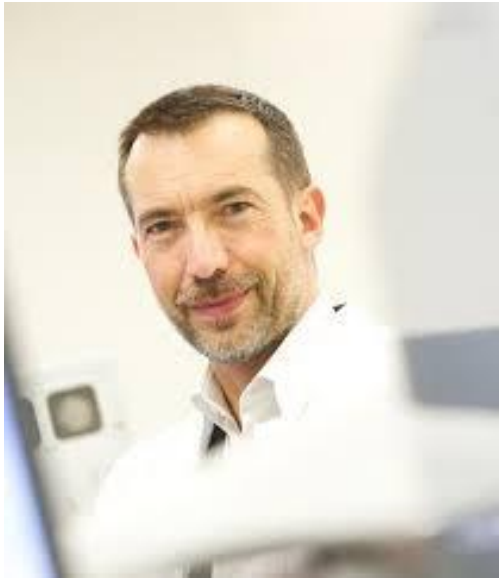
Jerome Galon, Prof., PhD.

CANCER CROSSLINKS

Current position:

- Research Director at INSERM; Head of the INSERM Integrative Cancer Immunology Laboratory, Cordeliers Research Center, Paris, France



Focus of work:

- Integrative cancer immunology
- Immunoscore and worldwide task force

Specific expertise / current research interest:

- Gain a global understanding of the tumor microenvironment and the dynamics of the host-immune response using high-throughput methods, novel technologies, and integrative biology approaches
- Implementation of Immunoscore into routine clinical practice

9th Cancer Crosslinks 2017

Oslo, Norway

January 26th, 2017

Cancer & Inflammation

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, Janssen*

Participation to Scientific Advisory Boards:

- *BMS, MedImmune, Astra Zeneca, Novartis, Definiens, IObiotech, ImmunID, Northwest Biotherapeutics, Actelion, Amgen, Kite Pharma*

Consultant :

- *BMS, Roche, GSK, Nanostring, Compugen, Mologen*

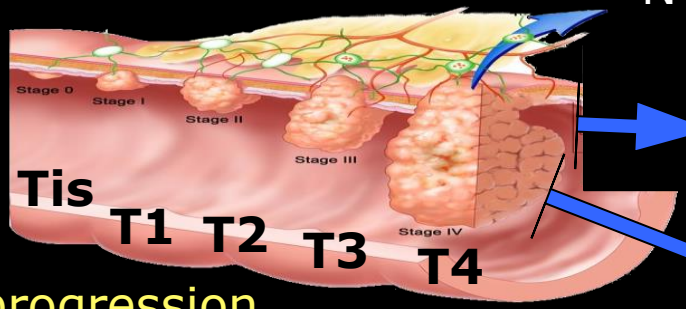
Definition of cancer

1) A tumor cell DNA disease – Cell-centric paradigm

2) Due to the acquisition of secondary key behavioral characteristics following tumor genomic changes (Hanahan & Weinberg, *Cell* 2000, 2011)

Tumor invasion

N-Stage



Early-metastasis
(venous emboli)

T-Stage

M-Stage

Tumor progression

Tumor grade differentiation
Tumor aggressiveness
(driver mutations, CIN, MSI, CIMP…)

-> Tumor recurrence

-> Tumor aggressiveness, progression, invasion and recurrence define early and late stage cancers, and the severity of the disease

Novel paradigm

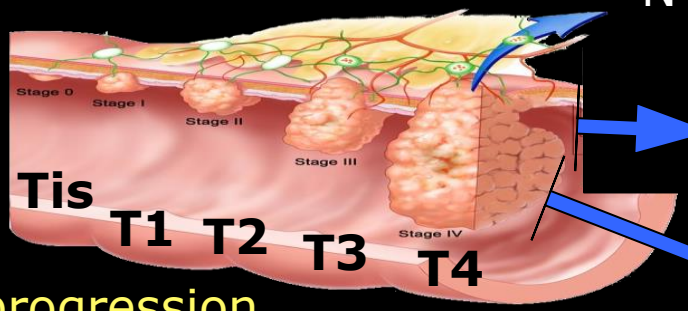
"Hot" Tumor

Immunoscore
Immune contexture

"Cold" Tumor

Tumor invasion

N-Stage



Early-metastasis
(venous emboli)

T-Stage

M-Stage

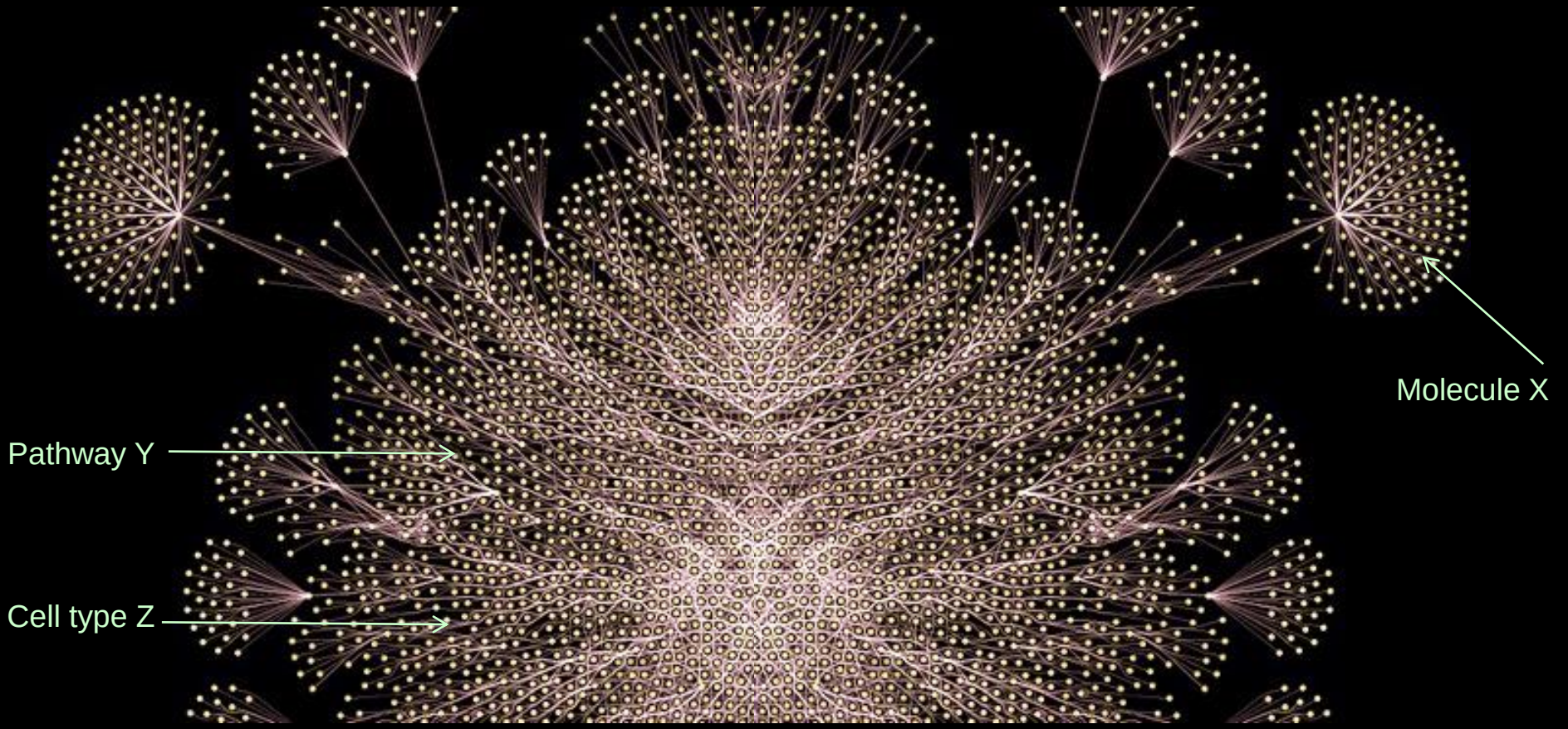
Tumor progression

Tumor grade differentiation
Tumor aggressiveness
(driver mutations, CIN, MSI, CIMP…)

-> Tumor recurrence
-> death

- ✓ Tumor progression, invasion and recurrence are dependent on pre-existing immunity and on Immunoscore
- ✓ Pre-existing immunity is determining the fate and survival of the patient
- ✓ Pre-existing immunity is determining the likelihood of response to immunotherapy

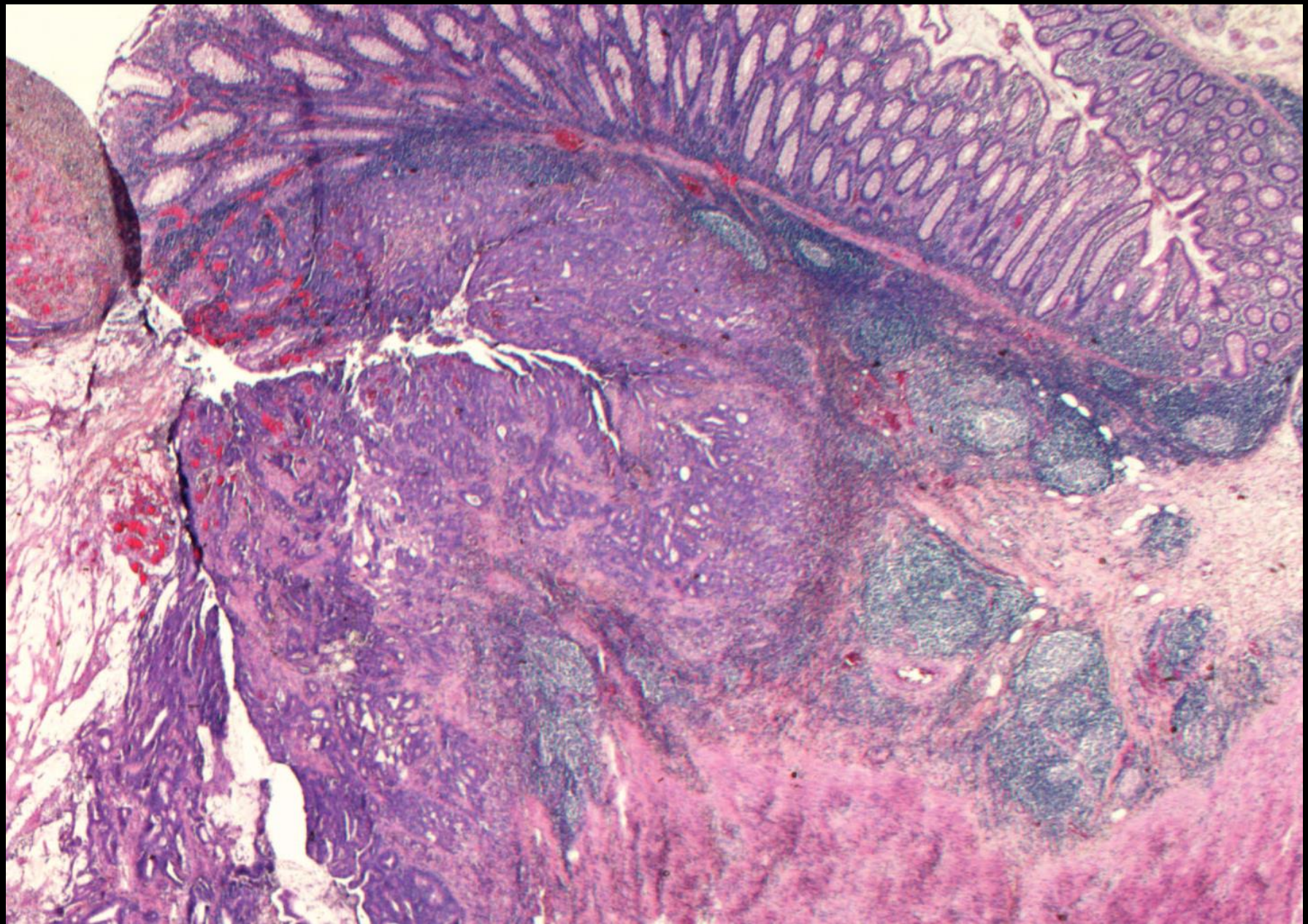
Cancer is one of the most complex biological system of all



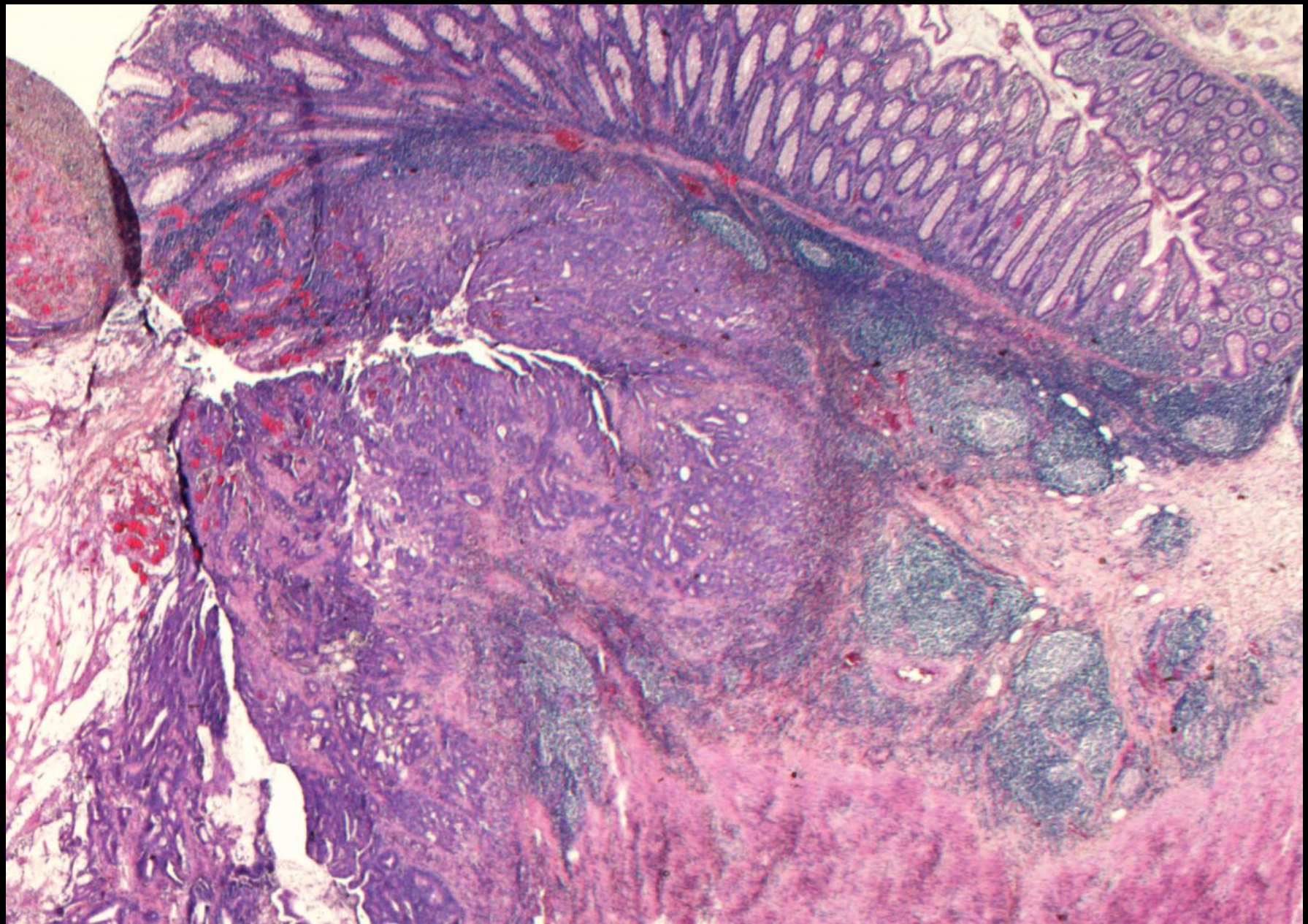
"*The whole is greater than the sum of its parts*", Aristotle

-> Systems biology in human cancer

Tumor microenvironment



Tumor microenvironment



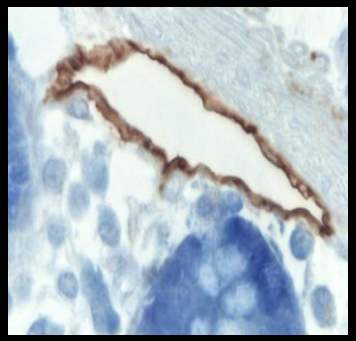
The tumor microenvironment is highly complex…

and it can be decipher

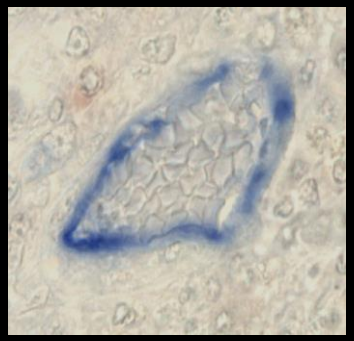
Question

Role of immune infiltrates in the promotion/control of early-metastatic invasion (VELIPI) in human cancers ? Impact on clinical outcome ?

