

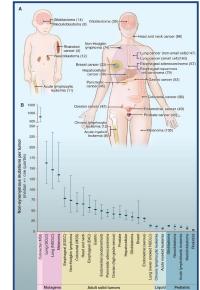
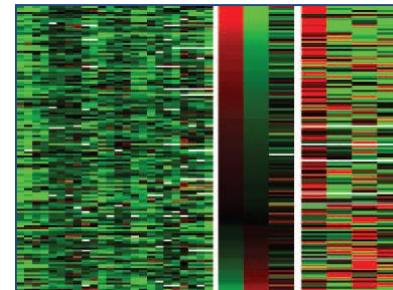
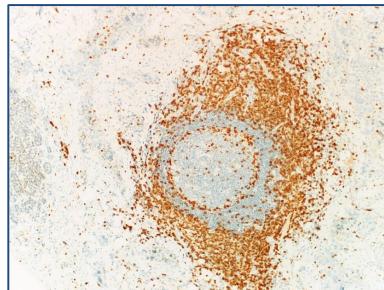
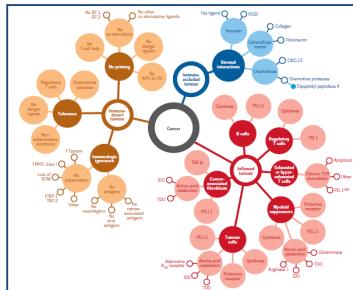
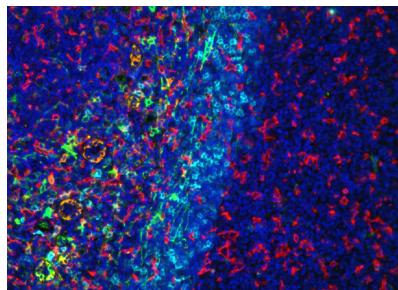


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8-10 ΟΚΤΩΒΡΙΟΥ 2021
ΗΡΑΚΛΕΙΟ ΚΡΗΤΗΣ

ΔΙΟΡΓΑΝΩΣΗ
ΠΑΓΚΡΗΤΙΑ
ΕΝΩΣΗ
ΥΓΕΙΑΣ



17:00-19:00

Cancer Immunology and Immunotherapy Chair: P. Verginis – P. Foukas

- Tumor microenvironment (P. Foukas)
- Immunotherapy in cancer: Resistance mechanisms in solid tumors (O. Tsitsiloni)
- Colon cancer (I. Souglakos)
- Bone marrow malignancies - Immunotherapy (C. Pontikoglou)

Περικλής Γ. Φούκας
Β' Εργαστήριο Παθολογικής Ανατομικής,
Ιατρικής Σχολής ΕΚΠΑ,
Π.Γ.Ν Αττικόν



Outline

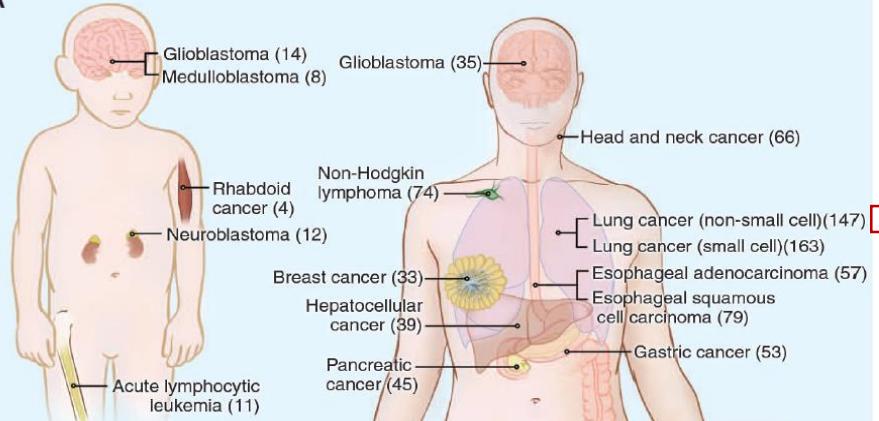
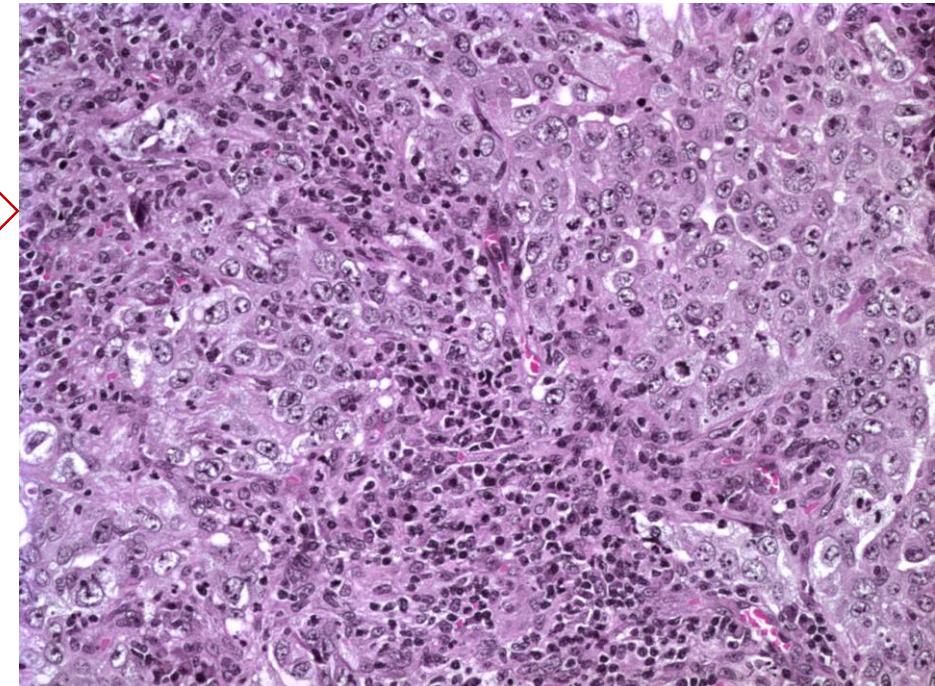
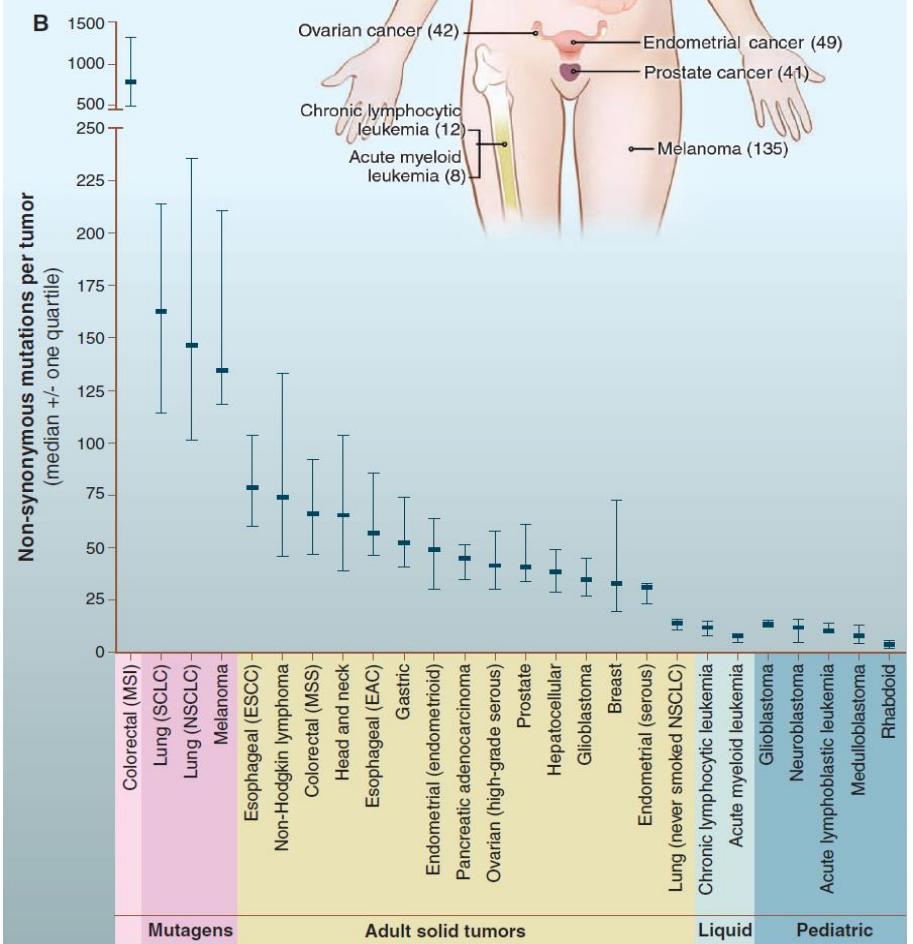
- Introduction to the Tumor Immune Microenvironment (TIME)
 - Prognostic / Predictive value
- Mechanisms regulating TIME
- Evaluation / Methodologies
- Turning-up the heat



Outline

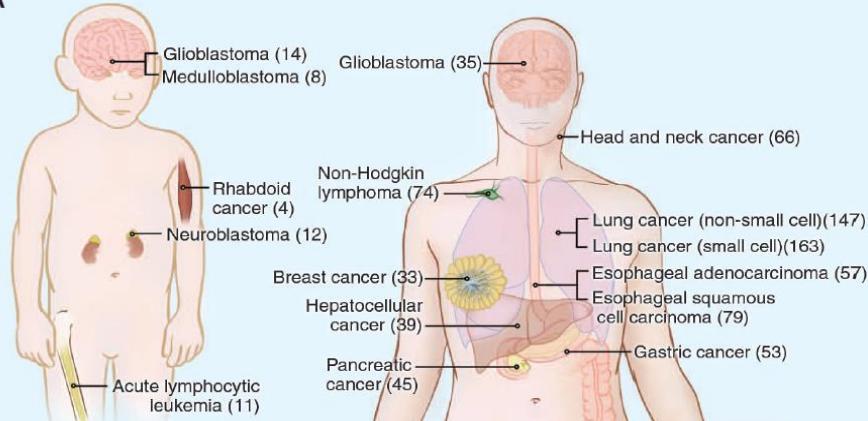
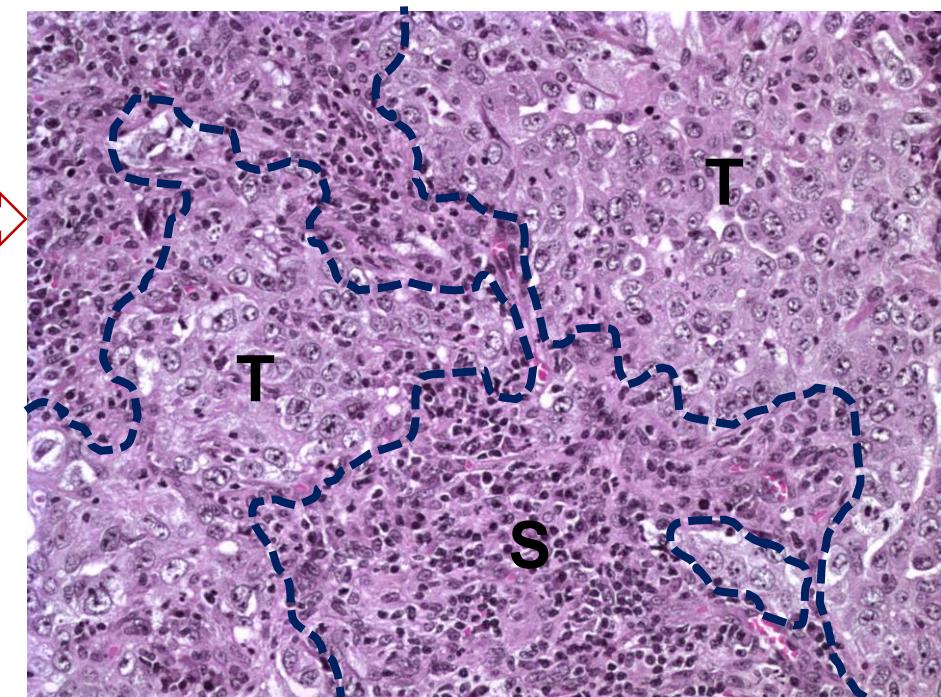
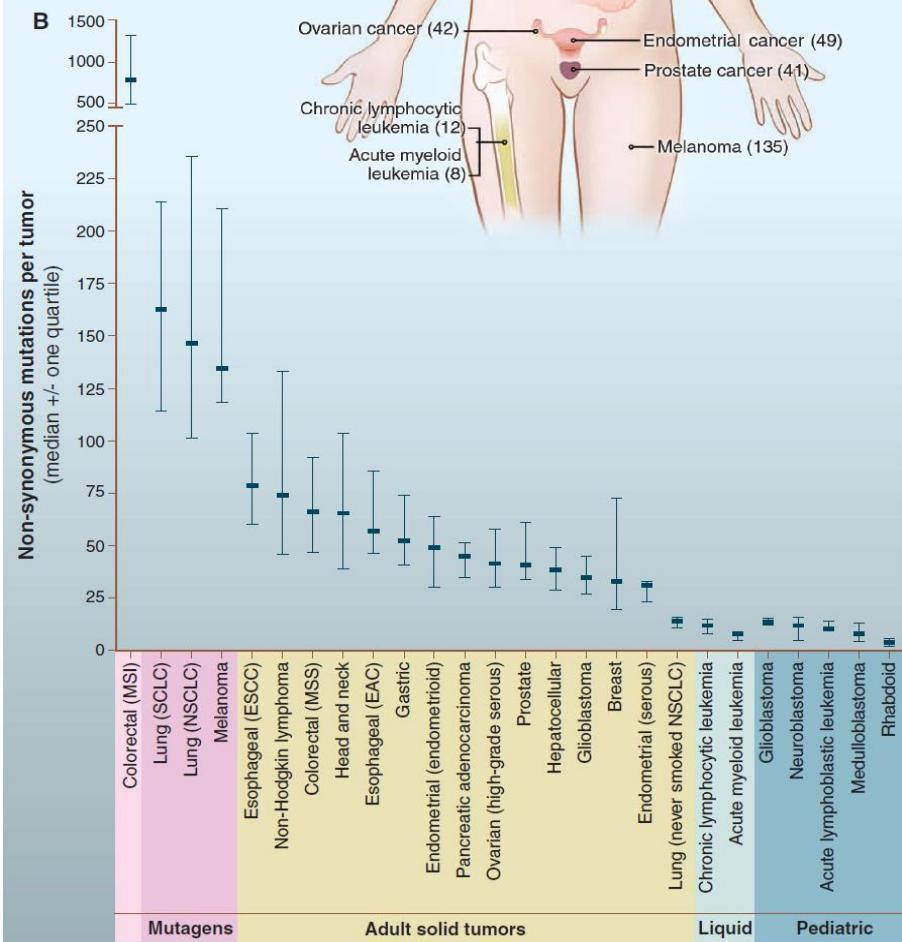
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A**B**

Cancer Genome Landscapes

Bert Vogelstein, Nickolas Papadopoulos, Victor E. Velculescu, Shabin Zhou,
Luis A. Diaz Jr., Kenneth W. Kinzler*

A**B**

Cancer Genome Landscapes

Bert Vogelstein, Nickolas Papadopoulos, Victor E. Velculescu, Shabin Zhou,
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Tumor Immunology and Tumor Evolution: Intertwined Histories

Jérôme Galon^{1,*} and Daniela Bruni¹

Immunity 52, January 14, 2020

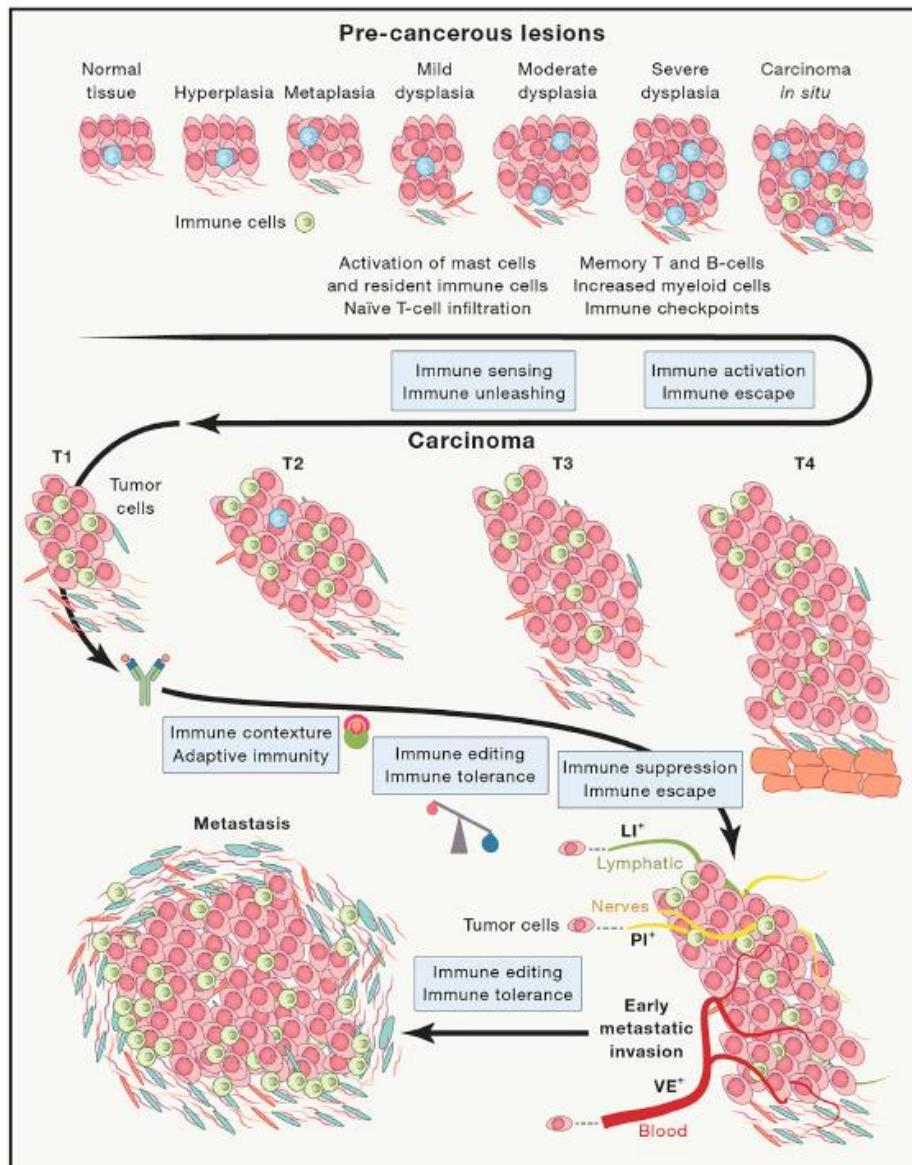
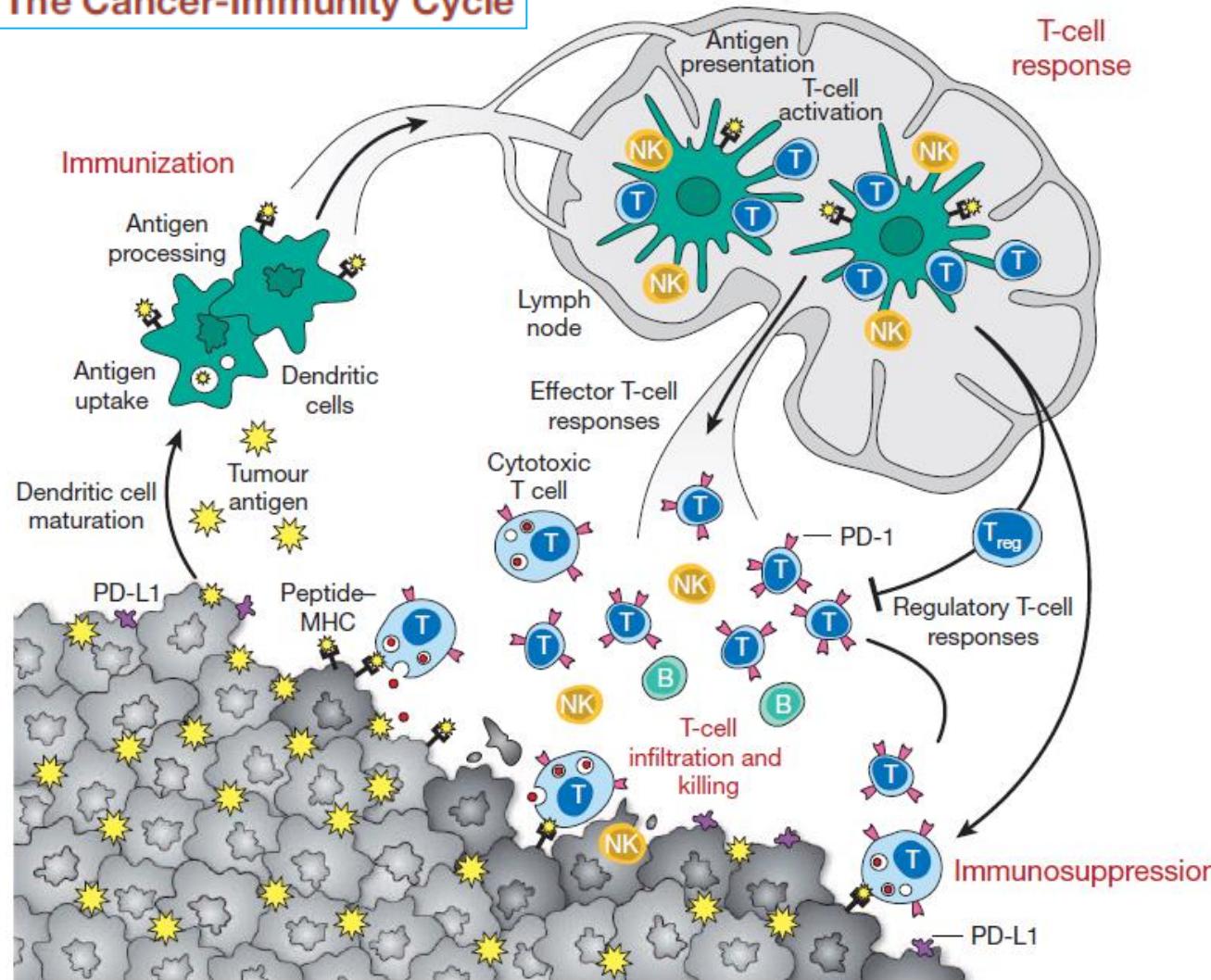


Figure 2. History of Cancer: from Pre-cancerous Lesions, to Primary Tumors, to Metastases

Cancer immunotherapy comes of age

Ira Mellman¹, George Coukos² & Glenn Dranoff³

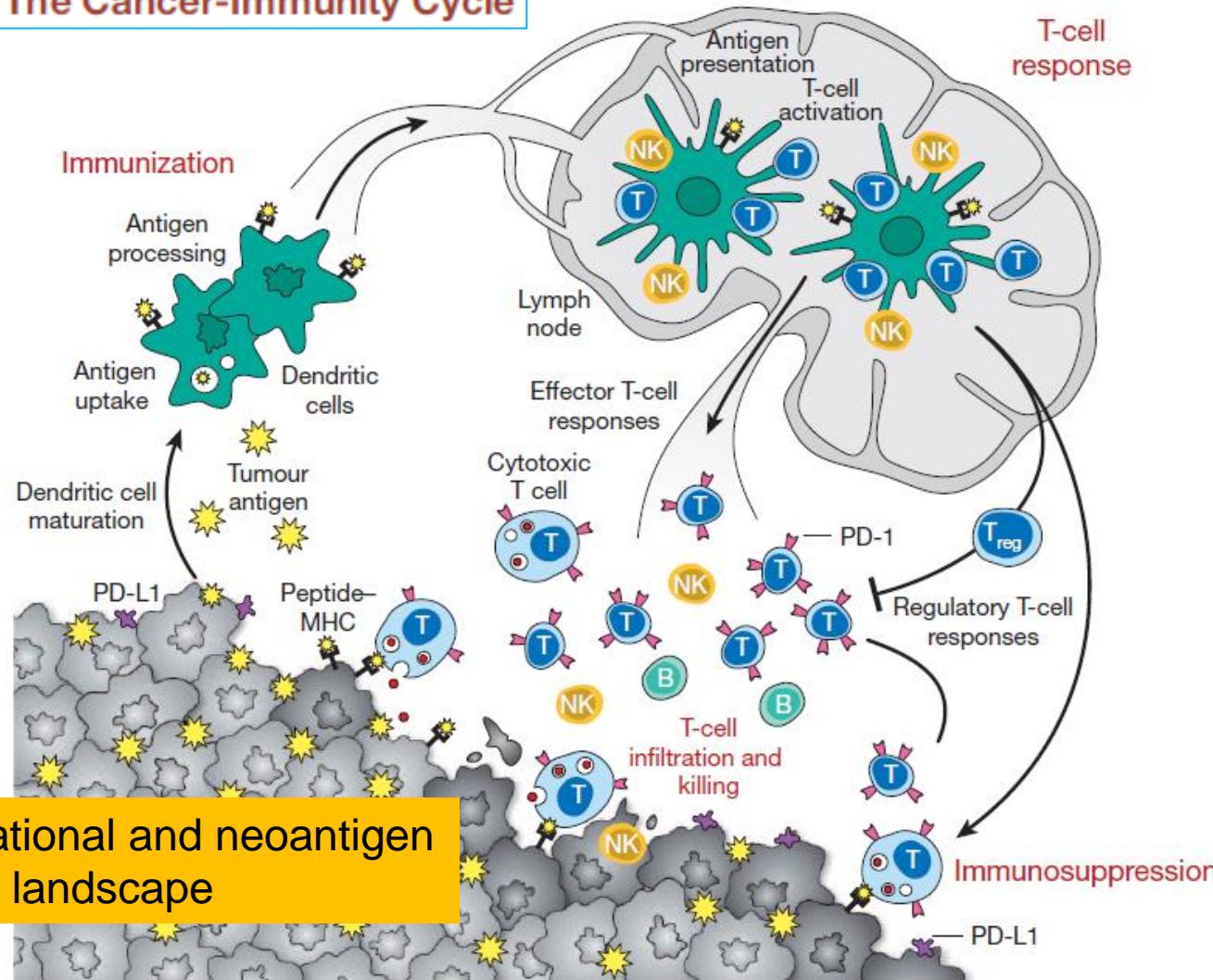
The Cancer-Immunity Cycle



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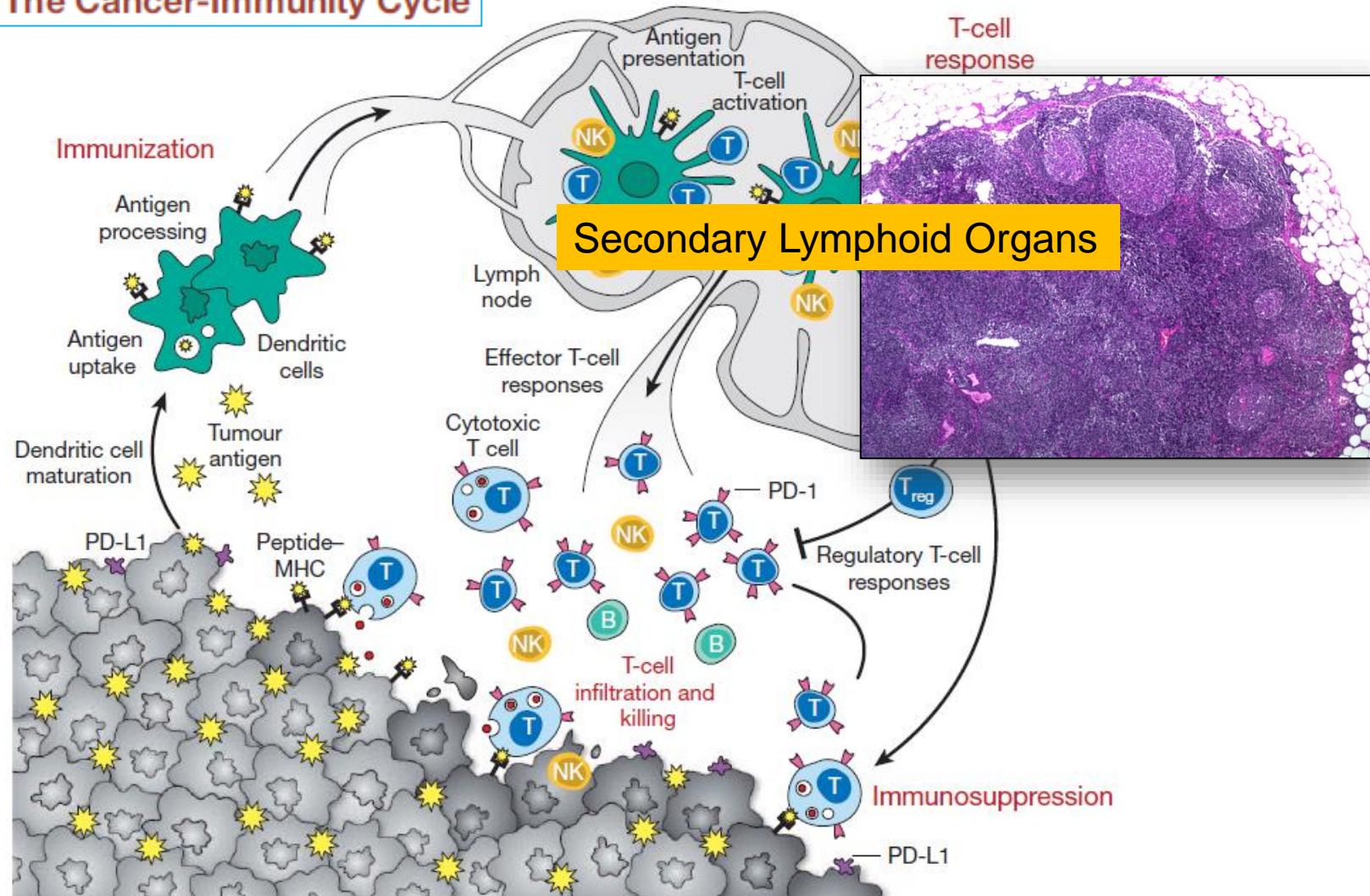
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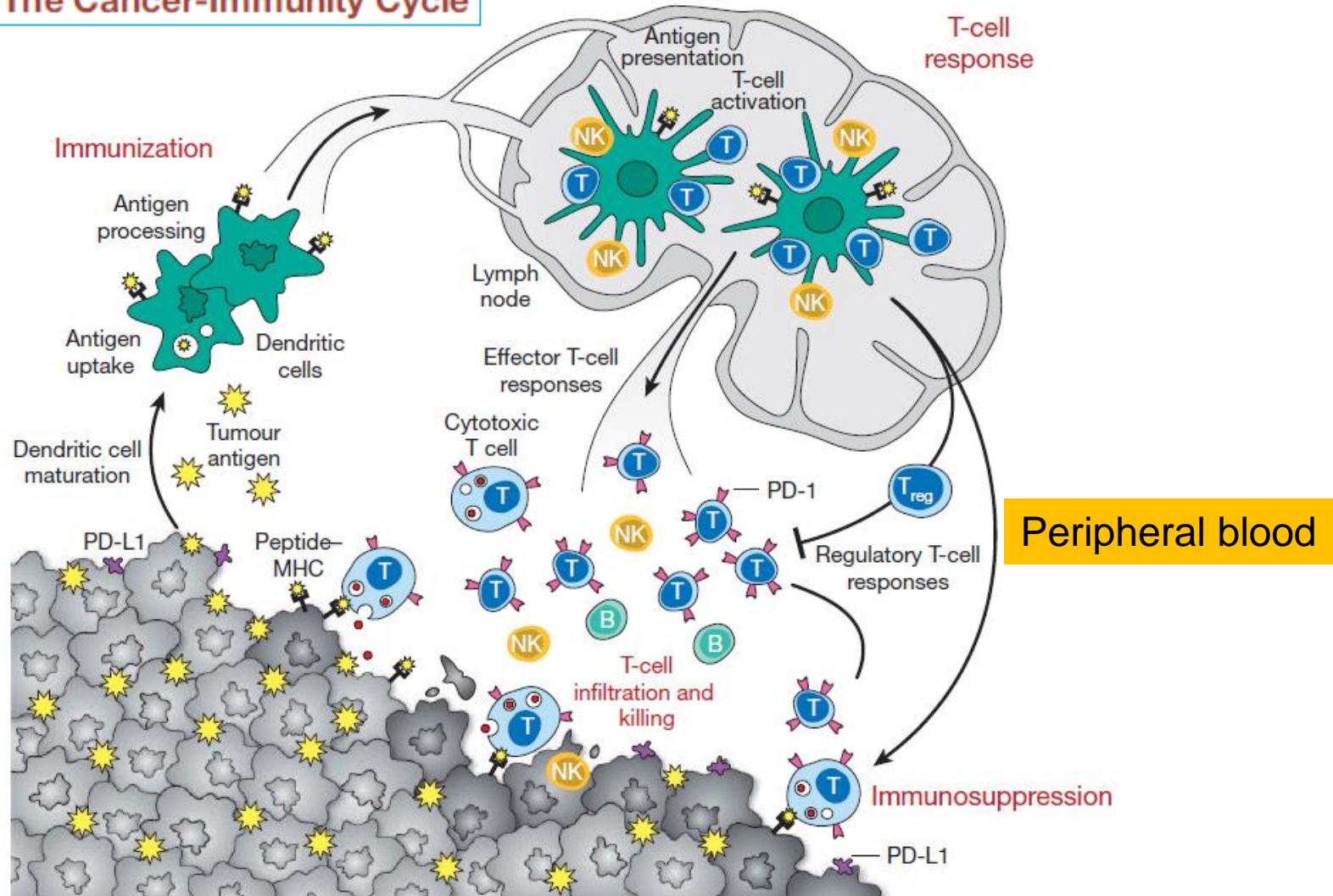
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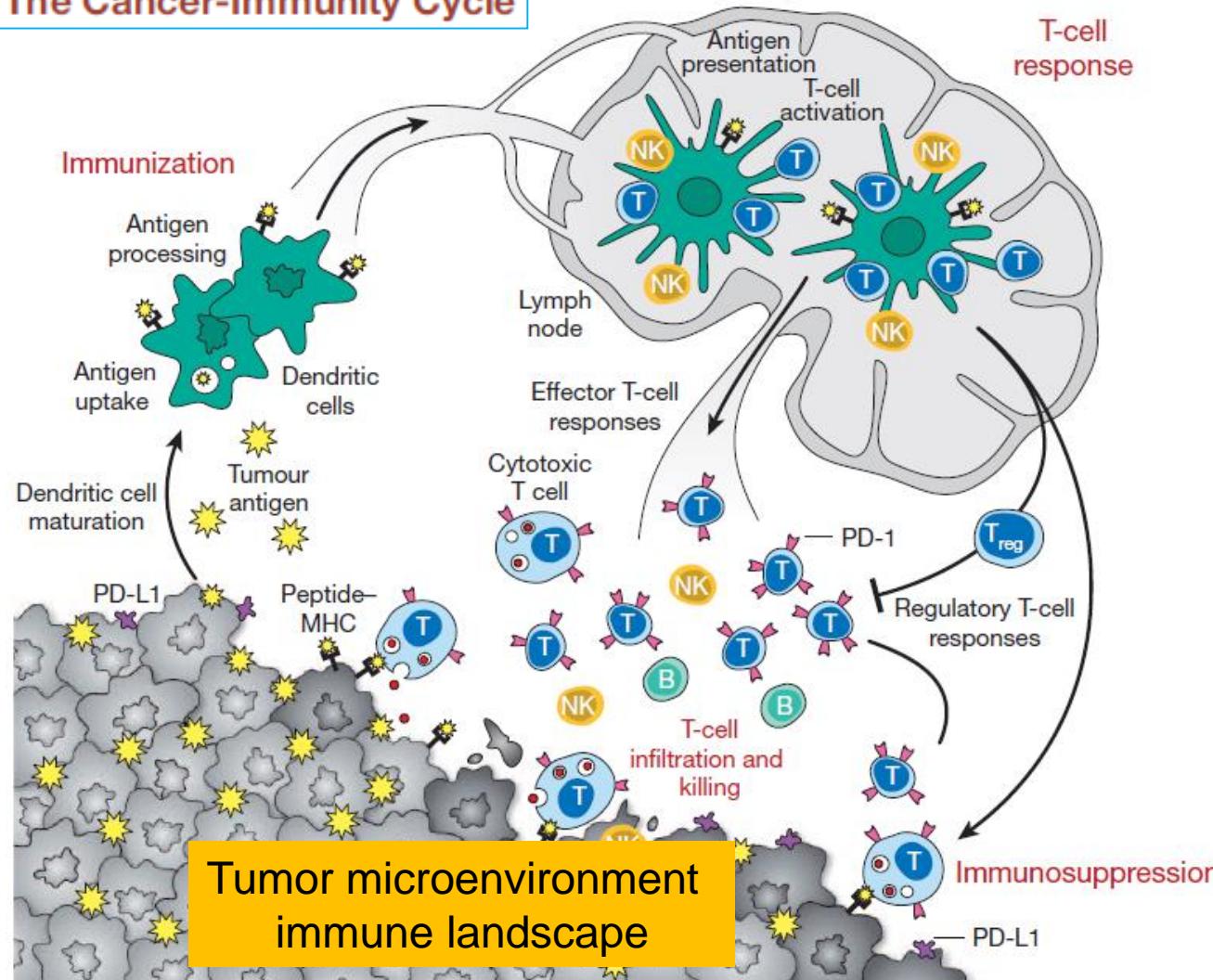
The Cancer-Immunity Cycle



