Immune mechanisms in breast cancer

SUNIL BADVE, MD, FRCPATH

JOSHUA EDWARDS PROFESSOR,

DEPARTMENT OF PATHOL & LAB MED

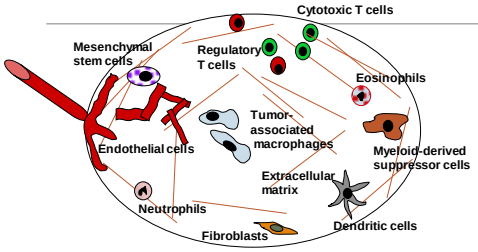
PROFESSOR, INTERNAL MEDICINE.

INDIANA UNIVERSITY, IN. USA.

Agenda

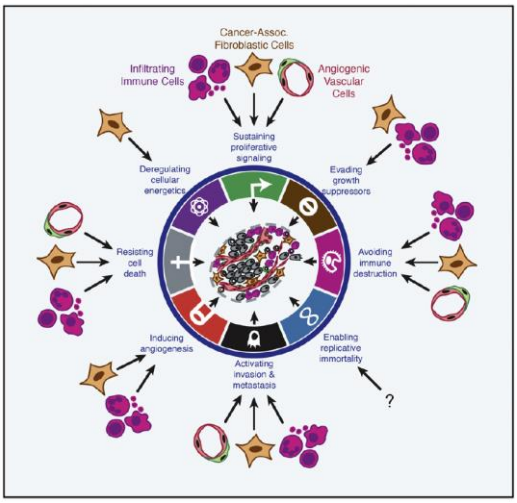
- Tumor infiltrating lymphocytes
- PDL-1 and immunotherapy
- Predictors of immune response

Current focus of pathologic evaluation is cancer cell centric



Tumor progression
Invasion & Metastasis
Immunosuppression

Tumor inhibition
Anti-tumor immune response

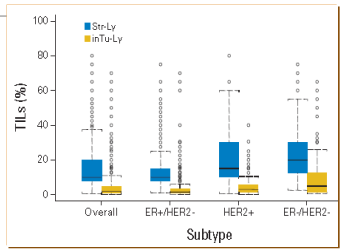


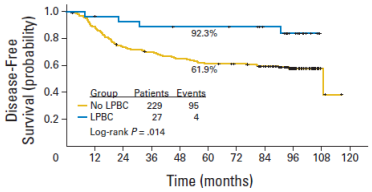
Stroma and infiltrating immune cells can also impact prognosis

Hanahan D and Coussens L. Cancer Cell 2012

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

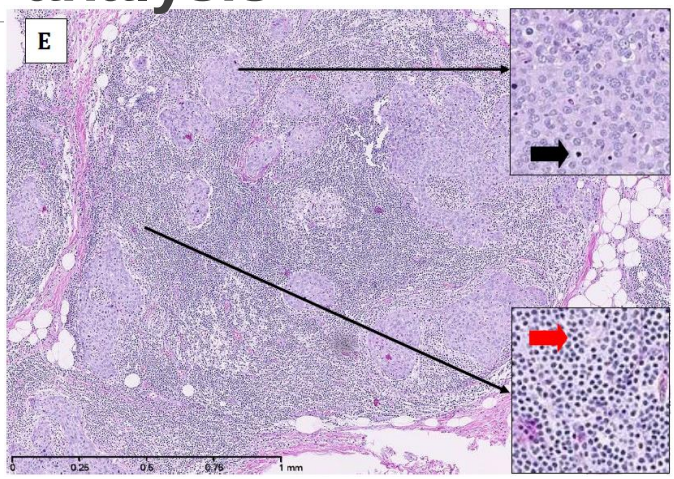




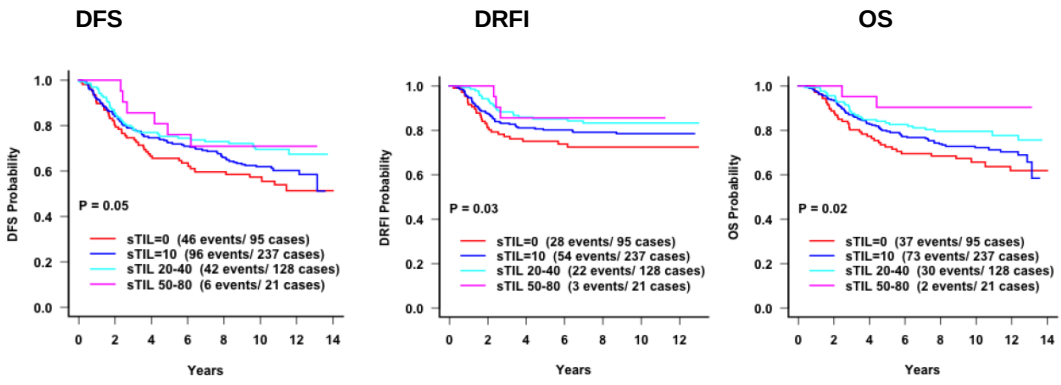
Loi et al, JCO 2013

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



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
	Lum A: PR+, HER2-; Ki67 (low <20%)	
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)