959 colorectal cancer patients

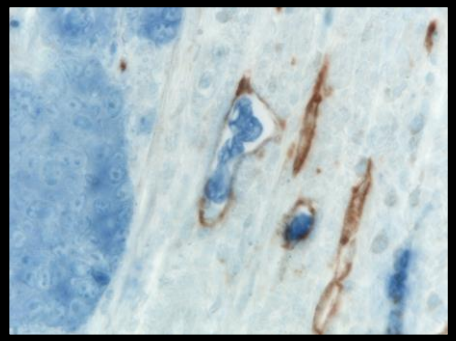


Lymphatic vessels (PDPN)



blood vessels (Endoglin)

Lymphatic emboli / Tumor



immune

? ? ?

promotion — Detrimental

No impact

control — Beneficial

Venous Emboli (VE)
Lymphatic Invasion (LI)
Perineural Invasion (PI)

Immunologists *Cancer biologists* ← *Thinking in the field in 2000's*

-> Global analysis of tumor microenvironment in Human tumors

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

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

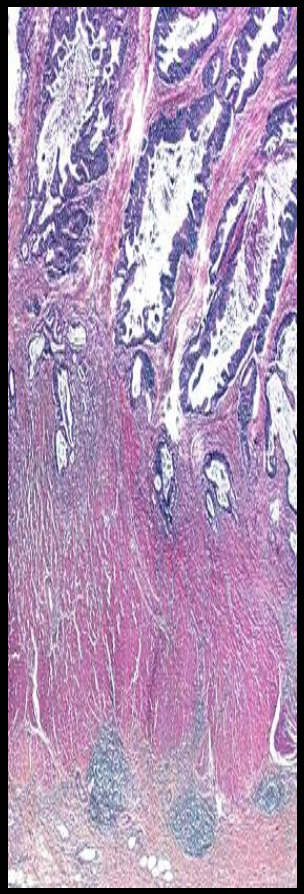
The foundation of a new concept

Immune contexture ⟷ **Immunoscore**

✓ Quantification of immune cell densities (n=415 Patients, 6640 IHC) revealed the major positive role of cytotoxic and memory T cells for patient's survival

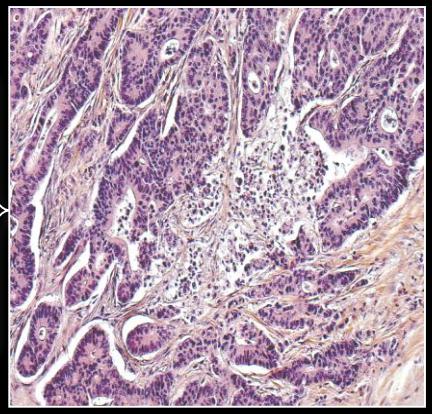
Galon J et al. ***Science*** 2006

Immune cells are present within the tumor

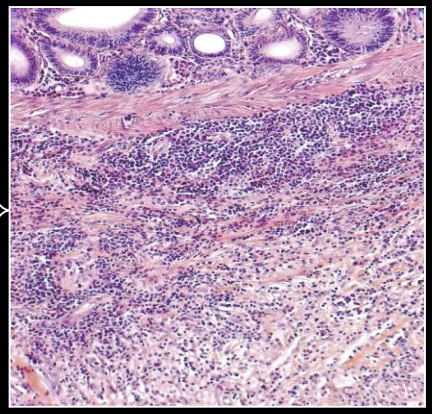


H&E sections

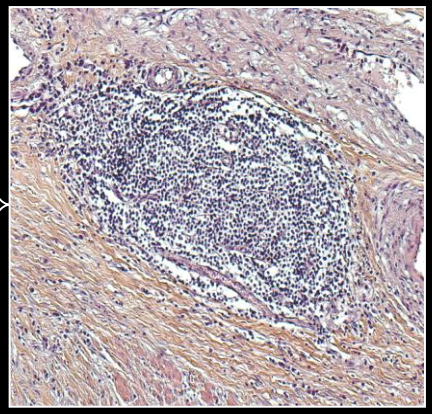
Center

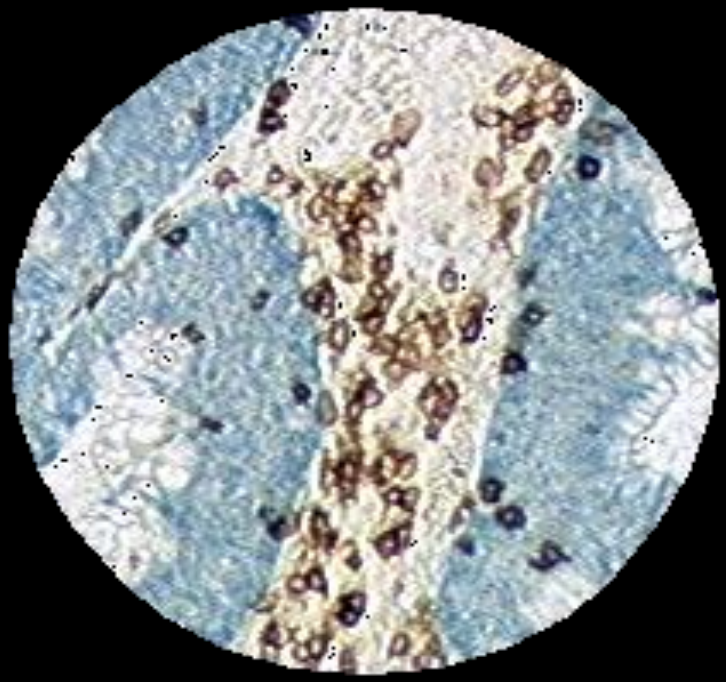


Margin



TLS



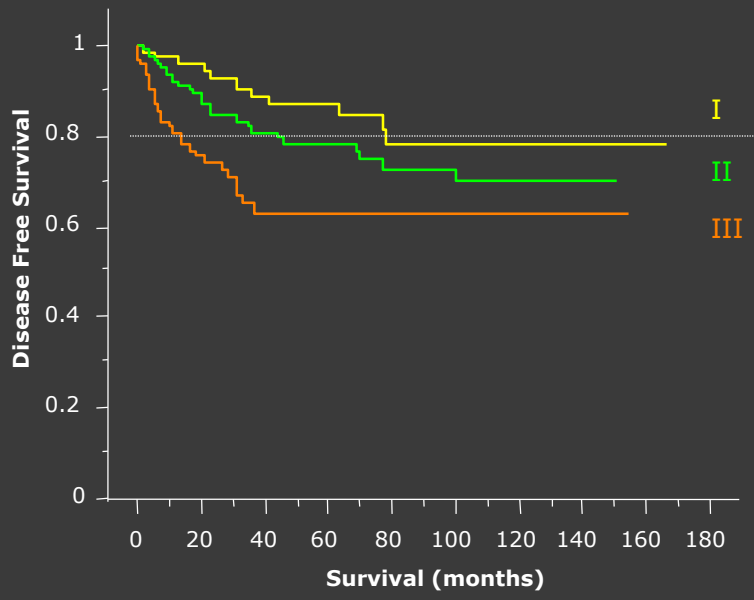


Tumor (blue)
CD3 T cells (brown)

Importance of the distribution of the adaptive immune reaction compared to tumor invasion

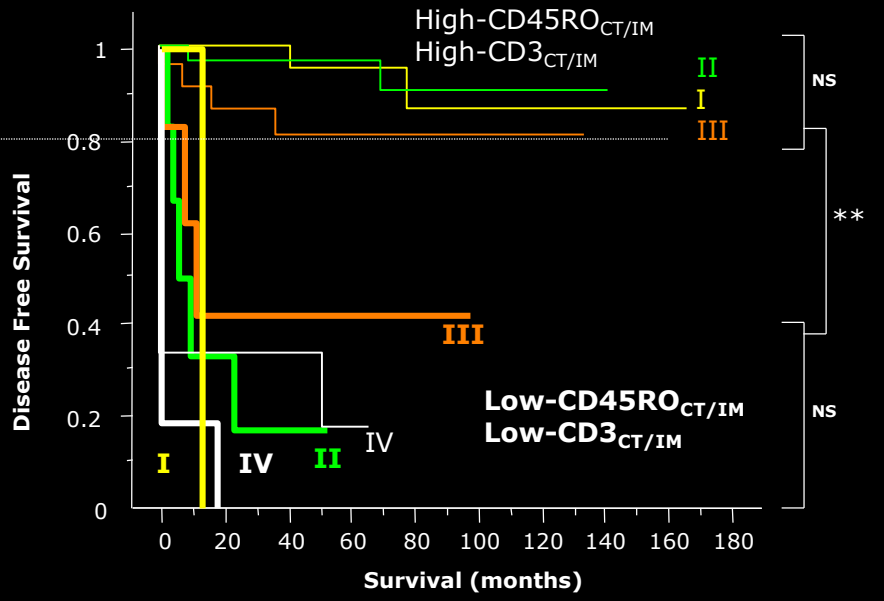
Tumor Histopathologic Findings

AJCC/UICC-TNM
Current prognosis classification



Immune cells analysis

$CD3_{CT}/CD3_{IM}$ evaluation
plus
$CD45RO_{CT}/CD45RO_{IM}$ evaluation



-> Coordinated adaptive immune reaction more than tumor invasion predicts clinical outcome

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

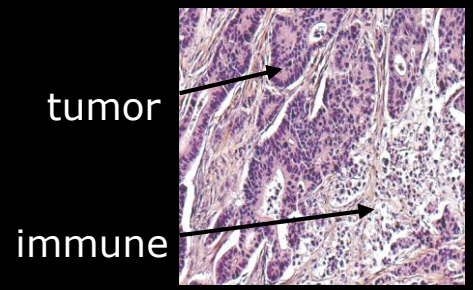
Novel Paradigm

COX multivariate analysis (OS) in **all** stages I, II, III patients

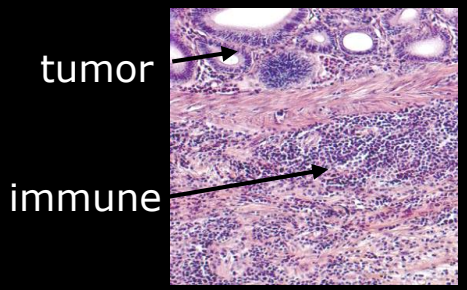
Parameter	*HR*	*P* value
• T-stage	1.2	0.25
• N-stage	1.4	0.15
• Differentiation	1.1	0.84
• Immunoscore	1.9	0.00001

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

"***Immune Contexture***" : **nature**, immune **functional** orientation, **density**, and **location** within distinct tumor regions, of a natural *in situ* immune reaction



Center of the tumor (CT)



Invasive margin (IM)

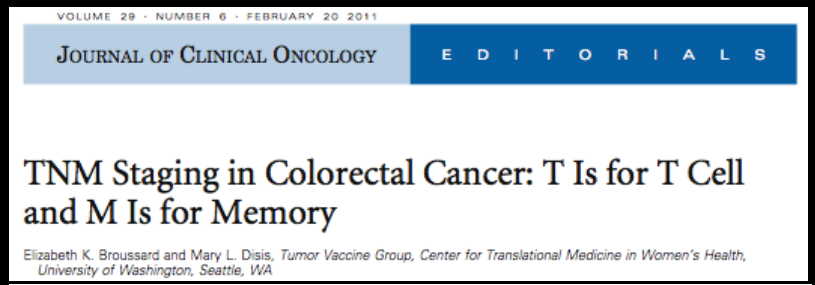
Galon J et al. ***Science*** 2006

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

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

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


VOLUME 29 · NUMBER 6 · FEBRUARY 20 2011

JOURNAL OF CLINICAL ONCOLOGY — E D I T O R I A L S

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

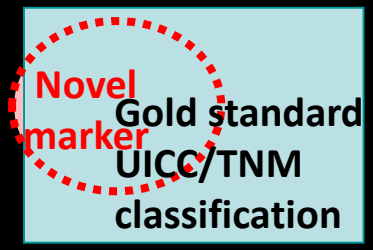
- ✓ Tumor progression, invasion and recurrence are dependent on the immune contexture and Immunoscore
- ✓ Pre-existing immunity is determining the fate and survival of the patient

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

Assessment of a novel marker for prognosis

multivariate analysis (COX)

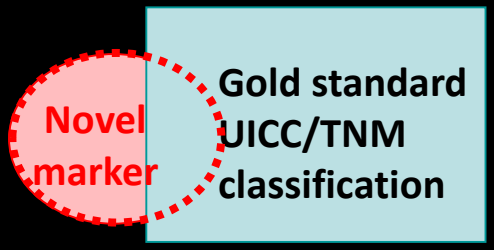
Not good marker



P: ns

No improvement for prediction

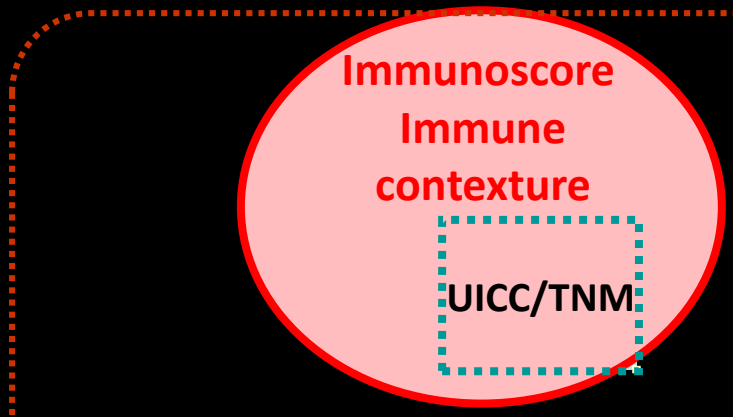
Good marker



P<0.05

Better accuracy for prediction +

Novel concept



P<0.0001

Novel concept for prediction +++

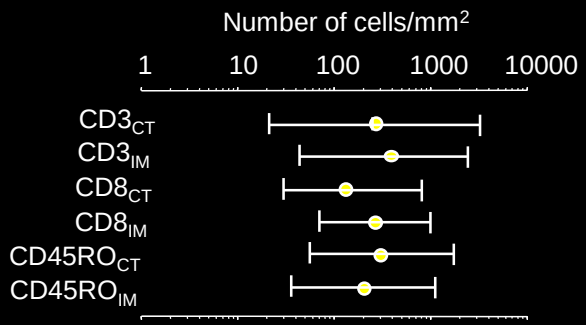
Immune Contexture

Parameters with major positive impact on patient' survival

Type

- Cytotoxic T cells (CD3, CD8)
- Memory T cells (CD45RO)

Density



Functional orientation

T_H1 cells

Associated factors

IFNG	GZMA	CX3CL1	MADCAM1	CXCL13
IL12	GZMB	CXCL9	ICAM1	IL21
TBX21	GZMH	CXCL10	VCAM1	
IRF1	PRF	CCL5		IL15
STAT1	GLNY	CCL2		

(T_H17, T_{reg}, T_H2 cells have variable impact on survival depending on tumor types)

Location

- Center of the Tumor (CT)
- Invasive Margin (IM)

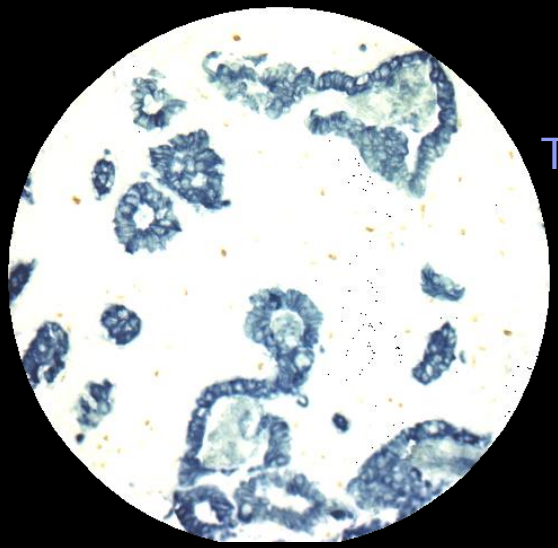
TLS (Presence and quality)

Pages F ***NEJM*** 2005 ; Galon J. ***Science*** 2006 ; Galon J. ***Cancer Res***. 2007 ; Camus M. ***Cancer Res*** 2009 ; Pages F ***JCO*** 2011; Mlecnik B ***Gastroenterology*** 2011; Tosolini M. ***Cancer Res*** 2011 ; Mlecnik B ***JCO*** 2011; Fridman H. ***Nat Rev Cancer*** 2012 ; Galon J. ***Immunity*** 2013 ; Bindea G. ***Immunity*** 2013 ; Mlecnik B ***Science TM*** 2014 ; Mlecnik B ***Science TM*** 2016 ; Church S ***Immunity*** 2015 ; Mlecnik B ***Immunity*** 2016

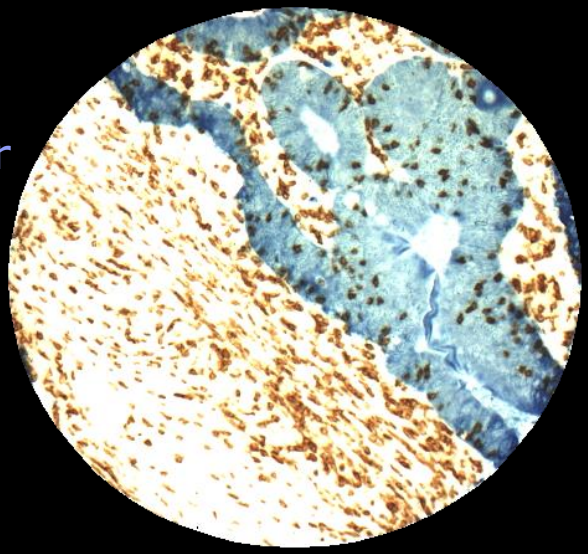
Mechanistic understanding of the tumor microenvironment using integrative biology

Deciphering the tumor immune microenvironment: Clinical implications

"Cold" Tumor
I 0



CD3
Tumor



"Hot" Tumor
I 4

Clinical implications

Predictions

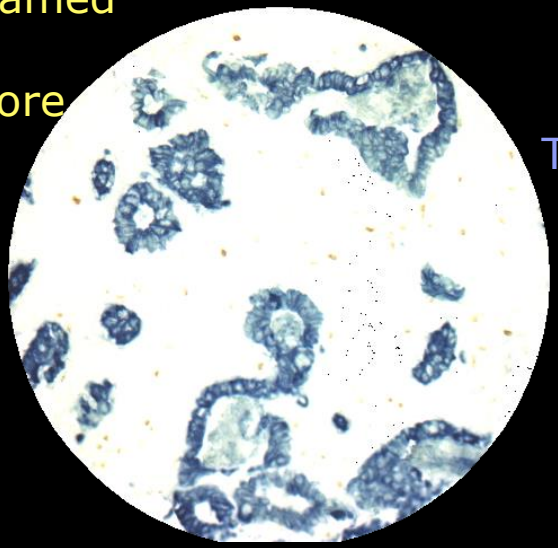
Need T-cell priming
Cancer vaccine
CAR-T cells

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

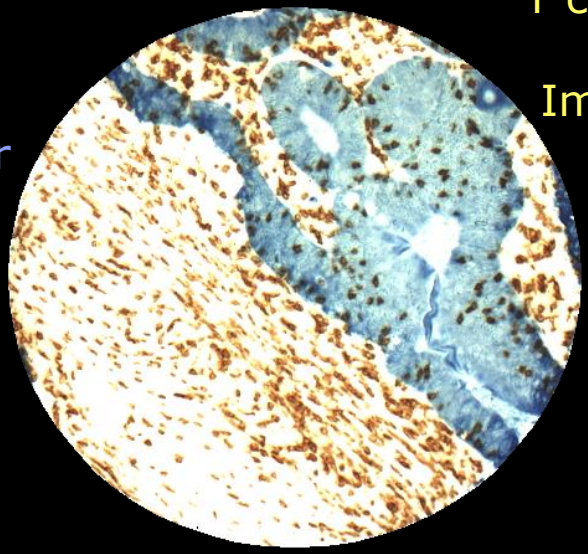
But it is not as simple since biology is complex and is not dichotomized in good & bad

Deciphering the tumor immune microenvironment: Clinical implications

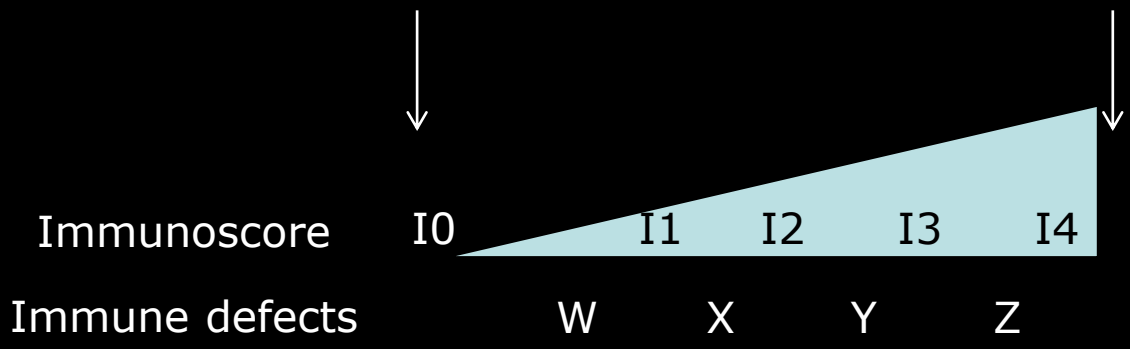
"Non-T cell inflamed" Tumor Immunoscore I 0



