Biomarkers in Breast Cancer

D.Cohen, MD, PhD, Dept. of Pathology Leiden University Medical Center, d.cohen@lumc.nl

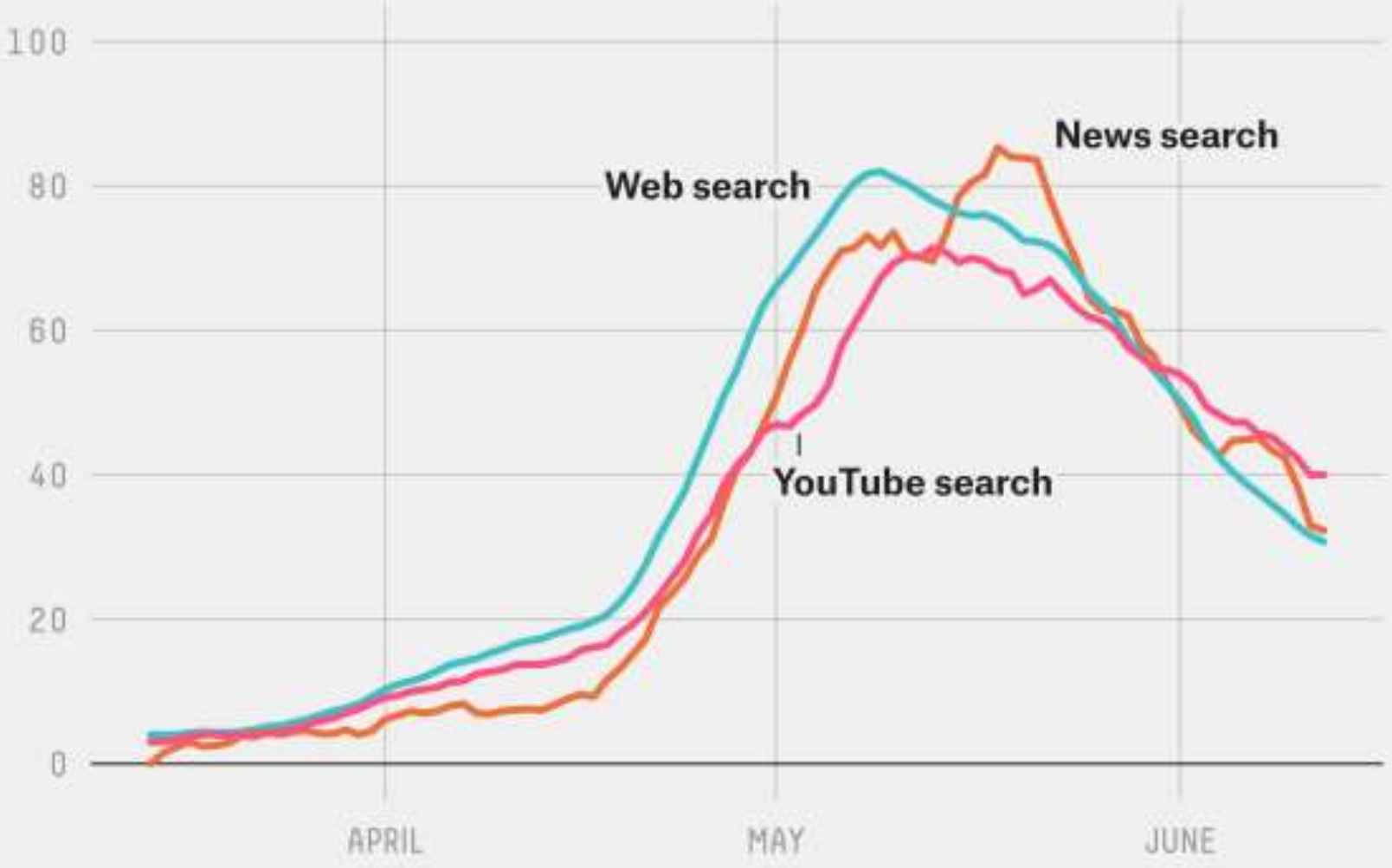
The rise of the fidget spinner

Is the controversial device reaching peak popularity?

Social data analysis via @Brandwatch | 1 March - 25 May 2017

Search them, see them on video, spin 'em

One-week rolling average of Google Trends search interest in fidget toys from March 14 to June 12, where 100 is peak interest for each category



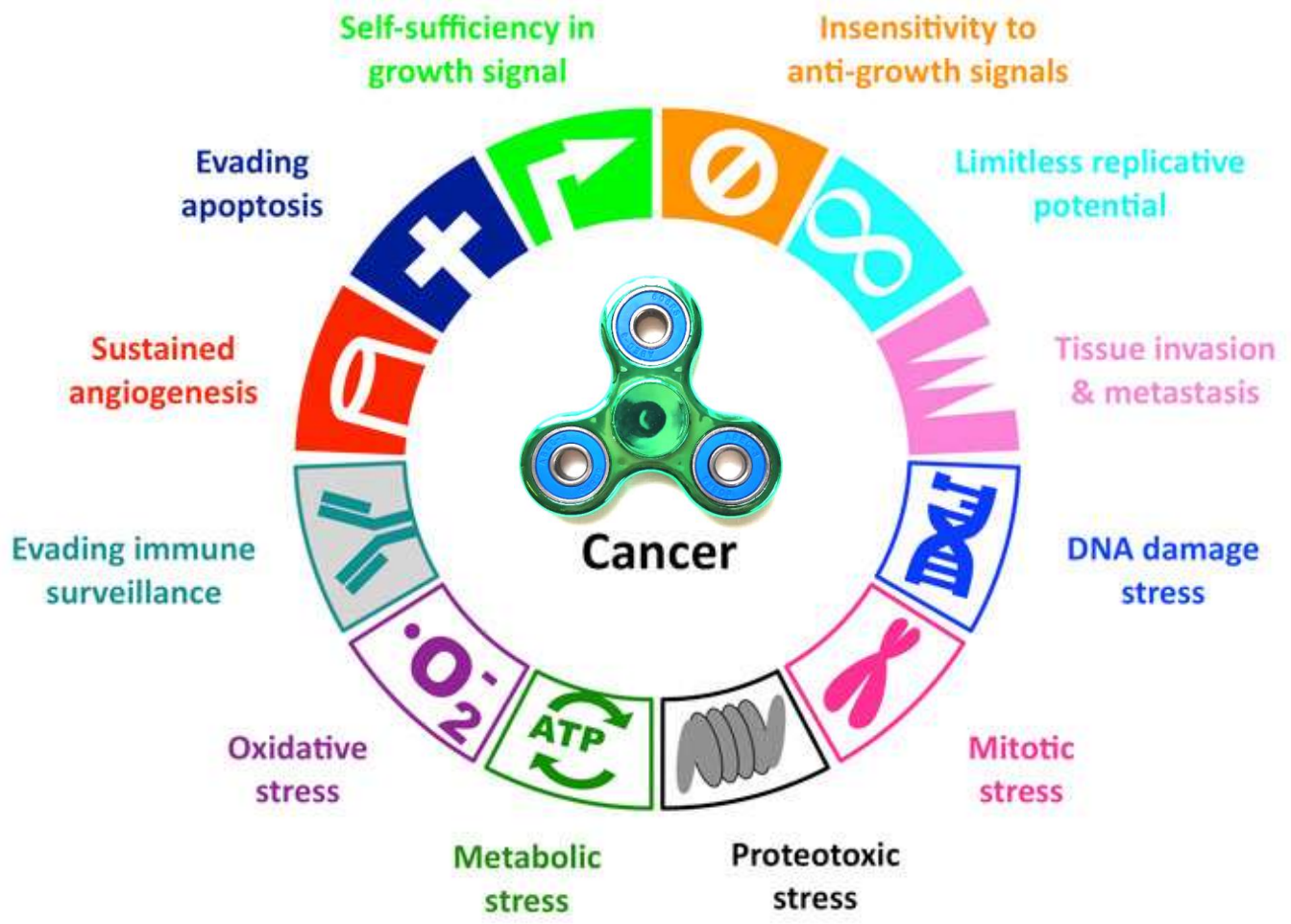
FiveThirtyEight

SOURCE: GOOGLE TRENDS

Most biomarkers become extinct

=

Prognostic markers in Breast Cancer (IHC): >10.000 ‘fidget spinners’ around on pubmed



Why biomarkers?

- PROGNOSTIC → WHO SHOULD BE TREATED?

- PREDICTIVE → WHICH TREATMENT / RESPONSE

Clark GM. Breast Cancer Res Treat. 1994;30:117-26

- PROGNOSTIC → WHO SHOULD BE TREATED?
- PREDICTIVE → WHICH TREATMENT / RESPONSE

Breast Cancer Biomarker Timeline

Staging

Grading

Typing

Protein detection, ER, PR, Ki67, Ker5/6, Her2 etc

Transcriptomics, RNA analysis

NGS, DNA analysis

Liquid biopsy

1957

1980

21th century

The nano-tech 10's

The roaring 20's and beyond

Staging

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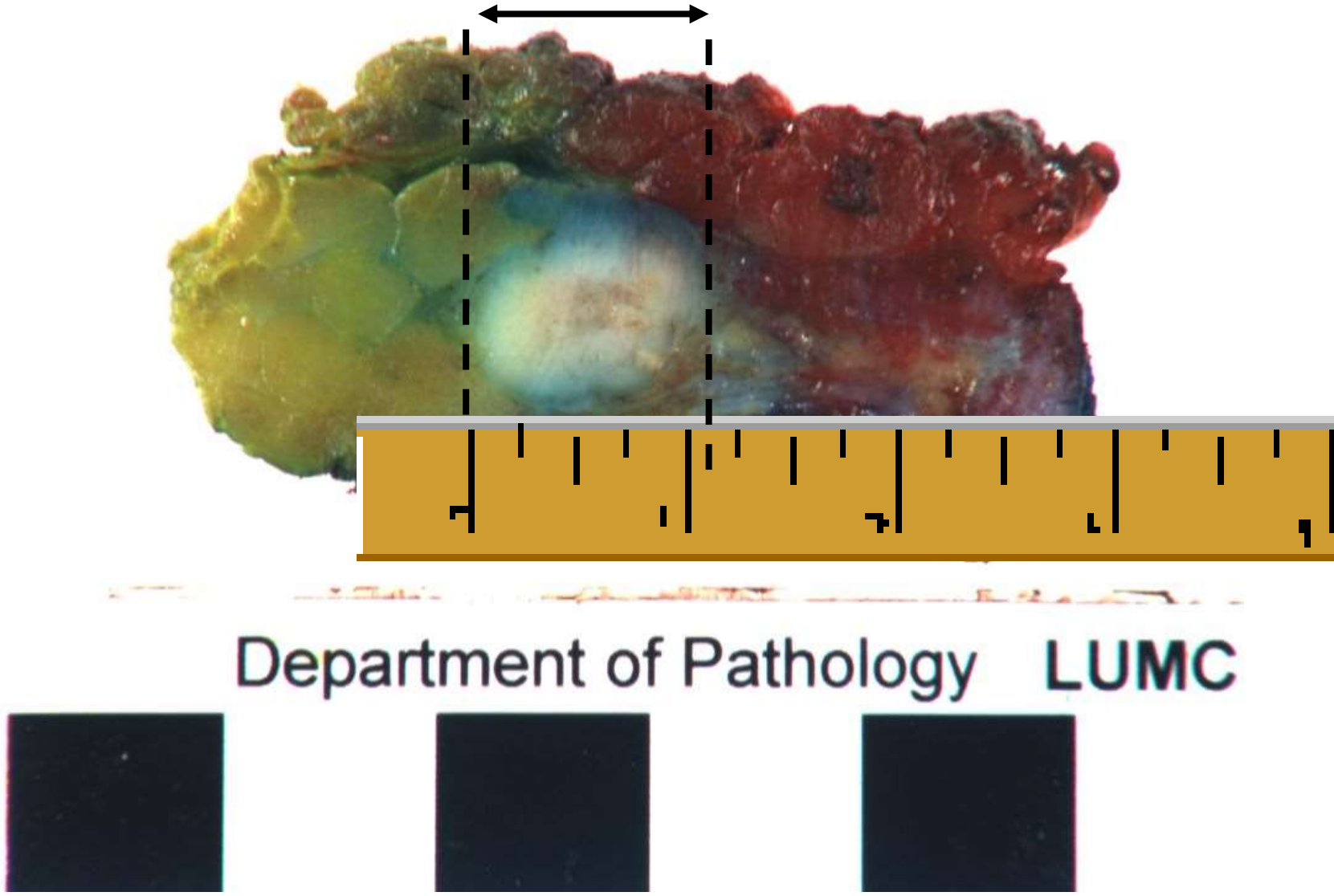
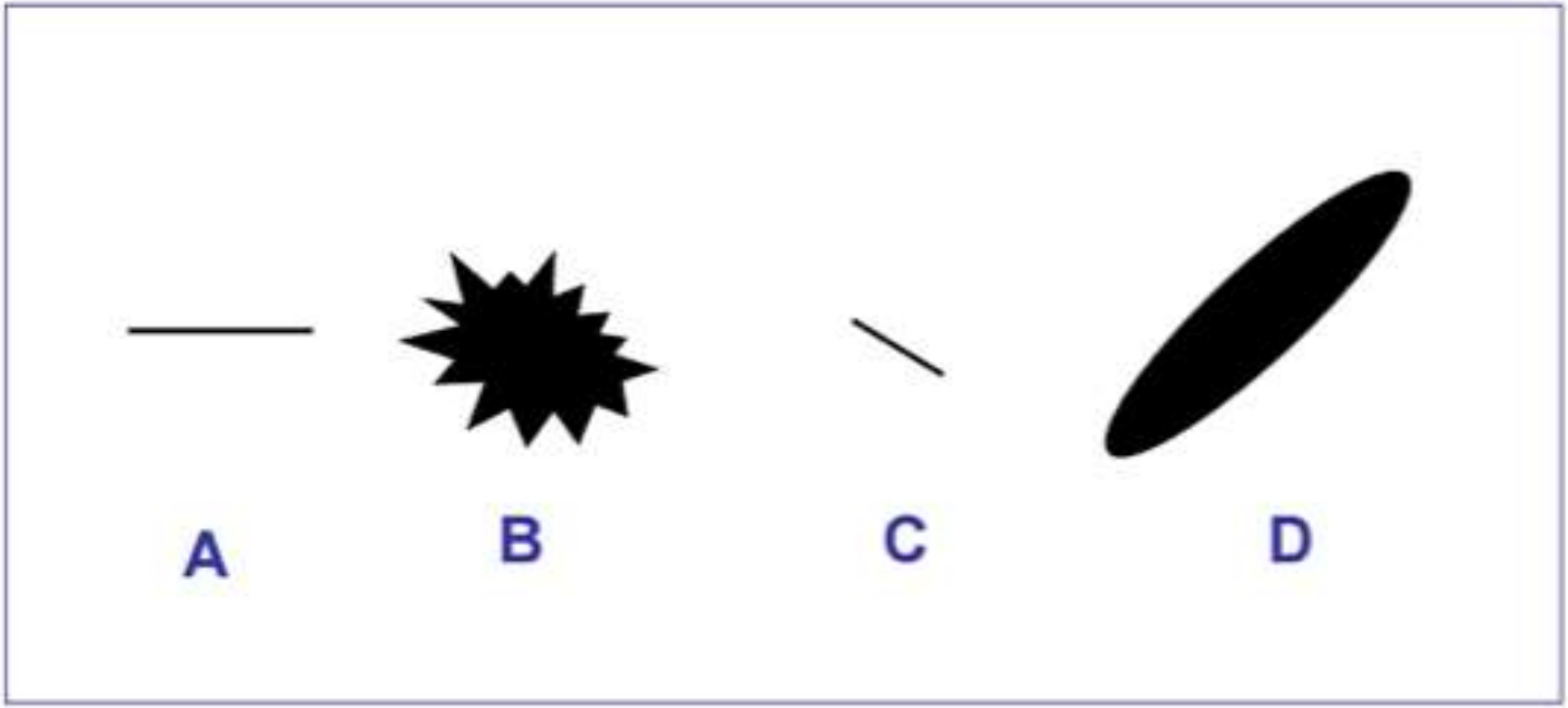
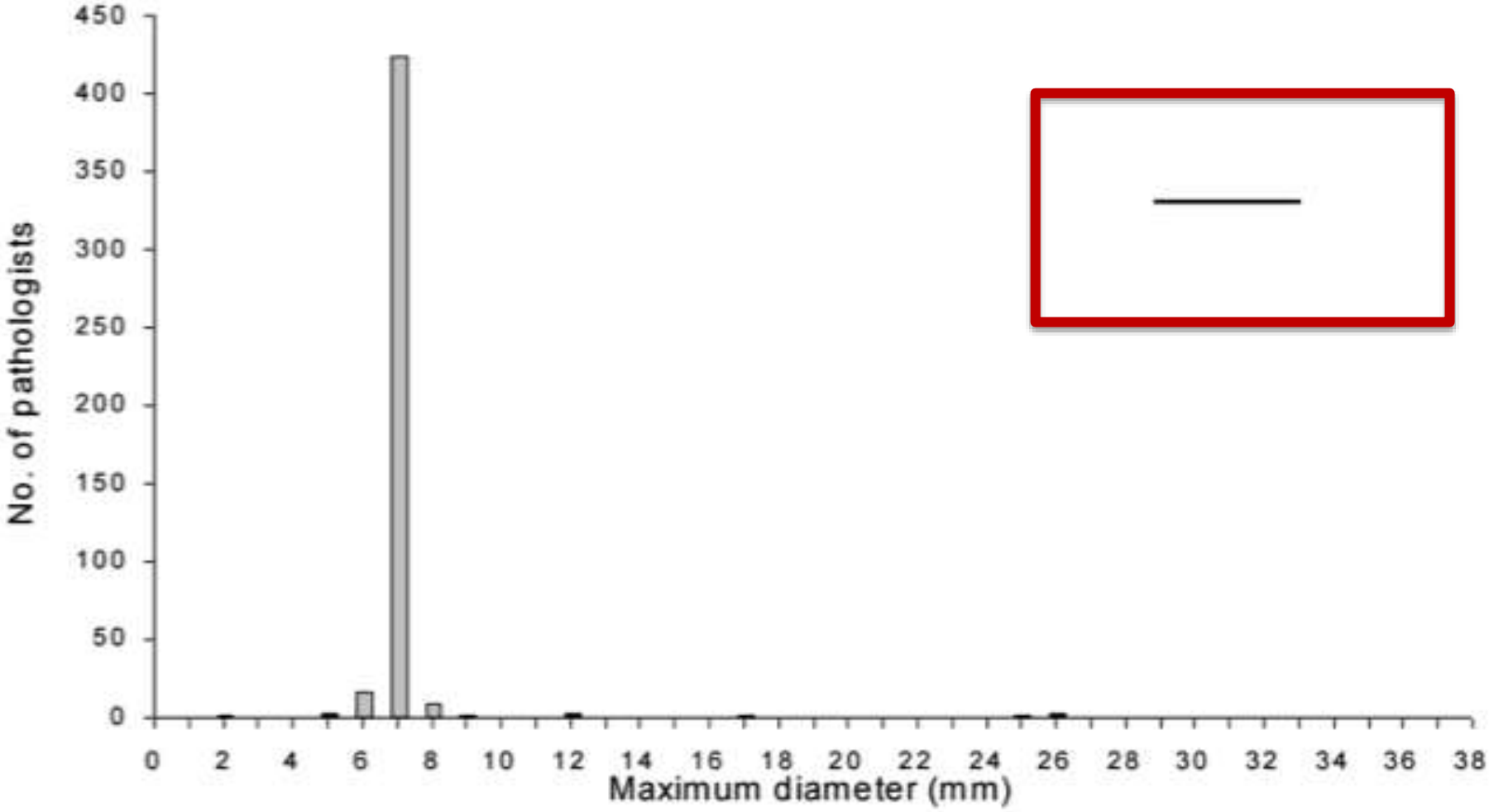
Lateral
1
2
3
4
5
A
B
C
D
F
G
H
I
J
K
L
M
N
O
P
6
7
8
9
Superior
Posterior
Anterior
Inferior
Medial

Tumor-size. Anyone can do that, right?




