Tumor infiltrating lymphocytes

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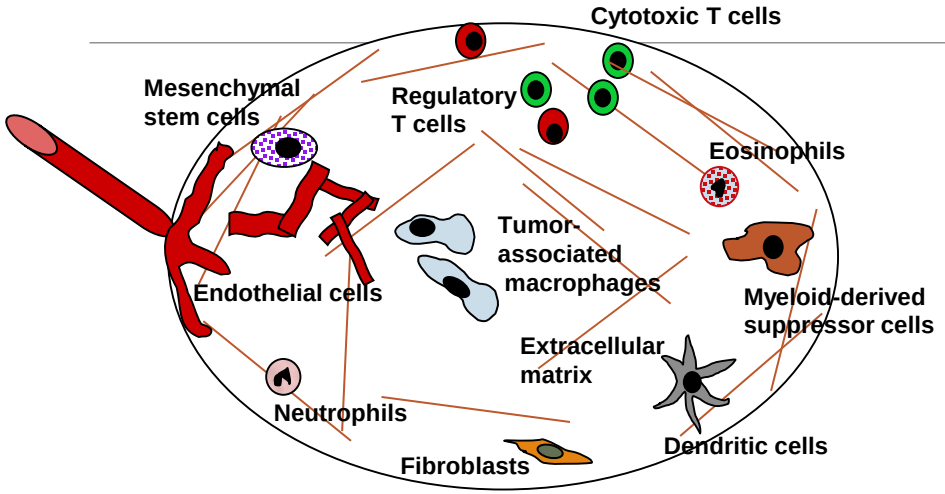
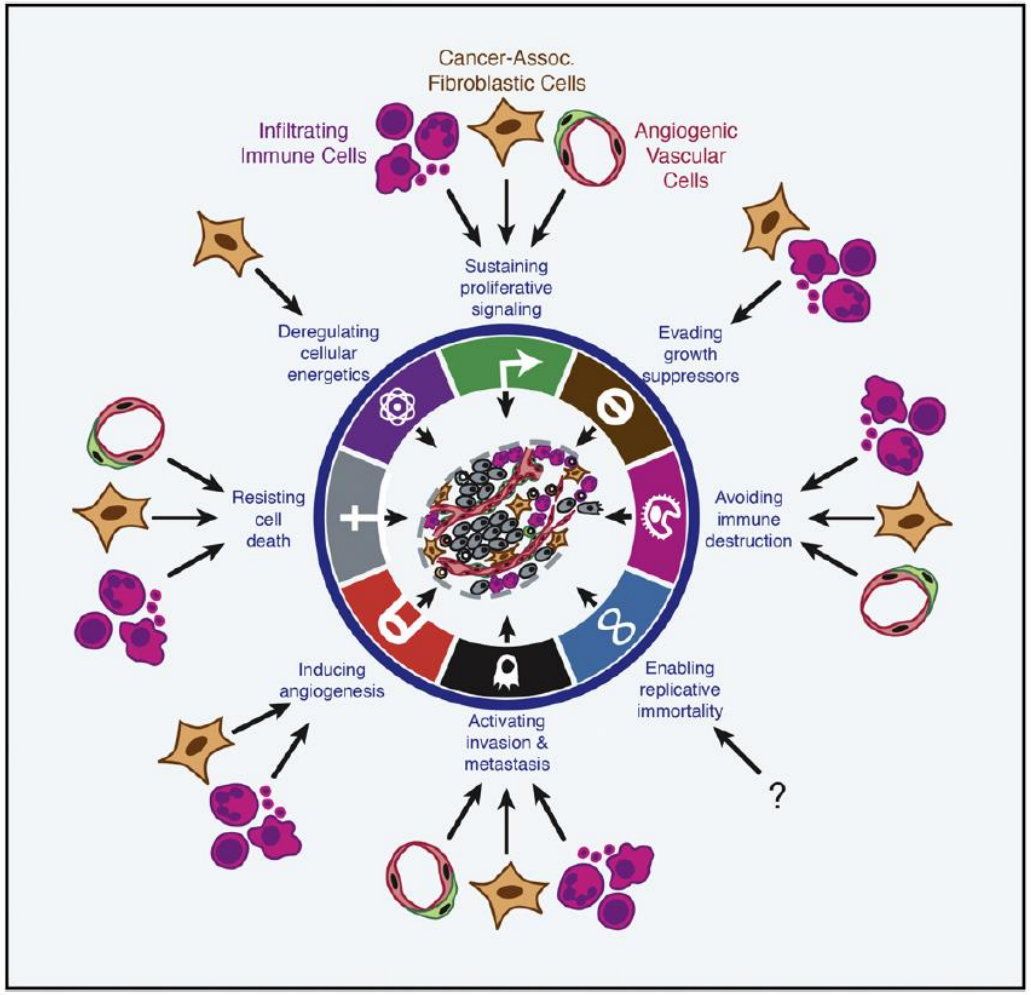
Current focus of pathologic evaluation is cancer cell centric



Tumor progression
Invasion & Metastasis
Immunosuppression

Tumor inhibition
Anti-tumor immune response



Stroma and infiltrating immune cells can also impact prognosis

Hanahan D and Coussens L. Cancer Cell 2012

Lymphocyte Infiltrates as a Prognostic Variable in Female Breast Cancer

S. Aaltomaa, P. Lipponen, M. Eskelinen, V.-M. Kosma, S. Marin, E. Alhava and K. Syrjänen

Eur J Cancer. 1992

498 patients, H&E sections at baseline

Lymphocytic infiltrate ~ correlation with high grade, high proliferation tumors

- Did not differentiate between intra-epithelial (iTILs) and stromal (sTILs)
- Hormone receptor expression not tested

Lymphocytic infiltrate ~ correlation with outcome?

