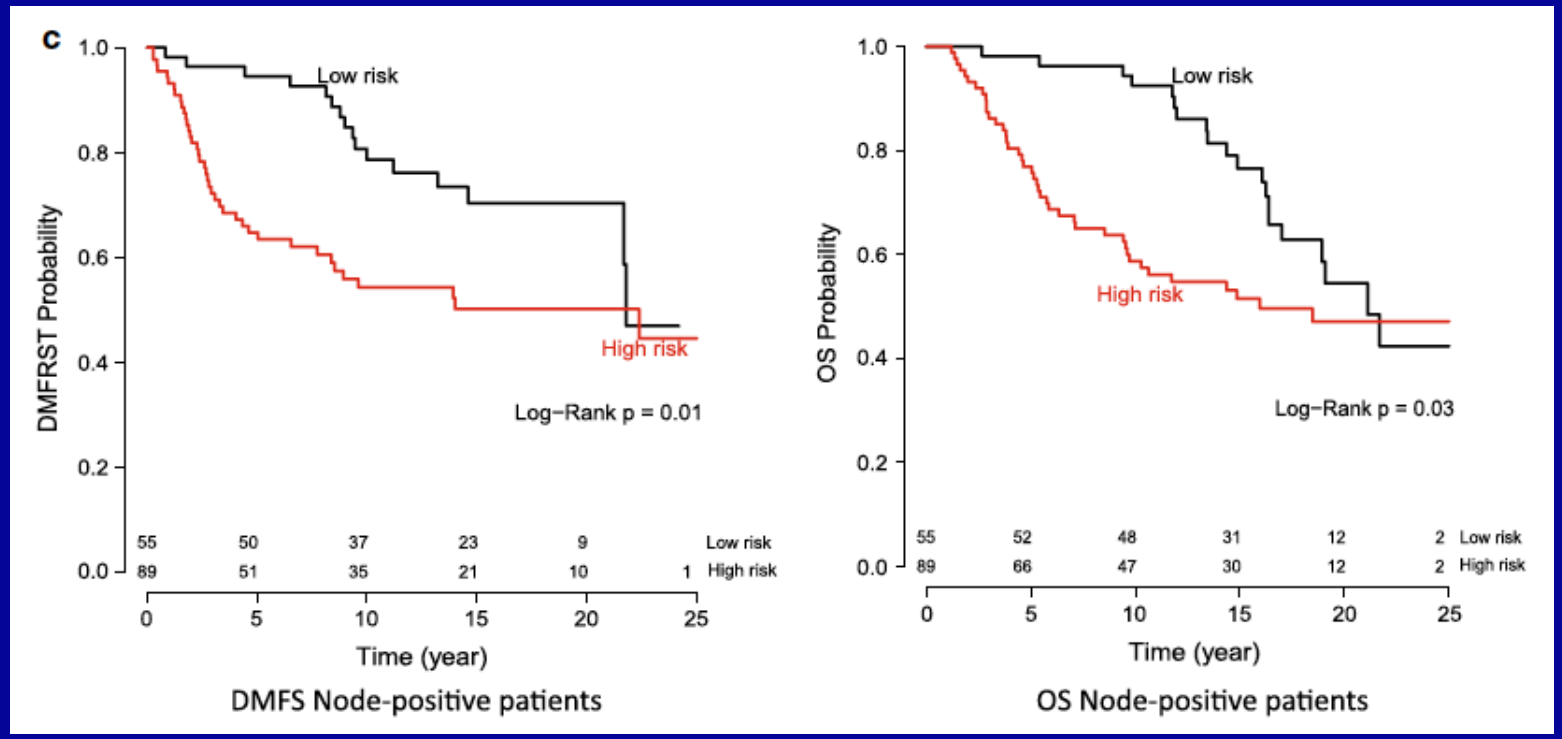
Node-Negative



Drukker et al, Br Cancer Res Treat 2014;143:587-93

70-Gene Mammaprint Assay
Very Long-Term

Node-Positive



Drukker et al, Br Cancer Res Treat 2014;143:587-93

Summary

Prognostic biology can be time-dependent

- **Initial 5 years = Proliferation**
- **Years 5 to 10 = Endocrine**

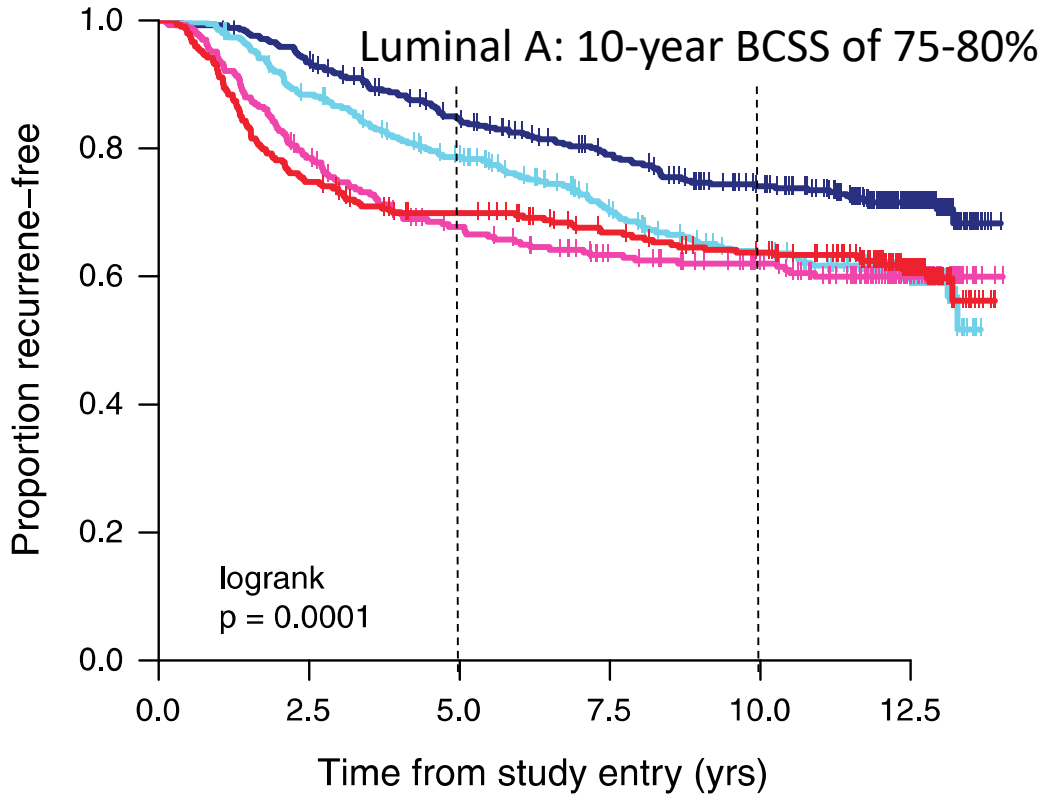
Performance of prognostic tests is context-dependent

- **All the boats (large & small) rise on the incoming tide**
    - **Burden of disease retains importance**

Residual Risk and Prediction: Chemotherapy +/- Endocrine Therapy

Stage II-III Breast Cancer: Long-term Residual Risk

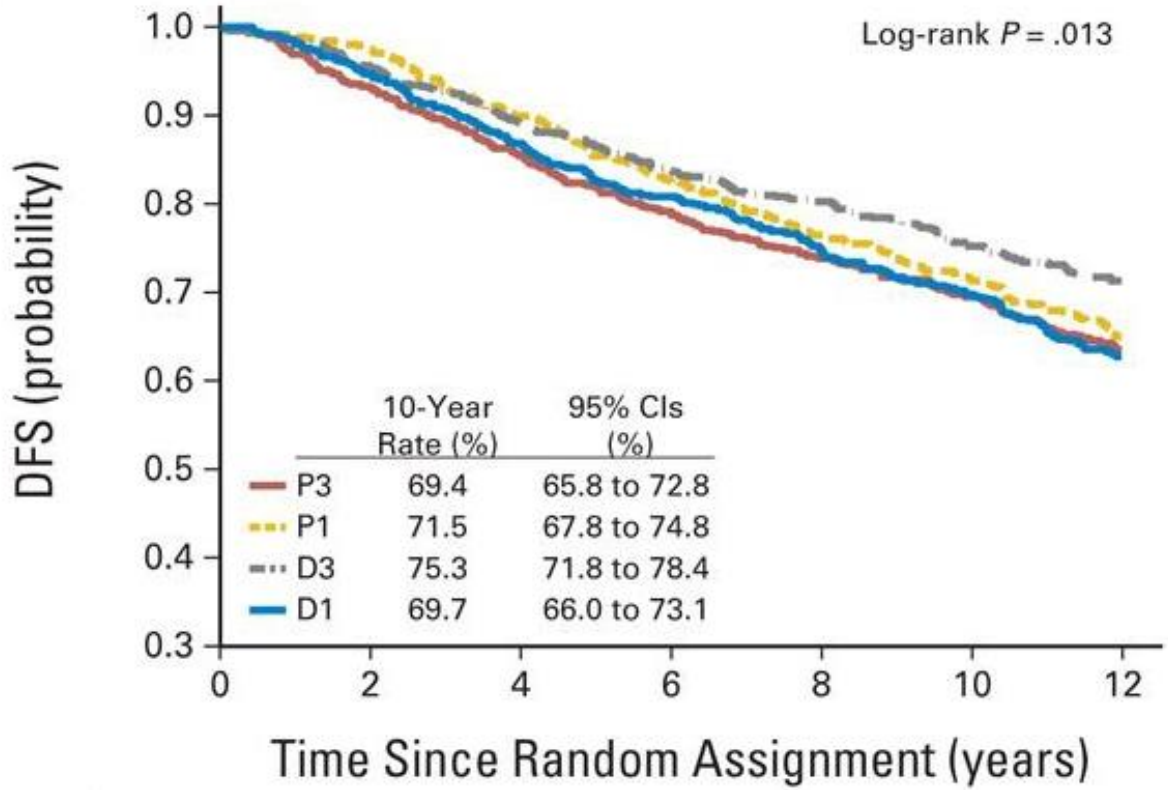
CALGB-9741, AC/T chemo, all subtypes



	Number at risk					
LuminalA	414	385	335	293	256	125
LuminalB	338	295	253	203	172	81
Her2Enriched	266	207	171	146	126	57
BasalLike	293	219	195	173	159	69

