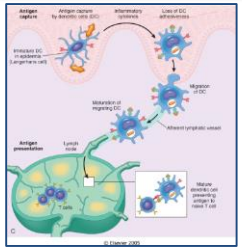
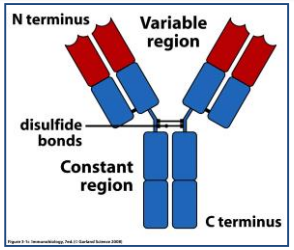
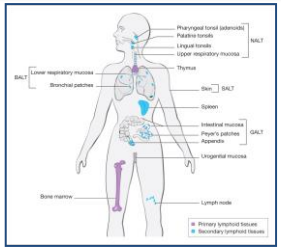
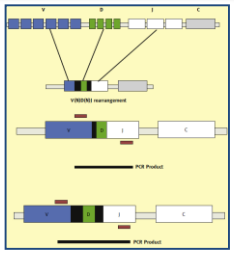
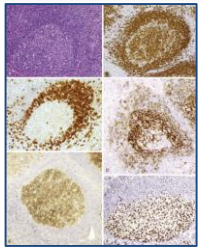
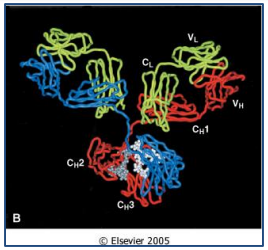
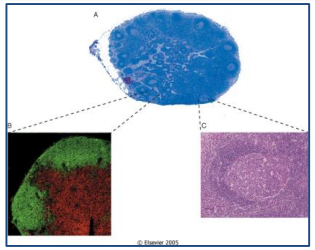



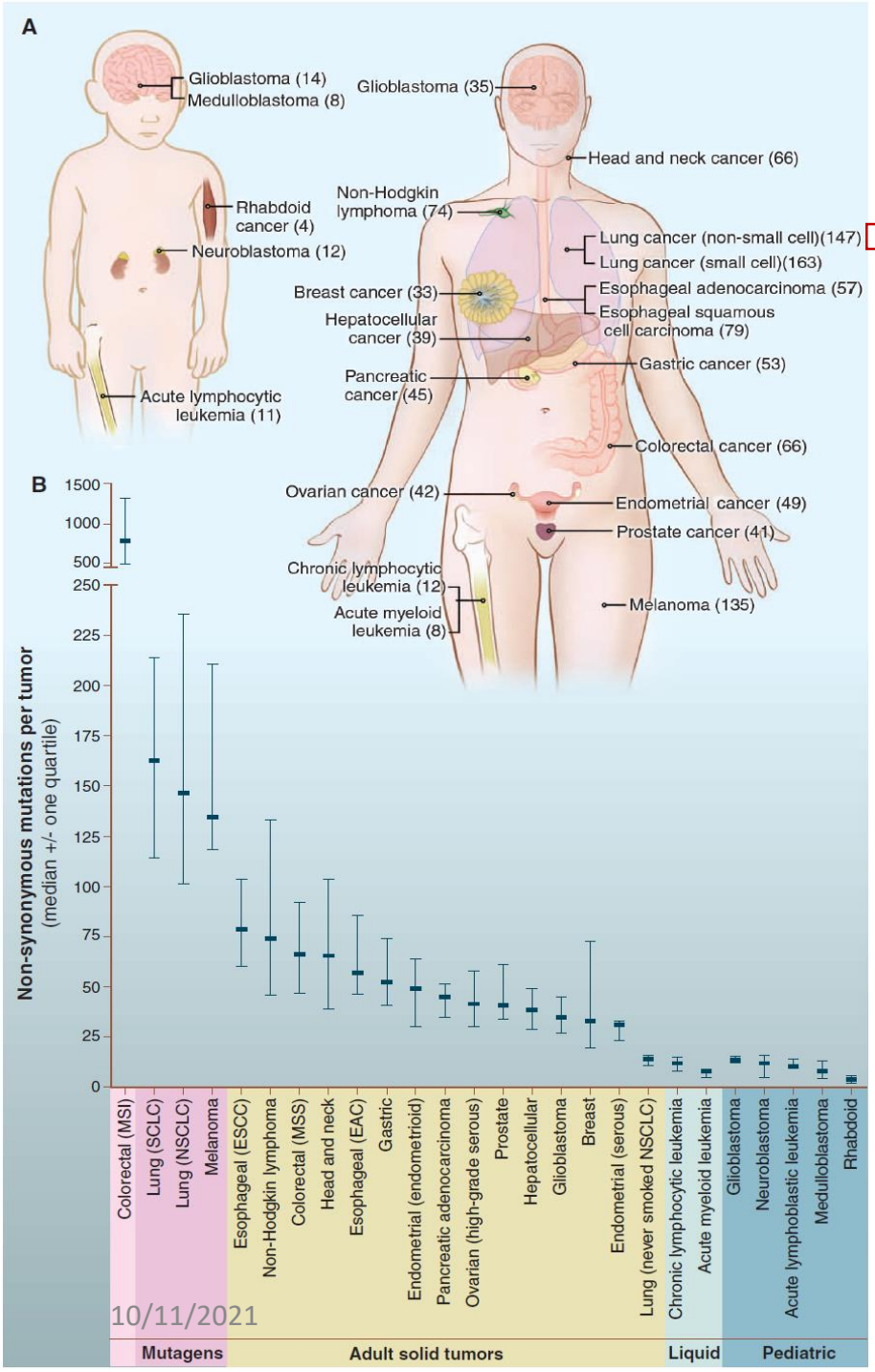


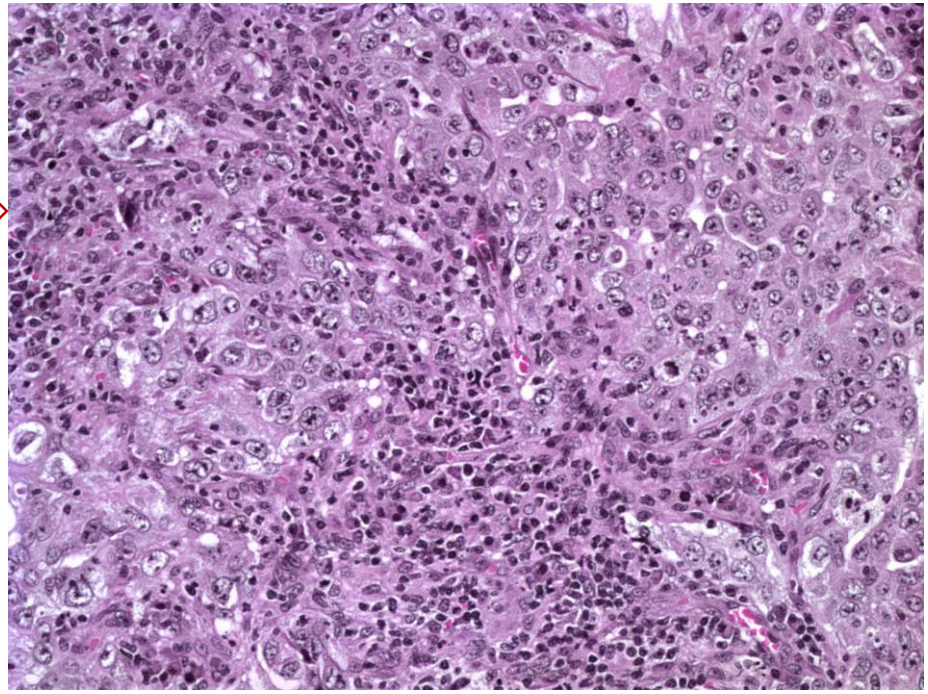
ΜΙΚΡΟΠΕΡΙΒΑΛΛΟΝ, ΑΝΟΣΙΑΚΗ ΑΠΑΝΤΗΣΗ ΣΤΗ ΝΕΟΠΛΑΣΙΑ

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

Outline

- Intoduction to the Tumor Immune Microenvironment (TIME)
  - Prognostic / Predictive value
  - Methodologies
- Mechanisms regulating TIME
- TIME and neoplastic evolution

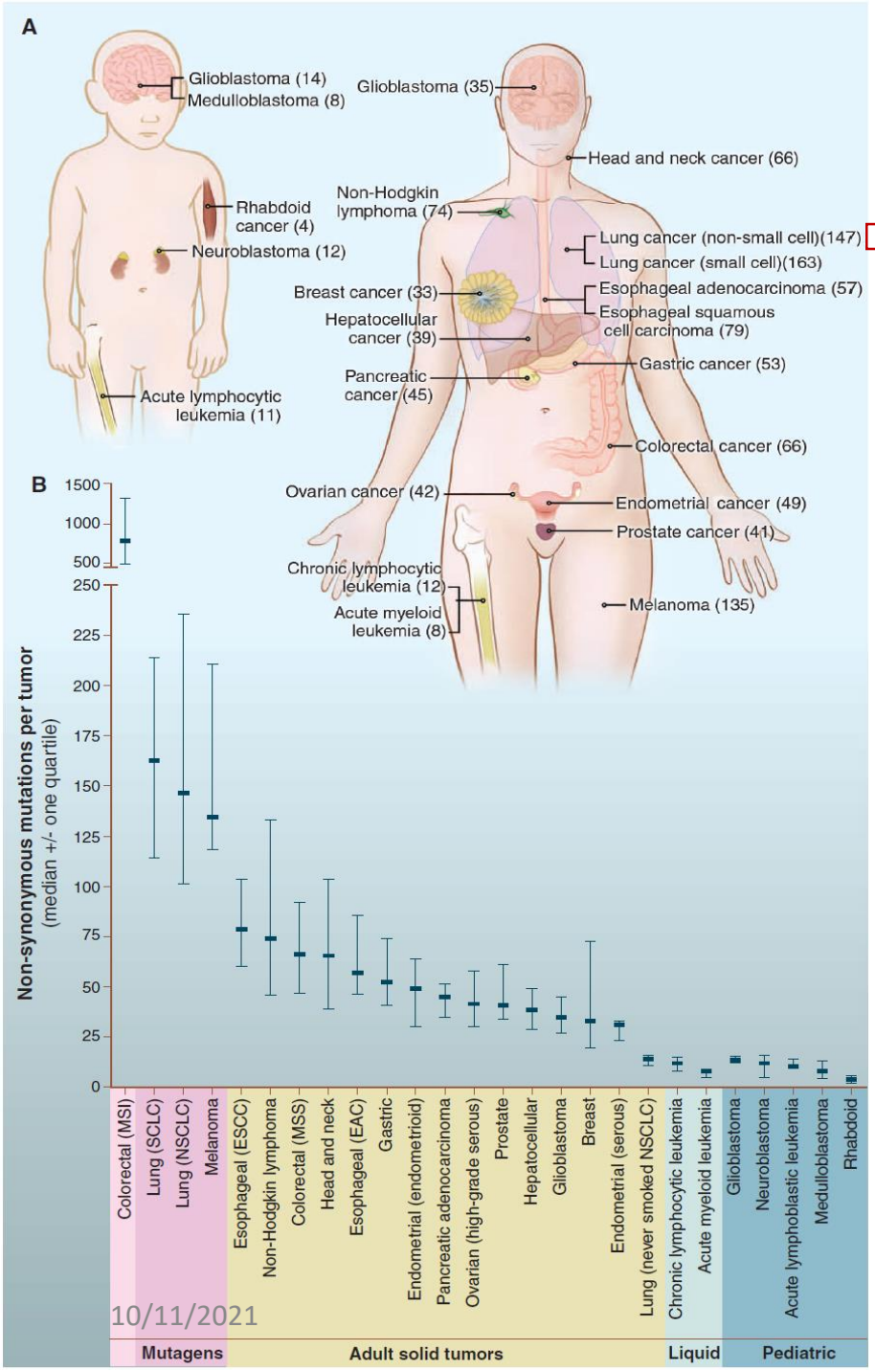


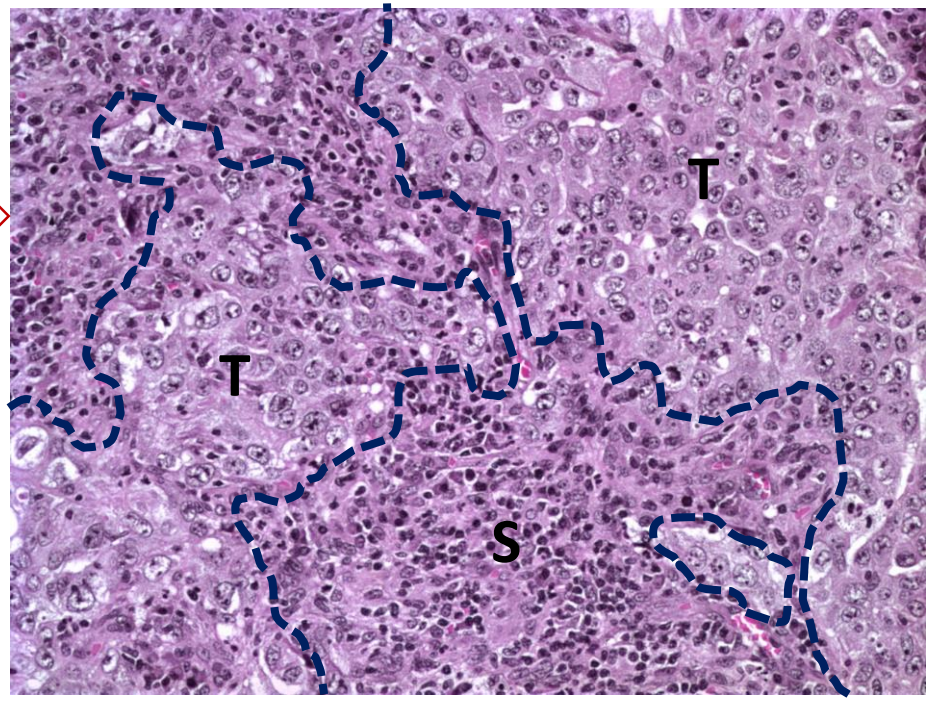


Cancer Genome Landscapes

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

SCIENCE VOL 339 29 MARCH 2013





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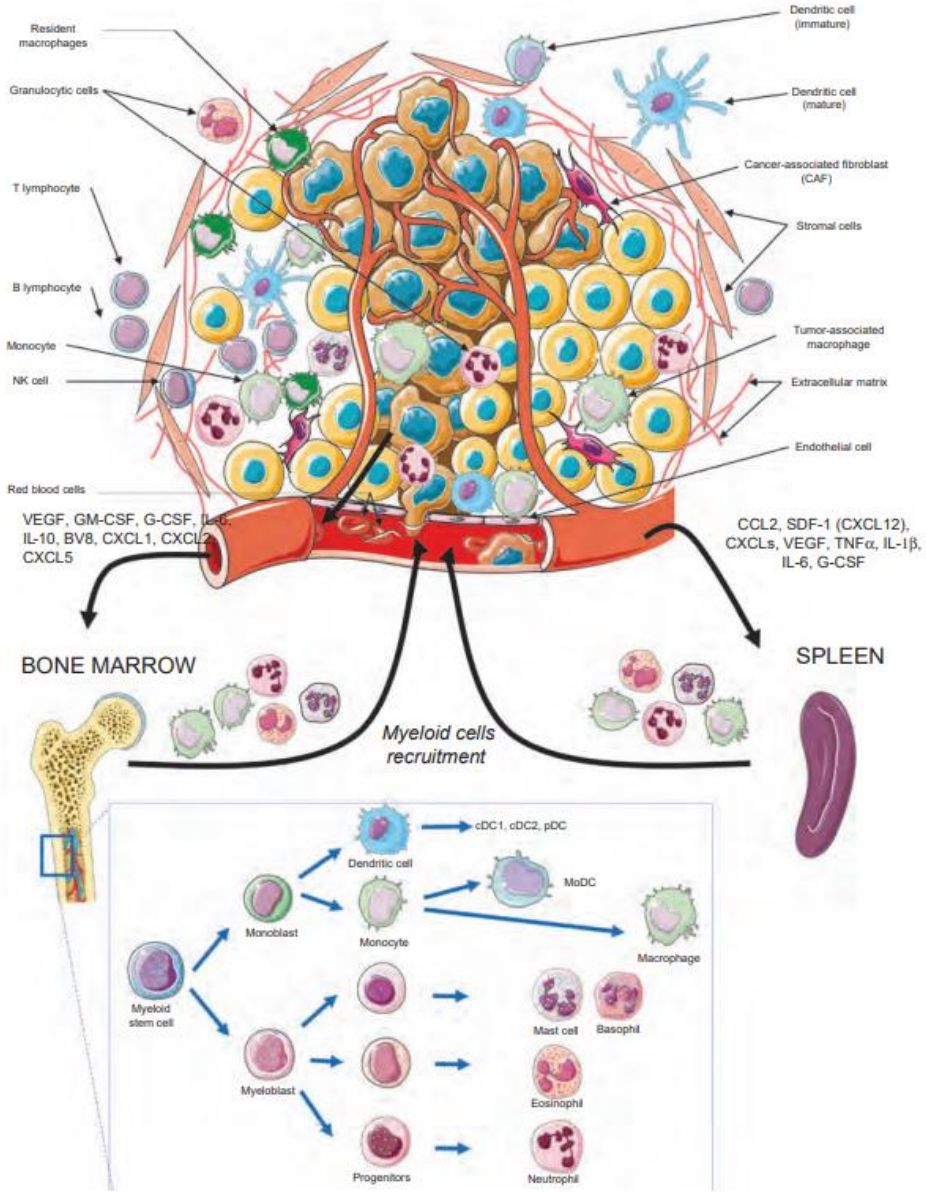
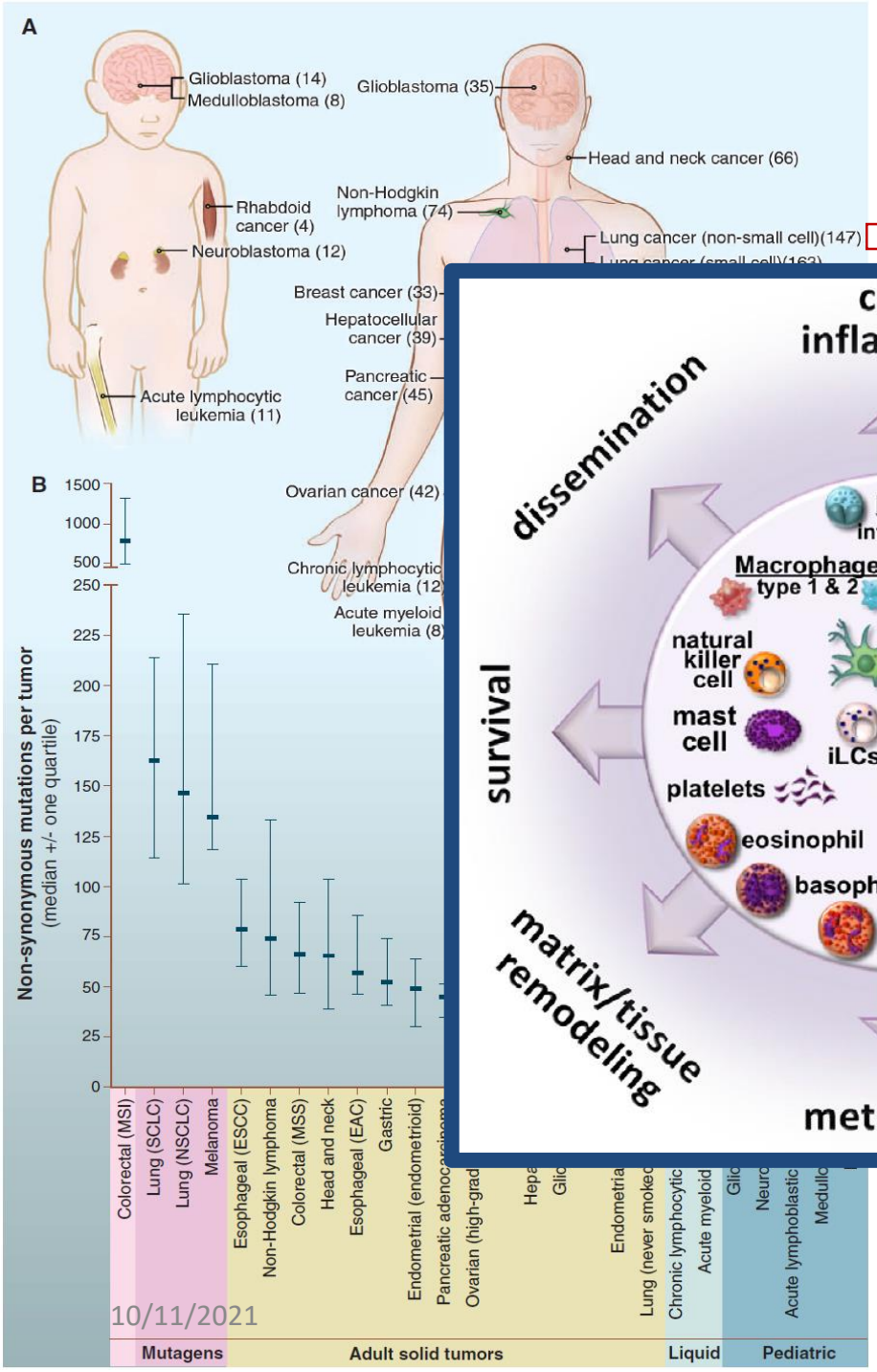
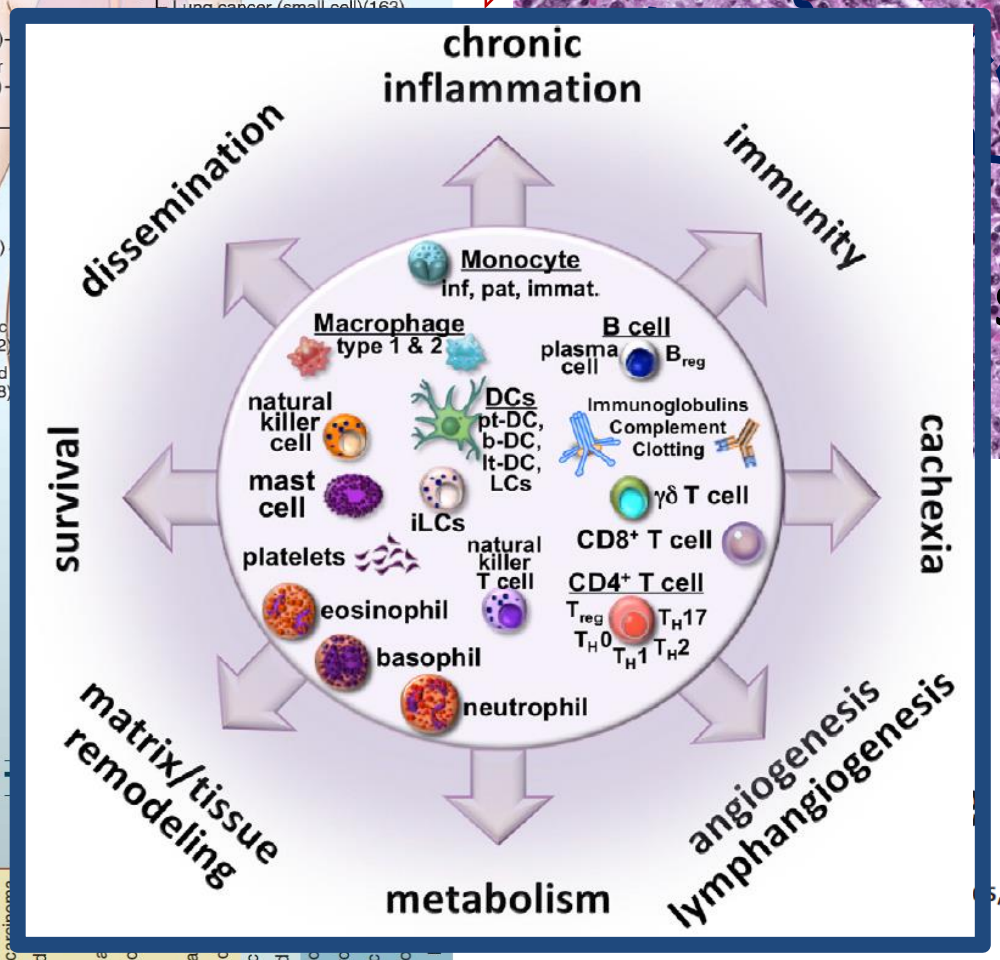
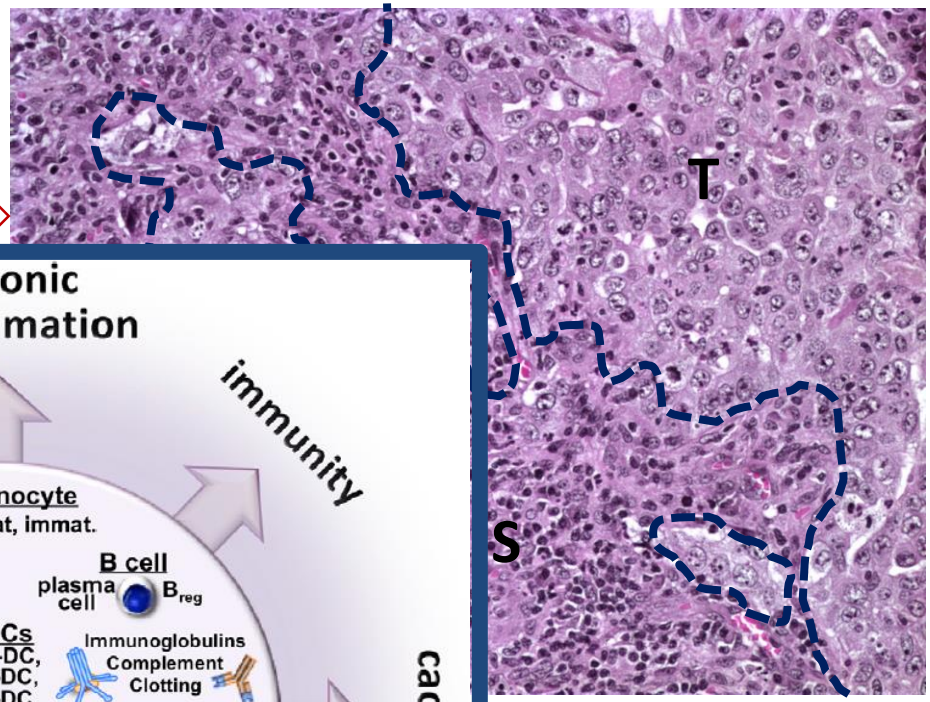


Figure 34.1 From HSC to tumor-infiltrating myeloid cells. The TME consists of numerous components originating from mesenchymal, epithelial, and hematopoietic origins. Within established tumors, both innate and adaptive immune subsets are present where the balance between pro- and antitumorigenic actors is key to malignancy. Various immune cell subsets (such as tumor-associated macrophages, DCs, mast cells, eosinophils, neutrophils with immunosuppressive functions) contribute to fuel tumor progression. These cells are generated during induced myelopoiesis in bone marrow in response to secreted factors by TMEs where a spectrum of myeloid subsets reside. In tumor-bearing context, splenic myelopoiesis is mediated through a distinct hematopoiesis progenitor response where generated cells migrate to TMEs and maintain immunosuppressive environments by producing protumoral factors in a complex regulatory network.



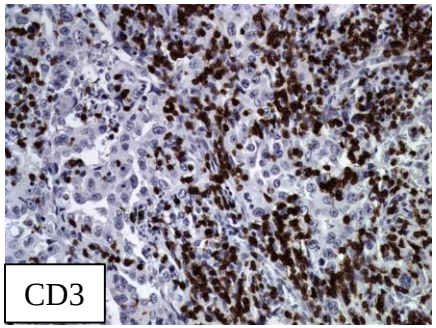




Landscapes

s, Victor E. Velculescu, Shibin Zhou,

SCIENCE VOL 339 29 MARCH 2013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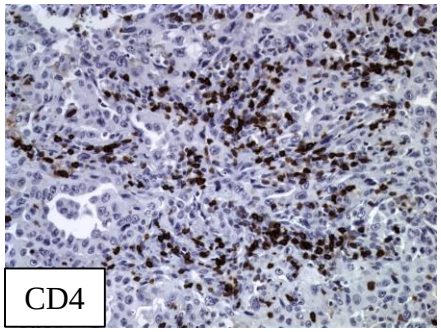
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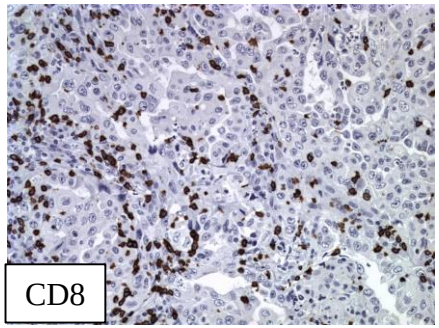
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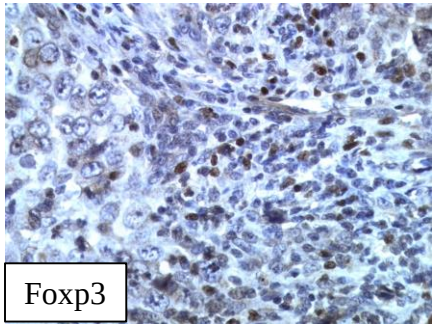
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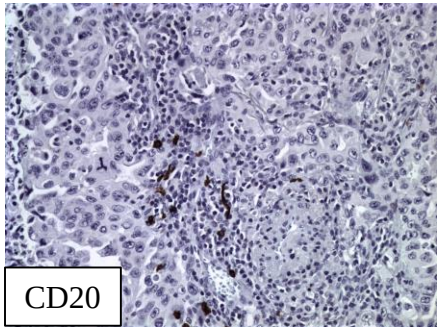
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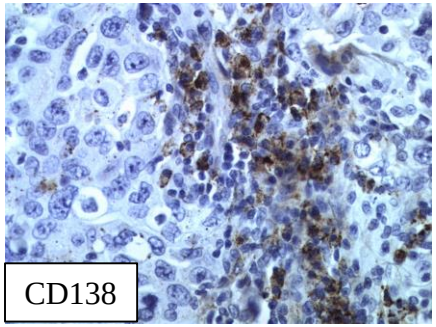
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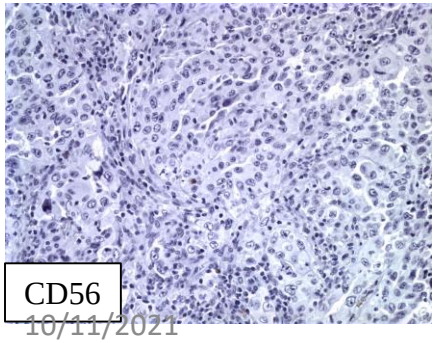
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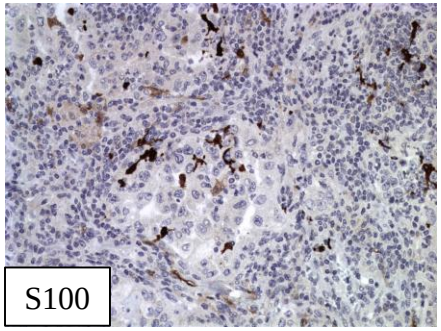
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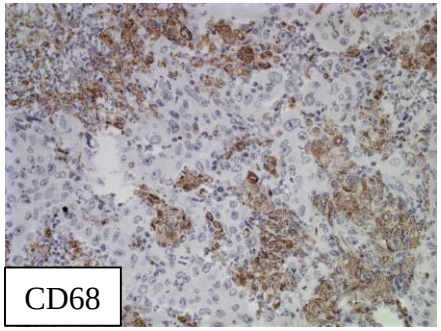

CD68

Immune evasion before tumour invasion in early lung squamous carcinogenesis

Céline Mascaux[1,2,3,4,14,15,18]*, Mihaela Angelova[5,6,7,8,16,18], Angela Vasaturo[5,6,7,8], Jennifer Beane[2], Kahkeshan Hijazi[2], Geraldine Anthoine[1], Bénédicte Buttard[5,6,7,8], Françoise Rothe[9], Karen Willard-Gallo[10], Annick Haller[11,17], Vincent Ninane[12], Arsène Burny[13], Jean-Paul Sculier[1], Avi Spira[2] & Jérôme Galon[5,6,7,8]*

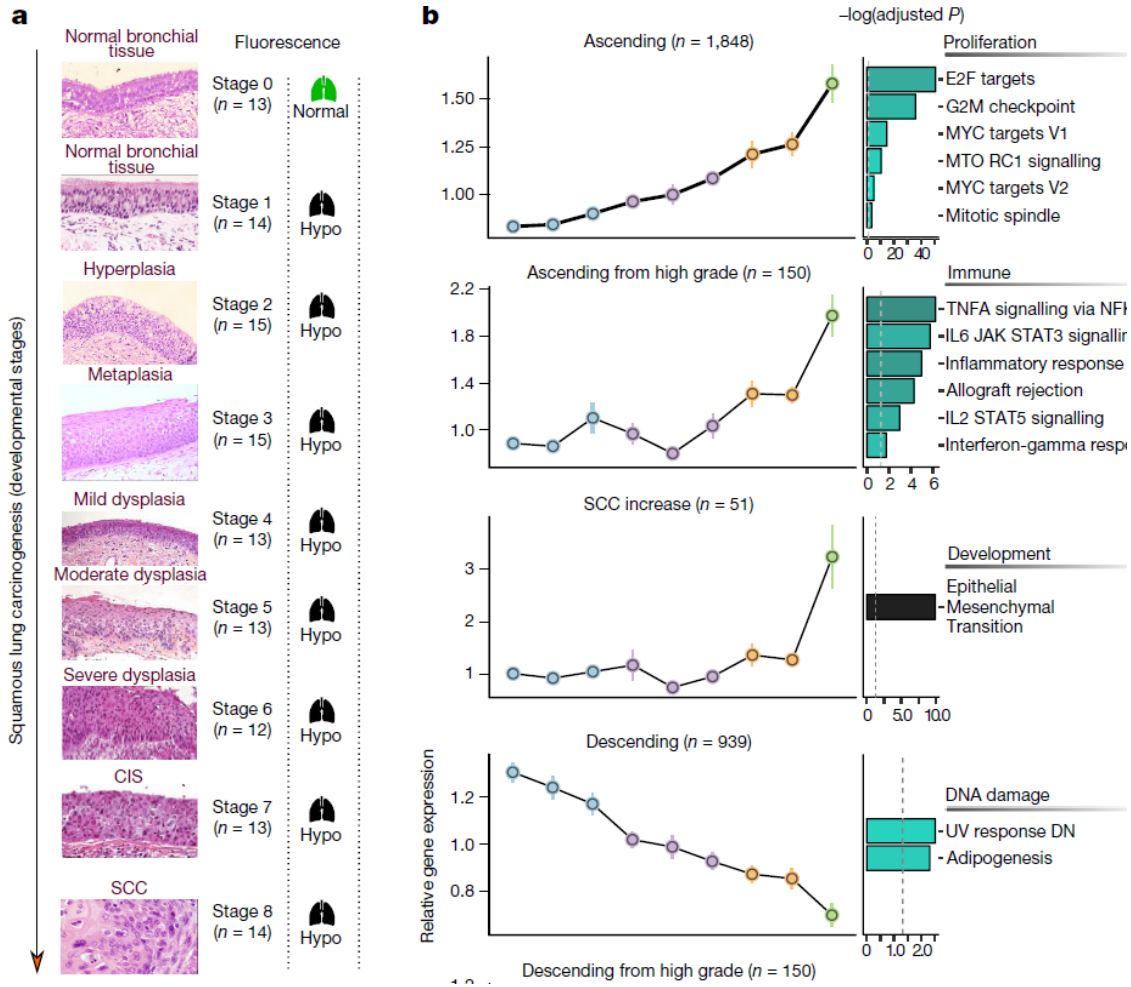


Fig. 1 | Temporal order of cancer hallmarks during carcinogenesis. **a**, Nine morphological stages of development. The normal tissues were split into stage 0 (normal fluorescence) and stage 1 (hypo-fluorescent; hypo) on the basis of fluorescence bronchoscopy. CIS, in situ carcinoma. **b**, Seven modules of co-expressed genes were identified with weighted gene-correlation network analysis. The gene-expression measurement represents the relative abundance of each gene compared to reference RNA from bronchial biopsies from 16 people who had never smoked, derived with two-colour gene-expression microarrays (mean ± s.e.m.). Over-representation analysis linked cancer hallmarks with several gene modules (hypergeometric test, FDR ≤ 0.05). Adjusted *P* values are shown as bar plots after $-\log_{10}(P)$ transformation. **c**, Genes representing

Immune evasion before tumour invasion in early lung squamous carcinogenesis

Céline Mascaux[1,2,3,4,14,15,18*], Mihaela Angelova[5,6,7,8,16,18], Angela Vasaturo[5,6,7,8], Jennifer Beane[2], Kahkeshan Hijazi[2], Geraldine Anthoine[1], Bénédicte Buttard[5,6,7,8], Françoise Rothe[9], Karen Willard-Gallo[10], Annick Haller[11,17], Vincent Ninane[12], Arsène Burny[13], Jean-Paul Sculier[1], Avi Spira[2] & Jérôme Galon[5,6,7,8*]

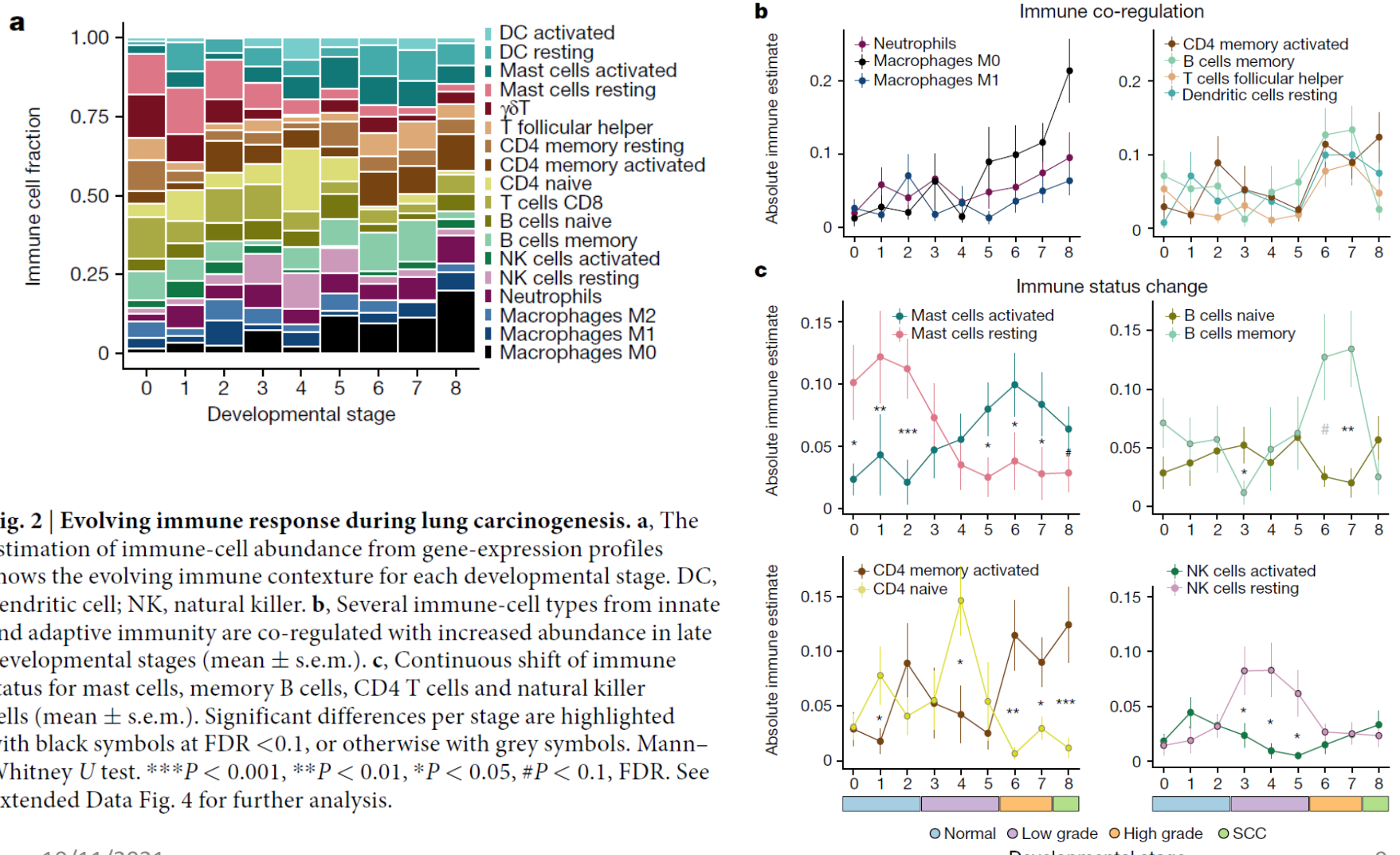
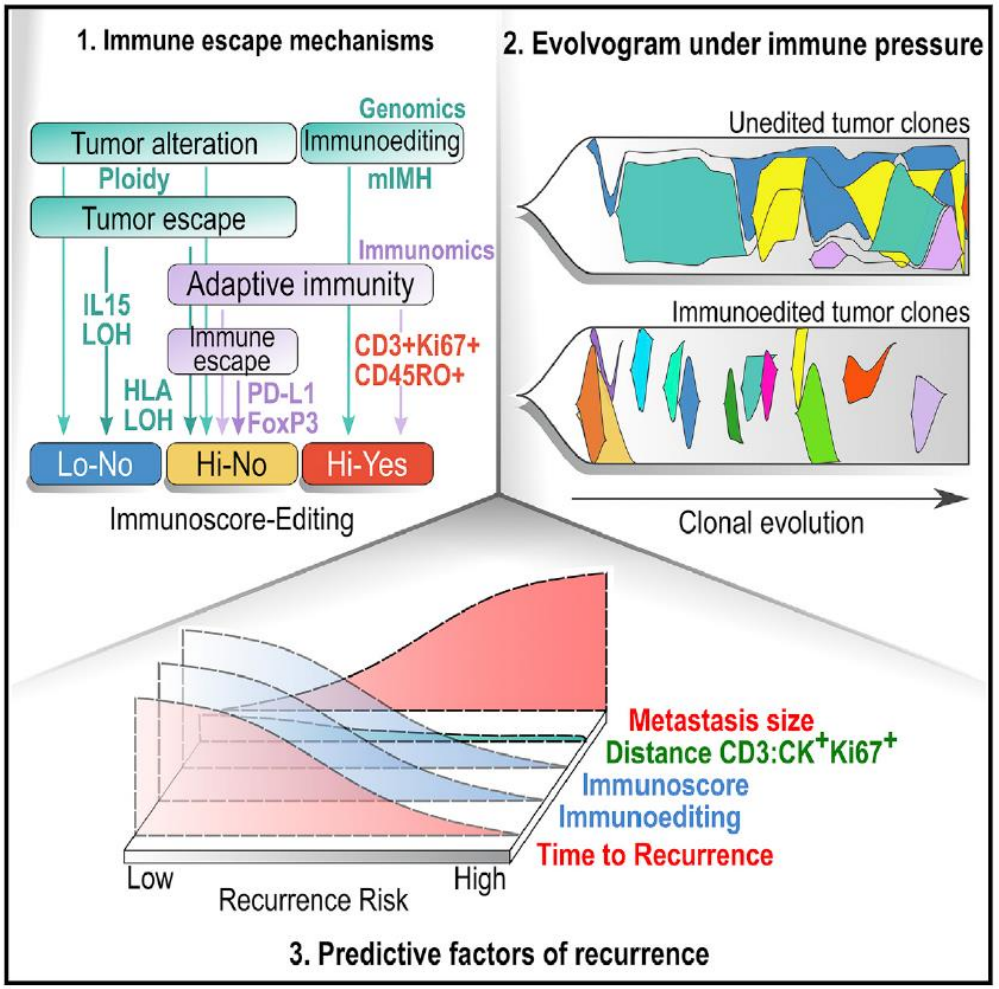


Fig. 2 | Evolving immune response during lung carcinogenesis. a, The estimation of immune-cell abundance from gene-expression profiles shows the evolving immune contexture for each developmental stage. DC, dendritic cell; NK, natural killer. **b**, Several immune-cell types from innate and adaptive immunity are co-regulated with increased abundance in late developmental stages (mean ± s.e.m.). **c**, Continuous shift of immune status for mast cells, memory B cells, CD4 T cells and natural killer cells (mean ± s.e.m.). Significant differences per stage are highlighted with black symbols at FDR <0.1, or otherwise with grey symbols. Mann–Whitney *U* test. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, #$P < 0.1$, FDR. See Extended Data Fig. 4 for further analysis.

