Immunological fingerprint in breast cancer patients – what does that mean?

"I-O development: from early to late clinical development - Successes and failures along the way"
OCC, April 6th 2016

The molecular pathologists' perspective

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and
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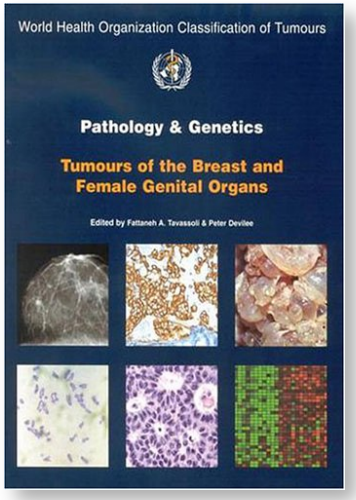
Oslo University Hospital

The Norwegian Cancer Association
Forskningsrådet
Oslo University Hospital

Breast cancer, the diagnosis



- 17 different types of breast cancer is recognized by histologic appearance (WHO)
  - ~60% is Ductal carcinoma not otherwise specified
  - 10-15% is Lobular carcinoma
- No major importance in clinical decisions

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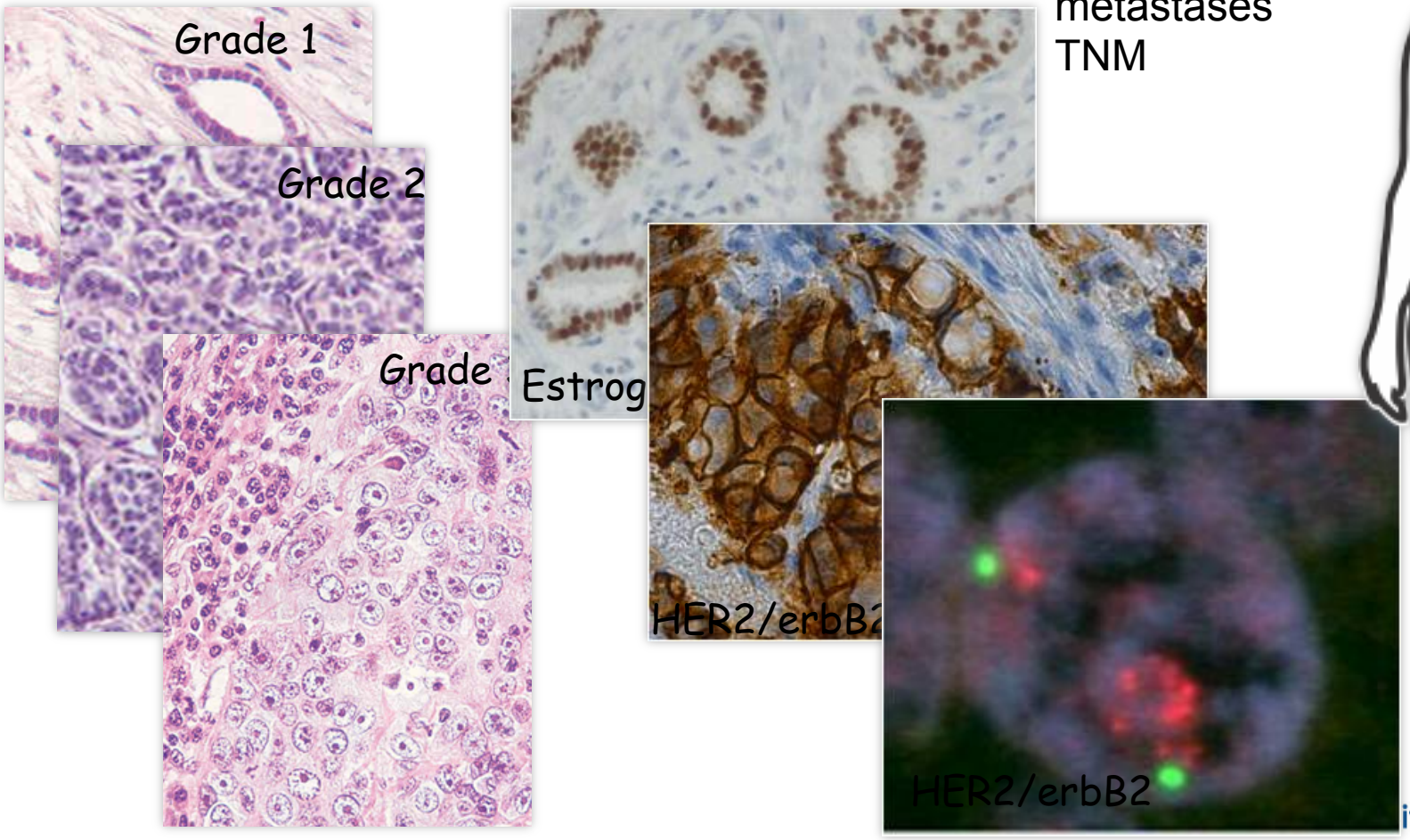
Grouping of breast cancer

Grade

ER/PgR, Ki67, HER2

Size, nodal involvment, metastases TNM

Age

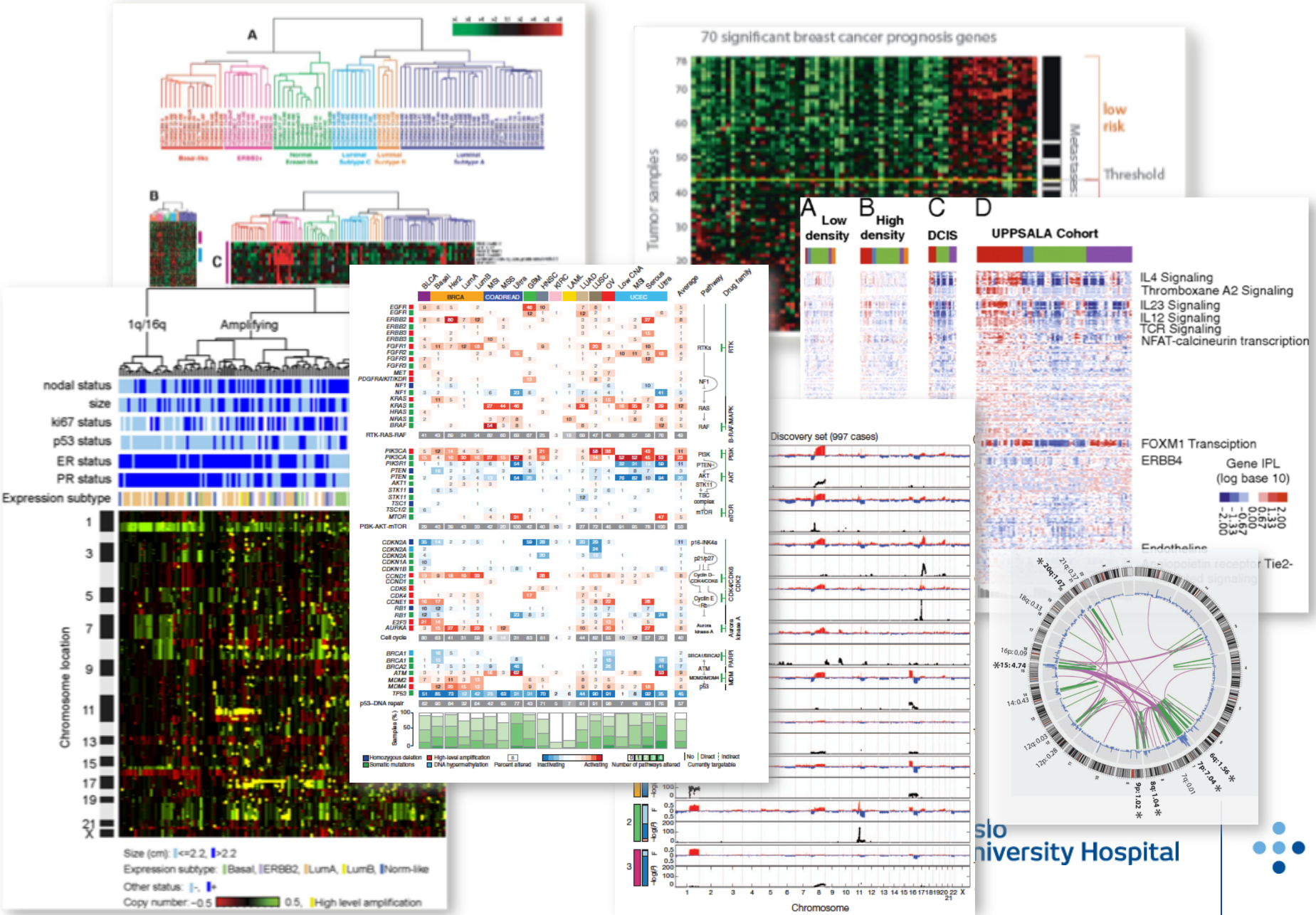


Norwegian guidelines, adjuvant systemic treatment 01.09.15

Oversikt NBCGs retningslinjer for adjuvant systemisk behandling. *Gjelder fra 01.09.15*

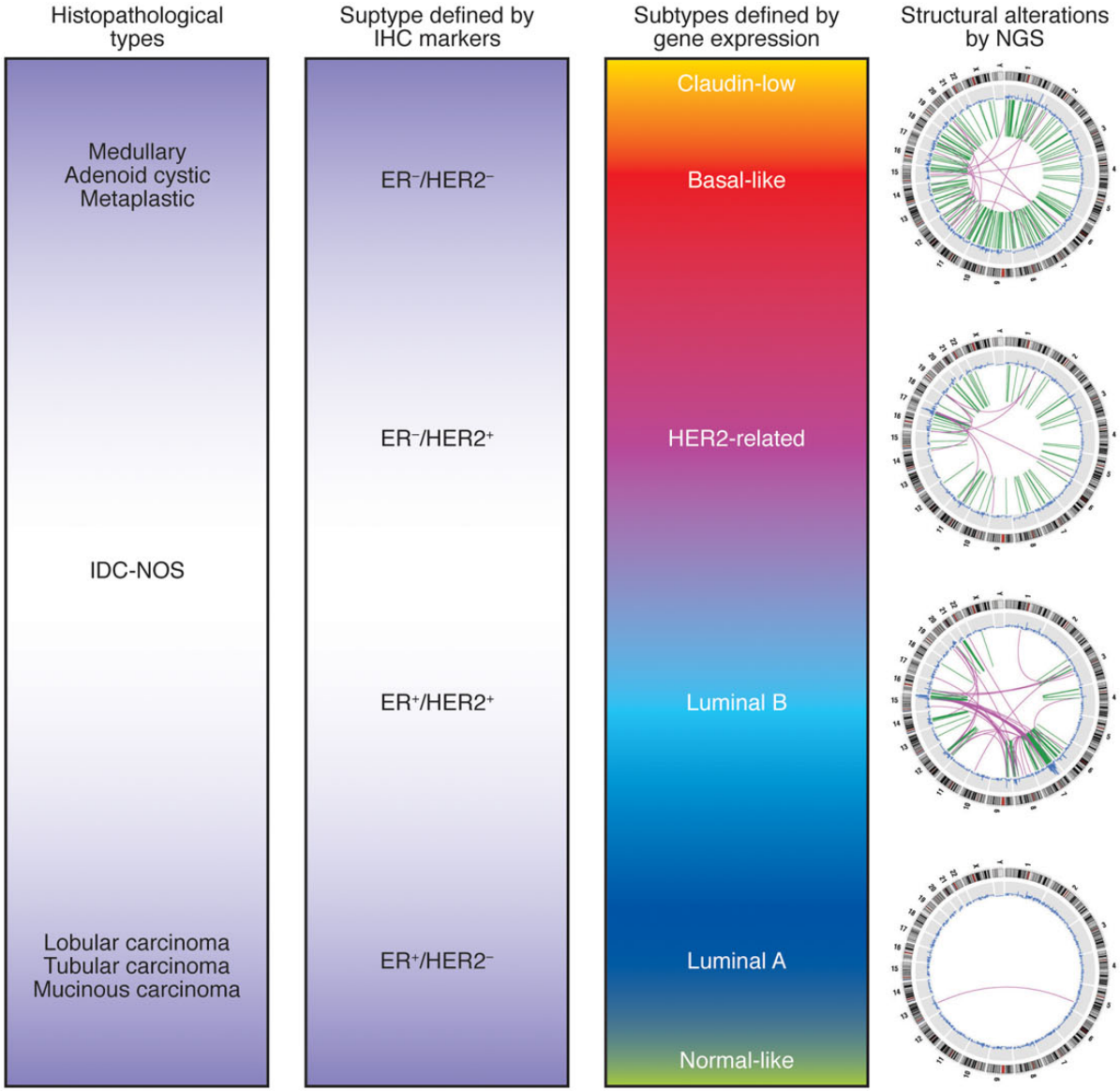
Hoved-gruppe	Subgruppering ved undersøkelser av tumor	Ytterligere subgruppering	Type terapi	Kommentar	Terapikategori
		pT1a-b pN0	Ingen behandling	Ved G3 og <35 år bør endokrin behandling vurderes	Generelt meget liten absolutt nytte av behandling
HR+ HER2-	Alle følgende karakteristika til stede: HR+≥10% og Hotspot Ki67<30% og pN0-1	pT1a-b pN0	Ingen behandling		
		pT1c pN0 grad 1	Ingen behandling		
		HR+≥50% og en av følgende: 1) pT2 pN0 og grad 1 2) pN1 og grad 1 3) pT1c-T2 pN0 og grad 2 og Ki67<15% 4) pN1 og grad 2 og Ki67<15%	Kun endokrin behandling Zoledronsyre ved alder ≥55år		
		pT1c-pT2 pN0 eller pT1-2 pN1, med en av følgende: 1) Grad 3 2) Grad 2 og Ki67 ≥15-<30% 3) HR+ ≥10-<50%	EC90 x 4 etterfulgt av endokrin behandling Zoledronsyre ved alder ≥55år		
	Alle følgende til stede: HR+≥10% og Hotspot Ki67<30% og pN2-3 og grad 1-2		EC90 x 4 etterfulgt av endokrin behandling Zoledronsyre ved alder ≥55år		
	Alle følgende til stede: HR+ 1-10% og Hotspot Ki67 <30% og pN0-1		EC90 x 4 etterfulgt av endokrin behandling Zoledronsyre ved alder ≥55år		
	En av følgende til stede: 1) Hotspot Ki67 ≥30% eller 2) Grad 3 og pN2-3 eller 3) HR+ ≥1-<10% og pN2-3		EC90 x 4 → taxan etterfulgt av endokrin behandling Zoledronsyre ved alder ≥55år		
	Begge følgende til stede: pN1 grad 3 (uavhengig av Ki67)		EC90 x 4 → taxan Zoledronsyre ved alder ≥55år		Optimalisert kjemoterapi
	pN2-3 (uavhengig av andre faktorer)		EC90 x 4 → taxan Zoledronsyre ved alder ≥55år		Optimalisert kjemoterapi

