Immunoscore Update

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Knight Cancer Institute, OHSU, Portland, Oregon , USA

I have Consultant/Advisory Roles or Research support/Grant to disclose.

Bristol-Myers Squibb, MannKind, Aduro, Immunophotonics, Dendreon, 3M, Ventana/Roche, Nodality, Definiens, Janssen/Johnson & Johnson, PerkinElmer, MedImmune/AstraZeneca, Viralytics, Immune Design, Macrogenics

Yes, I have a Leadership Position / Stock Ownership to disclose.

UbiVac, UbiVac-CMV, Insys Ther

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The practice of oncology is undergoing a transformation.

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The practice of oncology is undergoing a transformation.

Why?

New Paradigm

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- The immune system is the "agent" that improves outcome and **CURES** people with metastatic cancer.

New Paradigm

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- The immune system is the "agent" that improves outcome and **CURES** people with metastatic cancer.

- **Fundamental shift in our understanding of cancer.**

New Paradigm

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- The immune system is the "agent" that improves outcome and **CURES** people with metastatic solid cancer.
- **Fundamental shift in our understanding of cancer.**

CONTROVERSIAL...

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Breaking Through Cancer's Shield

Is the cure for cancer inside you?

Immunotherapy Cancer Drug Data Show Promise in Prolonging Lives

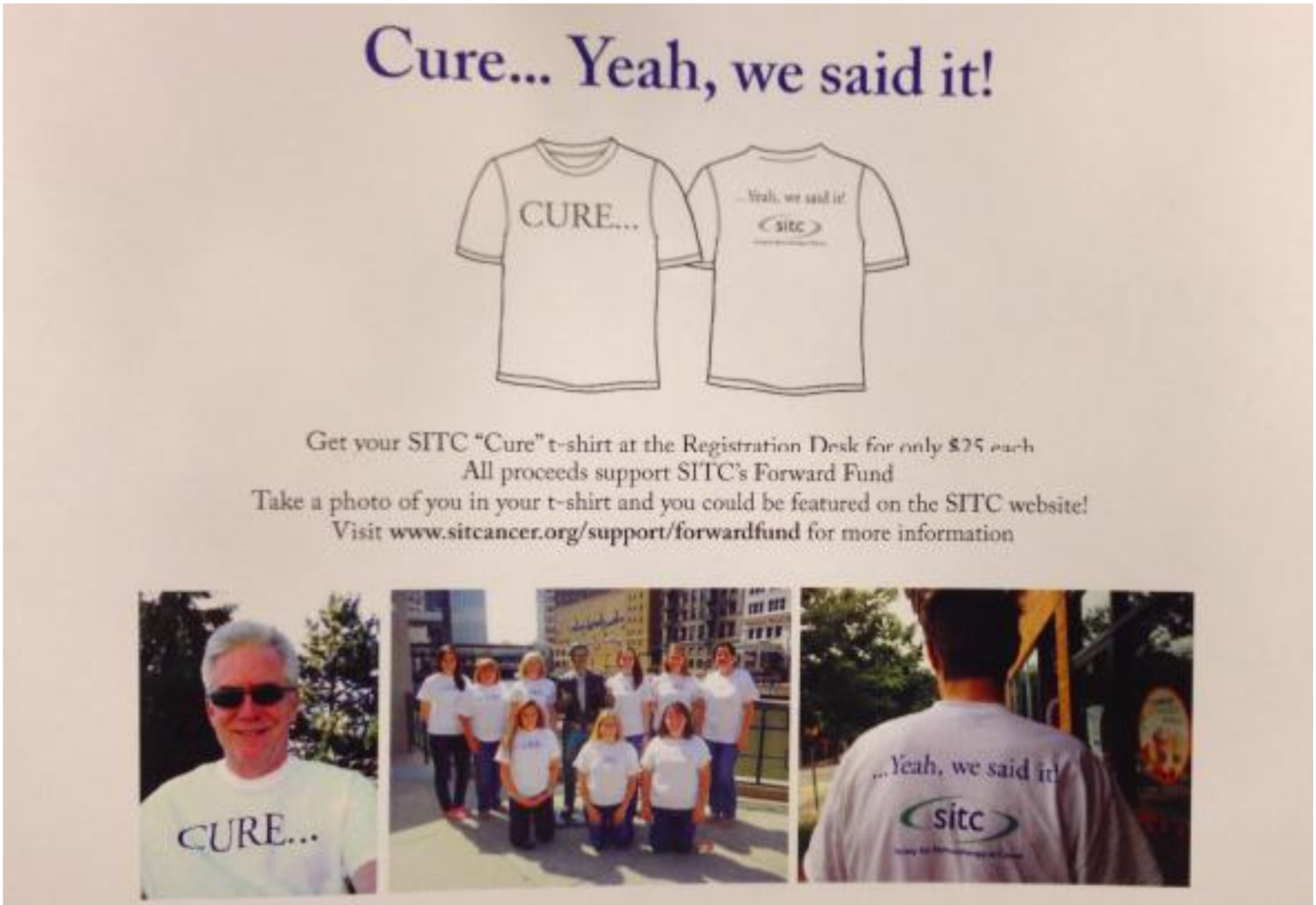
by Veronica Smith

Researchers report progress in cancer immunotherapy

Source: The New York Times, Los Angeles Times

SITC Immunoscore Taskforce!

Cure…. Yeah, we said it!!!!!



CURE

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- **IL-2 +/- TIL**
- **Anti-CTLA-4**
- **Anti-PD-1 / anti-PD-L1**
- **Chimeric Antigen Receptor (CAR)**

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†]

The role of the adaptive immune response in controlling the growth and recurrence of human tumors has been controversial. We characterized the tumor-infiltrating immune cells in large cohorts of human colorectal cancers by gene expression profiling and in situ immunohistochemical staining. Collectively, the immunological data (the type, density, and location of immune cells within the tumor samples) were found to be a better predictor of patient survival than the histopathological methods currently used to stage colorectal cancer. The results were validated in two additional patient populations. These data support the hypothesis that the adaptive immune response influences the behavior of human tumors. In situ analysis of tumor-infiltrating immune cells may therefore be a valuable prognostic tool in the treatment of colorectal cancer and possibly other malignancies.

Landmark Article

-> **Top 0.1%** most frequently cited research article in all disciplines (ESI, Essential Science Indicators)

29 SEPTEMBER 2006 VOL 313 SCIENCE

Jerome Galon and Franck Pagès used digital imaging and objectively assessed immune infiltrates – IM vs Tumor (Science 2006).

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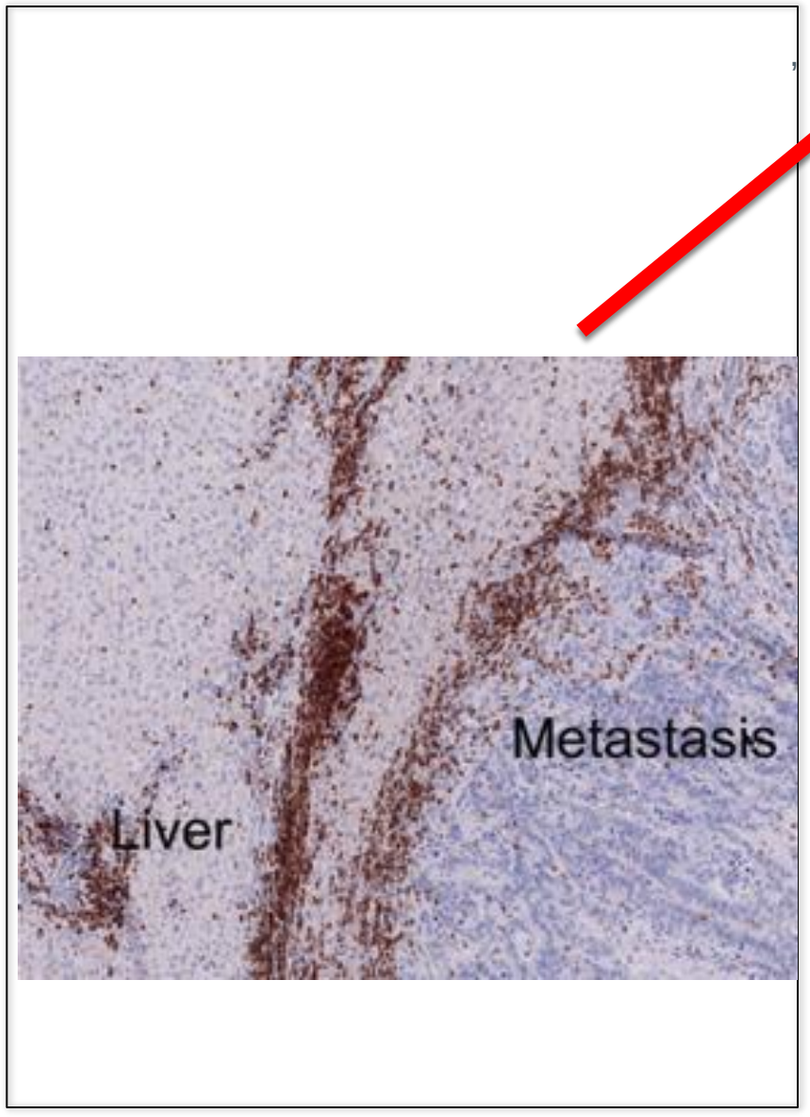
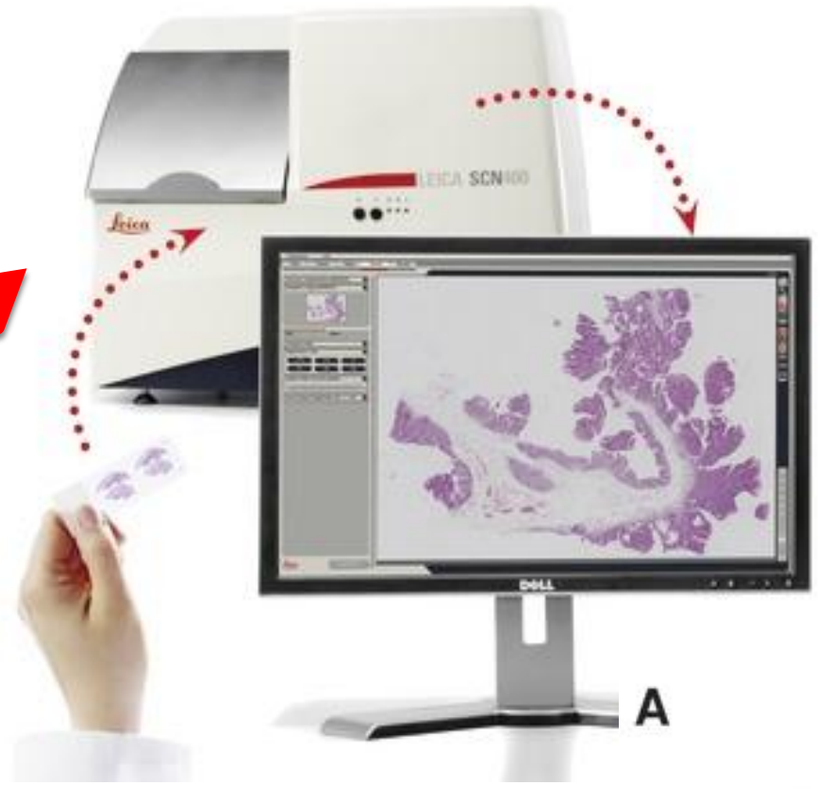
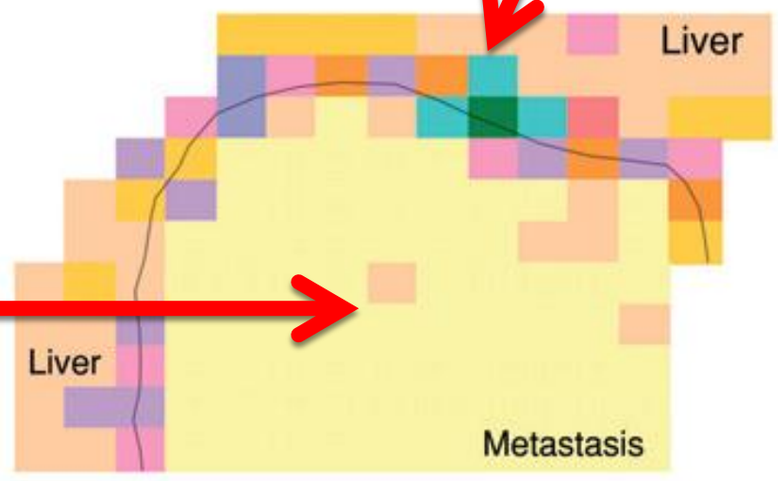