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

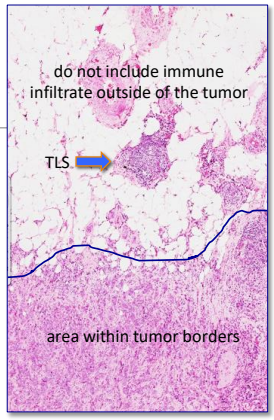
Salgado et al

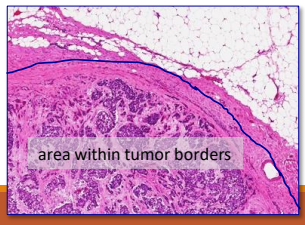
Step 1: Define area for TIL evaluation

Only TILs within the borders of the invasive tumors are evaluated

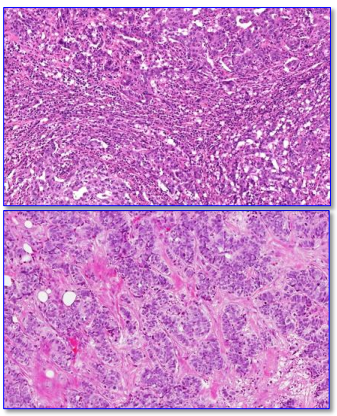
The invasive edge is included in the evaluation, but not reported separately

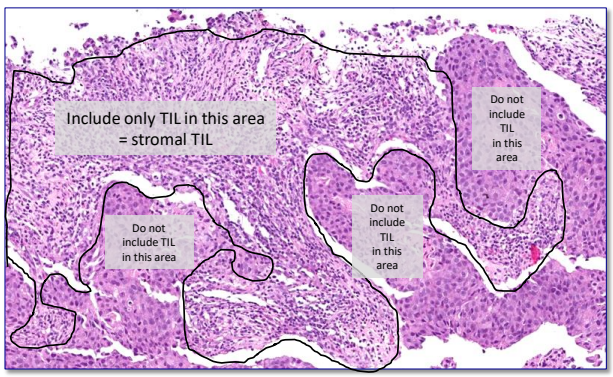
Immune infiltrates outside of the tumor borders, e.g. in adjacent normal tissue or DCIS are not included





Step 2: Scan the slide with focus on stromal TIL

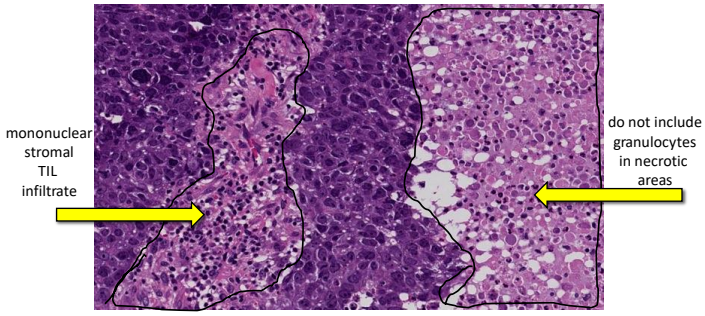




Step 3: Determine type of inflammatory infiltrate

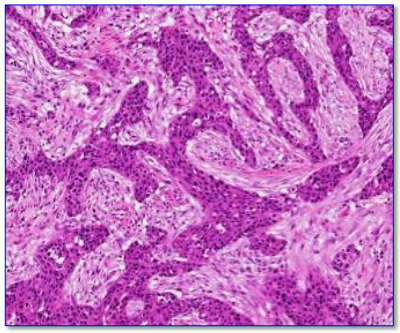
Include only mononuclear infiltrate (lymphocytes & plasma cells)

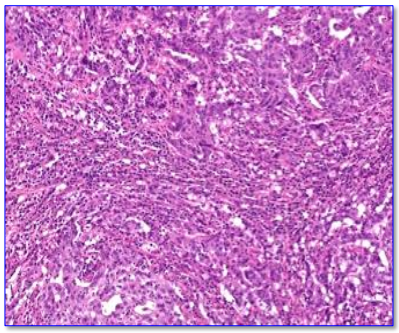
Do not include granulocytic infiltrate in areas of tumor necrosis

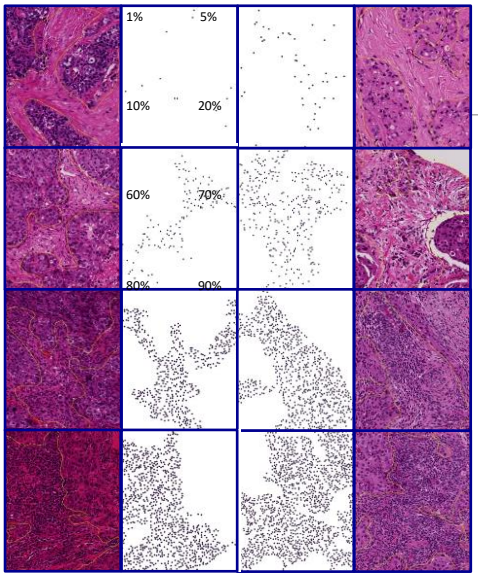


Step 4: As a first approach, include tumor in one of three groups based on low magnification and assess % stromal TILs (continue with Step 5 for percentage)

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.

original report

Tumor-Infiltrating Lymphocytes and Prognosis: A Pooled Individual Patient Analysis of Triple-Negative Breast Cancers

Sherene Loi, MD[1]; Damien Drubay, PhD[2,3]; Sylvia Adams, MD[4]; Giancarlo Pruneri, MD[5]; Pru
Magali Lacroix-Triki, MD[2]; Heikki Joensuu, MD[7]; Maria Vittoria Dieci, MD[8,9]; Sunil Badve, M
Robert Gray, PhD[12]; Elisabetta Munzone, MD[13]; Jerome Lemonnier, PhD[6]; Christos Sotiriou,
Pirkko-Liisa Kellokumpu-Lehtinen, MD[15]; Andrea Vingiani, MD[16]; Kathryn Gray, PhD[12]; Fabri
Roberto Salgado, MD[1,18]; and Stefan Michiels, PhD[2,3]