Liu et al. NPJ Breast Cancer 2016

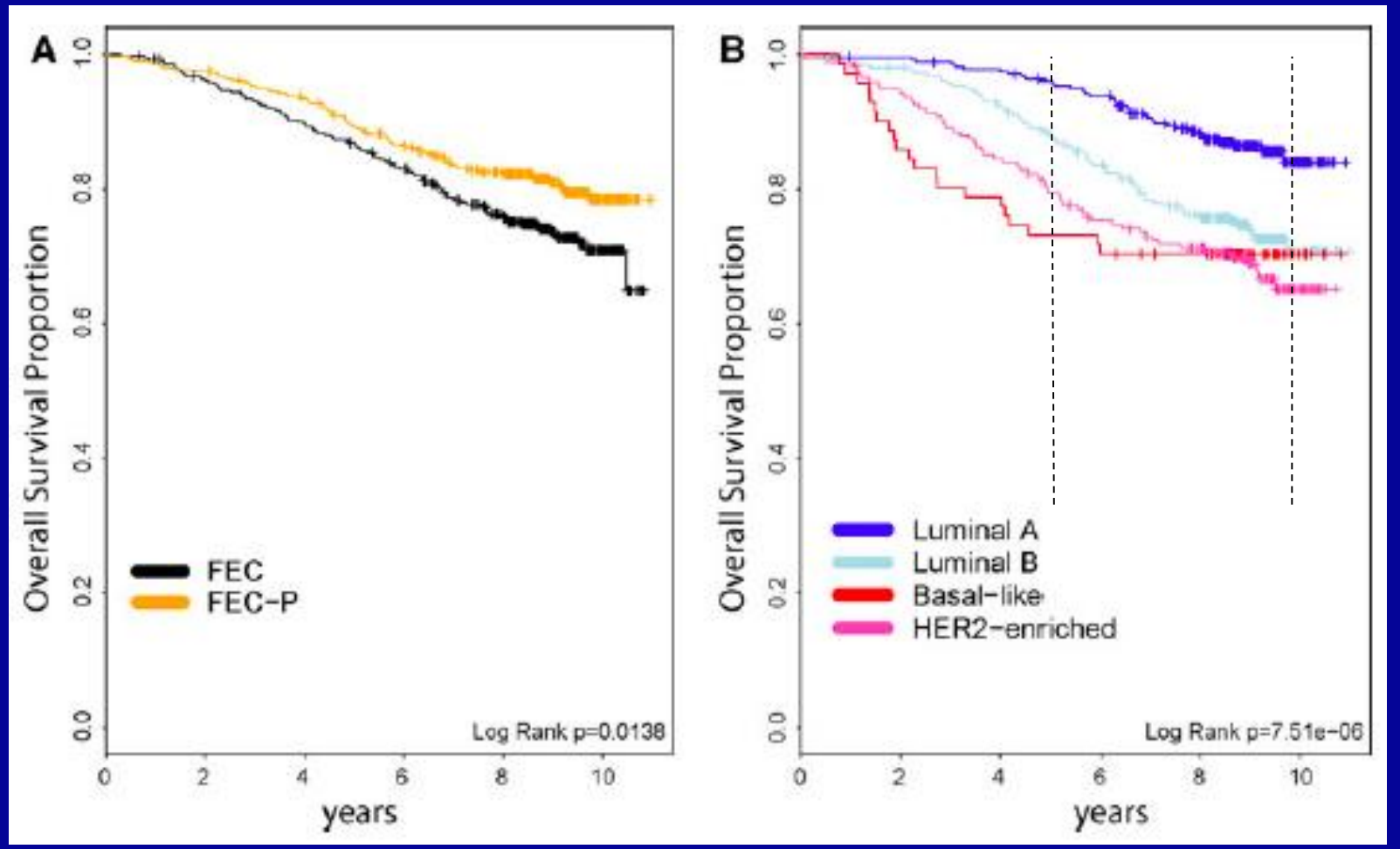
ECOG E-1199, AC/T chemo, HR+/HER2-



No. at risk							
P3	715	653	572	505	452	377	197
P1	706	673	600	525	458	385	189
D3	731	683	621	562	513	436	223
D1	735	683	599	523	458	381	187

Sparano et al. JCO 2015

GEICAM 9906: FEC +/- weekly Paclitaxel, Node-Positive Breast Cancer

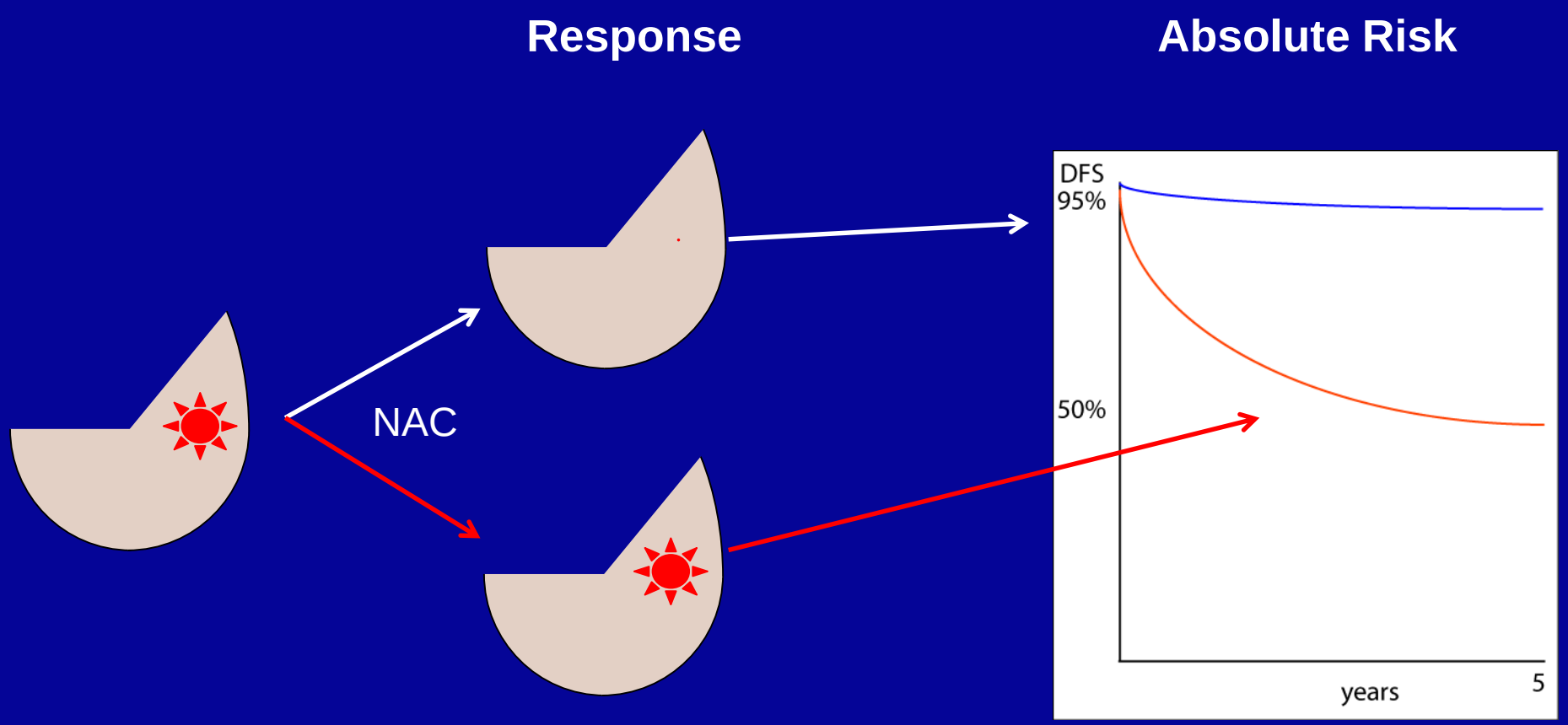


≈ 85% @ 10 years

PAM50 Intrinsic Subtypes did not predict benefit from paclitaxel

Martin et al. Br Can Res Treat 2013;138:457-66

Prediction of Chemotherapy Response and Survival

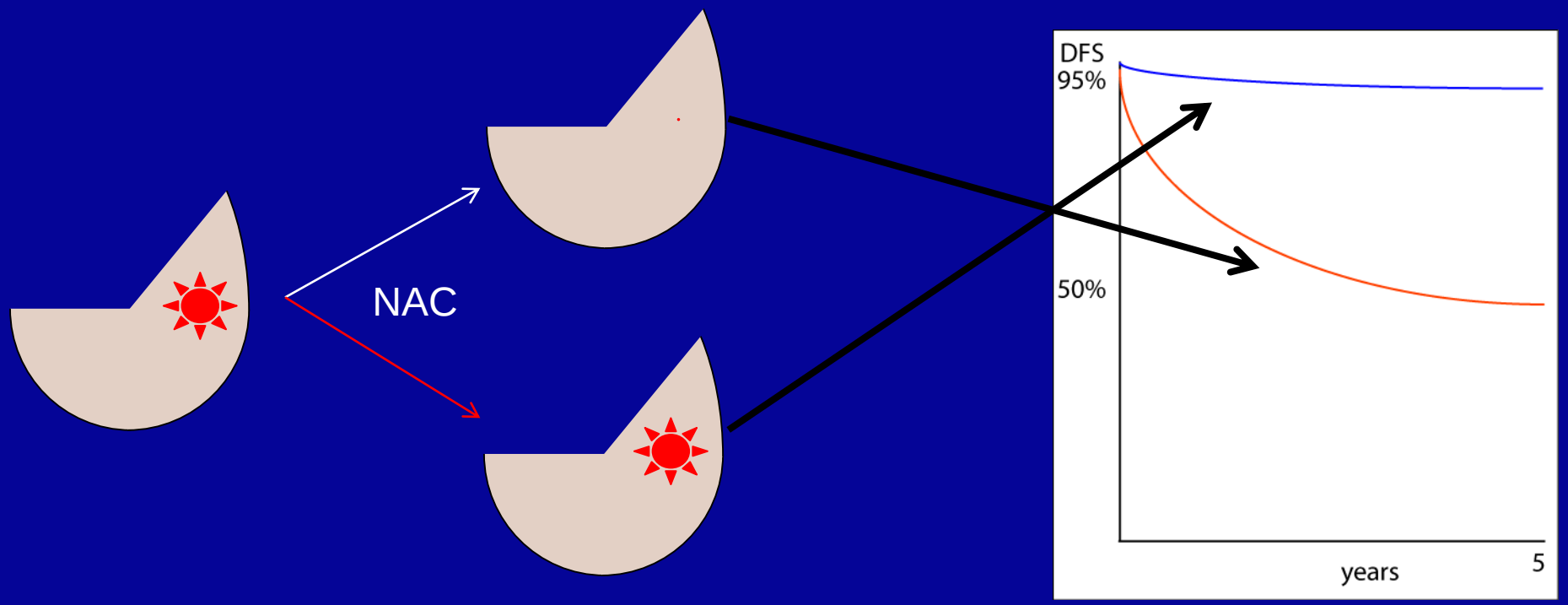


Model depends on alignment of BOTH the predicted response outcome and the absolute survival probability

Prognostic Biomarkers: Paradox

Predicted Response

Absolute Risk



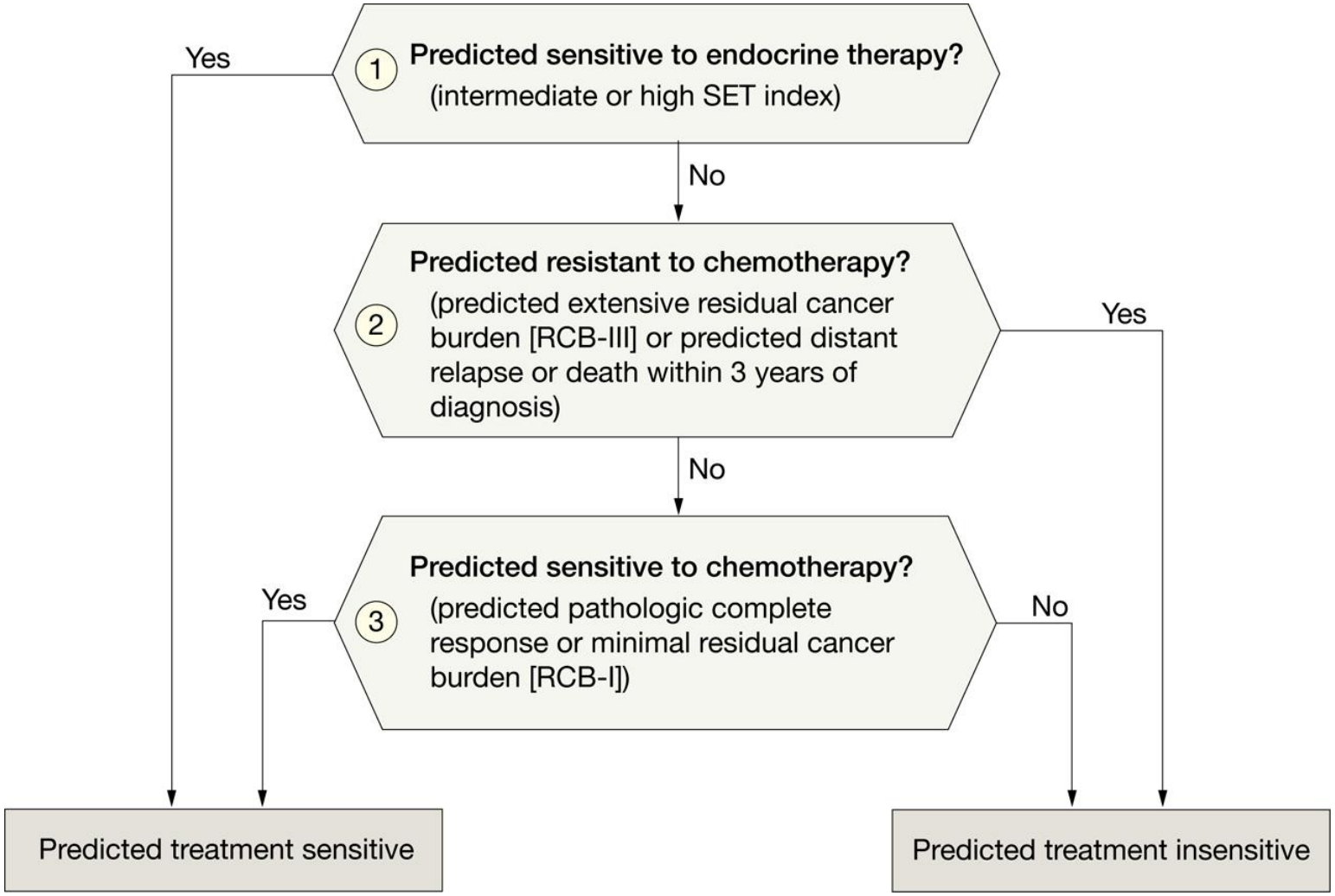
Indolent tumor biology associated with poor response but good prognosis.

