Diagnostic and therapeutic implications of tumour-infiltrating lymphocytes in breast cancer

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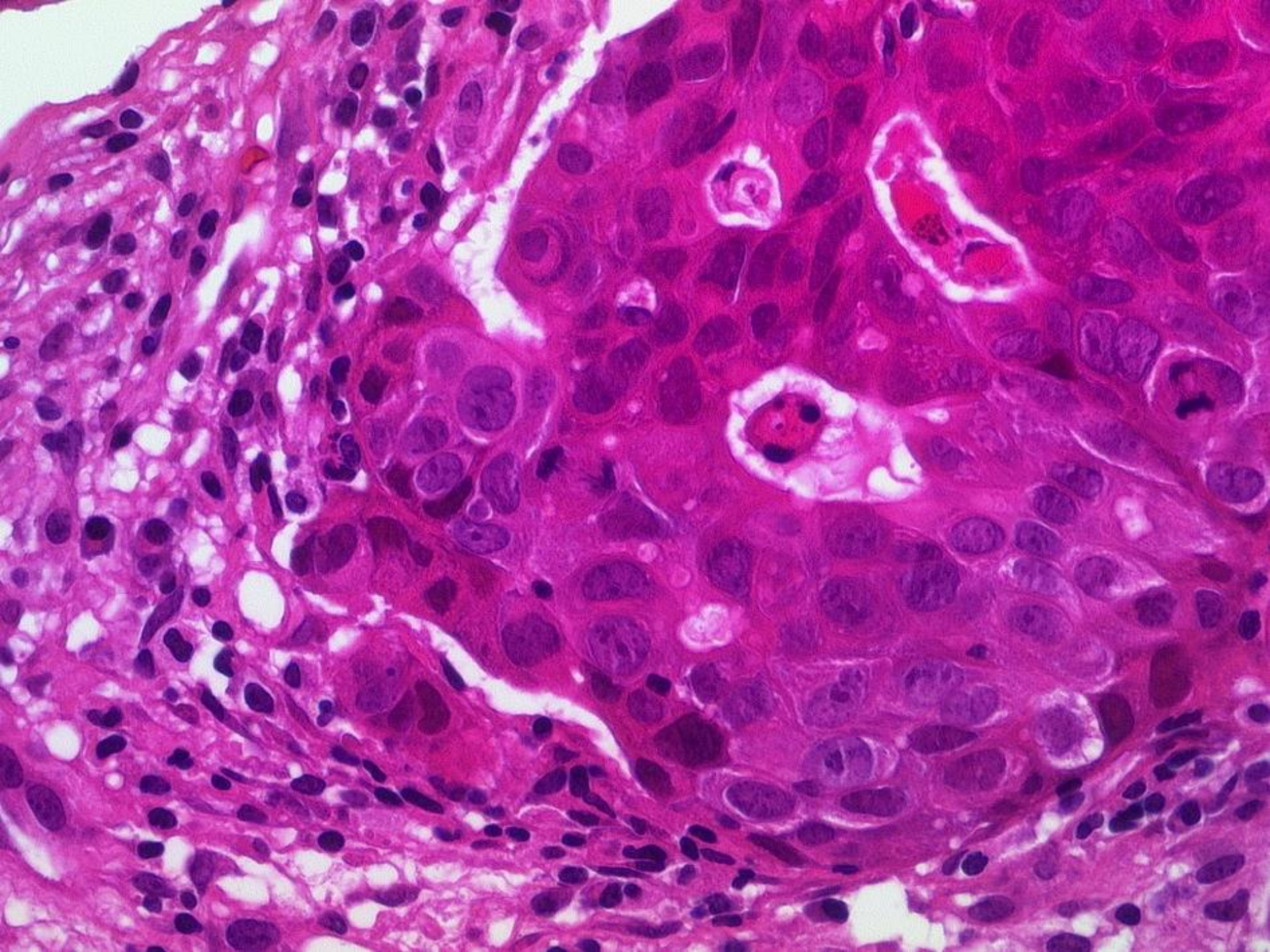
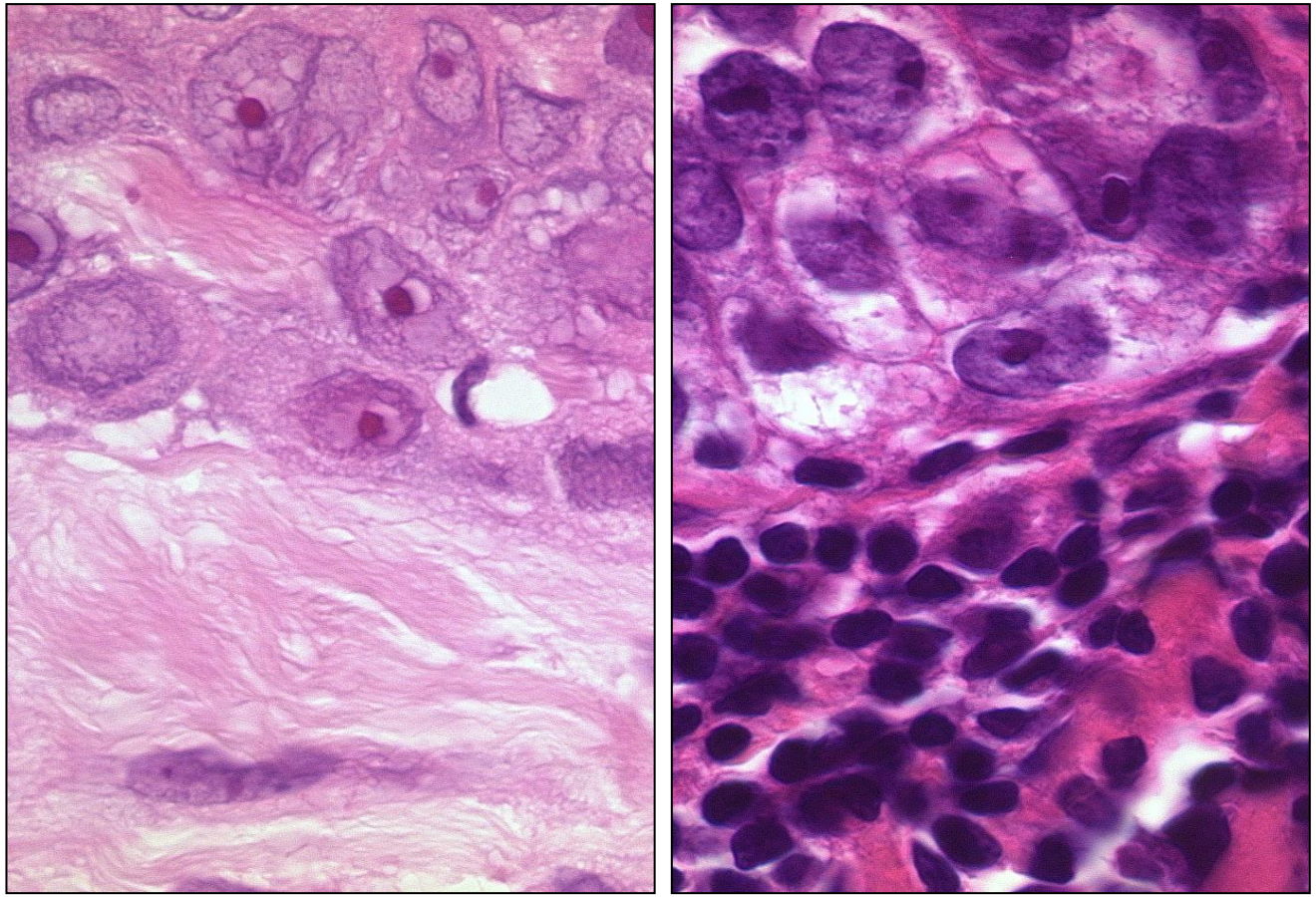
Disclosure slide

- Sividon Diagnostics: research funding, co-founder and shareholder



Heterogenous immune infiltrate in breast cancer

Lymphocyte-predominant breast cancer
(LPBC = more lymphocytes than tumor cells)