Cancer immunotherapy comes of age

Ira Mellman¹, George Coukos² & Glenn Dranoff³

The Cancer-Immunity Cycle





Novel technologies and emerging biomarkers for personalized cancer immunotherapy

Jianda Yuan^{1*}, Priti S. Hegde², Raphael Clynes³, Periklis G. Foukas^{4,5}, Alexandre Harari⁴, Thomas O. Kleen⁶, Pia Kvistborg⁷, Cristina MacCall⁸, Holden T. Maecker⁹, David B. Page¹⁰, Harlan Robins¹¹, Wenru Song¹², Edward C. Stack¹³, Ena Wang¹⁴, Theresa L. Whiteside¹⁵, Yingdong Zhao¹⁶, Heinz Zwierzina¹⁷, Lisa H. Butterfield¹⁸ and Bernard A. Fox^{10*}

Novel immune monitoring assays for biomarker discovery and personalized cancer immunotherapy

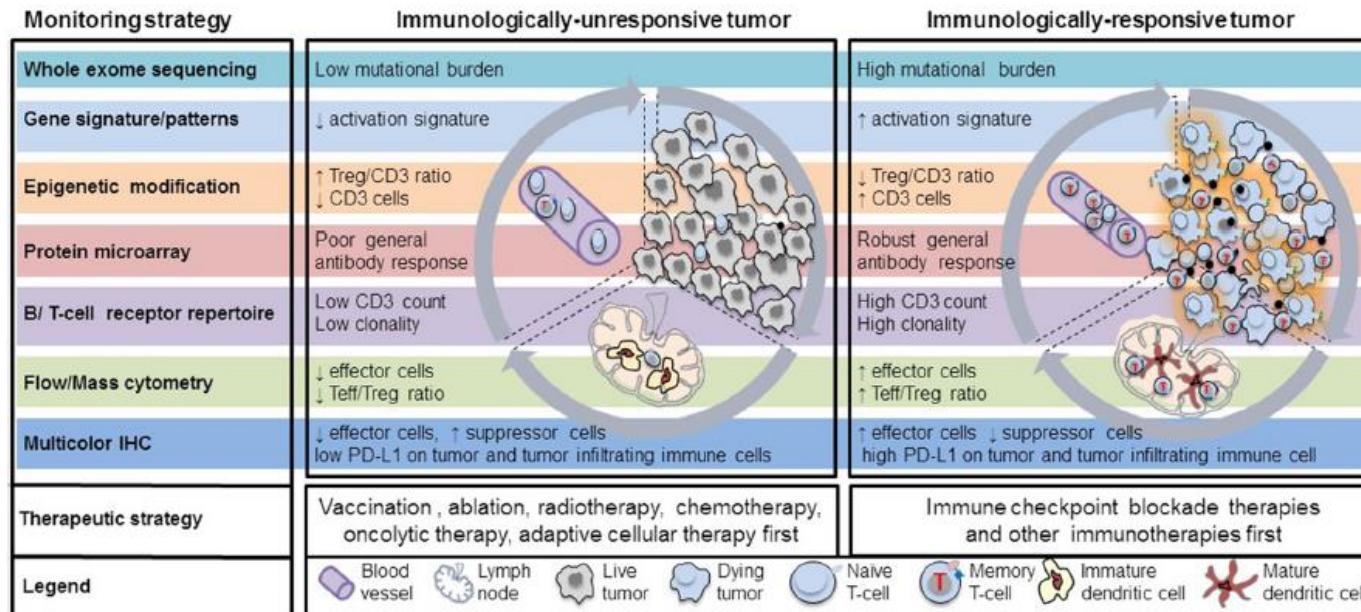
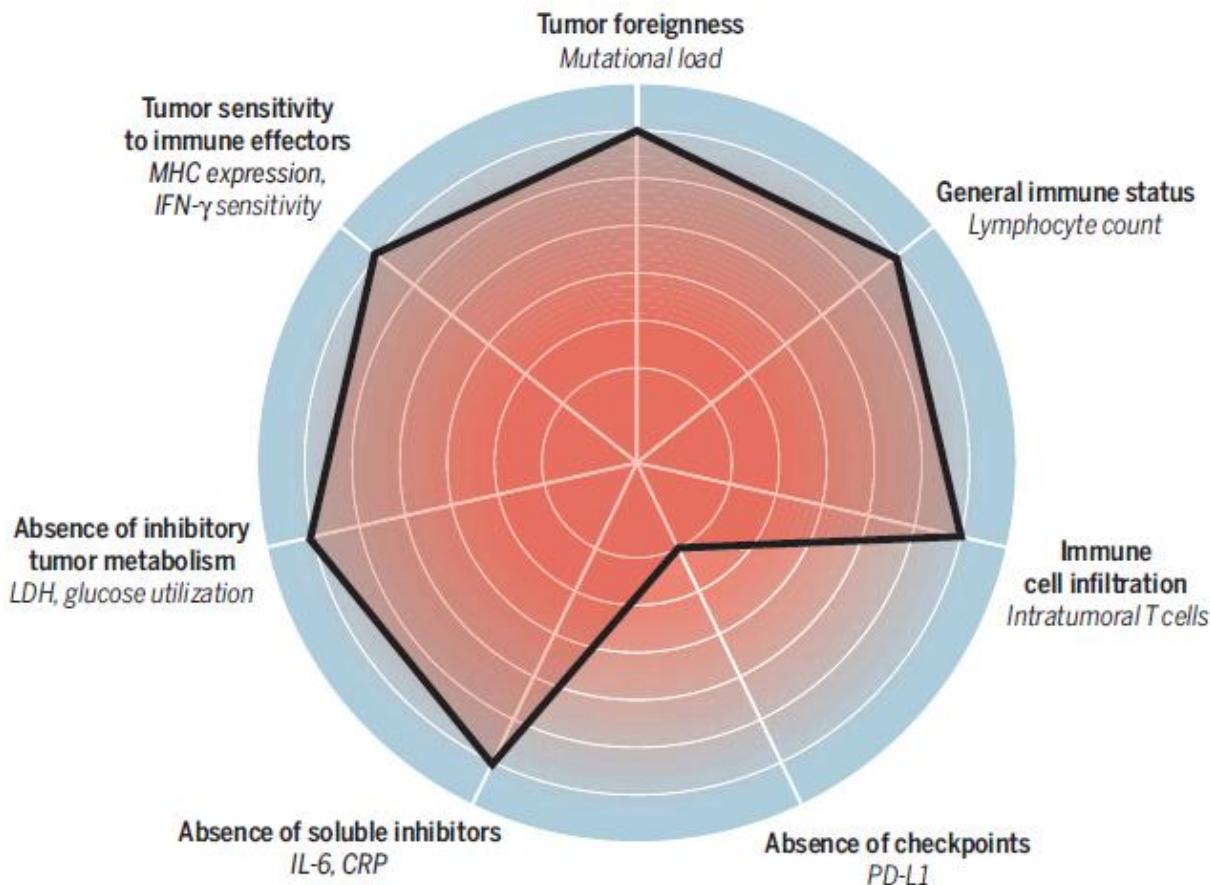


Fig. 1 High-throughput immune assessment for biomarker discovery and personalized cancer immunotherapy. Immunologically-ignorant and immunologically-responsive tumors are classified by the presence of immune cells in the tumor microenvironment. Potential biomarkers identified from high-throughput technologies can further differentiate these tumors by the mutation load, gene/protein/antibody signature profile, phenotype and function of immune cells, and can also provide clinical strategies for personalized cancer immunotherapies. The new and innovative technologies that can be utilized to identify potential biomarkers include whole exome sequencing, gene signature, epigenetic modification, protein microarray, B/T cell receptor repertoire, flow/mass cytometry and multicolor IHC. Arrows indicate a decrease (↓) or increase (↑).

The “cancer immunogram”

Visualizing the state of cancer-immune system interactions may spur personalized therapy

By Christian U. Blank,^{1,2} John B. Haanen,^{1,2}
Antoni Ribas,³ Ton N. Schumacher²

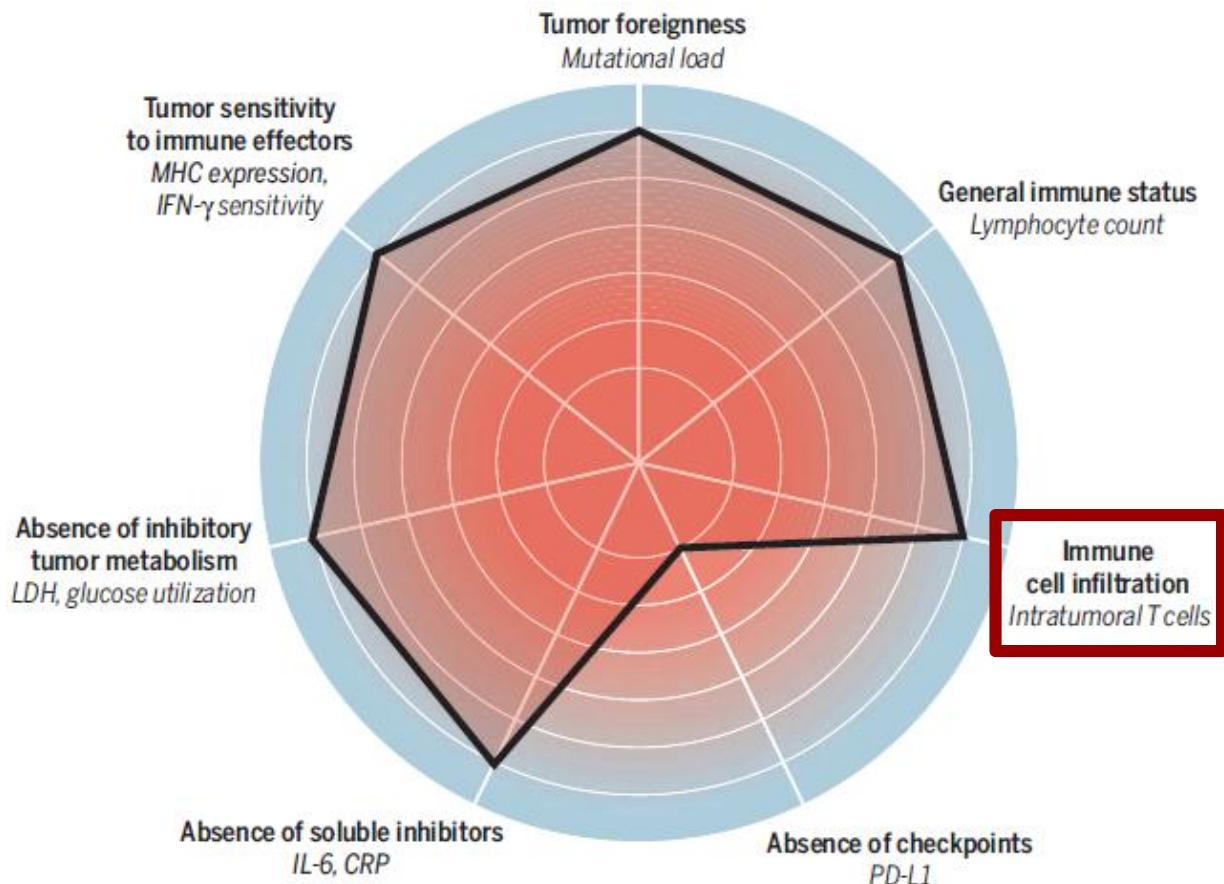


The cancer immunogram. The radar plot depicts the seven parameters that characterize aspects of cancer-immune interactions for which biomarkers have been identified or are plausible. Potential biomarkers for the different parameters are shown in italics. Desirable states are located in blue; progressively undesirable states are shown in the red gradient. The black line connecting the data values for each parameter represents a plot for a single hypothetical patient. In the case shown, it may be argued that single-agent PD-1 blockade, rather than combined PD-1 and CTLA-4 blockade, could be a first treatment of choice. For details on this case and other hypothetical patient cases, see (2).

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Elements of cancer immunity and the cancer-immune set point

Daniel S. Chen¹ & Ira Mellman¹

19 JANUARY 2017 | VOL 541 | NATURE | 321

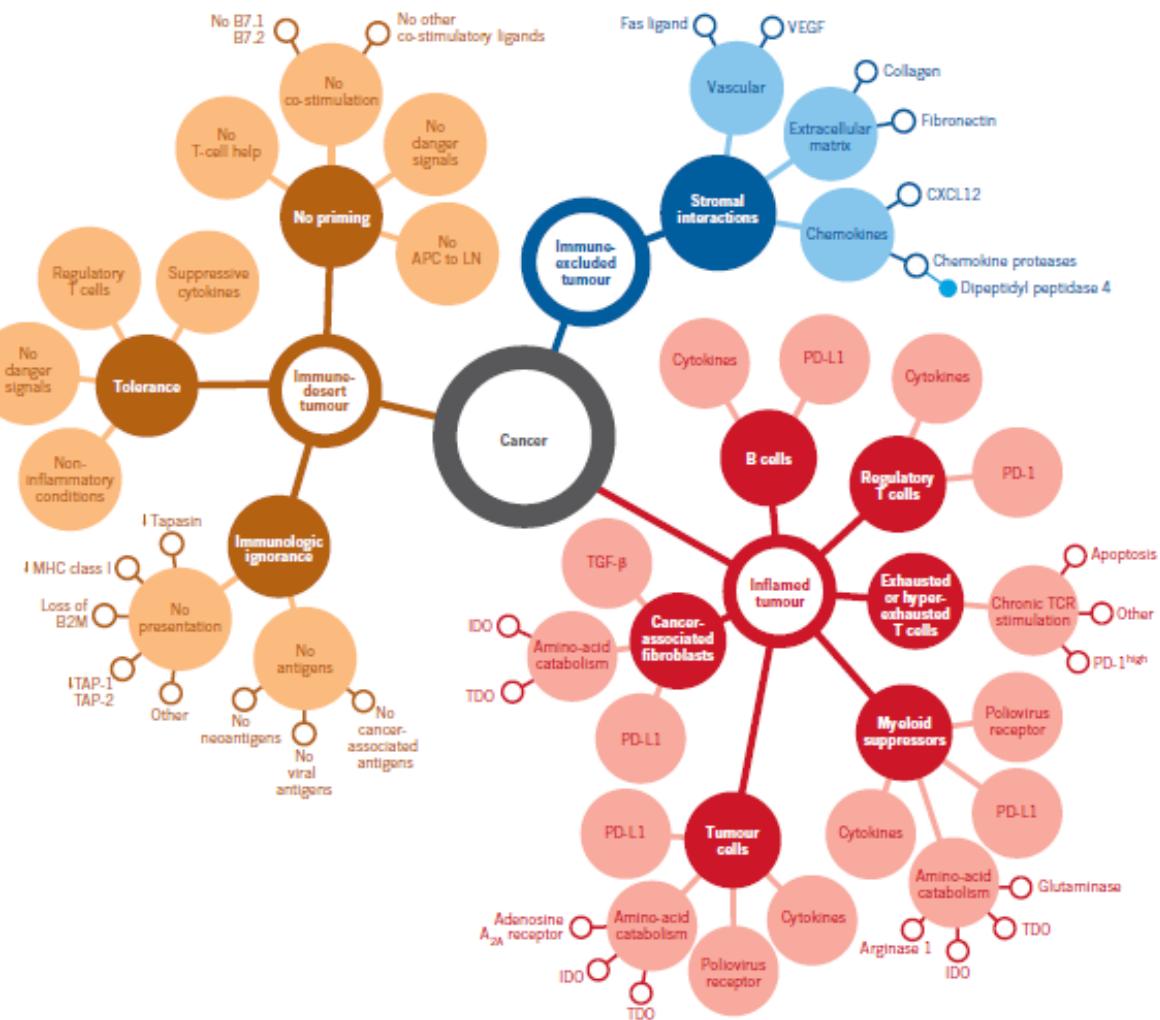


Figure 3 | Cancer-immune phenotypes. Anticancer immunity in humans can be segregated into three main phenotypes: the immune-desert phenotype (brown), the immune-excluded phenotype (blue) and the inflamed phenotype (red). Each is associated with specific underlying biological mechanisms that may prevent the host's immune response from eradicating the cancer. A tumour that is characterized as an immune desert can be the result of immunological ignorance, the induction of tolerance or a lack of appropriate T-cell priming or activation. Immune-excluded tumours may reflect a specific chemokine state, the presence of particular vascular factors or barriers, or specific stromal-based inhibition. Inflamed tumours can demonstrate infiltration by a number of subtypes of immune cells, including immune-inhibitory regulatory T cells, myeloid-derived suppressor cells, suppressor B cells and cancer-associated fibroblasts. Tumour-infiltrating lymphocytes that express CD8 may also demonstrate a dysfunctional state such as hyporeactivity. Tumour cells in inflamed tumours can also express inhibitory factors, downregulating MHC class I molecule expression or other pathways that de-sensitize them to anticancer immunity. APC, antigen-presenting cell; B2M, β -2-microglobulin; IDO, indoleamine 2,3-dioxygenase; LN, lymph node; TAP, transporter associated with antigen processing; TDO, tryptophan 2,3-dioxygenase; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

Elements of cancer immunity and the cancer-immune set point

Daniel S. Chen¹ & Ira Mellman¹

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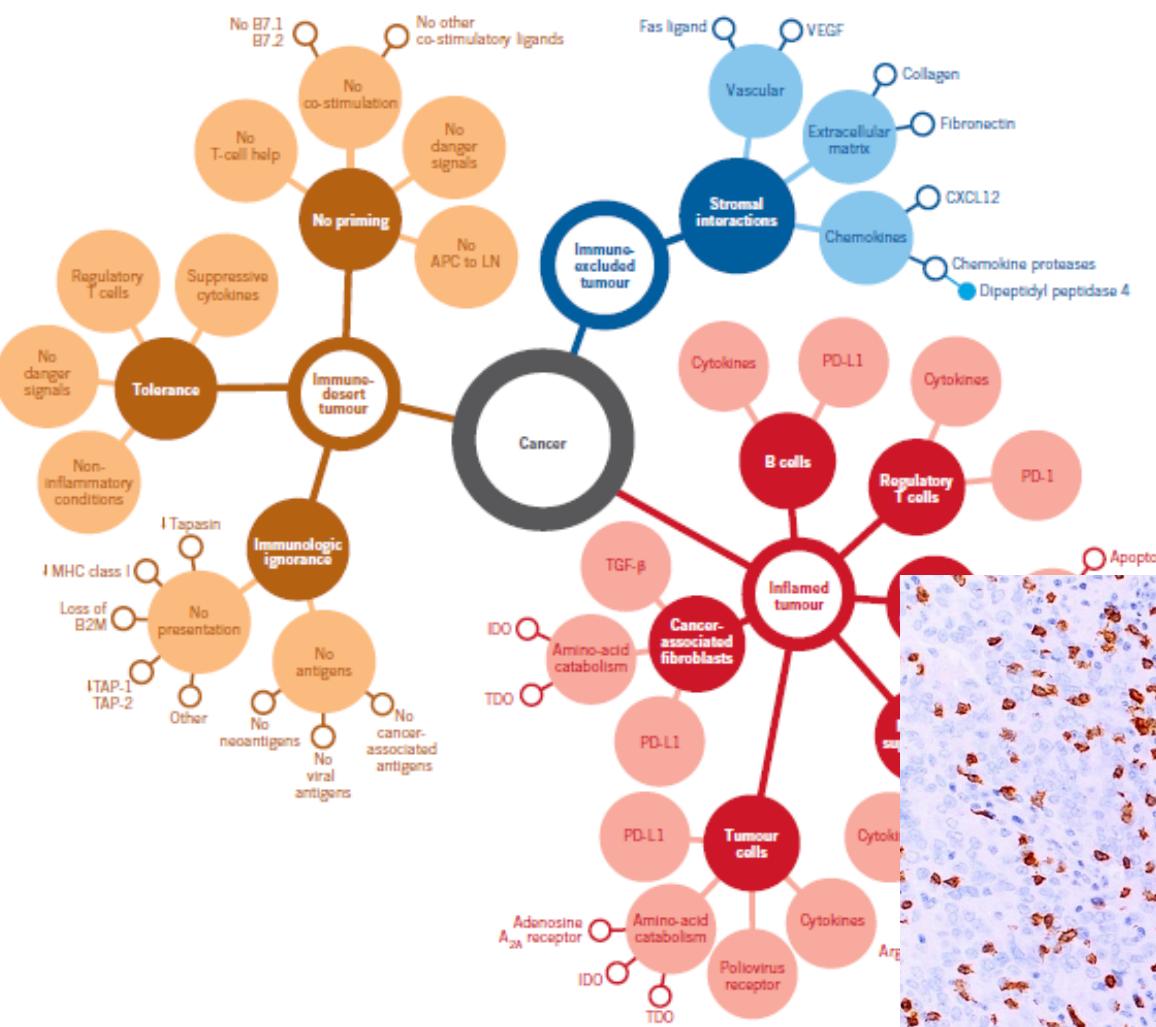
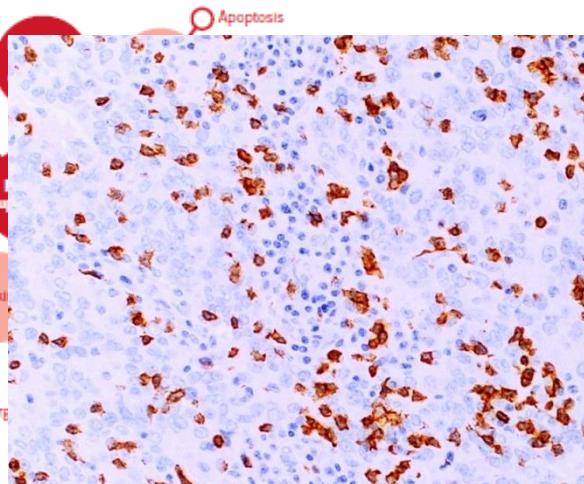


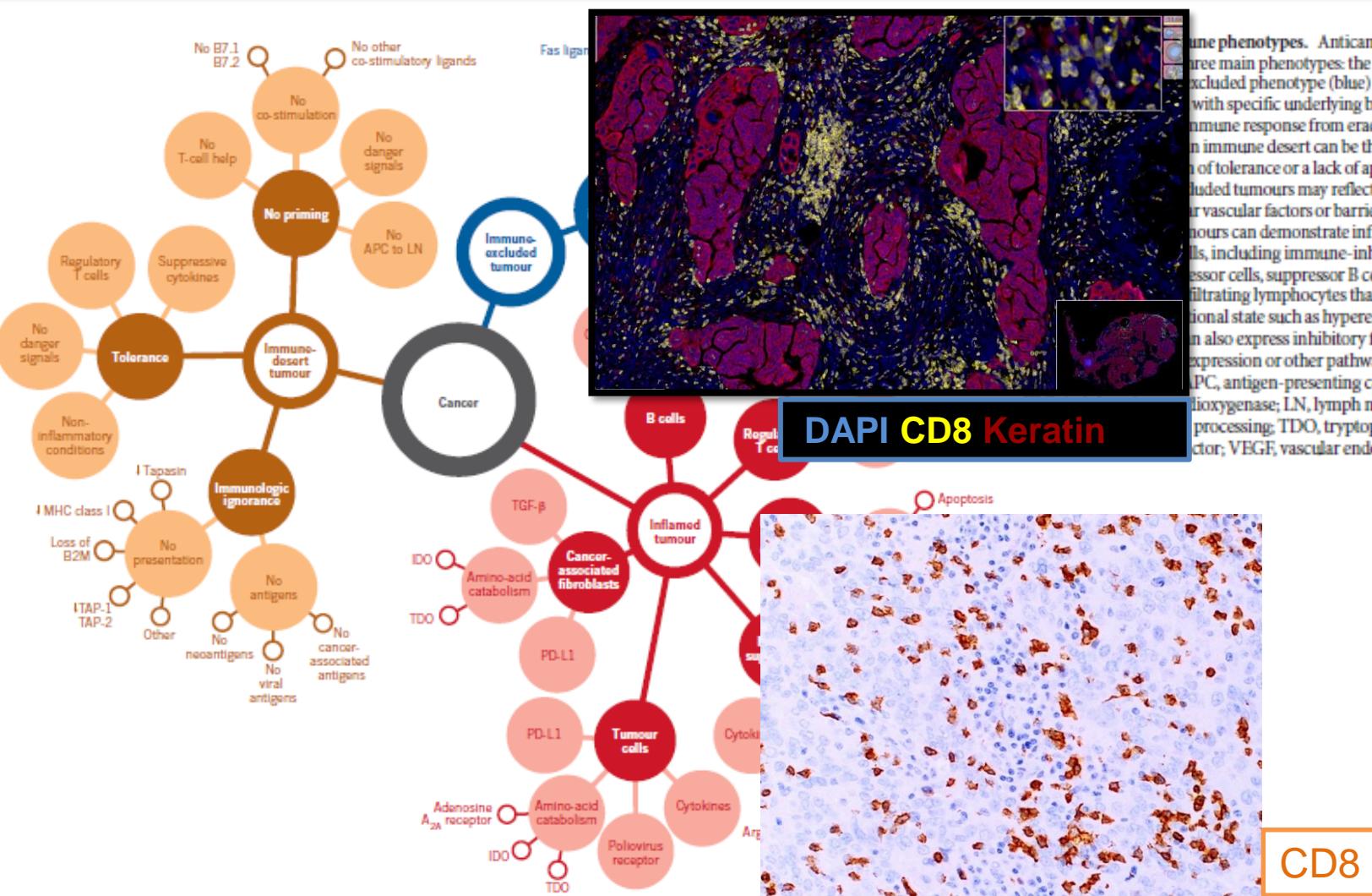
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Elements of cancer immunity and the cancer-immune set point

Daniel S. Chen¹ & Ira Mellman¹

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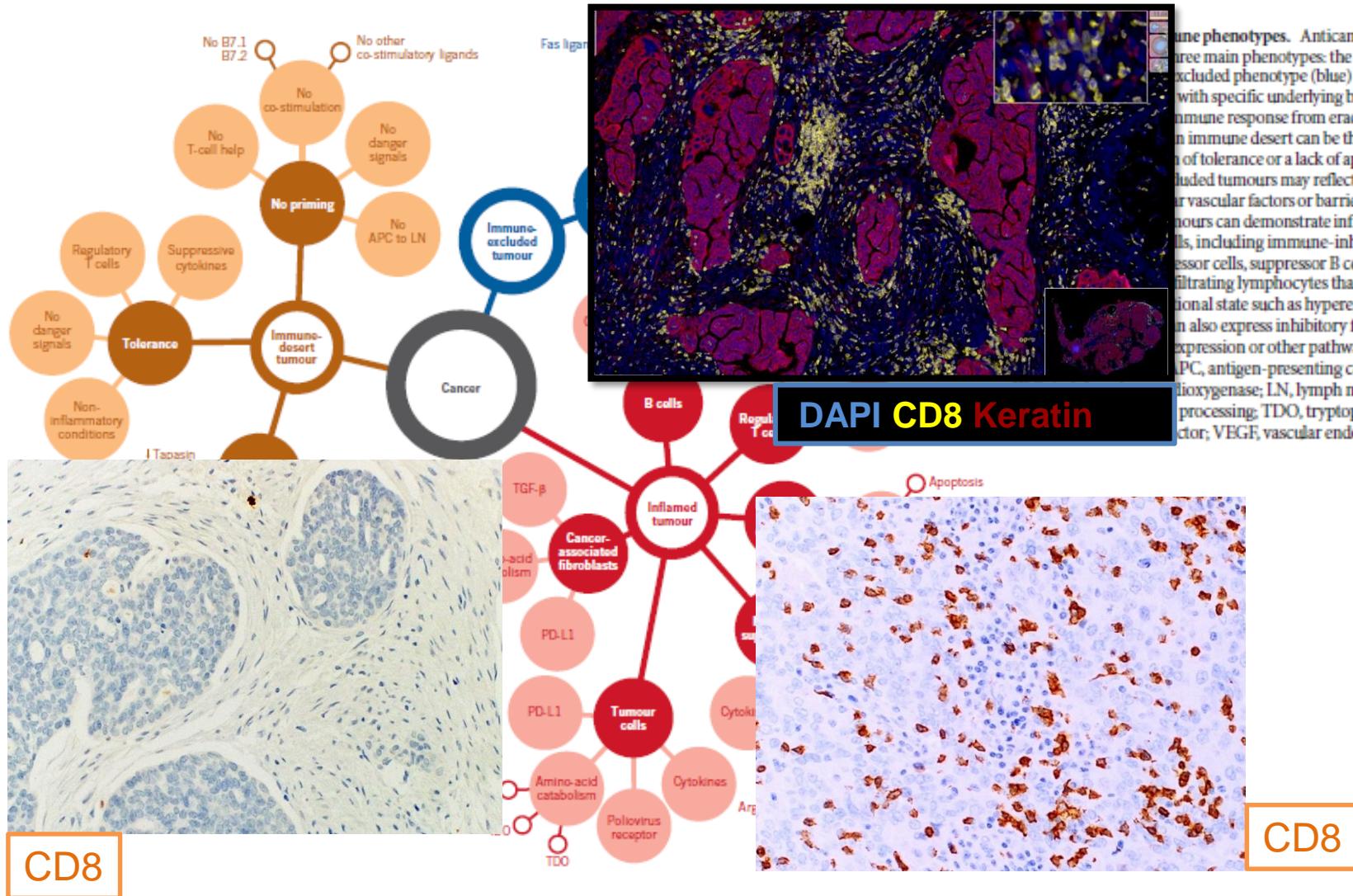


une phenotypes. Anticancer immunity in humans can result in three main phenotypes: the immune-desert phenotype (blue) and the inflamed phenotype (red), with specific underlying biological mechanisms that determine the immune response from eradicating the cancer. A tumour can be in an immune desert or inflamed state, or excluded from the immune response. An immune desert can be the result of immunological tolerance or a lack of appropriate T-cell priming or activation. Excluded tumours may reflect a specific chemokine state, or lack of vascular factors or barriers, or specific stromal-based mechanisms. Inflammation can demonstrate infiltration by a number of cells, including immune-inhibitory regulatory T cells, myeloid-derived suppressor cells, suppressor B cells and cancer-associated fibroblasts, or infiltrating lymphocytes that express CD8. This state may be a functional state such as hyperexhaustion. Tumour cells can also express inhibitory factors, downregulating T-cell expression or other pathways that de-sensitize them to T-cell attack. APC, antigen-presenting cell; B2M, β-2-microglobulin; IDO, indoleamine 2,3-dioxygenase; LN, lymph node; TAP, transporter associated with antigen processing; TDO, tryptophan 2,3-dioxygenase; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

Elements of cancer immunity and the cancer-immune set point

Daniel S. Chen¹ & Ira Mellman¹

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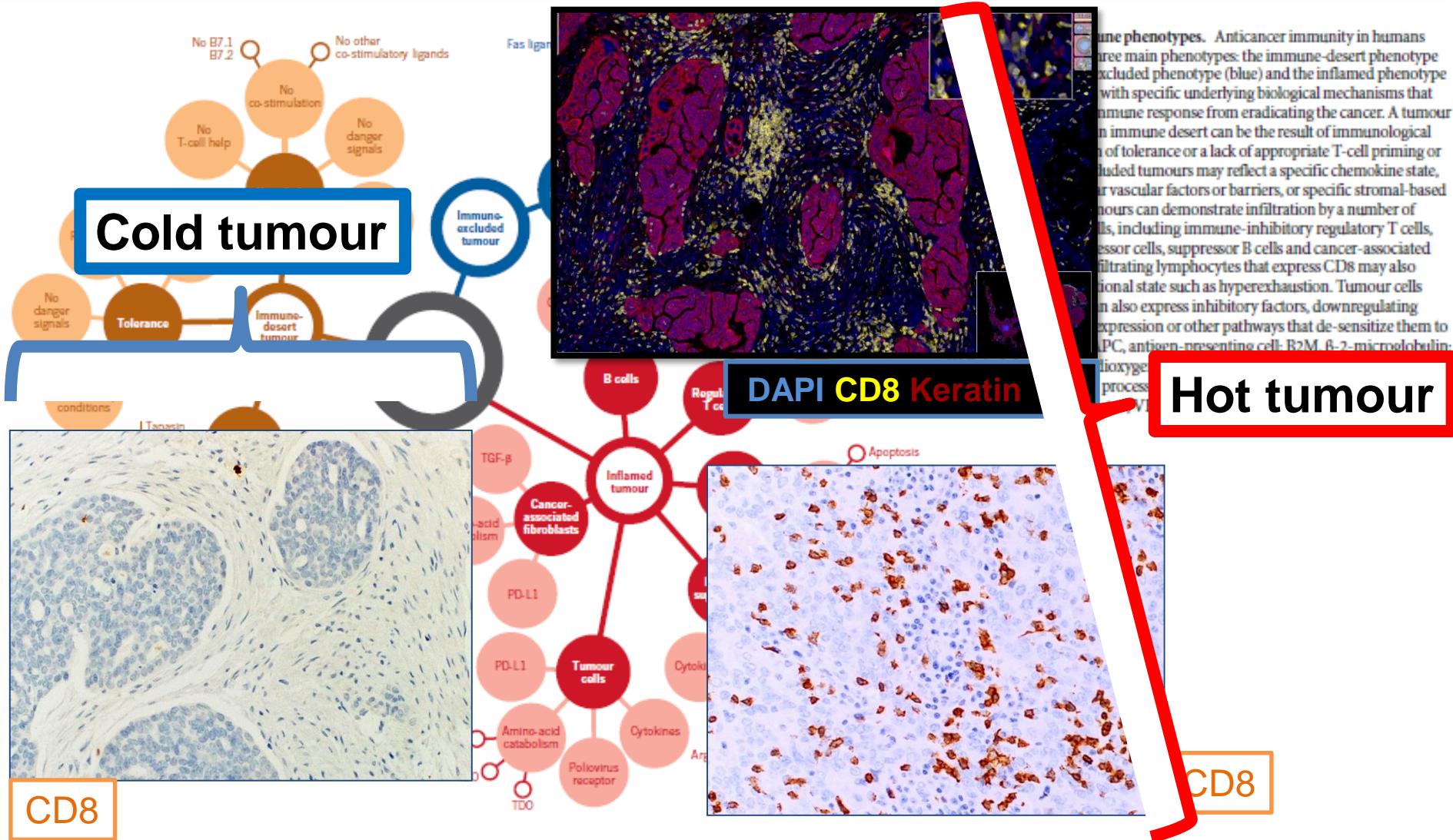


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Elements of cancer immunity and the cancer-immune set point

Daniel S. Chen¹ & Ira Mellman¹

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Turning up the heat on non-immunoreactive tumours: opportunities for clinical development