Invasive Margin

Tumor

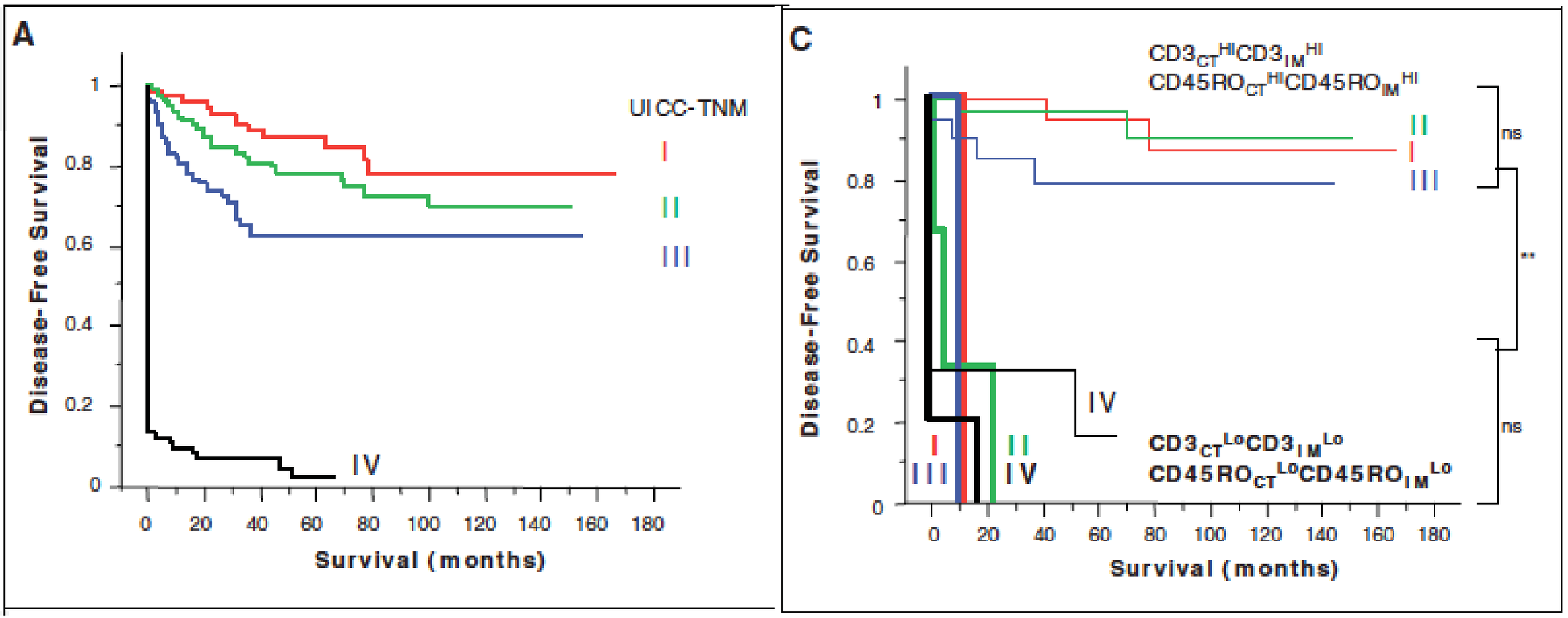


Halama N, et al. Can Res 71:5670 2011

Coordinated adaptive immune response more than tumor invasion predicts outcome.

UICC-TNM Staging system

$CD3_{CT}CD3_{IM}$ evaluation ± $CD45RO_{CT}CD45RO_{IM}$ evaluation



29 SEPTEMBER 2006 VOL 313
SCIENCE

VOLUME 29 · NUMBER 6 · FEBRUARY 20 2011

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Histopathologic-Based Prognostic Factors of Colorectal Cancers Are Associated With the State of the Local Immune Reaction

Bernhard Mlecnik, Marie Tosolini, Amos Kirilovsky, Anne Berger, Gabriela Bindea, Tchao Meatchi, Patrick Bruneval, Zlatko Trajanoski, Wolf-Herman Fridman, Franck Pagès, and Jérôme Galon

Patients and Methods

We studied the intratumoral immune infiltrates in the center of the tumor and in the invasive margin of 599 specimens of stage I to IV colorectal cancers from two independent cohorts. We analyzed these findings in relation to the degree of tumor extension and to the frequency of recurrence.

Conclusion

Assessment of $CD8^+$ cytotoxic T lymphocytes in combined tumor regions provides an indicator of tumor recurrence beyond that predicted by AJCC/UICC-TNM staging.

Multivariate proportional hazard COX analysis among all patients with AJCC/UICC-TNM Stage I/II/III colorectal cancer

According to clinical parameters and immune parameters

COX analysis for DFS	HR	Log Rank *P*-values
Tumor (T) stage	1.24	0.29
N Stage	1.31	0.17
Gender	1.47	0.18
Number of total lymph nodes	1.13	0.68
Histological grade	0.69	0.29
Mucinous Colloide	1.29	0.47
Occlusion	1.03	0.94
Perforation	4.03	0.0084
Immune Score	**0.65**	**0.0003**

According to AJCC/UICC-TNM classification and immune score

COX analysis	DFS		OS		DSS	
	HR	*P*-value	HR	*P*-value	HR	*P*-value
AJCC/UICC-TNM	1.38	0.09 ns	1.18	0.29 ns	1.43	0.10 ns
Immune Score	0.64	**<0.0001**	0.71	**<0.0001**	0.63	**<0.0001**

-> Validation in 2 independent cohorts of colorectal cancer patients

Mlecnik et al. ***J Clin Oncol*** 2011

VOLUME 29 · NUMBER 6 · FEBRUARY 20 2011

JOURNAL OF CLINICAL ONCOLOGY

EDITORIALS

TNM Staging in Colorectal Cancer: T Is for T Cell and M Is for Memory

Elizabeth K. Broussard and Mary L. Disis, *Tumor Vaccine Group, Center for Translational Medicine in Women's Health, University of Washington, Seattle, WA*

SITC Immunoscore Taskforce!

Cancer classification using the Immunoscore: a worldwide task force

Galon *et al.*

BioMed Central

Galon *et al. Journal of Translational Medicine* 2012, **10**:205
http://www.translational-medicine.com/content/10/1/205

SITC Immunoscore Taskforce!

GOALS:

- Validate immunoscore as a prognostic biomarker

- Modify TNM Classification. AJCC

SITC Immunoscore Taskforce!

Steering Committee

Jerome Galon

Bernie Fox

Paolo Ascierto

Carlo Bifulco

Franco Marincola

SITC Immunoscore Taskforce!

