SITC Winter school, Houston, TX, USA
January 16th, 2020

Tumor microenvironment cell analyses: immunopathology

Jérôme GALON

INSERM, Laboratory of Integrative Cancer Immunology, Cordeliers Research Center, Paris, France

Disclosures

Co-founder and chairman of the scientific advisory board:

- *HalioDx*

Collaborative Research Agreement (grants) :

- *Perkin-Elmer, IObiotech, MedImmune, Astra Zeneca, Janssen, Imcheck Therapeutics*

Participation to Scientific Advisory Boards:

- *BMS, MedImmune, Astra Zeneca, Novartis, Definiens, Merck Serono, IObiotech, ImmunID, Nanostring, Illumina, Northwest Biotherapeutics, Actelion, Amgen, Catalym, Merck MSD*

Consultant :

- *BMS, Roche, GSK, Compugen, Mologen, Gilead, Sanofi*

Research *versus* clinical routine

Research purposes

- ✓ Complexity
- ✓ Dynamics
- ✓ Multiplex
- ✓ Discovery

Routine clinical purposes

- ✓ Simple
- ✓ Robust
- ✓ Reproducible
- ✓ Clinical Utility
- ✓ Guidelines
- ✓ EMA/FDA approval
- ✓ CLIA certification
- ✓ Reimbursment

Cancer patient

Current cancer classification
Tumor cell characteristics

Immune-based classification
Host immune response

T-stage

N-stage

M-stage

Tumor cell extension and invasion

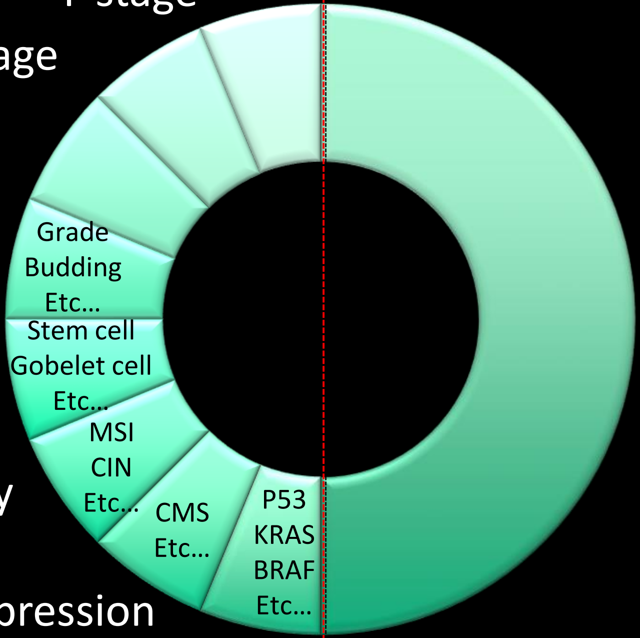
Anatopathology
Tumor Morphology

Tumor cell of origin

Tumor Molecular pathway

Tumor Gene expression

Tumor Mutation status



Currently NONE

Angell & Galon *Clin Cancer Res*. 2019

Concepts in Immuno-oncology

"Contexture: the act of assembling parts into a whole; an arrangement of interconnected parts"

Concept

"***Immune Contexture***" :

✓Type
✓Quality
✓Quantity
✓Spatial

✓Complexity
✓Dynamics

Research purposes

Concept, not a simple Biomarker

"***Immunoscore***" :

✓Digital pathology
✓Quantitative
✓Location

✓Simple
✓Powerful

Routine clinical purposes

Galon J et al. ***Science*** 2006
Galon J et al. ***Cancer Res***. 2007

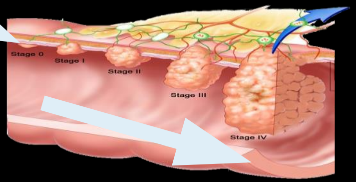
The Immune landscape and the importance of the immune contexture

Oncogenensis

Pre-cancer lesions

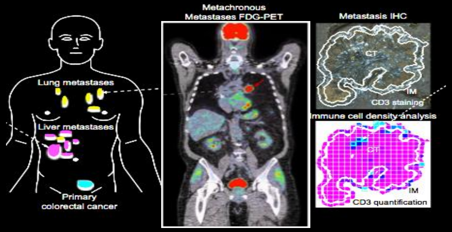
VELIPI+ Dissemination

N+ Invasion



Progression T-stage

M+

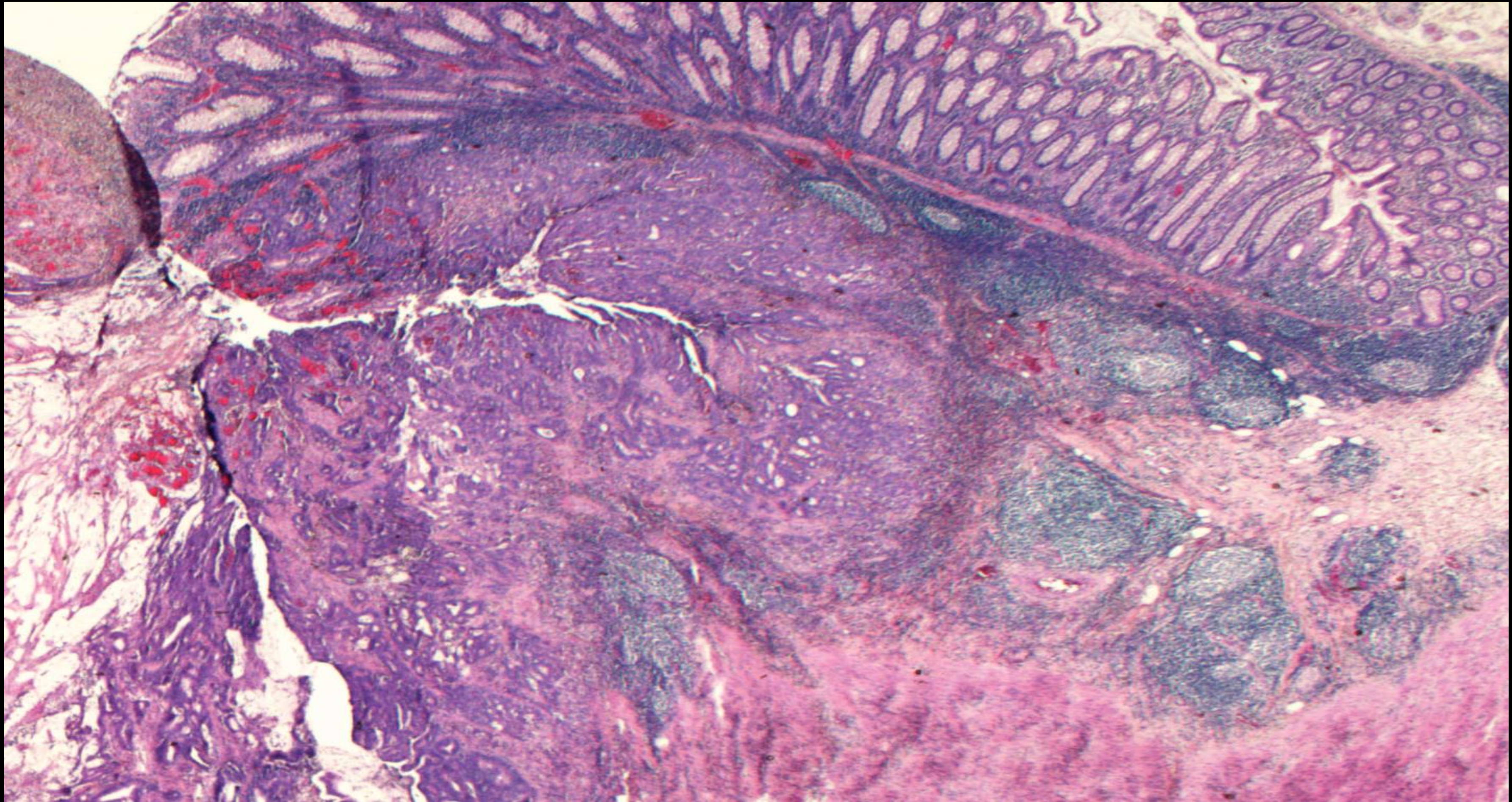


Prognosis

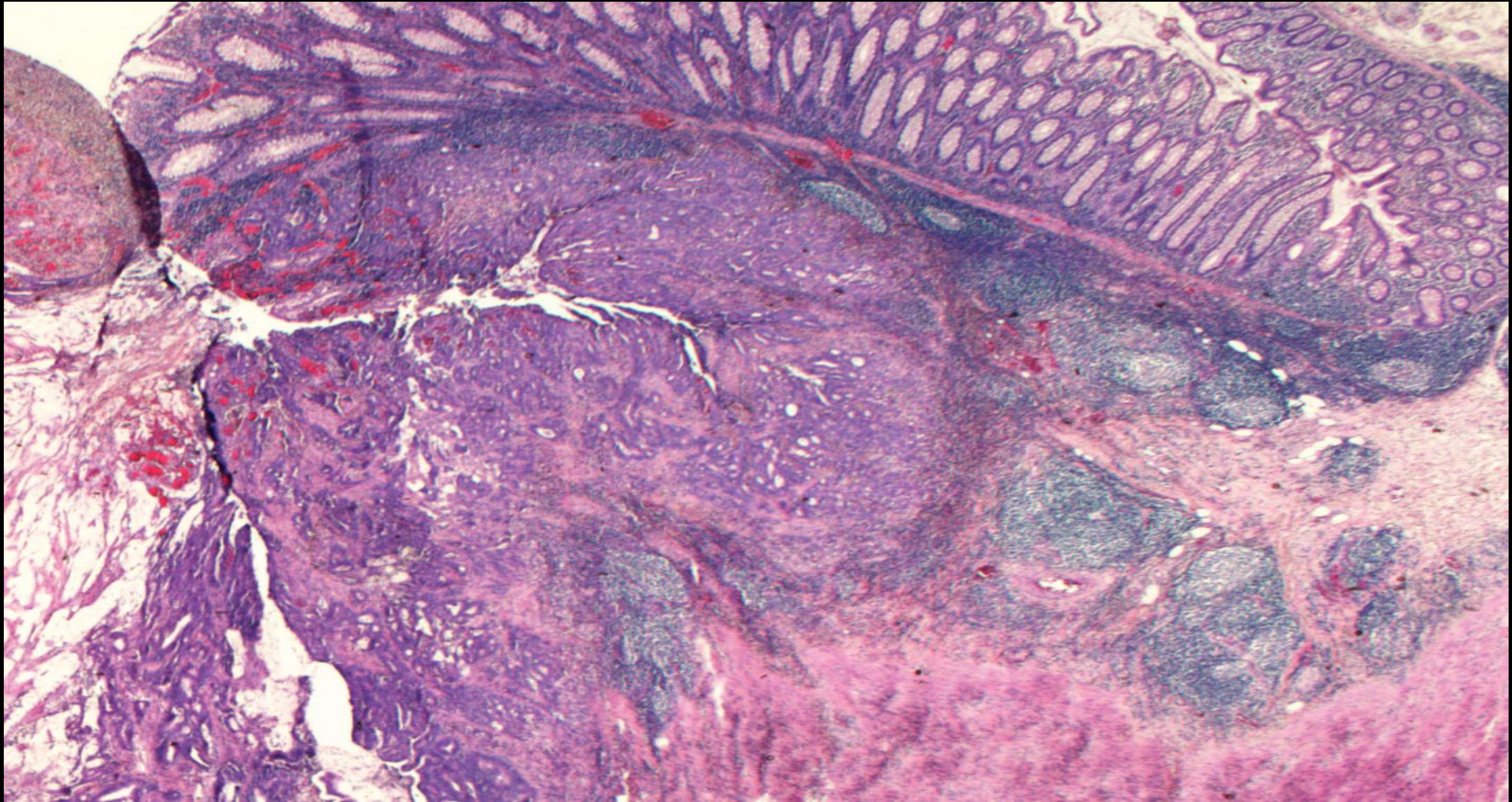
Recurrence
Death

Immunotherapy

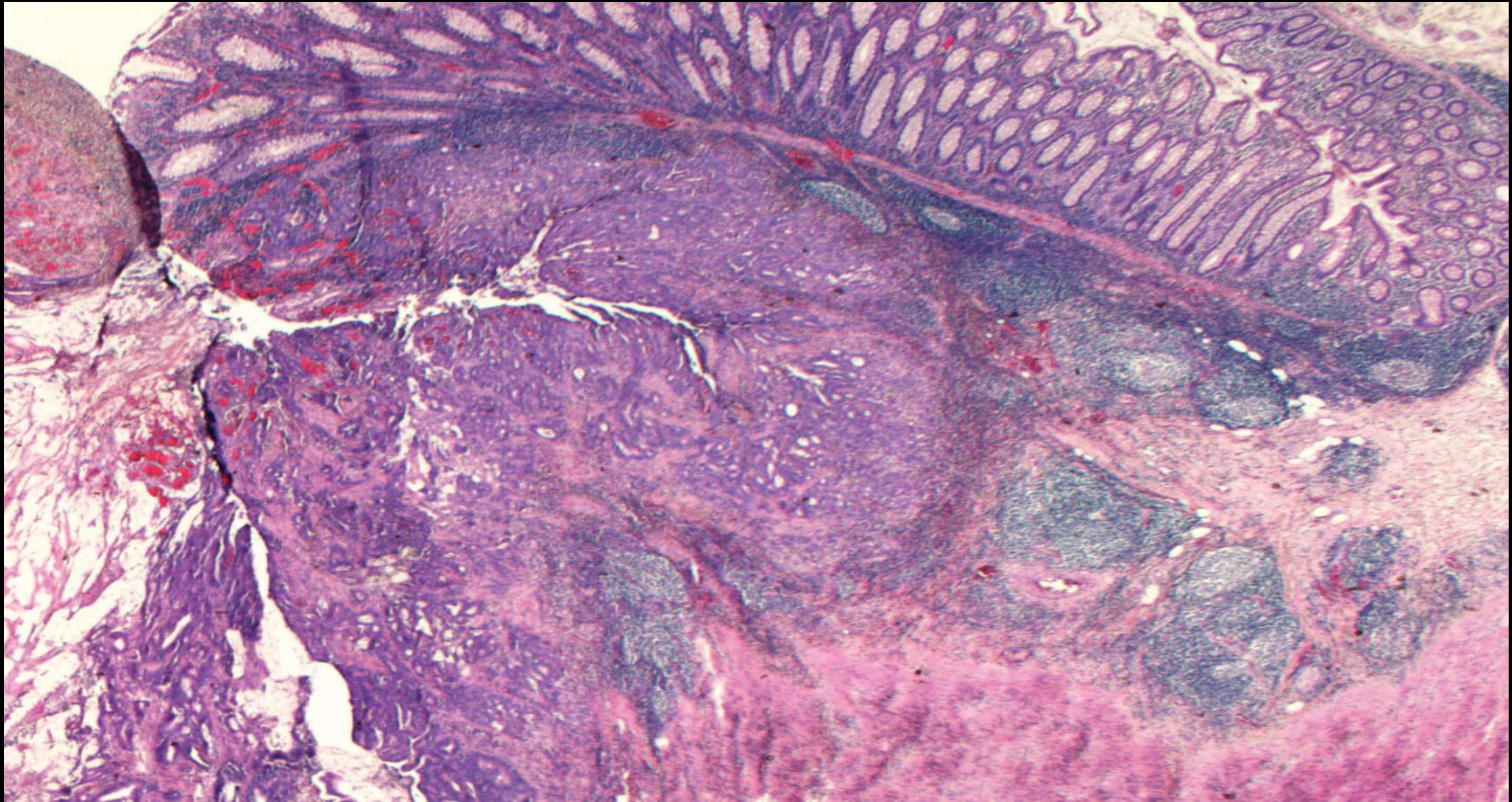
Tumor microenvironment



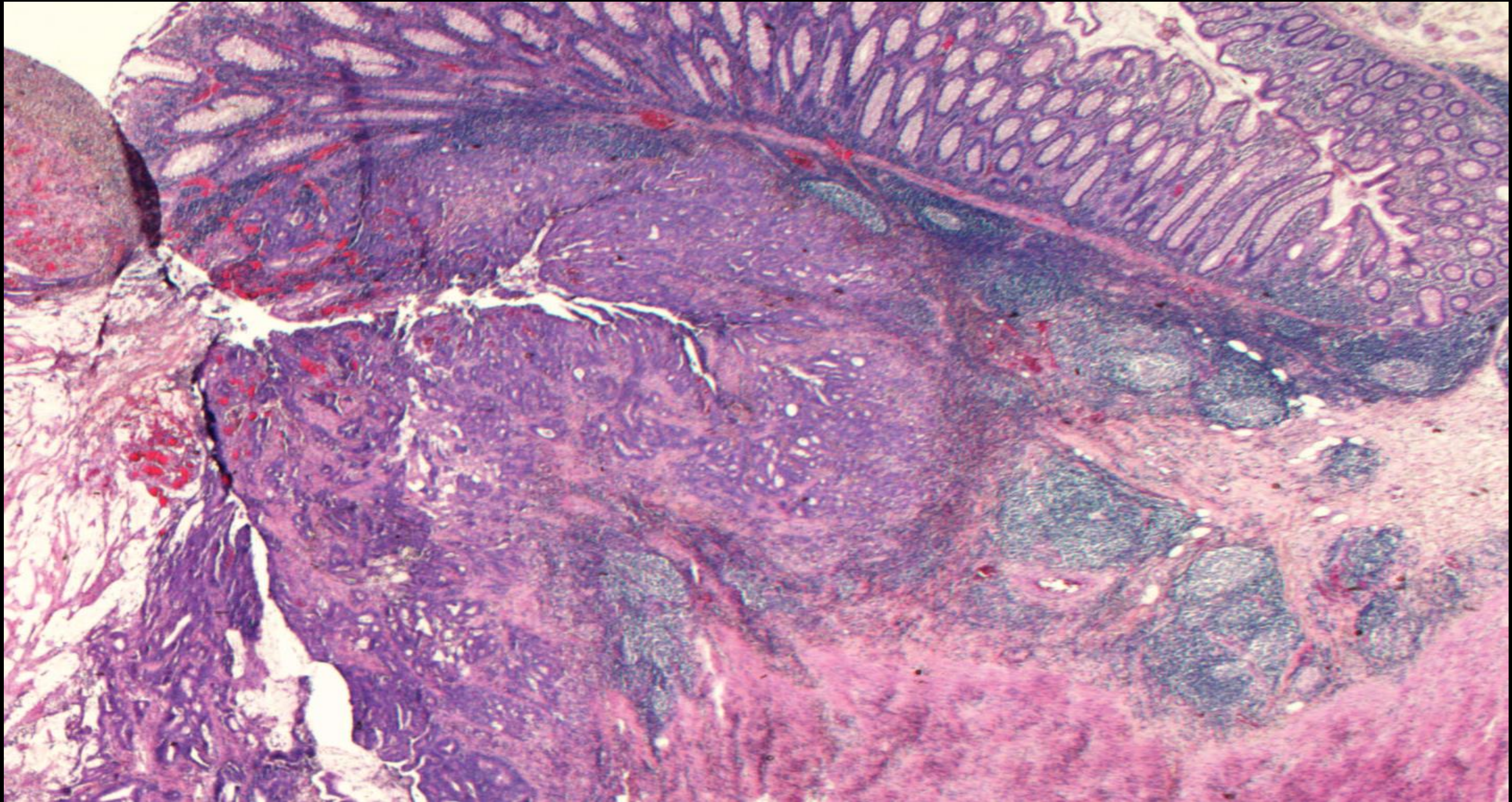
Tumor microenvironment



Tumor microenvironment



Tumor microenvironment



Exploring tumor microenvironment using single cell data

Deconvolution of expression data from mixture of cells to infer single cell population results

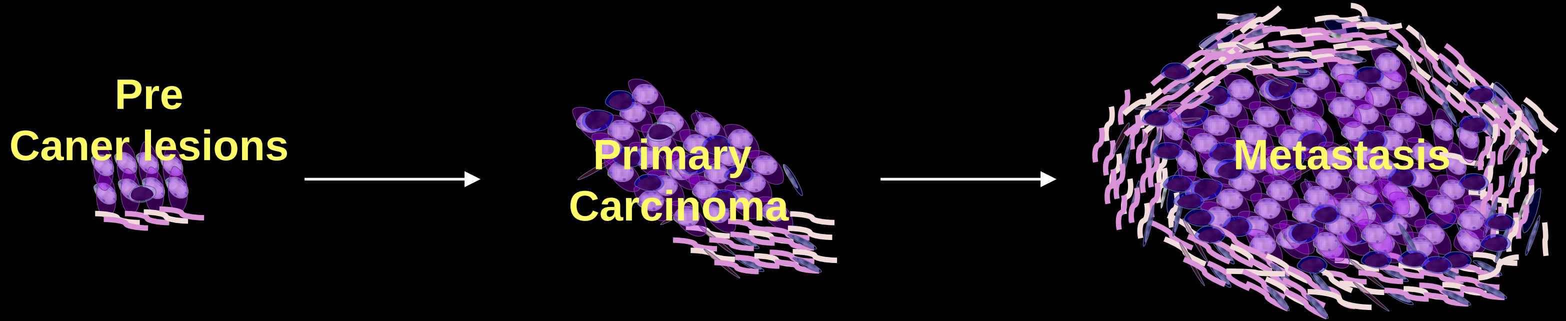
- ✓ Tumor cell clones
- ✓ Immune cell subpopulations
- ✓ Bioinformatic software
- ✓ Need single cell validation

Single cell analysis from isolated cells

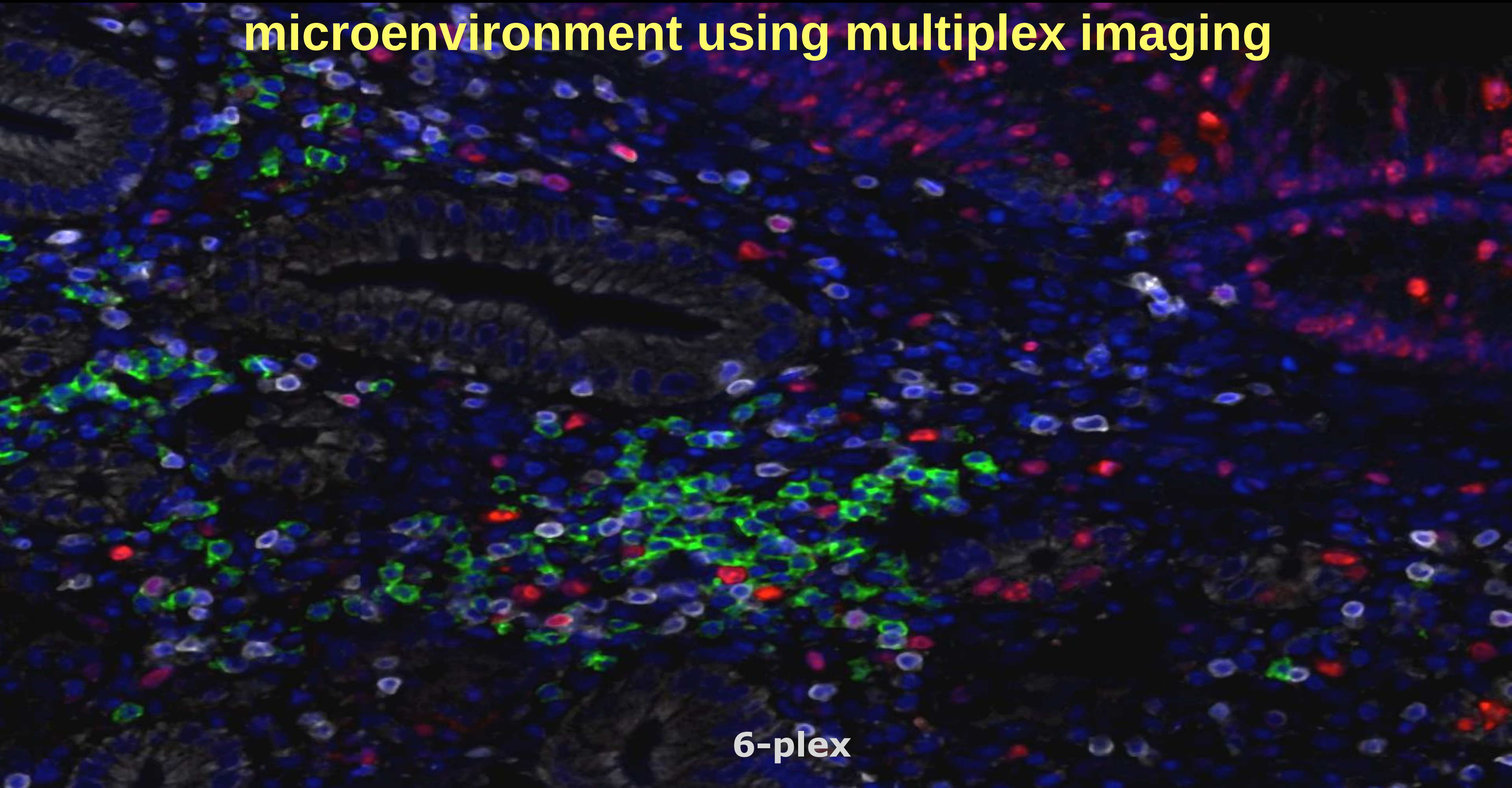
- ✓ FACS phenotyping (10-30 markers/cell)
- ✓ CyTOF phenotyping (30-50 markers/cell)
- ✓ scExomeSeq (genome)
- ✓ scRNAseq (genome)