CD3 Tumor

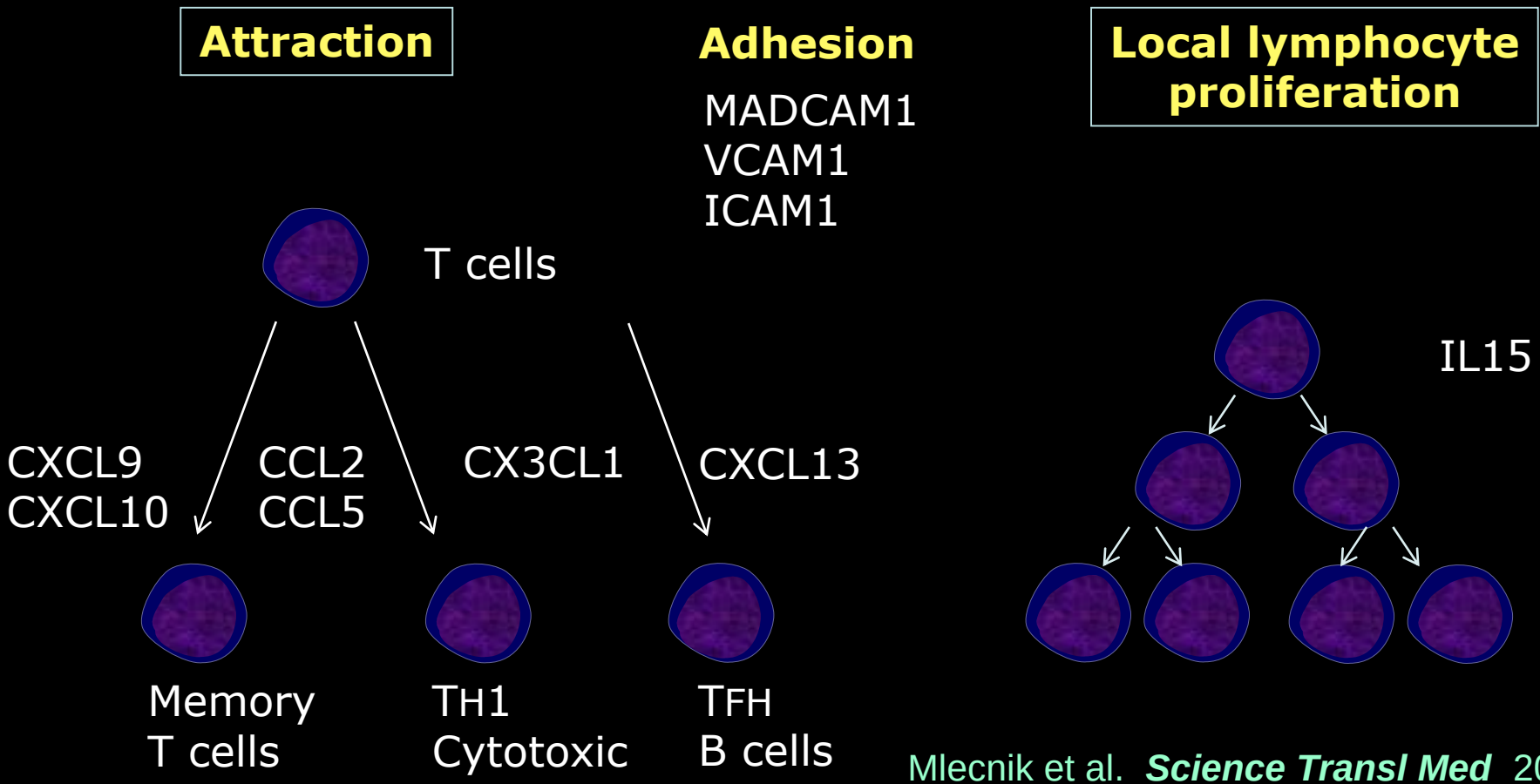


"T cell inflamed" Tumor Immunoscore I 4



-> Immunotherapies based on Immunoscore and immune defects

Mechanisms associated with T cells infiltration



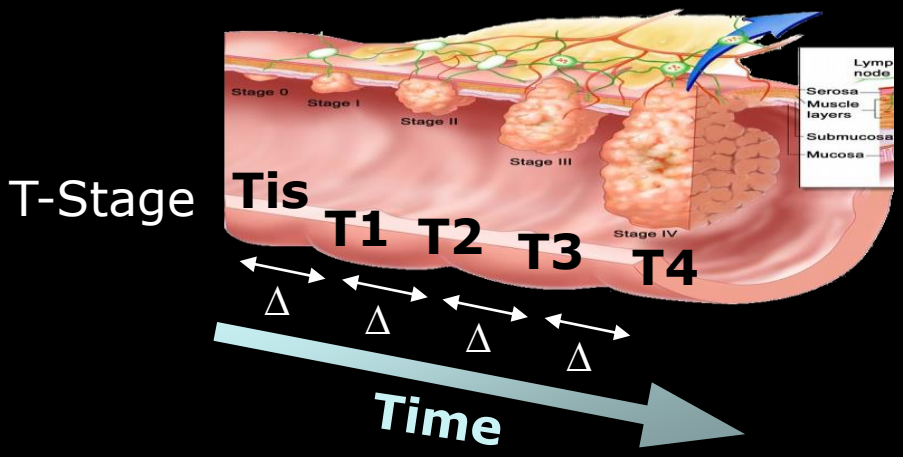
Mlecnik et al. ***Science Transl Med*** 2014

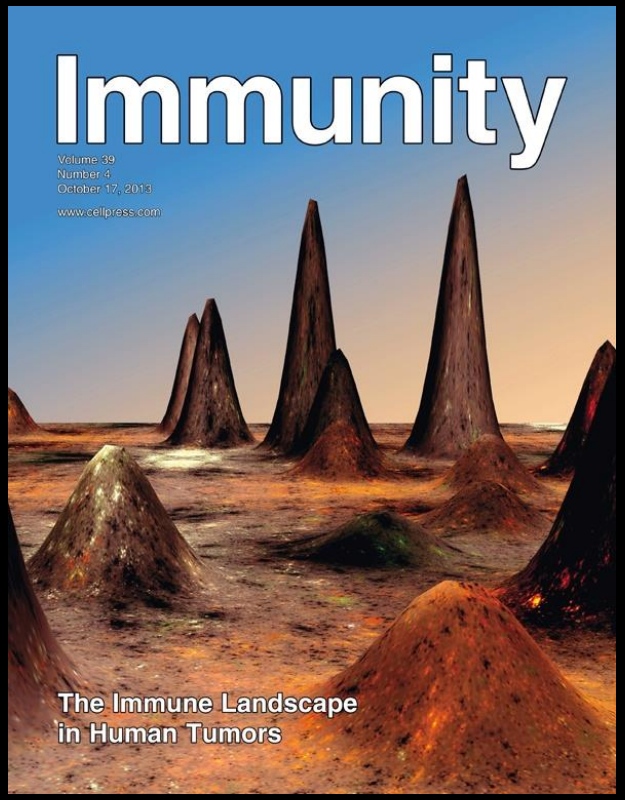
Mlecnik et al. ***Gastroenterology*** 2010

Bindea et al. ***Immunity*** 2013

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

- Evolution of the tumor microenvironment with tumor progression?
- Immune escape mechanisms in human tumors?



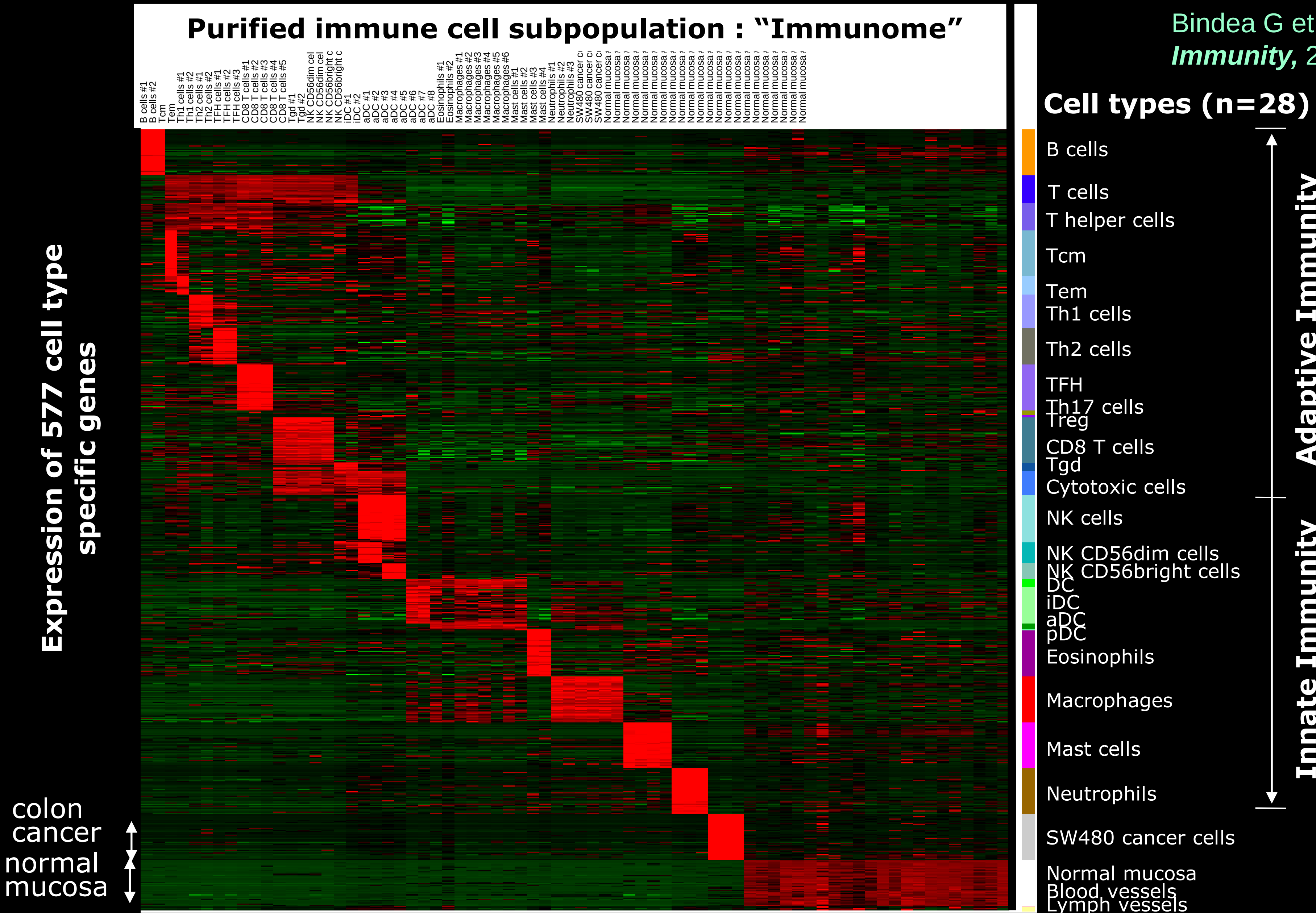


-> **Spatio-temporal dynamics**
of the immune response with tumor progression

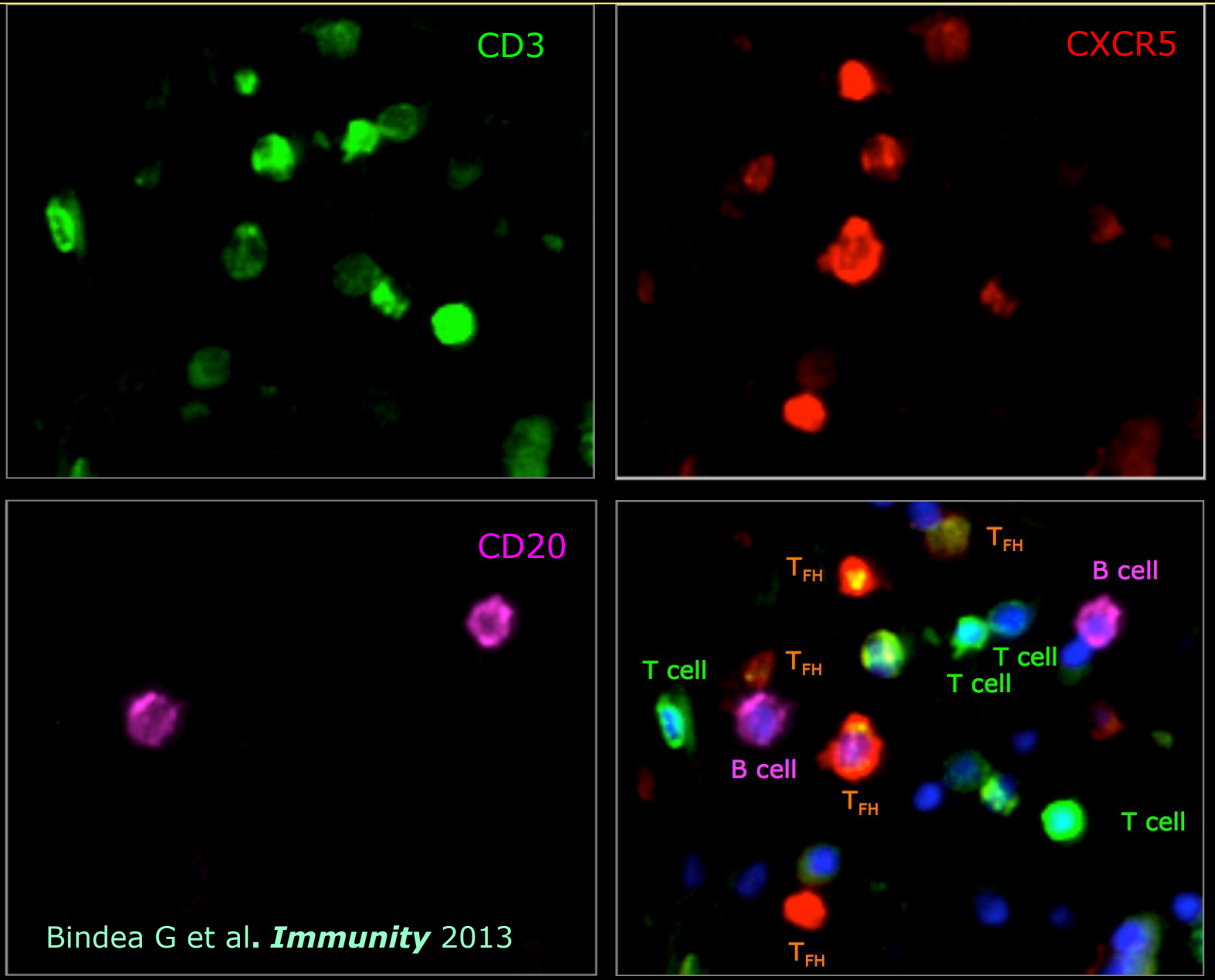
Bindea G et al. ***Immunity,*** 2013

"Immunome" of purified immune cell subpopulations

Bindea G et al.
Immunity, 2013

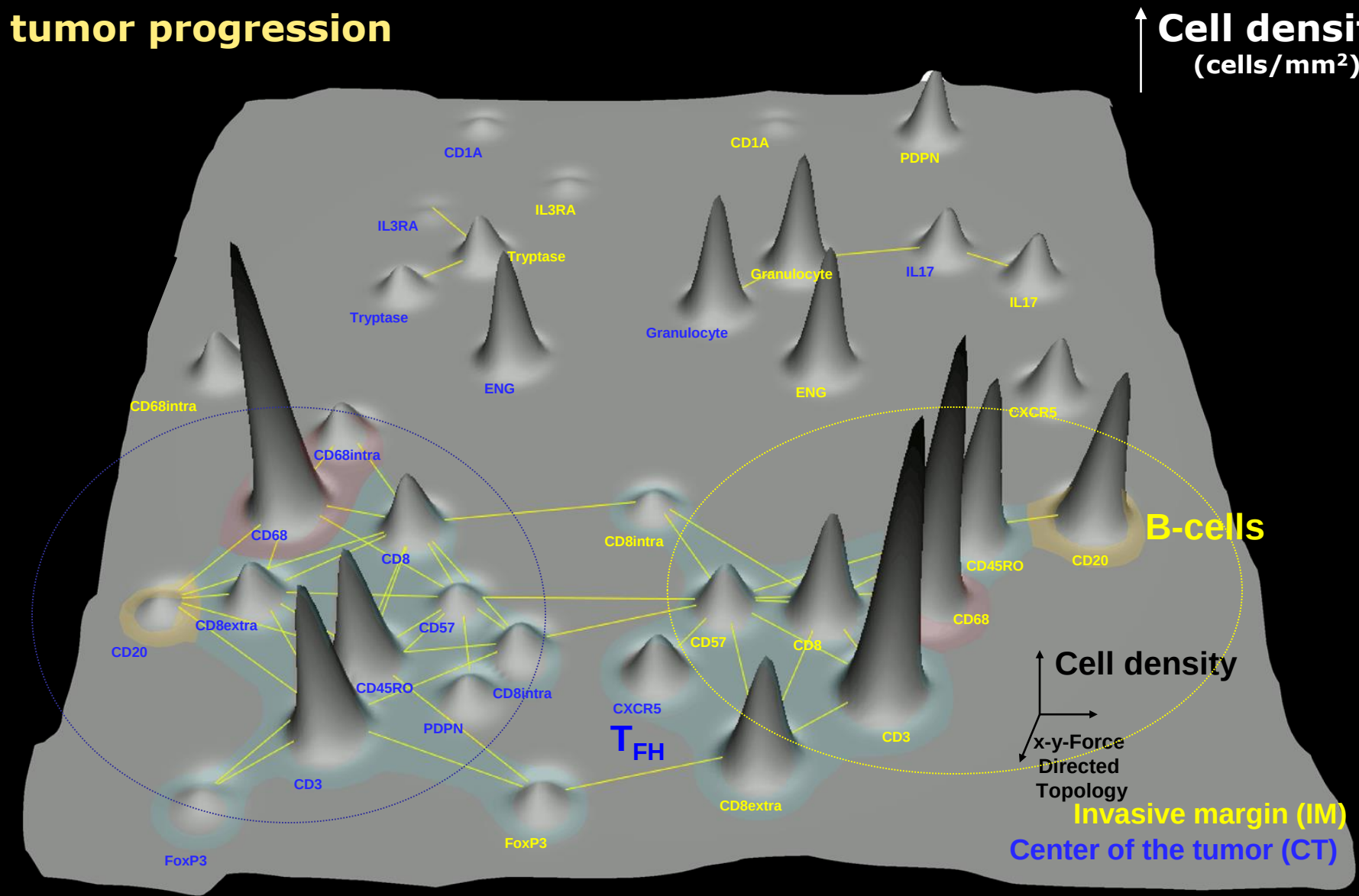


Intratumor B cells, T cells and T_{FH} cells



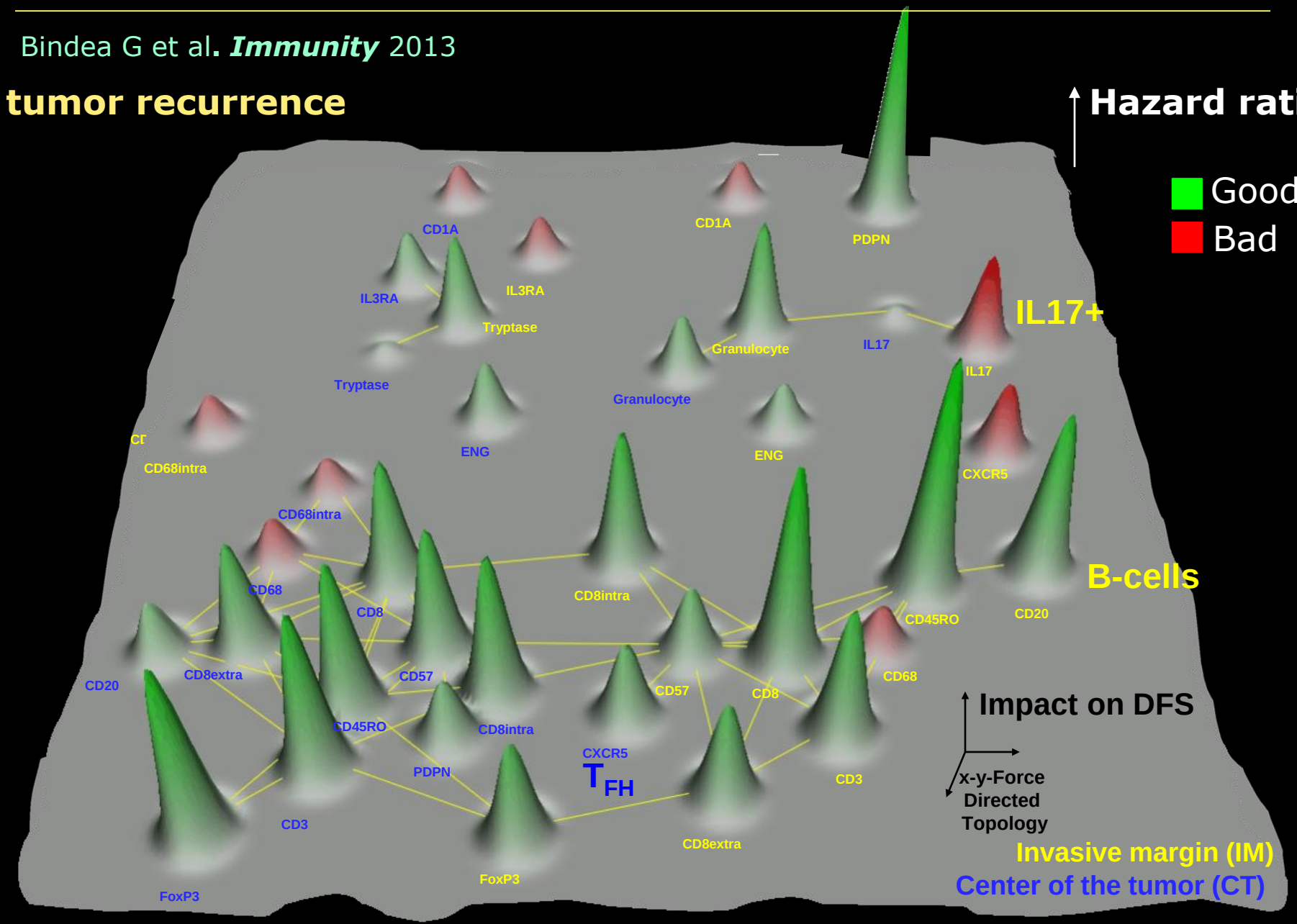
Understanding the evolution of the immune response with tumor progression : The immune landscape