Summary of the Chemopredictive Properties of Molecular Classification/Prognostic Tests

Test	Subset	Chemosensitive Group	Response: PPV for pCR	Survival: relative
Grade	all	High grade	≈ 30%	worse
Ki67	all	High proliferation	≈ 30%	worse
Recurrence Score	HR+/HER2-	High	≈ 20-30%	similar
Mammaprint	HR+/HER2-	High Risk	≈ 20%	worse
PAM50	HR+/HER2-	Lum B	≈ 50%	worse
PAM50	TNBC	Basal-like	≈ 50%	similar
GGI	all	High grade	≈ 50%	worse
DLDA-30	all	Predicted pCR	≈ 50%	worse

Proof of Concept Strategy For T ➔ A Chemotherapy +/- Endocrine Therapy

Developed Separately For ER+/HER2- and ER-/HER2- Cancers (N = 310)



Hatzis, C. et al. JAMA 2011;305:1873-1881

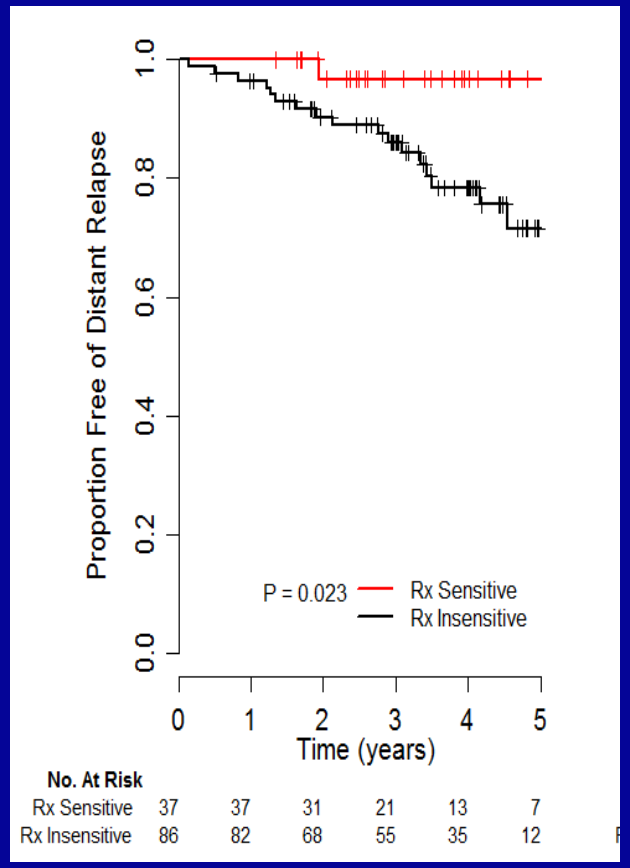
Copyright restrictions may apply.

Response & Survival Outcomes in Validation Cohort

N = 198 Chemotherapy +/- Endocrine Therapy (99% Stage II-III)

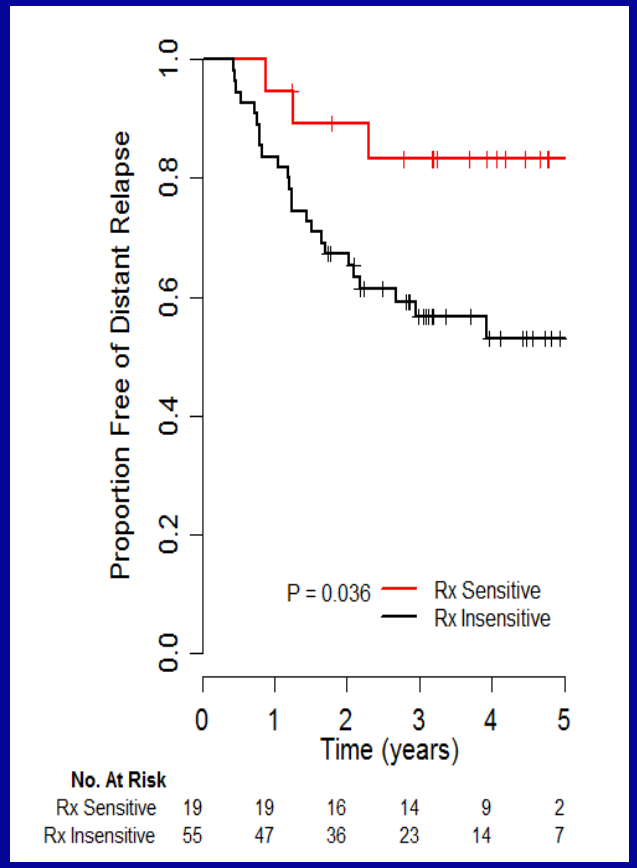
HR+ / HER2-

PPV for response 42% (95% CI 15-72)



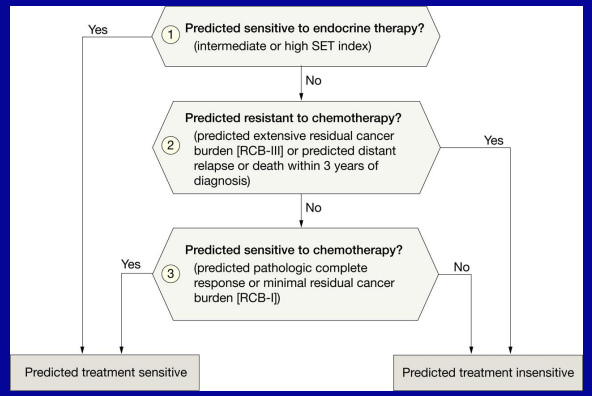
HR- / HER2- (TNBC)

PPV for response 83% (95% CI 36-100)



Hatzis et al. JAMA 2011;305:1873-1881

TNBC: Logistic Algorithm vs. Random Forest Algorithm to Combine Gene Expression Signatures (GES)