A
B
C
D


No. of pathologists
450
400
350
300
250
200
150
100
50
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36 38
Maximum diameter (mm)

"...Wie de Macro niet eert is de Micro niet weerd..."

MCB Gorsira , Leiden

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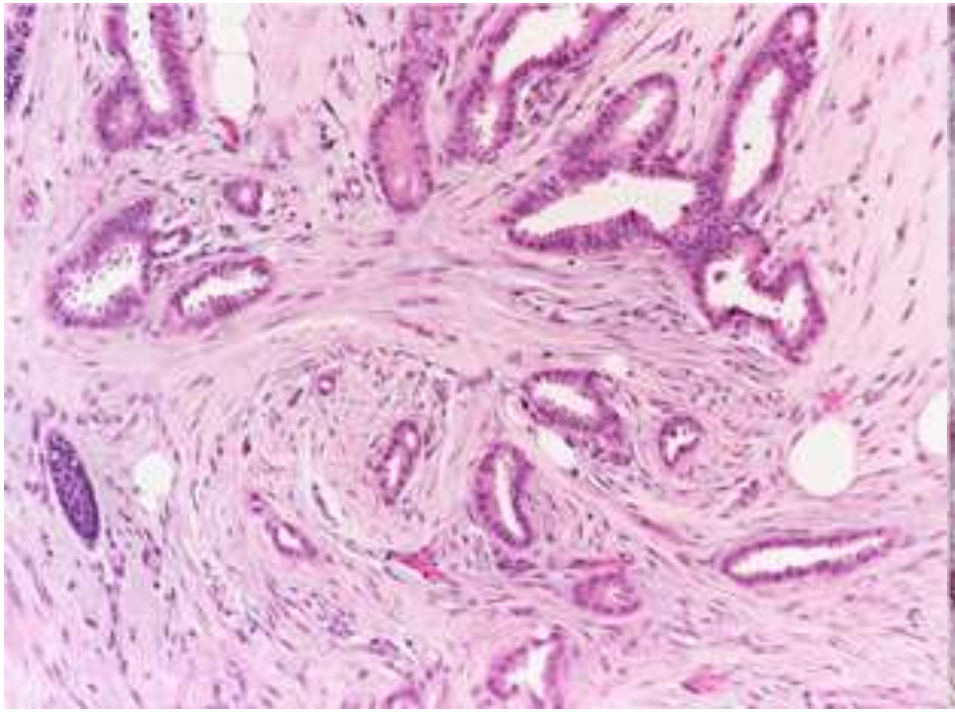
HISTOLOGICAL GRADING AND PROGNOSIS IN BREAST CANCER

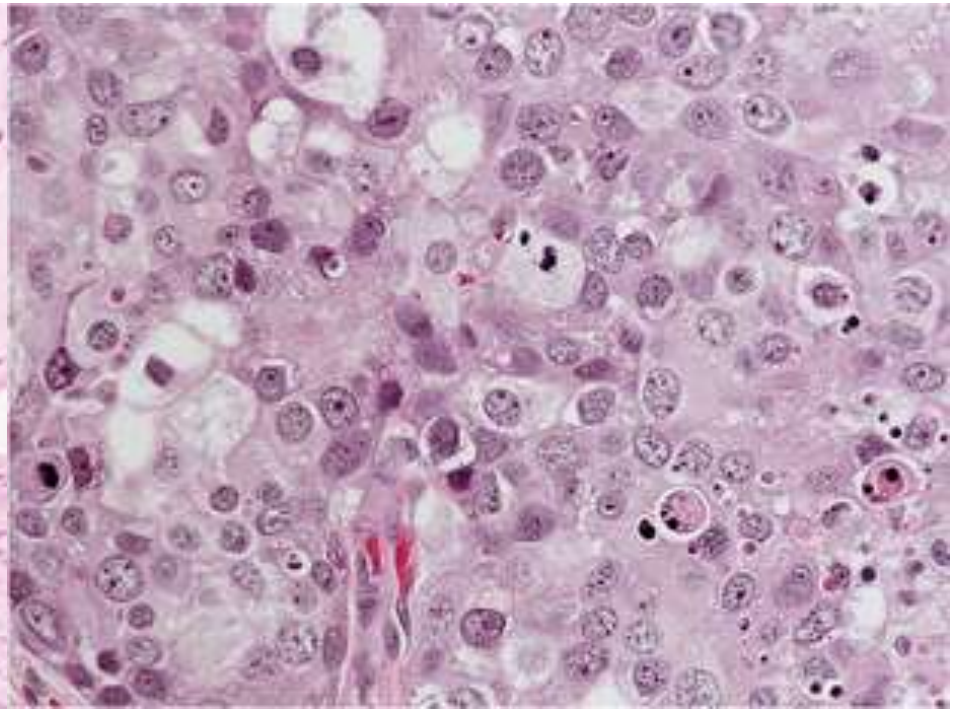
A Study of 1409 Cases of which 359 have been Followed for 15 Years

H. J. G. BLOOM and W. W. RICHARDSON

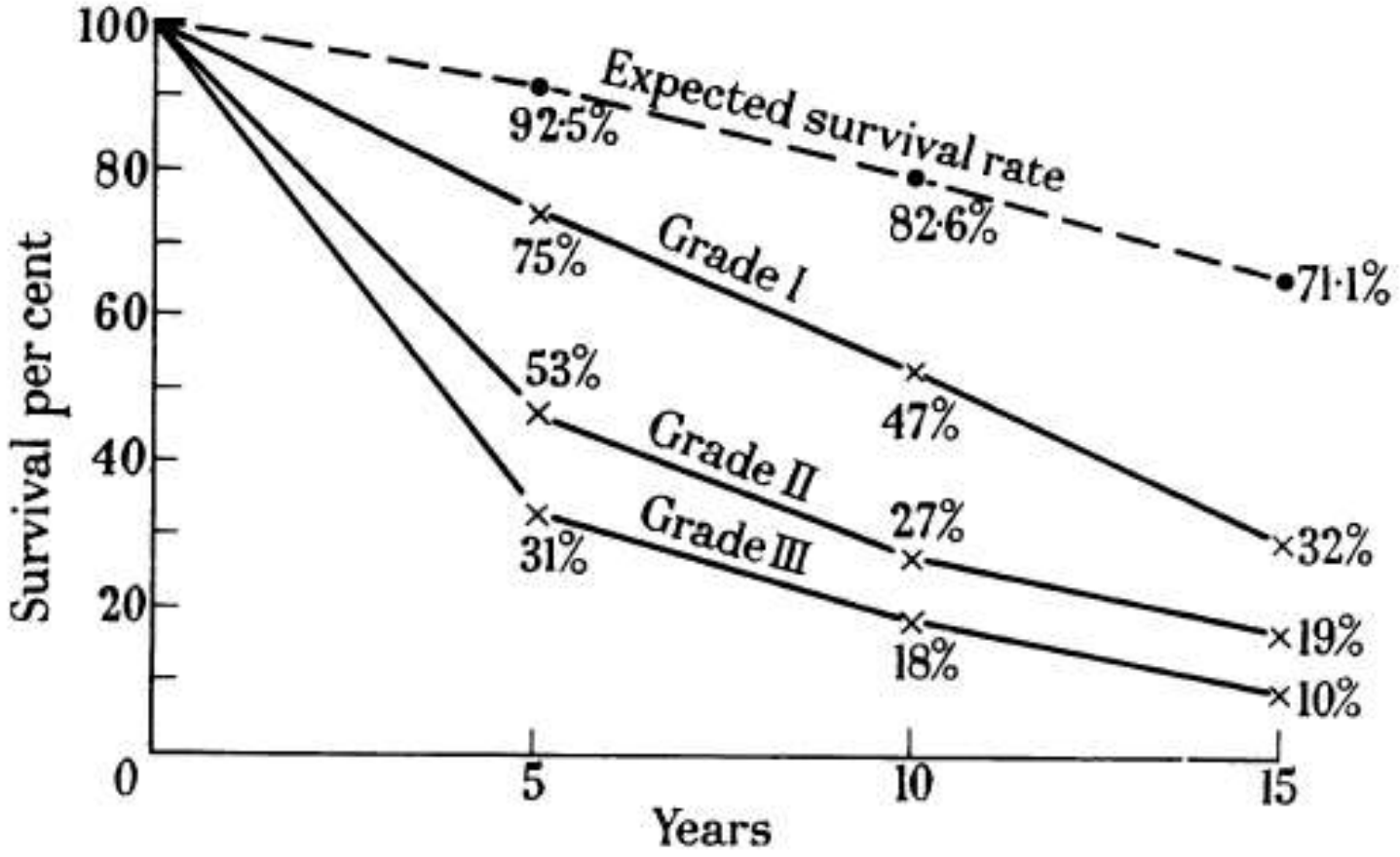
From the Meyerstein Institute of Radiotherapy and the Bland-Sutton Institute of Pathology of the Middlesex Hospital, London, W.1