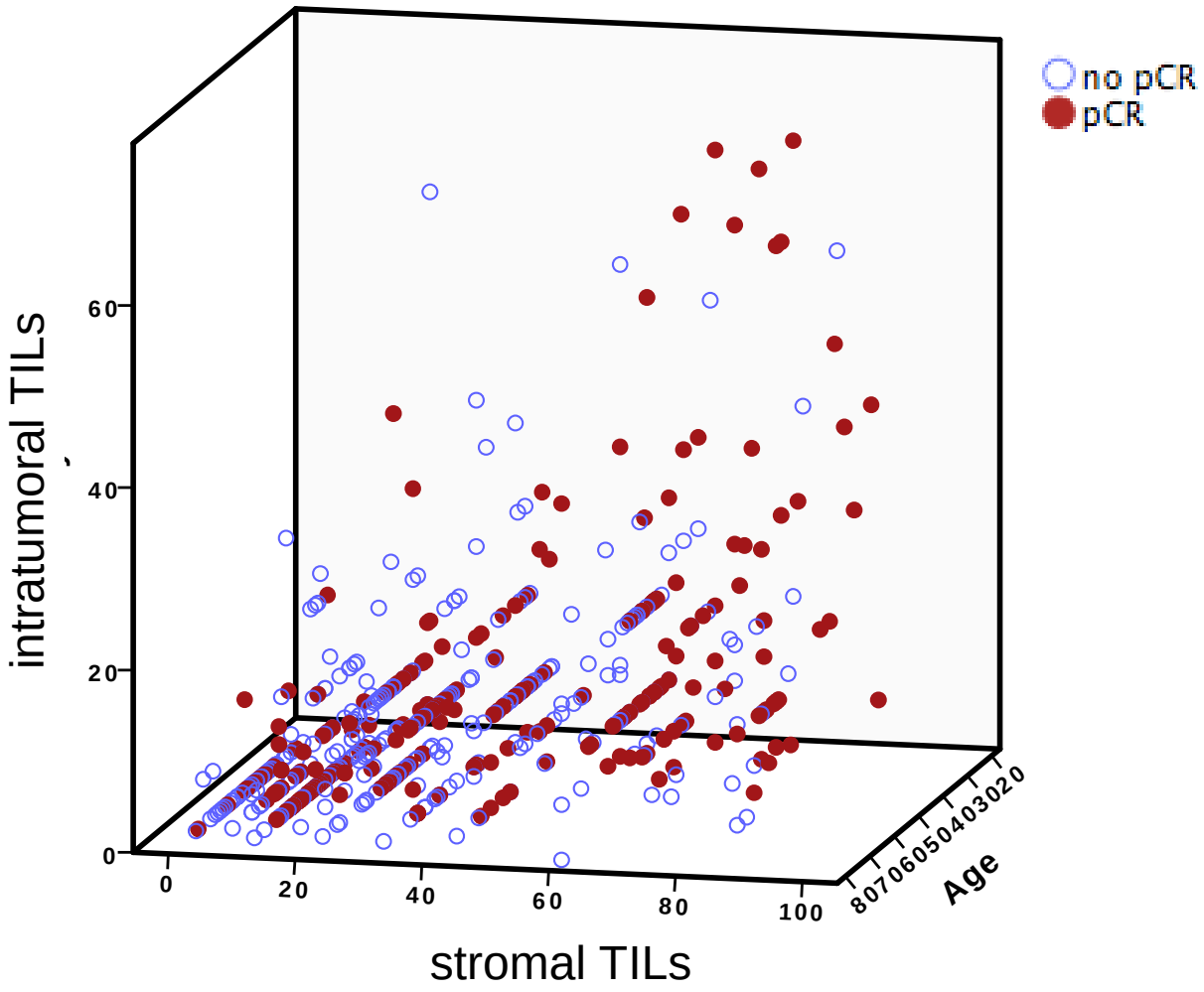


Tumor-associated lymphocytes

Clinical relevance

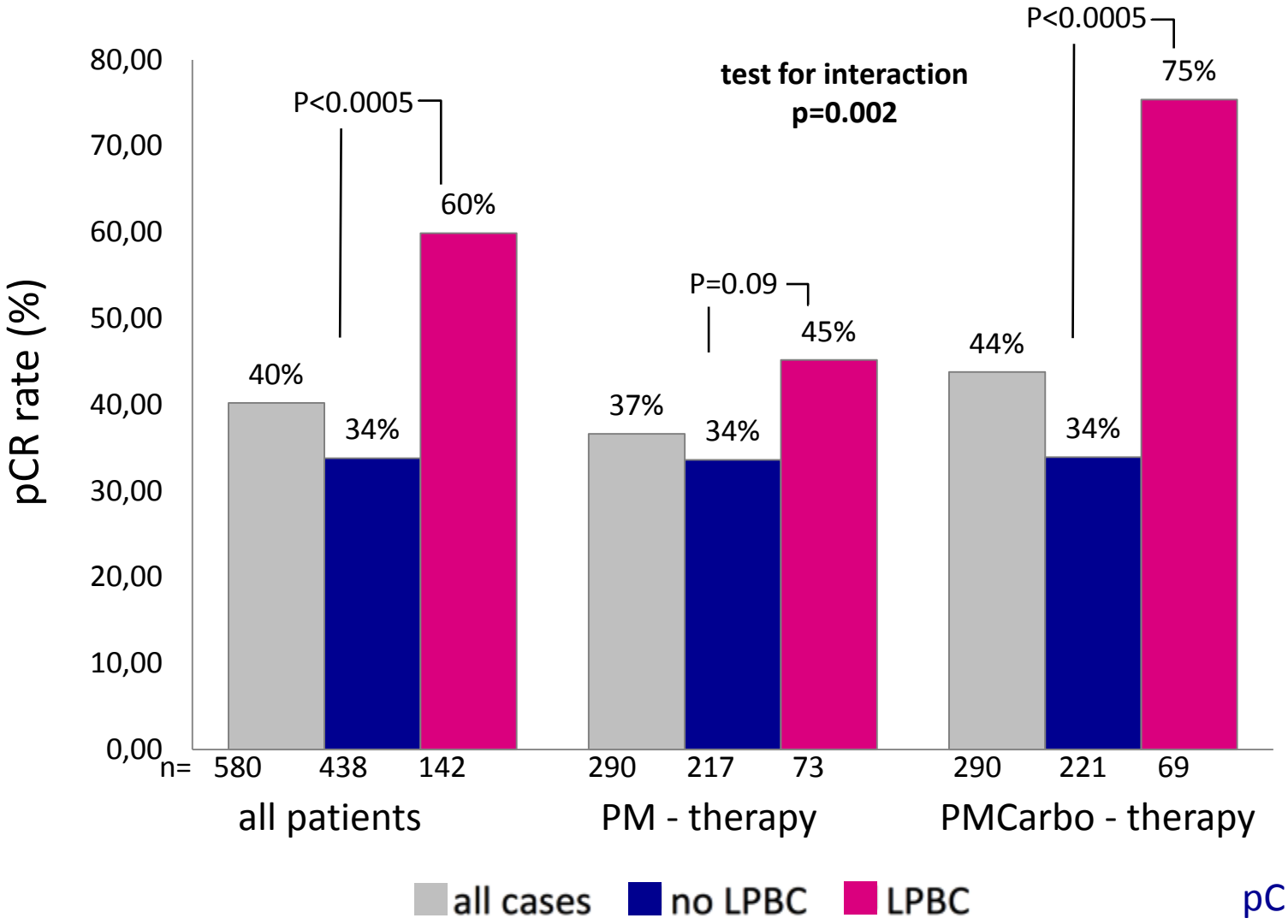
TILs and chemotherapy response in GeparSixto

n=580



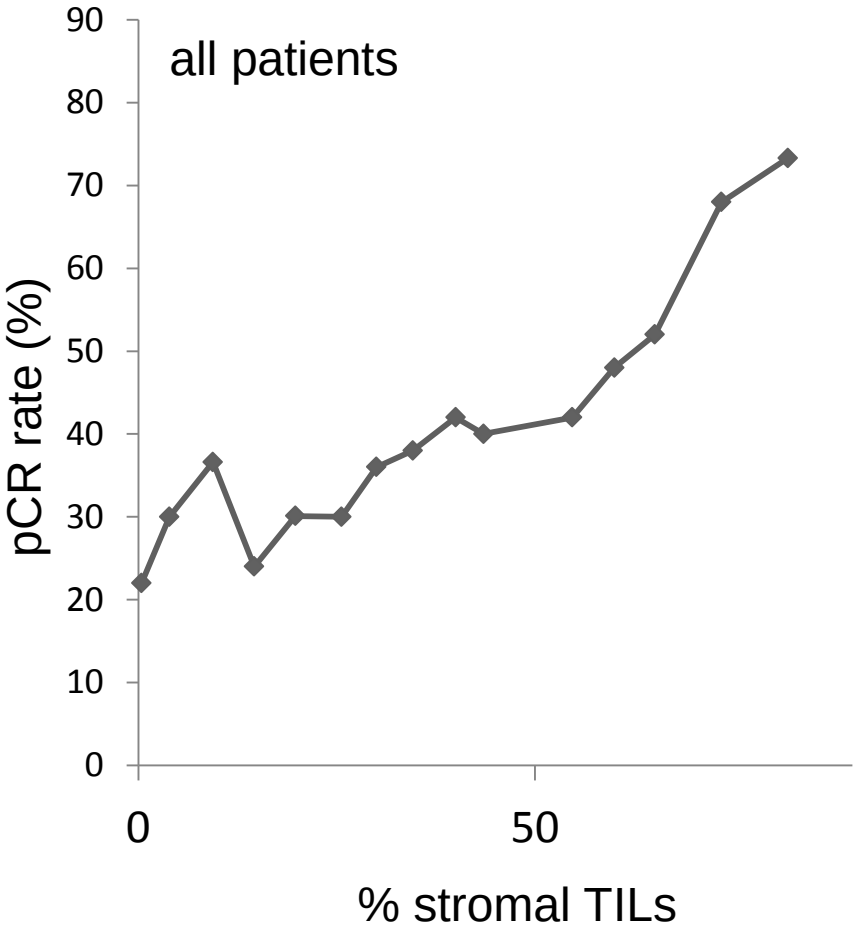
GBG GERMAN BREAST GROUP

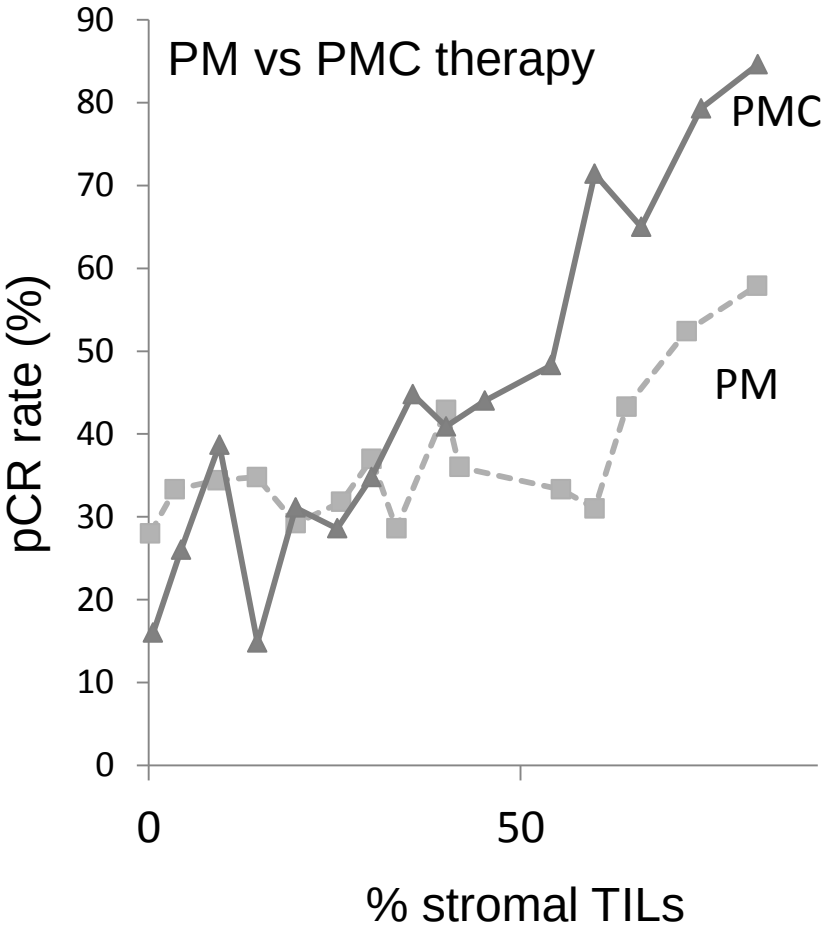
pCR rates in GeparSixto: LPBC vs non-LPBC



pCR: ypT0ypN0

STEPP analysis – pCR rate in GeparSixto





Clinical evaluation of TILs in breast cancer

Neoadjuvant n=2216

Training	Validation 1	2	3	4
GeparDuo neoadjuv. EC-Doc (n=218) JCO, 2010	**Gepartrio neoadjuv.** TAC (n=840) JCO, 2010	**GeparQuinto HER2-** (prospective) EC-Doc (n=313)	**GeparQuinto HER2+ EC-DOC Lap vs. Trast.(n=250)**	**GeparSixto (prospective) TNBC and HER2+ (n=595)**

Adjuvant TNBC n=737

Ono et al, 2012 neoadjuvant, TNBC: n=96

BIG2-98 adjuvant n=2009, TNBC: n=256 (Loi et al, JCO, 2013)