Evolution of Metastases in Space and Time under Immune Selection

Mihaela Angelova,[1] Bernhard Mlecnik,[1,2] Angela Vasaturo,[1] Gabriela Bindea,[1] Tessa Fredriksen,[1] Lucie Lafontaine,[1] Bénédicte Buttard,[1] Erwan Morgand,[1] Daniela Bruni,[1] Anne Jouret-Mourin,[3] Catherine Hubert,[3] Alex Kartheuser,[3] Yves Humblet,[3] Michele Ceccarelli,[4,5] Najeeb Syed,[6] Francesco M. Marincola,[7,8] Davide Bedognetti,[9,10] Marc Van den Eynde,[1,3,10] and Jérôme Galon[1,11,*]



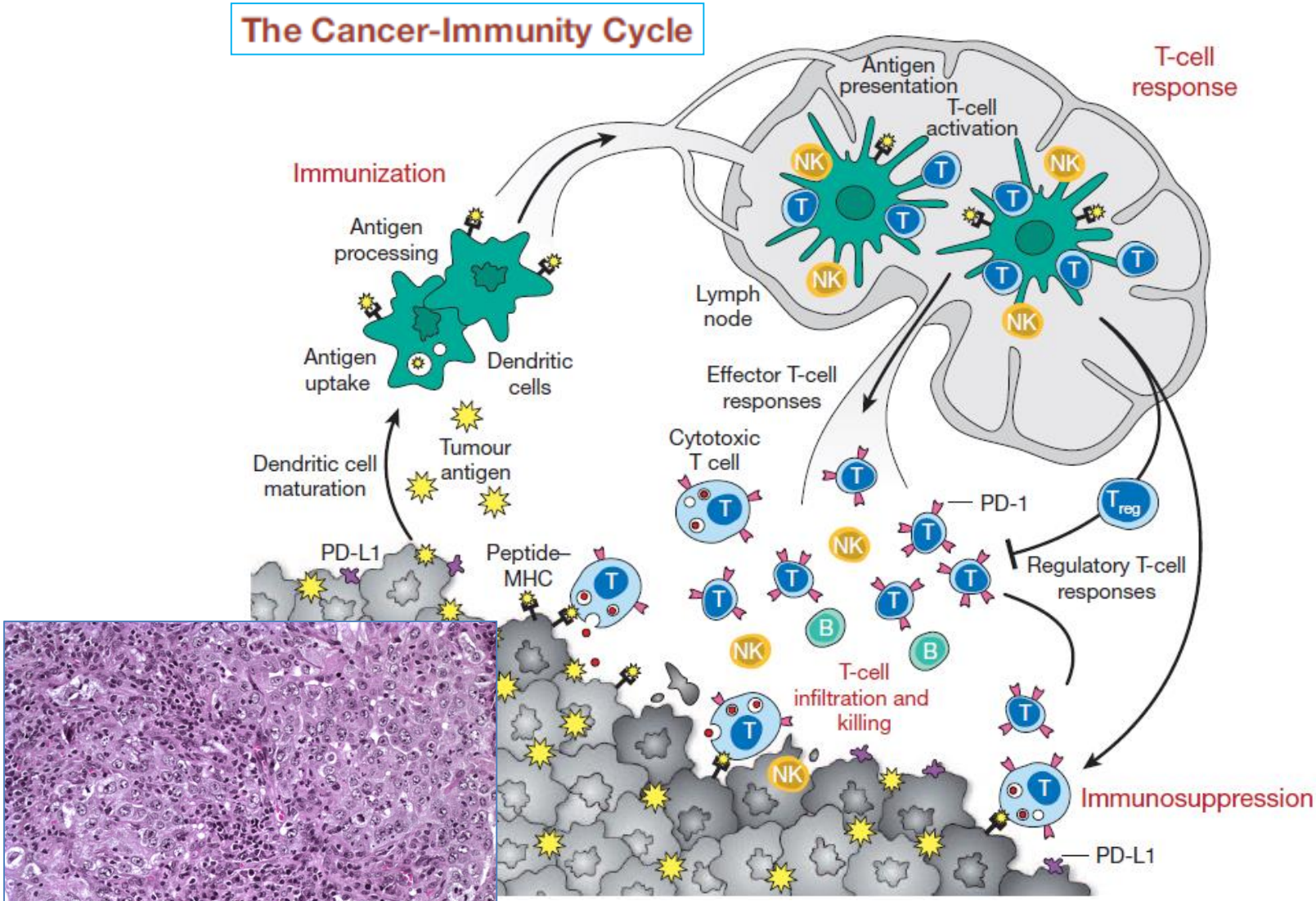
Highlights

- Different escape mechanisms delineated by lack of adaptive immunity or immunoediting
- Non-recurrent clones are immunoedited; progressing clones are immune privileged
- Immunoediting and Immunoscore are predictive factors of metastasis recurrence
- Parallel selection model describes clonal immunoediting and tumor evolution

Cell *175*, 751–765, October 18, 2018

Cancer immunotherapy comes of age

Ira Mellman[1], George Coukos[2] & Glenn Dranoff[3]



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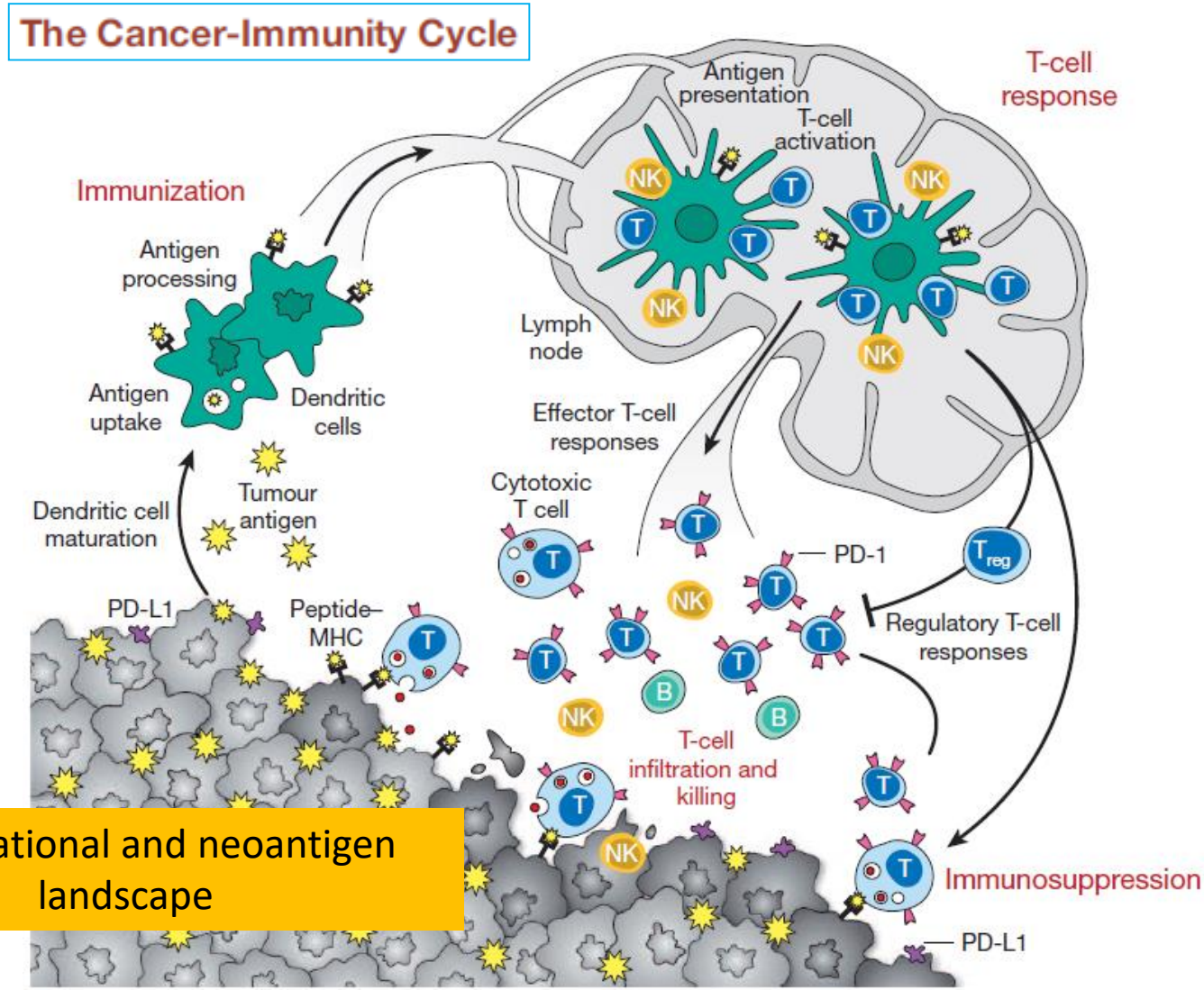

Tumor mutational and neoantigen landscape

Cancer immunotherapy comes of age

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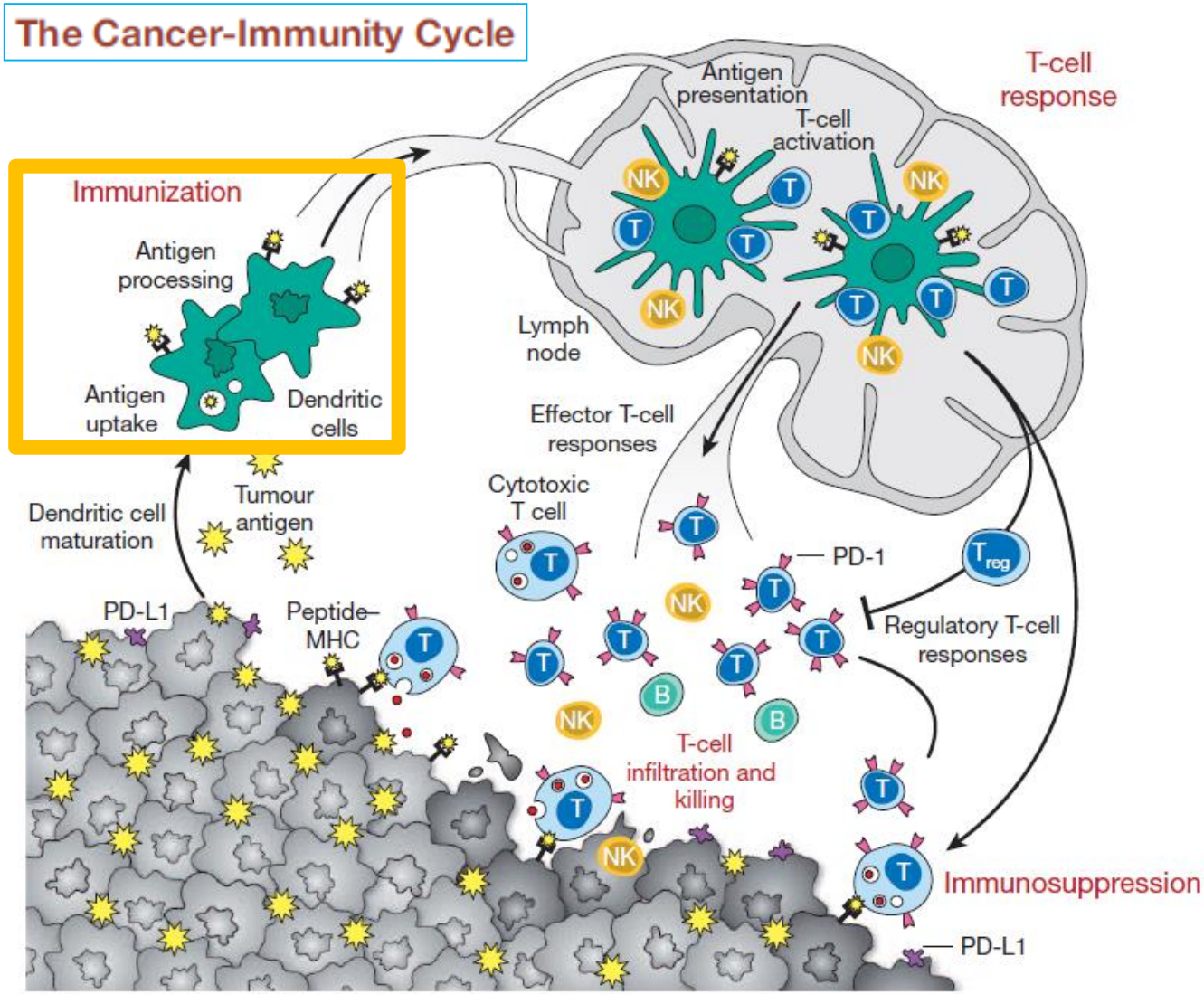

Dendritic Cells: Master Regulators of the Immune Response

Ira Mellman

Cancer Immunol Res; 1(3); 145–9. ©2013

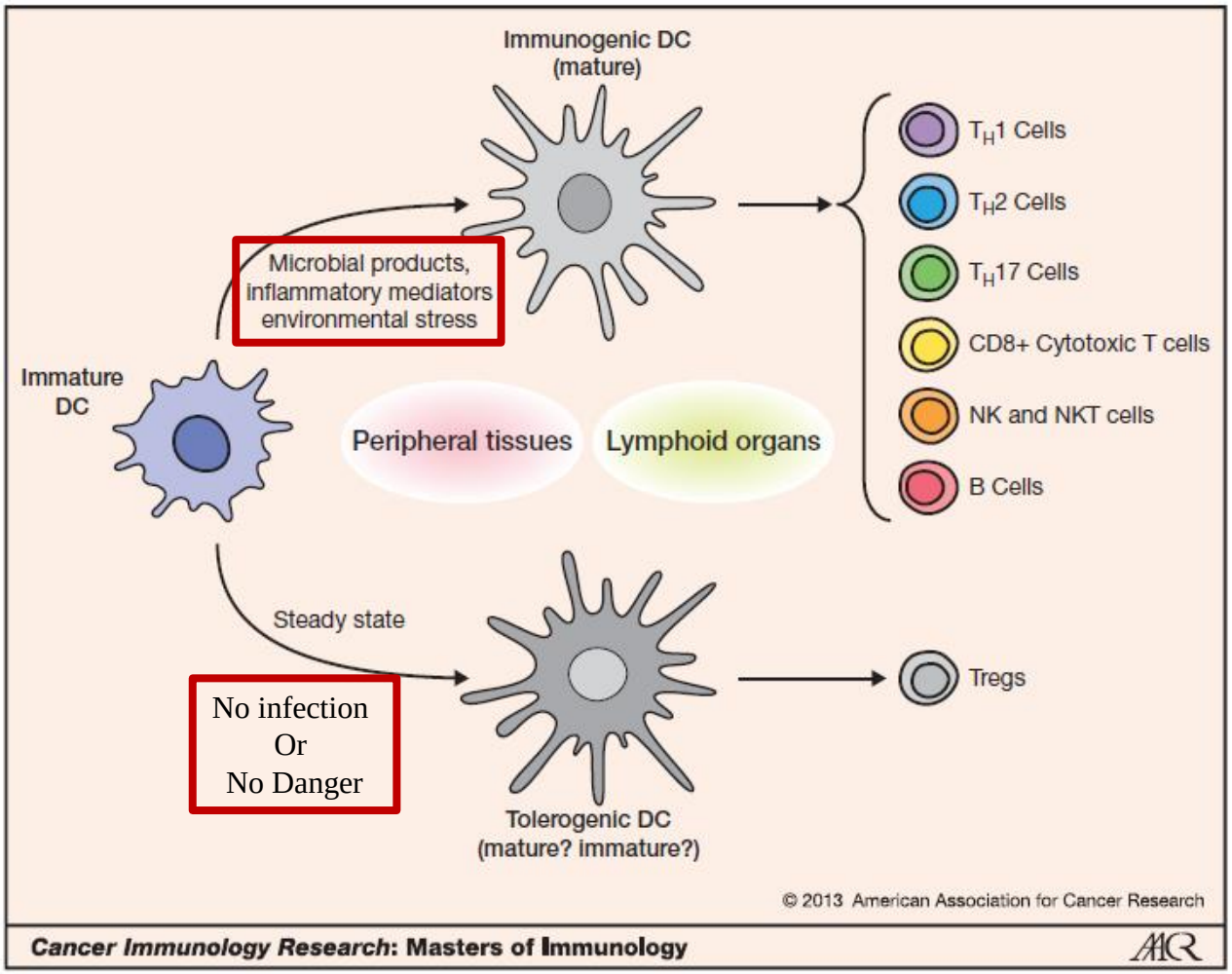

Figure 1. Functional consequences of DC maturation. Depending on the type of microbial, inflammatory, or steady-state signals a resting DC is exposed to, it is converted into a tolerogenic or immunogenic state that programs the development of distinct subsets of T cells, each with distinct functions adapted to the signal detected by the dendritic cell. Different arrays of cytokines released by these differentially matured dendritic cells play an important role in determining T-cell outcomes. It is possible that some of the variation in T-cell outcomes reflects the roles of distinct DC populations, although these must still respond as indicated to microbial or steady-state signals.

Transcriptional determinants of tolerogenic and immunogenic states during dendritic cell maturation

Bryan Vander Lugt,[1] Jeremy Riddell,[4] Aly A. Khan,[6,7] Jason A. Hackney,[2] Justin Lesch,[3] Jason DeVoss,[3] Matthew T. Weirauch,[4] Harinder Singh,[5] and Ira Mellman[1]

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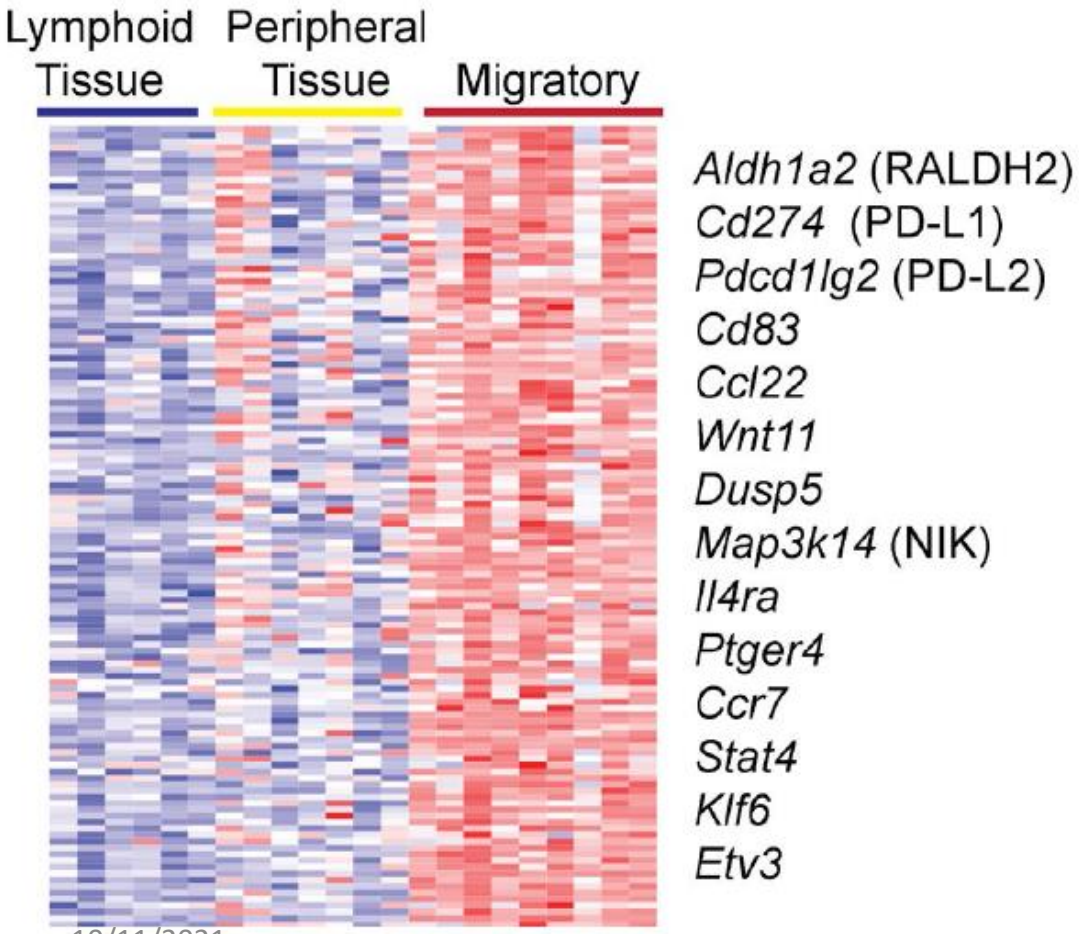
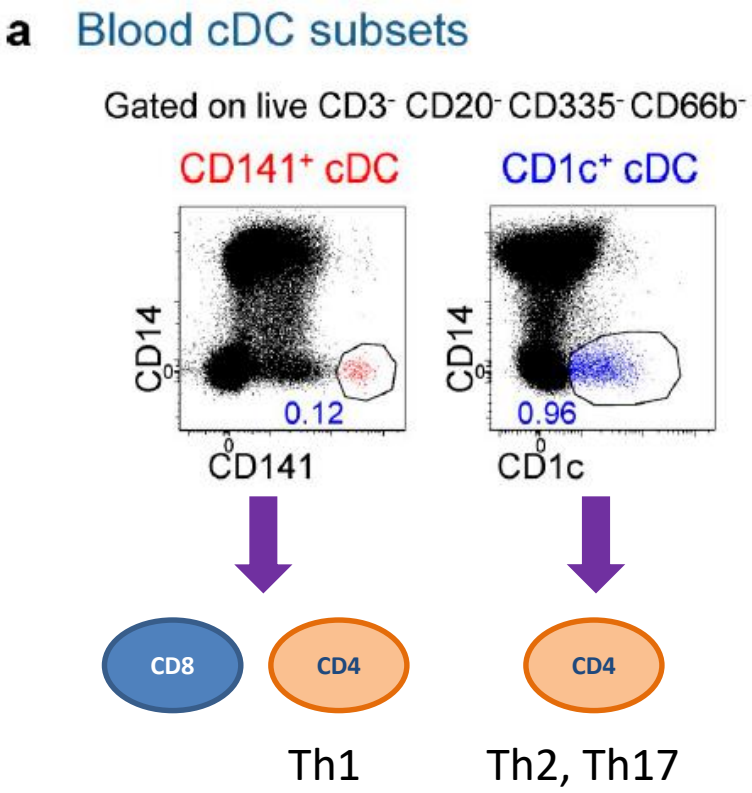
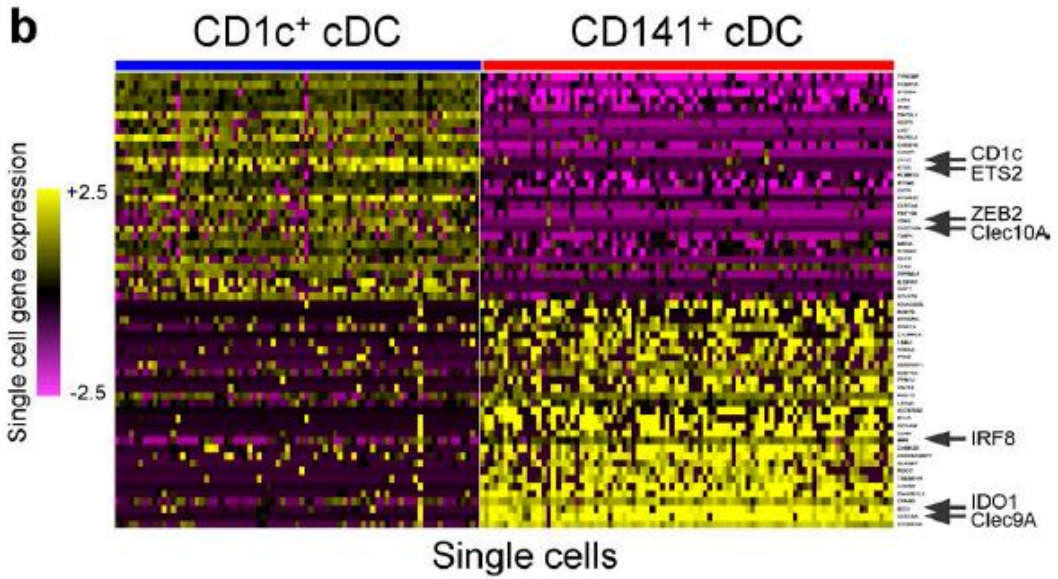


Human dendritic cells (DCs) are derived from distinct circulating precursors that are precommitted to become CD1c$^+$ or CD141$^+$ DCs

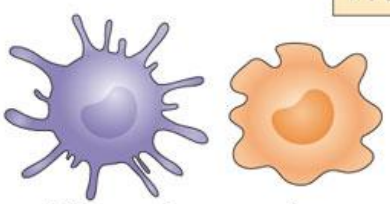
Gaëlle Breton,[1] Shiwei Zheng,[2,5] Renan Valieris,[4] Israel Tojal da Silva,[4] Rahul Satija,[2,5] and Michel C. Nussenzweig[1,3]

J. Exp. Med. 2016 Vol. 213 No. 13 2861–2870



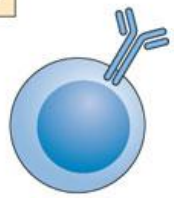


Professional APCs



DCs and macrophages

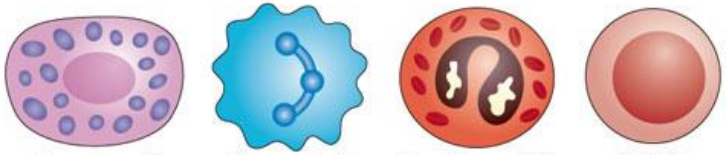
Key features
- Phagocytic
- Express receptors for apoptotic cells, DAMPs and PAMPs
- Localize to tissues
- Localize to T cell zone of lymph nodes following activation (DCs)
- Constitutively express high levels of MHC class II molecules and antigen processing machinery
- Express co-stimulatory molecules following activation



B cells

Key features
- Internalize antigens via BCRs
- Constitutively express MHC class II molecules and antigen processing machinery
- Express co-stimulatory molecules following activation

Atypical APCs



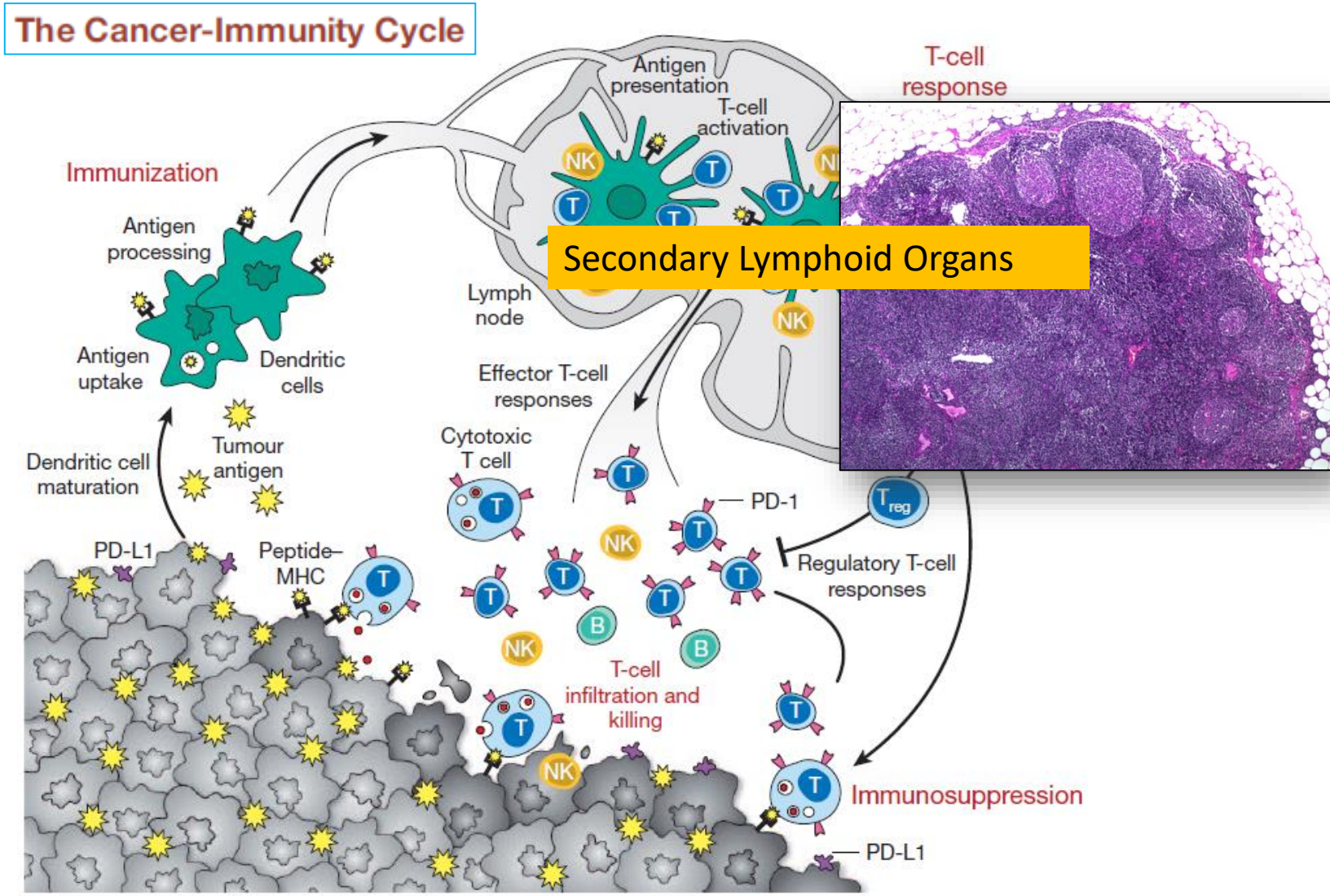
Mast cells | Basophils | Eosinophils | ILC3s

Key features
- Inducible expression of MHC class II molecules
- Antigen-presenting functions limited to specific immune environments (especially type 2 immune settings)
- Lack of compelling evidence that they can activate naive $CD4^+$ T cells in an antigen-specific manner

Nature Reviews | Immunology

Cancer immunotherapy comes of age

Ira Mellman[1], George Coukos[2] & Glenn Dranoff[3]



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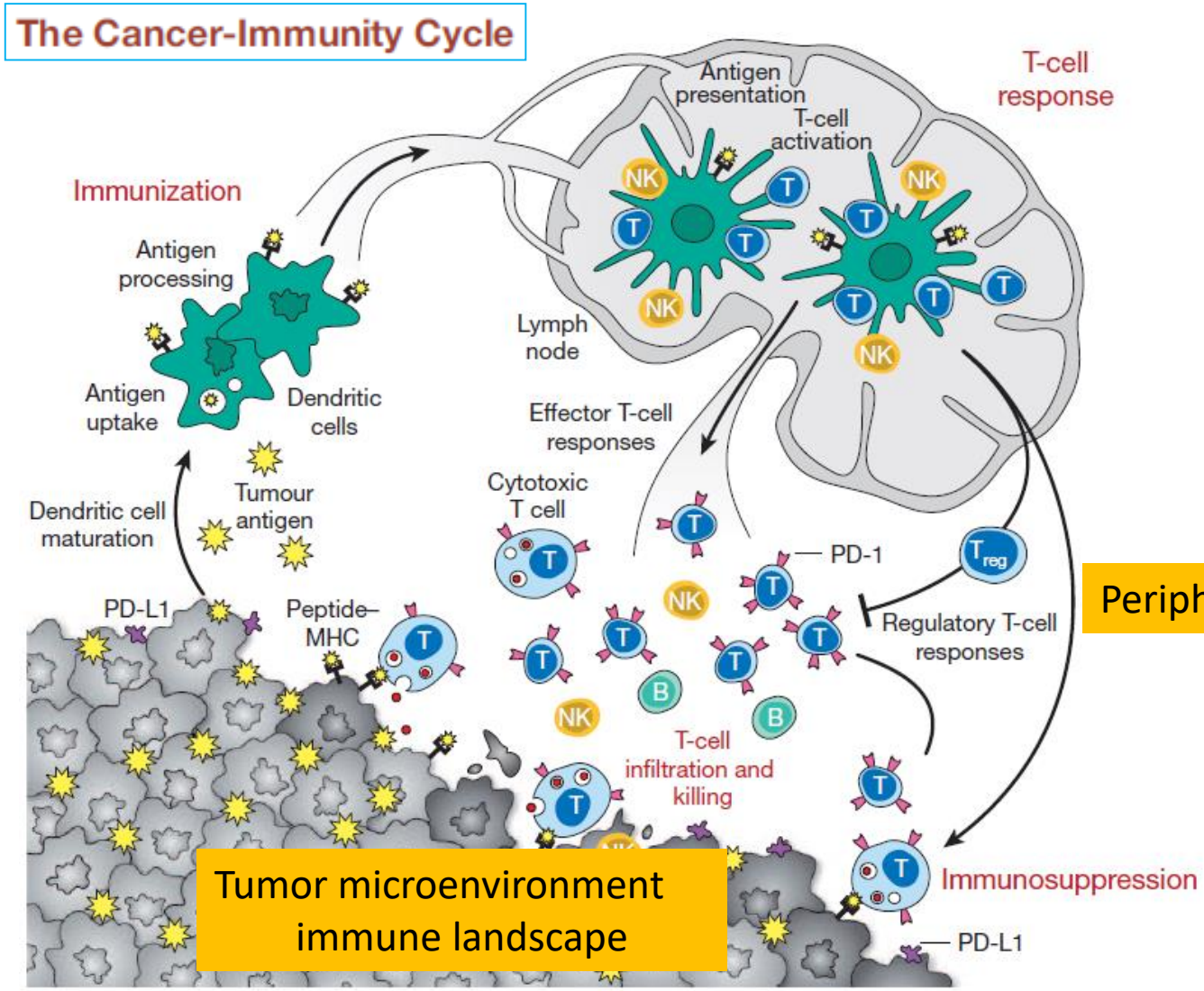


REVIEW Open Access

Novel technologies and emerging biomarkers for personalized cancer immunotherapy

Jianda Yuan[1*], Priti S. Hegde[2], Raphael Clynes[3], Periklis G. Foukas[4,5], Alexandre Harari[4], Thomas O. Kleen[6], Pia Kvistborg[7], Cristina Maccalli[8], Holden T. Maecker[9], David B. Page[10], Harlan Robins[11], Wenru Song[12], Edward C. Stack[13], Ena Wang[14], Theresa L. Whiteside[15], Yingdong Zhao[16], Heinz Zwierzina[17], Lisa H. Butterfield[18] and Bernard A. Fox[10*]

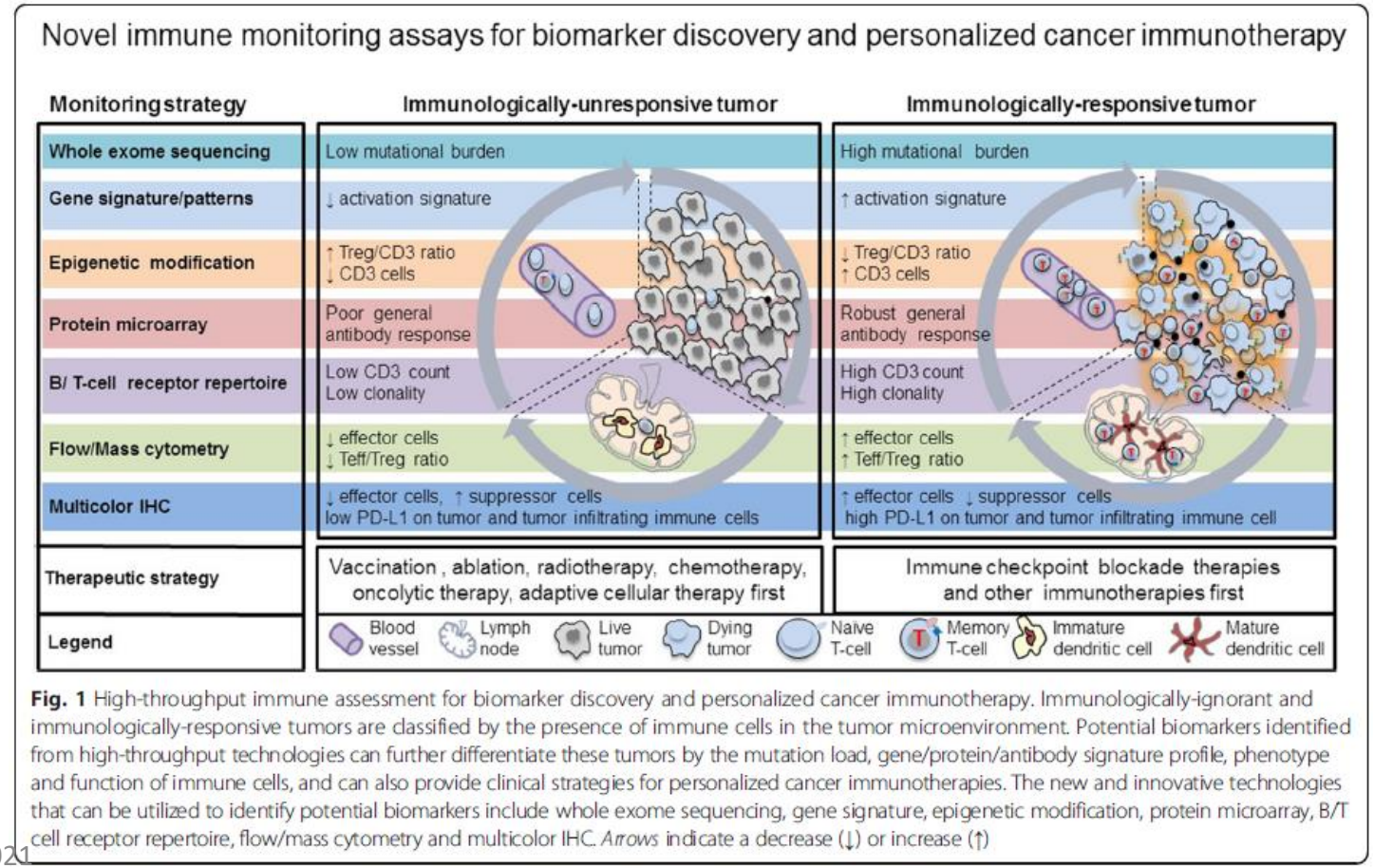


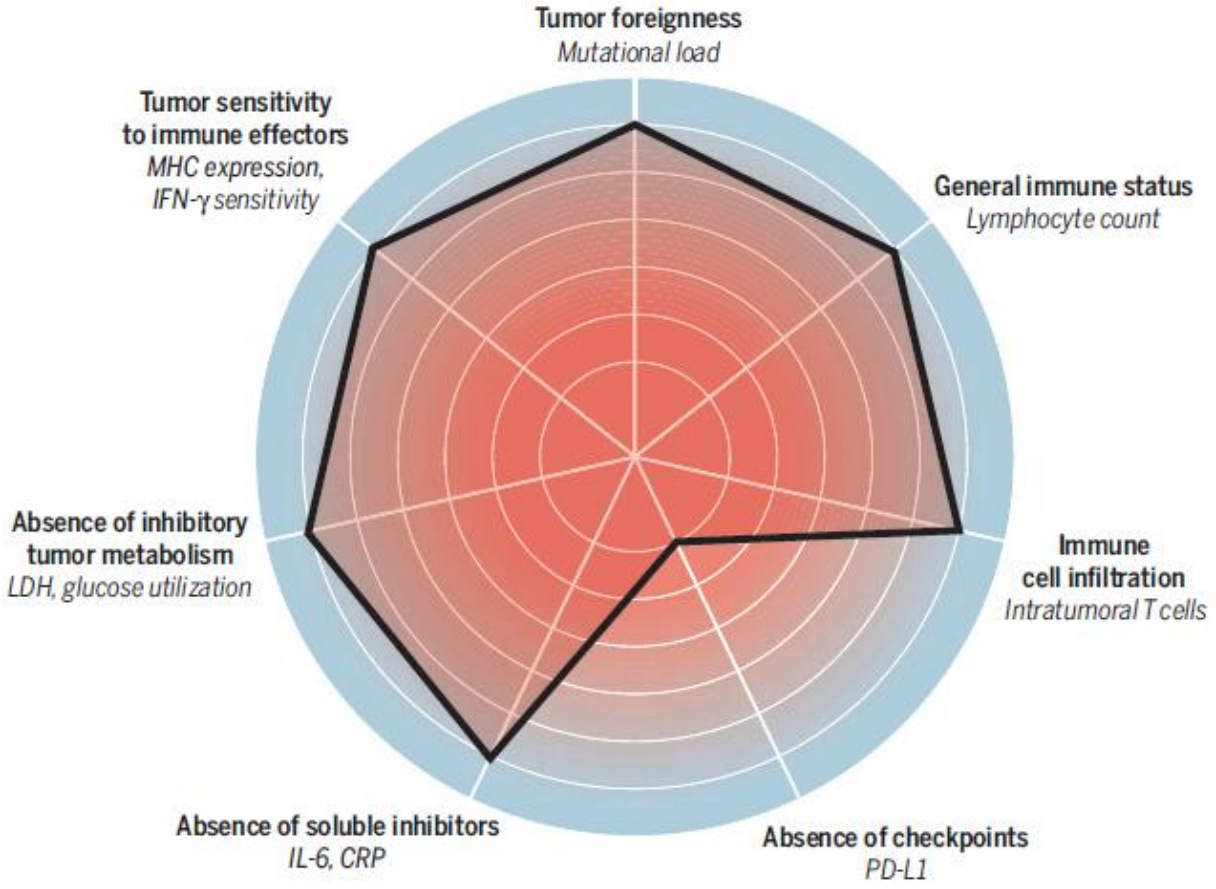
Fig. 1 High-throughput immune assessment for biomarker discovery and personalized cancer immunotherapy. Immunologically-ignorant and immunologically-responsive tumors are classified by the presence of immune cells in the tumor microenvironment. Potential biomarkers identified from high-throughput technologies 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 (↑)

CANCER IMMUNOLOGY

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. Schumacher[2]**



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*).

Science **352** (6286), 658-660.

Elements of cancer immunity and the cancer–immune set point

Daniel S. Chen[1] & Ira Mellman[1]

19 JANUARY 2017 | VOL 541 | NATURE | 321

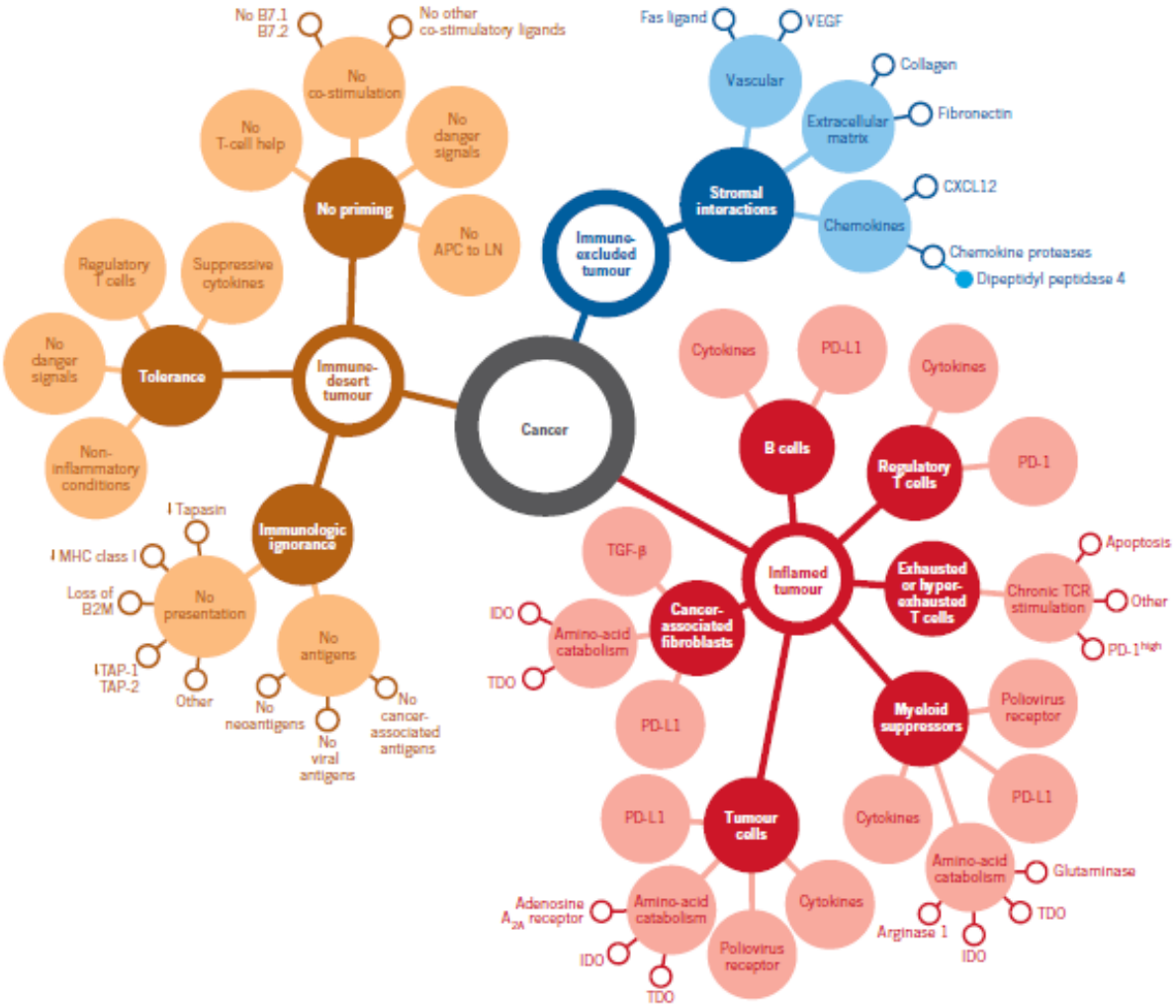


Figure 3 | Cancer-immune phenotypes. Anticancer immunity in humans can be segregated into three main phenotypes: the immune-desert phenotype (brown), the immune–excluded phenotype (blue) and the inflamed phenotype (red). Each is associated with specific underlying biological mechanisms that may prevent 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 hyperexhaustion. 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.

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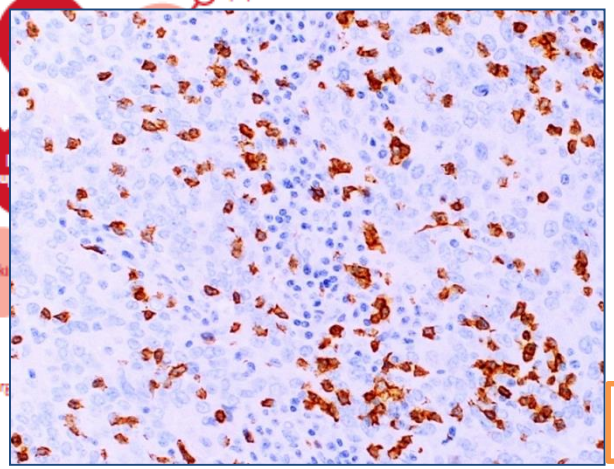

CD8

Elements of cancer immunity and the cancer–immune set point

Daniel S. Chen[1] & Ira Mellman[1]

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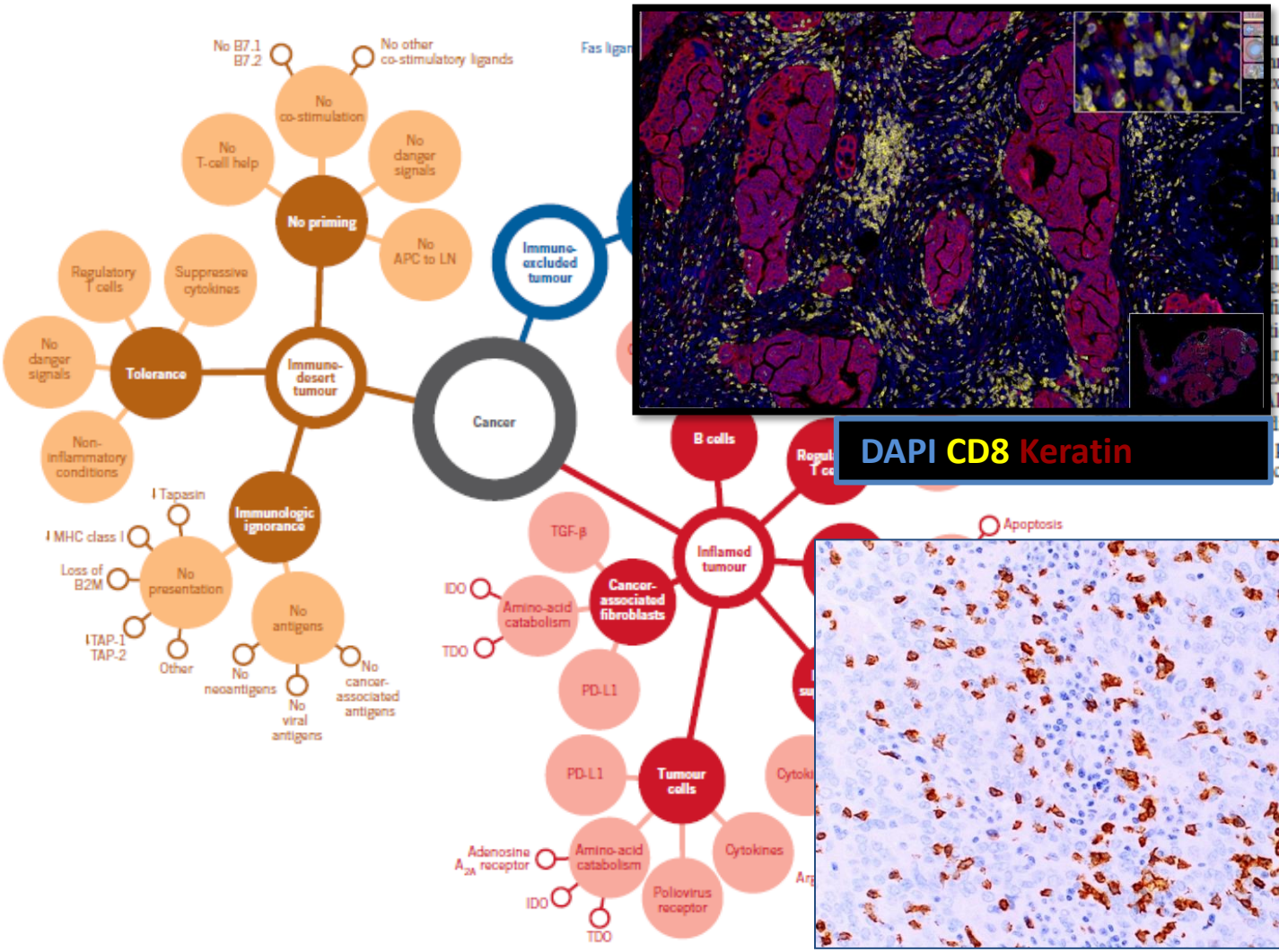

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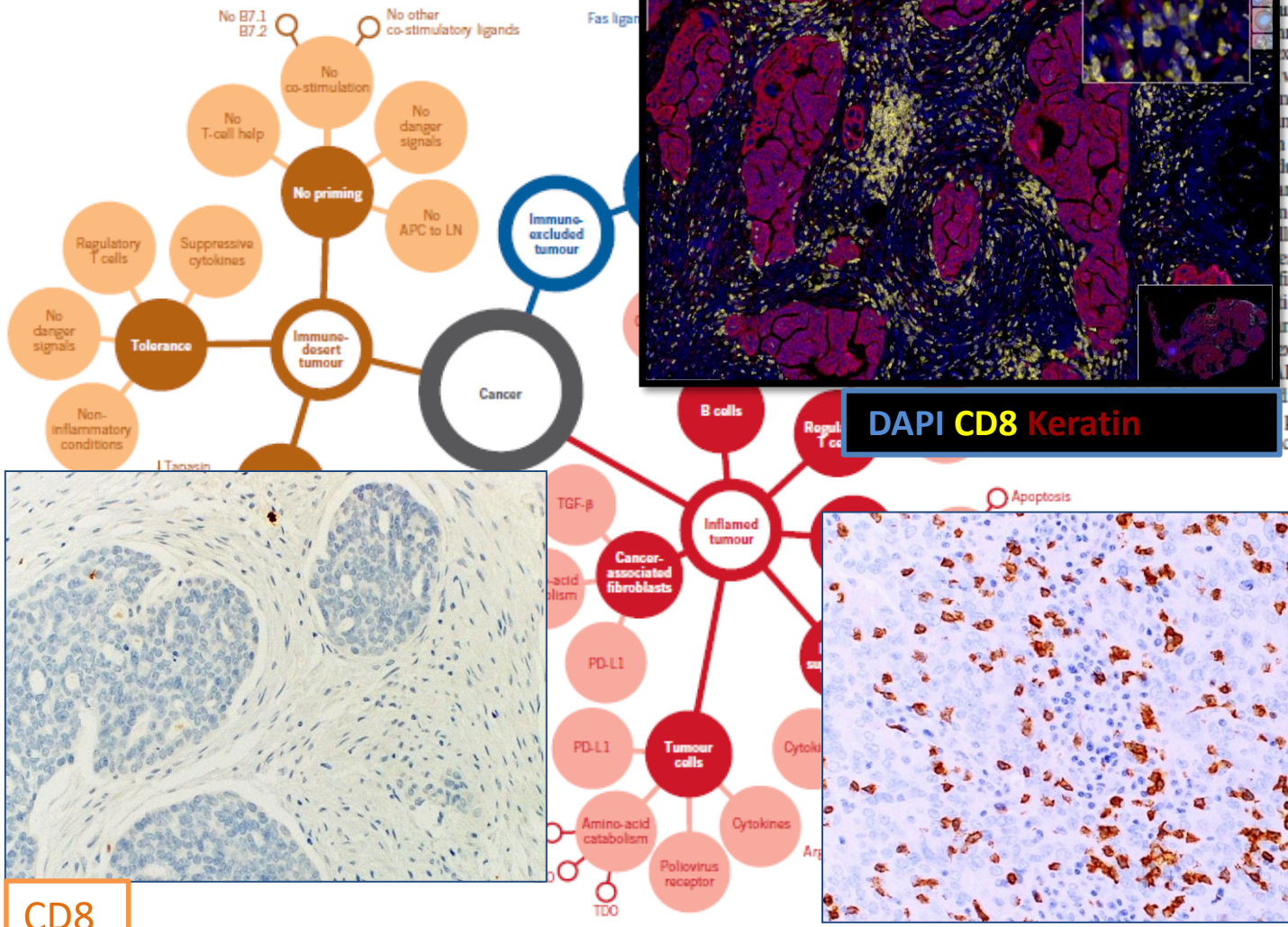