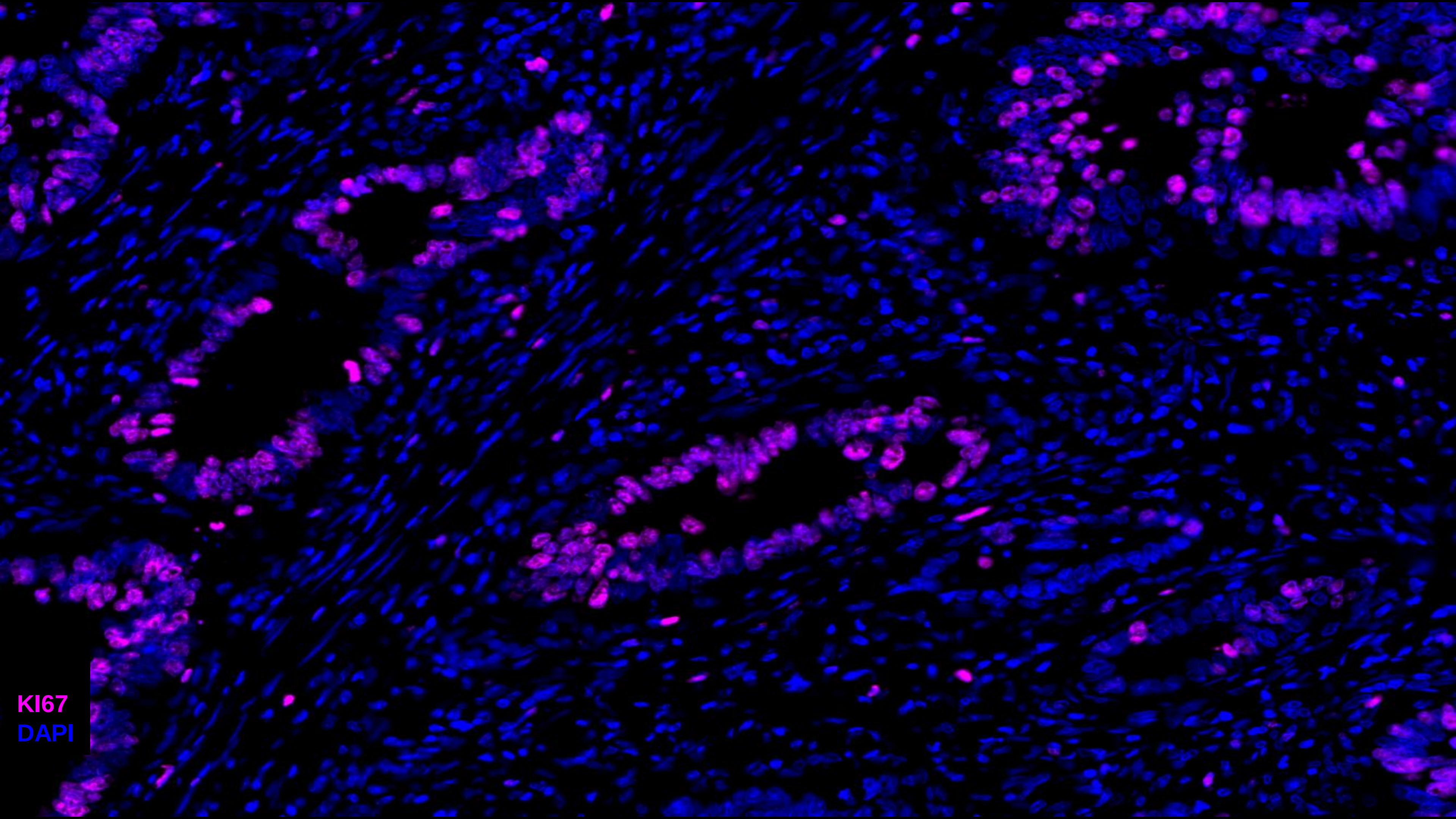
In situ Single cell analysis from tissue

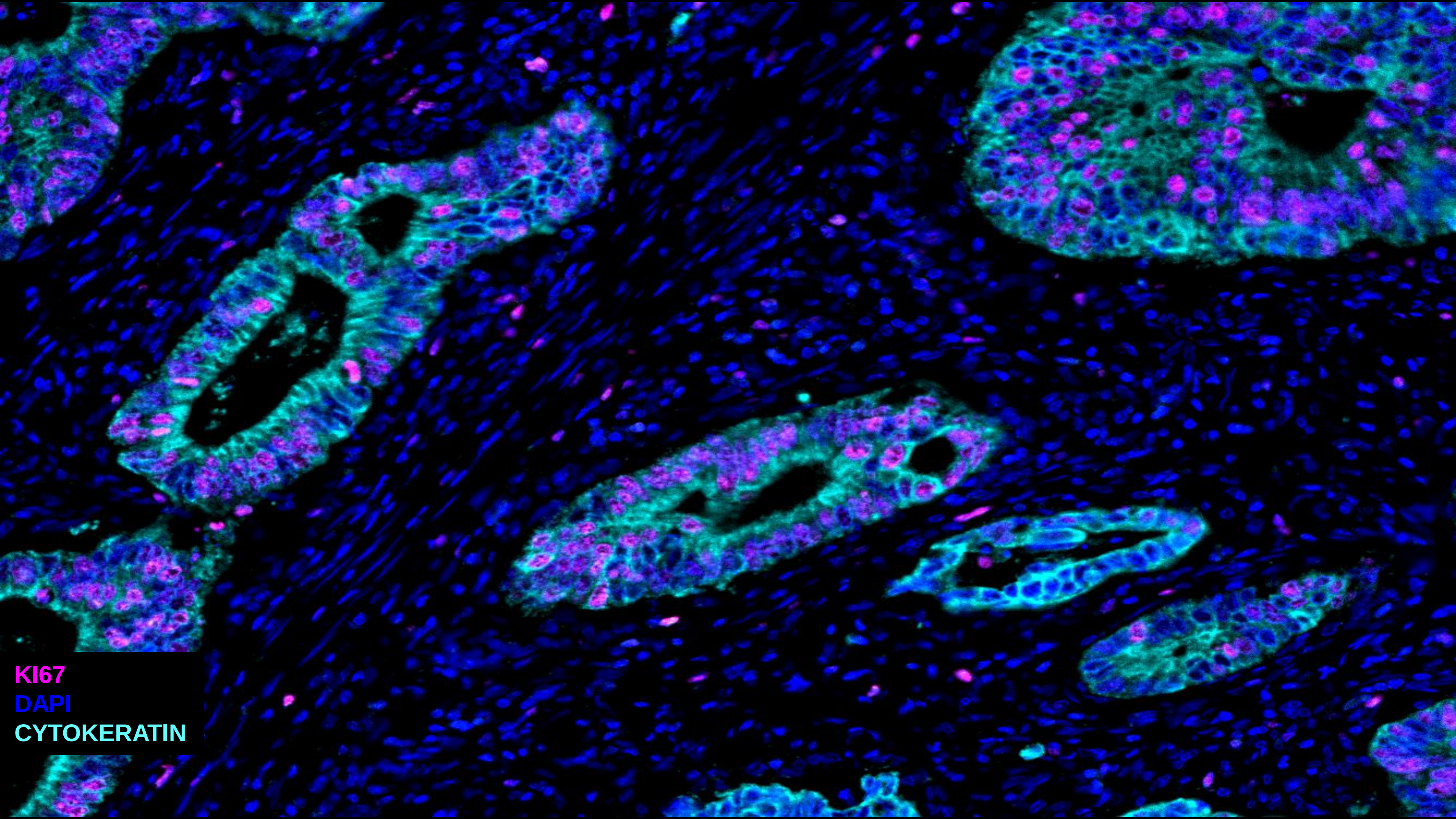
- ✓ IHC (1-10 markers/cell)
- ✓ Multiplex IF/multispectral (4-9 markers/cell)
- ✓ Hyperion phenotyping (30-50 markers/cell)
- ✓ Barecoded DSP (40 markers/cell)
- ✓ Barecoded DSP (700 genes/cell)
- ✓ scRNAseq in situ (genome)

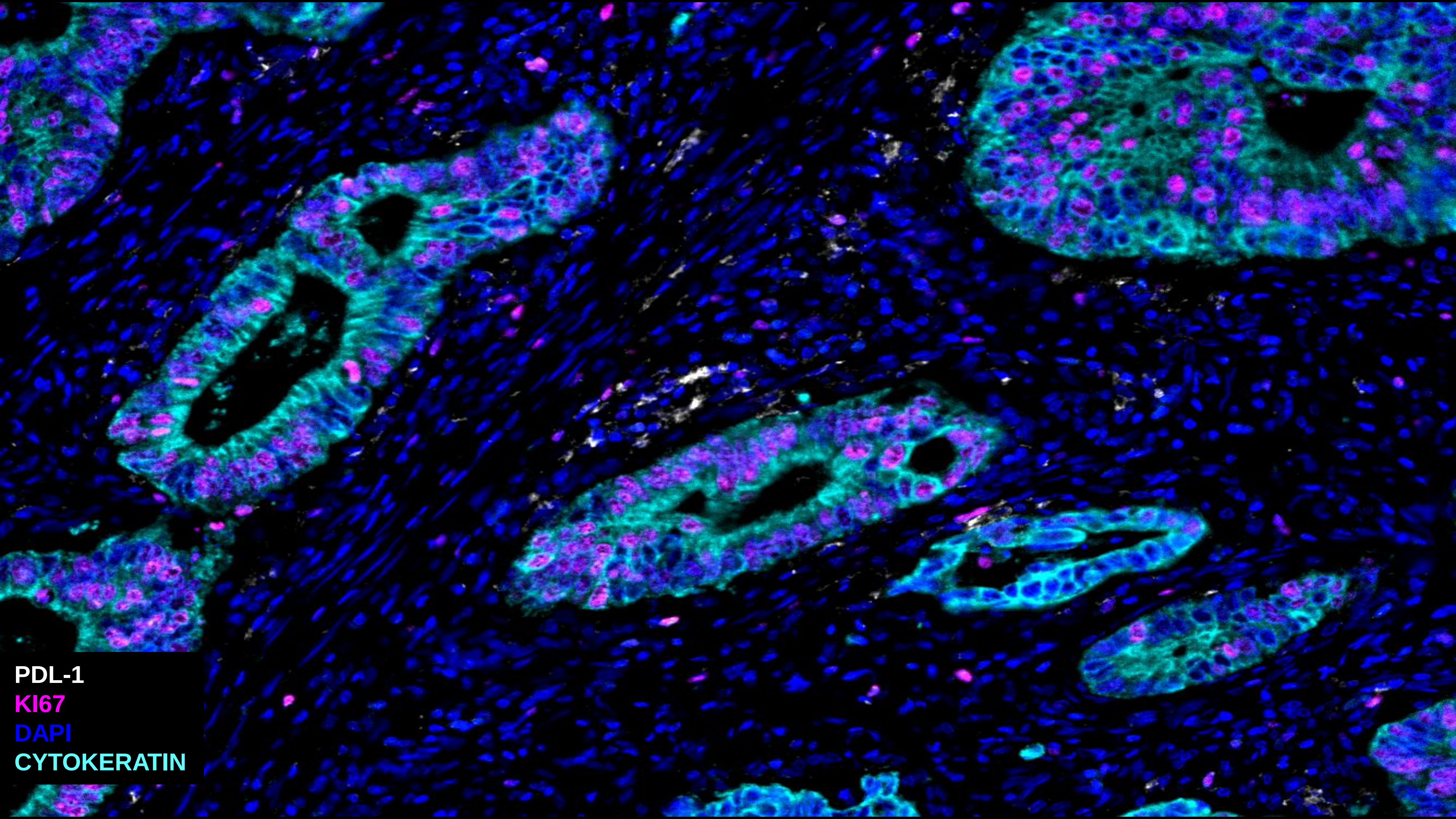


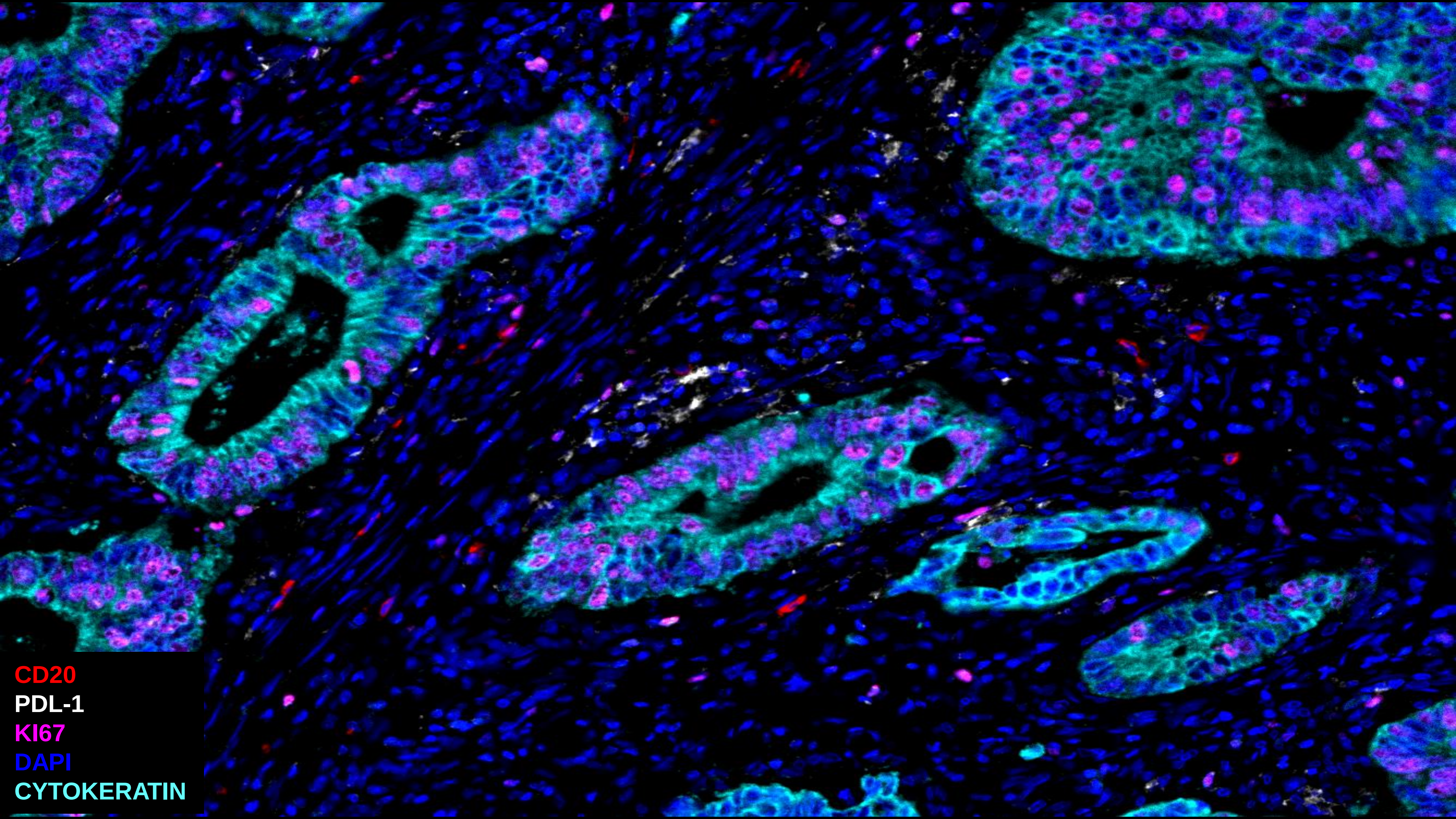
Deeper spatial resolution of the tumour immune microenvironment using multiplex imaging

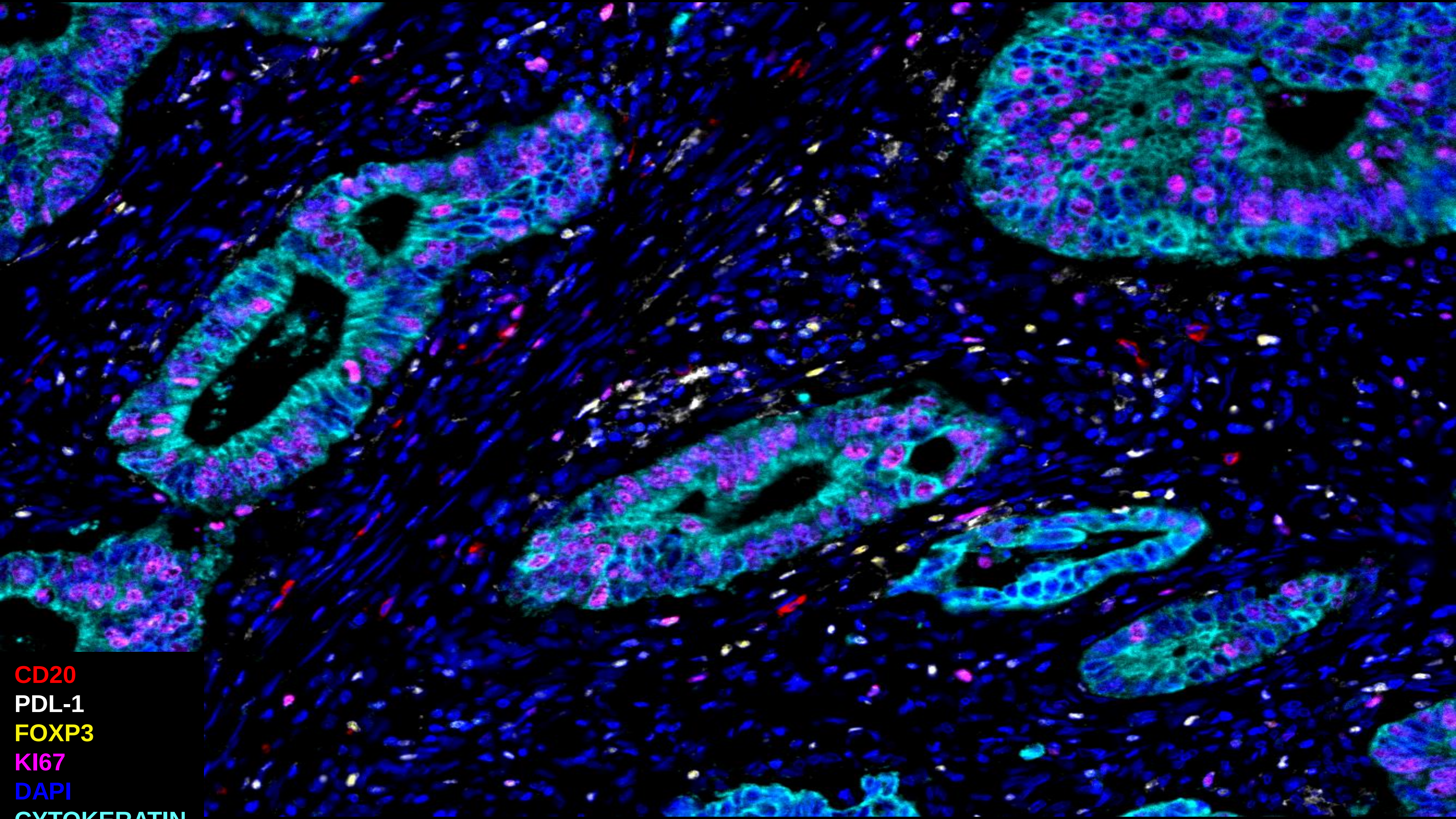


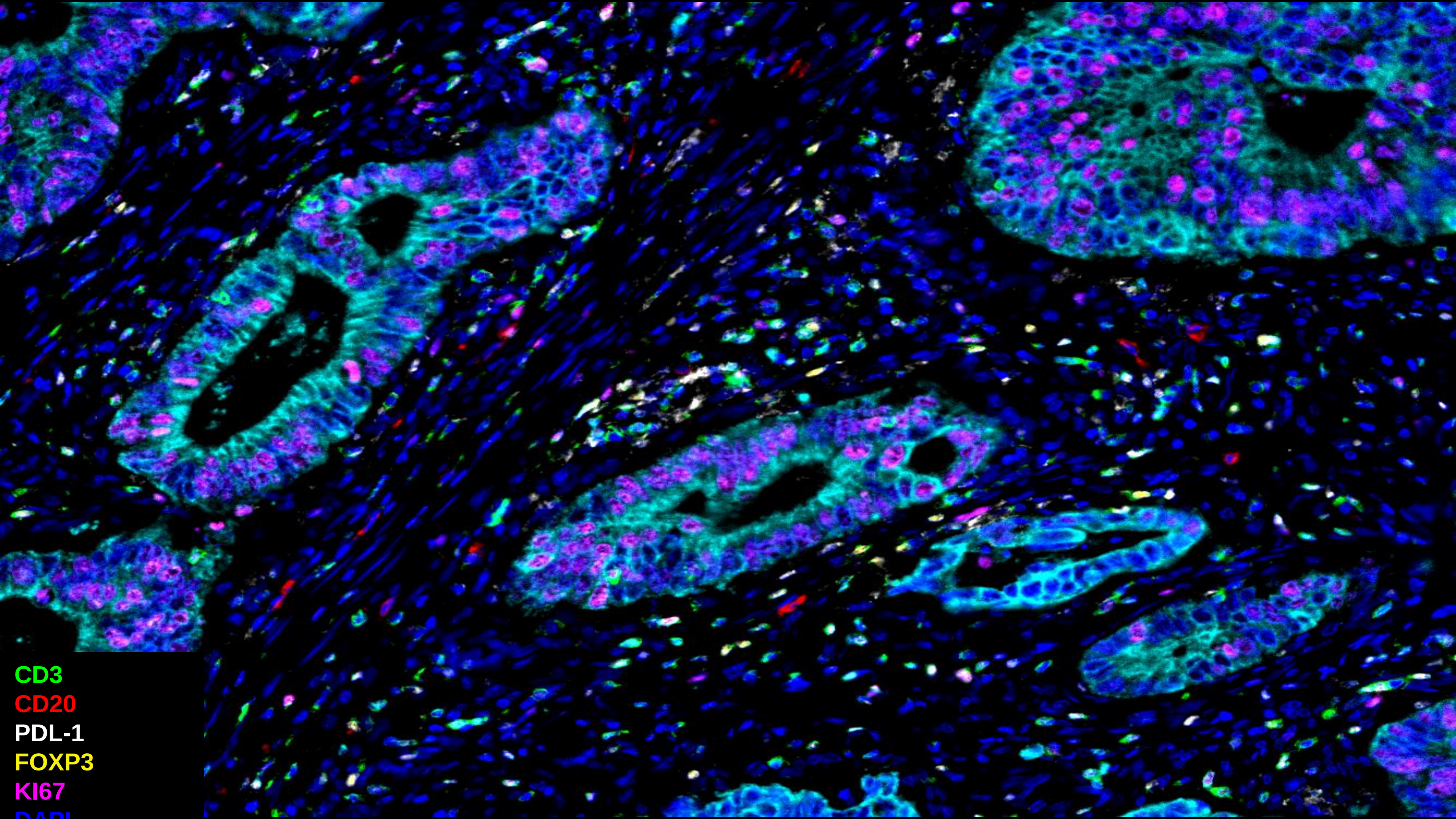

KI67
DAPI


KI67
DAPI
CYTOKERATIN


PDL-1
KI67
DAPI
CYTOKERATIN

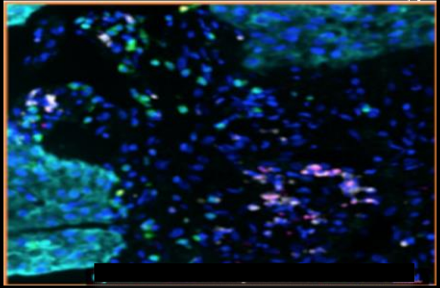

CD20
PDL-1
KI67
DAPI
CYTOKERATIN


CD20
PDL-1
FOXP3
KI67
DAPI
CYTOKERATIN

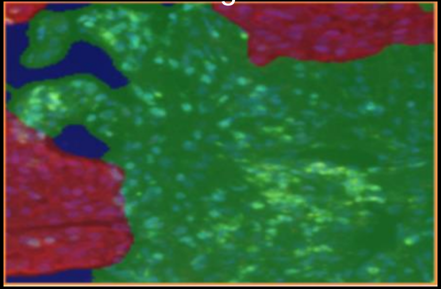

CD3
CD20
PDL-1
FOXP3
KI67

Multispectral analysis using 7-Plex phenotyping

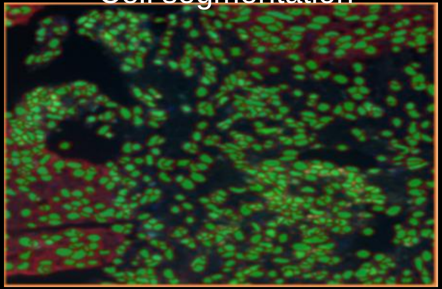
Fluorescent unmixed image



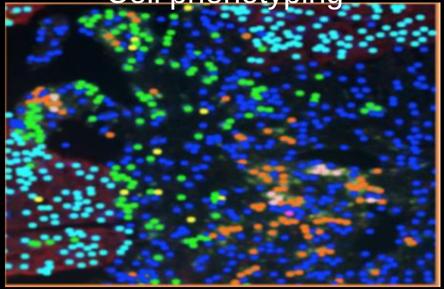
Tissue segmentation

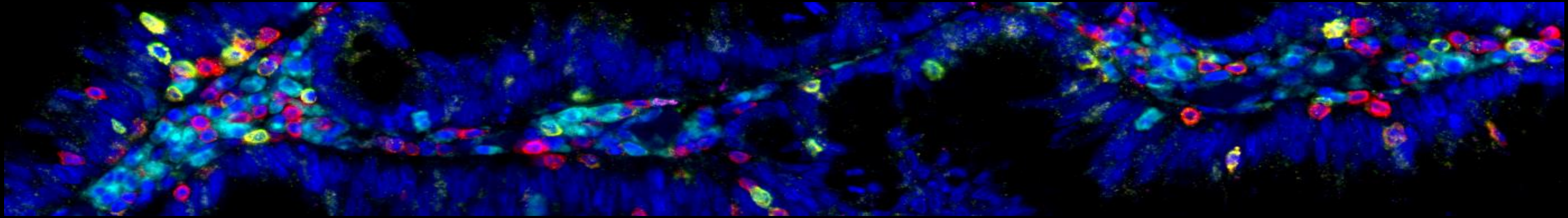


Cell segmentation

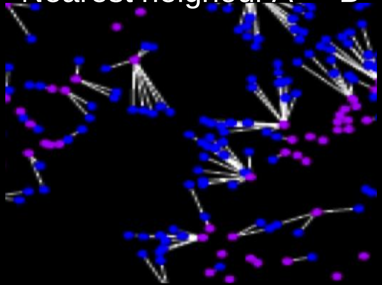


Cell phenotyping

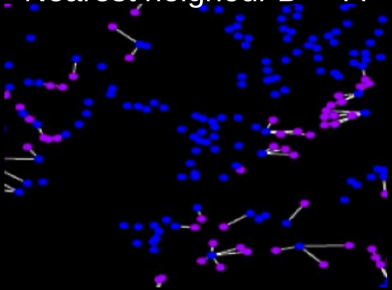




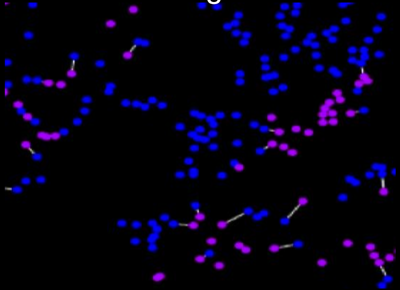
Nearest neighour A -> B



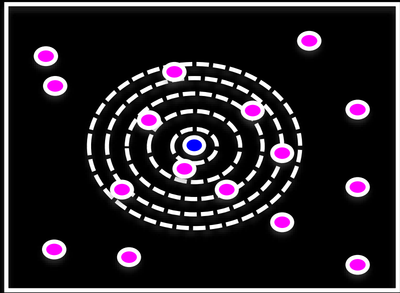
Nearest neighour B -> A



Mutual neighours



G fraction



Immunity
Review
CellPress

Tumor Immunology and Tumor Evolution: Intertwined Histories

Jérôme Galon[1,*] and Daniela Bruni[1]
[1]INSERM, Laboratory of Integrative Cancer Immunology, Equipe Labellisée Ligue Contre le Cancer, Sorbonne Université, Sorbonne Paris Cité, Université Paris Descartes, Université Paris Diderot; Centre de Recherche des Cordeliers, F-75006 Paris, France
*Correspondence: jerome.galon@crc.jussieu.fr

Galon & Bruni ***Immunity*** 2020

What is the importance of the pre-existing immunity within tumors ? Does it matter ?