Molecular subclassification of breast cancer...



Molecular subtypes

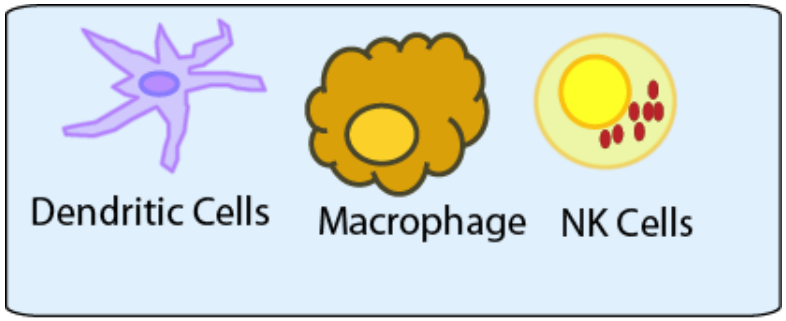
A relationship between phenotypic and genomic subtypes



Russnes et al. JCI 2011

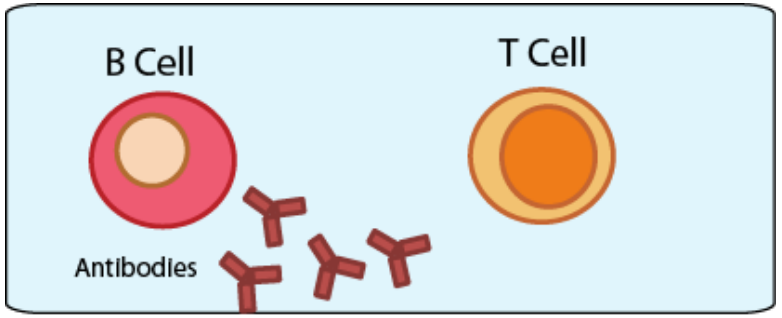
Local Immune response

Innate Immunity



- First line of defense
- Present in tissue
- Recruite immune cells to sites of infection (inflammation)
- Engulf pathogens or cell debris
- Present antigens
- Activates the adaptive system

Adaptive Immunity



- Clonal expansion
- Antibodies or cell receptors target specific antigens
- May kill targeted cells

Courtesy, Inga H. Rye

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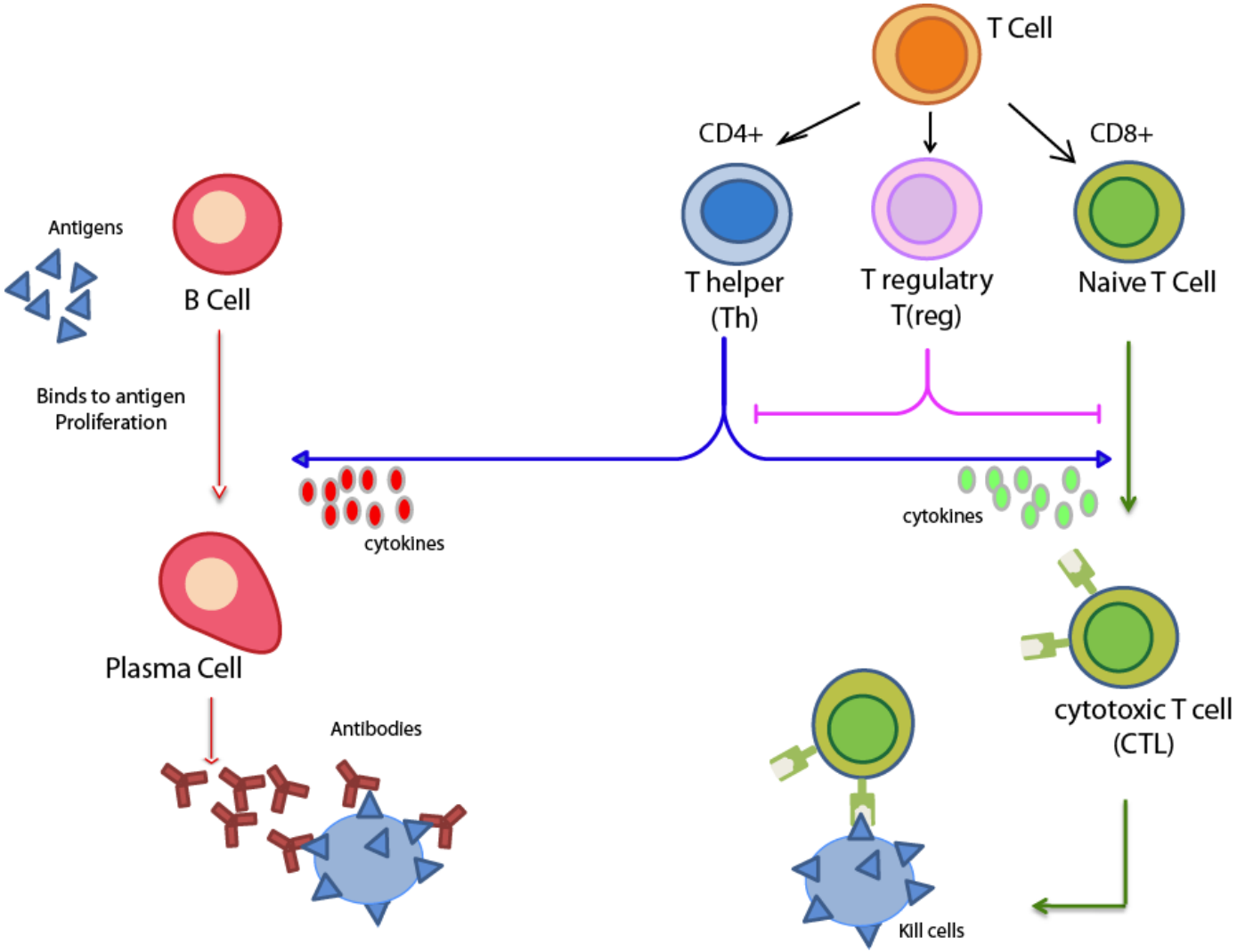
Adaptive immunity

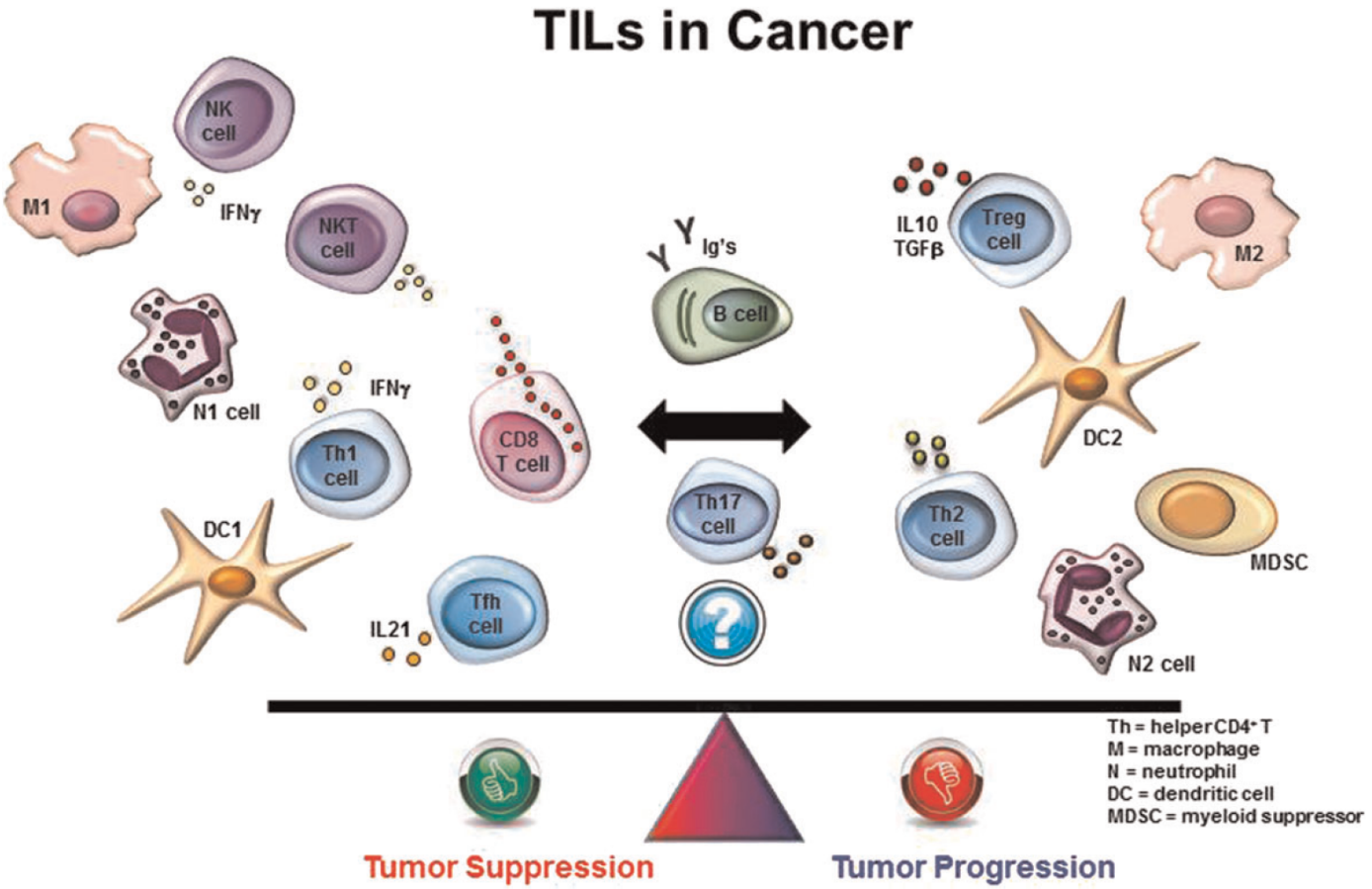


Courtesy, Inga H. Rye

TILs; tumour infiltrating lymfocytes

- Association with outcome first documented in 1922 *(Sistrunk and Maccarty, Ann. Surg 1922)*
- Tumors with oncogene amplifications had more frequent increased numbers of TILs *(Tang et al. J Cell. Biochem 1990)*
- Infiltrative TILs capable of cytolytic activity and cytokine secretion *(Schwartzenstruber et al. 1991 and 1992, Tanaka et al. 1991)*