ECOG 2197 and ECOG 1199 adjuvant Adams et al., SABCS 2013, TNBC: n=481

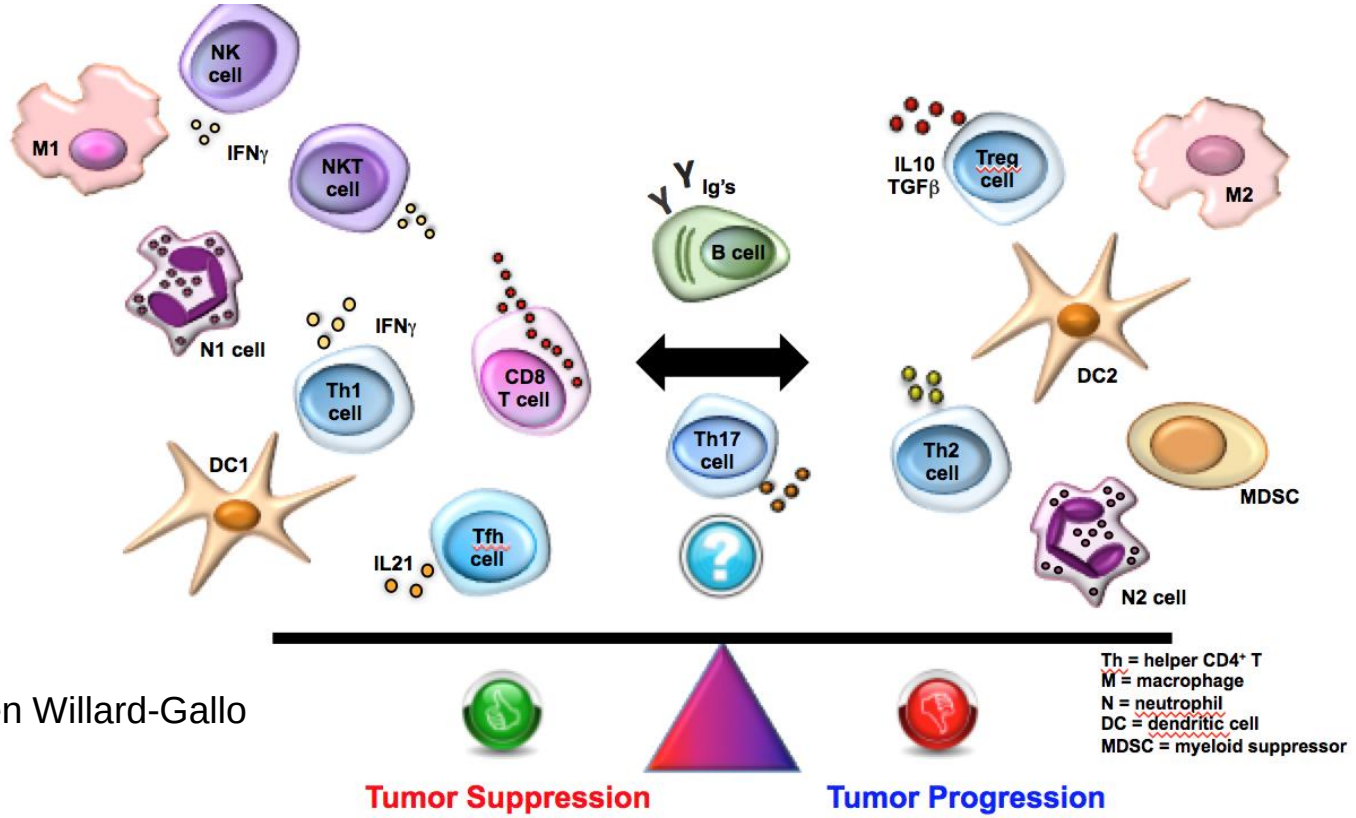
Tumor-associated lymphocytes- clinically relevant questions

- Clinical validity: Results of clinical biomarker studies
  - prediction of response to neoadjuvant therapy
  - improved prognosis
  - relevant subtypes (TNBC, HER2+, luminal?)

  - consistent results in several studies

TILs vs. molecular markers

- „Counting little blue cells in the tumor tissue.“
- Can this reflect the complexity of the immune system?

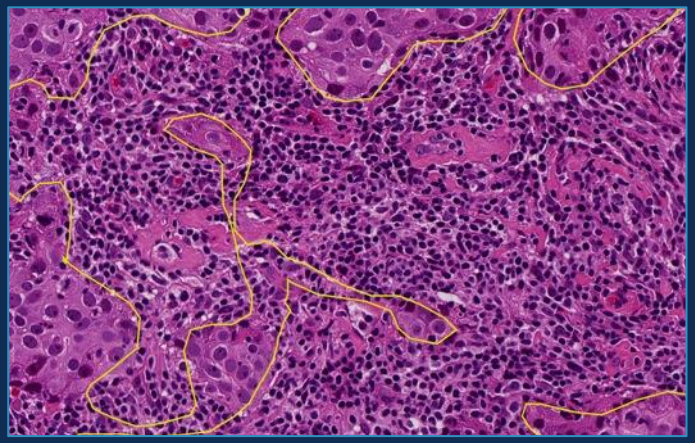


Karen Willard-Gallo

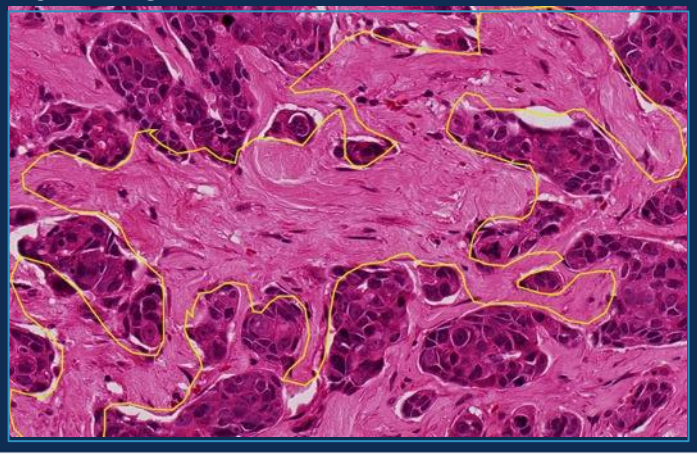
Further molecular characterization of immune infiltrate

morphological classification

Lymphocyte-predominant breast cancer (LPBC)
= more that 60% TILs



non-LPBC



molecular characterization

Hypothesis:

Immunosuppressive regulators:

PD1, PDL1,

CTLA4, IDO1, FOXP3

Immune activation:

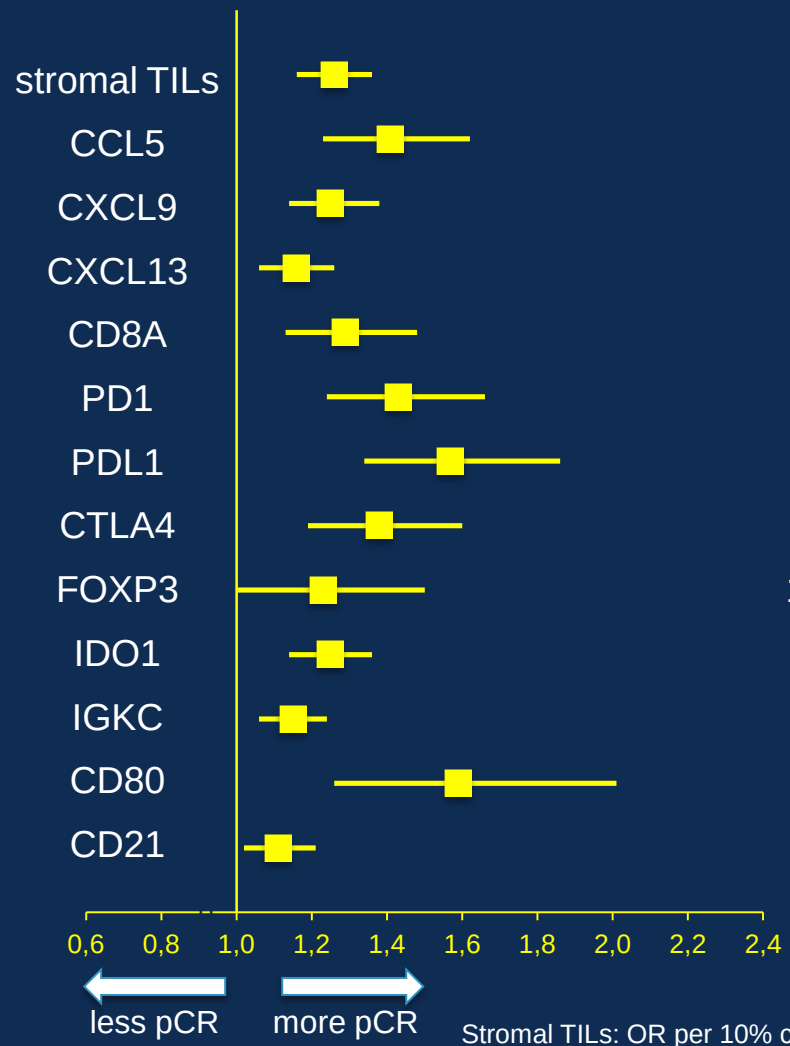
T-Cells: CD8A, CCL5

B-Cells: IGKC, CD21, CD80

Chemoattractants:

CXCL9, CXCL13

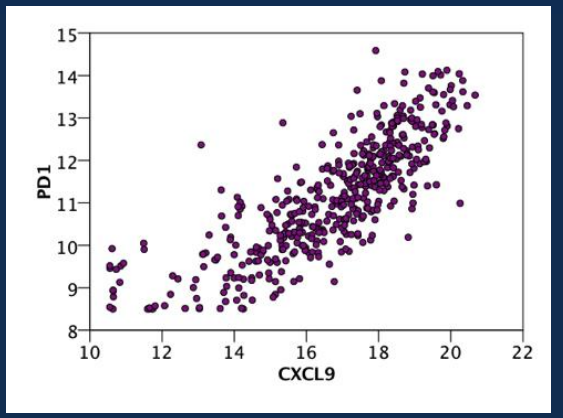
Immune markers were significantly linked to increased pCR rates – all cases (n=481)