- Not in entire cohort
- Not in tumors with low proliferation
- **Significant predictor of RFS and BS in highly proliferating tumors (M/V index > 15/mm2)**

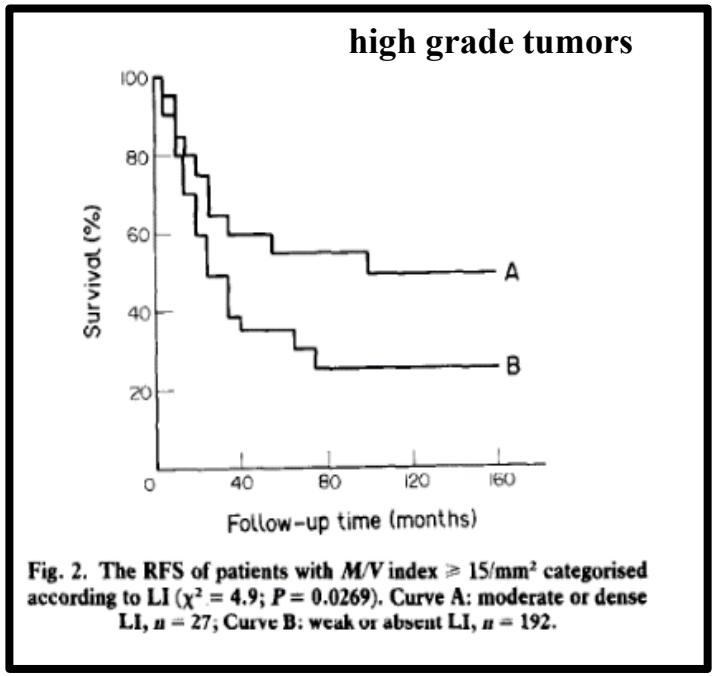
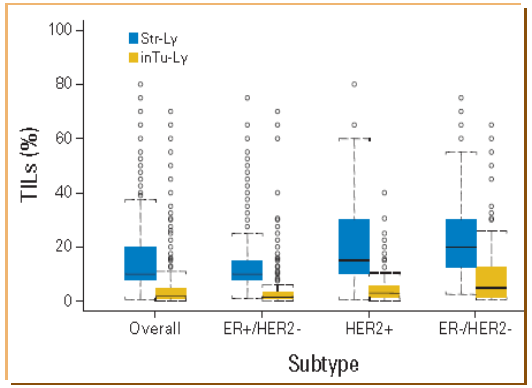
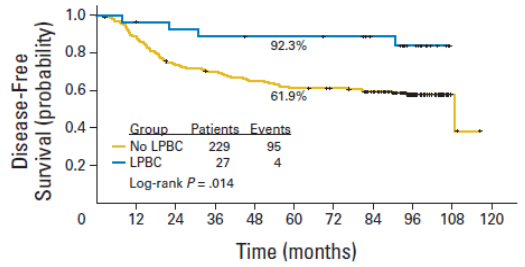


Fig. 2. The RFS of patients with *M/V* index ≥ 15/mm² categorised according to LI ($\chi^2 = 4.9$; $P = 0.0269$). Curve A: moderate or dense LI, $n = 27$; Curve B: weak or absent LI, $n = 192$.

TIL are prognostic in TNBC treated with adjuvant chemotherapy in BIG 02-98

- Randomized Phase III, 2009 patients (256 TNBC), all LN+, A→CMF vs AC→CMF
- H&E TILs on full sections
- Highest TIL counts in TNBC and HER2+BC
- Correlation of TIL with outcome only in TNBC, not in overall population or ER+ BC
- Continuous: Reduction of risk for recurrence and death was seen for every 10% increment in stromal/intratumoral TIL
- Binary: Tumors with >/=50% TIL (LPBC) best outcome