Different types of immune response



Salgado et al, Ann of Oncology 2015

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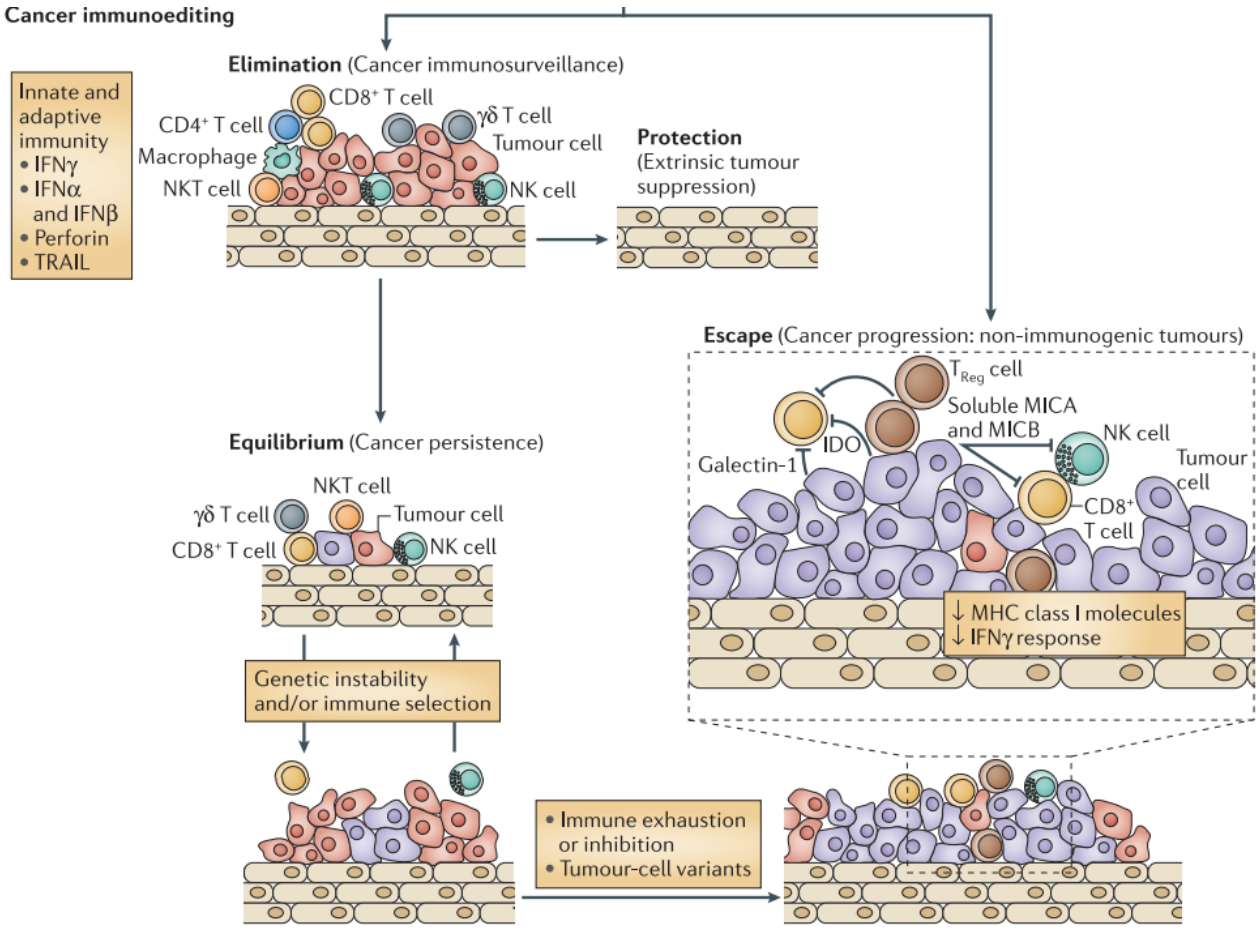
Immune response vary during cancer development



Dunn et al., Nature reviews 2006

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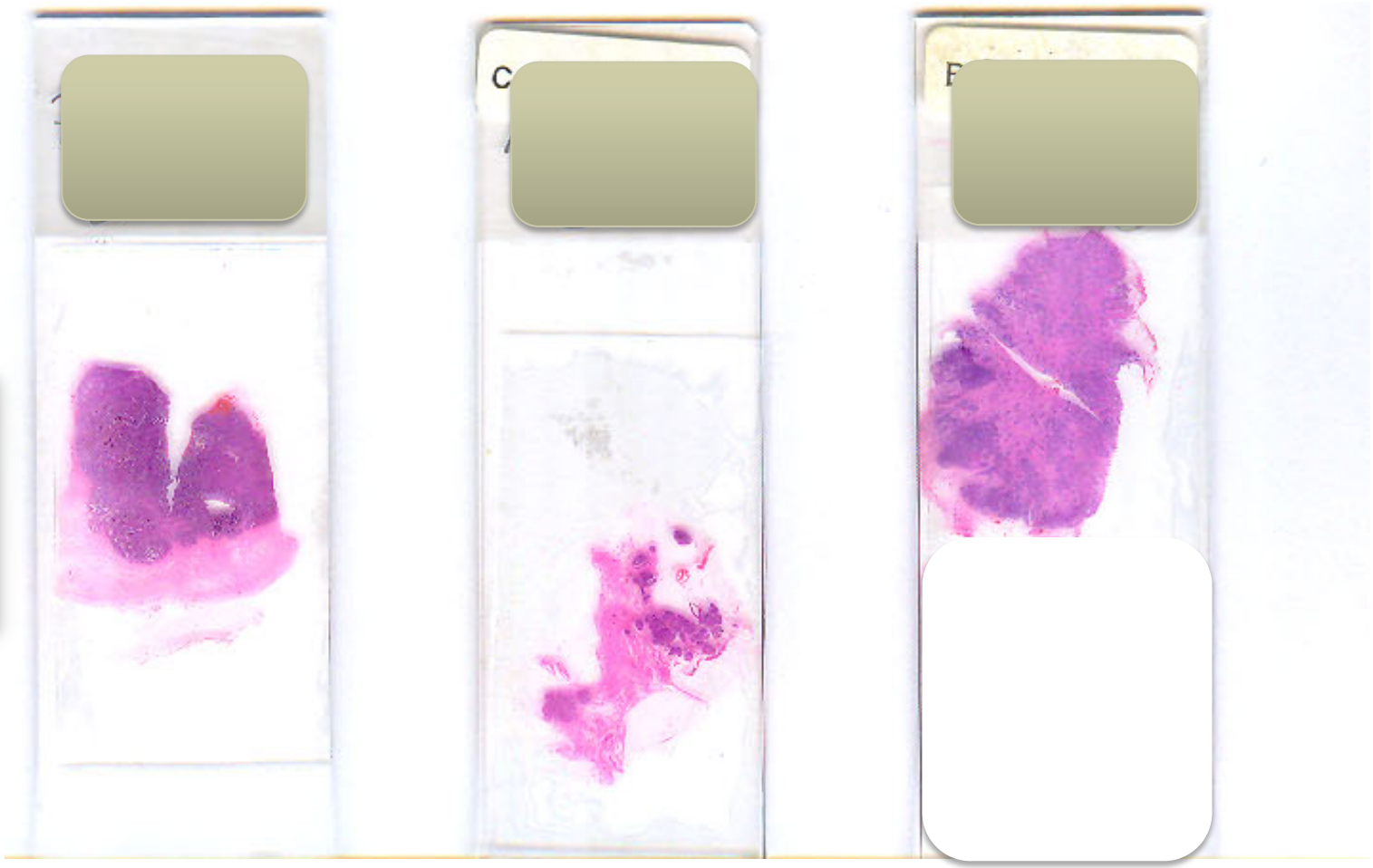
Immune response in tissue



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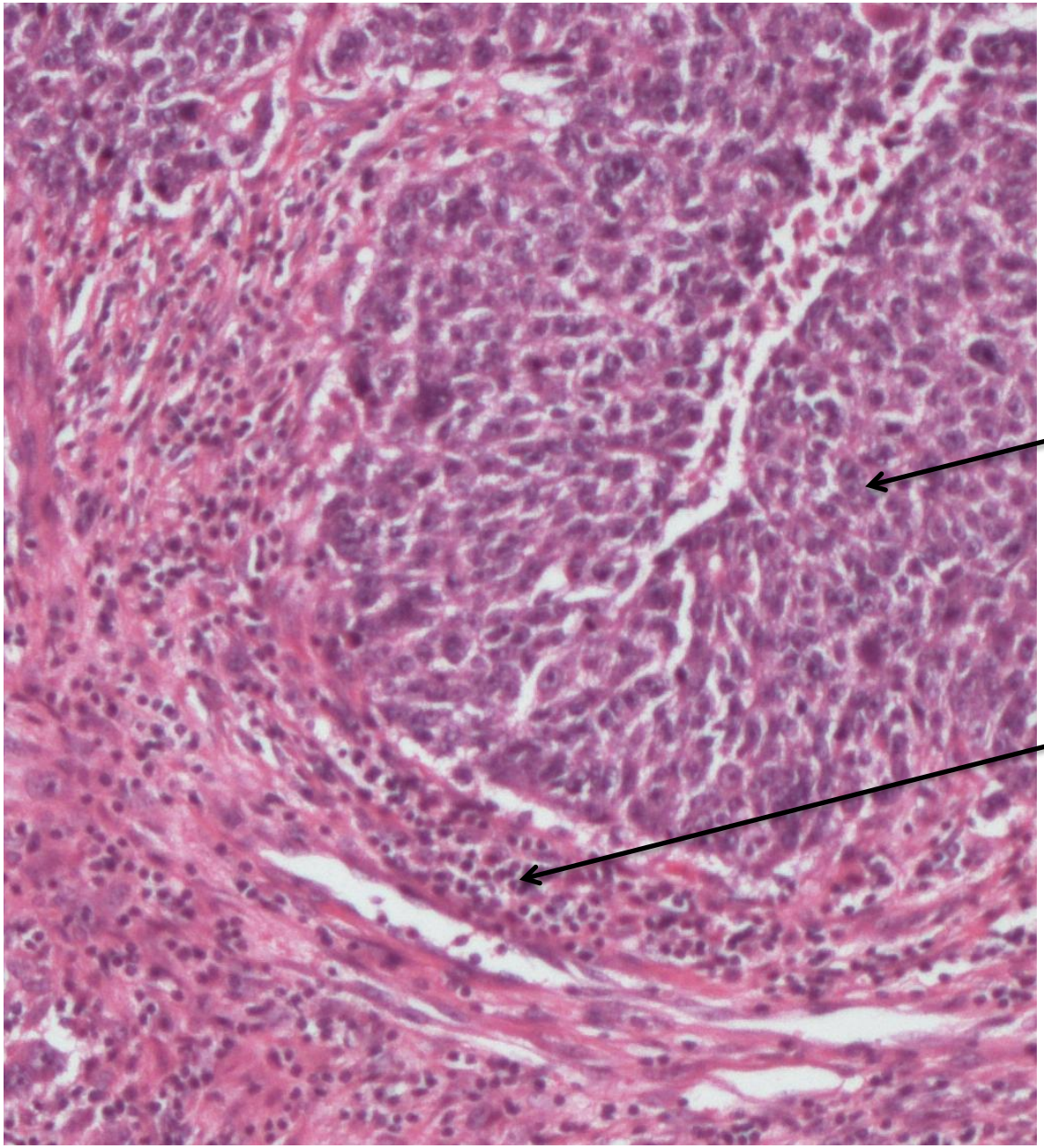
Tumor #1