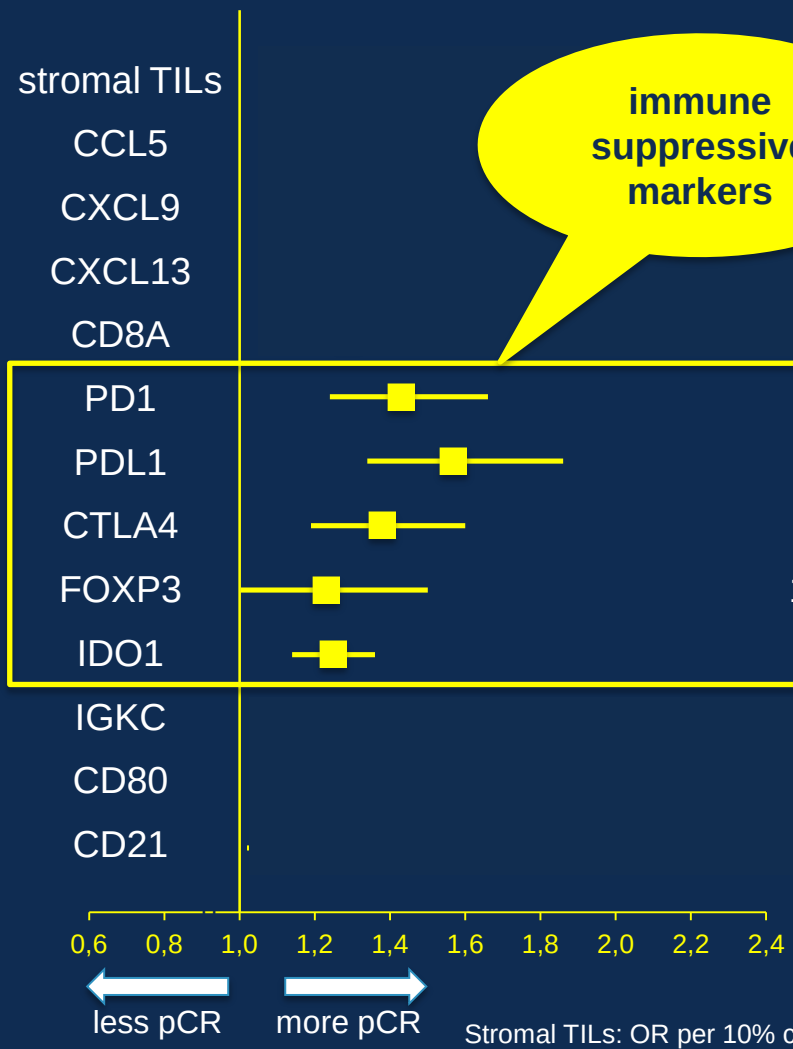


	univariate OR (95% CI)	univariate p-value	multivariate p-value	interacti. w. CbTx p-value
stromal TILs	1.26 (1.16-1.36)	0.00000001	0.0000005	0.007
CCL5	1.41 (1.23-1.62)	0.000001	0.00001	0.002
CXCL9	1.25 (1.14-1.38)	0.000006	0.003	0.09
CXCL13	1.16 (1.06-1.26)	0.001	0.006	ns
CD8A	1.29 (1.13-1.48)	0.0002	0.001	0.01
PD1	1.43 (1.24-1.66)	0.000001	0.00002	0.02
PDL1	1.57 (1.34-1.86)	0.00000003	0.000001	0.09
CTLA4	1.38 (1.19-1.60)	0.00001	0.0001	0.06
FOXP3	1.23 (1.003-1.50)	0.05	0.02	ns
IDO1	1.25 (1.14-1.36)	0.0000005	0.00003	0.03
IGKC	1.15 (1.06-1.24)	0.0004	0.002	ns
CD80	1.59 (1.26-2.01)	0.0001	0.0002	ns
CD21	1.11 (1.02-1.21)	0.01	ns	ns

Stromal TILs: OR per 10% change, mRNA markers: OR per 1 dCt value (l≈ doubling of mRNA)

Immune markers were significantly linked to increased pCR rates – all cases (n=481)





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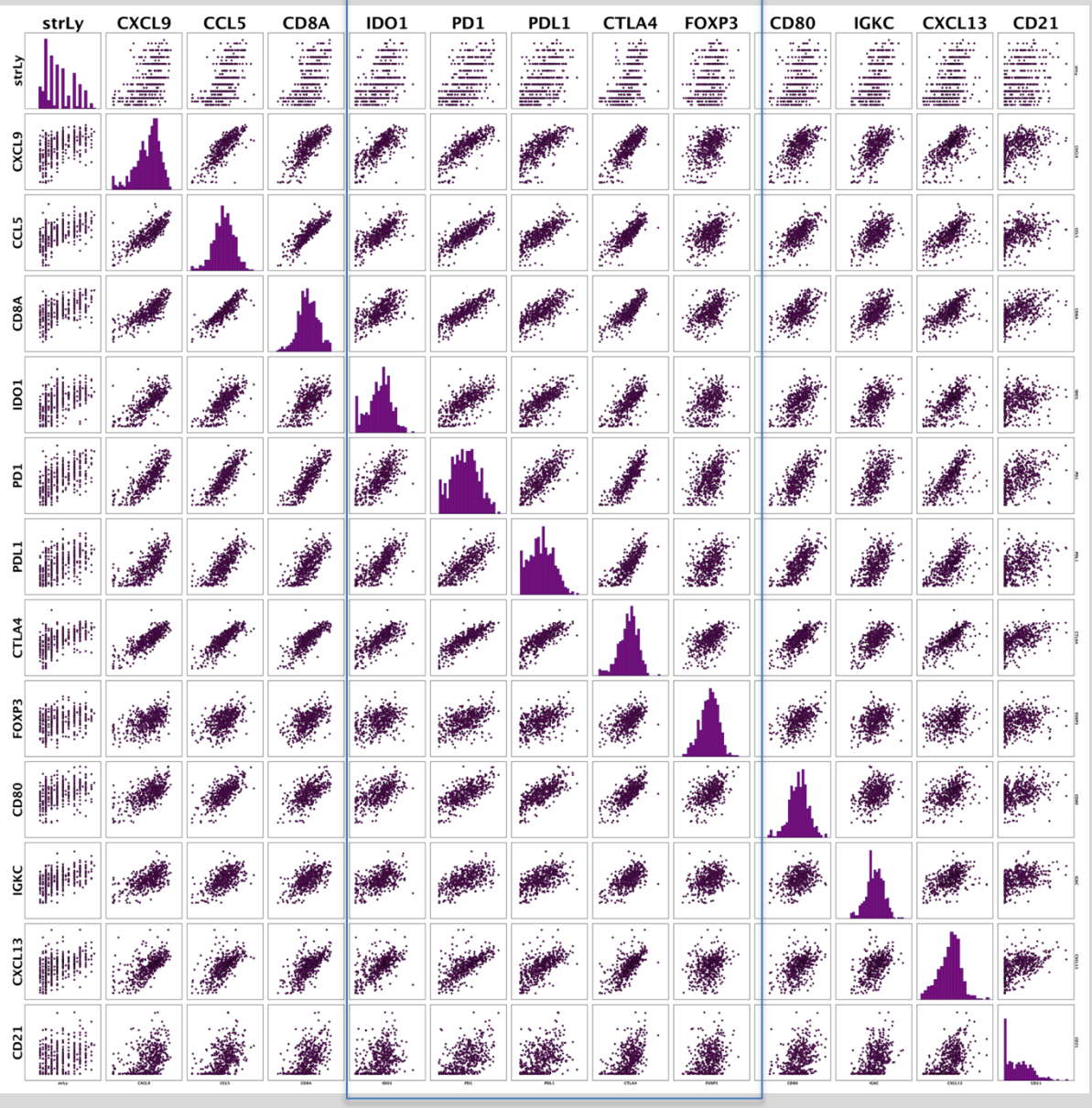
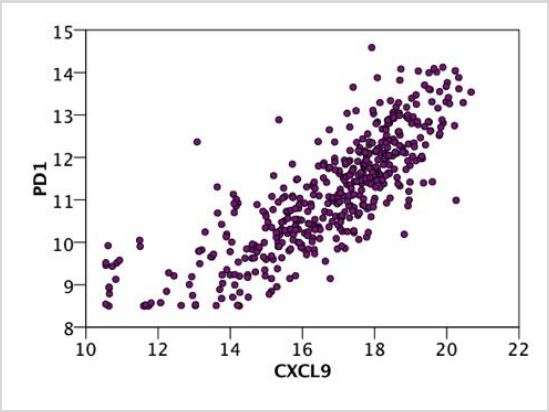
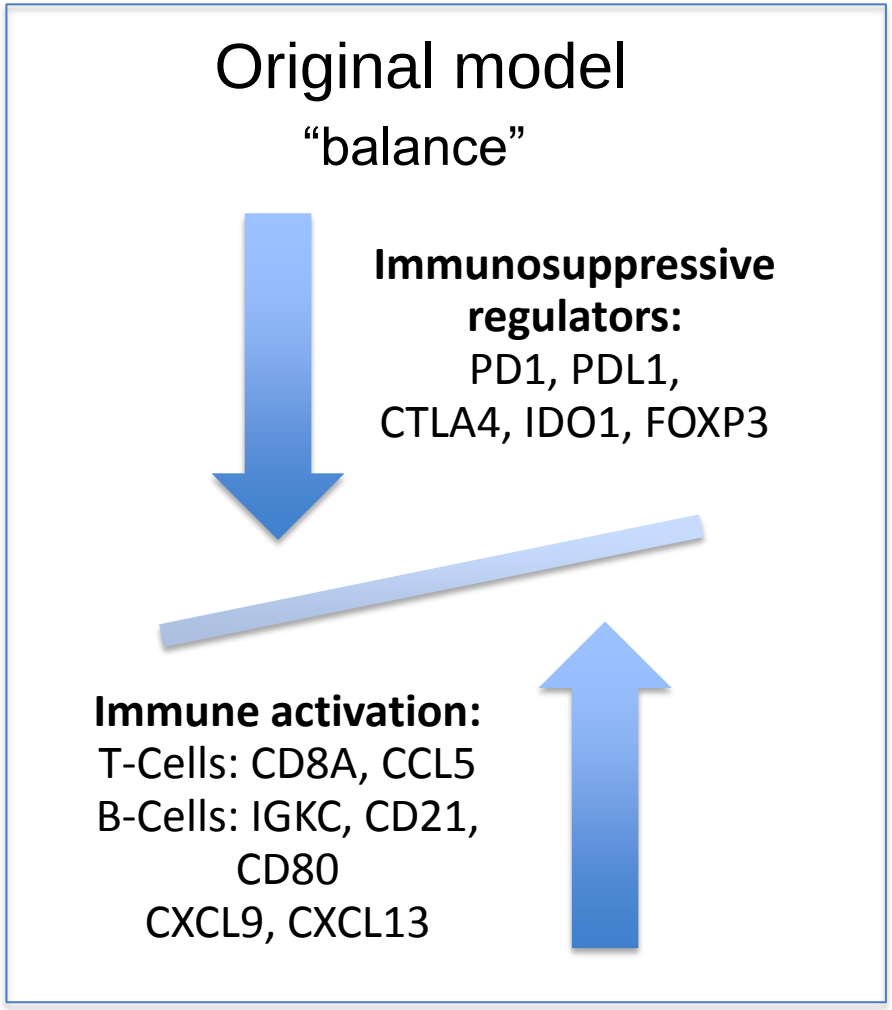
“immunosuppressive”





GeparSixto n=481

Models for immune interaction in breast cancer



Models for immune interaction in breast cancer

Original model

"balance"

Immunosuppressive regulators:
PD1, PDL1,
CTLA4, IDO1, FOXP3

Immune activation:
T-Cells: CD8A, CCL5
B-Cells: IGKC, CD21,
CD80
CXCL9, CXCL13

Modified model

"feedback loop"

activation

Immune activation:
CD8A, CCL5,
IGKC, CD21,
CD80
CXCL9, CXCL13

Immuno-suppressive regulators:
PD1, PDL1,
CTLA4,
IDO1, FOXP3

inhibition

Immune biomarkers vs. immune subtypes

Immune biomarkers

- investigate key immunological molecules or combinations
- determine pro- and anti-immune activation states
- approach based on knowledge about the function of the immune system

- so far no clear clinically relevant pro- and anti-immune groups
- most markers reflect the presence of immune cells