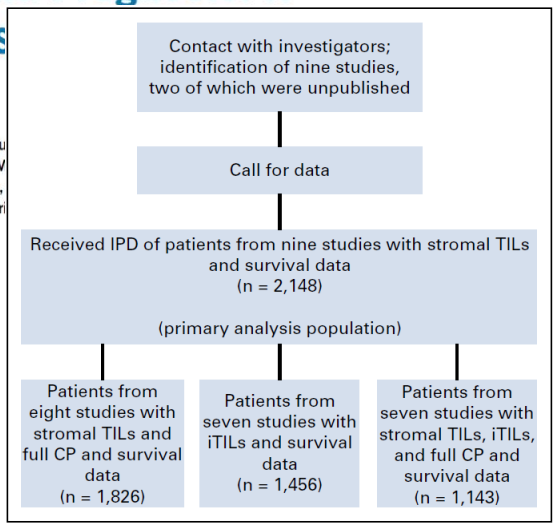
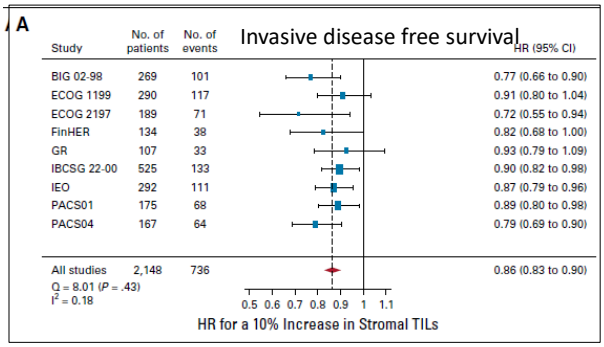
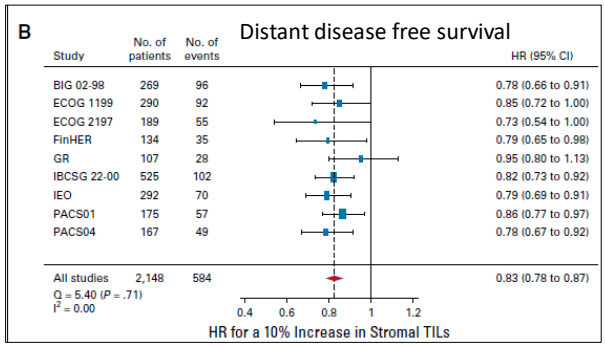


FIG 1. Study flow chart. CP, clinicopathologic; IPD, individual participant data; iTILs, intratumoral tumor-infiltrating lymphocytes; TILs, tumor-infiltrating lymphocytes.





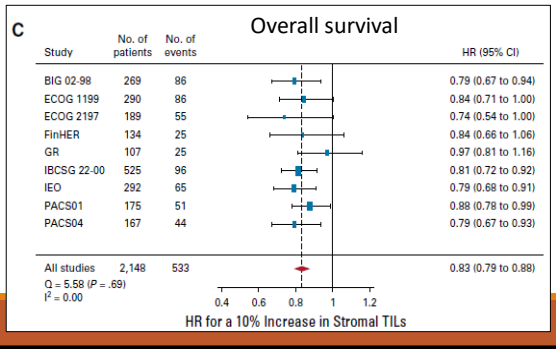
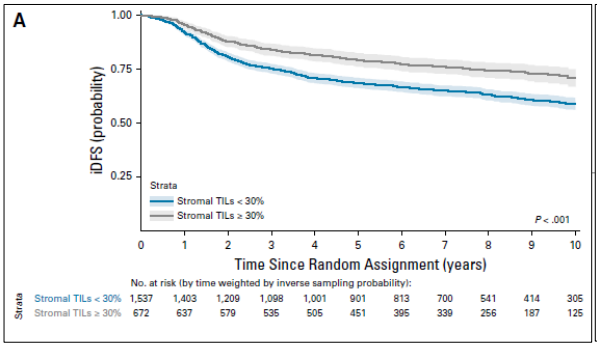
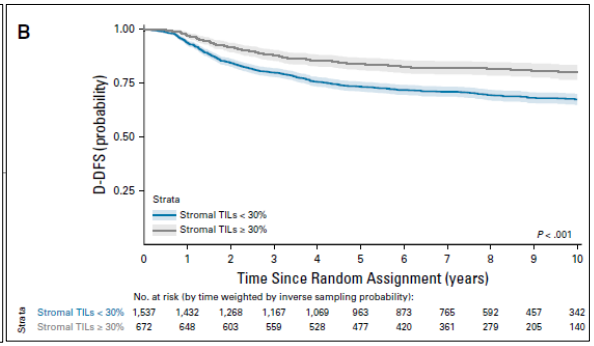