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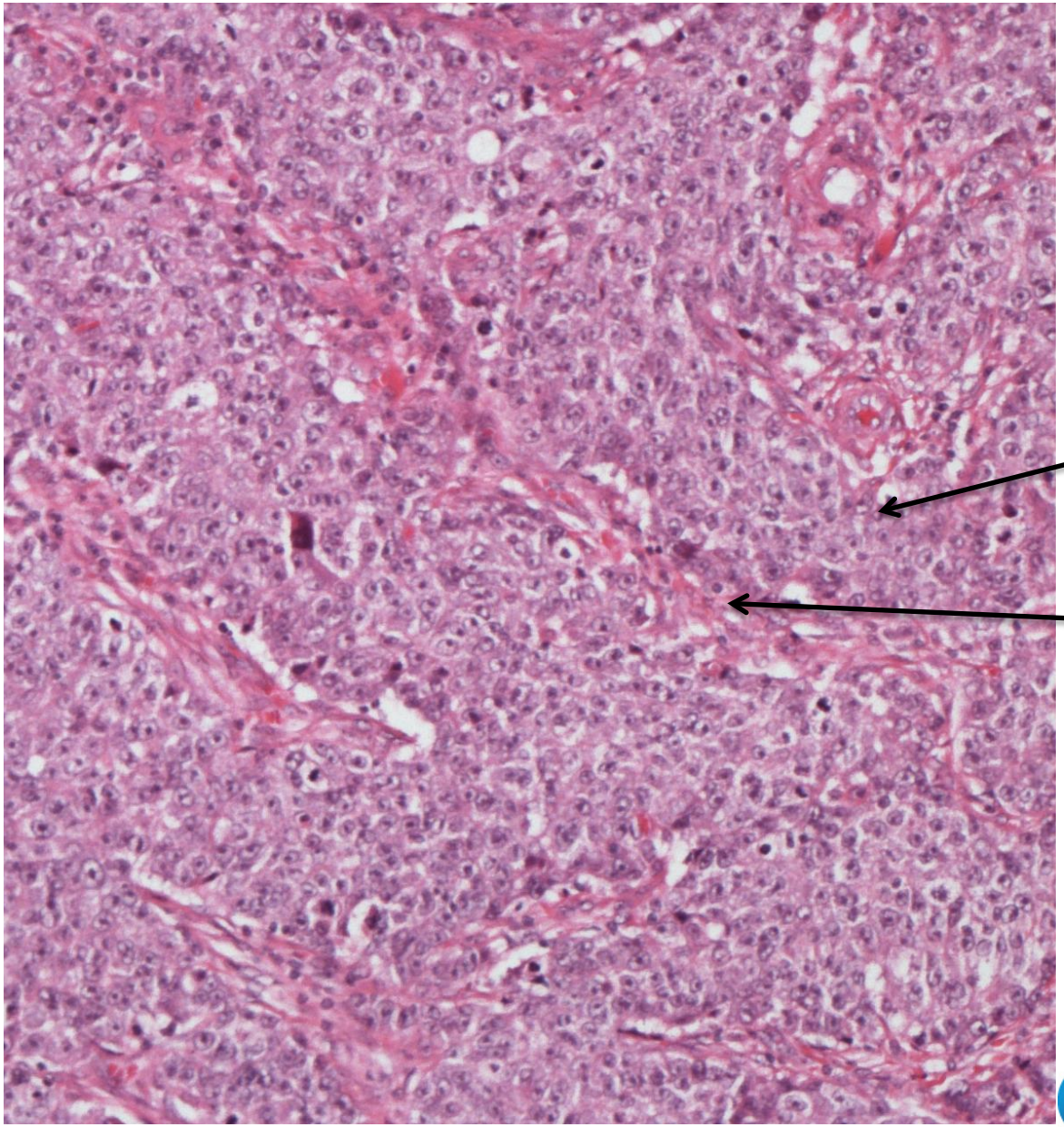
Tumor #1



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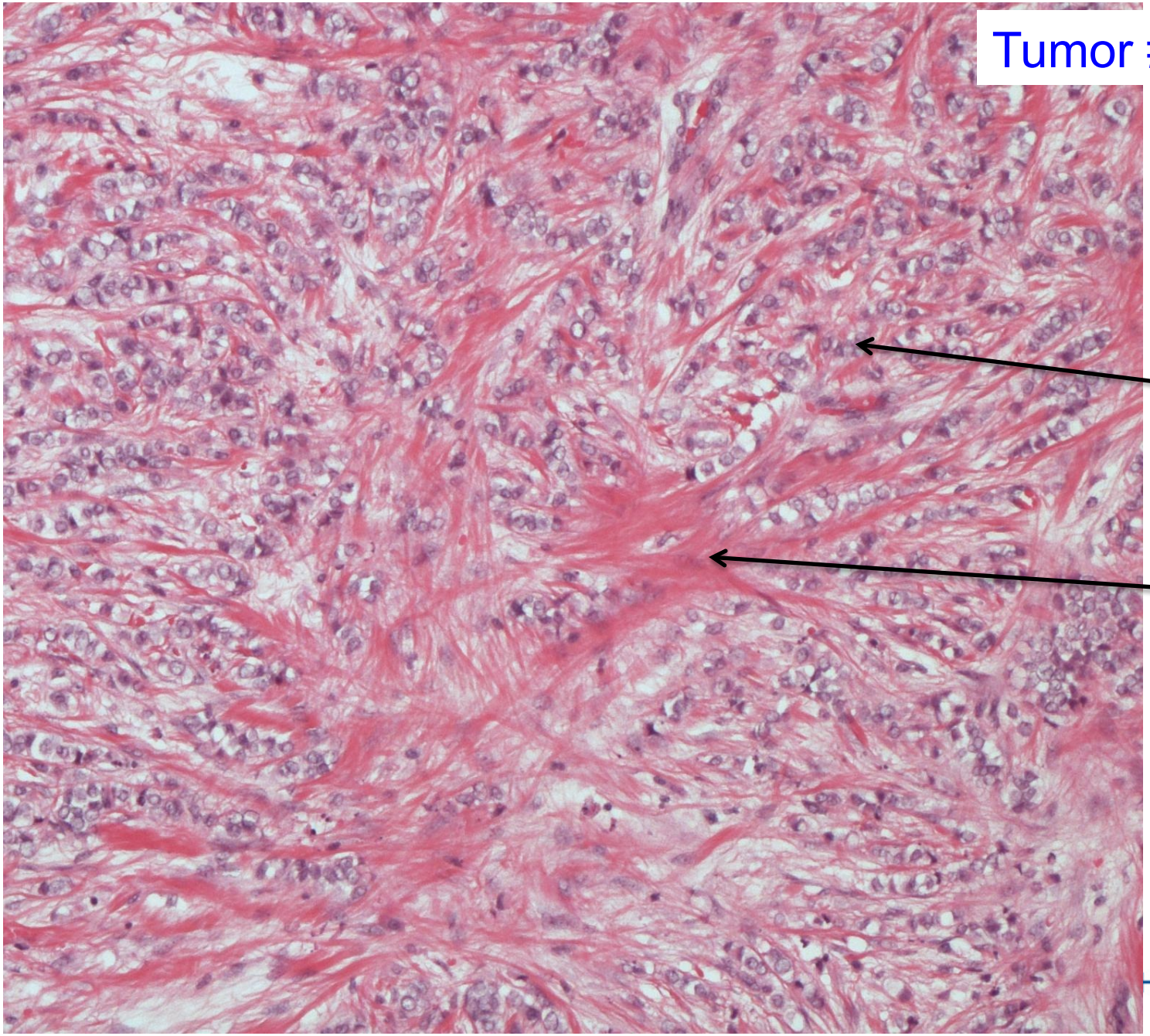
Tumor #2



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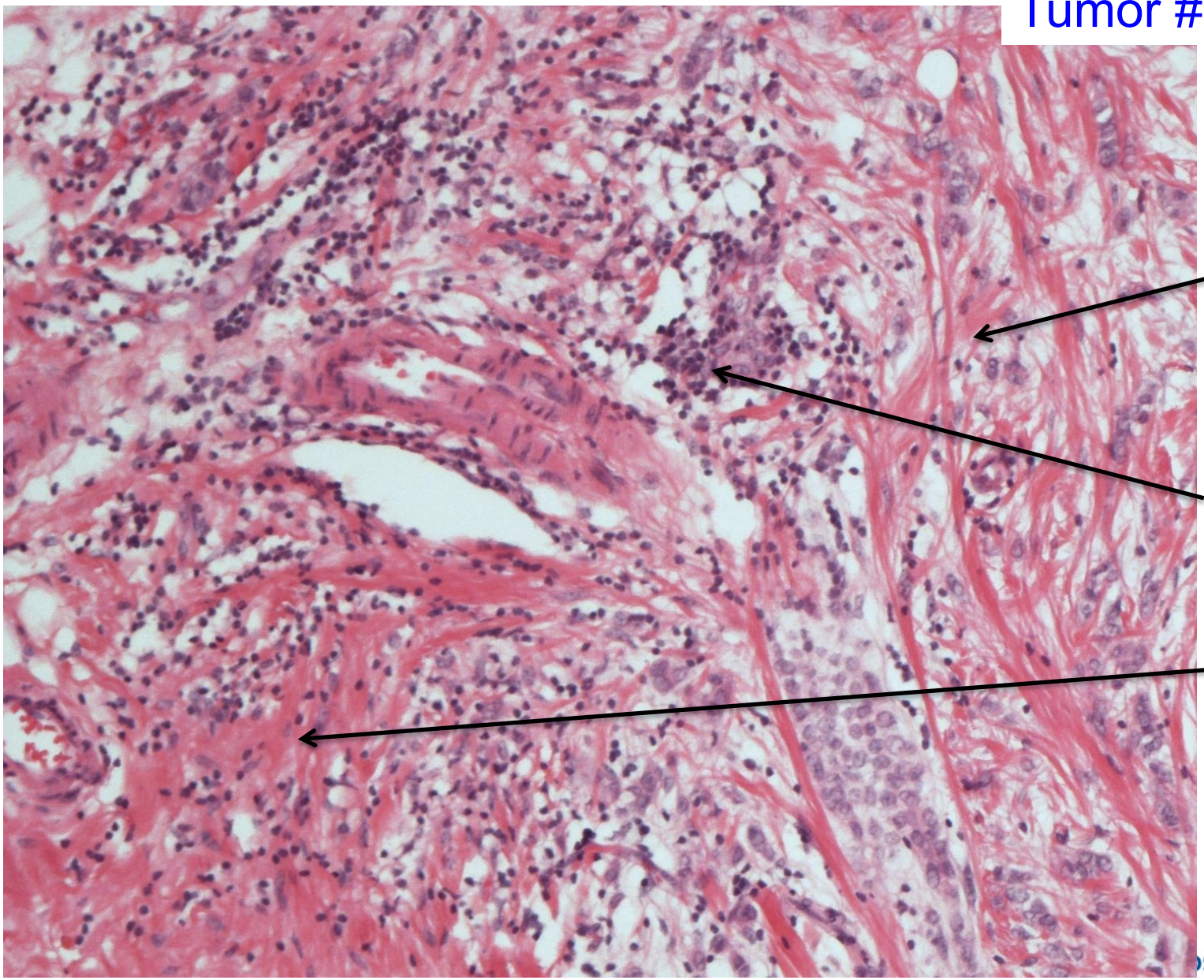
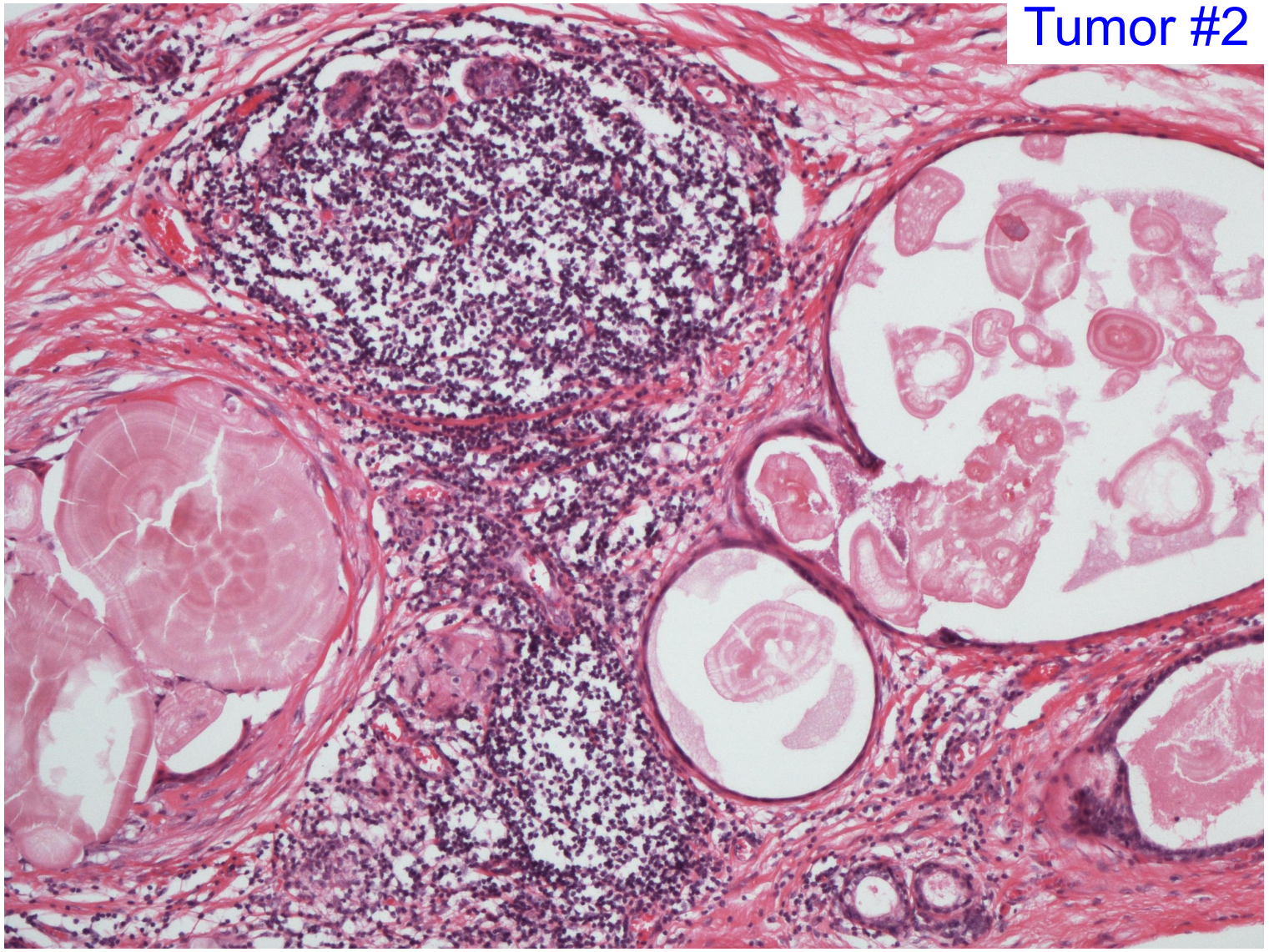
Tumor #2