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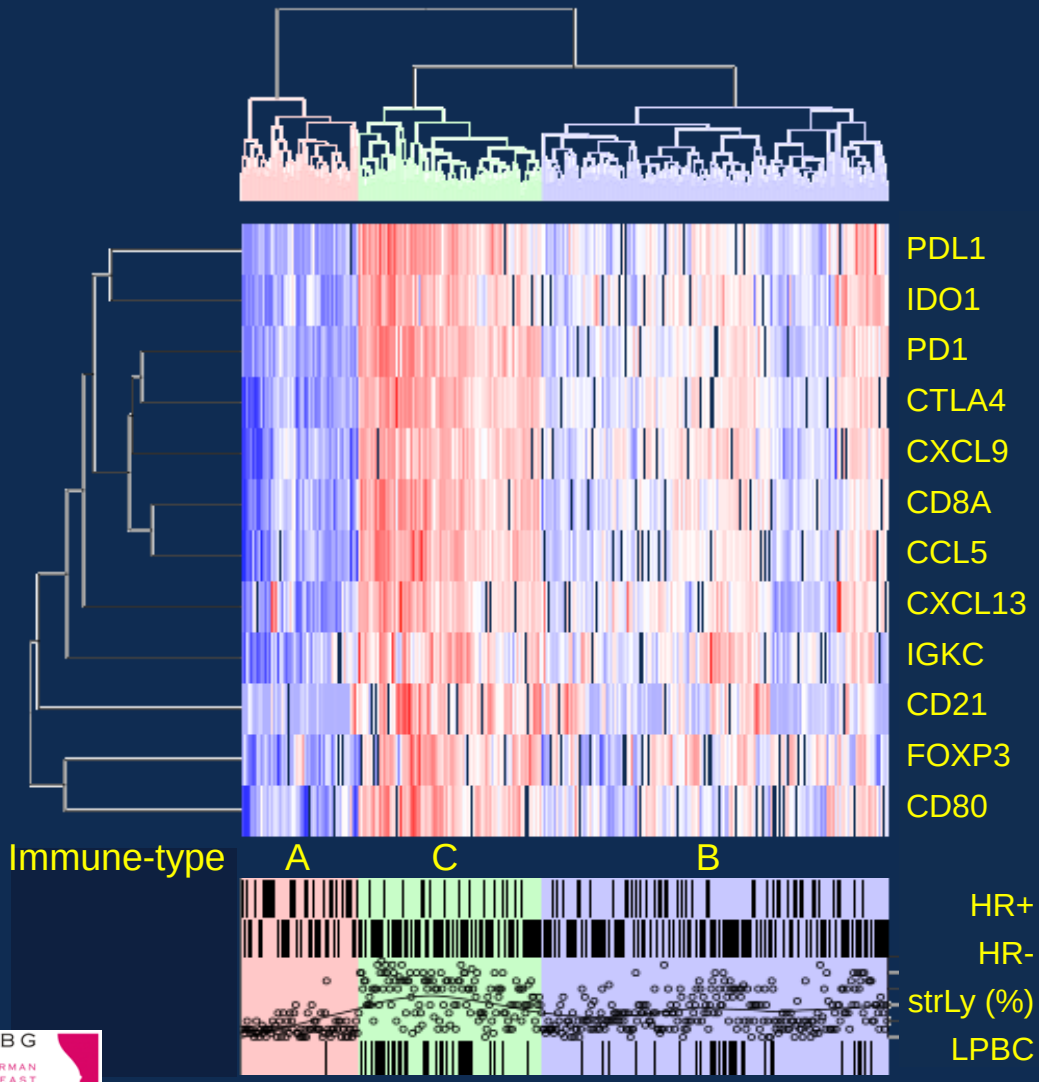
- most markers reflect the presence of immune cells

Immune subtypes

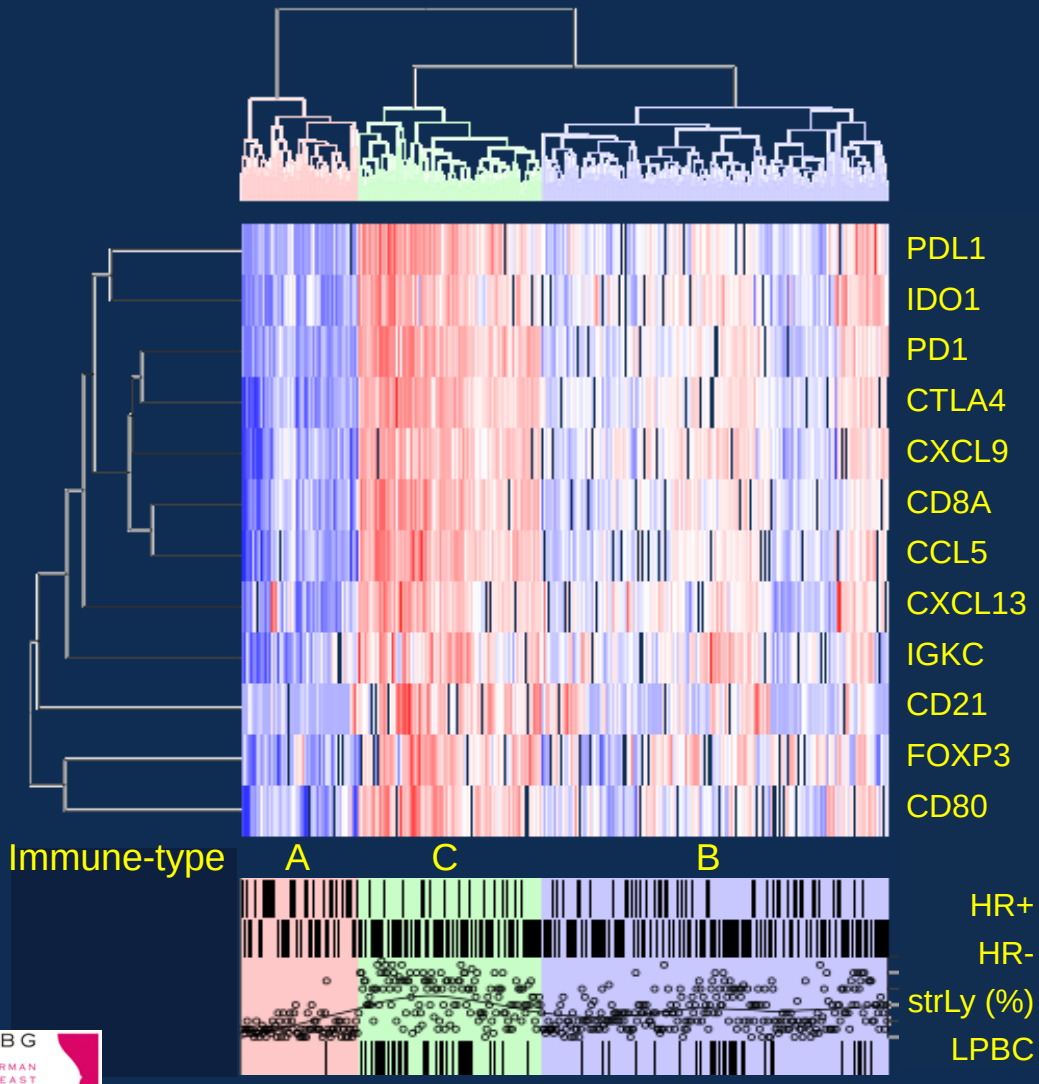
- the immunogenicity of individual tumors is different
- this can be monitored by analysis of
  - TILs
  - immune signatures
- determine groups for therapeutic stratification
- different immunogenicity
  - poor
  - moderate
  - strong

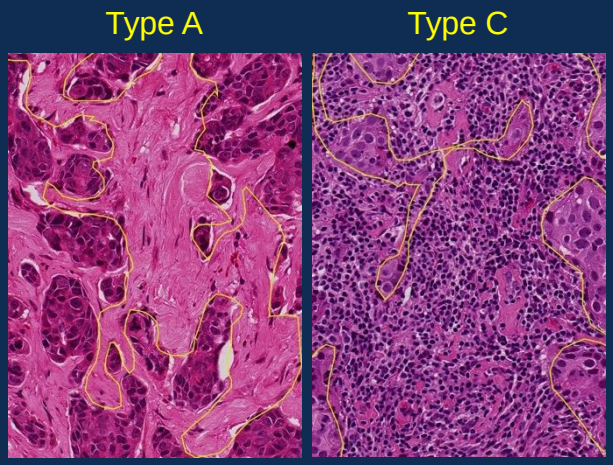
The immunological heterogeneity of breast cancer

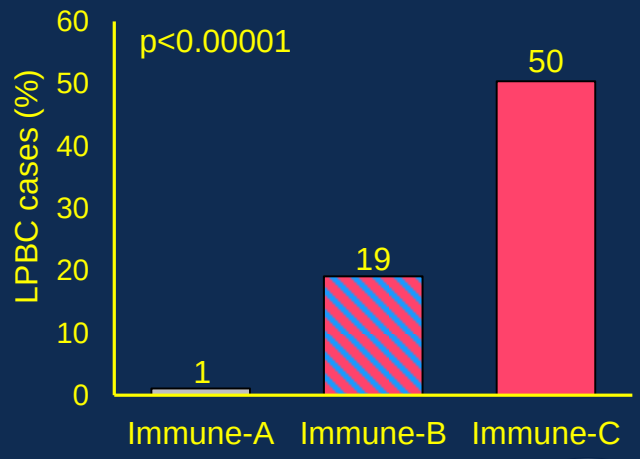
Three different immune subtypes by unsupervised hierarchical clustering (481 tumors, 12 immune genes)



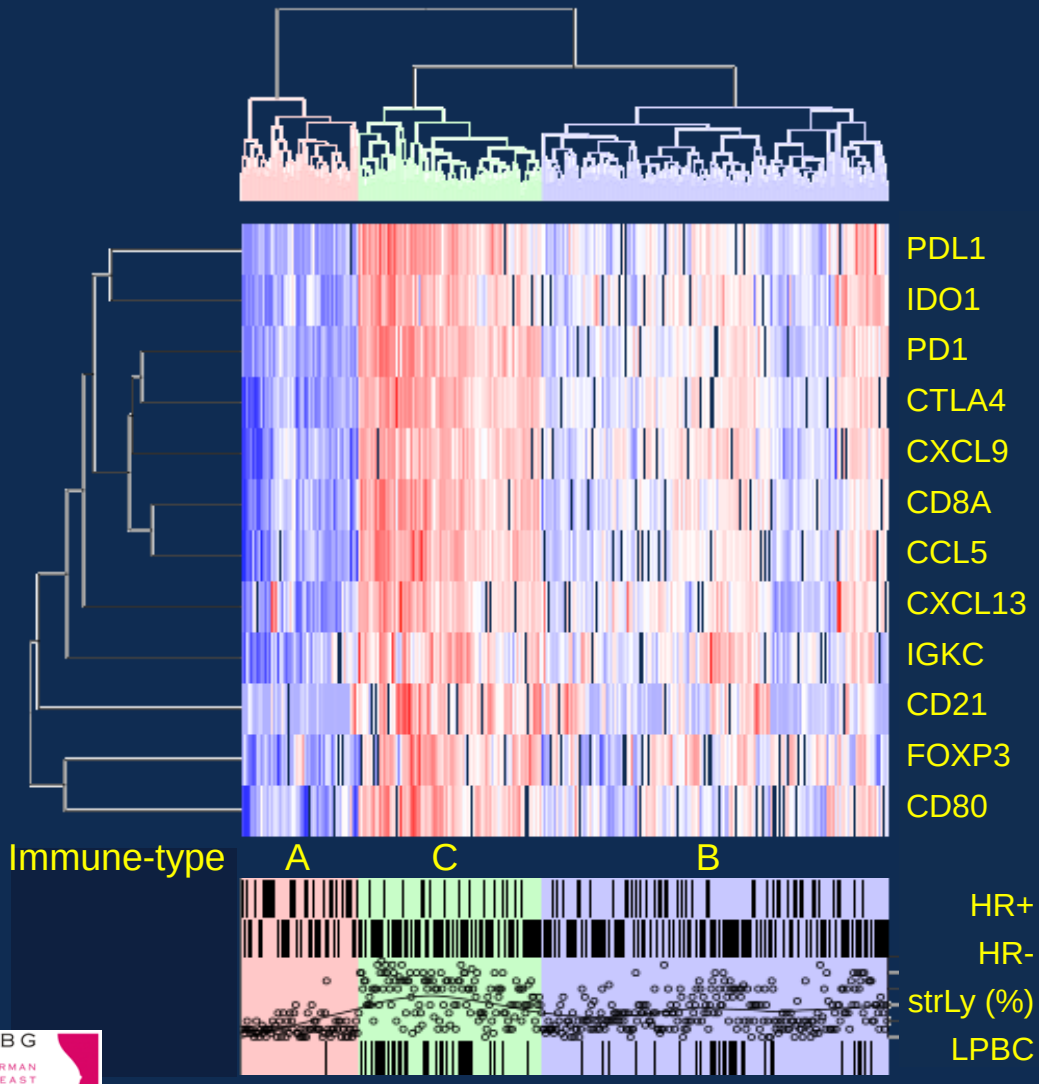
Three different immune subtypes: correlation with TIL morphology