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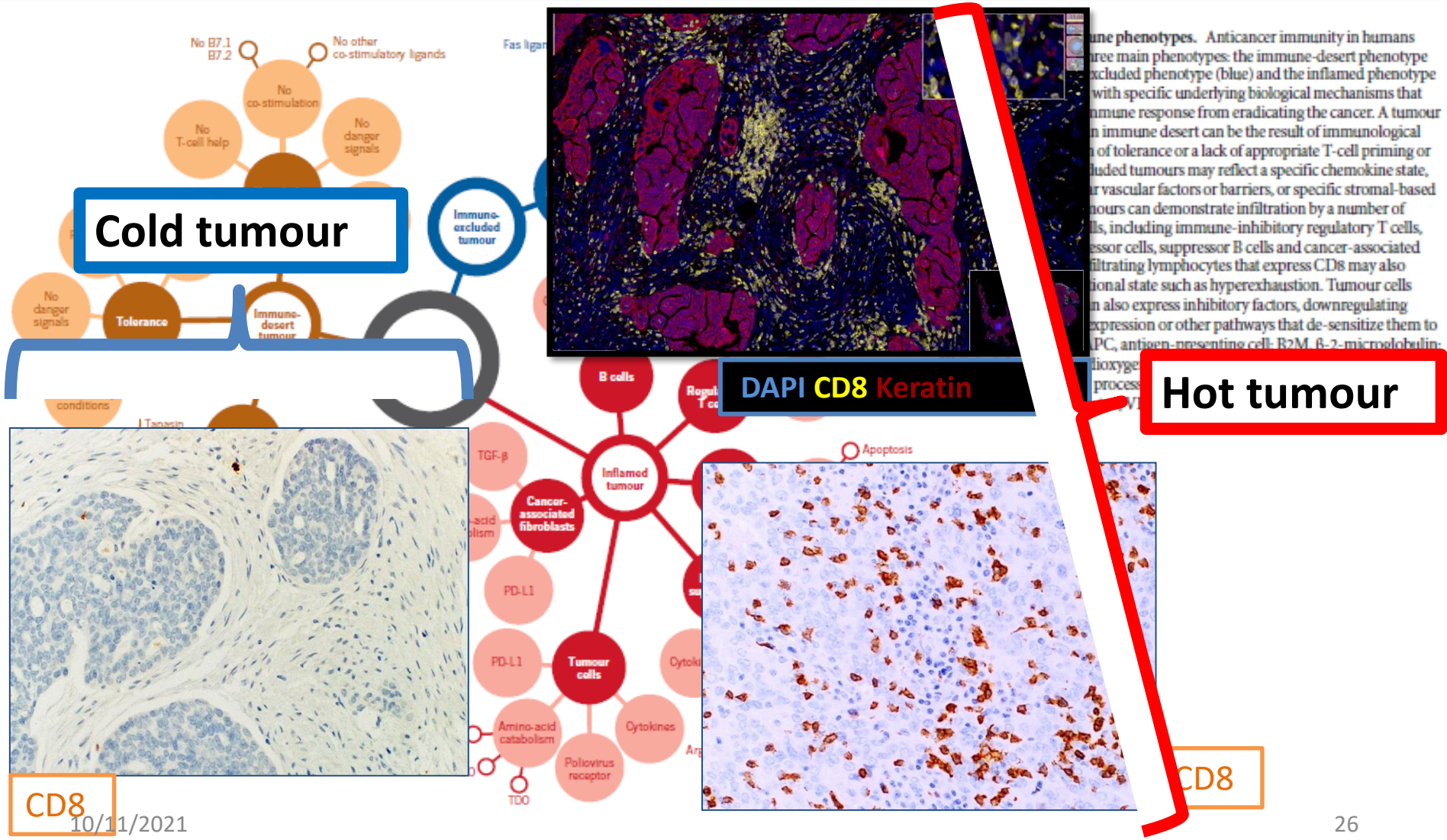

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Host tissue determinants of tumour immunity

Hélène Salmon[1,2,3], Romain Remark[4], Sacha Gnjatic[1,2,5] and Miriam Merad[1,2]**

NATURE REVIEWS | CANCER

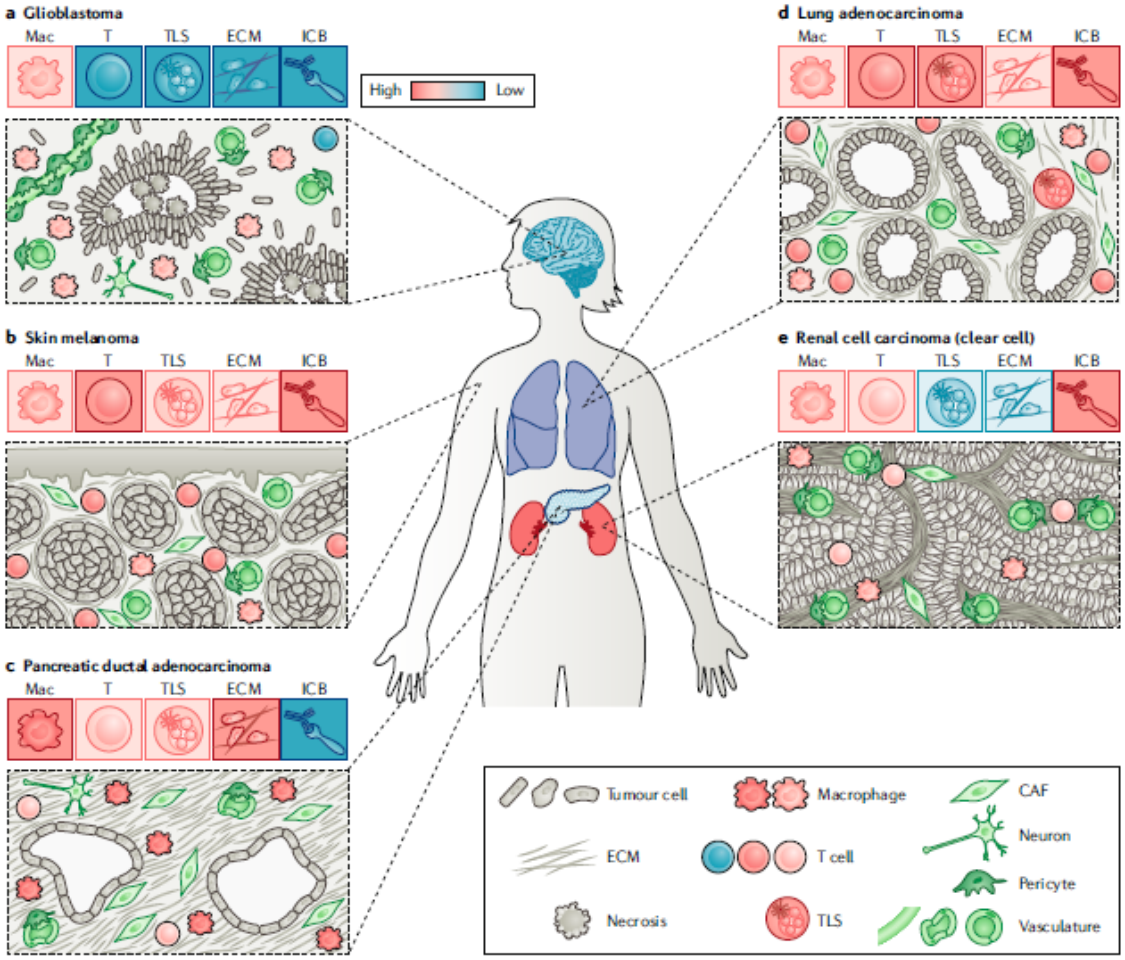

Fig. 1 | **The cellular and architectural heterogeneity of the tumour microenvironment at distinct cancer sites.** Schematics of representative histological patterns of glioblastoma (part **a**), skin melanoma (part **b**), pancreatic ductal adenocarcinoma (part **c**), lung adenocarcinoma (part **d**) and clear-cell renal cell carcinoma (part **e**) are shown. Tumour lesions at distinct tissue sites display different tumour mass organization, stroma to tumour ratios and levels of fibrotic reaction. In addition to neoplastic cells, each tumour microenvironment contains cells derived from both circulating cells and local cells such as fibroblasts, pericytes and endothelial cells that may differentially impact antitumour immune responses across cancer sites. For each tumour type, a colour-coded heatmap (red: high; blue: low) shows the level of dominance of macrophage or lymphocyte infiltrate, presence of tertiary lymphoid structures (TLSs), matrix deposition and response to immune checkpoint blockade (ICB). CAF, cancer-associated fibroblast; ECM, extracellular matrix; Mac, macrophage; T, T cell.

Outline

- Intoduction to the Tumor Immune Microenvironment (TIME)
  - **Prognostic /** Predictive value
  - Methodologies
- Mechanisms regulating TIME
- TIME and neoplastic evolution

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 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†]

29 SEPTEMBER 2006 VOL 313 SCIENCE

The immune contexture in human tumours: impact on clinical outcome

Wolf Herman Fridman, Franck Pagès, Catherine Sautès-Fridman and Jérôme Galon

NATURE REVIEWS | CANCER VOLUME 12 | APRIL 2012

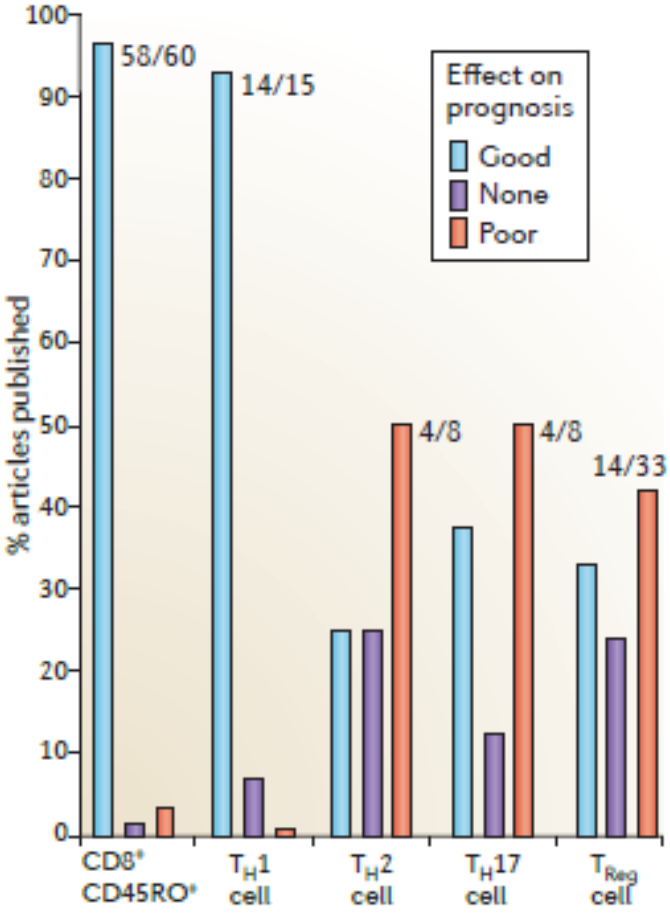

Figure 2 | **The association of immune cell infiltrates with prognosis in various types of cancer.** The analysis of 124 published articles studying the impact of cytotoxic T cells, memory T cells, regulatory T (T_{Reg}) cells and T helper (T_H) cell subpopulations with regard to prognosis of cancer patients (20 different cancer types were analysed) is represented. 'Good' means that the cell type is associated with a good prognosis, 'none' means that there was no correlation and 'poor' means that the cells are associated with a poor prognosis. Please also refer to TABLE 1 for references.

The immune contexture in cancer prognosis and treatment

NATURE REVIEWS | CLINICAL ONCOLOGY

Wolf H. Fridman[1–3], Laurence Zitvogel[4], Catherine Sautès–Fridman[1–3] and Guido Kroemer[2,3,5–8]

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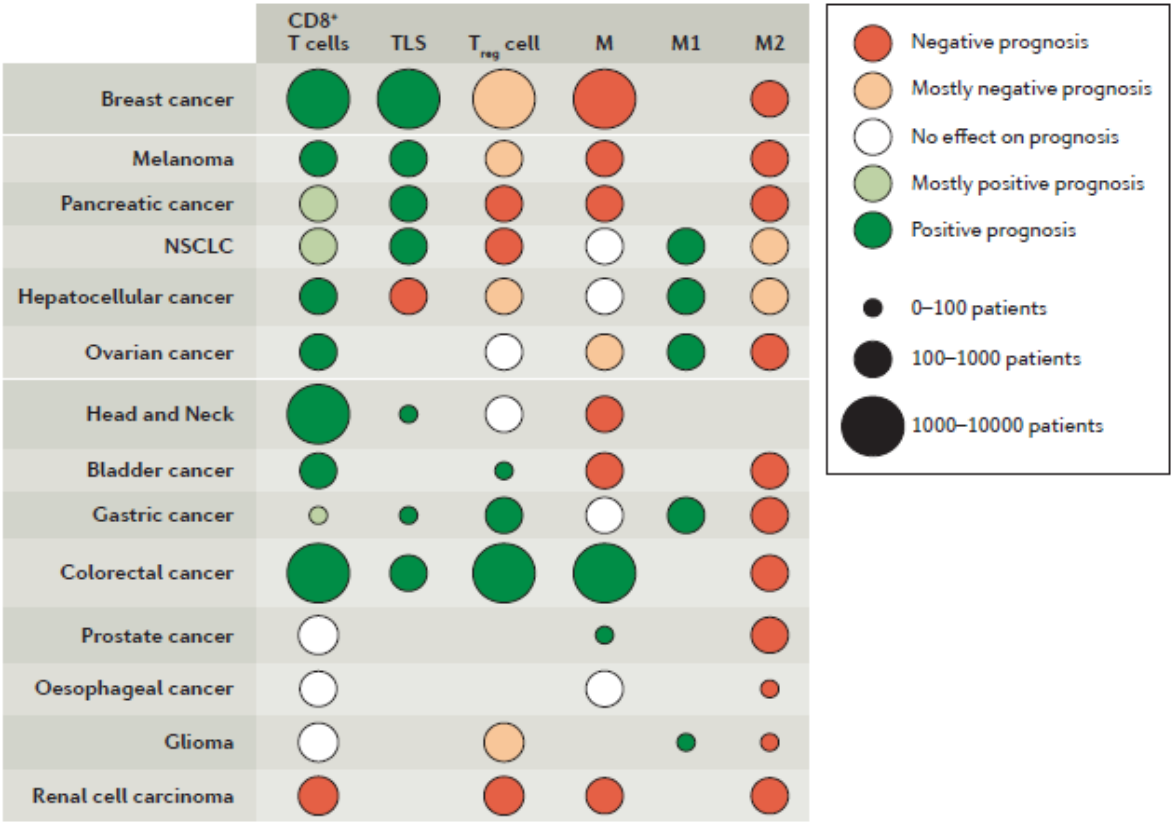

Figure 3 | **Effects of the immune infiltrate on the prognosis of patients with cancer.** Data from 200 studies (cited in Supplementary information S1 (table) and in the main-text), involving approximately 25,000 patients were analysed regarding the relevance of CD8+ T cells, tertiary lymphoid structures (TLSs), regulatory T cells (T_{reg}), CD68+ macrophages (M) and, more specifically, macrophages of an M1 or M2 subtype to overall survival outcomes. Bold colours indicate a positive (green) or a negative (red) prognostic association following analysis of all relevant studies; lighter colours indicate a predominantly positive (light green) or negative (orange) prognostic association in the majority of studies analysed. White circles indicate no statistically significant correlation, or that a dubious prognostic association was observed in a similar number of studies. The size of the circles indicates the number of patients enrolled in the studies: small circles indicate 0–100 patients, medium-sized circles indicate 100–1,000 patients and large circles indicate 1,000–10,000 patients.

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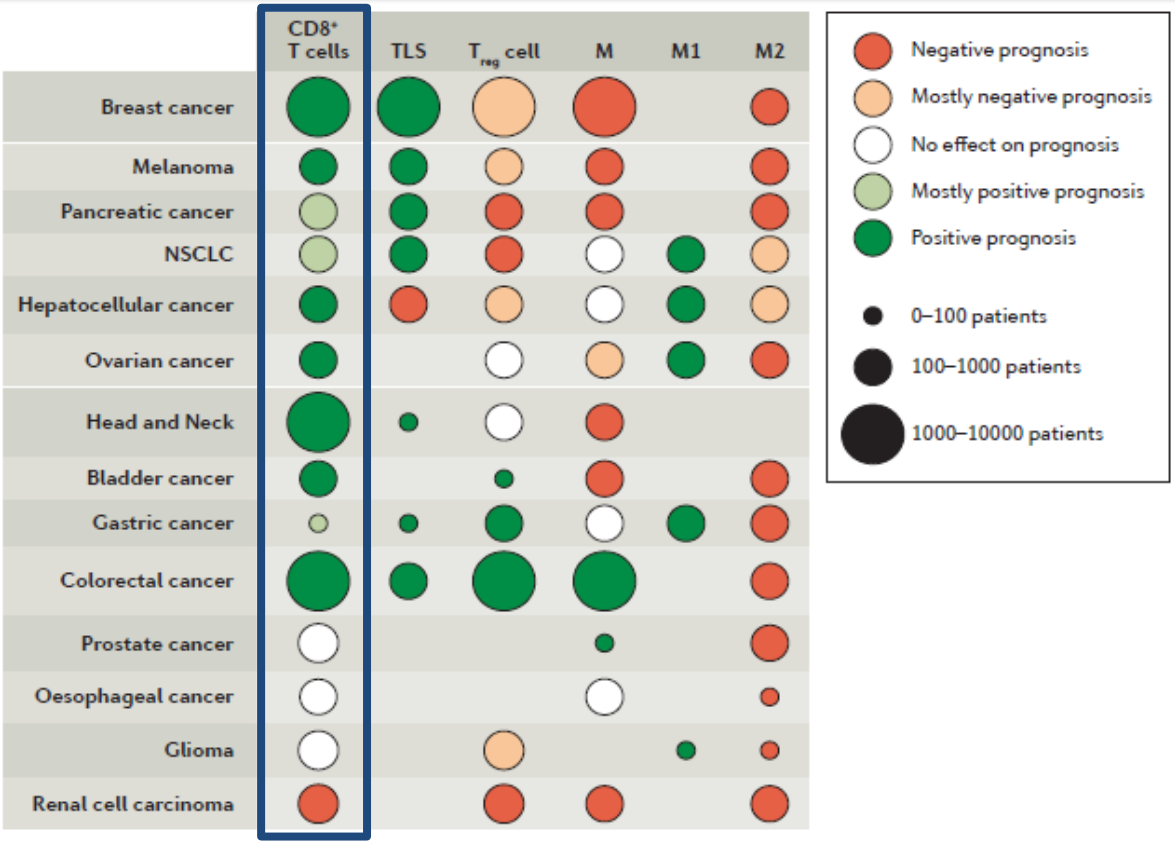

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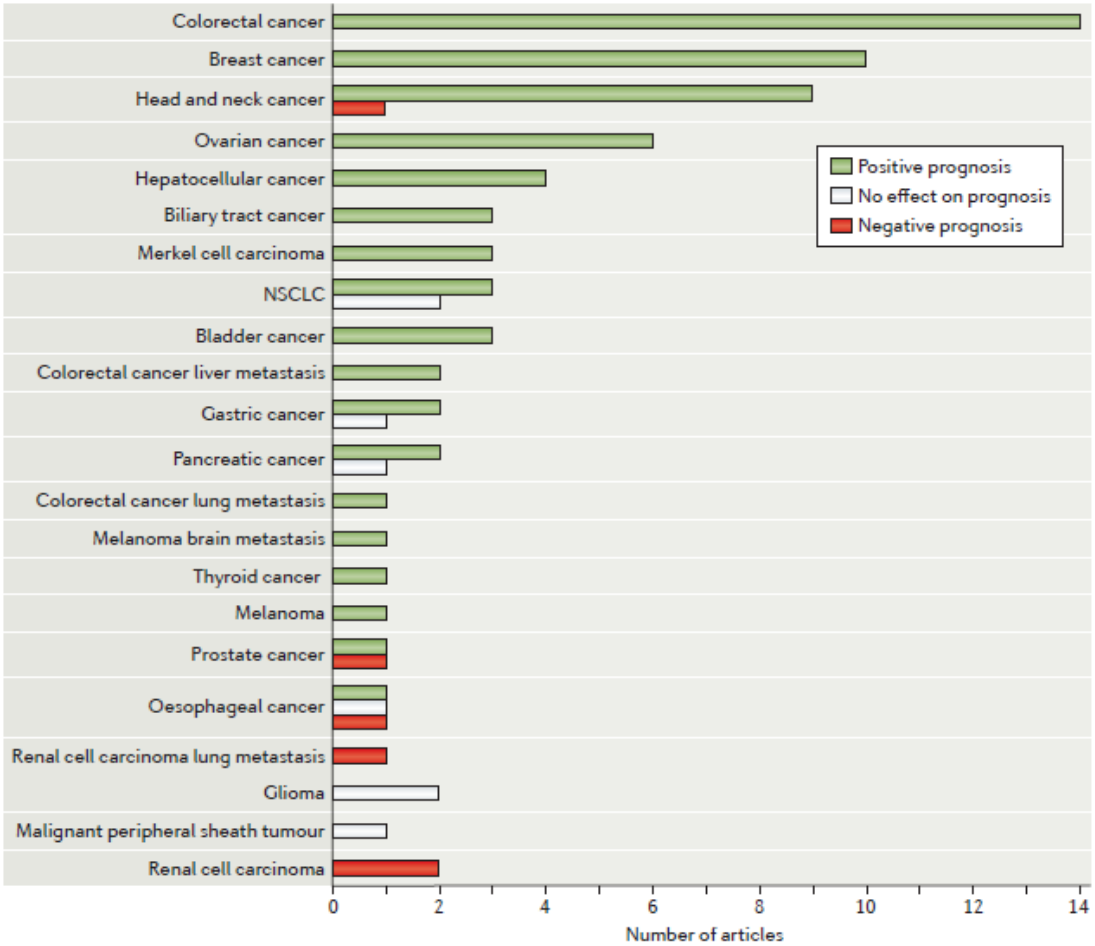

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The immune contexture in cancer prognosis and treatment

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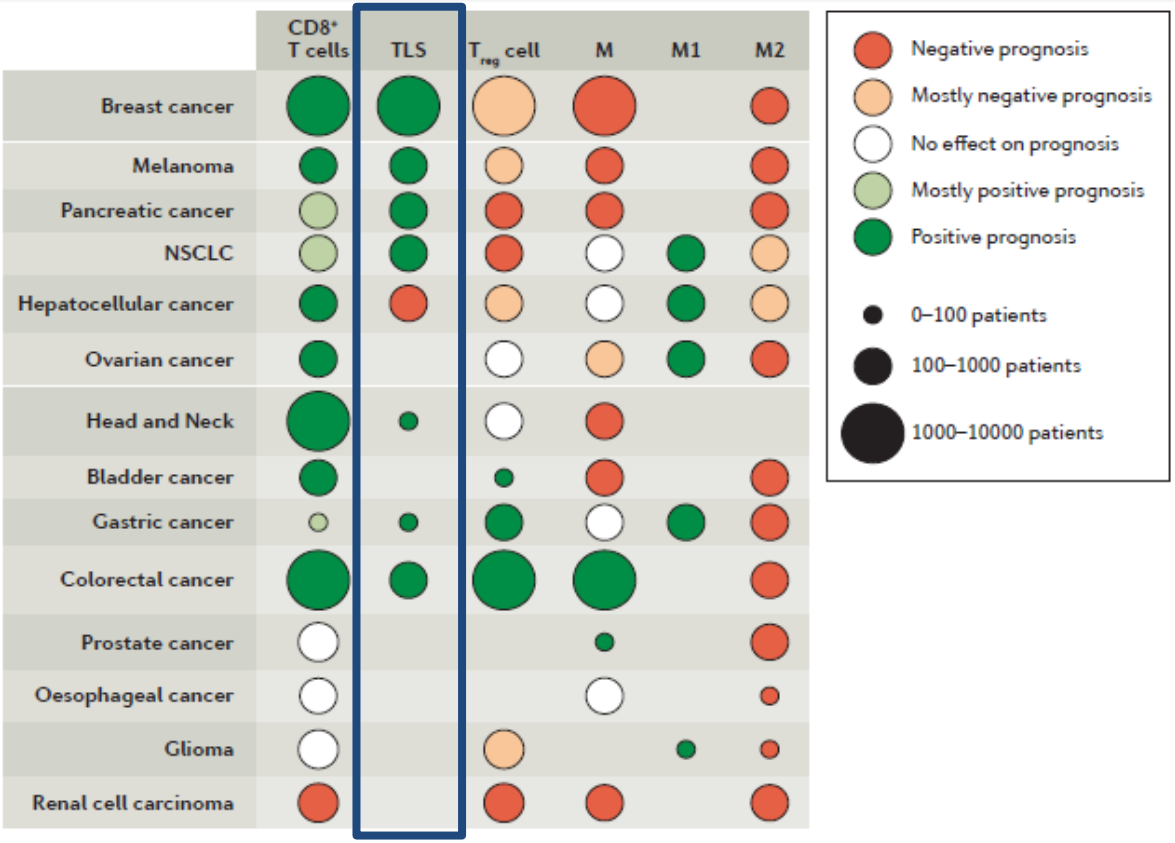

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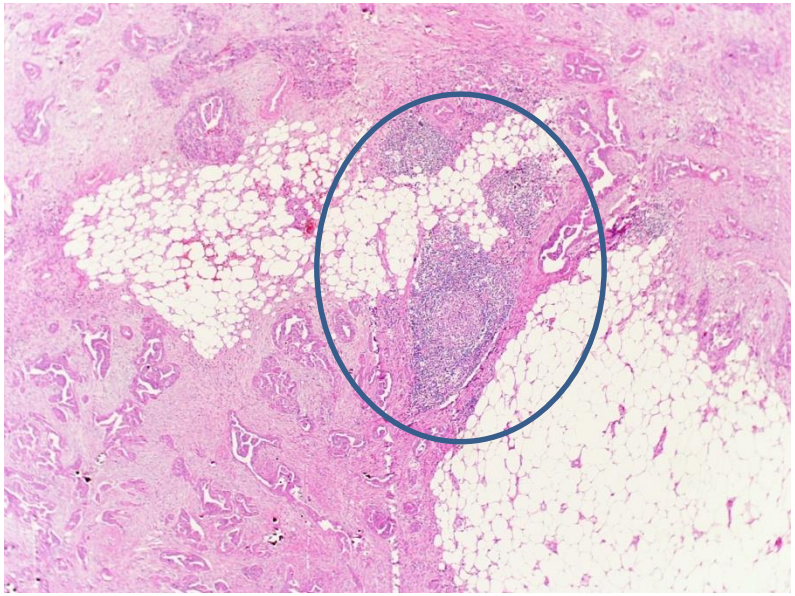
OncoImmunology 2:12, e26836; December 2013; © 2013 Landes Bioscience

Characteristics of tertiary lymphoid structures in primary cancers

Jérémy Goc[1,2,3], Wolf-Herman Fridman[1,2,3], Catherine Sautès-Fridman[1,2,3], and Marie-Caroline Dieu-Nosjean[1,2,3,*]

[1]The Laboratory of Immune Microenvironment and Tumors; INSERM U872; Cordeliers Research Center; Paris, France;
[2]University Pierre and Marie Curie; UMRS872; Paris, France; [3]University 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 agregates/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 naive 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
	T cells, B cells, mature DCs, HEVs	146 patients	grade I to III	Martinet et al., 2013
Colorectal carcinoma	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	Bergomas et al., 2011
	T cells, B cells (including B cell precursors), FDCs	21 patients	grade 0 to IVA	Coppola et al., 2011
	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 liver metastasis	mature DCs	70 patients	ND	Miyagawa et al., 2004
Colorectal carcinoma lung metastasis	T cells, B cells, mature DCs	140 patients	stage IV	Remark et al., 2013
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
	T cells, B cells, HEVs	5 patients	ND	Martinet et al., 2011
Melanoma	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
	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
Mucosal-Associated Lymphoid Tissue lymphoma	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.

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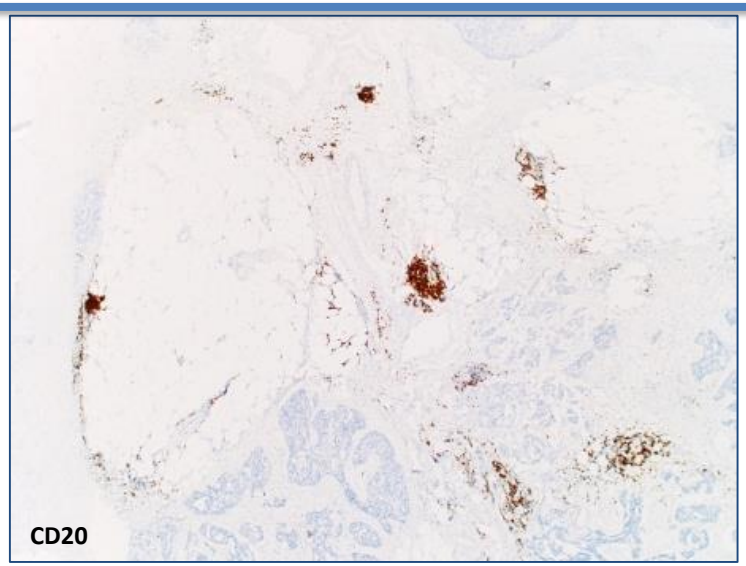
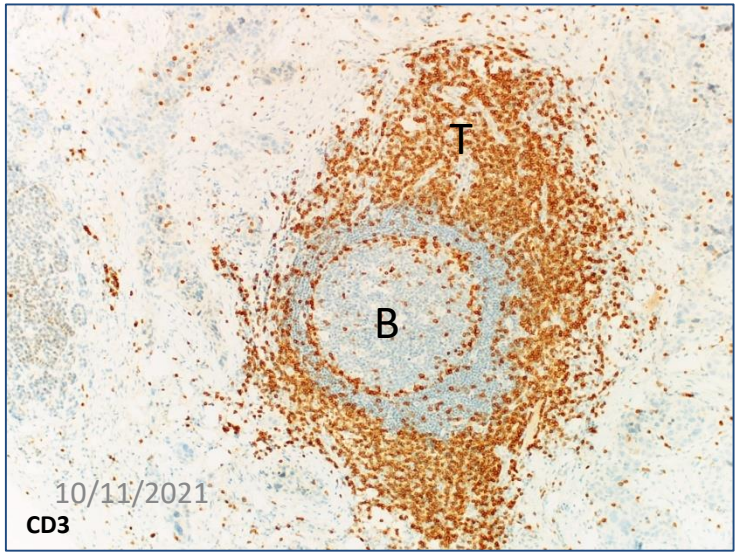




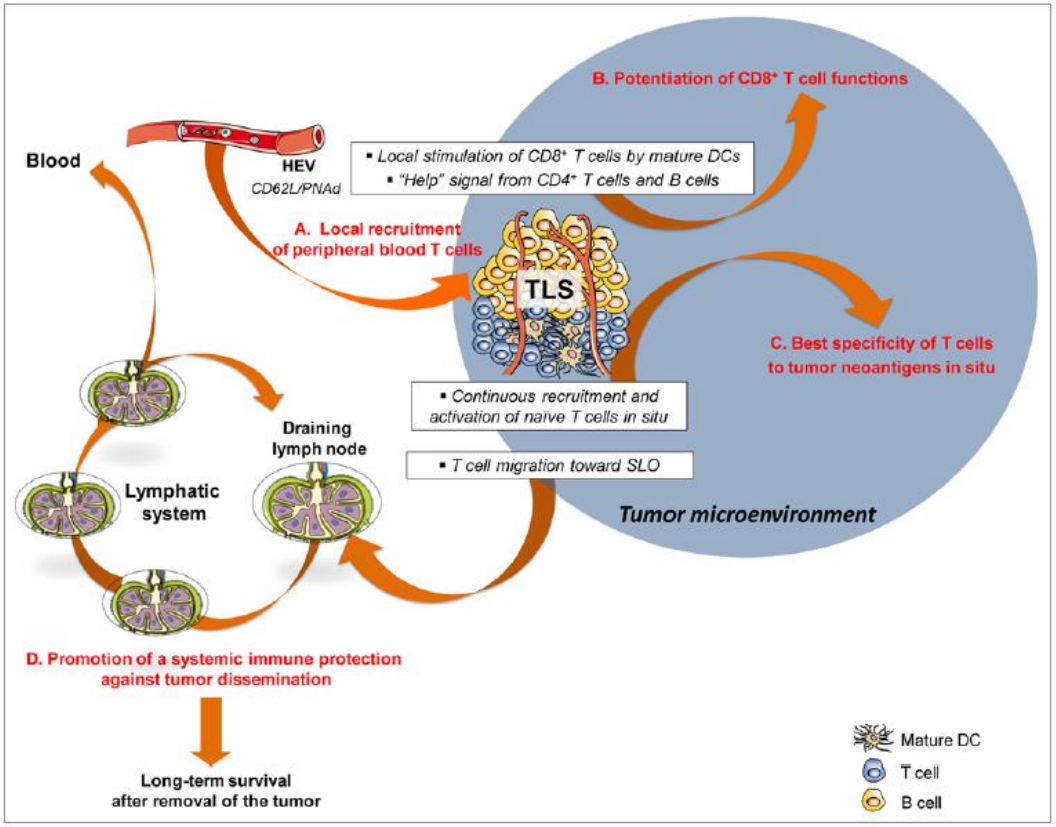
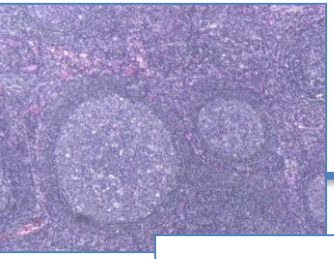
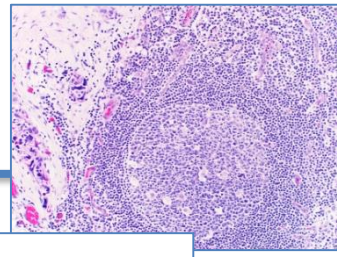
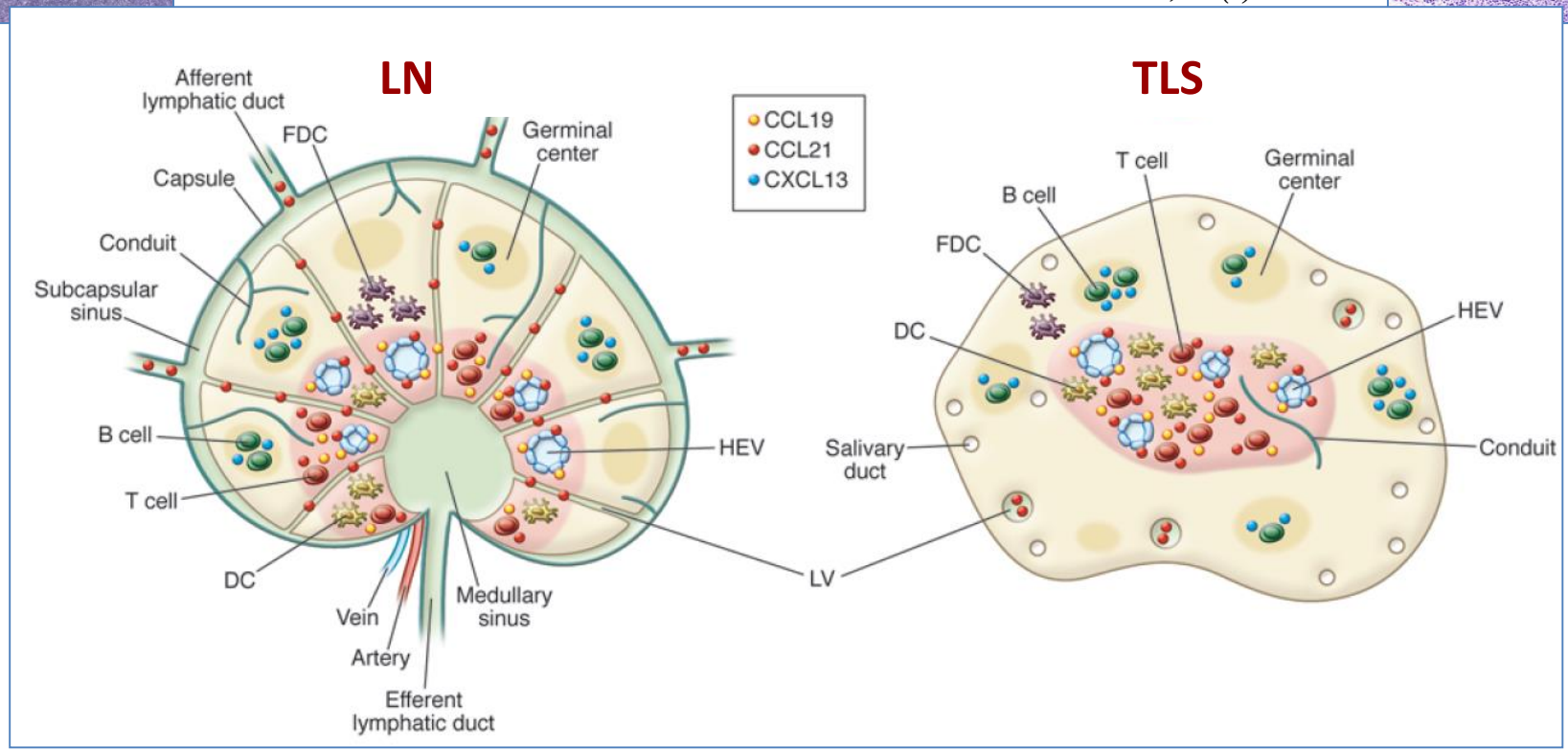


Figure 1. Role of tertiary lymphoid structures in the initiation of local and systemic protective immune responses against primary neoplastic lesions and metastases. DC, dendritic cell; HEV, high endothelial venule; SLO, secondary lymphoid organ; TLS, tertiary lymphoid structure.



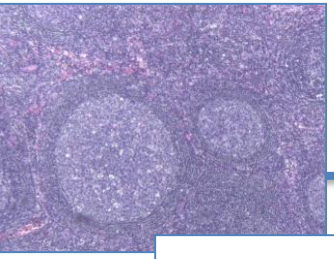


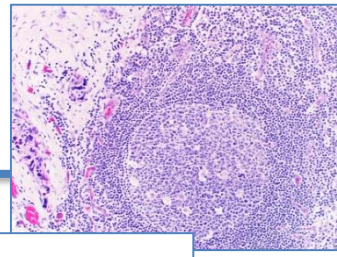
Ruddle NH. J Clin Invest. 2014; 124(3):953-959



Similarities

- Cellular content and organization
- Stromal components
- Lymphoid chemokines
- Vasculature (HEVs, LVs)





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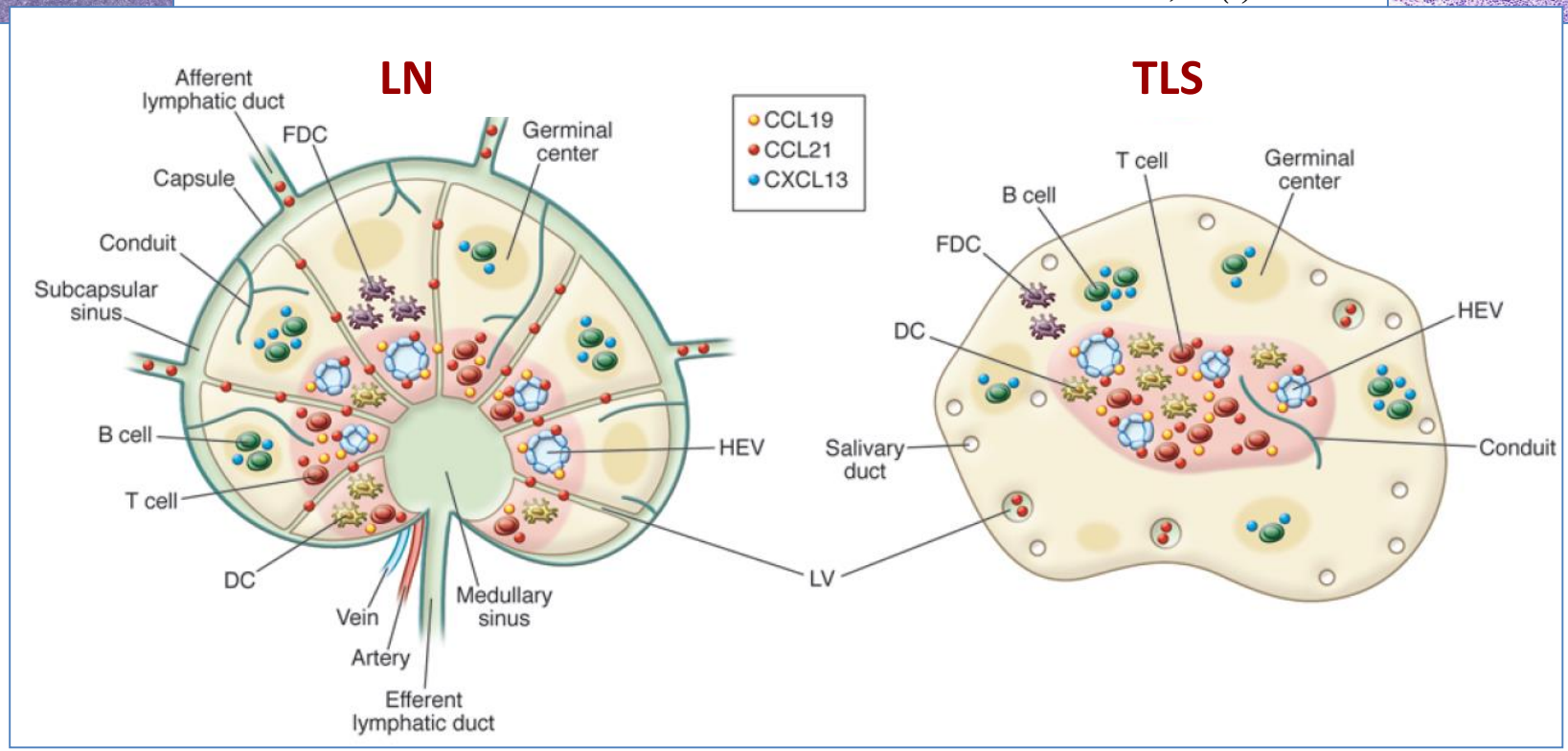


Differences

- Ontogeny, predetermined locations

- Ectopic to canonical lymphoid organs.
- Arise in response to inflammation
- No capsule (free diffusion of antigen?)

Tertiary Lymphoid Structures in Cancers: Prognostic Value, Regulation, and Manipulation for Therapeutic Intervention

*Catherine Sautès-Fridman[1,2,3], Myriam Lawand[1,2,3], Nicolas A. Giraldo[1,2,3], Hélène Kaplon[1,2,3], Claire Germain[1,2,3], Wolf Herman Fridman[1,2,3] and Marie-Caroline Dieu-Nosjean[1,2,3]**

Frontiers in Immunology October 2016 | Volume 7

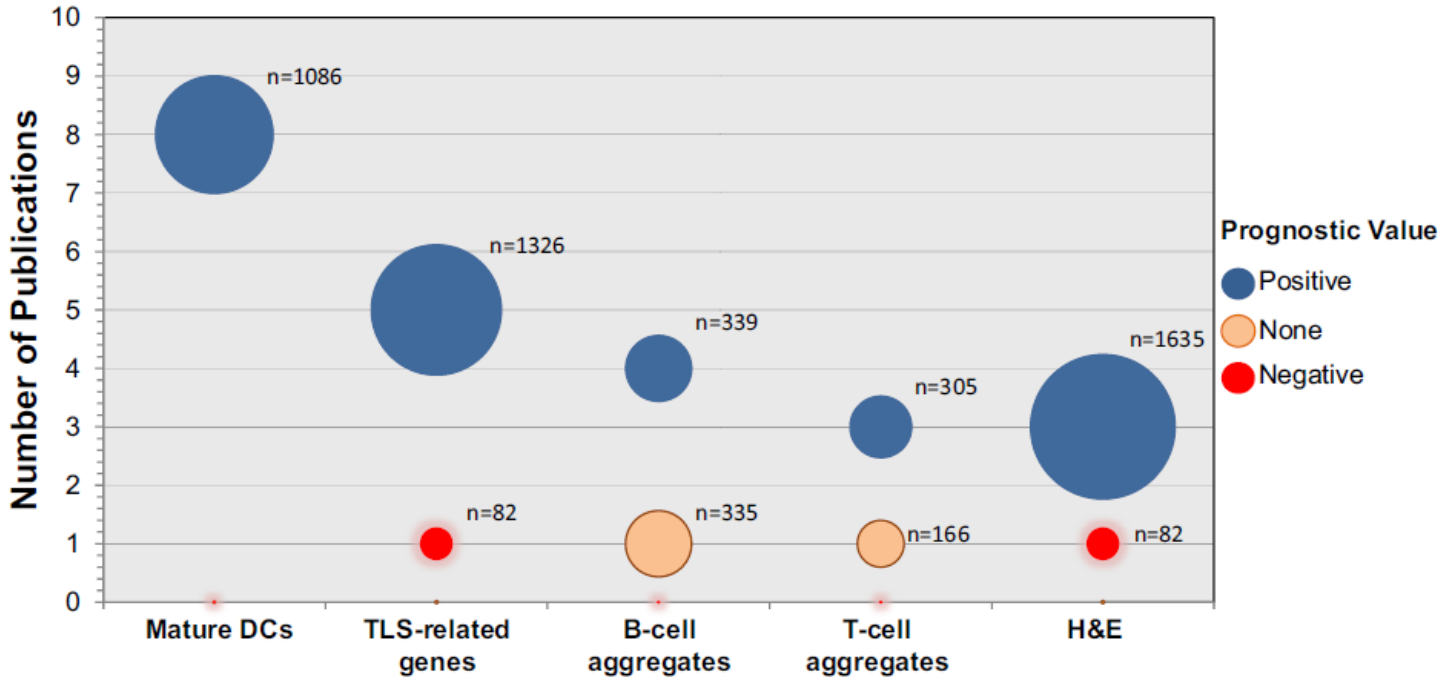


FIGURE 1 | Prognostic value of TLS-associated biomarkers in primary and metastatic cancers. The number of publications studying the impact of mature DCs, TLS-related gene signatures, B-cell aggregates, T cell aggregates, or H&E with regard to prognosis in human cancers is represented (12 cancer types have been included). Blue, orange, and red circles represent an association with good, none, and poor prognosis, respectively. The diameter of the circles represents the total number of tumors (*n*) that have been analyzed on these studies.

Cancer Research

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

Karīna Siliņa[1], Alex Soltermann[2], Farkhondeh Movahedian Attar[1], Ruben Casanova[2], Zina M. Uckeley[1], Helen Thut[1], Muriel Wandres[1], Sergejs Isajevs[3,4], Phil Cheng[5], Alessandra Curioni-Fontecedro[6], Periklis Foukas[7,8], Mitchell P. Levesque[5], Holger Moch[2], Aija Linē[9], and Maries van den Broek[1]

Cancer Res; 78(5); 1308–20.