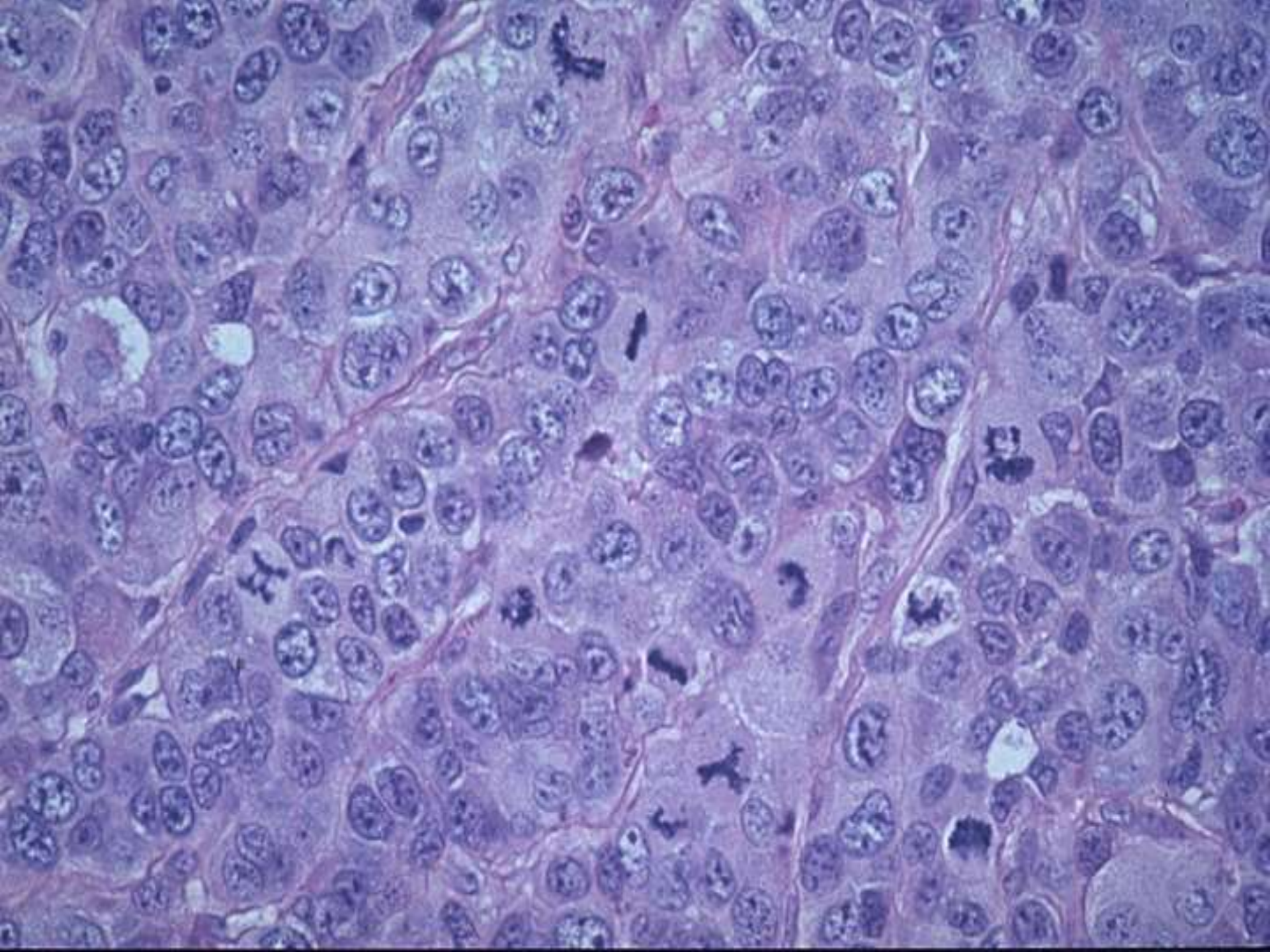
Received for publication July 29, 1957



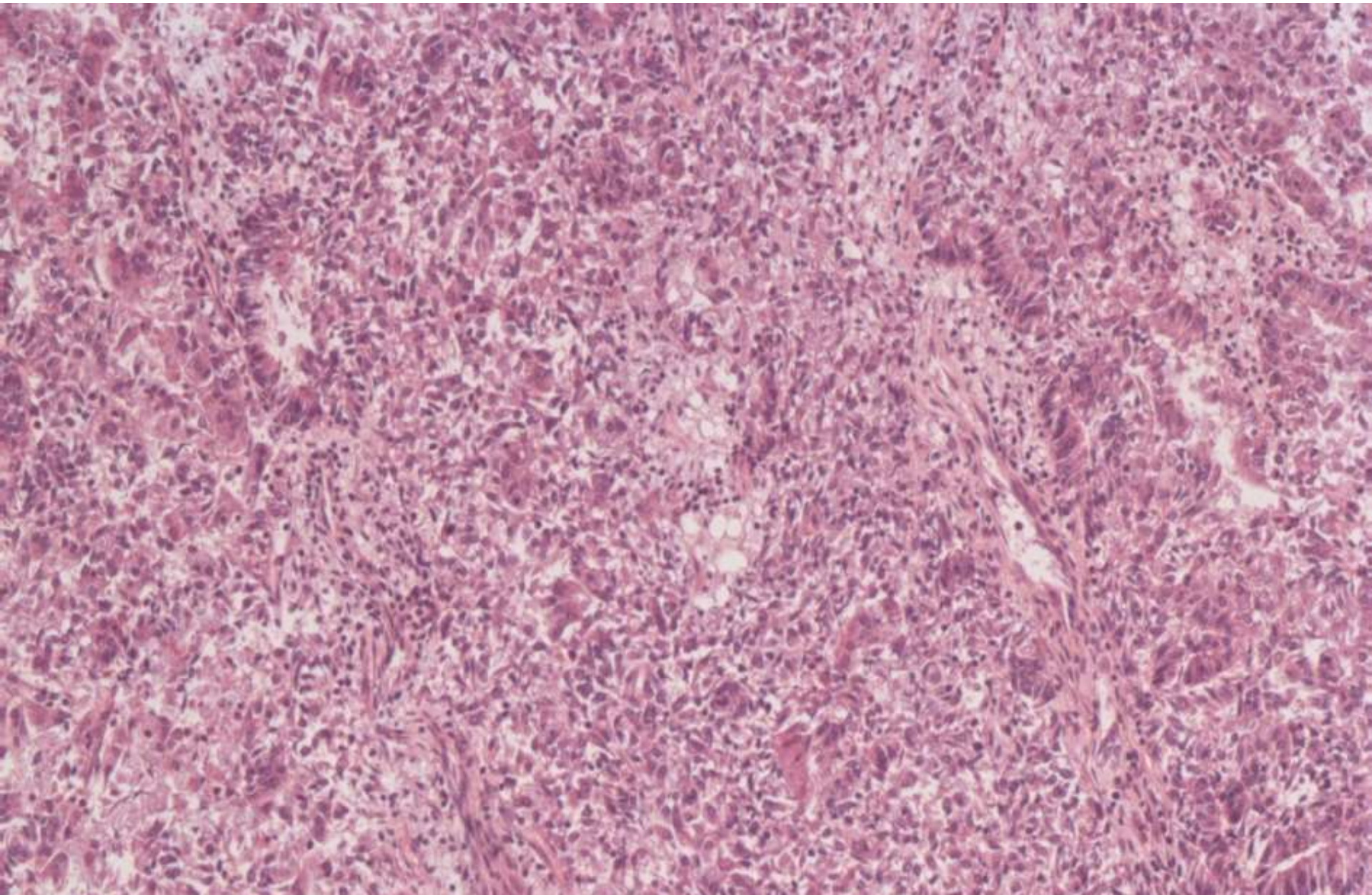


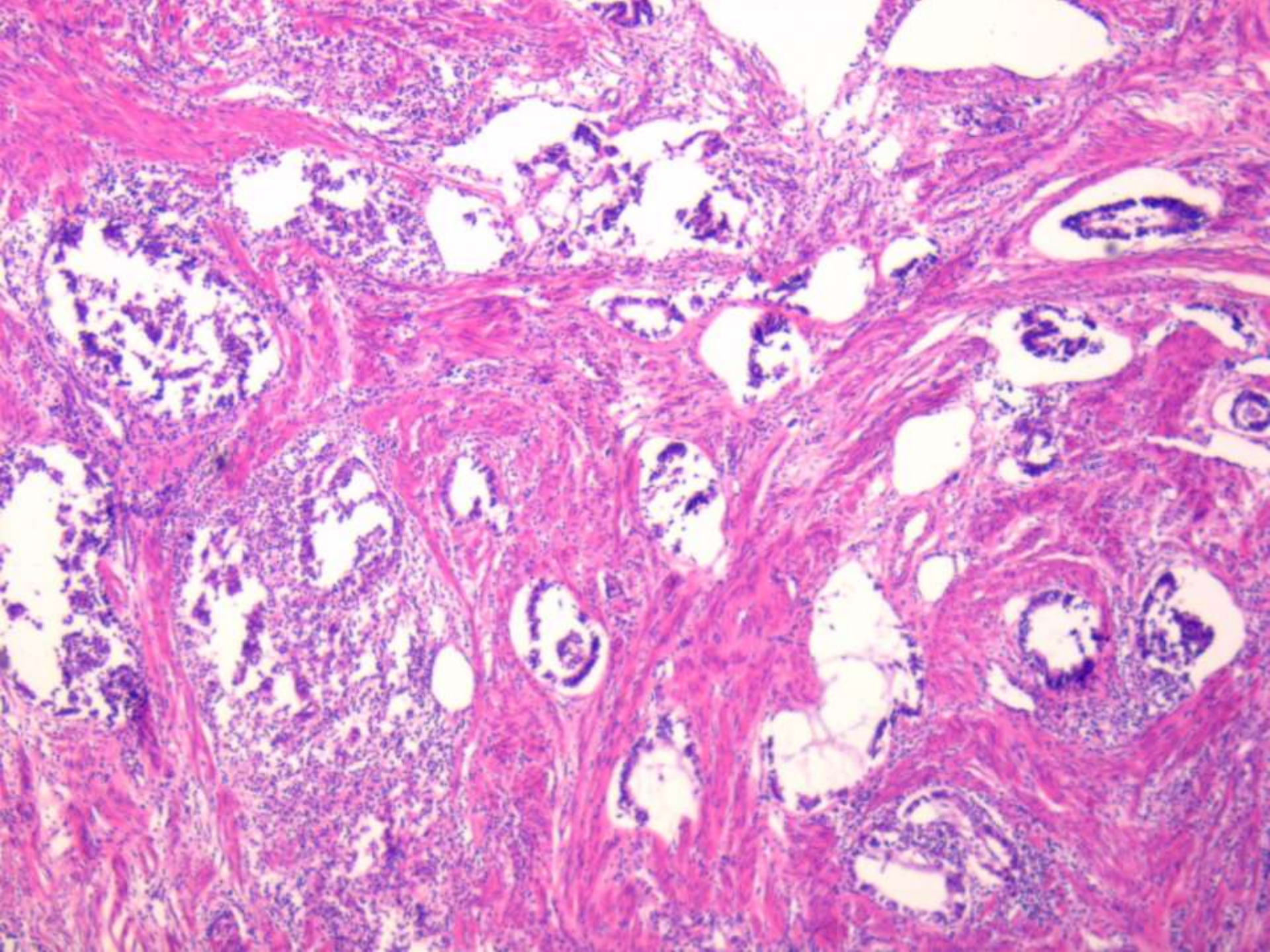
Survival related to grade in breast cancer

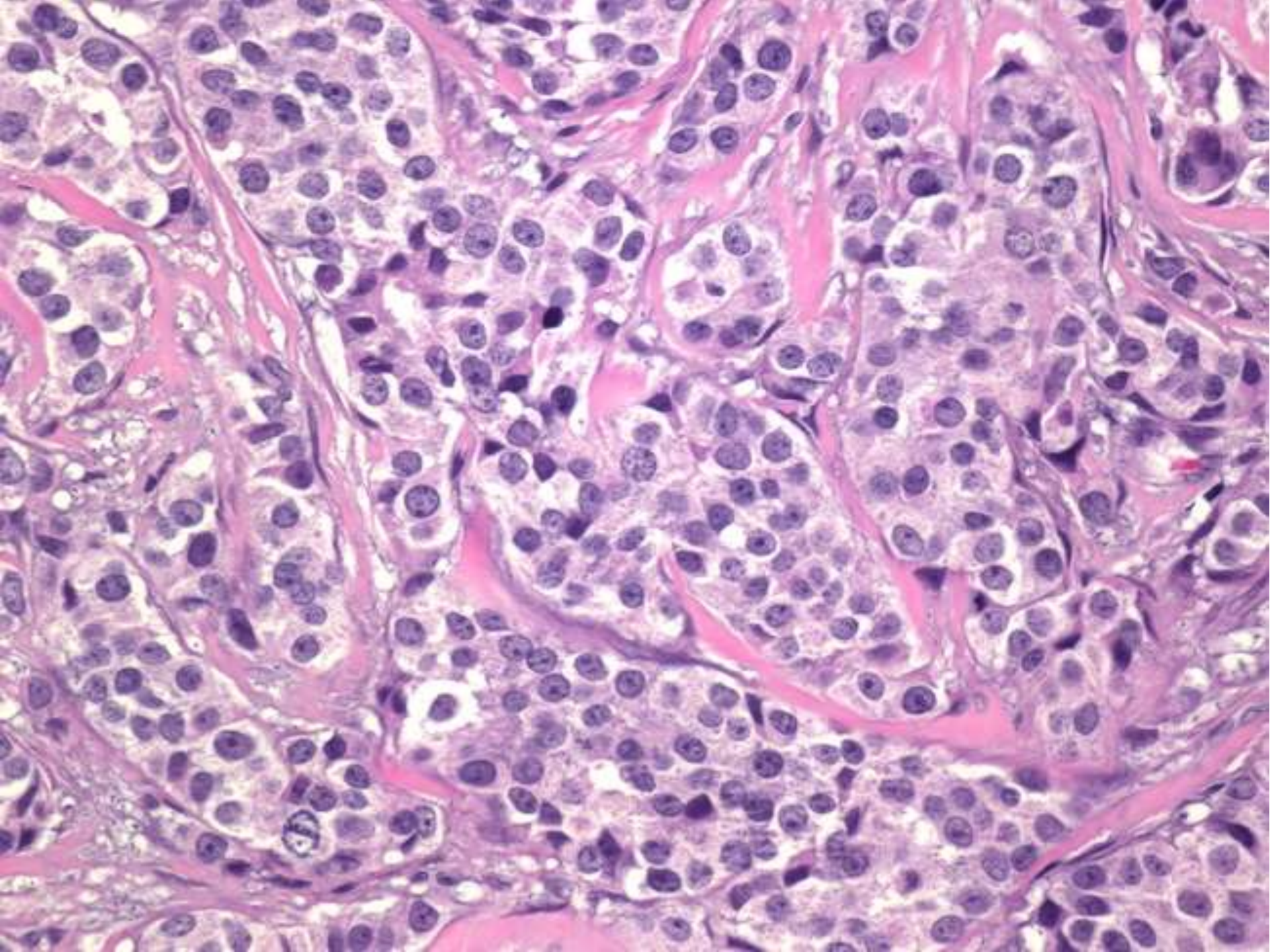




Poor fixation. Anno 2017….







Annals of Oncology 21: 40–47, 2010
doi:10.1093/annonc/mdp273
Published online 21 July 2009

The impact of inter-observer variation in pathological assessment of node-negative breast cancer on clinical risk assessment and patient selection for adjuvant systemic treatment

J. M. Bueno-de-Mesquita[1], D. S. A. Nuyten[2], J. Wesseling[1], H. van Tinteren[3], S. C. Linn[4] & M. J. van de Vijver[1,5*]

Departments of [1]Pathology; [2]Radiation Oncology; [3]Biometrics; [4]Division of Medical Oncology, The Netherlands Cancer Institute and [5]Department of Pathology, Amsterdam Medical Centre, Amsterdam, The Netherlands

Table 4. Impact (clinical relevance) of inter-observer variation on clinical risk assessment

Guideline	Grade	Total	Discordance based on initial examination versus central review		Kappa
			n	%	
CBO [missing 5 (1%)]	2	328	69	21	0.54
	1 and 3	361	33	9	0.82
AO [missing 5 (1%)]	2	328	36	11	0.75
	1 and 3	361	18	5	0.89
St Gallen [missing 6 (1%)]	2	327	42	13	n.a.[a]
	1 and 3	361	42	12	0.713
NPI [missing 5 (1%)]	2	328	56	17	0.58
	1 and 3	361	39	11	0.78

[a]Grade 2 tumours are always intermediate/high-risk tumours based on the St Gallen guidelines.
AO, adjuvant! online; NPI, nottingham prognostic index; n.a., not applicable.

Interobserver variatie ~ **30%**

Staging

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Protein detection, ER, PR, Ki67, Ker5/6, Her2 etc

Transcriptomics, RNA analysis

NGS, DNA analysis

Liquid biopsy

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The roaring 20's and beyond

ON THE TREATMENT OF INOPERABLE CASES OF CARCINOMA OF THE MAMMA: SUGGESTIONS FOR A NEW METHOD OF TREATMENT, WITH ILLUSTRATIVE CASES.[1]

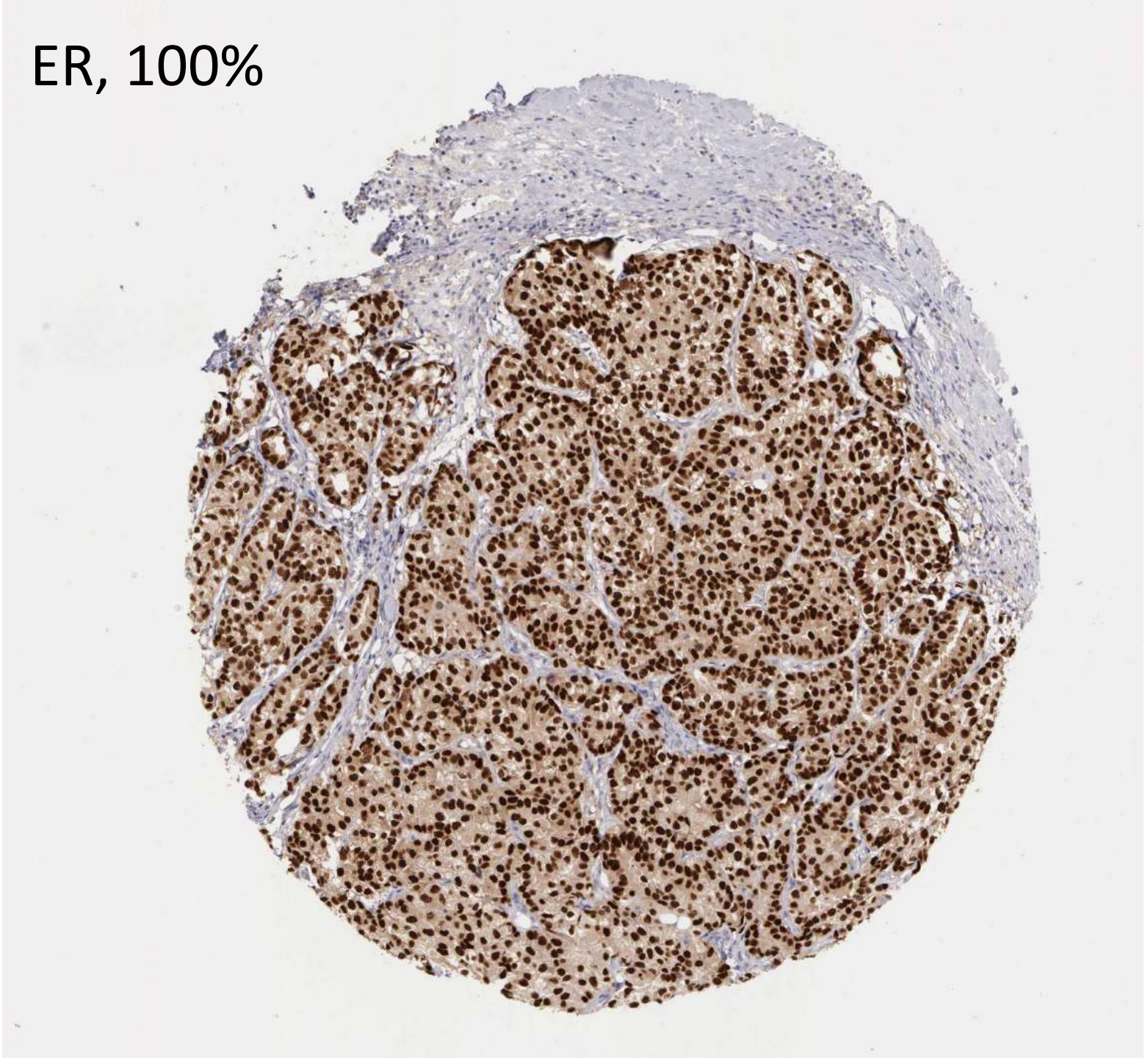
By GEORGE THOMAS BEATSON, M.D. Edin.,

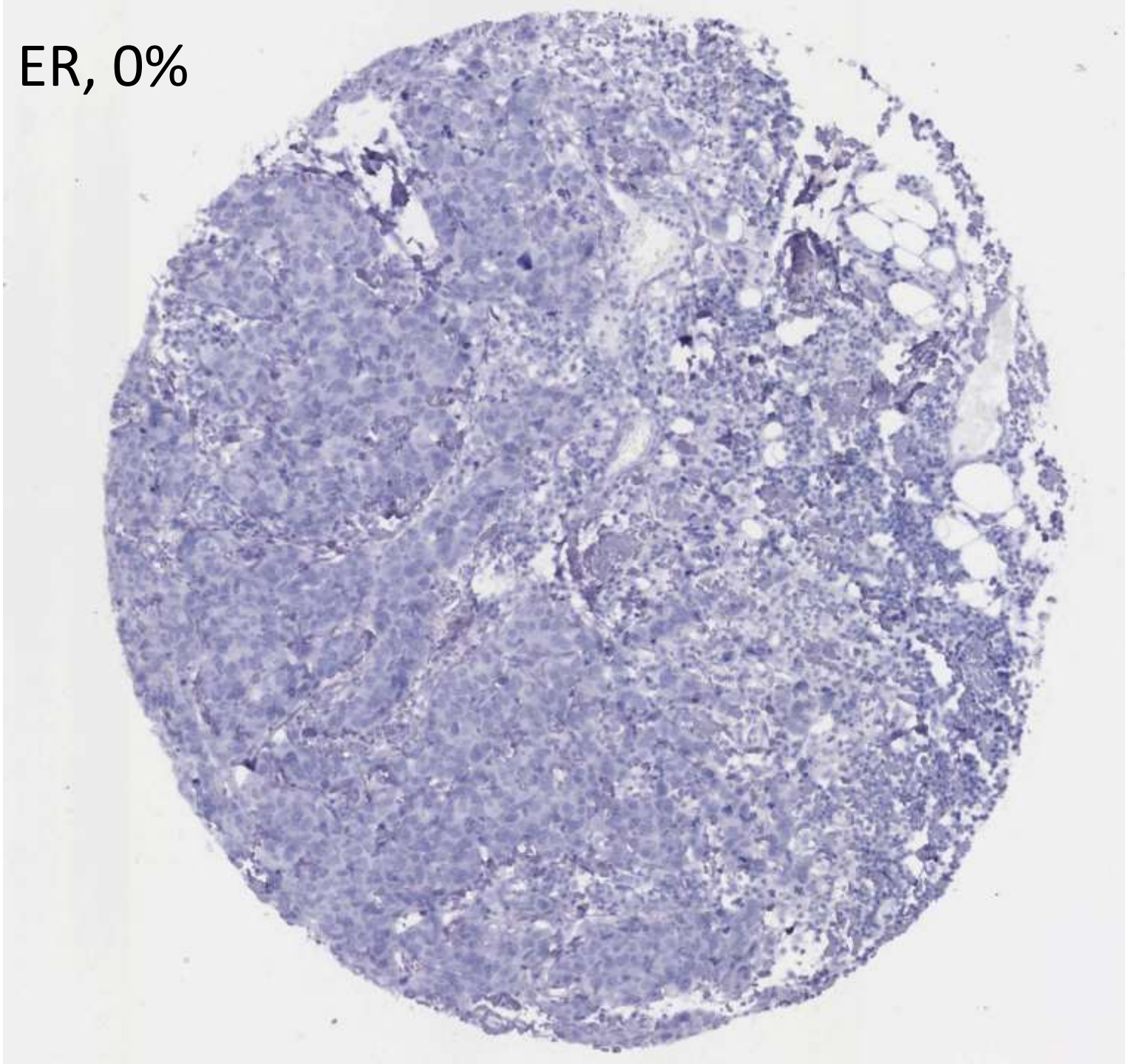
SURGEON TO THE GLASGOW CANCER HOSPITAL; ASSISTANT SURGEON GLASGOW WESTERN INFIRMARY; AND EXAMINER IN SURGERY TO THE UNIVERSITY OF EDINBURGH.