cN-

GES: TILs

GES: RCB-III
or
RCB-II & relapse

GES: pCR/RCB-I
&
no relapse

Kappa values: 0.24 - 0.34

Triplicate Runs
23 TNBCs

Kappa values: 0.63 - 0.91

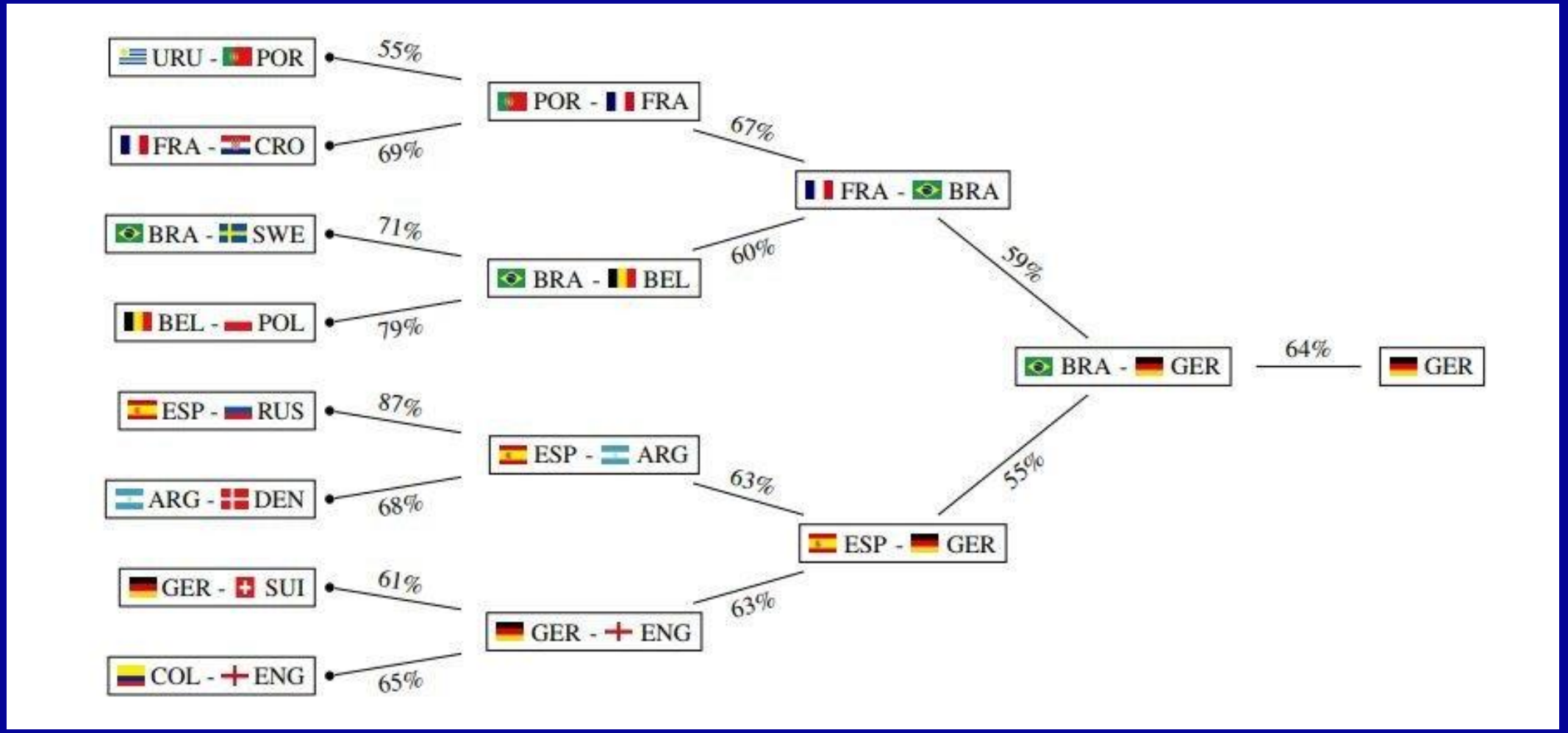
PPV: 0.60 - 0.65

Validation Cohort in TNBC
Predicting pCR/RCB-I

PPV: 0.60 - 0.65

Machine Learning Algorithms To Combine Predictions

Example: Random Forest Approach

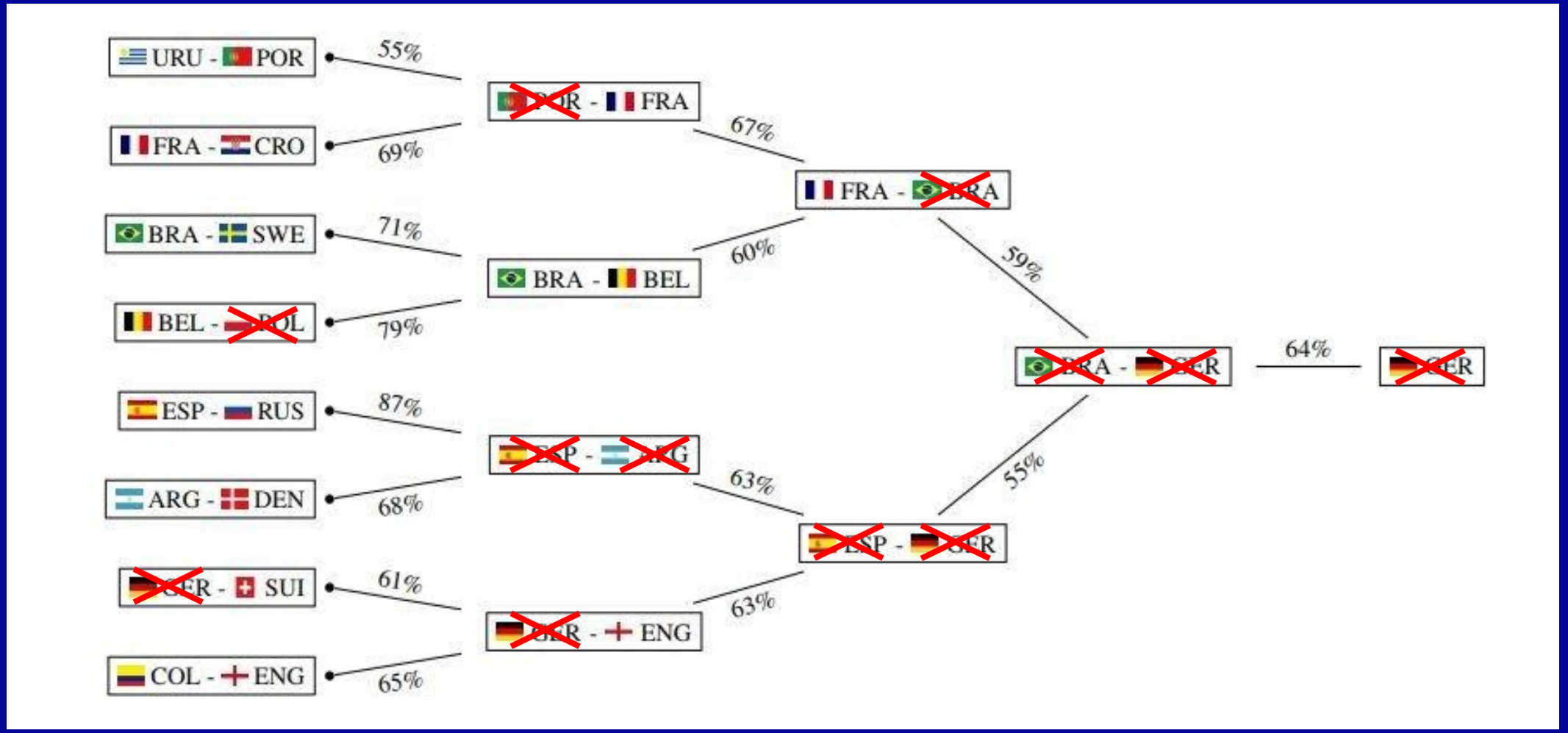


Groll et al. Technical University of Dortmund, MIT Technology Review, June 12, 2018

Ref: arxiv.org/abs/1806.03208 : Prediction Of The FIFA World Cup 2018 – A Random Forest Approach With An Emphasis On Estimated Team Ability Parameters

Machine Learning Algorithms To Combine Predictions

Example: Random Forest Approach

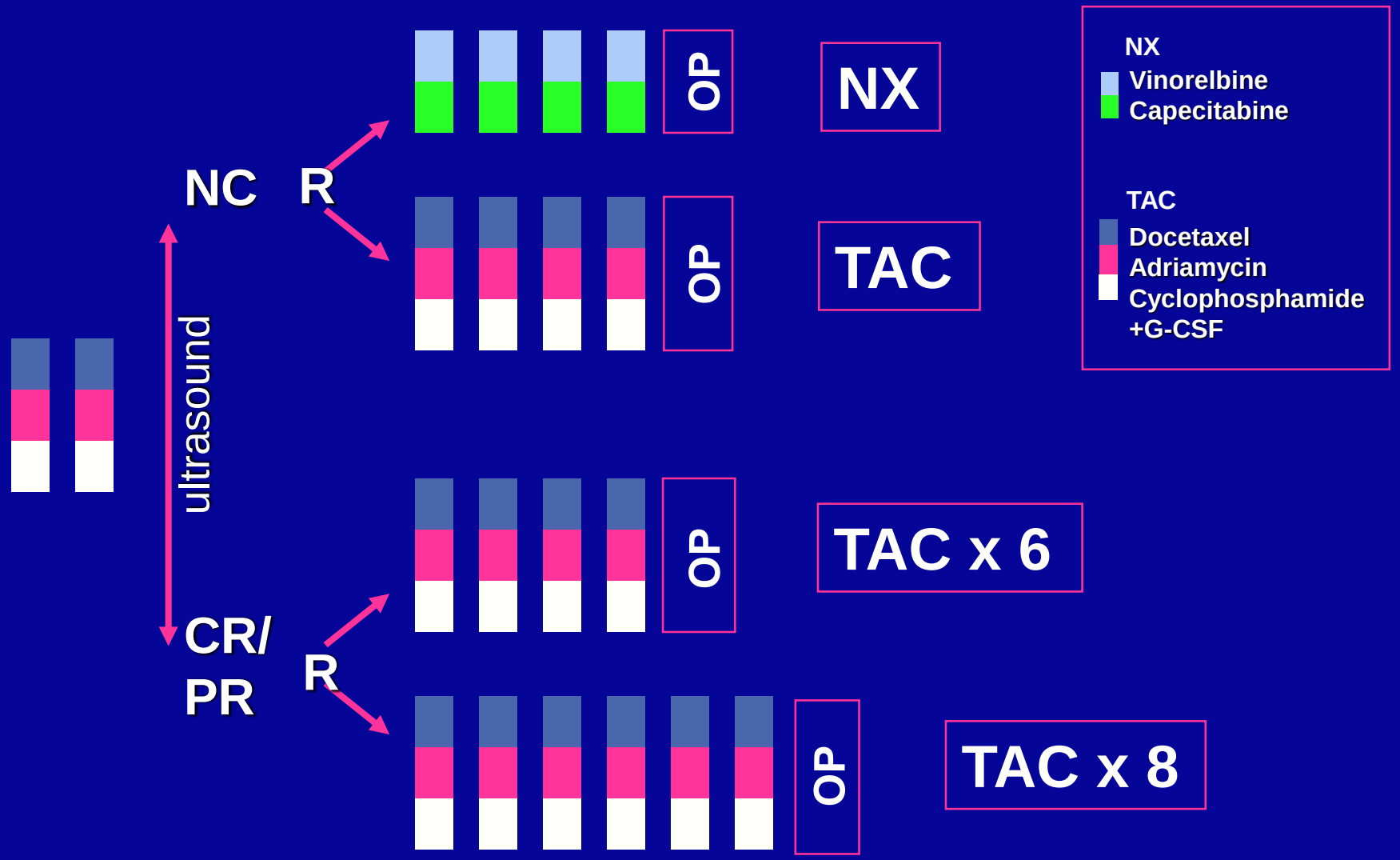


Groll et al. Technical University of Dortmund, MIT Technology Review, June 12, 2018

Ref: arxiv.org/abs/1806.03208 : Prediction Of The FIFA World Cup 2018 – A Random Forest Approach With An Emphasis On Estimated Team Ability Parameters

Using Radiologic Response To Guide Neoadjuvant Chemotherapy: GEPAR-TRIO Trial

Core biopsy:
uni/bilateral
cT2-4
cN0-3
size ≥ 2 cm*



*excluding low risk (T2 + ER/PR pos. + cNO + G1/2 + > 35y.)

von Minckwitz G et al. JCO 2013;31:3623-3630

Consequences of Response Guidance, by HR Status: GEPAR-TRIO Trial

Table 2. ORs for pCR and HRs for DFS Between Response-Guided Compared With Conventional Chemotherapy and HRs for pCR According to Hormone Receptor Status and Phenotypes

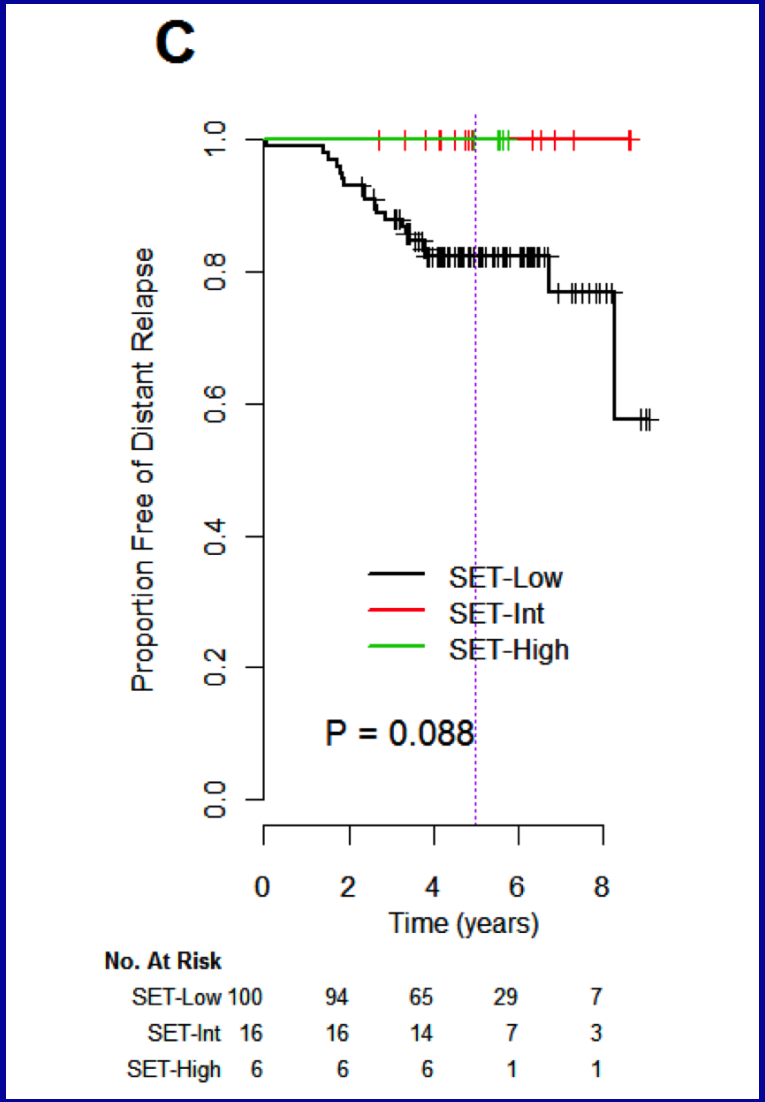
				pCR		DFS			
		pCR Rate		Conventional *v* Response Guided		pCR *v* No pCR		Conventional *v* Response Guided	
Subgroups by HR and Phenotype*	No. of Patients	No.	%	OR	*P*	HR	*P*	HR	*P*
HR									
Positive	1,295	105	8.2	1.34	.155	1.53	.122	0.56	< .001
Negative	717	238	33.2	1.17	.330	4.89	< .001	0.94	.663
Test for interaction							.004		.008

von Minckwitz G et al. JCO 2013;31:3623-3630

SET Index: ER-related Gene Expression w/o Proliferation

ER+, Stage II-III
66% clinically LN+
Neoadjuvant T/FAC chemotherapy
Surgery / XRT
Adjuvant Tam &/or AI
(N=122)

Association With Pathologic Response		
SET Class	pCR / RCB-I	Chi-square test
Low	35 / 100	NS
Intermediate / High	6 / 22	



Symmans et al. JCO 2010;28:4111-19

Response from Chemotherapy and Predicted SET

ER+, neoadjuvant T/FAC, adjuvant Tam &/or AI (N=122)

Cox Regression Model for DRFS			
Factor	HR	95% CI	P value
RCB Index	2.07	1.20 - 3.60	0.009
SET Index	0.19	0.05 - 0.69	0.011
RCB * SET	1.49	0.99 - 2.24	0.054

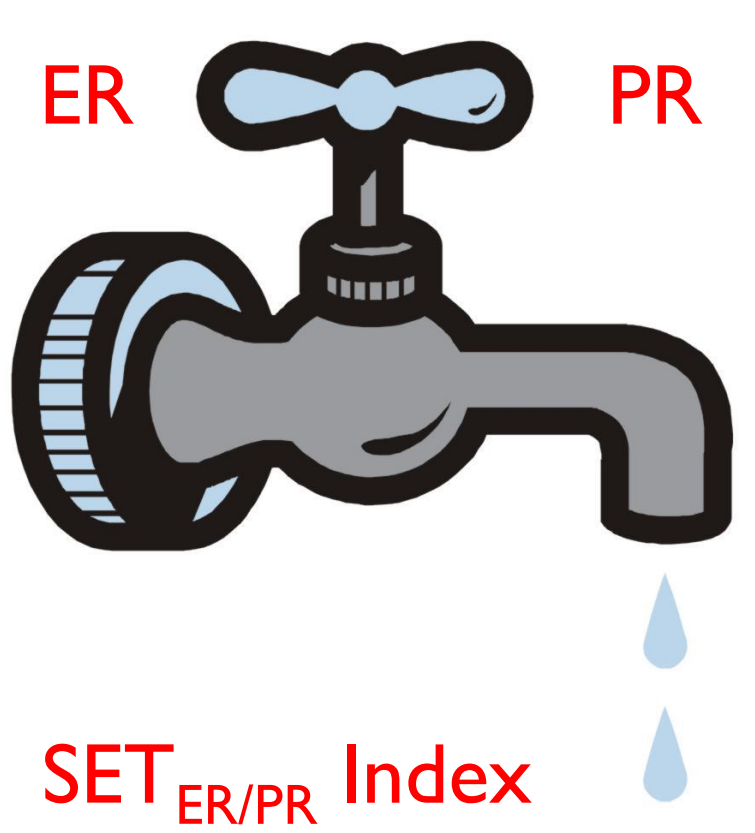
RCB index = Residual Cancer Burden in breast and regional lymph nodes after completion of chemotherapy