Tumor #2



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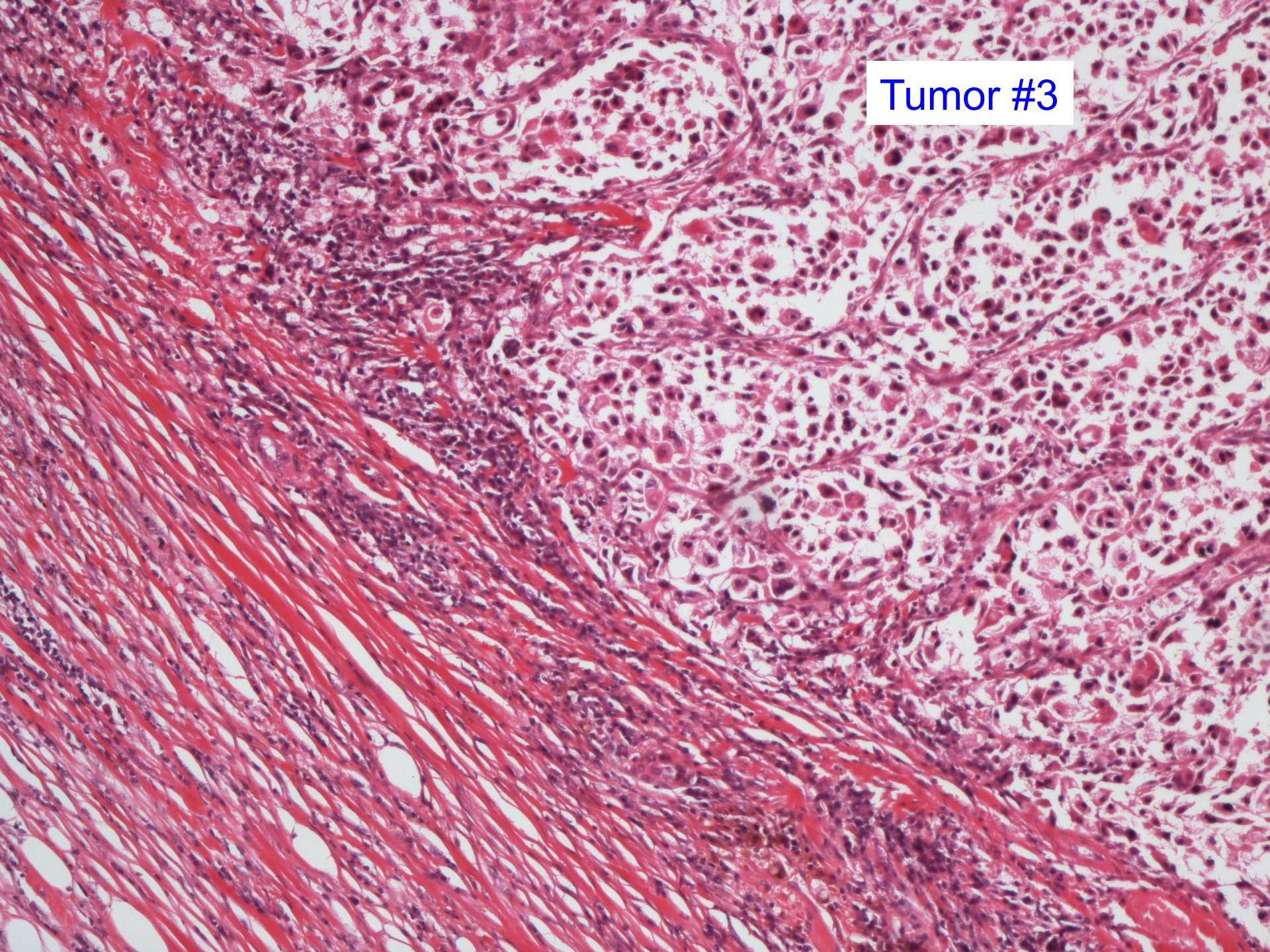

Tumor #3

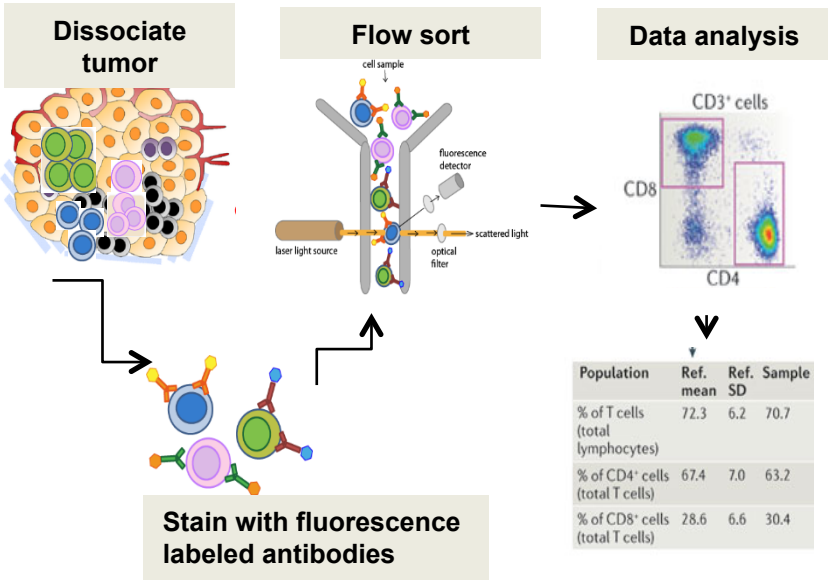
Immune response in tissue

- Varies between tumors
- Varies within tumors
- Morphology alone cannot reveal type of lymphocytes

Methodology for immuno phenotyping of solid tumors

- Flow cytometry (needs dissociation, “bulk” tumor)
- Cell morphology (microscopy)
- IHC, selected markers (microscopy)
- Phenotype by molecular analyses, gene expression/ protein signatures (“bulk” tumor)

- *NB: tissue preservation is of major importance*



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Bias in sampeling

- "Fresh" tissue piece for research is selected prior to microscopic examination
- Methodology using FFPE tissue will secure selection of representative part of tumor by dissection

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Assessment by morphology

reviews

Annals of Oncology 26: 259–271, 2015
doi:10.1093/annonc/mdu450
Published online 11 September 2014

The evaluation of tumor-infiltrating lymphocytes (TILs) in breast cancer: recommendations by an International TILs Working Group 2014

R. Salgado[1,2,†], C. Denkert[3,†], S. Demaria[4,†], N. Sirtaine[5], F. Klauschen[3], G. Pruneri[6], S. Wienert[3], G. Van den Eynden[7], F. L. Baehner[8,9], F. Penault-Llorca[10], E. A. Perez[11], E. A. Thompson[12], W. F. Symmans[13], A. L. Richardson[14,15], J. Brock[15,16], C. Criscitiello[17], H. Bailey[8], M. Ignatiadis[18], G. Floris[19], J. Sparano[20], Z. Kos[21], T. Nielsen[22], D. L. Rimm[23], K. H. Allison[24], J. S. Reis-Filho[25], S. Loibl[26], C. Sotiriou[18], G. Viale[27], S. Badve[28], S. Adams[4,†], K. Willard-Gallo[29,†] & S. Loi[30*,†]

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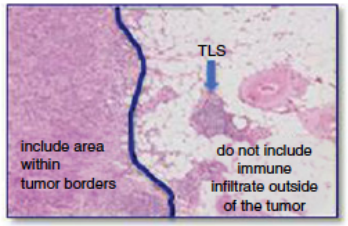
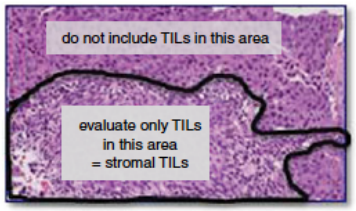
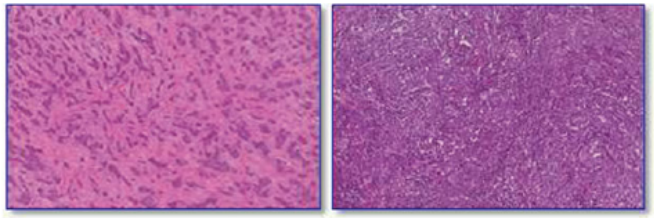
Step 1: Select tumor area



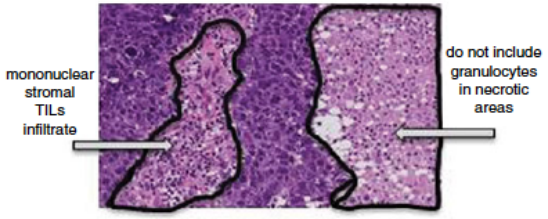
Step 2: Define stromal area



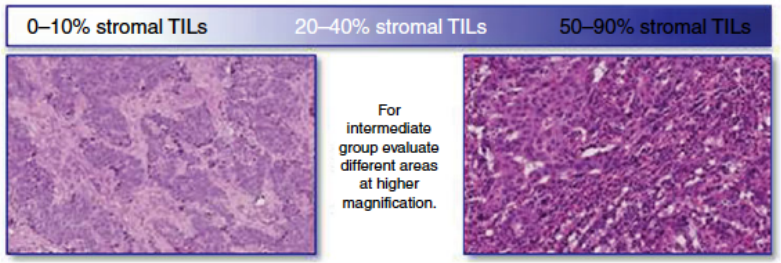
Step 3: Scan at low magnification



Step 4: Determine type of inflammatory infiltrate



Step 5: Assess the percentage of stromal TILs
(examples of percentages shown in figure 4)



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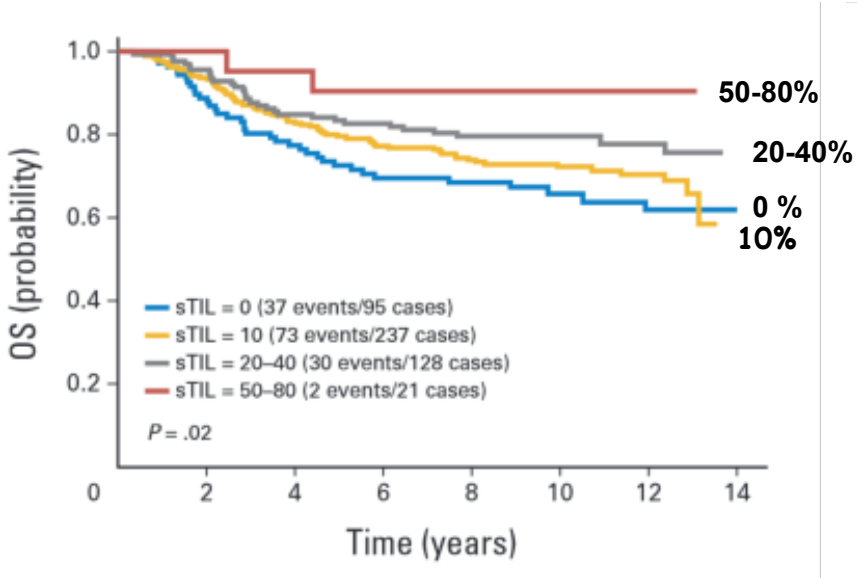
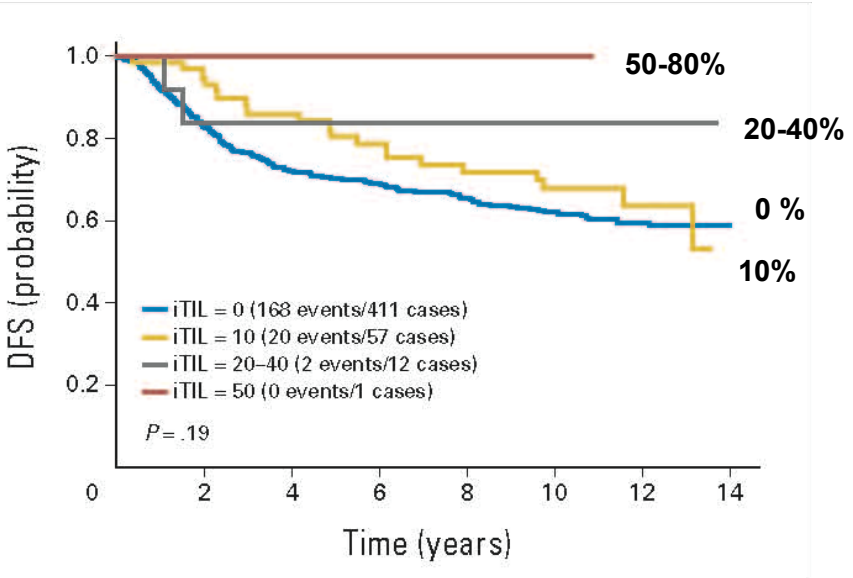
Prognostic information in Triple Negative Breast Tumors