MacCarty WC, Mahle AE.
Relation of differentiation and lymphocytic infiltration to postoperative longevity in gastric carcinoma.
J Lab Clin Med 1921 ; 6:473.

A Novel Paradigm for Cancer

Science AAAS

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†]

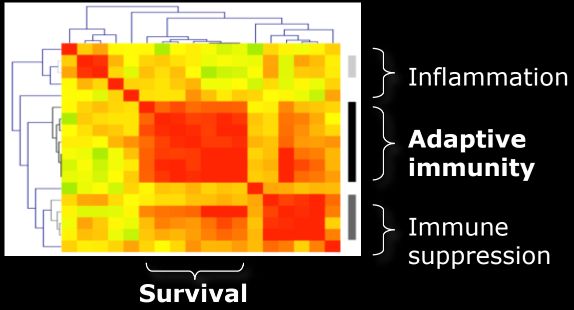
29 SEPTEMBER 2006 VOL 313 SCIENCE www.sciencemag.org

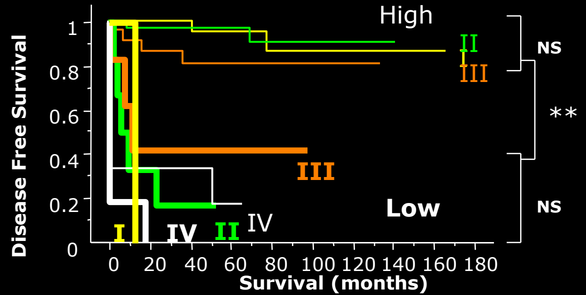
The foundation a new concept

Immune contexture

- ✓ Gene expression profiling
- ✓ Qualitative immune signature

- ✓ Immunohistochemistry (IHC)
- ✓ Digital Pathology
- ✓ Quantitative immune cell infiltration





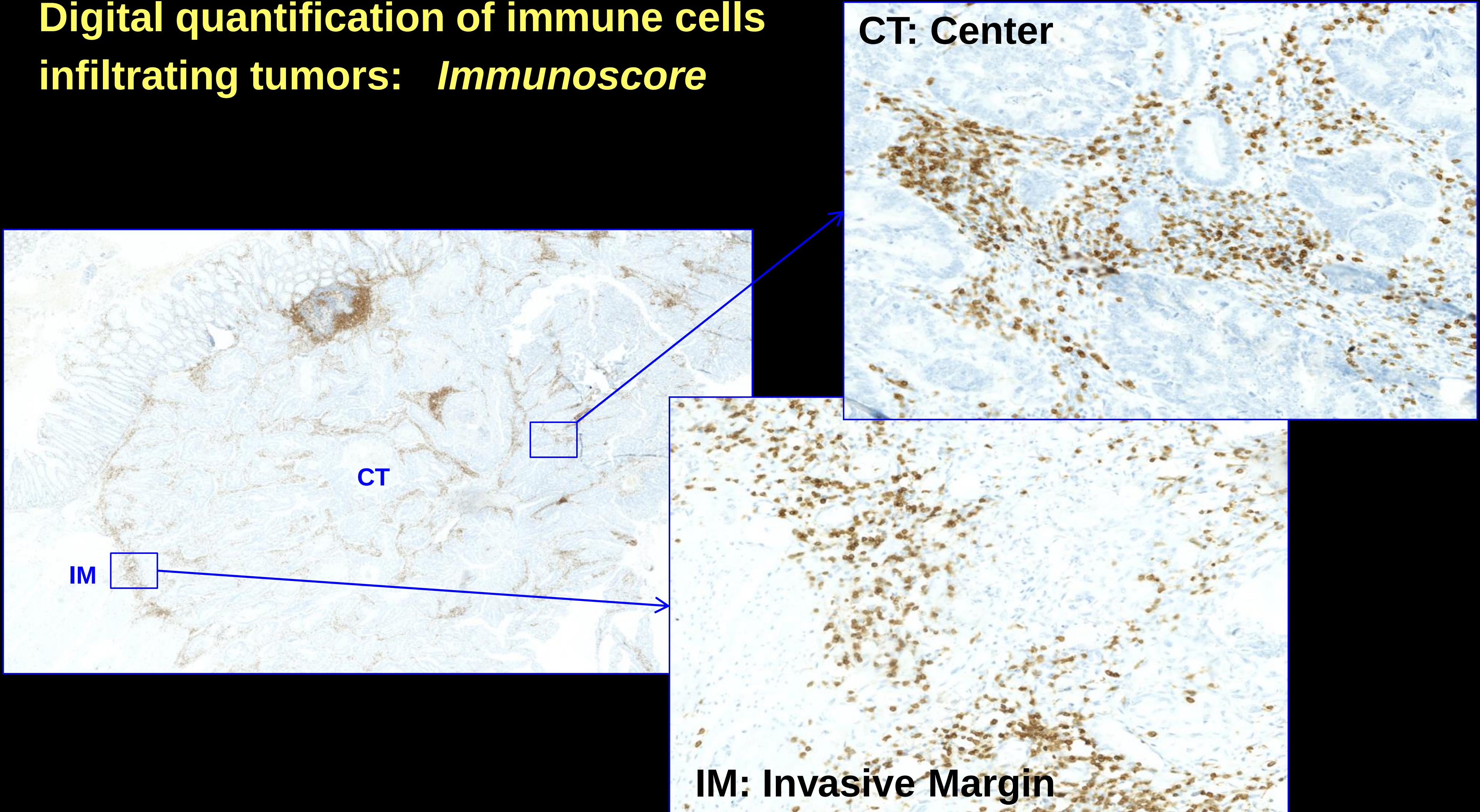
Optimized Immunosign

Quality

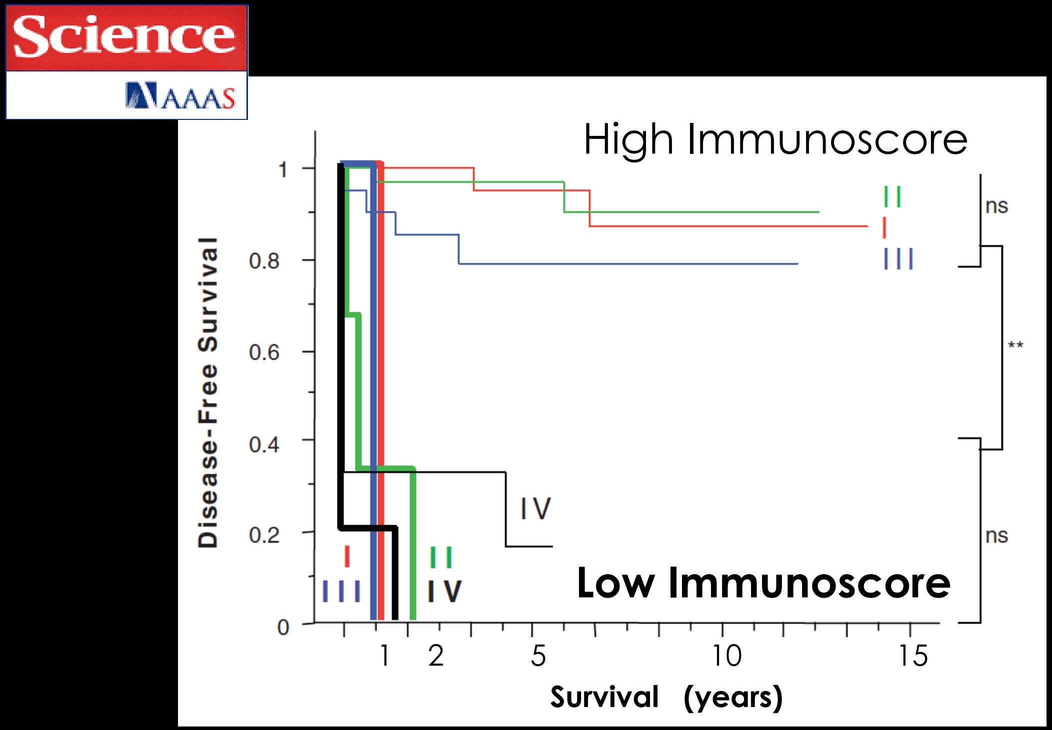
Type/Density/Location

Galon J et al. ***Science*** 2006

Digital quantification of immune cells infiltrating tumors: *Immunoscore*



Immunoscore: a novel paradigm for cancer



- ✓ High Immunoscore
- ✓ Inflammed tumors
- ✓ Strong pre-existing adaptive immunity

- ✓ Low Immunoscore
- ✓ Non-Inflammed tumors
- ✓ Weak/absent pre-existing adaptive immunity

Coordinated adaptive immune reaction (Immunoscore) more than tumor invasion predicts clinical outcome

Galon et al. ***Science*** 2006

Science AAAS

A Novel Paradigm for Cancer

Multivariate Cox Analysis

Parameters	*HR*	*P* value
• T-stage	1.2	0.25
• N-stage	1.4	0.15
• Differentiation	1.1	0.84
• **Immunoscore**	1.9	0.00001

"***Immune Contexture***" :

Cells -> ✓**Type**

Quantity -> ✓**Density**

Spatial -> ✓**Location**

-> **Immunoscore**

Quality -> ✓Immune **functional** orientation

-> **Immunosign**

Galon J et al. ***Science*** 2006

Assessment of a novel marker for prognosis

multivariate analysis (COX)

Not good marker

Novel marker

Gold standard UICC/TNM classification

P: ns

No improvement for prediction

Good marker

Novel marker

Gold standard UICC/TNM classification

$P<0.05$

Better accuracy for prediction +

Novel concept

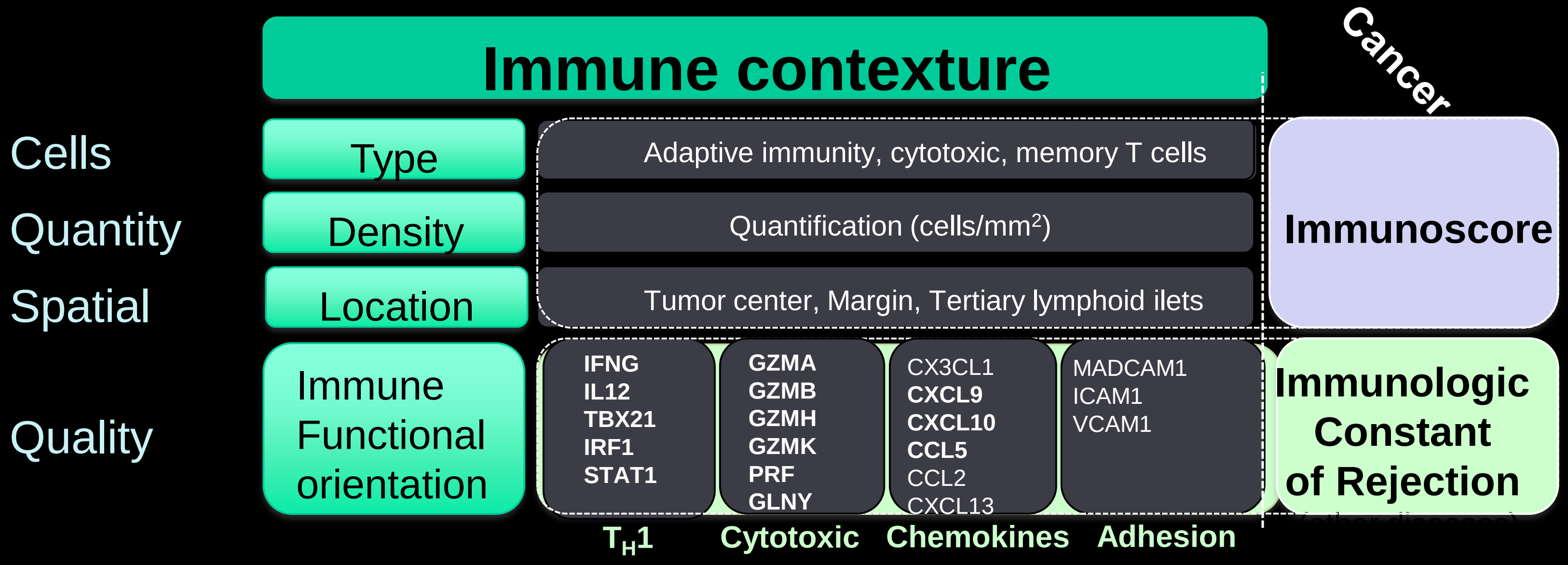
Immunoscore Immune contexture

AJCC/TNM

$P<0.0001$

Novel concept for prediction +++

The overlap between the immunologic constant of rejection, the immune contexture and the Immunoscore



Galon et al. ***Science*** 2006
Galon J et al. ***Cancer Res*** 2007
Galon J et al. ***Immunity*** 2013

JOURNAL OF CLINICAL ONCOLOGY
Official Journal of the American Society of Clinical Oncology

Cox Multivariate analysis including Immunoscore

COX analysis for DPS	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
Immunoscore	0.65	0.0003

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*

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

Galon J et al. ***Science*** 2006. Mlecnik B et al. ***JCO*** 2011

"TNM staging: T is for T cell and M is for Memory"

JOURNAL OF CLINICAL ONCOLOGY
Official Journal of the American Society of Clinical Oncology

Editorial: Broussard et al. *JCO* 2011

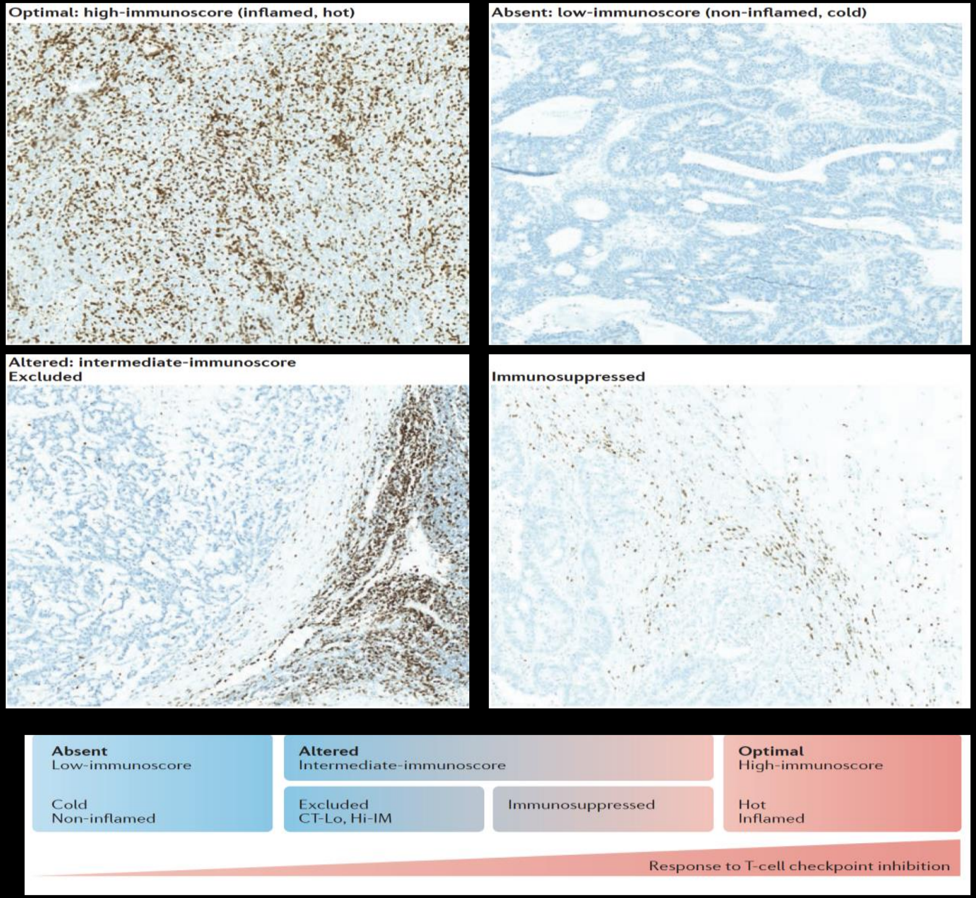
Multivariate Analysis

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
Immunoscore	0.64	<0.0001	0.71	<0.0001	0.63	<0.0001

Galon et al. *Science* 2006, Mlecnik et al. *JCO* 2011

- ✓ An immune classification of cancer
- ✓ The power of the pre-existing immunity
- ✓ The possibility to unleash the immune response with immunotherapy

Essential role of the pre-existing immunity: The Immune contexture



Major immune categories of tumors

- ✓ Immune infiltrated (Hot)
- ✓ Altered: Immune-excluded
- ✓ Altered: Immune suppressed
- ✓ Immune desert (Cold)

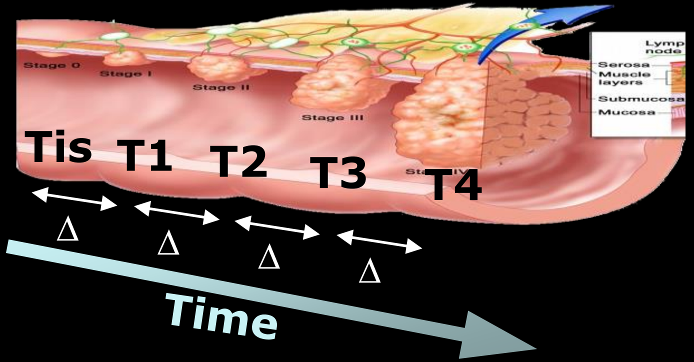
Original publications
Galon et al. ***Science*** *2006*
Camus & *Galon* ***Cancer Res*** 2009

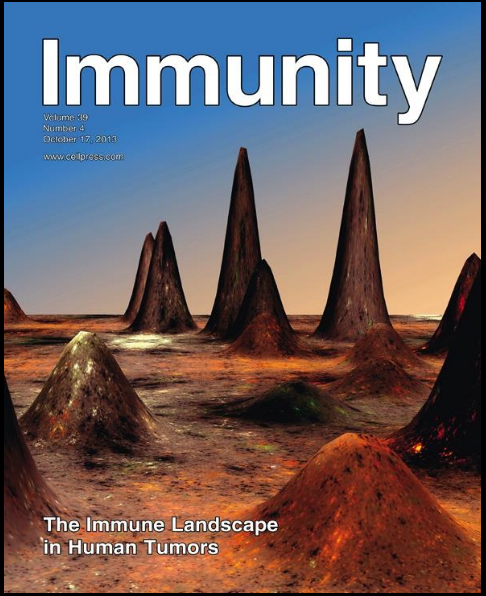
Review
Galon J. & Bruni D.
Nature Reviews Drug Discovery 2019

Understanding the evolution of the immune response with tumor progression using systems biology

- **The Immune Landscape in human cancer**
- Evolution of the tumor microenvironment with tumor progression?
- Immune escape mechanisms in human tumors?

T-Stage

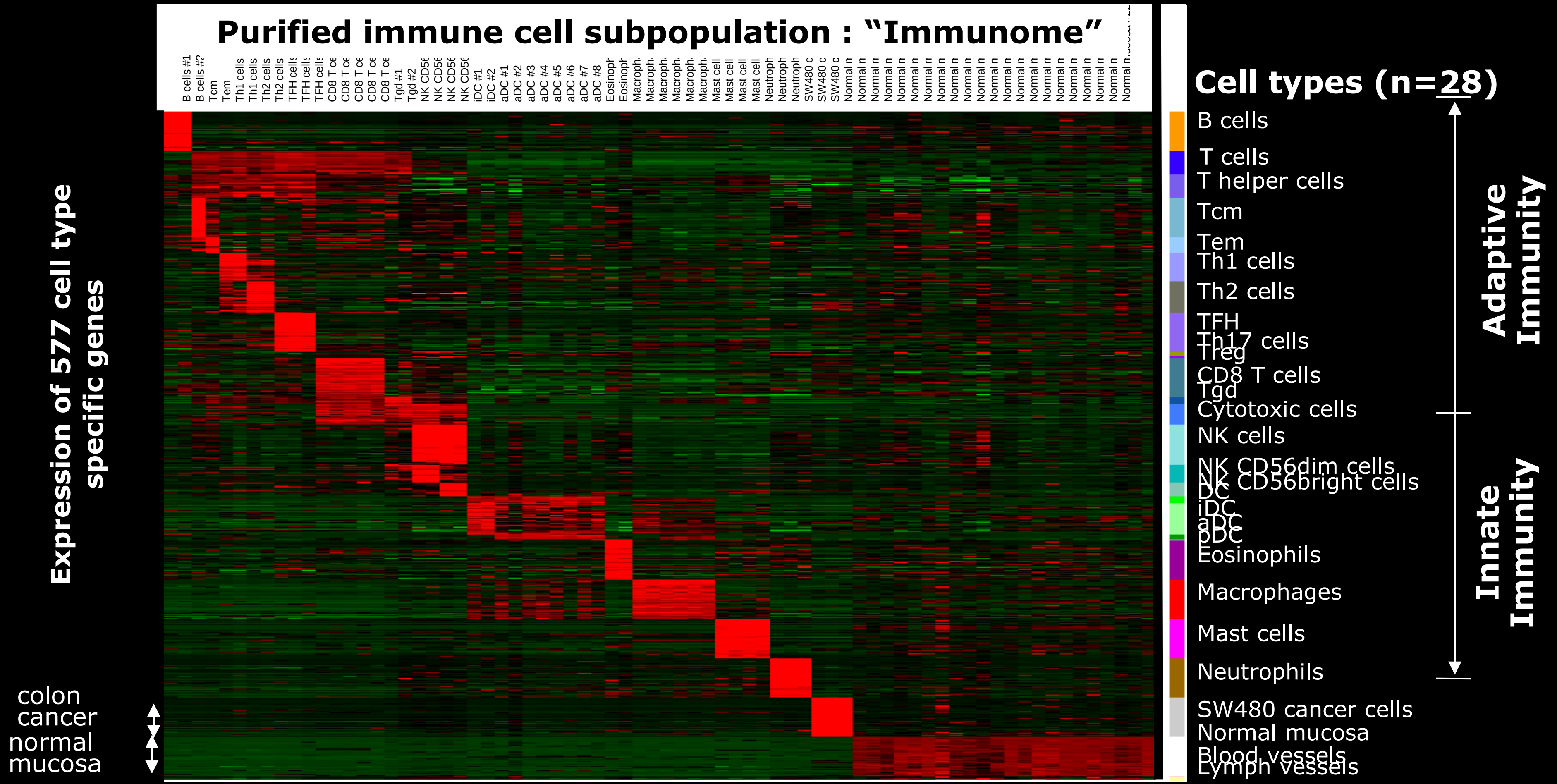




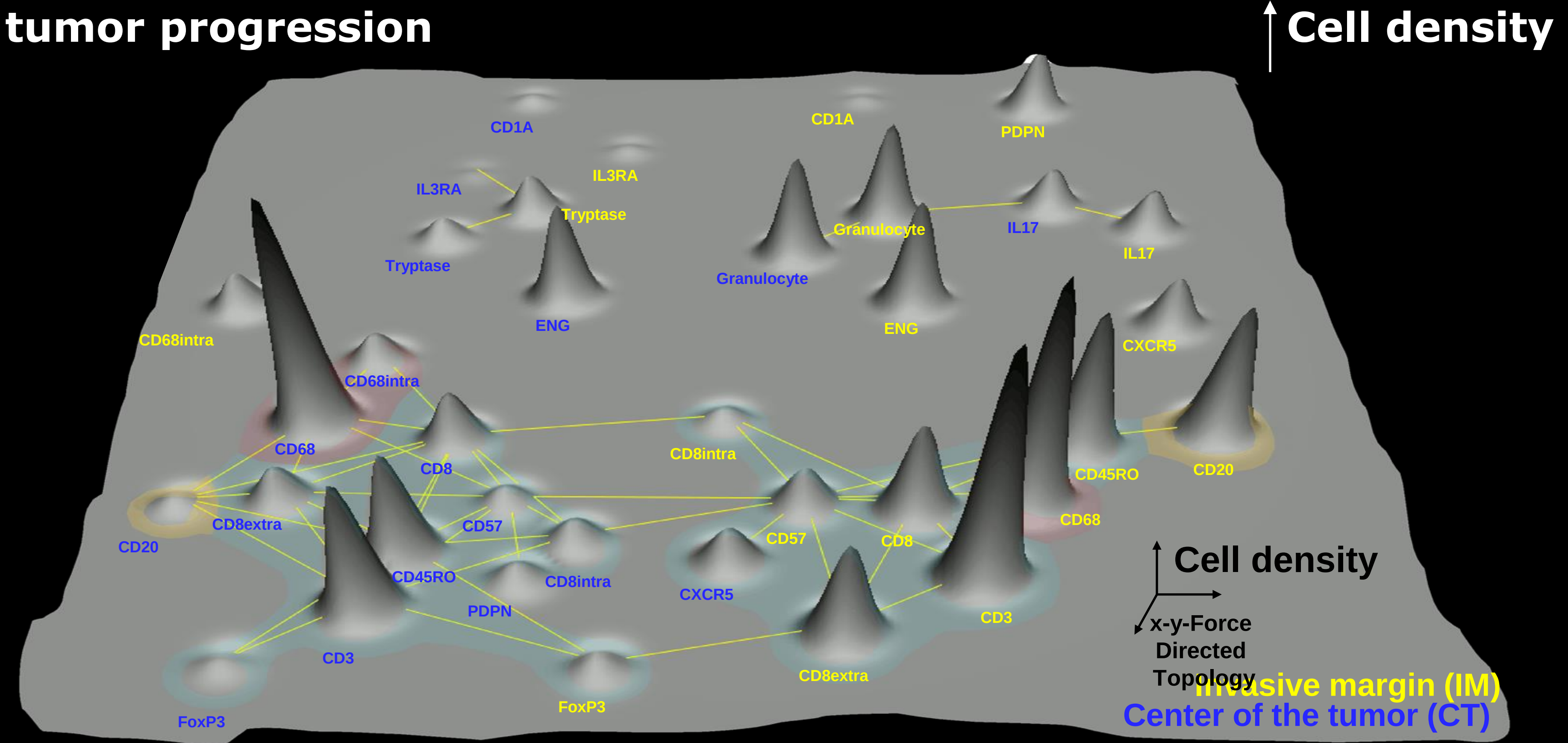
-> **Spatio-temporal dynamics**
of the immune response with tumor progression