(*Concluded from page 107*).

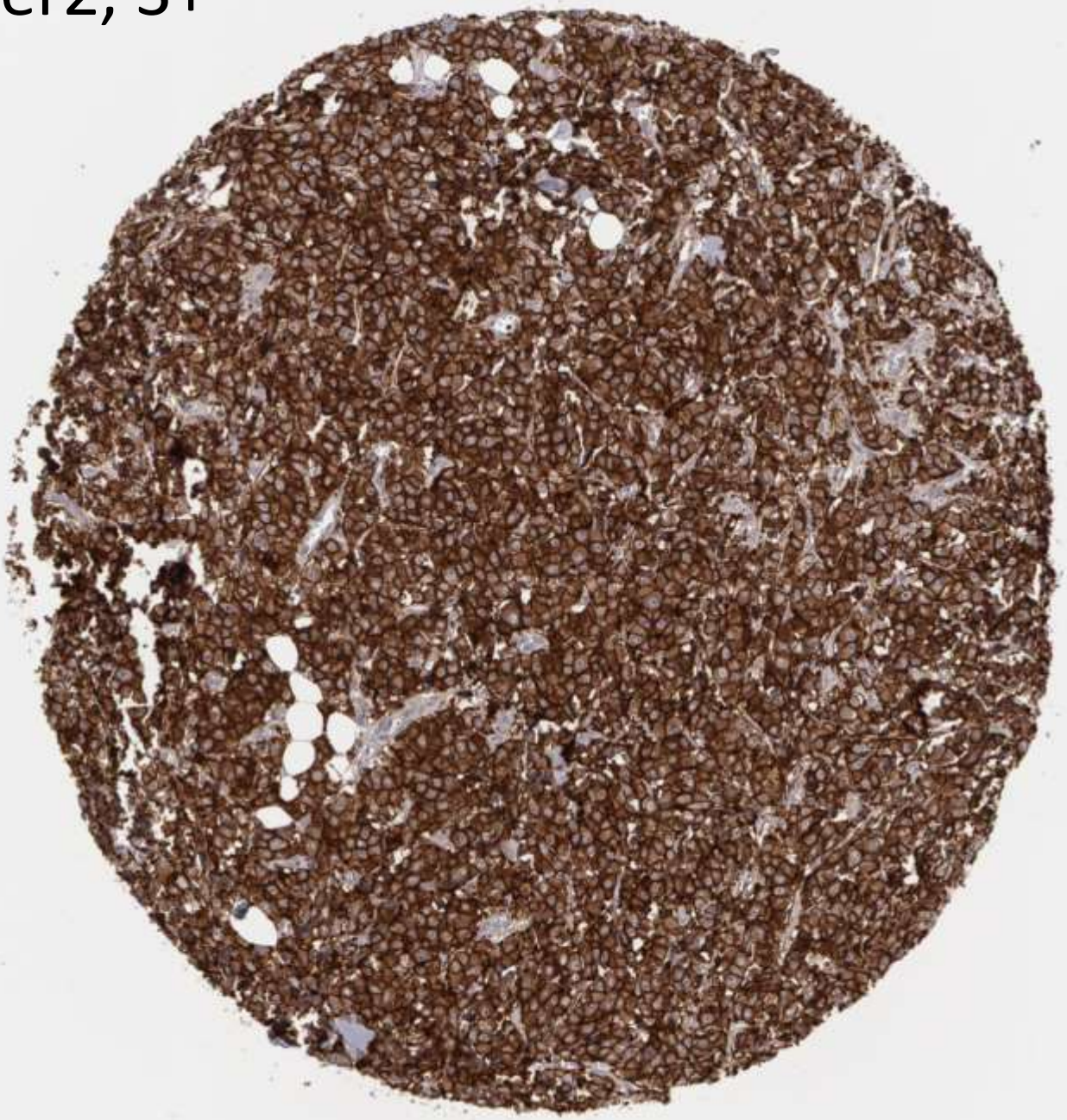
THE next case that I wish to bring under notice is that of a married woman aged forty years, with no family, who was admitted to the Glasgow Cancer Hospital on Sept. 2nd, 1895, suffering from a large tumour of the right mamma. It had

ER, 100%

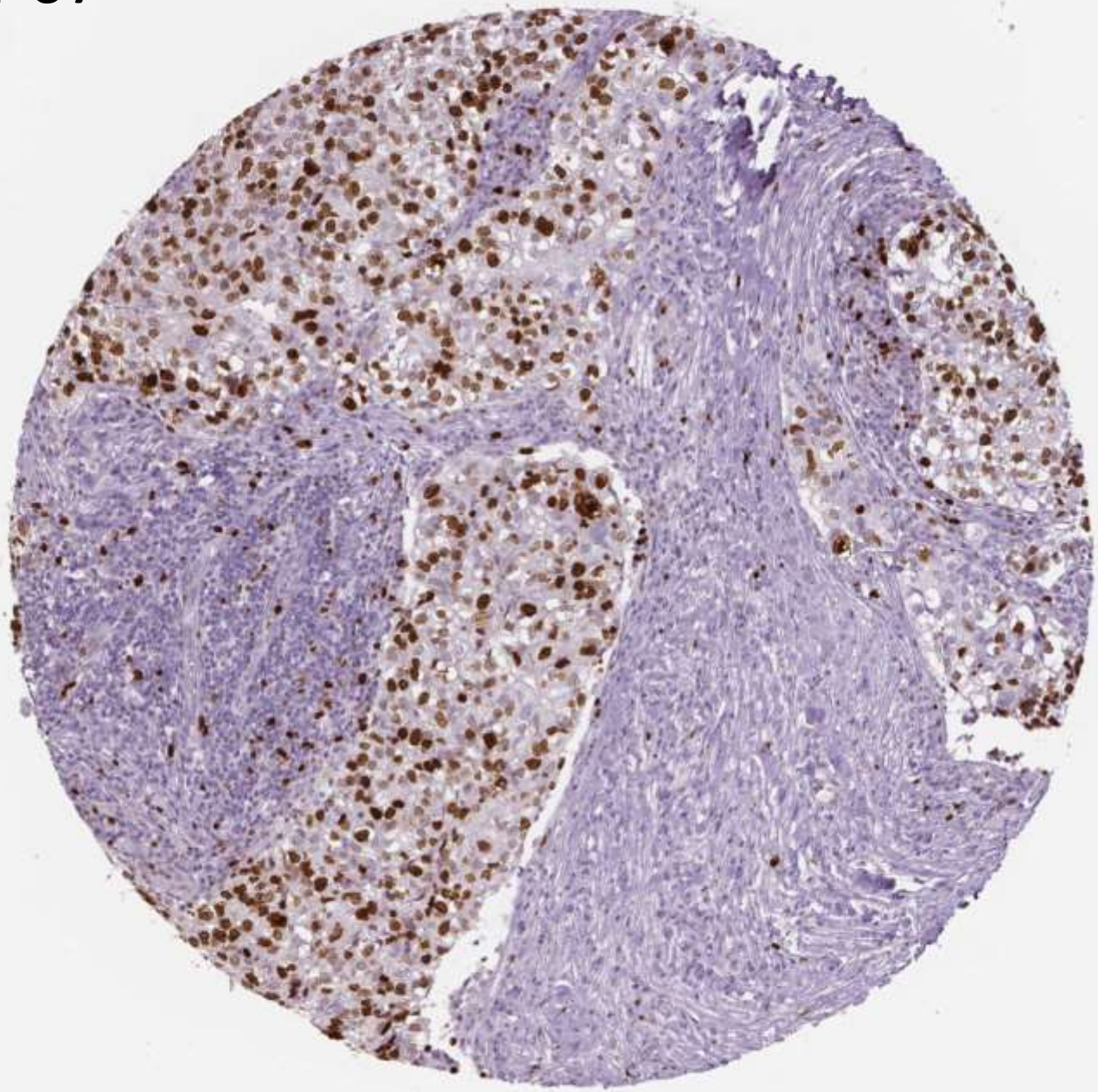


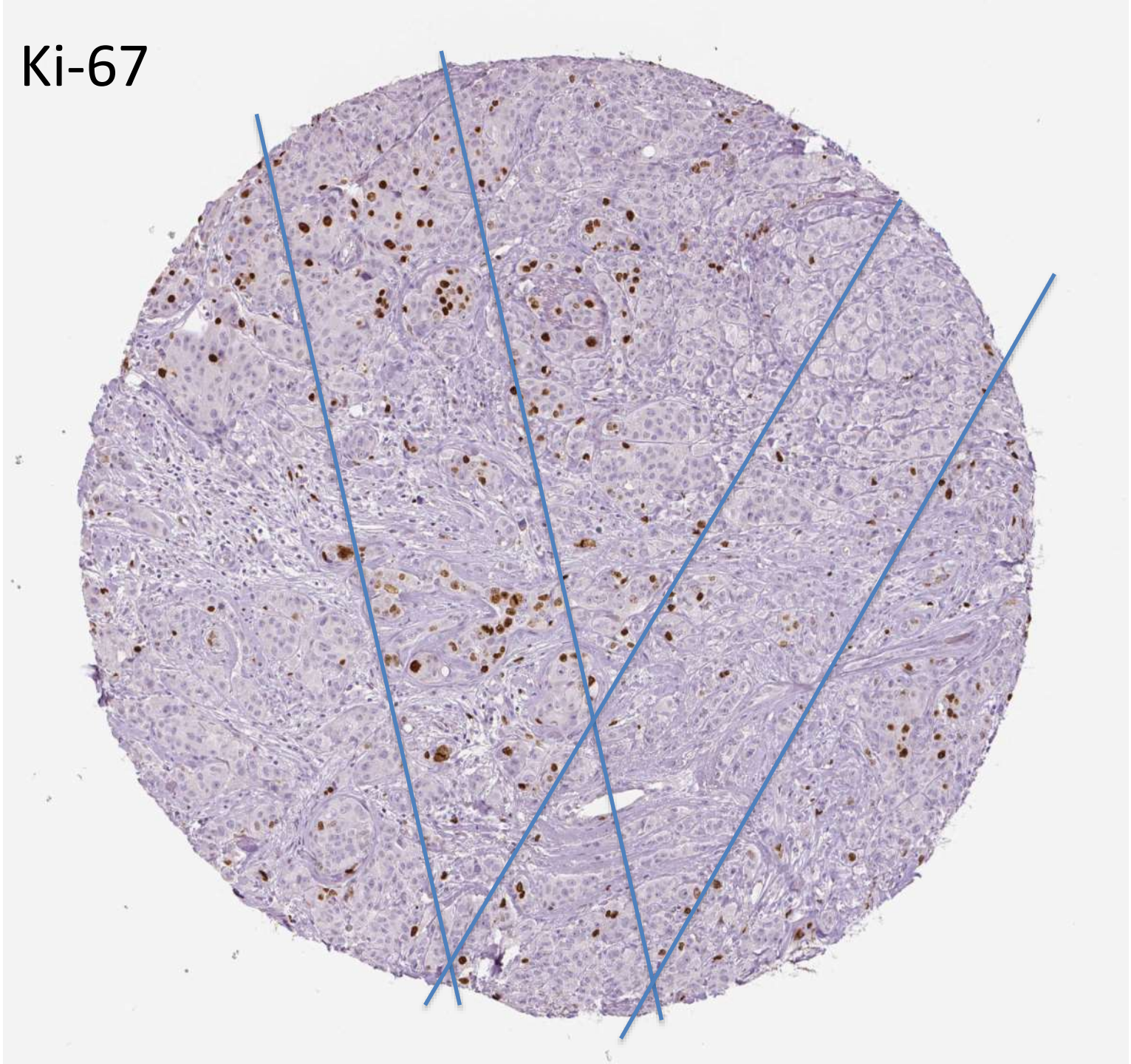

ER, 0%

Her2, 3+



Ki-67




Ki-67

Journal of Pathology and Translational Medicine 2016; 50: 129-137
http://dx.doi.org/10.4132/jptm.2015.12.24

■ ORIGINAL ARTICLE ■

Interobserver Variability of Ki-67 Measurement in Breast Cancer

In conclusion, our nationwide thirty-center study of Ki-67 interobserver variability showed that interobserver variability in measuring this critical biomarker is high. Although direct com-

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Protein detection, ER, PR, Ki67, Ker5/6, Her2 etc

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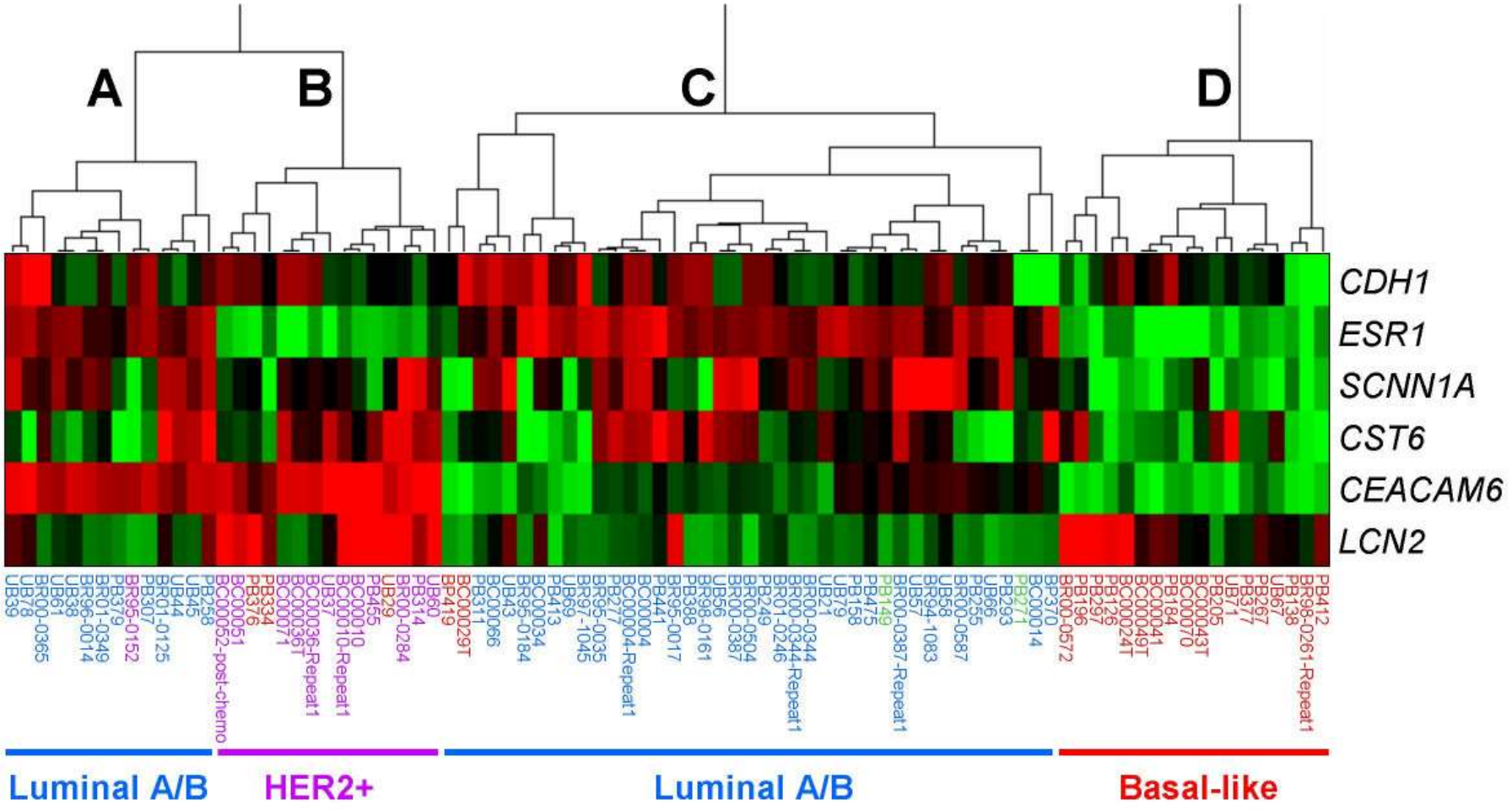
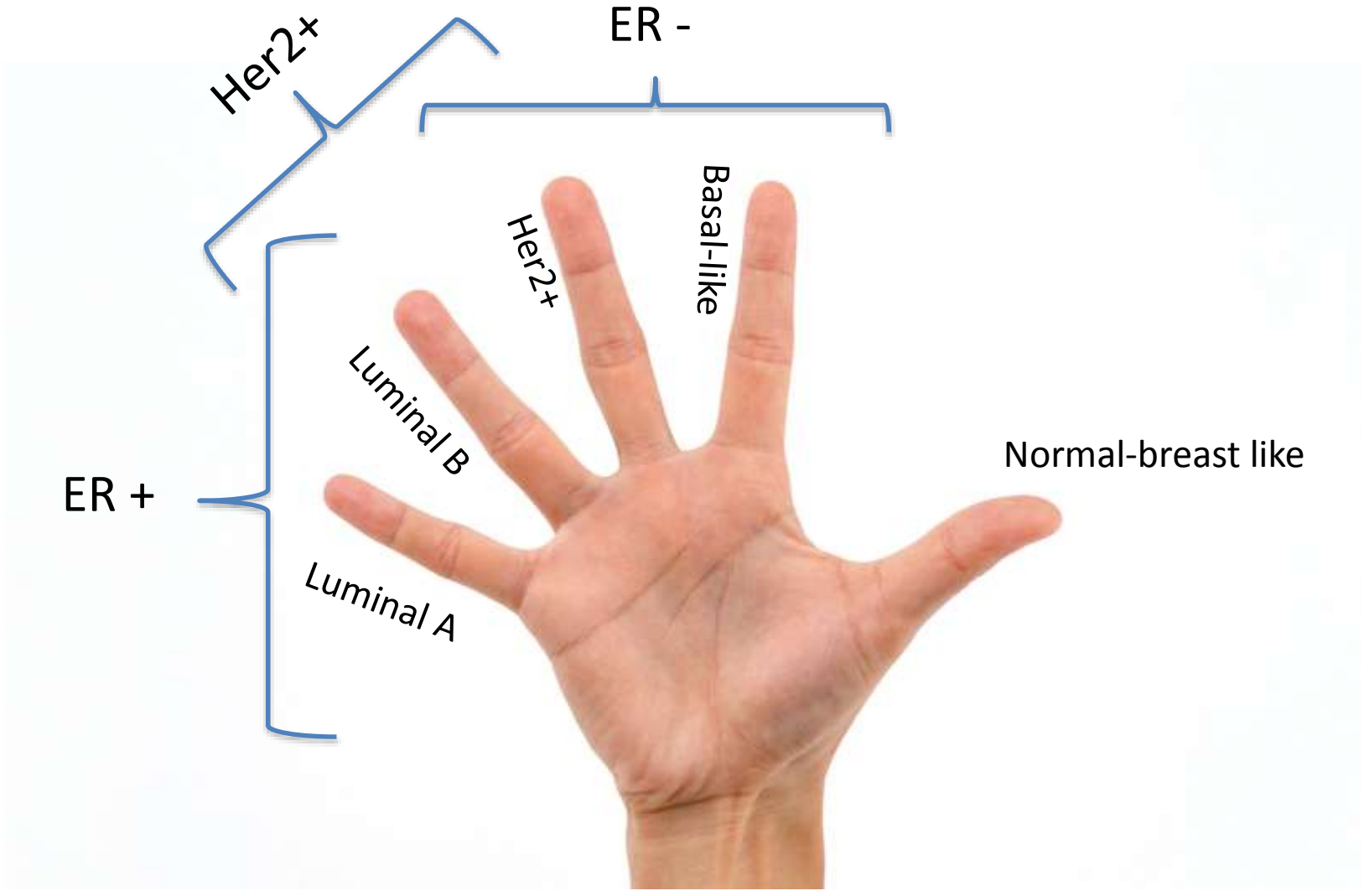
21th century: The genomic era: TRANSCRIPTOMICS