SET index = genomic predicted Sensitivity to Endocrine Therapy

Symmans et al. JCO 2010;28:4111-19

Sensitivity to Endocrine Therapy: $SET_{ER/PR}$ Index

ER-Related Transcription in Primary Disease



Gene expression correlated with expression of ER & PR

- Exclude proliferation-related genes
- Select if robust to pre-analytical and analytical conditions
- Measured relative to reference genes

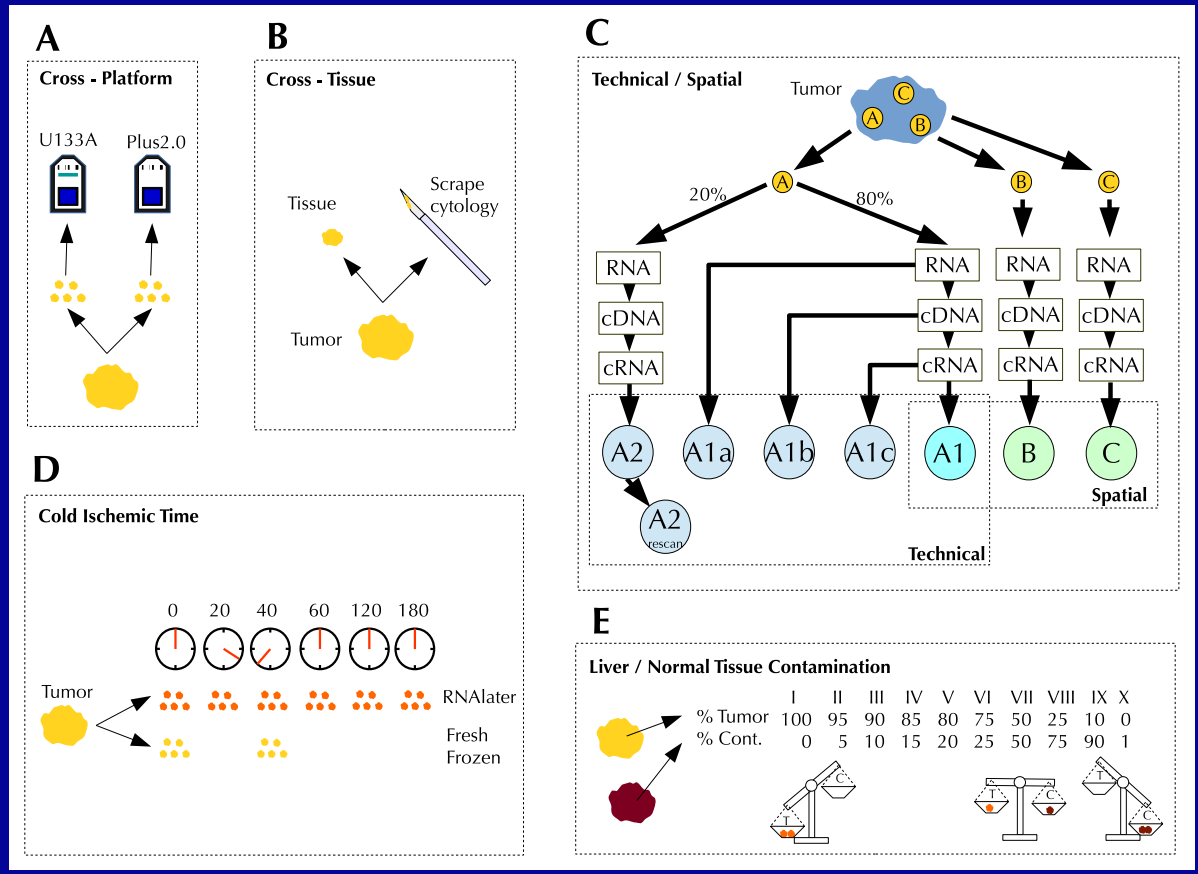
165 → 18

Translation to assay for use with FFPE samples

- Simple workflow
- Affordable technology
- Robust technical measurements

based on *Symmans et al. JCO 2010;28:4111-19*

Biospecimen Studies To Filter Genes for the SET2,3 Test



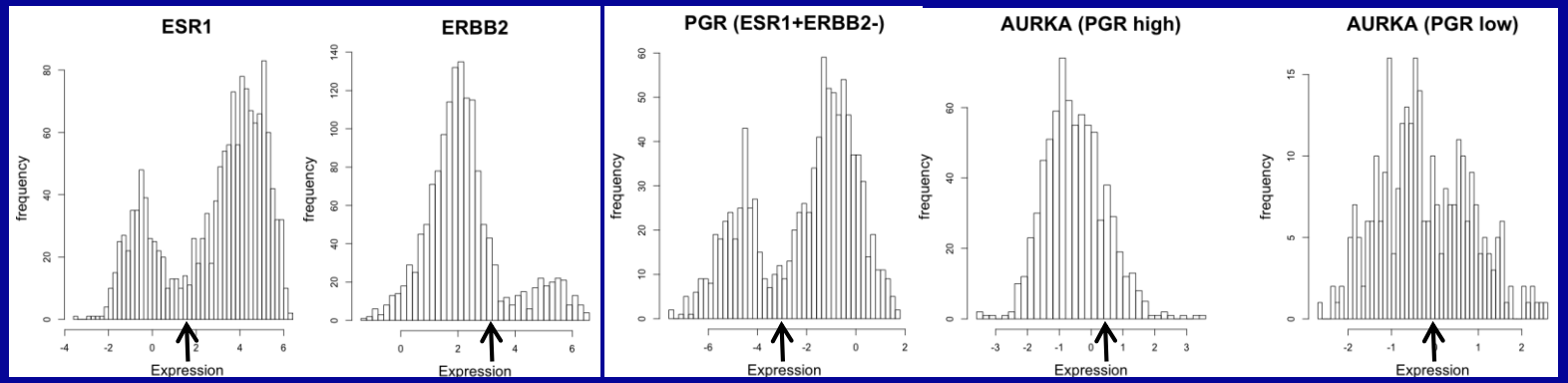
$SET_{ER/PR}$ Genes	Reference Genes	RNA4		
ABAT, ADCY1, AZGP1, CA12, CD2, CD3D, DNAJC12, ESR1, KCNE4, MAPT, MRPS30, NAT1, NPY1R, PDZK1, QDPR, SCUBE2, SLC39A6, STC2	*AK2, APPBB2, ATP5J2, DARS, LDHA, TRIM2, UBE2Z, UGP2, VDAC2, WIPF2*	*ESR1, ERBB2, PGR, AURKA*	pT	pN

$SET_{ER/PR}$ Index ⟷ Prognostic Risk

Bruno Sinn et al. manuscript in preparation

SET2,3 Test: Risk-Adjusted Cutpoints for the $SET_{ER/PR}$ Index

RNA4



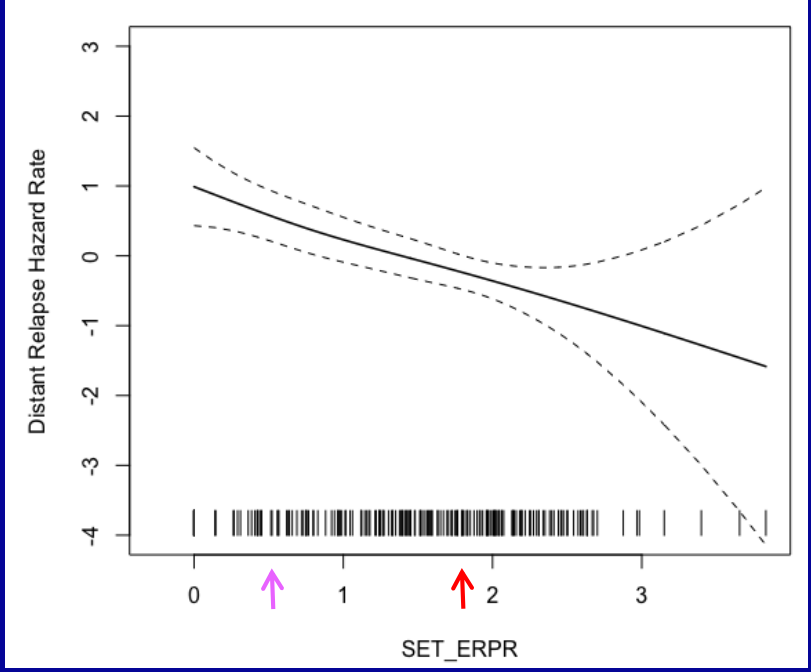
Risk Score

cN Stage: cN- (0 votes), cN+ (2 votes)

cT Stage: cT1-2 (0 votes), cT3-4 (1 vote)

RNA4: low risk (0 votes), not low risk (1 vote)

Low Risk if ≤ 1 vote, High Risk if ≥2 votes



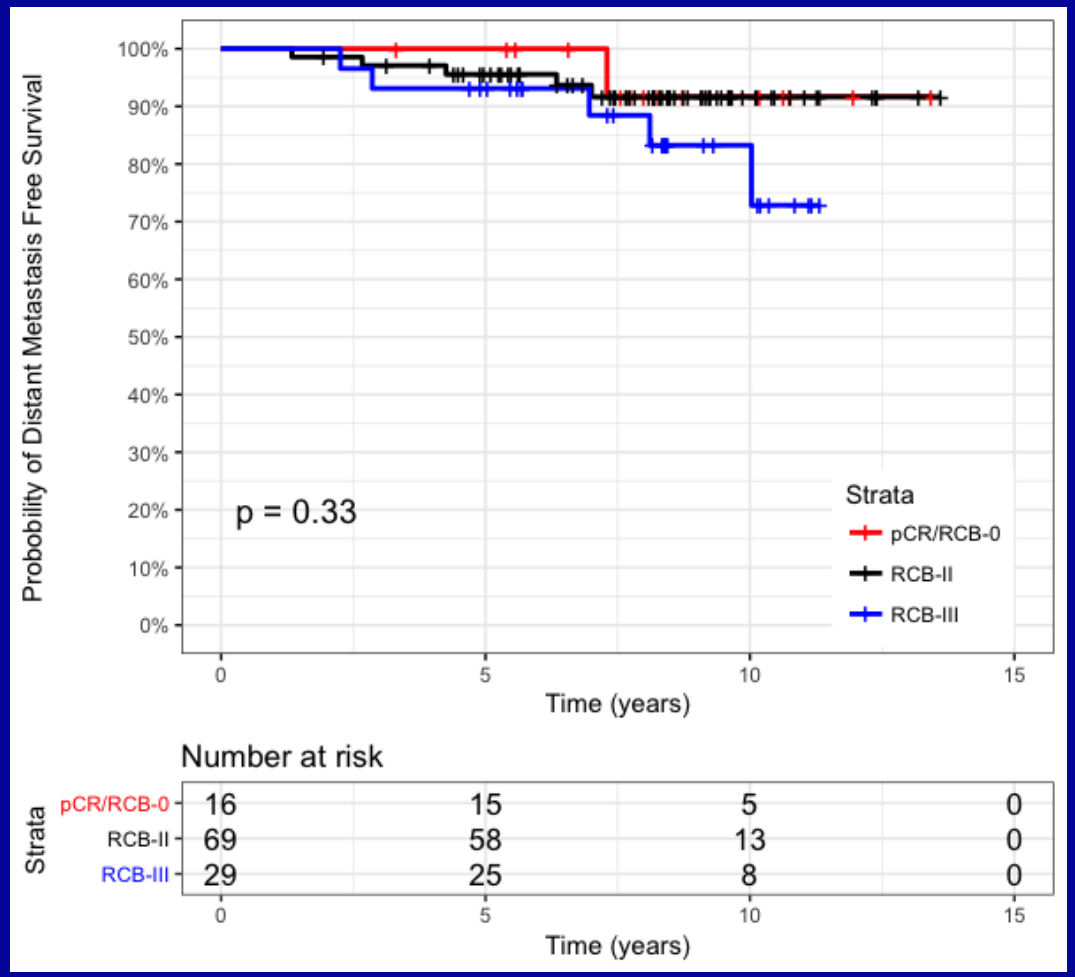
$SET_{ER/PR}$ Index Cutpoint = 0.5 if Low Risk