Bindea G et al. ***Immunity*, 2013**

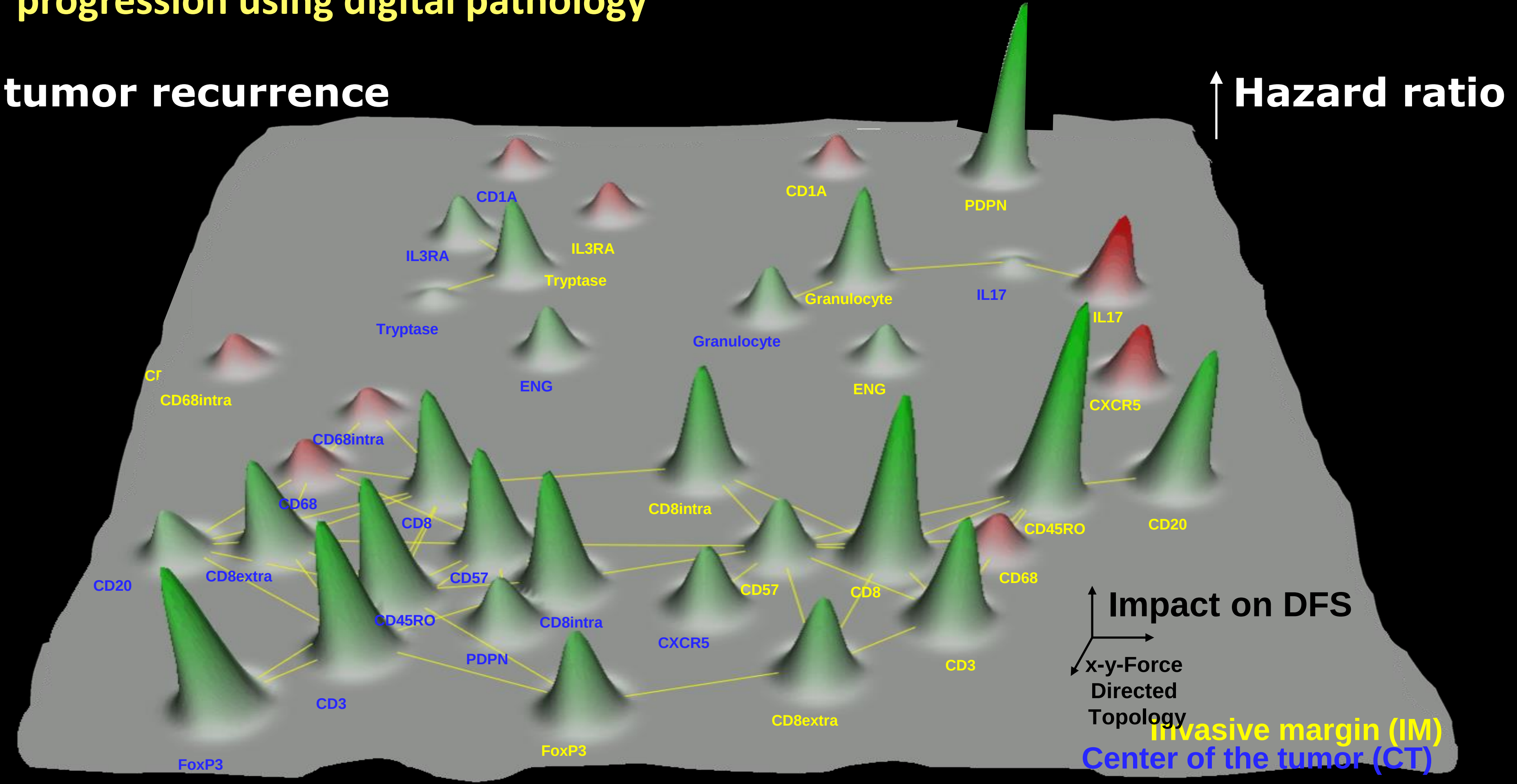
“Immunome” of purified immune cell subpopulations



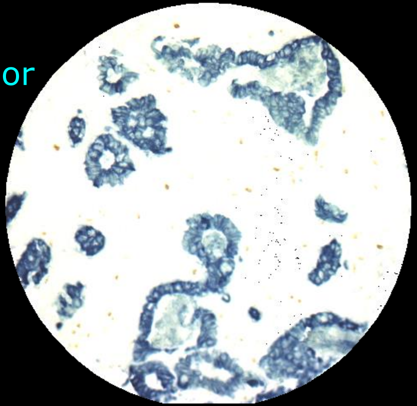
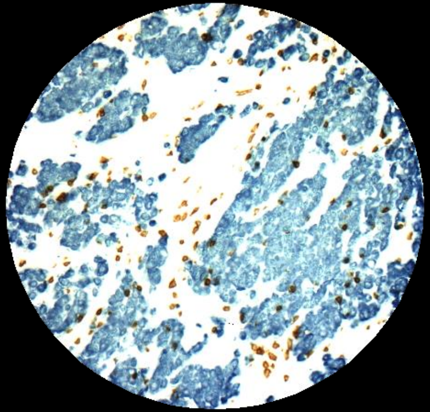
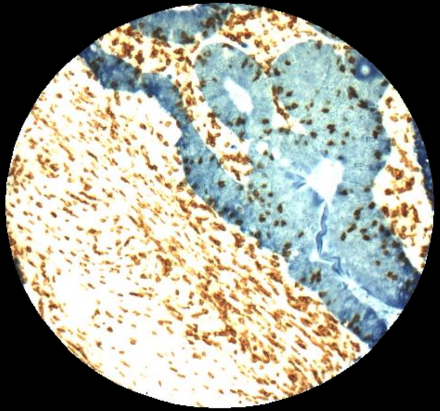
Understanding the evolution of the immune response along with tumor progression using digital pathology



Understanding the evolution of the immune response along with tumor progression using digital pathology



Is the quantification of the pre-existing immunity with Immunoscore clinically relevant ?

Patient 1 (weak)	Patient 2 (moderate)	Patient 3 (strong)
		

CD3
Tumor

	Patient 1 (weak)	Patient 2 (moderate)	Patient 3 (strong)
Immunoscore CD3/CD8 Center/Margin	**I 0**	**I 2**	**I 4**
Median OS (death)	< 2 years	4.9 years	> 15 years

Colorectal cancer classifications

Tumor cell extension and invasion	T-STAGE	N-STAGE	M-STAGE

Ways to classify	Morphology	Cell of origin	Molecular pathway	Mutation status	Gene expression
Tumor cell characteristics	Mucinous	Enterocyte	CIN	BRAF	CMS1
	Medullary	Goblet-like	MSI	APC	CMS2
	Adeno. NOS	Transit-amplifying-R	CIMP	KRAS	CMS3
	Serrated	Transit-amplifying-S		TP53	CMS4
	Signet ring cell	Inflammatory		CTNNB1	
	Micropapillary	Stem-like			
	Cribriform comedo - type				

Host immune response	**Immunoscore**	CD3+ T cells	CD8+ T cells	Density	Location (CT, IM)

Galon et al. *J Pathol*. 2014

The Immunoscore as a New Possible Approach for the Classification of Cancer

World Immunotherapy Council inaugural meeting (Feb 2012)

Support (moral) from the World Immunotherapy Council (WIC), and support from societies including, EATI, BDA, CCIC, CIC, CRI, CIMT, CSCO, TIBT, DTIWP, ESCII, NIBIT, JACI, NCV-network, PIVAC, ATTACK, TVACT…

Worldwide Immunoscore consortium (PI: J Galon)

(17 countries: >3000 Stage I/II/III Colon cancer patients)

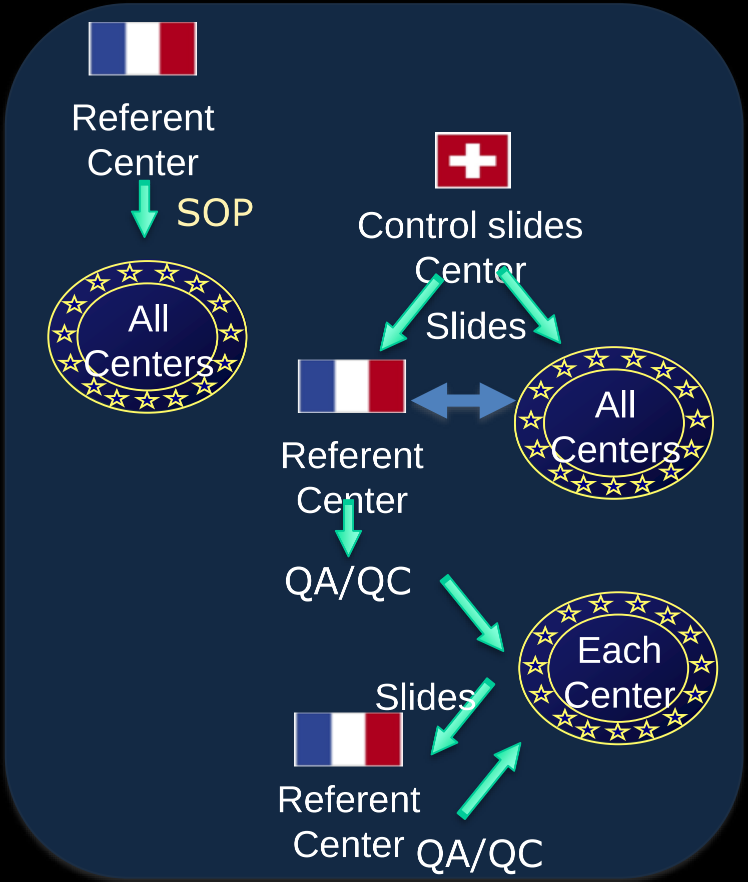
Assay harmonization

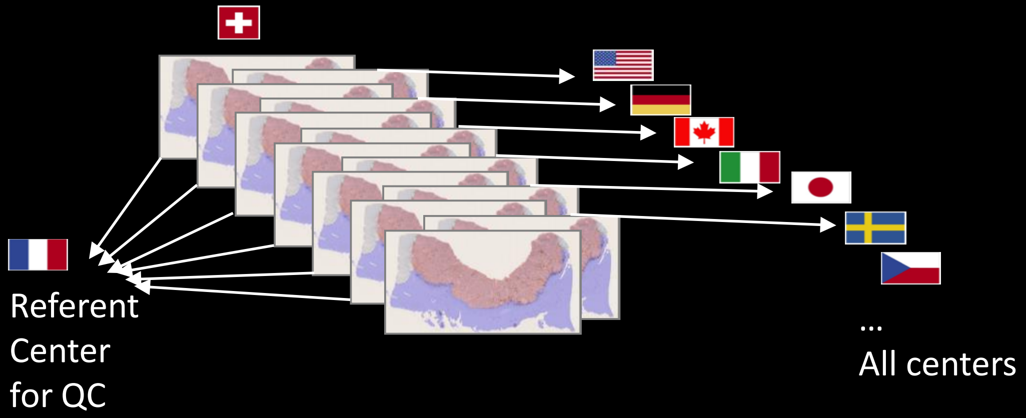
Immunoscore meetings :

- Feb 2012, Italy
- Dec 2012, Italy
- Nov 2013, SITC, USA
- Dec 2013, Italy
- Jan 2014, Qatar
- Jul 2014, Paris, France
- Nov 2014, SITC, USA
- Nov 2015, SITC, USA
- Dec 2015, Italy
- Feb 2016, USCAP, USA
- April 2016, USA
- Nov 2016, SITC, USA
- Dec 2016, Italy
- Feb 2017, USCAP, USA
- Dec 2017, Italy

Worldwide Immunoscore consortium (PI: J Galon)

Study design

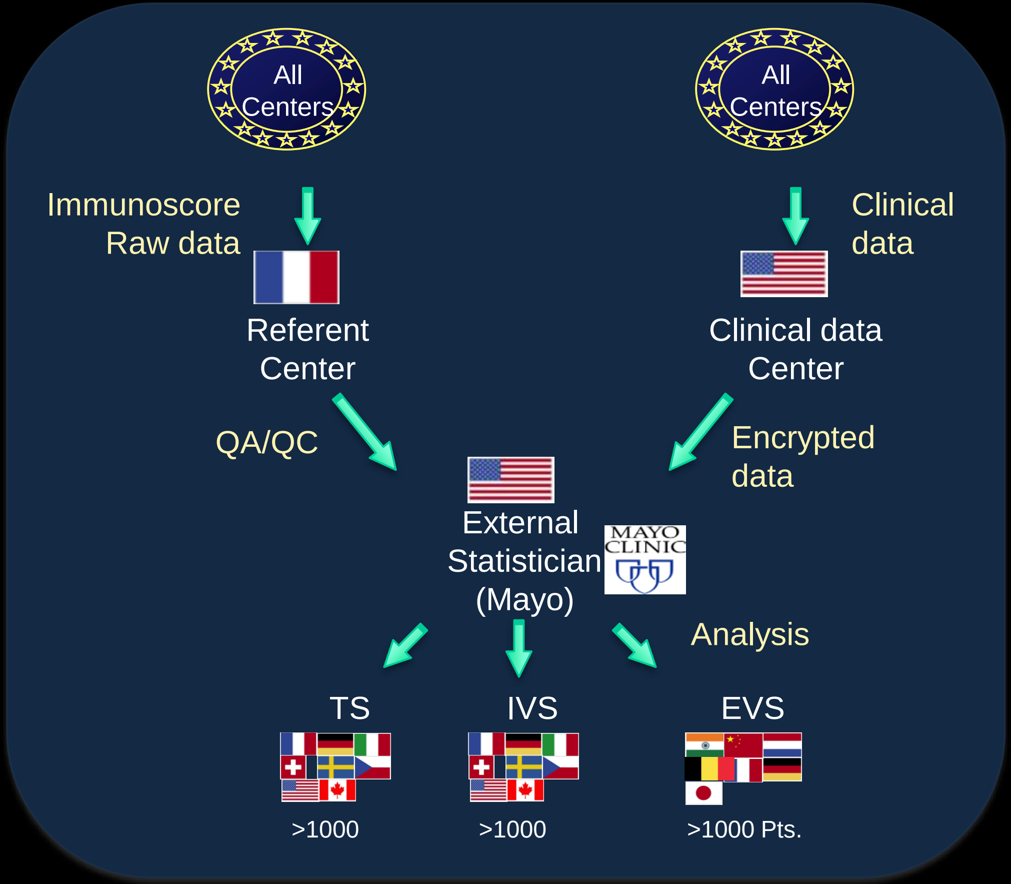




Harmonization

Worldwide Immunoscore consortium (PI: J Galon)

Study design



IMMUNOSCORE : METHODS

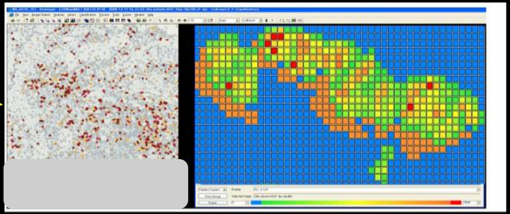
-> Standardized Operating Procedure
-> Today’s tools for modern pathologists

IHC automate

High-resolution scanner

whole slide quantification

Digital pathology



Software (INSERM, HalioDx, Definiens)

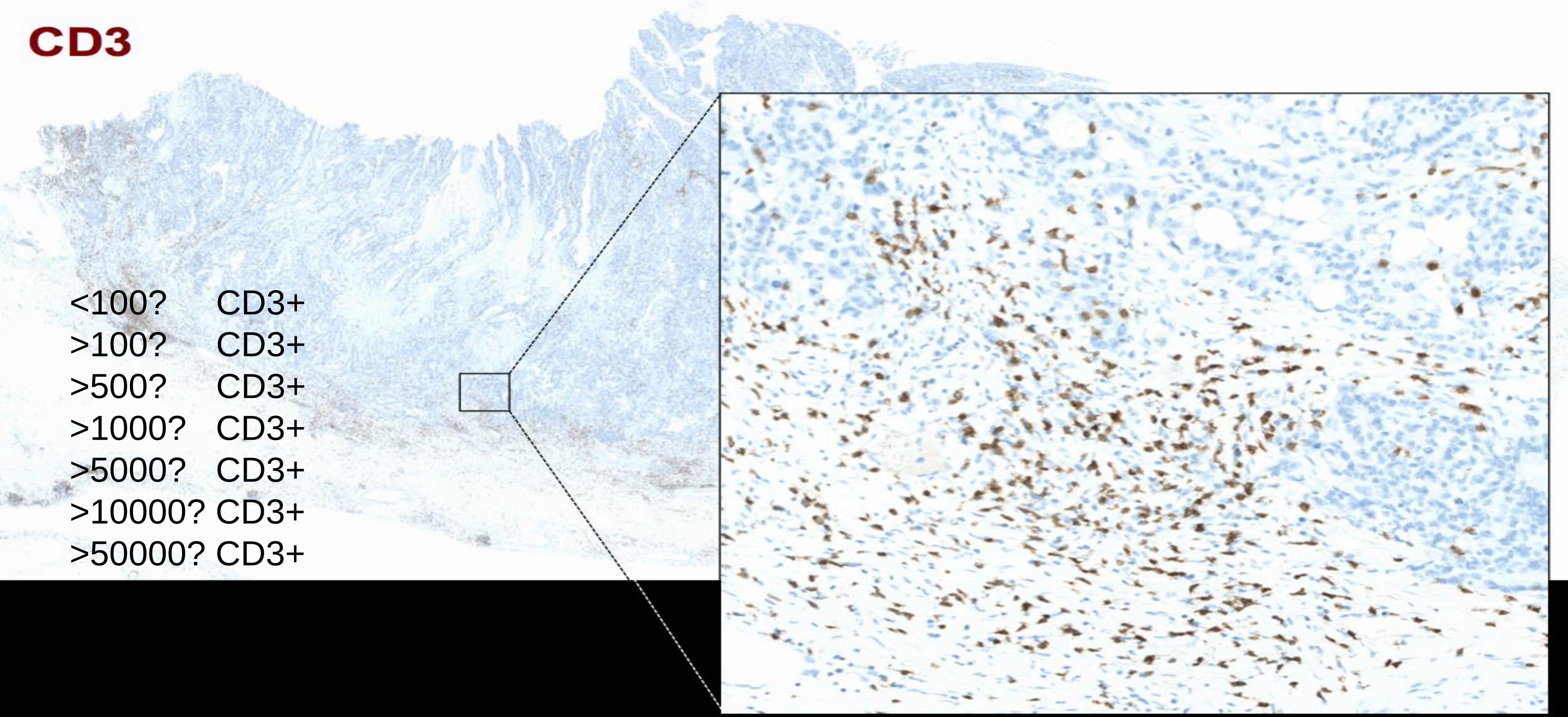
-> Quantitative Immuno-Pathology

Immunoscore

Galon J et al. ***J. Transl. Med.*** 2012
Galon J et al. ***J. Pathol.*** 2014
Pages F et al. ***Lancet*** 2018

Immunoscore using whole slide FFPE

Routine whole slide stainings & full image quantification



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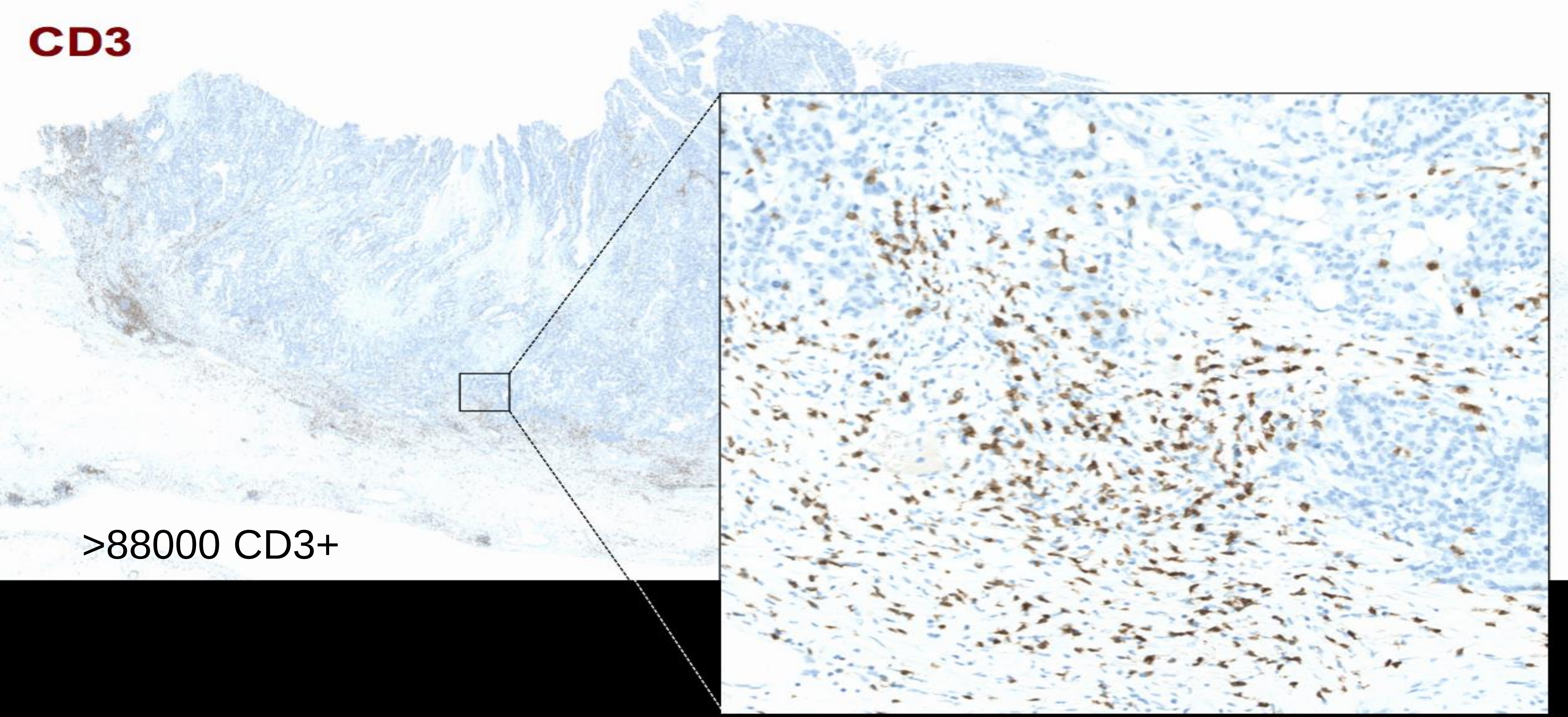


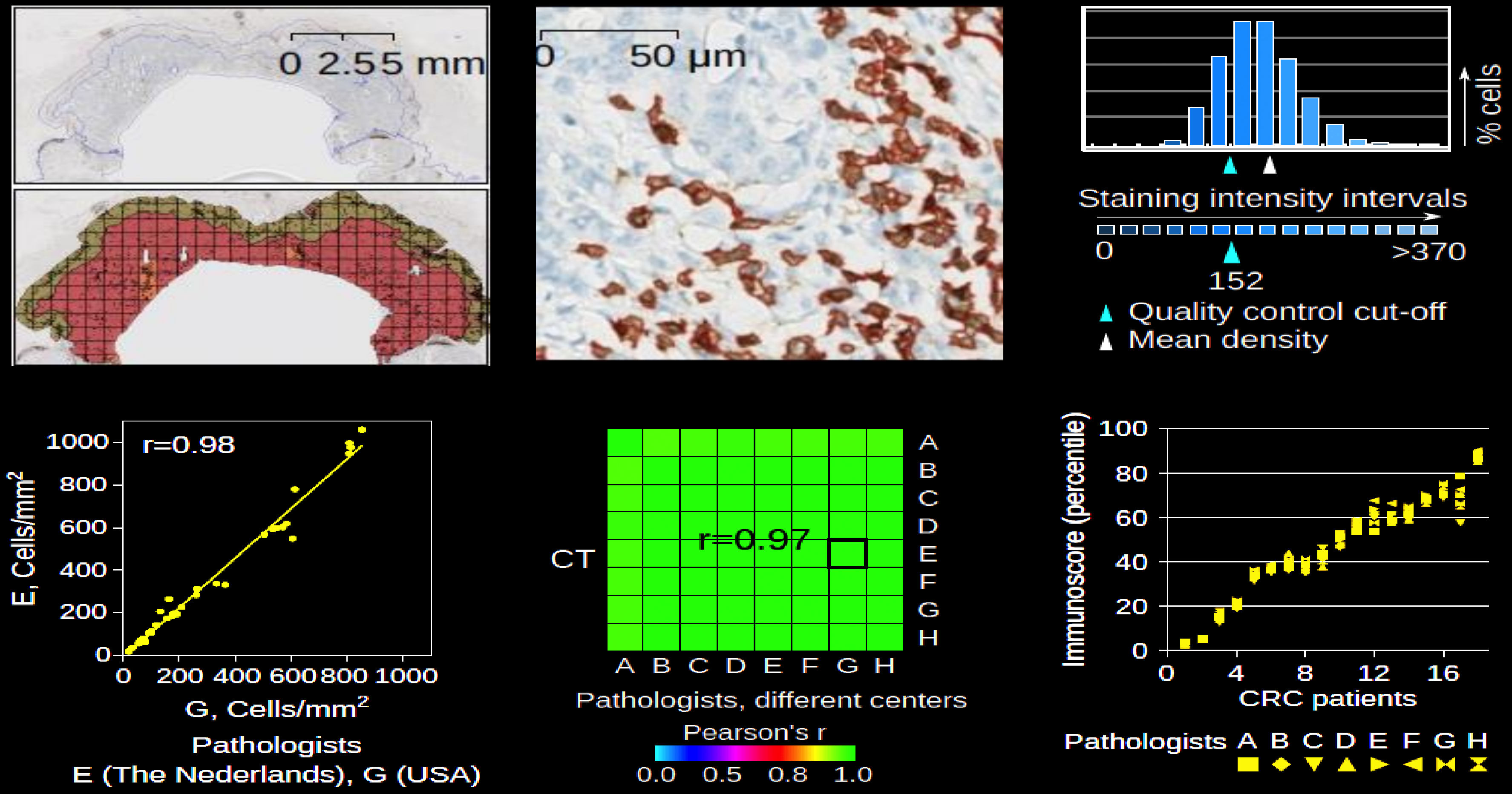
THE LANCET

International validation of the consensus Immunoscore for the classification of colon cancer: a prognostic and accuracy study