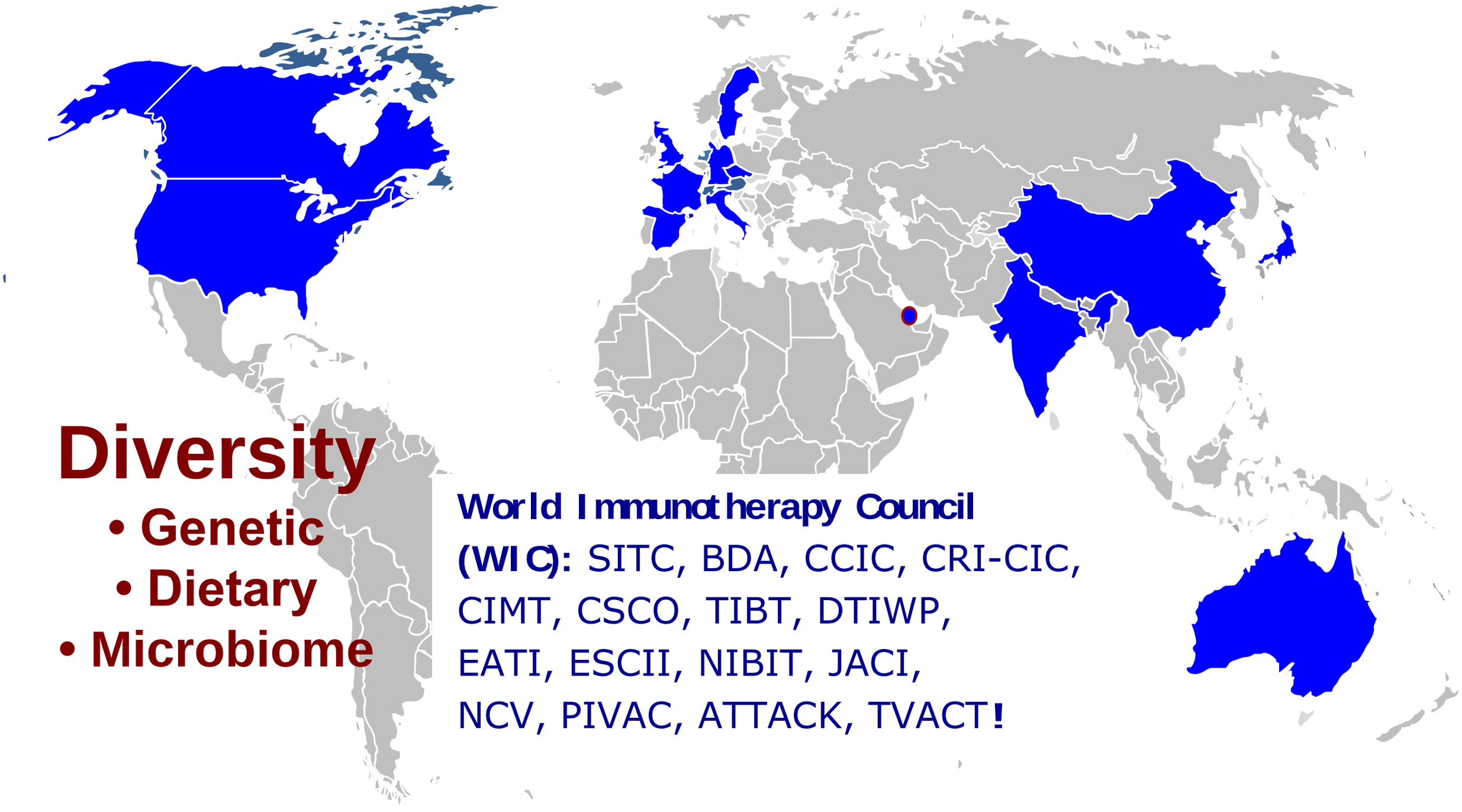
Steering Committee

Jerome Galon
Bernie Fox
Paolo Ascierto
Carlo Bifulco
Franco Marincola

Daniel J. Sargent

SITC Immunoscore Taskforce!

November 2014 - 17 Countries



Diversity

- Genetic
- Dietary
- Microbiome

World Immunotherapy Council (WIC): SITC, BDA, CCIC, CRI-CIC, CIMT, CSCO, TIBT, DTIWP, EATI, ESCII, NIBIT, JACI, NCV, PIVAC, ATTACK, TVACT!

Similar to TCGA --- Searchable database
Images and outcomes

SITC managed:
Not for profit
Professional Member Organization.

SITC Immunoscore Taskforce!

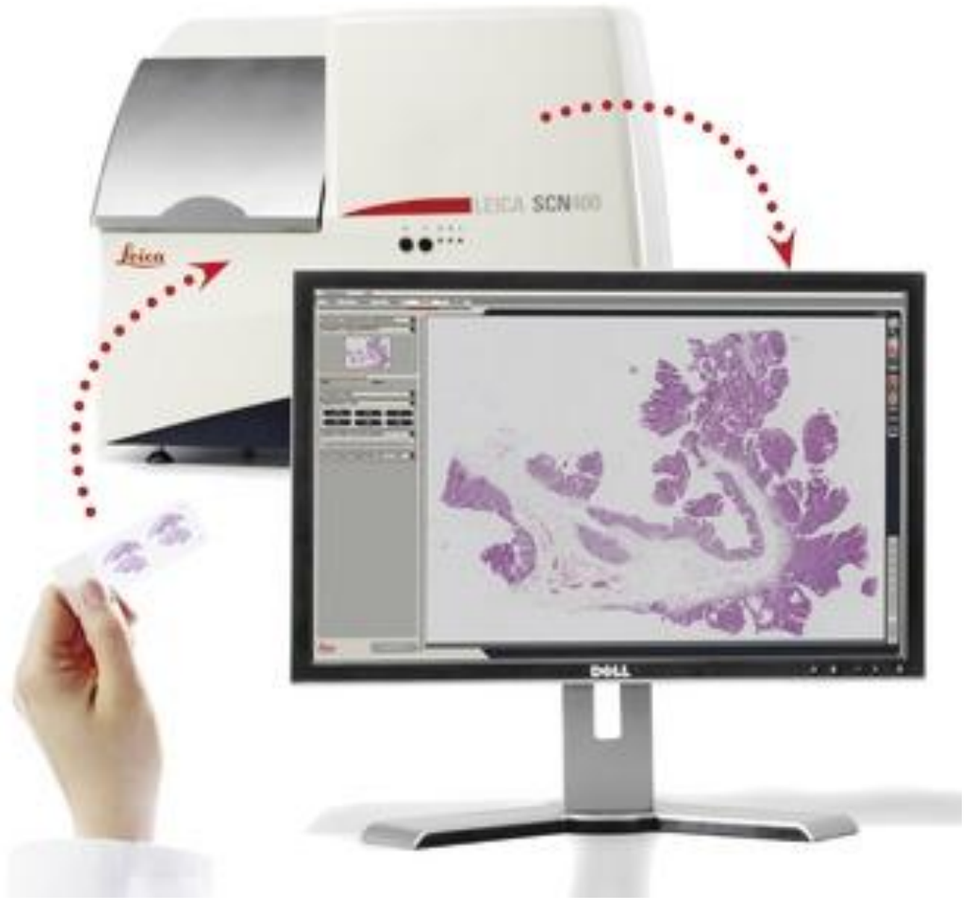
STATUS – November 2014

- Control slides cut and distributed to centers
  - Allesandro Lugli, Bern - **DONE**

- Centers stained (INSERM SOP) and imaged slides
  - Jerome Galon and Franck Pages - **DONE**

- Analyze images with Immunoscore plug-in - **NOW**
- Upload images – (Asia, N.A., Europe) -- **NOW**
- Determination of cut-points and statistical analysis

,



Slow acceptance Of Digital Path

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SITC Immunoscore Taskforce!

2014 REVIEW ISSUE
FREE ONLINE

The Journal of Pathology
Pathology in Drug Discovery and Development

Towards the introduction of the Immunoscore in the classification of malignant tumors.

Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, Lagorce C, Lugli A, Zlobec I, Hartmann A, Bifulco C, Nagtegaal ID, Palmqvist R, Masucci GV, Botti G, Tatangelo F, Delrio P, Maio M, Laghi L, Grizzi F, Asslaber M, D'Arrigo C, Vidal-Vanaclocha F, Zavadova E, Chouchane L, Ohashi PS, Hafezi-Bakhtiari S, Wouters BG, Roehrl M, Nguyen L, Kawakami Y, Hazama S, Okuno K, Ogino S, Gibbs P, Waring P, Sato N, Torigoe T, Itoh K, Patel PS, Shukla SN, Wang Y, Kopetz S, Sinicrope FA, Scripcariu V, Ascierto PA, Marincola FM, **Fox BA**, Pagès F.