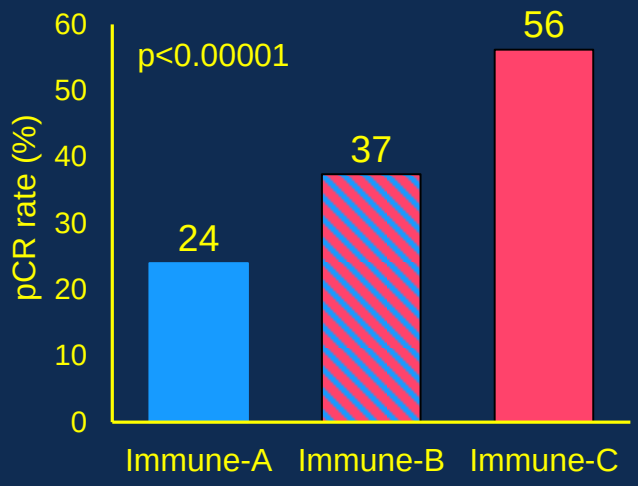






Three different immune subtypes: correlation with response rate



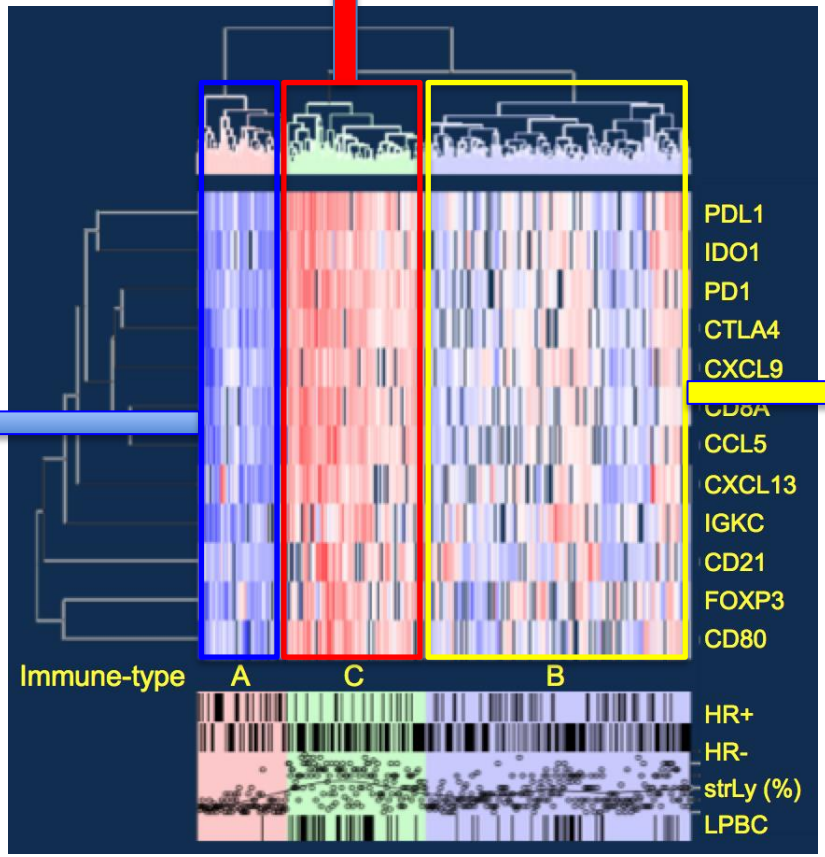


Therapeutic strategies for different types of immune reactions

immunogenic effects of chemotherapy present
can they be enhanced by checkpoint inhibition?

- strong immune reaction, but tumor still growing
- good prognosis with chemotherapy

- no evidence of immune activation
- immune therapy approaches not useful?



- partial immune activation
- immune heterogeneity
- immune escape?
- enhancement of response by immune therapy / checkpoint inhibition?

Comparison of immune mRNAs and TILs for response prediction

	all cases	TNBC	HER2+
	p-value for immune mRNA	p-value for immune mRNA	p-value for immune mRNA
CCL5	0.04		
CXCL9			
CXCL13			
CD8A			
PD1	0.09		
PDL1	0.005	0.04	0.06
CTLA4			
FOXP3			
IDO1	0.05	0.08	
IGKC			
CD80	0.07	0.005	
CD21			

Exploratory multivariate analysis including TILs, mRNA markers and clinical markers:

- TILs are significant in all analyses
- immune mRNAs are only significant in selected analyses

TILs contain similar information as immune mRNAs

Analytical validity and strategies for standardization

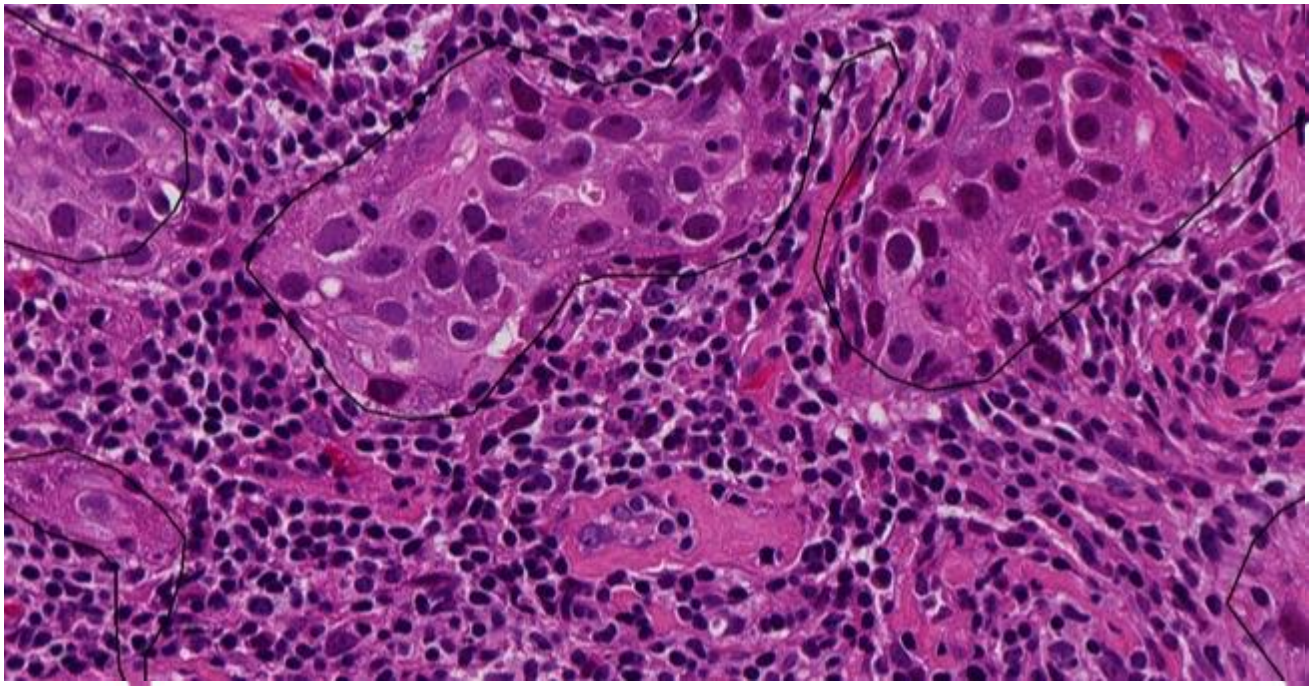
- We do not observe an improved prediction with molecular markers...
- ... if we measure TILs by H&E at the same time.
- Focus on TILs using H&E sections.

Predefined parameters for TIL evaluation

intratumoral TILs = direct contact to tumor cells

stromal TILs = between the tumor cells

LPBC = Lymphocyte-predominant breast cancer
„more lymphocytes than tumor cells“ (≥60% TILs)



Denkert C, Loibl S, et al. J Clin Oncol. 2010, Issa-Nummer et al. PLOSone 2013.

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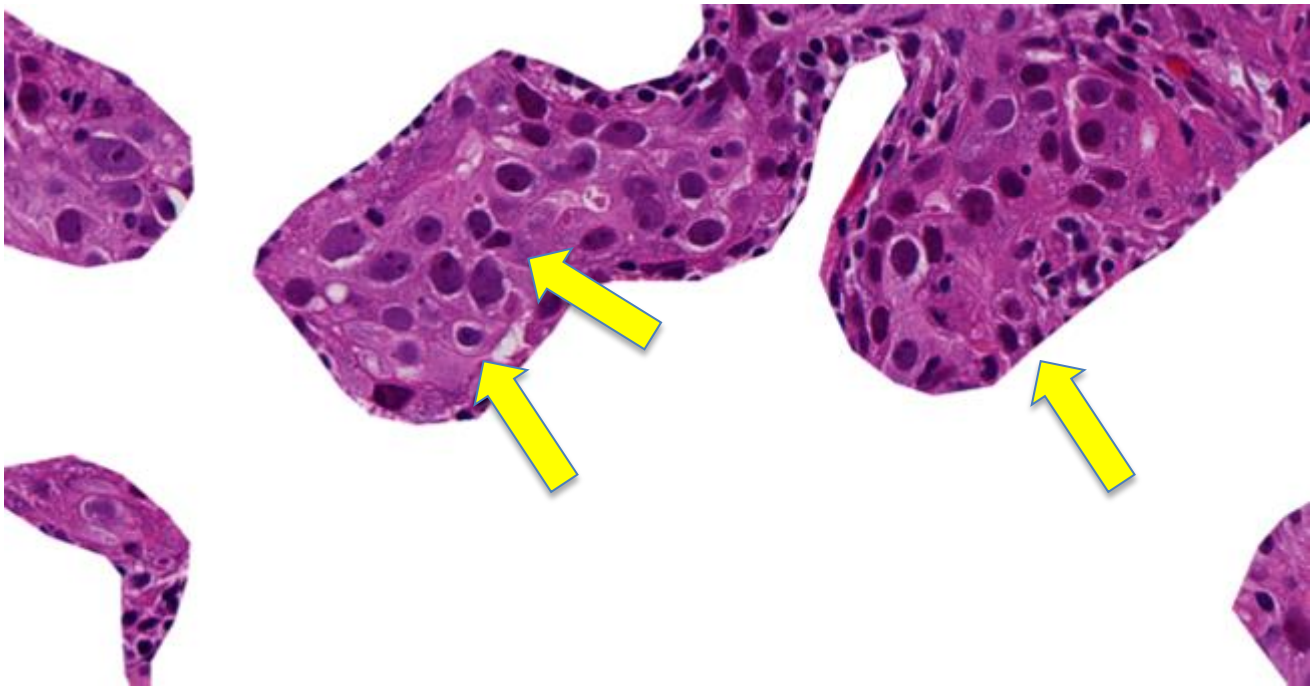