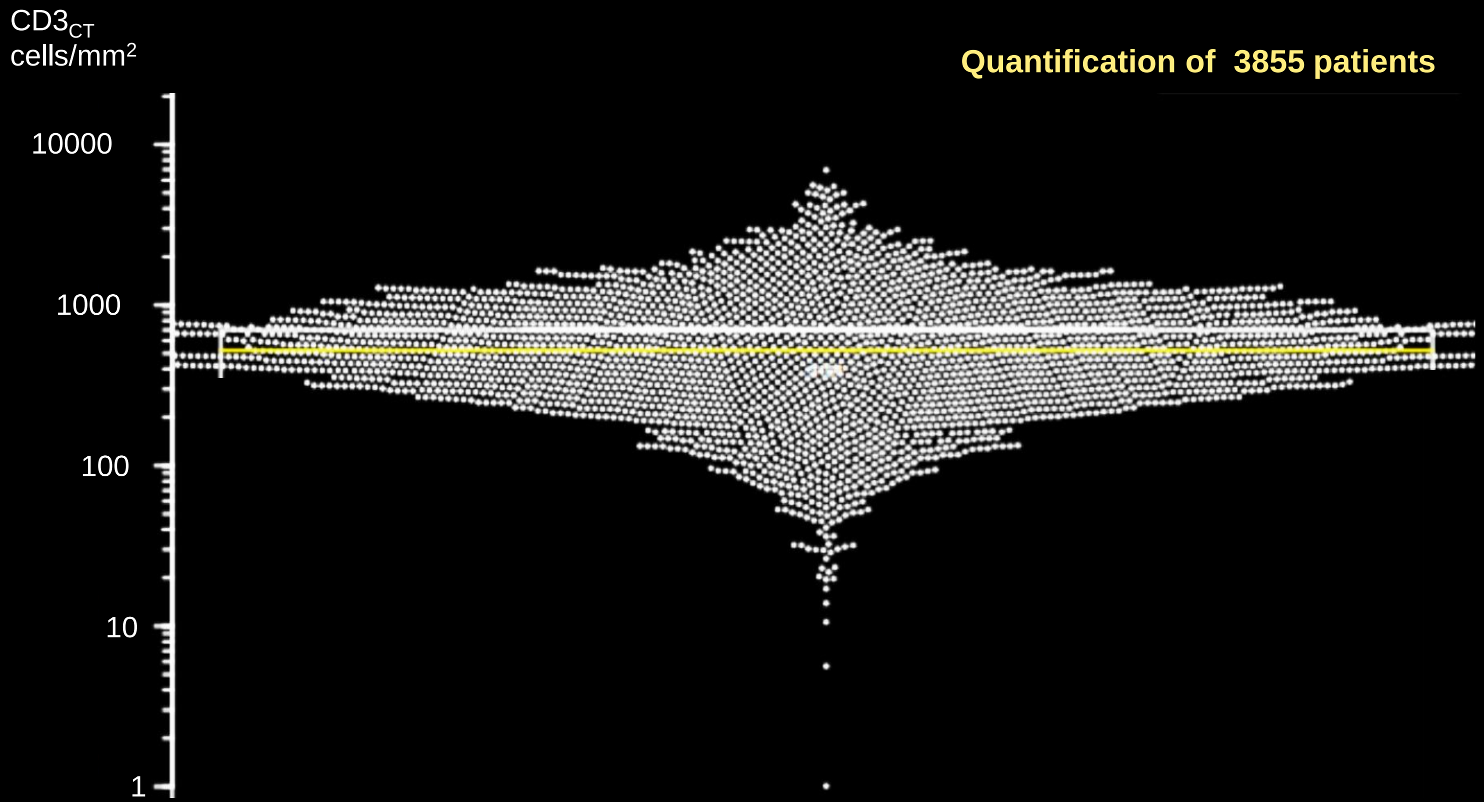
Franck Pagès, Bernhard Mlecnik, Florence Marliot, Gabriela Bindea, Fang-Shu Ou, Carlo Bifulco, Alessandro Lugli, Inti Zlobec, Tilman T Rau, Martin D Berger, Iris D Nagtegaal, Elisa Vink-Börger, Arndt Hartmann, Carol Geppert, Julie Kolwelter, Susanne Merkel, Robert Grützmann, Marc Van den Eynde, Anne Jouret-Mourin, Alex Kartheuser, Daniel Léonard, Christophe Remue, Julia Y Wang, P Bavi, Michael H A Roehrl, Pamela S Ohashi, Linh T Nguyen, SeongJun Han, Heather L MacGregor, Sara Hafezi-Bakhtiari, Bradly G Wouters, Giuseppe V Masucci, Emilia K Andersson, Eva Zavadova, Michal Vocka, Jan Spacek, Lubos Petruzelka, Bohuslav Konopasek, Pavel Dundr, Helena Skalova, Kristyna Nemejcova, Gerardo Botti, Fabiana Tatangelo, Paolo Delrio, Gennaro Ciliberto, Michele Maio, Luigi Laghi, Fabio Grizzi, Tessa Fredriksen, Bénédicte Buttard, Mihaela Angelova, Angela Vasaturo, Pauline Maby, Sarah E Church, Helen K Angell, Lucie Lafontaine, Daniela Bruni, Carine El Sissy, Nacilla Haicheur, Amos Kirilovsky, Anne Berger, Christine Lagorce, Jeffrey P Meyers, Christopher Paustian, Zipei Feng, Carmen Ballesteros-Merino, Jeroen Dijkstra, Carlijn van de Water, Shannon van Lent-van Vliet, Nikki Knijn, Ana-Maria Mușină, Dragos-Viorel Scripcariu, Boryana Popivanova, Mingli Xu, Tomonobu Fujita, Shoichi Hazama, Nobuaki Suzuki, Hiroaki Nagano, Kiyotaka Okuno, Toshihiko Torigoe, Noriyuki Sato, Tomohisa Furuhata, Ichiro Takemasa, Kyogo Itoh, Prabhu S Patel, Hemangini H Vora, Birva Shah, Jayendrakumar B Patel, Kruti N Rajvik, Shashank J Pandya, Shilin N Shukla, Yili Wang, Guanjun Zhang, Yutaka Kawakami, Francesco M Marincola, Paolo A Ascierto, Daniel J Sargent, Bernard A Fox, Jérôme Galon*

Pages et al. *The Lancet* 2018

Immunoscore quality controls



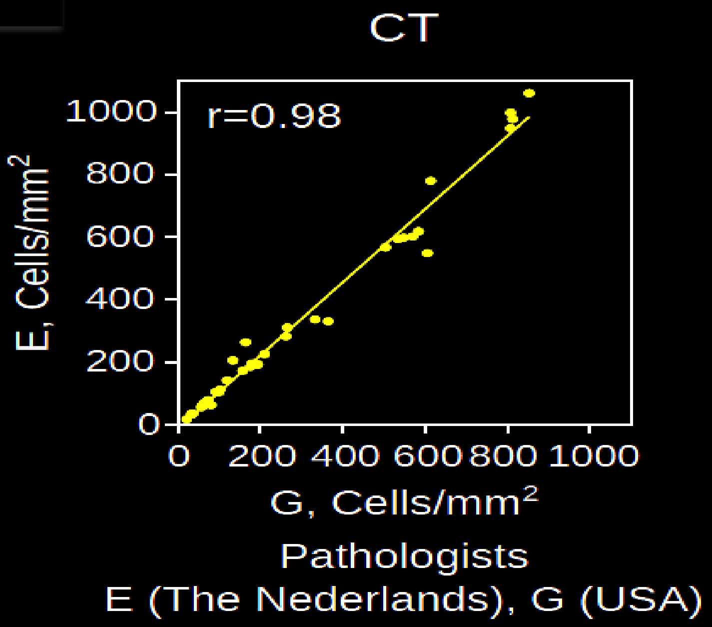
Densities of $CD3_{CT}$ (cells/mm^2) within tumors

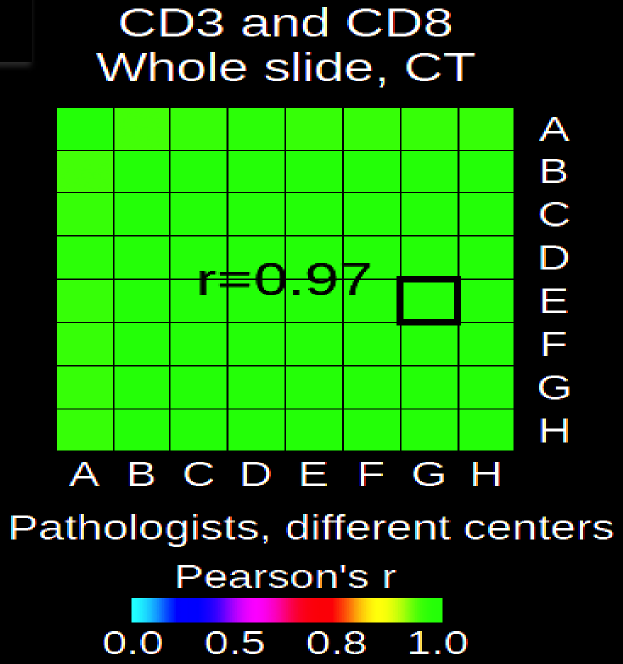


- ✓ Whole slide quantification within the CT region
- ✓ Similar quantification were performed for $CD3_{CT}$, $CD3_{IM}$, $CD8_{CT}$, $CD8_{IM}$

Immunoscore quality controls

Correlation between 8 pathologists from different countries using the software for the digital quantification of Immunoscore



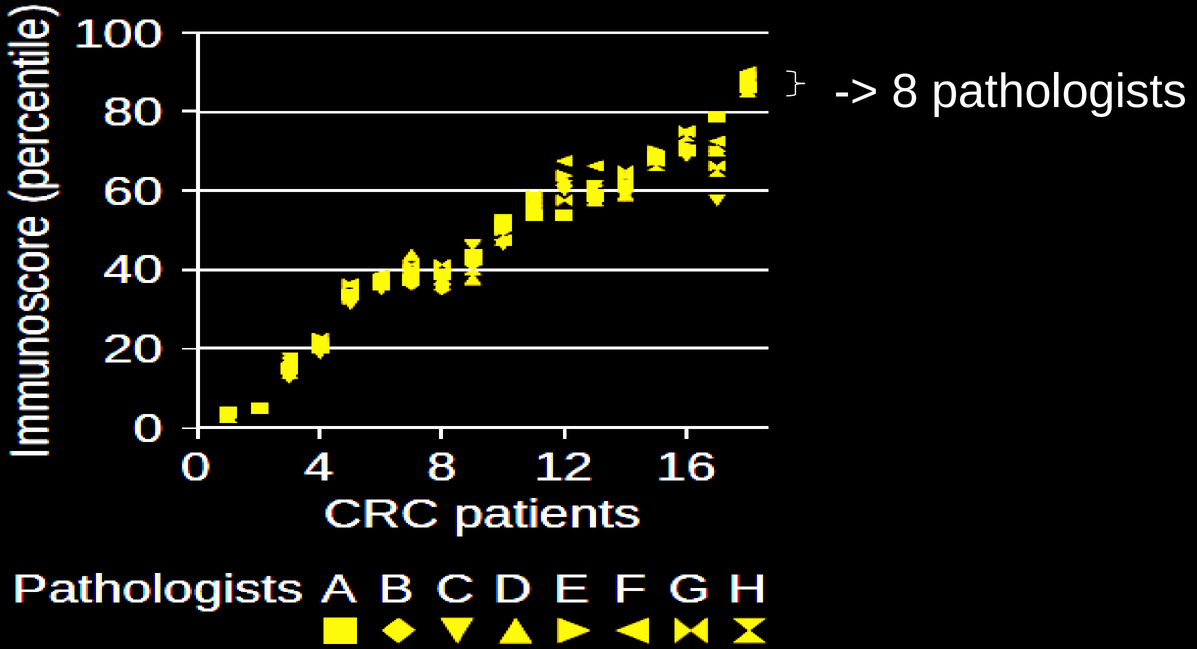


Very good correlation (R=0.97, *P*<0.0001) between independent digital quantification of Immunoscore (Immunoscore software) by 8 pathologists

Pages et al. *The Lancet* 2018

Immunoscore quality controls

Immunoscore quantification with digital pathology performed by 8 independent pathologists

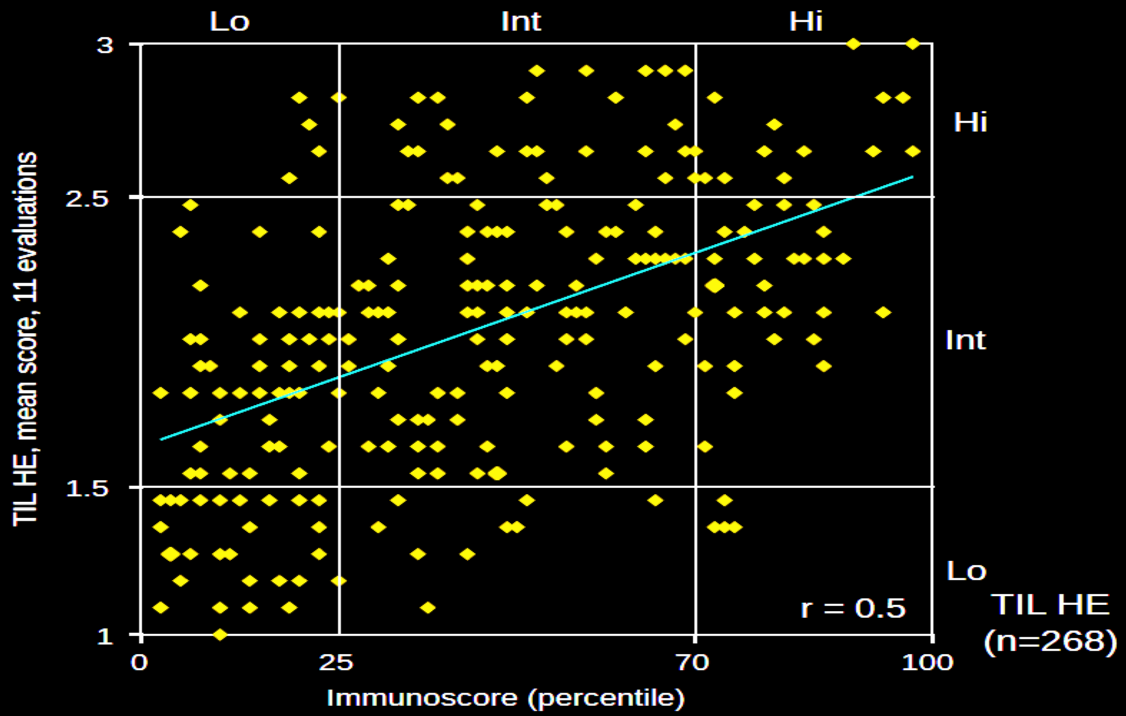


Very good concordance between independent digital quantification of Immunoscore (Immunoscore software) by 8 pathologists

Pages et al. *The Lancet* 2018

TIL evaluation on H&E slides quality controls

Comparison between and 11 independent TIL evaluation on H&E slides (mean score) and Immunoscore quantification by digital pathology

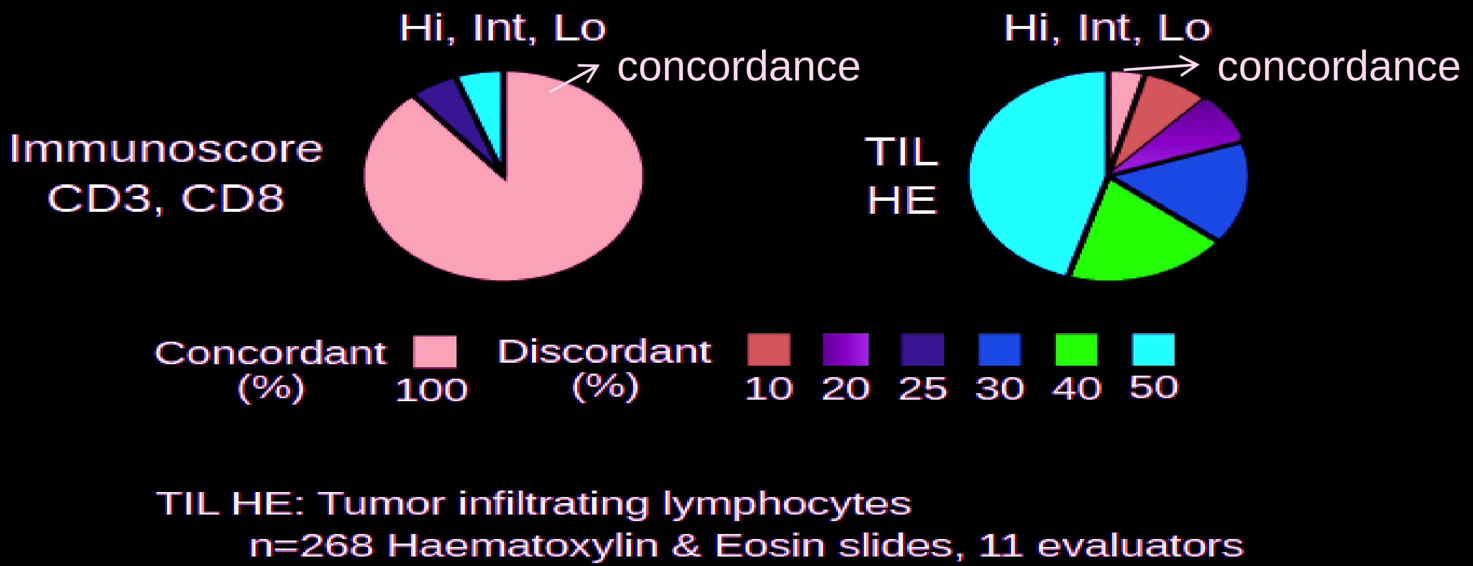


Poor correlation between Immunoscore quantification and TIL evaluation on H&E

Pages et al. *The Lancet* 2018

Immunoscore quality controls

Comparison of the concordance between independent evaluation of TIL on H&E slides and Immunoscore quantification

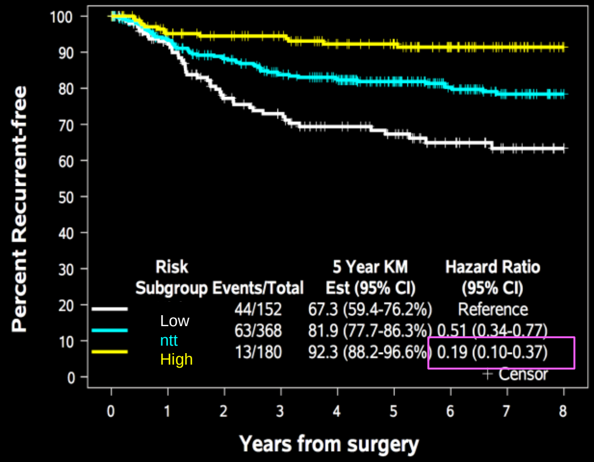


- Discordance between 11 independent TIL evaluation on H&E slides
- Concordance between 8 independent Immunoscore quantification by digital pathology
- High Robustness and reproducibility of the Immunoscore quantification

Pages et al. *The Lancet* 2018

Time to recurrence for Immunoscore (High/Int/Low)

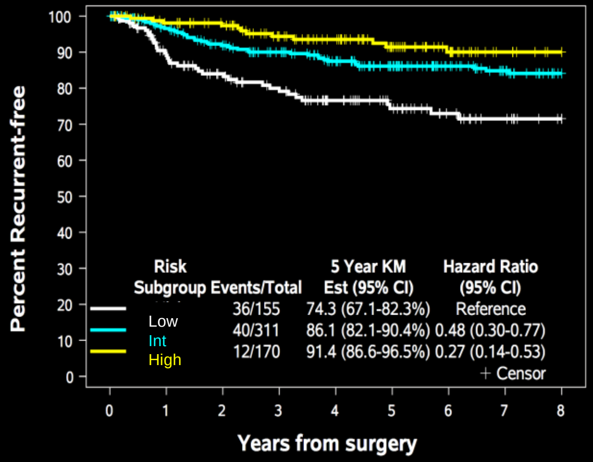
TS



Subgroup					
Low	152	92	71	48	31
Int	368	269	218	144	92
High	180	140	118	86	53

***P*< 0.0001**
HR (0-2)= 0.19
C-index= 0.64

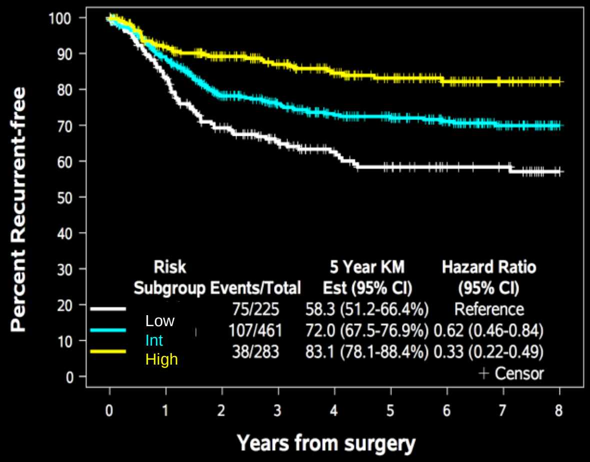
IVS



Subgroup					
Low	155	109	79	52	34
Int	311	248	204	139	104
High	170	139	104	64	41

***P*= 0.0001**
HR (0-2)= 0.27
C-index= 0.63

EVS



Subgroup					
Low	225	120	75	53	35
Int	461	268	191	142	76
High	283	182	129	84	51

***P*< 0.0001**
HR (0-2)= 0.33
C-index= 0.60

Primary and Secondary objectives are reached
Immunoscore **3 groups** (**and 2 or 5 groups**) predicted time to recurrence on Training Set (TS), and on 2 independent validation sets (IVS and EVS), blinded to clinical outcome.

Multivariate anlayses for Immunoscore

Multivariate Overall Survival (OS) analysis stratifed by center

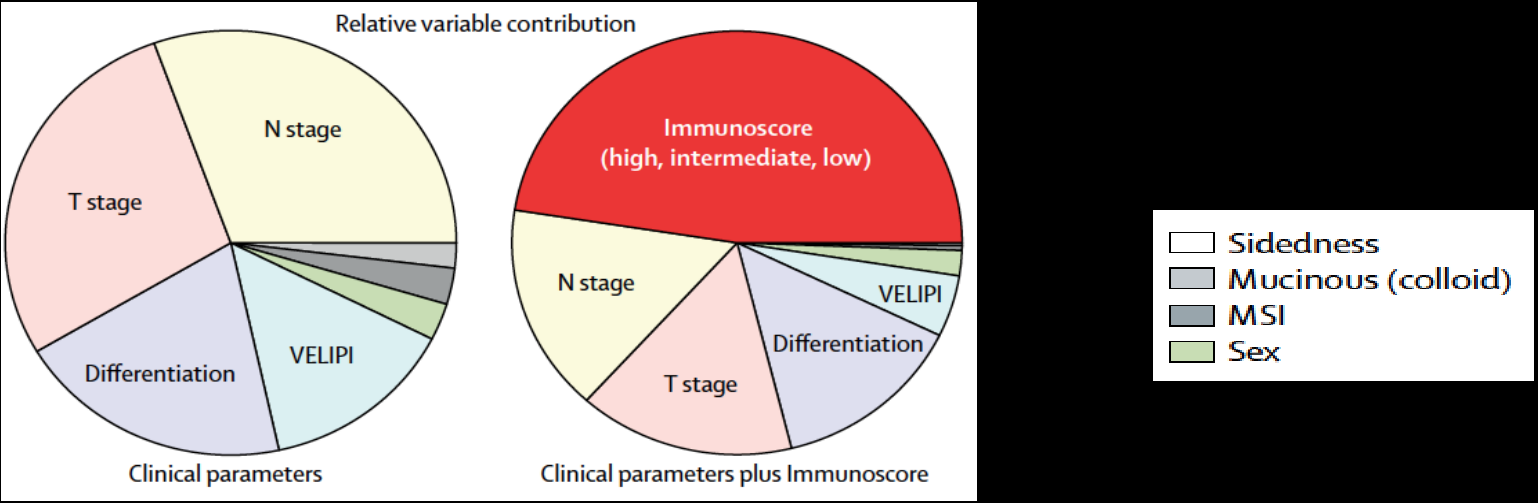
Individual Parameters	Hazard ratio (95%CI)	P-value
Gender Female *vs* Male	0.90 (0.72-1.12)	0.34
T Stage T2 *vs* T1	1.49 (0.62-3.57)	0.37
T Stage T3 *vs* T1	1.91 (0.84-4.38)	0.12
T Stage T4 *vs* T1	**2.36 (1.01-5.55)**	**0.0484**
N Stage N1 *vs* N0	1.16 (0.89-1.52)	0.28
N Stage N2 *vs* N0	**1.58 (1.15-2.17)**	**0.0052**
MSI Status MSI *vs* MSS	0.93 (0.68-1.27)	0.64
VELIPI Yes *vs* No	1.20 (0.94-1.54)	0.15
Diferentiation moderate *vs* Well	0.91 (0.66-1.24)	0.54
Diferentiation poor-undif *vs* Well	1.37 (0.9-2.08)	0.14
Mucinous (Colloid) Yes *vs* No	1.02 (0.78-1.33)	0.87
Sidedness distal *vs* proximal	0.96 (0.76-1.21)	0.74
Immunoscore Int *vs* Lo	**0.67 (0.52-0.86)**	**0.0014**
Immunoscore Hi *vs* Lo	**0.47 (0.33-0.65)**	**<0.0001**

- ✓ Cox multivariate regression model for OS stratified by center, combining Immunoscore with T-stage, N-stage, gender, VELIPI, histological grade, mucinous-colloide type, sideness, and microsatellite status (MSI).
- ✓ Immunoscore is the most significant parameter in multivariate analysis

The Lancet 2018

Relative variable contribution to risk

Chi squared proportion (χ^2) test for clinical parameters



Cox Multivariate

All patients

Immunoscore	**P-values**	***c-index***
2 groups	<0.0001	0.73 (0.66-0.80)
3 groups	<0.0001	0.73 (0.67-0.80)
5 groups	<0.0001	0.73 (0.67-0.80)

Pages et al. ***The Lancet*** 2018

Characteristics of good biomarkers

Hurdles for biomarker	**Immunoscore quantification**
• Routine	☑
• Feasible	☑
• Simple	☑
• Rapid	☑
• Robust	☑
• Objective	☑
• Specific	☑
• Reproducible	☑
• Quantitative	☑
• Standardized	☑
• Powerful	☑
• Pathology-based	☑

International validation of the consensus Immunoscore for the classification of colon cancer:

irAEs: immune-related Adverse Effects.

irRC: immune-related Response Criteria
(Wolchock et al. Clin Can Res 2009).

irRECIST: immune-related Response Evaluation Criteria In Solid Tumor
(Wong et al. NEJM 2017).

