Society for Immunotherapy of Cancer

Advances in Cancer Immunotherapy™

TIL as a biomarker

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Disclosures

- **Research support (to UW)**: *BMS, EMD-Serono, Immune Design, Merck, Novartis, Oncosec, Nantkwest, Exicure, Nektar, Amphivena, Checkmate, Xencor.*

- **Advisory Board**: *Genentech, BMS, EMD-Serono, Sanofi-Genzyme, Castle Biosciences*

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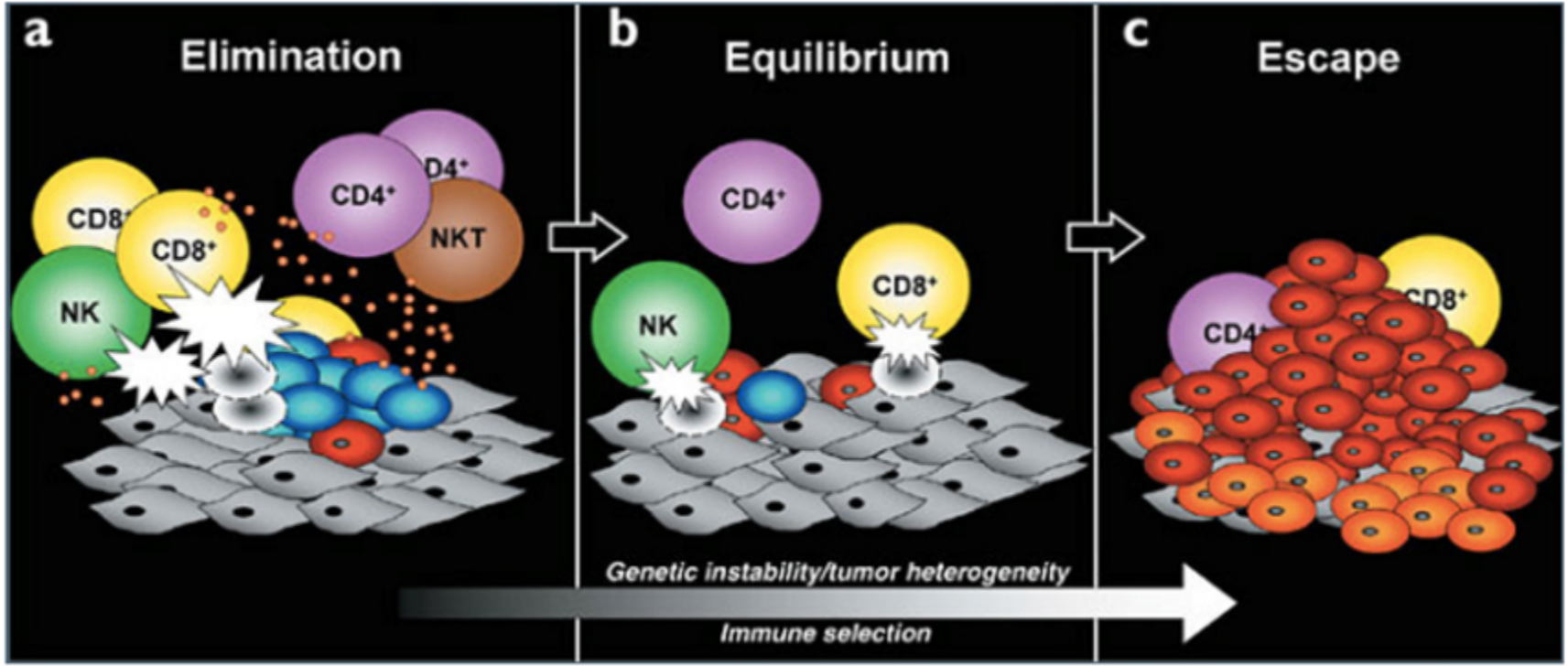
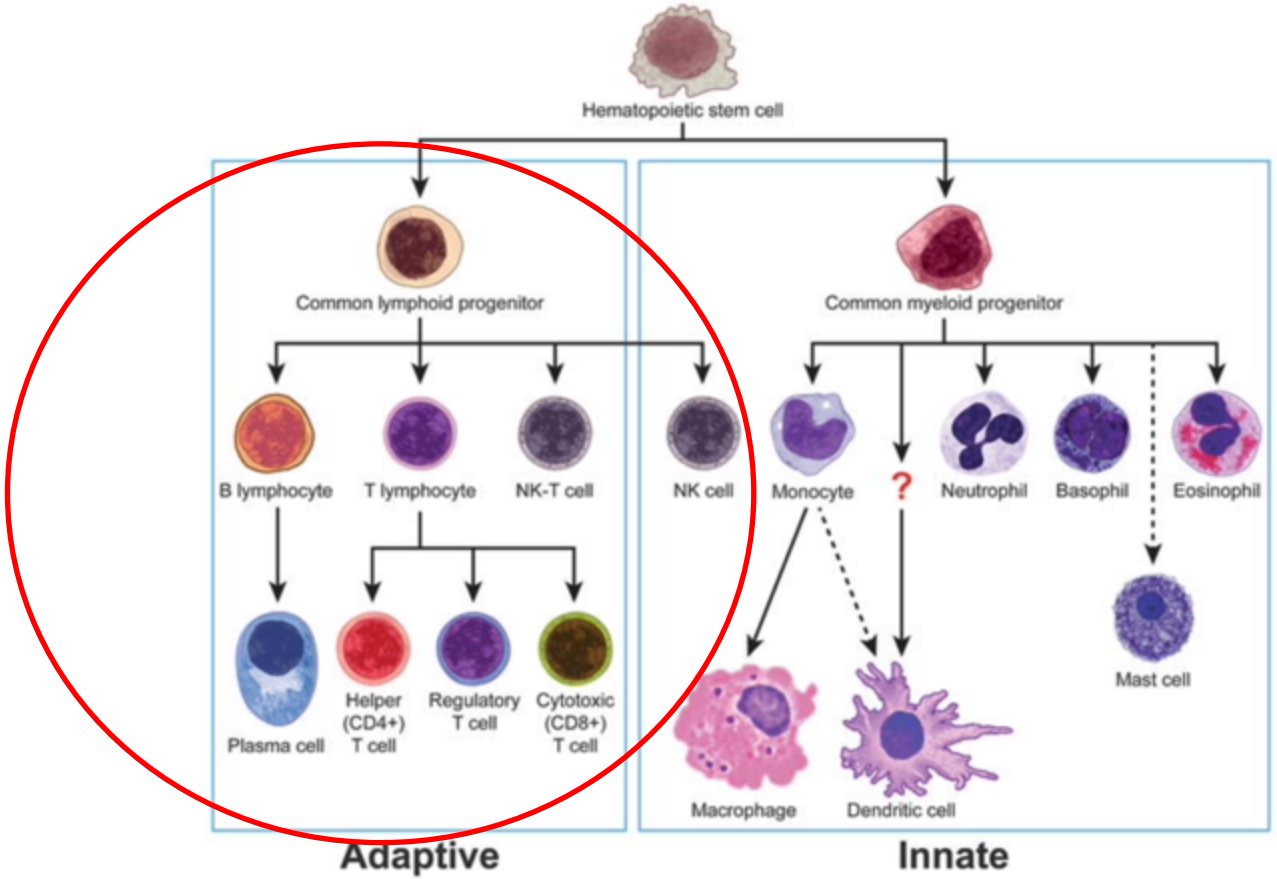
Cancer vs Immune System: The 3 Es



Nature Immunology, 3, 991-998; 2002

TILs: Tumor Infiltrating Lymphocytes



TILs: Advantages as a biomarker

- Simpler and Inexpensive (as compared to mIHC, GEP, scRNAseq etc)
- Can be used on FFPE tissue (e.g. archival samples from RCTs)
- Needs a light microscope plus trained pathologist; no extra setup needed
- Proven to be reproducible (e.g. excellent inter-observer concordance for stromal-TILs in TNBC)
- Tracks well with other immune biomarkers, such as PD-L1

{Loi S et al. ESMO. 2021}

TILs as Prognostic Biomarker

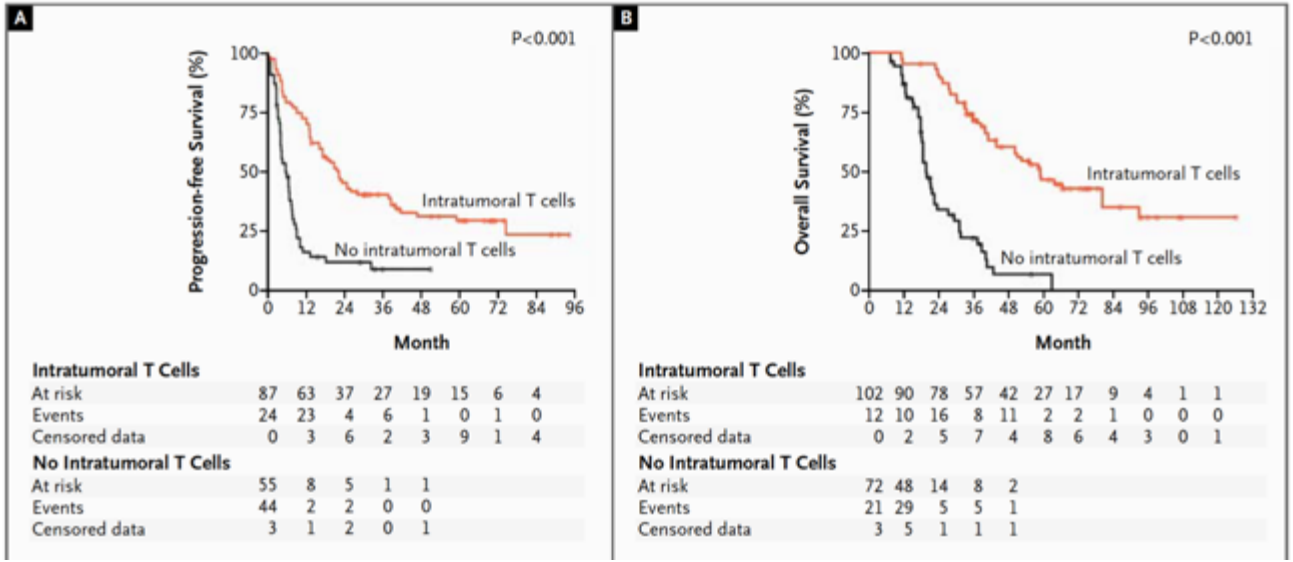
TILs have prognostic impact in numerous cancers, including Ovarian, Colorectal, Melanoma, HCC, Breast, Lung *et cetera*