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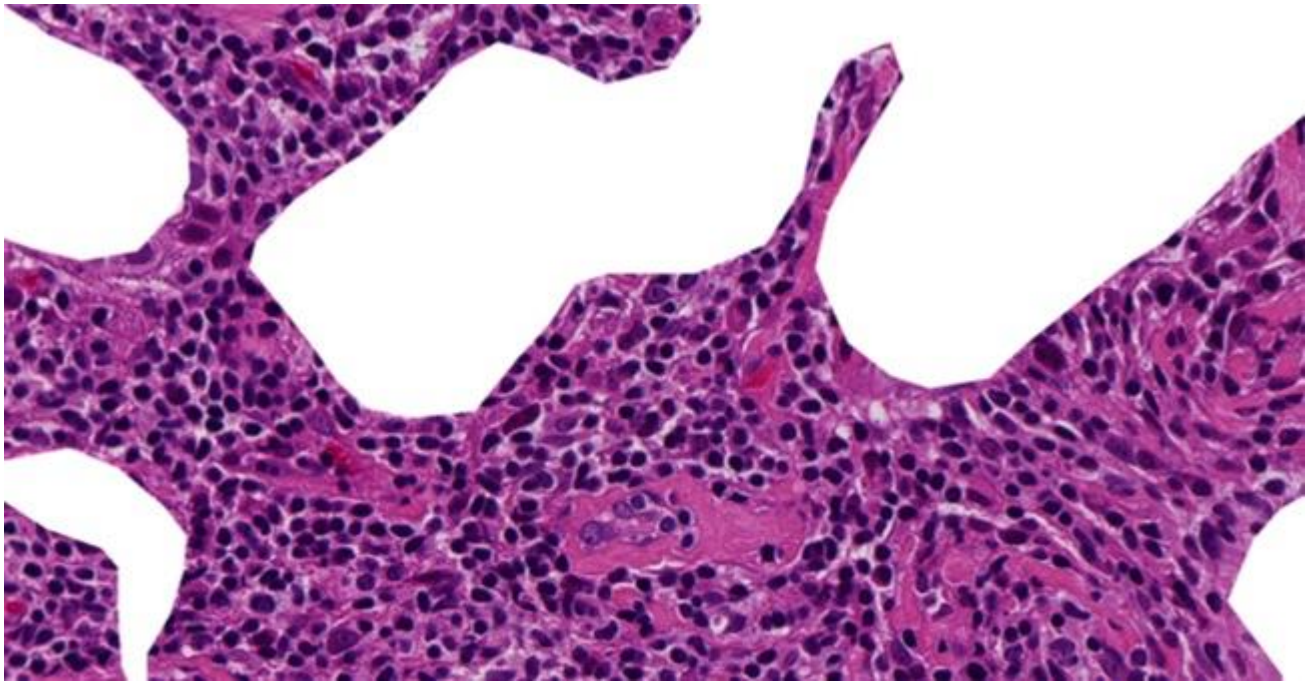
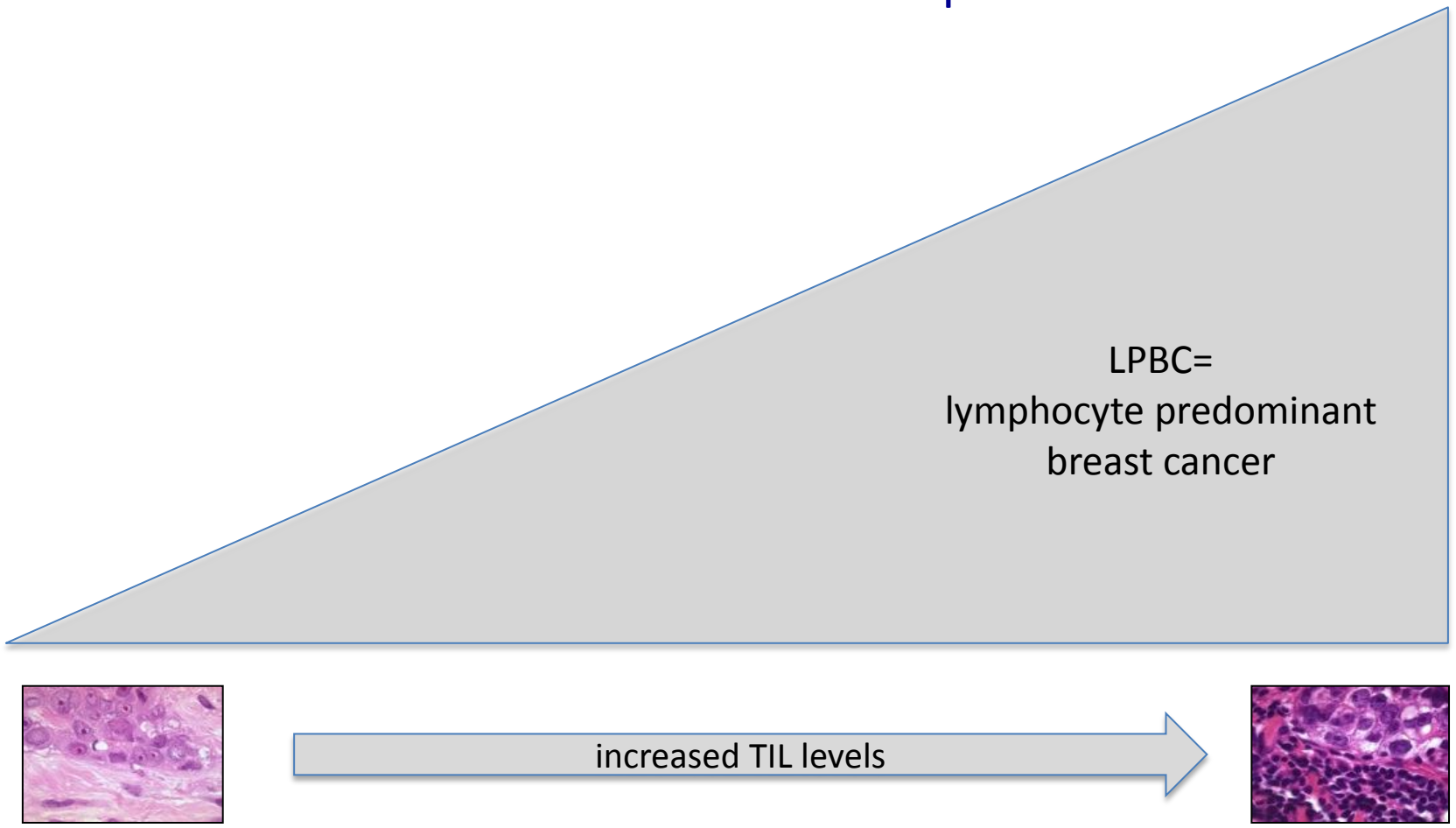


Best parameter: stromal TILs

Denkert C, Loibl S, et al. J Clin Oncol. 2010, Issa-Nummer et al. PLOSone 2013.

Tumor-associated lymphocytes are a continuous parameter



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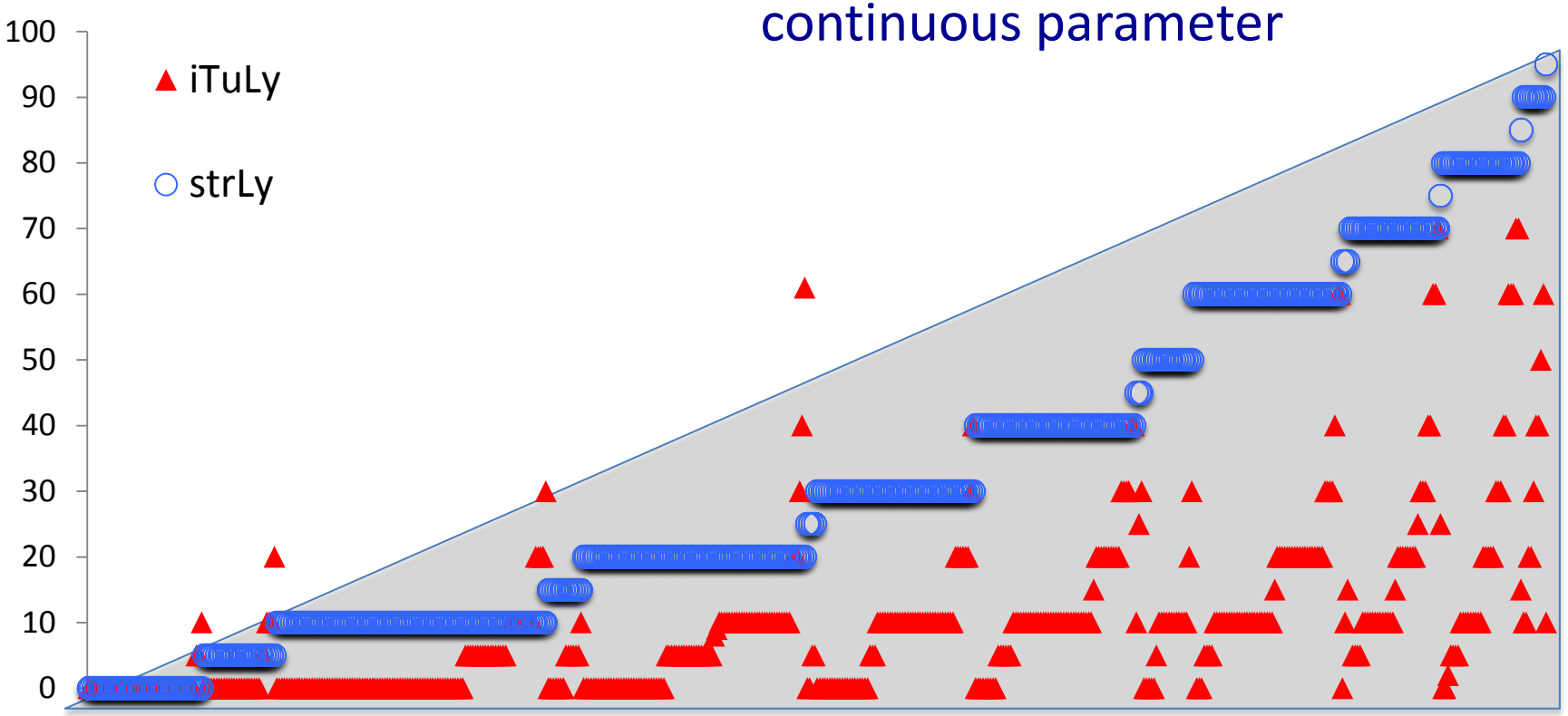
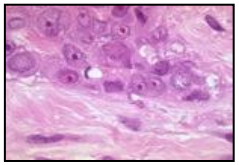
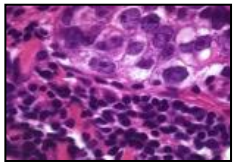