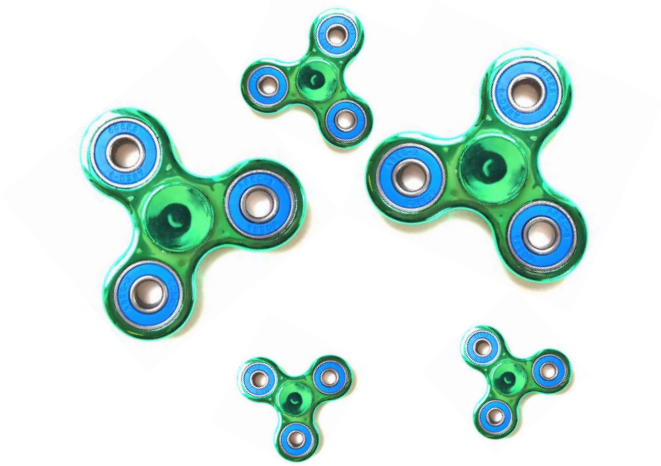


Perou et al. Nature 2000; 406:747–752.
Sorlie et al. Proc Natl Acad Sci USA 2001;98:10869–10874.
Sorlie et al. Proc Natl Acad Sci USA 2003;100:8418–8423.
Hu et al. BMC Genomics 2006;7:96.


Her2+
ER -
ER +
Luminal A
Luminal B
Her2+
Basal-like
Normal-breast like

Multigene signature panels (spinners?)

Microarray and RT-PCR based assays



- **21 gene signature (Oncotype Dx)**

- 70 gene signature (MammaPrint)

- 76 gene signature (Rotterdam)

- 50 genes: Risk of Recurrence (ROR) score (Prosigna)

- 8 genes (Endopredict) & Epclin

- **5 genes (Molecular grade index)**

- 2 gene ratio (H/I™)

} 7 gene assay (THEROS The Breast Cancer Index)

- 97 gene: Genomic grade index (MapQuant Dx)

- 14 genes (BreastOncPx)

- 14 gene signature (Celera Metastasis Score™)

-186 gene signature (Invasiveness Gene Signature)

Recurrence Score® Result Uses 21 key Genes Linked to Critical Molecular Pathways

16 BREAST CANCER RELATED GENES

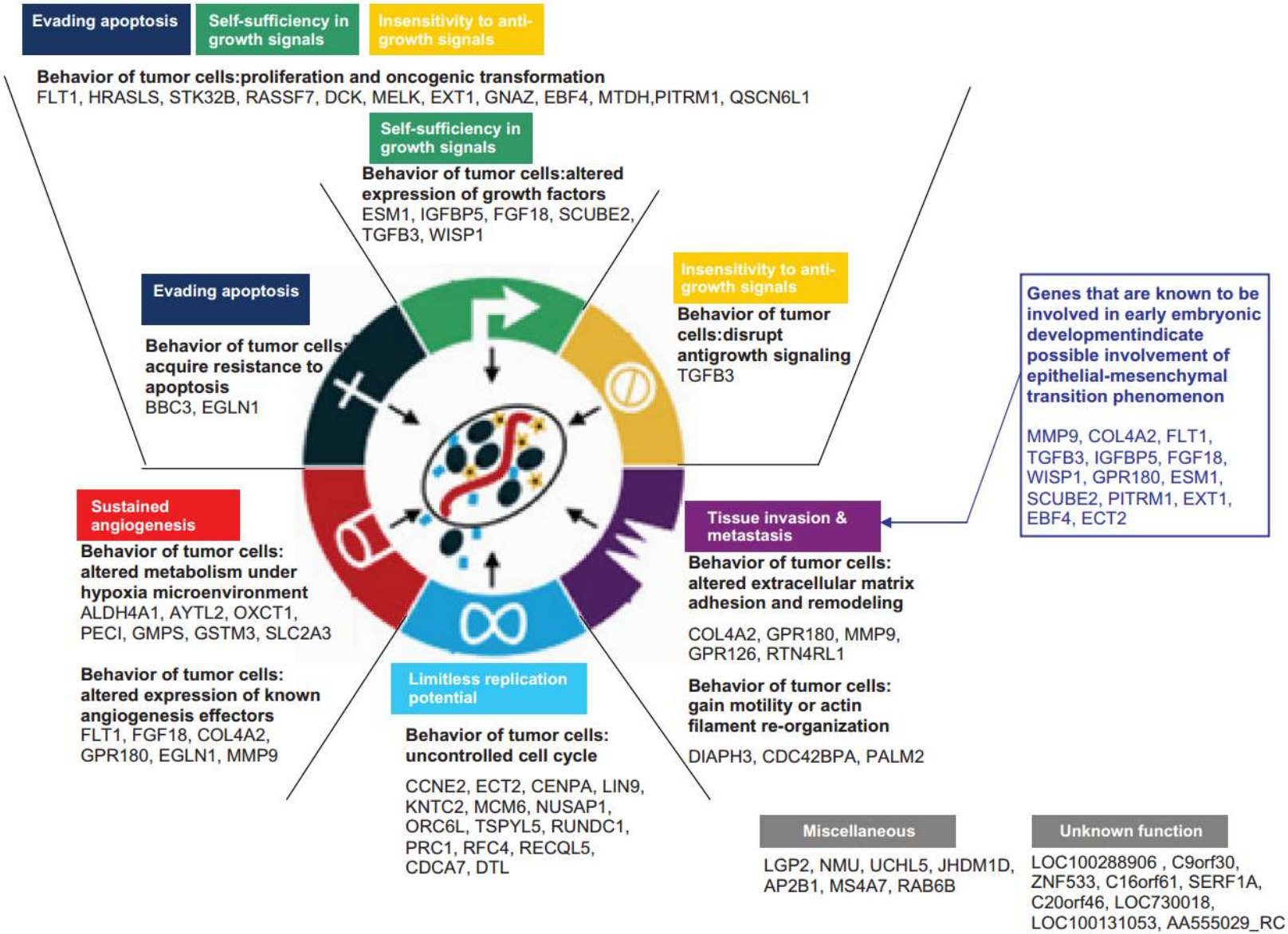
Estrogen	Proliferation	HER2	Invasion	Others
ER PR Bcl2 SCUBE2	Ki-67 STK15 Survivin Cyclin B1 MYBL2	GRB7 HER2	Stromelysin 3 Cathepsin L2	CD68 GSTM1 BAG1

5 REFERENCE GENES

Beta-actin	GAPDH	RPLPO	GUS	TFRC

Paik S, et al. *N Engl J Med*. 2004;351:2817-2826.

Mammaprint genes



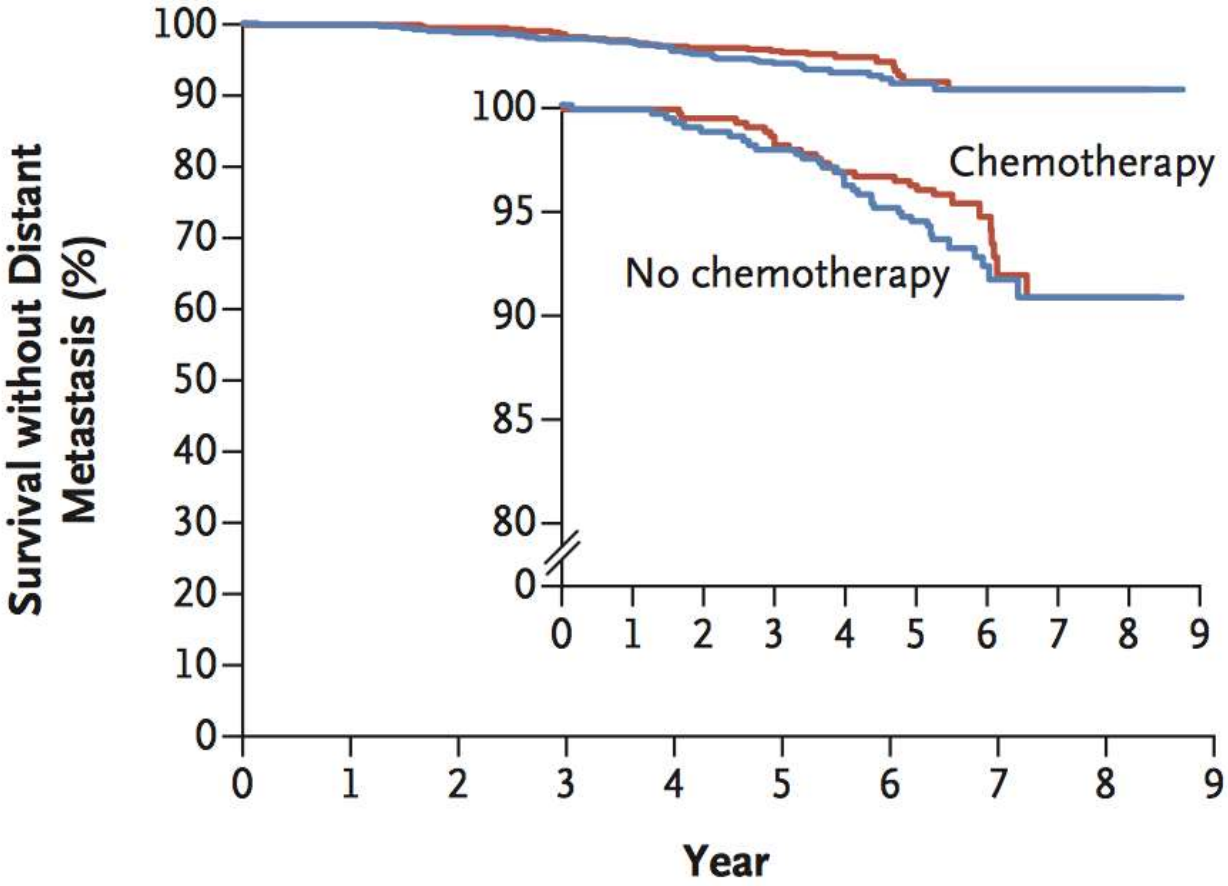
The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 AUGUST 25, 2016 VOL. 375 NO. 8

70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer

F. Cardoso, L.J. van't Veer, J. Bogaerts, L. Slaets, G. Viale, S. Delaloge, J.-Y. Pierga, E. Brain, S. Causeret, M. DeLorenzi, A.M. Glas, V. Golfinopoulos, T. Goulioti, S. Knox, E. Matos, B. Meulemans, P.A. Neijenhuis, U. Nitz, R. Passalacqua, P. Ravdin, I.T. Rubio, M. Saghatchian, T.J. Smilde, C. Sotiriou, L. Stork, C. Straehle, G. Thomas, A.M. Thompson, J.M. van der Hoeven, P. Vuylsteke, R. Bernards, K. Tryfonidis, E. Rutgers, and M. Piccart, for the MINDACT Investigators*

A **High Clinical Risk, Low Genomic Risk**



No. at risk

Chemotherapy	749	714	698	677	611	346	145	41	3
No chemotherapy	748	727	708	696	655	424	160	41	4

Ultralow risk

The ability to identify patients with ultralow-risk disease can allow clinicians to make bold recommendations, suggested Dr Esserman.

"You can really say to someone, 'You're not going to die of this disease. And we don't have to be aggressive up front and treat you with everything," she said in an article posted on the npr.org website.

Esserman et al, JAMA oncology, june 2017

OncotypeDX vs Mammaprint

OncotypeDX	**Mammaprint**
21 genes	70 genes
qPCR	microarray
Prognostic & predictive	Prognostic

- Number of overlapping genes: 1

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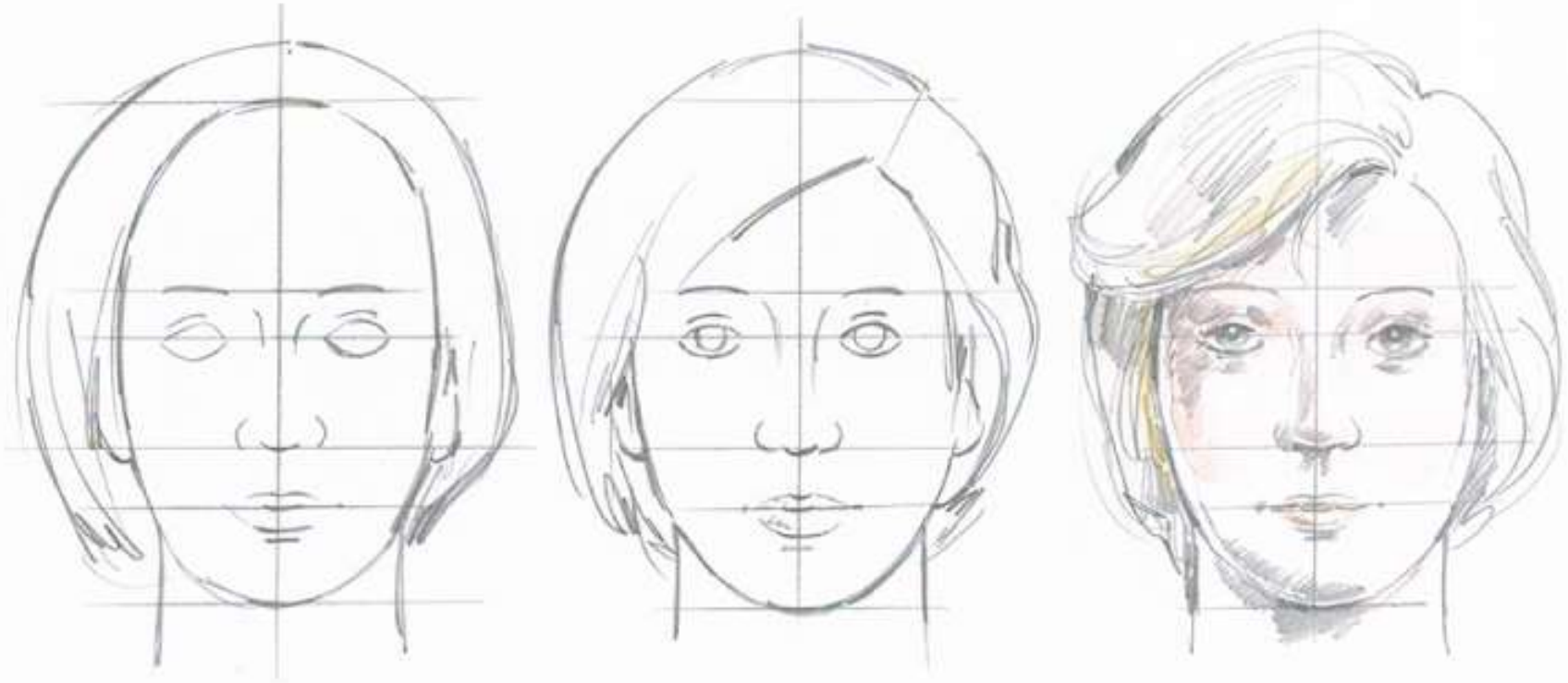
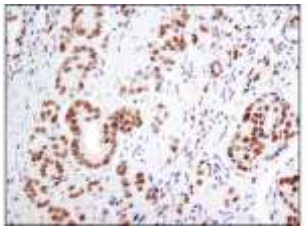
Driver-mutations and ‘mutational portret/signature’



- Basal like 1
- Basal like 2
- Immunomodulatory
- Mesenchymal stem-cell like
- Luminal androgen Receptor



Lehman et al, J Clin Invest. 2011

TNBCtype

A Subtyping Tool for Triple-negative Breast Cancer

>> Menu

- Prediction
- Help
- View Your Result
- Contact
- Terms of Use

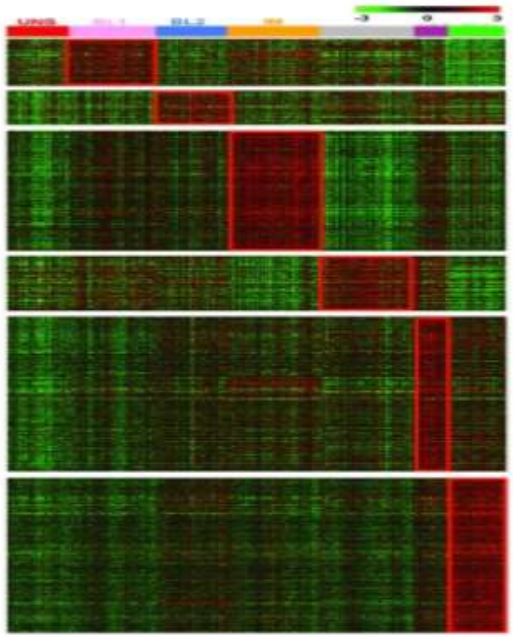
Upload Your Data

Your email address(*required)

Choose a file to upload(Example File|Help)
(*Maximun File Size: 200M)

Kies bestand | geen bestand geselecteerd

Analysis | Cancel



Introduction

Triple-negative breast cancer (TNBC) is a heterogeneous breast cancer group, and identification of its subtypes is essential for understanding the biological characteristics and clinical behaviors of TNBC as well as for developing personalized treatments. Based on 3,247 gene expression profiles from 21 breast cancer data sets, we discovered six TNBC subtypes including 2 basal-like (**BL1** and **BL2**), an immunomodulatory (**IM**), a mesenchymal (**M**), a mesenchymal stem–like (**MSL**), and a luminal androgen receptor (**LAR**) subtype from 587 TNBC samples with unique gene expression patterns and ontologies. Cell line models representing each of the TNBC subtypes also displayed different sensitivities to targeted therapeutic agents.