Strong arguments for introducing a "I" for Immune into the classification of cancer: TNM-I

Exemple: Standard of care in colon cancer

	Surgery	Chemotherapy	
Stage I	X		
Stage II Low-risk	X		
Stage II high-risk	X	No recommendation Often done	
Stage III Low-risk	X	X	Short course
Stage III High-risk	X	X	Long course
Stage IV	+/-	X	+ targeted therapy (immunotherapy in MSI patients)

Immunoscore in early-stage colon cancer

Stage I

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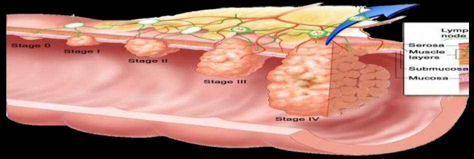
Immunoscore in Stage II colon cancer



Secondary Objective:
Time to recurrence for Immunoscore (High/Int/Low) in Stage II

Stage II patients (n=1433)

HR(0-2)= **0.36** (0.23-0.56)

***P*< 0.0001**

Objective is reached

Pre-sprecified consensus Immunoscore predicted time to recurrence in all Stage II colon cancer

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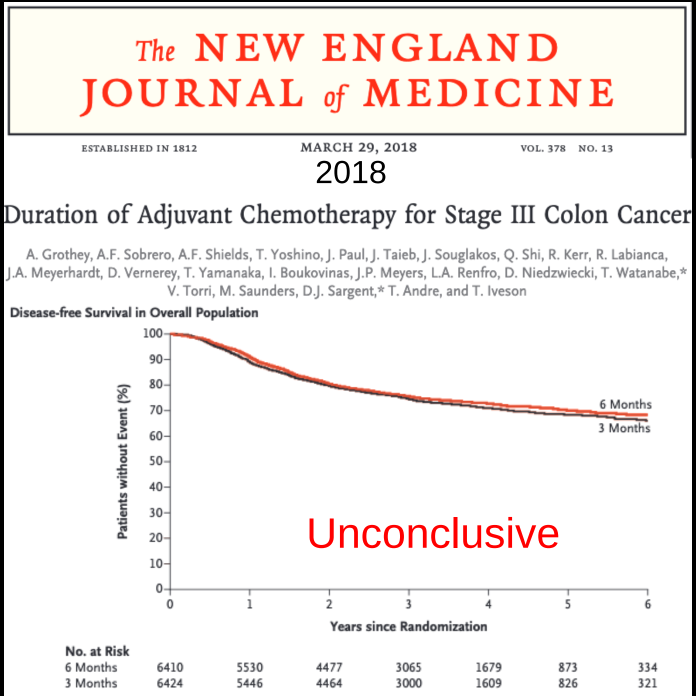
Immunoscore in locally advanced colon cancer

Stage III

Immunity and chemotherapeutic Efficacy

Phase 3 randomized study of stage III colon cancer patients (IDEA)

3 *vs* 6 months of chemotherapy


The NEW ENGLAND JOURNAL of MEDICINE

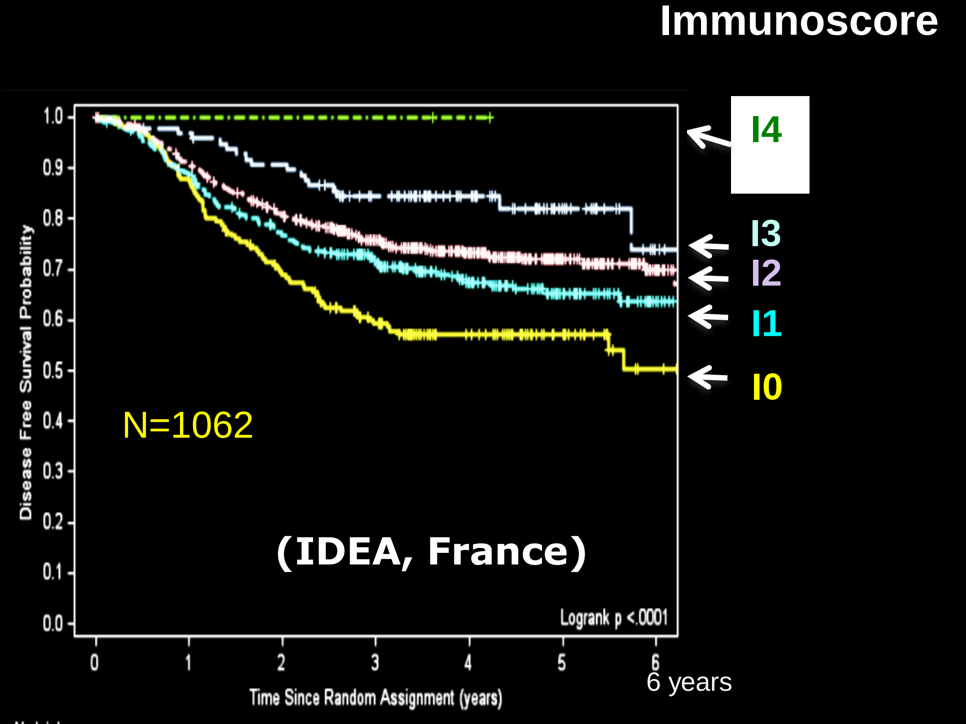
ESTABLISHED IN 1812 MARCH 29, 2018 VOL. 378 NO. 13

2018

Duration of Adjuvant Chemotherapy for Stage III Colon Cancer

A. Grothey, A.F. Sobrero, A.F. Shields, T. Yoshino, J. Paul, J. Taieb, J. Souglakos, Q. Shi, R. Kerr, R. Labianca, J.A. Meyerhardt, D. Vernerey, T. Yamanaka, I. Boukovinas, J.P. Meyers, L.A. Renfro, D. Niedzwiecki, T. Watanabe,* V. Torri, M. Saunders, D.J. Sargent,* T. Andre, and T. Iveson

No. at Risk							
6 Months	6410	5530	4477	3065	1679	873	334
3 Months	6424	5446	4464	3000	1609	826	321

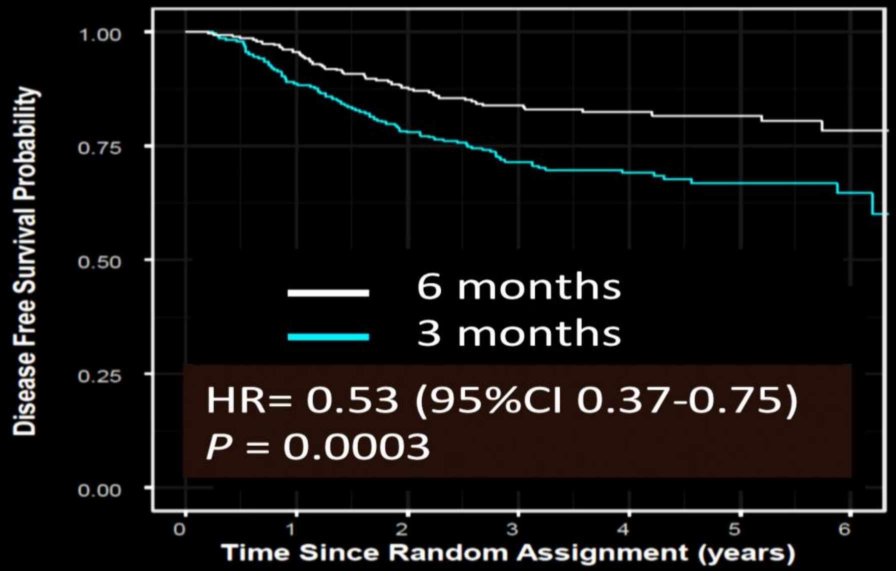


Clinical Utility (1): Immunoscore for defines patients at high-risk and NO risk in Stage III

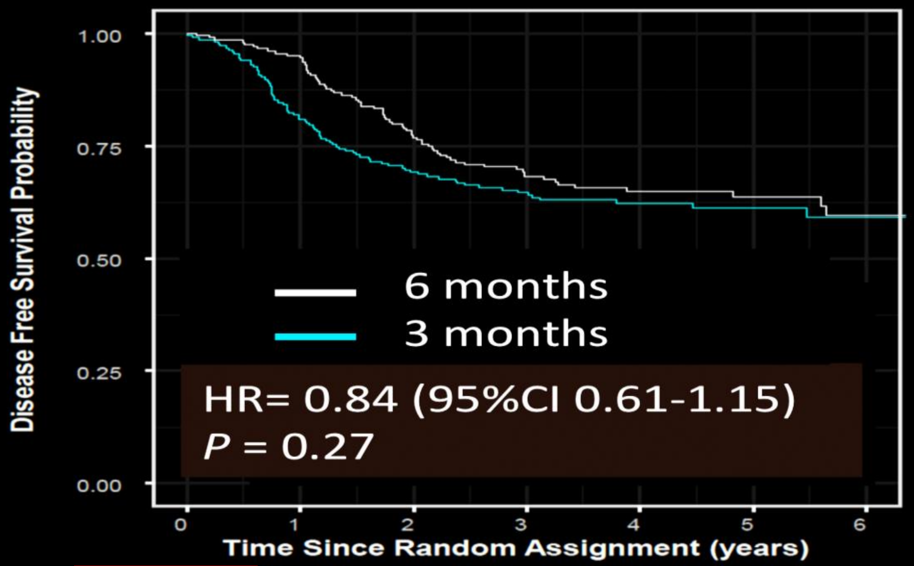
Phase 3 randomized study of stage III colon cancer patients (IDEA) 3 *vs* 6 months of chemotherapy (n=1062)

All Stage III treated with FOLFOX

High Immunoscore



Low Immunoscore



Clinical Utility (2): High Immunoscore significantly **predicts** response to 6 months FOLFOX chemotherapy in all Stage III patients

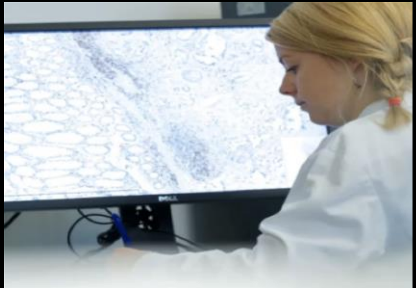
ASCO 2019

Immunoscore

CE-IVD, EU

Marseille France, January 2018
Richmond, USA, January 2019

Wet lab:
Stainer / Scanner

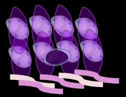


Digital Pathology:
Immunoscore Analyzer

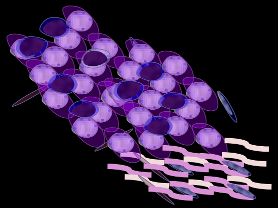
Immunoscore is CE-IVD (In Vitro Diagnostic for clinical use) in colon cancer

The continuum of cancer immunosurveillance

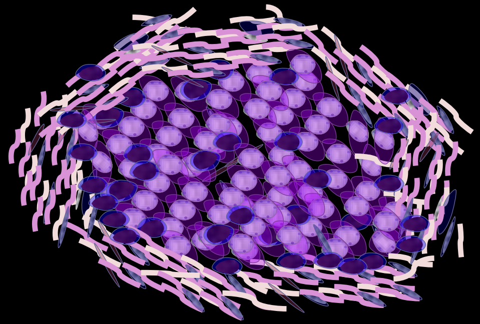
Pre-cancerous lesions



Primary Carcinoma



Metastasis



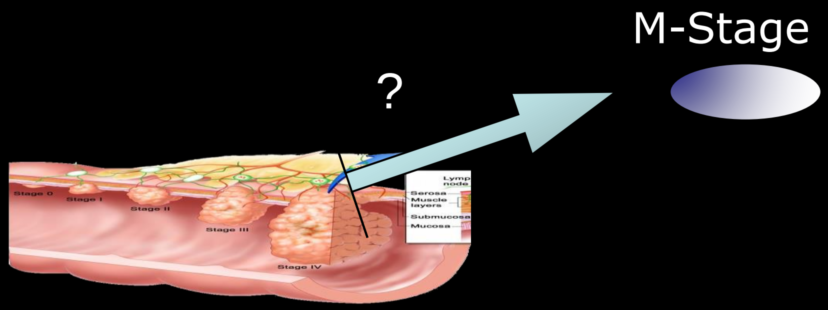
Mascaux C. … Galon J.
Nature 2019

Pagès F. … Galon J.
Lancet 2018

Van den Eynde. … Galon J.
Cancer Cell 2018

Angelova M. … Galon J.
Cell 2018

What are the parameters associated with the dissemenation to distant metastasis? What is driving metastasis ?



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Effector Memory T Cells, Early Metastasis, and Survival in Colorectal Cancer

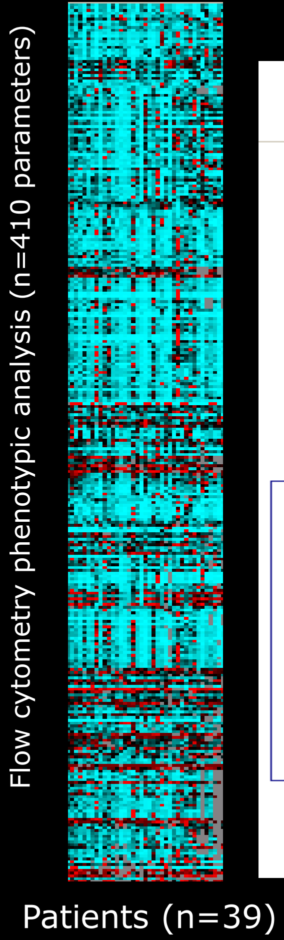
Franck Pagès, M.D., Ph.D., Anne Berger, M.D., Ph.D., Matthieu Camus, M.Sc., Fatima Sanchez-Cabo, Ph.D., Anne Costes, B.S., Robert Molidor, Ph.D., Bernhard Mlecnik, M.Sc., Amos Kirilovsky, M.Sc., Malin Nilsson, B.S., Diane Damotte, M.D., Ph.D., Tchao Meatchi, M.D., Patrick Bruneval, M.D., Ph.D., Paul-Henri Cugnenc, M.D., Ph.D., Zlatko Trajanoski, Ph.D., Wolf-Herman Fridman, M.D., Ph.D., and Jérôme Galon, Ph.D.*

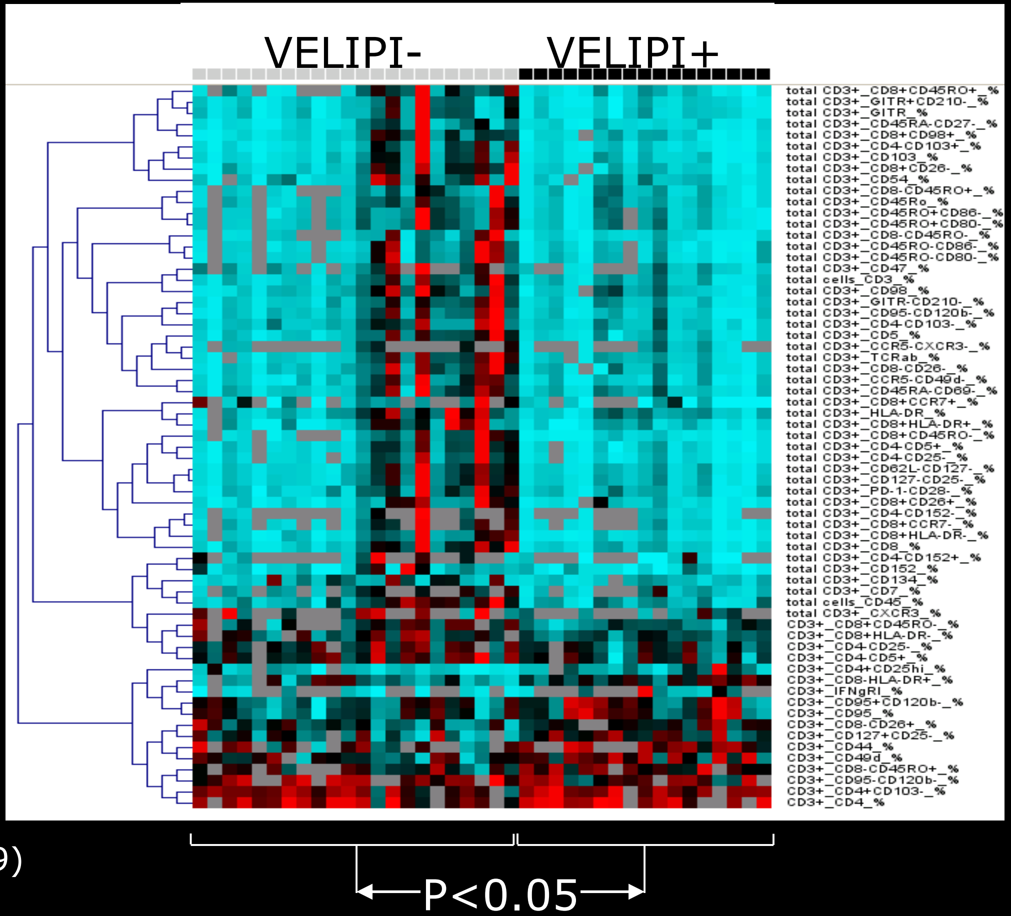
Memory T cells, in particular, T_{EM} correlate with the absence of early-metastatic invasion, and improved clinical outcome in colorectal carcinoma.

Pagès F, et al. ***N Engl J Med.*** 2005

Pagès F & Galon J. ***N Engl J Med.*** 2006

Large-scale investigation of infiltrating immune cells





410 FACS parameters Analyzed

65 significant parameters

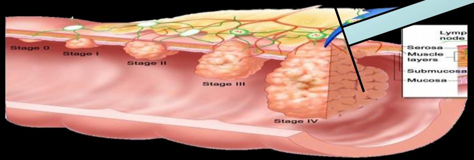
- T cells
- Activation
- Migration
- Differentiation
  - . Th1
  - . **Memory cells**

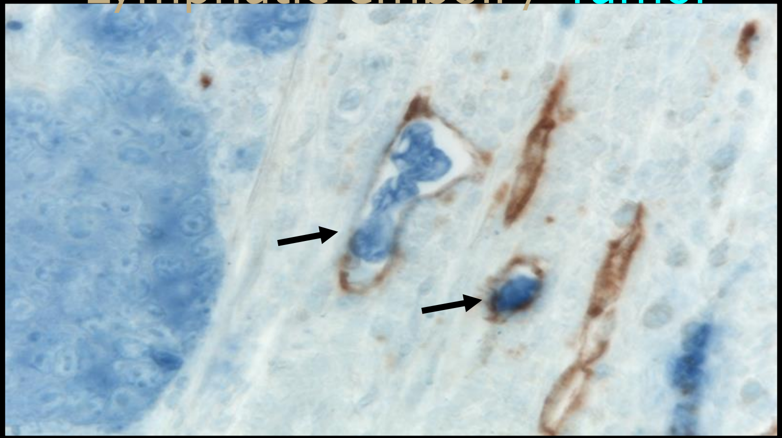
What are the mechanisms of early-metastatic dissemenation ?

VELIPI: Venous Emboli, Lymphatic Invasion, Perineural Invasion

Lymphatic emboli / Tumor

?





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Pagès F, et al. ***N Engl J Med***. 2005

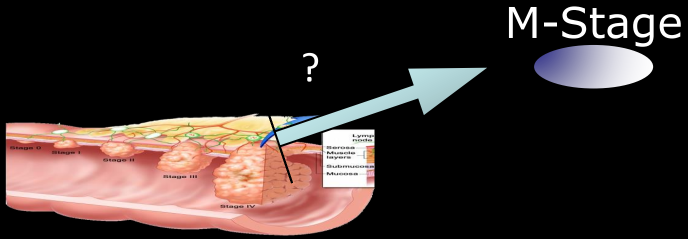
Pagès F & Galon J. ***N Engl J Med***. 2006

RESEARCH ARTICLE

CANCER

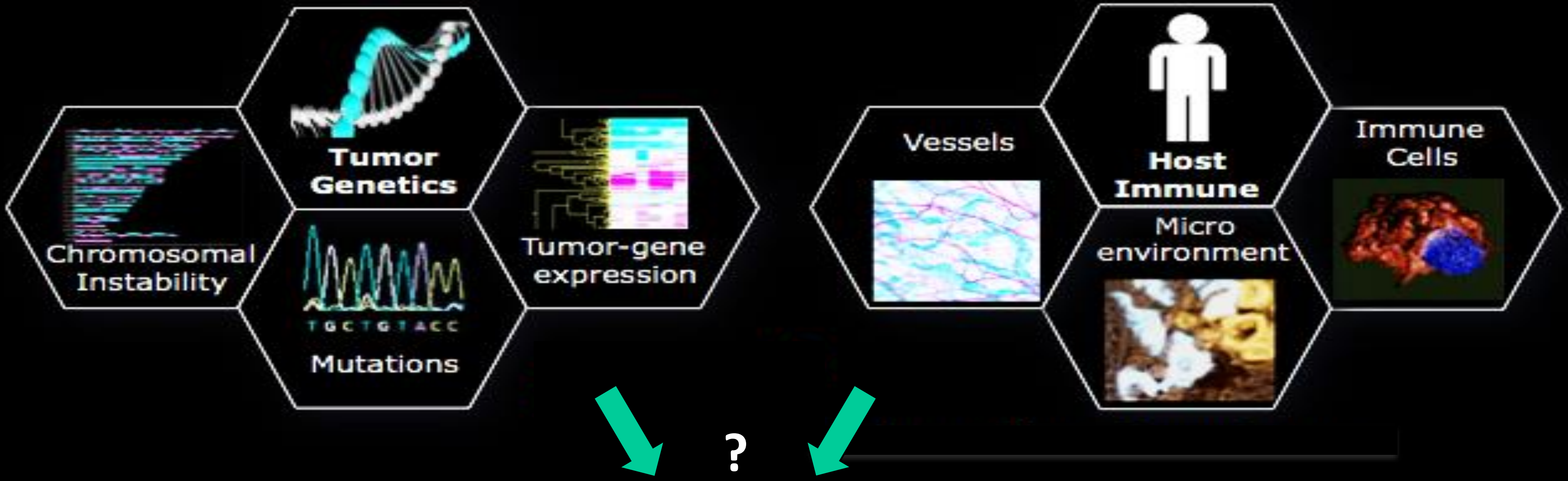
The tumor microenvironment and Immunoscore are critical determinants of dissemination to distant metastasis

Bernhard Mlecnik,[1,2,3*] Gabriela Bindea,[1,2,3*] Amos Kirilovsky,[1,2,3*] Helen K. Angell,[1,2,3,4] Anna C. Obenauf,[5] Marie Tosolini,[1,2,3] Sarah E. Church,[1,2,3] Pauline Maby,[1,2,3] Angela Vasaturo,[1,2,3] Mihaela Angelova,[1,2,3] Tessa Fredriksen,[1,2,3] Stéphanie Mauger,[1,2,3] Maximilian Waldner,[6] Anne Berger,[7] Michael R. Speicher,[5] Franck Pagès,[1,2,3,8] Viia Valge-Archer,[9] Jérôme Galon[1,2,3†]



ONLINE COVER: Protecting Against Metastasis. Notre Dame de Paris gargoyles guard over the city of Paris to frighten off and protect from any evil or harmful spirits. In this issue of Science Translational Medicine, Mlecnik et al. describe the protective role of cytotoxic immune infiltrate, Immunoscore, and lymphatic vessels against metastatic invasion in human cancer. These results support the use of T cell based immunotherapy at early stage disease.

What drives metastasis?

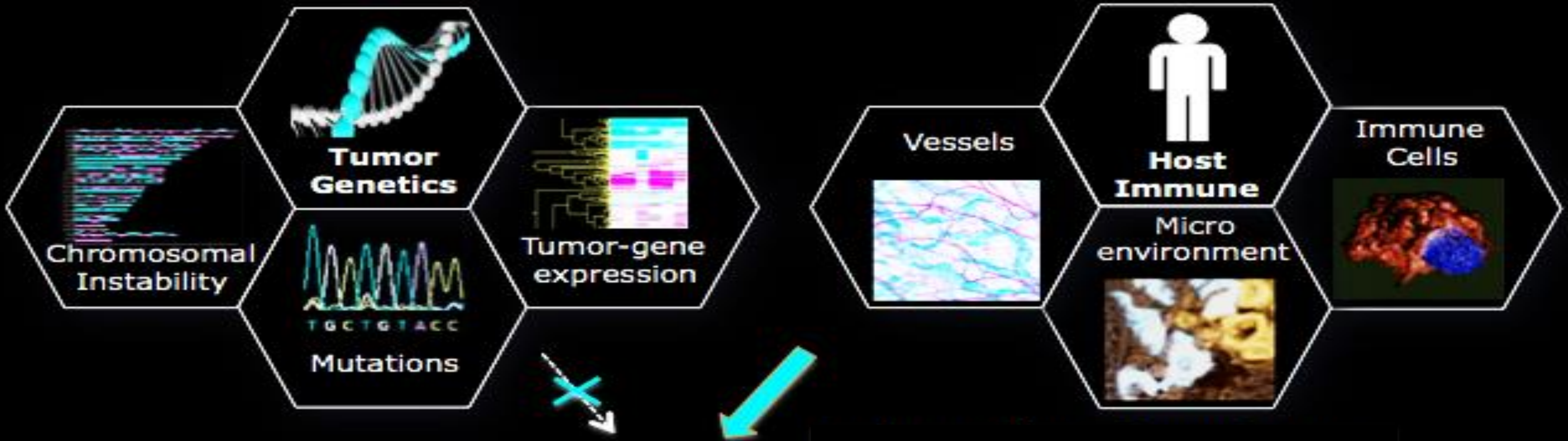


Tumor cell dissemination to distant metastasis

M1 stage

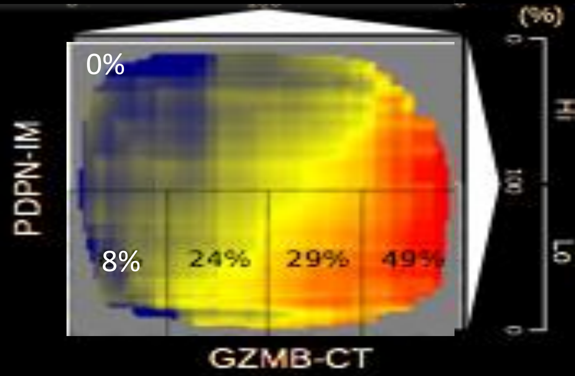
Mlecnik et al. *Science Transl Med*. 2016

What drives metastasis?



M1 stage

Lymph vessels (IM)
GZMB+ (CT)

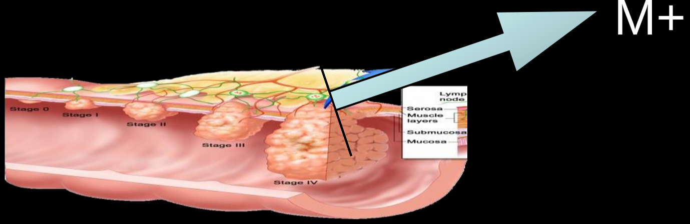


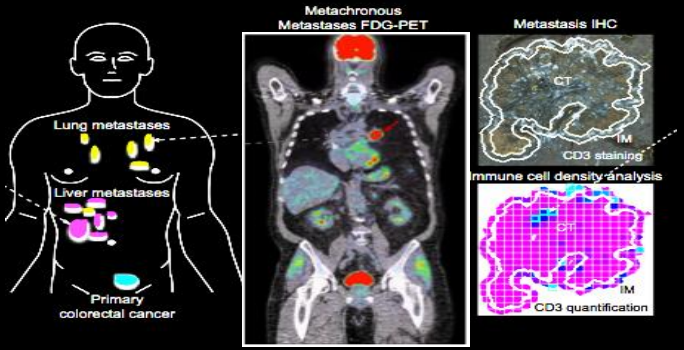
Mlecnik et al. *Science Transl Med*. 2016

Is there an immune escape at the metastatic stage ?