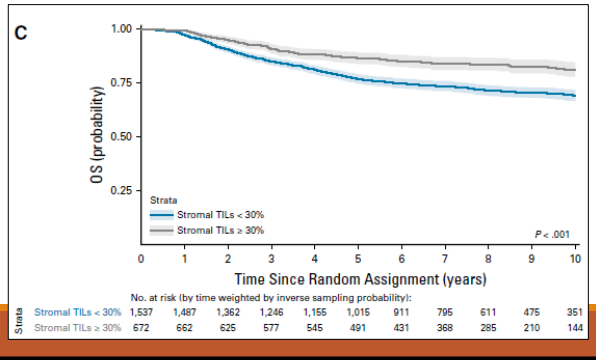
Table 2: Multivariable survival analyses adjusted for s TILs, age, tumor size, number of positive nodes, histological grade and treatment

n=1826	IDFS (608 events)		DDFS (482 events)		OS (438 events)	
Variables	HR [95%CI]	p	HR [95%CI]	p	HR [95%CI]	p
Stromal TILs (per10%increments)	0.87 [0.83; 0.91]	$< 10^{-6}$	0.83 [0.79; 0.88]	$< 10^{-6}$	0.84 [0.79; 0.89]	$< 10^{-6}$
Age (/years)	1.00 [1.00; 1.01]	0.394	1.00 [1.00; 1.01]	0.323	1.01 [1.00; 1.01]	0.300
Tumor size category						
T1 (≤2cm)	1	reference	1	reference	1	reference
T2 (2-5cm)	1.30 [1.10; 1.55]	$< 10^{-2}$	1.46 [1.20; 1.78]	$< 10^{-3}$	1.44 [1.17; 1.77]	$< 10^{-3}$
T3 (≥5cm)	1.63 [1.18; 2.25]	$< 10^{-2}$	1.73 [1.22; 2.46]	$< 10^{-2}$	1.61 [1.11; 2.33]	0.012
Positive nodes, N0 vs N1 vs N2	1.07 [1.06; 1.08]	$< 10^{-6}$	1.08 [1.07; 1.09]	$< 10^{-6}$	1.08 [1.07; 1.09]	$< 10^{-6}$
Histological grade						
Grade 1 or 2	1	reference	1	reference	1	reference
Grade 3	1.05 [0.86; 1.30]	0.617	1.16 [0.91; 1.48]	0.233	1.17 [0.91; 1.49]	0.223
Treatment						
Anthracycline	1	reference	1	reference	1	reference
Anthracycline + Taxane	1.04 [0.87; 1.24]	0.704	0.98 [0.80; 1.21]	0.877	1.03 [0.84; 1.27]	0.771

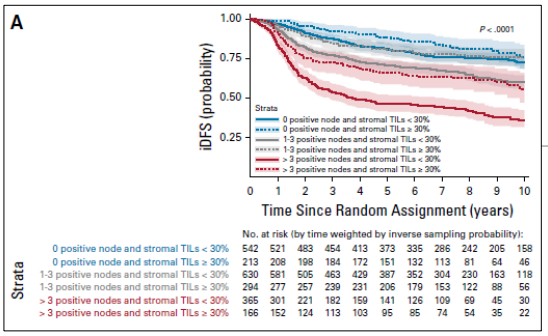
Loi et al JCO 2019

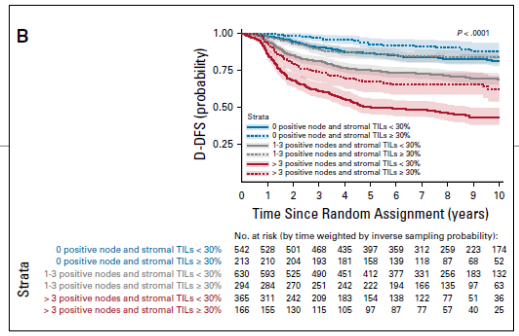


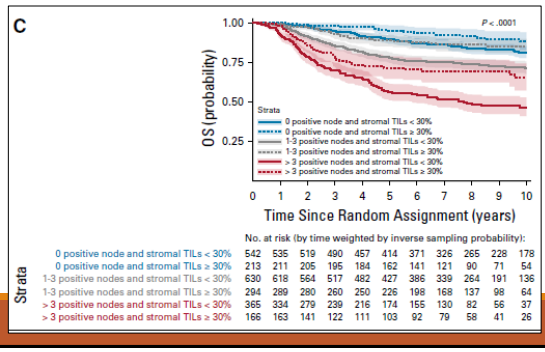




Entire population



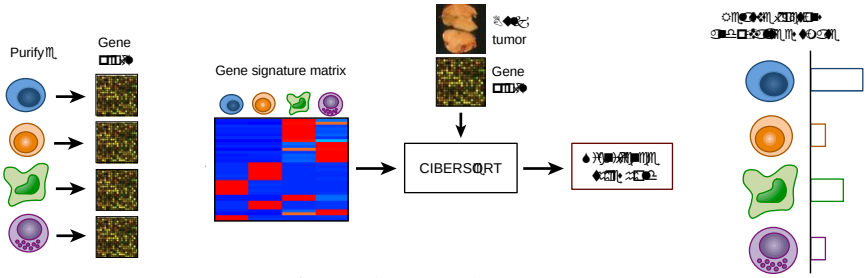




By nodal status

Loi et al JCO 2019

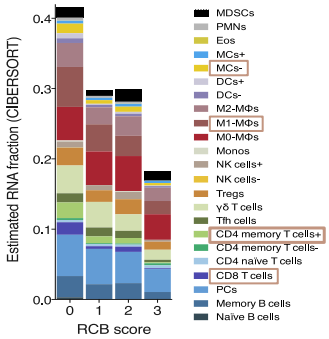
What are TILs?