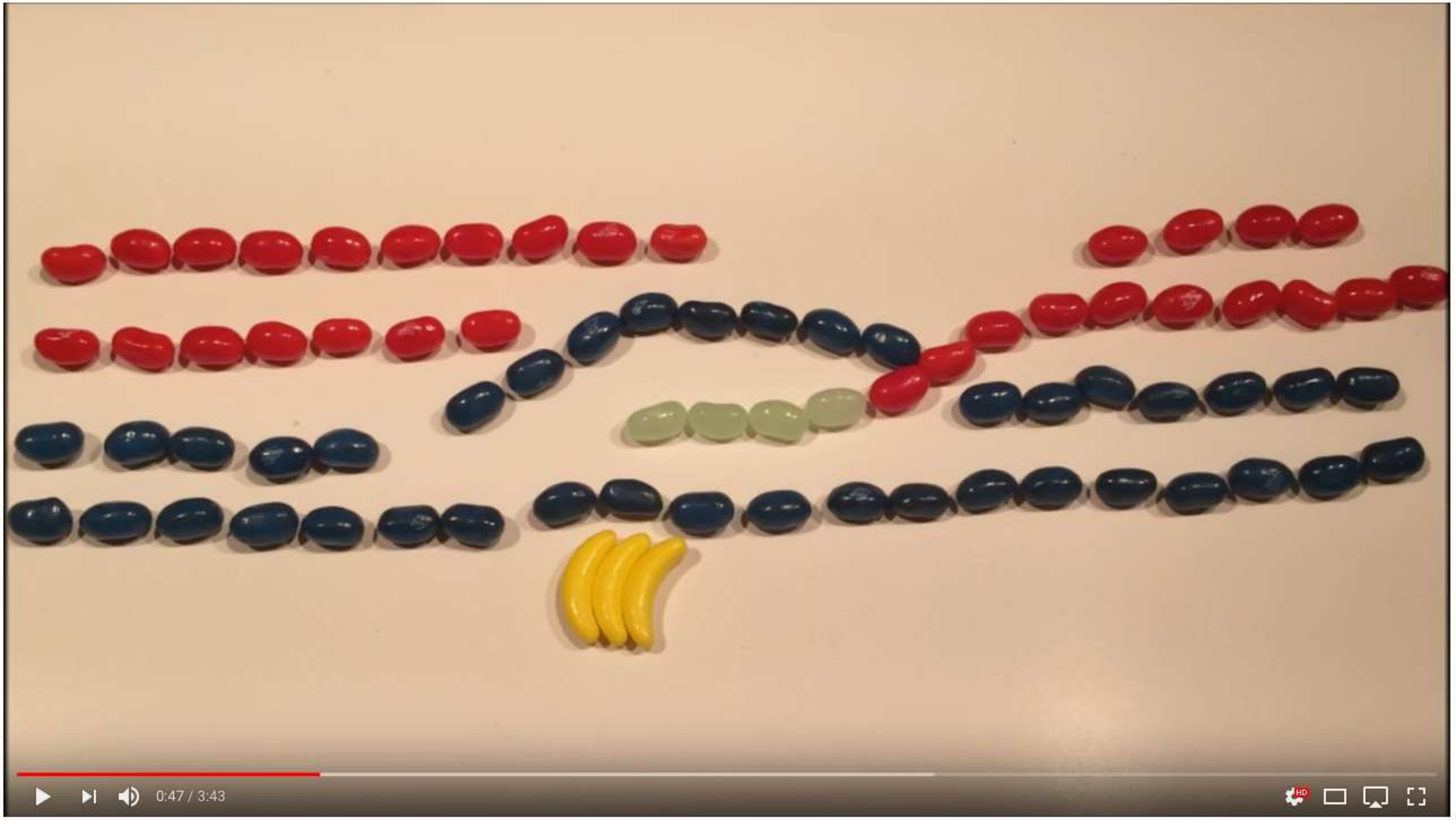
It is important to classify the TNBC into subtypes for further genomic research and clinical applications. We developed a web-based prediction tool for candidate TNBC samples using our gene expression meta data and classification methods. Given a gene expression data matrix, this tool will display for each candidate sample the predicted subtype, the corresponding correlation coefficient, and the permutation p-value.

The input data is a genome-wide gene expression matrix in a .csv file (please check the help section for details). We highly recommend pre-processing and normalizing the raw data for TNBC samples only. The distinctions between TNBC subtypes are relatively subtle compared with the dramatic difference between TNBC and ER positive breast cancer samples at the transcriptome level. If we normalize TNBC gene expressions with the ER positive samples, the gene expression signals driven by ER could disturb the TNBC gene expression normalization, thus affecting the final prediction results. Thus we have implemented a quality control step in **TNBCtype** program, to identify ER-positive samples. In the event that a sample does not pass the ER-filter, the user will be notified to remove the possible ER-positive sample and redo the normalization procedures.

Citation

TNBCtype: A Subtyping Tool for Triple-Negative Breast Cancer
Xi Chen, Jiang Li, William H. Gray, Brian D. Lehmann, Joshua A. Bauer, Yu Shyr, Jennifer A. Pietenpol
Cancer Informatics, 2012:11 147-156, doi:10.4137/CIN.S9983

Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies
Brian D. Lehmann, Joshua A. Bauer, Xi Chen, Melinda E. Sanders, A. Bapsi Chakravarthy, Yu Shyr, Jennifer A. Pietenpol
J Clin Invest. 2011; 121(7):2750–2767 doi:10.1172/JCI45014



Homologous Recombination and BRCA1

587 weergaven

8 0 DELEN

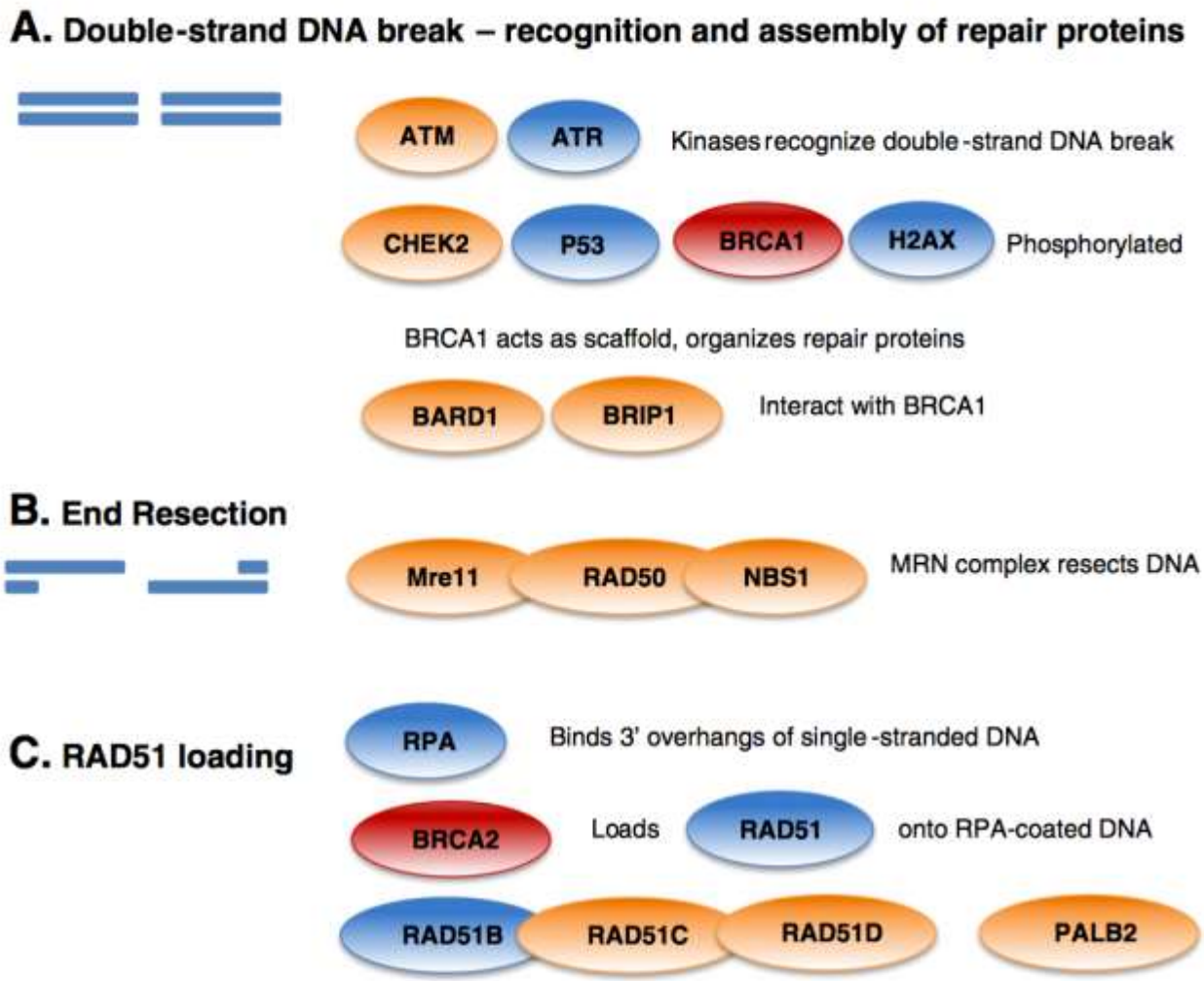
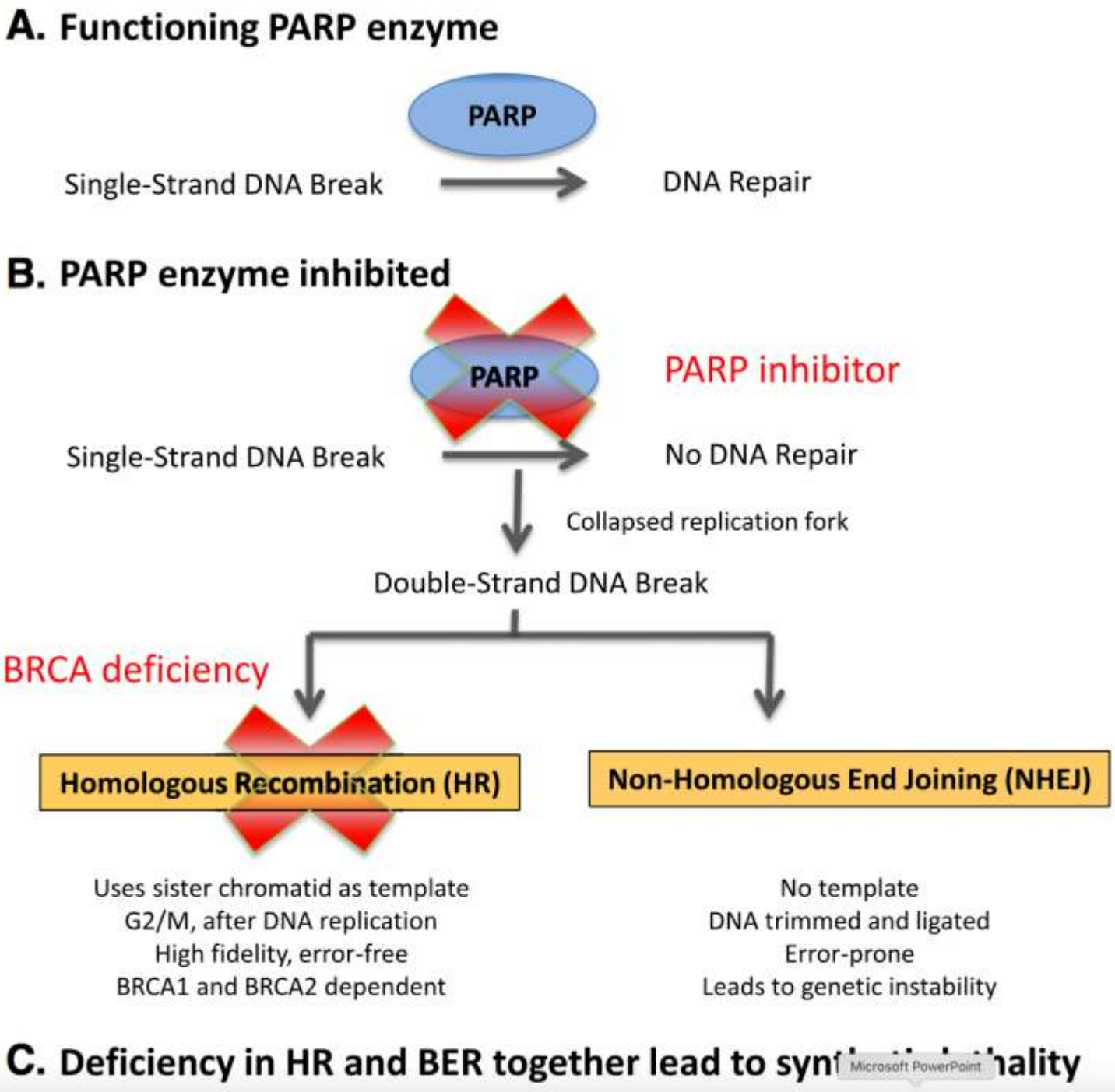

A. Double-strand DNA break – recognition and assembly of repair proteins
ATM
ATR
Kinases recognize double-strand DNA break
CHEK2
P53
BRCA1
H2AX
Phosphorylated
BRCA1 acts as scaffold, organizes repair proteins
BARD1
BRIP1
Interact with BRCA1
B. End Resection
Mre11
RAD50
NBS1
MRN complex resects DNA
C. RAD51 loading
RPA
Binds 3' overhangs of single-stranded DNA
BRCA2
Loads
RAD51
onto RPA-coated DNA
RAD51B
RAD51C
RAD51D
PALB2
D. Strand Invasion – RAD51 nucleoprotein filament invades homologous DNA
E. DNA Synthesis and Repair

Table 1
Homologous recombination genes linked to hereditary breast and ovarian cancer susceptibility.