$SET_{ER/PR}$ Index Cutpoint = 1.8 if High Risk

SET2,3 Classes: Prognostic Influence of Response To NAC

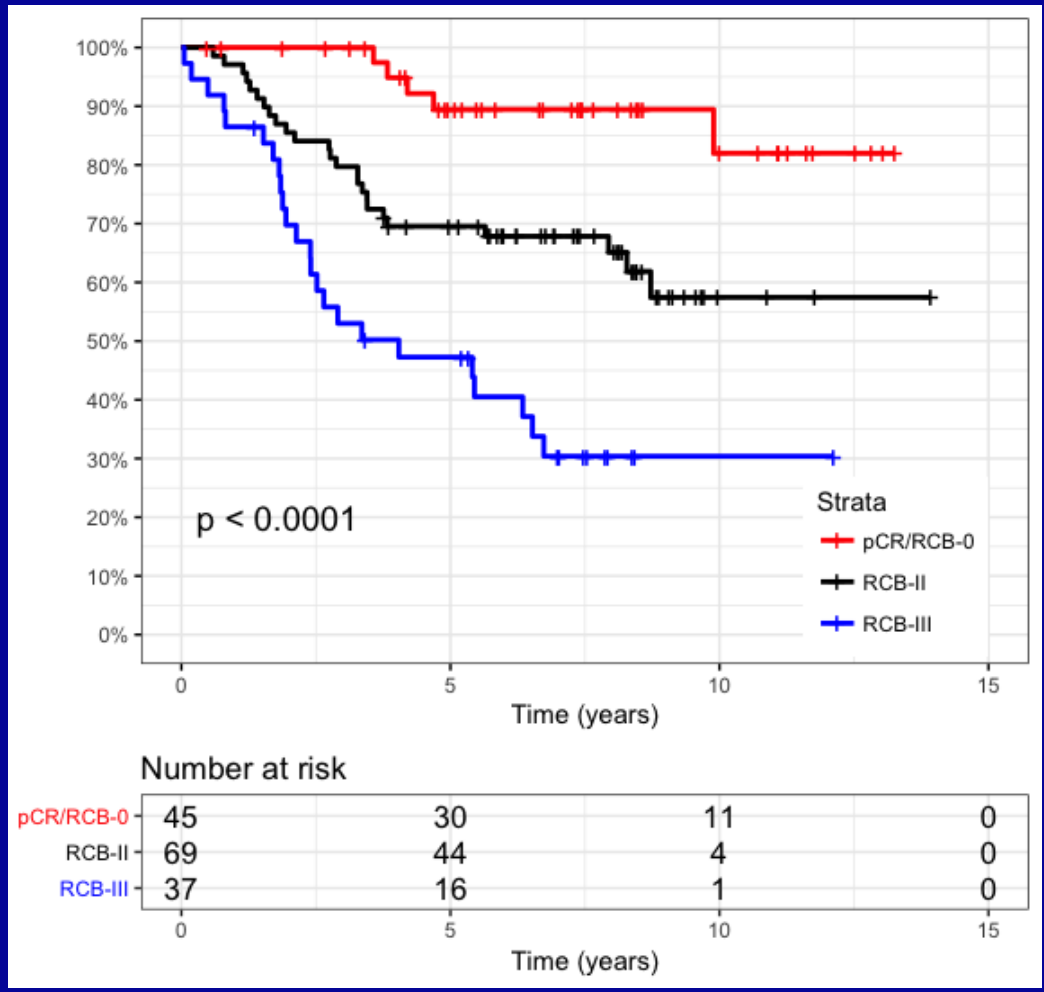
43%

High SET2,3 (low risk, n= 114)



57%

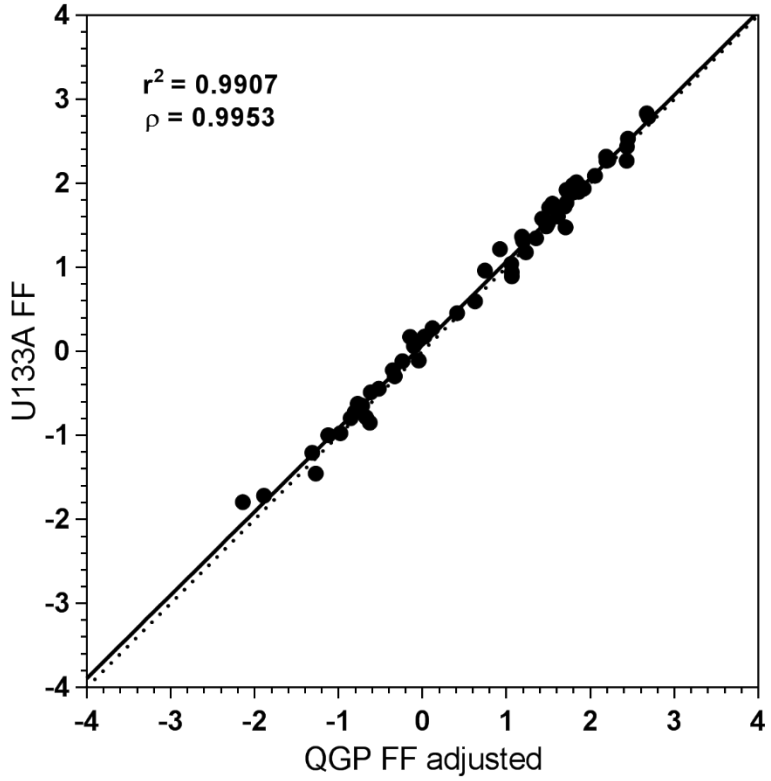
Low SET2,3 (high risk, n= 151)



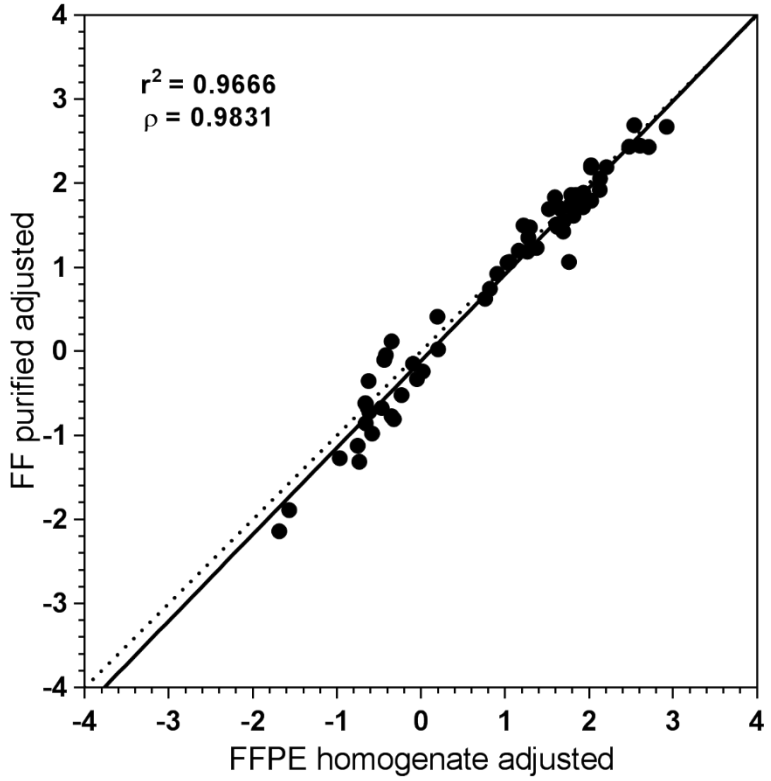
Manuscript in preparation

Translation of SET2,3 to a Customized Test Format QGP

Inter-Platform
Using Fresh Frozen Samples



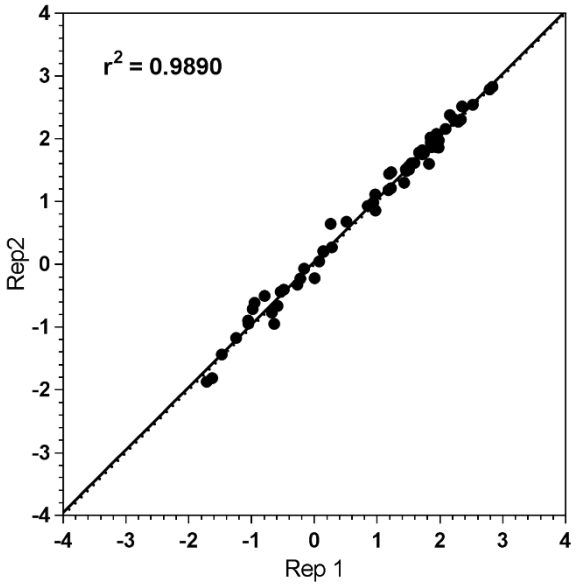
Sample Fixation
Fresh Frozen vs FFPE



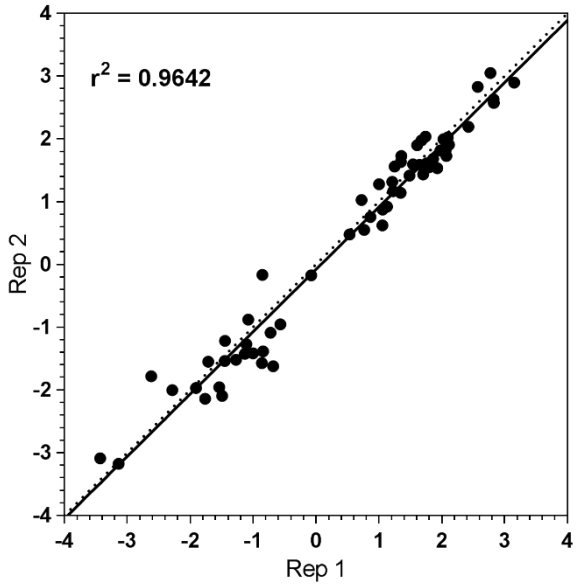
QuantiGene Plex (QGP) assay using Luminex MagPix device, lysis homogenate from unstained tissue section

Technical Reproducibility of SET2,3 Assay

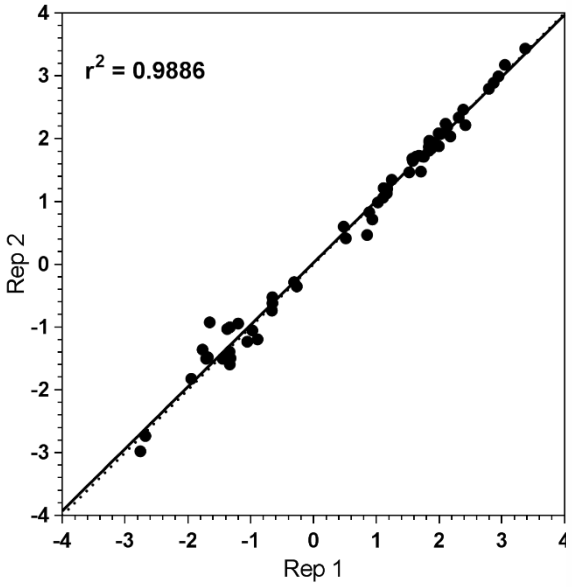
U133A Microarrays
RNA from fresh frozen tissue