Stromal lymphocytes



Intratumor lymphocytes



n=481, triple negative breast tumors, adjuvant treated with doxorubicin + docetaxel/ cyclophosphamid

Adams et al, JCO 2014

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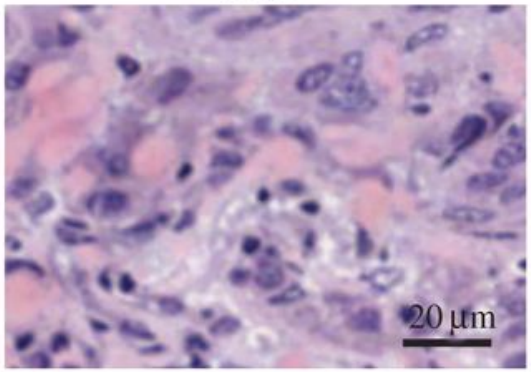
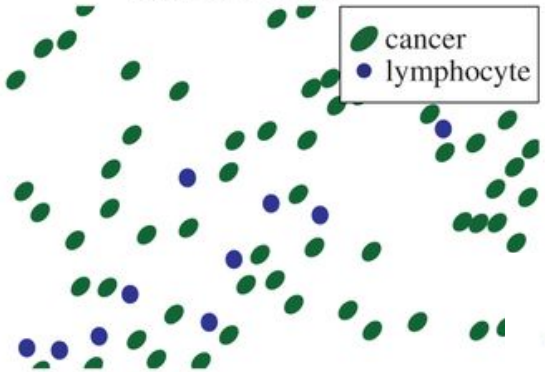
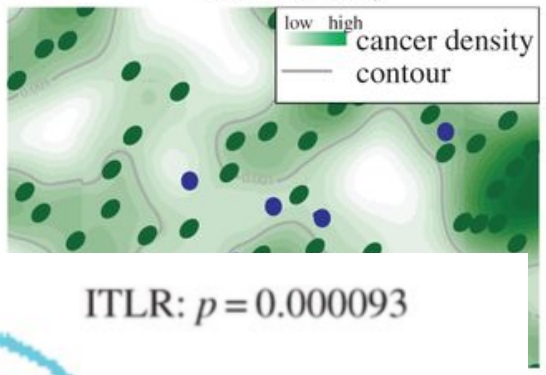
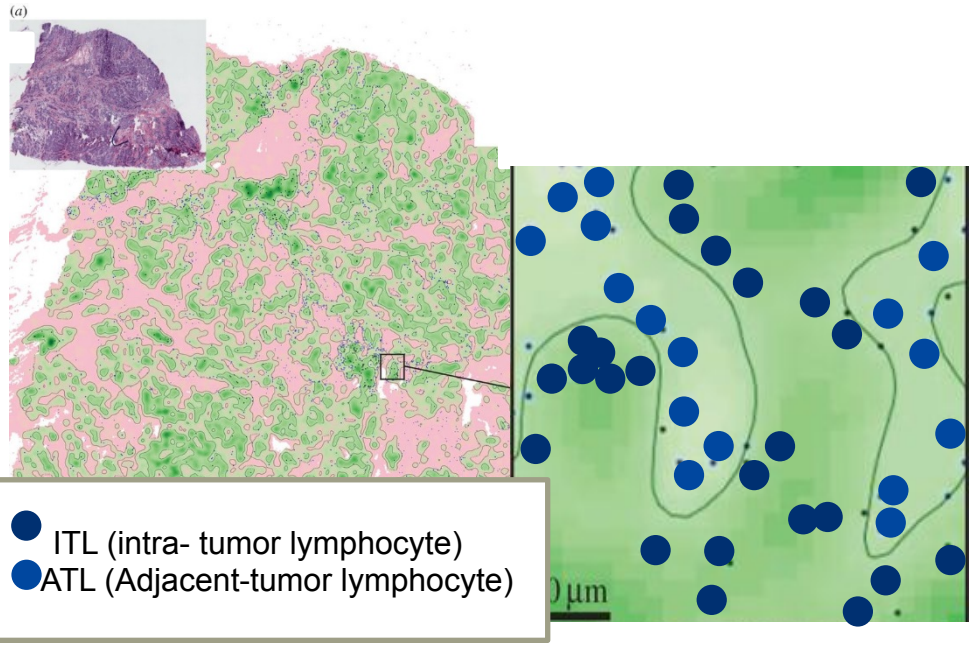
Digital imaging of tissue sections

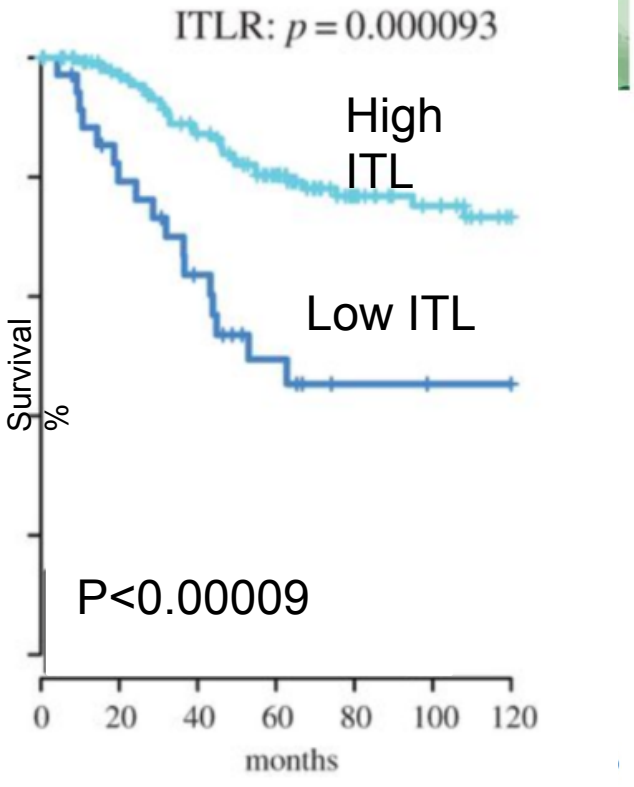








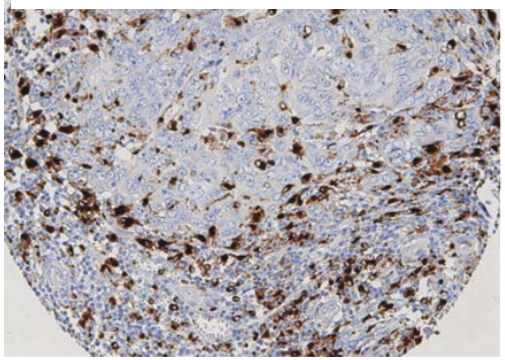
ITL (intra- tumor lymphocyte)
ATL (Adjacent-tumor lymphocyte)