Stage IV

Immunoscore in Stage IV metastatic colon cancer

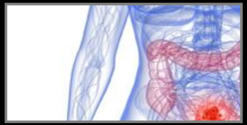




Metastasis analysis

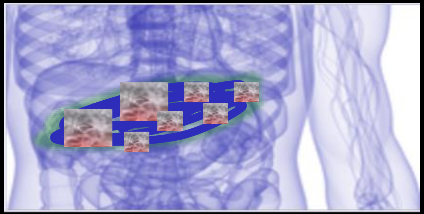
One primary tumor

Colorectal cancer



Multiple metastatic sites

Liver Metastasis



Lung Metastasis

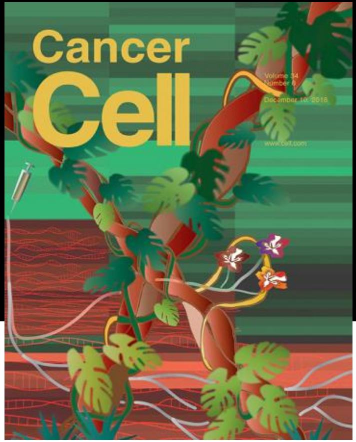
N=603 metastases

- Immunoscore within multiple metastases at different sites

Mlecnik et al. ***JNCI*** 2018

Van den Eynde M. *et al.* ***Cancer Cell 2018***

Metastasis analysis



CellPress

Cancer Cell
Article

The Link between the Multiverse of Immune Microenvironments in Metastases and the Survival of Colorectal Cancer Patients

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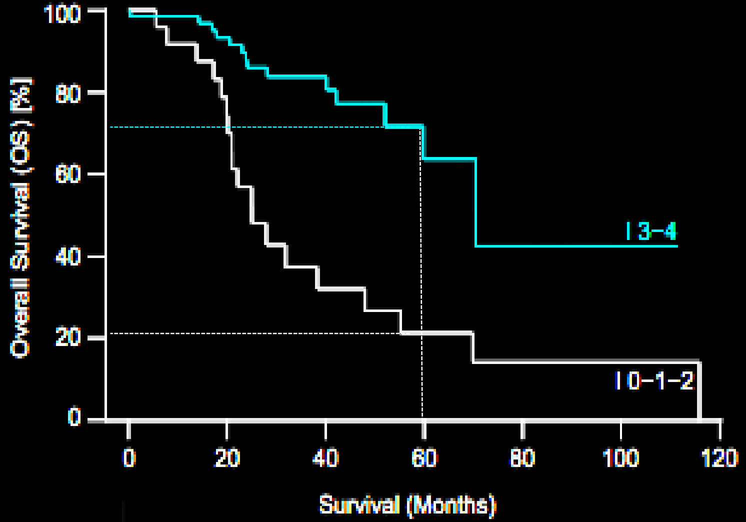
- Immunoscore within multiple metastases at different sites

Van den Eynde et al. ***Cancer Cell*** 2018

High-Immunoscore within metastasis predicts prolonged survival

Lung metastaes

Liver metastaes



High-Immunoscore

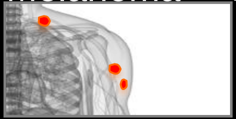
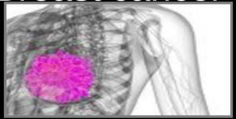
Low-Immunoscore

- Similar Immunoscore frequency in lung and liver metastaes

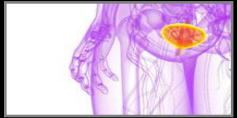
Mlecnik et al. ***JNCI*** 2018

Metastasis analysis

Multiple primary tumors

Melanoma

Breast cancer

Kidney cancer

Lung cancer



Other cancers

One metastatic site

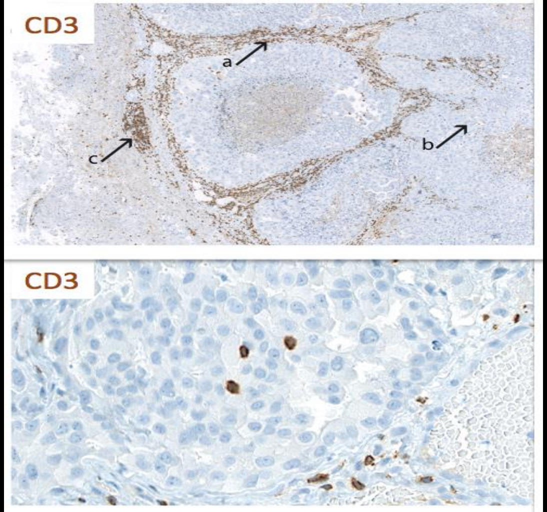
Brain Metastasis

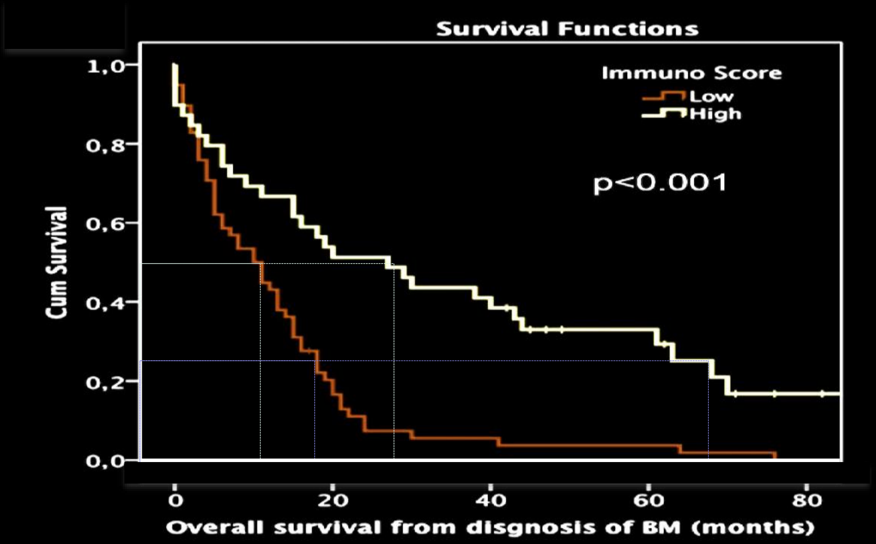
➢ Immunoscore within brain metastasis

Berghoff A. *et al.* ***OncoImmunol.*** 2016

Immunoscore in brain metastasis and survival

Immunoscore quantification (CD3, CD8, in CT and IM regions) within Brain Metastases (n=116 patients)





50% OS
25% OS

Immunoscore predicts overall survival and long-term survival in patients with Brain Metastases

Berghoff A. *et al.* ***OncoImmunol.*** 2016

What drives metastasis ?

What are the metastatic escape mechanisms ?

A Novel theory of cancer evolution ?

Current theories of cancer evolution

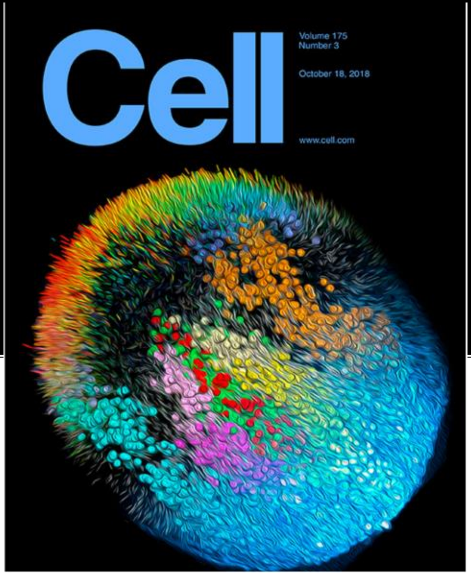
Models

LINEAR	NEUTRAL	BIG-BANG	BRANCHED
			

Immune pressure from Darwinian selection

NO	**NO**	**NO**	**NO**

- The 4 proposed theories of cancer evolution
- All theories are tumor cell-centric. None involves a role of the immune system.



Article

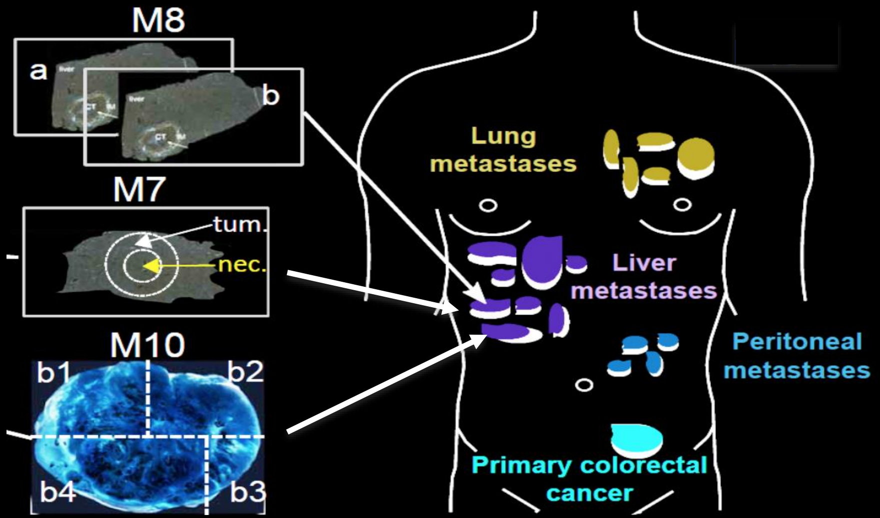
Cell

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,*]

Angelova M. *et al.* ***Cell*** 2018

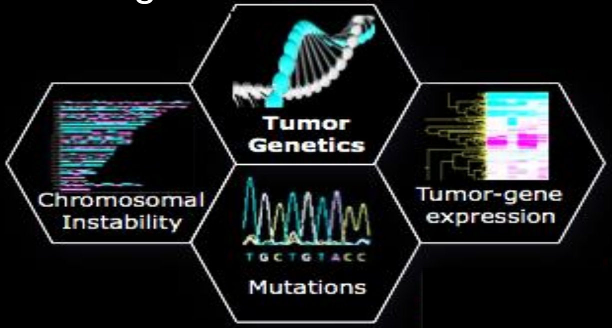
What drives metastasis?

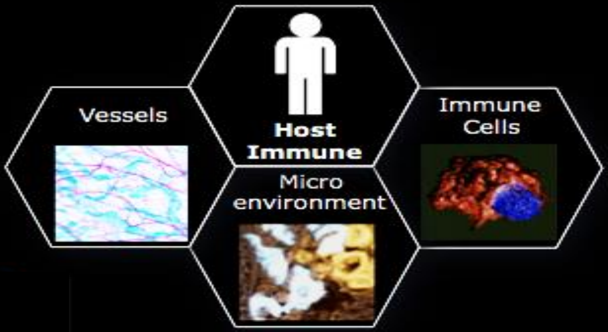


Primary tumors
↓
Synchronous metastases
↓
Metachronous metastases
↓
Metachronous metastases
↓
Metachronous metastases
↓

> 11 years

Multi-Omics technologies



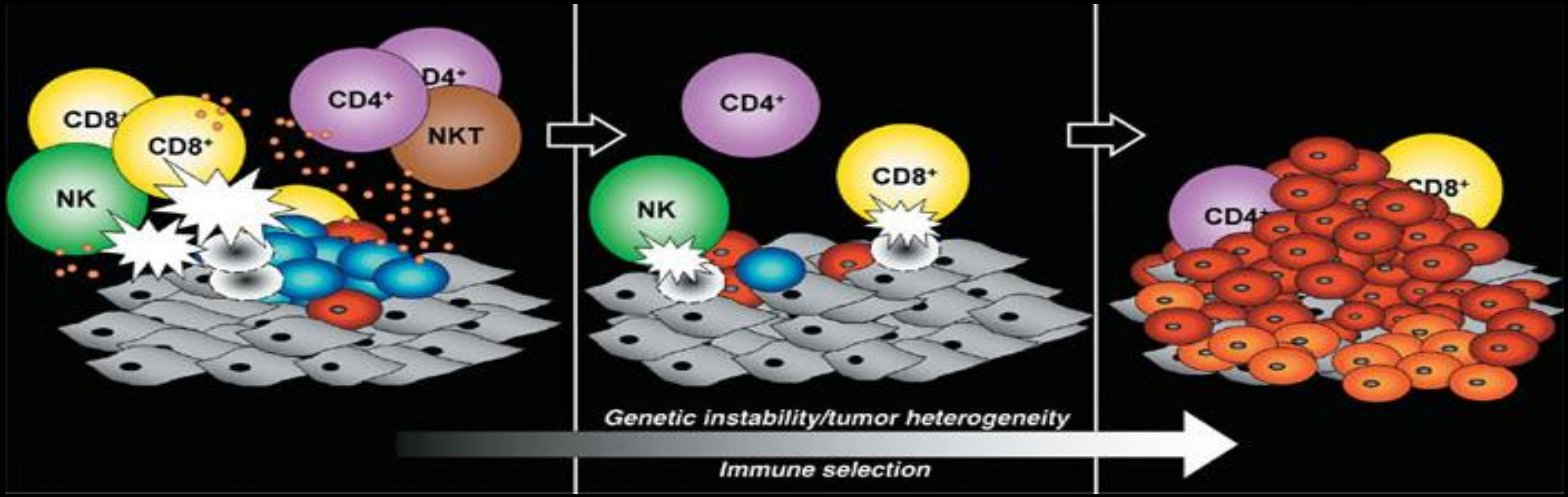


Genomics of primary tumors and metastases

✓ Highly heterogeneous genomic patterns between metastases

Immunoediting of cancer cells

Schreiber et al. *Science* 2011



Elimination

refers to effective immune surveillance for clones that express TSA

Shankaran et al. *Nature* 2001
Immunosurveillance RAG-/- STAT1-/-

Equilibrium

refers to the selection for resistant clones (red)

Koebel et al. *Nature* 2007
Immunoediting / Equilibrium

Escape

refers to the rapid proliferation of resistant clones in the immunocompetent host

Matsushita et al. *Nature* 2012
Immunoediting / Escape

Tumor and microenvironment evolution: immunoediting in Human

Genetic evidence for immunoediting in tumors and tumor-intrinsic resistance to cytolytic activity
Rooney MS et al. *Cell* 2015

Demonstration of the existence of immunoediting in Human with genetic evidence for missense and frameshift mutations
Mlecnik B et al. *Immunity* 2016

Anti-PD1 immunotherapy induces changes in the mutational burden of tumors, with loss of certain neoantigens, clonal T-cell expansion, and changes in immune contexture (mechanistic signature)
Riaz N et al. *Cell* 2017

First demonstration that Immunoscore and immunoediting in Human shape the evolution of specific tumor clones. Darwinian selection of immune-escape variant tumor clones throught parallel immune selection model.
Angelova M et al. *Cell* 2018

Observed compared to expected frameshift and missense epitopes (immunogenic mutations) using ExomeSeq data

Genetic analysis of missense and frameshift immunogenic mutations (epitopes)

- ✓ ExomeSeq
- ✓ RNAseq
- ✓ Mutations detection
- ✓ Variant calling
- ✓ HLA haplotypes prediction
- ✓ Epitopes prediction
- ✓ HLA / TCR peptide binding prediction
- ✓ Immunogenicity scores

Silent mutations

↕

PanCancer rate (n=3659 Pts.)

↕

Non-immunogenic mutations

↕

Expected immunogenic mutations

↕

Observed immunogenic mutations

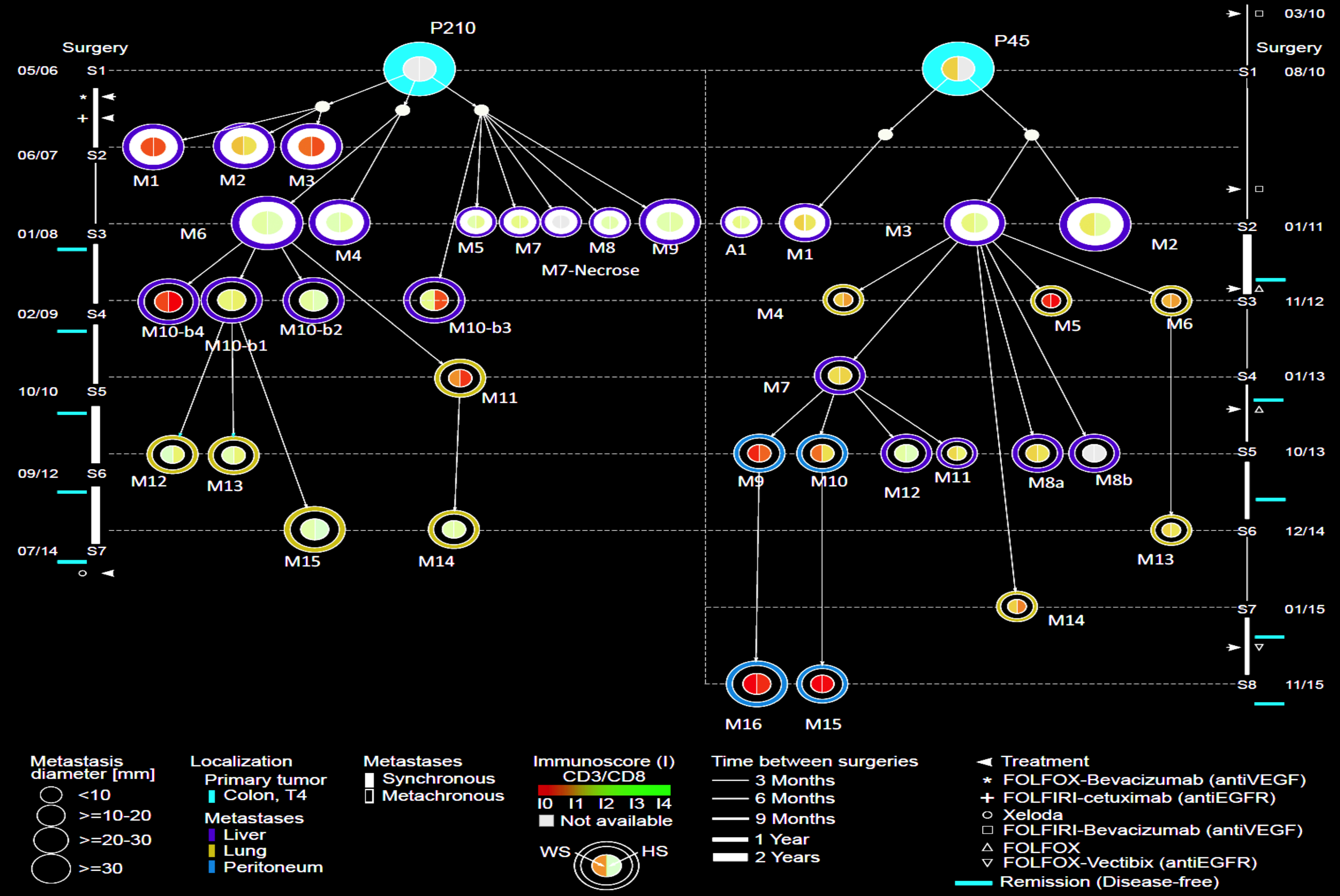
Clonal dissemination – Parent/child-relationship

Primary tumors

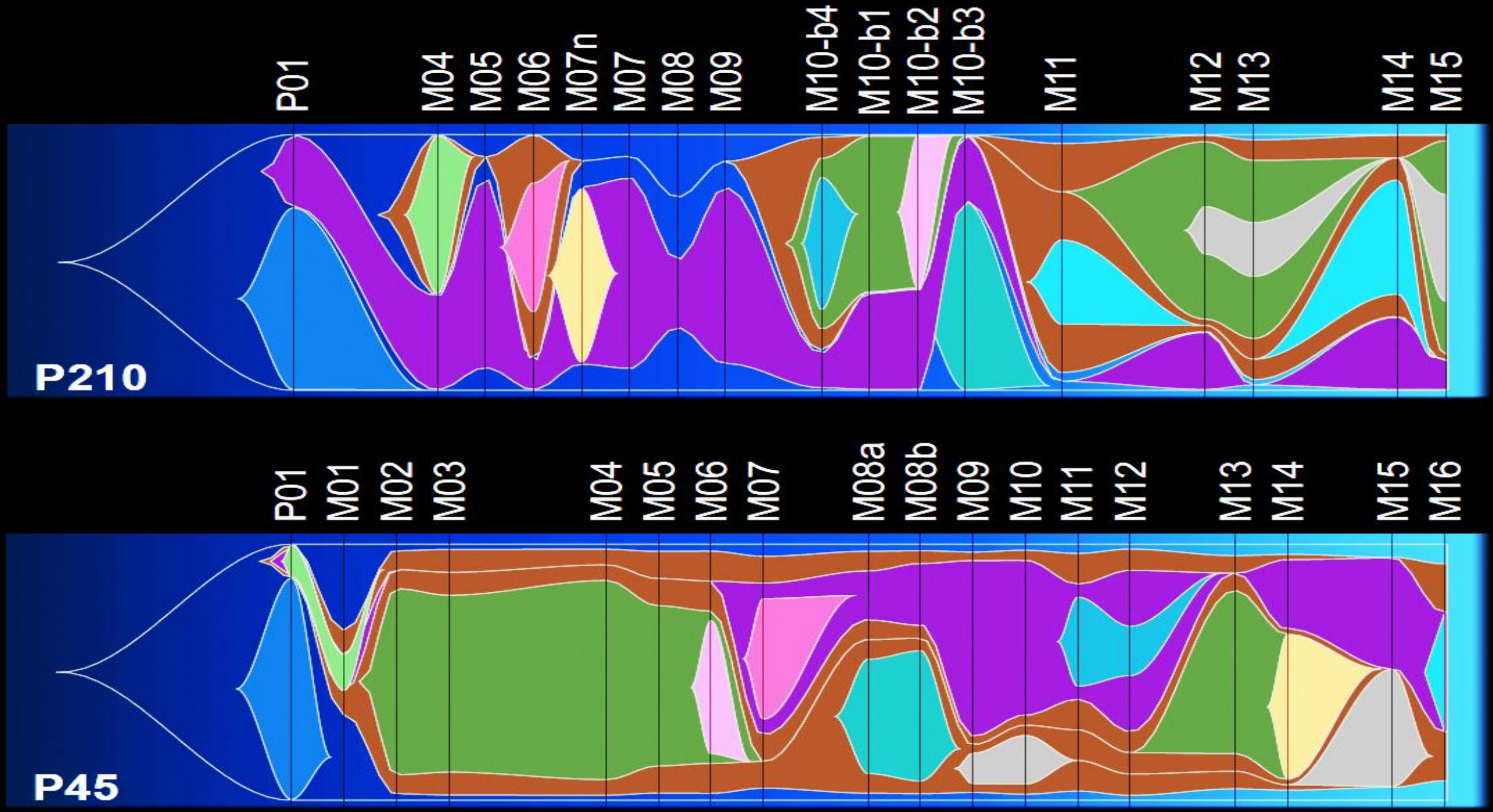
Synchronous meta

Metachronous meta

11 years



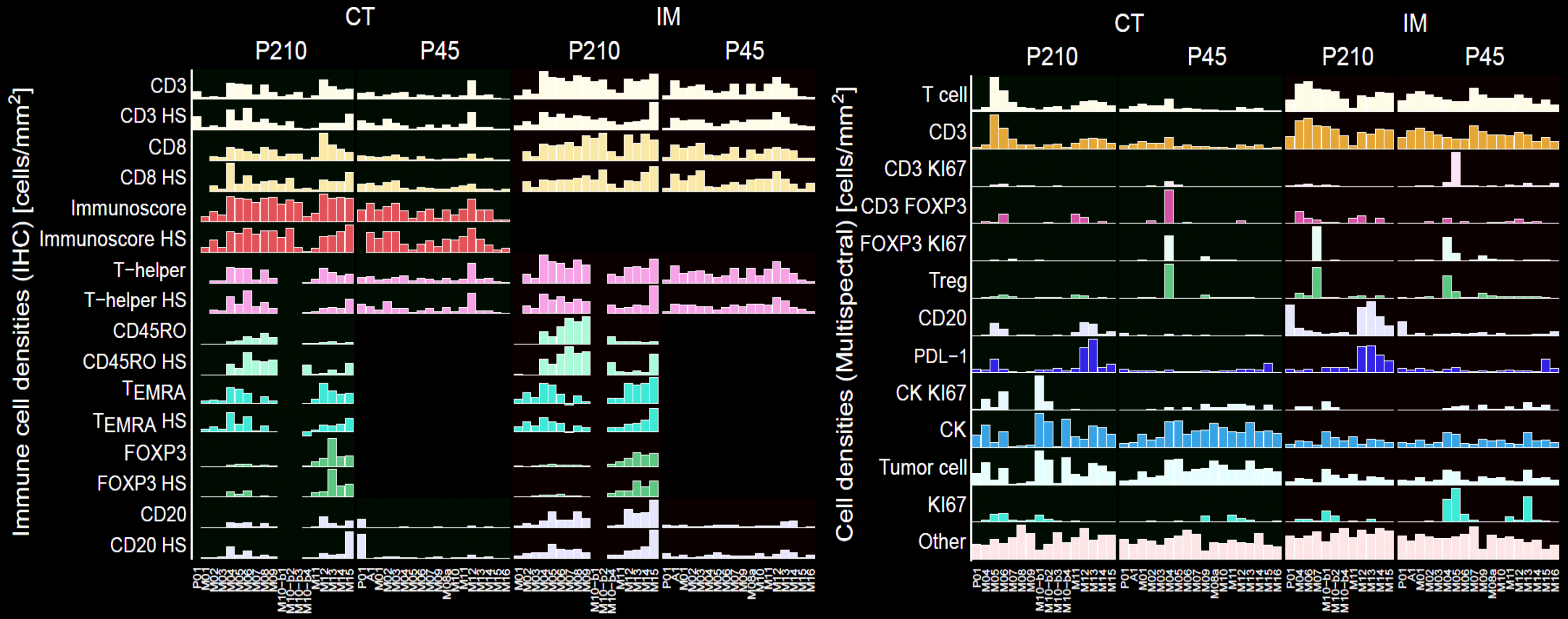
Evolvogram of tumor clones



- ✓ Clonal evolution and cancer evolvogram
- ✓ Non-recurrent clones are immunoedited. Progressing clones are immune privileged

Immune microenvironment

Immune cell densities (cells/mm^2)