GeparSixto – sorted by increased TIL levels

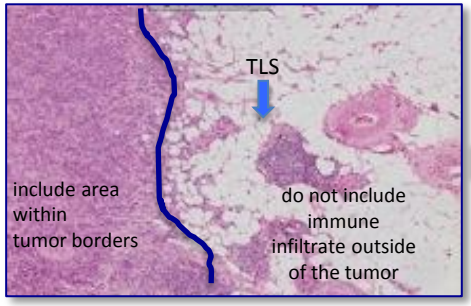


MADRID 2014 ESMO congress

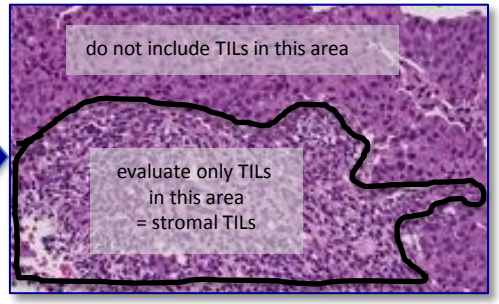
Standardization of TIL-evaluation in Breast Cancer

Salgado, Denkert et al., Annals of Oncology 2014

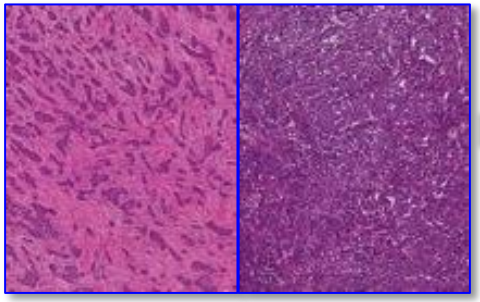
1: select tumor area



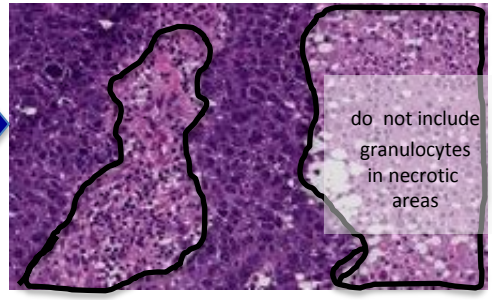
2: define stromal area



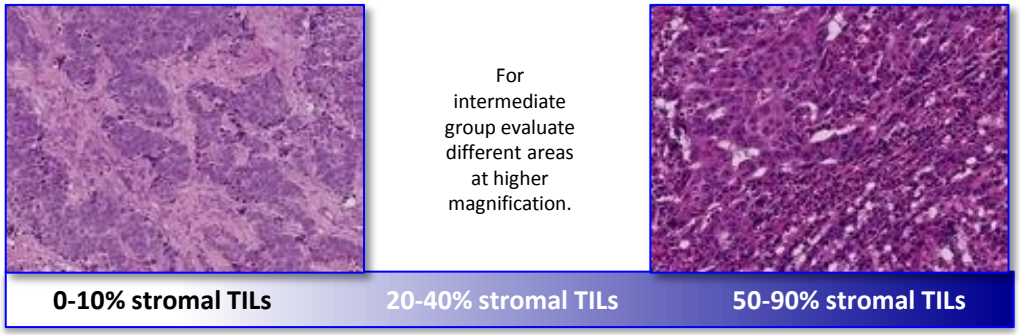
3: scan at low magnification



4: exclude granulocytes



5: assess range of stromal TILs



Standardized evaluation of Tumor-Infiltating Lymphocytes (TIL) in Breast Cancer for daily clinical and research practice or clinical trial setting

A tutorial prepared by the International Working Group for TIL in breast cancer - 2014

Carsten Denkert
Roberto Salgado
Sandra Demaria

MADRID 2014 ESMO congress

Standardization of TIL-evaluation in Breast Cancer

Salgado, Denkert et al., Annals of Oncology 2014

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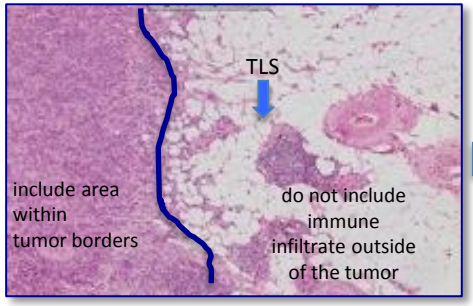

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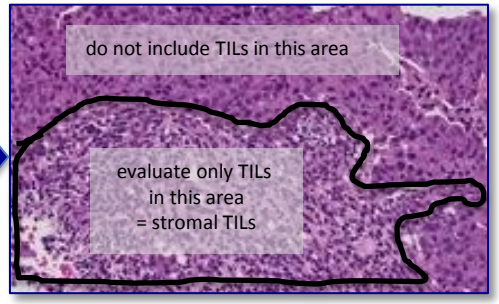

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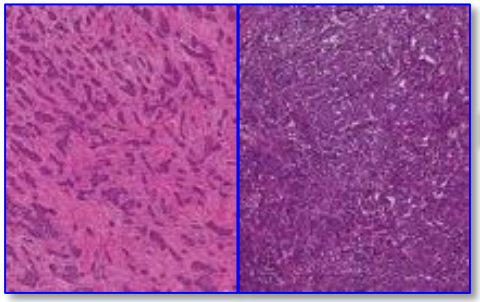

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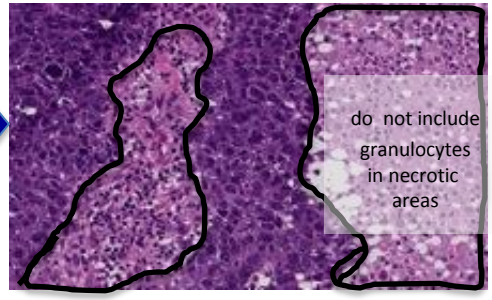

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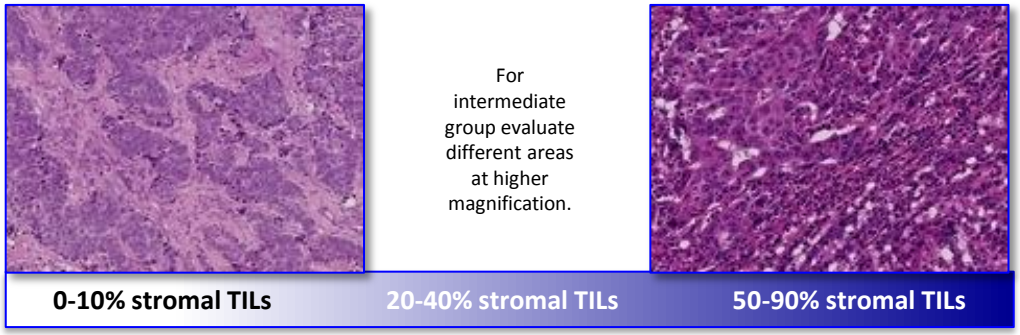
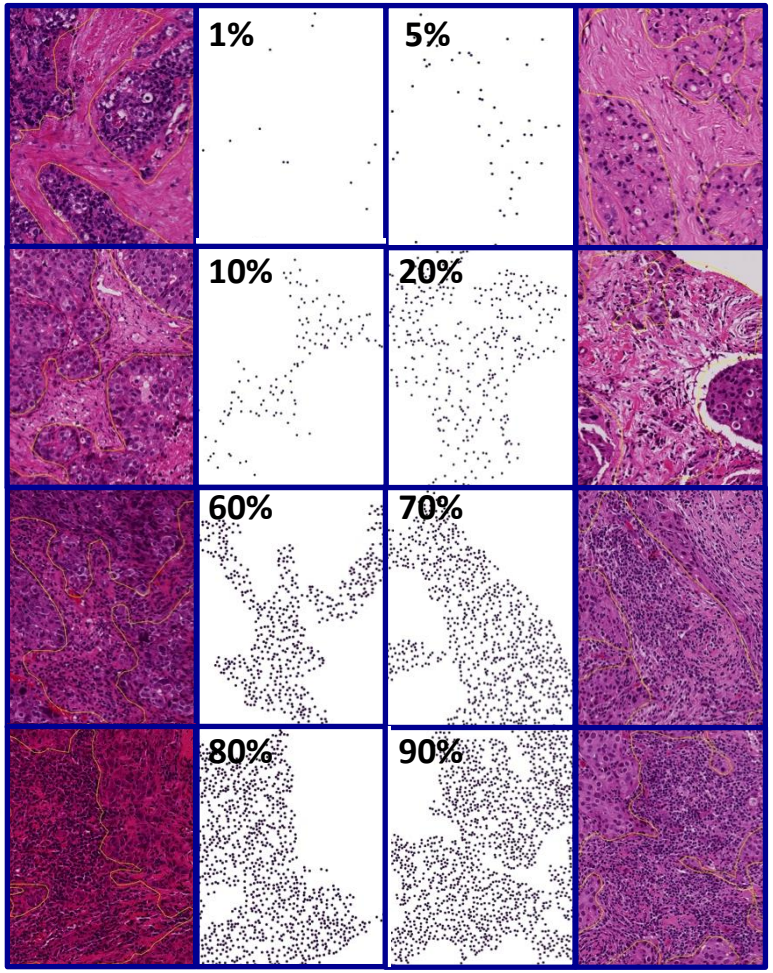


Step 6: determine percentage of TILs (in 5-10% steps)



1st TIL breast cancer ring trial

- kickoff meeting scheduled for SABCS 2014
- evaluation of digital slides by different pathologists
- determination of concordance and interclass correlation coefficient
- development of image analysis approaches

Tumor-associated lymphocytes – options for clinical utility

- Conclusions for clinical practice
  - immune signals are strong and easily detectable
  - but there is no clear clinical utility so far
- Option 1: neoadjuvant carboplatin in TNBC
  - high complete response rates in GeparSixto with increased TILs
  - might be an additional factor for therapy decisions
  - validation in other Platin trials pending
  - GeparOcto: dose-dense conventional vs. dose-dense carboplatin
- Option 2: HER2 positive BC
  - trastuzumab effect dependent on TILs (Finher)
  - other validations pending
- Option 3: immune therapies ... prediction of response

Summary – immune infiltration in breast cancer

1. TILs are a predictive marker for response to neoadjuvant therapy – several studies with >2000 patients.
2. TILs are prognostic in TNBC (n>700, two studies).
3. Interaction with therapy:
   - In Geparsixto, the predictive effect of TILs for pCR was particularly high in patients treated with carboplatin.
   - In Finher, the effect of trastuzumab was increased in TIL+ tumors.
   - Validation studies are needed for both questions.
4. Stromal TILs are the most useful parameter.
5. Immune marker signatures are correlated with the presence of immune cells
   - no clear pro- and anti-immune signatures
6. The next steps:
   - include TILs in clinical trial parameters (as well as routine histology).
   - clinical studies for immune checkpoint inhibitors

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GBG GERMAN BREAST GROUP

AGO-B BREAST STUDY GROUP

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