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Intratumoral T Cells, Recurrence, and Survival in Epithelial Ovarian Cancer

INTRATUMORAL T CELLS IN OVARIAN CANCER

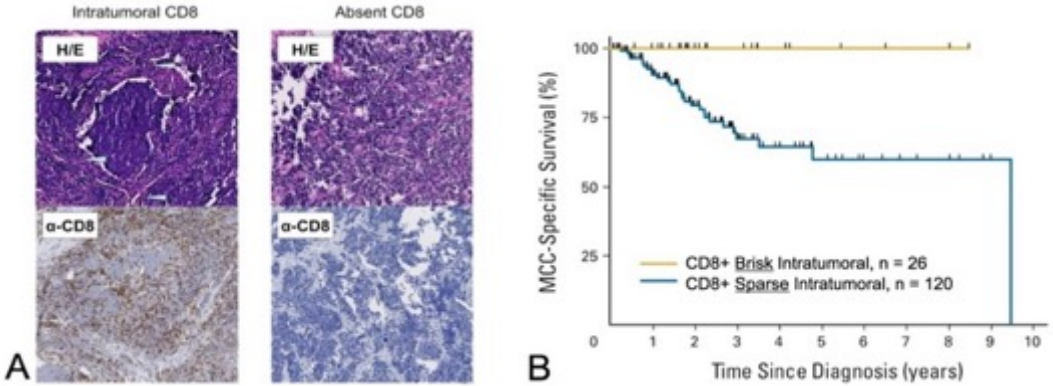


{Zhang L et al, *NEJM*, 2003}

Transcriptome-Wide Studies of Merkel Cell Carcinoma and Validation of Intratumoral CD8+ Lymphocyte Invasion As an Independent Predictor of Survival

Kelly G. Paulson, Jayasri G. Iyer, Andrew R. Tegeder, Renee Thibodeau, Janell Schelter, Shinichi Koba, David Schrama, William T. Simonson, Bianca D. Lemos, David R. Byrd, David M. Koelle, Denise A. Galloway, J. Helen Leonard, Margaret M. Madeleine, Zsolt B. Argenyi, Mary L. Disis, Juergen C. Becker, Michele A. Cleary, and Paul Nghiem

The NEW ENGLAND JOURNAL of MEDICINE



ORIGINAL ARTICLE

PD-1 Blockade with Pembrolizumab in Advanced Merkel-Cell Carcinoma

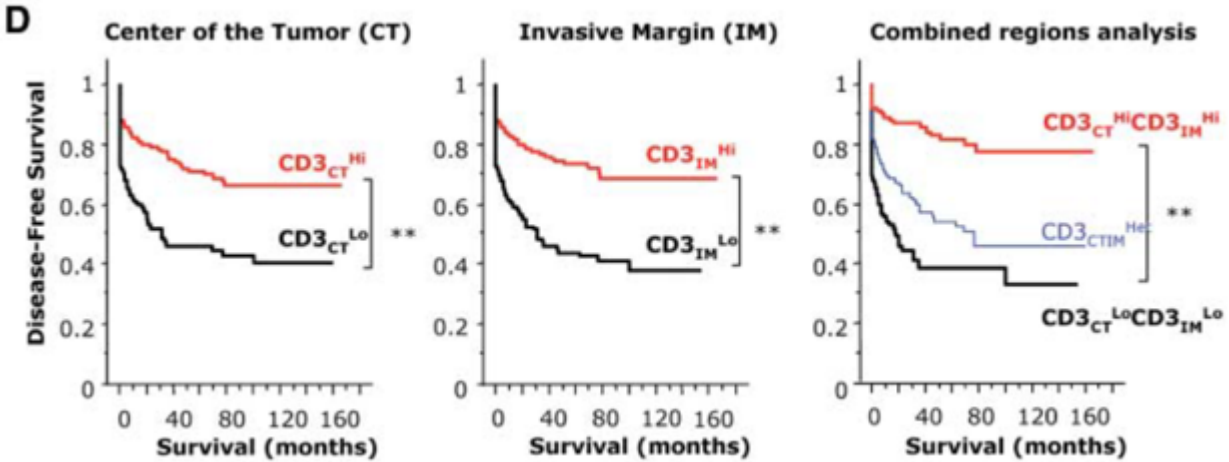
Paul T. Nghiem, M.D., Ph.D., Shailender Bhatia, M.D., Evan J. Lipson, M.D., Ragini R. Kudchadkar, M.D., Natalie J. Miller, B.A., Lakshmanan Annamalai, D.V.M., Ph.D, Sneha Berry, M.S., Elliot K. Chartash, M.D., Adil Daud, M.B., B.S., Steven P. Fling, Ph.D., Philip A. Friedlander, M.D., Harriet M. Kluger, M.D., Holbrook E. Kohrt, M.D., Ph.D.,* Lisa Lundgren, M.S., Kim Margolin, M.D., Alan Mitchell, M.Sc., Thomas Olencki, D.O., Drew M. Pardoll, M.D., Ph.D., Sunil A. Reddy, M.D., Erica M. Shantha, M.D., William H. Sharfman, M.D., Elad Sharon, M.D., M.P.H., Lynn R. Shemanski, Ph.D., Michi M. Shinohara, M.D., Joel C. Sunshine, M.D., Ph.D., Janis M. Taube, M.D., John A. Thompson, M.D., Steven M. Townson, Ph.D., Jennifer H. Yearley, D.V.M., Ph.D., Suzanne L. Topalian, M.D., and Martin A. Cheever, M.D.

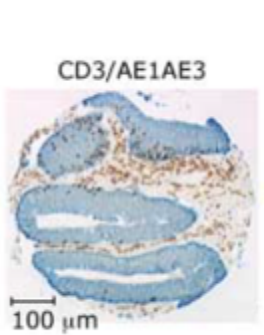
Figure: Intratumoral (not peritumoral) CD8+ infiltration is associated with improved MCC survival

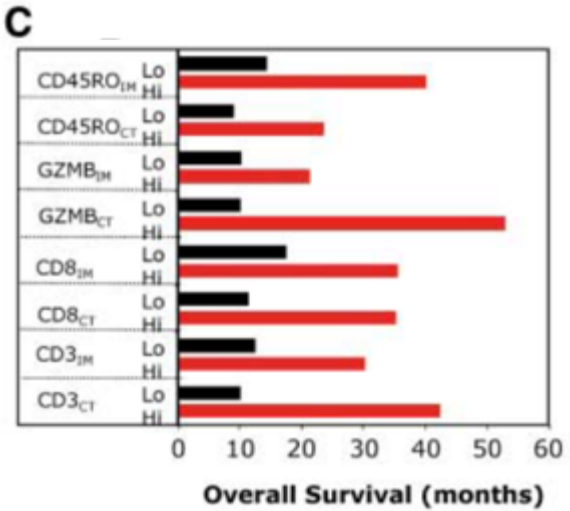
{Paulson et al, *J Clin Oncol*, 2011}

Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon,[1*†] Anne Costes,[1] Fatima Sanchez-Cabo,[2] Amos Kirilovsky,[1] Bernhard Mlecnik,[2] Christine Lagorce-Pagès,[3] Marie Tosolini,[1] Matthieu Camus,[1] Anne Berger,[4] Philippe Wind,[4] Franck Zinzindohoué,[5] Patrick Bruneval,[6] Paul-Henri Cugnenc,[5] Zlatko Trajanoski,[2] Wolf-Herman Fridman,[1,7] Franck Pagès[1,7†]







{Galon J et al, *Science*, 2006}

Immunoscore in CRC

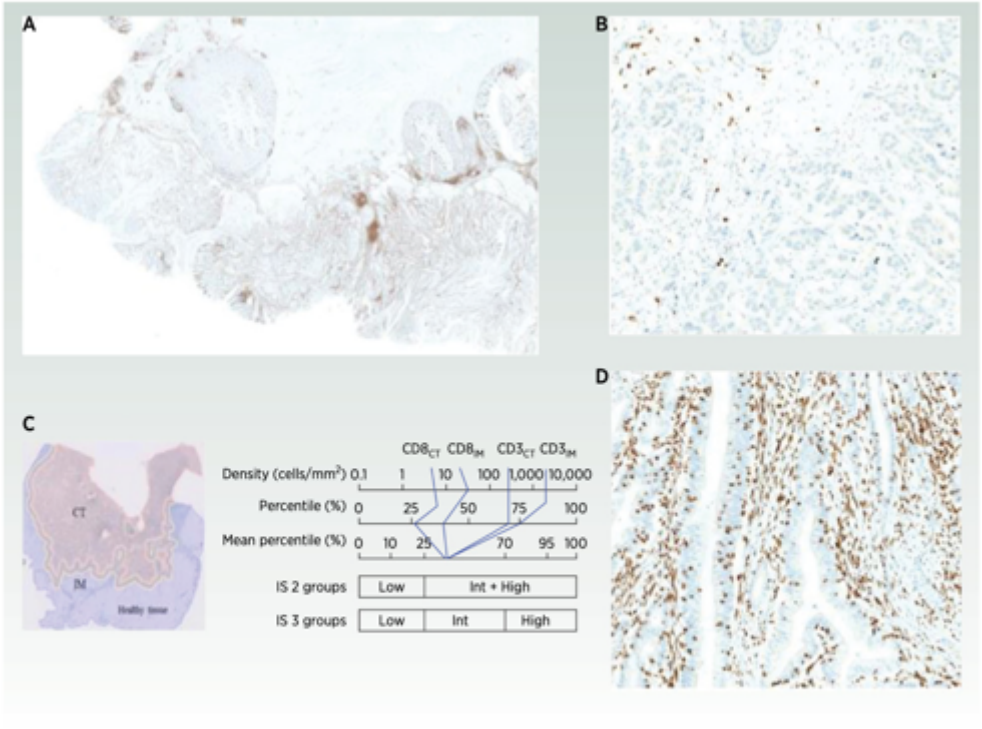
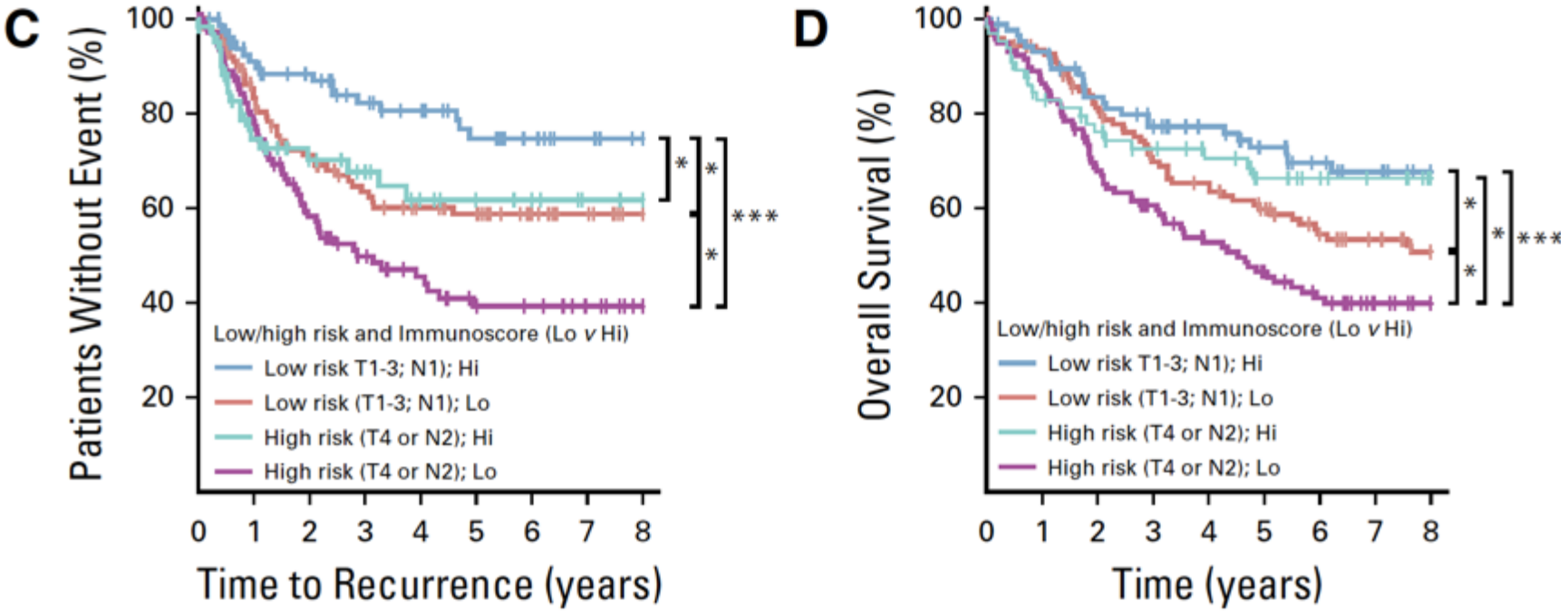