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



Understanding the evolution of the immune response with tumor progression : The immune landscape

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

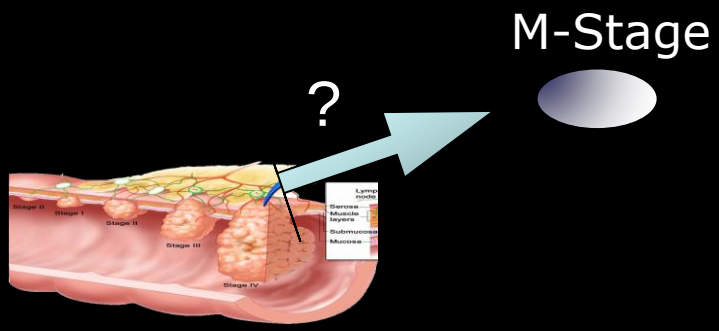


RESEARCH ARTICLE

CANCER

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

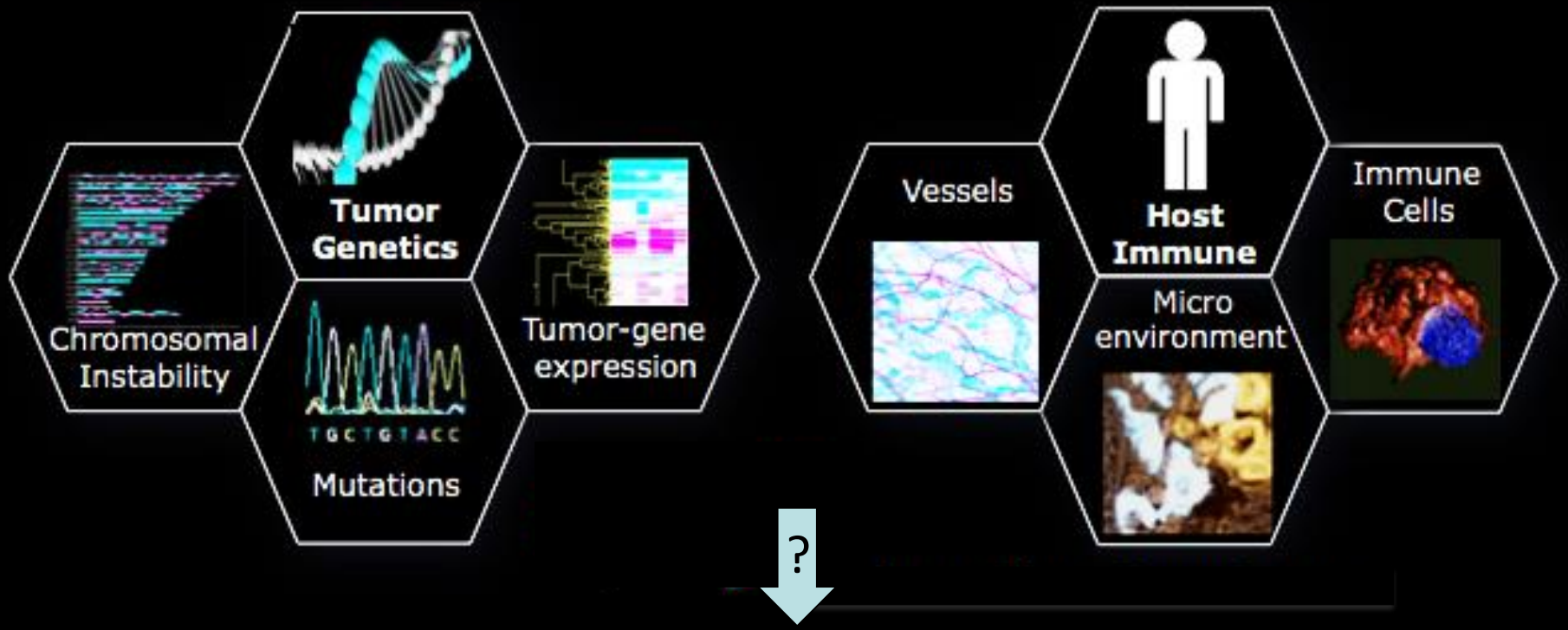
Bernhard Mlecnik,[1,2,3]* Gabriela Bindea,[1,2,3]* Amos Kirilovsky,[1,2,3]* Helen K. Angell,[1,2,3,4] Anna C. Obenauf,[5] Marie Tosolini,[1,2,3] Sarah E. Church,[1,2,3] Pauline Maby,[1,2,3] Angela Vasaturo,[1,2,3] Mihaela Angelova,[1,2,3] Tessa Fredriksen,[1,2,3] Stéphanie Mauger,[1,2,3] Maximilian Waldner,[6]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



n= 838 patients

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?

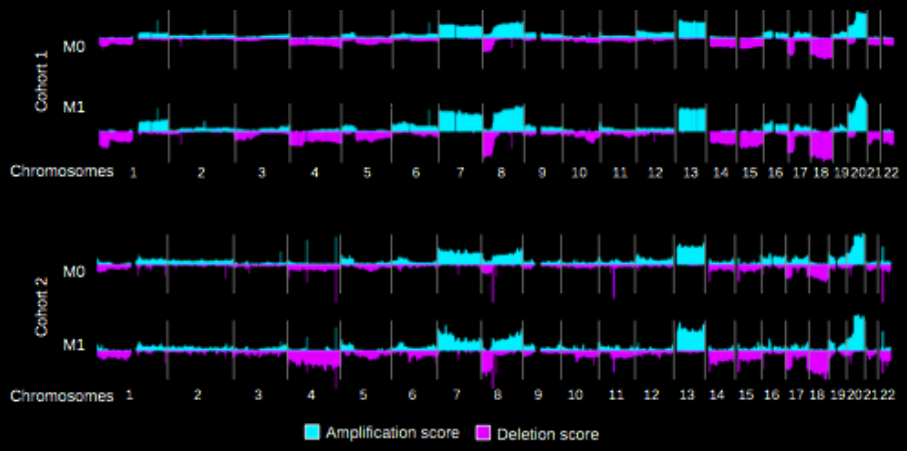


M1 stage

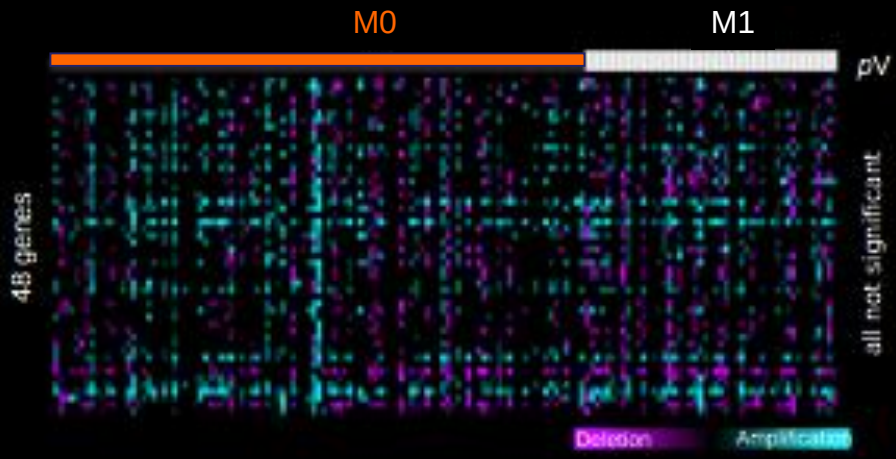
Mlecnik et al. *Science Transl Med*. 2016

Genomic alterations in tumors

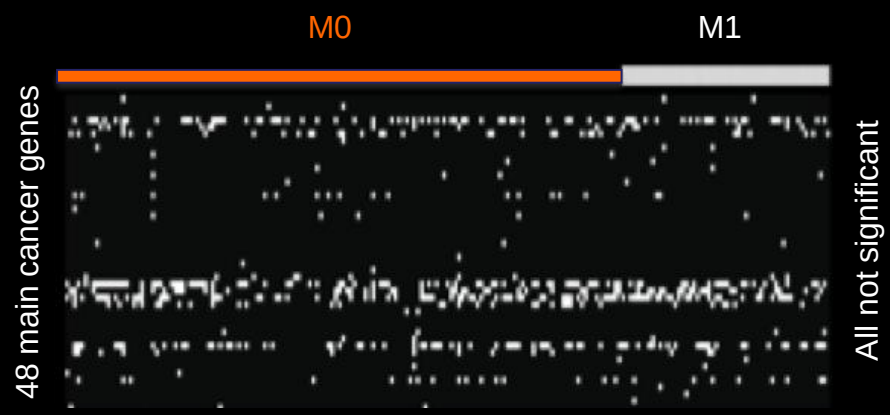
Chromosomal instability pattern (CIN)



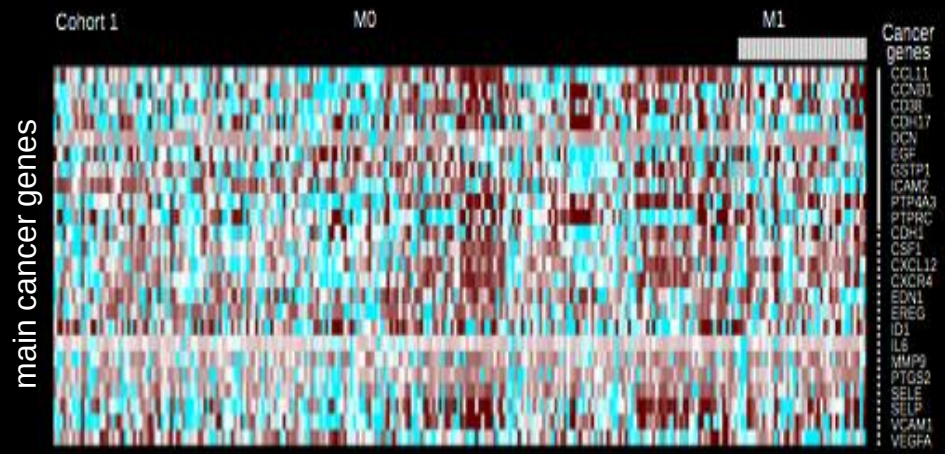
Chromosomal instability pattern in M0/M1



Mutation pattern

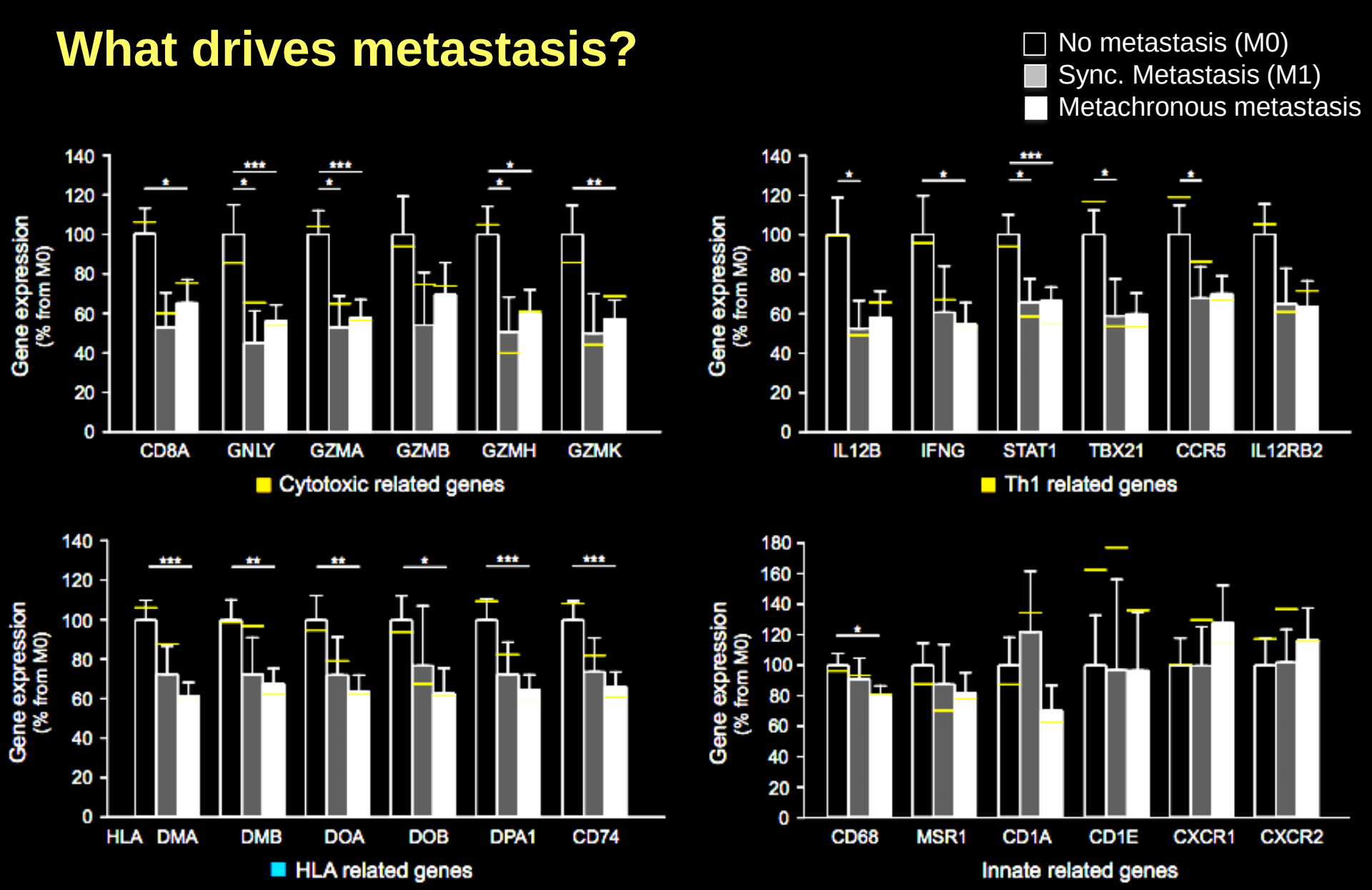


Tumor gene expression pattern



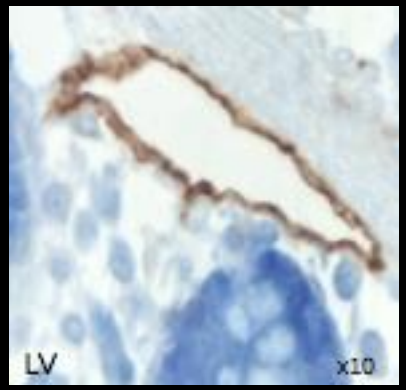
-> No significant difference between M0 and M1 cancer patients (except FBXW7 mutation)

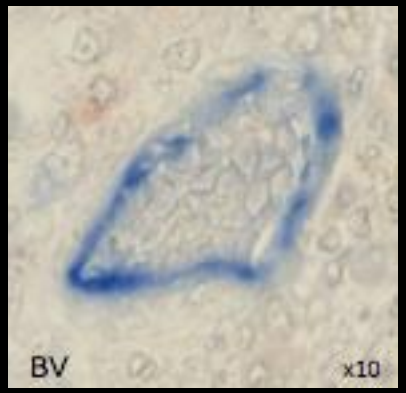
What drives metastasis?

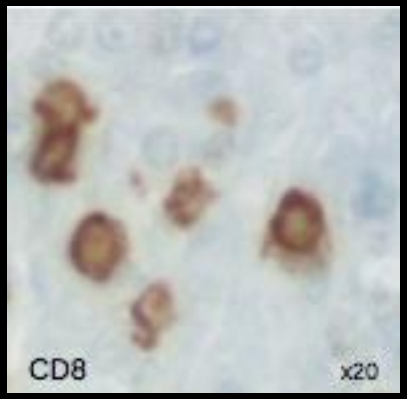


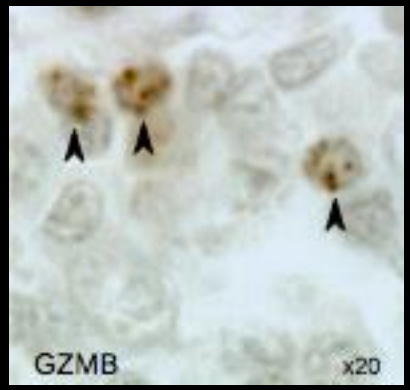
- Cytotoxic, Th1, HLA-Cl2 gene expression are significantly decreased in primary tumors from patients with synchronous

Blood and Lymph vessels, CD8+ cell and GZMB+ cell densities within primary tumors