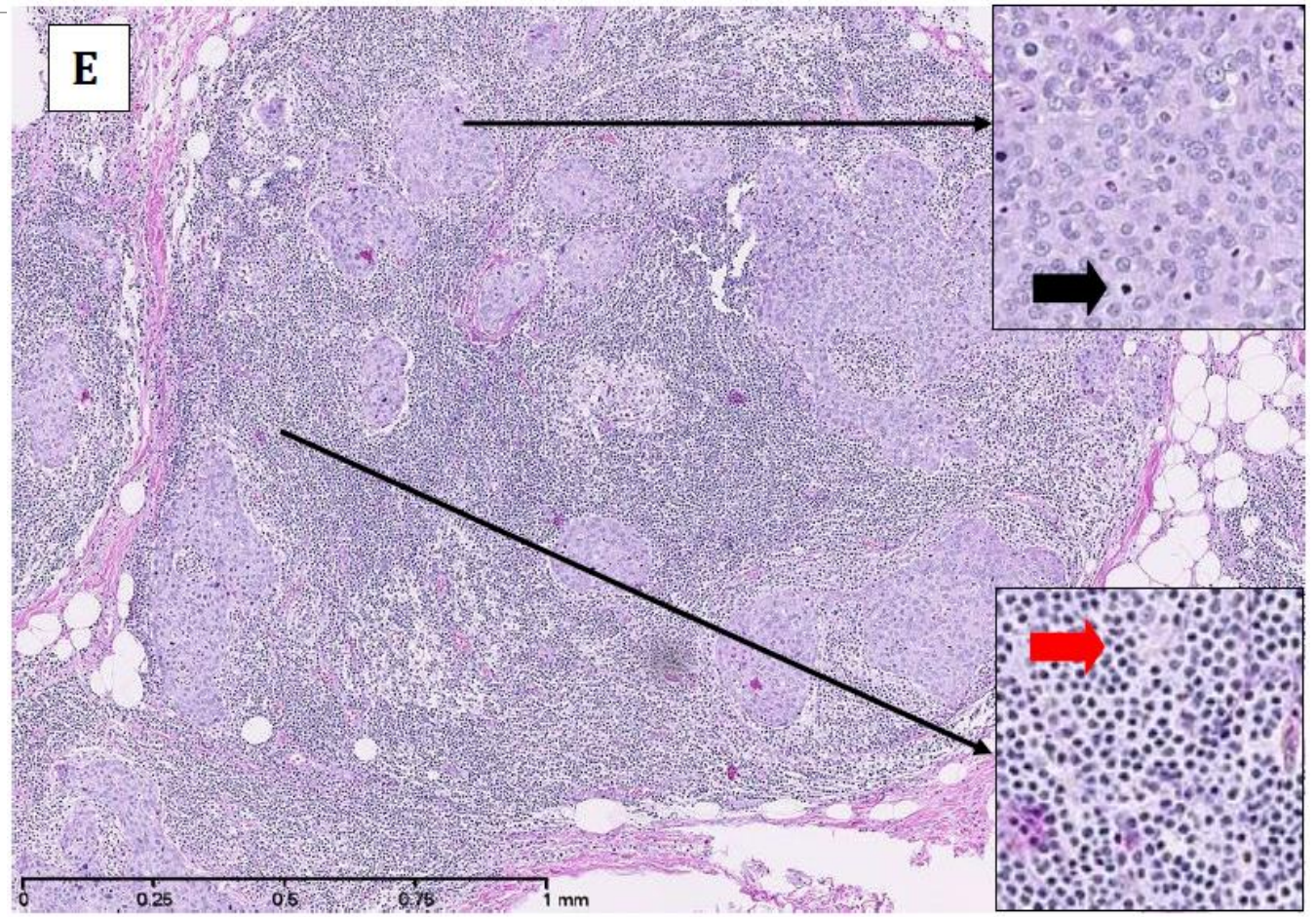




Loi et al, JCO 2013

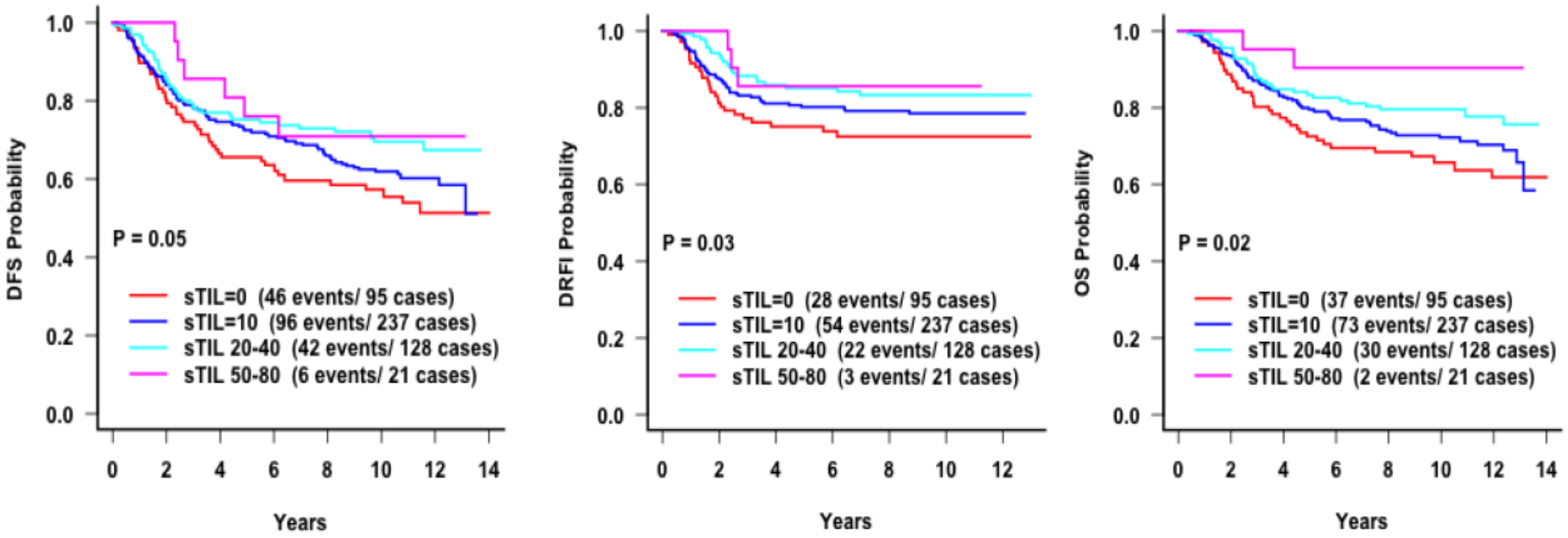
ECOG 1199-2197 Study: Histopathologic analysis

- Full H&E stained section
- 2 breast cancer pathologists by consensus, grading in deciles
- * analytic validity data TBD
- Intraepithelial TIL (iTIL) in direct contact with tumor cells (black arrow)
- Stromal TIL (sTIL) % of tumor stroma containing lymphocytes not in direct contact with tumor cells (red arrow)
- “Lymphocyte-predominant breast cancer” (LPBC): >/= 50 iTIL or sTIL (arbitrary cut-off)



Prognostic value of stromal TIL in TNBC

DFS **DRFI** **OS**



Grouped as 0 vs. 10 vs. 20-40 vs. 50-80; p-values are for comparison of the 4 groups

ECOG : Summary

Study	Loi et al	Adams et al
Randomized Ph III trial	BIG 02-98	E2197, E1199
TNBC cases	256	481
Median follow-up	8 years	10.6 years
Methods	REMARK	REMARK
	H&E full section	H&E full section
	2 pathologists independently	2 pathologists jointly
	Analyzed in 10% increments + binary	Analyzed in 10% increments + binary
Median %	20 sTIL, 5 iTIL	10 sTIL, 0 iTIL
LPBC	10.6%	4.4%
	HR 0.31 (p=0.02, DFS)	HR 0.58 (p=0.18, DFS)
Intraepi TIL, 10% increase	HR 0.83 (p=0.1, DFS)	HR 0.72 (p=0.06)
	HR 0.73 (p=0.03, OS)	HR 0.64 (p=0.08)
Stromal TIL, 10% increase	HR 0.84 (p=0.02, DFS)	HR 0.86 (p=0.02, DFS)
	HR 0.82 (p=0.02, OS)	HR 0.81 (p=0.01, OS)
	HR 0.85 (p=0.02, DFS multivariate)	HR 0.84 (p=0.005, DFS multivariate)
	HR 0.83 (p=0.02, OS multivariate)	HR 0.79 (p=0.003, OS multivariate)

Pooled individual patient data analysis of tumor infiltrating lymphocytes (TILs) in primary triple negative breast cancer (TNBC) treated with anthracycline-based chemotherapy

Sherene Loi, Damien Drubay, Sylvia Adams, Prudence A Francis, Heikki Joensuu, Maria Vittoria Dieci, Sunil Badve, Sandra Demaria, Robert Gray, Martine J Piccart, Pirkko-Liisa Kellokumpu-Lehtinen, Fabrice Andre, Carsten Denkert, Roberto Salgado, Stefan Michiels.

San Antonio Breast Cancer Symposium, December 8-12, 2015

Clinical Trials Pooled

Study	Original Trial Reference	Number of TNBC pts	Treatment	Definition TNBC
BIG 2-98	Francis *et al*, JNCI 2008	269	A-T- CMF vs A-CMF AT- CMF vs AC-CMF	ER<1%
ECOG 1199	Sparano *et al*, NEJM 2008	291	AC-q3w taxol/docetaxel AC-q1w taxol/docetaxel	ER<10%
ECOG 2197	Goldstein *et al*, JCO 2008	190	AC vs AT	ER<1%
FinHER	Joensuu *et al*, NEJM 2006	134	FEC-V vs FEC-Docetaxel	ER<10%
Gustave Roussy	Arriagada *et al*, Acta Oncologica 2005	107	FEC*6	ER<10%
total		991		

Multivariate Cox Analyses (adjusted)

	iDFS (events 362)	D-DFS (events 308)	OS (events 278)
Stromal TILs (per 10%)	**0.88 (0.79-0.97) p=0.01**	0.87 (0.79-0.97) p=0.01	0.88 (0.79-0.99) p=0.03
InTu TILs (per 10%)	**0.86 (0.72-1.02) p=0.08**	0.85 (0.70-1.03) p=0.09	0.86 (0.71-1.05) p=0.13
Age* (yrs)	**1.01 (1.001-1.02) p=0.04**	1.01 (1.002-1.03) p=0.02	1.02 (1.005-1.03) p=0.006
Tumor size* (cm)	**1.08 (1.03-1.14) p=0.004**	1.08 (1.03-1.15) p=0.003	1.09 (1.03-1.16) p=0.003
Positive nodes			
1-3	**1.70 (1.27-2.33) p<0.001**	2.01 (1.42-2.84) p<0.001	1.95 (1.36-2.81) p<0.001
>3	**3.42 (2.40-4.87) p<0.001**	4.04 (2.72-5.99) p<0.001	4.19 (2.76-6.35) p<0.001