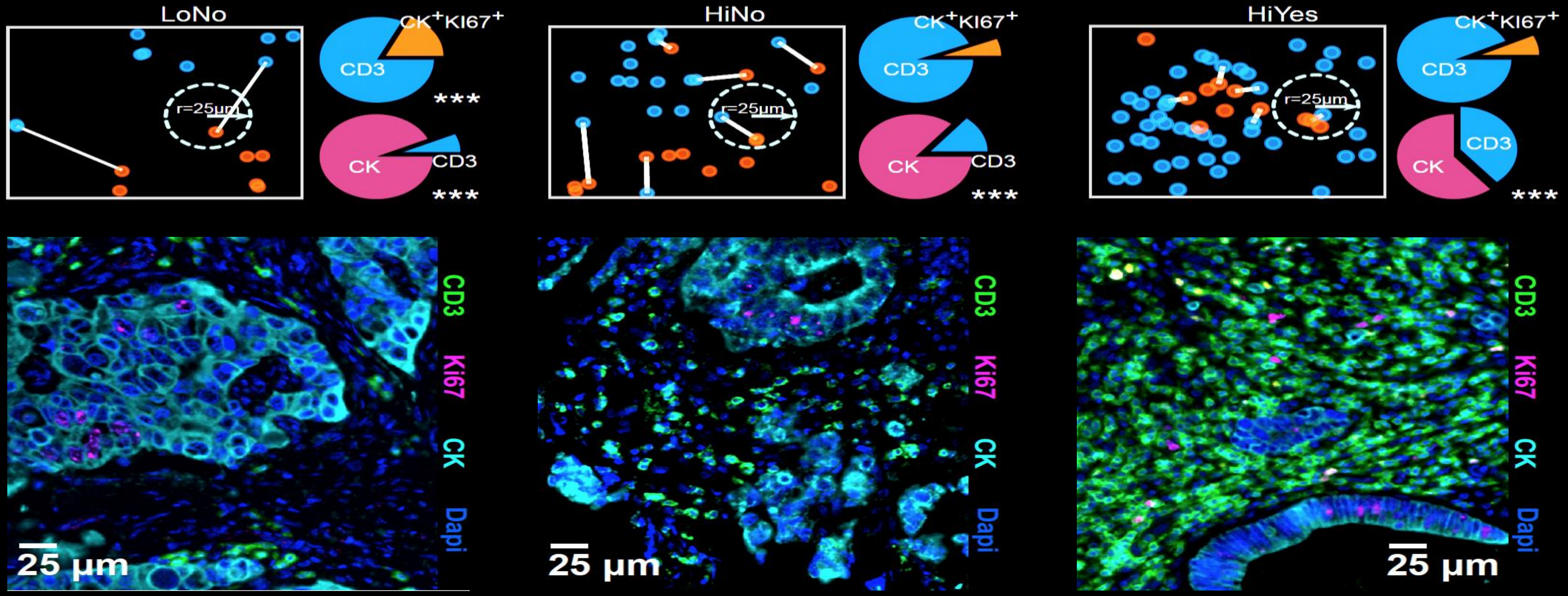


✓ Highly heterogeneous Immunomics patterns and immune cell infiltration between metastases

Angelova M. et al. *Cell* 2018

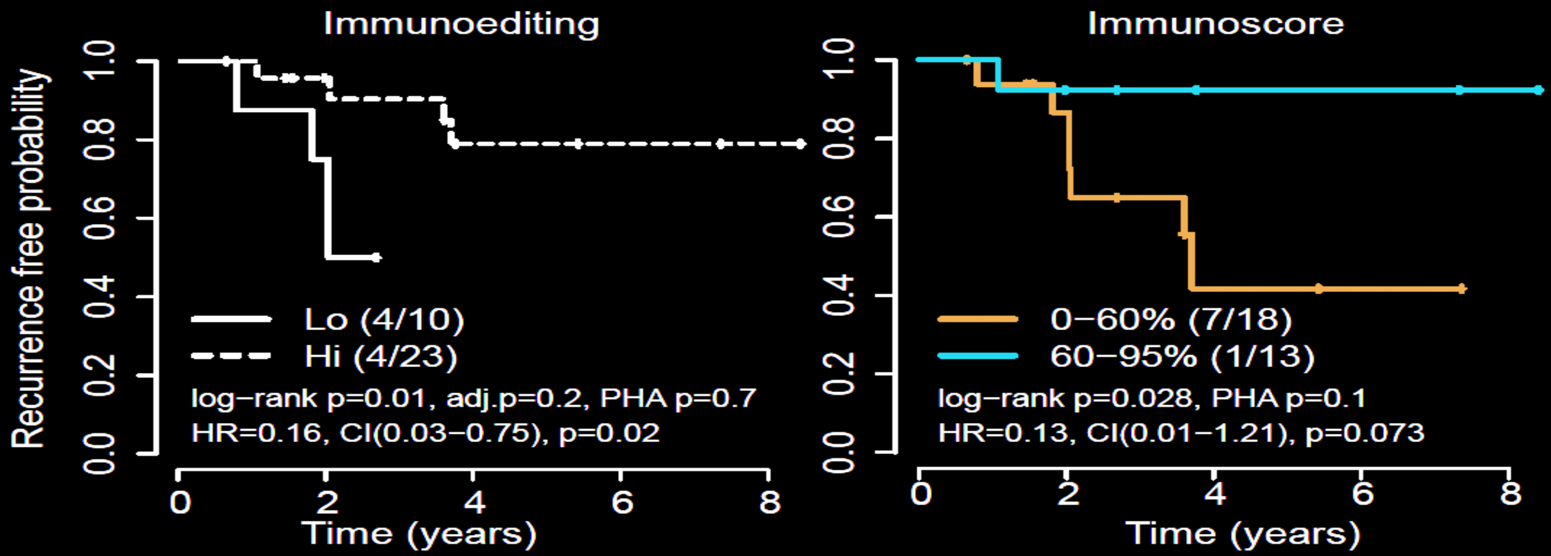
What drives metastasis?

Spatial mapping of the metastatic microenvironment



- ➢ Distance between CD3 + cells and tumor cells Ki67+ are associated with Immunoscore and Immunoediting groups, and with metastasis recurrence.

Metastasis recurrence

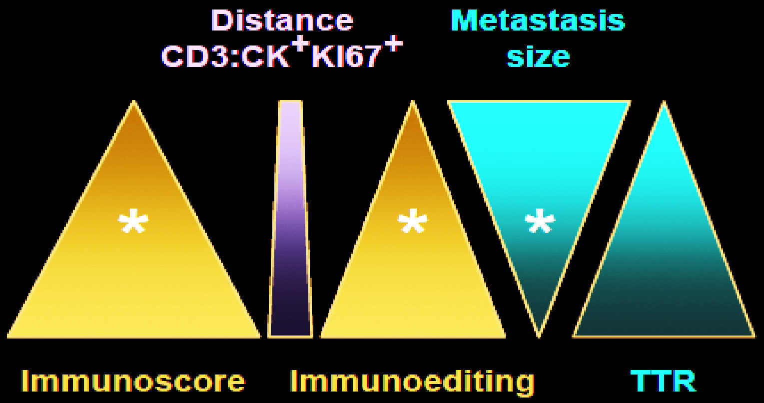


- ✓ Immunoediting and Immunoscore are associated with metachronous metastasis recurrence

Angelova M. et al. *Cell* 2018

Multivariate analysis of all genomics and immunomics parameters

	Df	First recurrence		Multiple recurrences	
Excluded variable		AIC	log(HR)	AIC	log(HR)
<none>		43.3		124	
CD3 to CK+KI67+ mutual neighbor distance (Hi)	1	43.7	-2.2	124.1	-1.6
Immunoscore (>60%)	1	46.2	**-3.1**	124.8	**-1.8**
Immunoediting (Low)	1	48.1	**-3.1**	133	**-1.9**
Meta Size (log)	1	45.9	**2.5**	133.7	**2.6**



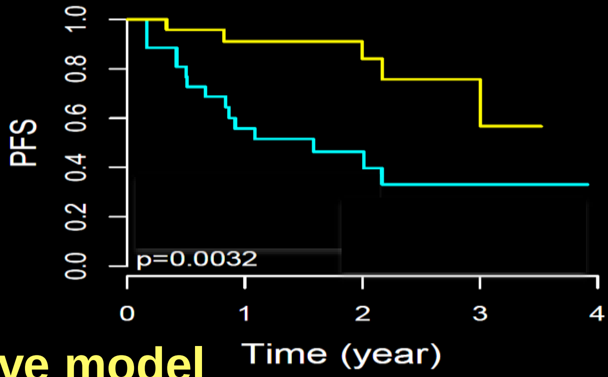
- ✓ Cox multivariate analysis revealed 4 parameters associated with metastatic dissemination:
- ✓ Immunoscore, Immunoediting, the distance between CD3 T-cells and Ki67+ tumor cells, and the size of the parent metastasis

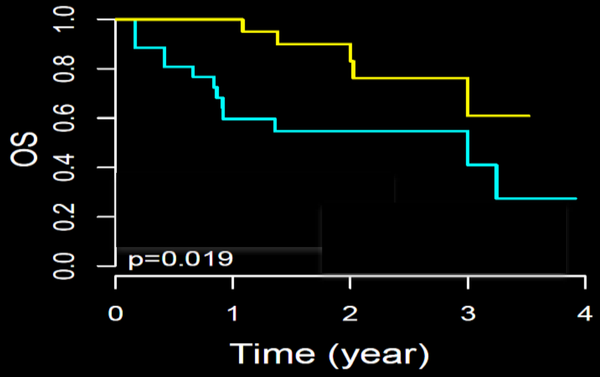
Angelova M. et al. *Cell* 2018

Validation Study

CRC Primary tumor recurrence (n=132 patients)

Immunoediting

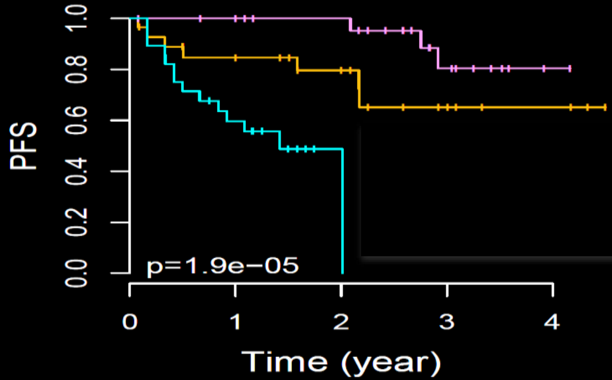




Immunoediting

No Immunoediting

Predictive model

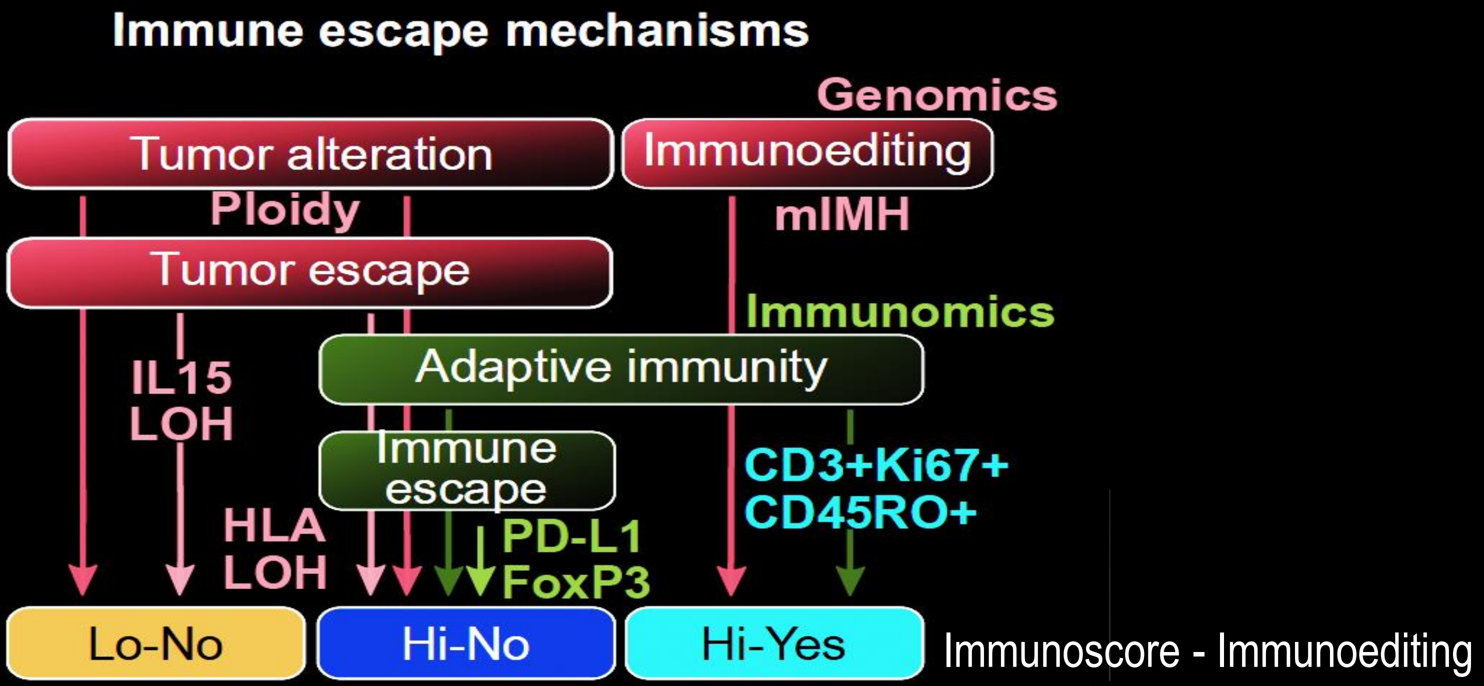


Predictive model
Recurrence probability
Low
Int
High

- **Immunoediting and Predictive model are predictive factors of recurrence.**

Angelova M. et al. *Cell* 2018

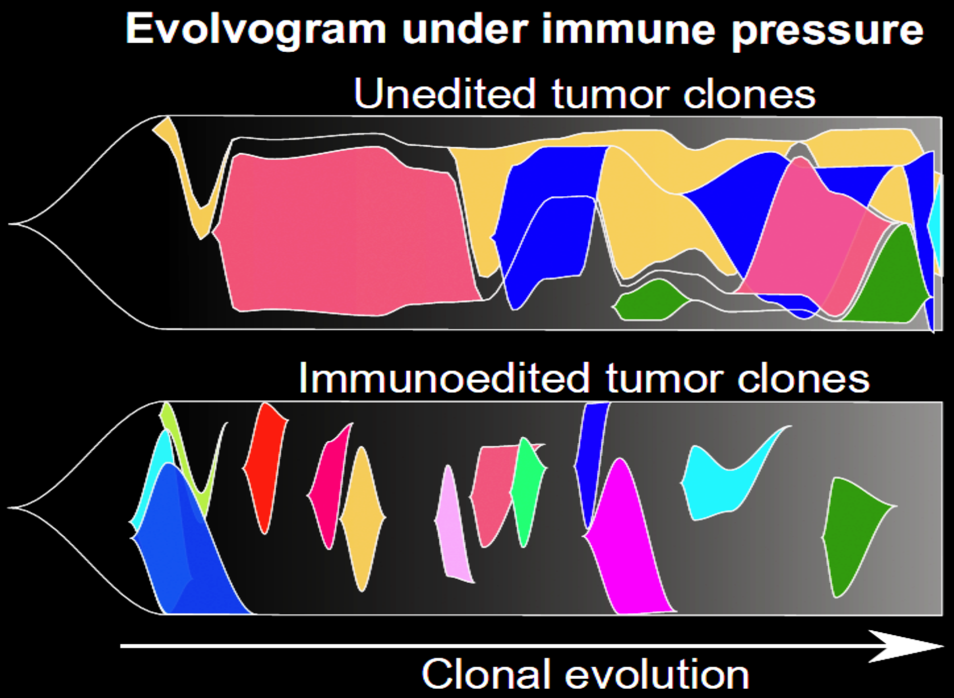
What drives metastasis? Conclusions



- Different escape mechanisms delineated by lack of adaptive immunity or immunoediting.

Angelova M. et al. *Cell* 2018

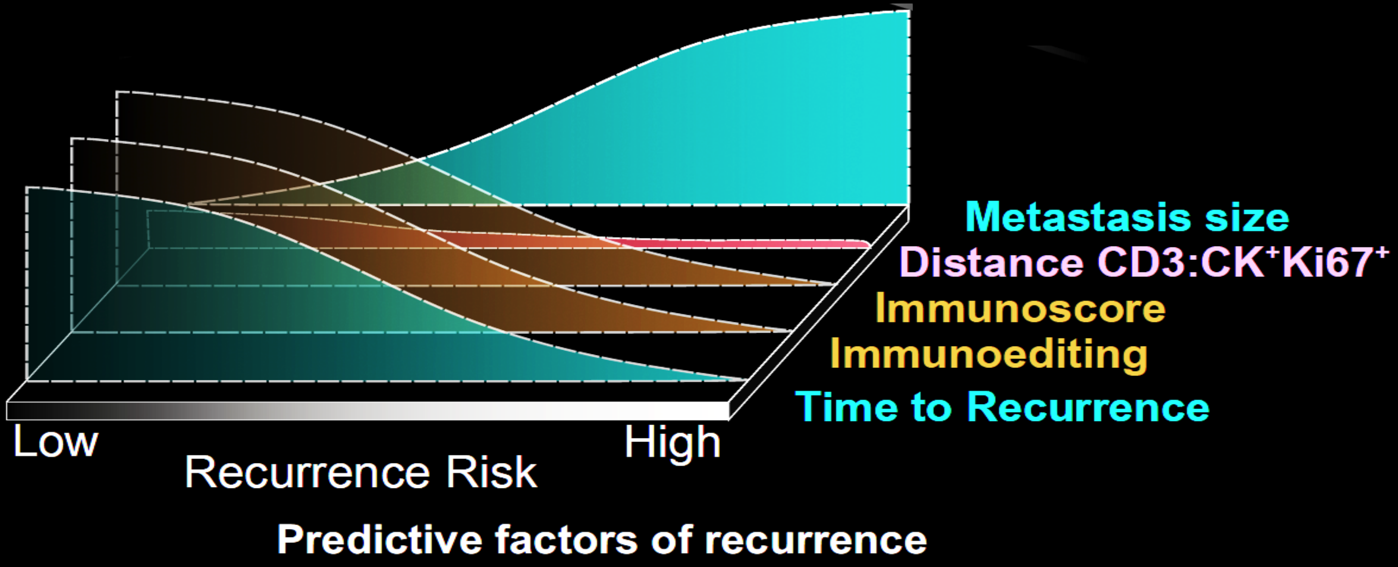
What drives metastasis? Conclusions (2)



- Multiverse of metastases evolution in space and time under immune selection
- Evolution of tumor clones is linked to the intra-metastatic immune contexture.
- Non-recurrent clones are immunoedited. Progressing clones are immune privileged.

Angelova M. et al. *Cell* 2018

What drives metastasis? Conclusions (3)



- Parallel selection model describes tumor evolution during the metastatic process.
- Immunoediting and Immunoscore are predictive factors of metastasis recurrence.
- Distance between CD3 + cells and tumor cells Ki67+ and metastasis size are also associated metastasis recurrence.

Angelova M. et al. *Cell* 2018

A Novel theory of cancer evolution

Models

LINEAR | NEUTRAL | BIG-BANG | BRANCHED | SELECTION





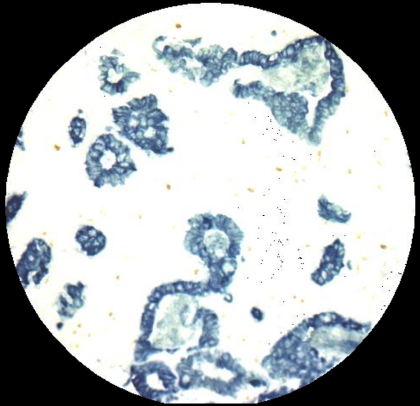
Immune pressure from Darwinian selection

NO | **NO** | **NO** | **NO** | **YES**

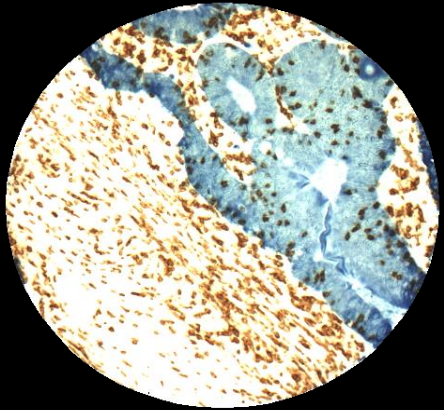
- **Parallel immune selection model**
- Dynamic interaction of tumor-cells with immune-cells and Darwinian selection of immune escape variant, with parallel evolution and multiverse of metastases.

Deciphering the tumor immune microenvironment: Clinical implications

"Cold" Tumor
I 0



CD3
Tumor



"Hot" Tumor
I 4

Clinical implications

Predictions

Need T-cell priming
Cancer vaccine

Response to immunotherapies
(CTLA4, PD1, PDL1, …)

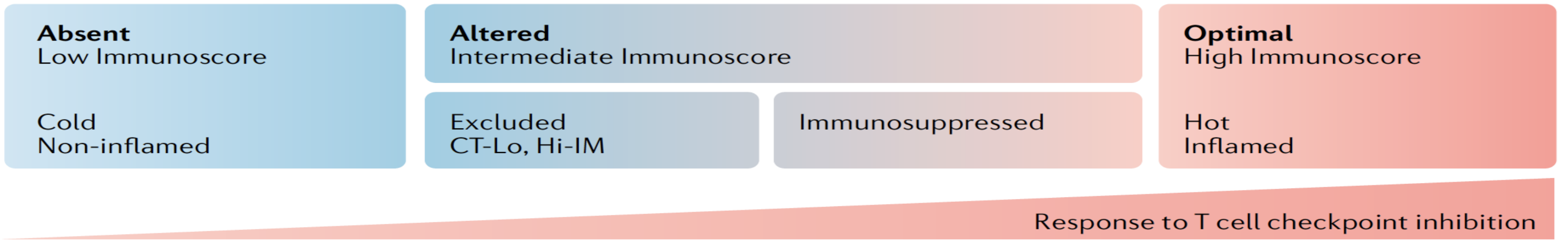
But it is not as simple since biology is complex and is not dichotomized in good & bad

NATURE REVIEWS | DRUG DISCOVERY

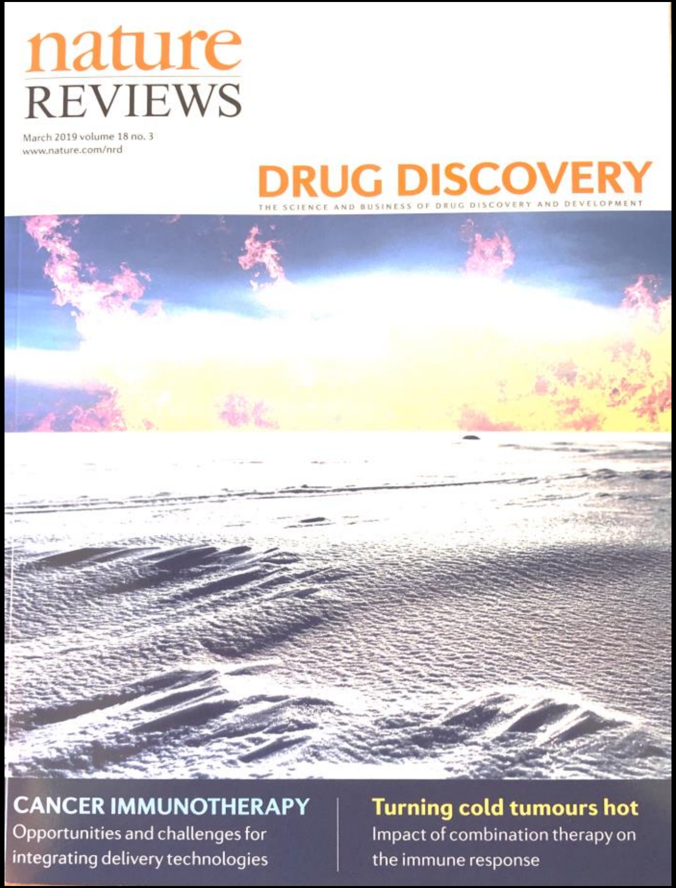
Approaches to treat immune hot, altered and cold tumours with combination immunotherapies

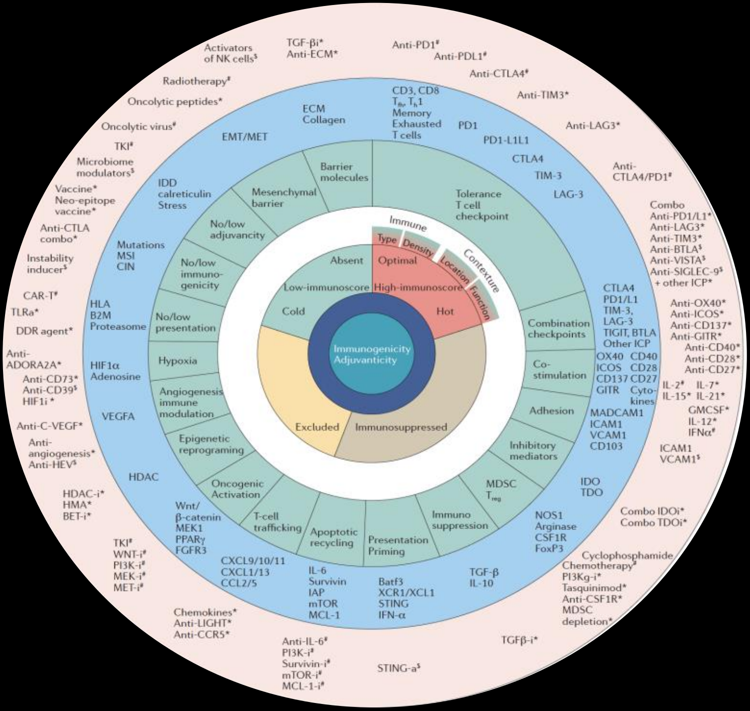
Jérôme Galon * *and Daniela Bruni*

2019



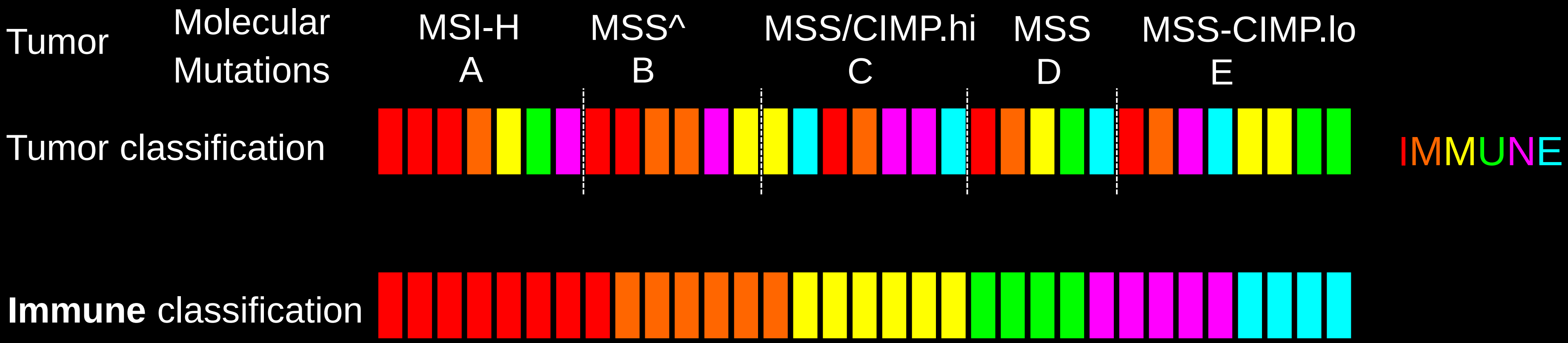
Treating hot, altered and cold immune tumors with immunotherapy





Galon J. & Bruni D.
Nature Reviews Drug Discovery 2019

Stratification of cancer based on the immune status



-> Importance of having standardized immune Assays

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