J Pathol 2014; **232:** 199–209

FOR THE ONCOLOGY SPECIALIST

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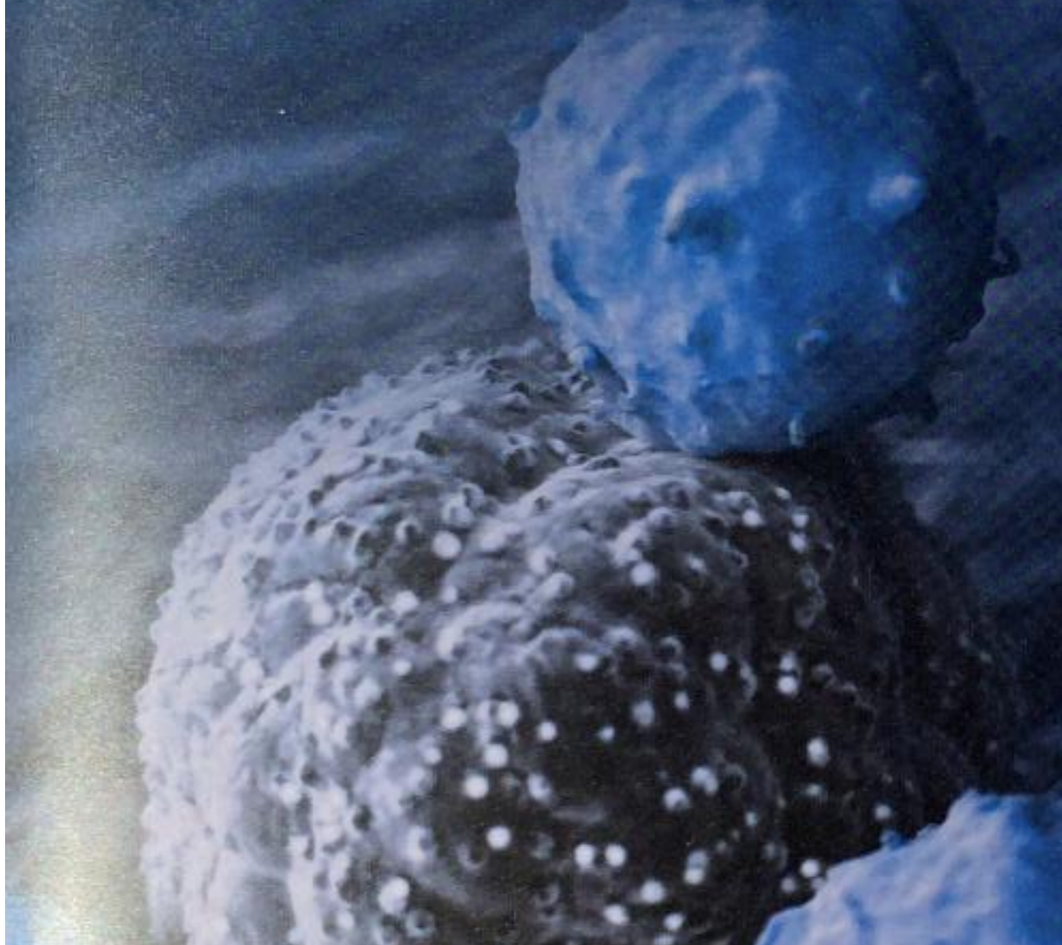
New Paradigms Emerge for Translating Immunotherapy Into Broad Clinical Use



Adverse Events Report

A snapshot of findings from recent reports, articles, and abstracts

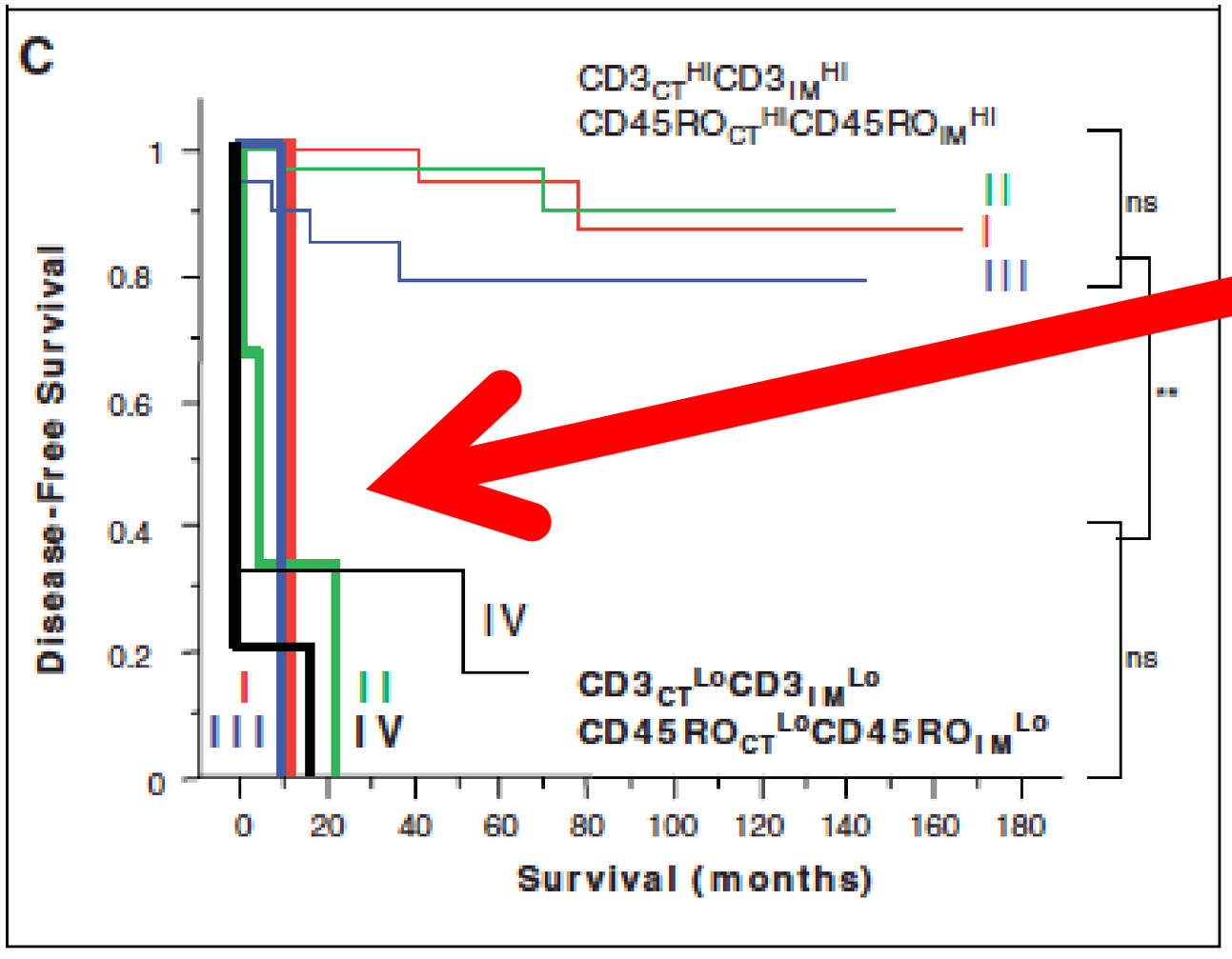
Discovery Dilemma: Narrow "Superiority" Standard Is an Inferior Way to Evaluate Novel Therapies

By Maurie Markman, MD

Failed Studies Provide Clues in Renal Cell Carcinoma

Suc

Stratify stage I or II patients for adjuvant trials.



- **Drs. Chris Heery and Jeffery Schlom (NCI)**

- **Planning to stratify Immunoscore negative patients for a cancer vaccine trial.**

- **Can we use this to identify patients at high risk of recurrence for other cancers?**

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Immunoscore: Defined

For colon cancer the answer may be as simple as Immunoscore (CD3 and CD8).

- Similar for other cancers?
- Or more complex?

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Immunoscore: Defined

For colon cancer the answer may be as simple as Immunoscore (CD3 and CD8).

- Similar for other cancers?
- Or more complex?

Likely more complex and variable!

Ascierto et al. Journal of Translational Medicine 2013, 11:54
http://www.translational-medicine.com/content/11/1/54

JOURNAL OF TRANSLATIONAL MEDICINE

EDITORIAL Open Access

The additional facet of immunoscore: immunoprofiling as a possible predictive tool for cancer treatment

Paolo A Ascierto[1*], Mariaelena Capone[1], Walter J Urba[2], Carlo B Bifulco[2], Gerardo Botti[1], Alessandro Lugli[3], Francesco M Marincola[4], Gennaro Ciliberto[1], Jérôme Galon[5,6,7] and Bernard A Fox[2,8]

Table 1 Differences between immunoscore and immunoprofiling

	Immunoscore	Immunoprofiling
	Prognostic/Predictive(?)	**Prognostic/Predictive(?)**
Number of immune markers	2-4	1 – Several
Immunoscore markers	CD3/CD8	
Immunoscore-like markers	CD3/CD8/CD20/FoxP3	Immune gene signatures
	CD3/CD8/CD45RO	Multiplex assays
	CD4/CD8/CD68	CD137, Galectin1, LAG-3, OX40, PD-
	CD3/CD8/CD20, CD3/GZMB	
	CD8/FoxP3	
	CD8/IL17	
	(others)	
Possible application	• Staging in colorectal cancer (already tested)	• Prognostic assay
	• Staging in Melanoma, Breast cancer, Ovarian cancer, NSCLC, Prostate cancer, Pancreatic cancer, Head & Neck cancer (to be defined).	• Predictive assay

Not just colon cancer: More than 100 publications - Association between immune infiltrate and outcome

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Z%3&#$(&2' 3%	P- - : [CCCSCC[]	! "P- - : [CCUCCT] ! "J - 23[CCW]	! "P- - : [[K] ! "J - 23[CCW]		! "J - 23[KT] ! "V- - 0%[WX]
Z7&: : 3%(&2' 3%	P- - : [CCXCCY]				P- - : [RX]
? 9&%&2(&2' 3%	P- - : [CKQSCKK]	P- - : [CKRCK[]	V- - 0%[KR]	P- - : [CKU]	! "P- - : [RRTC] ! "V- - 0%[T]
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Fridman WH., et al., Nature Reviews Cancer, March 15, 2012

Clinical Trial Design

What should “we” be doing?