** TILs, tumor size and age treated as continuous variables*

Using Wald tests

Stromal TILs ≥20% in node negative TNBC patients have excellent 5 yr D-DFS estimated by Kaplan-Meier

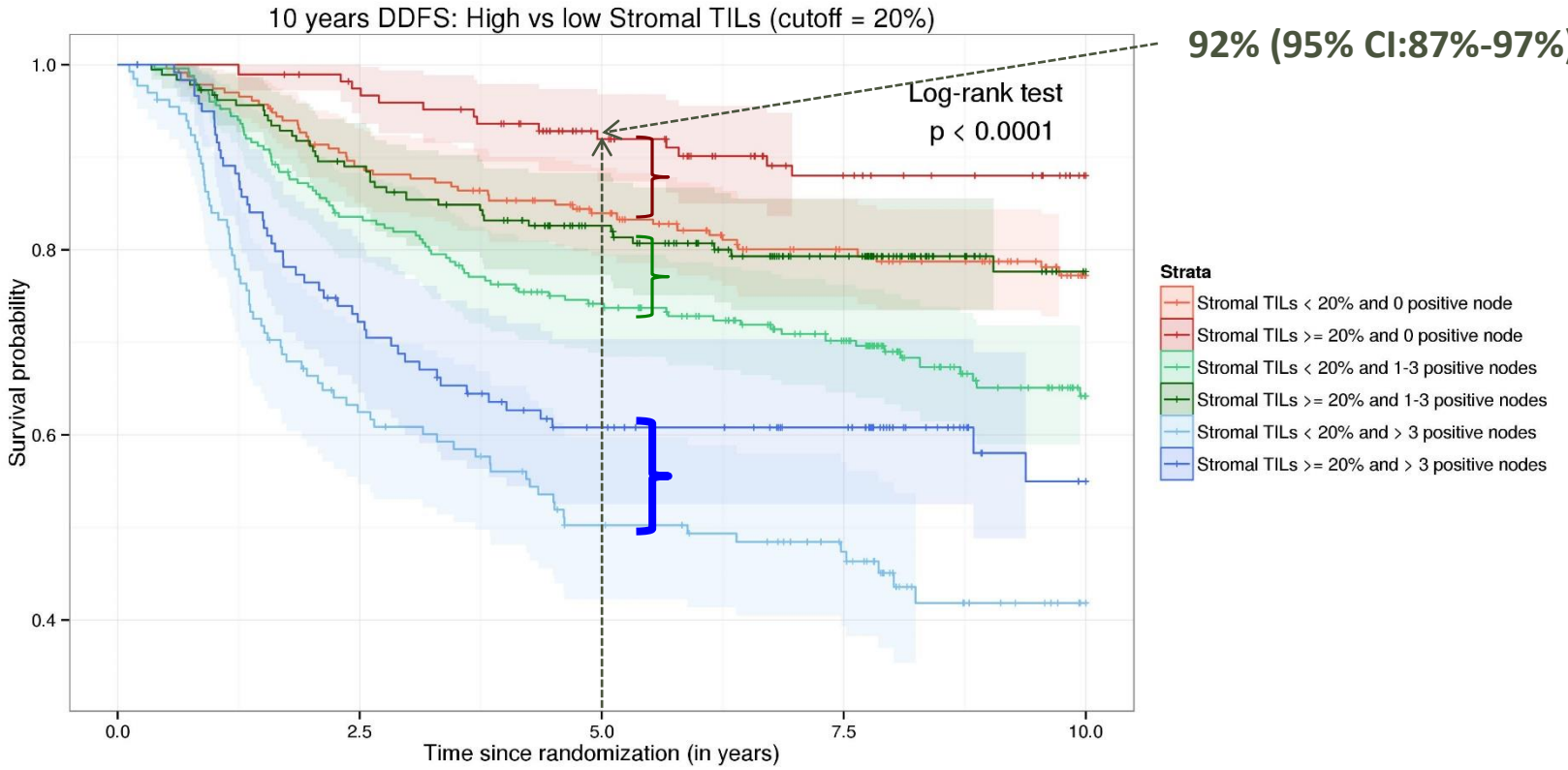


Table 3 | **Adjuvant trials in which TILs have been assessed**

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. Nat Rev Clin Oncol 2015

Method for evaluation of TILs

All the initial studies performed independently

- No agreement on the scoring system

(yet) TILs are clinically significant