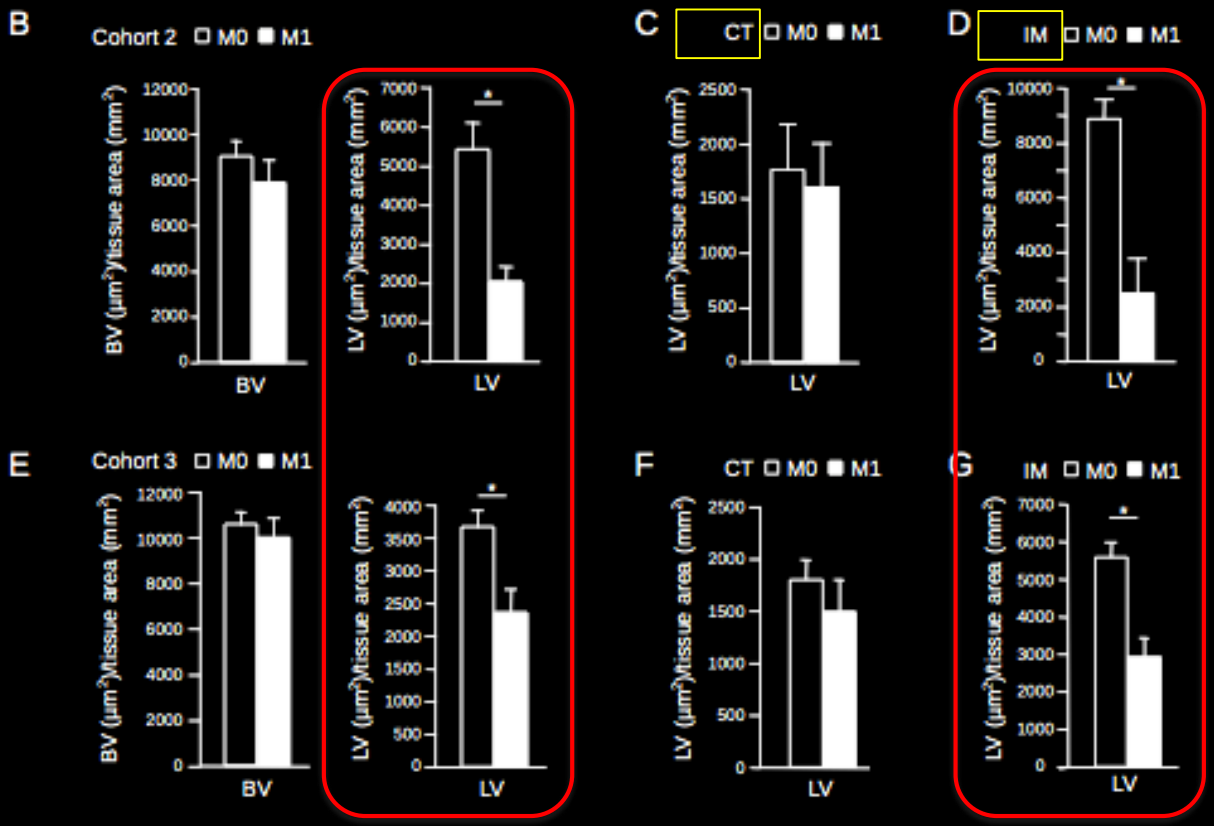




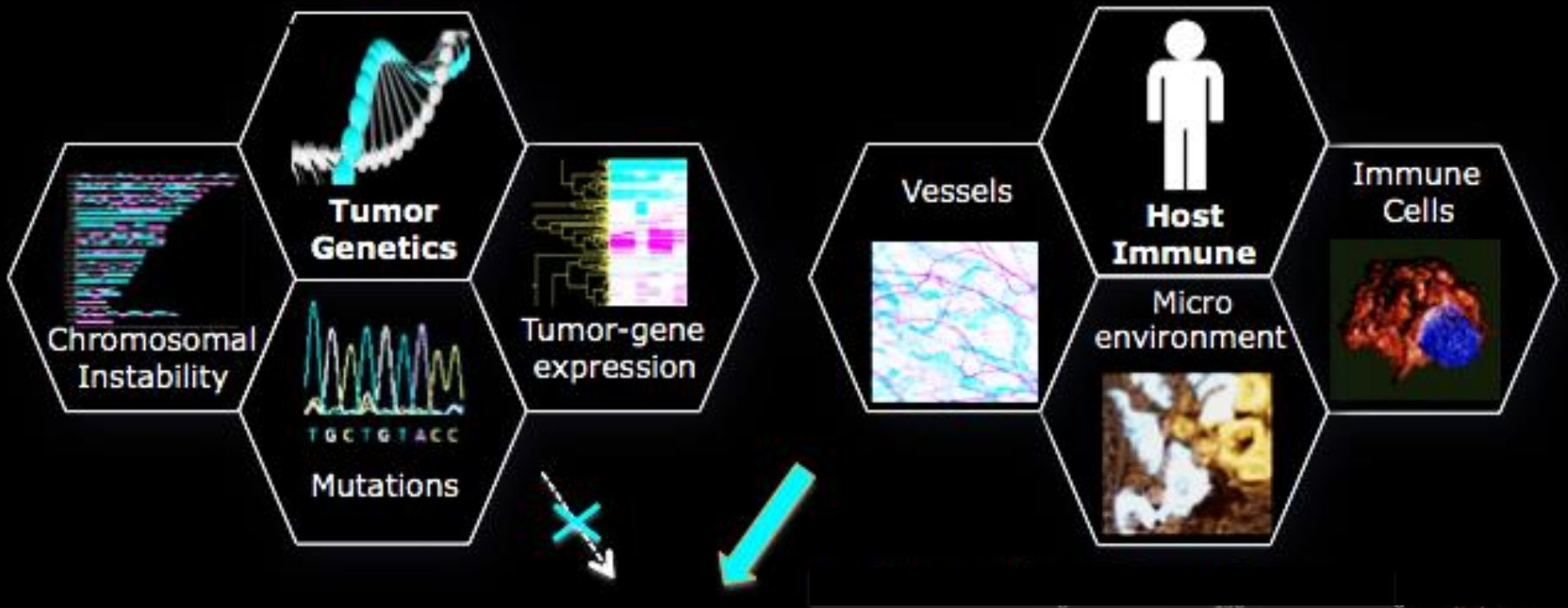


Cohort 1
(n=108)

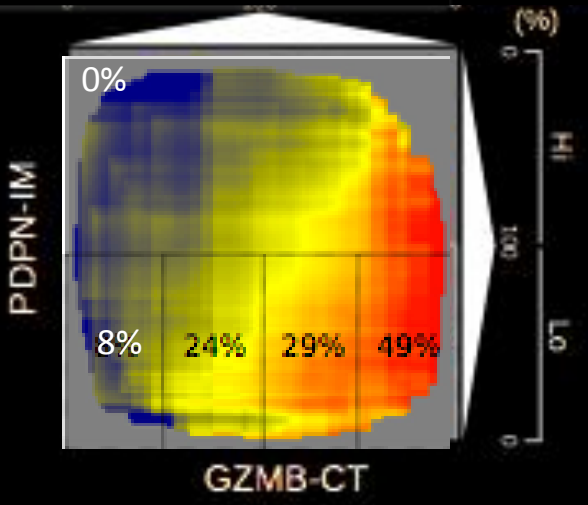
Cohort 2
(n=415)



What drives metastasis?



M1 stage



Lymph vessels (IM)
GZMB+ (CT)

Mlecnik et al. *Science Transl Med*. 2016

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

PD-1 Blockade in Tumors with Mismatch-Repair Deficiency

D.T. Le, J.N. Uram, H. Wang, B.R. Bartlett, H. Kemberling, A.D. Eyring, A.D. Skora, B.S. Luber, N.S. Azad, D. Laheru, B. Biedrzycki, R.C. Donehower, A. Zaheer, G.A. Fisher, T.S. Crocenzi, J.J. Lee, S.M. Duffy, R.M. Goldberg, A. de la Chapelle, M. Koshiji, F. Bhaijee, T. Huebner, R.H. Hruban, L.D. Wood, N. Cuka, D.M. Pardoll, N. Papadopoulos, K.W. Kinzler, S. Zhou, T.C. Cornish, J.M. Taube, R.A. Anders, J.R. Eshleman, B. Vogelstein, and L.A. Diaz, Jr.

CellPress

Immunity
Article

Integrative Analyses of Colorectal Cancer Show Immunoscore Is a Stronger Predictor of Patient Survival Than Microsatellite Instability

Bernhard Mlecnik,[1,2,3,19] Gabriela Bindea,[1,2,3,19] Helen K. Angell,[1,2,3,4] Pauline Maby,[1,2,3,5] Mihaela Angelova,[1,2,3,6] David Tougeron,[5,7,8] Sarah E. Church,[1,2,3] Lucie Lafontaine,[1,2,3] Maria Fischer,[6] Tessa Fredriksen,[1,2,3] Maristella Sasso,[1,2,3] Amélie M. Bilocq,[1,2,3] Amos Kirilovsky,[1,2,3] Anna C. Obenauf,[9] Mohamad Hamieh,[5] Anne Berger,[1,10] Patrick Bruneval,[11] Jean-Jacques Tuech,[12] Jean-Christophe Sabourin,[13] Florence Le Pessot,[13] Jacques Mauillon,[13,14] Arash Rafii,[15] Pierre Laurent-Puig,[2,16] Michael R. Speicher,[9] Zlatko Trajanoski,[6] Pierre Michel,[7] Richard Sesboüe,[5] Thierry Frebourg,[5,16] Franck Pagès,[1,2,3,17] Viia Valge-Archer,[4,18] Jean-Baptiste Latouche,[5,8] and Jérôme Galon[1,2,3,*]

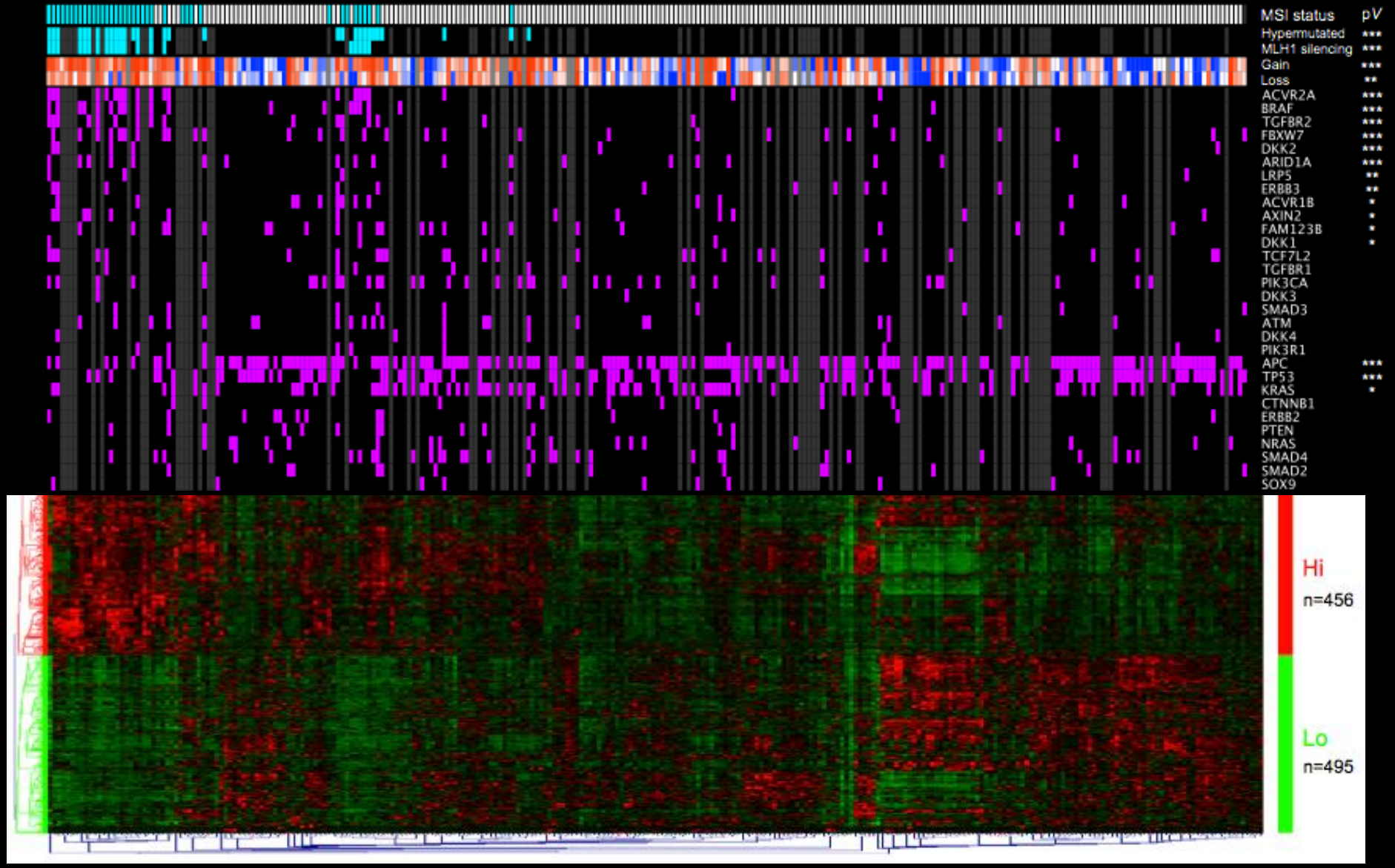
TCGA CRC cohort: n=270 patients

Inserm cohort: n=689 patients

Mlecnik et al. *Immunity* 2016

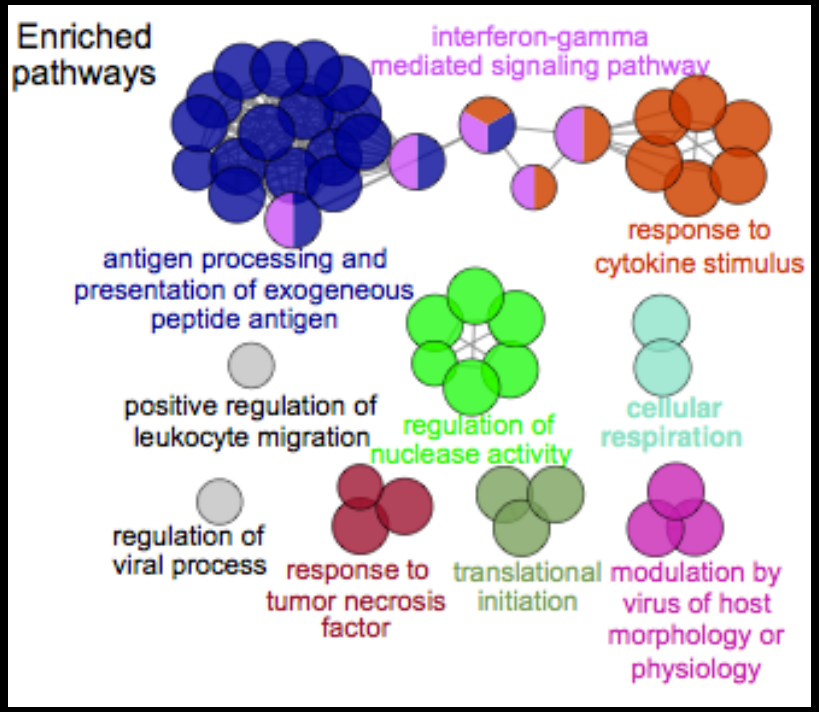
Specific genotype and gene profiling in MSI-H patients

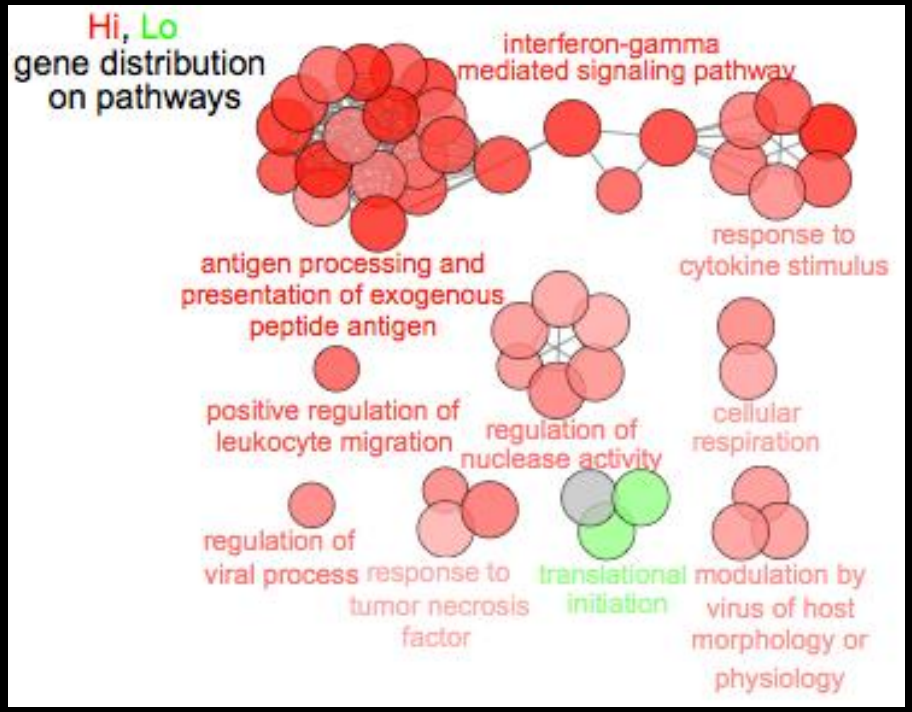
Chromosomal instability, mutation patterns, and gene expression profiling in 270 MSI-H and MSS patients



Most of over-expressed genes in MSI-H patients correspond to immune-related genes

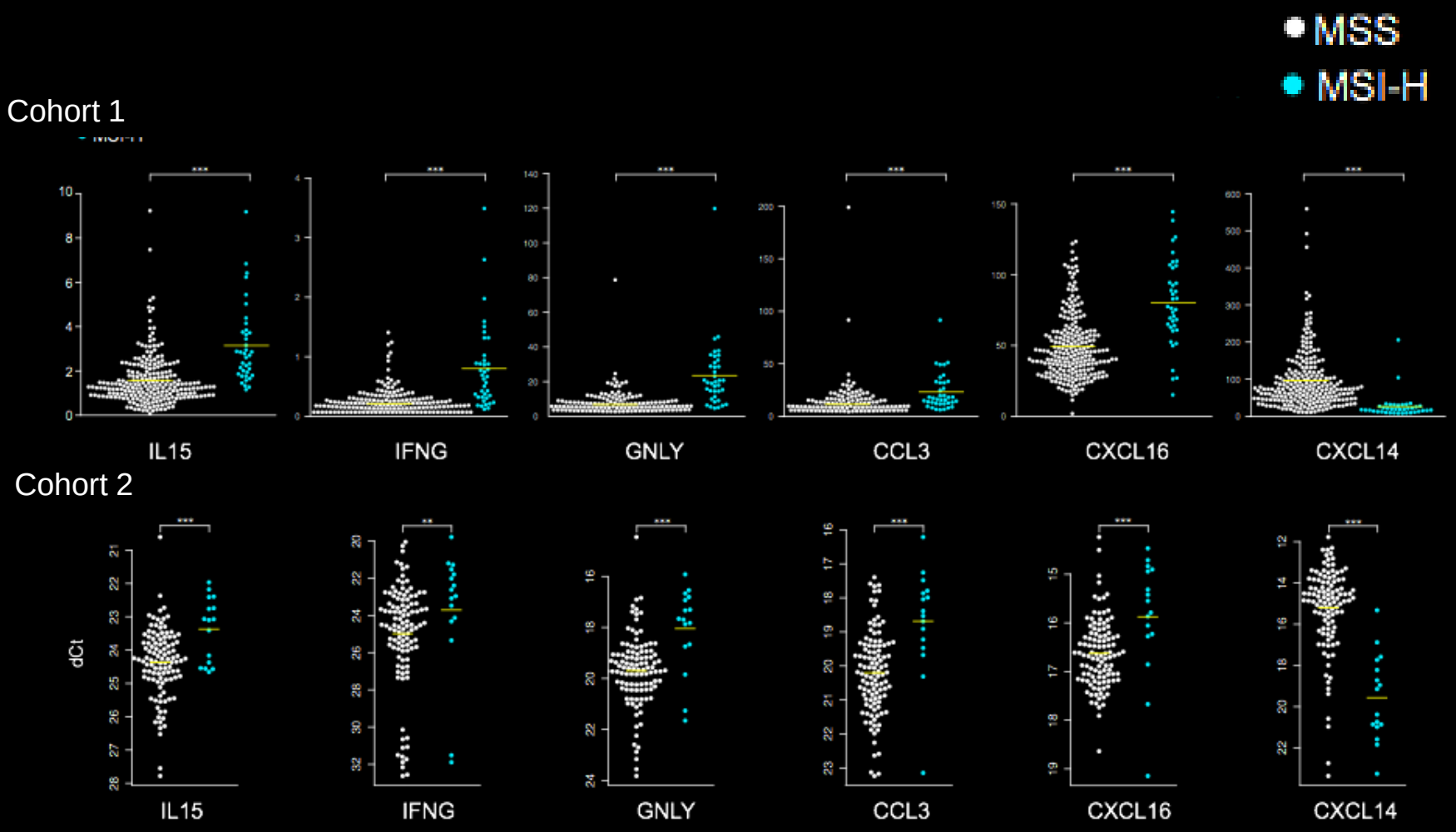
Gene functions and gene distribution analysis using ClueGO software