Maria Ochoa de Olza, Blanca Navarro Rodrigo, Stefan Zimmermann, George Coukos

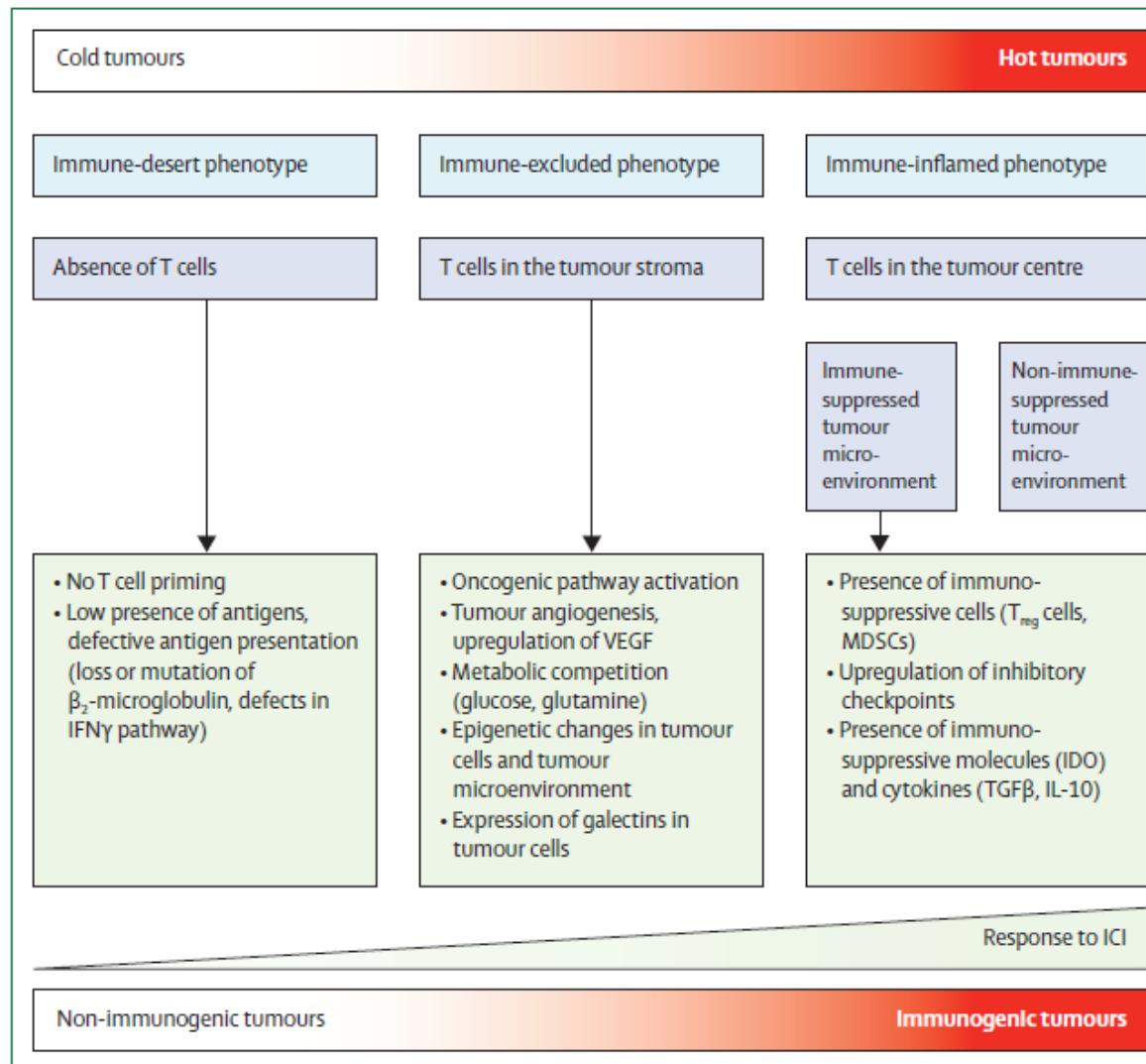


Figure 1: Immune phenotypes and their underlying mechanisms

Turning up the heat on non-immunoreactive tumours: opportunities for clinical development

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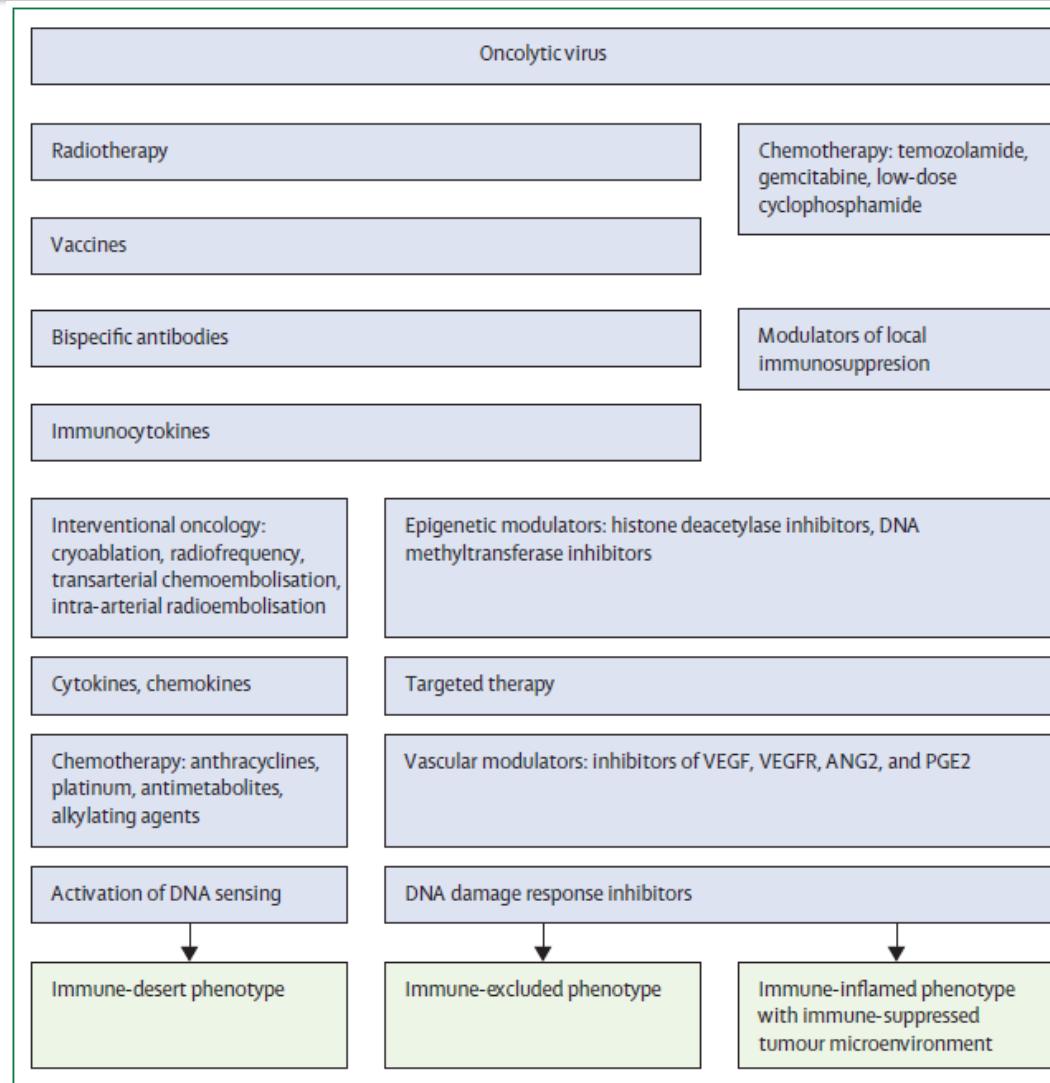


Figure 2: Proposal of therapeutic strategies according to immune phenotype
ANG2=angiopoietin 2. PGE2=prostaglandin E₂. VEGFR=VEGF receptor.

The natural (spontaneous) adaptive immune responses of cancer patients have been shown to influence their survival

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

Lin Zhang, M.D., Jose R. Conejo-Garcia, M.D., Ph.D.,
Dionyssios Katsaros, M.D., Ph.D., Phyllis A. Gimotty, Ph.D.,
Marco Massobrio, M.D., Giorgia Regnani, M.D.,
Antonis Makrigiannakis, M.D., Ph.D., Heidi Gray, M.D.,
Katia Schlienger, M.D., Ph.D., Michael N. Liebman, Ph.D.,
Stephen C. Rubin, M.D., and George Coukos, M.D., Ph.D.

N Engl J Med 2003;348:203-13.

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 Mlecník,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}†

29 SEPTEMBER 2006 VOL 313 SCIENCE

Tumor Immunology and Tumor Evolution: Intertwined Histories

Jérôme Galon^{1,*} and Daniela Bruni¹

Immunity 52, January 14, 2020

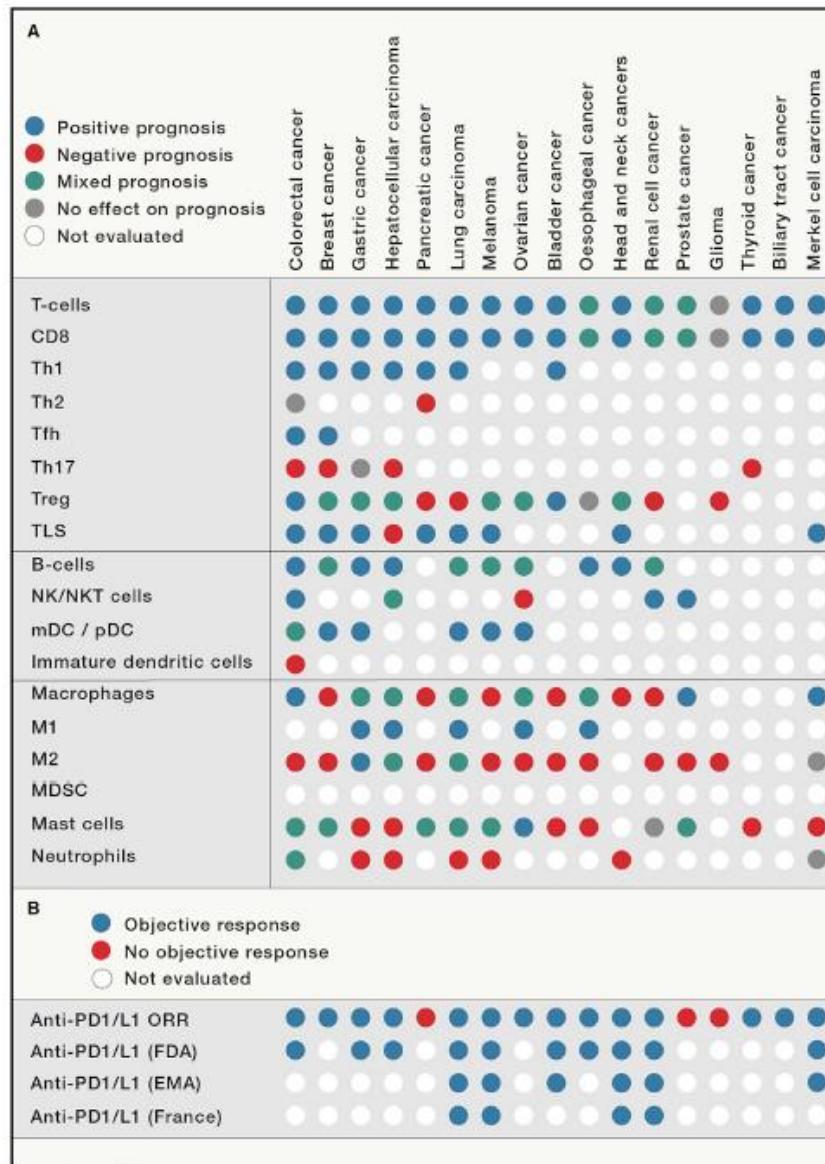


Figure 3. Prognostic Effect of Immune Cells in Solid Cancer

Tumor Immunology and Tumor Evolution: Intertwined Histories

Jérôme Galon^{1,*} and Daniela Bruni¹

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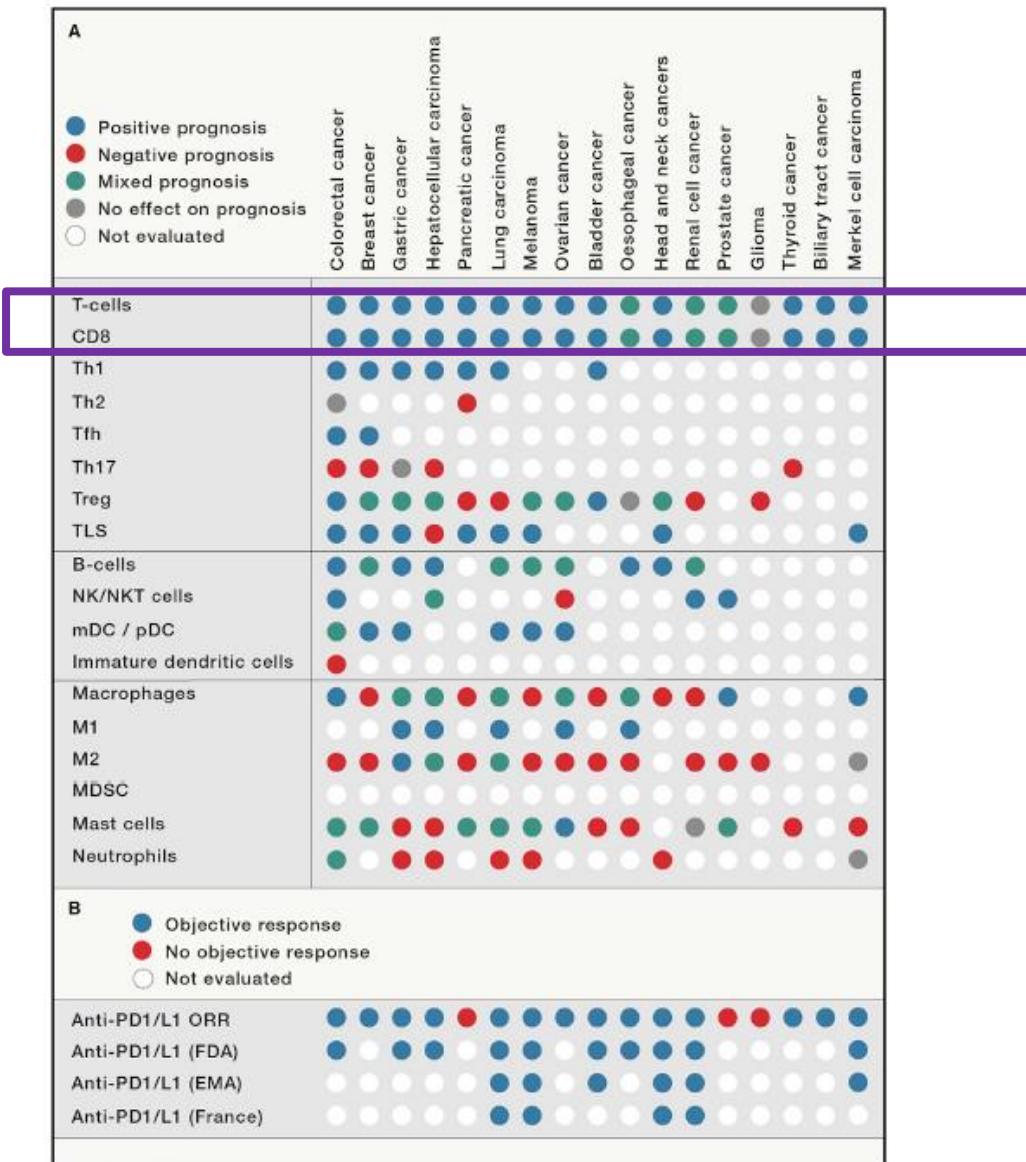


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CD4+

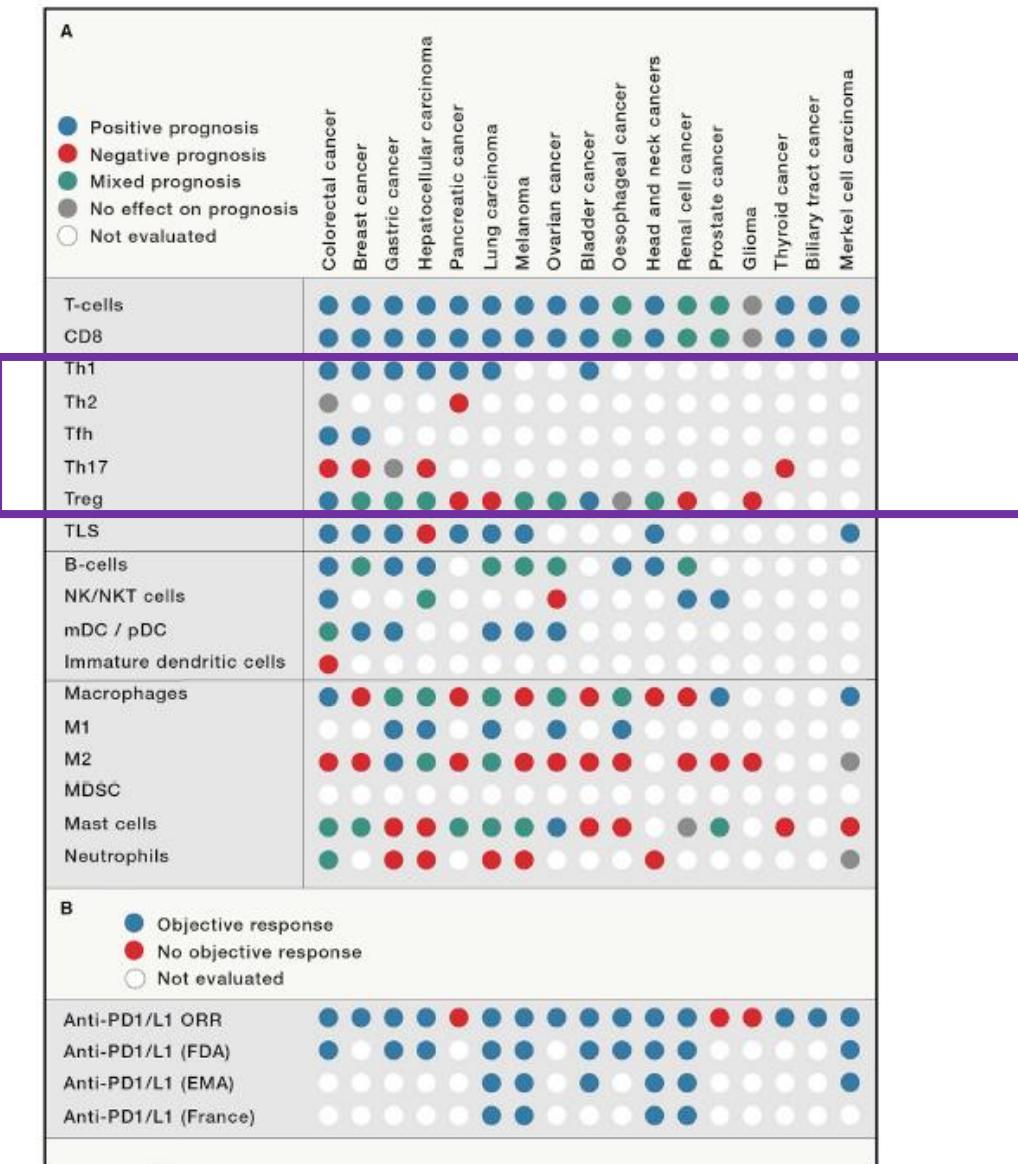


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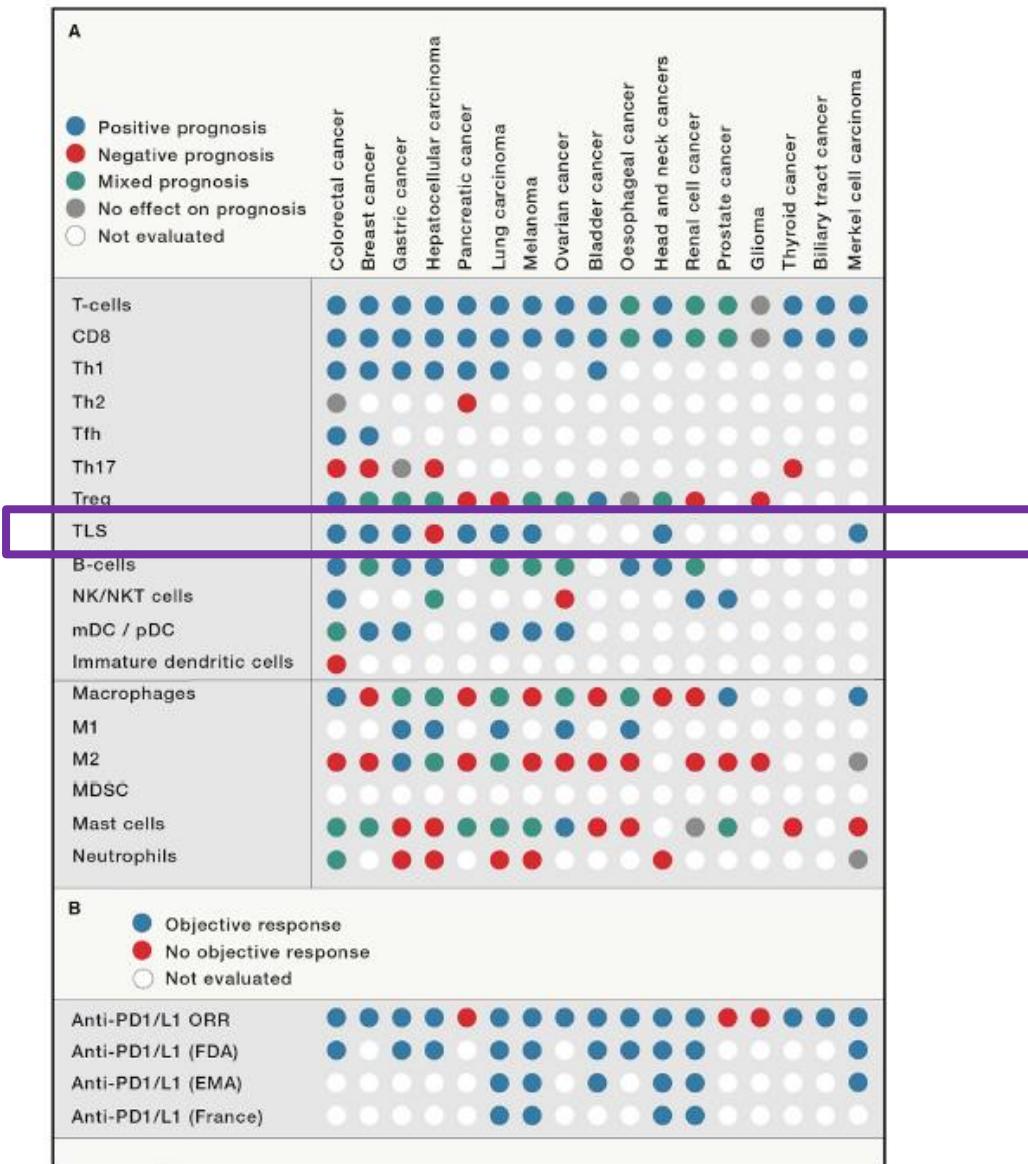


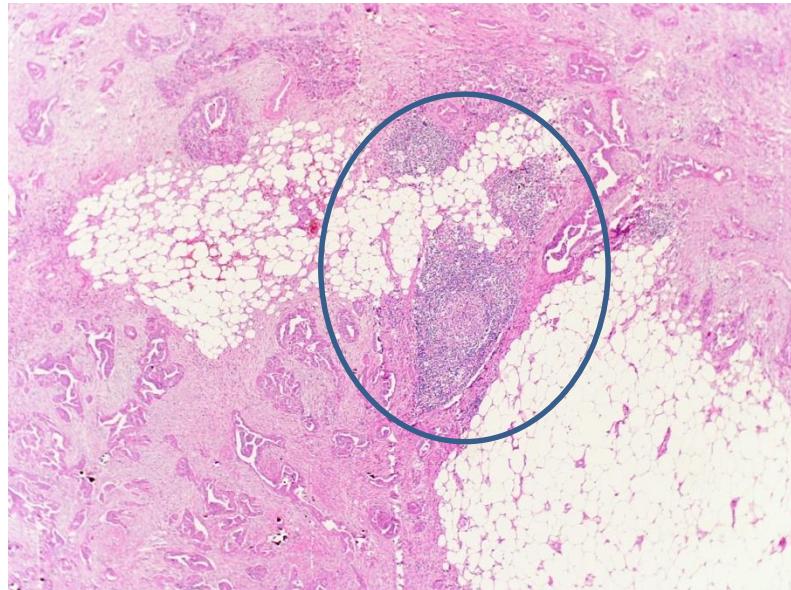
Figure 3. Prognostic Effect of Immune Cells in Solid Cancer

Characteristics of tertiary lymphoid structures in primary cancers

Jérémie Goc^{1,2,3}, Wolf-Herman Fridman^{1,2,3}, Catherine Sautès-Fridman^{1,2,3}, and Marie-Caroline Dieu-Nosjean^{1,2,3,*}

¹The Laboratory of Immune Microenvironment and Tumors; INSERM U872; Cordeliers Research Center; Paris, France;

²University Pierre and Marie Curie; UMRS872; Paris, France; ³University Paris Descartes; UMRS872; Paris, France



Ectopic lymphoid formations found in:

- **chronic infections,**
- **autoimmune diseases,**
- **chronic allograft rejection and**
- **solid cancers**

Table 1. Studies reporting the presence of tertiary lymphoid structures in human neoplasms

Cancers	Cellular composition of lymphoid aggregates/TLS	Studied cases	Stage of the disease	References
Breast carcinoma	T cells (including CD4 ⁺ T cells), mature DCs	32 patients	carcinoma in situ to grade III	Bell et al., 1999
	T cells, B cells (GC B cells and naïve B cells), FDCs	3 patients	grade II to III	Coronella et al., 2002
	T cells, B cells, PCs, FDCs	4 patients	ND	Nzula et al., 2003
	lymphocytes (hematoxylin counterstaining)	191 patients	grade II to III	Gobert et al., 2009
	T cells, B cells, HEVs	146 patients	grade I to III	Martinet et al., 2011
	T cells (Tfh, CD4 ⁺ T cells and few CD8 ⁺ T cells), B cells (GC B cells), FDCs	70 patients	grade I to III	Gu-Trantien et al., 2013
Colorectal carcinoma	T cells, B cells, mature DCs, HEVs	146 patients	grade I to III	Martinet et al., 2013
	T cells (including CD4 ⁺ T cells, memory T cells, few CD8 ⁺ T cells), B cells, mature DCs	17 patients	ND	Suzuki et al., 2002
	T cells, mature DCs	40 patients	grade I to IV	McMullen et al., 2010
	T cells, B cells, FDCs	ND	ND	Bergamas et al., 2011
	T cells, B cells (including B cell precursors), FDCs	21 patients	grade 0 to IVA	Coppola et al., 2011
Colorectal carcinoma liver metastasis	T cells, B cells, HEVs	5 patients	ND	Martinet et al., 2011
	T cells, B cells, mature DCs	25 patients	ND	Remark et al., 2013
Colorectal carcinoma lung metastasis	mature DCs	70 patients	ND	Miyagawa et al., 2004
Lung carcinoma	T cells (including CD4 ⁺ T cells and few CD8 ⁺ T cells), B cells (including GC B cells), mature DCs, FDCs	74 patients	stage I to II	Dieu-Nosjean et al., 2008
	no NK cells	86 patients	stage I to III	Platonova et al., 2011
	T cells (including memory T cells and few naïve T cells), mature DCs, HEVs	15 patients	stage I to III	de Chaisemartin et al., 2011
Melanoma	T cells, B cells, HEVs	5 patients	ND	Martinet et al., 2011
	memory T cells, mature DCs	82 patients	stage IA to IIIA	Ladányi et al., 2007
	T cells (including CD4 ⁺ and CD8 ⁺ T cells, rare FoxP3 ⁺ cells), B cells, mature DCs	21 patients	stage IV	Messina et al., 2012
	T cells, B cells, HEVs	18 patients	ND	Martinet et al., 2012
Mucosal-Associated Lymphoid Tissue lymphoma	T cells (including CD8 ⁺ T cells), B cells (including AID ⁺ GC B cells), mature DCs, FDCs, HEVs	29 patients	stage IIIB to IV	Cipponi et al., 2012
	T cells, B cells (including naïve B cells, AID ⁺ GC B cells, marginal zone B cells, malignant B cells), FDCs	18 patients	low grade	Bombardieri et al., 2007
	T cells, B cells (including naïve B cells, AID ⁺ GC B cells, marginal zone B cells, malignant B cells), FDCs	20 patients	ND	Barone et al., 2008
Ovary carcinoma	T cells, B cells, HEVs	18 patients	ND	Martinet et al., 2011
Renal cell carcinoma	T cells, B cells, mature DCs	24 patients	ND	Remark et al., 2013
Renal cell carcinoma lung metastasis	T cells, B cells, mature DCs	52 patients	stage IV	Remark et al., 2013

Abbreviations: DC, dendritic cell; FDC, follicular DC; GC, germinal center; HEV, high endothelial venule; ND, not determined; TLS, tertiary lymphoid structure.

Characteristics of tertiary lymphoid structures in primary cancers

Jérémie Goc^{1,2,3}, Wolf-Herman Fridman^{1,2,3}, Catherine Sautès-Fridman^{1,2,3}, and Marie-Caroline Dieu-Nosjean^{1,2,3,*}

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²University Pierre and Marie Curie; UMRS872; Paris, France; ³University Paris Descartes; UMRS872; Paris, France

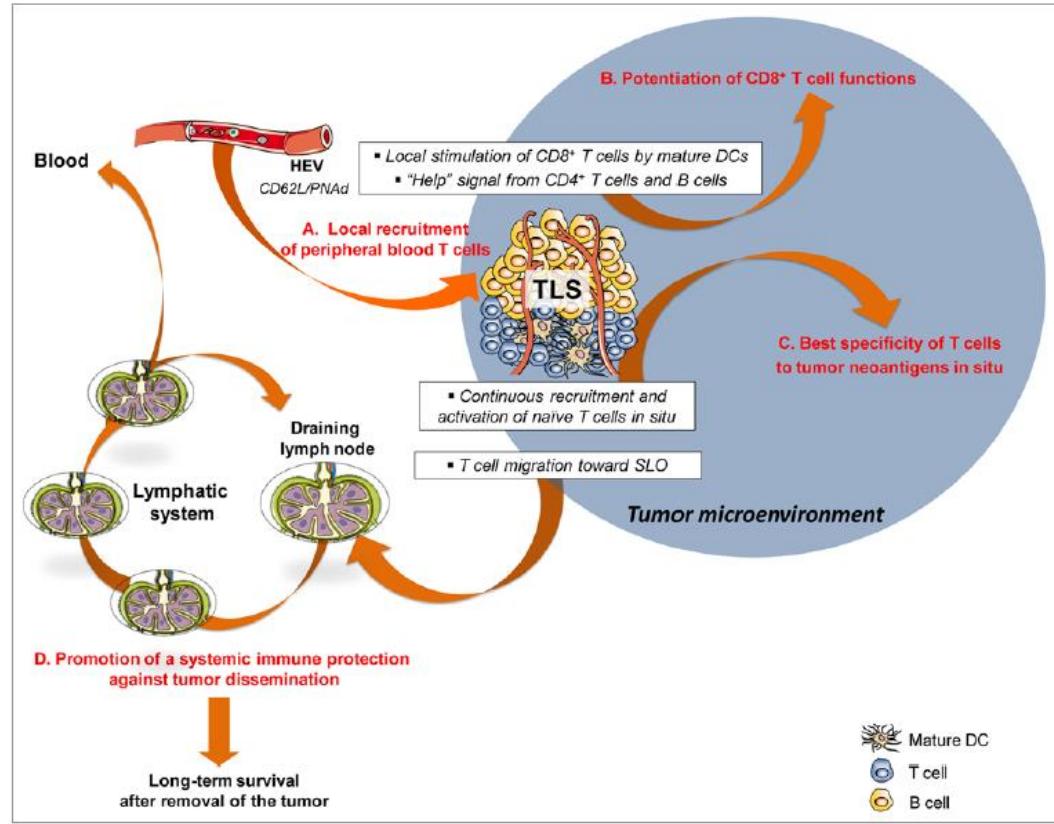
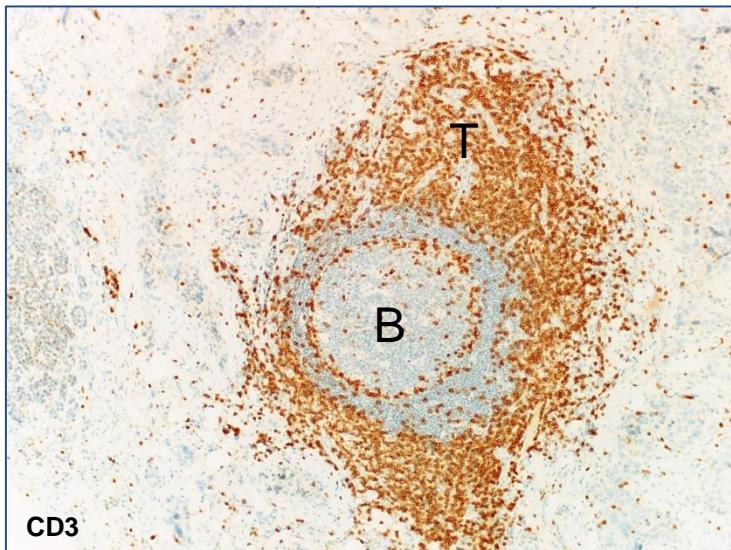
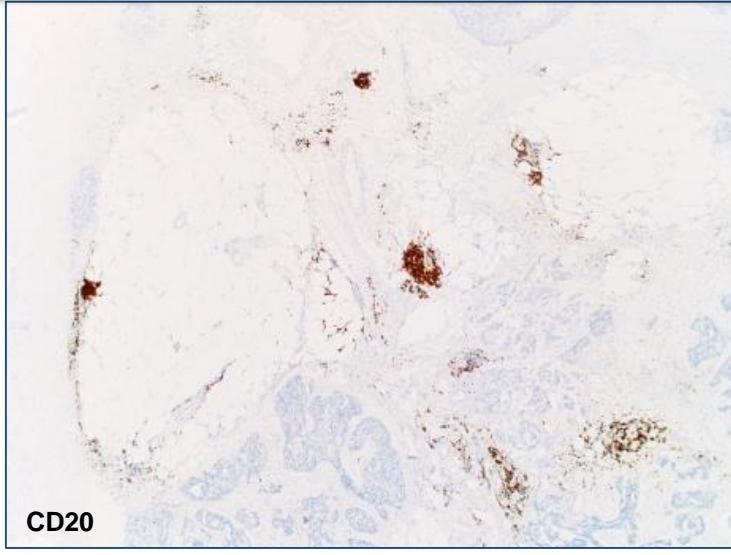


Figure 1. Role of tertiary lymphoid structures in the initiation of local and systemic protective immune responses against primary neoplastic lesions and metastases. DC, dendritic cell; HEV, high endothelial venule; SLO, secondary lymphoid organ; TLS, tertiary lymphoid structure.

Germinal Centers Determine the Prognostic Relevance of Tertiary Lymphoid Structures and Are Impaired by Corticosteroids in Lung Squamous Cell Carcinoma

Karīna Siliņa¹, Alex Soltermann², Farkhondeh Movahedian Attar¹, Ruben Casanova², Zina M. Uckley¹, Helen Thut¹, Muriel Wandres¹, Sergejs Isajevs^{3,4}, Phil Cheng⁵, Alessandra Curioni-Fontecedro⁶, Periklis Foukas^{7,8}, Mitchell P. Levesque⁵, Holger Moch², Aija Linē⁹, and Maries van den Broek¹

Cancer Res; 78(5); 1308–20.

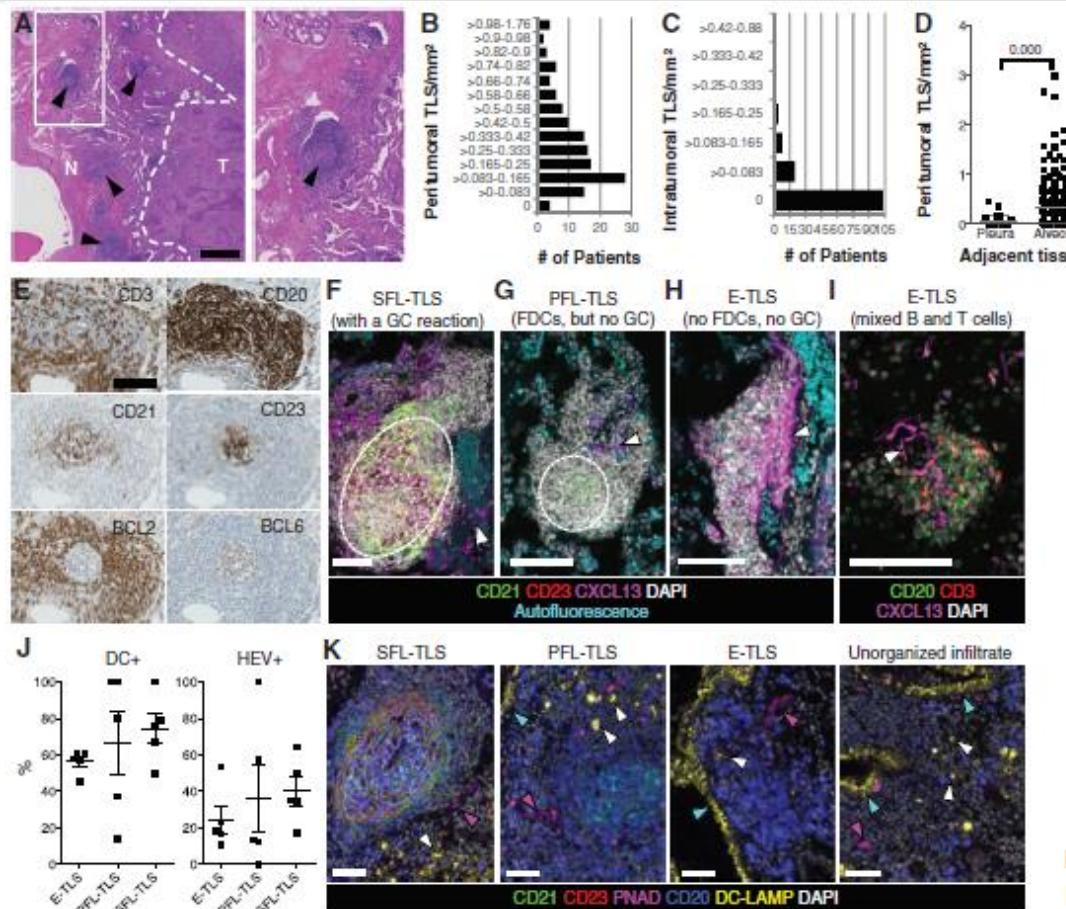


Figure 1.
Development of LSCC-associated TLS.