Figure 2.

A, Example of whole-slide CD3 staining. **B,** Example of staining for a low Immunoscore patient. **C,** Digital representation of tissue areas including CT, tumor center; IM, invasive margin; and adjacent excluded healthy tissue. Cell density cutoffs also indicated for CD3 and CD8. (CD3CT, CD8CT, CD3IM, and CD8IM). **D,** Example of for a high Immunoscore patient.

{Angell HK et al, *CCR*, 2020}

Prognostic impact of Immunoscore in stage III CRC



{Mlecnik B et al, *J Clin Oncol*, 2020}

Multivariate overall survival analysis stratified by center			
Events/Total: 341/760; C-index (95%CI): 0.59 (0.51 to 0.67)			
	HR (95% CI)	CoxPH	Wald *P*
Sex Female *v* Male	0.89 (0.72 to 1.11)	.2167	.2964
T stage T3 *v* T1-2	1.11 (0.73 to 1.69)	.5842	.6304
T stage T4 *v* T1-2	1.28 (0.80 to 2.04)	.9914	.3010
N stage N2 *v* N1	1.40 (1.12 to 1.76)	.0009	.0032
Sidedness distal *v* proximal	0.85 (0.68 to 1.07)	.4068	.1705
MSI status MSI *v* MSS MSI	1.15 (0.78 to 1.70)	.0602	.4750
Status unknown *v* MSS	0.92 (0.58 to 1.46)	.6441	.7240
Immunoscore Int (25%-70%) *v* Lo (0%-25%)	0.72 (0.56 to 0.91)	.5020	.0071
Immunoscore Hi (70%-100%) *v* Lo (0%-25%)	0.58 (0.41 to 0.80)	.9781	.0012
Events/Total: 342/763; C-index (95%CI): 0.57 (0.53-0.6)			
	HR (95% CI)	CoxPH	Wald *P*
Histopathological classification			
Risk high (T4 or N2) *v* Low (T1-T3,N1)	1.4 (1.12 to 1.74)	.0178	.0025
Immunoscore classification			
Immunoscore Int (25%-70%) *v* Lo (0%-25%)	0.71 (0.56 to 0.91)	.5263	.0062
Immunoscore Hi (70%-100%) *v* Lo (0%-25%)	0.61 (0.44 to 0.85)	.7204	.0031

{Mlecnik B et al, *J Clin Oncol*, 2020}

TNM-Immune (CRC)

	HR (95% CI)	Wald p value
Female vs male	0·90 (0·72–1·12)	0·3400
T stage		
T2 vs T1	1·49 (0·62–3·57)	0·3686
T3 vs T1	1·91 (0·84–4·38)	0·1238
T4 vs T1	2·36 (1·01–5·55)	0·0484
N stage		
N1 vs N0	1·16 (0·89–1·52)	0·2770
N2 vs N0	1·58 (1·15–2·17)	0·0052
VELIPI (yes vs no)	1·20 (0·94–1·54)	0·1488
Differentiation		
Moderate vs well	0·91 (0·66–1·24)	0·5403
Poor-undifferentiated vs well	1·37 (0·9–2·08)	0·1421
Mucinous (colloid) type (yes vs no)	1·02 (0·78–1·33)	0·8741
Sidedness distal vs proximal	0·96 (0·76–1·21)	0·7362
Immunoscore		
Intermediate vs low	0·67 (0·52–0·86)	0·0014
High vs low	0·47 (0·33–0·65)	<0·0001
MSI status (MSI vs MSS)	0·93 (0·68–1·27)	0·6356

The significance of the Cox multivariate regression model was evaluated with the Wald p value. None of the parameters violated the Cox proportional hazards assumption (all p value >0·05). Events per total number of patients was 341 deaths per 1107 total patients. C index 0·64 (95% CI 0·59–0·69). HR=hazard ratio. MSI=microsatellite instability. MSS=microsatellite stable. VELIPI=venous emboli, lymphatic invasion, perineural invasion.

Table 2: **Cox multivariate regression analysis of overall survival stratified by centre, combining Immunoscore with T stage, N stage, sex, VELIPI, histological grade, mucinous-colloid type, sidedness, and MSI status**

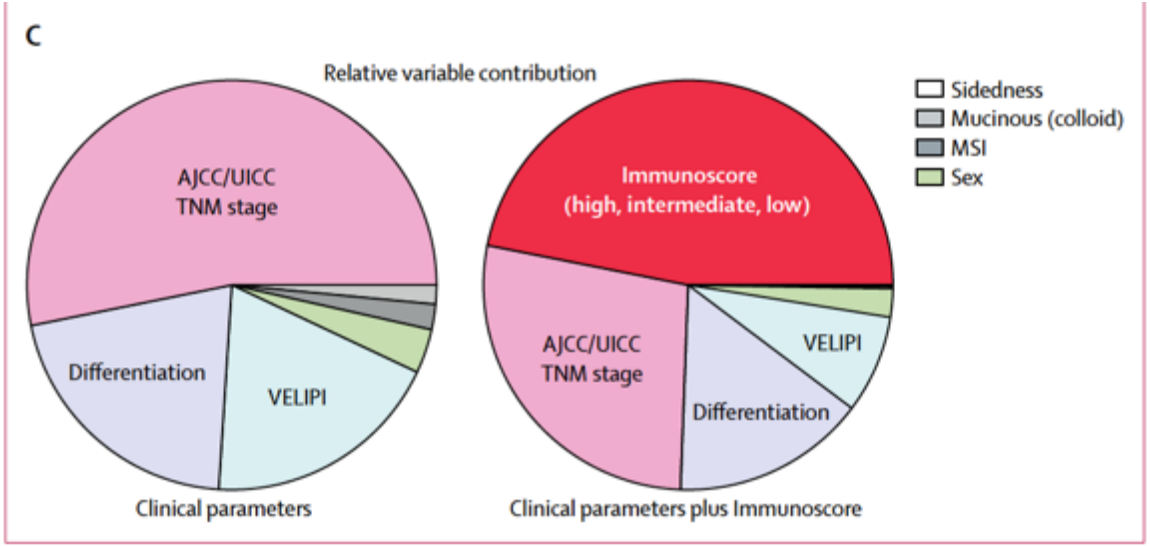


Figure 4: **Performance of Immunoscore compared with clinico-pathological parameters including AJCC/UICC TNM staging**