Mlecnik et al. *submitted*

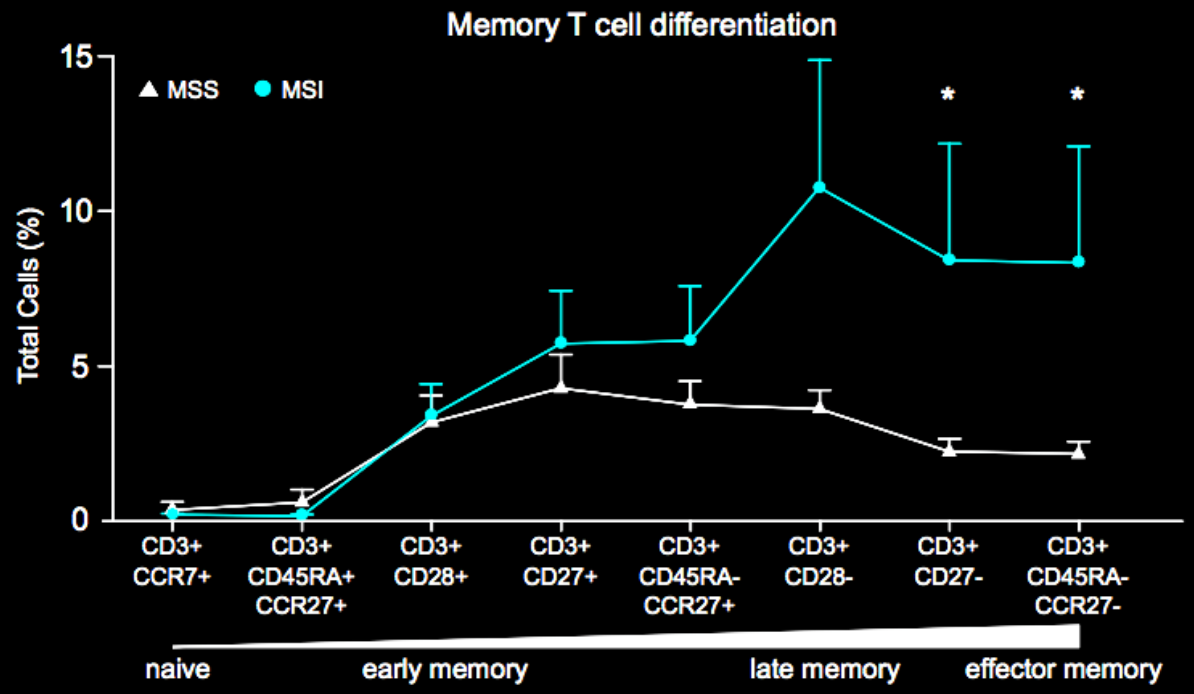
MSI-H patients have significantly more cytokines,Th1, cytotoxic and chemokines such as IL15, IFNG, GNLY, CCL3, CXCL16, and less CXCL14 in their tumors



Mlecnik et al. *Immunity* 2016

Patients with MSI-H have increased effector memory T-cells

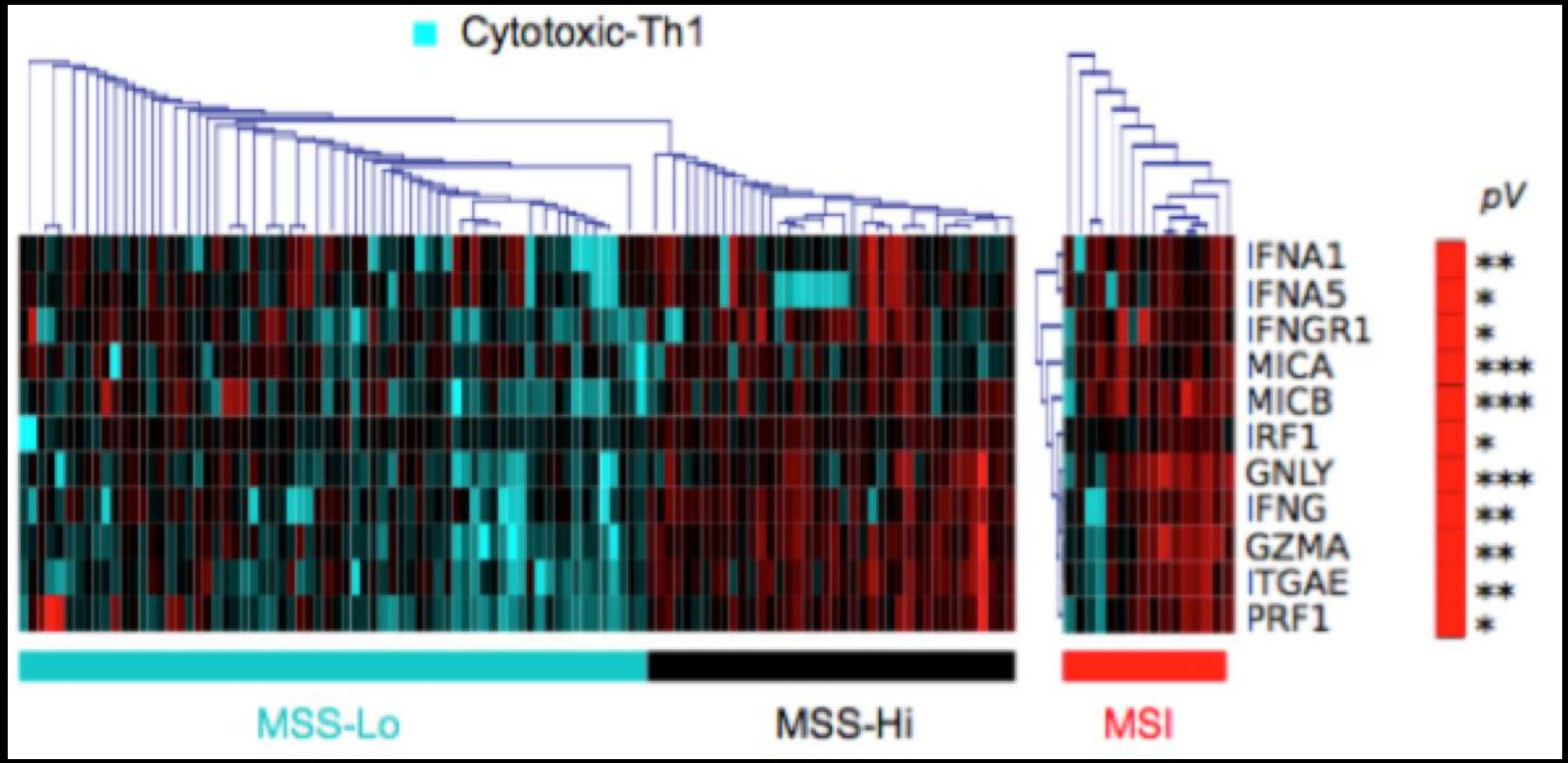
FACS analysis of intratumoral T-cells



Mlecnik et al. *In press*

Patients with MSI-H have statistically increased cytotoxic, Th1, gene expression

- ✓ A majority of MSI-H patients have increased cytotoxic, Th1, gene expression, however some do not
- ✓ A majority of MSS patients have low expression of cytotoxic, Th1, gene, however many have a high expression profile

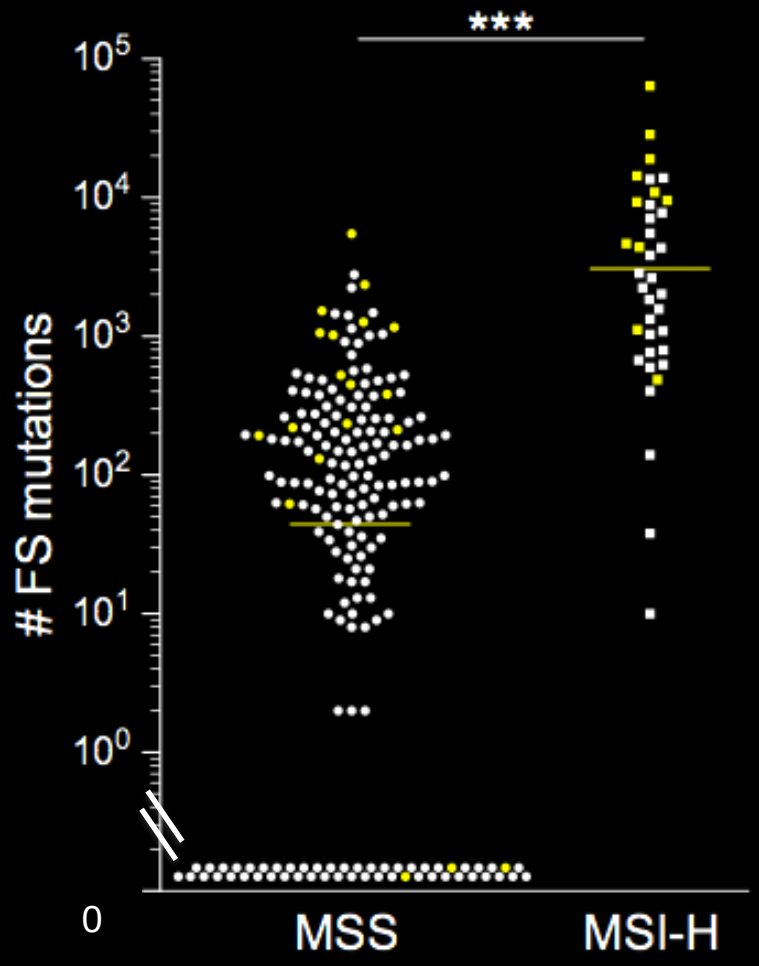


Mlecnik et al. *Immunity* 2016

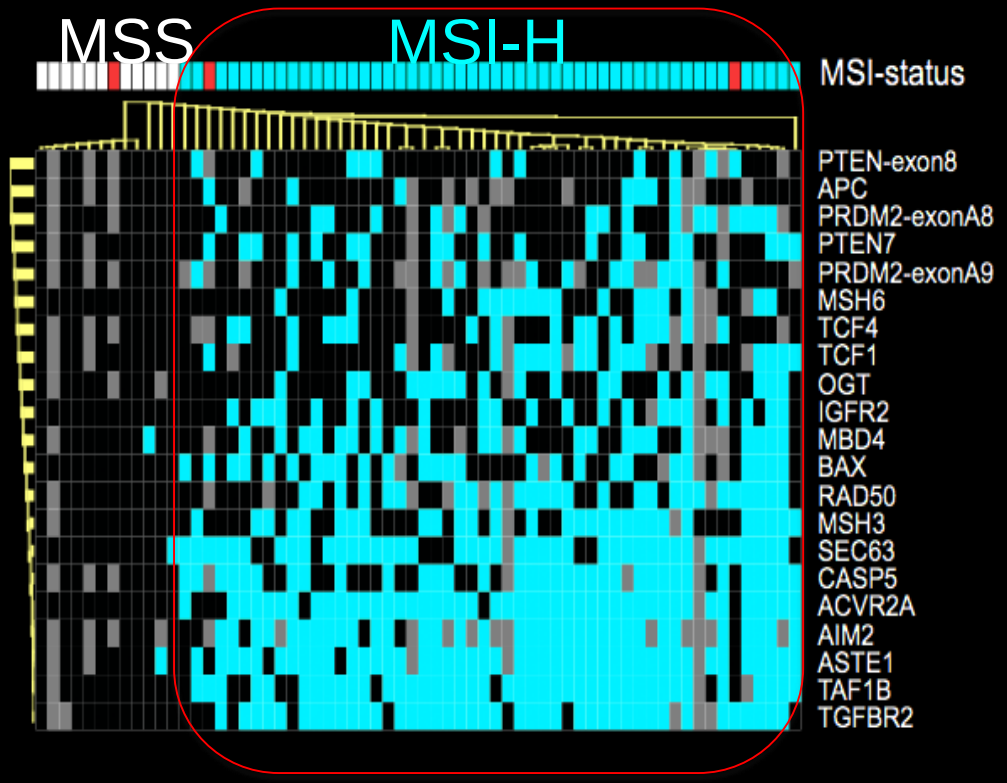
Patients with MSI-H have multiple Frameshift mutations (Fsmut)

● cohort 1
● cohort 2

ExomeSeq



Multiplex FSmut validation



Mlecnik et al. *Immunity* 2016

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

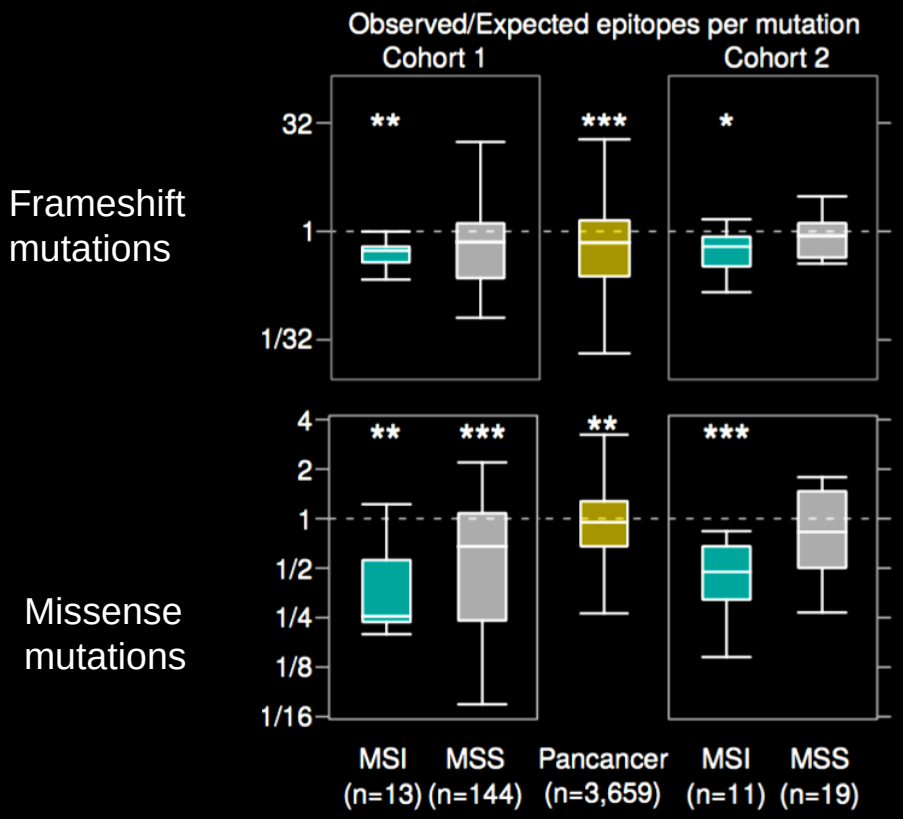
↕

Observed immunogenic mutations

Mlecnik et al. ***Immunity*** 2016

Patients with MSI-H have decreased observed compared to expected frameshift and missense epitopes

Genetic analysis of missense and frameshift immunogenic mutations (epitopes) compared to nonsense (silent) mutations



Silent mutations

↕

PanCancer rate (n=3659 Pts.)

↕

Non-immunogenic mutations

↕

Expected immunogenic mutations

↕

Observed immunogenic mutations

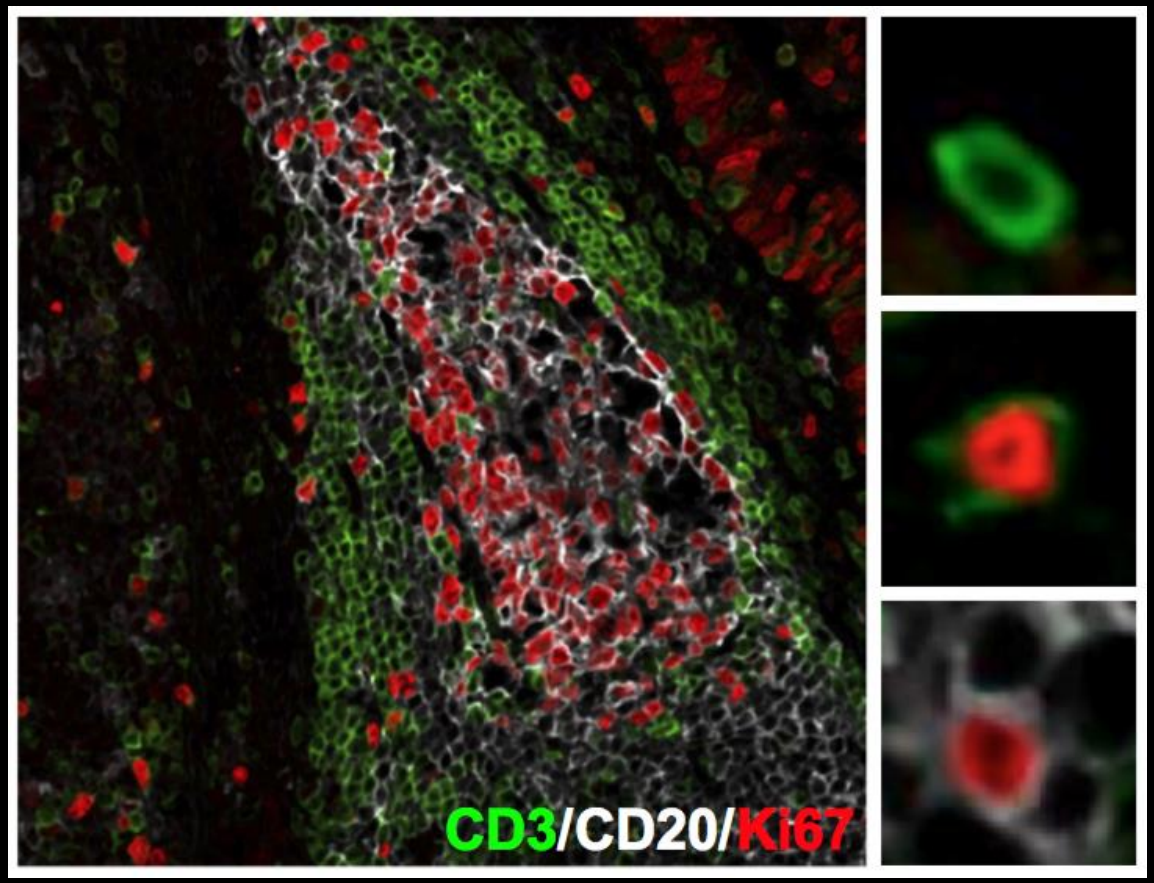
Genetic evidence of immunoediting

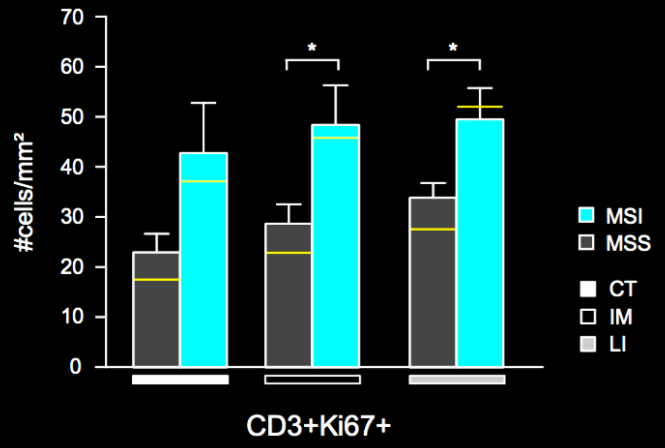
- Decreased in frameshift immunogenic mutations in MSI patients compared to silent mutations
- Less than expected number of missense immunogenic mutations in CRC patients, and particularly MSI patients compared to silent mutations