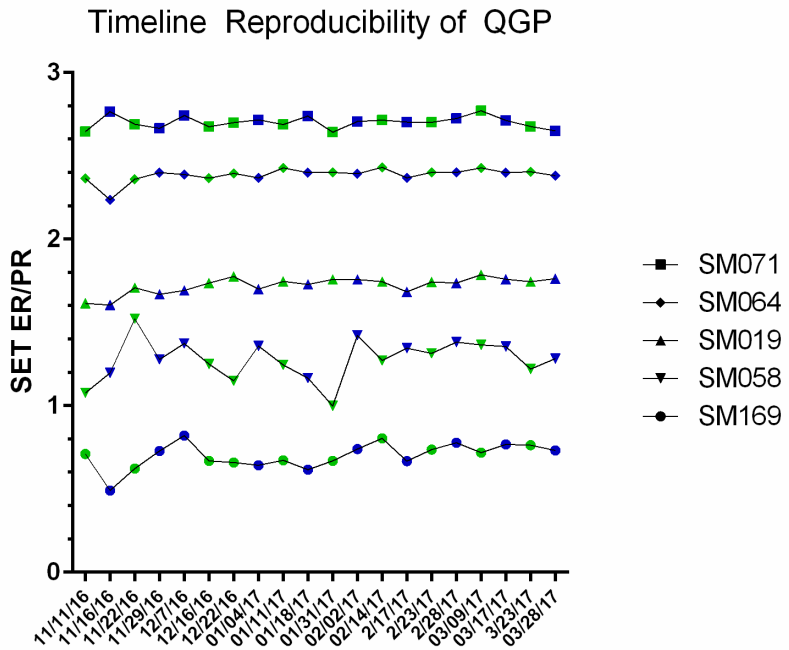


NANOSTRING
RNA from FFPE tissue



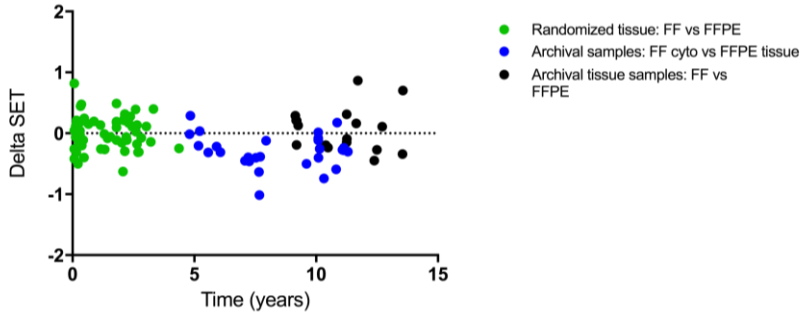
QuantiGene Plex
FFPE tissue slides

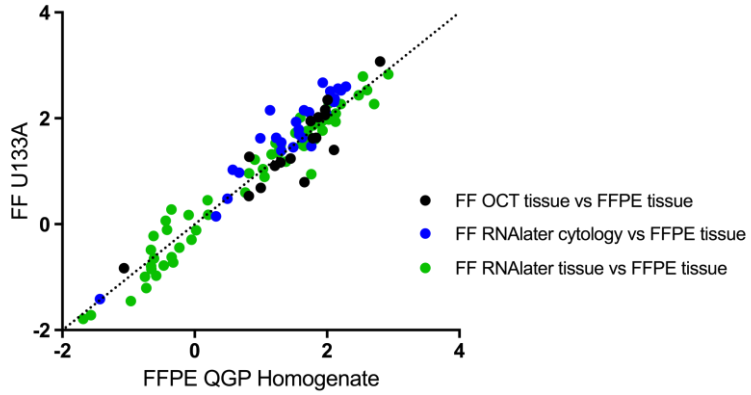




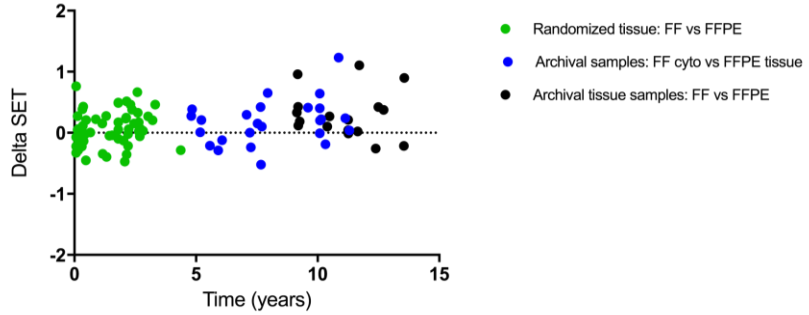
SET2,3 Assay Performance: Older FFPE & Frozen Samples

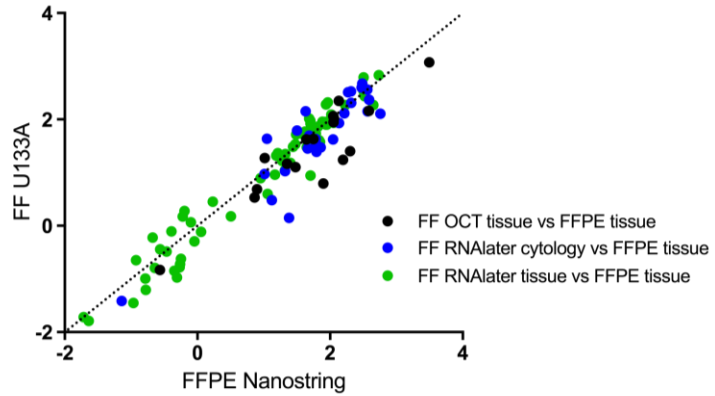
QuantiGene Plex using FFPE
Duration of tissue storage
Difference (delta) from frozen tissue U133A





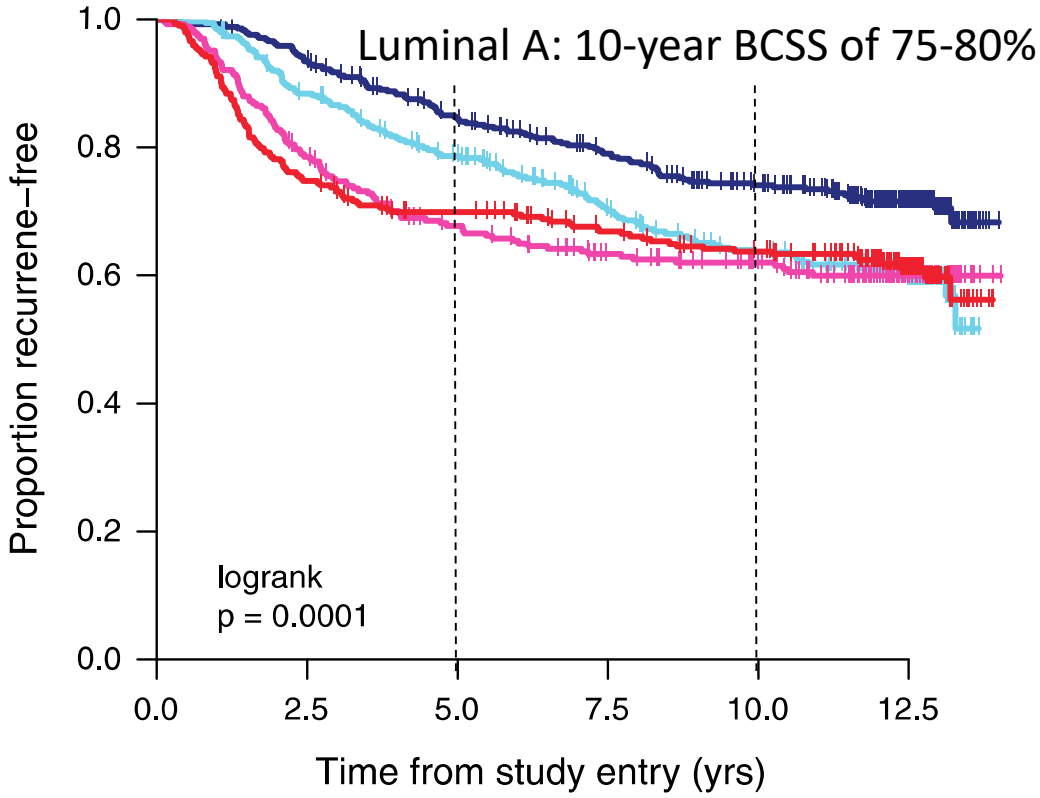
NANOSTRING using FFPE
Duration of tissue storage
Difference (delta) from frozen tissue U133A





Validation Strategy: Stratify Long-Term Risk

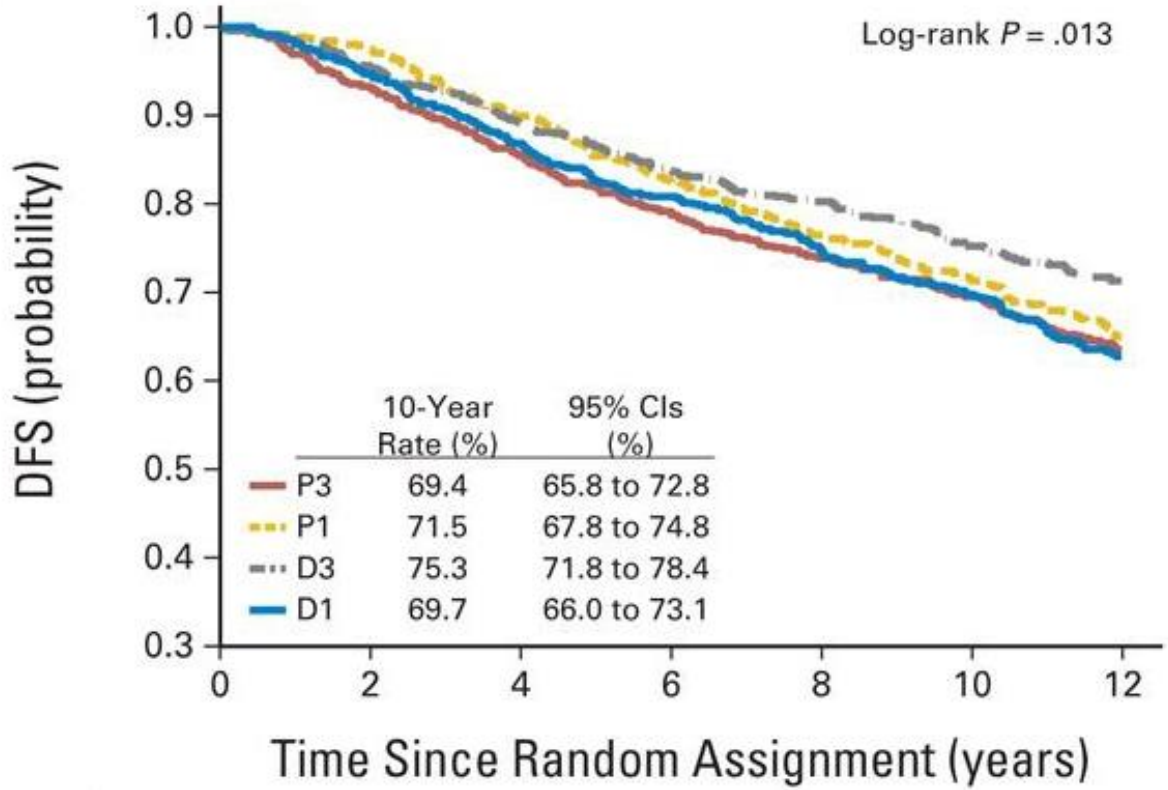
CALGB-9741, AC/T chemo, all subtypes



	Number at risk					
LuminalA	414	385	335	293	256	125
LuminalB	338	295	253	203	172	81
Her2Enriched	266	207	171	146	126	57
BasalLike	293	219	195	173	159	69