Need standardization of methods

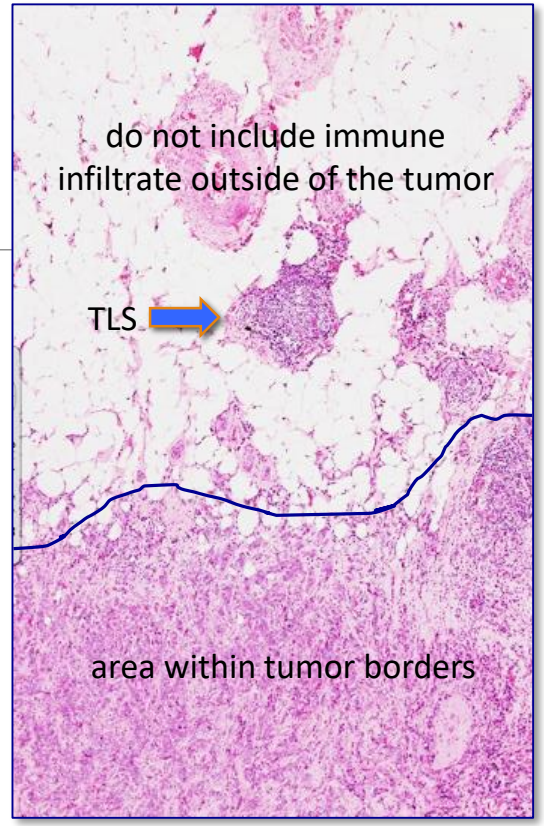
Salgado et al

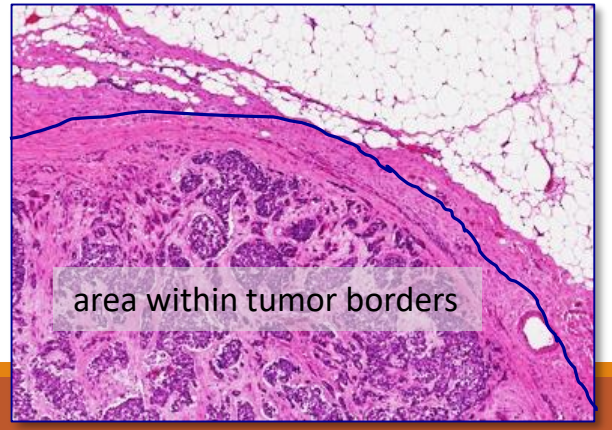
Step 1: Define area for TIL evaluation

Only TILs within the borders of the invasive tumors are evaluated

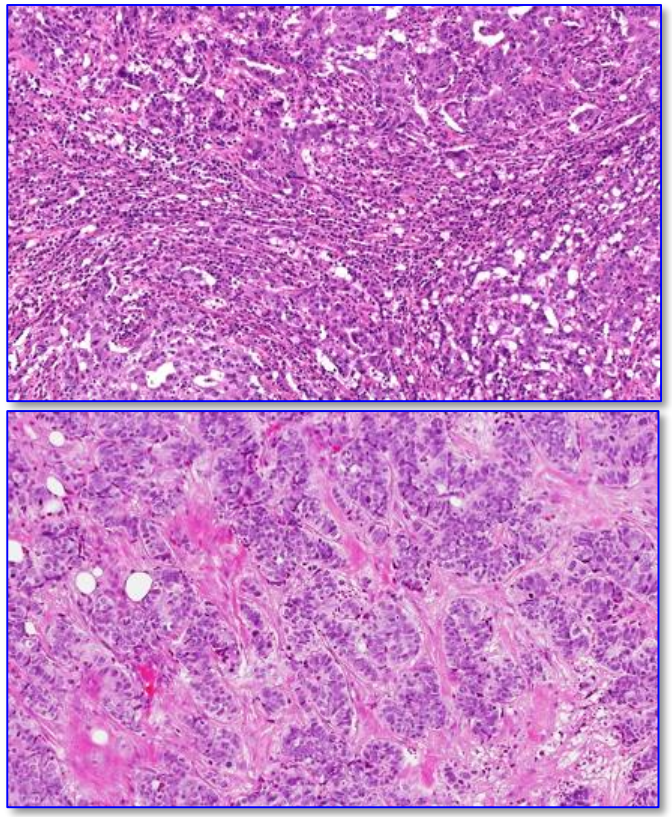
The invasive edge is included in the evaluation, but not reported separately

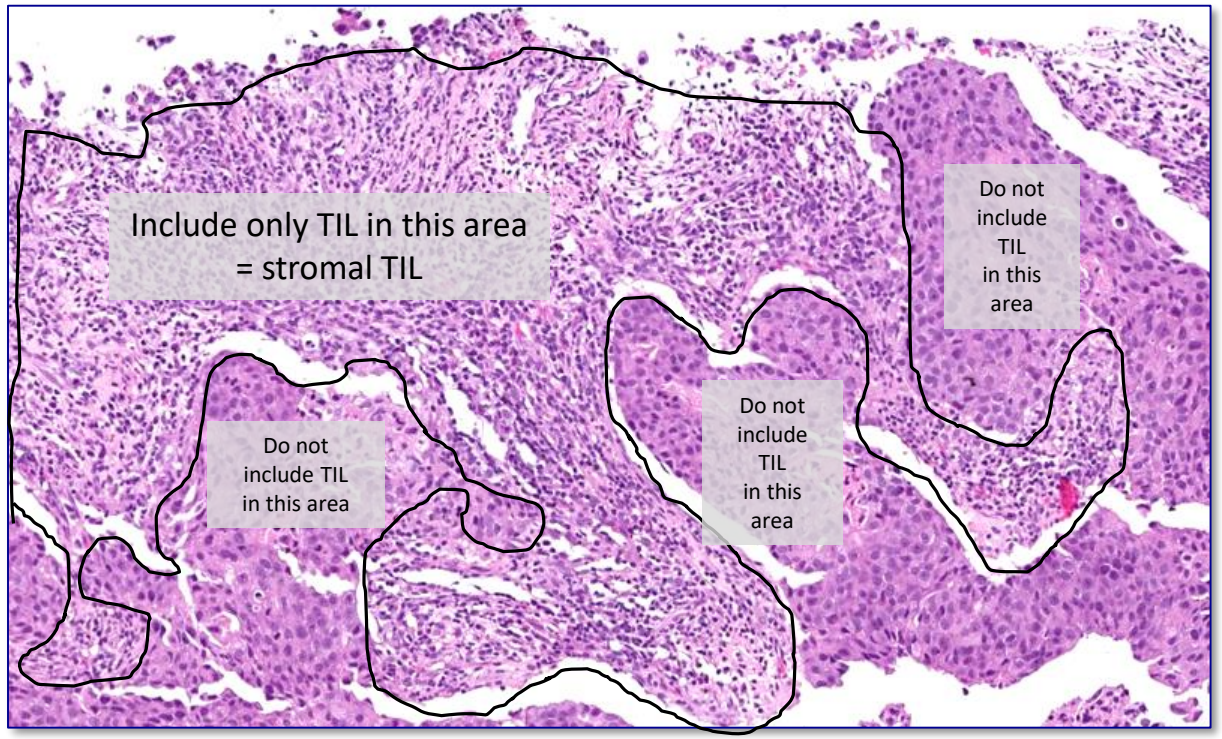
Immune infiltrates outside of the tumor borders, e.g. in adjacent normal tissue or DCIS are not included