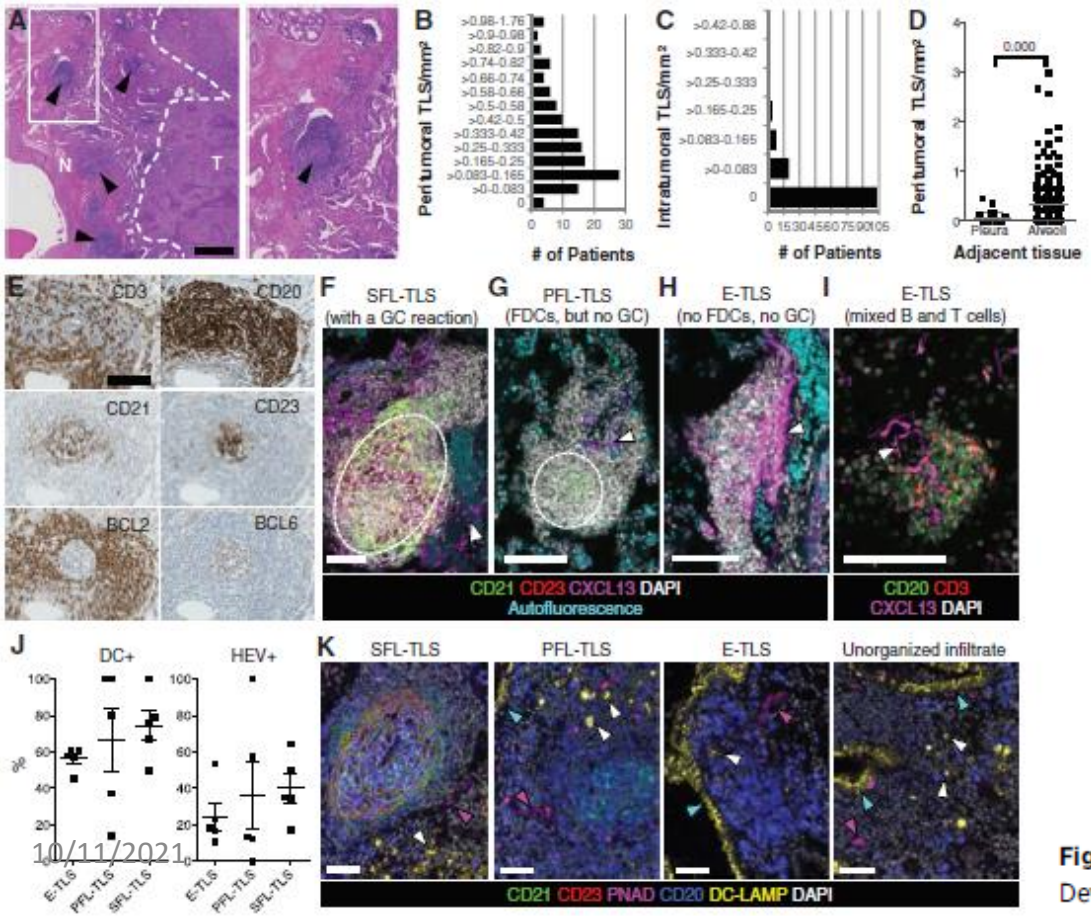
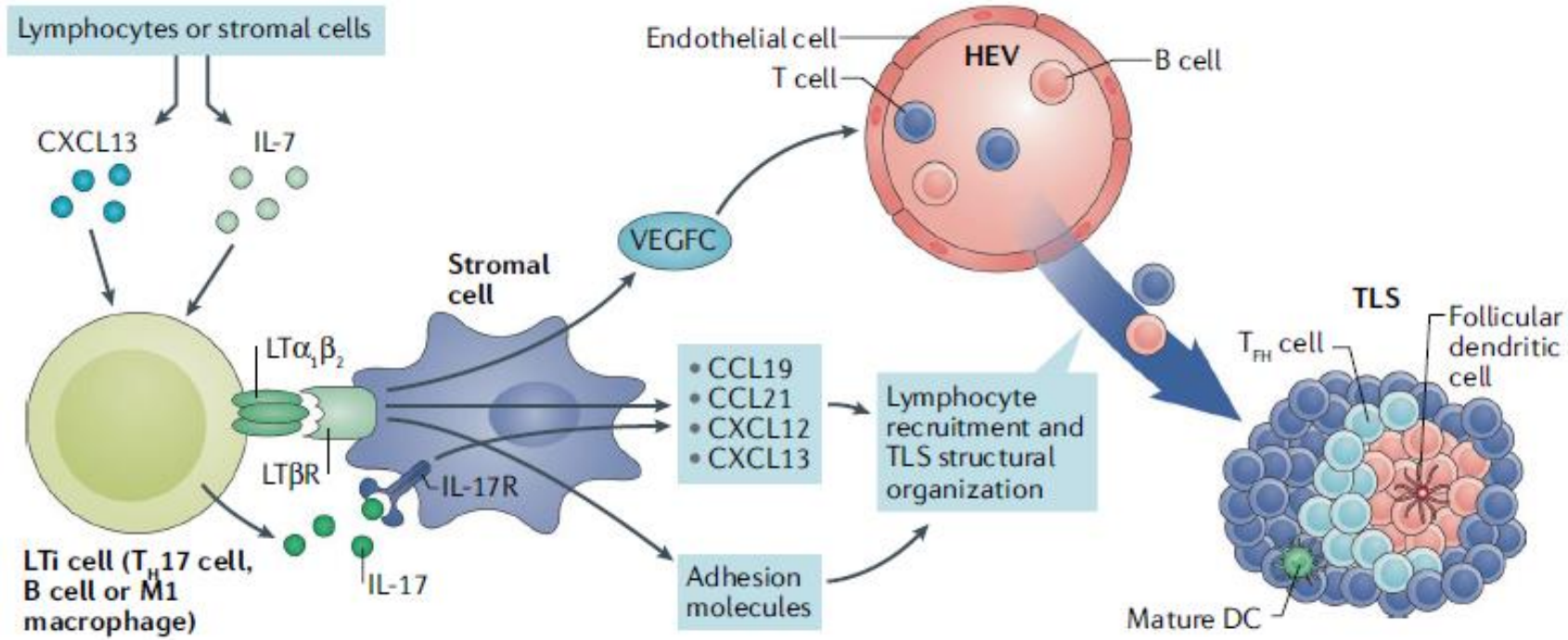


Figure 1.
Development of LSCC-associated TLS.

Tertiary lymphoid structures in the era of cancer immunotherapy

Catherine Sautès-Fridman[1], Florent Petitprez[1,2], Julien Calderaro[1,3,4] and Wolf Herman Fridman[1]*

Genesis of tertiary lymphoid structures



Nature Reviews Cancer **19**, 307–325(2019)

The immune contexture in cancer prognosis and treatment

Wolf H. Fridman[1–3], Laurence Zitvogel[4], Catherine Sautès–Fridman[1–3] and Guido Kroemer[2,3,5–8]

NATURE REVIEWS | CLINICAL ONCOLOGY

VOLUME 14 | DECEMBER 2017 | 717

Figure 3 | **Effects of the immune infiltrate on the prognosis of patients with cancer.** Data from 200 studies (cited in Supplementary information S1 (table) and in the main-text), involving approximately 25,000 patients were analysed regarding the relevance of CD8+ T cells, tertiary lymphoid structures (TLSs), regulatory T cells (T_{reg}), CD68+ macrophages (M) and, more specifically, macrophages of an M1 or M2 subtype to overall survival outcomes. Bold colours indicate a positive (green) or a negative (red) prognostic association following analysis of all relevant studies; lighter colours indicate a predominantly positive (light green) or negative (orange) prognostic association in the majority of studies analysed. White circles indicate no statistically significant correlation, or that a dubious prognostic association was observed in a similar number of studies. The size of the circles indicates the number of patients enrolled in the studies: small circles indicate 0–100 patients, medium-sized circles indicate 100–1,000 patients and large circles indicate 1,000–10,000 patients.

The immune contexture in cancer prognosis and treatment

Wolf H. Fridman[1–3], Laurence Zitvogel[4], Catherine Sautès–Fridman[1–3] and Guido Kroemer[2,3,5–8]

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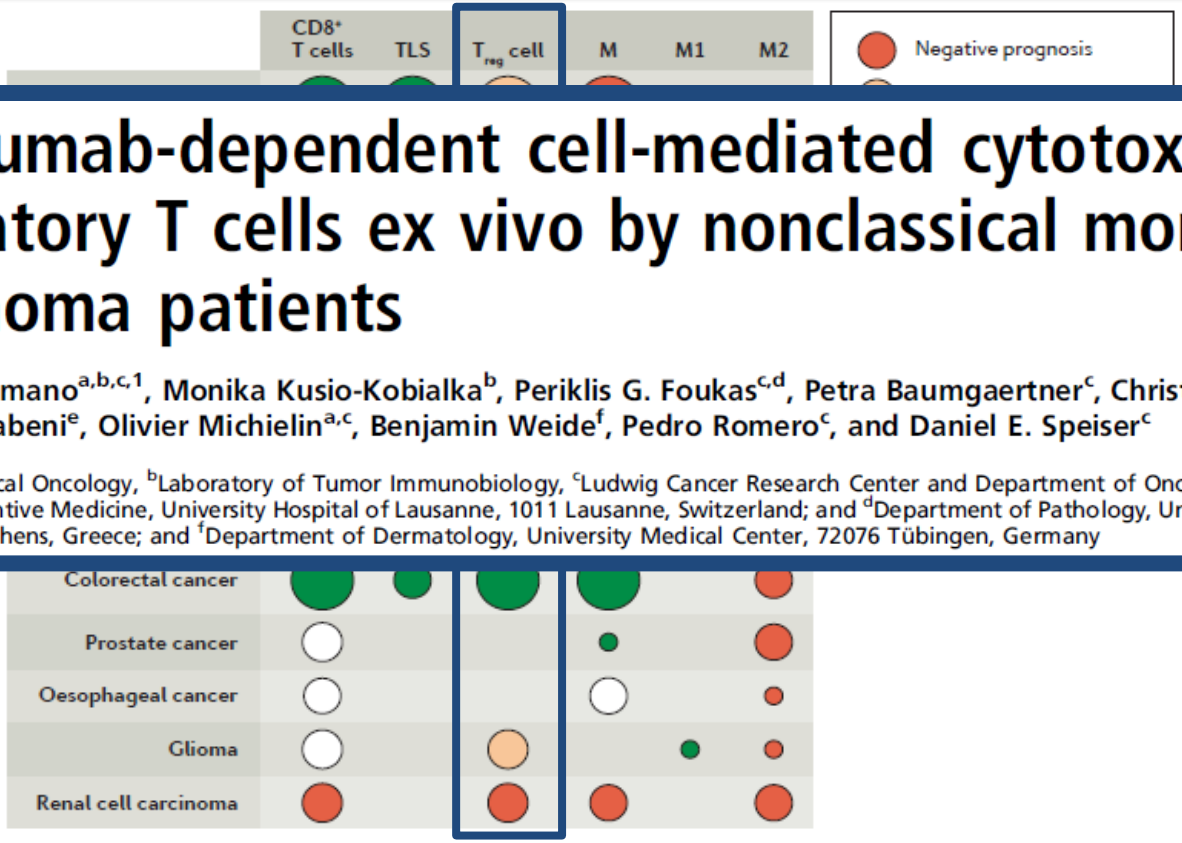

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PNAS

Ipilimumab-dependent cell-mediated cytotoxicity of regulatory T cells ex vivo by nonclassical monocytes in melanoma patients

Emanuela Romano[a,b,c,1], Monika Kusio-Kobialka[b], Periklis G. Foukas[c,d], Petra Baumgaertner[c], Christiane Meyer[c], Pierluigi Ballabeni[e], Olivier Michielin[a,c], Benjamin Weide[f], Pedro Romero[c], and Daniel E. Speiser[c]

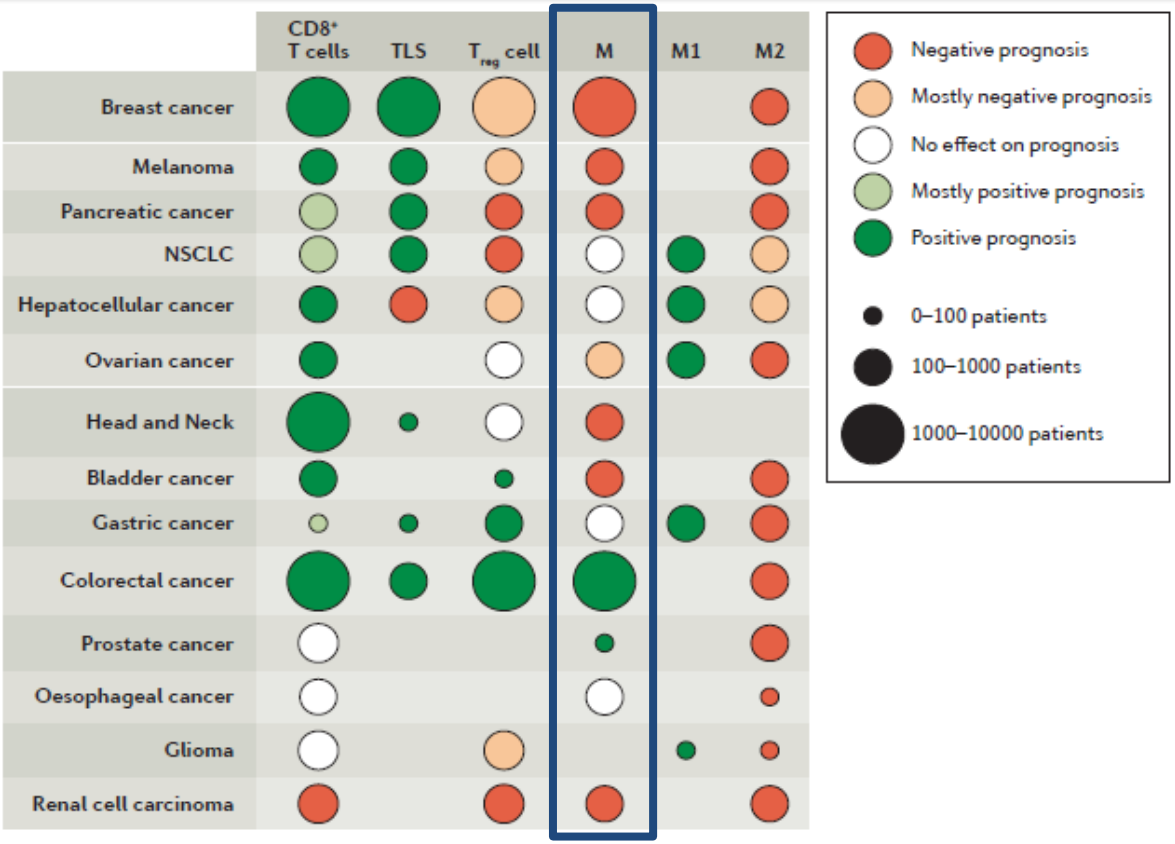
[a]Service of Medical Oncology, [b]Laboratory of Tumor Immunobiology, [c]Ludwig Cancer Research Center and Department of Oncology, and [e]Institute of Social and Preventive Medicine, University Hospital of Lausanne, 1011 Lausanne, Switzerland; and [d]Department of Pathology, University of Athens Medical School, 11527 Athens, Greece; and [f]Department of Dermatology, University Medical Center, 72076 Tübingen, Germany

The immune contexture in cancer prognosis and treatment

NATURE REVIEWS | CLINICAL ONCOLOGY

Wolf H. Fridman[1–3], Laurence Zitvogel[4], Catherine Sautès–Fridman[1–3] and Guido Kroemer[2,3,5–8]

VOLUME 14 | DECEMBER 2017 | 717



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The immune contexture in cancer prognosis and treatment

Wolf H. Fridman[1–3], Laurence Zitvogel[4], Catherine Sautès–Fridman[1–3] and Guido Kroemer[2,3,5–8]

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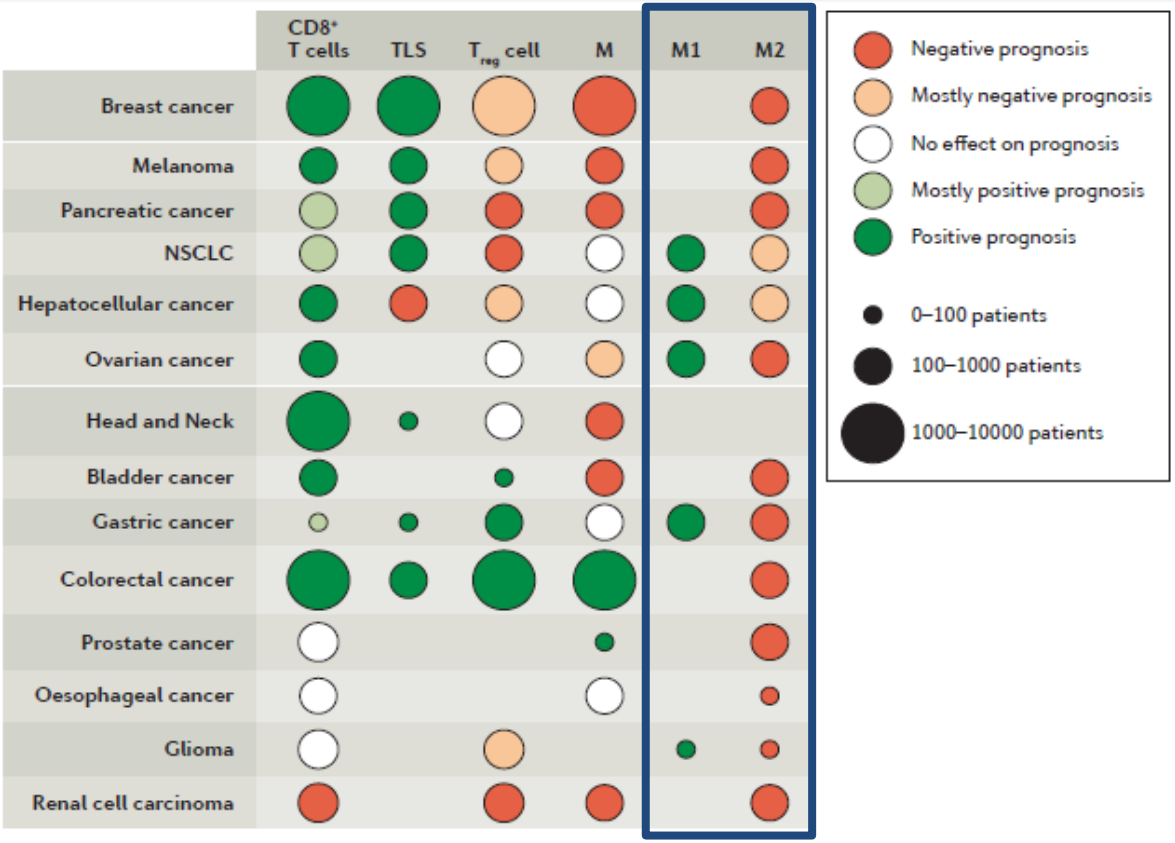

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Tumor-Associated Macrophages: From Mechanisms to Therapy

Roy Noy[1] and Jeffrey W. Pollard[1,2,*]

Immunity 41, July 17, 2014

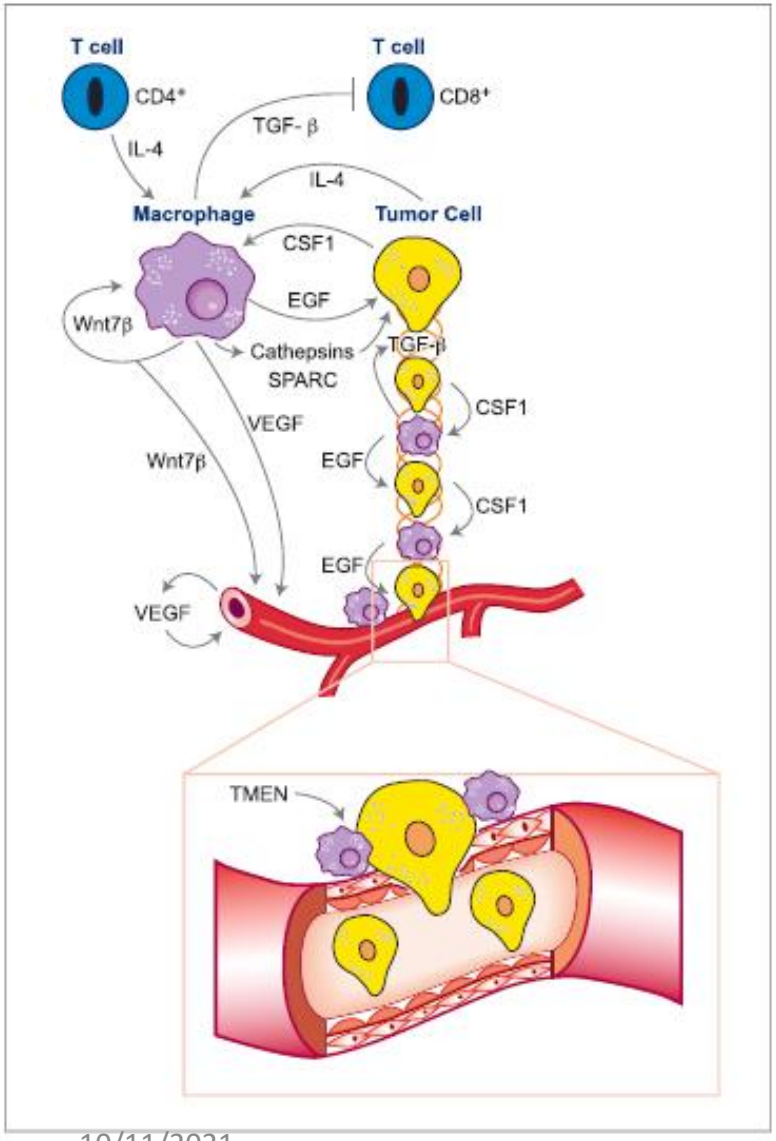


Figure 1. Tumor-Associated Macrophages in the Primary Tumor Promote Malignancy

In the primary tumor, microenvironment macrophages under the influence of IL-4 produced by $CD4^+$ T cells and tumors and WNT7b promote tumor cell invasion. This invasion is mediated via a paracrine loop involving tumor-synthesized CSF1 and macrophage-produced EGF that drives migration of tumor cells in lock-step with macrophages along collagen fibers that act as highways toward blood vessels. This process also requires TGFβ that drives an epithelial-mesenchymal transition (EMT) in the tumor cells that promotes migration and matrix remodeling via Cathepsins and matrix adhesion of tumor cells via SPARC. This streaming of tumor cells results in their pileup on the vessels where macrophages promote their intravasation into the circulation through a structure named the "Tumor Microenvironment of Metastasis" (TMEN). In addition to effect on tumor cell migration and invasion, $TIE2^+$ macrophages produce VEGF and WNT7b that stimulates angiogenesis in the tumor. Thus, there is an additive effect caused by macrophages of increased migration of tumor cells toward vessels and increased vascular targets that results in a large number of circulating tumor cells and thus increased malignancy. Macrophages also suppress cytotoxic T cell responses through the mechanisms described in Figure 2.

Tumor-Associated Macrophages: From Mechanisms to Therapy

Roy Noy[1] and Jeffrey W. Pollard[1,2,*]

Immunity 41, July 17, 2014

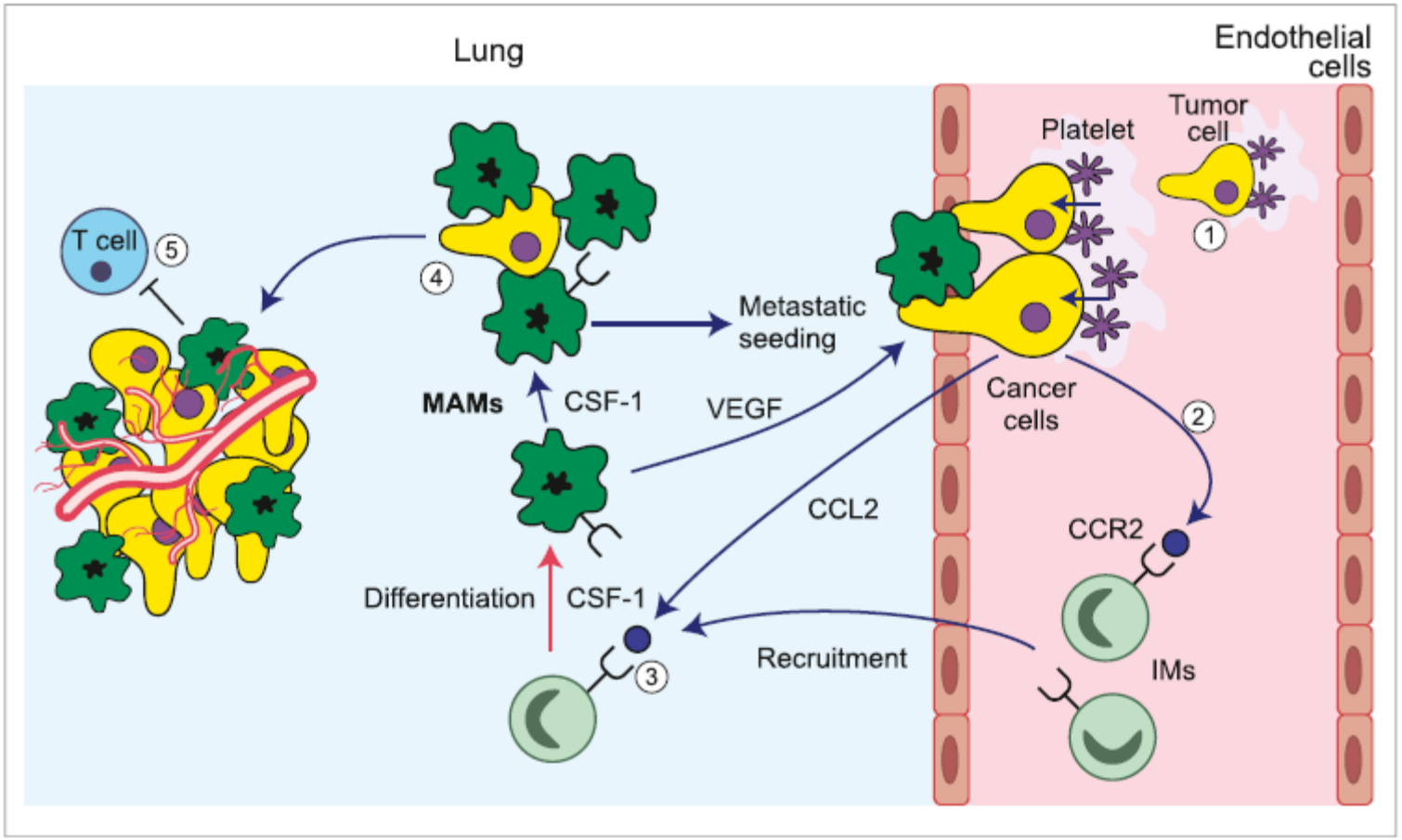


Figure 3. Macrophages Promote Metastasis

Arrest of tumor cells in the vasculature of target organs through the formation of microclots (1) results in CCL2-mediated recruitment of CCR2-expressing circulating inflammatory monocytes (2). These monocytes differentiate into metastasis-associated macrophages (MAMs) that mediate tumor cell extravasation via VEGF that increases vascular permeability (3). MAMS under the influence of CSF-1 further promote tumor cell survival (4) and persistent growth associated by angiogenesis and might also prevent T cell cytotoxicity (5).

Tumor-Associated Macrophages: From Mechanisms to Therapy

Roy Noy[1] and Jeffrey W. Pollard[1,2,*]

Immunity 41, July 17, 2014

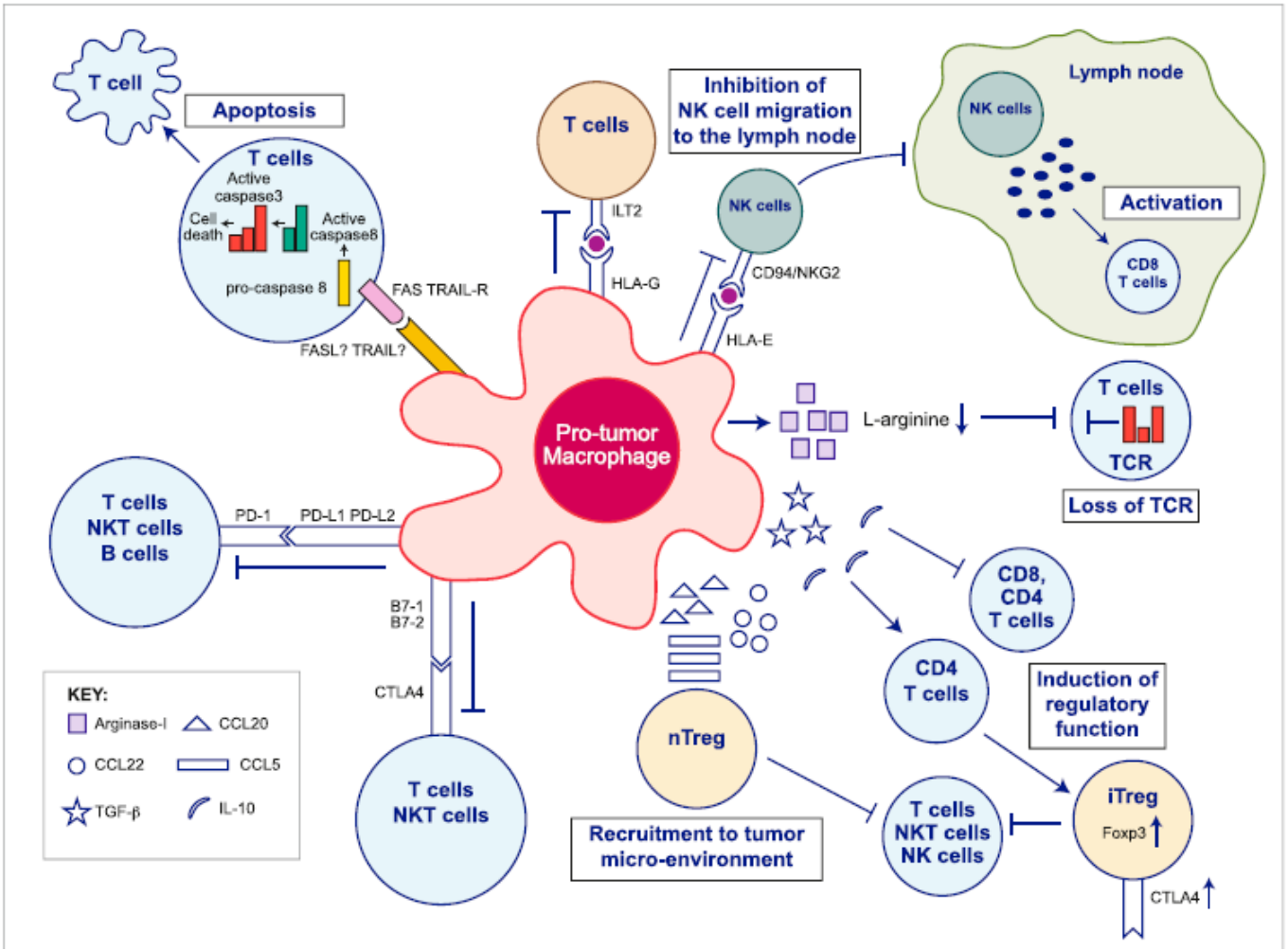


Figure 2. Protumor Macrophage Mechanisms of Effector Cells Inhibition

TAMs express an array of effector molecules that inhibit the antitumor immune responses; this includes cell surface receptors, cytokines, chemokines, and enzymes. Inhibition of immune responses by direct cell-to cell-contact is based on the interaction of TAMs receptors ligands with their counterpart death and/or inhibitory receptors expressed by the target immune effector cells. TAMs express the ligand receptors for PD-1 and CTLA-4 that upon activation suppress cytotoxic functions of T cell, NKT cells and NK cells. TAMs also express the ligand for the death receptors FAS and TRAIL that triggers caspase-dependent cell death (apoptosis) in target cells. TAMs also express the nonclassical HLA-G that inhibits T cell function through interaction with the costimulatory signal of T cells ILT2 and HLA-E that inhibit NK cells through CD94 (also known as NKG2). TAMs secrete the cytokines IL-10 and TGF-β that inhibit T cells effector functions and induce regulatory functions and chemokines CCL5, CCL20, and CCL22 that recruit nTreg cells. TAMs secrete Arginase I that inhibit TCR ζ chain re-expression in activated T cells by the depletion of L-arginine.

Outline

- Intoduction to the Tumor Immune Microenvironment (TIME)
  - Prognostic **/ Predictive value**
  - Methodologies
- Mechanisms regulating TIME
- TIME and neoplastic evolution

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

PFS

OS

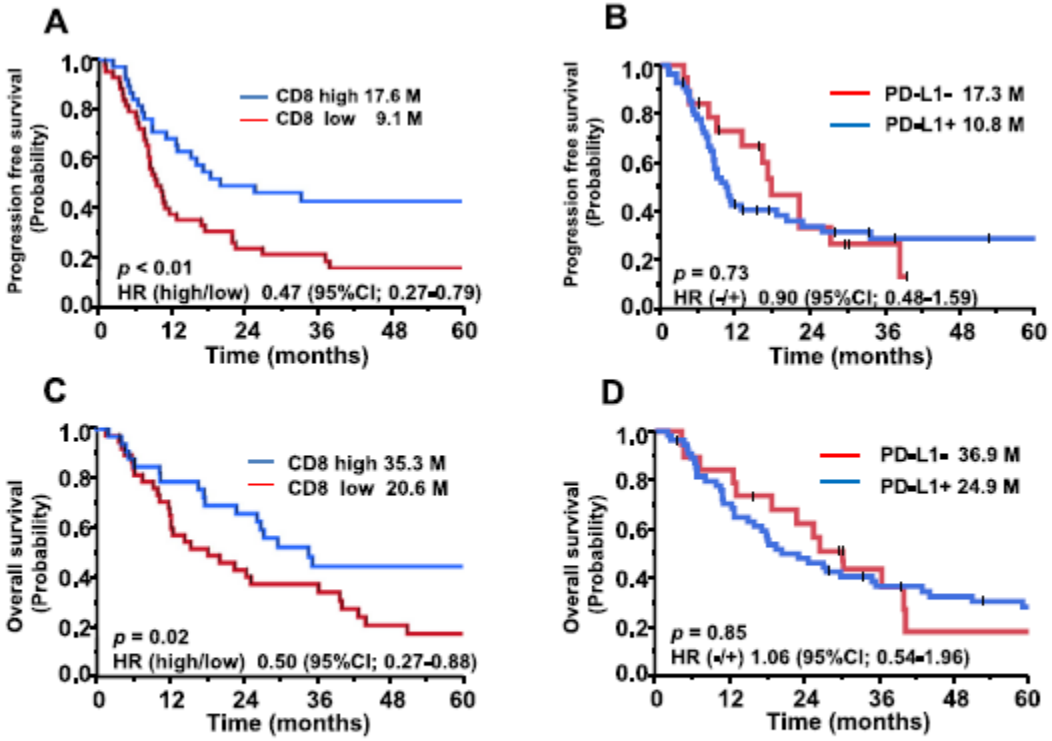


Fig. 2. Kaplan-Meier curves for progression-free survival (PFS) of stage III NSCLC patients with positive or negative for CD8+ TIL density (A) and PD-L1 expression (B). Kaplan-Meier curves for overall survival (OS) of stage III NSCLC patients with positive or negative for CD8+ TIL density (C) and PD-L1 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. Harview[1], Jennifer H. Yearley[3], I. Peter Shintaku[1], Emma J. M. Taylor[1], Lidia Robert[1], Bartosz Chmielowski[1,2], Marko Spasic[1], Gina Henry[1], Voicu Ciobanu[1], Alisha N. West[1], Manuel Carmona[1], Christine Kivork[1], Elizabeth Seja[1], Grace Cherry[1], Antonio J. Gutierrez[1], Tristan R. Grogan[1], Christine Mateus[4], Gorana Tomasic[4], John A. Glaspy[1,2], Ryan O. Emerson[5], Harlan Robins[5,6], Robert H. Pierce[3], David A. Elashoff[1,2], Caroline Robert[4] & Antoni Ribas[1,2]

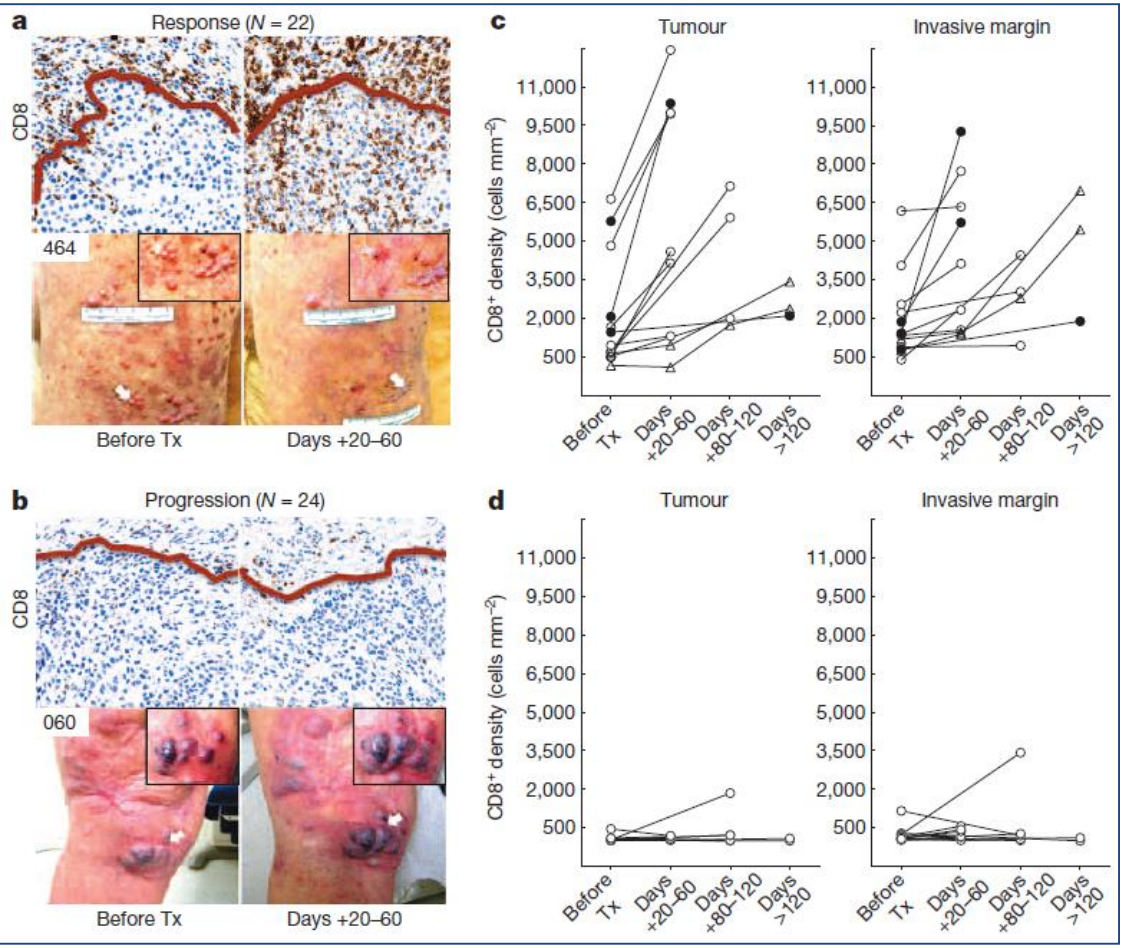


Figure 1 | Immunohistochemical analysis of CD8$^+$ T cells in samples obtained before and during pembrolizumab treatment. **a**, **b**, Examples of CD8 expression in melanoma tumours serially biopsied before PD-1 blocking treatment (Tx) and 20–60 days after treatment began (Days +20–60) from a patient in the response (**a**) and progression (**b**) groups. Red line separates tumour parenchyma (below line) and invasive margin (above line). Magnification, ×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.

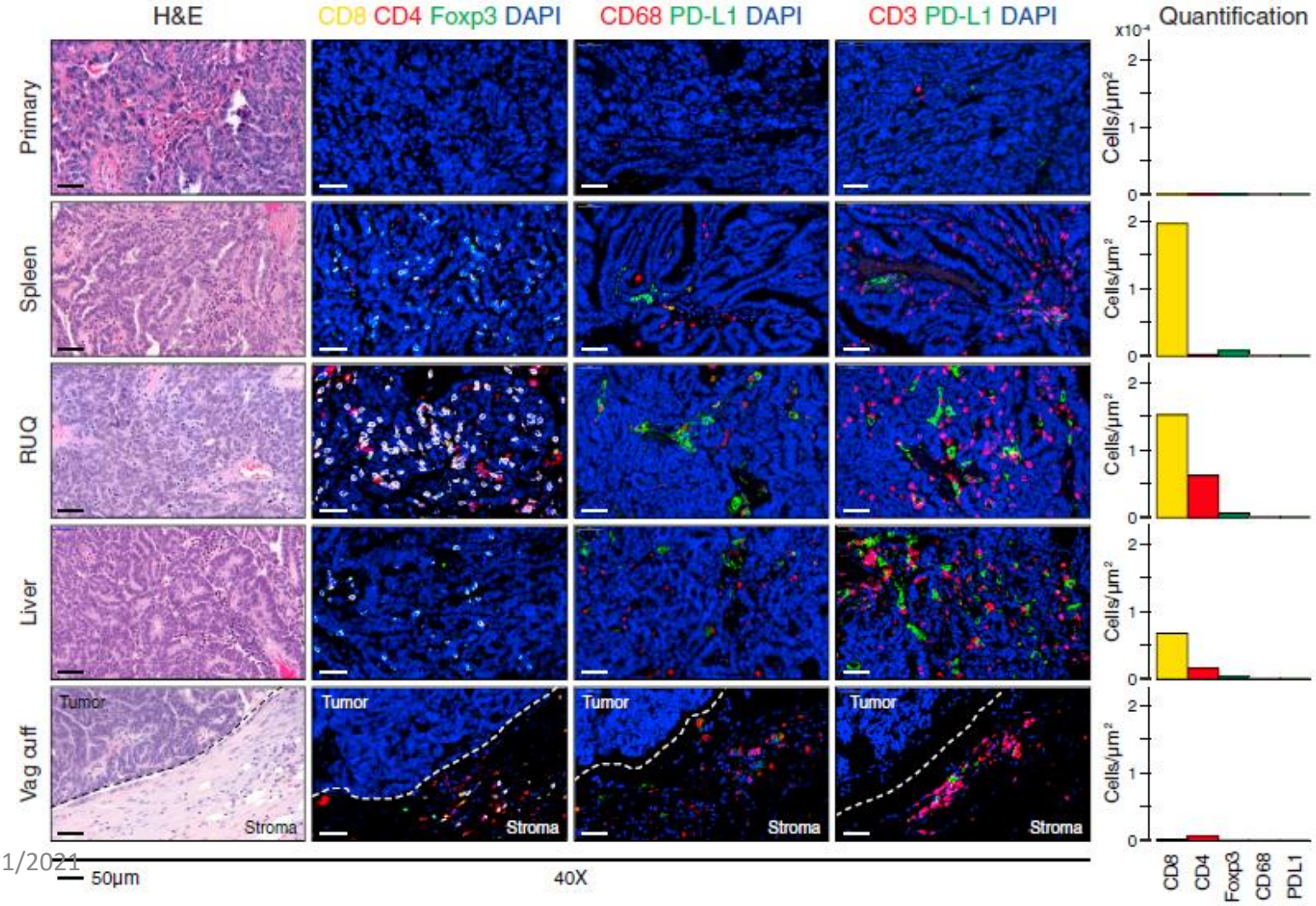
Outline

- Intoduction to the Tumor Immune Microenvironment (TIME)
  - Prognostic / Predictive value
  - **Methodologies**
- Mechanisms regulating TIME
- TIME and neoplastic evolution

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

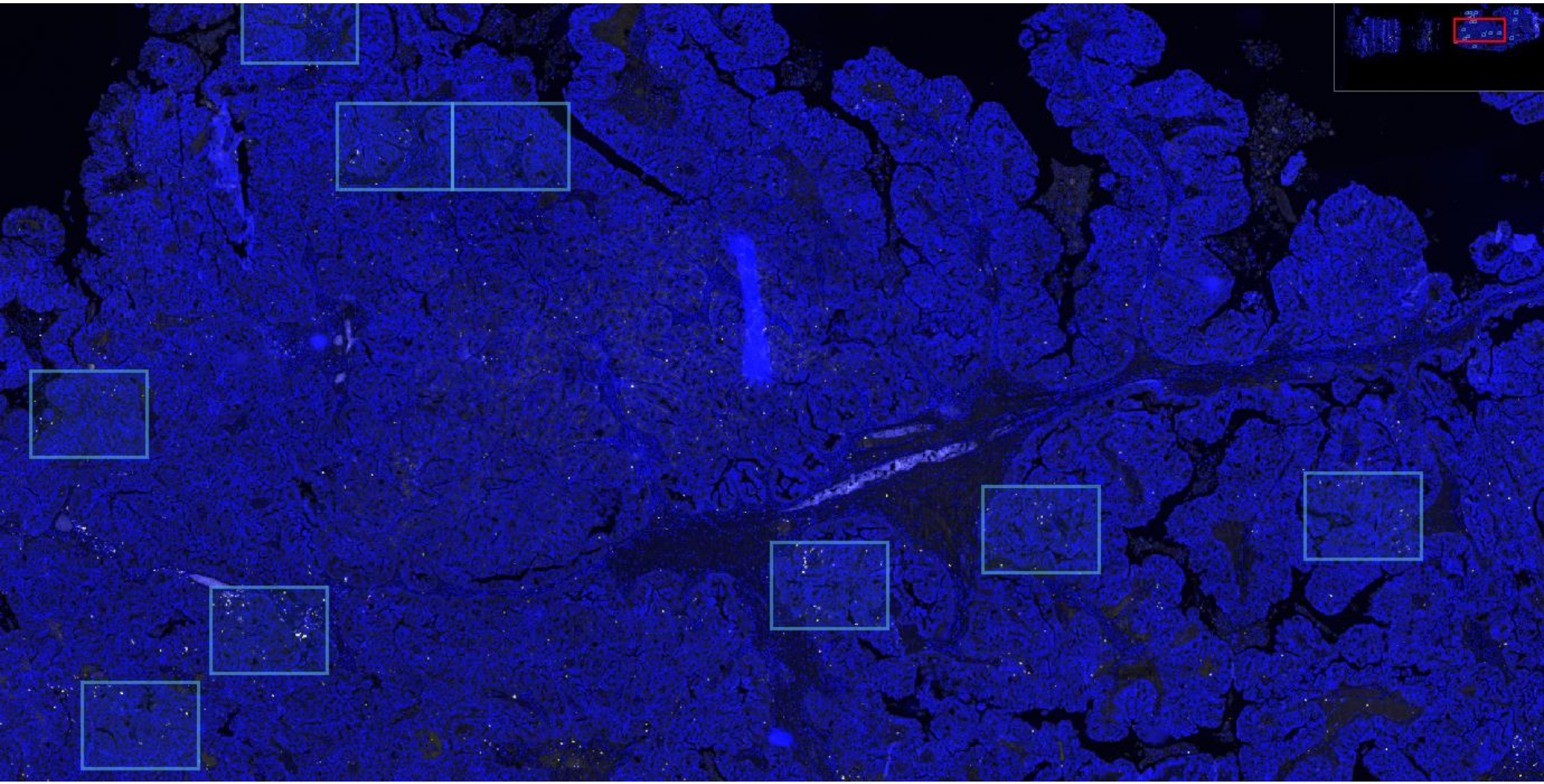
Alejandro Jiménez-Sánchez,[1] Danish Memon,[1,2] Stephane Pourpe,[10] Harini Veeraraghavan,[3] Yanyun Li,[4] Hebert Alberto Vargas,[5] Michael B. Gill,[1] Kay J. Park,[6] Oliver Zivanovic,[7] Jason Konner,[8] Jacob Ricca,[4] Dmitriy Zamarin,[4,10] Tyler Walther,[10] Carol Aghajanian,[8] Jedd D. Wolchok,[4,9,10,11,12] 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

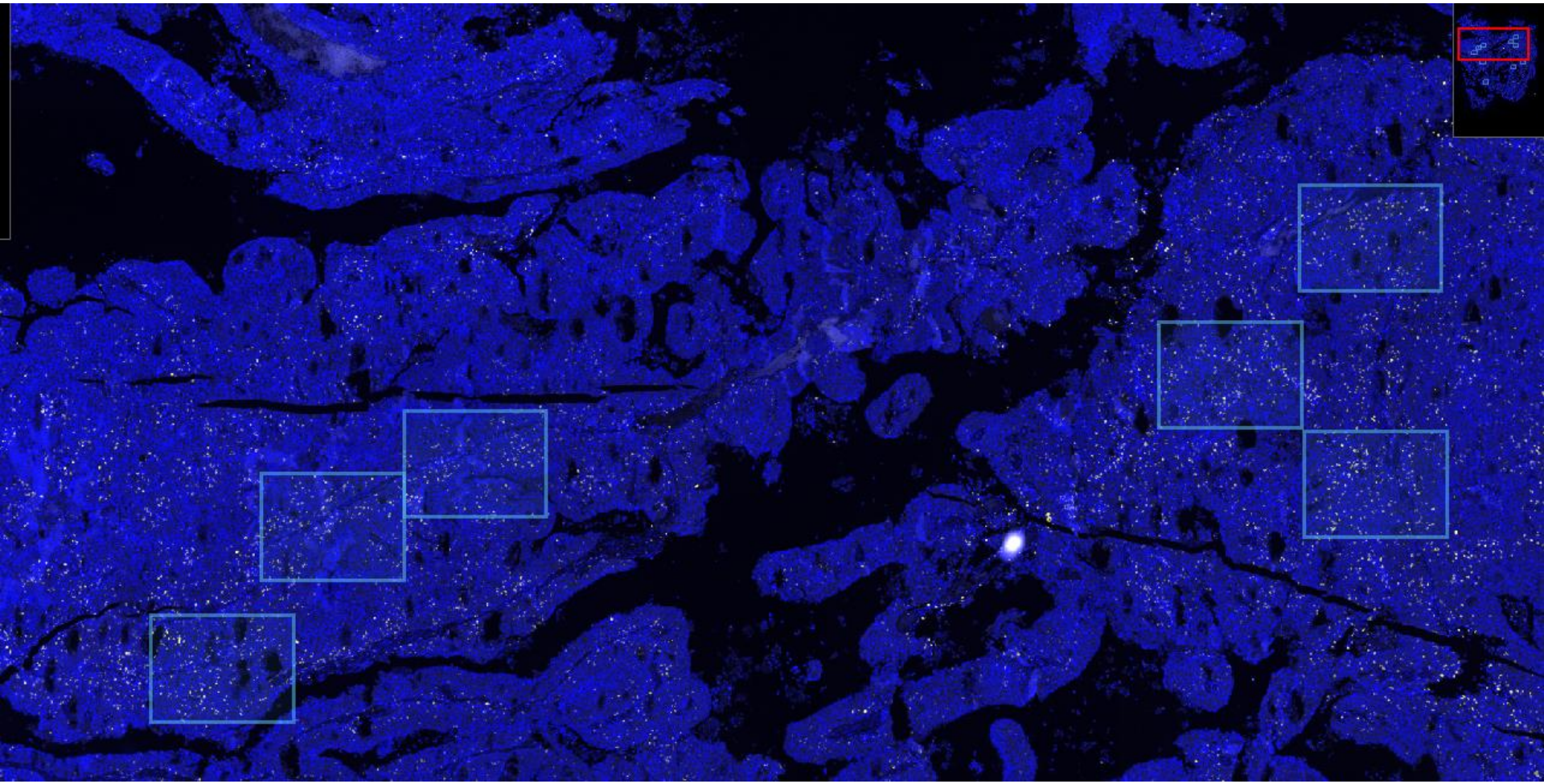
Low TILs



CD8 DAPI

Case 0100-Geico

High TILs



CD8 DAPI

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,*]

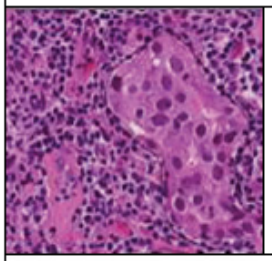
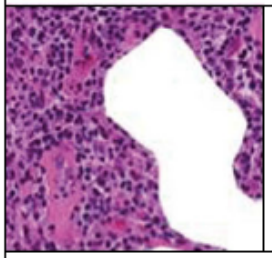
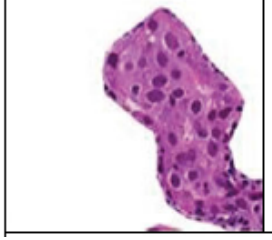
[a] Ovarian Cancer Research Center, University of Pennsylvania, Philadelphia, PA 19104, USA
[b] Department of Biostatistics and Epidemiology, University of Pennsylvania, Philadelphia, PA 19104, USA
[c] Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, PA 19104, 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.