{Pages F et al, *Lancet*, 2018}

TILs Quantification: Example

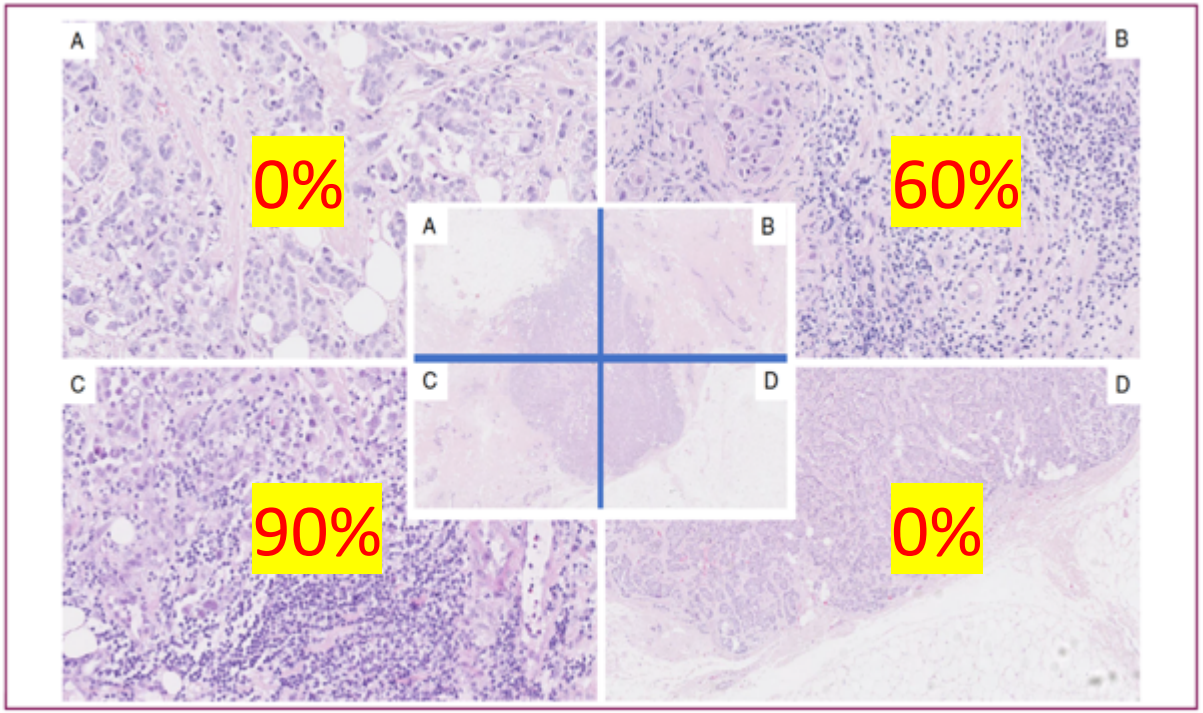
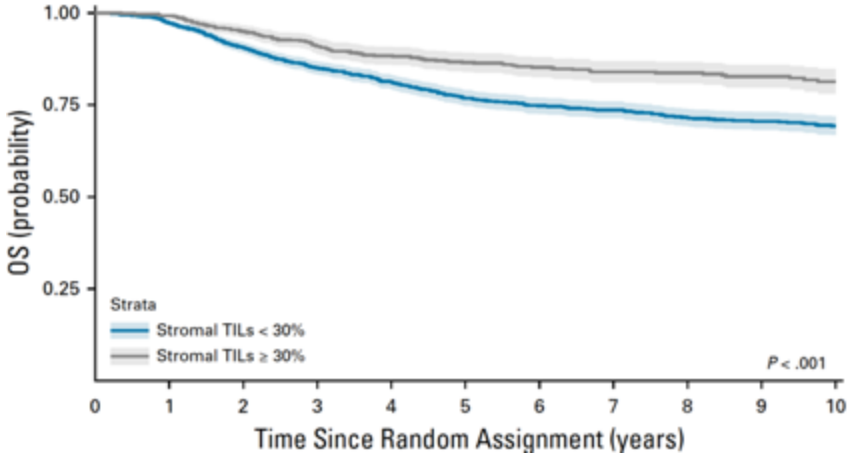


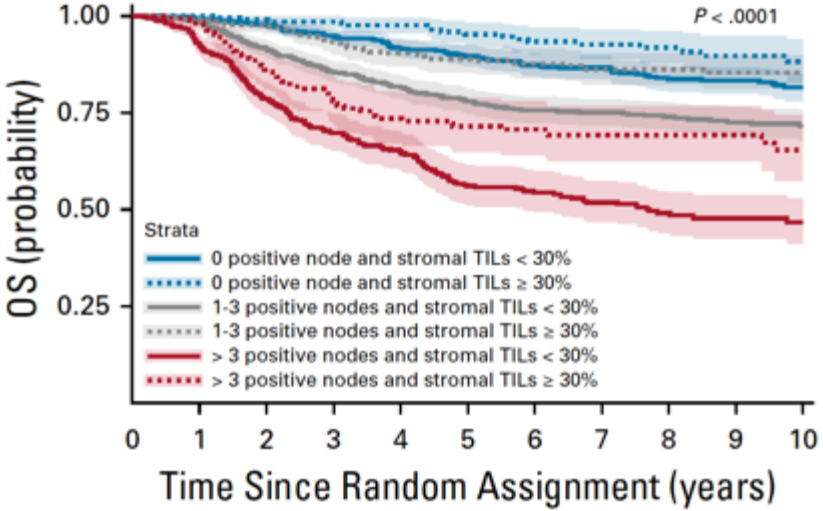
Figure 1. How to assess tumor-infiltrating lymphocytes (TILs) on a full-face hematoxylin and eosin-stained slide.
Panels A-D are regions of the full slide. The stromal score of TILs in zone A is 0%; in zone B it is 60%; in zone C it is 90% and in zone D it is 0%. The TIL score is averaged across each zone: 0% + 60% + 90% + 0% = 150%/4 = 37%, which is averaged by pathologists to 40%.

{Loi S et al. ESMO. 2021}

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

Sherene Loi, MD[1]; Damien Drubay, PhD[2,3]; Sylvia Adams, MD[4]; Giancarlo Pruneri, MD[5]; Prudence A. Francis, MD[1]; Magali Lacroix-Triki, MD[2]; Heikki Joensuu, MD[7]; Maria Vittoria Dieci, MD[8,9]; Sunil Badve, MD[10]; Sandra Demaria, MD[11]; Robert Gray, PhD[12]; Elisabetta Munzone, MD[13]; Jerome Lemonnier, PhD[6]; Christos Sotiriou, MD[14]; Martine J. Piccart, MD[14]; Pirkko-Liisa Kellokumpu-Lehtinen, MD[15]; Andrea Vingiani, MD[16]; Kathryn Gray, PhD[12]; Fabrice Andre, MD[2,3]; Carsten Denkert, MD[17]; Roberto Salgado, MD[1,18]; and Stefan Michiels, PhD[2,3]





{Loi S et al. JCO. 2019}

Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancers From Two Phase III Randomized Adjuvant Breast Cancer Trials: ECOG 2197 and ECOG 1199

Sylvia Adams, Robert J. Gray, Sandra Demaria, Lori Goldstein, Edith A. Perez, Lawrence N. Shulman, Silvana Martino, Molin Wang, Vicky E. Jones, Thomas J. Saphner, Antonio C. Wolff, William C. Wood, Nancy E. Davidson, George W. Sledge, Joseph A. Sparano, and Sunil S. Badve

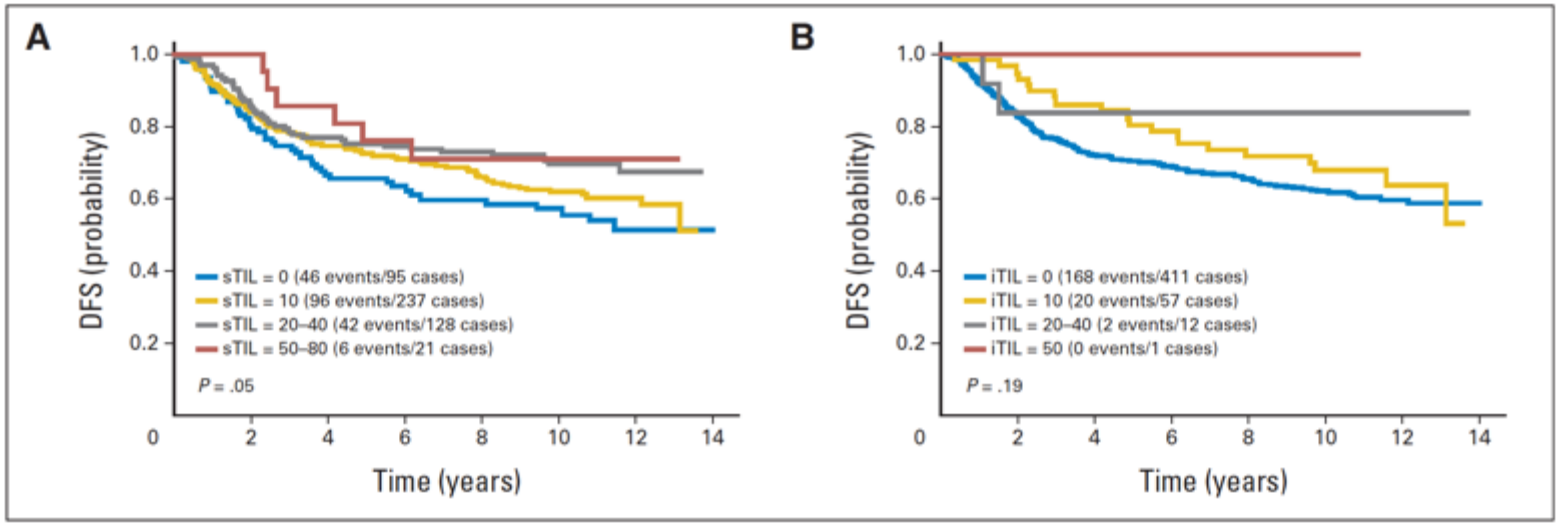


Fig 2. Prognostic value of tumor-infiltrating lymphocytes (TILs) in triple-negative breast cancer. Kaplan-Meier curves of estimated disease-free survival (DFS) for all patients for (A) stromal TIL (sTIL) score and (B) intraepithelial TIL (iTIL) score (grouped as 0 [defined as 0% to 1%] *v* 10 [2% to 10%] *v* 20 to 40 [11% to 40%] *v* 50 [41% to 50%] or *v* 50 to 80 [41% to 80%]); *P* values are for comparison of four groups.

{Adams S et al. J Clin Oncol. 2014}

Prognostic Value of Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancers From Two Phase III Randomized Adjuvant Breast Cancer Trials: ECOG 2197 and ECOG 1199

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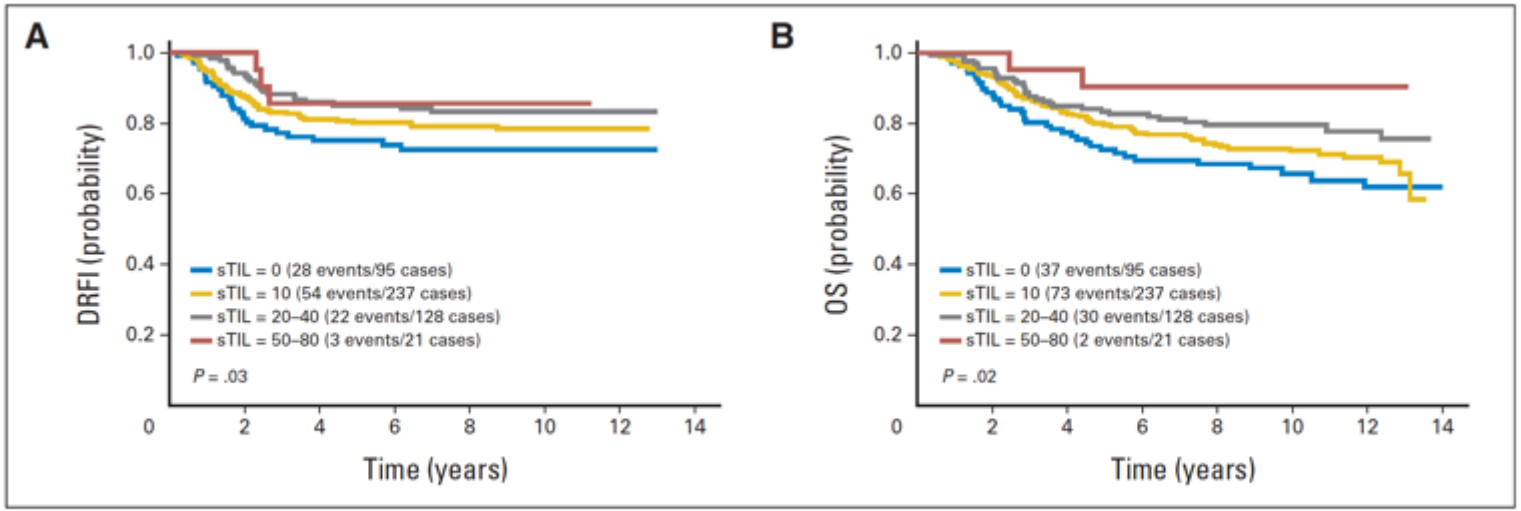


Fig 3. Prognostic value of stromal tumor-infiltrating lymphocytes (sTILs) in triple-negative breast cancer. Kaplan-Meier curves of estimated (A) distant recurrence–free interval (DRFI) and (B) overall survival (OS) for all patients for sTILs (grouped as 0 [defined as 0% to 1%] *v* 10 [2% to 10%] *v* 20 to 40 [11% to 40%] *v* 50 to 80 [41% to 80%]); *P* values are for comparison of four groups.