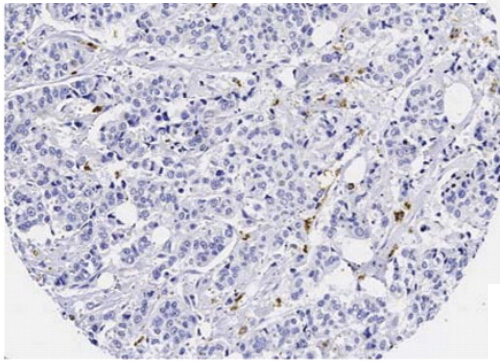
Yinyin Yuan, 2014

Assesment by immunohistochemistry

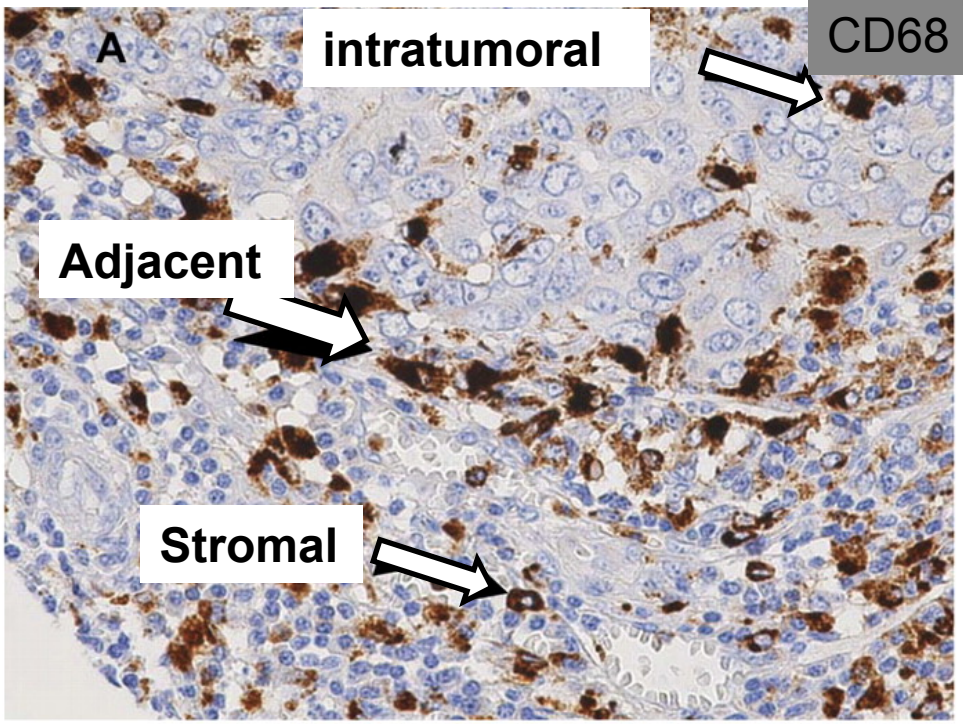
Dense macrophage infiltration



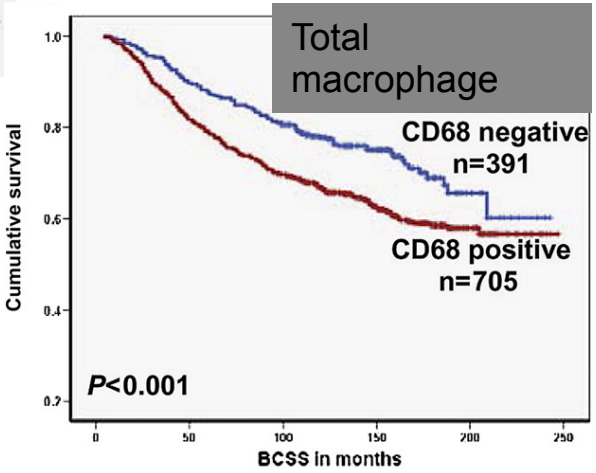
Sparse macrophage infiltration



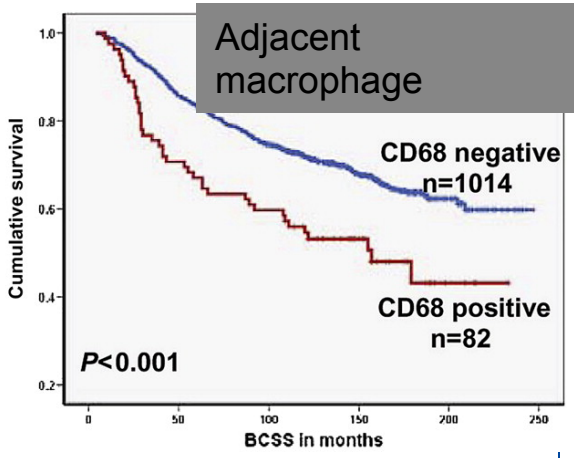
Macrophages CD68



Total macrophage

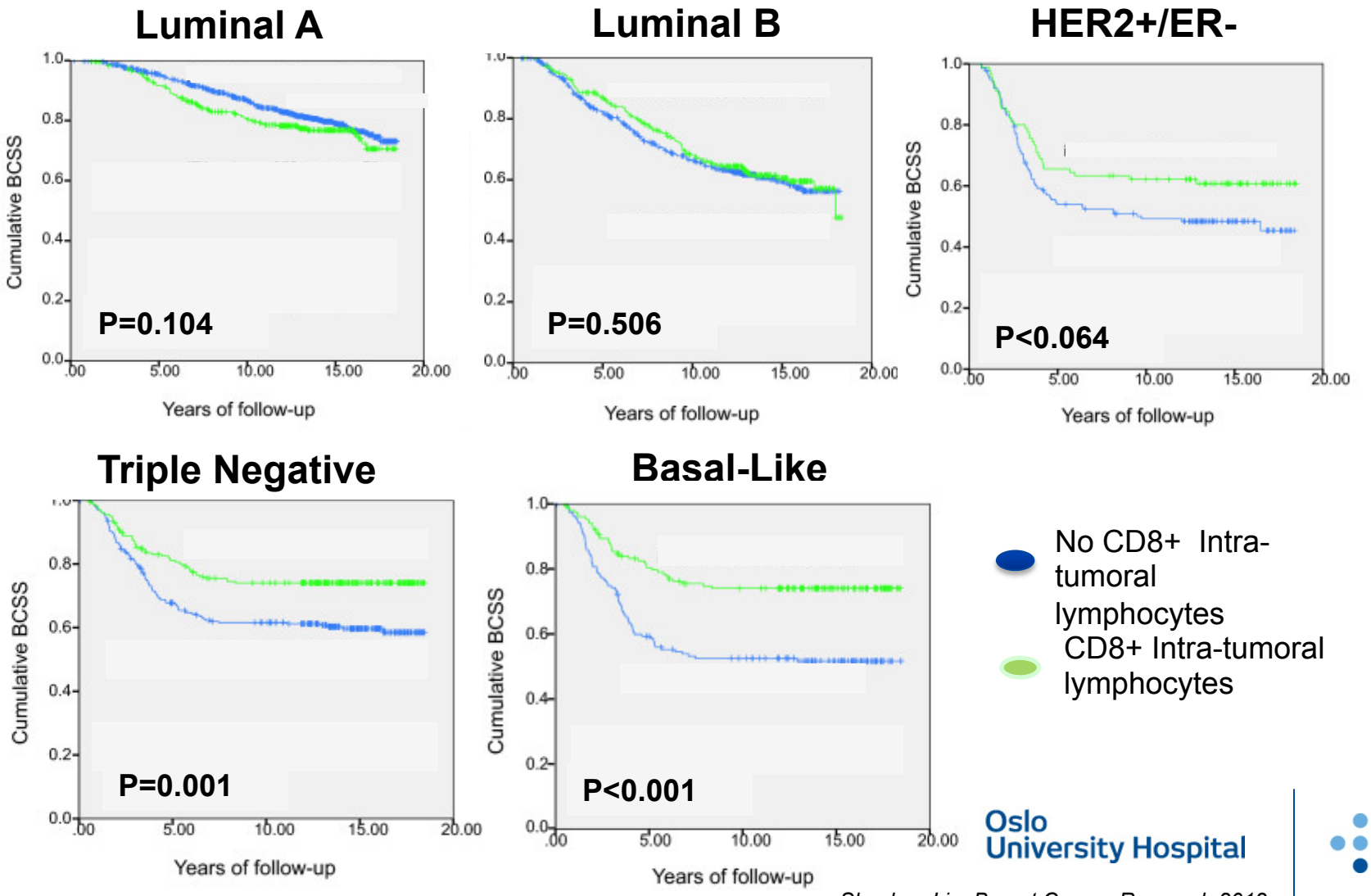


Adjacent macrophage



Mahmoud et al. J Clin Pathol 2012;65:159-163

Cytotoxic T cell (CD8+) infiltration in BC



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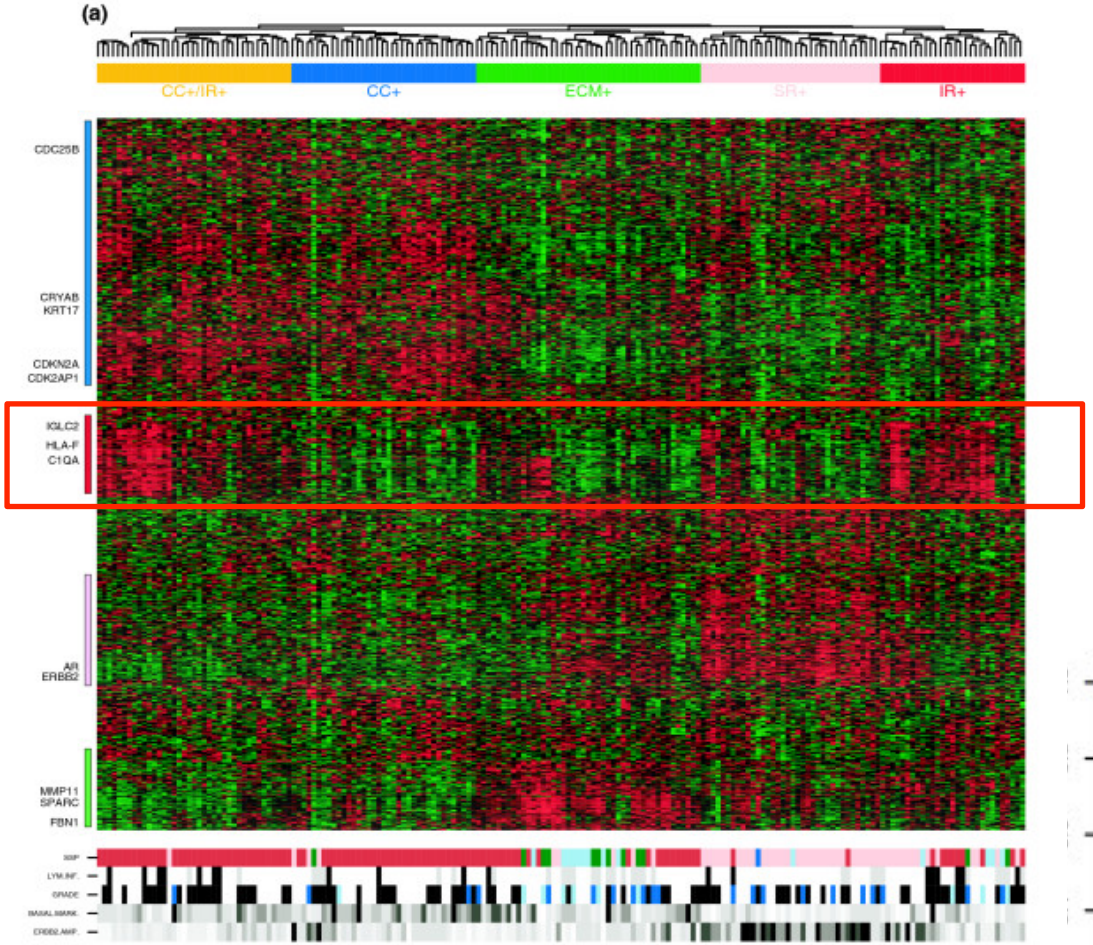
Shuzhen Liu, Breast Cancer Research 2012

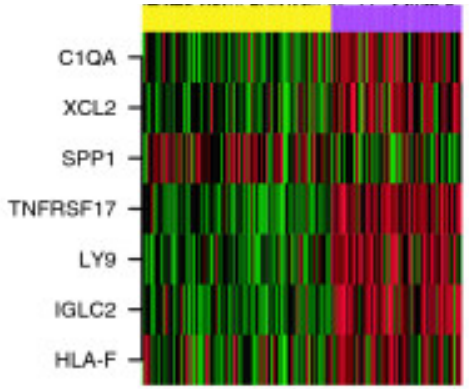
Assessment by gene expression

Immune response in ER negative tumors

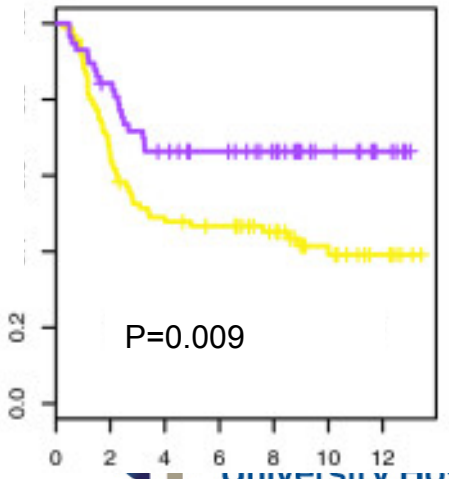


n=187

Immune Response



Time to distant metastasis

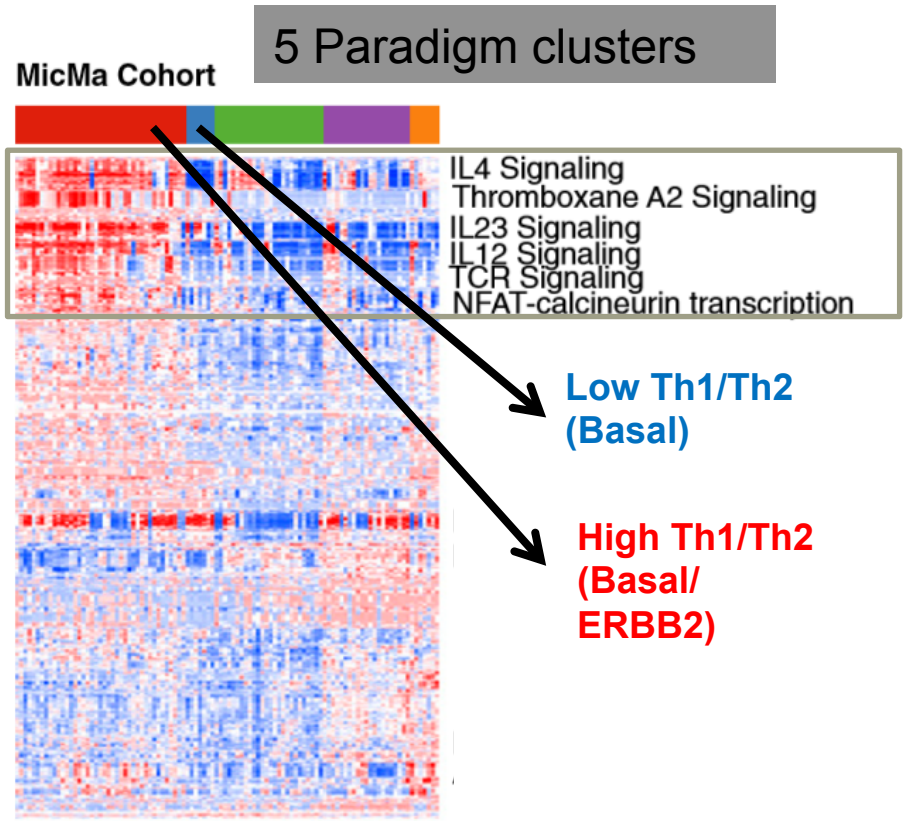


"High response signature"

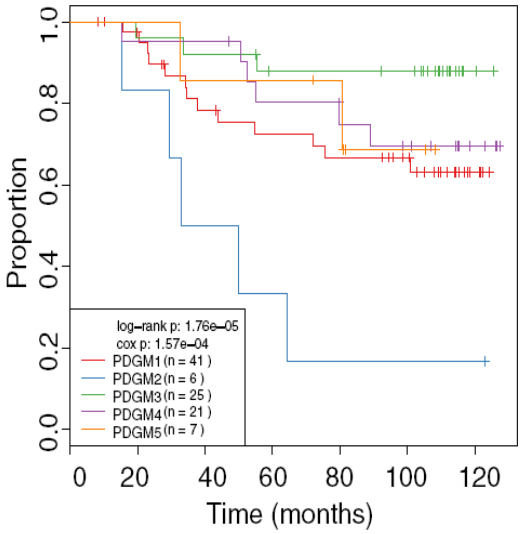
"Low response signature"

Teschendorff et al, 2007

Assessment by gene expression and CNA

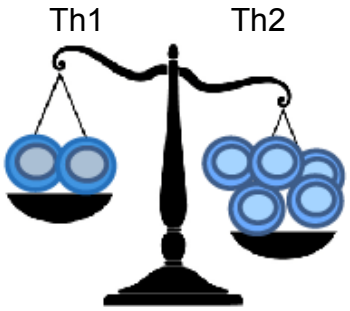


5 Paradigm clusters



High Th1/Th2 (Basal/ ERBB2)

Low Th1/Th2 (Basal)



Worse prognosis

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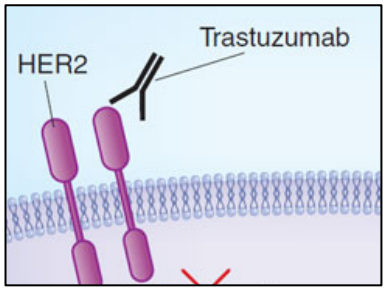
Kristensen VN et al, PNAS 2012;109

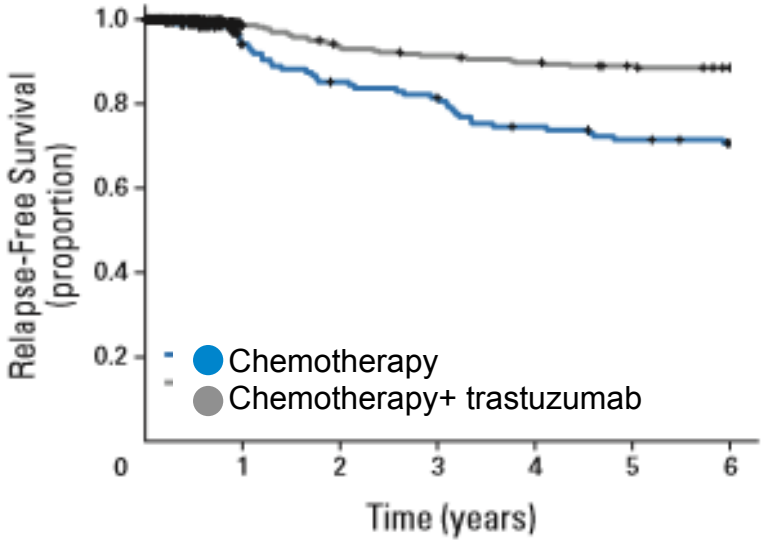
Predictive power

- Need to know tumor subtype and type of immune response
- Prediction of effect of “existing” adjuvant/neo-adjuvant therapy?
- Prediction of effect of immune modulating therapy/immune checkpoint inhibitors?
  - Specific markers?
  - Mutation burden?
  - Gene expression signatures?
- Vaccines?