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

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

Morphology	Definition and biological relevance	Diagnostic relevance
Lymphocyte-predominant breast cancer (LPBC)		
	Working category to describe tumors with "more lymphocytes than tymor 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 and more difficult to evaluate and do not provide additional predictive/prognostic information compared to stromal TILs.

Annals of Oncology 00: 1–13, 2014

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

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

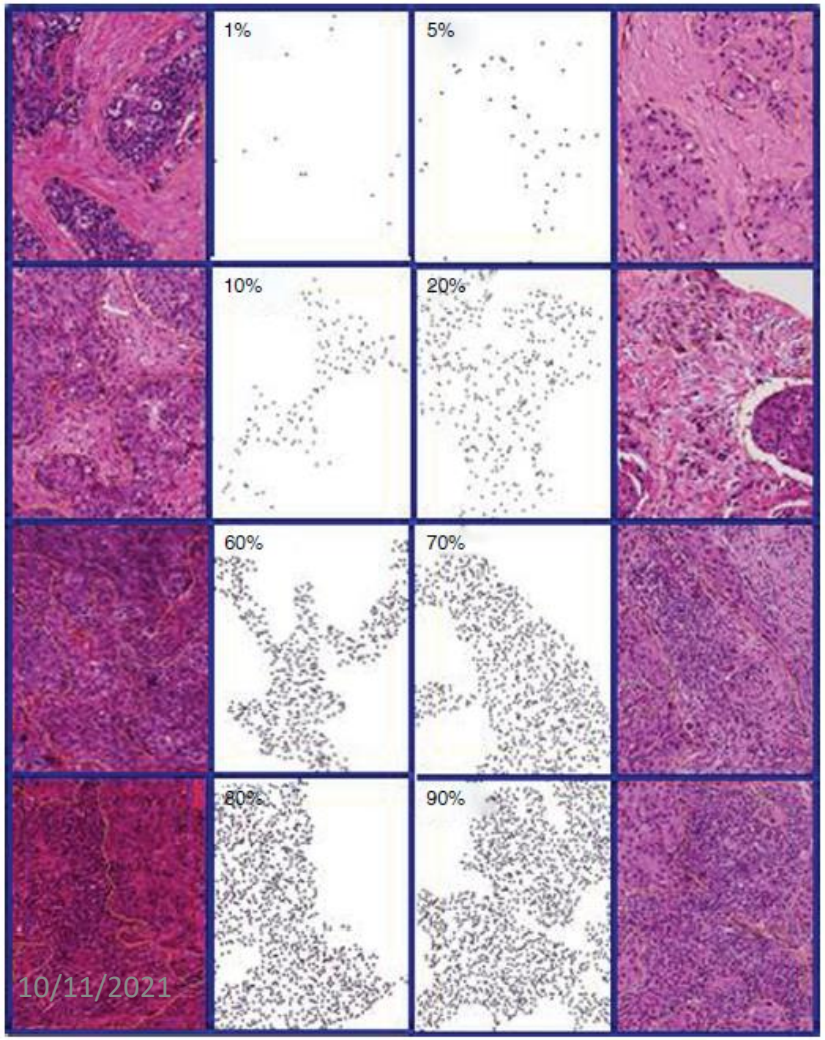
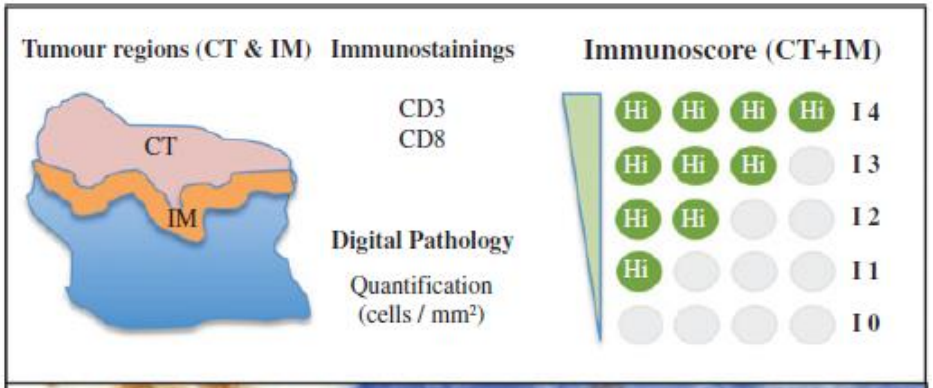
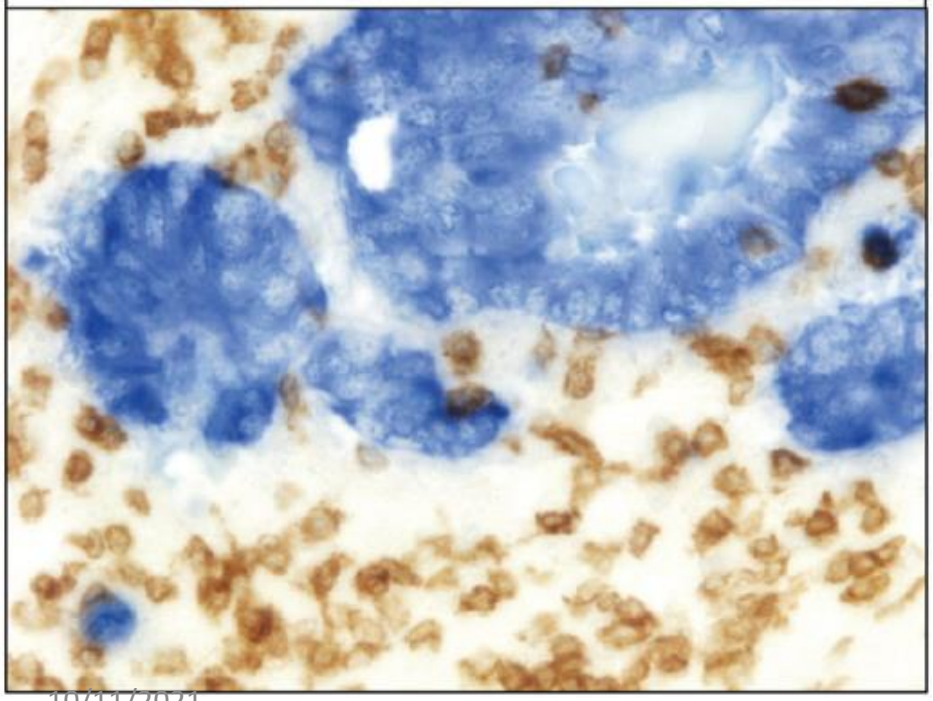


Figure 4. Standardization and guidelines for TILs assessment.

Annals of Oncology 00: 1–13, 2014

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



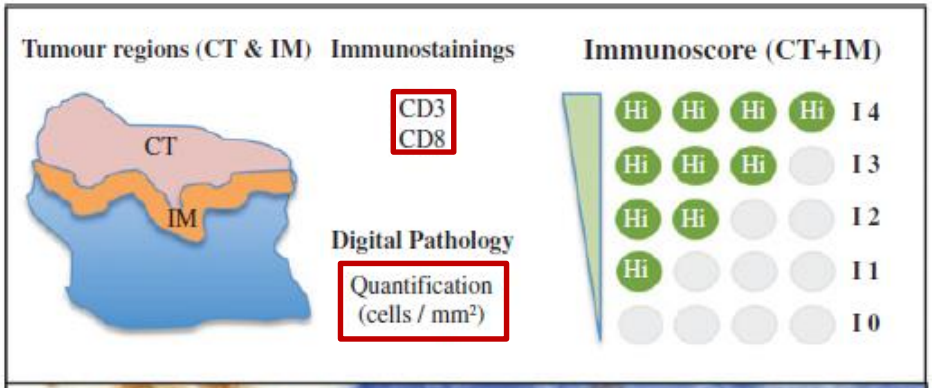


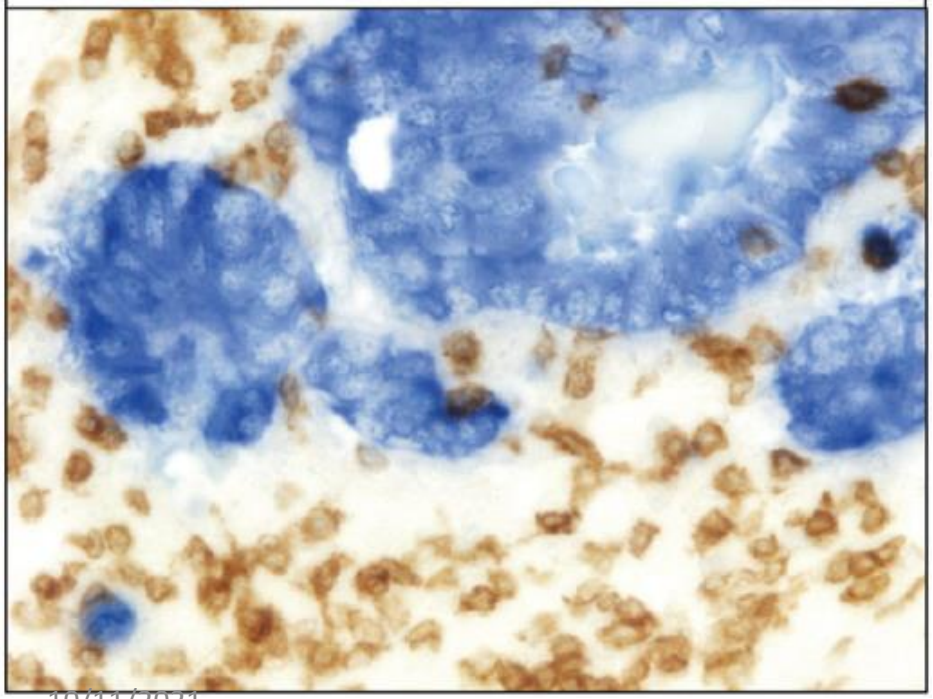
	Immune infiltration		Score
	CD3	CD8	(Hi = 1; Lo = 0)
CT:	Hi or Lo	+ Hi or Lo	= 0, 1, or 2
IM:	Hi or Lo	+ Hi or Lo	+ = 0, 1, or 2
			Immunoscore (I) = 0, 1, 2, 3, or 4

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

Galon J. et al J Pathol, 2014; 232: 199-209

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
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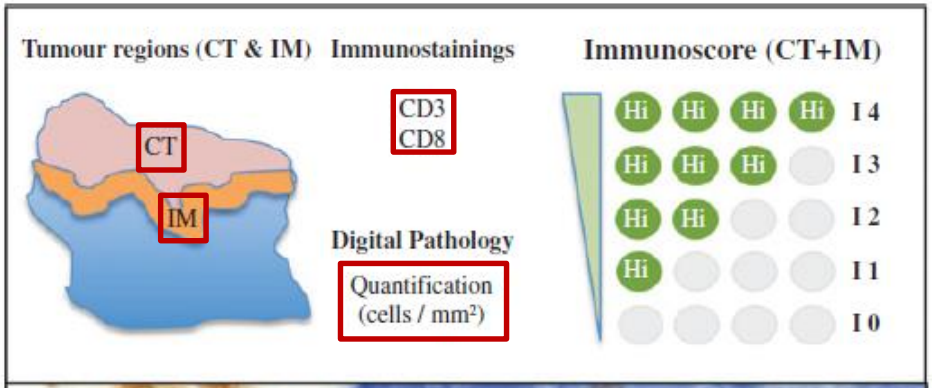
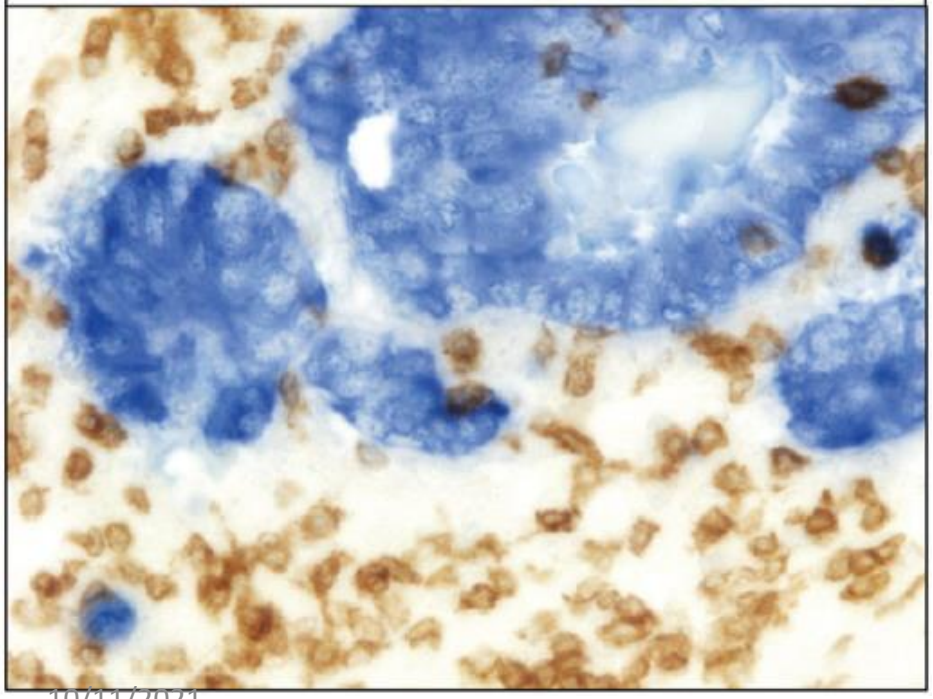
Galon J. et al J Pathol, 2014; 232: 199-209

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

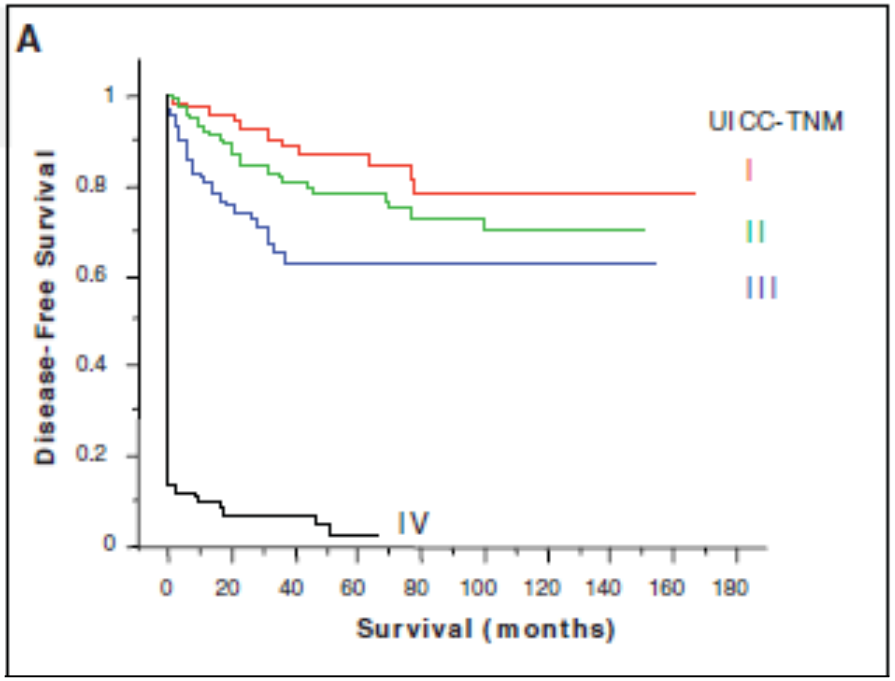
Galon J. et al J Pathol, 2014; 232: 199-209

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

UICC-TNM Staging system



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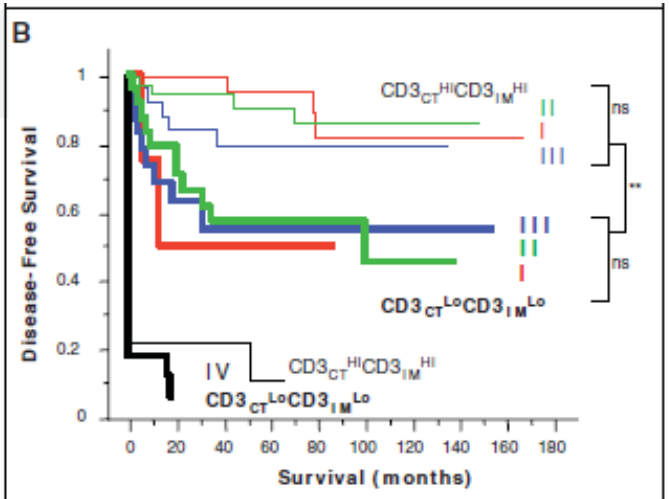
Tumor infiltrating immune cells

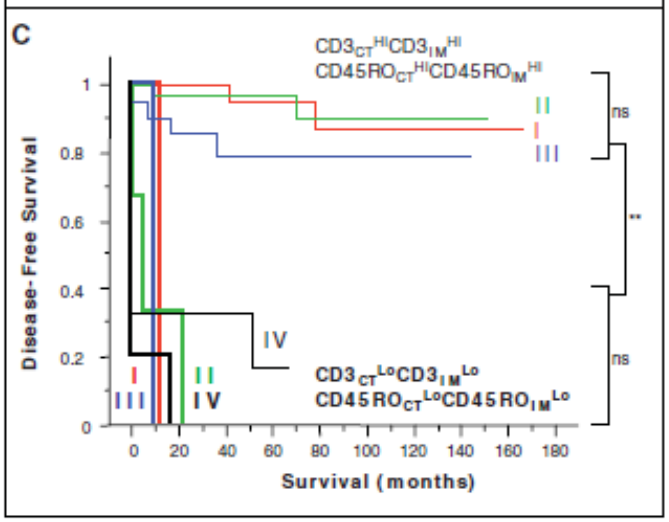
$CD3_{CT}CD3_{IM}$ evaluation



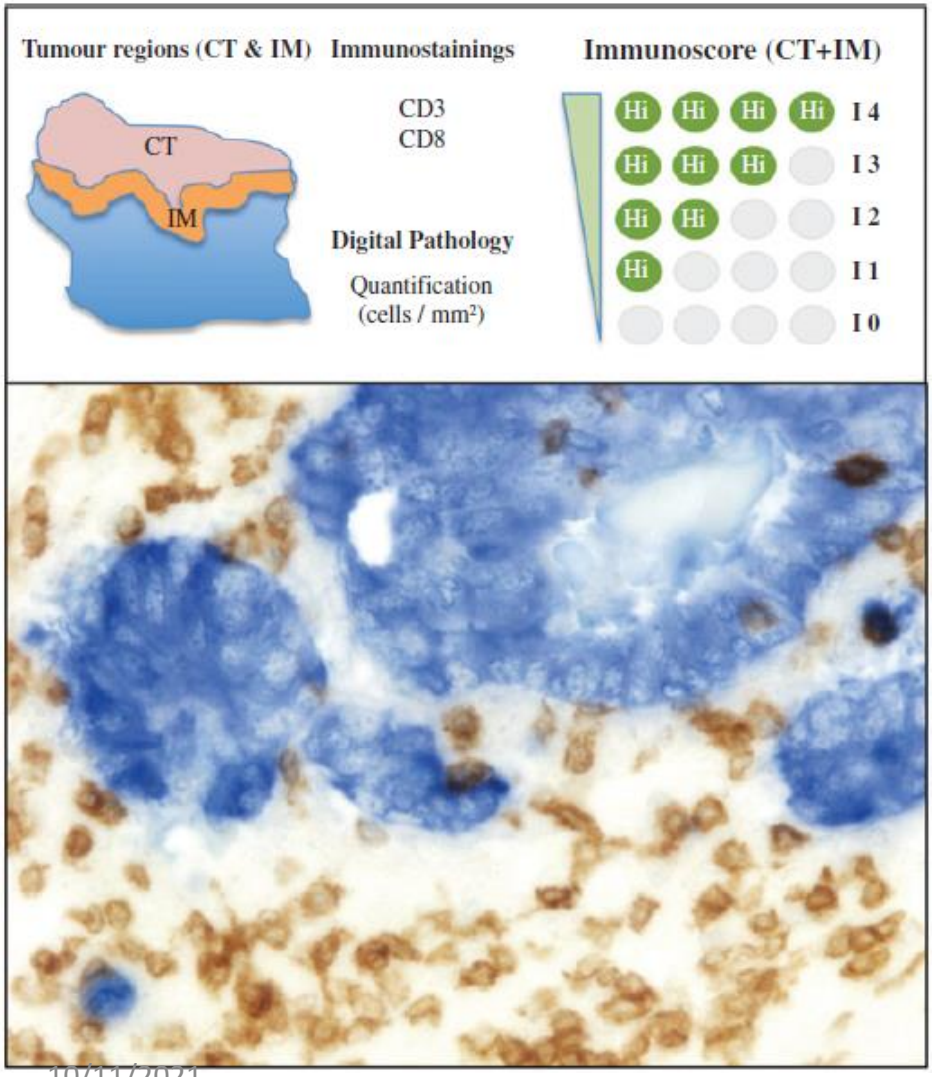
$CD3_{CT}CD3_{IM}$ evaluation

plus

$CD45RO_{CT}CD45RO_{IM}$ evaluation



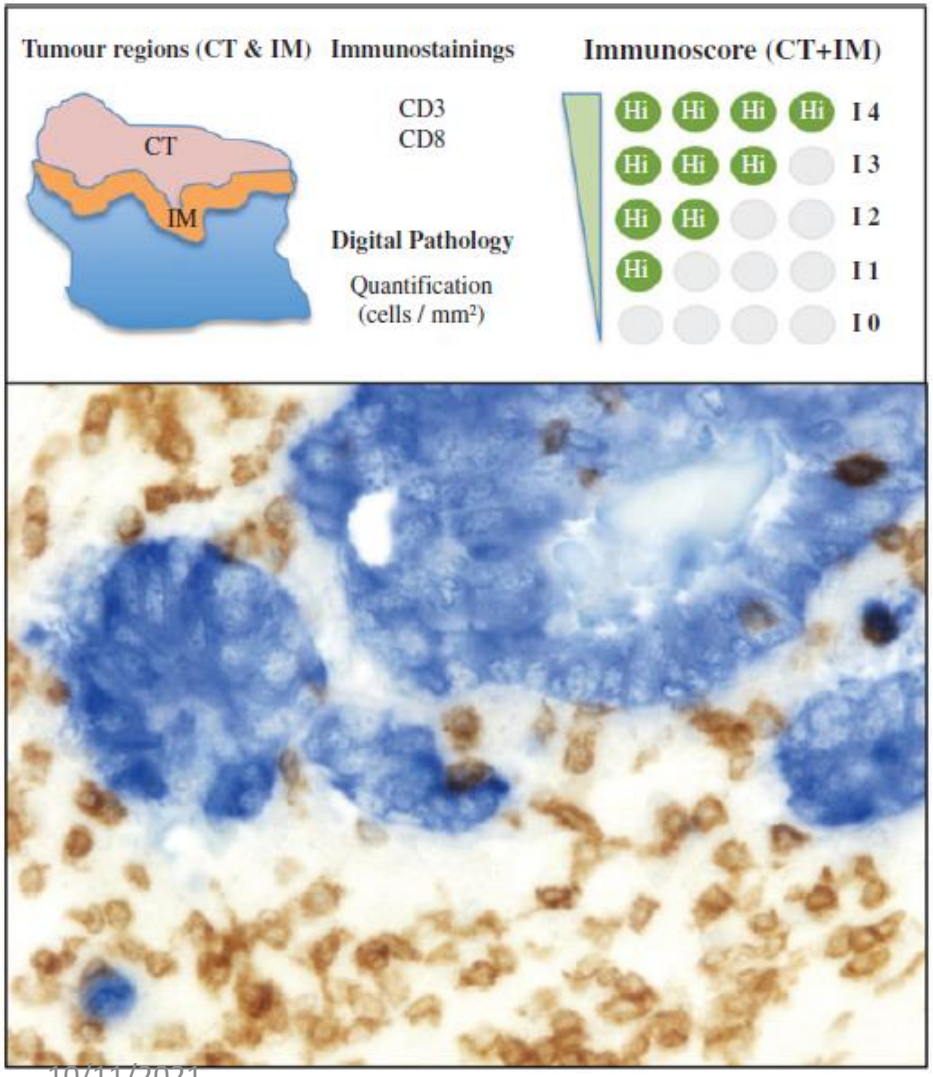
Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



Good biomarker	
Routine	☑
Feasible	☑
Simple	☑
Inexpensive	☑
Rapid	☑
Robust	☑
Reproducible	☑
Quantitative	☑
Standardized	☑
Pathology-based	☑
Powerful	☑

Galon J. et al J Pathol, 2014; 232: 199-209

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



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

Good biomarker	
Routine	☑
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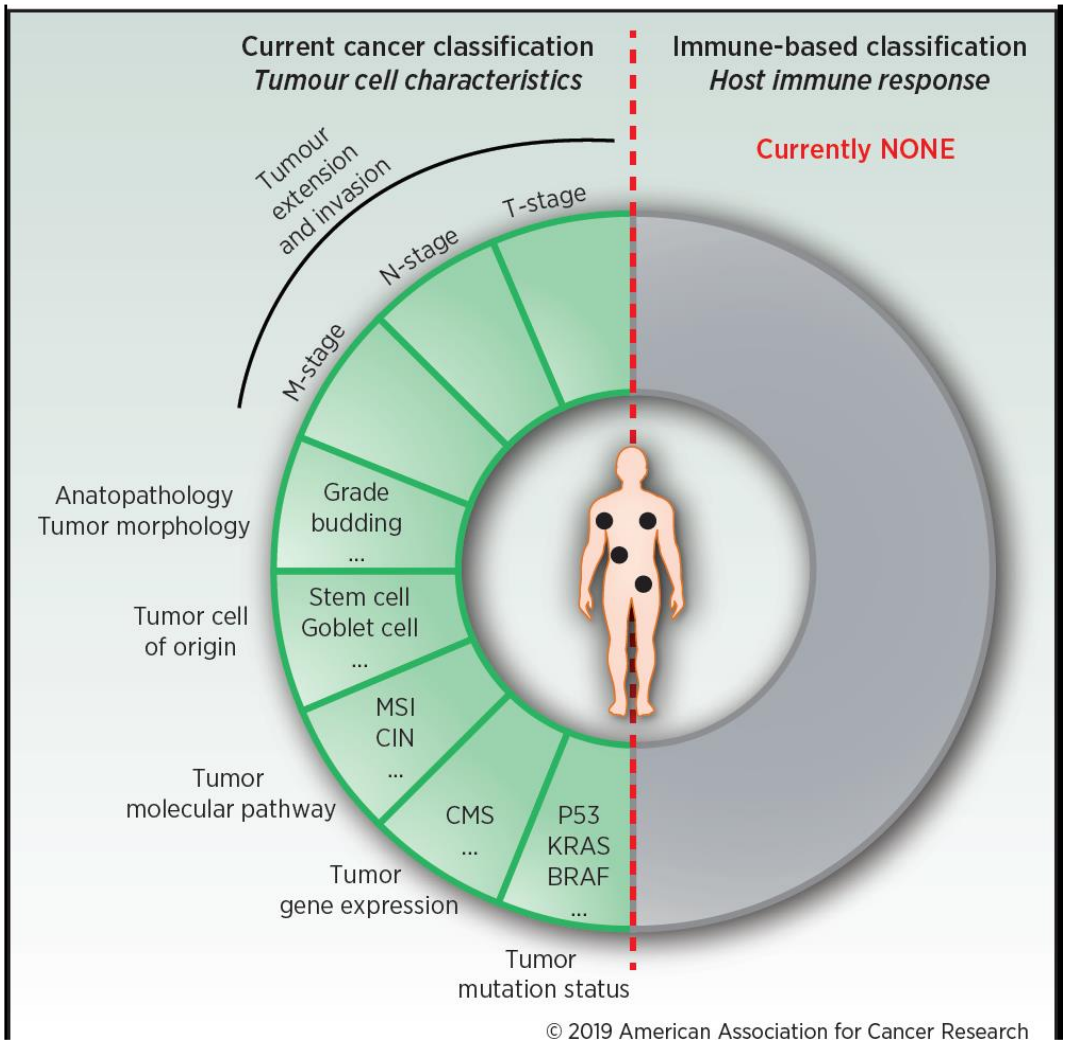
Quantitative	☑
Standardized	☑
Pathology-based	☑
Powerful	☑

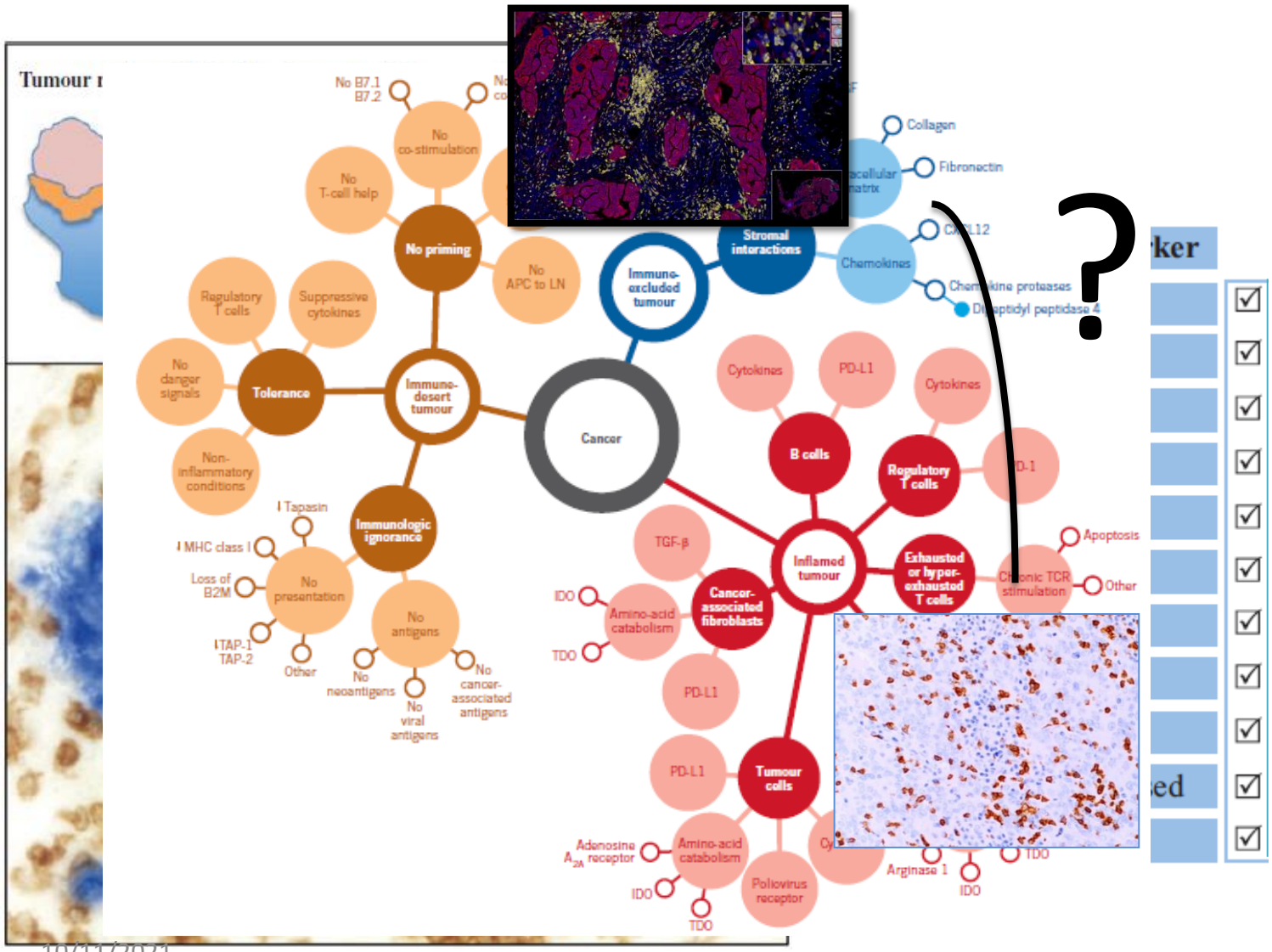
Galon J. et al J Pathol, 2014; 232: 199-209

The Immunoscore: Colon Cancer and Beyond

Helen K. Angell[1*], Daniela Bruni[2#], J. Carl Barrett[3], Ronald Herbst[4#], and Jérôme Galon[2]



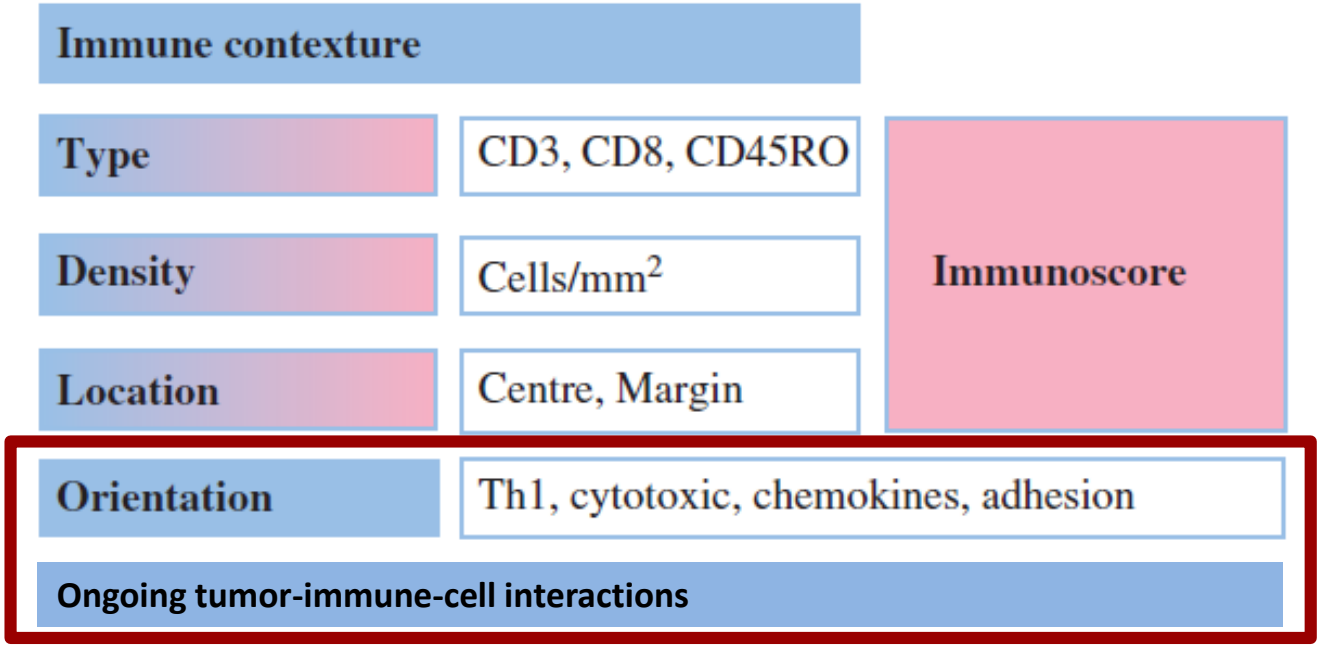
Towards the introduction of the 'Immunoscore' in the classification of malignant tumours



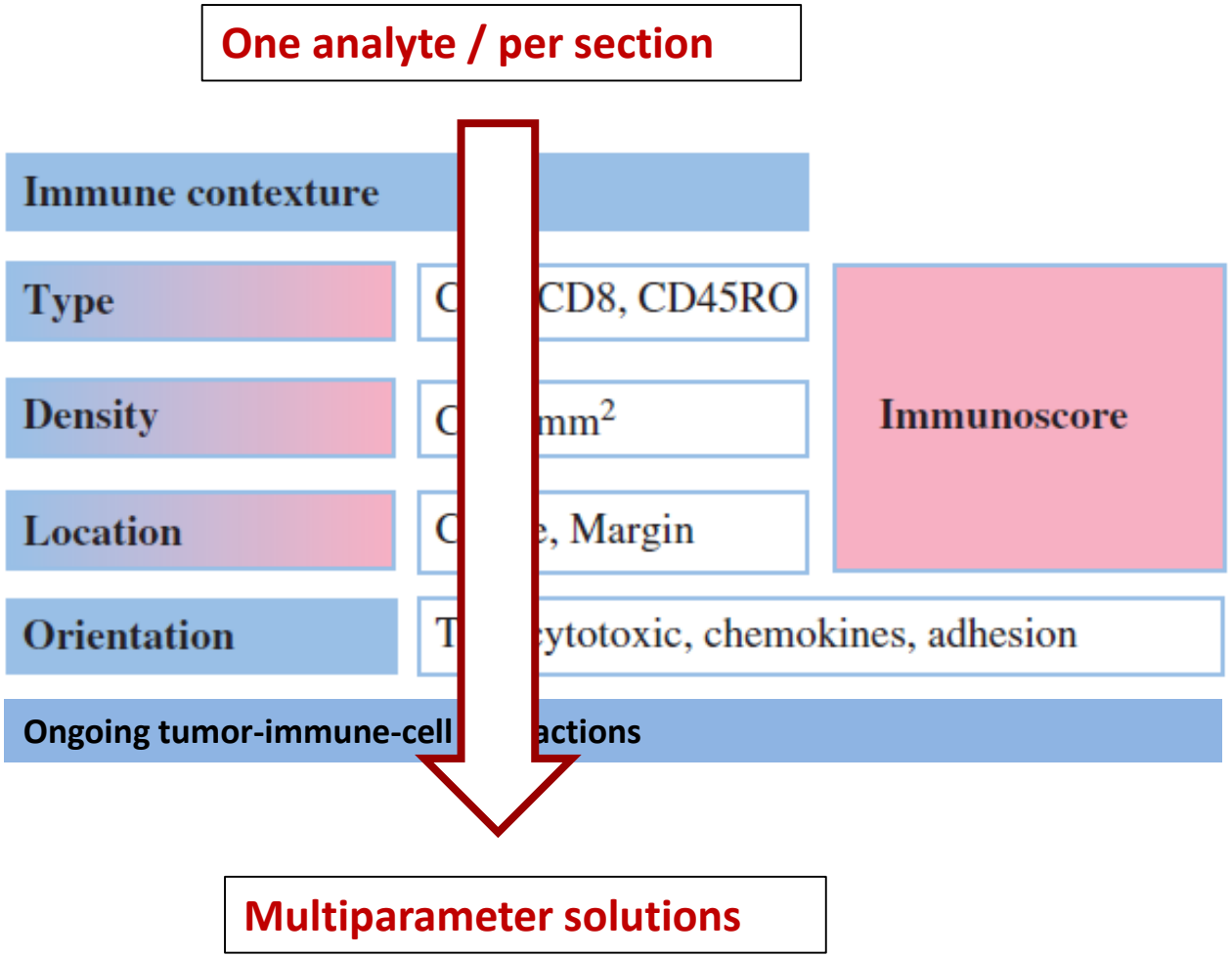
Galon J. et al J Pathol, 2014; 232: 199-209

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

One analyte / per section



Modified from: Galon J. et al J Pathol, 2014; 232: 199-209



Modified from: Galon J. et al J Pathol, 2014; 232: 199-209

Immune contexture		
Type	CD3, CD8, CD45RO	Immunoscore
Density	Cells/mm^2	
Location	Centre, Margin	
Orientation	Th1, cytotoxic, chemokines, adhesion	

Ongoing tumor-immune-cell interactions

Simple and powerful immune biomarkers

Immune monitoring technology primer: flow and mass cytometry

Holden T. Maecker[1*] and Alexandre Harari[2]

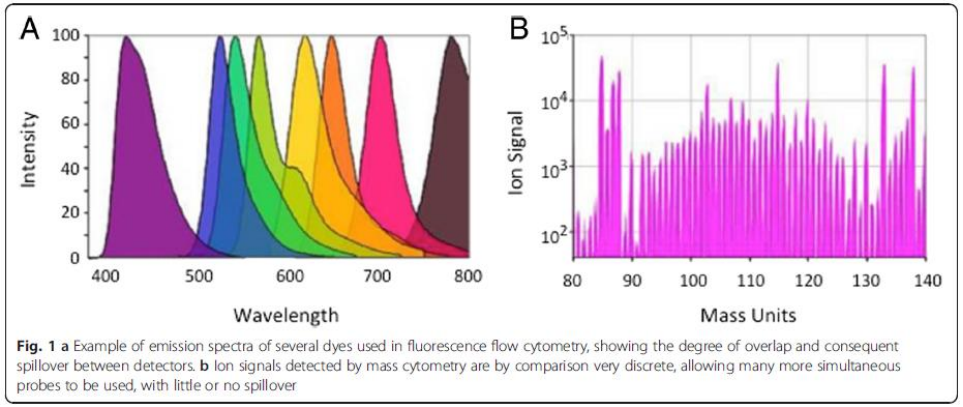
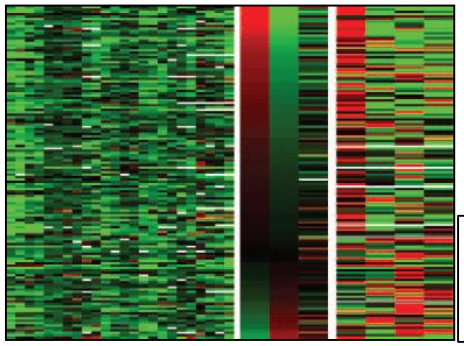


Fig. 1 **a** Example of emission spectra of several dyes used in fluorescence flow cytometry, showing the degree of overlap and consequent spillover between detectors. **b** Ion signals detected by mass cytometry are by comparison very discrete, allowing many more simultaneous probes to be used, with little or no spillover

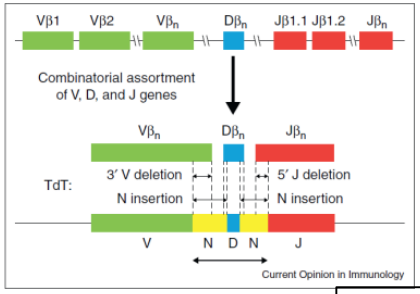
Journal for ImmunoTherapy of Cancer (2015) 3:44

Immune contexture		
Type	CD3, CD8, CD45RO	Immunoscore
Density	Cells/mm^2	
Location	Centre, Margin	
Orientation	Th1, cytotoxic, chemokines, adhesion	
Ongoing tumor-immune-cell interactions		

Simple and powerful immune biomarkers



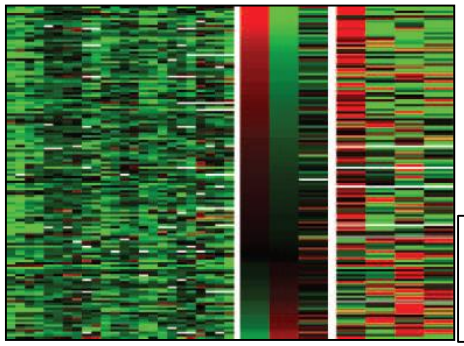
Gene expression signatures



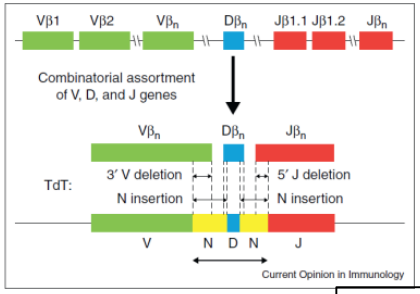
Immunosequencing

Immune contexture		
Type	CD3, CD8, CD45RO	Immunoscore
Density	Cells/mm^2	
Location	Centre, Margin	
Orientation	Th1, cytotoxic, chemokines, adhesion	
Ongoing tumor-immune-cell interactions		

Simple and powerful immune biomarkers



Gene expressions ignatures



Immunosequencing

Important histological context is lost

Stack *et al. Journal for ImmunoTherapy of Cancer* (2016) 4:9
DOI 10.1186/s40425-016-0115-3

Journal for ImmunoTherapy of Cancer

SHORT REPORT Open Access

Multiplexed tissue biomarker imaging

Edward C. Stack[1*], Periklis G. Foukas[2,3] and Peter P. Lee[4]