Step 2: Scan the slide with focus on stromal TIL

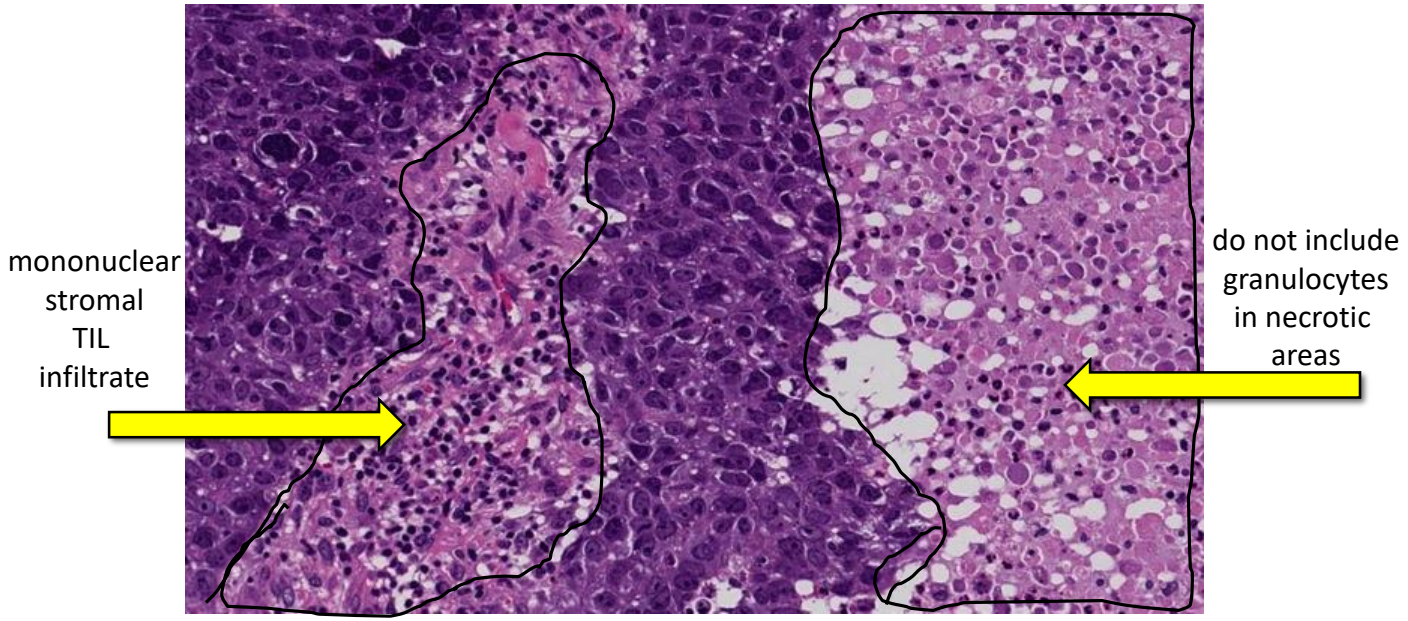




Step 3: Determine type of inflammatory infiltrate

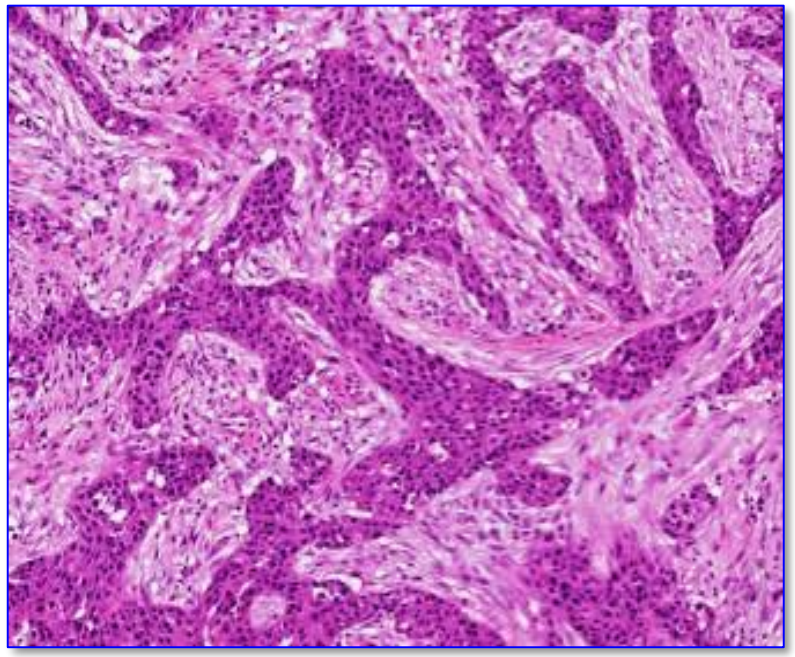
Include only mononuclear infiltrate (lymphocytes & plasma cells)

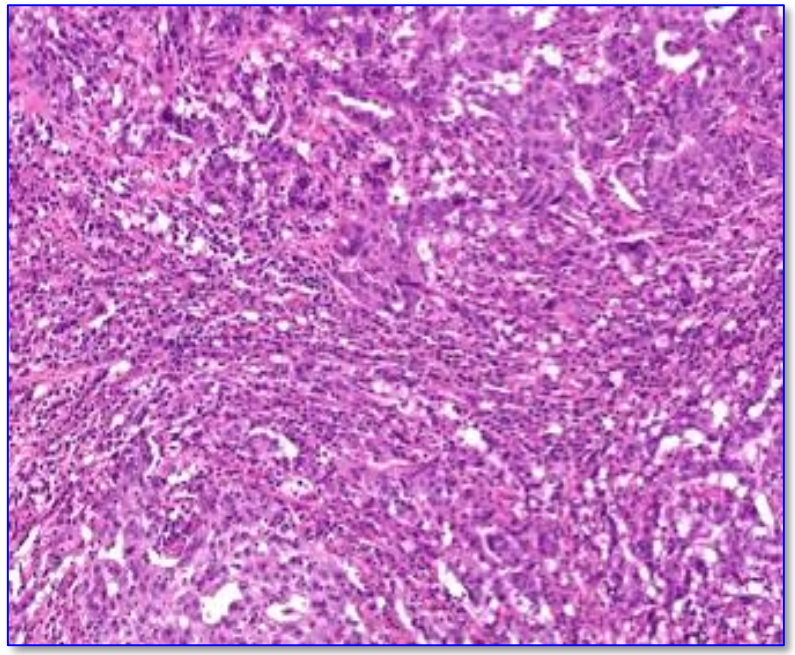
Do not include granulocytic infiltrate in areas of tumor necrosis