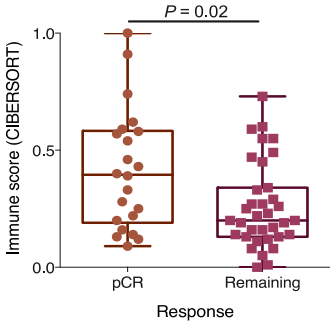
23 purified leukocyte subset signatures used to distinguish cell types

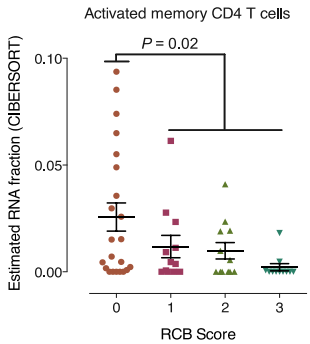
Naïve B Memory B Plasma cells	CD8 T CD4 Naïve T CD4 Memory T inact. CD4 Memory T act. CD4 Follicular helper T CD4 Regulatory T Gamma delta T	NK unstim. NK stim.	DCs unstim. DCs stim.	Mast cells unstim. Mast cells stim. Eosinophils Neutrophils	Monocytes M0 macrophages M1 macrophages M2 macrophages	MDSCs

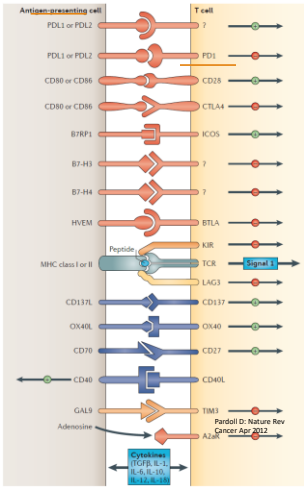
Total and subsets both matter!!



Higher Immune Score in patients with pCR







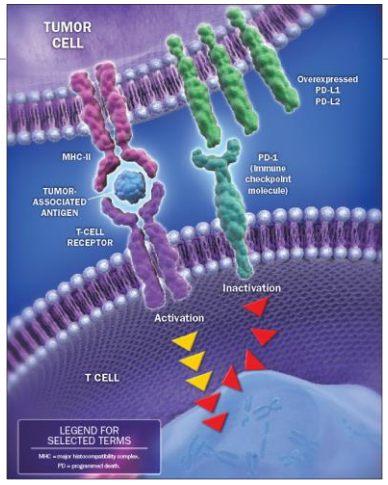
Complexity of molecules

Protumor vs inhibitory

Safety vs autoimmunity

Interactions

Role of the PD-1 Pathway in Cancer



- Programmed death 1 (PD-1) pathway is an immune checkpoint pathway that is expressed on the surface of activated T cells
- One of its ligands, PD-L1, is highly expressed on the surface of tumor cells
- Binding of PD-1 with PD-L1 inhibits T cell activation, allowing immunosuppression and neoplastic growth

Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12:252–264.

IMpassion130: Efficacy in immune biomarker subgroups from the global, randomized, double-blind, placebo-controlled, Phase III study of atezolizumab + *nab*-paclitaxel in patients with treatment-naive, locally advanced or metastatic triple-negative breast cancer

Leisha A. Emens,[1] Sherene Loi,[2] Hope S. Rugo,[3] Andreas Schneeweiss,[4] Véronique Diéras,[5] Hiroji Iwata,[6] Carlos H. Barrios,[7] Marina Nechaeva,[8] Luciana Molinero,[9] Anh Nguyen Duc,[10] Roel Funke,[9] Stephen Y Chui,[9] Amreen Husain,[10] Eric P. Winer,[11] Sylvia Adams,[12] Peter Schmid[13]

[1]UPMC Hillman Cancer Center, University of Pittsburgh, Pittsburgh, PA; [2]Peter MacCallum Cancer Centre, Melbourne, VIC, Australia; [3]University of California San Francisco Comprehensive Cancer Center, San Francisco, CA; [4]University Hospital Heidelberg, Heidelberg, Germany; [5]Department of Medical Oncology, Centre Eugène Marquis, Rennes, France; [6]Aichi Cancer Center Hospital, Aichi, Japan; [7]Department of Medicine, PUCRS School of Medicine, Porto Alegre, Brazil; [8]Arkhangelsk Regional Clinical Oncology Dispensary, Arkhangelsk, Russia; [9]Genentech, Inc., South San Francisco, CA; [10]F. Hoffmann-La Roche AG, Basel, Switzerland; [11]Dana-Farber Cancer Institute, Boston, MA; [12]New York University Langone Medical Center, New York, NY; [13]Barts Cancer Institute, Queen Mary University of London, London, UK

Phase III study IMpassion130[a]

Previously untreated metastatic or inoperable locally advanced TNBC[b]

N = 902 patients randomized

Double blind; no crossover

R 1:1

Stratification factors:
1. *Prior taxane use*
2. *Liver metastases*
3. ***PD-L1 on IC[c]***

Atezo + nab-P arm[d]