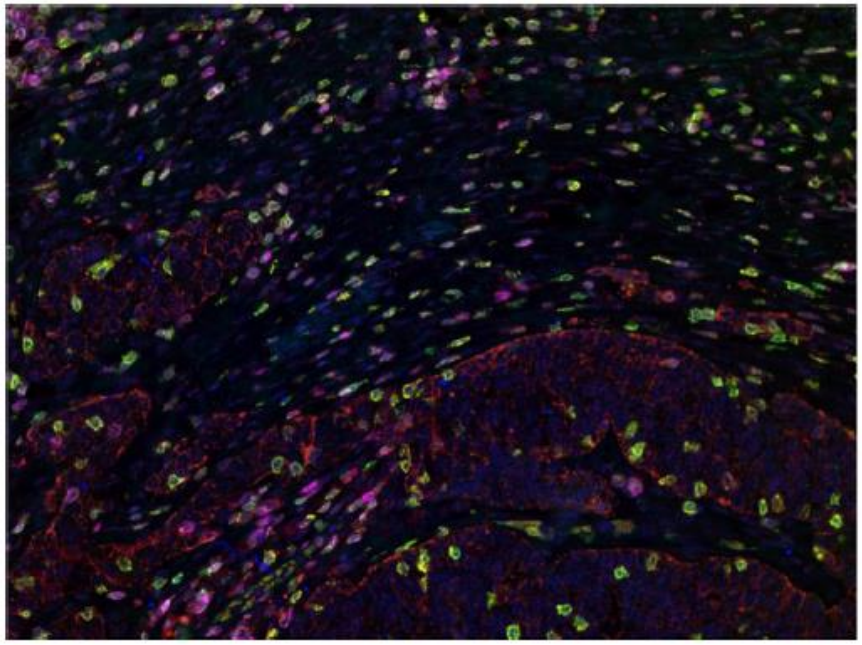
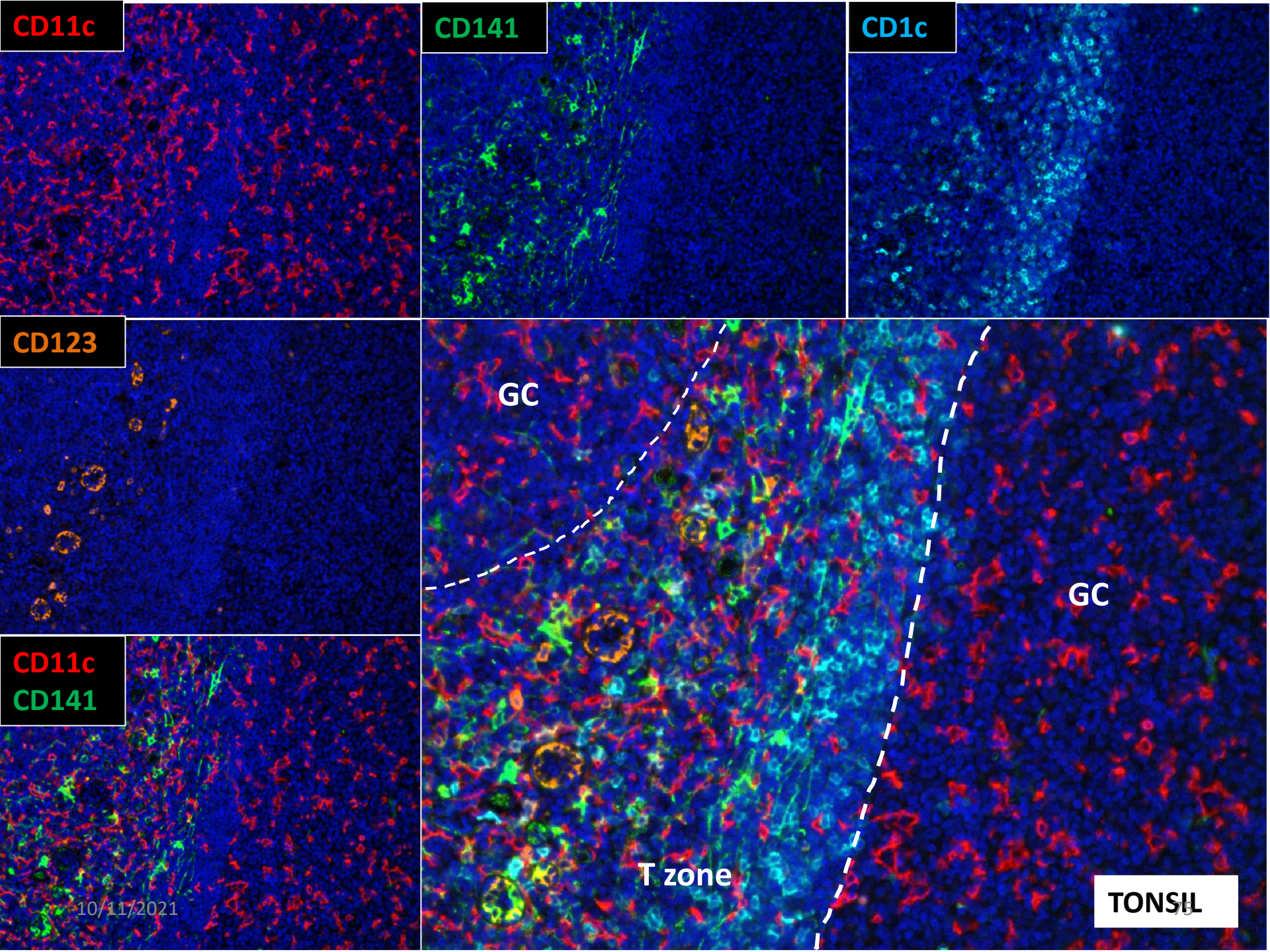


Fig. 1 Multiplexed Lymphocyte Assay in Ovarian Adenocarcinoma. Representative TSA multiplex of CD3 (green), CD4 (red), CD8 (yellow), CD45RO (magenta), Cytokeratins (brown) and DAPI (blue) in Ovarian Cancer. Multispectral imaging yields a composite image where each marker-associated dye can be reliable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

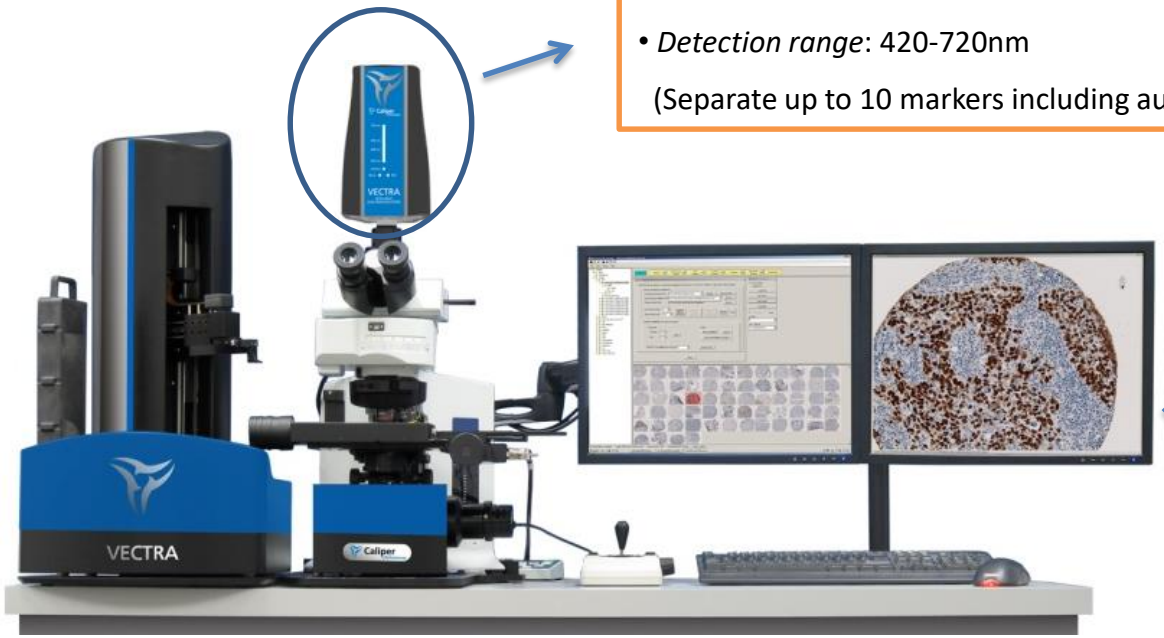

CD11c
CD141
CD1c
CD123
GC
GC
CD11c
CD141
T zone
TONSIL

Multispectral Imaging

CCD camera with Liquid Crystal Tunable Filter

- *Detection range*: 420-720nm

  (Separate up to 10 markers including autofluorescence)

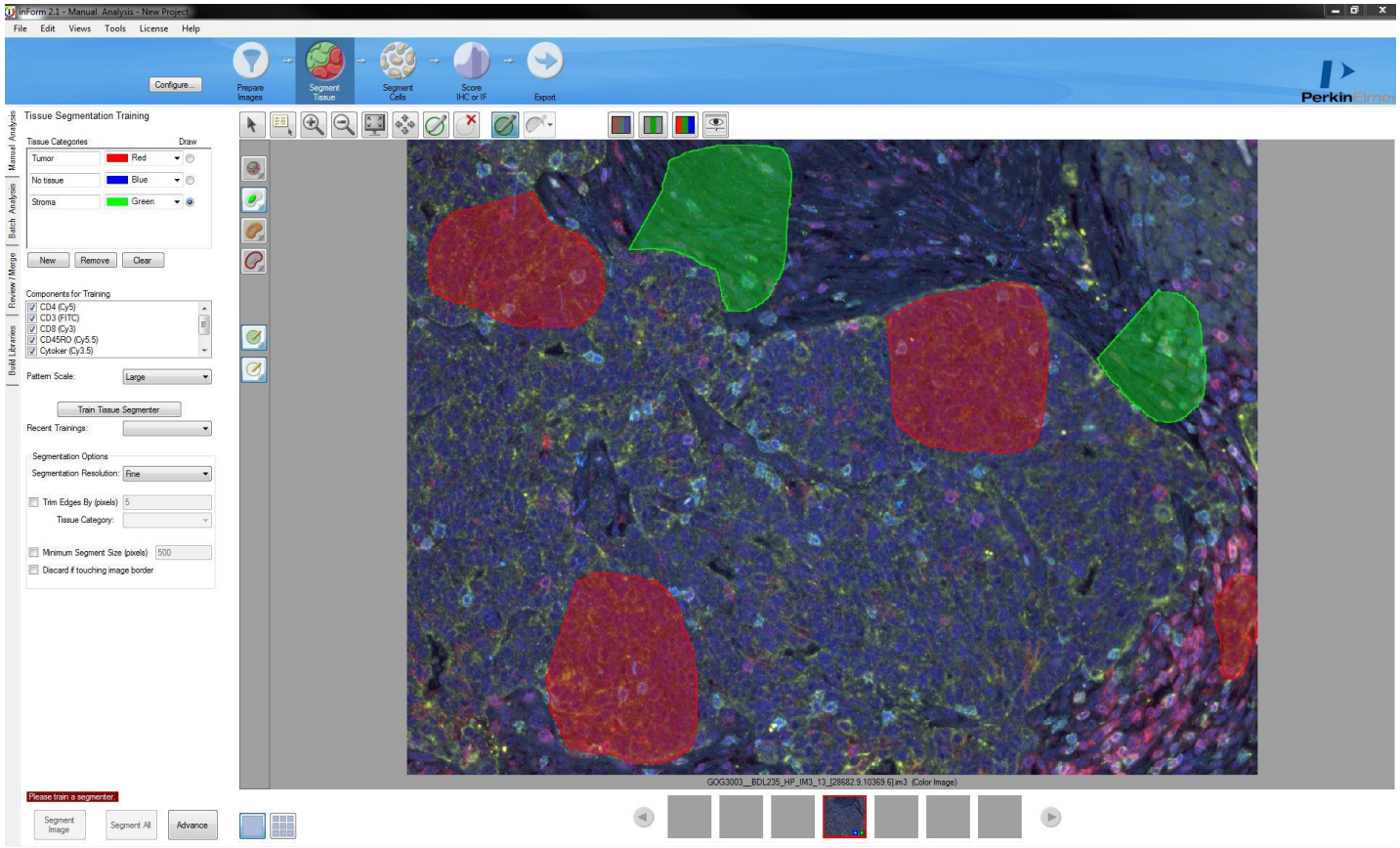


Analysis software

- Spectral unmixing
- Learn by examples algorithm
- Quantitative data output

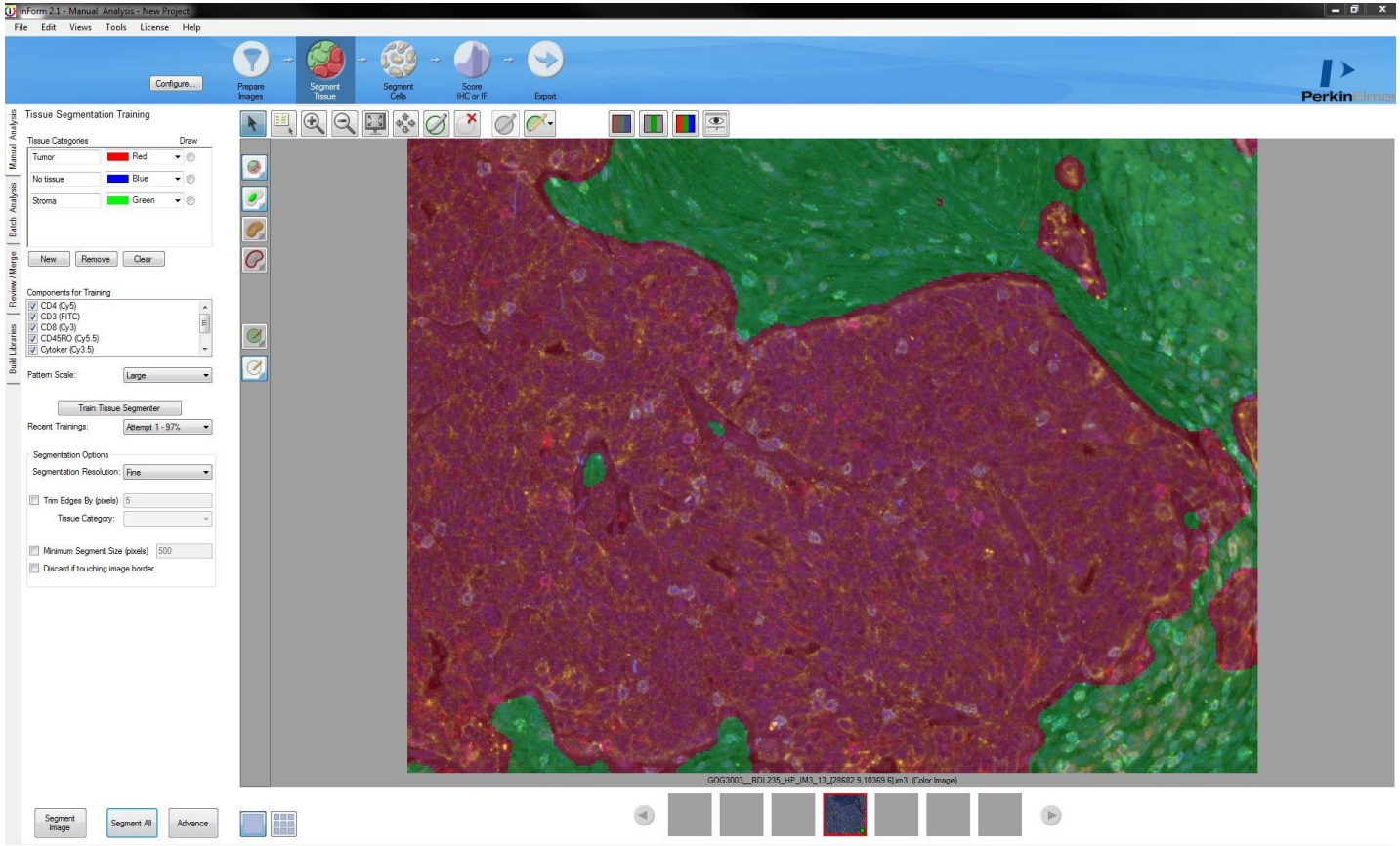
CASE_GACZNG

Tissue segmentation_train_algorithm



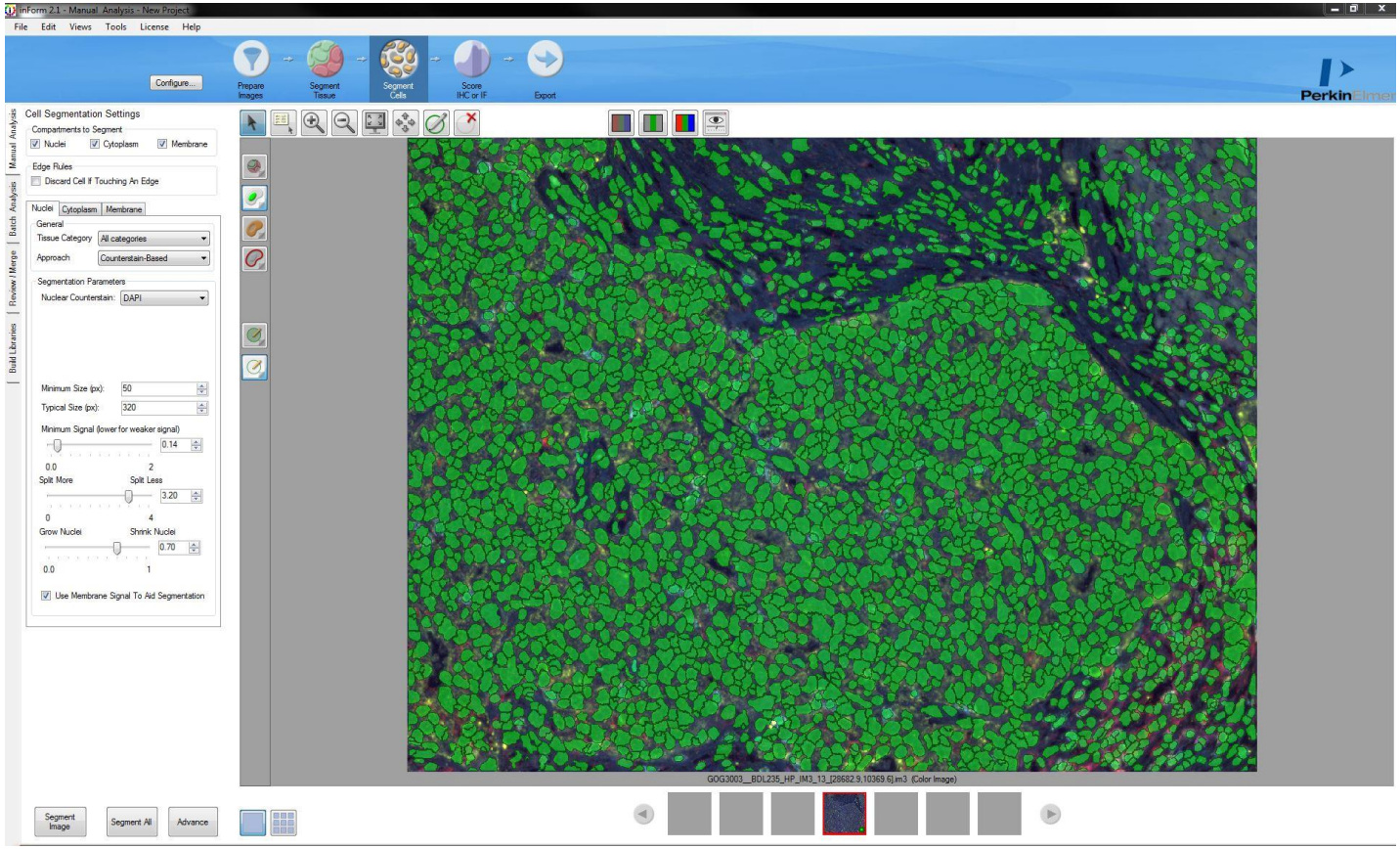
CASE_GACZNG

Tissue segmentation



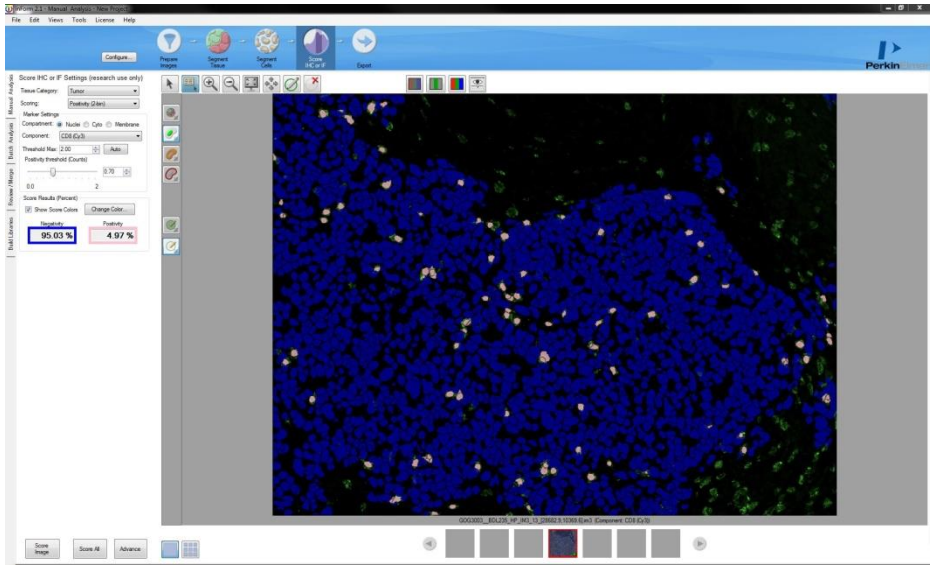
CASE_GACZNG

Cell segmentation

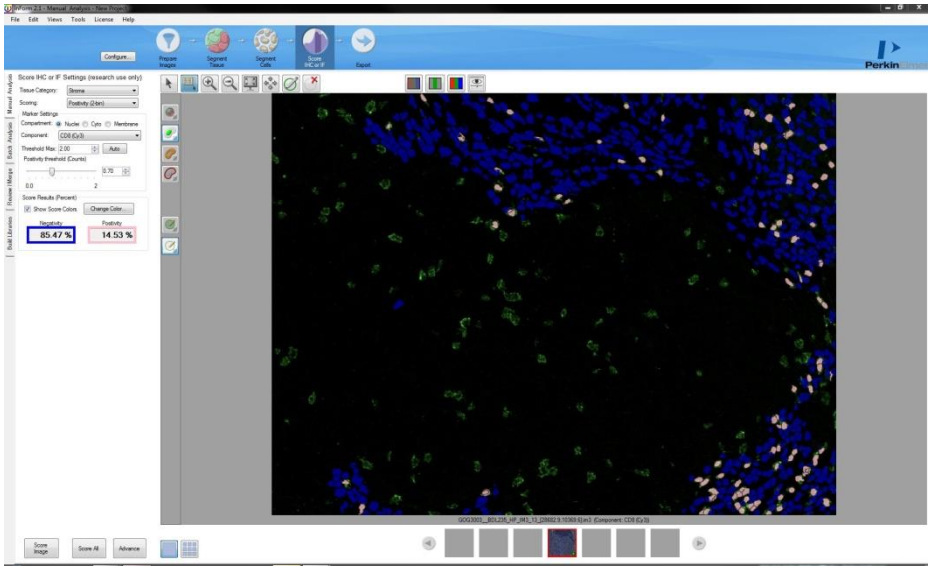


CASE_GACZNG

CD8_tumor



CD8_stroma



Panel : PD1 / Granzyme B / NFAT2c / CD8 / Keratins / DAPI

Panel : PD1 / Granzyme B / NFAT2c / CD8 / Keratins / DAPI

CD8+, PD1+, Granzyme B+ = terminal effector cells

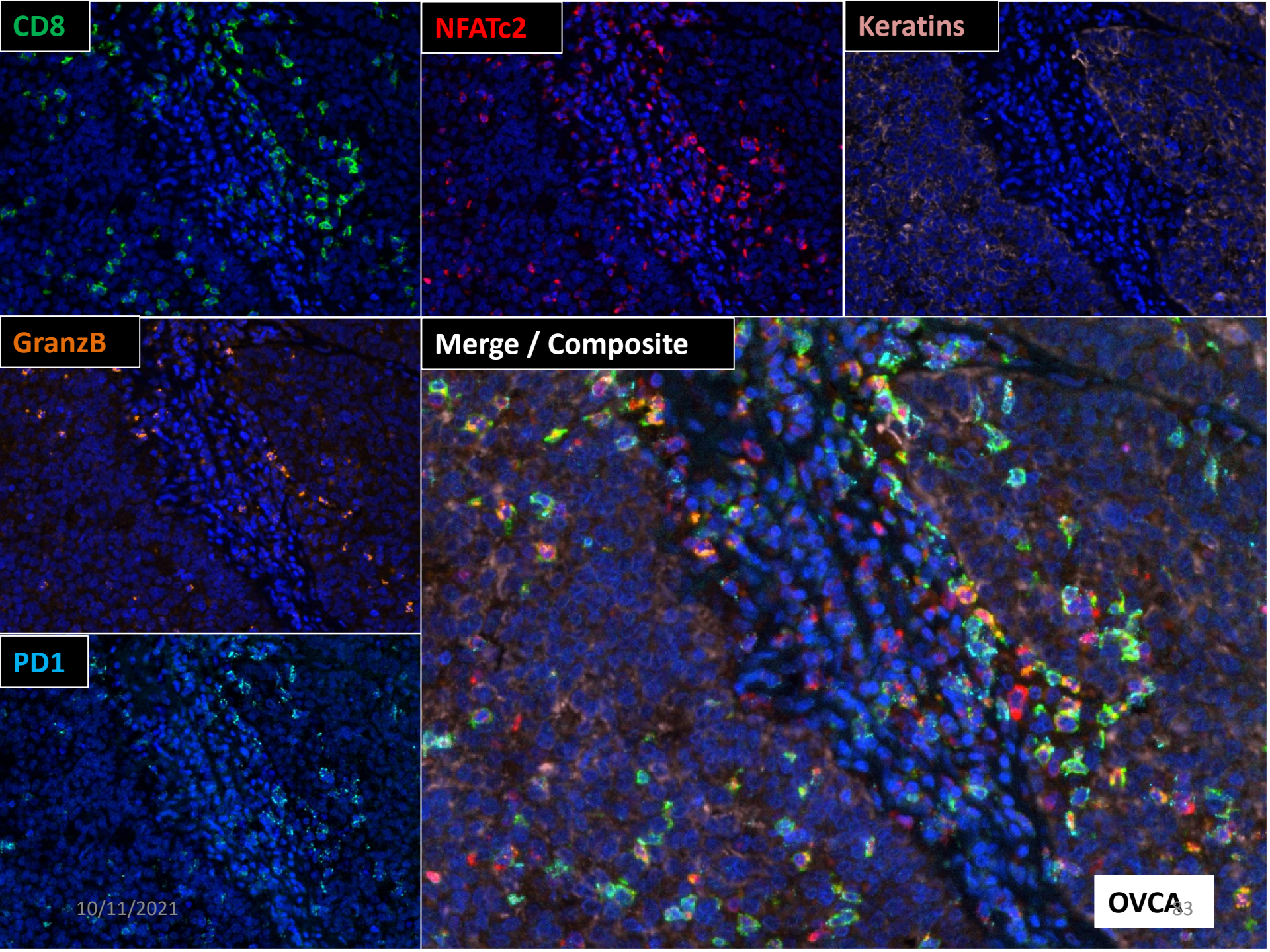
CD8+, PD1+, Granzyme B- =dysfunctional / exhausted cells

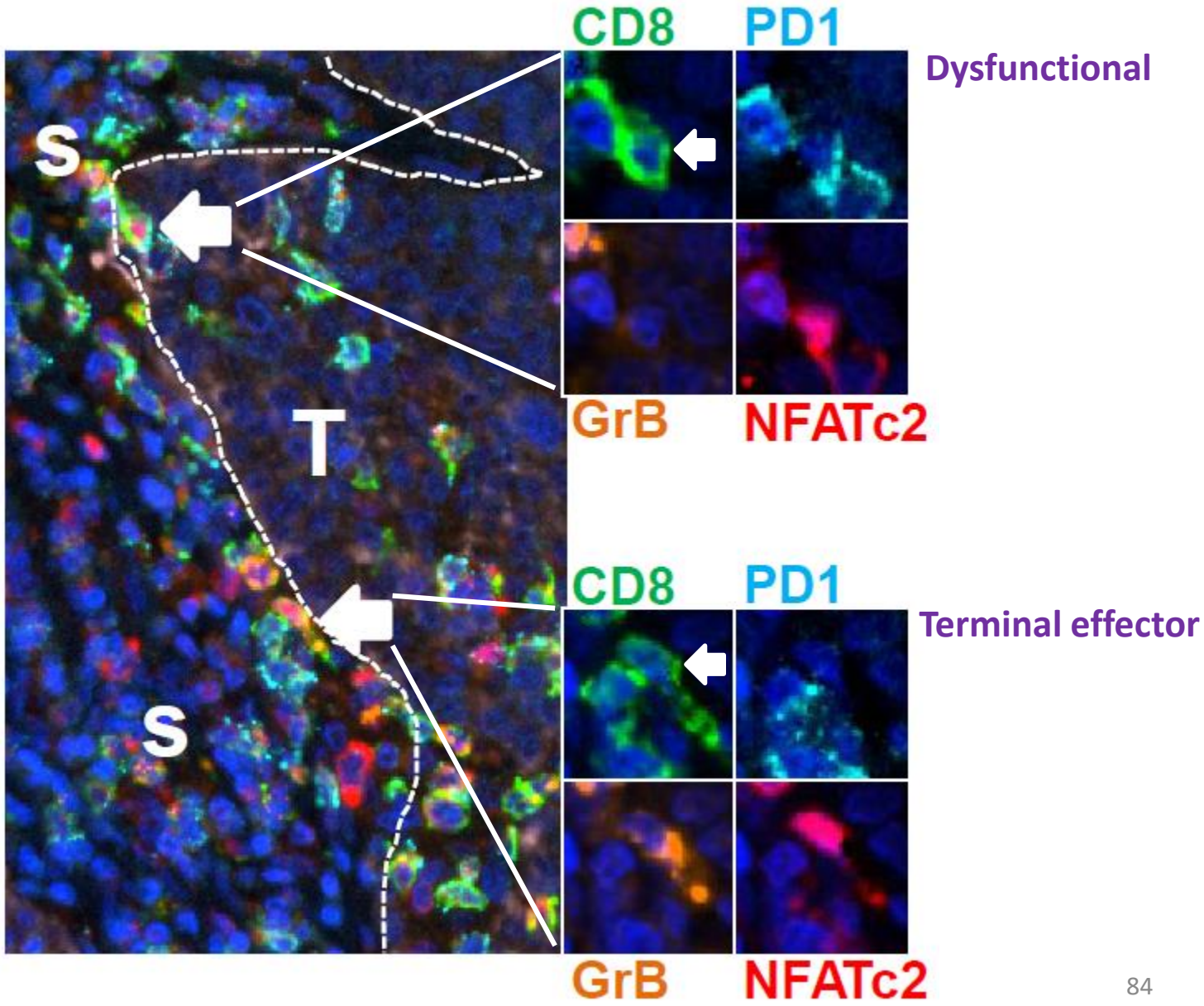
CD8+, nuclearNFAT2c+ = TCR signaling

Consensus nomenclature for CD8$^+$ T cell phenotypes in cancer

Lionel Apetoh[1,2,3,*], Mark J. Smyth[4], Charles G. Drake[5], Jean-Pierre Abastado[6], Ron N. Apte[7], Maha Ayyoub[8], Jean-Yves Blay[9,10], Marc Bonneville[11,12], Lisa H. Butterfield[13], Anne Caignard[14], Chiara Castelli[15], Federica Cavallo[16], Esteban Celis[17], Lieping Chen[18], Mario P. Colombo[19], Begoña Comin-Anduix[20], Georges Coukos[21], Madhav V. Dhodapkar[18], Glenn Dranoff[22], Ian H. Frazer[23], Wolf-Hervé Fridman[24], Dmitry I. Gabrilovich[25], Eli Gilboa[26], Sacha Gnjatic[27], Dirk Jäger[28], Pawel Kalinski[29], Howard L. Kaufman[30], Rolf Kiessling[31], John Kirkwood[32], Alexander Knuth[33], Roland Liblau[34,35,36,37], Michael T. Lotze[38], Enrico Lugli[39], Francesco Marincola[40], Ignacio Melero[41], Cornelis J. Melief[42], Thorsten R. Mempel[43], Elizabeth A. Mittendorf[44], Kunle Odun[45], Willem W. Overwijk[46], Anna Karolina Palucka[47], Giorgio Parmiani[48], Antoni Ribas[20], Pedro Romero[21], Robert D. Schreiber[49], Gerold Schuler[50], Pramod K. Srivastava[51], Eric Tartour[52], Danila Valmori[8,53], Sjoerd H. van der Burg[54], Pierre van der Bruggen[55], Benoît J. van den Eynde[55], Ena Wang[40], Weiping Zou[56], Theresa L. Whiteside[57], Daniel E. Speiser[21], Drew M. Pardoll[5], Nicholas P. Restifo[58], and Ana C. Anderson[59,*]

Oncoimmunology 4:4, e998538; February 1, 2015; © 2015 Taylor & Francis Group, LLC


CD8
NFATc2
Keratins
GranzB
Merge / Composite
PD1
OVCA


CD8
PD1
Dysfunctional
S
T
GrB
NFATc2
CD8
PD1
Terminal effector
S
GrB
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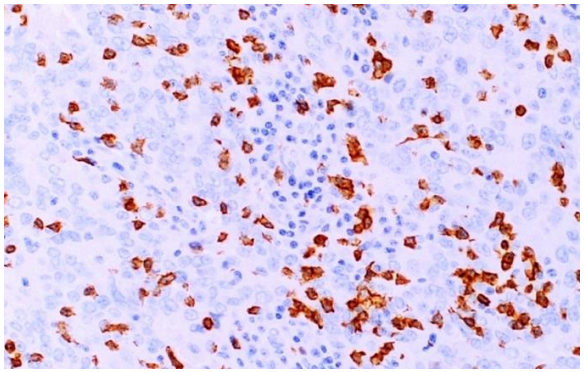
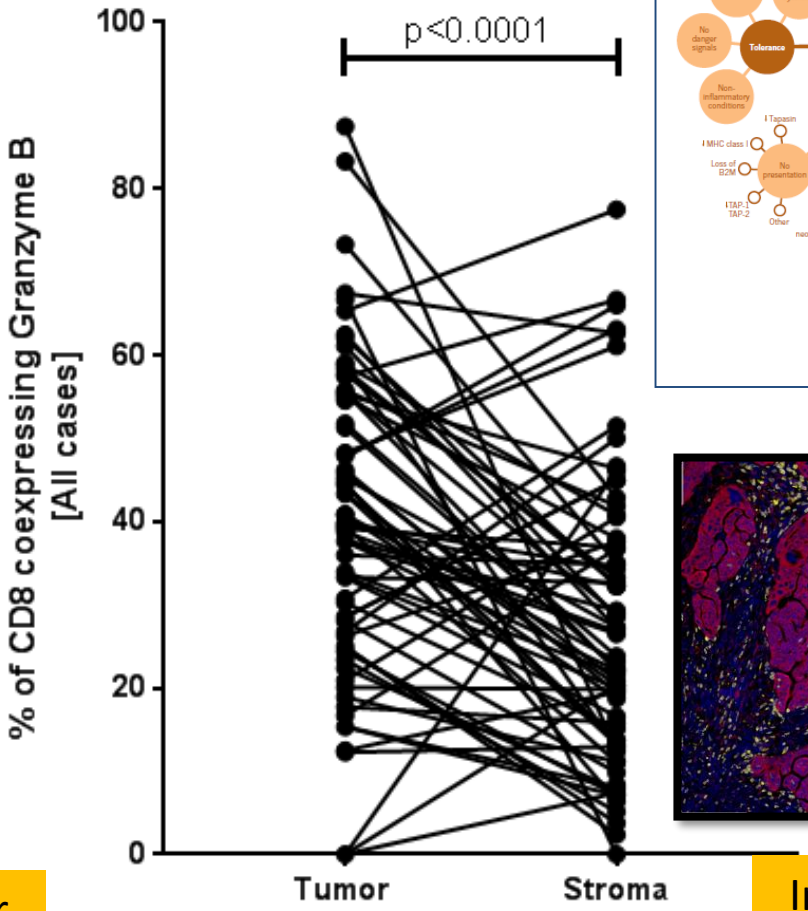
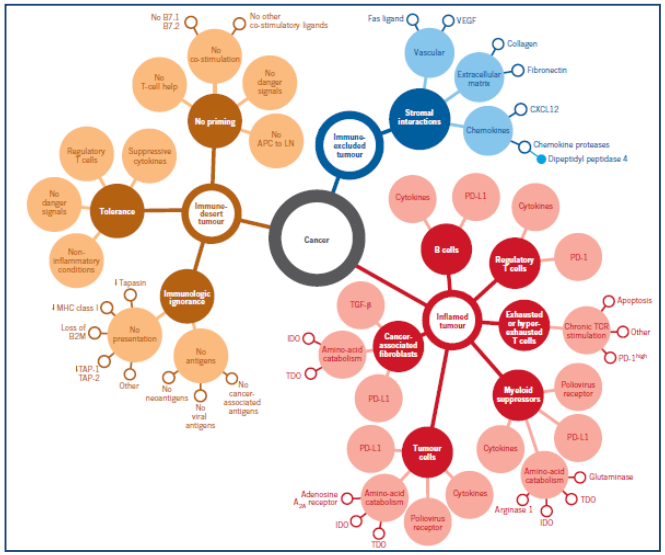
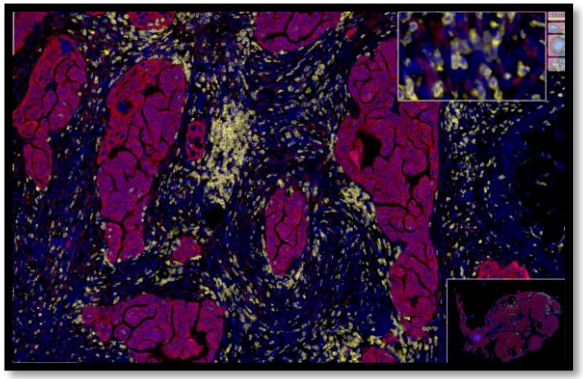




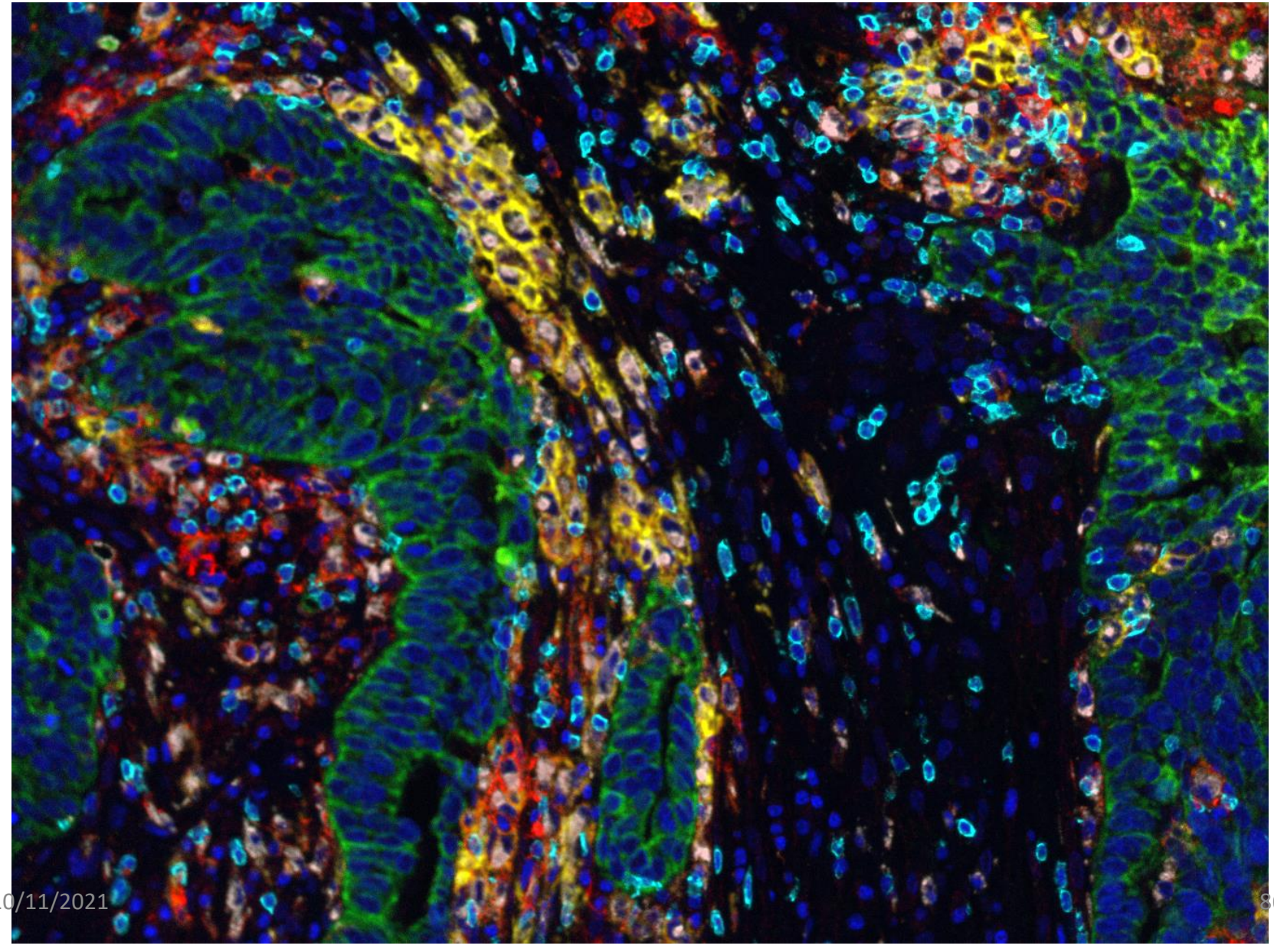




Inflamed tumor

Immune excluded tumor

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



Outline

- Intoduction to the Tumor Immune Microenvironment (TIME)
  - Prognostic / Predictive value
  - Methodologies
- **Mechanisms regulating TIME**
- TIME and neoplastic evolution

Understanding the tumor immune microenvironment (TIME) for effective therapy

Mikhail Binnewies[1], Edward W. Roberts[1], Kelly Kersten[1], Vincent Chan[2], Douglas F. Fearon[3], Miriam Merad[4], Lisa M. Coussens[5], Dmitry I. Gabrilovich[6], Suzanne Ostrand-Rosenberg[7,8], Catherine C. Hedrick[9], Robert H. Vonderheide[10], Mikael J. Pittet[11], Rakesh K. Jain[12], Weiping Zou[13], T. Kevin Howcroft[14], Elisa C. Woodhouse[14], Robert A. Weinberg[15*] and Matthew F. Krummel[1,2*]

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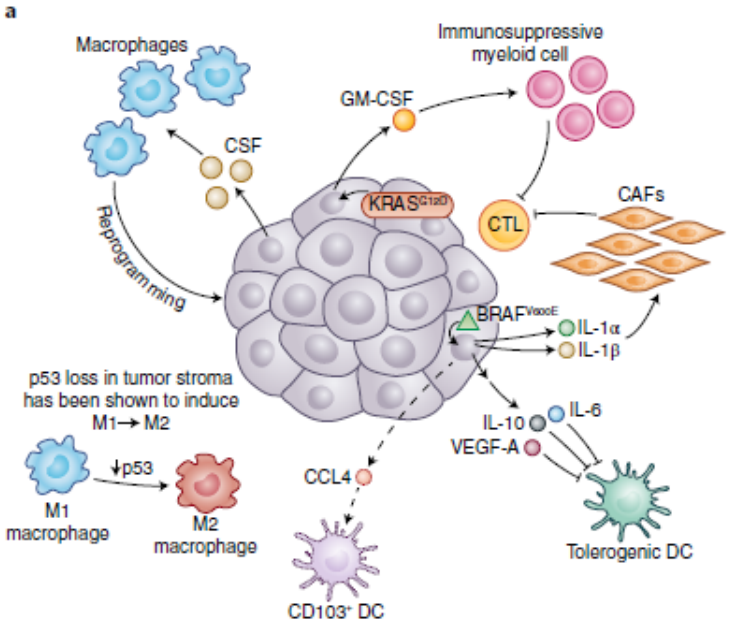

Fig. 2 | How tumor genotypes and phenotypes shape the TIME. a, Tumors are known to establish protumoral and immunosuppressive environments to support their growth and promote immune evasion. Central to building an immunosuppressive TIME are oncogenes and aberrant signaling pathways that lead to the production 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.

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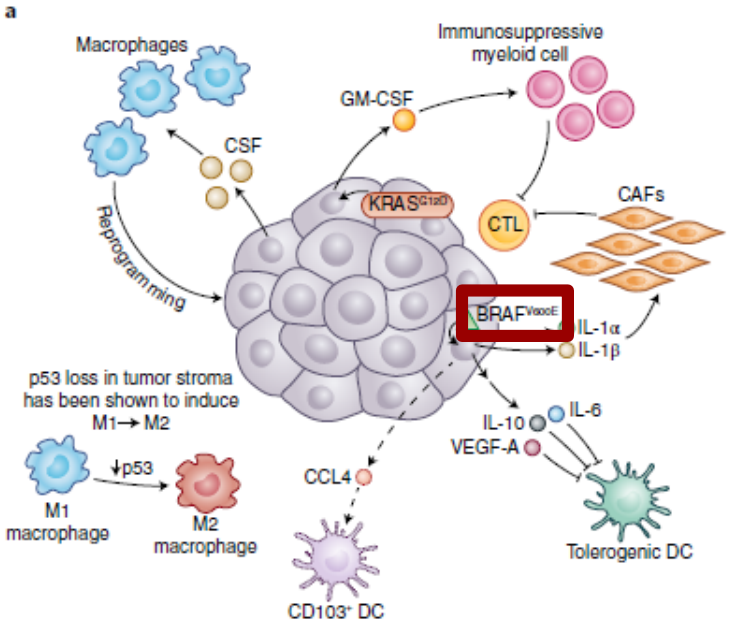

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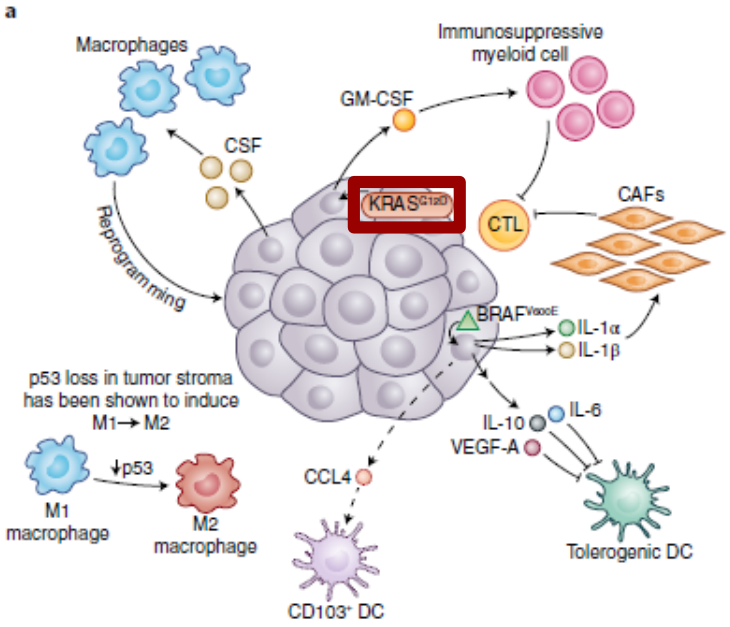

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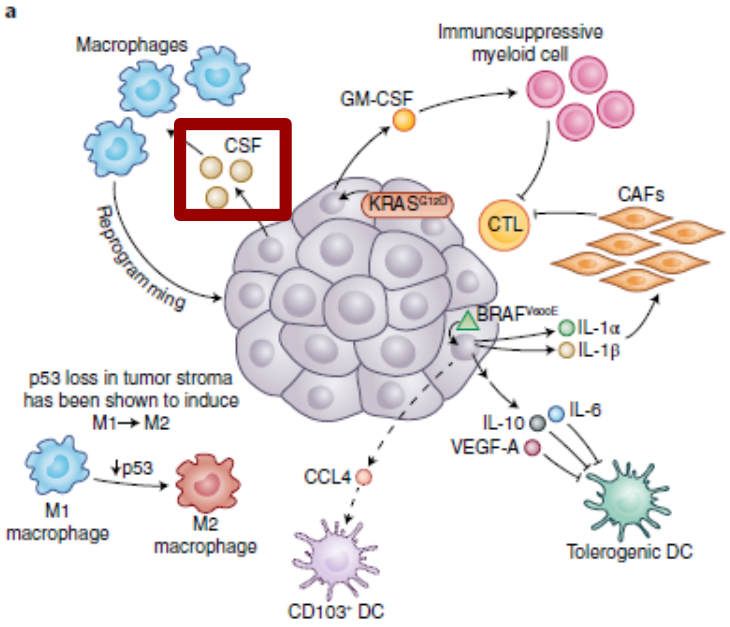

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T cell–induced CSF1 promotes melanoma resistance to PD1 blockade

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‡§]

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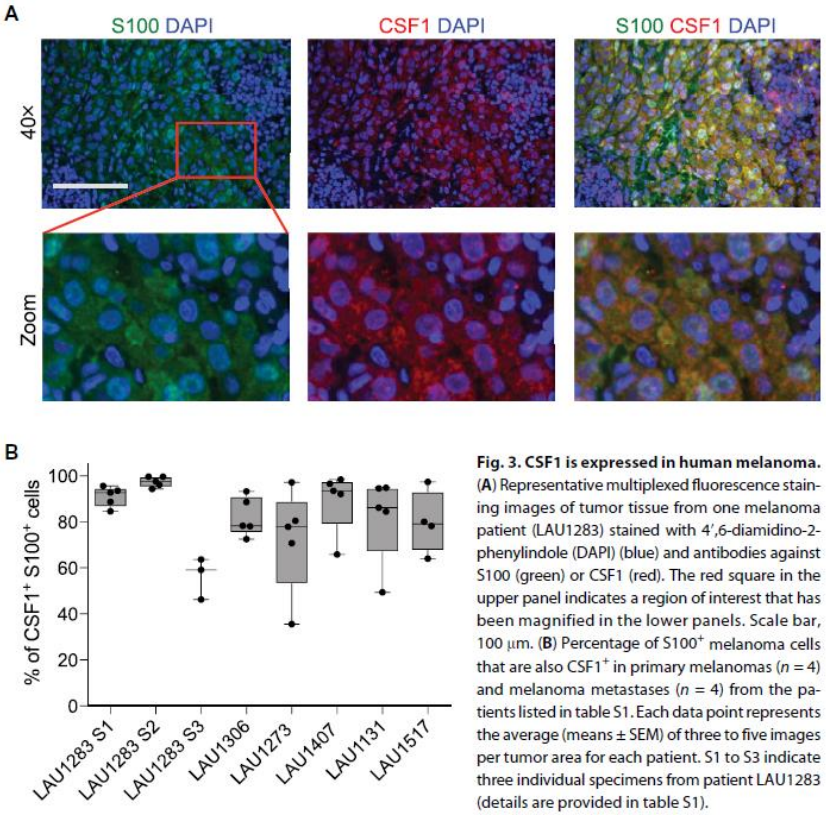
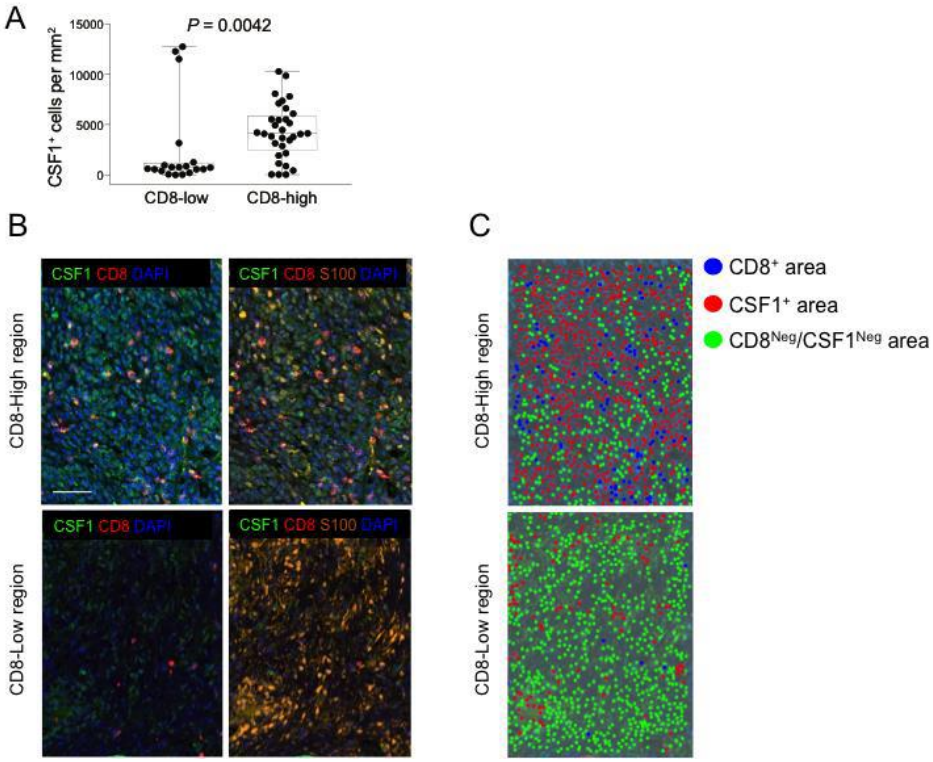


Fig. 3. CSF1 is expressed in human melanoma. (**A**) Representative multiplexed fluorescence staining images of tumor tissue from one melanoma patient (LAU1283) stained with 4′,6-diamidino-2-phenylindole (DAPI) (blue) and antibodies against S100 (green) or CSF1 (red). The red square in the upper panel indicates a region of interest that has been magnified in the lower panels. Scale bar, 100 μm. (**B**) Percentage of $S100^+$ melanoma cells that are also $CSF1^+$ in primary melanomas ($n = 4$) and melanoma metastases ($n = 4$) from the patients listed in table S1. Each data point represents the average (means ± SEM) of three to five images per tumor area for each patient. S1 to S3 indicate three individual specimens from patient LAU1283 (details are provided in table S1).