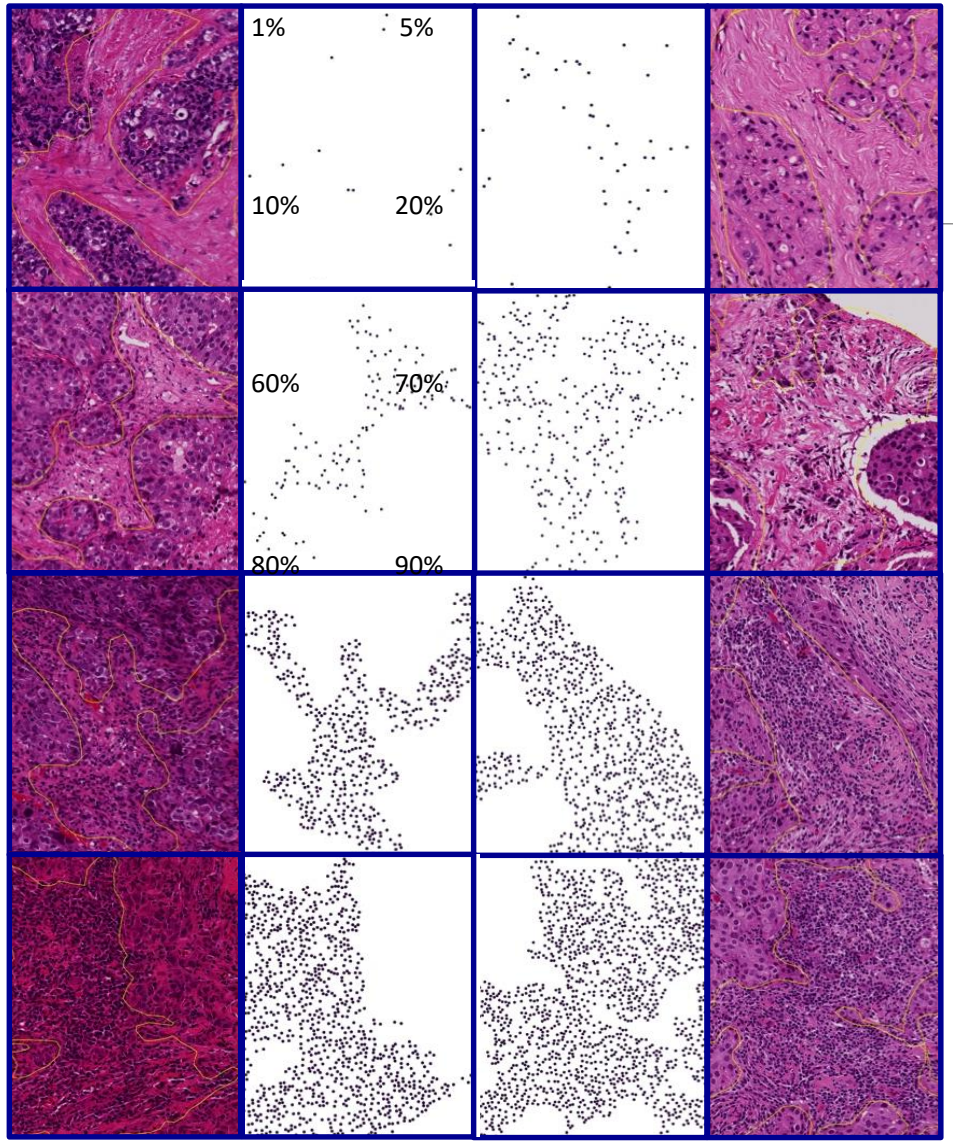


Step 4: As a first approach, include tumor in one of three groups based on low magnification and assess % stromal TILs (continue with Step 5 for percentage)

0-10% stromal TILs | 10-40% stromal TILs | 40-90% stromal TILs







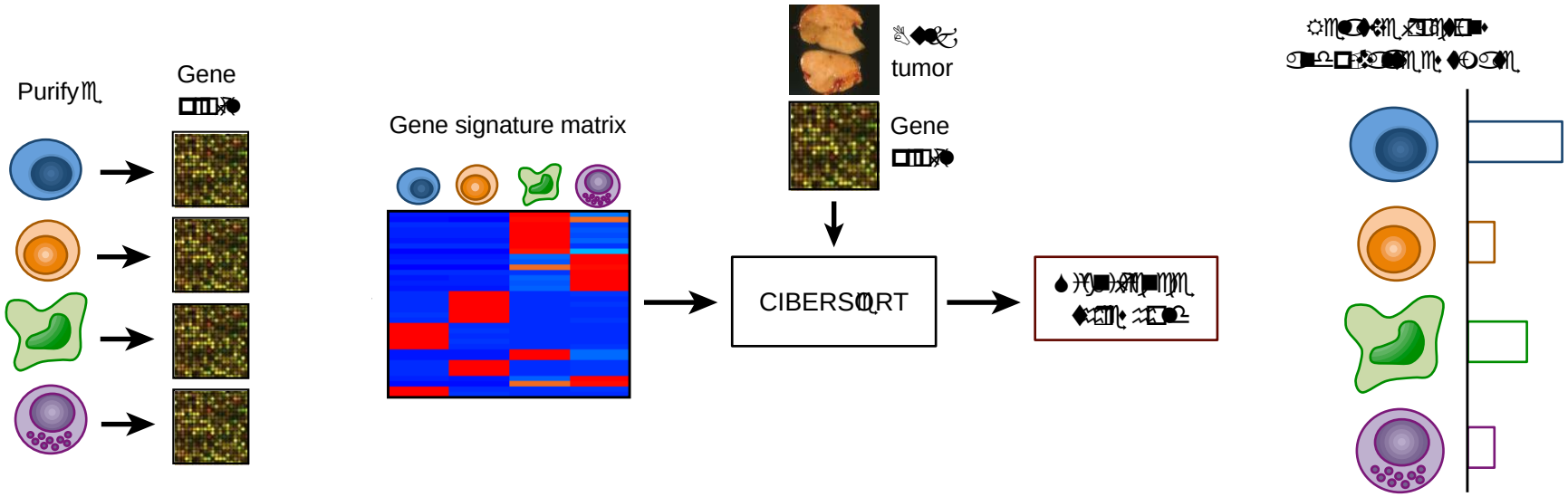
Step 5: Report percentage of stromal lymphocytes

Report the average of the stromal area, do not focus on hot spots.

For intermediate group evaluate different areas at higher magnification.

Please note that lymphocytes to not form solid aggregates, therefore even with 90-100% stromal TILs there will still be some space between the individual lymphocytes.

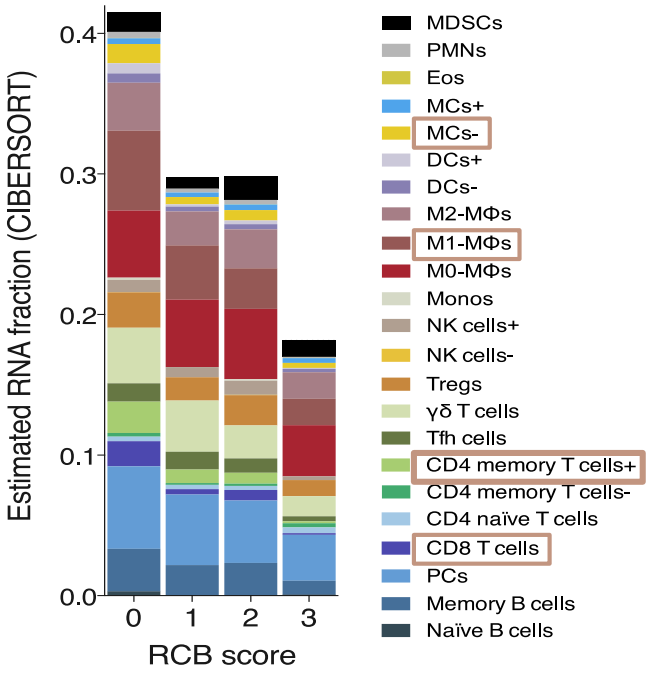
What are TILs?