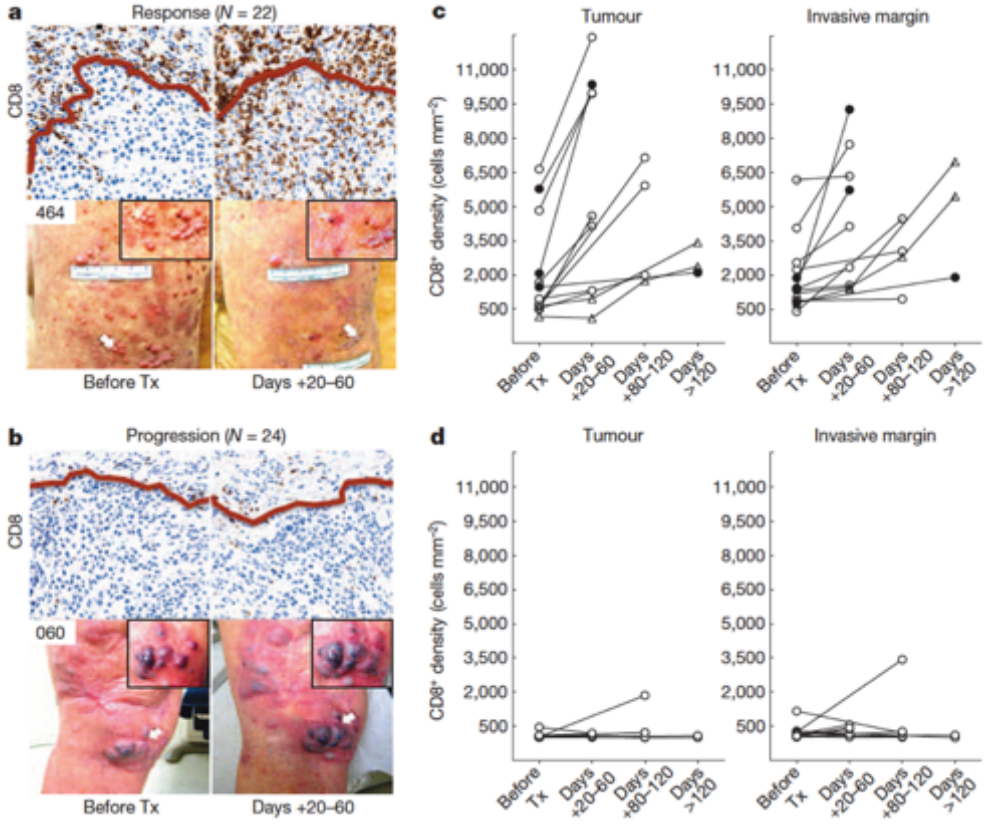
{Adams S et al. J Clin Oncol. 2014}

TILs as predictive biomarker

Predictive Biomarkers for ICI

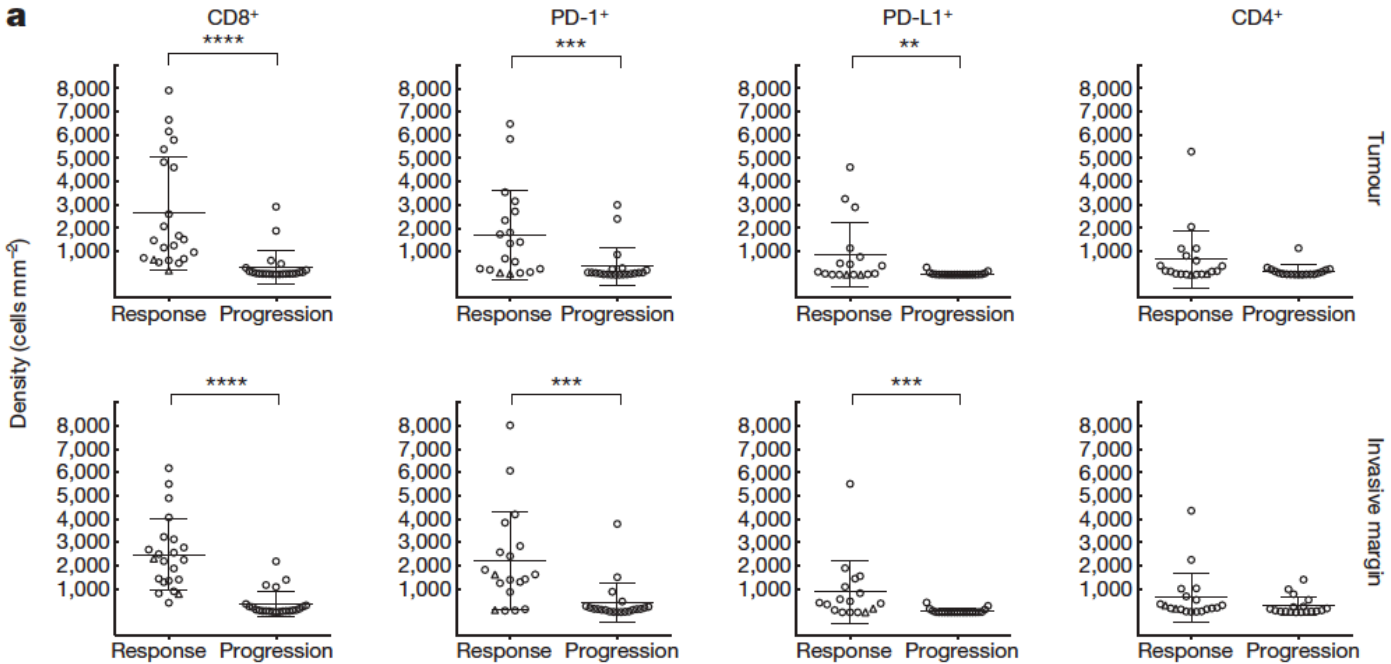
- **Pre-existing adaptive immune response** is considered a prerequisite for efficacy of PD-1/PD-L1 blockade.
- Most immunotherapy biomarkers (such as PD-L1, immune GEP, TILs) are measuring pre-existing immune activity in the TME.
- Ideal predictive biomarker goes beyond just enriching for responders and should allow a **binary decision** (Yes/No) in terms of choosing therapy. e.g. BRAF V600 mutation for selecting BRAFi in metastatic melanoma

Melanoma: CD8+ (at IM) correlates with response



{Tumeh PC et al. Nature. 2014}

Melanoma: TILs and PD-(L)1 mostly track together



{Tumeh PC et al. Nature. 2014}

Tumor mutational burden and immune infiltration as independent predictors of response to neoadjuvant immune checkpoint inhibition in early TNBC in GeparNuevo

T. Karn[1*], C. Denkert[2], K. E. Weber[3], U. Holtrich[1], C. Hanusch[4], B. V. Sinn[5], B. W. Higgs[6], P. Jank[2], H. P. Sinn[7], J. Huober[8], C. Becker[4], J.-U. Blohmer[5], F. Marmé[7], W. D. Schmitt[5], S. Wu[6], M. van Mackelenbergh[9], V. Müller[10], C. Schem[11], E. Stickeler[12], P. A. Fasching[13], C. Jackisch[14], M. Untch[15], A. Schneeweiss[16] & S. Loibl[3]