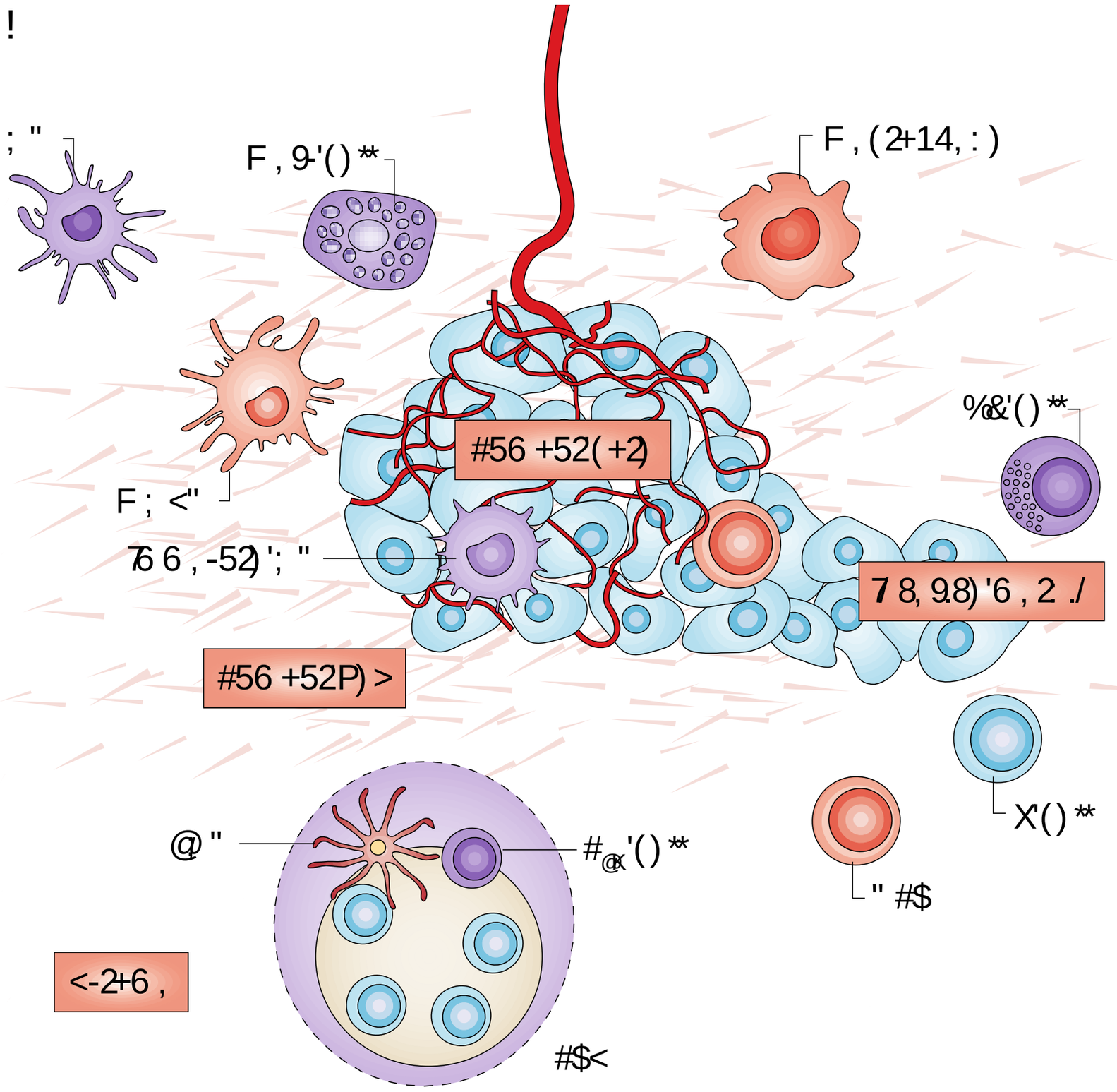
For every trial:

- Require cancer slides or blocks.
- Biopsy: pre, mid or post tx.

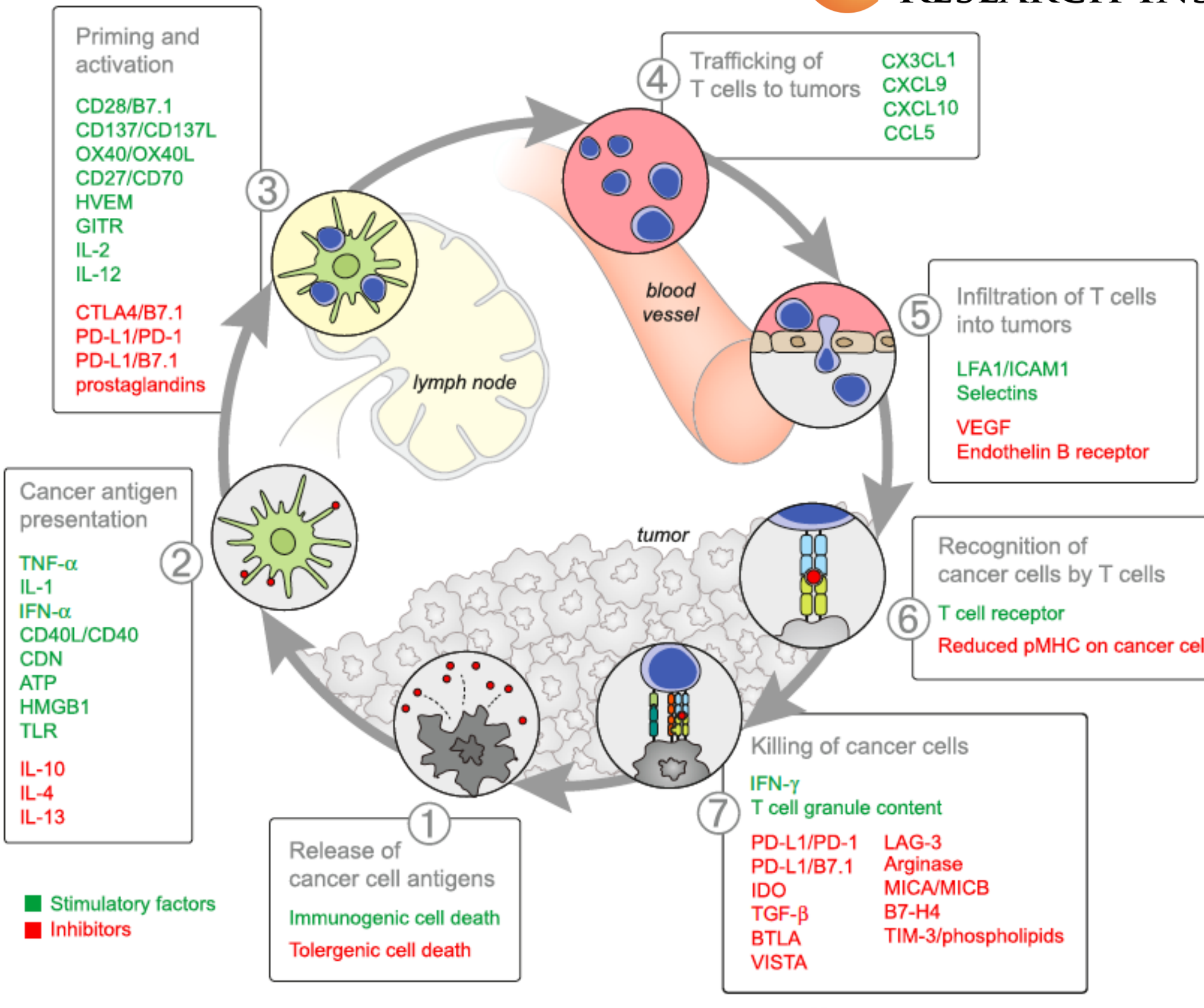
Why?

- MOA
- Stratify patients

Immunoscore – Immune Contexture:



Fridman WH., et al., Nature Reviews Cancer, March 15, 2012



Chen and Mellman Immunity 39, July 25, 2013

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Imaging Multiple Markers

On one slide

- Bright field
- Fluoresence

On multiple slides

- Co-registration

Imaging Multiple Markers

On one slide

- Bright field
- Fluoresence

On multiple slides

- Co-registration

Pros and Cons to each

Relationships

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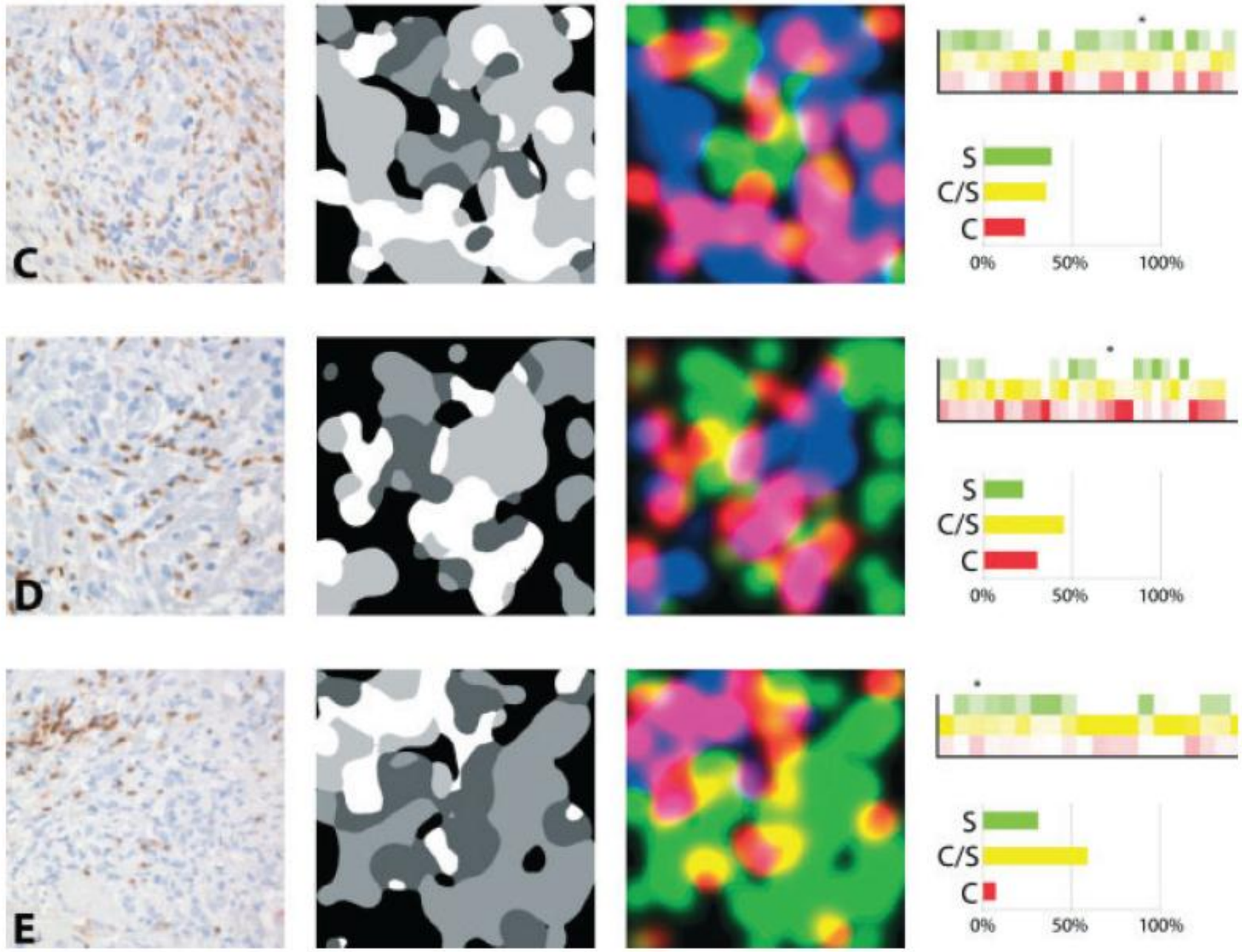