Tumor Immunology and Tumor Evolution: Intertwined Histories

Jérôme Galon^{1,*} and Daniela Bruni¹

Immunity 52, January 14, 2020

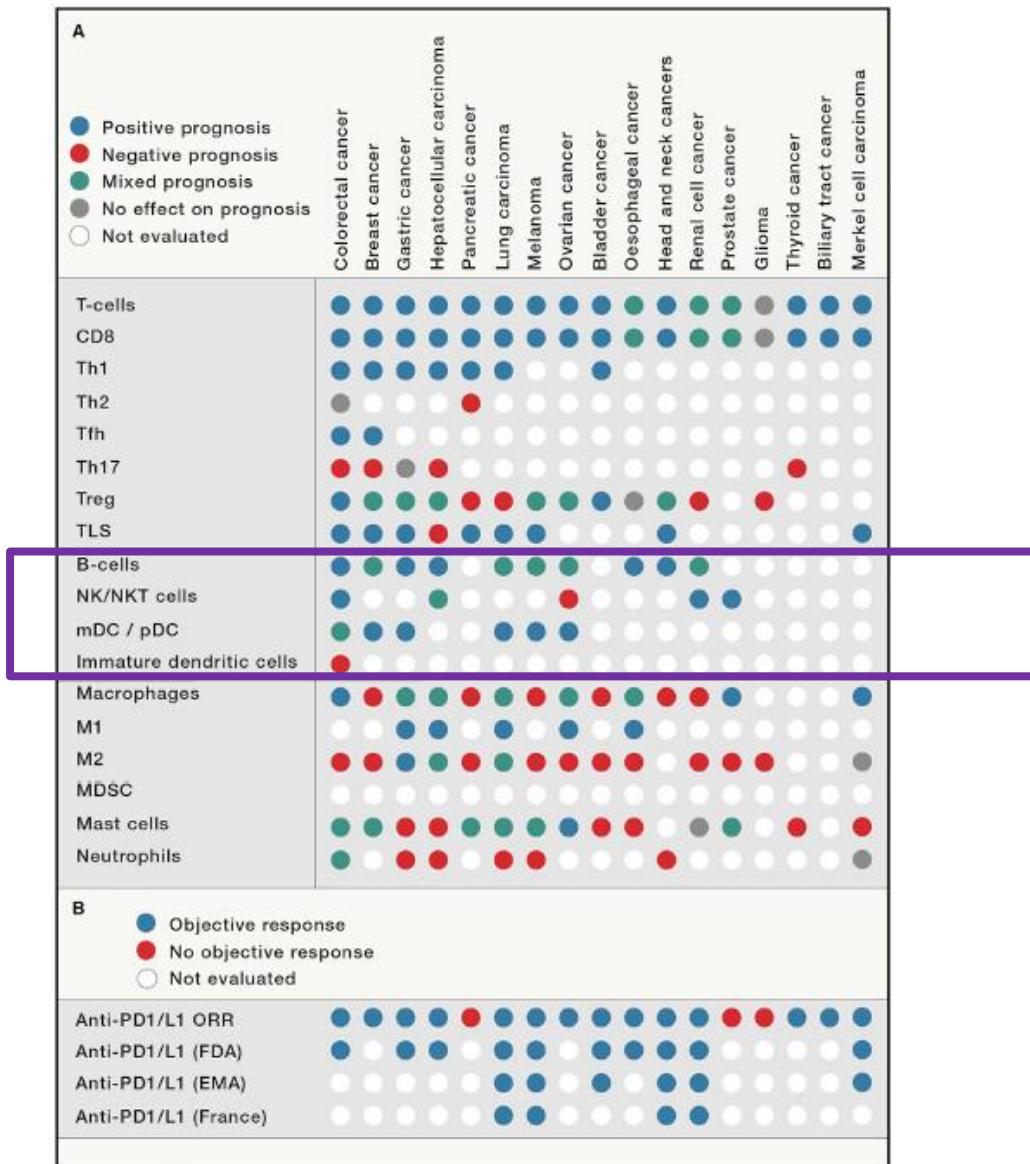


Figure 3. Prognostic Effect of Immune Cells in Solid Cancer

Tumor Immunology and Tumor Evolution: Intertwined Histories

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Immunity 52, January 14, 2020

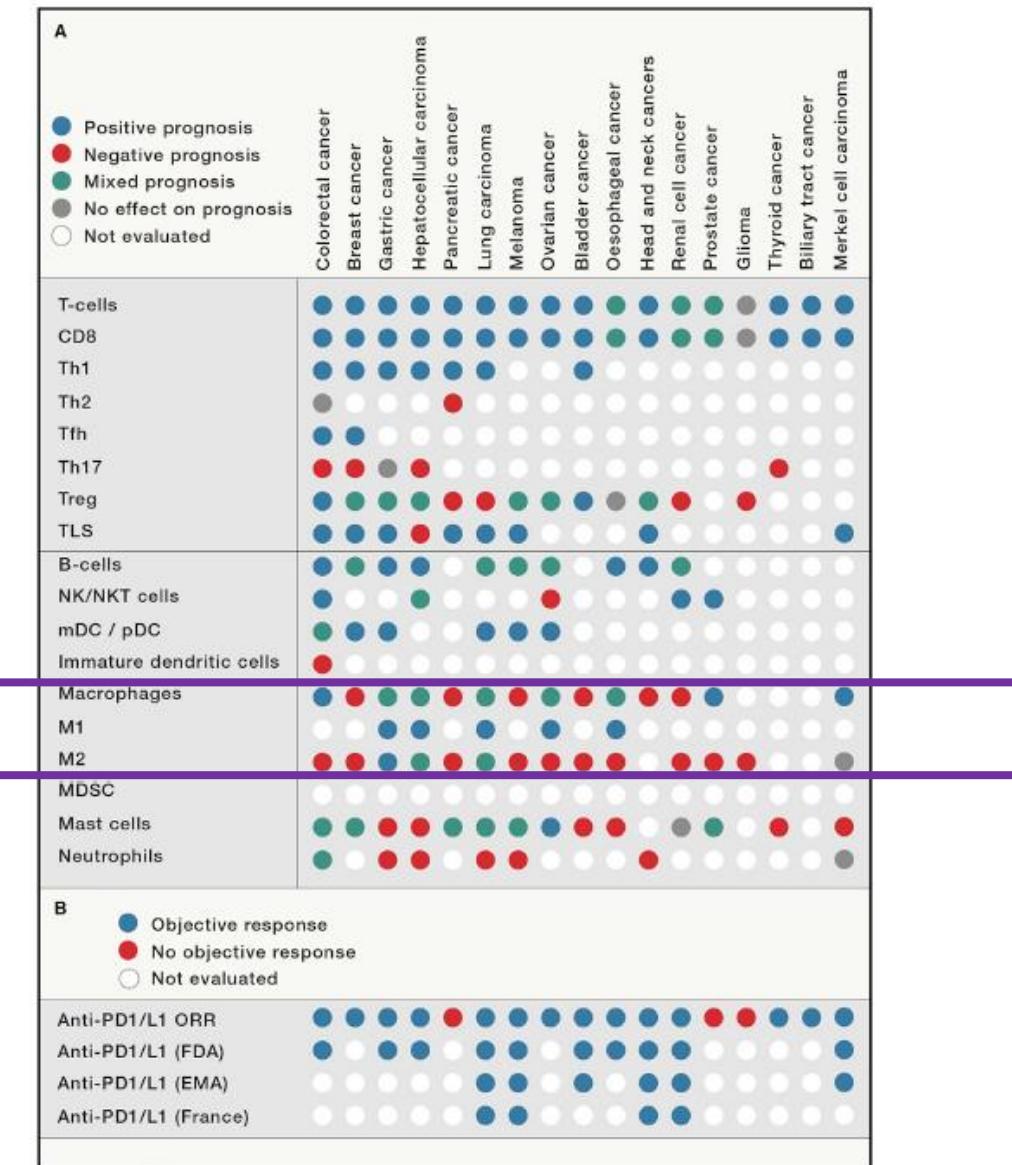


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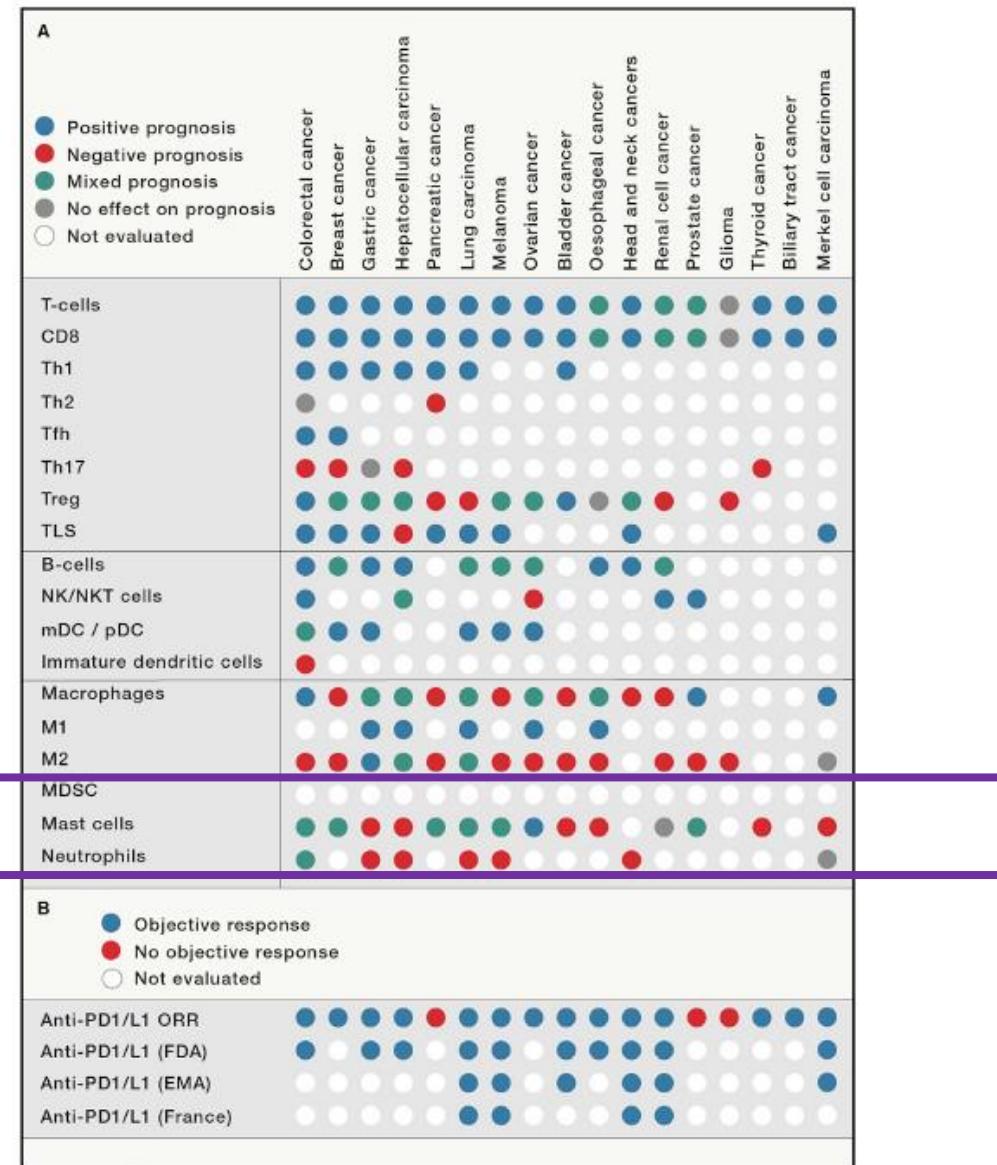


Figure 3. Prognostic Effect of Immune Cells in Solid Cancer

Outline

- Introduction to the Tumor Immune Microenvironment (TIME)
 - Prognostic / Predictive value
- Mechanisms regulating TIME
- Evaluation / Methodologies
- Turning-up the heat



Predictive relevance of PD-L1 expression combined with CD8+ TIL density in stage III non-small cell lung cancer patients receiving concurrent chemoradiotherapy

Takaaki Tokito ^a, Koichi Azuma ^{a,*}, Akihiko Kawahara ^b,
Hidenobu Ishii ^a, Kazuhiko Yamada ^a, Norikazu Matsuo ^a,
Takashi Kinoshita ^a, Naohisa Mizukami ^d, Hirofumi Ono ^c,
Masayoshi Kage ^b, Tomoaki Hoshino ^a

European Journal of Cancer 55 (2016) 7–14

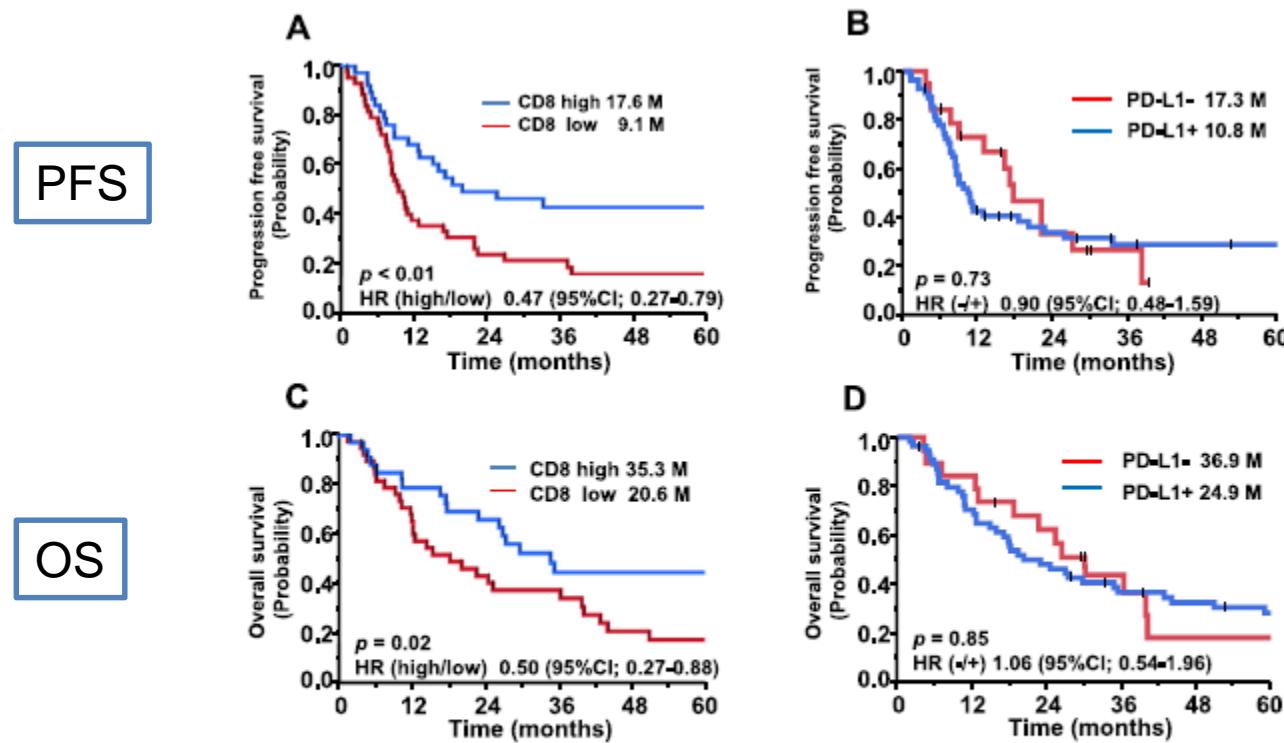


Fig. 2. Kaplan-Meier curves for progression-free survival (PFS) of stage III NSCLC patients with positive or negative for CD8+ TIL density (A) and PD-L1 expression (B). Kaplan-Meier curves for overall survival (OS) of stage III NSCLC patients with positive or negative for CD8+ TIL density (C) and PD-L1 expression (D). PD-L1, programmed cell death-ligand 1; TIL, tumour-infiltrating lymphocyte; NSCLC, non-small cell lung cancer; HR, hazard ratio; CI, confidence interval.

PD-1 blockade induces responses by inhibiting adaptive immune resistance

Paul C. Tumeh^{1,2}, Christina L. Harview¹, Jennifer H. Yearley³, I. Peter Shintaku¹, Emma J. M. Taylor¹, Lidia Robert¹, Bartosz Chmielowski^{1,2}, Marko Spasic¹, Gina Henry¹, Voicu Ciobanu¹, Alisha N. West¹, Manuel Carmona¹, Christine Kivork¹, Elizabeth Seja¹, Grace Cherry¹, Antonio J. Gutierrez¹, Tristan R. Grogan¹, Christine Mateus⁴, Gorana Tomasic⁴, John A. Glaspy^{1,2}, Ryan O. Emerson⁵, Harlan Robins^{5,6}, Robert H. Pierce³, David A. Elashoff^{1,2}, Caroline Robert⁴ & Antoni Ribas^{1,2}

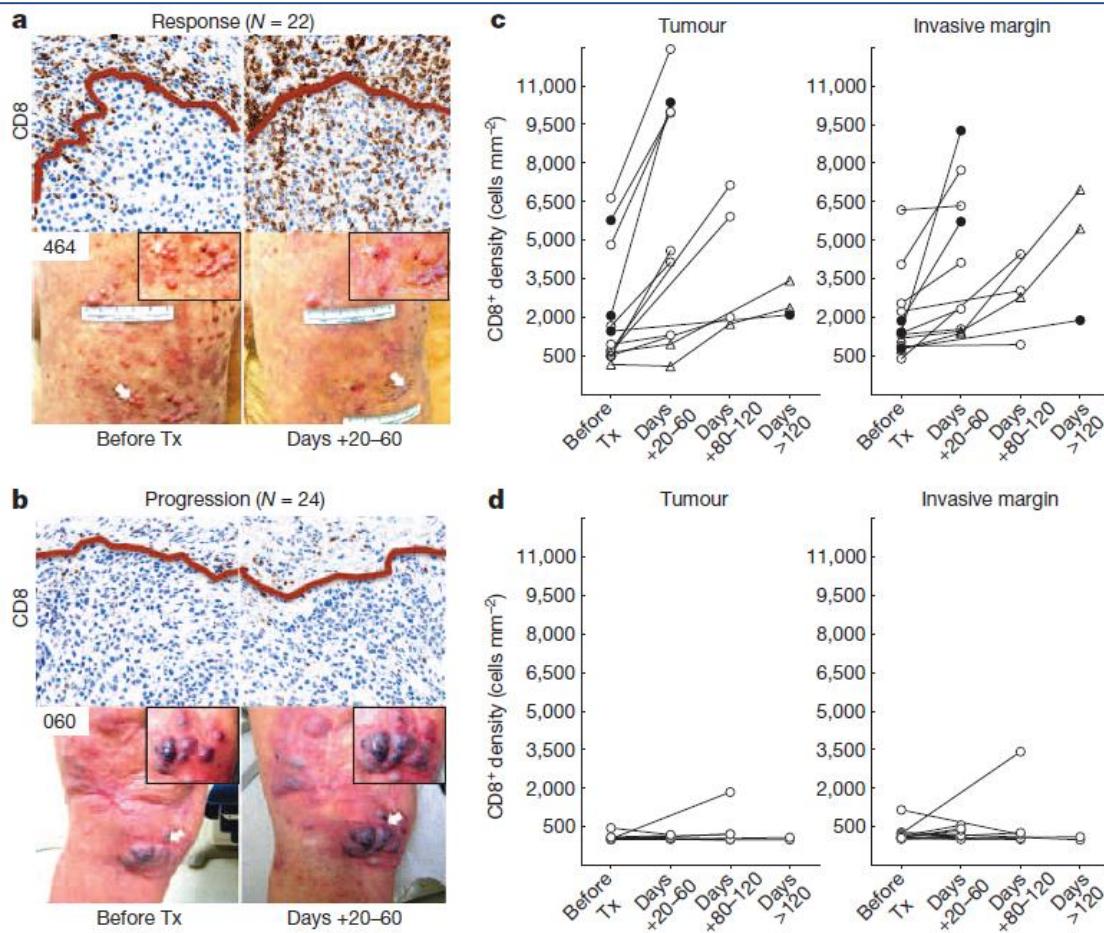


Figure 1 | Immunohistochemical analysis of CD8⁺ T cells in samples obtained before and during pembrolizumab treatment. **a, b,** Examples of CD8 expression in melanoma tumours serially biopsied before PD-1 blocking treatment (Tx) and 20–60 days after treatment began (Days +20–60) from a patient in the response (a) and progression (b) groups. Red line separates tumour parenchyma (below line) and invasive margin (above line). Magnification, $\times 20$. **c, d,** CD8⁺-cell density at the tumour parenchyma and invasive margin in samples from all responders (**c**; $n = 13$) and progressors (**d**; $n = 12$) who received a biopsy before and during treatment. Filled circle indicates complete response; open circle indicates partial response; triangle indicates delayed response.

Outline

- Introduction to the Tumor Immune Microenvironment (TIME)
 - Prognostic / Predictive value
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Understanding the tumor immune microenvironment (TIME) for effective therapy

Mikhail Binnewies¹, Edward W. Roberts¹, Kelly Kersten¹, Vincent Chan^{ID 2}, Douglas F. Fearon³,
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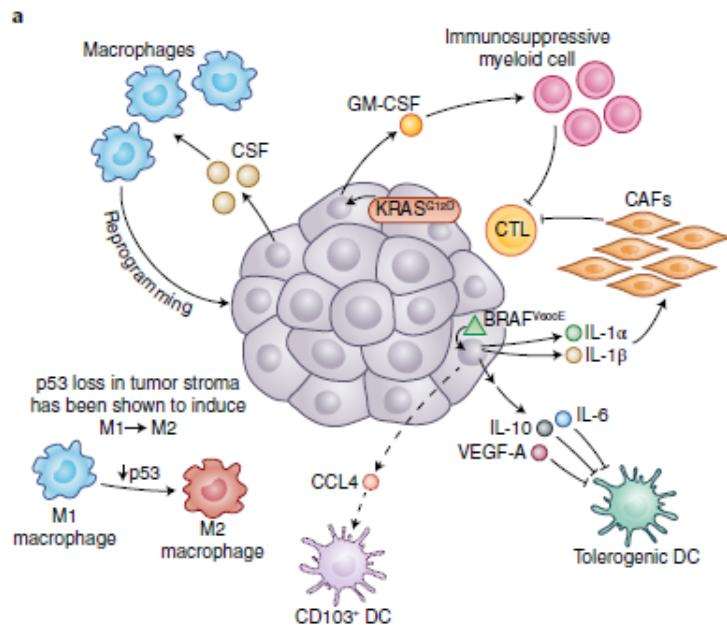


Fig. 2 | How tumor genotypes and phenotypes shape the TIME. a, Tumors are known to establish protumoral and immunosuppressive environments to support their growth and promote immune evasion. Central to building an immunosuppressive TIME are oncogenes and aberrant signaling pathways that lead to the production of cytokines and chemokines with potent effects. The tumor shown is representative of a spectrum of cancer types. In melanoma, **BRAF^{V600E}** (green triangle) has been shown to induce constitutive WNT/ β -catenin signaling, which in turn decreases production of CCL4, a chemokine important for the recruitment of CD103⁺ DCs. Additionally, **BRAF^{V600E}** has been shown to induce expression of factors such as IL-10 and IL-1 α , which can induce tolerogenic forms of DC and cancer-associated fibroblasts (CAFs), respectively. Oncogenic KRAS^{G12D} in PDAC leads to the secretion of GM-CSF, corresponding to increased development of CD11b⁺ myeloid cells with reported immunosuppressive function. Deficiency in p53 in hepatic stellate cells, a stromal population, leads to production of factors that polarize TAMs from the immunoactivating M1 phenotype to the immunosuppressive M2 phenotype. Interestingly, many tumors have been shown to secrete high levels of the monocyte/macrophage-promoting cytokine CSF-1.

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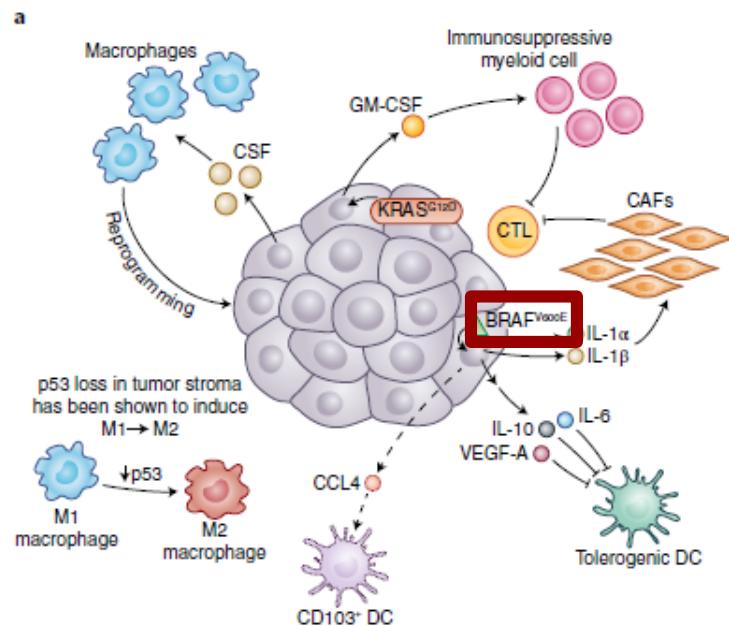


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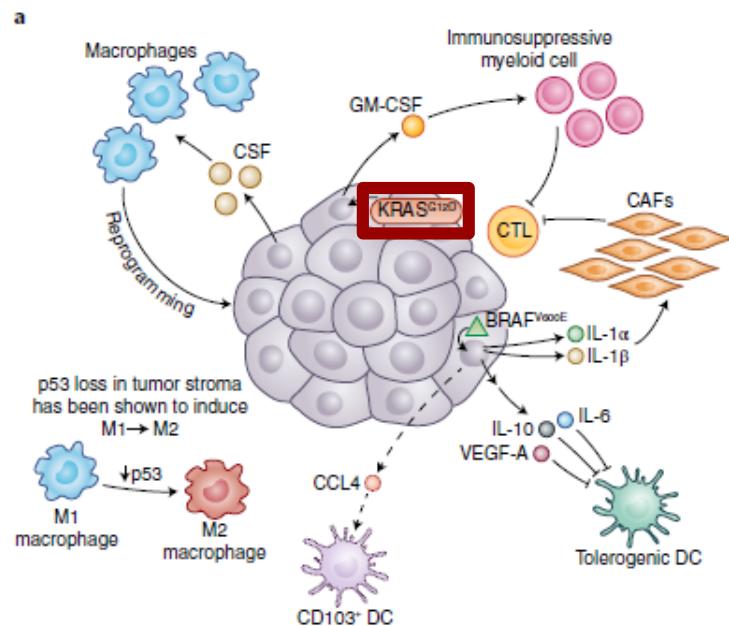


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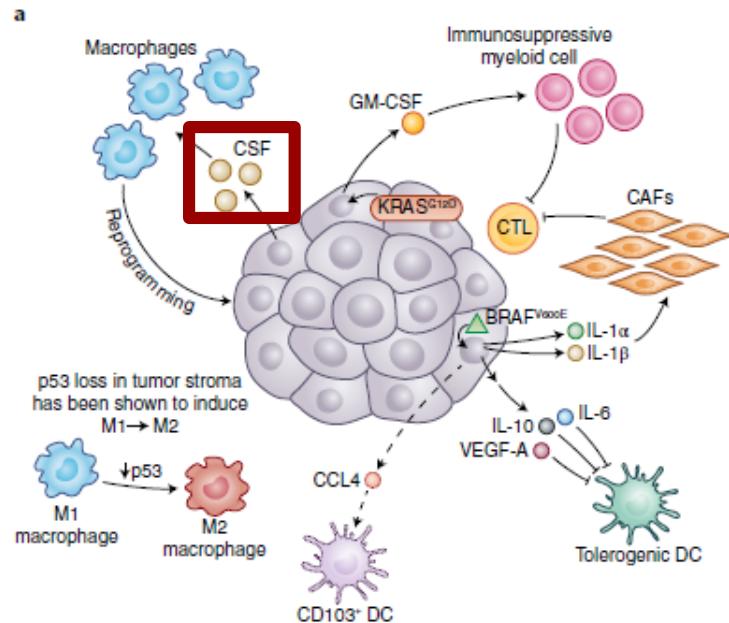
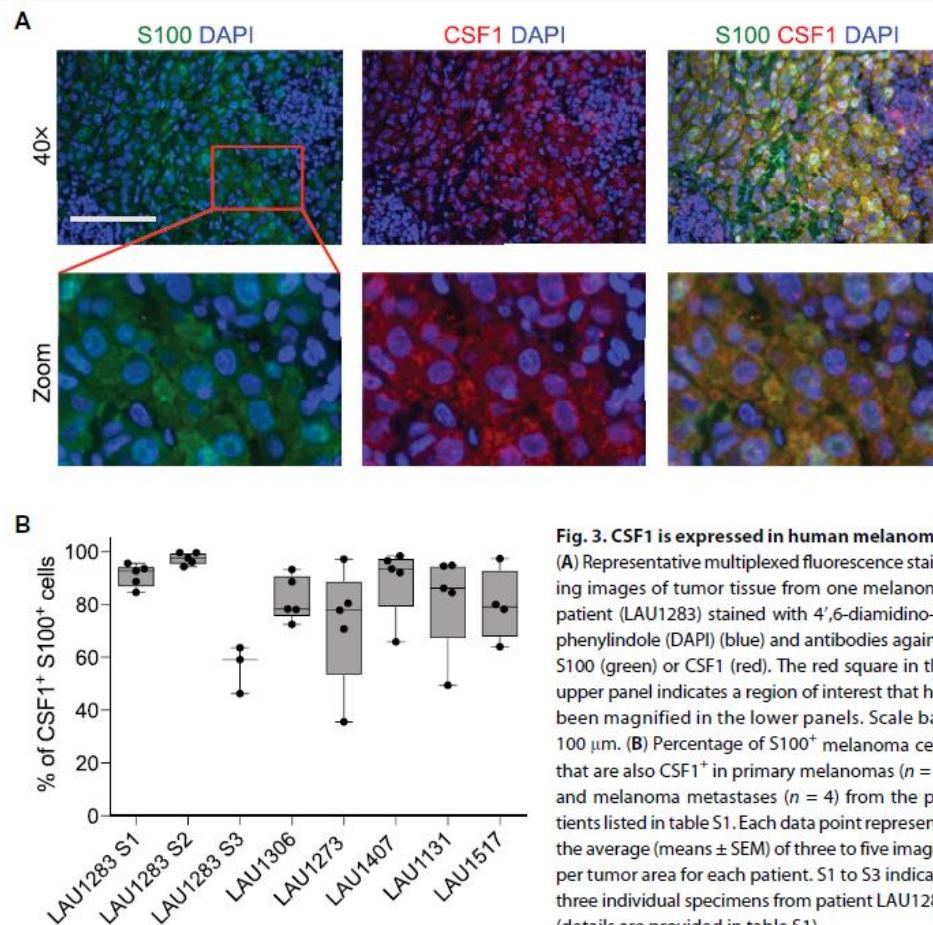


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T cell–induced CSF1 promotes melanoma resistance to PD1 blockade

Natalie J. Neubert,^{1*} Martina Schmittnaegel,^{2*} Natacha Bordry,^{1*} Sina Nassiri,² Noémie Wald,¹ Christophe Martignier,¹ Laure Tillé,¹ Krisztian Homicsko,^{1,2} William Damsky,³ Hélène Maby-El Hajjami,¹ Irina Klaman,⁴ Esther Danenberg,⁵ Kalliopi Ioannidou,¹ Lana Kandalaft,⁵ George Coukos,^{1,5} Sabine Hoves,⁴ Carola H. Ries,⁴ Silvia A. Fuertes Marraco,¹ Periklis G. Foukas,^{5†} Michele De Palma,^{2‡§} Daniel E. Speiser^{1‡§}

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

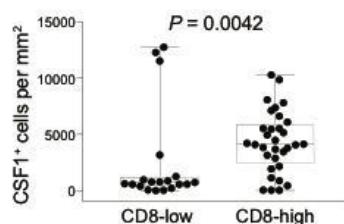


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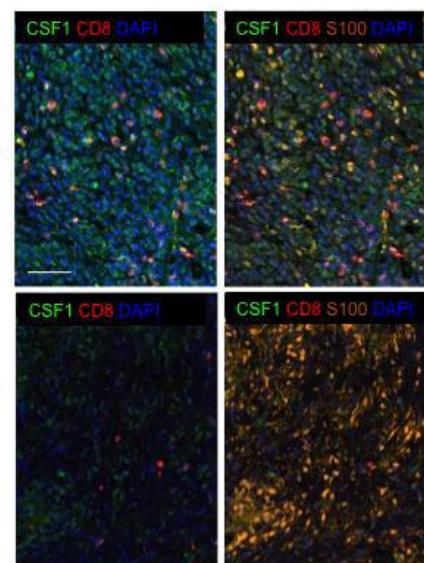
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SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

A



B



C

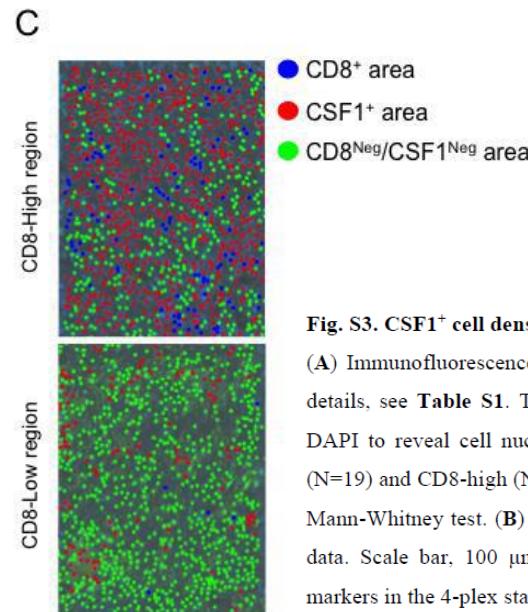


Fig. S3. CSF1⁺ cell density in high and low CD8⁺ cell-infiltrated tumor regions.

(A) Immunofluorescence analysis of primary tumors (N=3) and metastases (N=3) of cutaneous melanoma; for details, see Table S1. Tumor sections were stained with antibodies specific for CSF1, CD8 or S100, and with DAPI to reveal cell nuclei. CSF1-expressing cells were quantified in randomly selected (unpaired) CD8-low (N=19) and CD8-high (N=32) intratumoral regions. Error bars indicate min and max values. Statistical analysis by Mann-Whitney test. (B) Representative images of the immunofluorescence staining used for quantification of the data. Scale bar, 100 μ m. (C) Cell phenotype map identifying the cell populations defined by the individual markers in the 4-plex staining, overlaid on the raw image.