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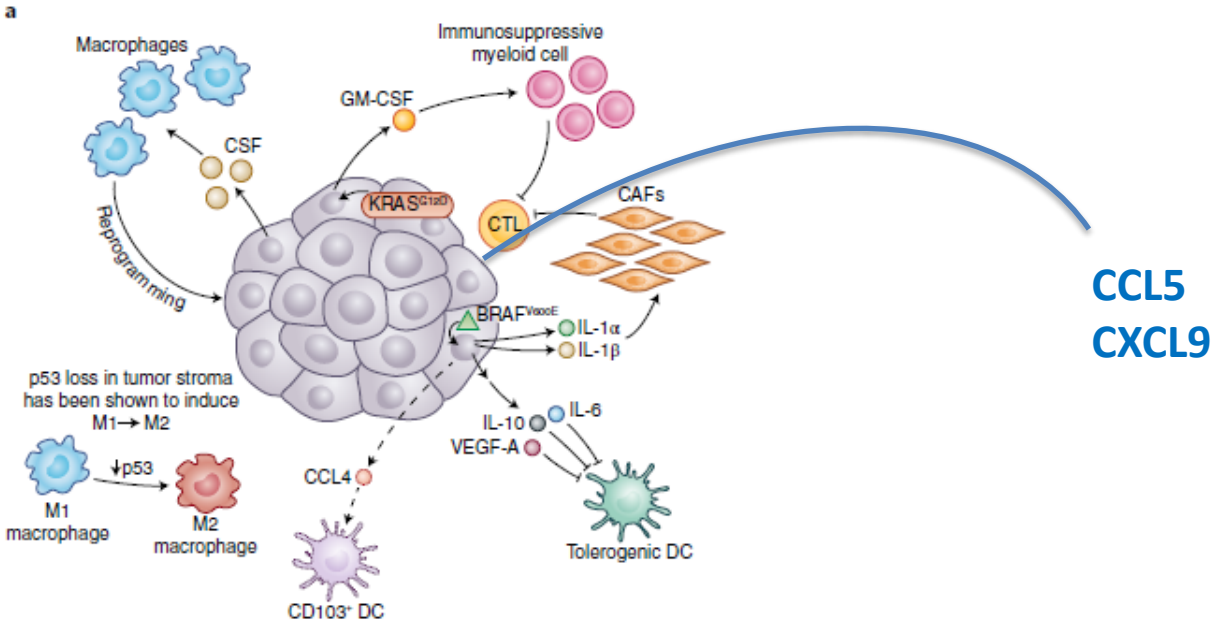

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Cooperation between Constitutive and Inducible Chemokines Enables T Cell Engraftment and Immune Attack in Solid Tumors

Cancer Cell
Article

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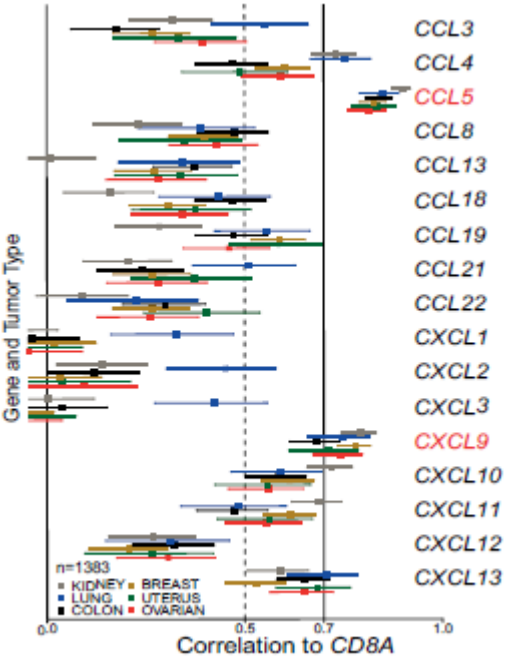
Cancer Cell *35*, 885–900, June 10, 2019



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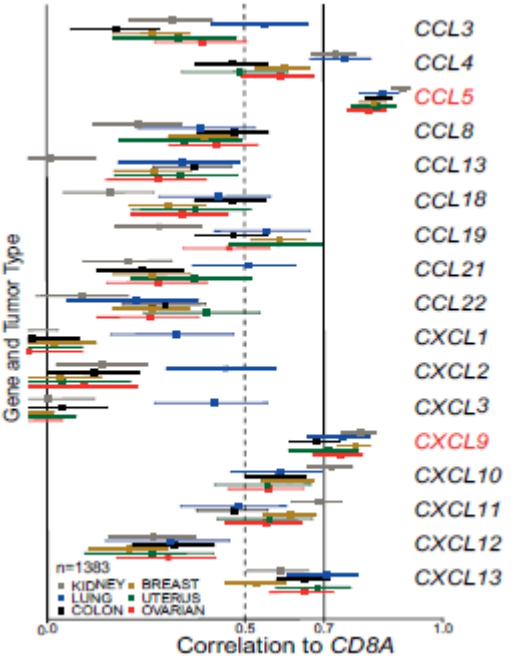

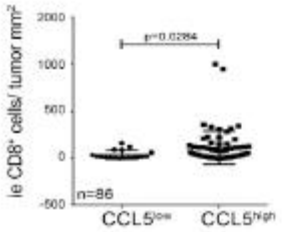

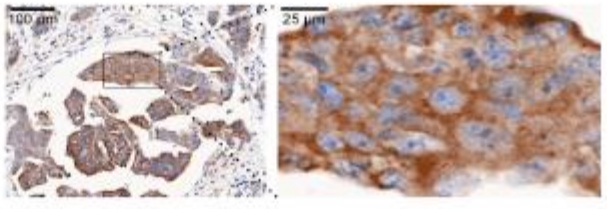

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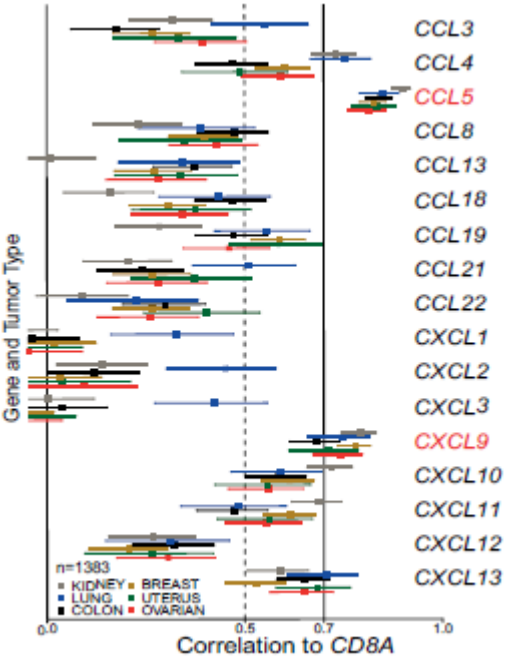

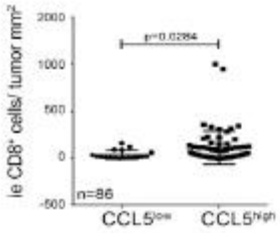

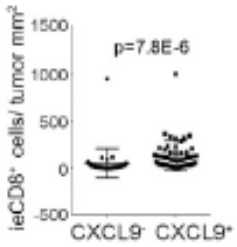

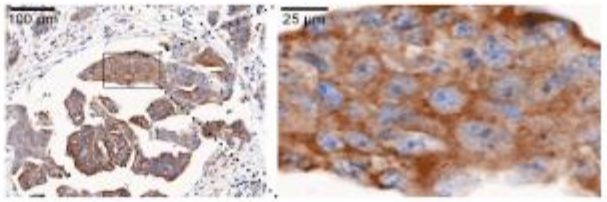

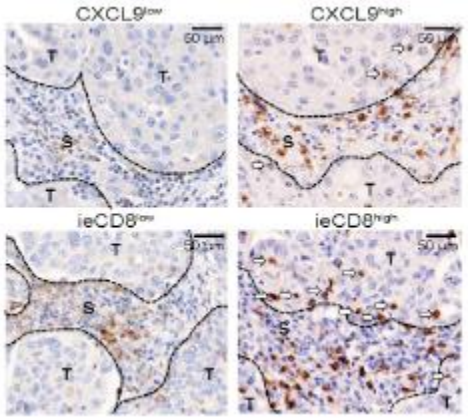

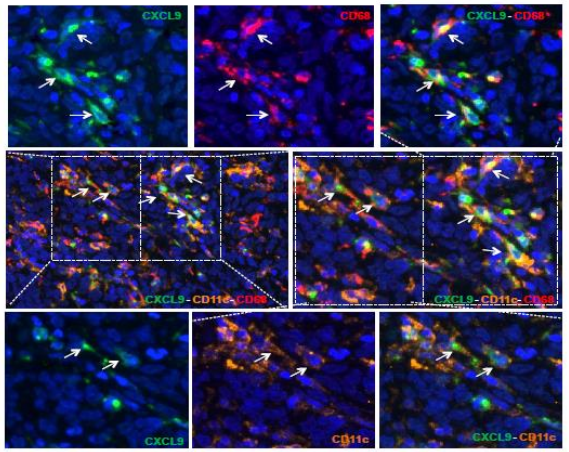

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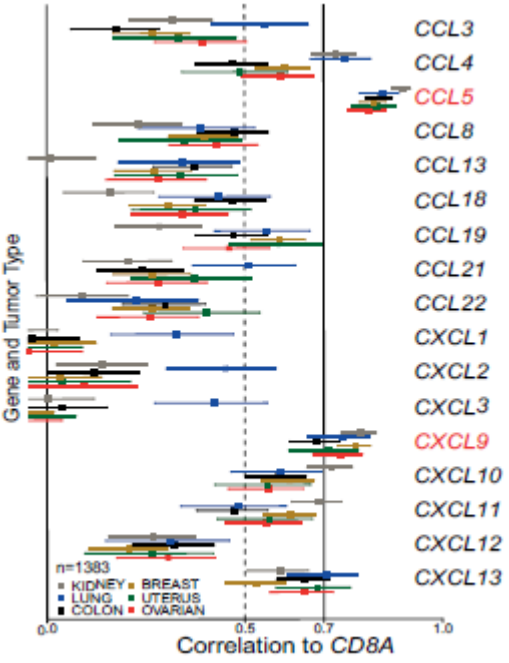

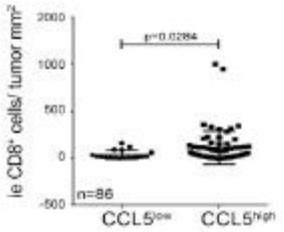

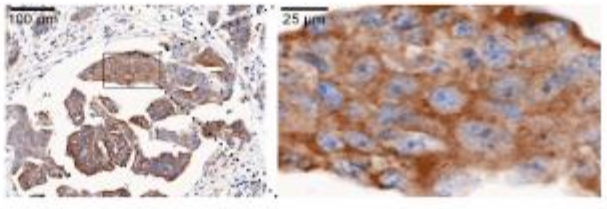

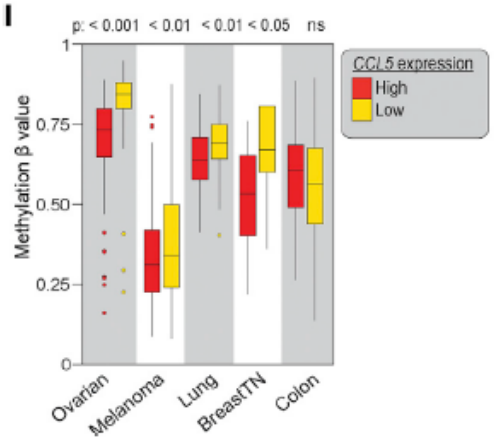

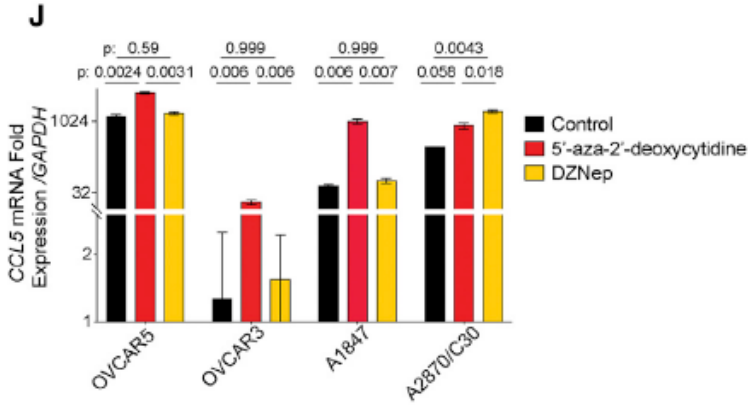

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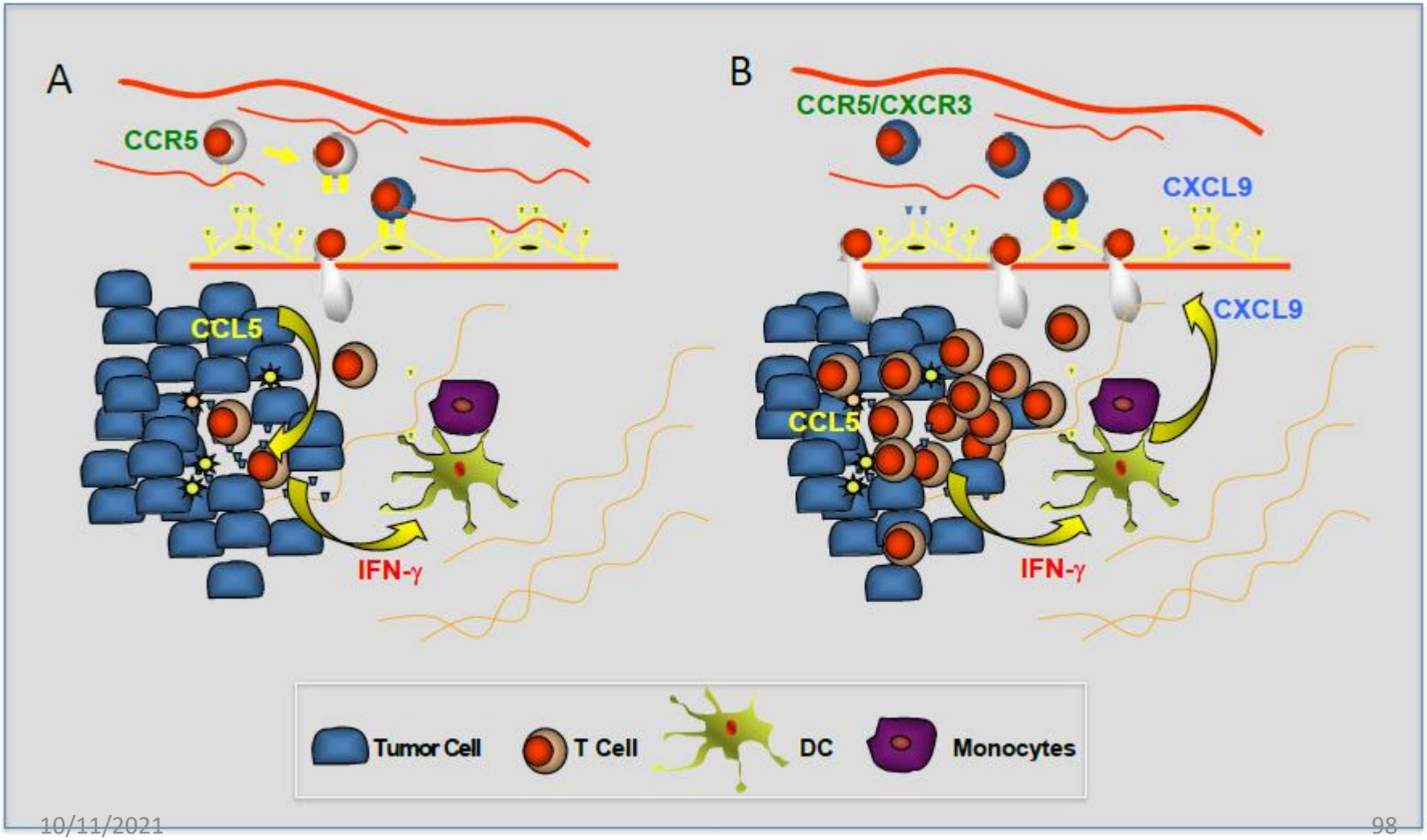
Cancer Cell
Article

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



Understanding the tumor immune microenvironment (TIME) for effective therapy

Mikhail Binnewies[1], Edward W. Roberts[1], Kelly Kersten[1], Vincent Chan[2], Douglas F. Fearon[3], Miriam Merad[4], Lisa M. Coussens[5], Dmitry I. Gabrilovich[6], Suzanne Ostrand-Rosenberg[7,8], Catherine C. Hedrick[9], Robert H. Vonderheide[10], Mikael J. Pittet[11], Rakesh K. Jain[12], Weiping Zou[13], T. Kevin Howcroft[14], Elisa C. Woodhouse[14], Robert A. Weinberg[15*] and Matthew F. Krummel[1,2*]

NATURE MEDICINE | VOL 24 | MAY 2018 | 541–550 |

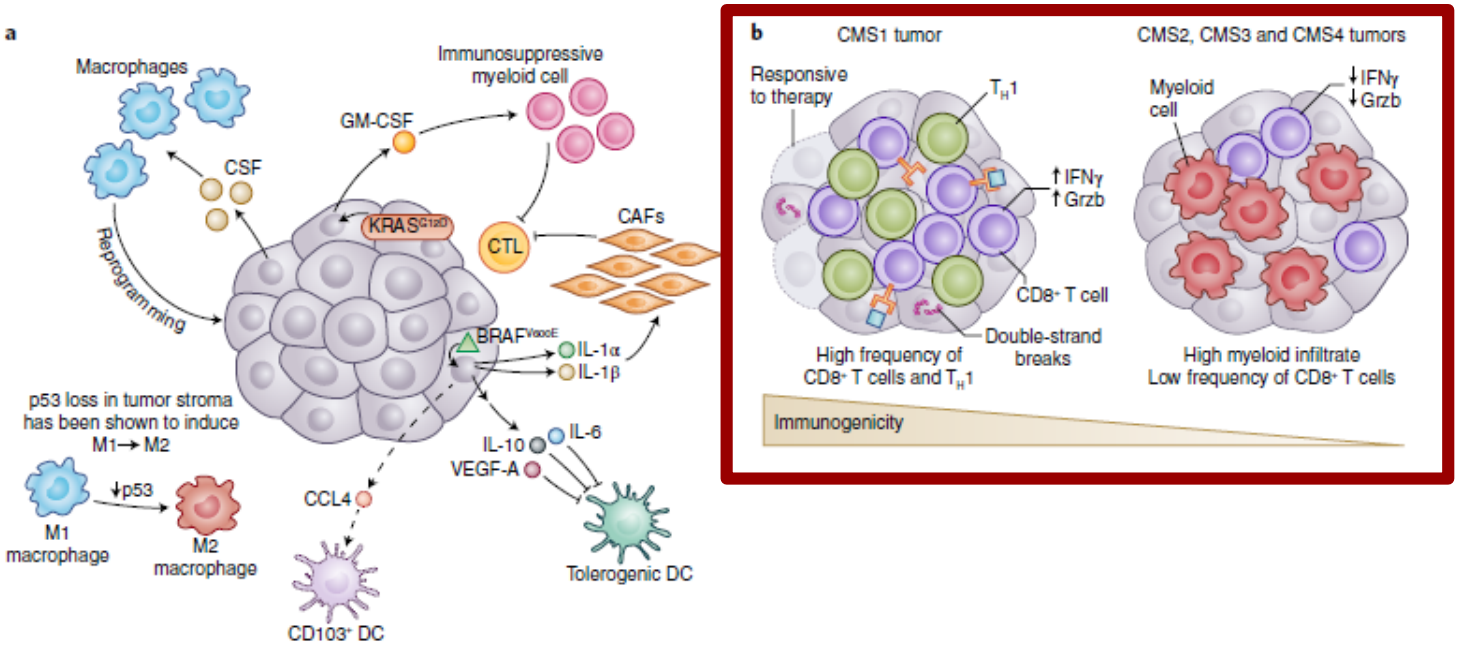
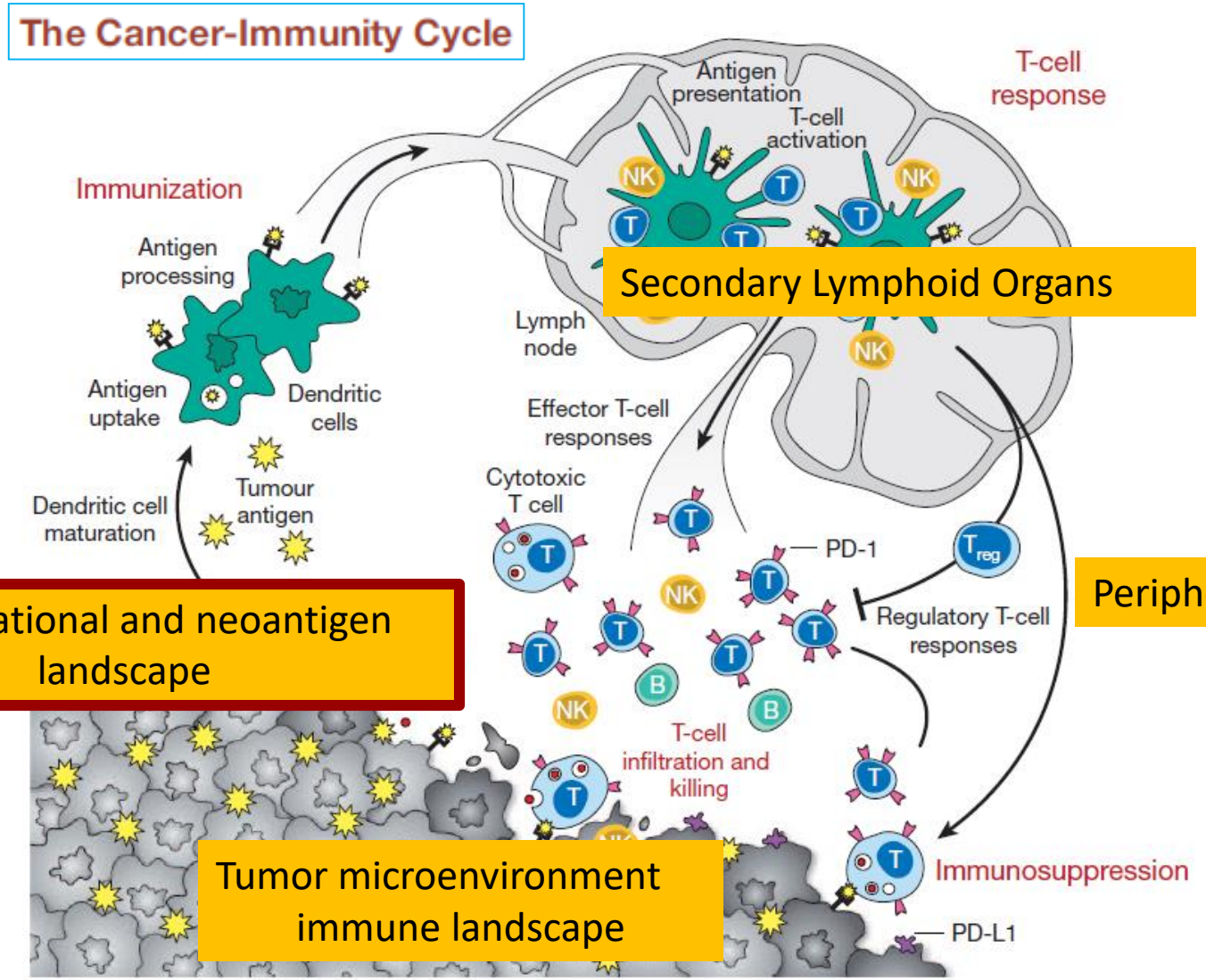


Fig. 2 | How tumor genotypes and phenotypes shape the TIME. a, Tumors are known to establish protumoral and immunosuppressive environments to support their growth and promote immune evasion. Central to building an immunosuppressive TIME are oncogenes and aberrant signaling pathways that lead to the production 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.

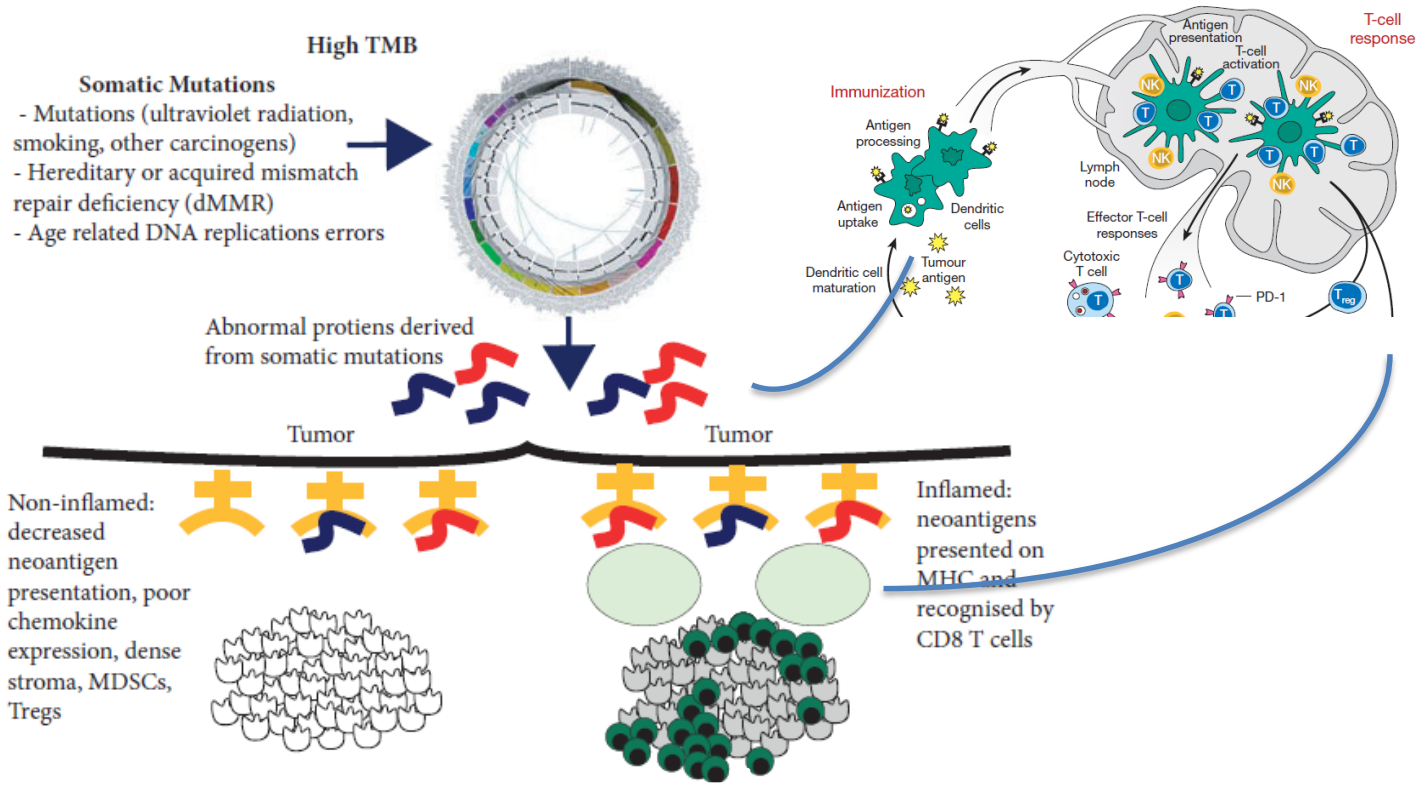
Cancer immunotherapy comes of age

Ira Mellman[1], George Coukos[2] & Glenn Dranoff[3]



Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic

T. A. Chan[1,2*], M. Yarchoan[3], E. Jaffee[3], C. Swanton[4], S. A. Quezada[5], A. Stenzinger[6,7] & S. Peters[8*]



Annals of Oncology 30: 44–56, 2019

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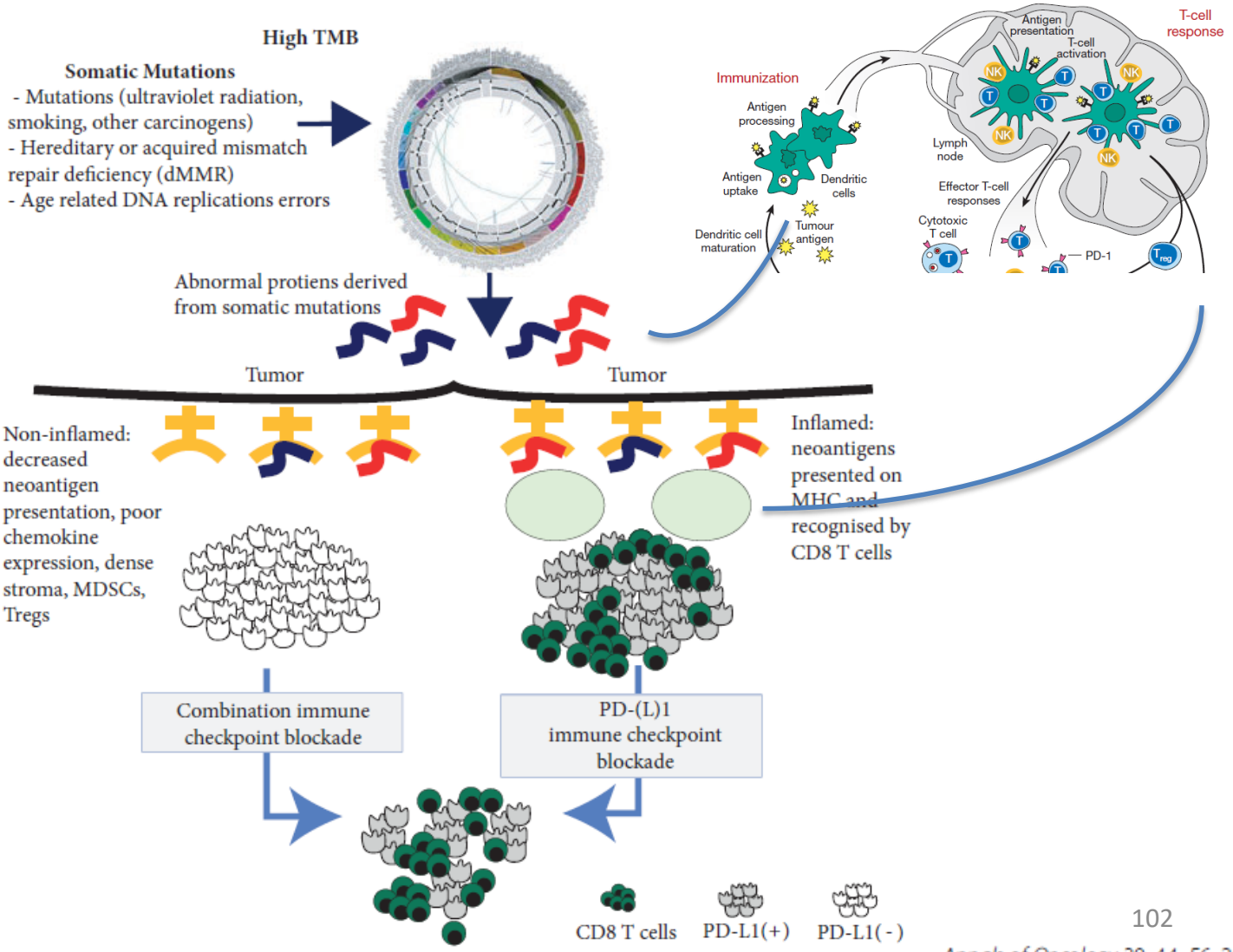

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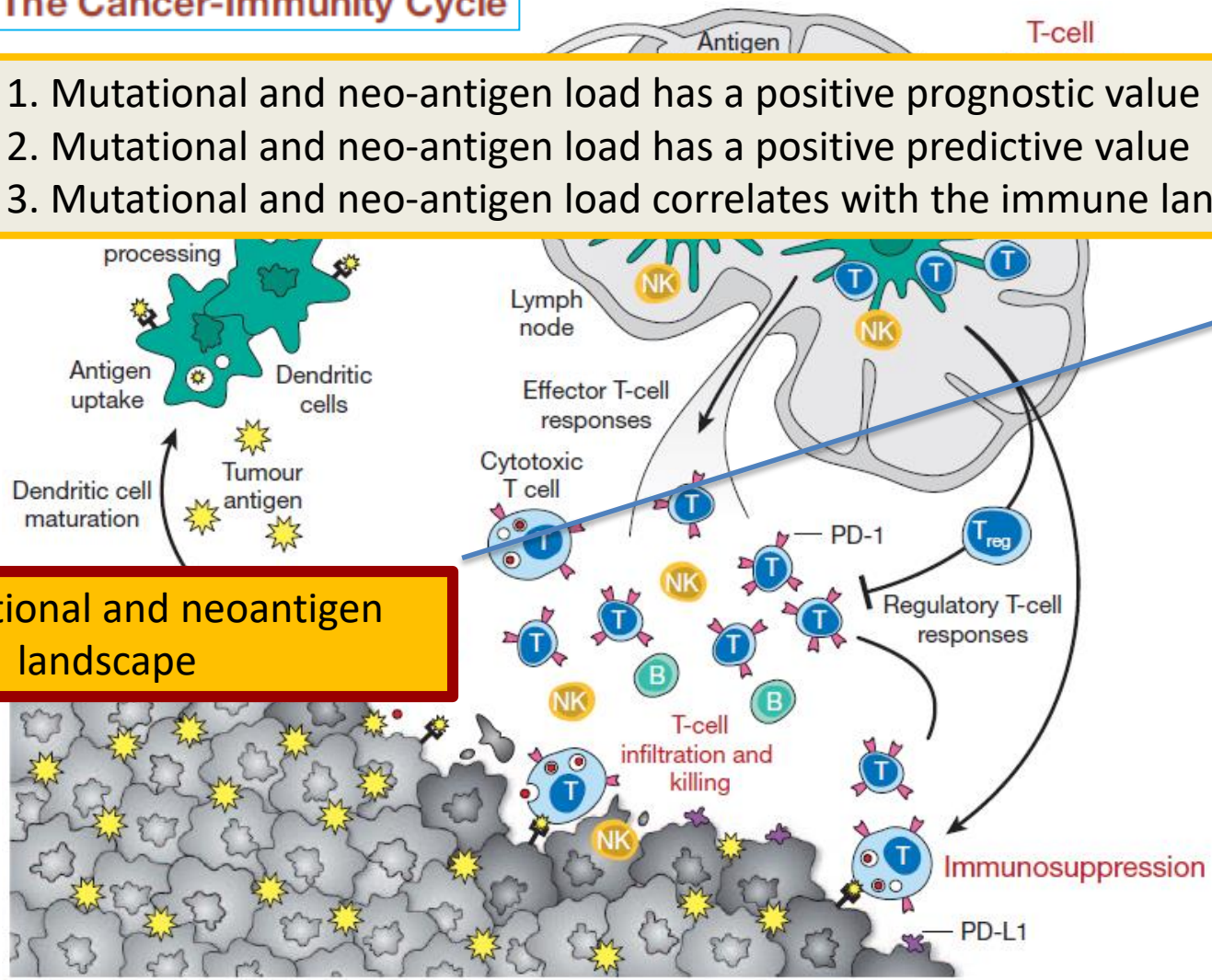
Cancer immunotherapy comes of age

Ira Mellman[1], George Coukos[2] & Glenn Dranoff[3]

1. Mutational and neo-antigen load has a positive prognostic value
2. Mutational and neo-antigen load has a positive predictive value
3. Mutational and neo-antigen load correlates with the immune landscape



Tumor mutational and neoantigen landscape

Outline

- Intoduction to the Tumor Immune Microenvironment (TIME)
  - Prognostic / Predictive value
  - Methodologies
- Mechanisms regulating TIME
- **TIME and neoplastic evolution**

How cancer evades immune system and whether and how the immune system shapes cancer genome evolution in humans

IFNγ and lymphocytes prevent primary tumour development and shape tumour immunogenicity

Vijay Shankaran*, Hiroaki Ikeda*, Allen T. Bruce*, J. Michael White*, Paul E. Swanson*, Lloyd J. Old† & Robert D. Schreiber*

NATURE | VOL 410 | 26 APRIL 2001

Adaptive immunity maintains occult cancer in an equilibrium state

Catherine M. Koebel[1], William Vermi[1,2], Jeremy B. Swann[3,4], Nadeen Zerafa[3], Scott J. Rodig[5], Lloyd J. Old[6], Mark J. Smyth[3,4]* & Robert D. Schreiber[1]*

Nature. 2007 Dec 6;450(7171):903-7.

THE THREE ES OF CANCER IMMUNOEDITING

Gavin P. Dunn,[1] Lloyd J. Old,[2] and Robert D. Schreiber[1]
[1]*Department of Pathology and Immunology, Center for Immunology, Washington University School of Medicine, St. Louis, Missouri 63110; email: schreiber@immunology.wustl.edu*
[2]*Ludwig Institute for Cancer Research, New York Branch at Memorial Sloan-Kettering Cancer Center, New York, NY 10021; email: lold@licr.org*

Annu. Rev. Immunol. 2004. 22:329–60

IFNγ and lymphocytes prevent primary tu… and shape

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Paul E. Swanson*, L…

Adaptive i… equilibrium

Catherine M. Koebel[1], …
Mark J. Smyth[3,4]* & Ro…

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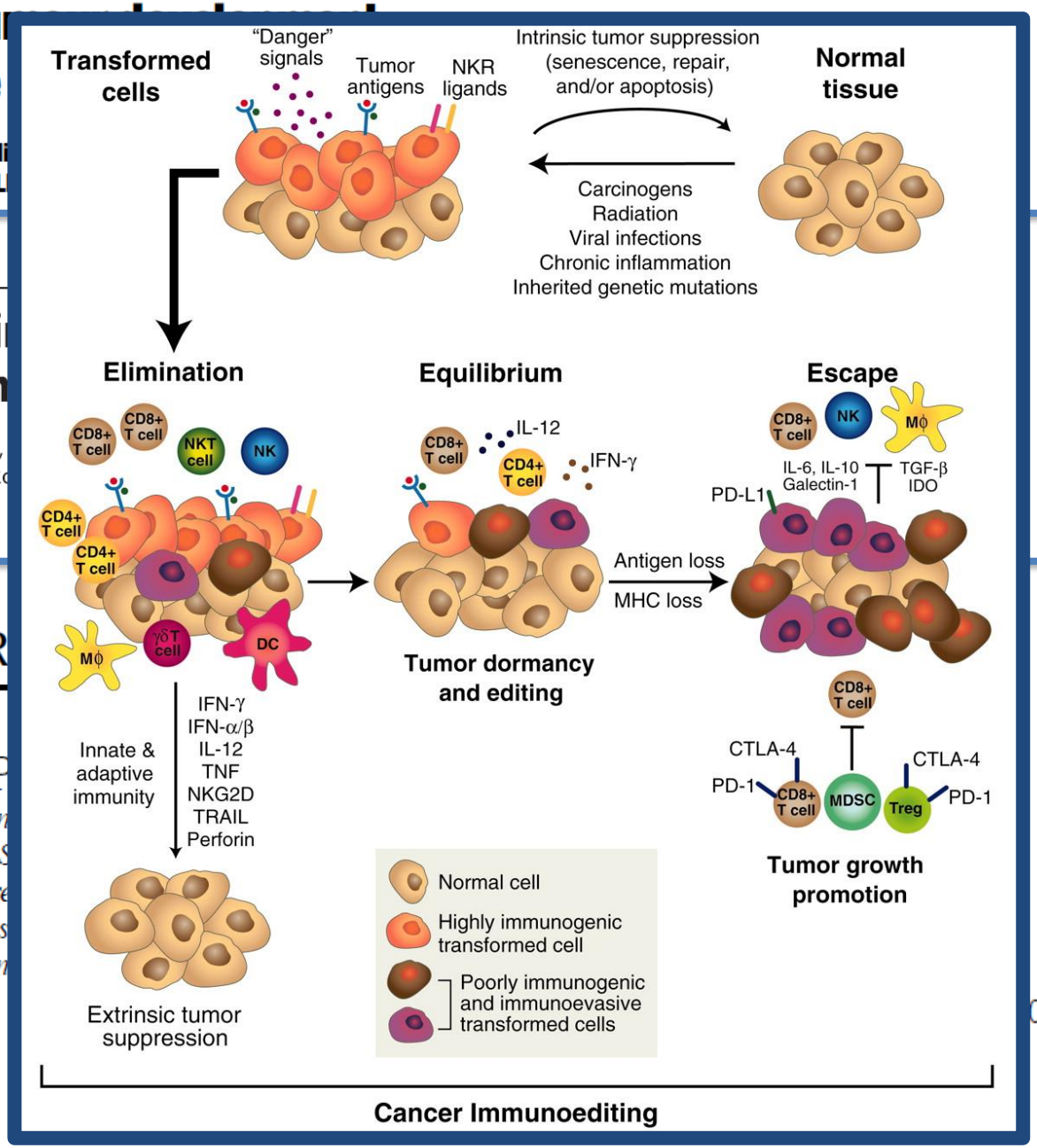


…04. 22:329–60

The causes and consequences of genetic heterogeneity in cancer evolution

Rebecca A. Burrell[1*], Nicholas McGranahan[1,2*], Jiri Bartek[3,4] & Charles Swanton[1,5]

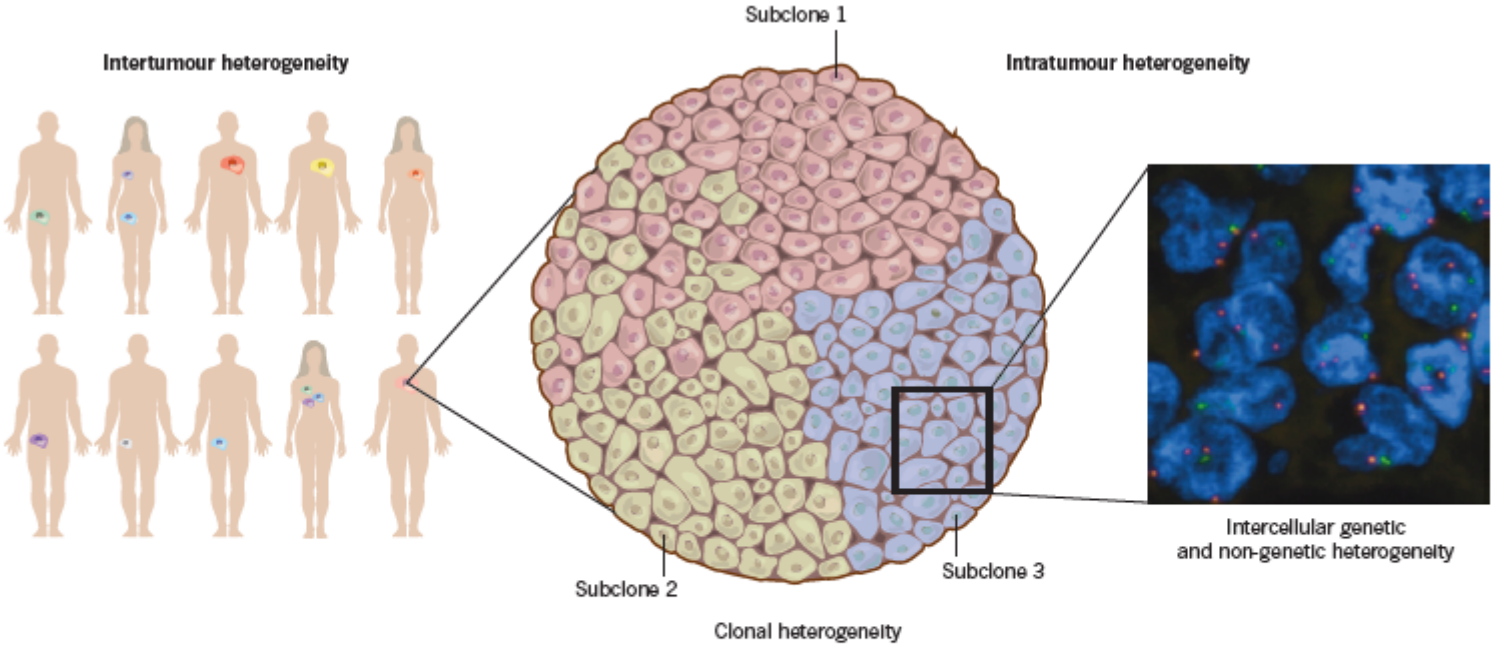
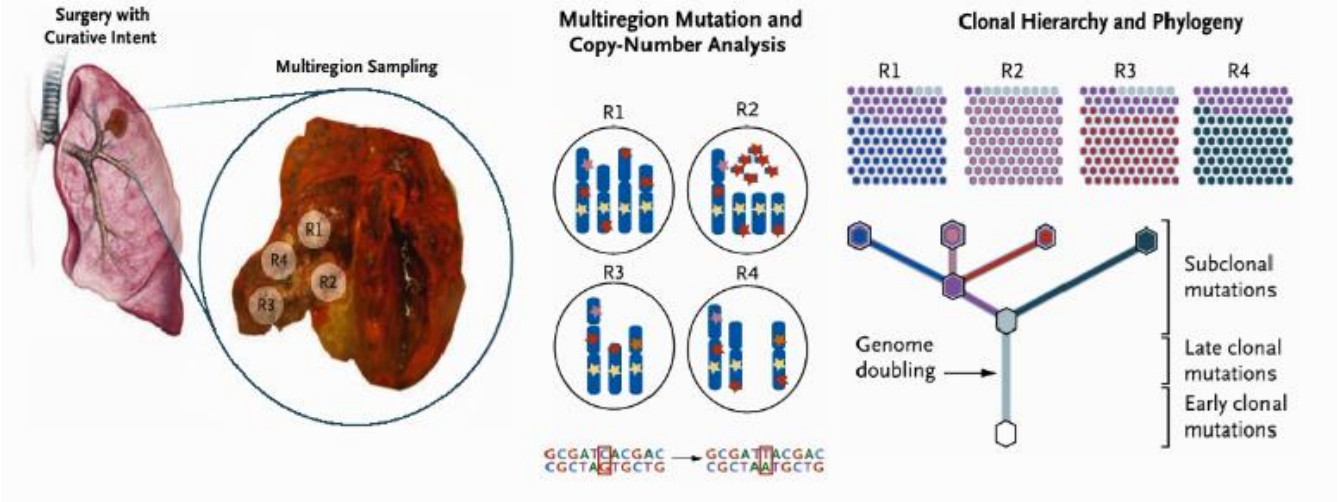
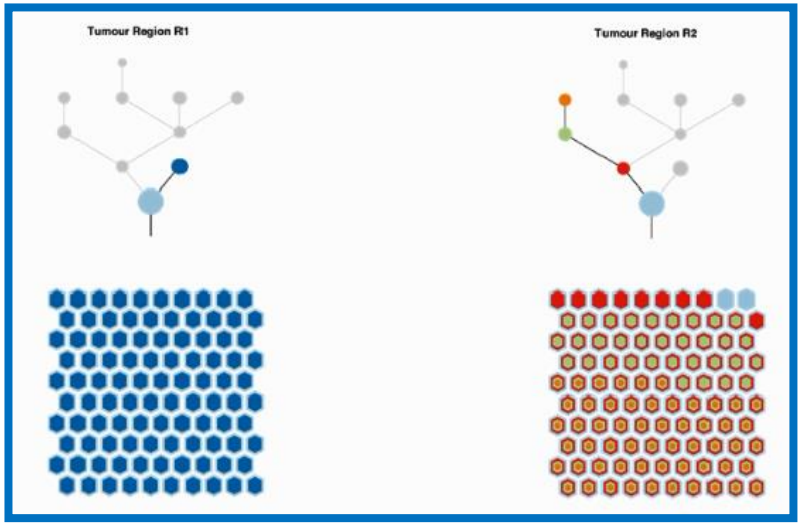


Figure 1 | Intertumour and intratumour heterogeneity. Genetic and phenotypic variation are observed between tumours of different tissue and cell types, as well as between individuals with the same tumour type (intertumour heterogeneity). Within a tumour, subclonal diversity may be observed (intratumour heterogeneity). Subclones may intermingle (as shown by subclones 1 and 2) or be spatially separated (as shown by subclone 3). Separation between subclones could reflect physical barriers such as blood vessels or micro-environmental changes. Tumour subclones may show differential gene expression due to both genetic and epigenetic heterogeneity. Within a subclonal population of tumour cells — shown here as a tumour section, hybridized to two fluorescent probes for the centromeres of two chromosomes (chromosome 2, red; chromosome 18, green) with DNA (blue) — there is intercellular genetic and non-genetic variation of, for example, chromosome copy number, somatic point mutations or epigenetic modifications that results in phenotypic diversity. Intercellular genetic heterogeneity is exacerbated by genomic instability, and may foster the emergence of tumour subclones. Genomic instability and tumour subclonal architecture may vary further over time if influenced by, for example, cancer treatment.