Mlecnik et al. ***Immunity*** 2016

Patients with MSI-H have increased intratumoral T-cell proliferation

Triple immunofluorescence quantification *in situ*





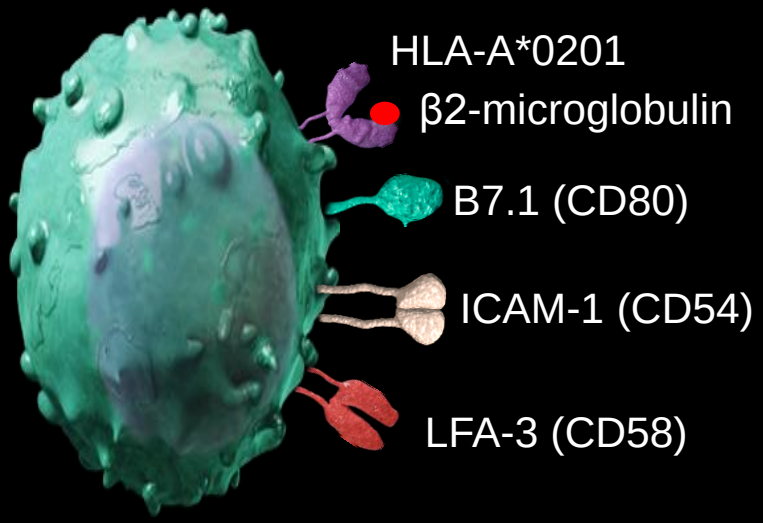
Increased CD3+Ki67+ cells in the center, invasive margin and tertiary lymphoid structures in patinets with MSI-H

Mlecnik et al. *Immunity* 2016

Functional T-cell assays

AAPC : Artificial APC

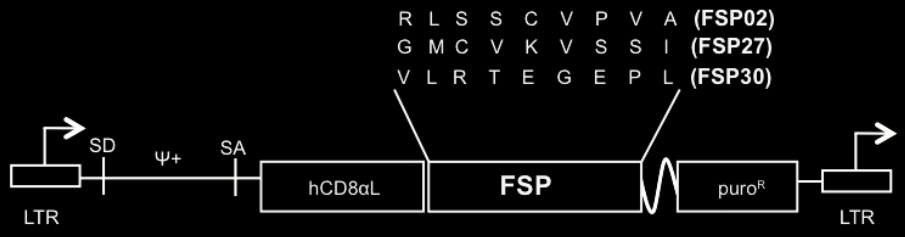
retroviral vectors encoding



+ T-cells from patients

T-cell stimulation

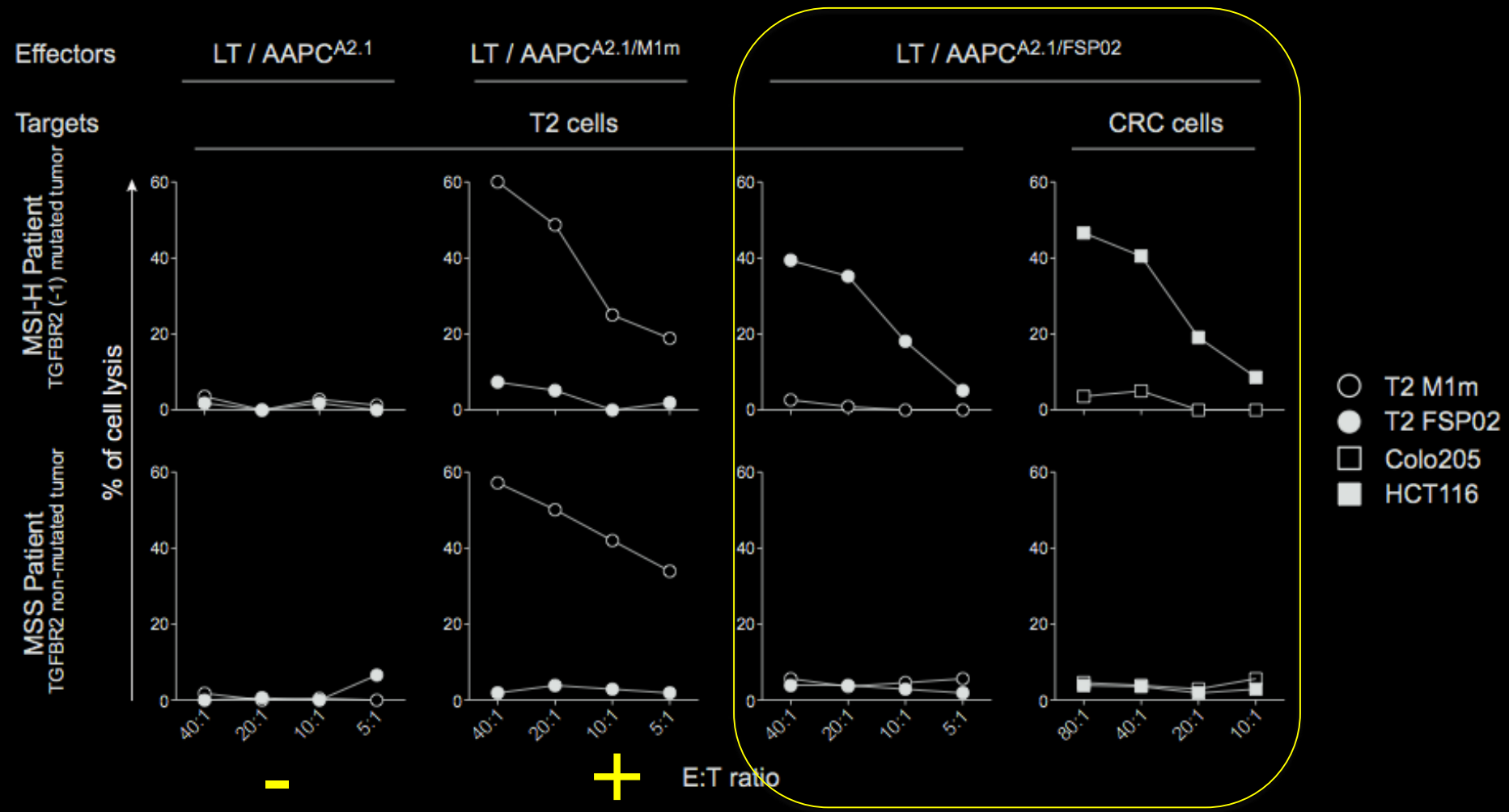
Transduced with a dicistronic vector encoding a puromycin resistance element and one frameshift peptide



Mutated TGFBR2 peptide

Cytotoxic assay: Target cancer cells labeled with ^{51}Cr

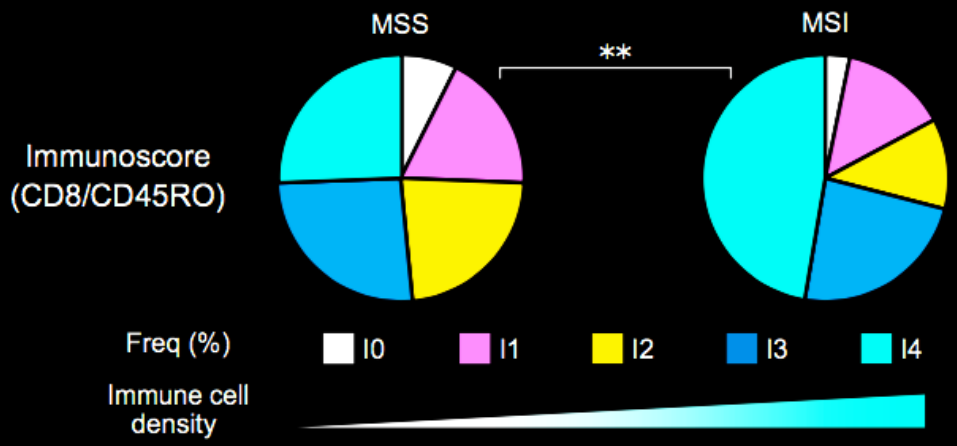
MSI-H patients with TGFBR2 FSmut have anti-TGFBR2-FSmut T-cells able to kill APC$^{A2.1/FSmutP2}$ cells



Mlecnik et al. *Immunity* 2016

Patients with MSI-H have increased frequency of High-Immunoscore

Immunoscore quantifying the densities (cells/mm^2) of CD8 and CD45RO T-cells in center and invasive margin of tumors (similar results with CD3/CD8)



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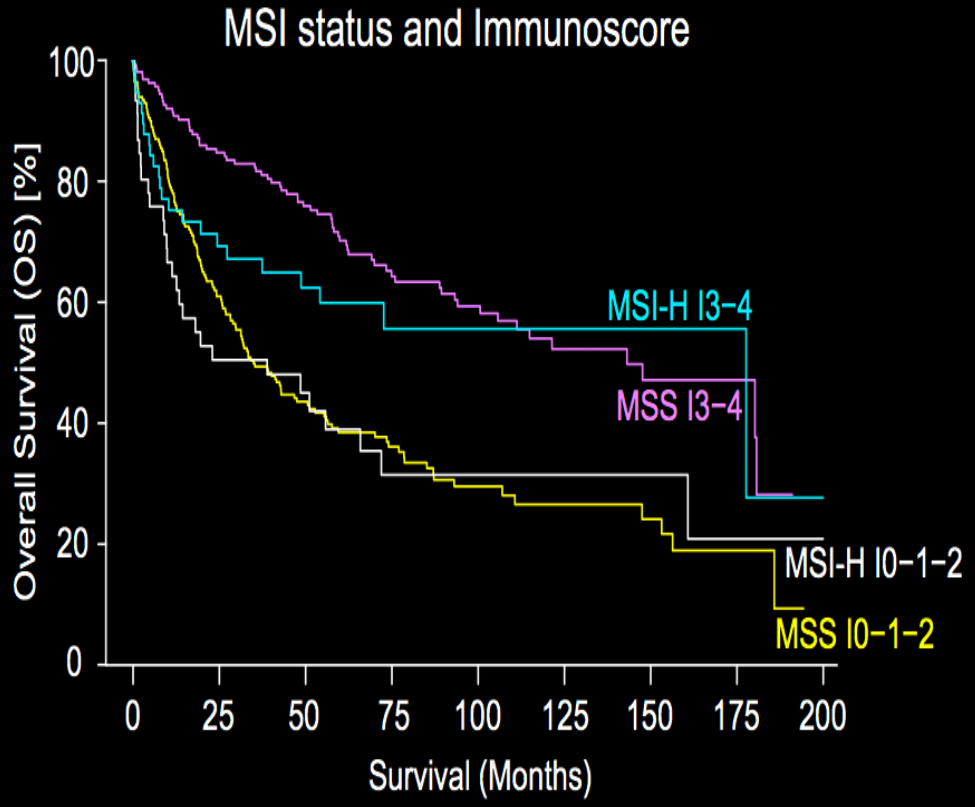
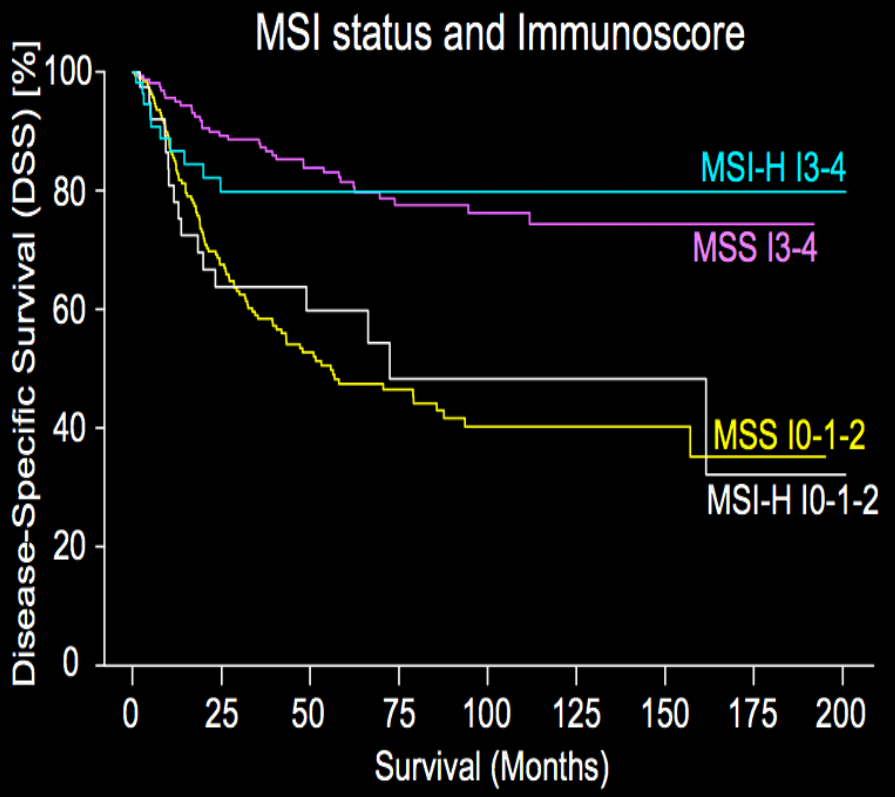
Immunoscore high (I3, I4) patients have prolonged survival regardless of the MSI status





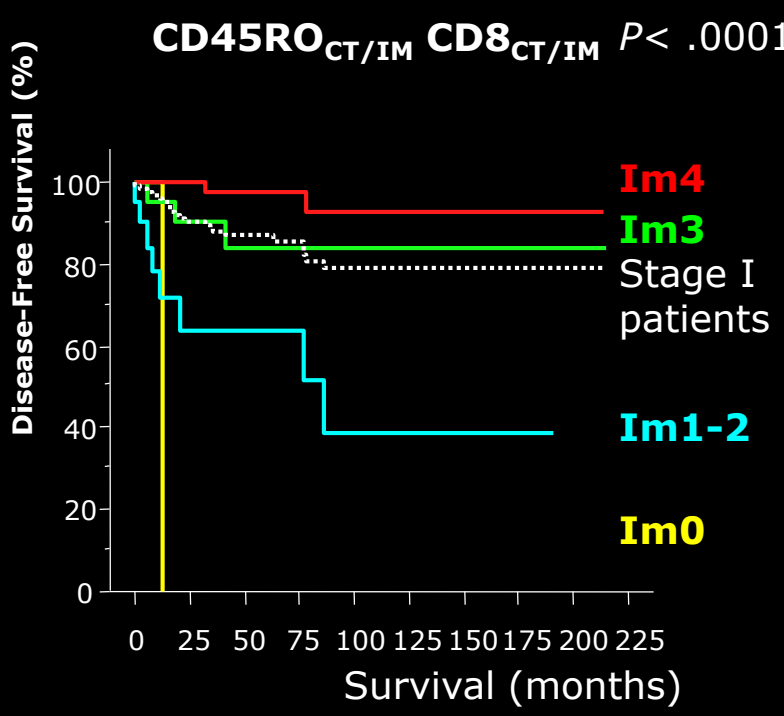
Cox multivariate analysis for DSS

markers	HR	P-value
MSI	1.00	0.99
N stage	1.32	0.27
VELIPI	0.56	0.024 *
Immunoscore	0.44	0.001 *

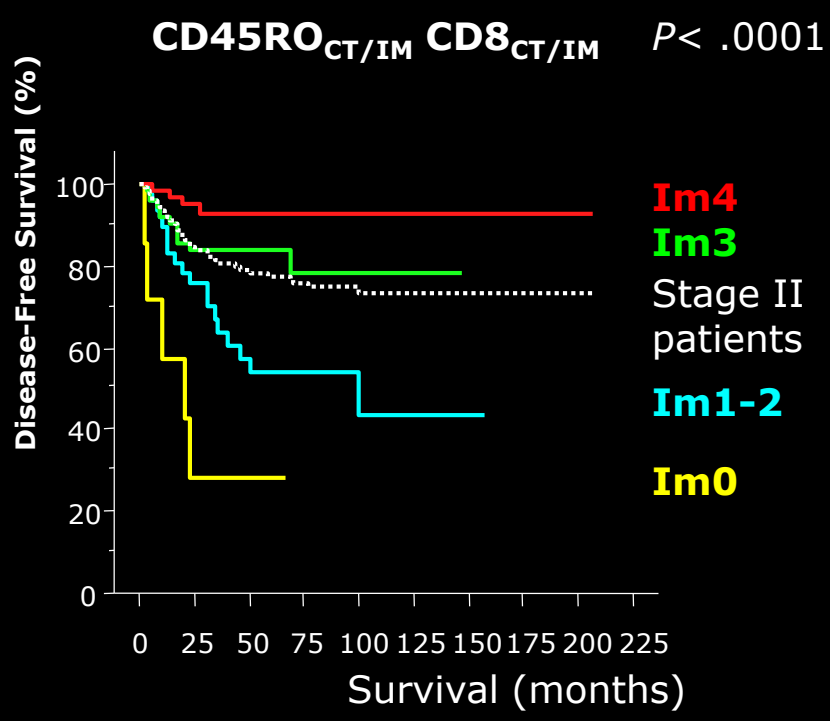
Mlecnik et al. *Immunity* 2016

Prognostic importance of the *in situ* immune reaction in patients with early-stage (Stage I/II) colorectal cancer