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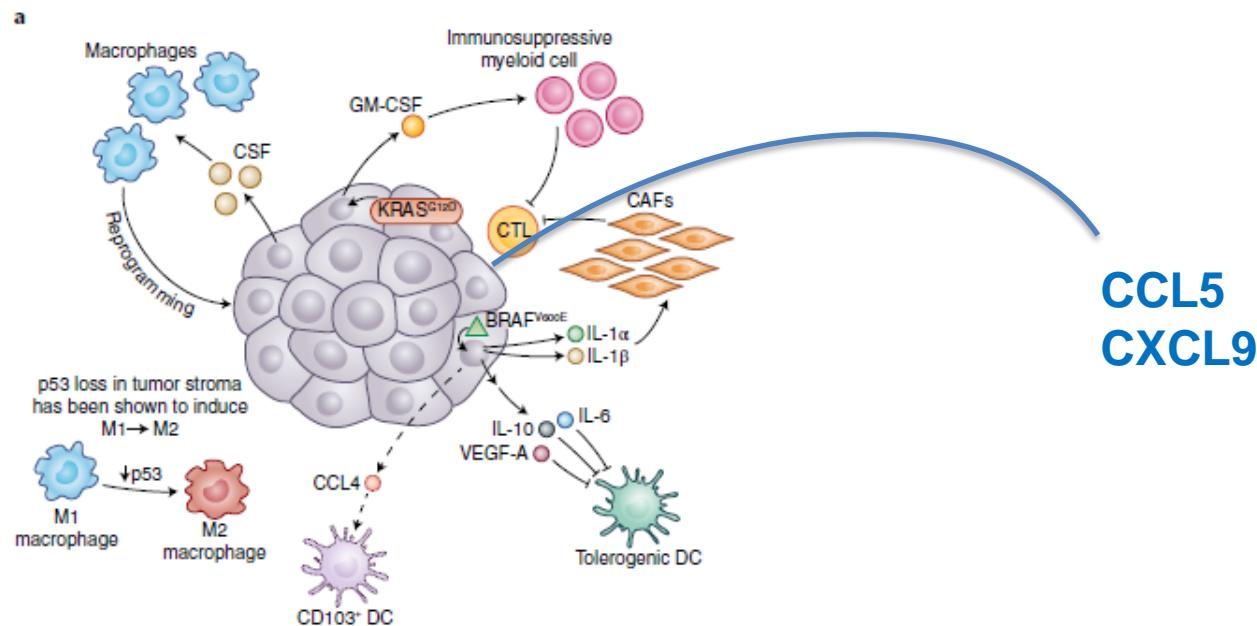
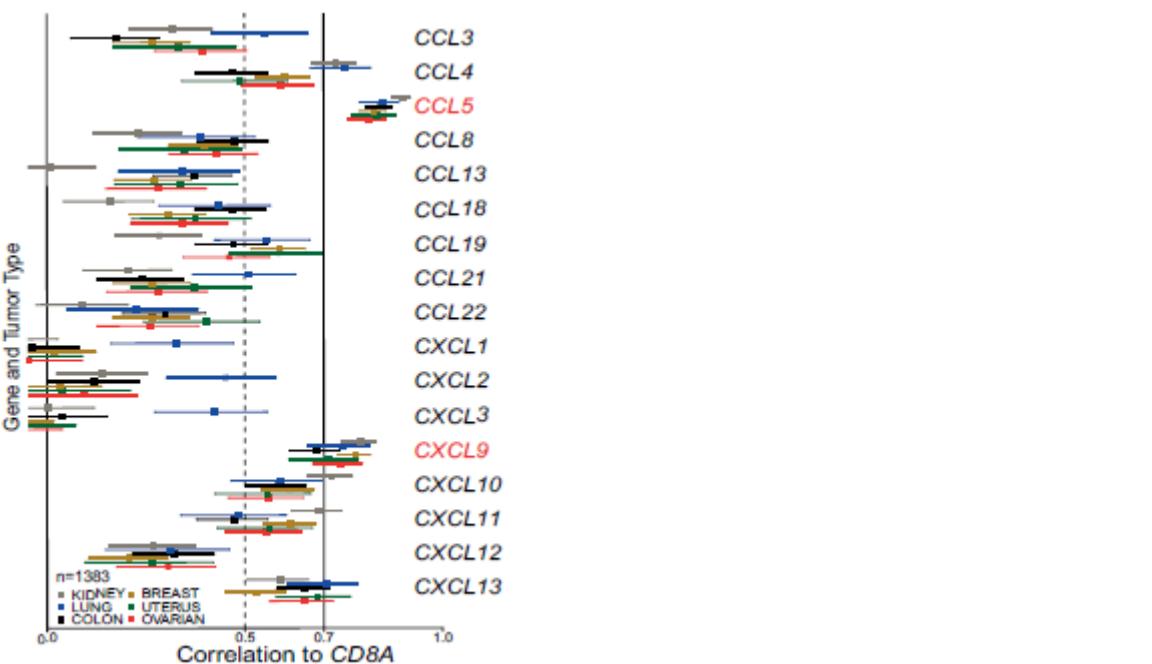


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Cooperation between Constitutive and Inducible Chemokines Enables T Cell Engraftment and Immune Attack in Solid Tumors

Denarda Dangaj,¹ Marine Bruand,¹ Alizée J. Grimm,¹ Catherine Ronet,¹ David Barras,^{1,2} Priyanka A. Duttagupta,^{3,4} Evripidis Lanitis,¹ Jaikumar Duraiswamy,^{3,5} Janos L. Tanyi,³ Fabian Benencia,⁶ Jose Conejo-Garcia,⁷ Hena R. Ramay,^{2,8} Kathleen T. Montone,⁹ Daniel J. Powell, Jr.,³ Phyllis A. Gimotty,¹⁰ Andrea Facciabene,³ Donald G. Jackson,¹¹ Jeffrey S. Weber,¹² Scott J. Rodig,^{13,14} Stephen F. Hodi,¹⁴ Lana E. Kandalaft,¹ Melita Irving,¹ Lin Zhang,³ Periklis Foukas,^{1,15} Sylvie Rusakiewicz,¹ Mauro Delorenzi,^{1,2} and George Coukos^{1,16,*}

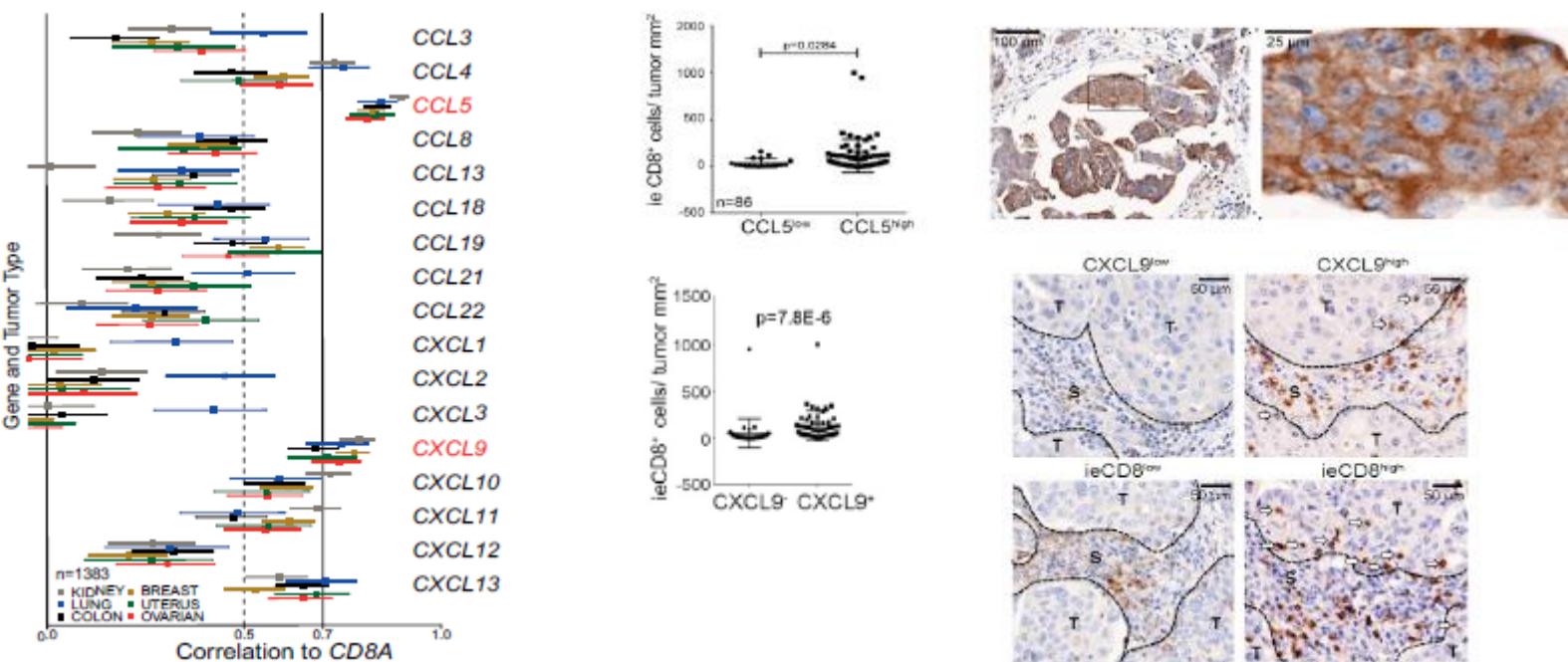
Cancer Cell 35, 885–900, June 10, 2019



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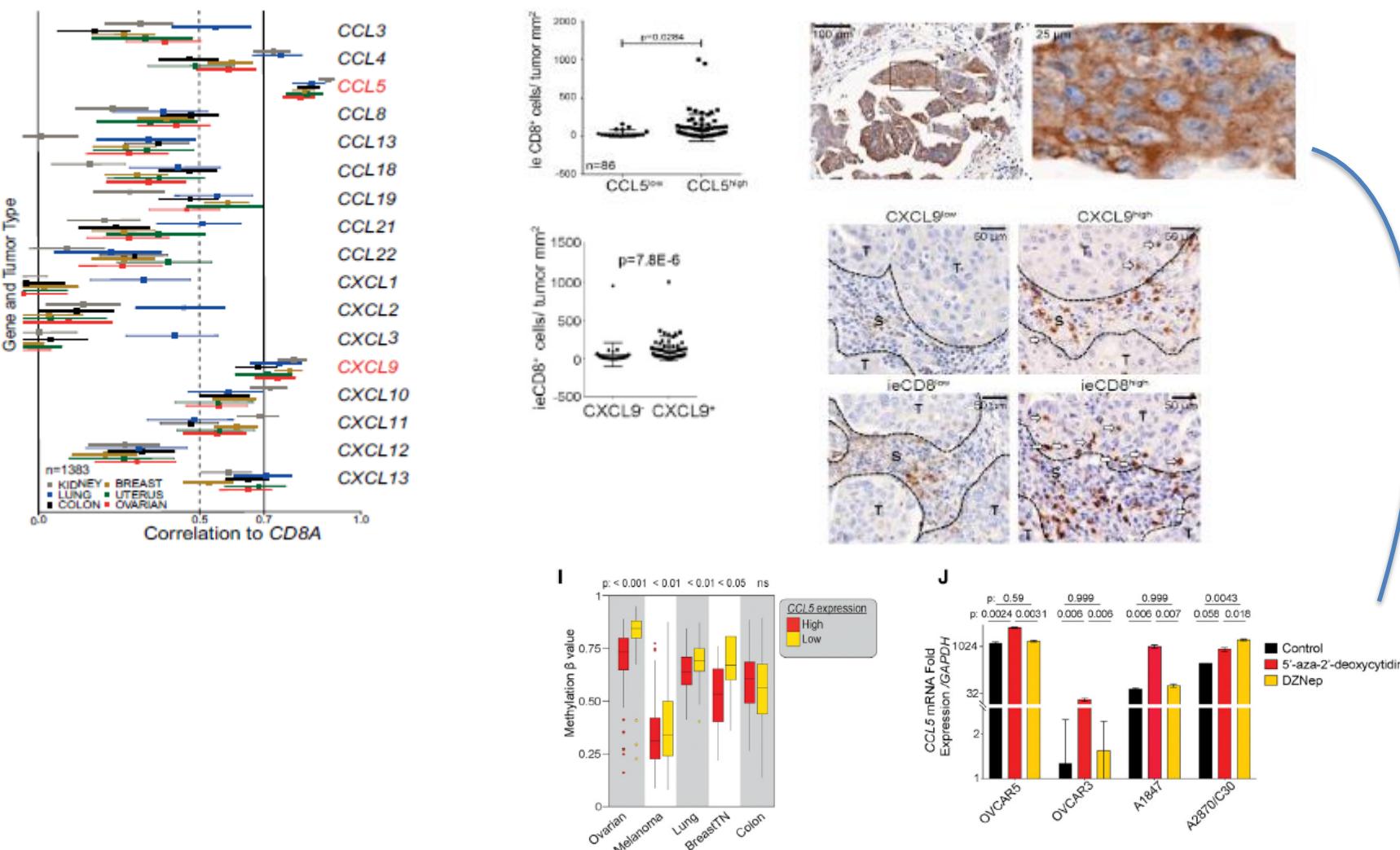
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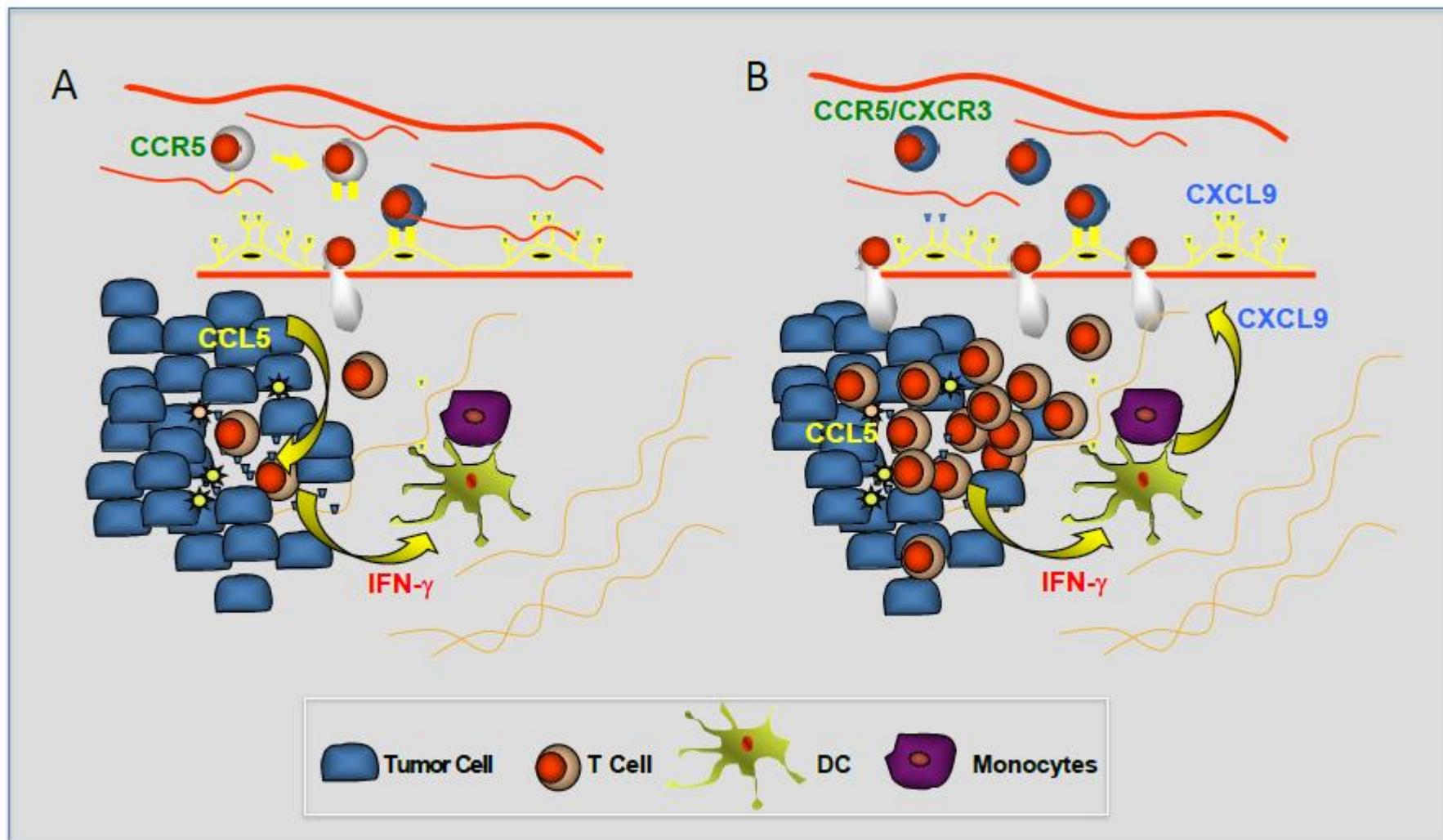
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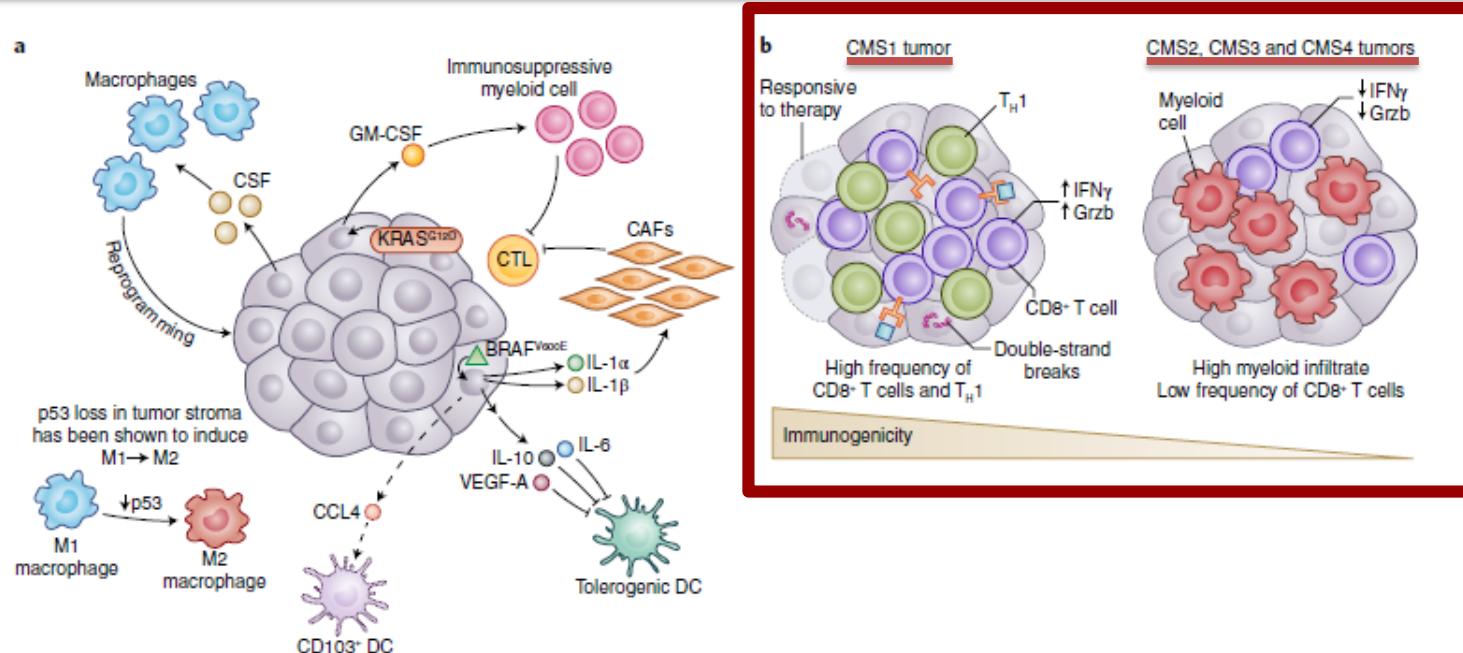


Fig. 2 | How tumor genotypes and phenotypes shape the TIME. **a**, Tumors are known to establish protumoral and immunosuppressive environments to support their growth and promote immune evasion. Central to building an immunosuppressive TIME are oncogenes and aberrant signaling pathways that lead to the production of cytokines and chemokines with potent effects. The tumor shown is representative of a spectrum of cancer types. In melanoma, BRAF^{V600E} (green triangle) has been shown to induce constitutive WNT/β-catenin signaling, which in turn decreases production of CCL4, a chemokine important for the recruitment of CD103⁺ DCs. Additionally, BRAF^{V600E} has been shown to induce expression of factors such as IL-10 and IL-1α, which can induce tolerogenic forms of DC and cancer-associated fibroblasts (CAFs), respectively. Oncogenic KRAS^{G12D} in PDAC leads to the secretion of GM-CSF, corresponding to increased development of CD11b⁺ myeloid cells with reported immunosuppressive function. Deficiency in p53 in hepatic stellate cells, a stromal population, leads to production of factors that polarize TAMs from the immunoactivating M1 phenotype to the immunosuppressive M2 phenotype. Interestingly, many tumors have been shown to secrete high levels of the monocyte/macrophage-promoting cytokine CSF-1. **b**, The mutational landscape of tumors can profoundly affect the quality and character of the TIME. In CRC, there are four consensus molecular subtypes (CMS1-4). CMS1 is defined by defects in DNA mismatch repair leading to microsatellite instability or hypermutation rates. Because of the abundance of possible neoepitopes, CTL infiltration is generally high, and CTls display gene expression patterns indicative of an ongoing immune response. Patients with CMS1 tumors have generally more favorable outcomes with checkpoint-blockade treatment than do patients with CMS2-4. Although there are differences in the histological and immunological character of CMS2, 3 and 4 CRC subtypes, they are generally less immune infiltrated, as is suggestive of antigenically cold tumors.

Outline

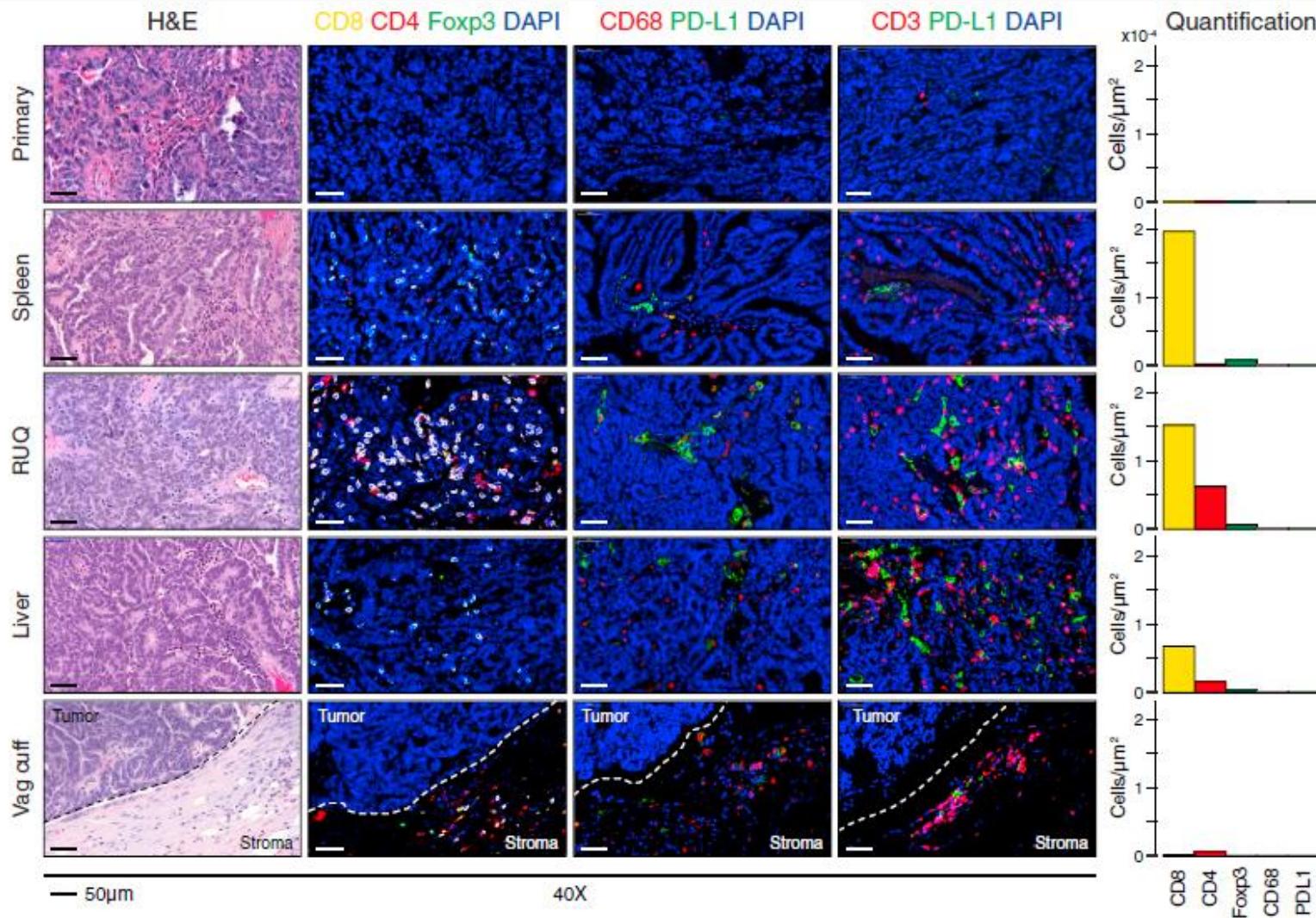
- Introduction to the Tumor Immune Microenvironment (TIME)
 - Prognostic / Predictive value
- Mechanisms regulating TIME
- Evaluation / Methodologies
- Turning-up the heat



Heterogeneous Tumor-Immune Microenvironments among Differentially Growing Metastases in an Ovarian Cancer Patient

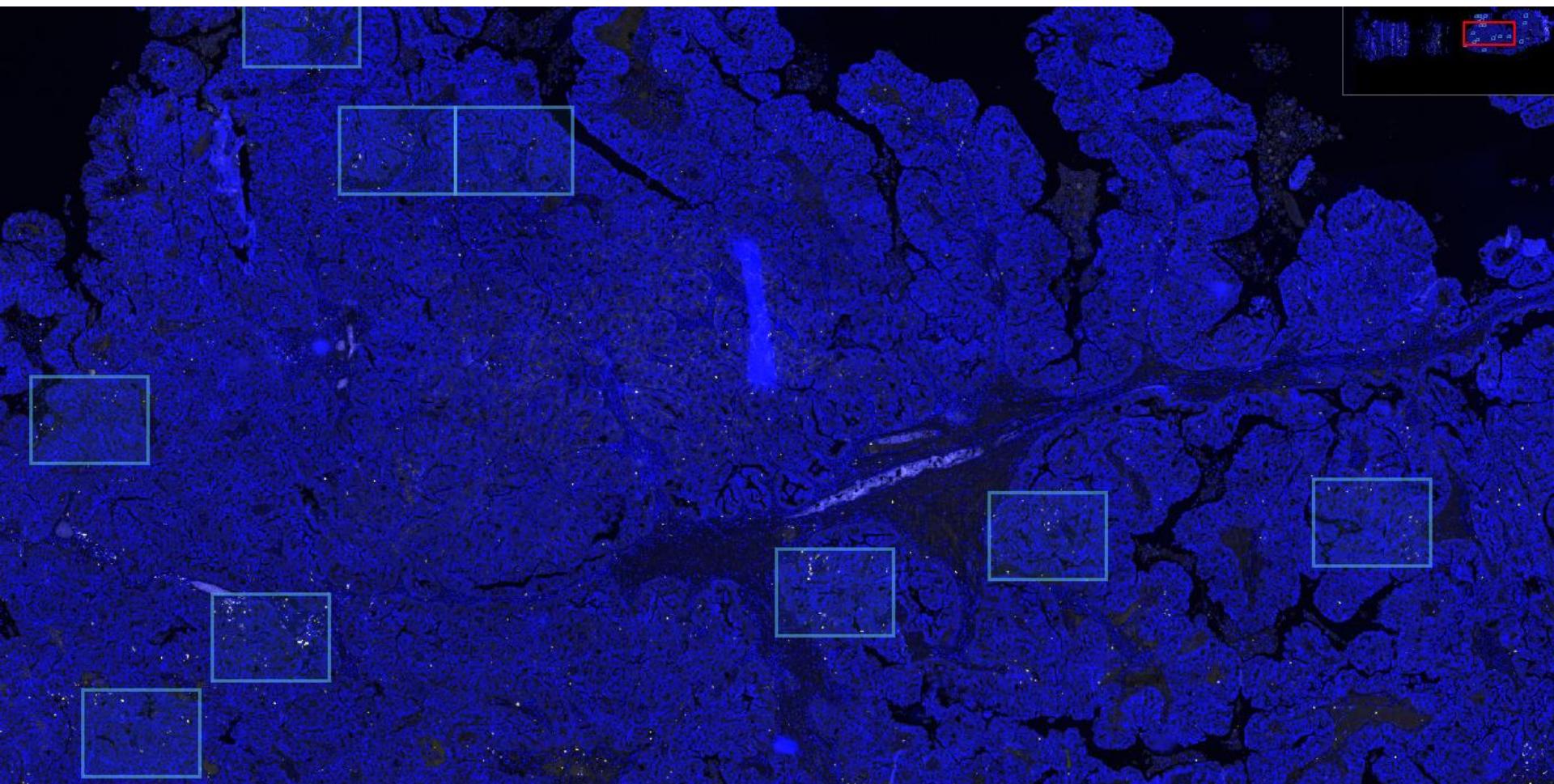
Alejandro Jiménez-Sánchez,¹ Danish Memon,^{1,2} Stephane Pourpe,¹⁰ Harini Veeraraghavan,³ Yanyun Li,⁴ Hebert Alberto Vargas,⁵ Michael B. Gill,¹ Kay J. Park,⁶ Oliver Zivanovic,⁷ Jason Konner,⁸ Jacob Ricca,⁴ Dmitriy Zamarin,^{4,10} Tyler Walther,¹⁰ Carol Aghajanian,⁸ Jedd D. Wolchok,^{4,9,10,11,12} Evis Sala,⁵ Taha Merghoub,⁴ Alexandra Snyder,^{10,11,13,*} and Martin L. Miller^{1,*}

Cell 170, 927–938, August 24, 2017



Case 0095-Geico

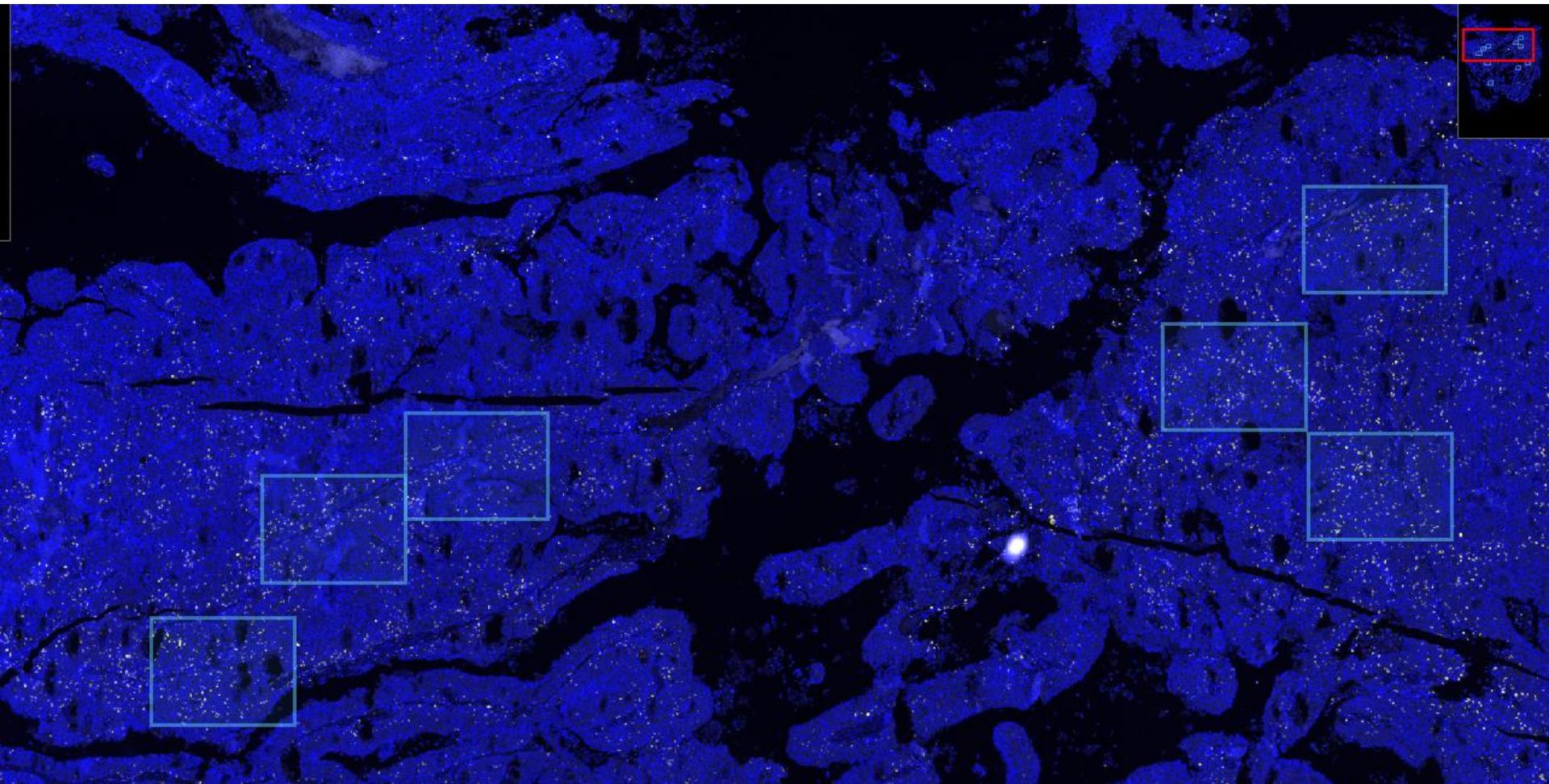
Low TILs



CD8 DAPI

Case 0100-Geico

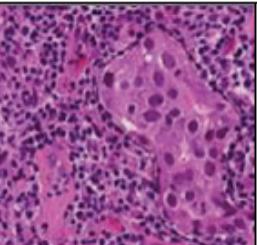
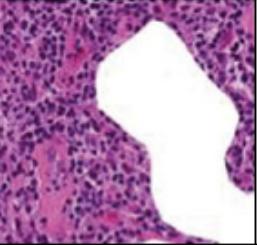
High TILs



CD8 DAPI

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. Sirtaine⁵, F. Klauschen³, G. Pruner⁶, S. Wienert³, G. Van den Eynden⁷, F. L. Baehner^{8,9}, F. Penault-Llorca¹⁰, E. A. Perez¹¹, E. A. Thompson¹², W. F. Symmans¹³, A. L. Richardson^{14,15}, J. Brock^{15,16}, C. Criscitiello¹⁷, H. Bailey⁸, M. Ignatiadis¹⁸, G. Floris¹⁹, J. Sparano²⁰, Z. Kos²¹, T. Nielsen²², D. L. Rimm²³, K. H. Allison²⁴, J. S. Reis-Filho²⁵, S. Loibl²⁶, C. Sotiriou¹⁸, G. Viale²⁷, S. Badve²⁸, S. Adams^{4,†}, K. Willard-Gallo^{29,†} & S. Loi^{30*,†}

Morphology	Definition and biological relevance	Diagnostic relevance
Lymphocyte-predominant breast cancer (LPBC)		
	Working category to describe tumors with "more lymphocytes than tumor cells".	Definitions vary across studies with stromal TILs of 50–60% used as a threshold. LPBC can be used for predefined subgroup analyses and for description of tumors with a particularly high immune infiltrate, however, keep in mind that TILs are a continuous parameter and the threshold for LPBC is still arbitrary.
Stromal TILs		
	Indicator of increased accumulation of immune-cells in tumor tissue	Stromal TILs have been shown to be predictive for increased response to neoadjuvant chemotherapy as well as improved outcome after adjuvant chemotherapy. Based on current data, this parameter is the best parameter for characterization of TILs.
Intratumoral TILs		
	TILs with direct cell-cell contact with carcinoma cells, might be an indicator of direct cell-based anti-tumor effects.	Several studies have shown that intratumoral TILs are more difficult to evaluate and do not provide additional predictive/prognostic information compared to stromal TILs.

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

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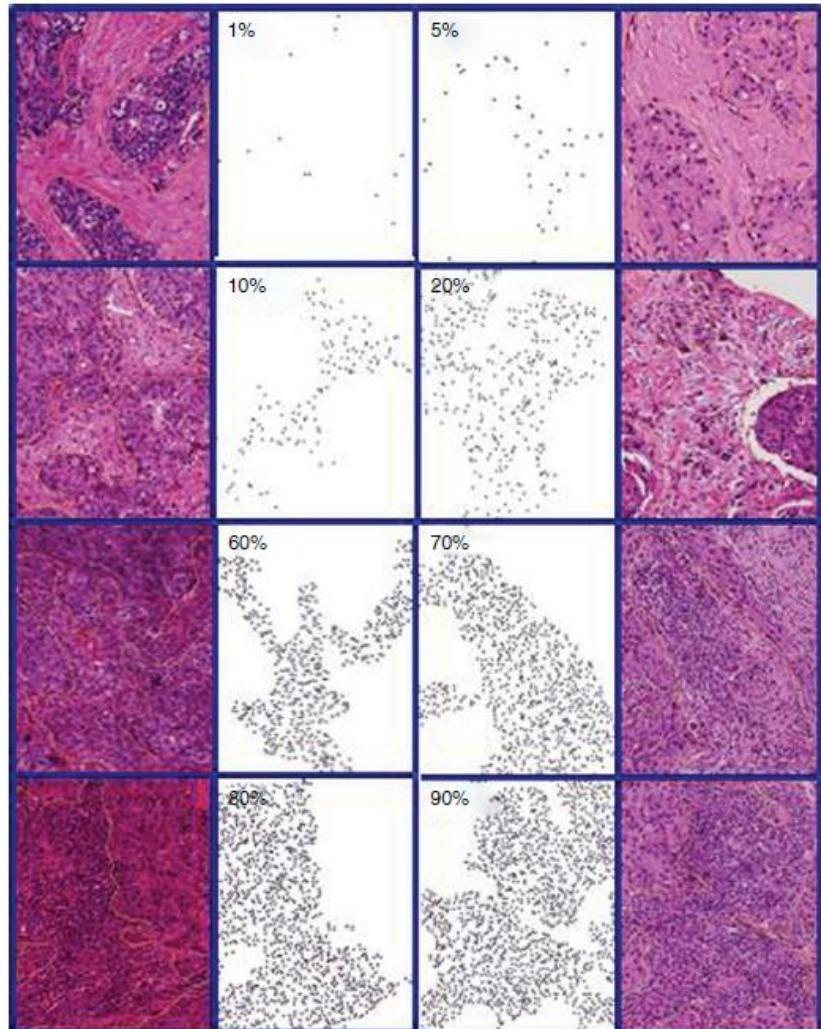


Figure 4. Standardization and guidelines for TILs assessment.