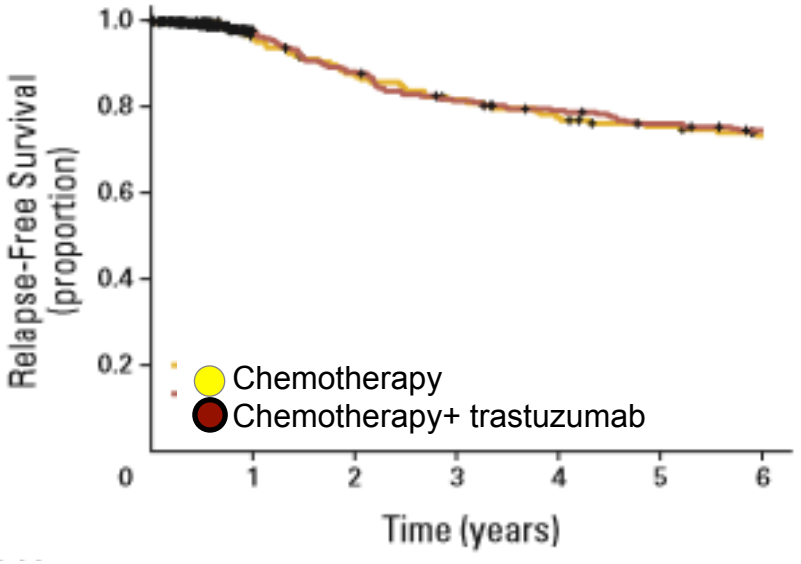


Immune cells in HER2+ tumors predicts response to trastuzumab

Immune enriched tumors



Non-enriched tumors



n=1282, early stage HER2+ breast cancer patients

Perez et al, 2015

Oslo University Hospital

Predictive power, adjuvant treated BC

Table 3 | **Adjuvant trials in which TILs have been assessed**

Trial analysed	Trial type	Treatment	TILs assessment	Population	*n*	Recurrence end points
BIG 2-98 (REF. 18)	Adjuvant Prospective RCT	Doxorubicin Cyclophosphamide CMF Docetaxel	Stromal on H&E	$ER^+/HER2^-$	1,079	Not significant
				$HER2^+$	297	Not significant
				TNBC	256	For each 10% increment of sTILs: DFS, HR = 0.84 (95% CI 0.74–0.98, *P* = 0.025)
FinHER[38]	Adjuvant Prospective RCT	Docetaxel Vinorelbine FEC Trastuzumab	Stromal on H&E	$ER^+/HER2^-$	591	Not significant
				$HER2^+$	209	Not significant
				TNBC	134	For each 10% increment of sTILs: DDFS, HR = 0.79 (95% CI 0.64–0.98, *P* = 0.032)
E2197 and E1199 (REF. 39)	Adjuvant Prospective RCT	Doxorubicin Cyclophosphamide Docetaxel	Stromal on H&E	TNBC	481	For each 10% increment of sTILs: DFS, HR = 0.84 (95% CI 0.74–0.95, *P* = 0.005)
SEARCH, BCCA, NBCS, NEAT[19]	Prospective Observational RCT (NEAT)	Various, not standardised No trastuzumab	IHC for CD8 in stroma (sCD8) IHC for CD8 in tumour (iCD8)	ER^+ (including $HER2^+$)	8,775	Presence versus absence of iCD8: Breast cancer-specific survival, HR = 0.95 (95% CI 0.85–1.07, *P* = 0.43)
				$ER^-/HER2^+$ TNBC	3,591	Presence versus absence of sCD8: Breast cancer-specific survival, HR = 0.79 (95% CI 0.67–0.93, *P* = 0.004)
NeoALTTO[40]	Neoadjuvant Prospective RCT	Trastuzumab Lapatinib Paclitaxel FEC	Stromal on H&E	$HER2^+$	387	3% decrease in rate of recurrence (event free survival) for every 1% increase in TILs *P* = 0.002

Trials overall include a total of 15,800 patients. BIG, Breast International Group; CMF, cyclophosphamide, methotrexate, 5-fluorouracil; DDFS, distant disease-free survival; DFS, disease-free survival; ER, oestrogen receptor; FEC, 5-fluorouracil, epirubicin, cyclophosphamide; H&E, haematoxylin and eosin; HR, hazard ratio; IHC, immunohistochemistry; PR, progesterone receptor; RCT, randomized controlled trial; sTIL, stromal TIL; TIL, tumour-infiltrating lymphocyte; TNBC, triple-negative breast cancer.

Savas et al., Nature Review Clinical Oncology, April 2016

Oslo University Hospital

Predictive power, neo-adjuvant treated BC

Table 4 | **Neoadjuvant trials that have assessed TILs**

Trial and treatments	Subtype	n	TILs assessment	Outcome	Multivariate analysis
GeparDuo[24] Doxorubicin Docetaxel Cyclophosphamide	All	218	sTILs and iTILs on H&E	>60% sTILs: pCR 41.7% <60% sTILS: pCR 9.3%	OR 1.38 of pCR per 10% iTILs (95% CI 1.08–1.78, *P*=0.012)
GeparTrio[24] Doxorubicin Docetaxel Cyclophosphamide Vinorelbine Capecitabine	All	840	sTILs and iTILs on H&E	>60% sTILs: pCR 40% <60% sTILs: pCR 13.9%	OR 1.21 of pCR per 10% iTILs (95% CI 1.08–1.35, *P*=0.001)
GeparQuattro[46] Epirubicin Cyclophosphamide Docetaxel Capecitabine Trastuzumab	HER2+	156	sTILs on H&E	>50% sTILs: pCR 47.4% <50% sTILs: pCR 31.7%	OR 1.16 of pCR per 10% sTILs (95% CI 1.01–1.32, *P*=0.038)
GeparQuinto[43] Epirubicin Cyclophosphamide Taxane Everolimus	ER+ and TNBC	313	sTILs and iTILs on H&E	>60% sTILs: pCR 36.6% <60% sTILs: pCR 14.3% (*P*<0.001)	OR 1.2 of pCR per 10% sTILs (95% CI 1.0–1.3, *P*=0.01)
GeparSixto[31] Paclitaxel Liposomal Doxorubicin Carboplatin Bevacizumab Trastuzumab	HER2+ and TNBC	580	sTILs and iTILs on H&E	>60% sTILs: pCR 59.9% <60% sTILs: pCR 33.8% (*P*<0.001) Significant test for interaction between increased TILs and response to carboplatin therapy	OR 1.2 of pCR per 10% sTILs (95% CI 1.11–1.29, *P*<0.001) OR 2.66 of pCR for >60% versus <60% sTILs (95% CI 1.76–4.02, *P*<0.001)
EORTC 10994 and BIG 00-01 (REF. 44) FEC Docetaxel	ER-	111	gTILs	High gTILs: pCR 74.2% Low gTILs: pCR 31.3%	OR 6.42 of pCR for high versus low gTILs (95% CI 2.08–19.83, *P*=0.001)
CHER-LOB[50] Trastuzumab Paclitaxel FEC	HER2+	105	sTILs and iTILs on H&E	>60% sTILs: pCR 59% <60% sTILs: pCR 27% (*P*<0.015)	Not reported

Trials overall include a total of 2,323 patients. ER, oestrogen receptor; FEC, 5-fluorouracil, epirubicin, cyclophosphamide; gTIL, gene-expression surrogate TIL; H&E, haematoxylin and eosin; iTIL, intratumoural TIL; OR, odds ratio; pCR, pathological complete response; sTIL, stromal TIL; TIL, tumour-infiltrating lymphocyte.

Can pre-surgery treatment mobilize an anti-tumor immune response even early stage BC patients could benefit from?

Savas et al., Nature Review Clinical Oncology, April 2016

Oslo University Hospital

Conclusion

- Immunological response differ between breast cancer subtypes
- Immunological response is influenced by some types of standard treatment

- Clinical trials must assess both the molecular fingerprint of cancer cells as well as the immunological!
- Tissue selection and methodology is essential

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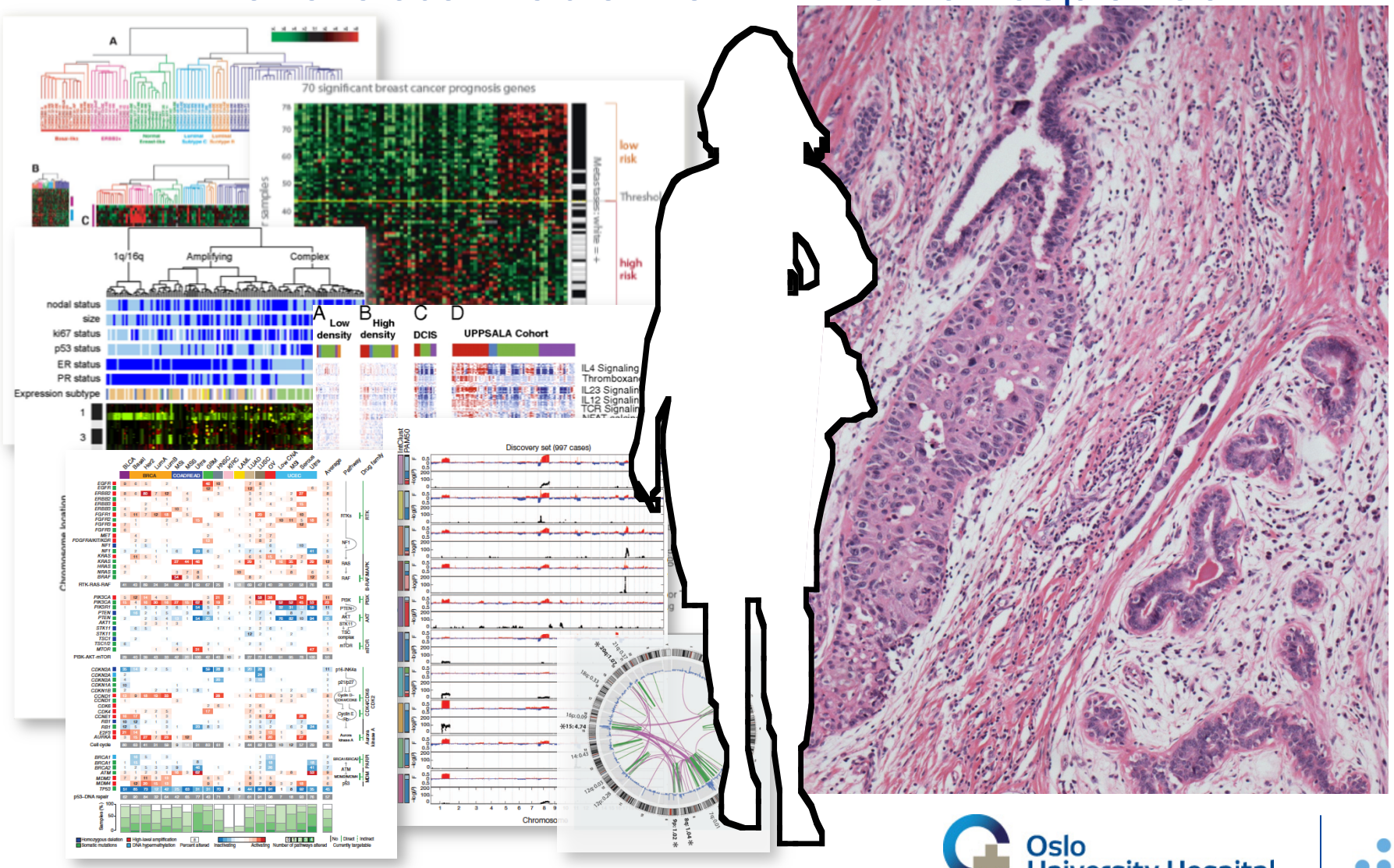
Integrating tumor derived knowledge with characterization of immune response



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