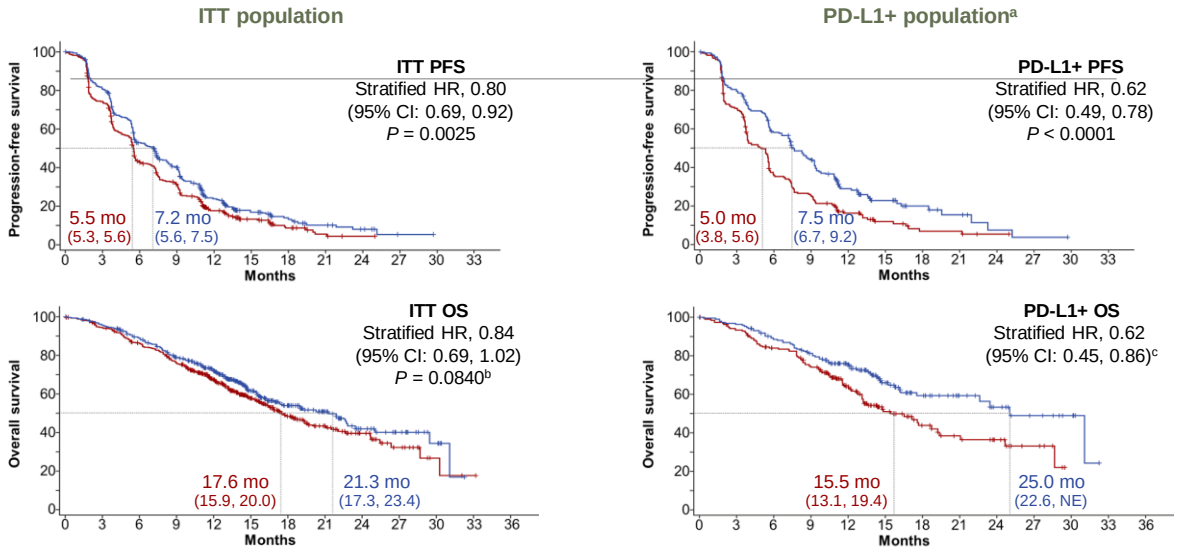
ITT population: n = 451
PD-L1 IC+ patients: n = 185 (41%)

Plac + nab-P arm[d]

ITT population: n = 451
PD-L1 IC+ patients: n = 184 (41%)

Key study endpoints
- Co-primary: PFS (ITT and PD-L1 IC+)
  OS (ITT and PD-L1 IC+)
- Secondary: ORR and DOR
- Safety and tolerability

[a] NCT02425891. [b] Locally evaluated per ASCO-CAP guidelines. Prior chemotherapy in the curative setting, including taxanes, allowed if treatment-free interval ≥ 12 mo.
[c] Centrally evaluated per VENTANA SP142 IHC assay (double blinded for PD-L1 status, PD-L1+: PD-L1 on ≥ 1% of IC). [d] Atezolizumab or placebo 840 mg IV on days 1 and 15 + *nab*-paclitaxel 100 mg/m² IV on days 1, 8 and 15 of 28-day cycle until RECIST v1.1 PD. 1. Schmid *N Engl J Med* 2018.



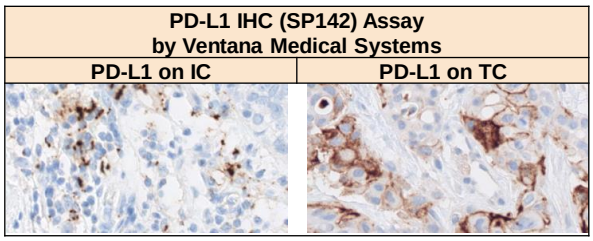
NE, not estimable.
Median follow-up (ITT): 12.9 months.
[a] PD-L1+: PD-L1 in ≥ 1% of IC. [b] Not significant. [c] Not formally tested per hierarchical study design.
1. Schmid *N Engl J Med* 2018. 2. Schmid ESMO 2018 [LBA1_PR].

Emens LA, et al. IMpassion130 biomarkers. SABCS 2018 (program #GS1-04)

25

PDL1 testing

- Pre-existing immune biology, including PD-L1 expression on TC, CD8+ T cells and stromal TILs, has also been associated with clinical benefit from anti–PD-L1/PD-1[1,2]
- In this exploratory analysis, we sought to evaluate whether this immune biology and *BRCA1/2* mutation status were associated with clinical benefit from atezolizumab + *nab*-paclitaxel
- Biomarkers were centrally analyzed in pre-treatment biopsies
  - PD-L1 on IC and TC by VENTANA SP142 IHC assay[a]
  - Intratumoral CD8+ T cells by IHC (Dako clone C8/144B) and stromal TILs by H&E[b]
  - *BRCA1/2* mutation status by FoundationOne assay

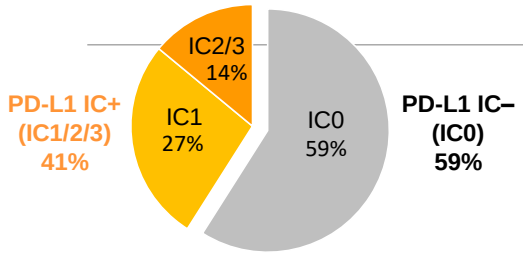


H&E, hematoxylin and eosin staining; IHC, immunohistochemistry.
[a] PD-L1 scoring: IC0: < 1%; IC1: ≥ 1% and < 5%; IC2: ≥ 5% and < 10%; IC3: ≥ 10%; TC–: < 1% PD-L1 on tumor cells; TC+: ≥ 1% PD-L1 on tumor cells.
[b] Pre-specified cutoffs for CD8 IHC and stromal TILs are based on references 1 and 2.
1. Adams *JAMA Oncol* 2018. 2. Denkert *Lancet Oncol* 2018.