Liu et al. NPJ Breast Cancer 2016

ECOG E-1199, AC/T chemo, HR+/HER2-



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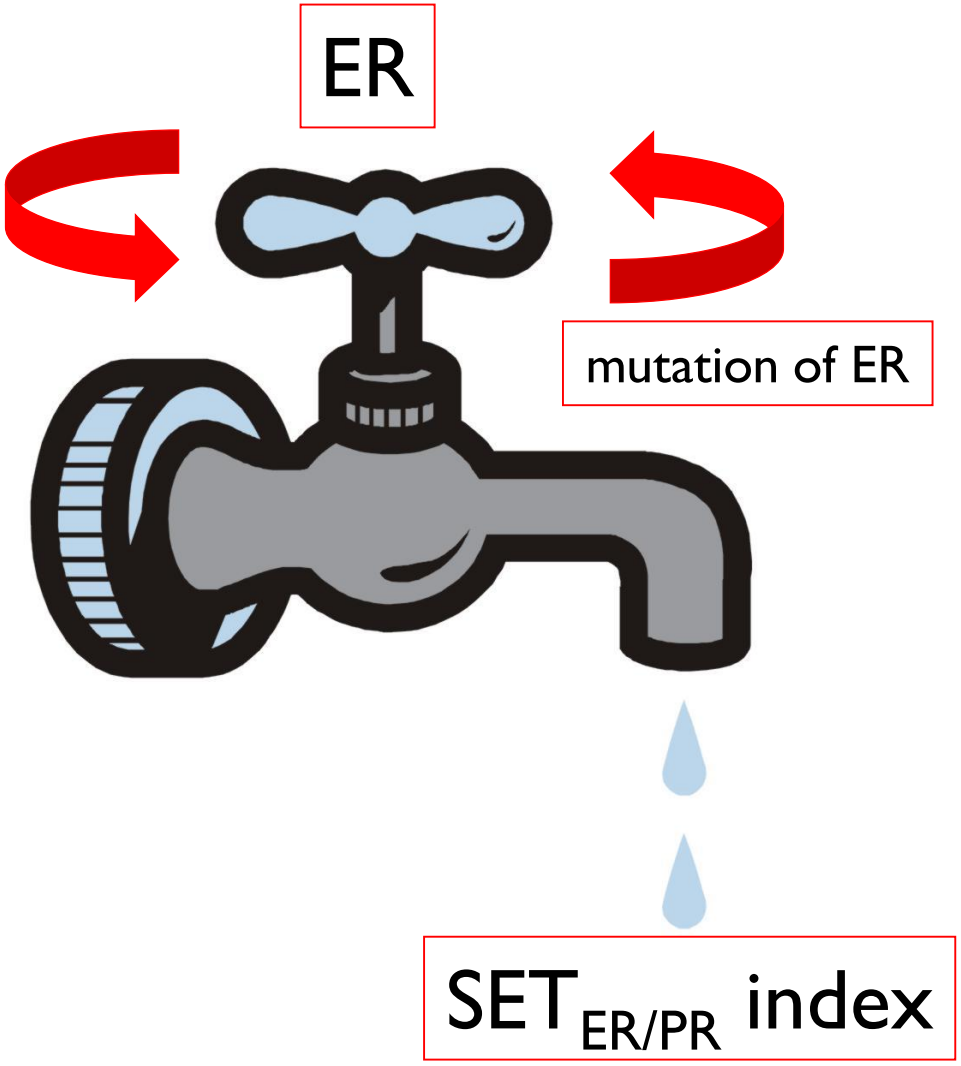
D3	731	683	621	562	513	436	223
D1	735	683	599	523	458	381	187

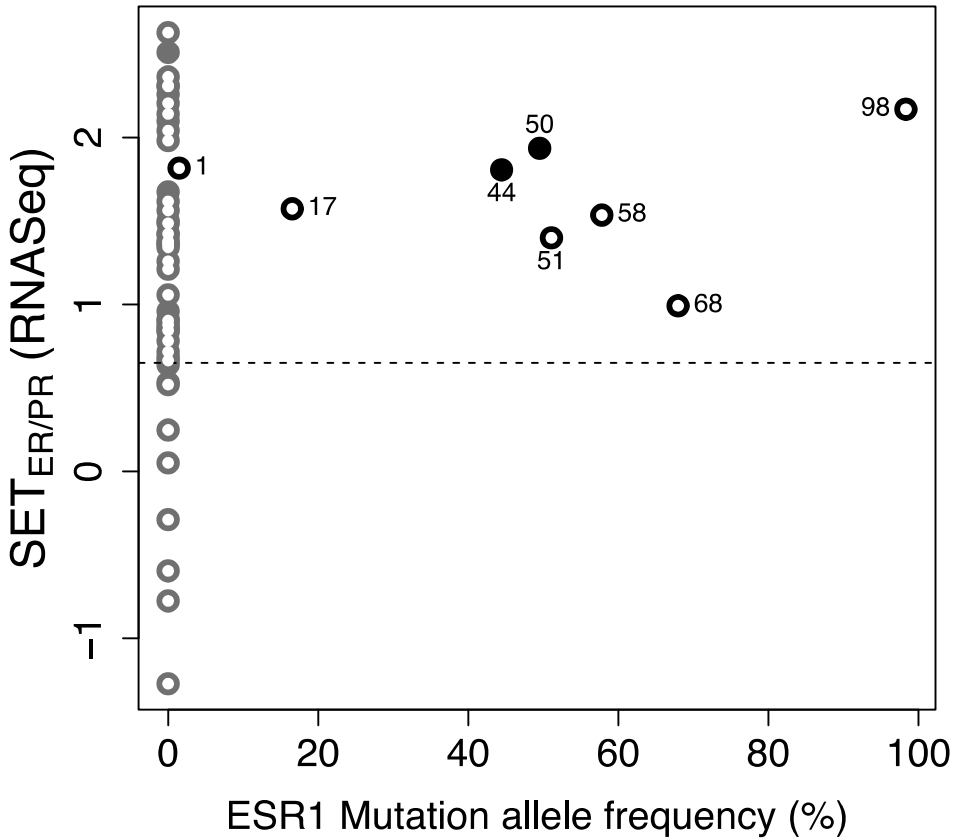
Sparano et al. JCO 2015

The next wave of treatments for Stage II-III HR+ breast cancer:

- Concurrent cdk4/6 inhibition with endocrine therapy
- Concurrent inhibition of PI3-kinase or pathway with endocrine therapy
- De-escalation to hormonal therapy alone
- Less intense / less toxic chemotherapy regimens (calibrated to lower risk)

Customized Transcriptomic Assay:
Quantify Mutant Transcript Load + Gene Expression Activity





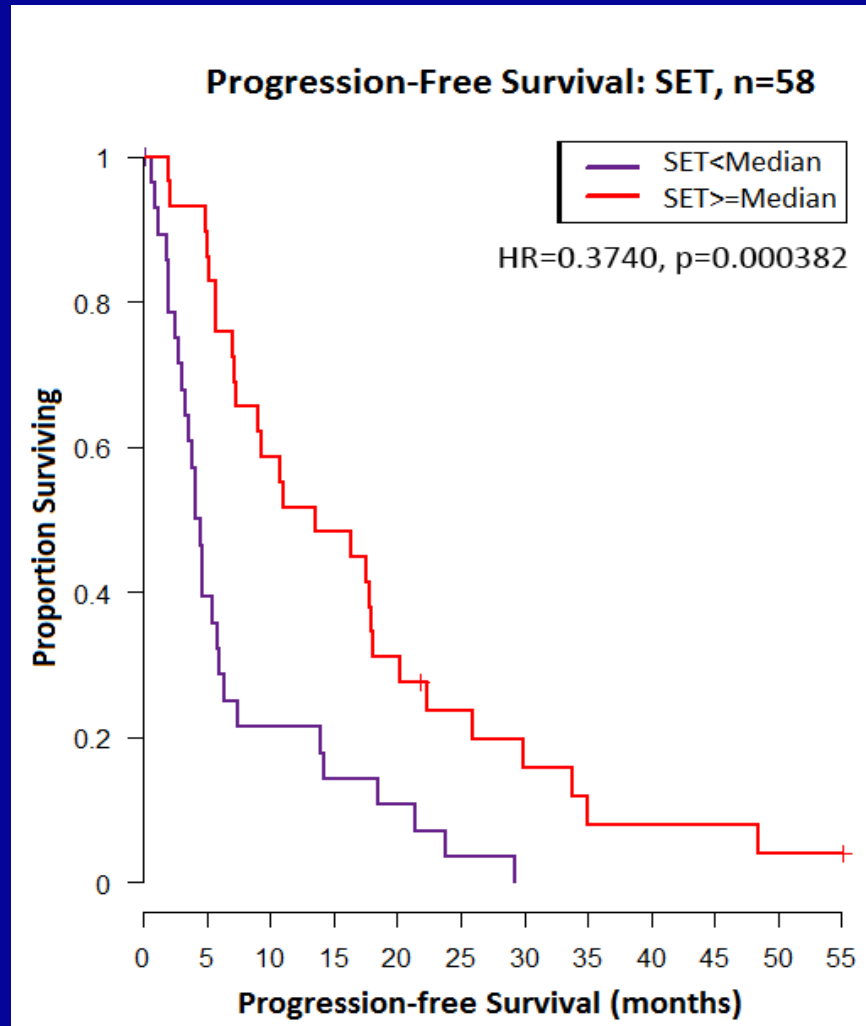
ESR1 LBD
SET index GES (n=18)

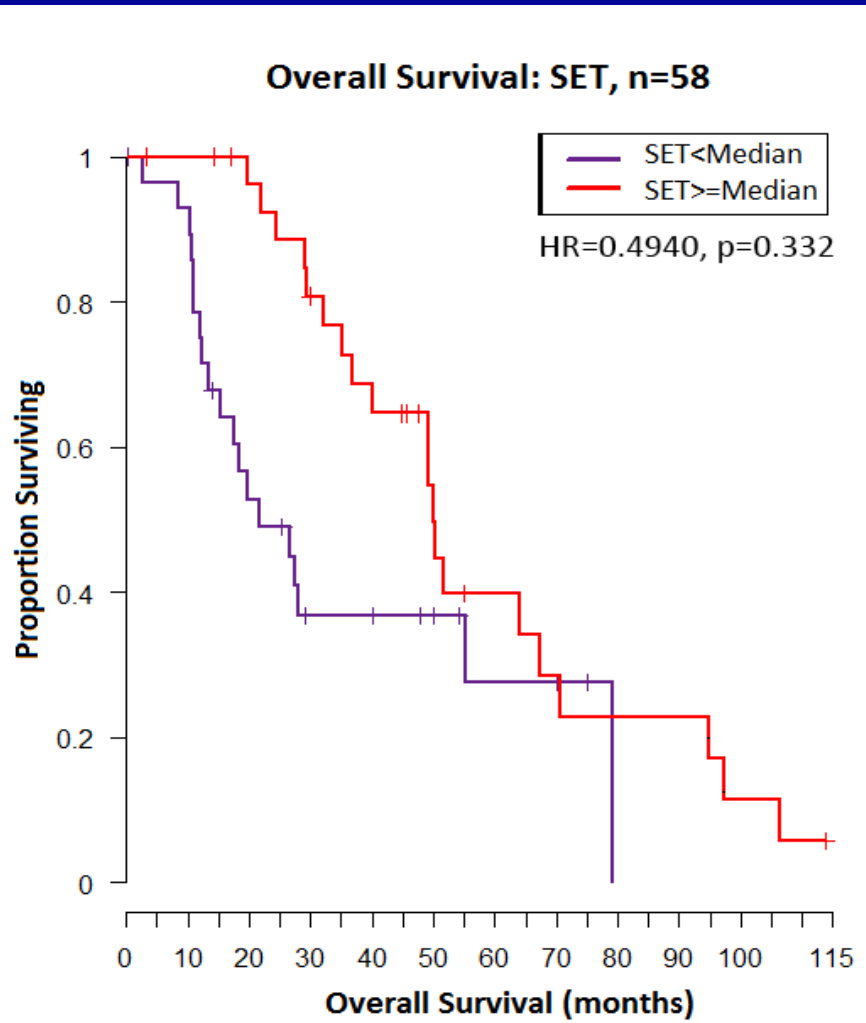
PIK3CA hotspots
PI3K index GES (n=10)

AKT1 hotspot
ERBB2 hotspot
FGFR1 expression

Reference genes (n=10)

Prognostic: Endocrine Treatment of Metastatic Breast Cancer





Independent of standard clinical and pathologic variables

Lau et al. Am Assoc Cancer Res 2016

Summary

Pathologic response to chemotherapy is meaningful in all subtypes

- **Standardized measurement of RCB is prognostic and generalizable**

HR+/HER2-

- **Residual risk is defined by c-Stage, prognostic biology, sensitivity to chemotherapy, endocrine therapy, other treatments**
  - **persists over the long-term**
- **Sequential synergy derived from chemotherapy followed by endocrine therapy**
- **Endocrine-related transcription is clinically relevant in all stages of disease**
  - **Biospecimen research helped us to define a robust biomarker for clinical studies**

TNBC

- **Extent of residual disease after chemotherapy is the most important prognostic information**
- **Predicting response/resistance to chemotherapy-based treatments is challenging**

Acknowledgements

MD Anderson Multidisciplinary Team: Surgeons, Medical Oncologists, Pathologists, Radiation Oncologists, Radiologists, Statistics and Bioinformatics

Research Laboratory: Chunxiao (Lily) Fu, Rosanna Lau, Rebekah Gould, Lili Du, Alex Trevarton, Tsung-Heng Tsai (former)

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and others