Gene	Hereditary breast cancer risk	Hereditary ovarian cancer risk
CHEK2	Am J Hum Genet 2004	Walsh, PNAS 2011
BRIP1	Seal, Nat Genet 2006	Rafnar, Nat Genet 2011
ATM	Renwick, Nat Genet 2006	Walsh, PNAS 2011
NBN	Steffen, Int J Ca 2006	Walsh, PNAS 2011
PALB2	Rahman, Nat Genet 2007	Walsh, PNAS 2011
RAD51C	Meindl, Nat Genet 2007	Meindl, Nat Genet 2010
BARD1	De Brakeleer, Hum Mutat 2010	Walsh, PNAS 2011
MRE11A	Damiola, Breast Ca Res 2014	Walsh, PNAS 2011
RAD50	Damiola, Breast Ca Res 2014	Walsh, PNAS 2011
RAD51D	n/a	Loveday, Nat Genet 2011

Den Brok et al, Precis Oncol 00, 2017


A. Functioning PARP enzyme
PARP
Single-Strand DNA Break
DNA Repair
B. PARP enzyme inhibited
PARP
PARP inhibitor
Single-Strand DNA Break
No DNA Repair
Collapsed replication fork
Double-Strand DNA Break
BRCA deficiency
Homologous Recombination (HR)
Non-Homologous End Joining (NHEJ)
Uses sister chromatid as template
G2/M, after DNA replication
High fidelity, error-free
BRCA1 and BRCA2 dependent
No template
DNA trimmed and ligated
Error-prone
Leads to genetic instability
C. Deficiency in HR and BER together lead to syn
hality
Microsoft PowerPoint

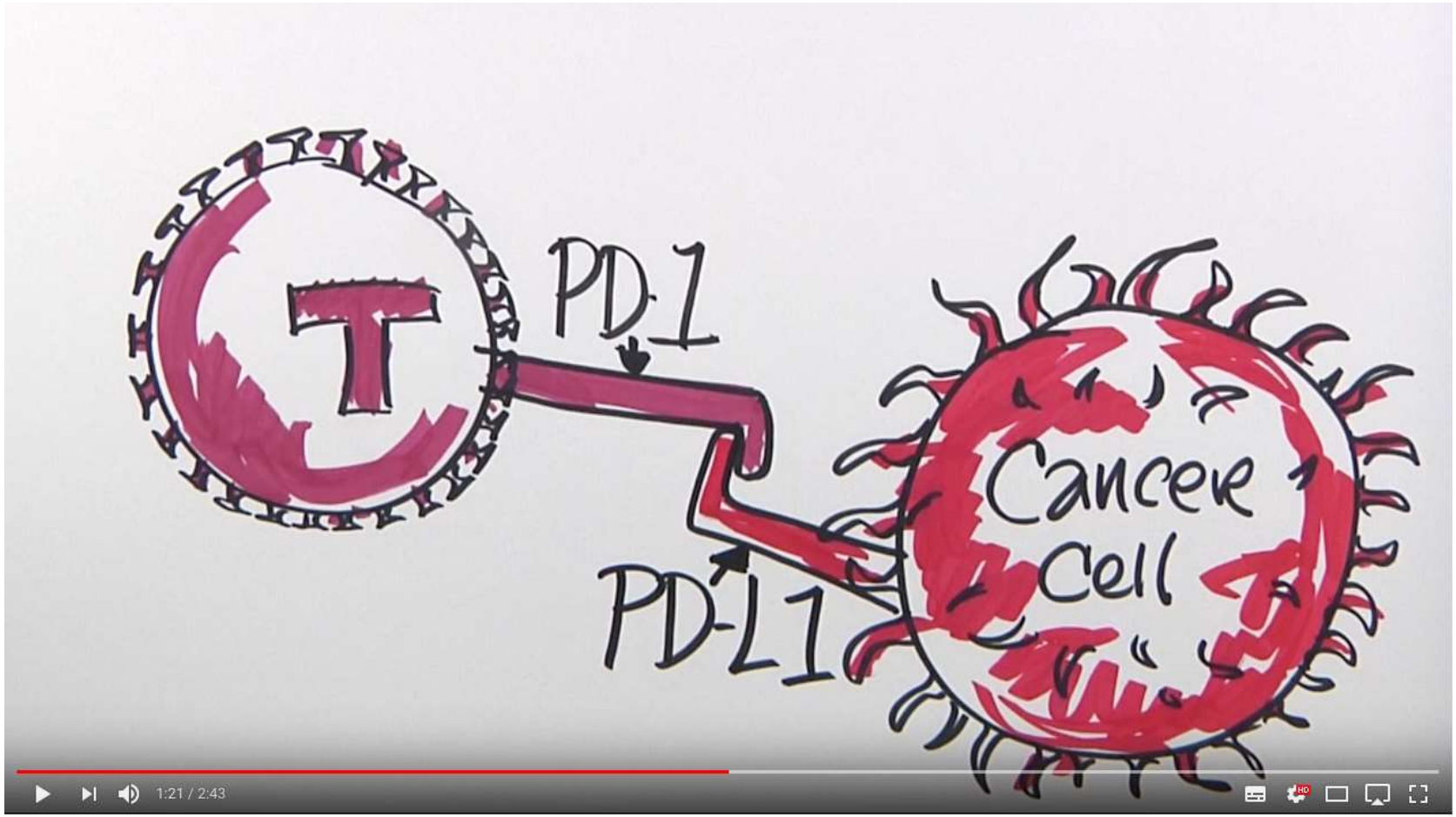
Optimal HRD-test→ we're not there yet…

Test for HR Deficiency	First Author	Study/Trial, Tissue Type	N	Primary Outcome	Treatment	Patient Population	Main Results
BRCA1-associated expression pattern using 69-gene LDA by qRT-PCR	Rodriguez[23]	Retrospective Archival frozen, FFPE	105	pCR	Neoadjuvant AC, FEC, taxane-based	TNBC	Defective DNA repair associated with higher pCR rates to anthracyclines and relative taxane resistance
77-gene BRCAness gene expression signature plus PARPi-7 signature	van t' Veer[24]	Exploratory analysis in an adaptive randomization trial (I-SPY II) Fresh tissue	115	pCR	Neoadjuvant standard chemotherapy ± veliparib, carboplatin, chemotherapy	HER2-negative locally advanced	DNA repair deficiency in 77 patients (38% of ER-positive and 95% of triple-negative) DNA repair deficiency associated with higher rates of pCR in V/C group
BRCA1 insufficiency by BRCA1, BRCA2 mutation, BRCA1 PM, BRCA1 mRNA	Sharma[28]	Retrospective FFPE	30	42 months RFS, OS	Neoadjuvant carboplatin, docetaxel, erlotinib	Stage II-III TNBC	BRCA1 insufficiency associated with better 42-month OS and RFS
BRCA1 PM	Sharma[29]	Retrospective FFPE	39	RFS, OS	Neoadjuvant/adjuvant chemotherapy (90% anthracycline, 69% taxane)	Stage I-III TNBC	BRCA1 PM in 30% and associated with worse RFS, OS
BRCA1-like aCGH classifier	Vollebergh[33]	Retrospective FFPE	230	RFS, OS	Adjuvant HD-PB ± standard anthracycline-based chemotherapy	Stage III, HER2-negative	18% BRCA1-like; BRCA1-like treated with HD-PB had improved RFS, no benefit in non-BRCA1-like treated with HD-PB
BRCA1-like aCGH based on copy number profiles	Schouten[33]	Retrospective FFPE	117	DFS, DDFS, OS	Adjuvant high-dose ifosfamide, epirubicin, carboplatin ± standard chemotherapy	High-risk stage II-III, any biomarker status	BRCA1-like associated with TNBC BRCA1-like treated with high-dose regimen had better DFS, DDFS, and OS No benefit in BRCA1-like negative.
BRCA1-like aCGH classifier by MLPA	Oonk[35]	Retrospective FFPE	101	5-year RFS	Adjuvant AC, FEC, TAC, CMF	TNBC	65% were BRCA1-like. No difference in 5-year RFS
BRCA1- and BRCA2-like aCGH classifier	Lips[36]	Retrospective	163	pCR	Neoadjuvant dose-dense AC	HER2-negative	BRCA1 dysfunction frequent in TNBC cohort but no difference in response to ddAC in BRCA1-like ± non-BRCA1-like
BRCA1 PM, BRCA1 mRNA, EMSY amplification		Pretreatment snap frozen					BRCA2-like frequent in ER-positive cohort and associated with better response to treatment

(Continued on following page)

Den Brok et al, Precis Oncol 00, 2017

Checkpoint inhibitors Breast Cancer (PDL1) ??



How is Immunotherapy Used to Fight Cancer? | Dana-Farber Cancer Institute | Science Illustrated

53.102 weergaven

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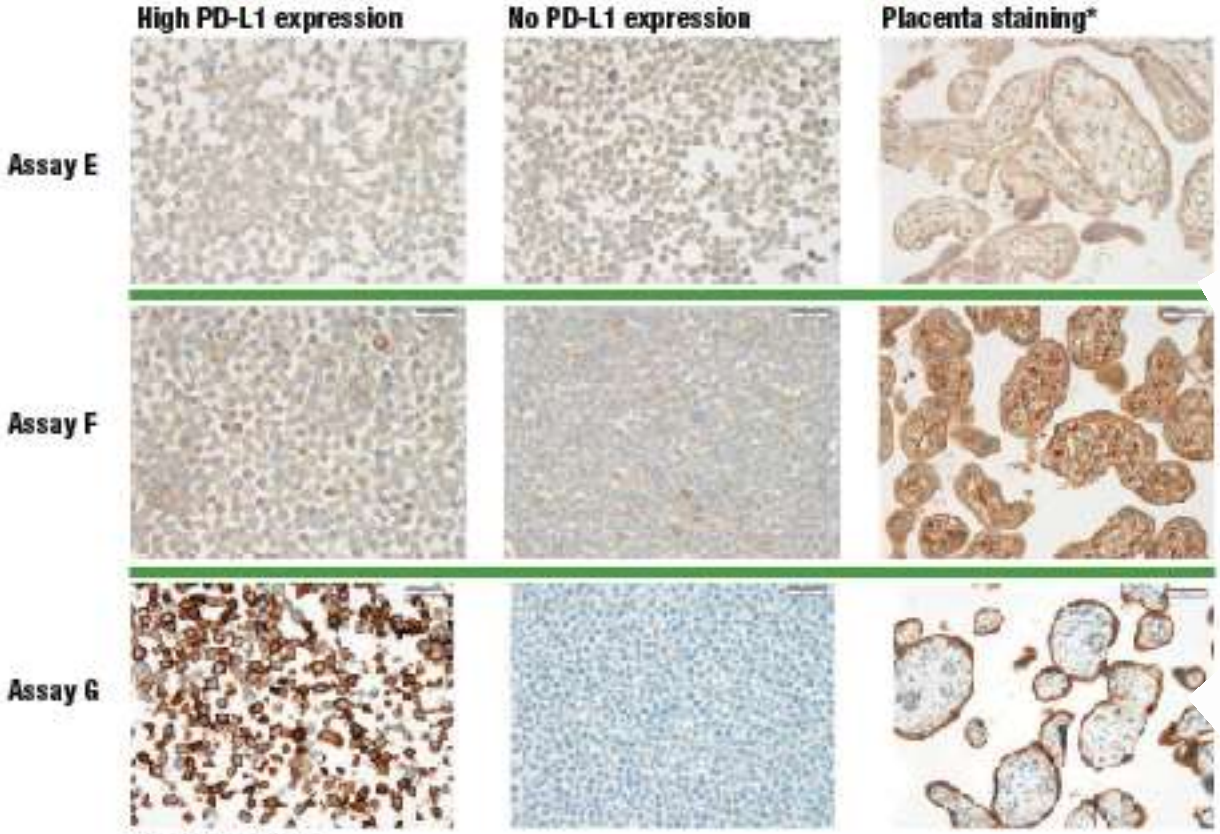
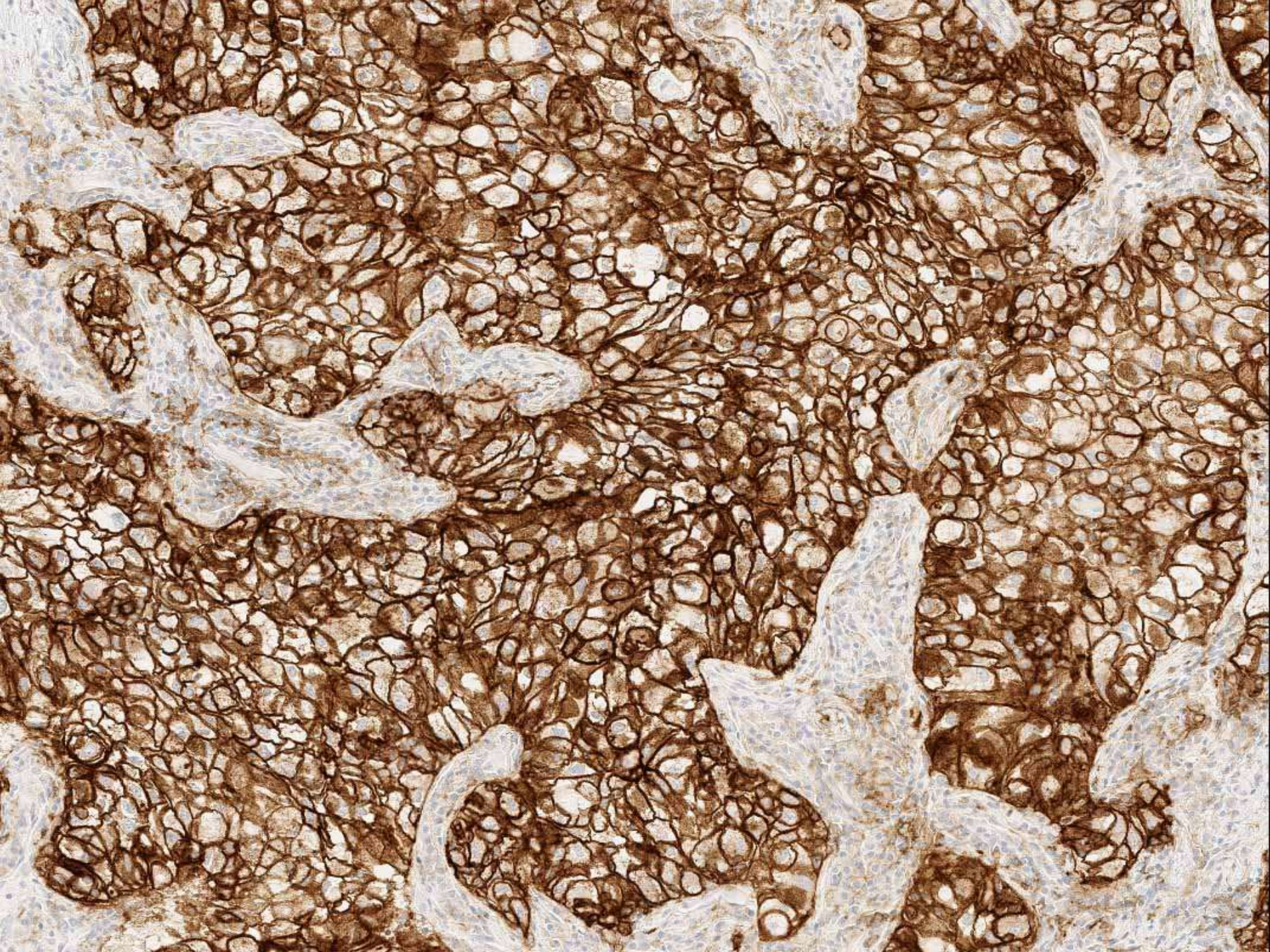
Fig. 6

Some PD-L1 IHC assays are neither sensitive nor specific when used on fixed specimens



*Syncytiotrophoblast

Chen DS. Presented at: IASLC Santa Monica Lung Meeting; Feb. 18–20, 2015; Santa Monica, Calif.