J Pathol 2013; **229**: 569–578

5-Plex Chromogenic Staining on Melanoma TMA – Collaboration / Istituto Nazionale Tumori, Napoli & EACRI

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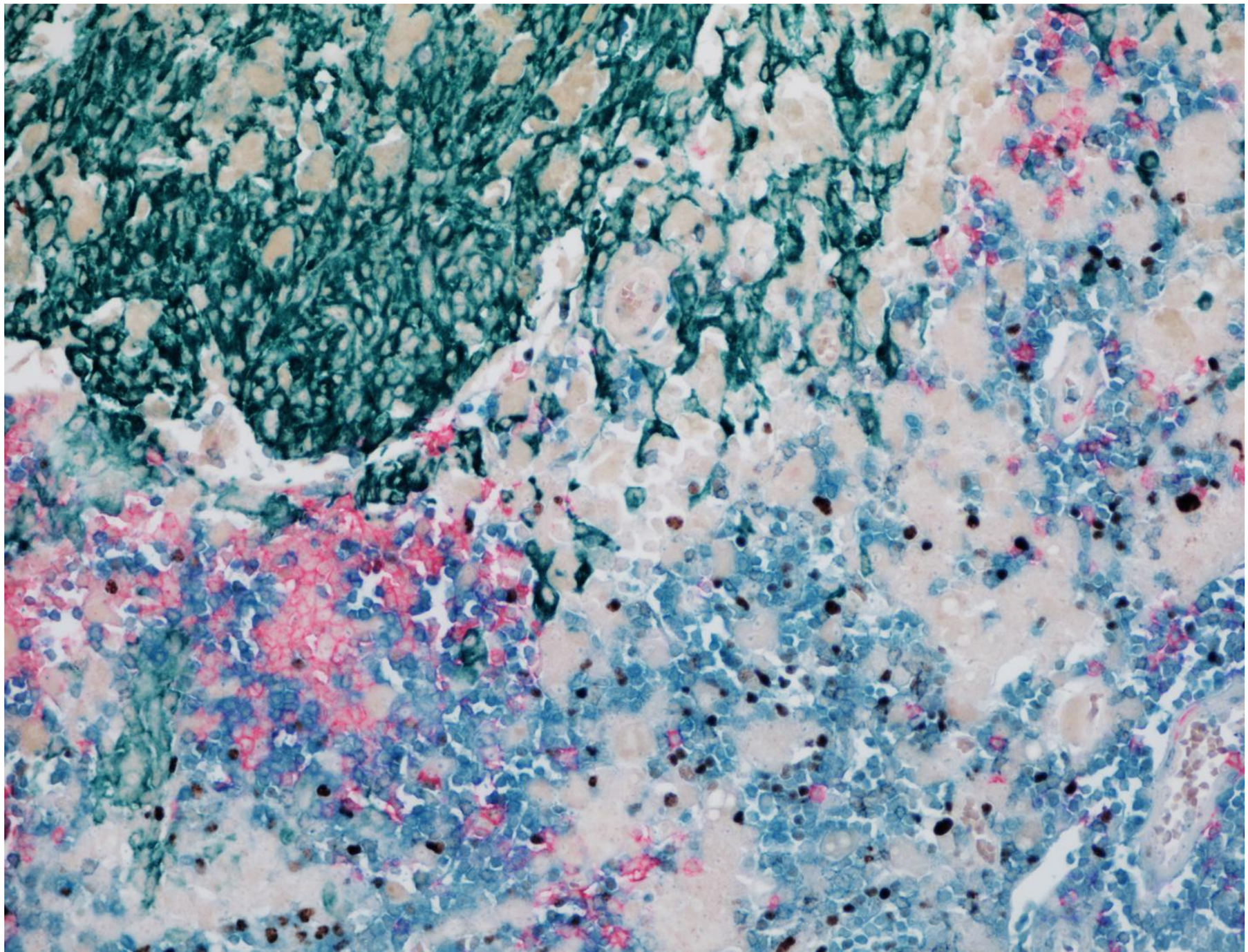

red = PD-L1
yellow = CD8
green = Foxp3
purple = CD20
aqua = melanoma cocktail
pink = CD163
blue = DAPI

Zipei Feng
Poster
163

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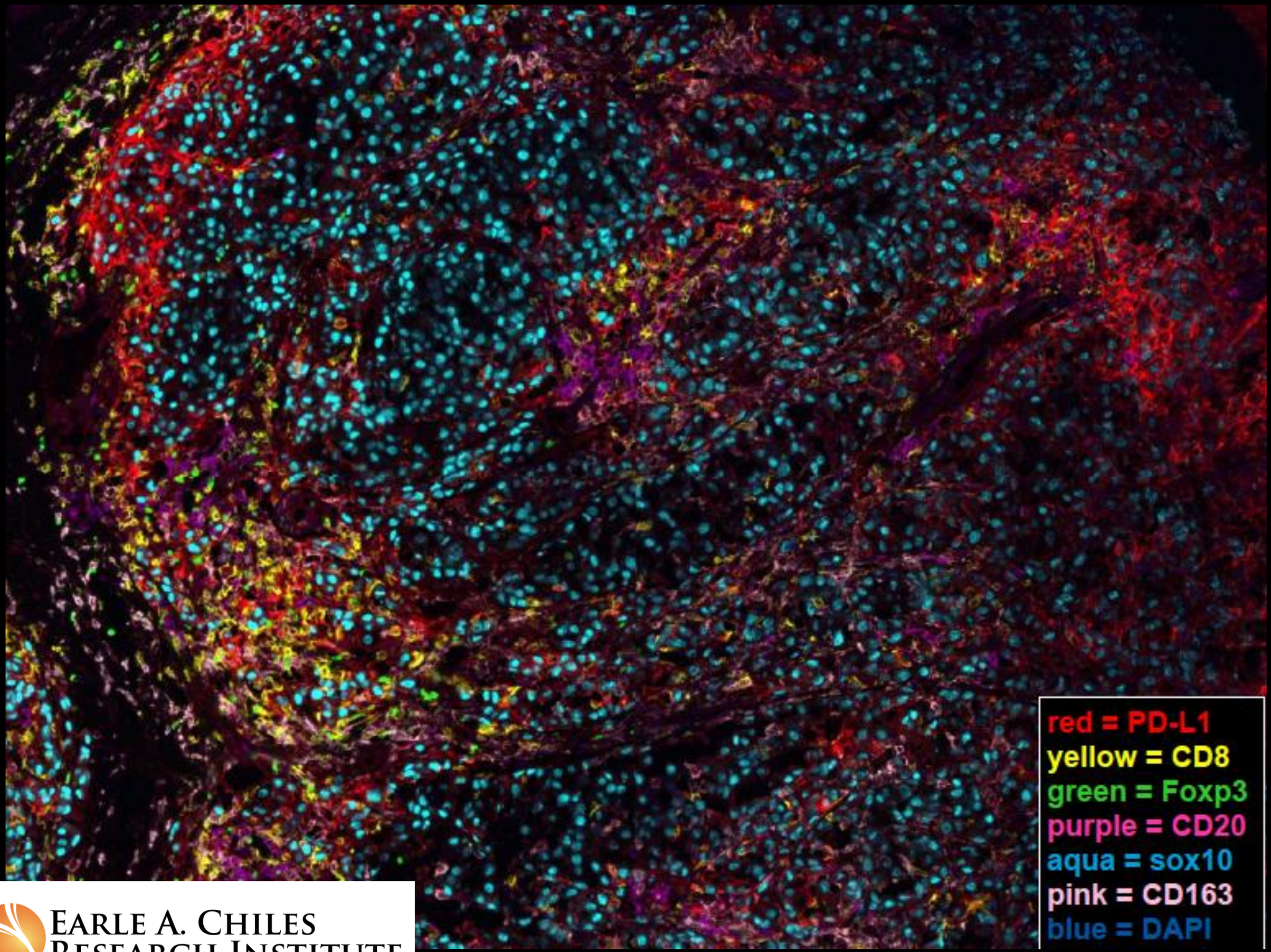