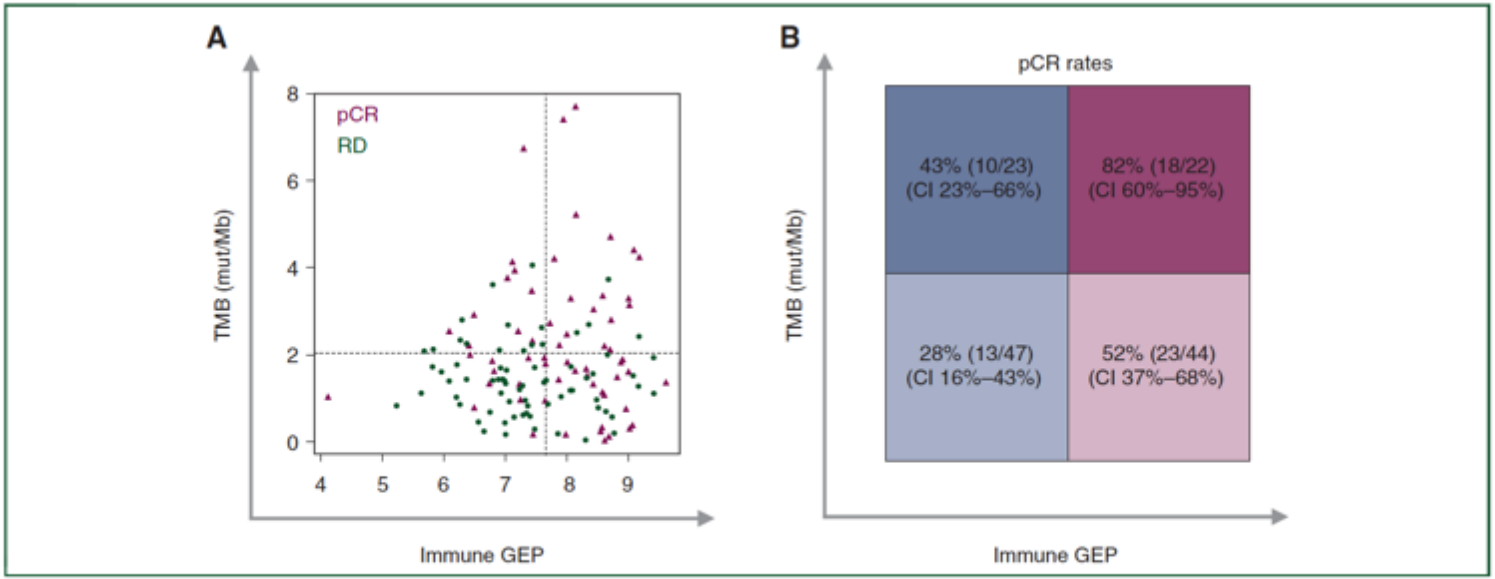
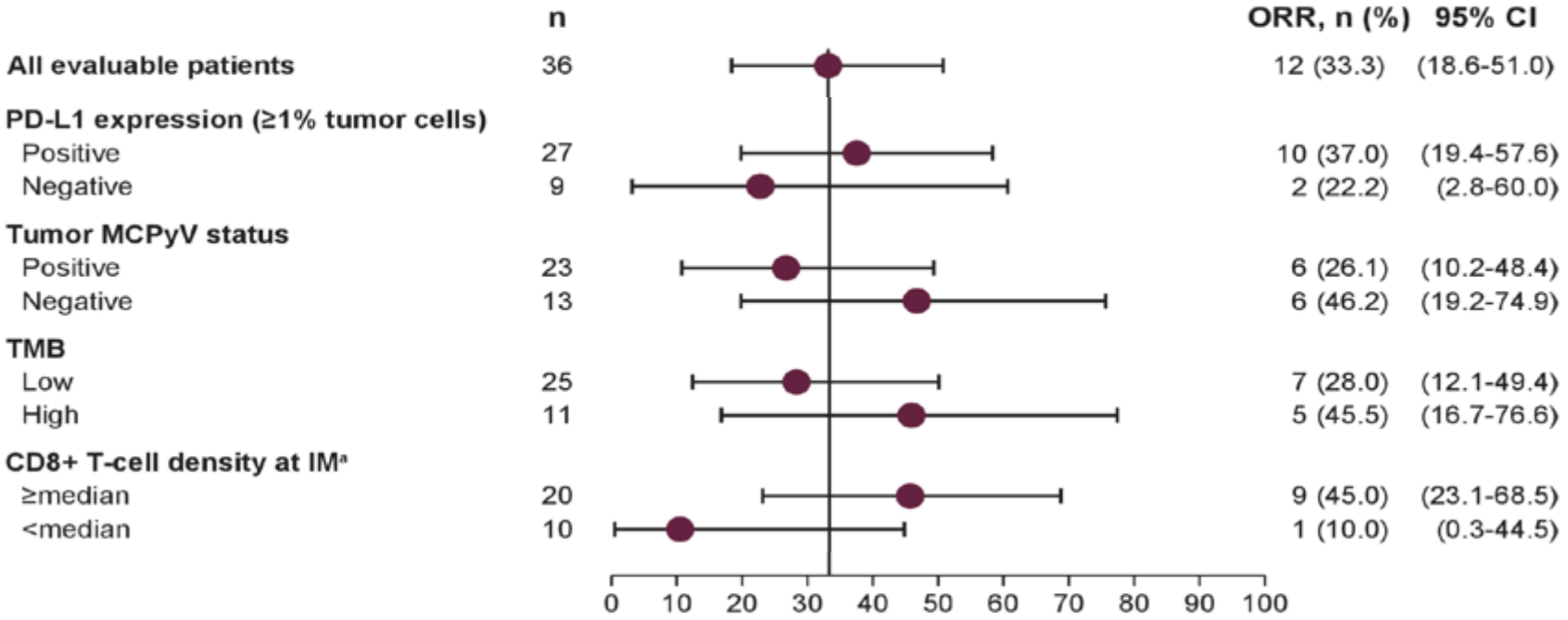


Figure 2. Joint relationship of tumor mutational burden (TMB) and immune gene expression profile (GEP) with pCR in GeparNuevo.
(A) Scatter plot of TMB and immune GEP in pretreatment biopsies of GeparNuevo patients colored by response [burgundy triangles, pathological complete remission (pCR); green circles, residual disease (RD)]. Cutoffs of median GEP and upper tertile of TMB are given by dashed vertical and horizontal lines, respectively. (B) pCR rates in percentages and 95% confidence intervals (CI) in subgroups defined by the cutoffs given as dashed lines in A.

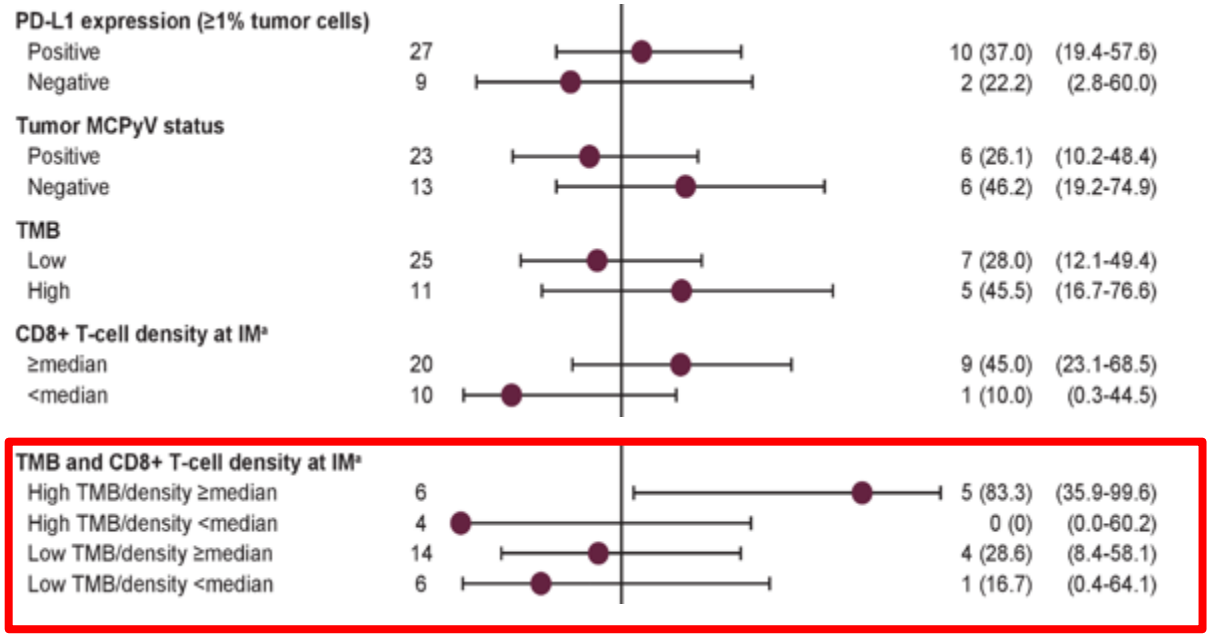
Currently, there are NO clinically useful biomarkers to predict ICI response to MCC



{D'Angelo SP, Bhatia S et al. *JITC* 2020}

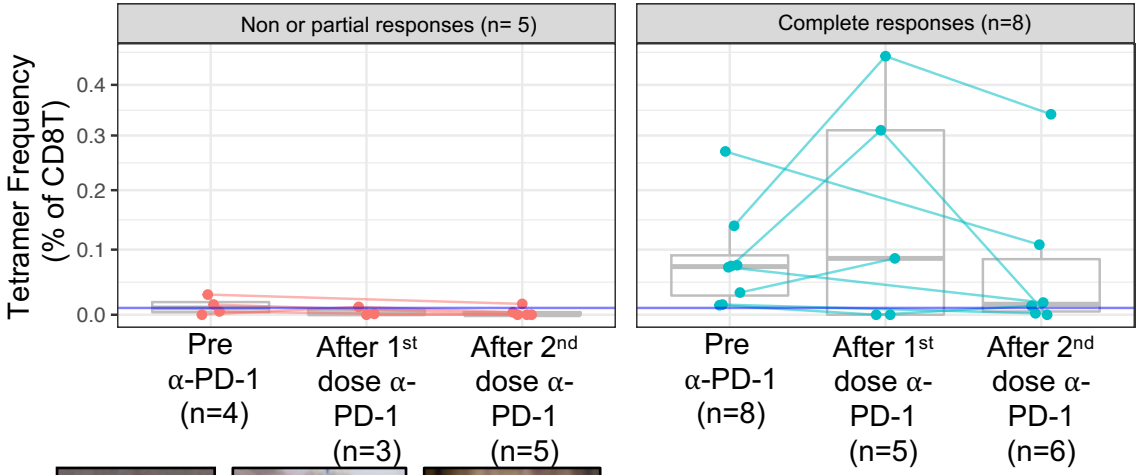
Combining biomarkers may improve predictive utility

ORR with Avelumab in metastatic Merkel cell carcinoma



{D'Angelo SP, Bhatia S et al. *JITC* 2020}

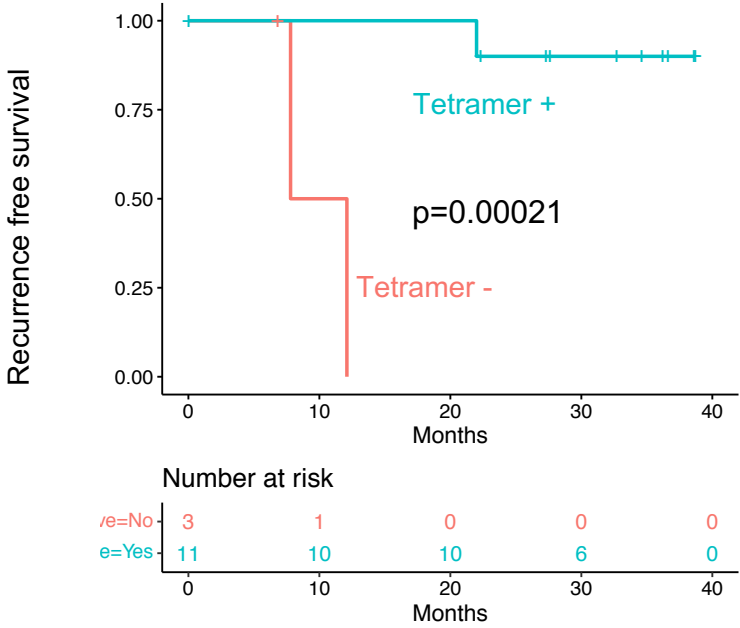
Frequency of peripheral MCPyV-specific CD8 T cells predicts anti-PD-1 response