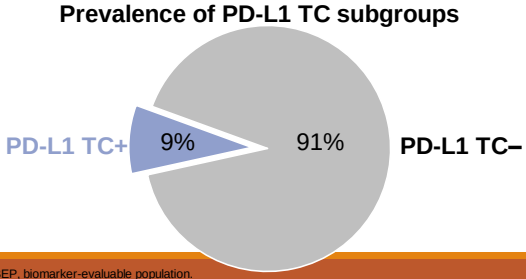
Emens LA, et al. IMpassion130 biomarkers. SABCS 2018 (program #GS1-04)

26

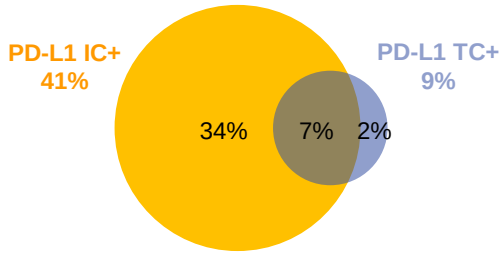
Prevalence of PD-L1 IC subgroups



Prevalence of PD-L1 TC subgroups



The majority of patients with expression of PD-L1 on TC are included within the PD-L1 IC+ population



BEP, biomarker-evaluable population.
BEP (TC): n = 900. PD-L1 scoring: IC0: < 1%; IC1: ≥ 1% and < 5%; IC2: ≥ 5% and < 10%; IC3: ≥ 10%; TC–: < 1% PD-L1 on tumor cells; TC+: ≥ 1% PD-L1 on tumor cells.

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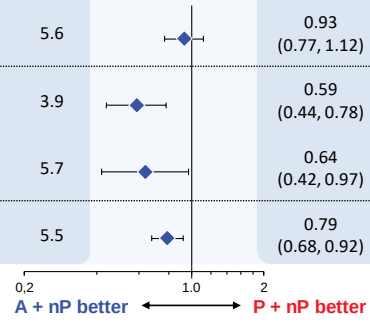
27

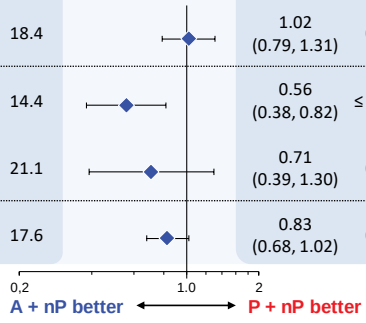
PFS

	PD-L1 IC Status	n	Median, mo A + nP	Median, mo P + nP	HR[a] (95% CI)	*P* value
Neg	IC0	532	5.6	5.6	0.93 (0.77, 1.12)	0.47
Pos	IC1	243	7.4	3.9	0.59 (0.44, 0.78)	≤ 0.005
Pos	IC2/3	125	9.3	5.7	0.64 (0.42, 0.97)	0.03
	All	900	7.2	5.5	0.79 (0.68, 0.92)	≤ 0.005



OS

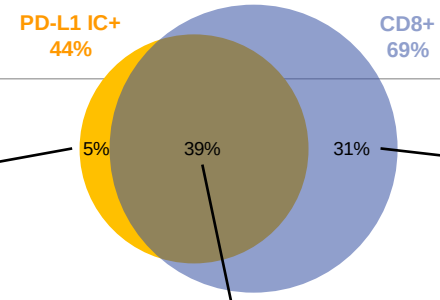
	PD-L1 IC Status	n	Median, mo A + nP	Median, mo P + nP	HR[a] (95% CI)	*P* value
Neg	IC0	532	18.9	18.4	1.02 (0.79, 1.31)	0.90
Pos	IC1	243	23.4	14.4	0.56 (0.38, 0.82)	≤ 0.005
Pos	IC2/3	125	25.0	21.1	0.71 (0.39, 1.30)	0.26
	All	900	21.3	17.6	0.83 (0.68, 1.02)	0.07



[a] Adjusted for prior taxane treatment and liver metastases.
A multivariate analysis was performed to account for imbalances in baseline characteristics between PD-L1 IC–expressing subgroups (IC1, IC2 and IC3).
IC0: < 1% PD-L1; IC1: ≥ 1% and < 5% PD-L1; IC2/3: ≥ 5% PD-L1. All *P* values are nominal. Data cutoff: April 17, 2018.

Emens LA, et al. IMpassion130 biomarkers. SABCS 2018 (program #GS1-04)

28



CD8–/PD-L1 IC+ (n = 37)

	HR (95% CI)	*P* Value
PFS	0.33 (0.13, 0.87)	0.03
OS	0.25 (0.06, 1.02)	0.05

CD8+/PD-L1 IC– (n = 220)

	HR (95% CI)	*P* Value
PFS	0.89 (0.66, 1.20)	0.45
OS	0.77 (0.50, 1.17)	0.21

CD8+/PD-L1 IC+ (n = 280)

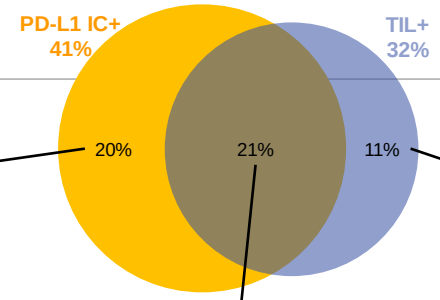
	HR (95% CI)	*P* Value
PFS	0.61 (0.46, 0.80)	≤ 0.005
OS	0.55 (0.38, 0.80)	≤ 0.005

- PD-L1 IC+ are enriched in CD8+ (*P* < 0.0001) and CD8+ are enriched in PD-L1 IC+ (*P* < 0.0001)[a]
- ***Patients with CD8+ tumors derived clinical benefit (PFS/OS) only if their tumors were also PD-L1 IC+***

BEP (CD8): n = 720. A CD8+ cutoff of 0.5% was selected based on Phase Ib study in TNBC (Adams *JAMA Oncol* 2018). All *P* values are nominal.
[a] Data derived from contingency table with Fisher exact tests.