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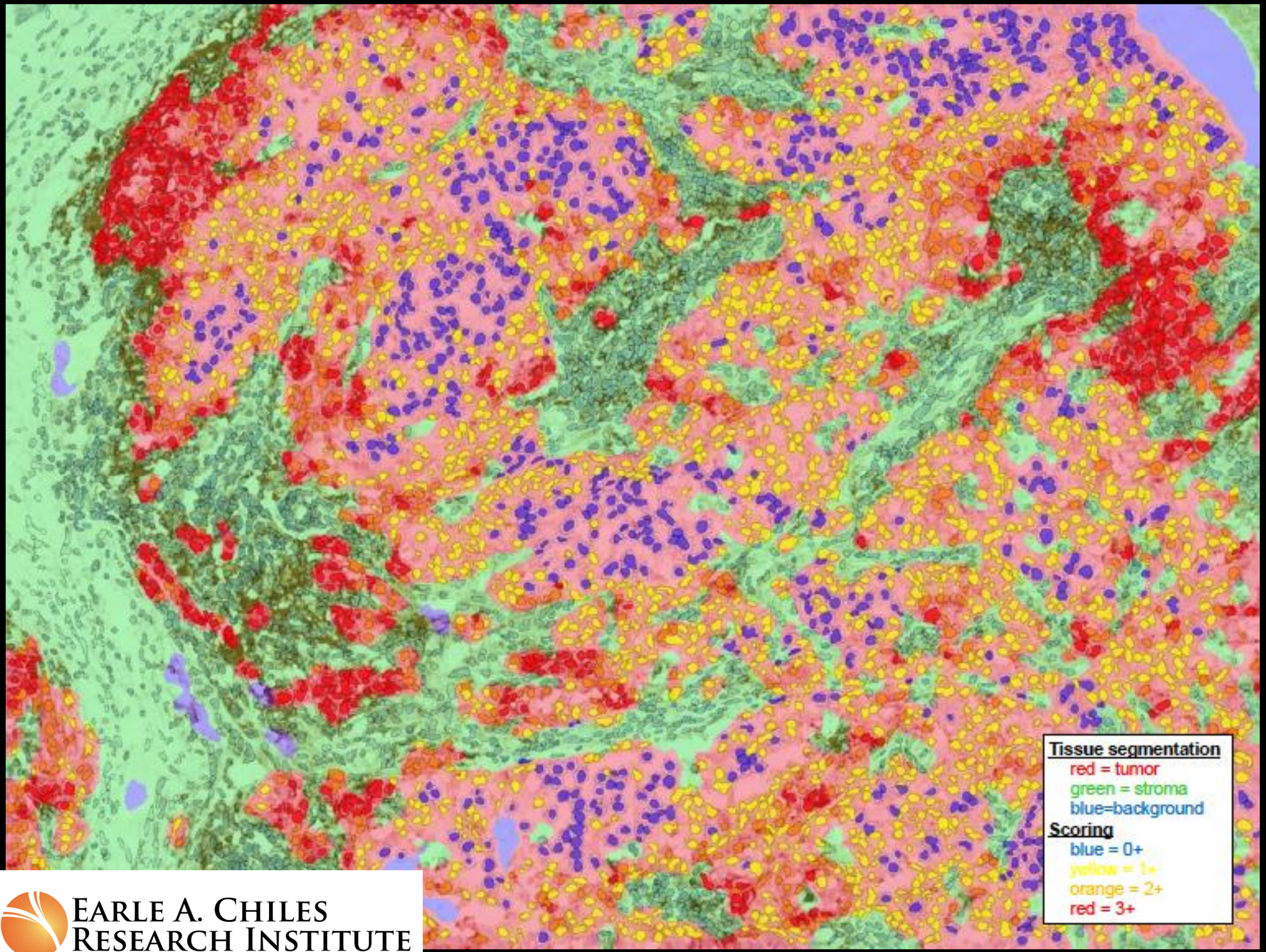

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Tissue segmentation

red = tumor
green = stroma
blue=background

Phenotyping

red = PD-L1+ tumor cell
aqua = PD-L1- tumor cell
green = regulatory T cell
yellow = cytotoxic T cell
purple = B cell
pink = macrophage
blue = other

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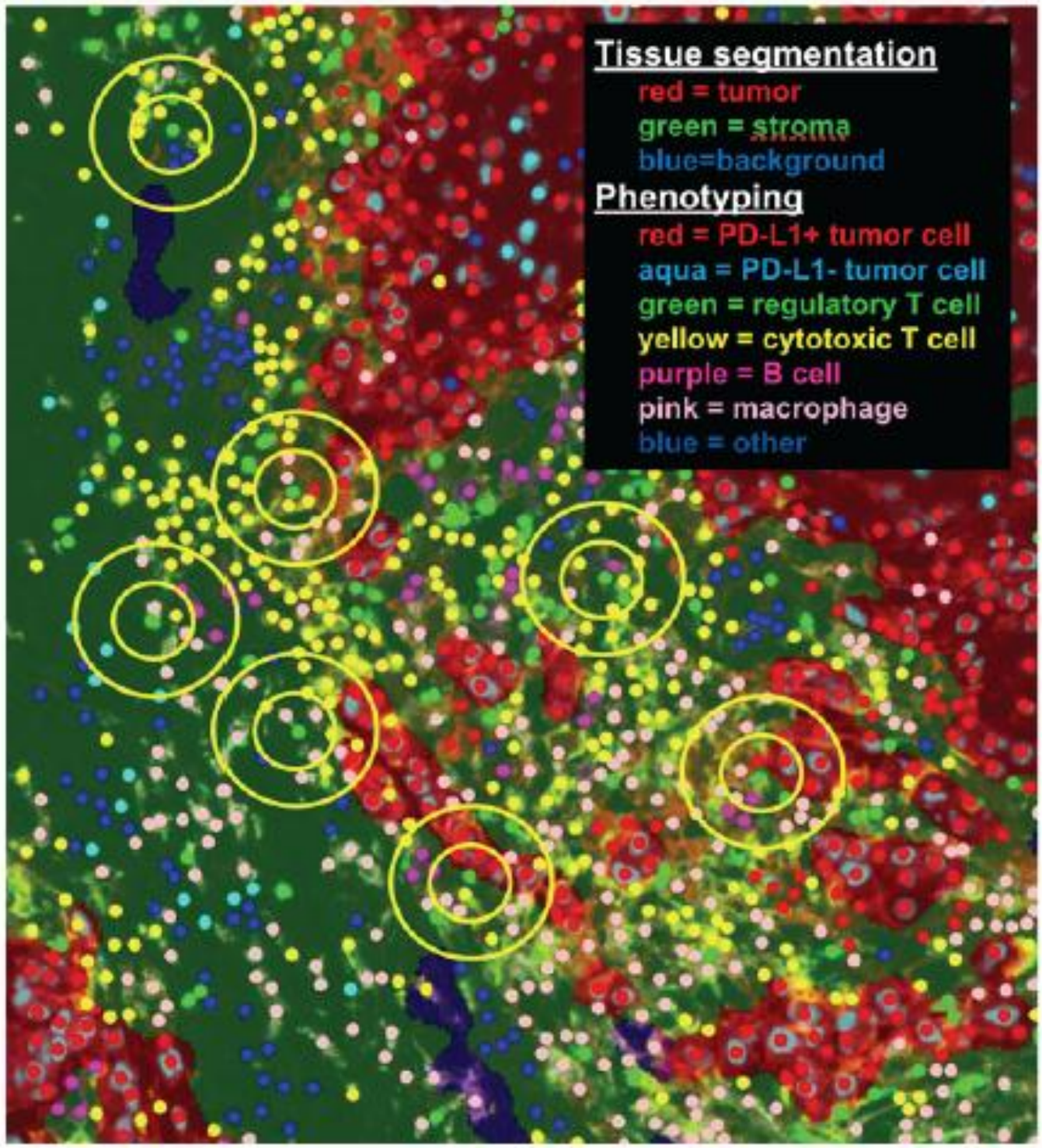
Relationships, e.g. T-regs

Example interaction distance measurement:

What are the average numbers of PD-L1+ tumor cells and cytotoxic T cells within 10 and 25 microns of regulatory T cells?

Calculations performed with R scripts. operating on inForm cell phenotype output files