Thomas Pulliam, PhD candidate

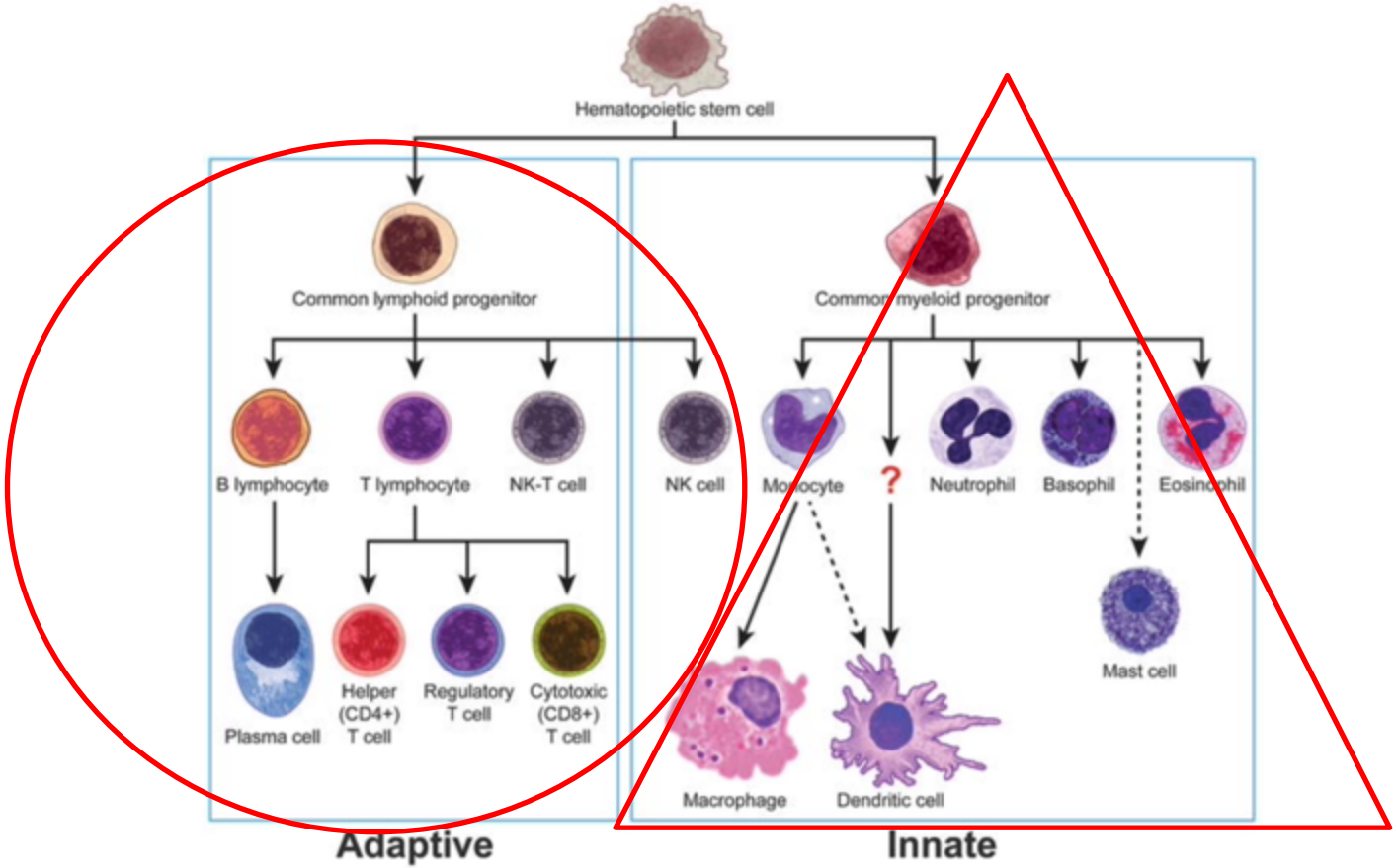
Lichen Jing, PhD

David Koelle, PhD



(Pulliam et al unpublished)

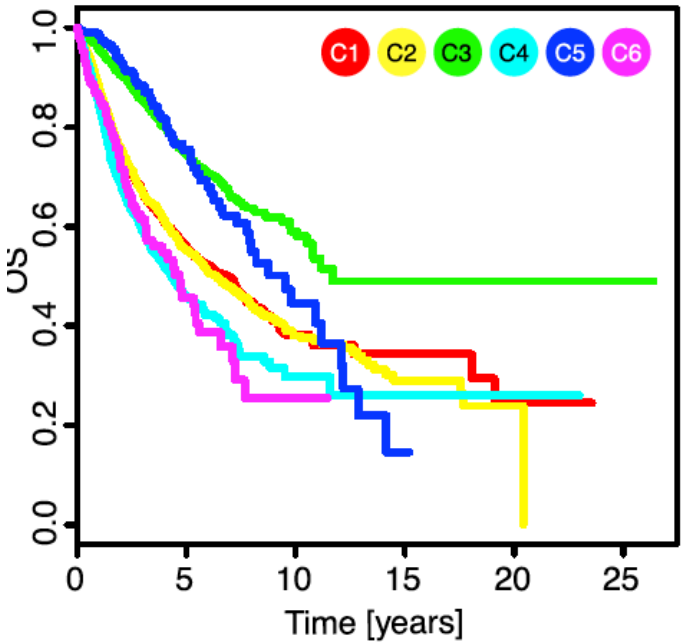
Tumor Immune Infiltrate: More than just lymphocytes!



The Immune Landscape of Cancer

Vésteinn Thorsson,[1,37,*] David L. Gibbs,[1,36] Scott D. Brown,[2] Denise Wolf,[3] Dante S. Bortone,[4] Tai-Hsien Ou Yang,[5] Eduard Porta-Pardo,[6,7] Galen F. Gao,[8] Christopher L. Plaisier,[1,9] James A. Eddy,[10] Elad Ziv,[11] Aedin C. Culhane,[12] Evan O. Paull,[13] I.K. Ashok Sivakumar,[14] Andrew J. Gentles,[15] Raunaq Malhotra,[16] Farshad Farshidfar,[17] Antonio Colaprico,[18] Joel S. Parker,[4] Lisle E. Mose,[4] Nam Sy Vo,[19] Jianfang Liu,[20] Yuexin Liu,[19] Janet Rader,[21] Varsha Dhankani,[1] Sheila M. Reynolds,[1] Reanne Bowlby,[2] Andrea Califano,[13] Andrew D. Cherniack,[8] Dimitris Anastassiou,[5] Davide Bedognetti,[22] Younes Mokrab,[22] Aaron M. Newman,[35] Arvind Rao,[19] Ken Chen,[19] Alexander Krasnitz,[23] Hai Hu,[20] Tathiane M. Malta,[24,25] Houtan Noushmehr,[24,25] Chandra Sekhar Pedamallu,[26]

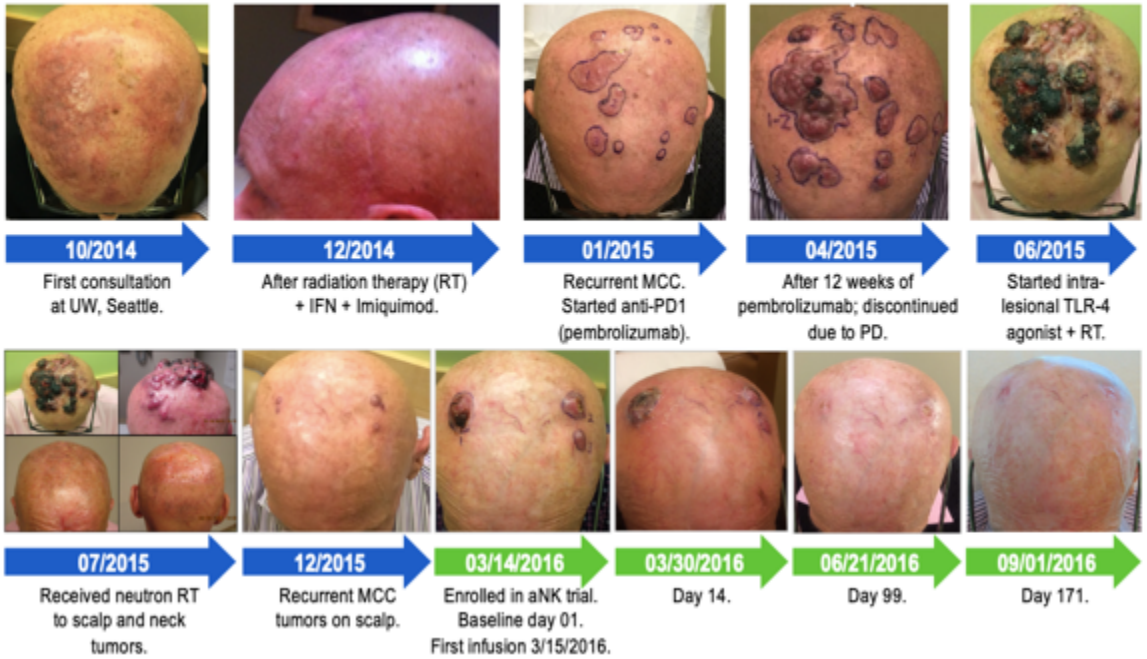
	Macrophage: lymphocyte	Th1:Th2	Proliferation	Intratumoral heterogeneity	Other
Wound healing	Balanced	Low	High	High	
IFN-γ dominant	Lowest	Lowest	High	Highest	Highest M1 and highest CD8 T cells
Inflammatory	Balanced	High	Low	Lowest	Highest Th17
Lymphocyte depleted	High	Minimal Th	Moderate	Moderate	
Immunologically quiet	Highest	Minimal Th	Low	Low	Highest M2
TGF-β dominant	High	Balanced	Moderate	Moderate	Highest TGF-β signature