Staging

Grading

Typing

Protein detection, ER, PR, Ki67, Ker5/6, Her2 etc

Transcriptomics, RNA analysis

NGS, DNA analysis

Liquid biopsy

1957

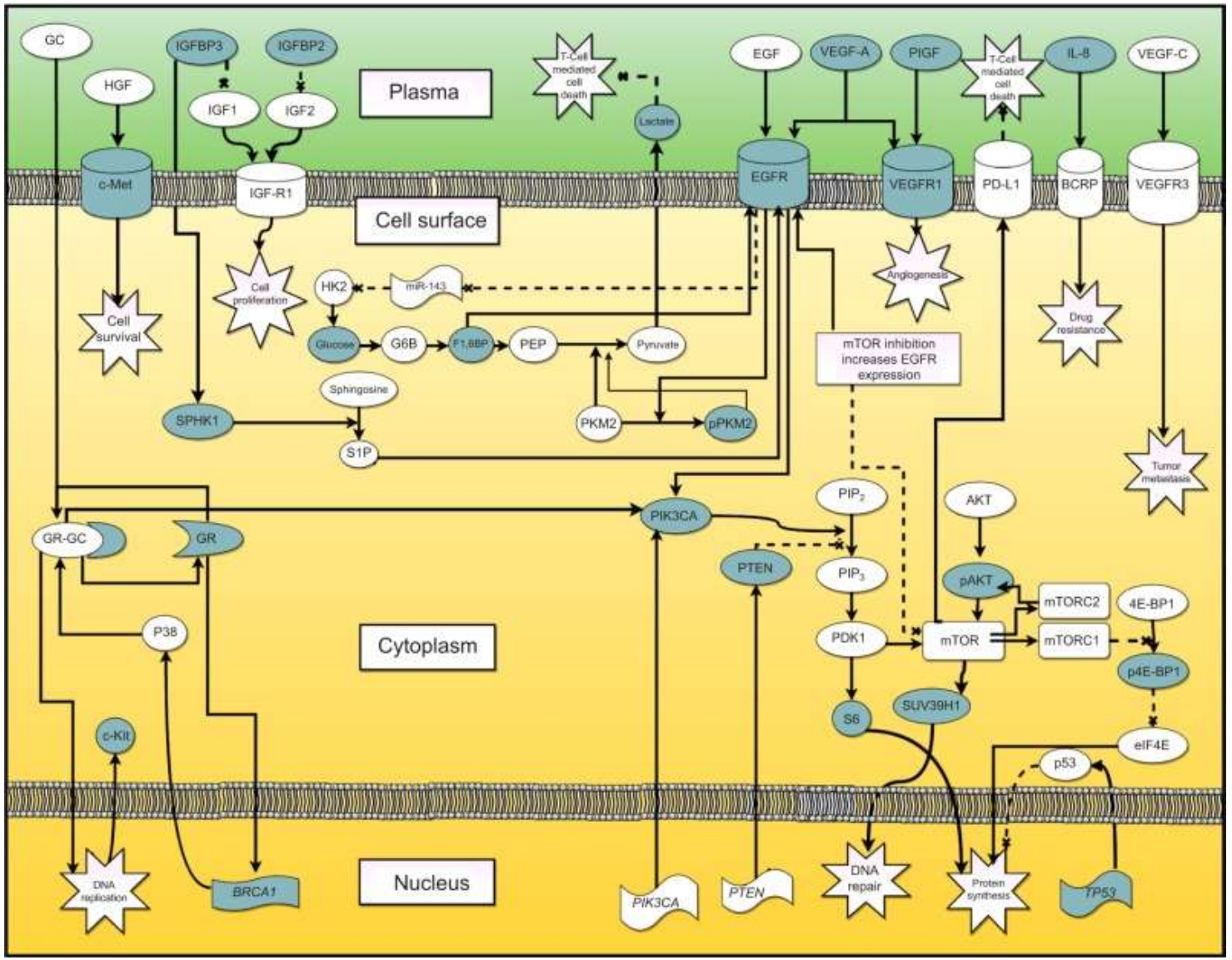
1980

21th century

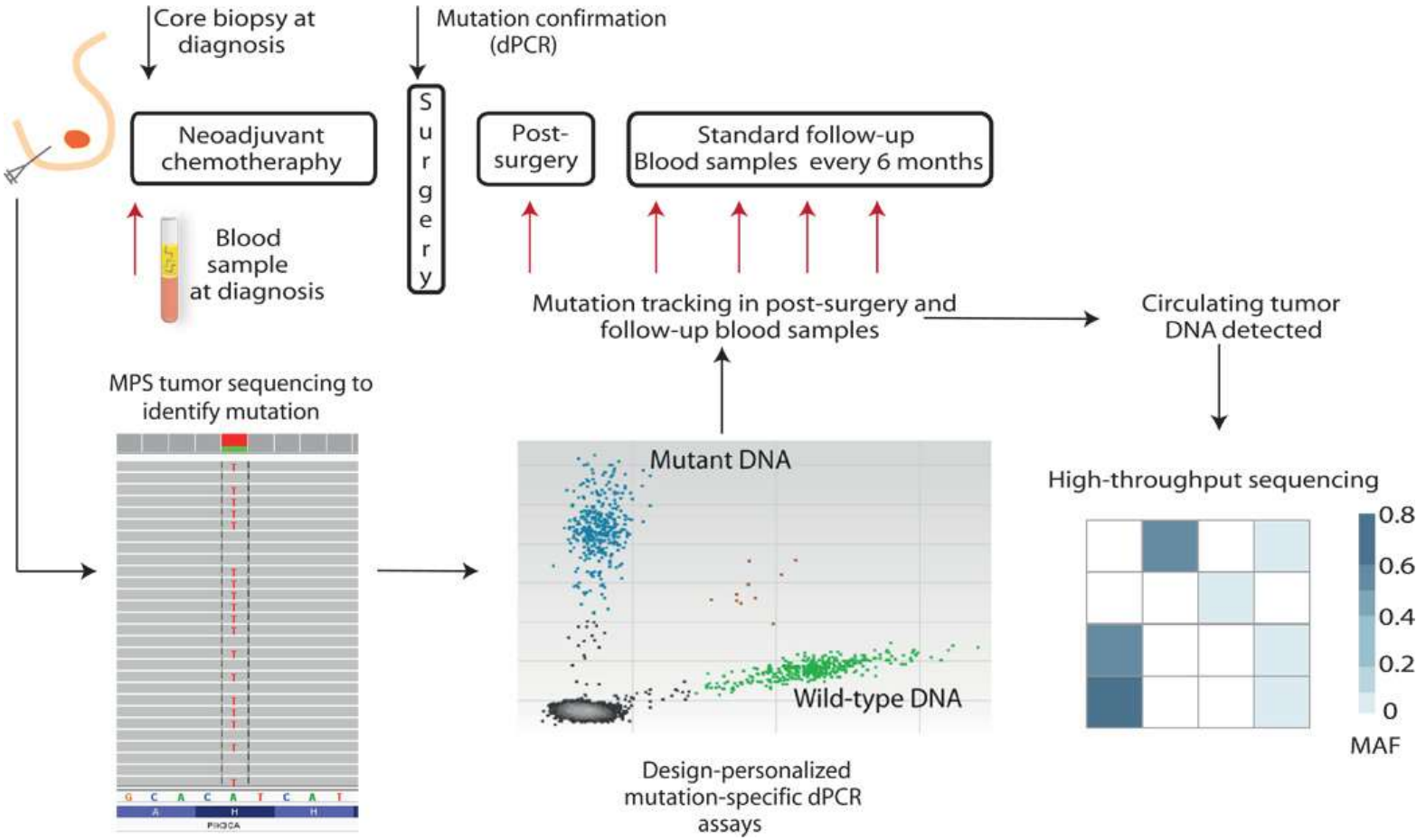
The nano-tech 10's

The roaring 20's and beyond

Targeted therapies in the pipeline?



Circulating Tumour DNA



- *Science Translational Medicine* 26 Aug 2015: Vol. 7, Issue 302, pp. 302ra133

TAKE HOME

- ✓ Breast cancer is an extremely heterogeneous disease
- ✓ Traditional biomarkers (grading/ER/PR/HER2/Ki-67) are relatively robust, **BUT** Intraobserver-variability is a serious problem
- ✓ Gene profiles can provide prognostic and predictive information in specific settings
  - HR-positive tumors, clinically high risk (20-25%): MammaPrint
  - Promising in BC with an ultra-lowrisk profile?
- ✓ TNBC: **not one** disease – molecular subtyping will be essential for selection of relevant therapy-regimes (standard AR-testing is 'nearby')

Summary

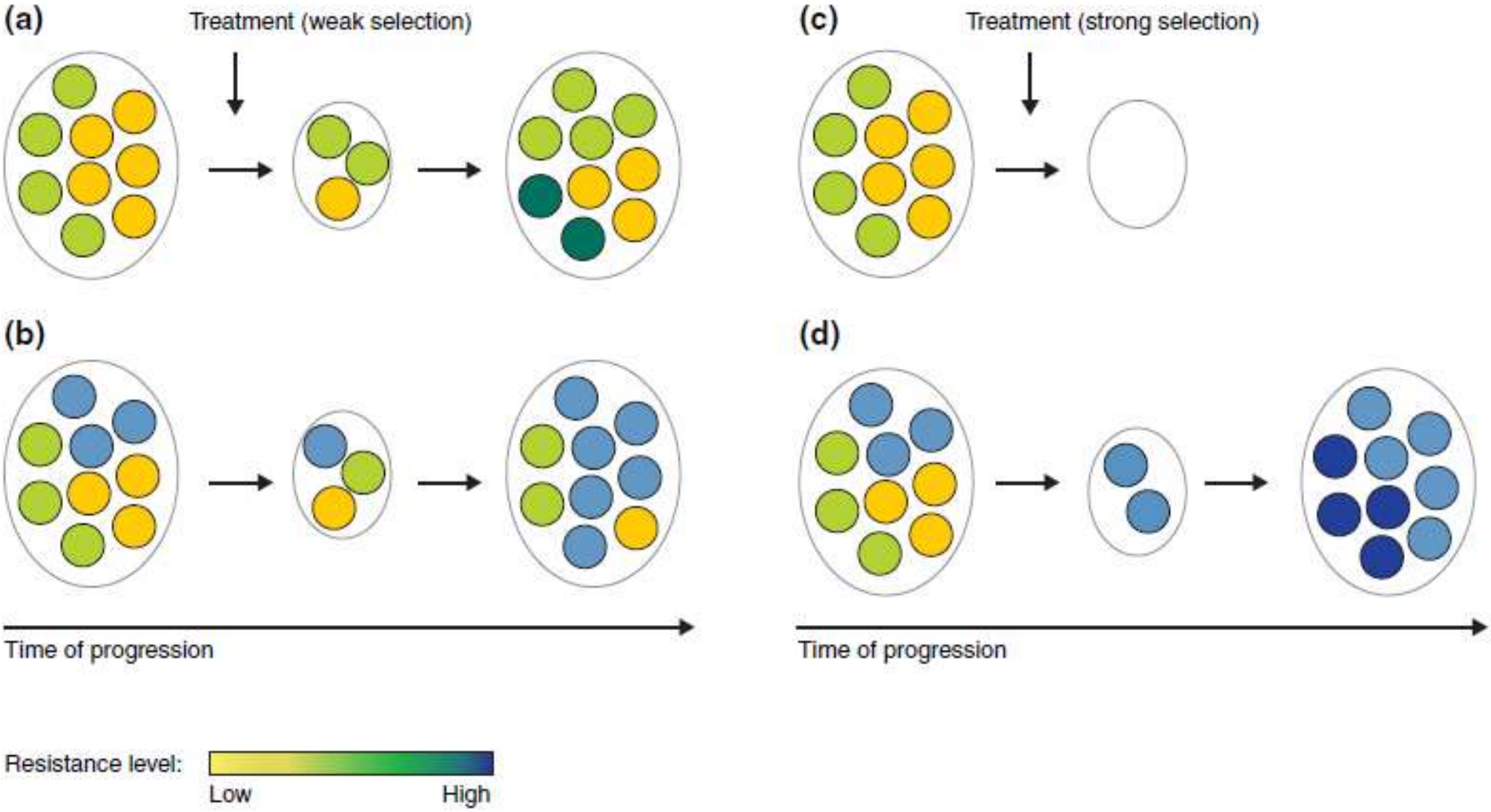
- ✓ HRD / BRCA-ness: No gold-standard test YET
- ✓ PDL1 IHC: high spinner-alert, but the best there is at the moment
- ✓ FUTURE: Integration of histology, IHC and Molecular profile in standard work-up
- ✓ Pathology has a central role – both analytical & coordination
- ✓ Decision making support systems will be essential
- ✓ It all starts with carefull tissue handling and FORMALIN fixing.

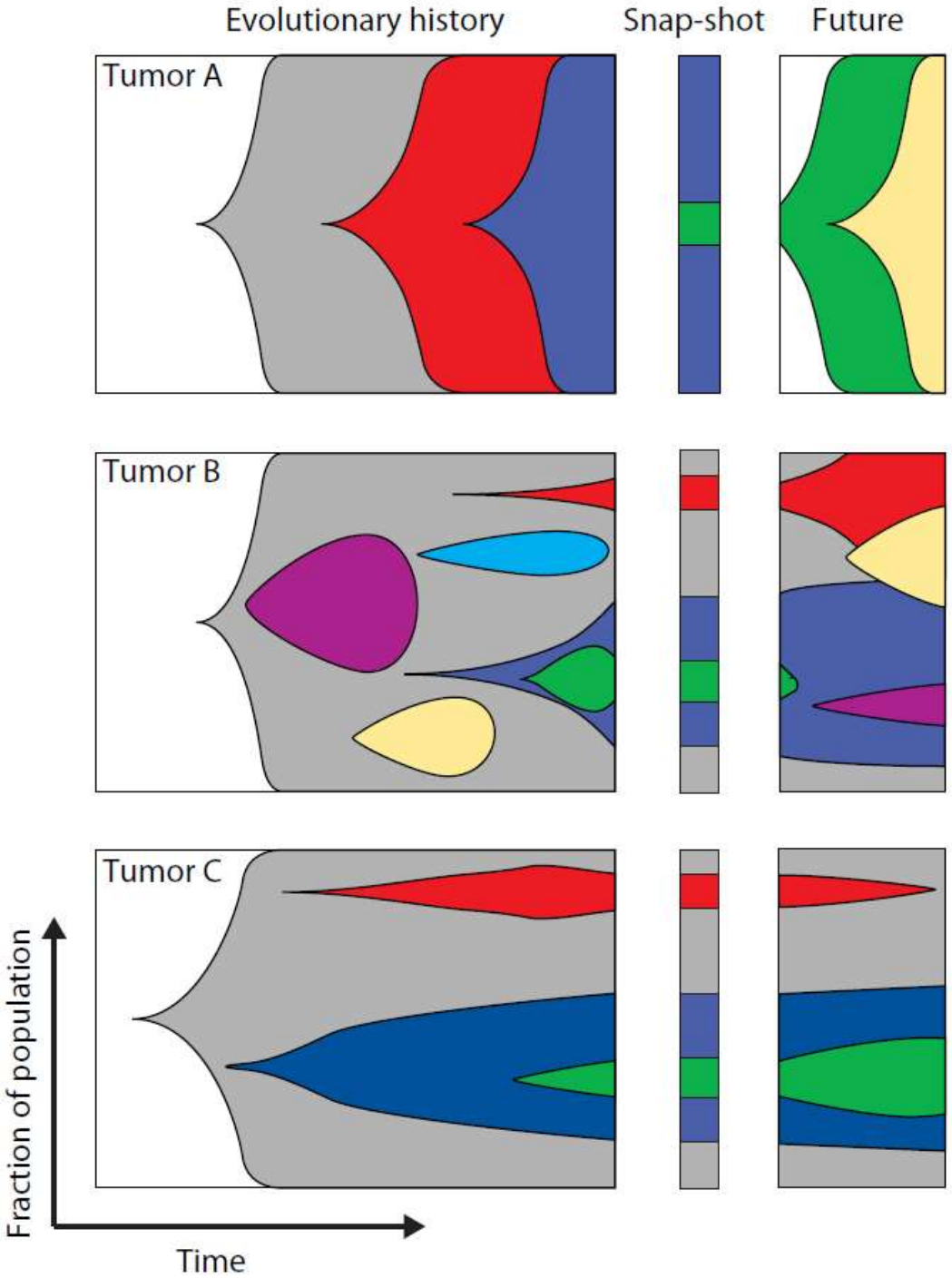
Thanks for your attention!




EXTRA SLIDES

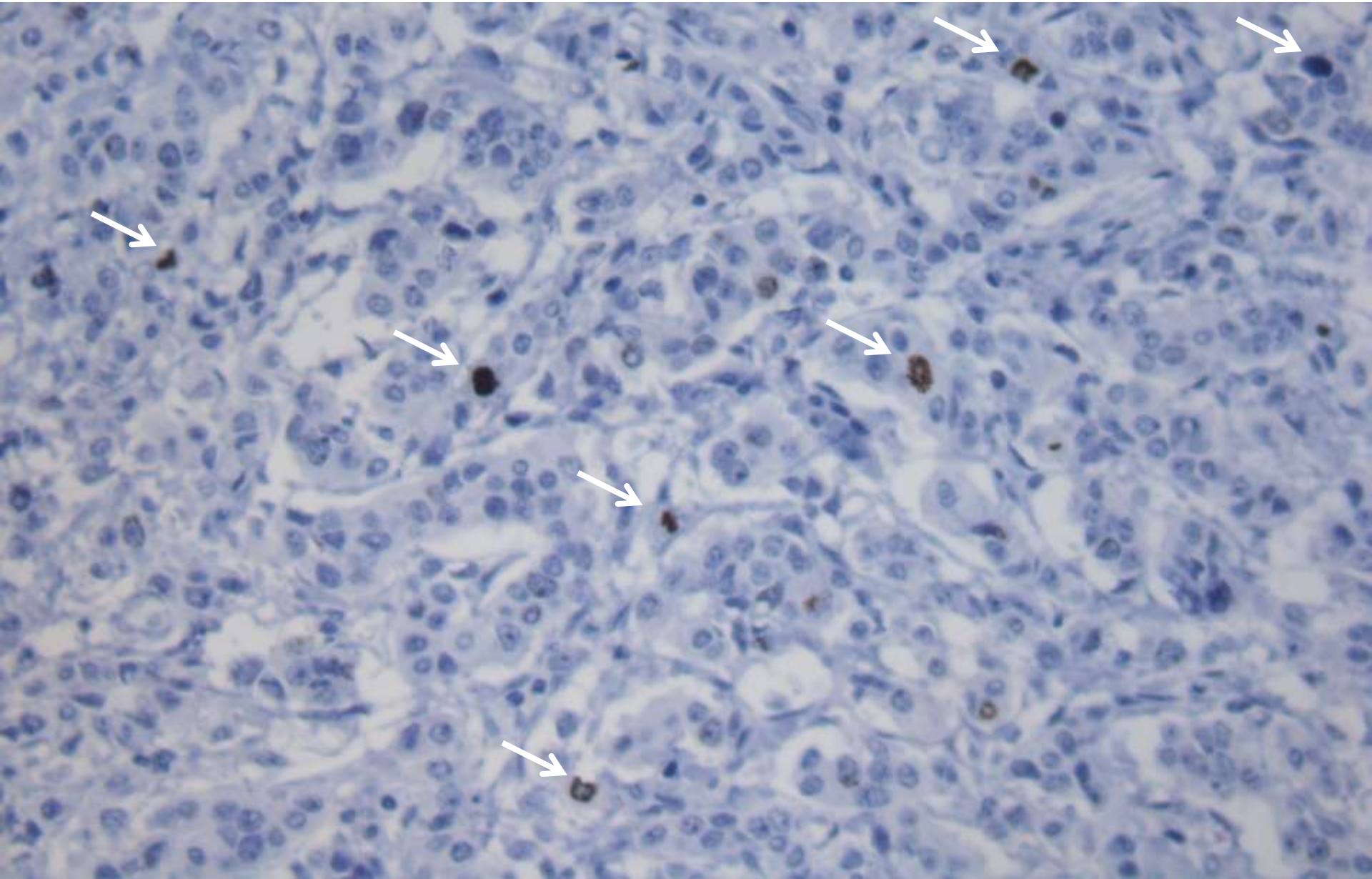
Heterogeneity





Heterogeneity

Histon-H3 – mitotic marker→



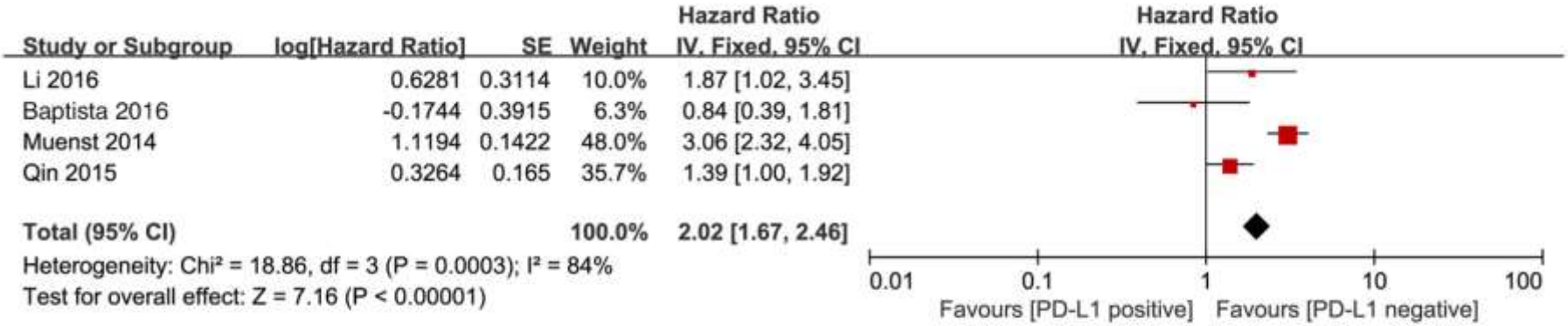


Figure 3: Forest plot describing subgroup analysis of the association between PD-L1 expression and OS after removal of Park et al study.