PanCancer Immune Profiling Panel: 770 Genes

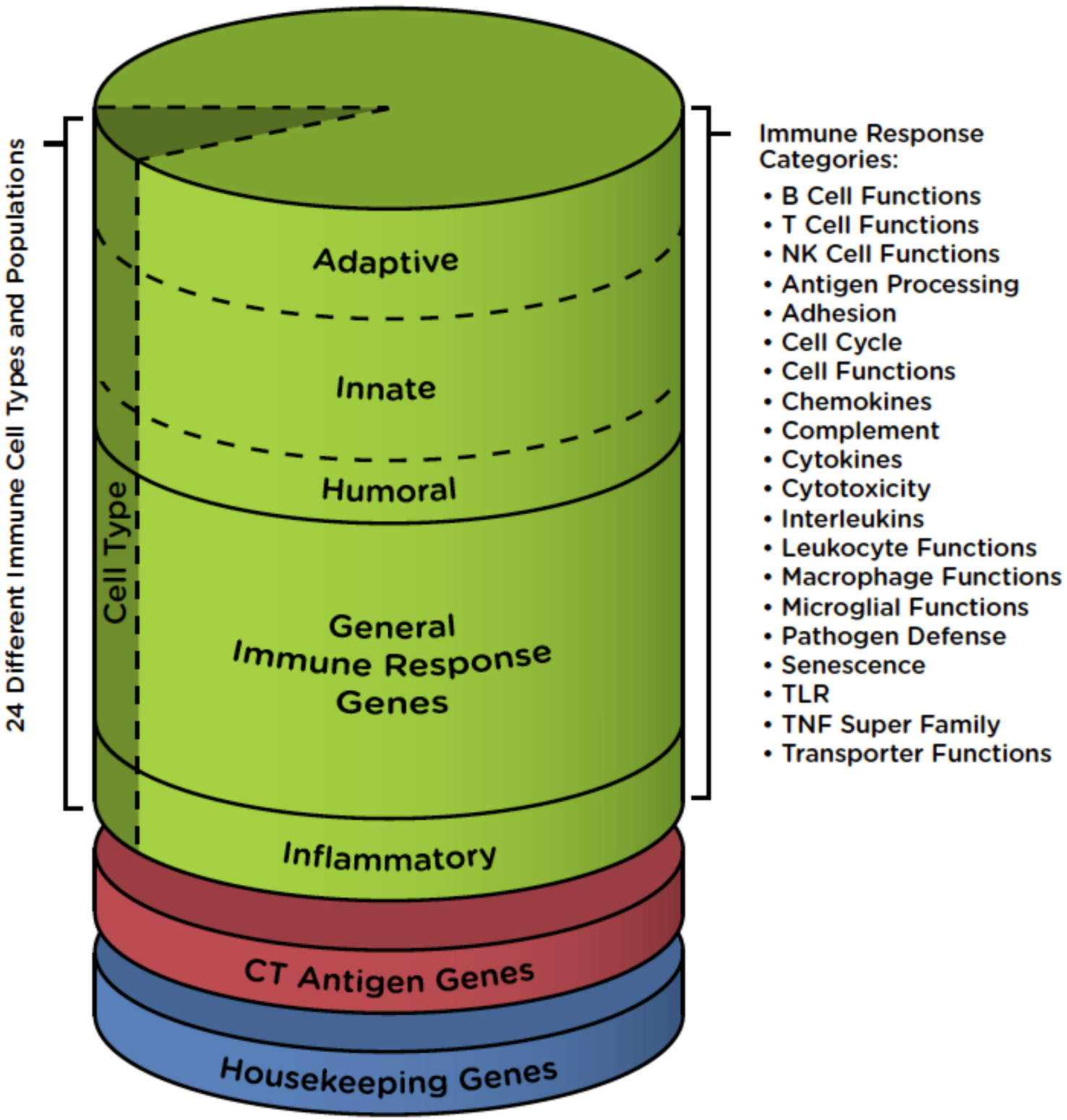
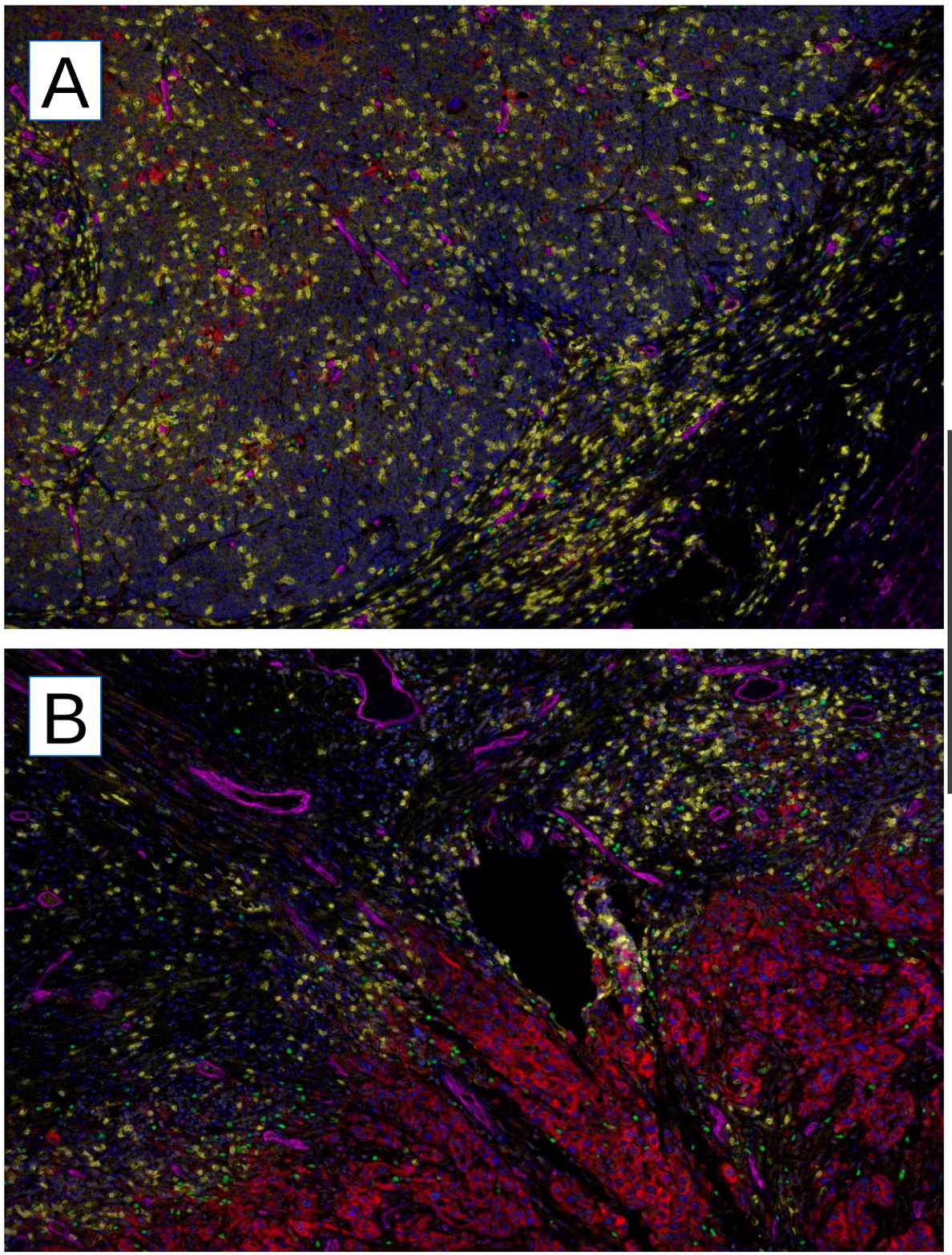
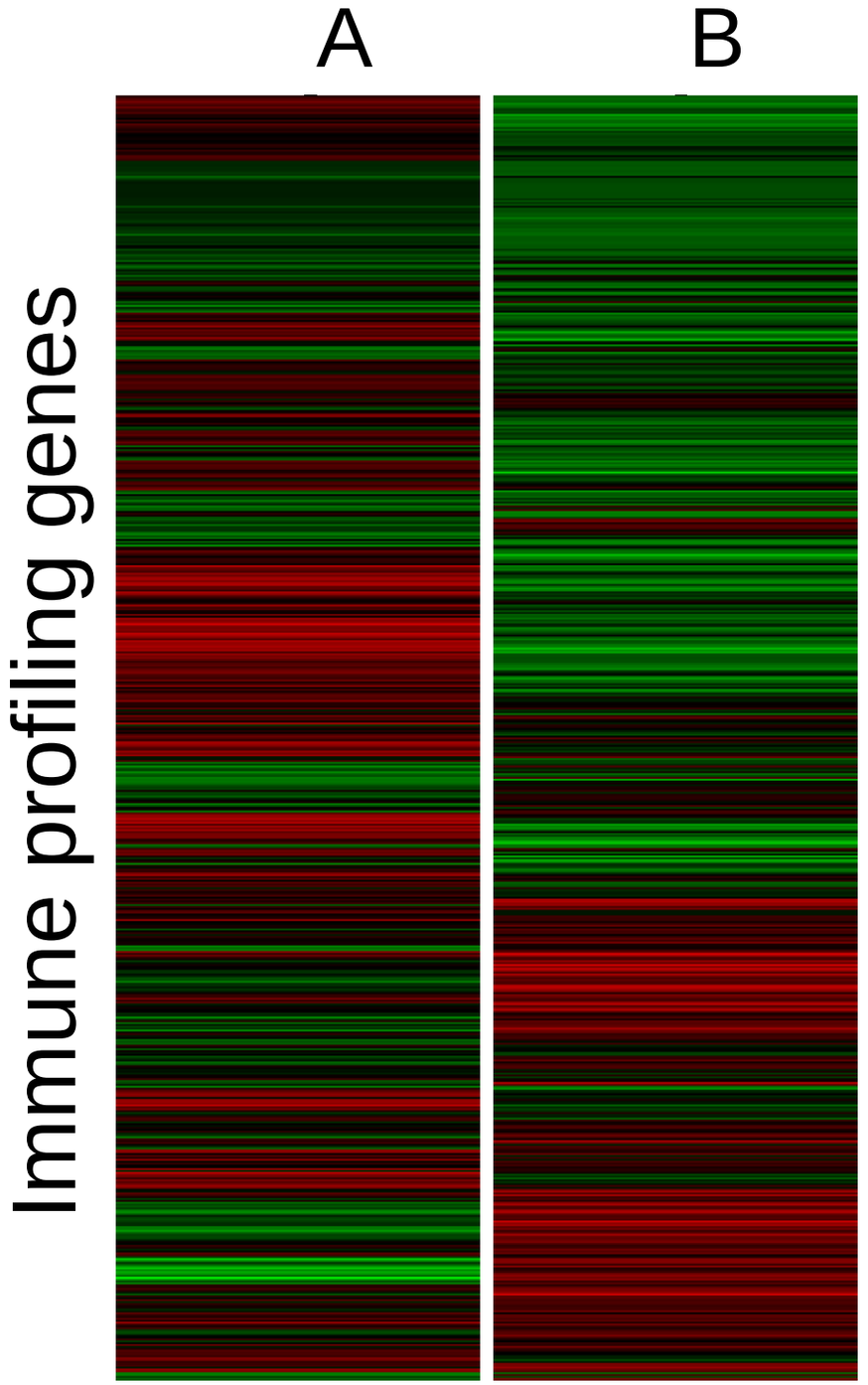


FIGURE 1: Distribution of genes included in the PanCancer Immune Profiling Panel, including genes for identifying Immune Cells (dark green), Immune Response genes (green), CT Antigens (red) and Housekeeping genes (blue). Biological process categories comprising the Immune Response genes are indicated on the right.

NanoString Gene Expression Immune Profiling Analysis Performed on Two OHNSCC



PD-L1
FoxP3
CD-3
DAPI



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Questions for the next 5 years:

1) What drives different anti-cancer immune responses in patients that appear otherwise similar for disease stage, age, gender?

   - Tumor landscape (bad actors)?
   - Mutatanome?
   - Microbiome?
   - Other?

SITC Immunoscore Taskforce

Acknowledgements:

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Cancer Classification using the Immunoscore: A Worldwide Task Force

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