{Thorsson V et al, *Immunity*, 2019}

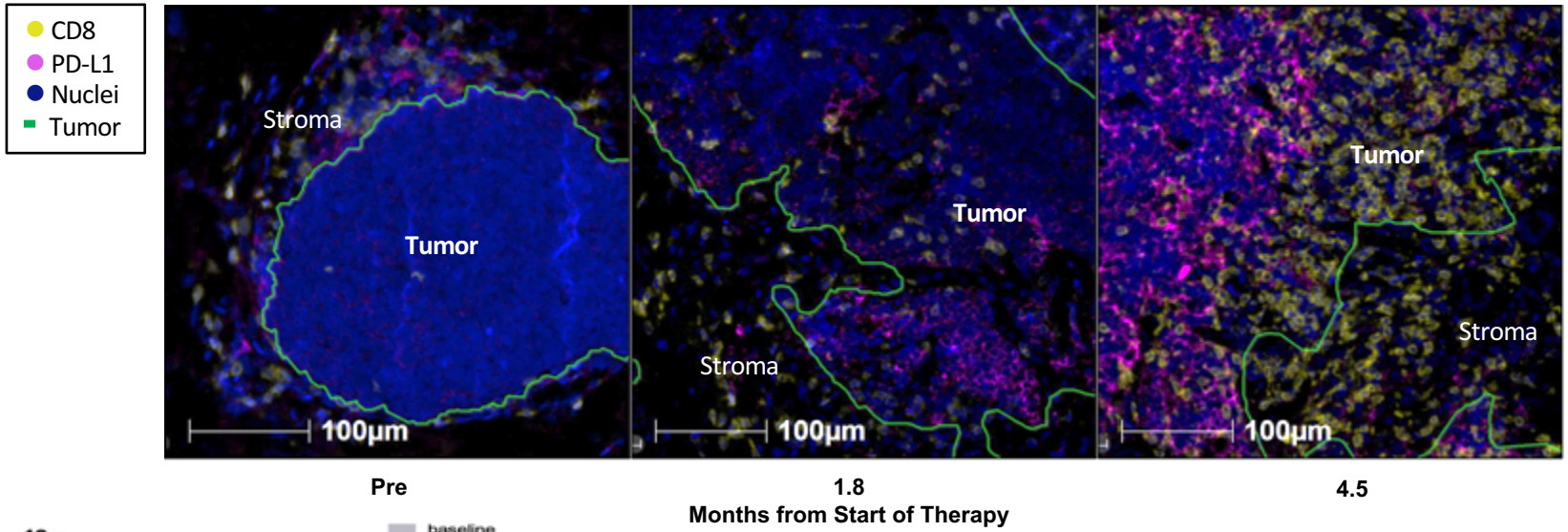
Reminder: Cancer Immunotherapy is not just ICIs

Fig 1: Response with aNK Cell Therapy in a Patient With MCC Refractory to Chemotherapy, Radiation Therapy, and PD-1 Blockade



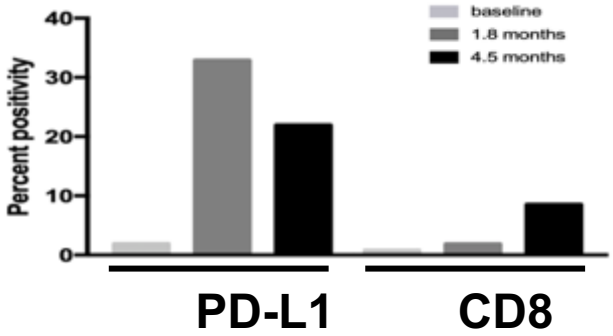
[Bhatia S *et al*; *Annual SITC meeting; 2019*]

TILs can have dynamic changes with therapy



Kimberly Smythe

Candice Church

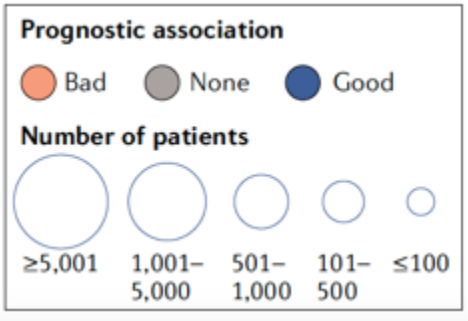


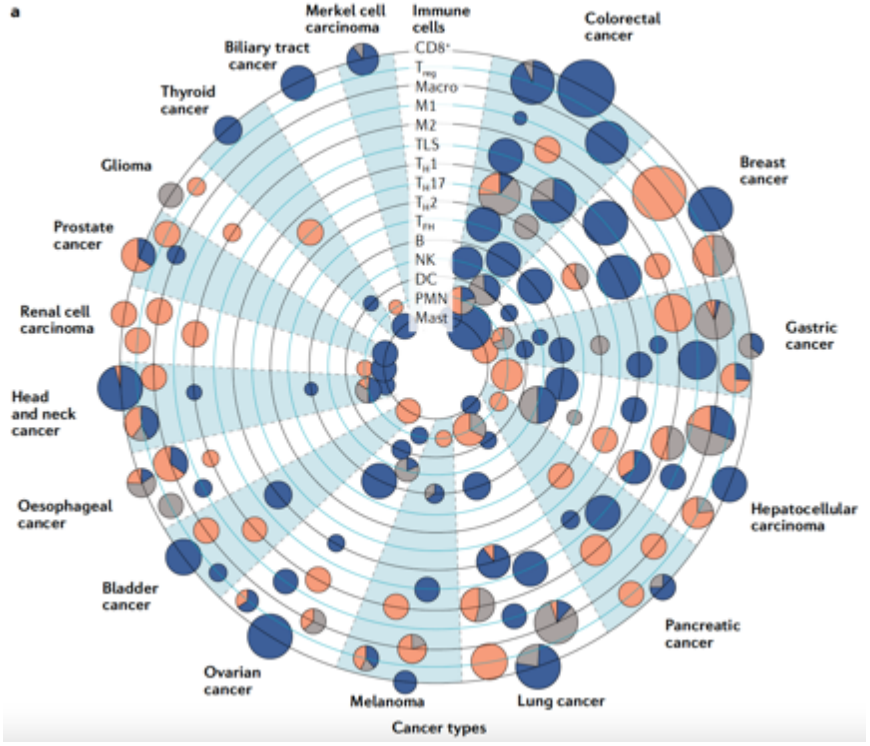
PD-1 refractory MCC patient: PD-L1 and Immune cell infiltration in the TME are increased after aNK Monotherapy and led to reversal of PD-1 refractoriness

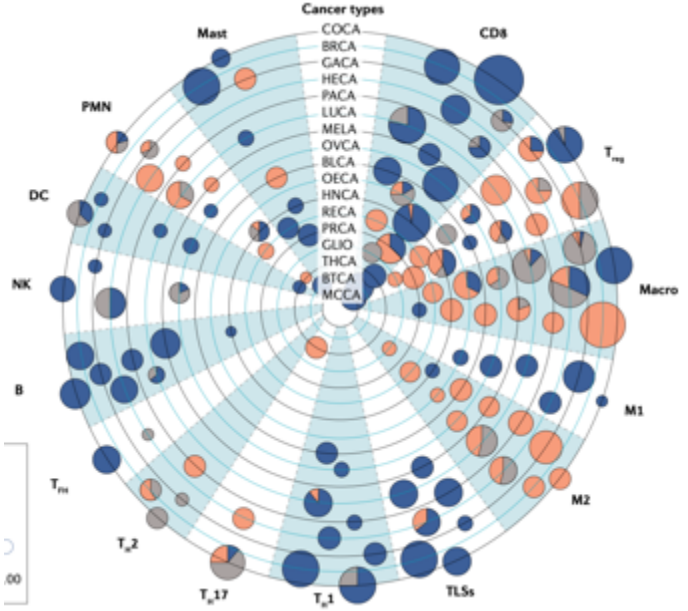
[Bhatia S *et al*; *Annual SITC meeting; 2019*]

The immune contexture and Immunoscore in cancer prognosis and therapeutic efficacy

Daniela Bruni[1,5], *Helen K. Angell*[2,5] *and Jérôme Galon*[1]✉

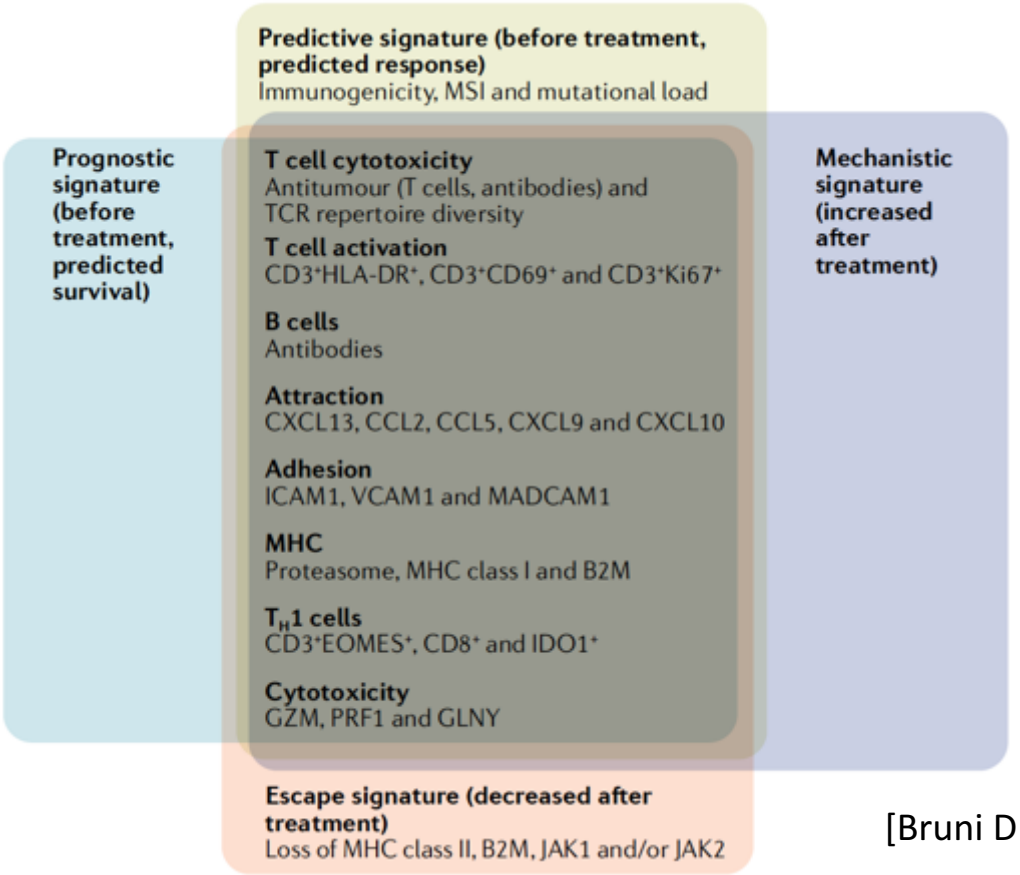






[Bruni D *et al*; *Nat Rev; 20120*]

Overlap between Immune Signatures



[Bruni D *et al*; *Nat Rev; 20120*]

TILs as a biomarker: Summary

- Inexpensive (needs a H&E slide); good reproducibility; tracks well with other immune markers such as PD-L1.
- Prognostic impact of TILs is well established in several cancer types; TNM-immunoscore is being adopted in colon cancer staging.
- However, tumor-immune interactions are complex, with multiple types of immune cells and signatures affecting outcomes.
- Data on TILs to predict responses to immunotherapy is emerging rapidly. Stay tuned!!