Emens LA, et al. IMpassion130 biomarkers. SABCS 2018 (program #GS1-04)

29



TIL–/PD-L1 IC+ (n = 176)

	HR (95% CI)	*P* Value
PFS	0.74 (0.54, 1.03)	0.07
OS	0.65 (0.41, 1.02)	0.06

TIL+/PD-L1 IC– (n = 94)

	HR (95% CI)	*P* Value
PFS	0.99 (0.62, 1.57)	0.97
OS	1.53 (0.76, 3.08)	0.24

TIL+/PD-L1 IC+ (n = 190)

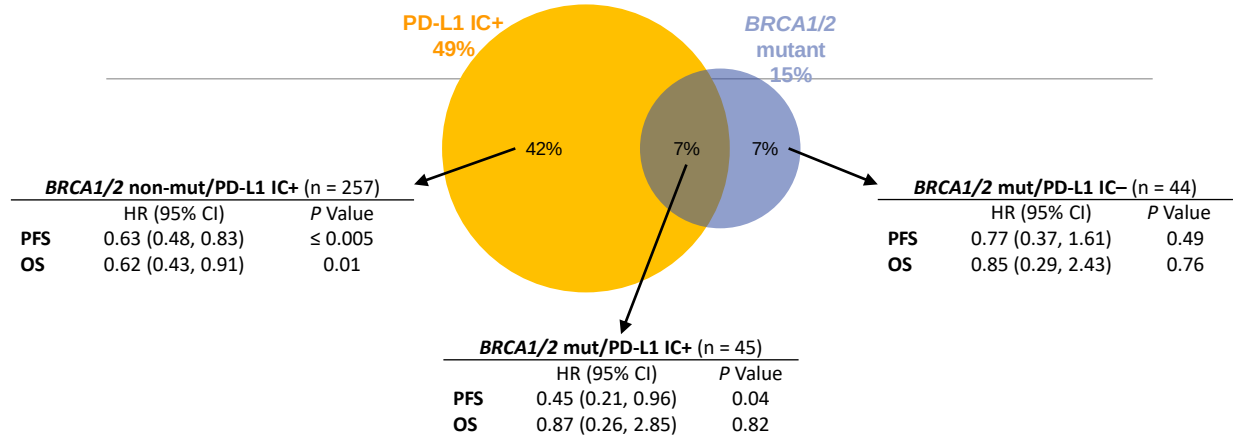
	HR (95% CI)	*P* Value
PFS	0.53 (0.38, 0.74)	≤ 0.005
OS	0.57 (0.35, 0.92)	0.02

- TIL+ were enriched for PD-L1 IC+ (*P* < 0.0001) but PD-L1 IC+ were not enriched for TIL+ (*P* = ns)[a]
- ***Patients with TIL+ tumors derived clinical benefit (PFS/OS) only if their tumors were also PD-L1 IC+***

BEP (TILs): n = 893. Cutoff of 10% was used to distinguish low vs intermediate/high levels of TILs (Denkert *Lancet Oncol* 2018). All *P* values are nominal.
[a] Data derived from contingency table with Fisher exact tests.

Emens LA, et al. IMpassion130 biomarkers. SABCS 2018 (program #GS1-04)

30



***BRCA1/2* non-mut/PD-L1 IC+** (n = 257)

	HR (95% CI)	*P* Value
PFS	0.63 (0.48, 0.83)	≤ 0.005
OS	0.62 (0.43, 0.91)	0.01

***BRCA1/2* mut/PD-L1 IC+** (n = 45)

	HR (95% CI)	*P* Value
PFS	0.45 (0.21, 0.96)	0.04
OS	0.87 (0.26, 2.85)	0.82

***BRCA1/2* mut/PD-L1 IC–** (n = 44)

	HR (95% CI)	*P* Value
PFS	0.77 (0.37, 1.61)	0.49
OS	0.85 (0.29, 2.43)	0.76

- *BRCA1/2* mutants and PD-L1 IC+ are independent from each other (*P* = ns)[a]
- ***Patients with BRCA1/2-mutant tumors derived clinical benefit (PFS/OS) only if their tumors were also PD-L1 IC+*[b]**

BEP (BRCA1/2): n = 612. Per FoundationOne *BRCA1/2* testing, *BRCA1/2* mutant: known and likely mutations. All *P* values are nominal.
[a] Data derived from contingency table with Fisher exact tests. [b] Data interpretation limited by small number of *BRCA1/2*-mutant patients.

Emens LA, et al. IMpassion130 biomarkers. SABCS 2018 (program #GS1-04)

31

Take home messages

TILs are prognostic (Methods are established/standardized)

PD1/PDL-1 directed therapies seems to have promise

- § In the Phase III IMpassion130 study, PD-L1 expression on IC is a predictive biomarker for selecting patients who clinically benefit from first-line atezolizumab + *nab*-paclitaxel treatment for mTNBC
  - PFS and OS benefit was observed in patients with a PD-L1 IC of ≥ 1% (by VENTANA SP142 IHC assay)
  - A treatment effect was not seen for adding atezolizumab to chemotherapy in the PD-L1–negative subgroup
- § PD-L1 expression on TC did not provide additional information beyond PD-L1 IC status
  - Prevalence of tumor-cell PD-L1 expression was low, and the majority of these tumors were also PD-L1 IC+
- § PD-L1 IC expression was the best predictor of clinical benefit as the patient subgroups with tumor-infiltrating immune cells (stromal TILs+) or cytotoxic T cells (CD8+) derived clinical benefit with atezolizumab + *nab*-paclitaxel if their tumors were also PD-L1 IC+
- § PFS and OS results were consistent regardless of *BRCA1/2* mutation status


Thank you
Clarian Health