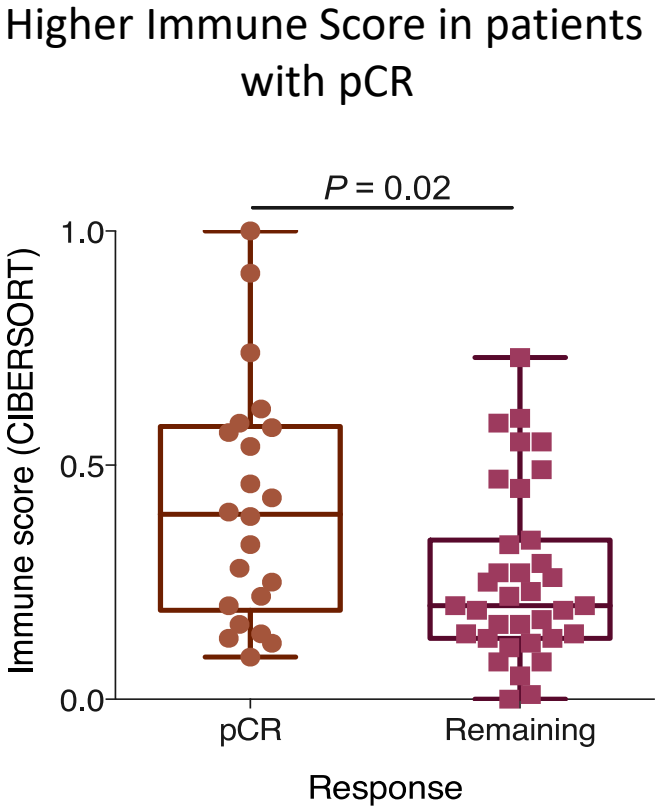


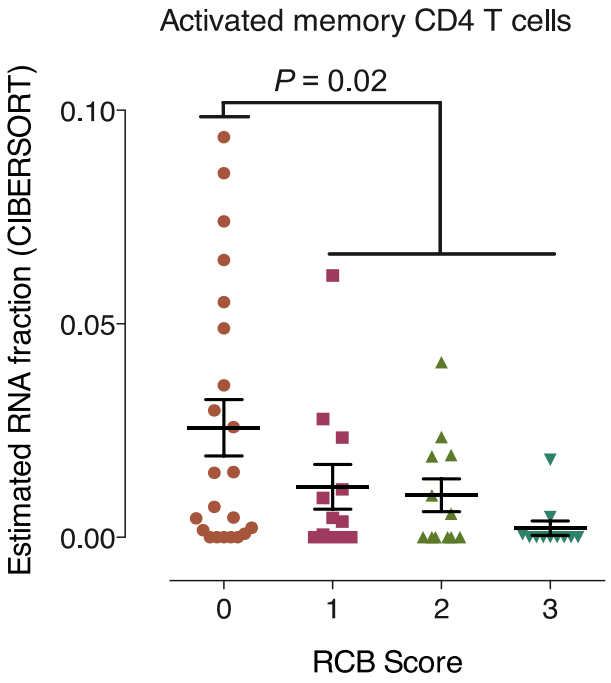
23 purified leukocyte subset signatures used to distinguish cell types

Naïve B Memory B Plasma cells	CD8 T CD4 Naïve T CD4 Memory T inact. CD4 Memory T act. CD4 Follicular helper T CD4 Regulatory T Gamma delta T	NK unstim. NK stim.	DCs unstim. DCs stim.	Mast cells unstim. Mast cells stim. Eosinophils Neutrophils	Monocytes M0 macrophages M1 macrophages M2 macrophages	MDSCs

Total and subsets both matter!!



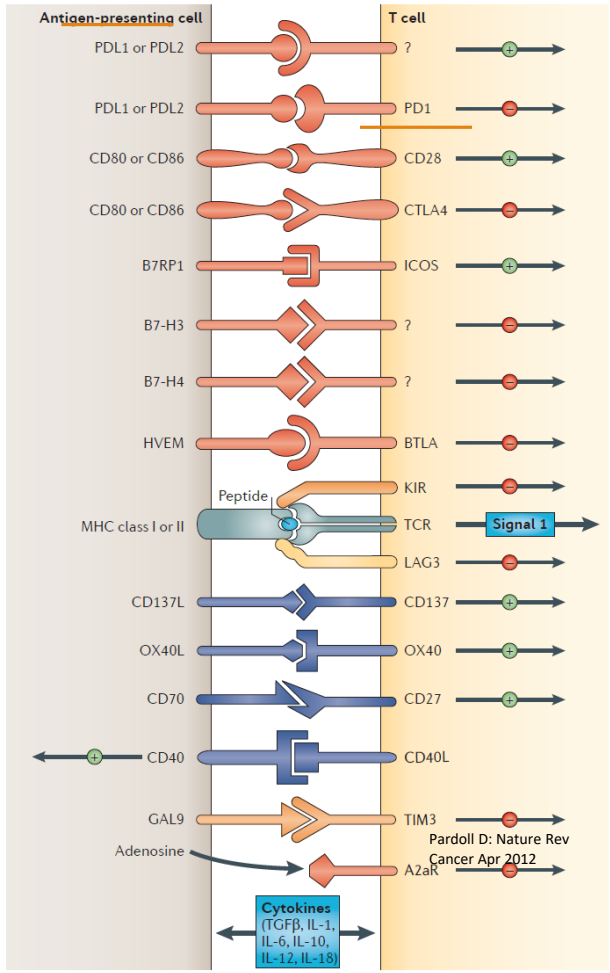




TILs and Immunotherapies

Immunity	Tumor	Therapeutic response to standard agents	Additional options?
Excellent	No	N/A	Preventive vaccine
Intermediate	Yes	↑ likelihood of response	Immunomodulatory therapies
			- Possible response to: anti-PD-1/PD-L1-2; CTLA-4, others
			- ? Add co-inhibitory targets (LAG-3, TIM-3, BTLA, 2B4, KLRG-1, CD160 etc)
Weak/low	Yes	↓ likelihood of response	Immunostimulation
			- Anti-vascular endothelium
			- Intratumoral cytokines, chemokines etc
			- Immunostimulatory Abs (CD137, CD40)

Complexity of molecules



Pardoll D: Nature Rev Cancer Apr 2012

Protumor vs inhibitory

Safety vs autoimmunity

Interactions

Take home messages

TILs are prognostic

- TNBC
- HER2+ (??)
- ER+ (no)

Methods are established/standardized

PD1/PDL-1 directed therapies seems to have promise


Thank you
Clarian Health