Prognostic significance of tumor-infiltrating T cells in ovarian cancer: A meta-analysis

Wei-Ting Hwang ^{a,b,1}, Sarah F. Adams ^{a,1}, Emin Tahirovic ^b, Ian S. Hagemann ^{c,2}, George Coukos ^{a,*}

^a Ovarian Cancer Research Center, University of Pennsylvania, Philadelphia, PA 19104, USA

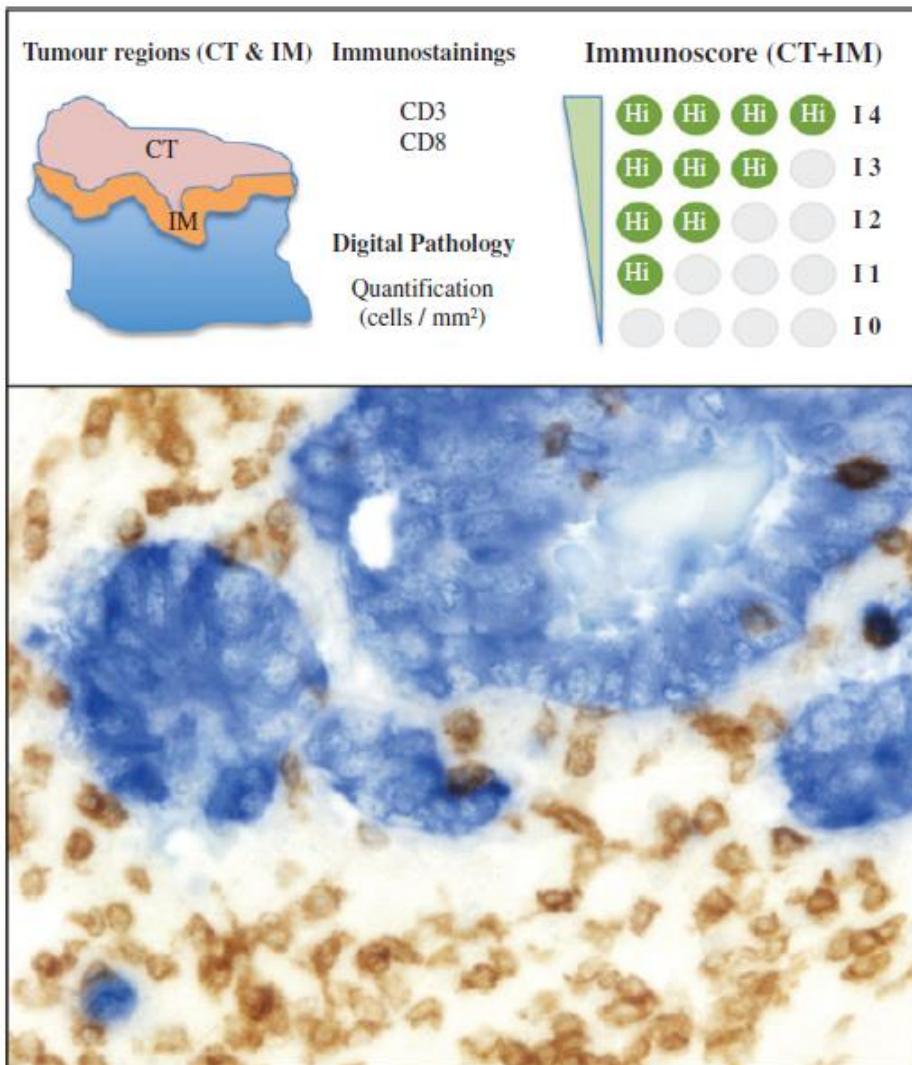
^b Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA 19104, USA

^c Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA

Gynecologic Oncology 124 (2012) 192–198

CD3 or CD8 expression, intraepithelial CD8 TIL showed a more consistent and stronger association with survival than CD3 TIL. Thus, CD8 staining should be used as the standard for evaluation of intraepithelial TIL in ovarian cancer specimens. Further, a significant difference was seen in the HRs based on scoring method used to evaluate TIL. While TIL scores represent an underlying continuous variable, a standardized measure of TIL positivity would facilitate future studies. Because significantly larger HRs were noted in studies that used greater than zero cut-offs (e.g., 3–10 cells/HPF) for a positive score, and 5 cells/HPF approximately represents the midpoint of those cut-off values, we propose that >5 CD8⁺ cells/200× HPF should be defined “TIL-positive” in ovarian tumors.

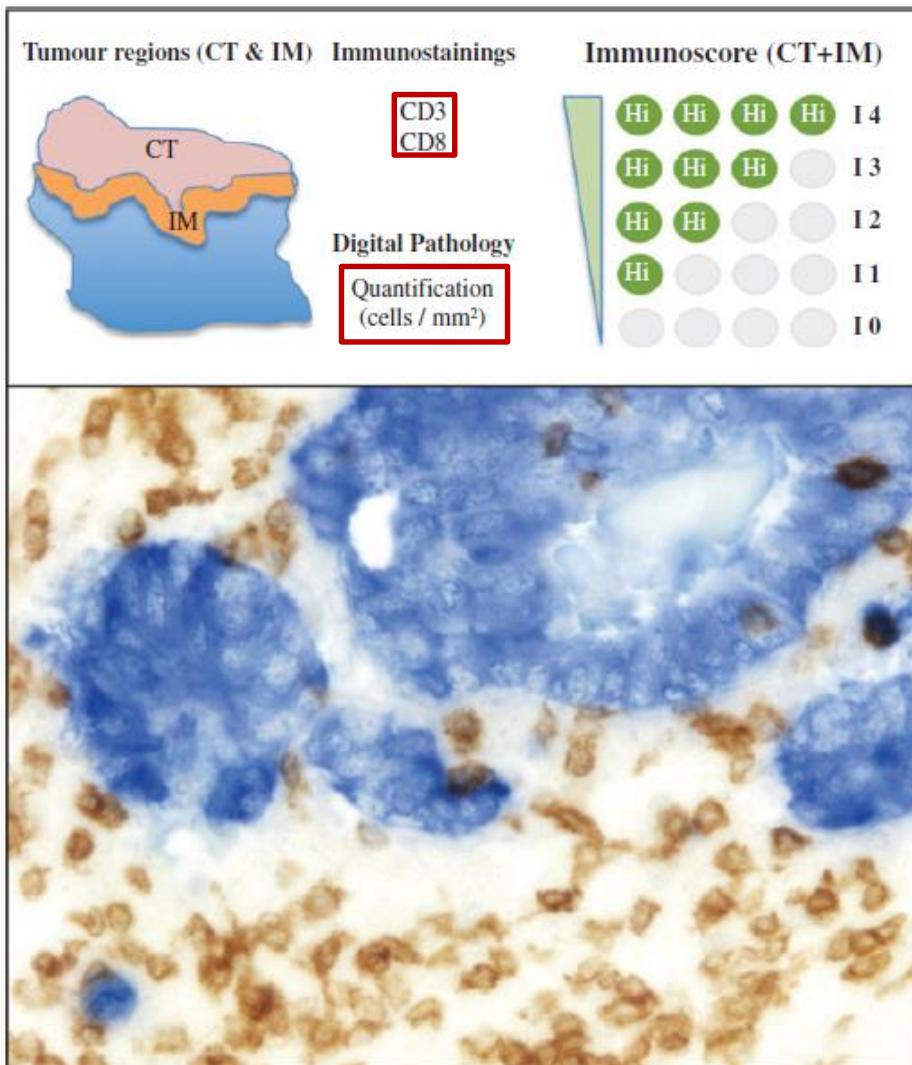
Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



	Immune infiltration	Score
CD3	CD8	(Hi = 1; Lo = 0)
CT:	Hi or Lo + Hi or Lo	= 0, 1, or 2 +
IM:	Hi or Lo + Hi or Lo	= 0, 1, or 2
Immunoscore (I) = 0, 1, 2, 3, or 4		

Cut off (Hi vs Lo)= the "minimum p value" approach

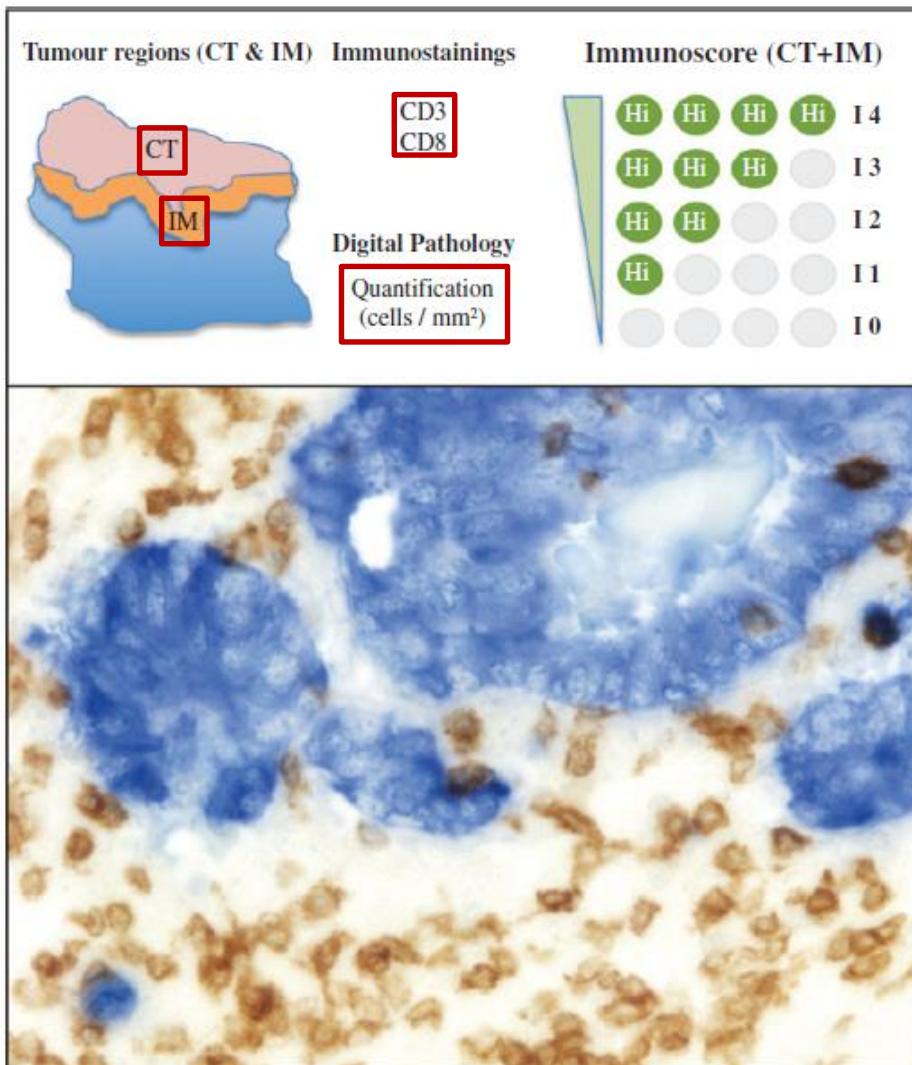
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Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



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Immunoscore (I) = 0, 1, 2, 3, or 4		

Cut off (Hi vs Lo)= the "minimum p value" approach

Implications of the tumor immune microenvironment for staging and therapeutics

Janis M Taube^{1,2,3}, Jérôme Galon^{4,5,6}, Lynette M Sholl⁷, Scott J Rodig⁷, Tricia R Cottrell², Nicolas A Giraldo^{1,2}, Alexander S Baras², Sanjay S Patel⁷, Robert A Anders², David L Rimm⁸ and Ashley Cimino-Mathews^{2,3}

MODERN PATHOLOGY (2018) 31, 214–234

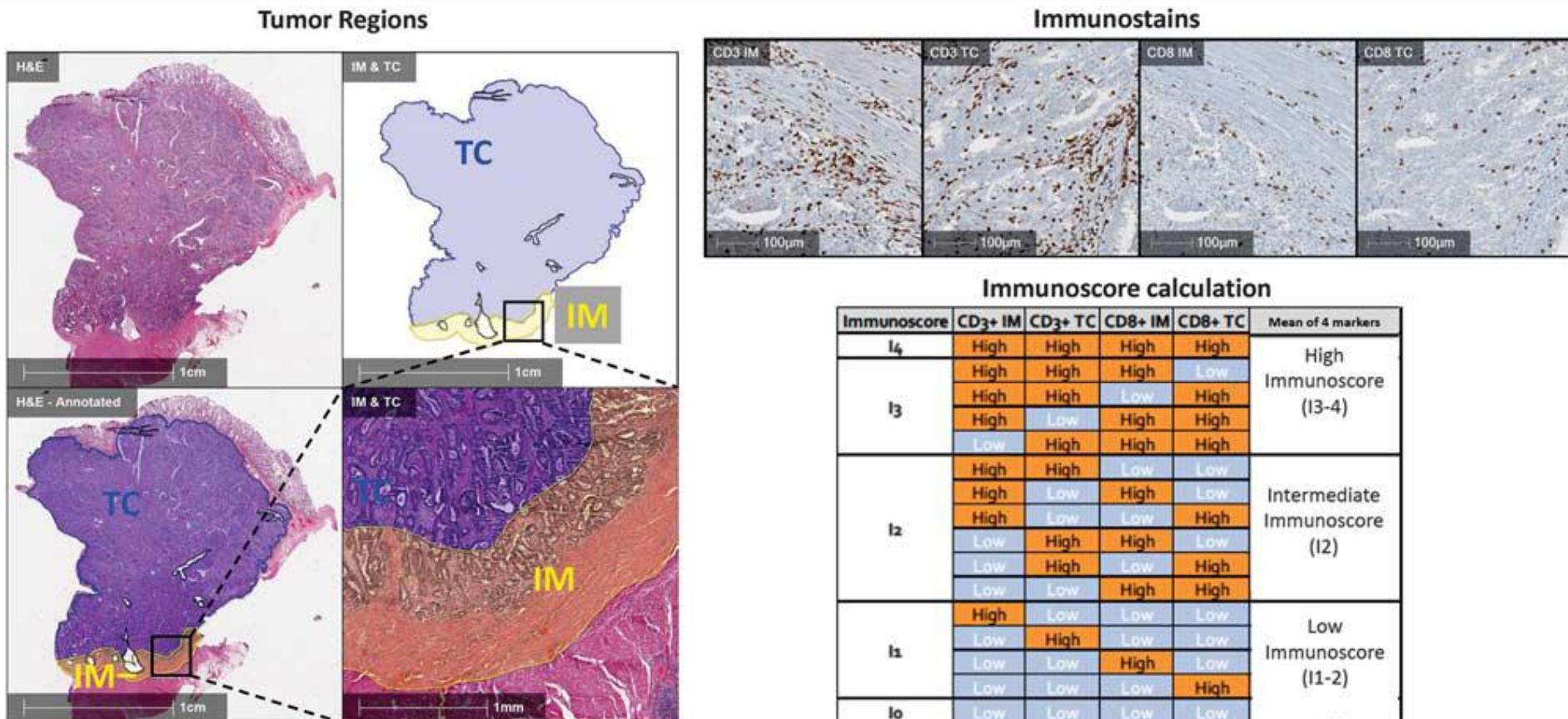


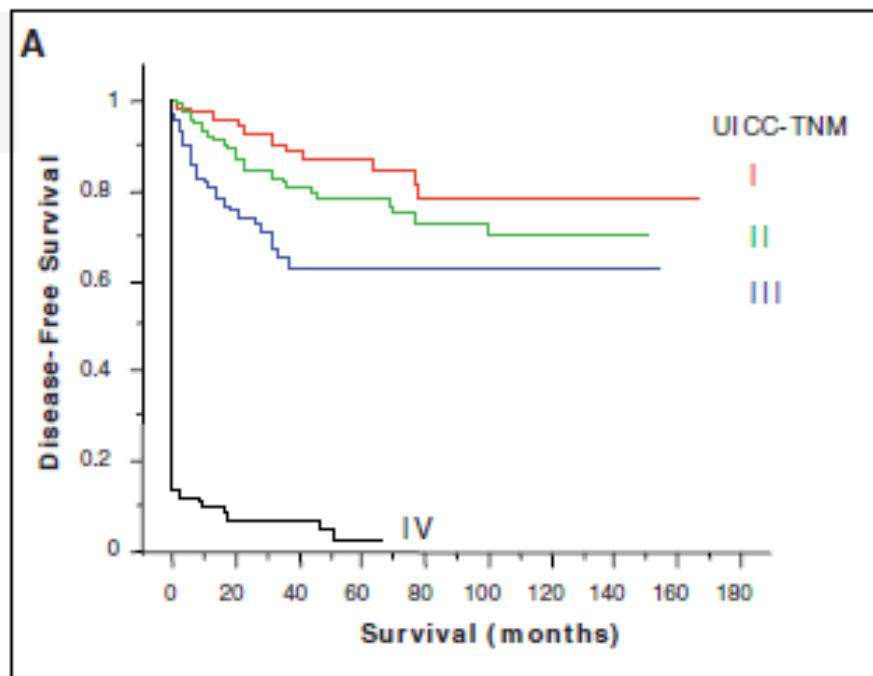
Figure 4 The Immunoscore is a standardized approach to characterizing T-cell infiltration of surgical pathology tumor specimen. Left panels: The border between the advancing tumor edge and normal tissue is annotated on a colorectal carcinoma specimen. A 500-μm distance on either side of this border is designated the ‘invasive margin’ (IM, yellow region). The remainder of the tumor is designated as the ‘tumor core’ (TC, blue region). Upper right panels: Immunohistochemistry for CD3 and CD8 is used to quantify cell densities for each of these immune cell subsets in both the invasive margin and tumor core. Lower right panels: The density of each region is labeled ‘high’ or ‘low’ density for each marker. The mean percentile of the four immune parameters is calculated, resulting in a possible Immunoscore ranging from I0 to I4, or three categories (Low, Intermediate, High). (Abbreviations: IM, invasive margin; TC, tumor core).

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} †

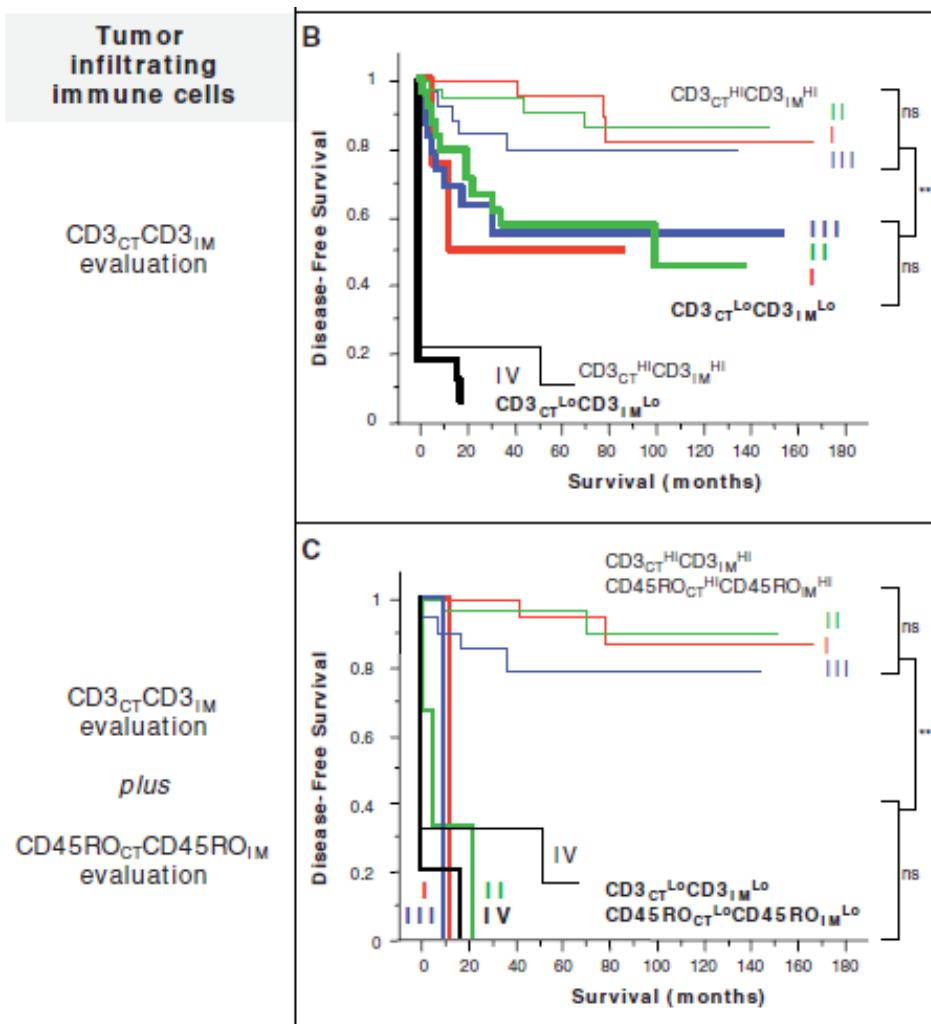
Tumor histopathology

UI CC-TNM Staging system

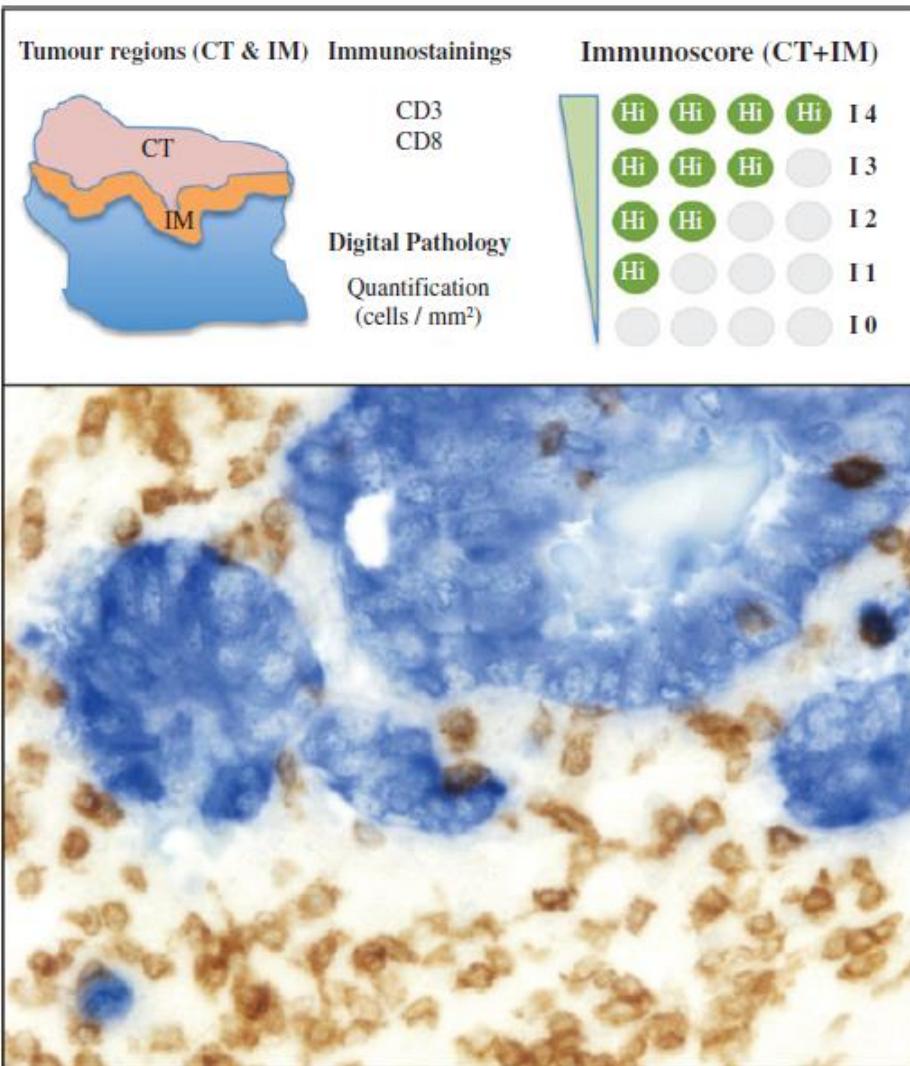


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Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



Good biomarker

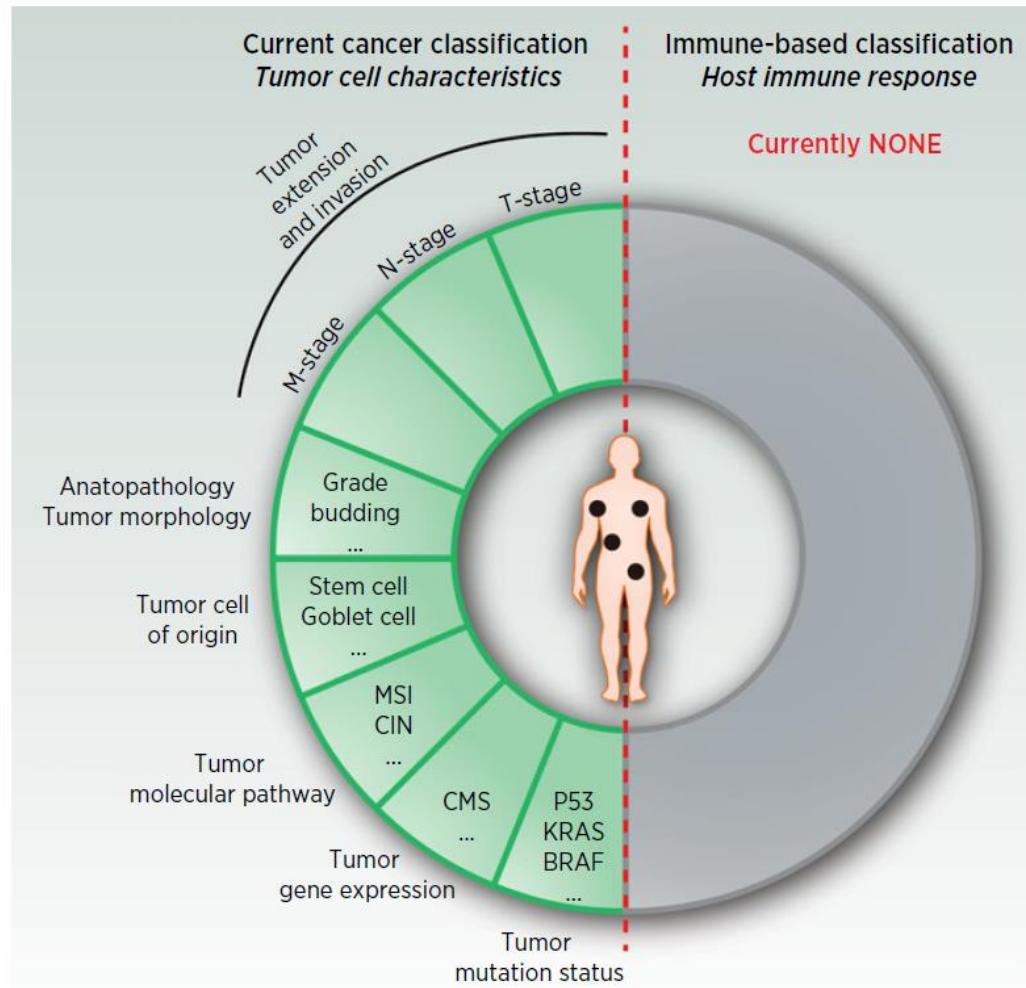
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The Immunoscore: Colon Cancer and Beyond

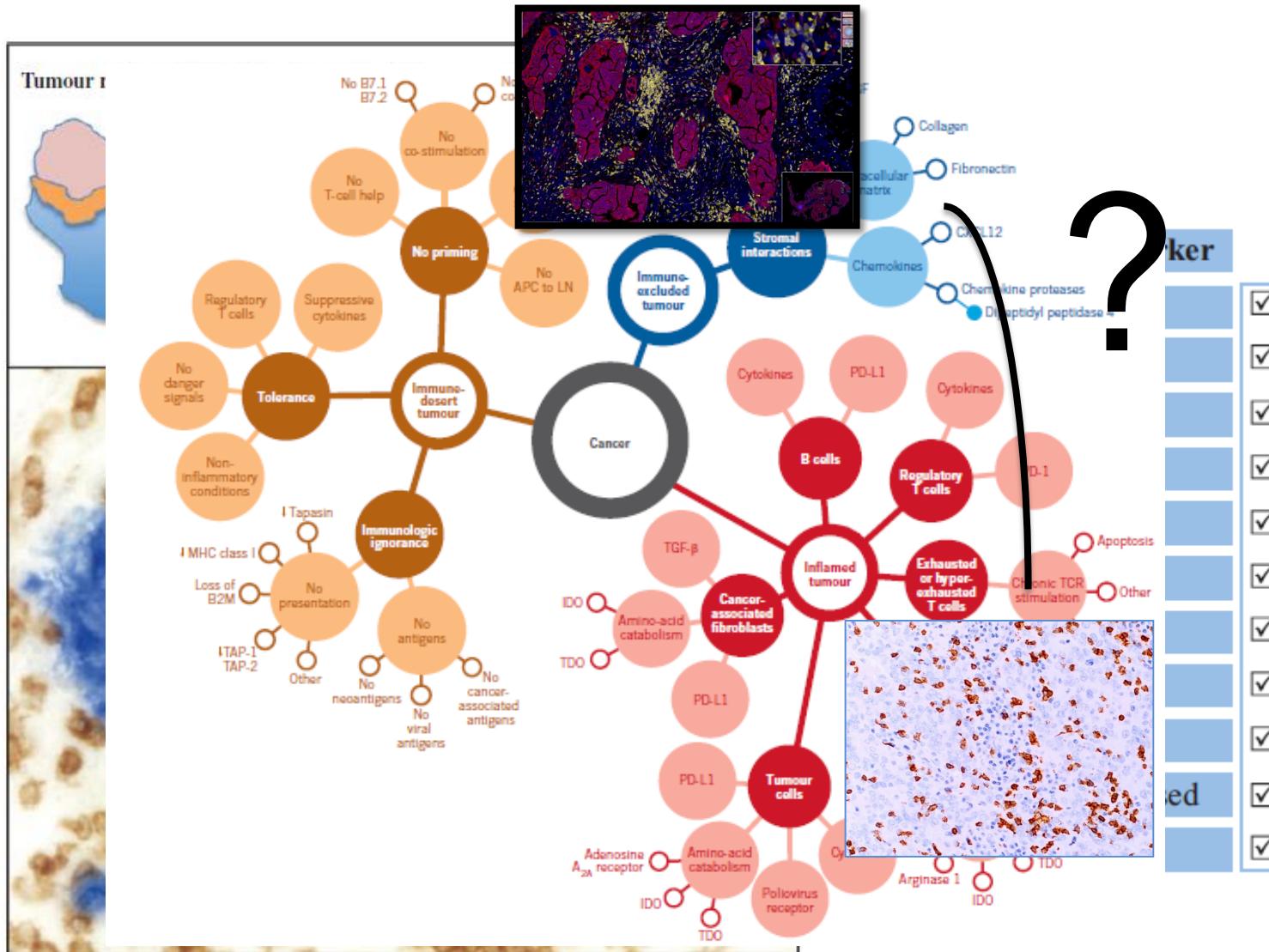
Helen K. Angell¹, Daniela Bruni², J. Carl Barrett³, Ronald Herbst⁴, and Jérôme Galon²

Figure 1.

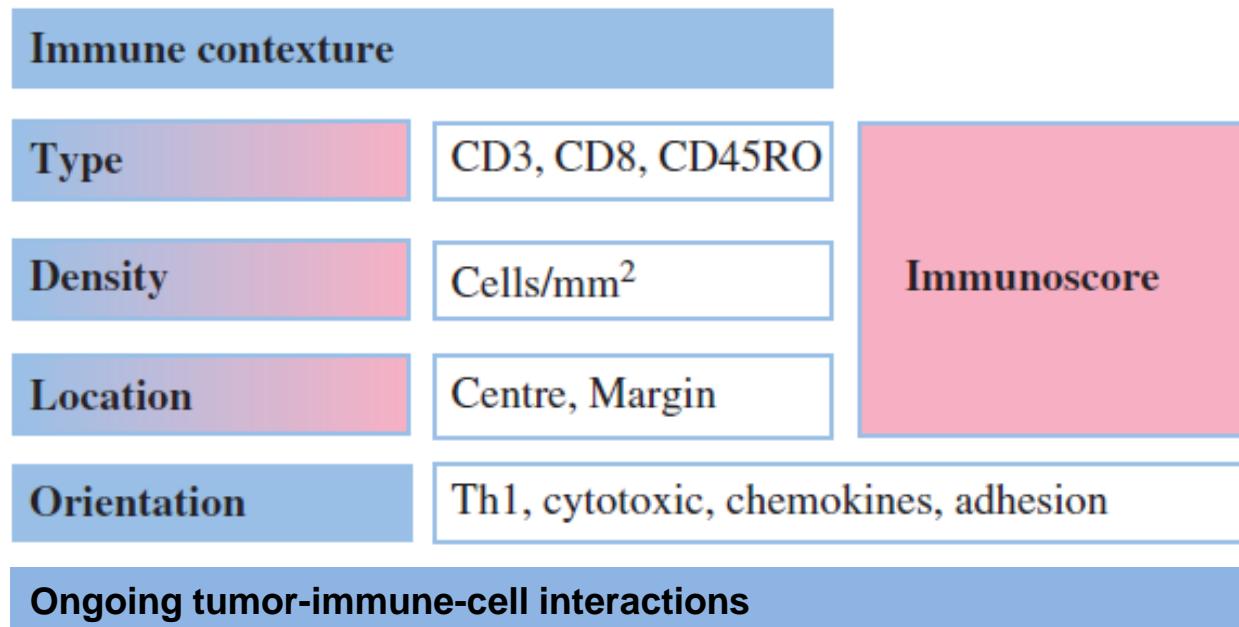
Pie chart represents current and possible cancer classification approaches. Tumors are currently classified based solely on tumor cell characteristics (left). These include the AJCC/UICC-TNM stratification system, tumor morphology (i.e., grade of differentiation; tumor budding; sidedness; location; venous emboli, lymphatic invasion, perineural invasion-VELPI), tumor cell of origin, deregulated molecular pathways, specific tumor signatures, and mutational status. Despite the existence of compelling evidence demonstrating the strength of immune-based classifications (such as that provided by the Immunoscore), current cancer classification does not include any immune parameter to date (right). CIN, chromosomal instability; CMS, consensus molecular subtypes; MSI, microsatellite instability.