Multiregion sampling to map tumour evolution

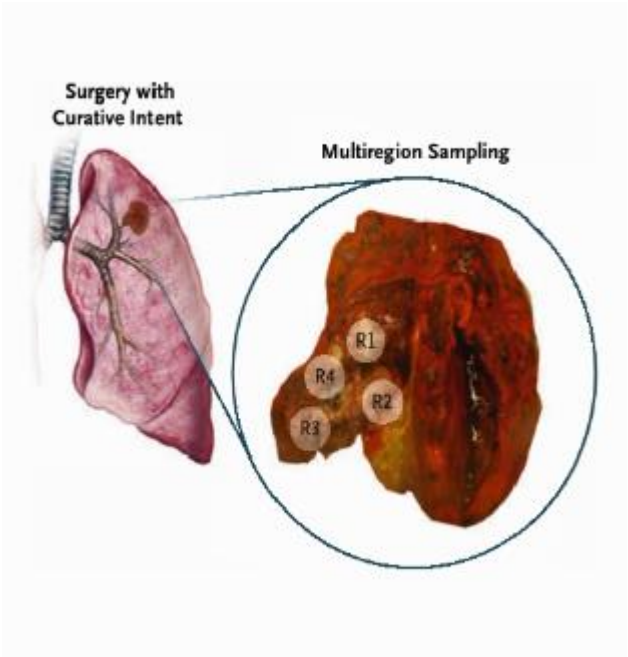




Nicholas McGranahan

Neoantigen-directed immune escape in lung cancer evolution

Rachel Rosenthal[1,2,3], Elizabeth Larose Cadieux[4,21], Roberto Salgado[5,6,21], Maise Al Bakir[3,21], David A. Moore[7,21], Crispin T. Hiley[1,3,21], Tom Lund[8,9,21], Miljana Tanić[10], James L. Reading[8,9], Kroopa Joshi[8,9], Jake Y. Henry[8,9], Ehsan Ghorani[8,9], Gareth A. Wilson[1,3], Nicolai J. Birkbak[1,3], Mariam Jamal-Hanjani[1], Selvaraju Veeriah[1], Zoltan Szallasi[11,12], Sherene Loi[6], Matthew D. Hellmann[13,14], Andrew Feber[15], Benny Chain[16,17], Javier Herrero[2], Sergio A. Quezada[1,8,9], Jonas Demeulemeester[4,18], Peter Van Loo[4,18], Stephan Beck[10], Nicholas McGranahan[1,19*], Charles Swanton[1,3*] & The TRACERx consortium[20]



Multi-region RNAseq
172 tumour regions, 64 patients

Multi-region pathology TIL estimates
234 tumour regions, 88 patients

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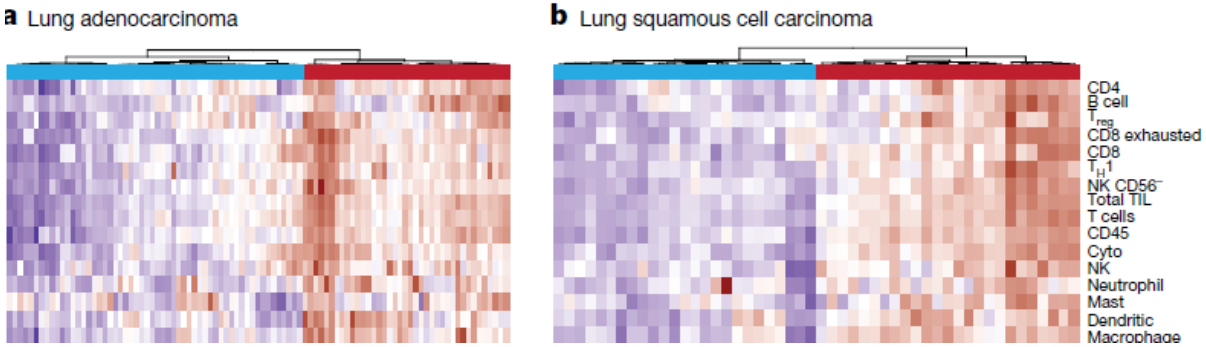

Fig. 1 | Heterogeneity of immune infiltration in NSCLC. a, **b**, TRACERx regions from lung adenocarcinoma (**a**) and lung squamous cell carcinoma (**b**) are shown, clustered by the level of estimated immune infiltrate. Each row represents an immune cell population, as estimated by the method used by Danaher et al.[11]. Immune populations are $CD4^+$ T cells (CD4), B cells, regulatory T cells (T_{reg}), exhausted $CD8^+$ T cells (CD8 exhausted), $CD8^+$ T cells (CD8), helper T cells (T_H1), natural killer $CD56^-$ cells (NK $CD56^-$), total TIL score (total TIL), total T cells (T cells), $CD45^+$ cells (CD45), cytotoxic cells (cyto), natural killer cells (NK), neutrophils, mast cells (mast), dendritic cells (dendritic) and macrophages. Each column represents a tumour region. Regions classified as having low levels of immune infiltration (low immune) are shown in blue; regions classified as having high levels of immune infiltration (high immune) are shown in red. If all the regions of a patient's tumour are classified as having low levels of immune infiltration, the patient is indicated in blue. If all the regions of a patient's tumour are classified as having high levels of immune infiltration, the patient is indicated in red. Patients who have tumours that contain heterogeneous levels of immune infiltration are indicated in orange. An example pathology image from a heterogeneous tumour is shown below each heat map to display a region with a high level of immune infiltration and a region with a low level of immune infiltration from the same tumour.

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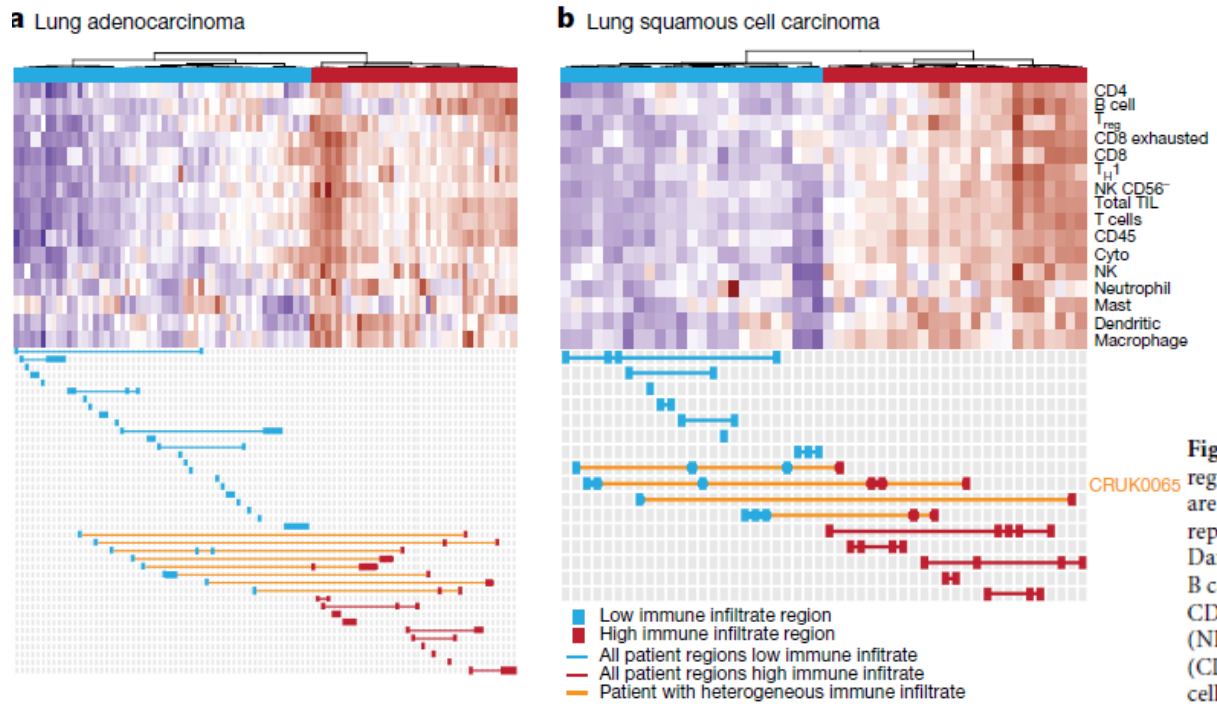

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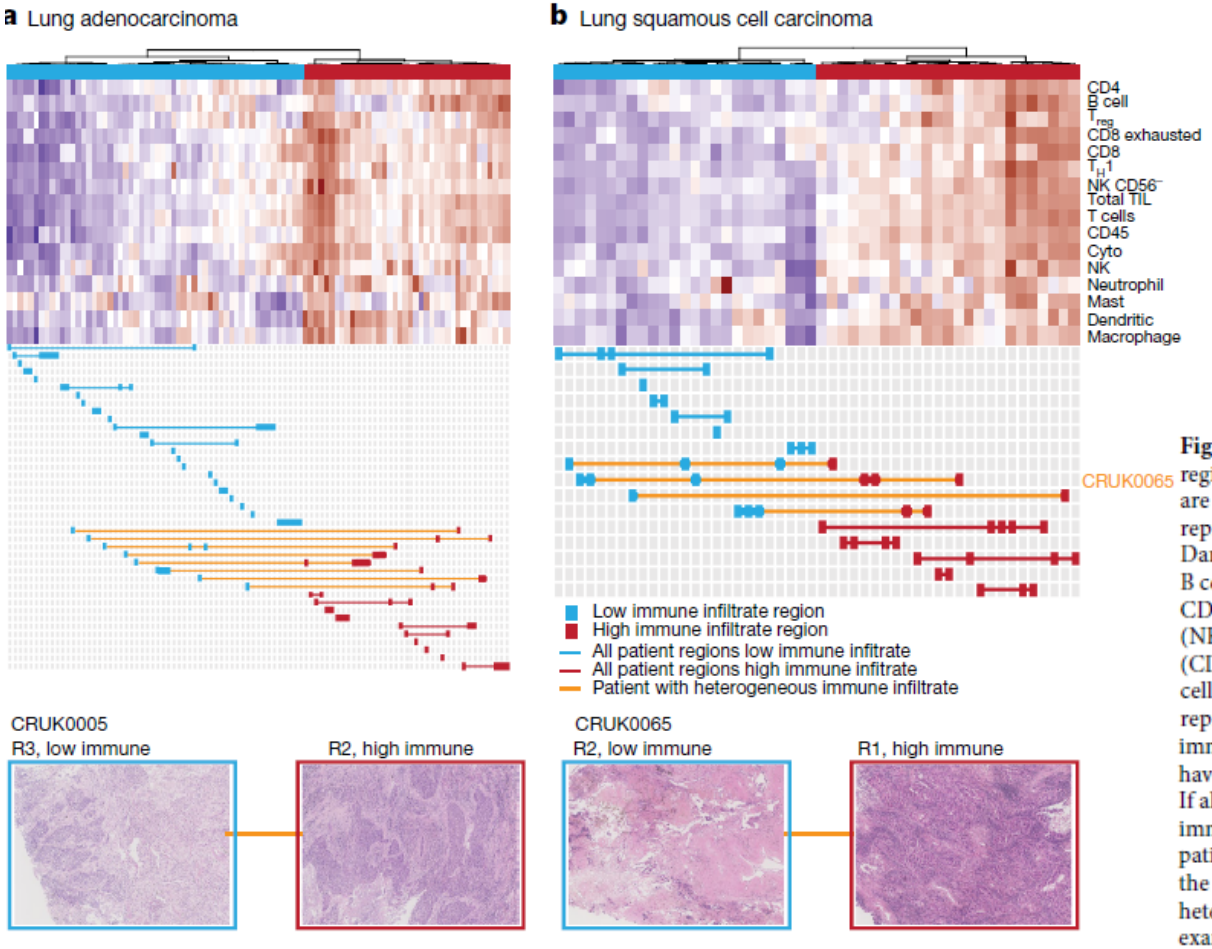

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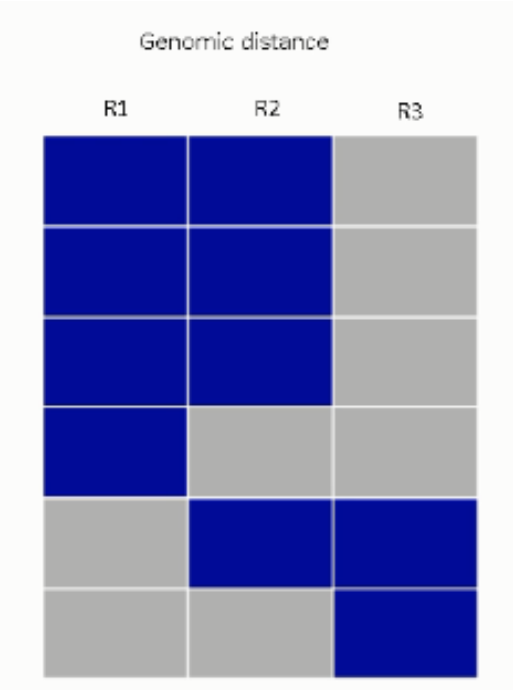
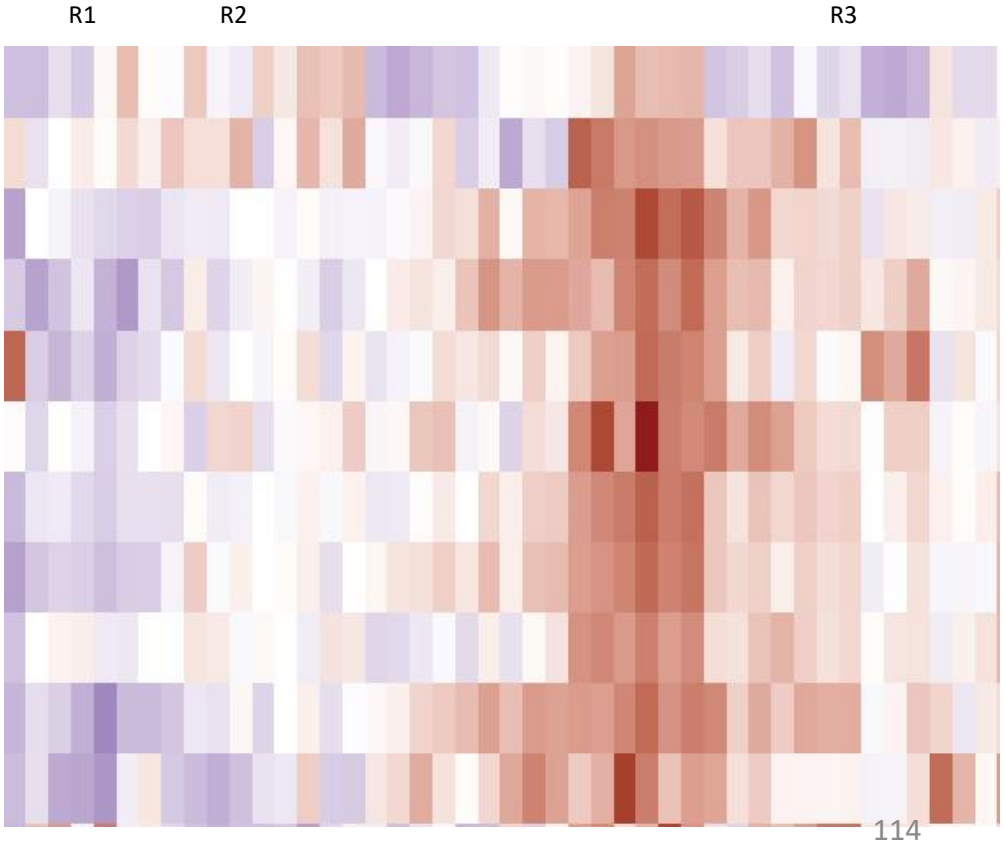
Is there any relationship between genomic heterogeneity and the TIME?



Immune landscape heatmap



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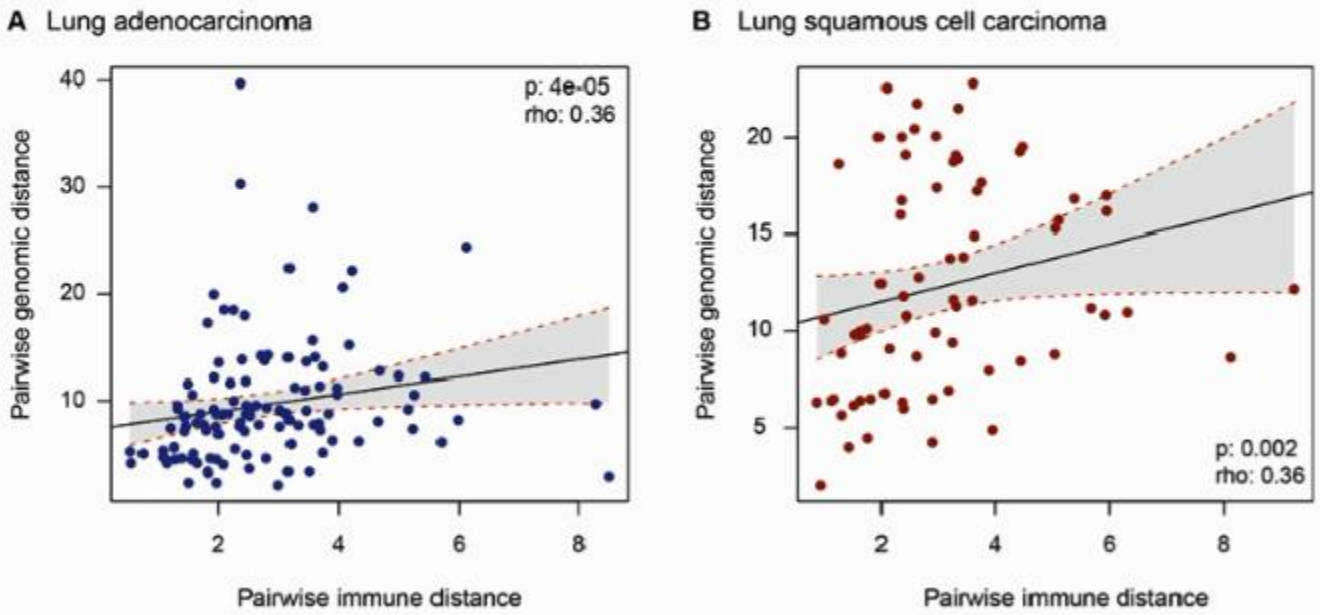
Genomically similar tumour regions share similar microenvironments



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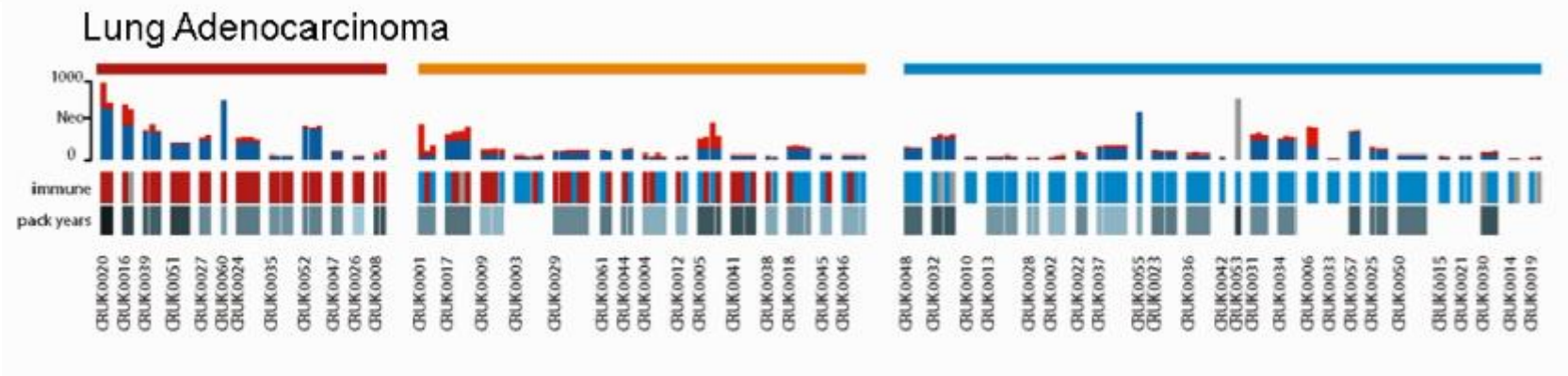
The heterogeneous neo-antigen landscape in NSCLC



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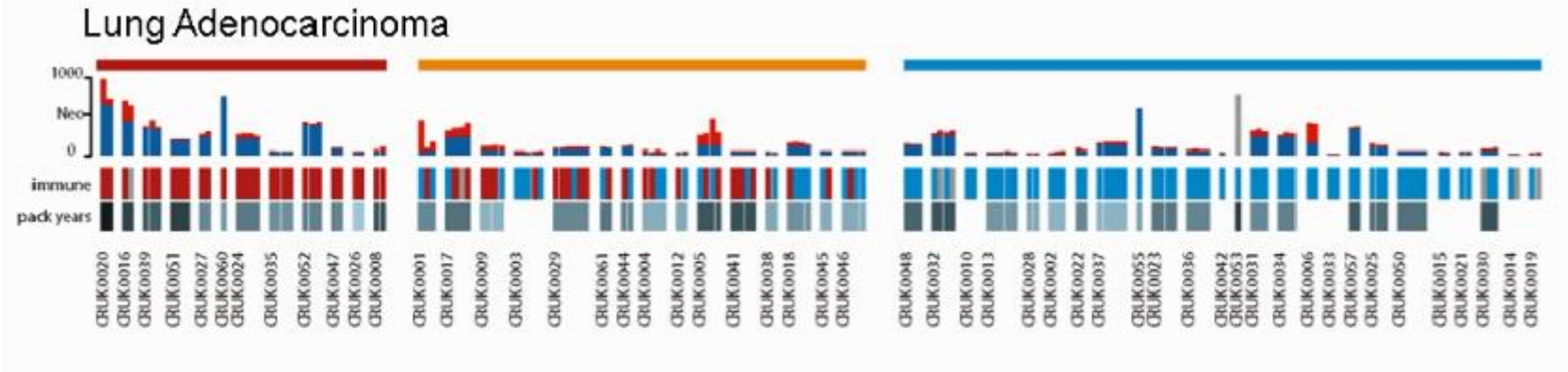
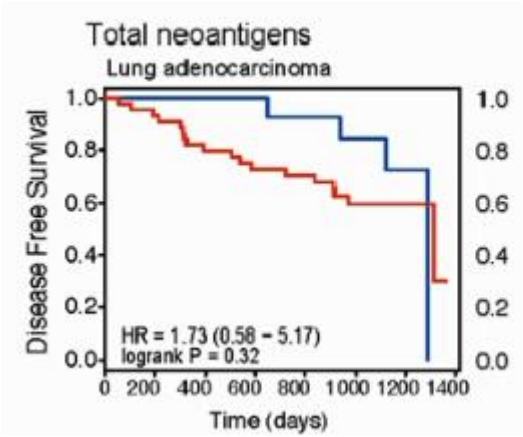
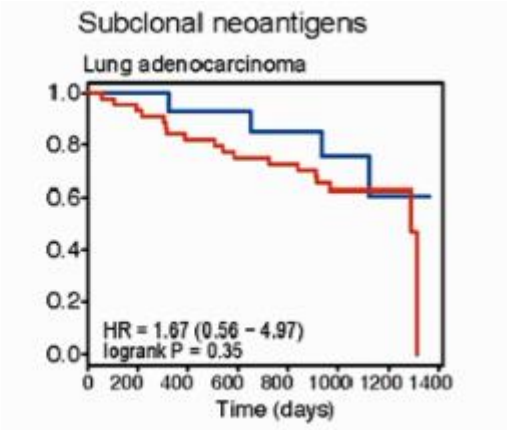
The heterogeneous neo-antigen landscape in NSCLC







Neoantigen-directed immune escape in lung cancer evolution

Rachel Rosenthal[1,2,3], Elizabeth Larose Cadieux[4,21], Roberto Salgado[5,6,21], Maise Al Bakir[3,21], David A. Moore[7,21], Crispin T. Hiley[1,3,21], Tom Lund[8,9,21], Miljana Tanić[10], James L. Reading[8,9], Kroopa Joshi[8,9], Jake Y. Henry[8,9], Ehsan Ghorani[8,9], Gareth A. Wilson[1,3], Nicolai J. Birkbak[1,3], Mariam Jamal-Hanjani[1], Selvaraju Veeriah[1], Zoltan Szallasi[11,12], Sherene Loi[6], Matthew D. Hellmann[13,14], Andrew Feber[15], Benny Chain[16,17], Javier Herrero[2], Sergio A. Quezada[1,8,9], Jonas Demeulemeester[4,18], Peter Van Loo[4,18], Stephan Beck[10], Nicholas McGranahan[1,19*], Charles Swanton[1,3*] & The TRACERx consortium[20]

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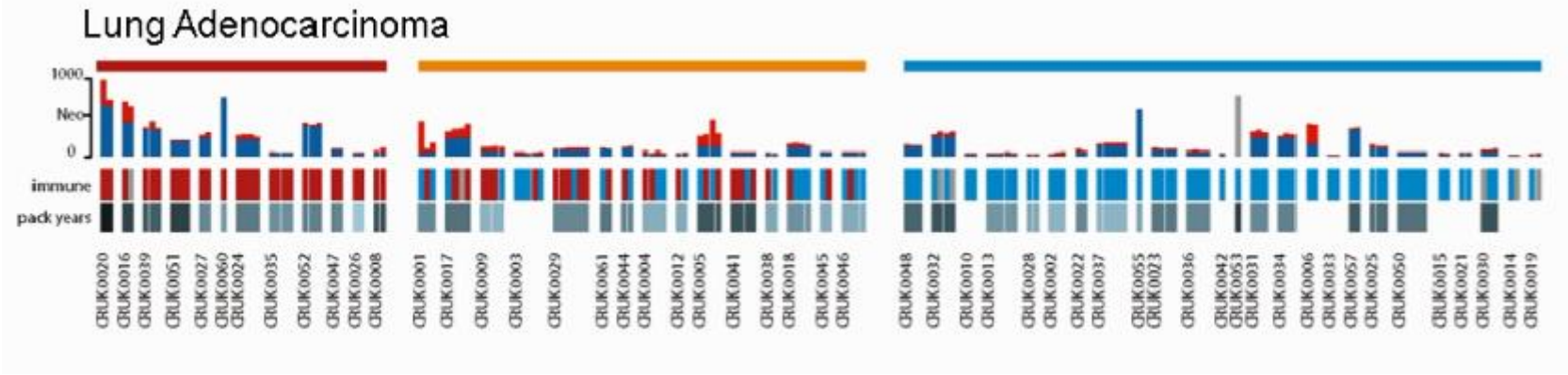

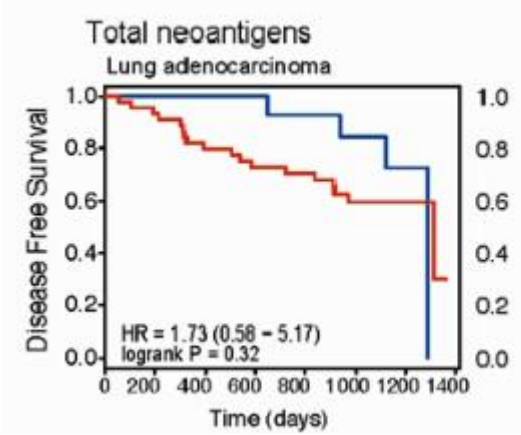

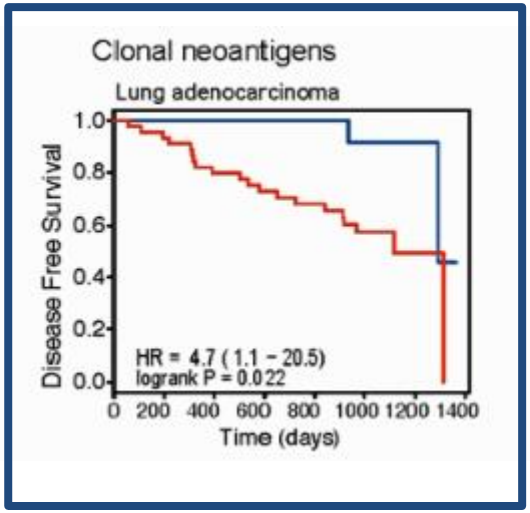

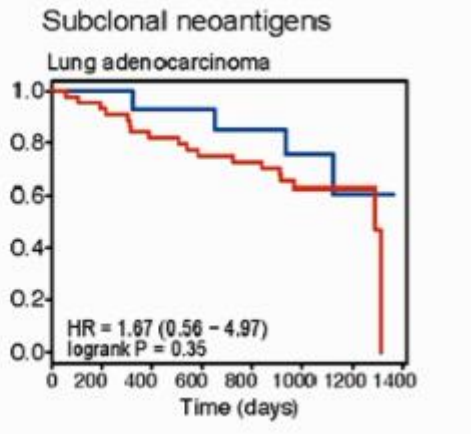

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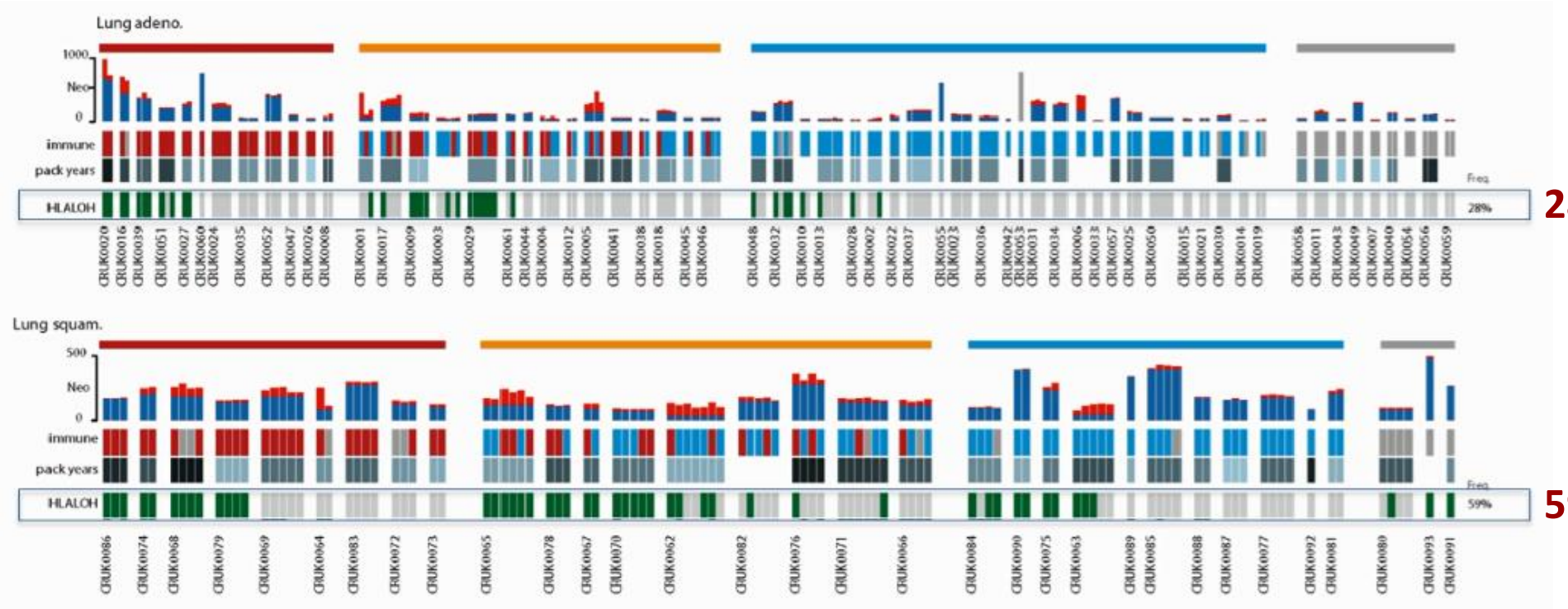
How tumours escape immune surveilance?



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Neoantigen-directed immune escape in lung cancer evolution

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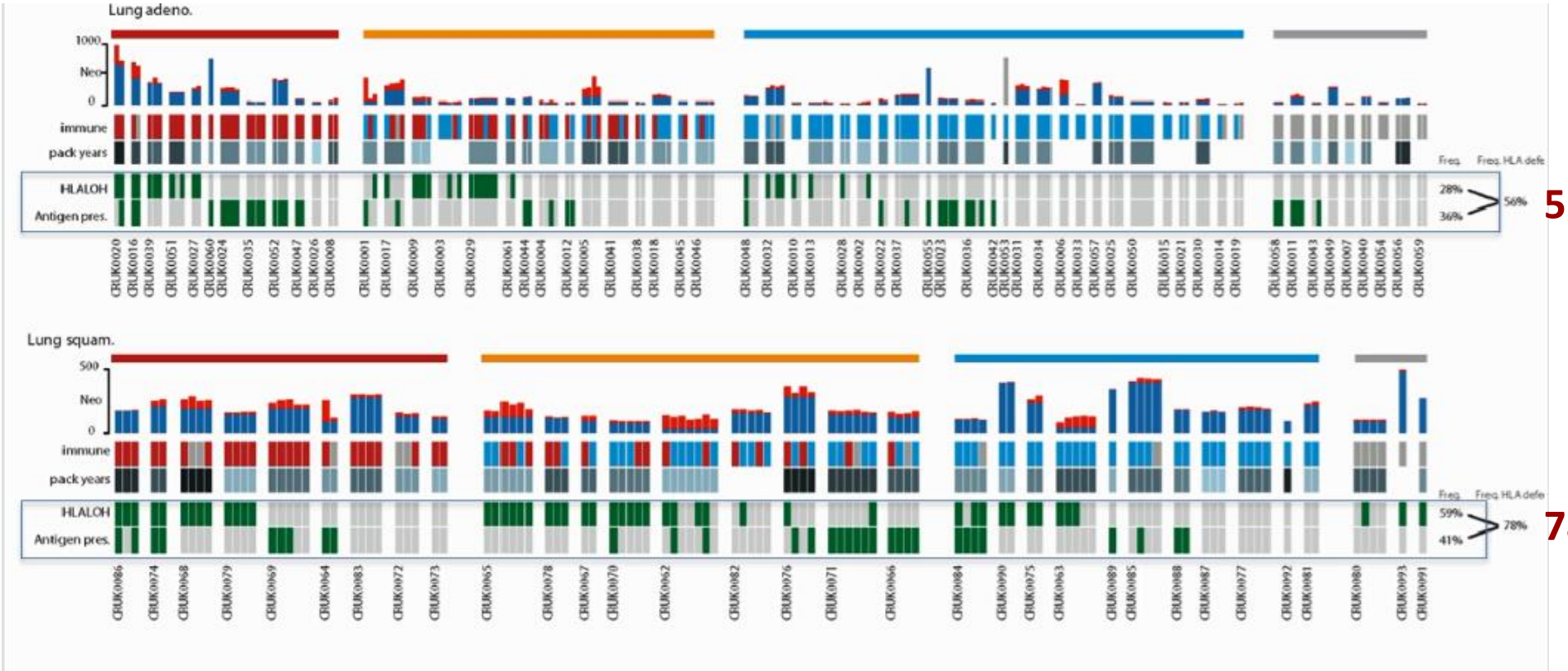

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Is there negative selection against neoantigens?

(only expressed peptides can result in neoantigens)

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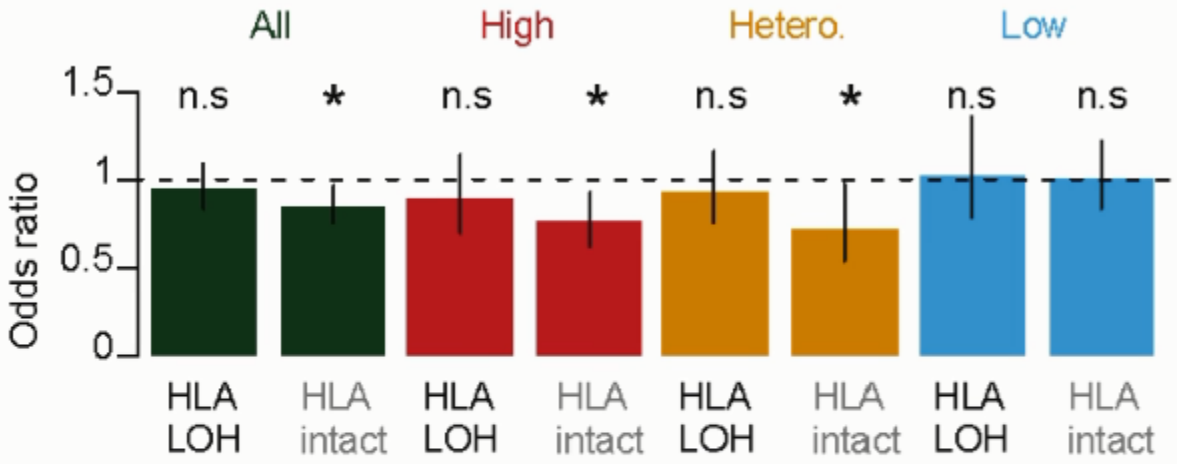

Depletion of expressed neoantigens in hot tumours with intact HLA alleles

Neoantigen-directed immune escape in lung cancer evolution

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Low immune evasion

High immune infiltration or no immune escape

- No immune editing
- No HLA LOH
- No antigen-processing defect

High immune evasion

Low/mixed immune infiltration and immune escape

- Immune editing /
- HLA LOH /
- Antigen-processing defect

Nicholas McGranahan

Neoantigen-directed immune escape in lung cancer evolution

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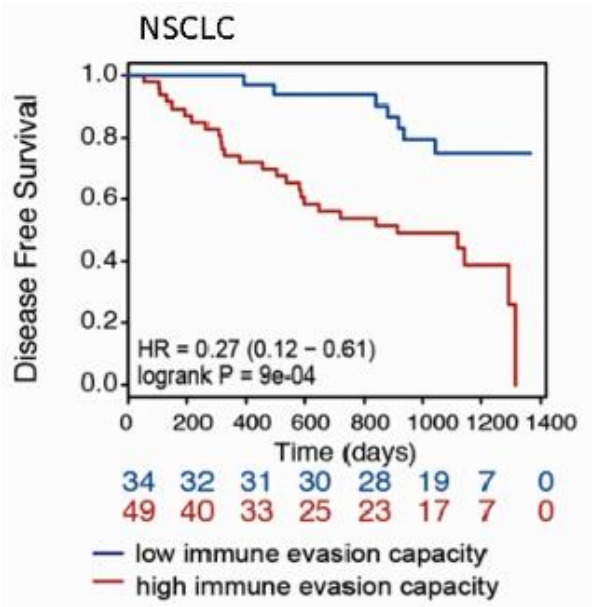

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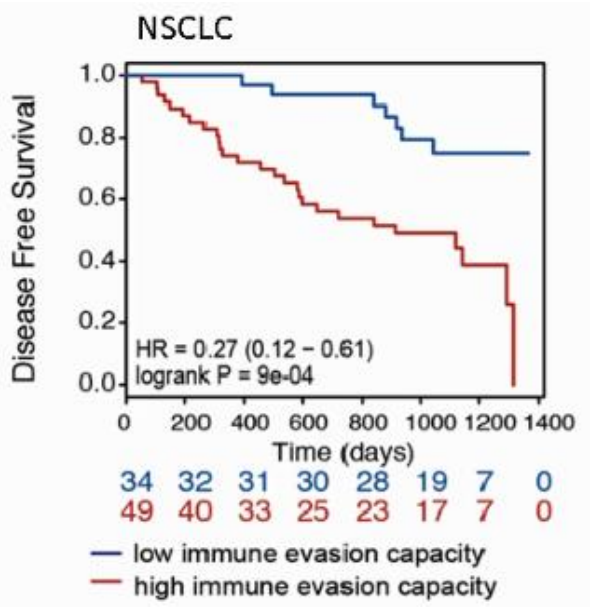
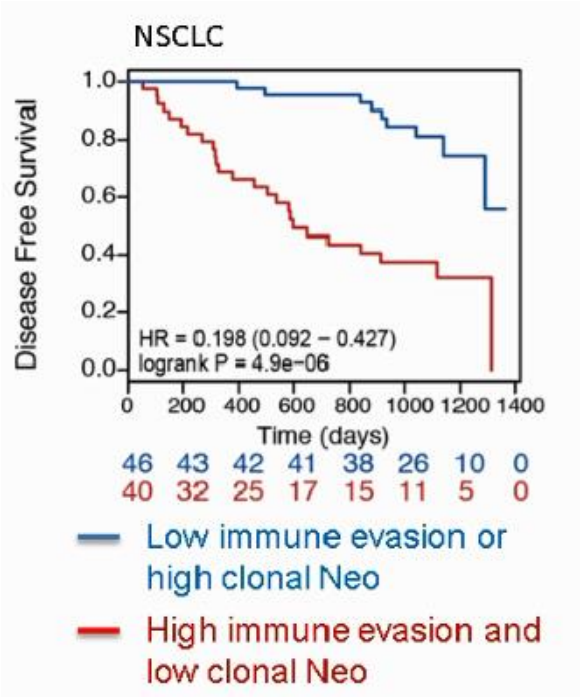
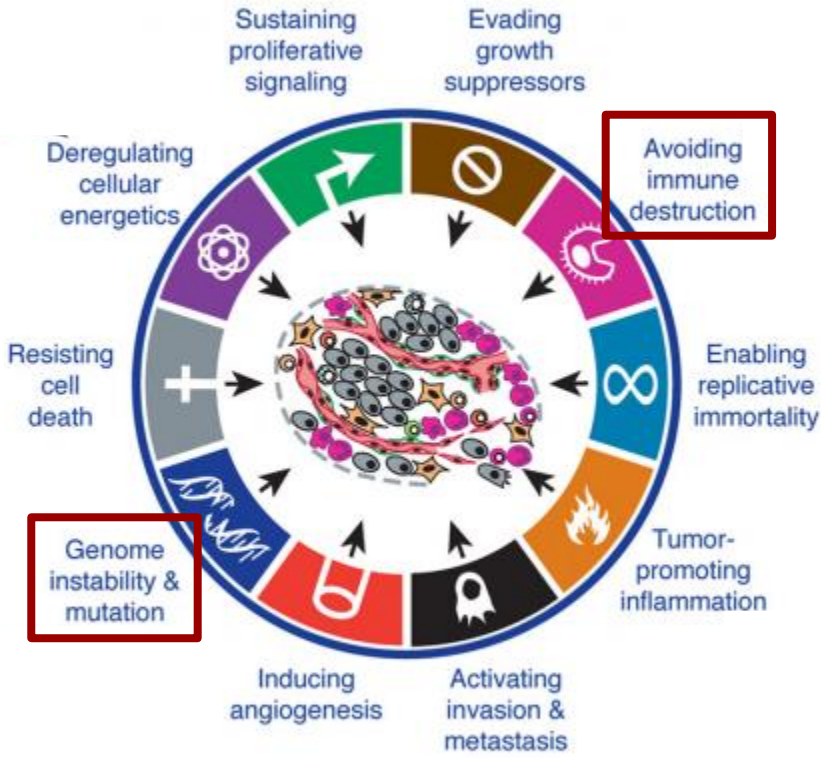




Hallmarks of Cancer: The Next Generation

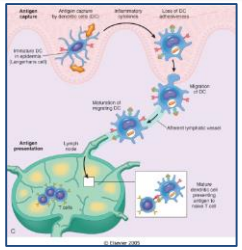
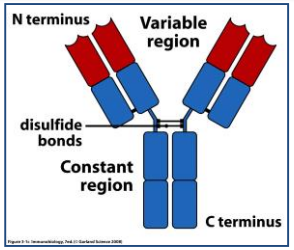
Douglas Hanahan[1,2,*] and Robert A. Weinberg[3,*]
[1]The Swiss Institute for Experimental Cancer Research (ISREC), School of Life Sciences, EPFL, Lausanne CH-1015, Switzerland
[2]The Department of Biochemistry & Biophysics, UCSF, San Francisco, CA 94158, USA
[3]Whitehead Institute for Biomedical Research, Ludwig/MIT Center for Molecular Oncology, and MIT Department of Biology, Cambridge, MA 02142, USA

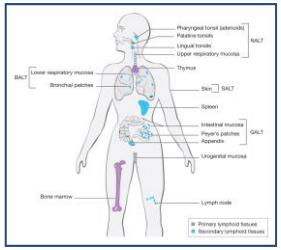
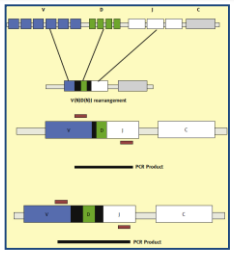
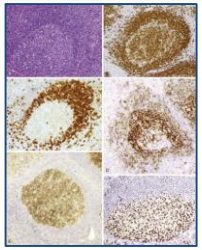
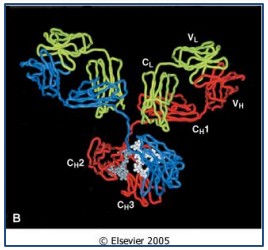
Cell *144*, March 4, 2011

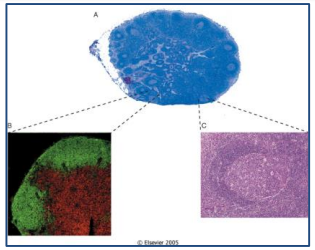


Conclusion

Close interaction between the immune and genomic landscape and emphasize the strong selection pressure that the immune system imposes upon tumour evolution



ΜΙΚΡΟΠΕΡΙΒΑΛΛΟΝ, ΑΝΟΣΙΑΚΗ ΑΠΑΝΤΗΣΗ ΣΤΗ ΝΕΟΠΛΑΣΙΑ

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

ΕΥΧΑΡΙΣΤΩ