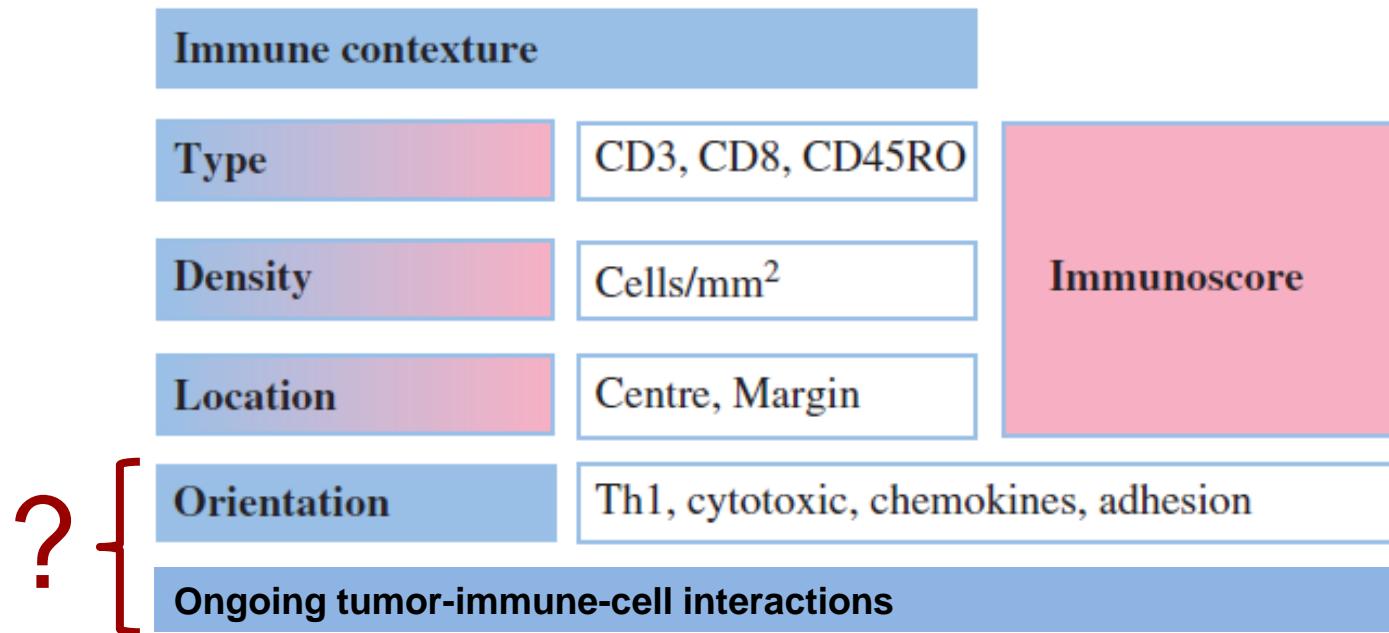
Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



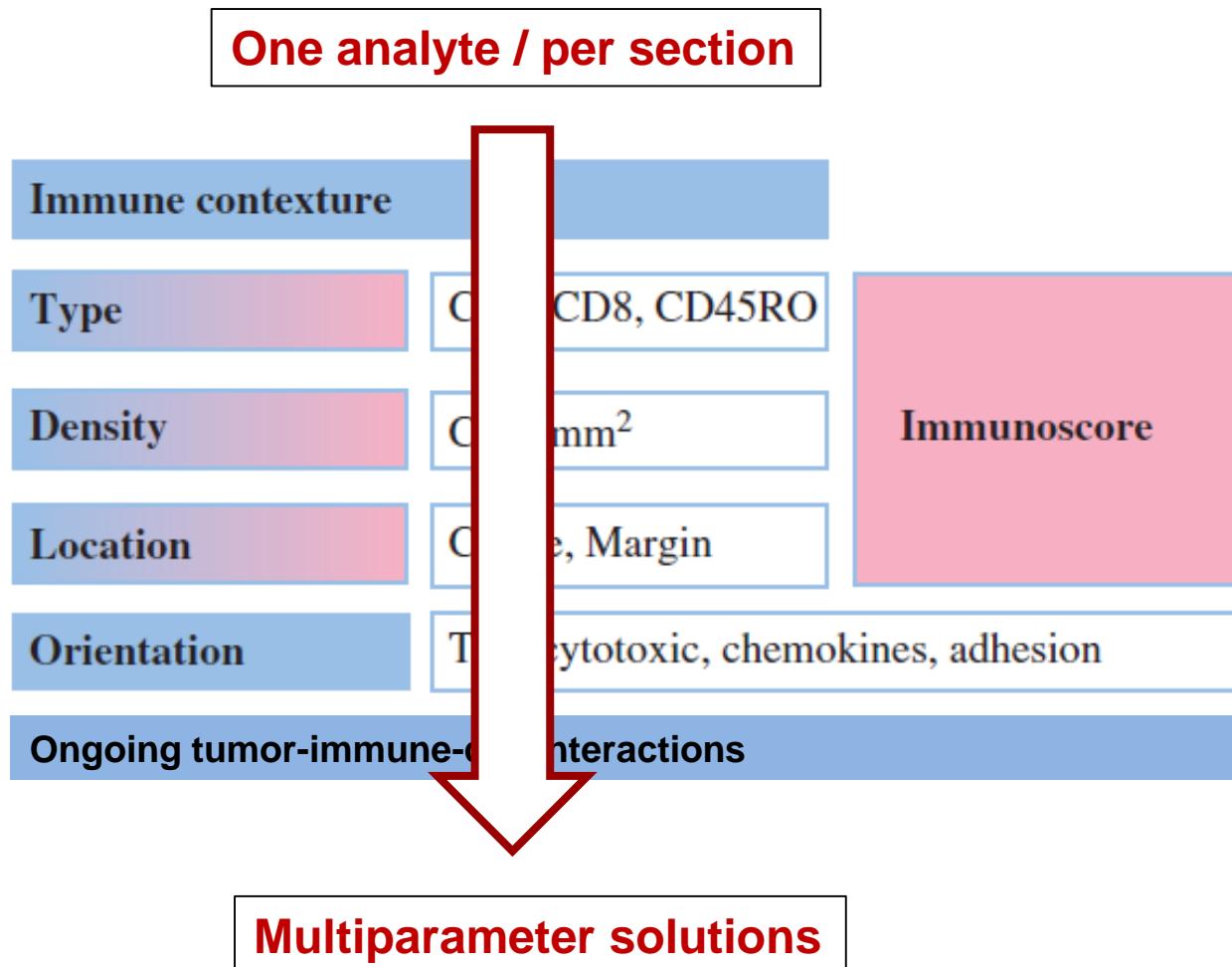
Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



SHORT REPORT

Open Access



Multiplexed tissue biomarker imaging

Edward C. Stack^{1*}, Periklis G. Foukas^{2,3} and Peter P. Lee⁴

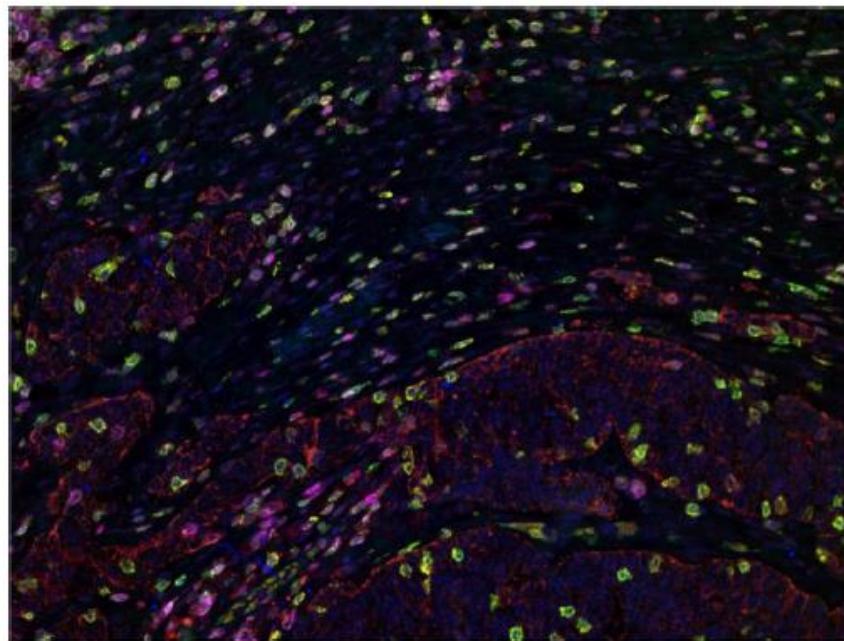
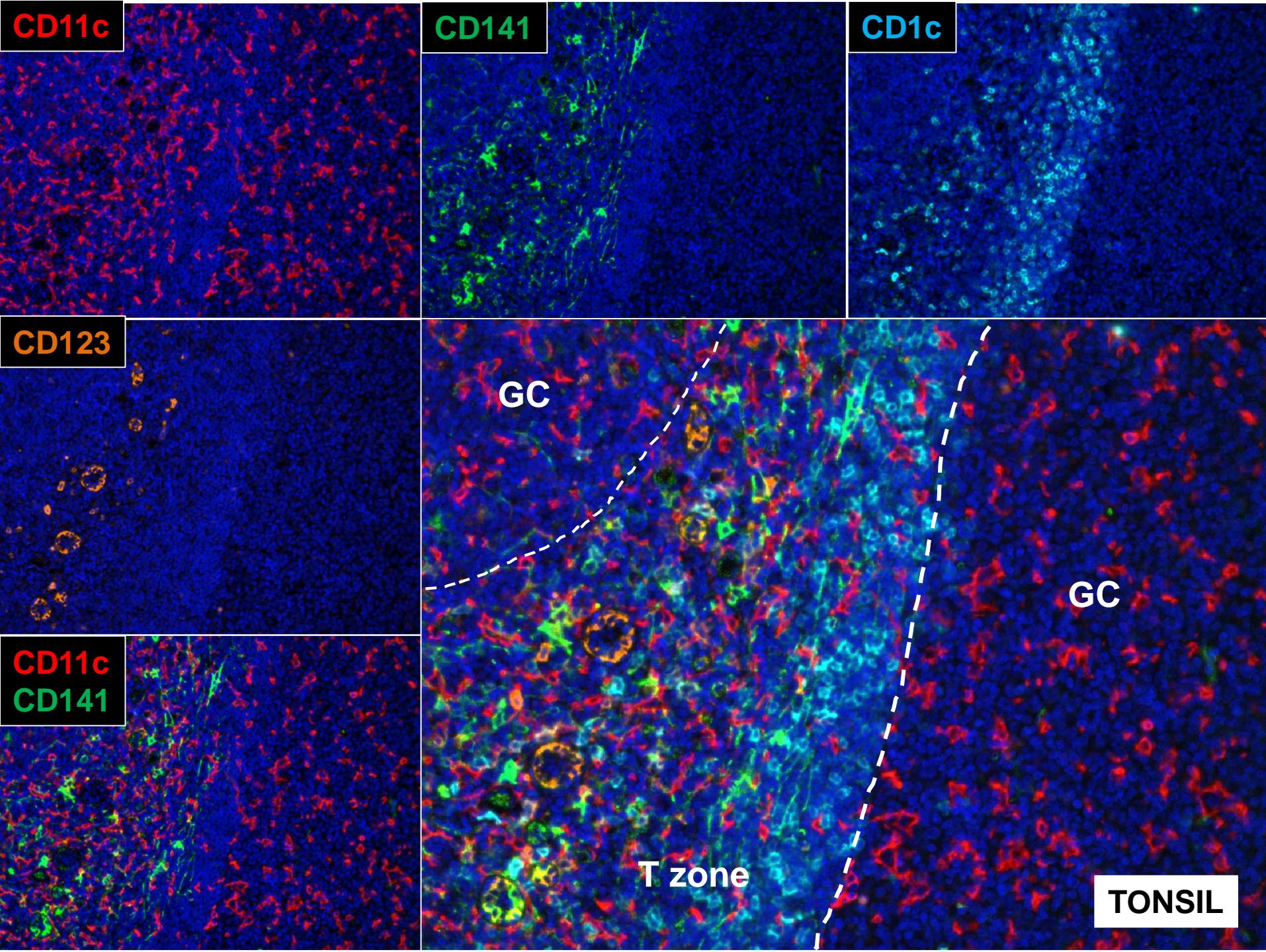
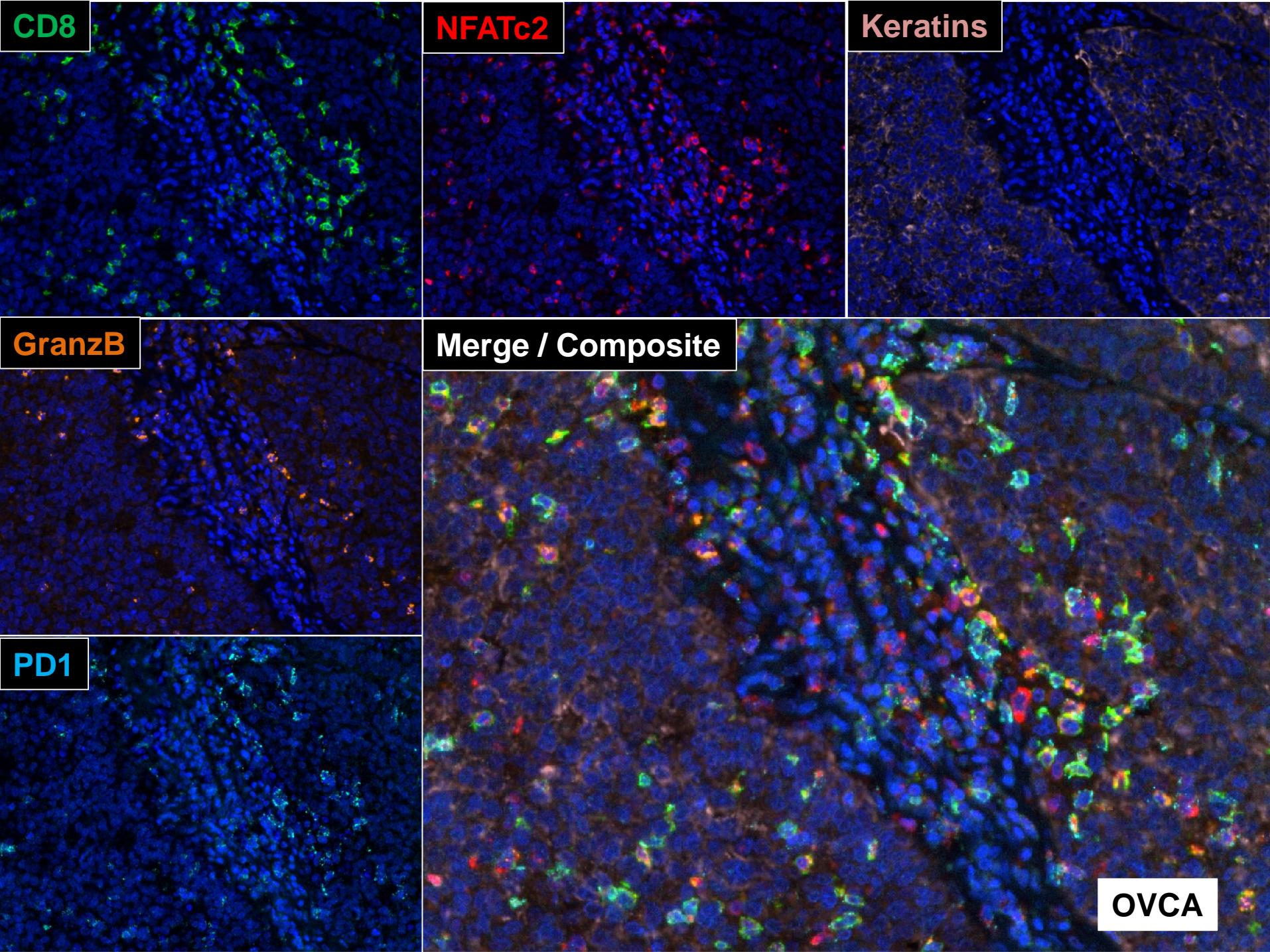
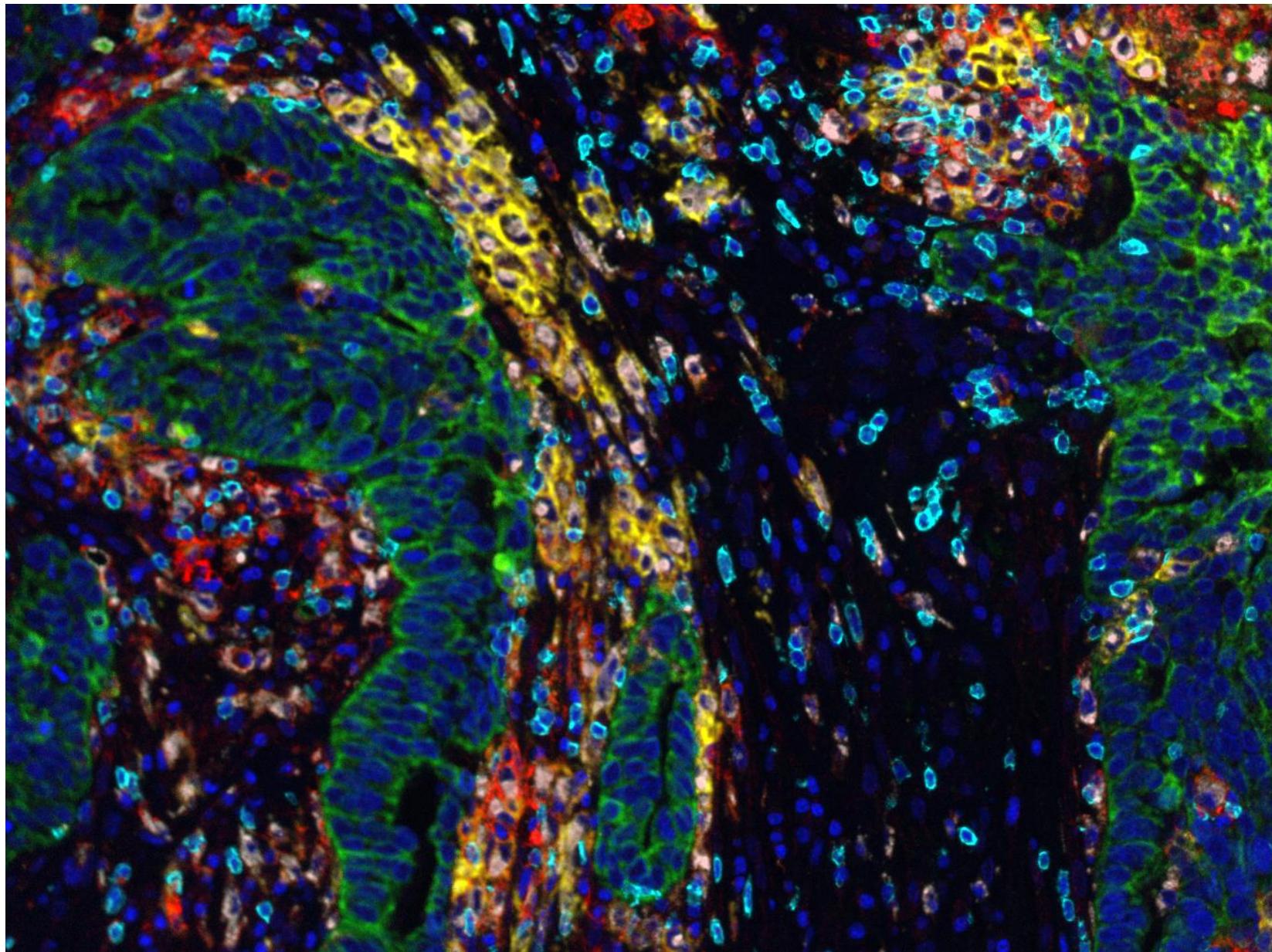


Fig. 1 Multiplexed Lymphocyte Assay in Ovarian Adenocarcinoma. Representative TSA multiplex of CD3 (green), CD4 (red), CD8 (yellow), CD45RO (magenta), Cytokeratins (brown) and DAPI (blue) in Ovarian Cancer. Multispectral imaging yields a composite image where each marker-associated dye can be reliably separated for accurate phenotypic and expression analyses





Panel: **PD-L1** / **CD68** / **CD11c** / **CD8** / **Keratins** / **DAPI**

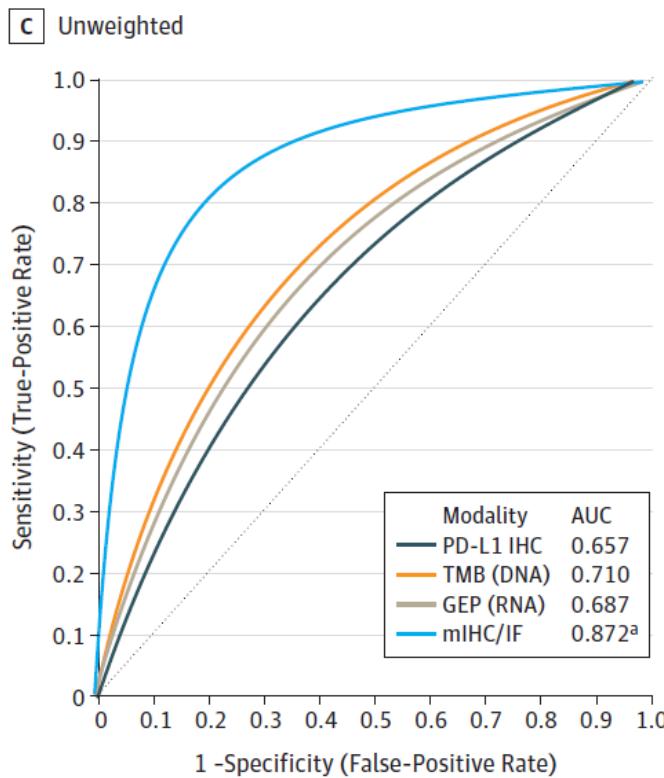
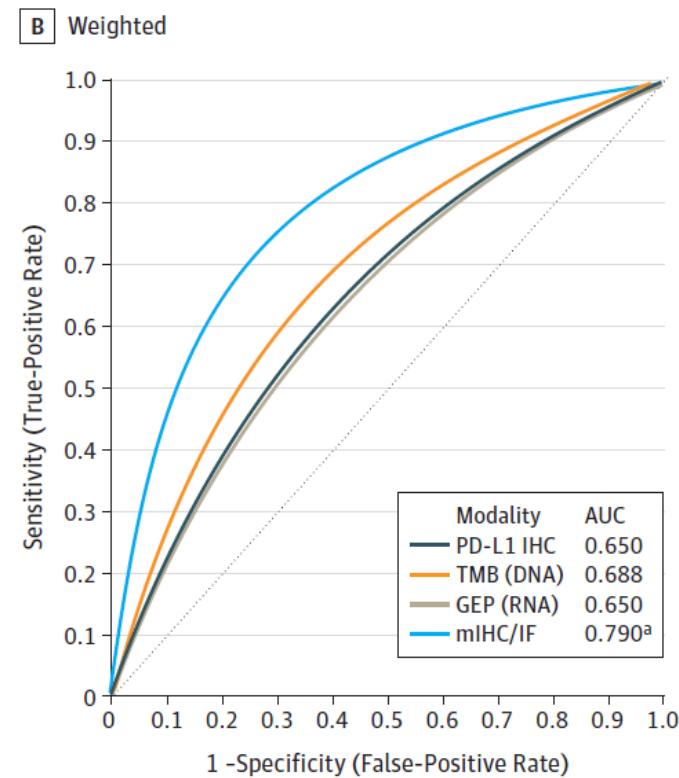


Comparison of Biomarker Modalities for Predicting Response to PD-1/PD-L1 Checkpoint Blockade

A Systematic Review and Meta-analysis

Steve Lu; Julie E. Stein, MD; David L. Rimm, MD, PhD; Daphne W. Wang, MS; J. Michael Bell; Douglas B. Johnson, MD; Jeffrey A. Sosman, MD; Kurt A. Schalper, MD, PhD; Robert A. Anders, MD, PhD; Hao Wang, PhD; Clifford Hoyt, MS; Drew M. Pardoll, MD, PhD; Ludmila Danilova, PhD; Janis M. Taube, MD

JAMA Oncol. 2019;5(8):1195-1204.



Linear regression models weighted (B) by the number of patients in each study and unweighted (C) (ie, each study treated equally) were used to generate summary receiver operating characteristic [sROC] curves for each assay modality. The multiplex immunohistochemistry/immunofluorescence (mIHC/IF) has a significantly higher area under the curve (AUC) than PD-L1 (programmed cell death ligand 1) IHC, tumor mutational burden (TMB), and gene expression profiling (GEP) by weighted approach and PD-L1 IHC and TMB by unweighted approach.

^a Indicates statistical significance ($P < .05$), Hanley and McNeil method.

Outline

- Introduction to the Tumor Immune Microenvironment (TIME)
 - Prognostic / Predictive value
- Mechanisms regulating TIME
- Evaluation / Methodologies
- Turning-up the heat



There are **5 mechanisms** of action that have the potential to turn cold tumours into so-called hot and inflamed tumours, hence increasing the tumour's responsiveness to immunotherapy:

- increasing local inflammation,
- neutralising immunosuppression at the tumour site,
- modifying the tumour vasculature,
- targeting the tumour cells themselves,
- increasing the frequency of tumour-specific T cells

Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy

Fernanda G. Herrera^{1,2,3}, Catherine Ronet¹, Maria Ochoa de Olza^{1,3*}, David Barras^{1*}, Isaac Crespo^{1*}, Massimo Andreatta¹, Jesus Corria-Osorio¹, Aodrenn Spill¹, Fabrizio Benedetti¹, Raphael Genolet¹, Angela Orcurto³, Martina Imbimbo³, Eleonora Ghisoni³, Blanca Navarro Rodrigo³, Dominik R. Berthold⁴, Apostolos Sarivalasis⁴, Khalil Zaman⁴, Rafael Duran⁵, Clarisse Dromain⁵, John Prior⁶, Niklaus Schaefer⁶, Jean Bourhis², Georgia Dimopoulos¹¹, Zoi Tsourtis¹¹, Marius Messemaekers¹⁰, Thomas Smith⁷, Sarah E. Warren⁷, Periklis Foukas⁸, Sylvie Rusakiewicz¹², Mikaël J. Pittet^{9,10}, Stefan Zimmermann³, Christine Sempoux¹¹, Urania Dafni¹², Alexandre Harari¹, Lana E. Kandalaft^{1,13}, Santiago J. Carmona¹, Denarda Dangaj Laniti¹, Melita Irving^{1*}, George Coukos^{1,3*#}.

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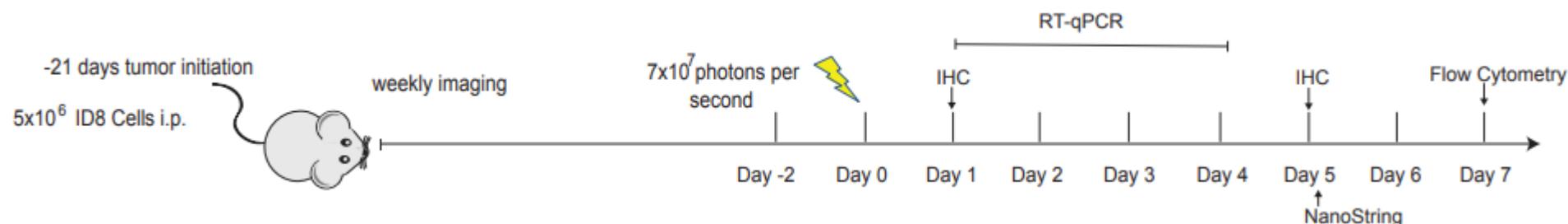
Q: Can low dose radiation reprogramme the tumor microenvironment of tumors with scarce immune infiltration and together with immunotherapy induce mobilization of (innate and/or adaptive) immunity?

Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy

Along with its direct tumoricidal effects, **hypofractionated (high-dose) radiation therapy** (RT) can mediate important immunomodulatory effects including:

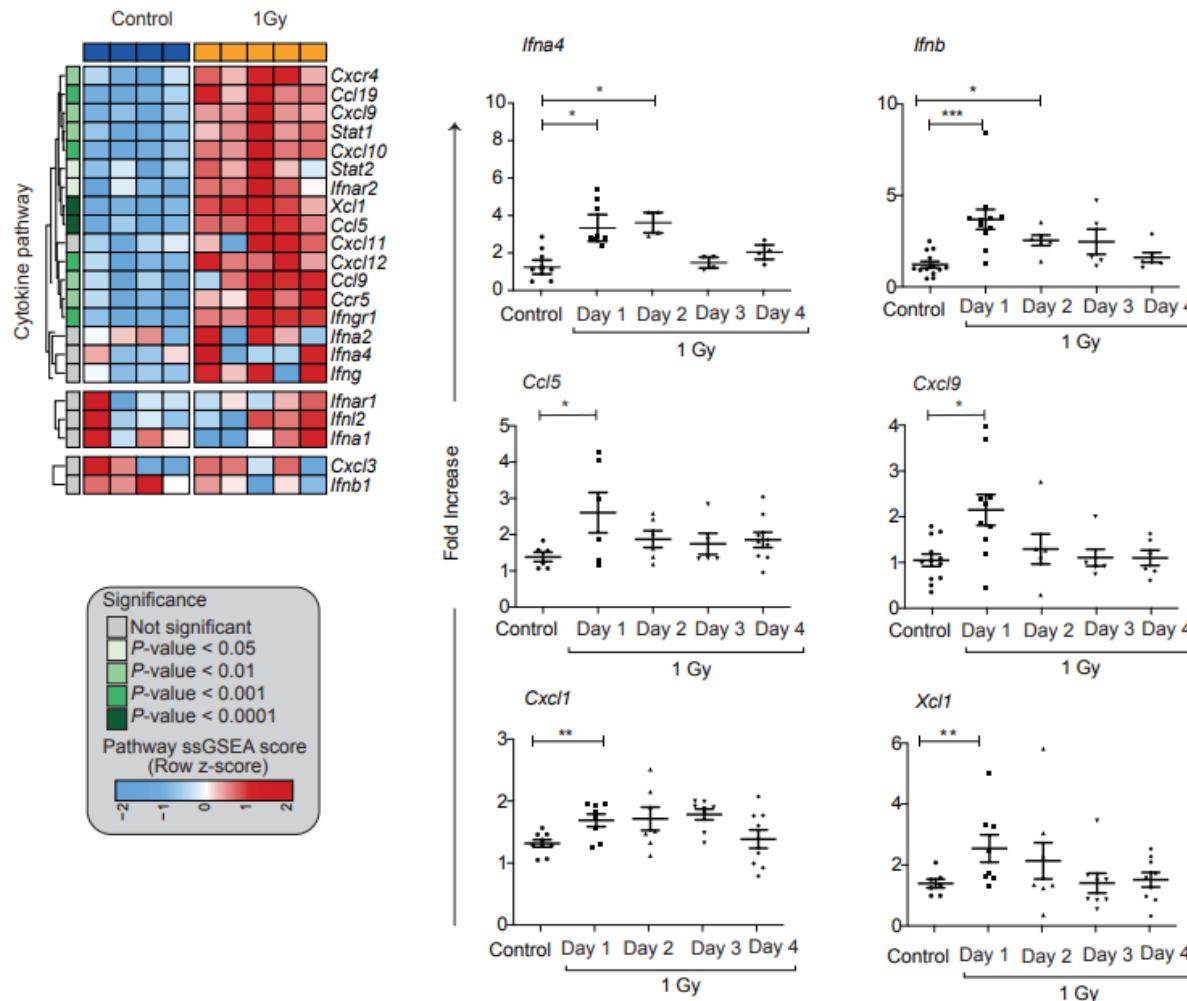
- (i) In situ vaccination through release of tumor-associated antigens
- (ii) the activation of dendritic cells (DCs)
- (iii) the release of danger signals and the upregulation of cytokines and chemokines
- (iv) normalization of the tumor vasculature
- (v) activate DNA sensing pathways in host and tumor cells, triggering production of type I interferon (IFN) and mobilizing innate and adaptive immunity
- (vi) abscopal effect

Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy



Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy

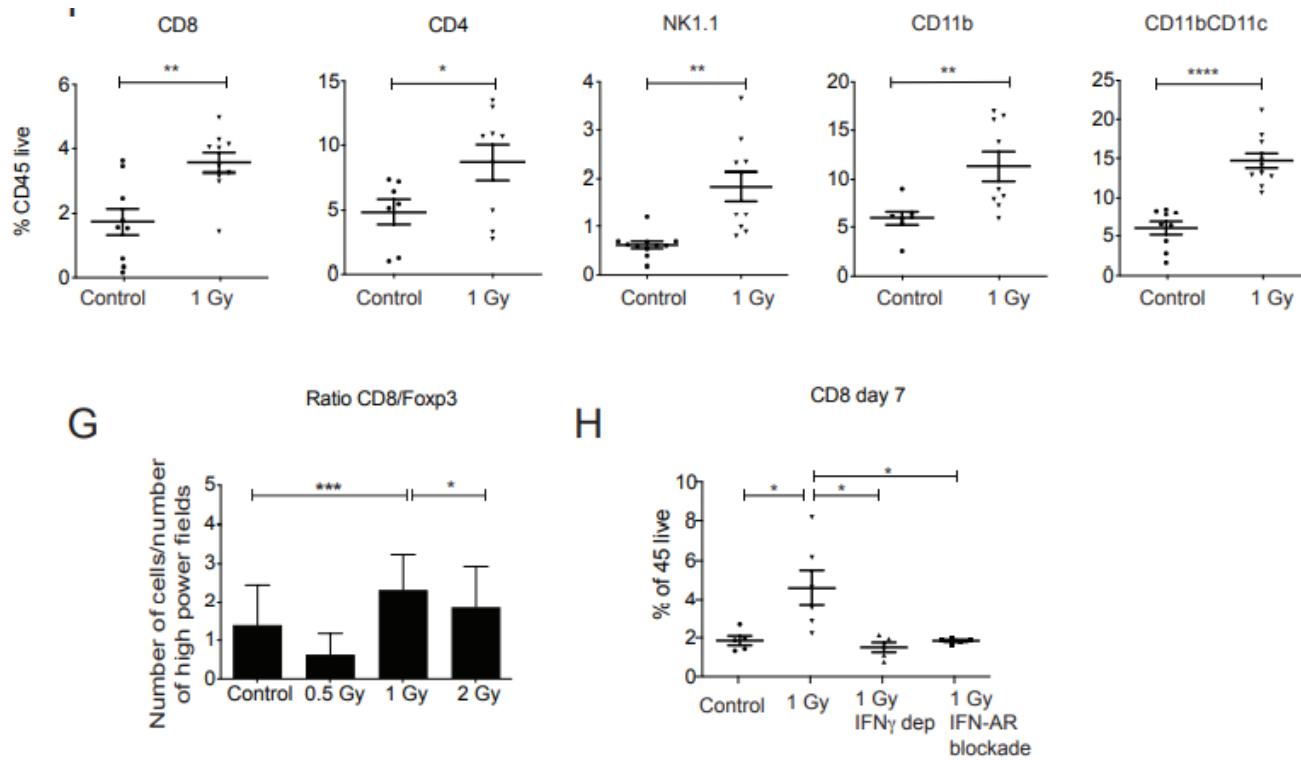
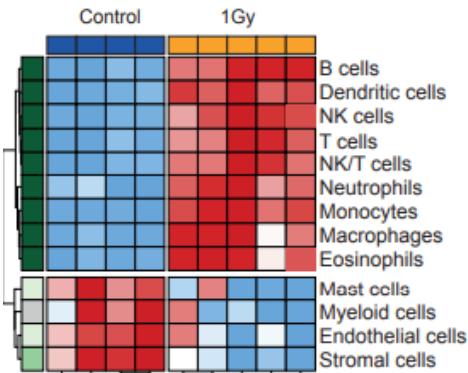
Low-dose whole abdominal radiotherapy induces immune-cell infiltration in advanced orthotopic ovarian cancer



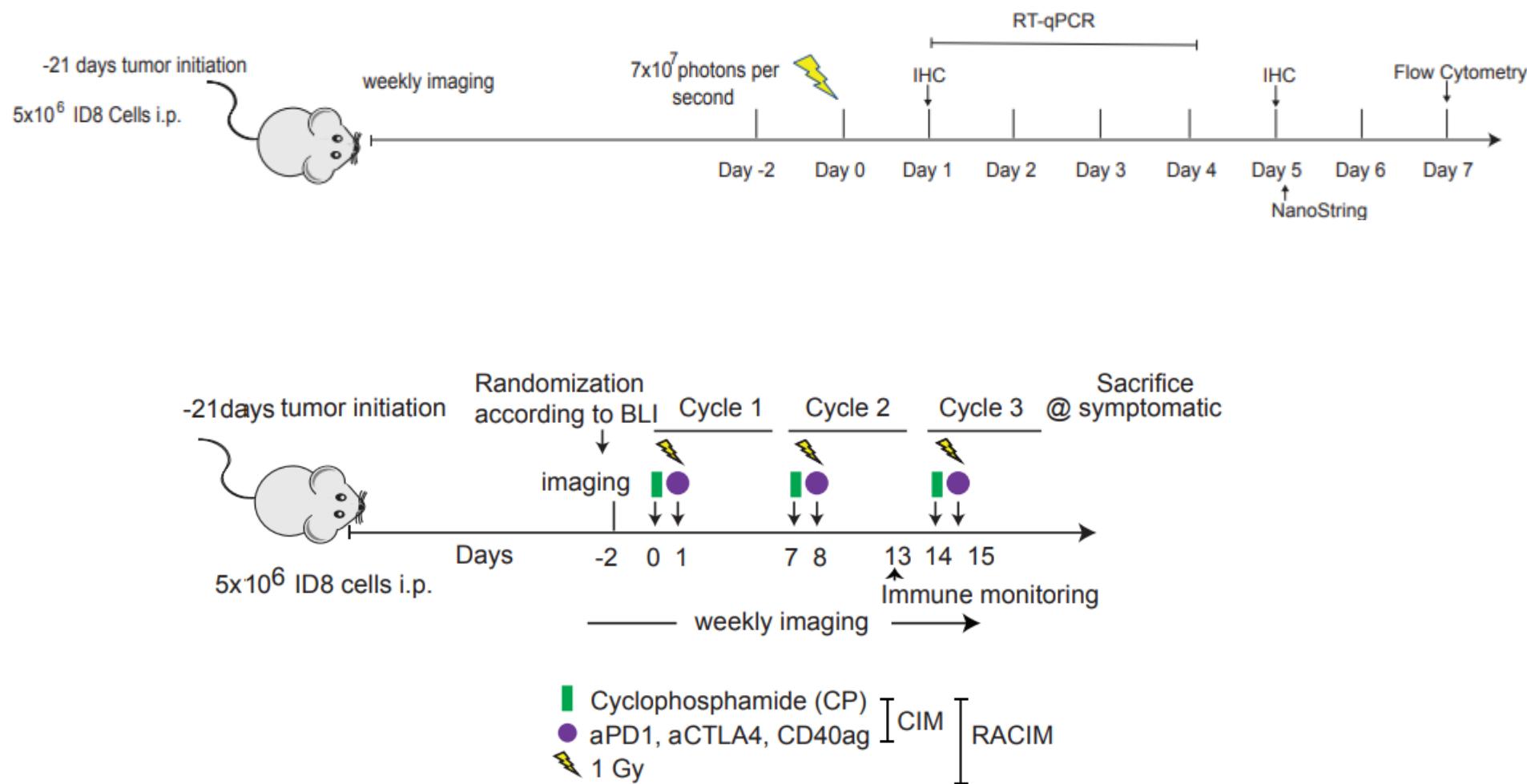
Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy

Low-dose whole abdominal radiotherapy induces immune-cell infiltration in advanced orthotopic ovarian cancer

Tumor

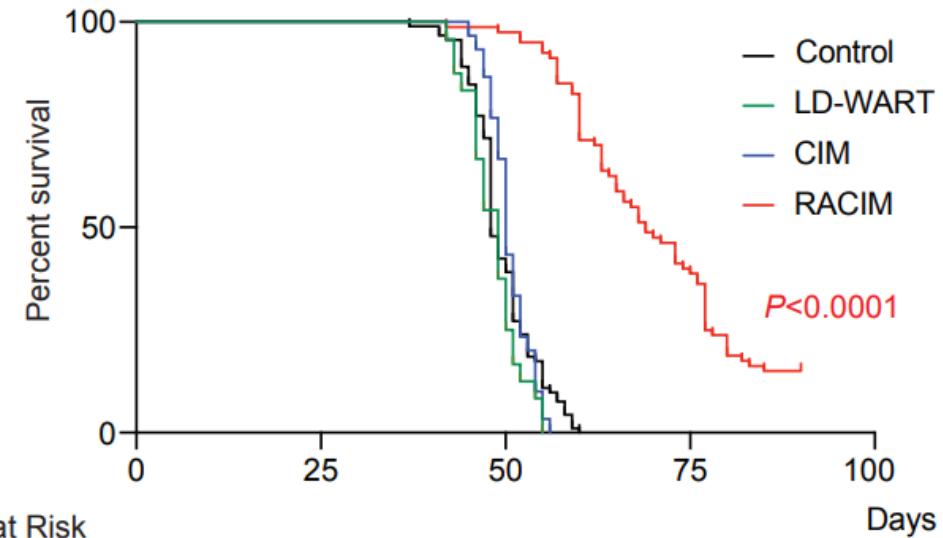
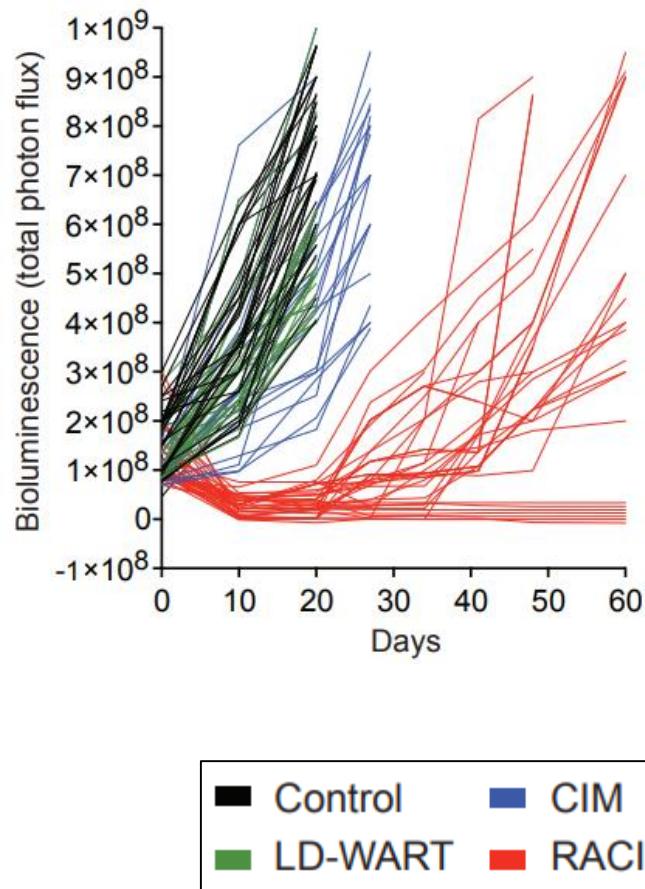


Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy



Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy

Metronomic radiotherapy confers tumor responsiveness to combinatorial immunotherapy

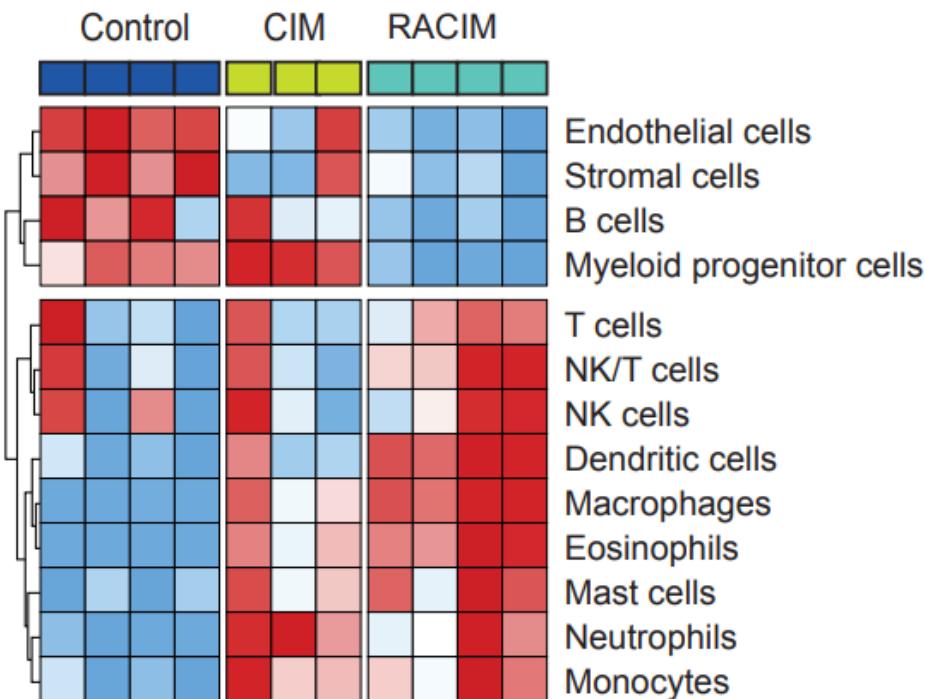


	No. at Risk				
Control	92	92	39	0	0
LD-WART	24	24	9	0	0
CIM	30	30	20	0	0
RACIM	80	80	79	32	12

Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy

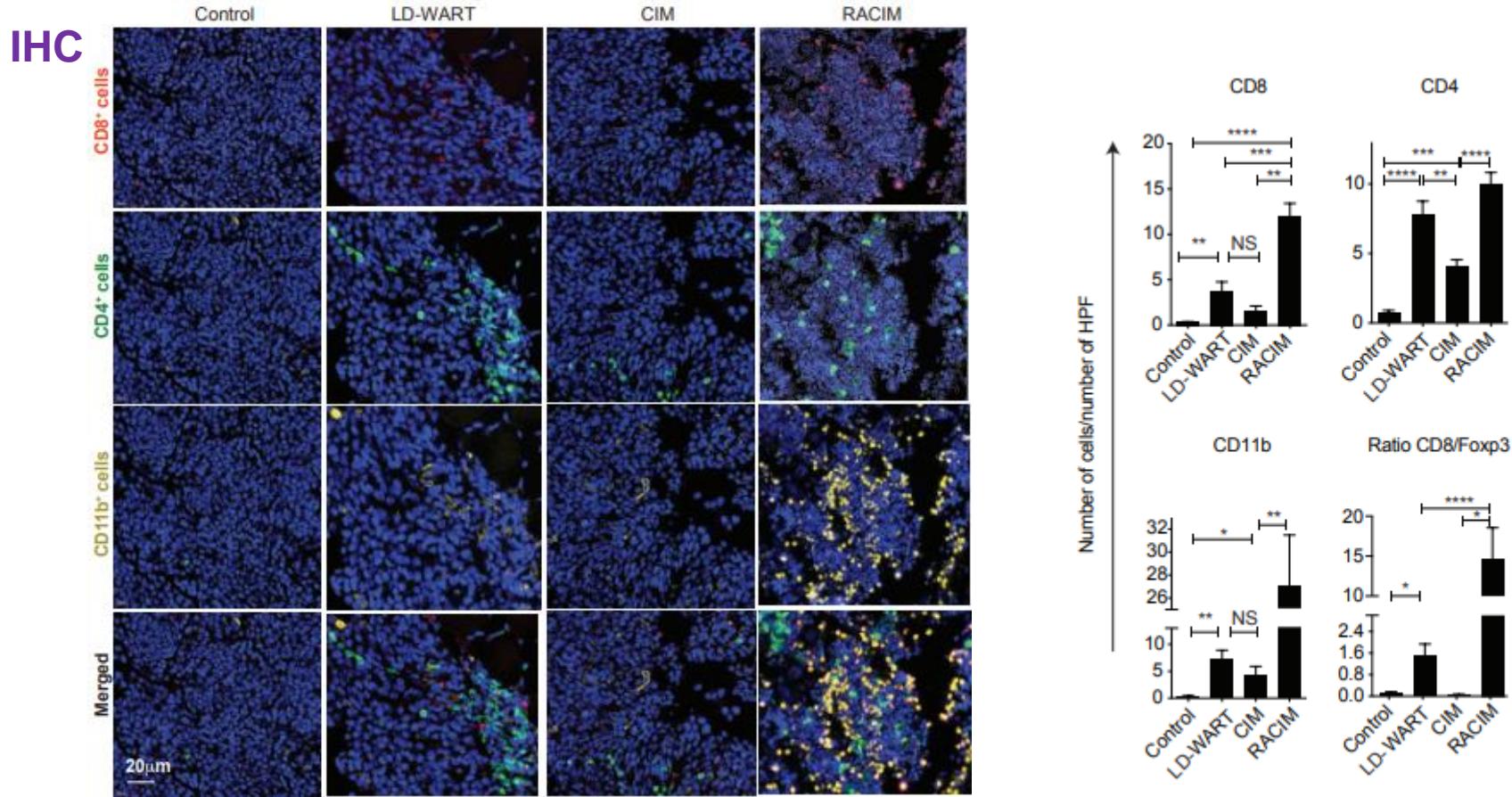
Metronomic radiotherapy confers tumor responsiveness to combinatorial immunotherapy

Nanostring



Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy

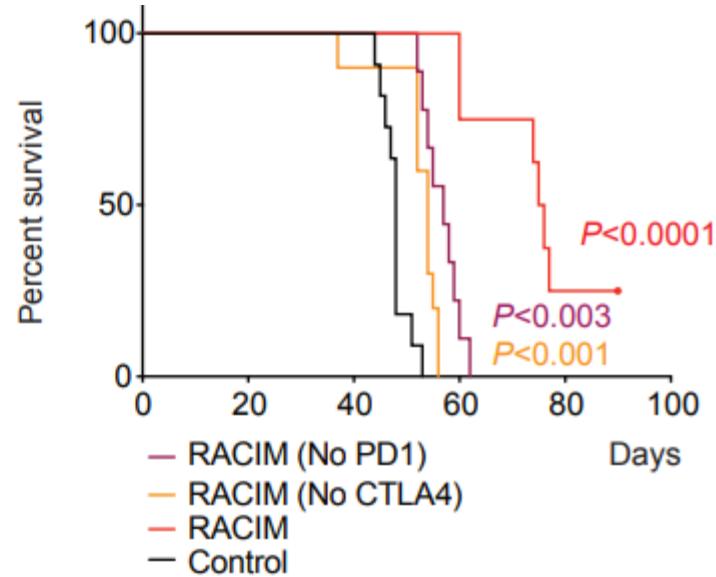
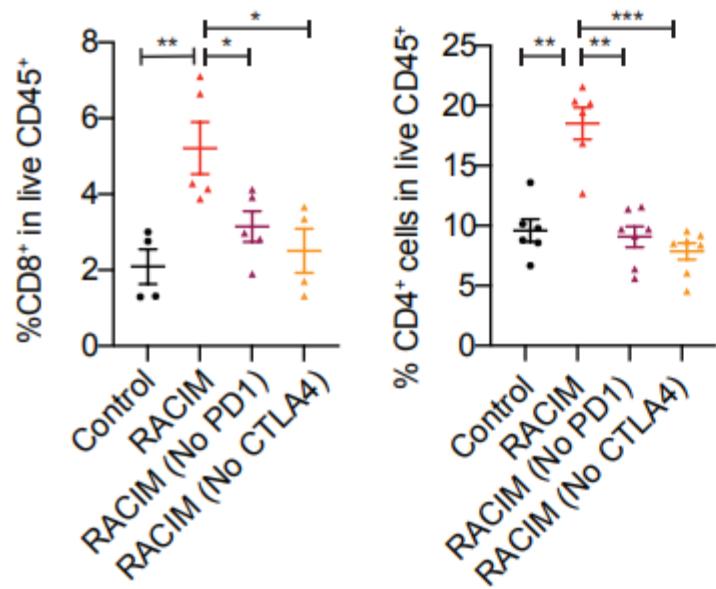
Metronomic radiotherapy confers tumor responsiveness to combinatorial immunotherapy



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Metronomic radiotherapy confers tumor responsiveness to combinatorial immunotherapy

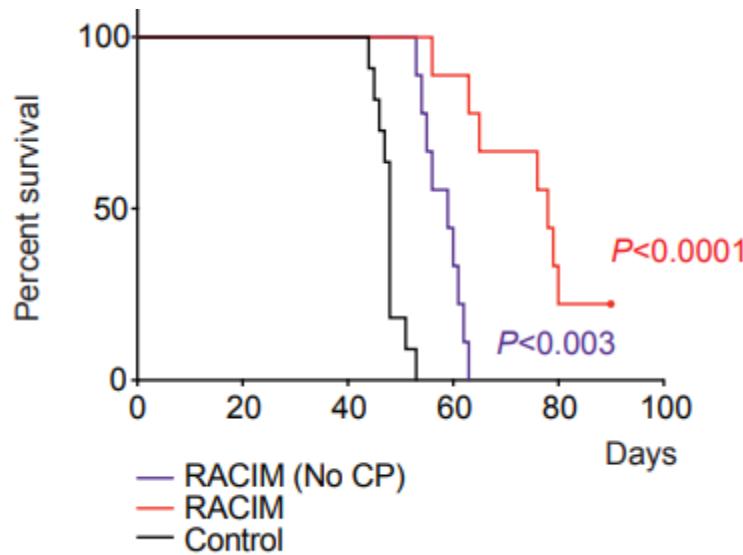
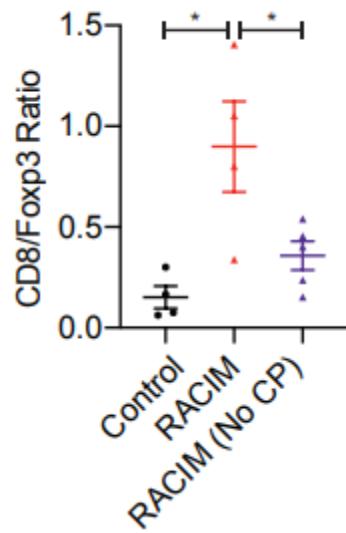
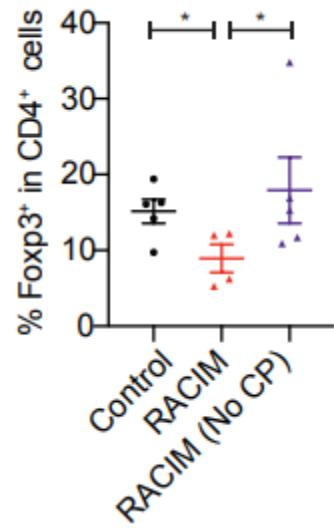
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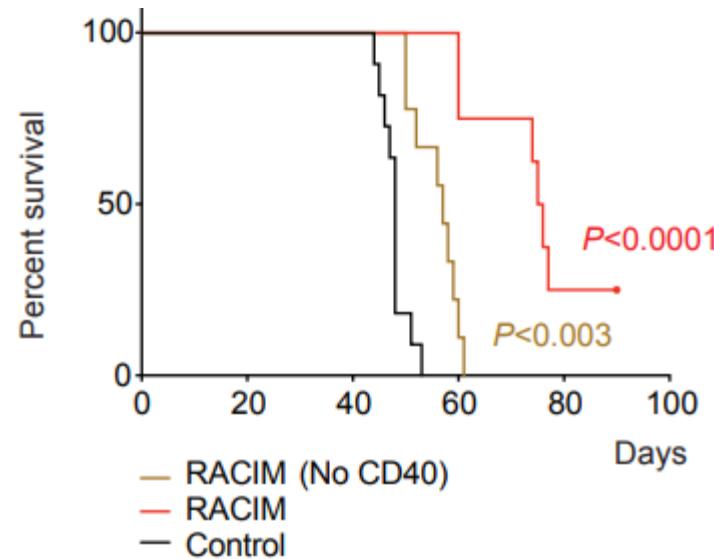
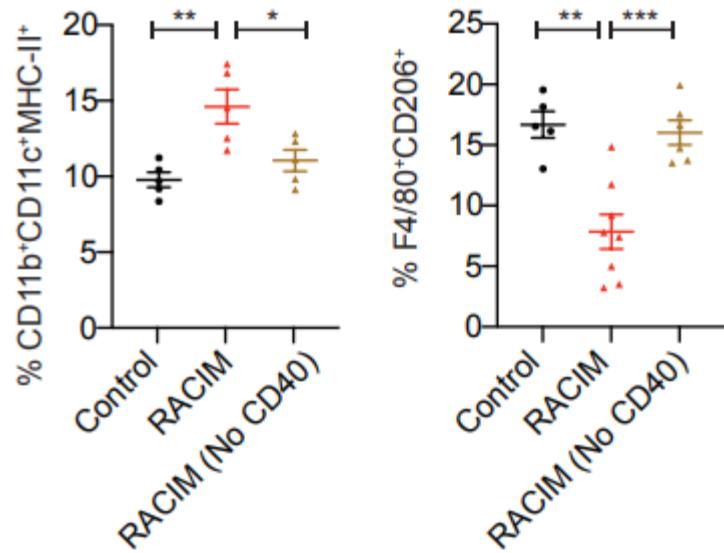
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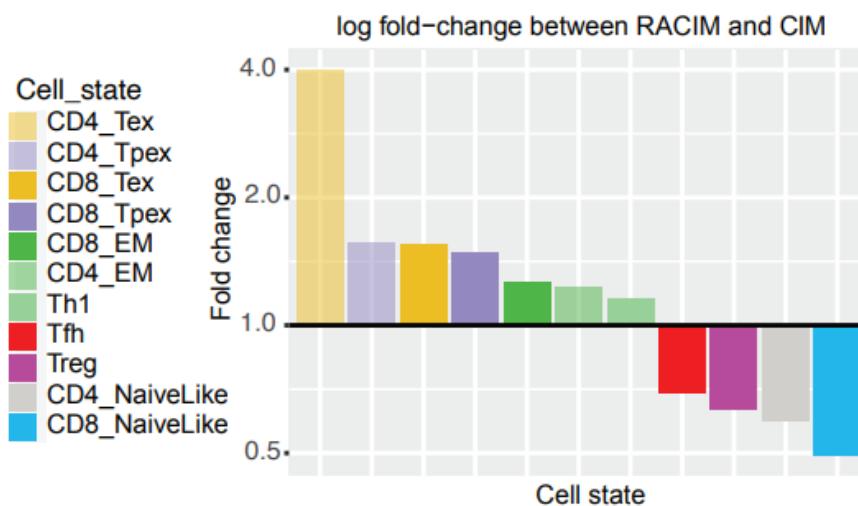
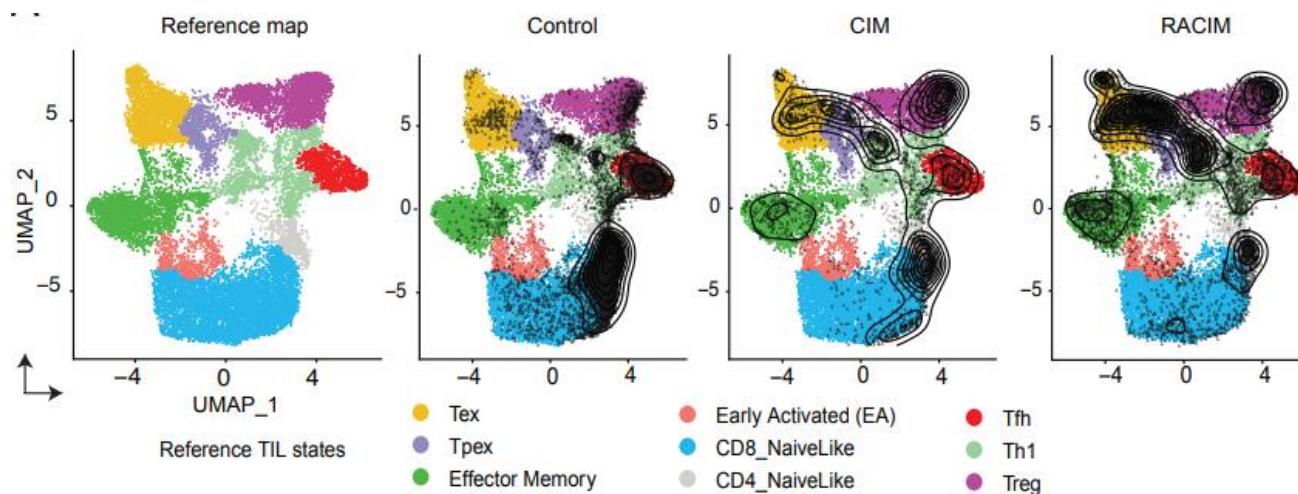
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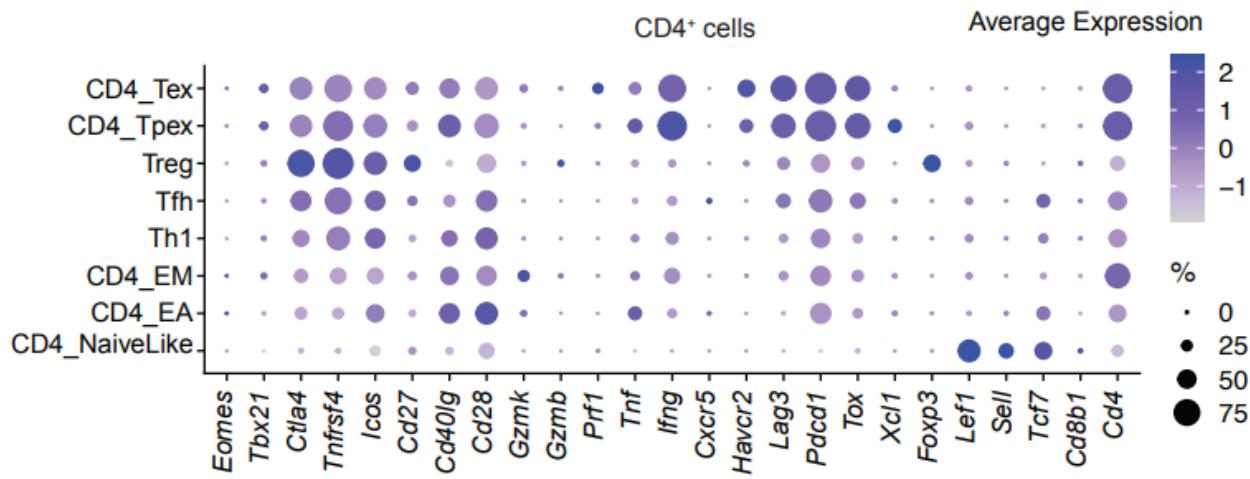
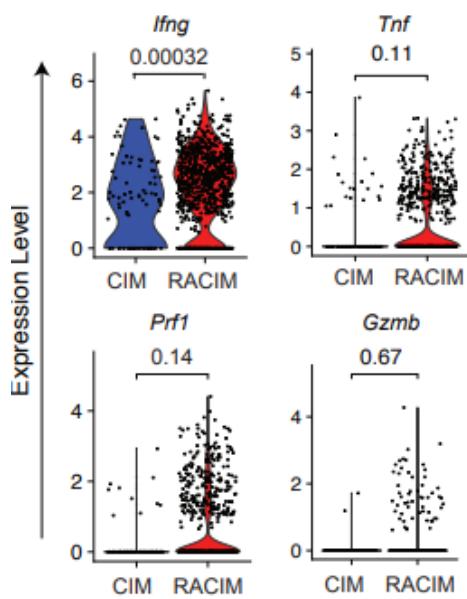
RACIM expands tumor-rejecting CD4+ and CD8+ TILs with activation and exhaustion features



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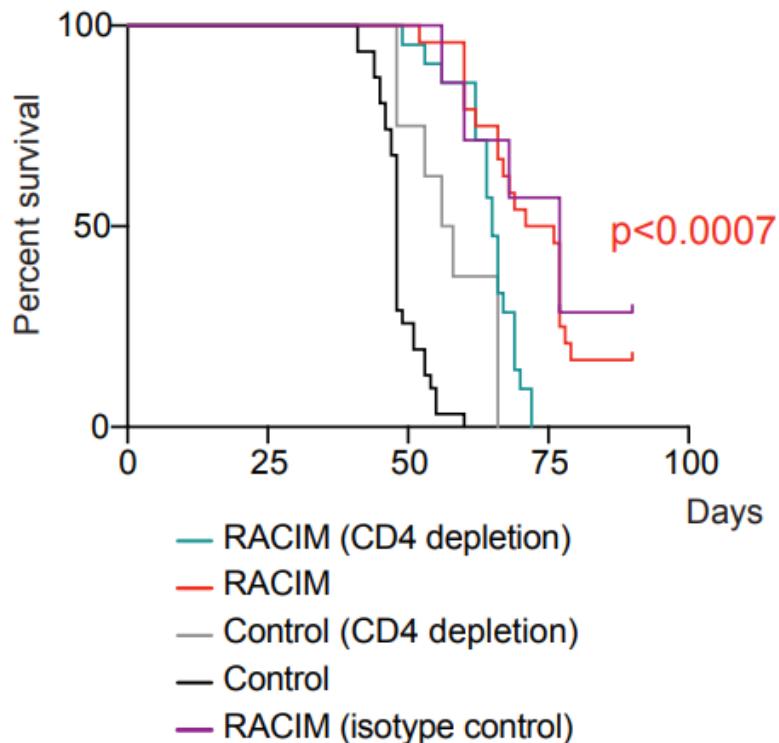
RACIM expands tumor-rejecting CD4+ and CD8+ TILs with activation and exhaustion features

CD4 Tex TILs



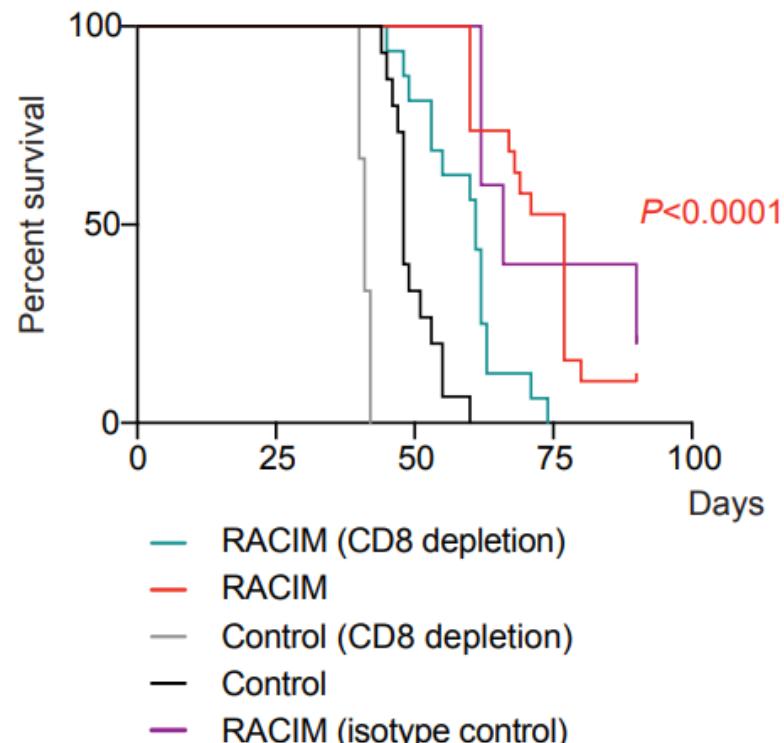
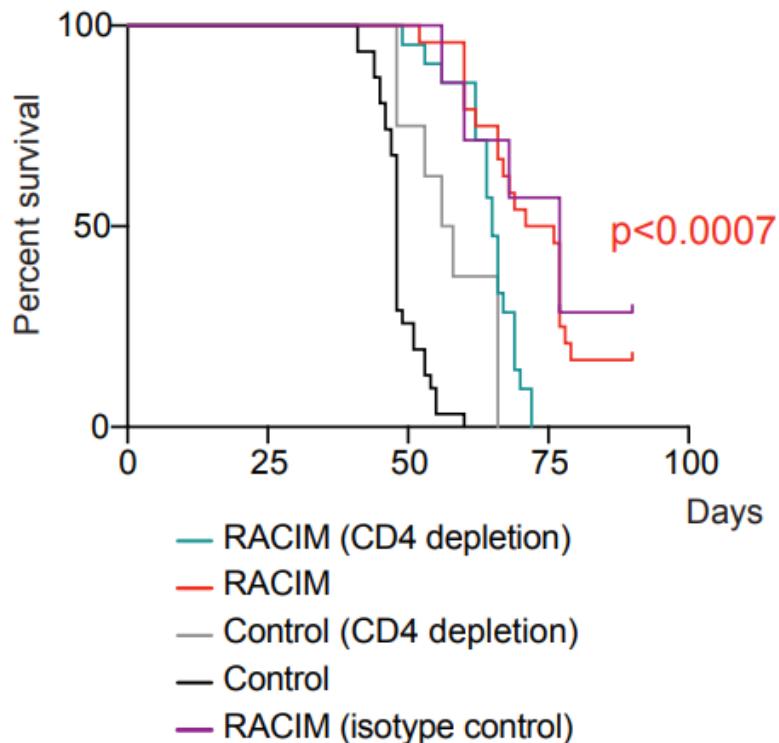
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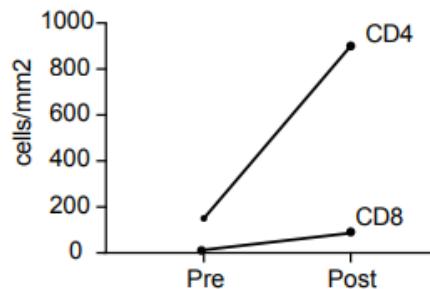
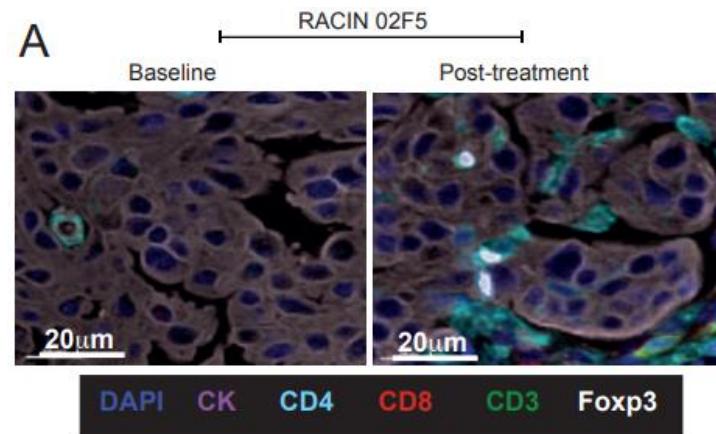
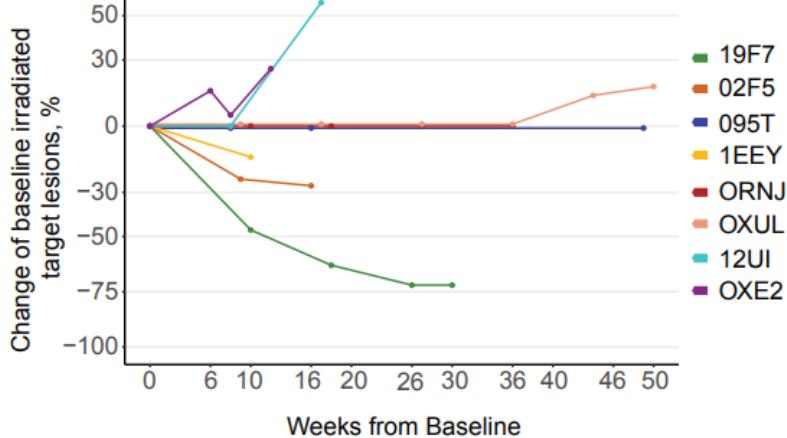
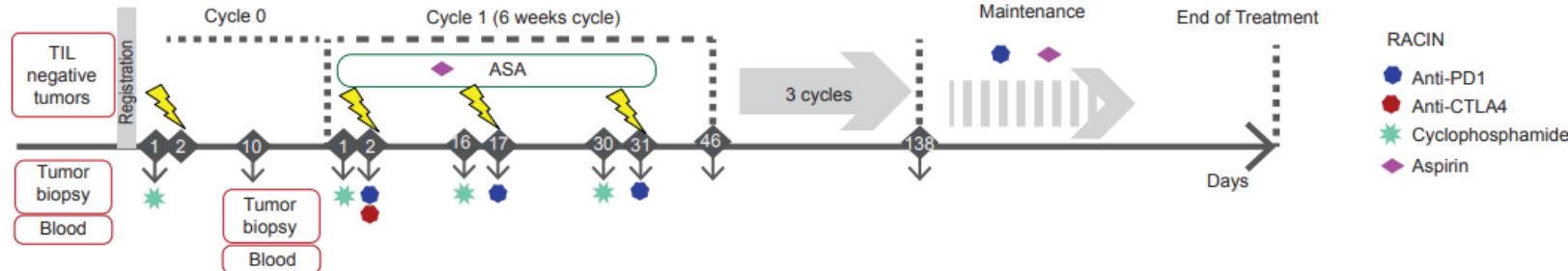
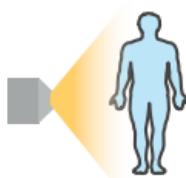
RACIM expands tumor-rejecting CD4+ and CD8+ TILs with activation and exhaustion features



Low Dose Radiotherapy Reverses Tumor Immune Desertification and Resistance to Immunotherapy

Immune desert tumors in humans are reprogrammed following low-dose radiotherapy

A



DO_CHUV_Lausanne

Prof. George Coukos, MD, PhD

CTE

Prof. Lana Kandalaft, PhD

Immune monitoring Lab

Alex Harari

Immune Landscape Lab

• *Sylvie Rusakiewics, PhD*

• *Esther Danenberg, MSc*

• *Ekaterina Fortis, MSc*

• *Pinelopi Chatziemmanuil, MSc*

• *Ioannidou Kalliopi, PhD*

• *Periklis Foukas*



Attikon University Hospital

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Panagiota Leou

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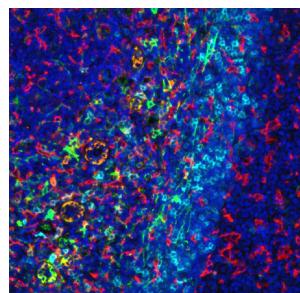


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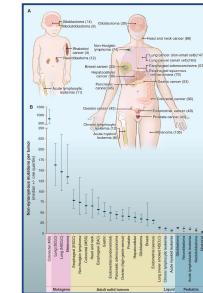
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ΥΓΕΙΑΣ



ΕΥΧΑΡΙΣΤΩ



17:00-19:00

Cancer Immunology and Immunotherapy Chair: P. Verginis – P. Foukas

- Tumor microenvironment (P. Foukas)
- Immunotherapy in cancer: Resistance mechanisms in solid tumors (O. Tsitsiloni)
- Colon cancer (I. Souglakos)
- Bone marrow malignancies - Immunotherapy (C. Pontikoglou)

Περικλής Γ. Φούκας
Β' Εργαστήριο Παθολογικής Ανατομικής,
Ιατρικής Σχολής ΕΚΠΑ,
Π.Γ.Ν Αττικόν