Stage I cancer



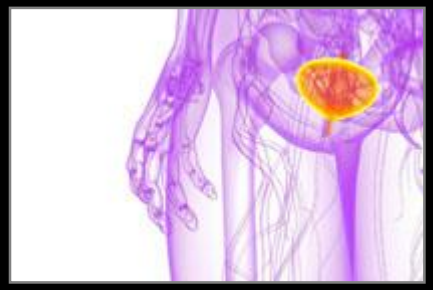
Stage II cancer



Pagès F et al. ***J. Clin. Oncol.*** 2009

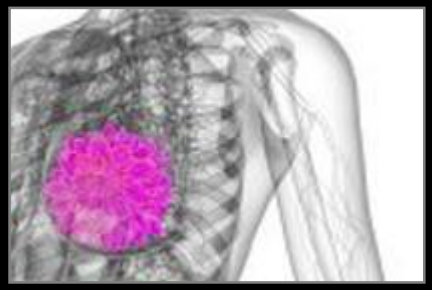
Impacting all cancers

Bladder cancer



Brain cancer

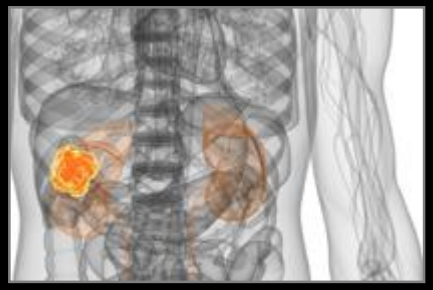
Breast cancer



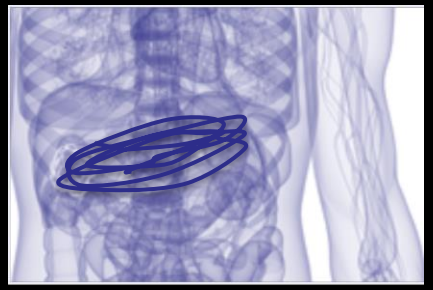
Cervical cancer

Colorectal cancer

Kidney cancer

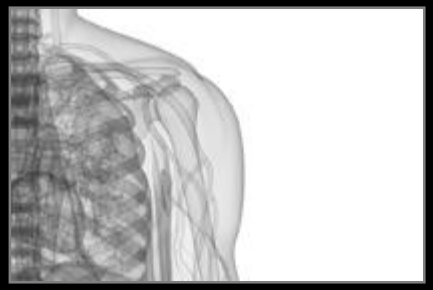


Liver cancer

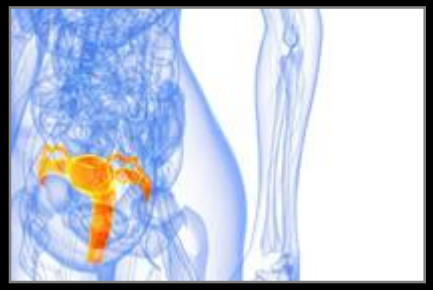


Lung cancer

Melanoma cancer

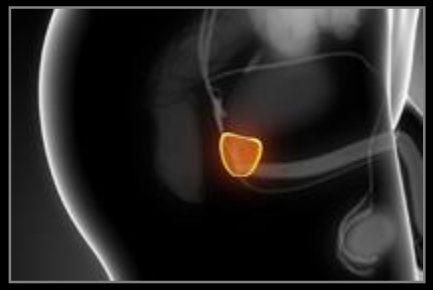


Ovarian cancer



Pancreatic cancer

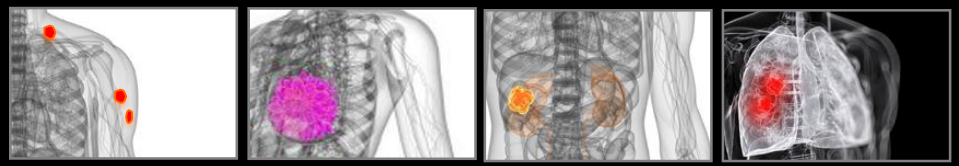
Prostate cancer

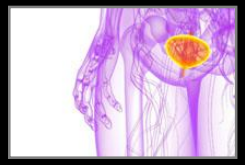


Metastasis analysis

Multiple primary tumors

Melanoma | Breast cancer | Kidney cancer | Lung cancer





Other cancers

One metastatic site

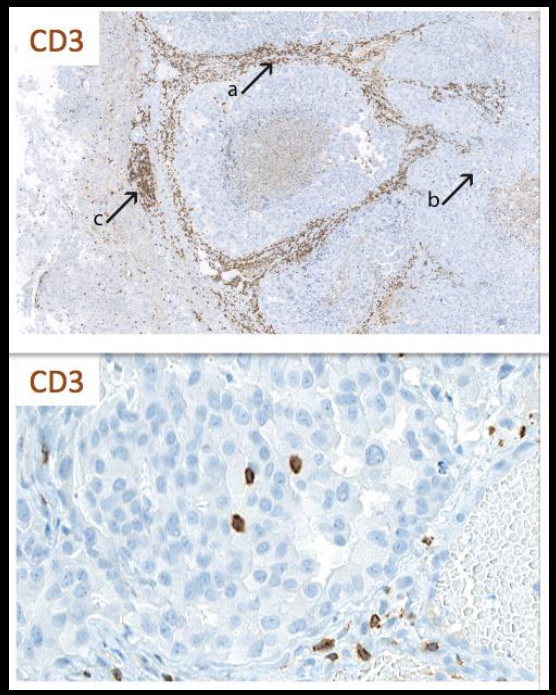
Brain Metastasis

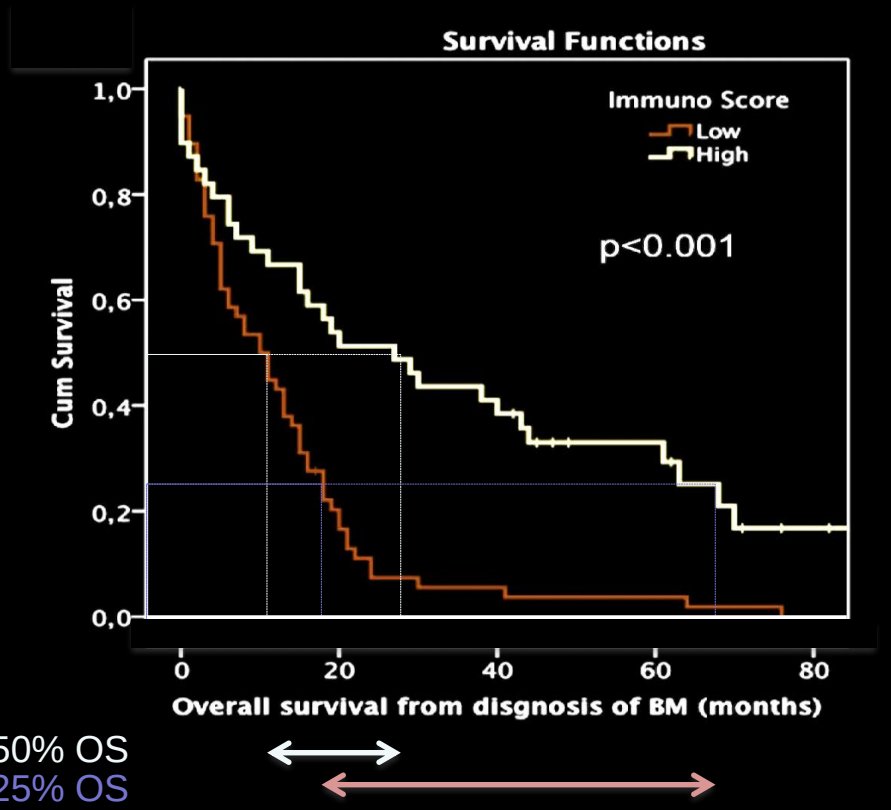
Berghoff A. *et al*. ***OncoImmunol.*** 2016

- Immunoscore within brain metastasis

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

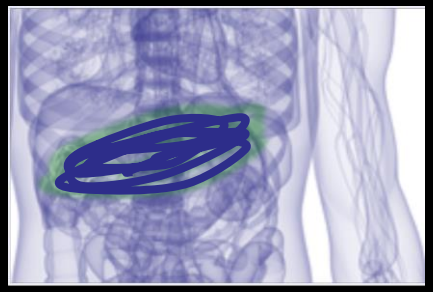
Metastasis analysis

One primary tumor

Colorectal cancer

Multiple metastatic sites

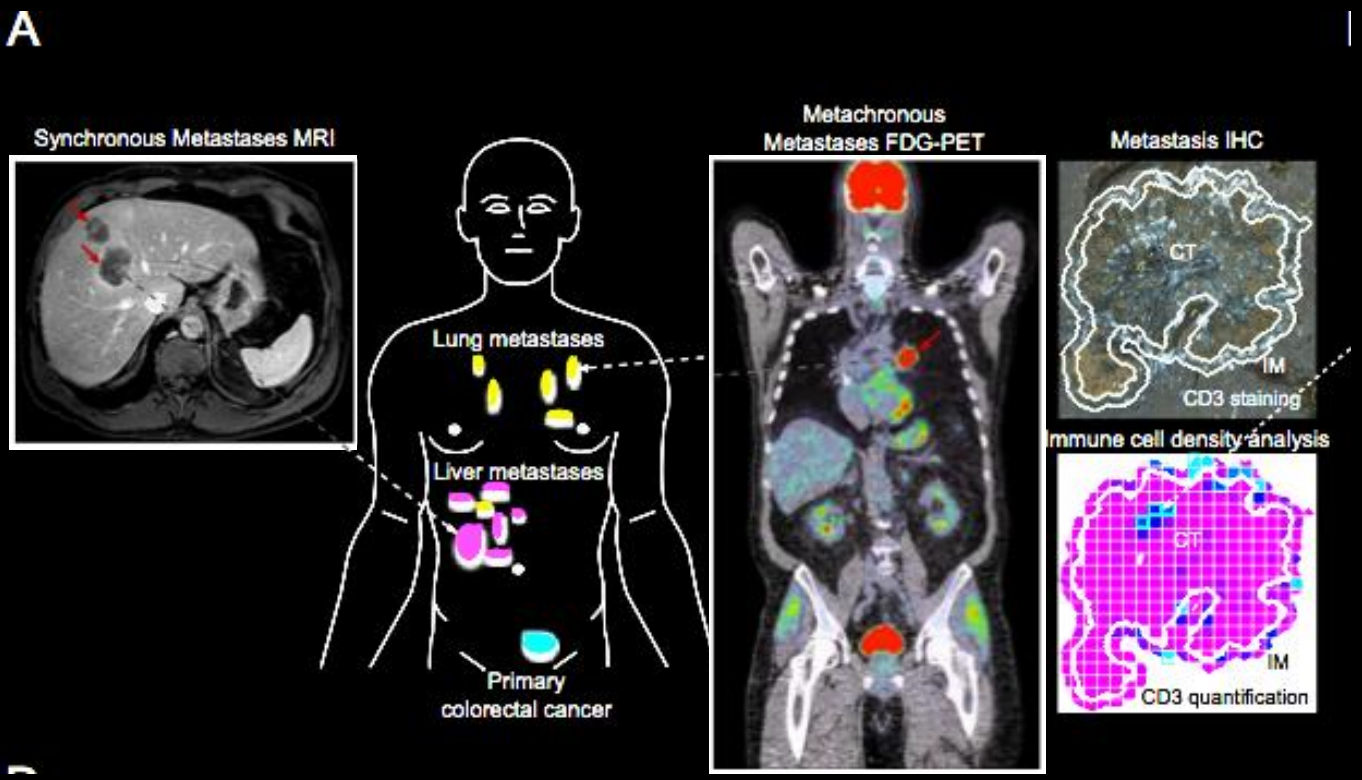
Liver Metastasis



Lung Metastasis

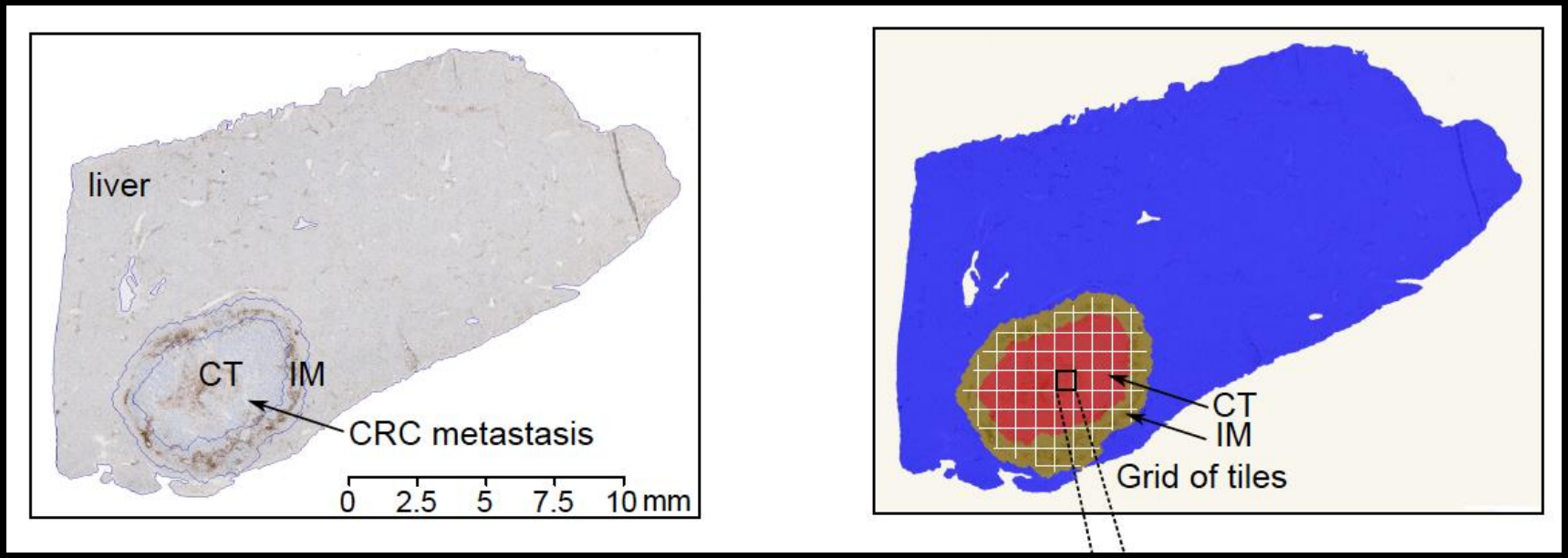
- Immunoscore within multiple metastases at different sites

Full-slide quantification of all metastases from CRC patients

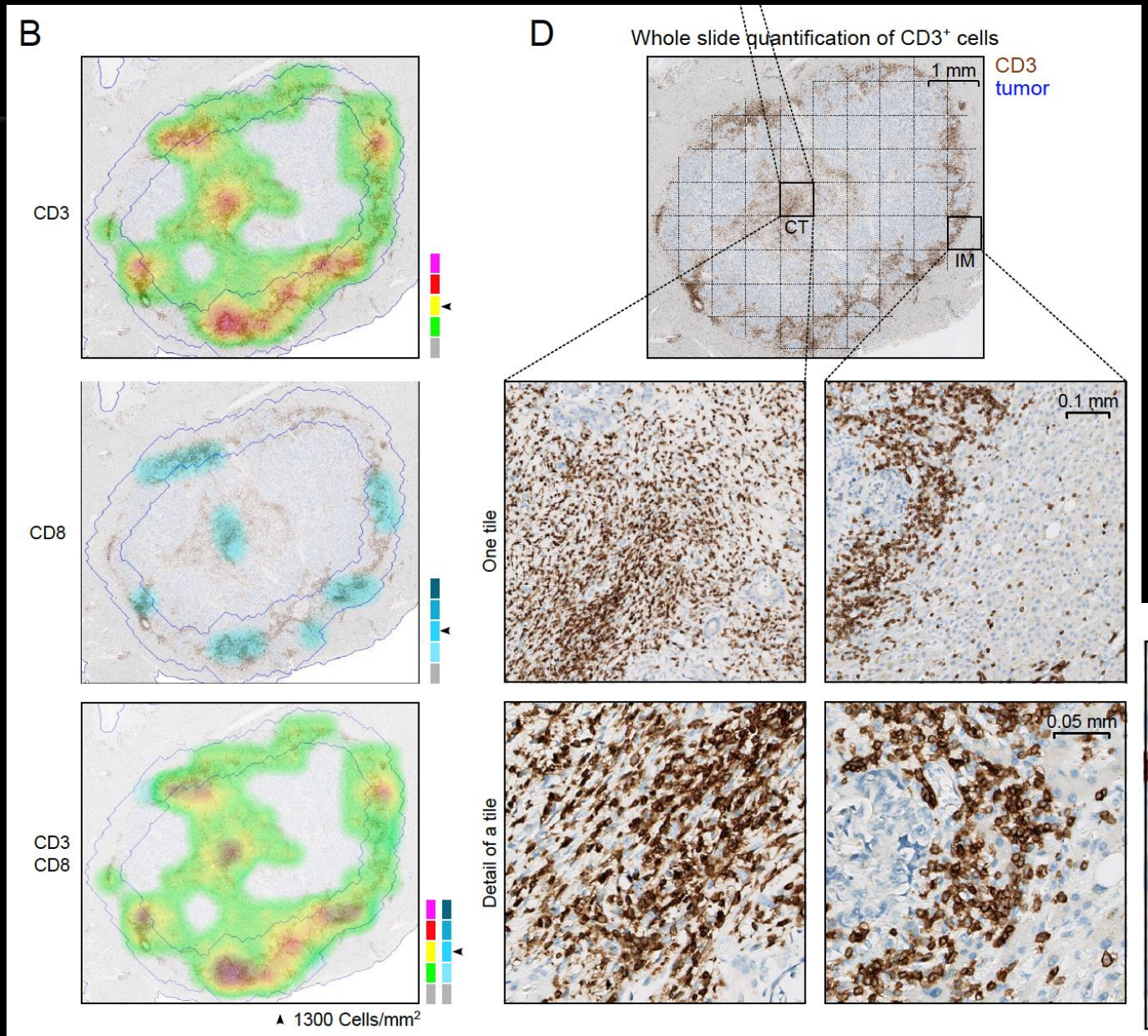


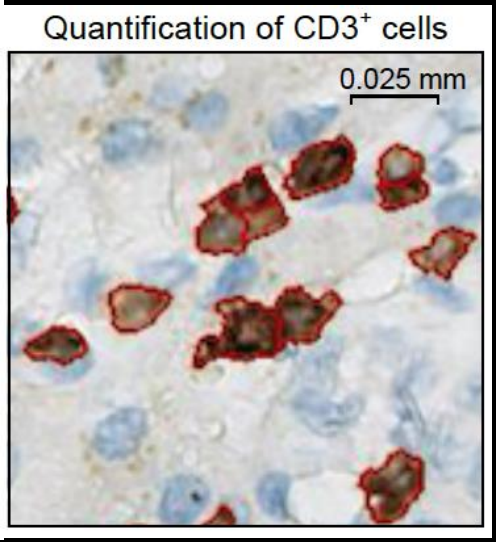
- ✓ All synchronous and metachronous metastases (liver or lung) resected
- ✓ 1 to 23 metastases resected per patient
- ✓ Full-slide quantification of immune cells infiltrating metastases and primary tumors
- ✓ Mutation analysis of 50 main cancer-driver genes

Immunoscore within metastases

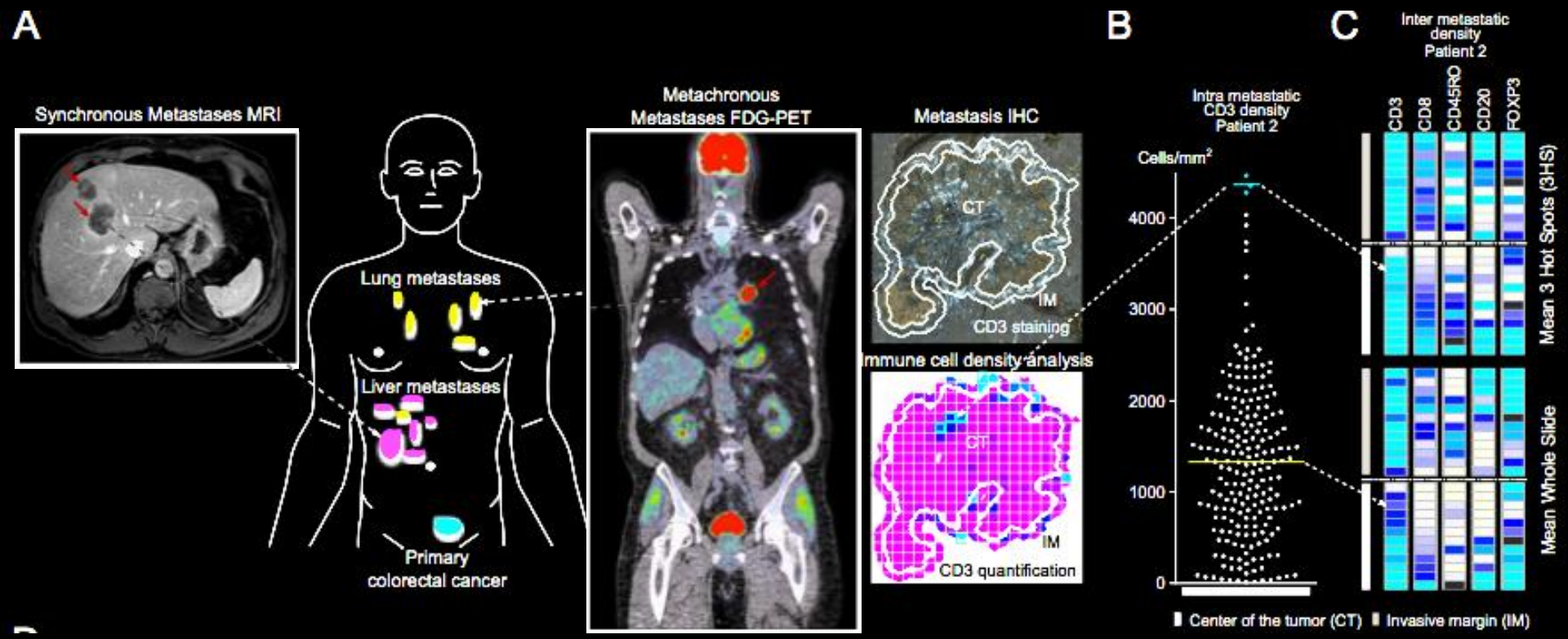


Immunoscore within metastases





Full-slide quantification of all metastases from CRC patients



- ✓ Up to 700 tiles quantified per metastasis
- ✓ Full-slide quantification of immune cells infiltrating CT and IM regions
- ✓ Quantification of CD3, CD8, CD54RO, CD20, FoxP3 (cells / mm^2)

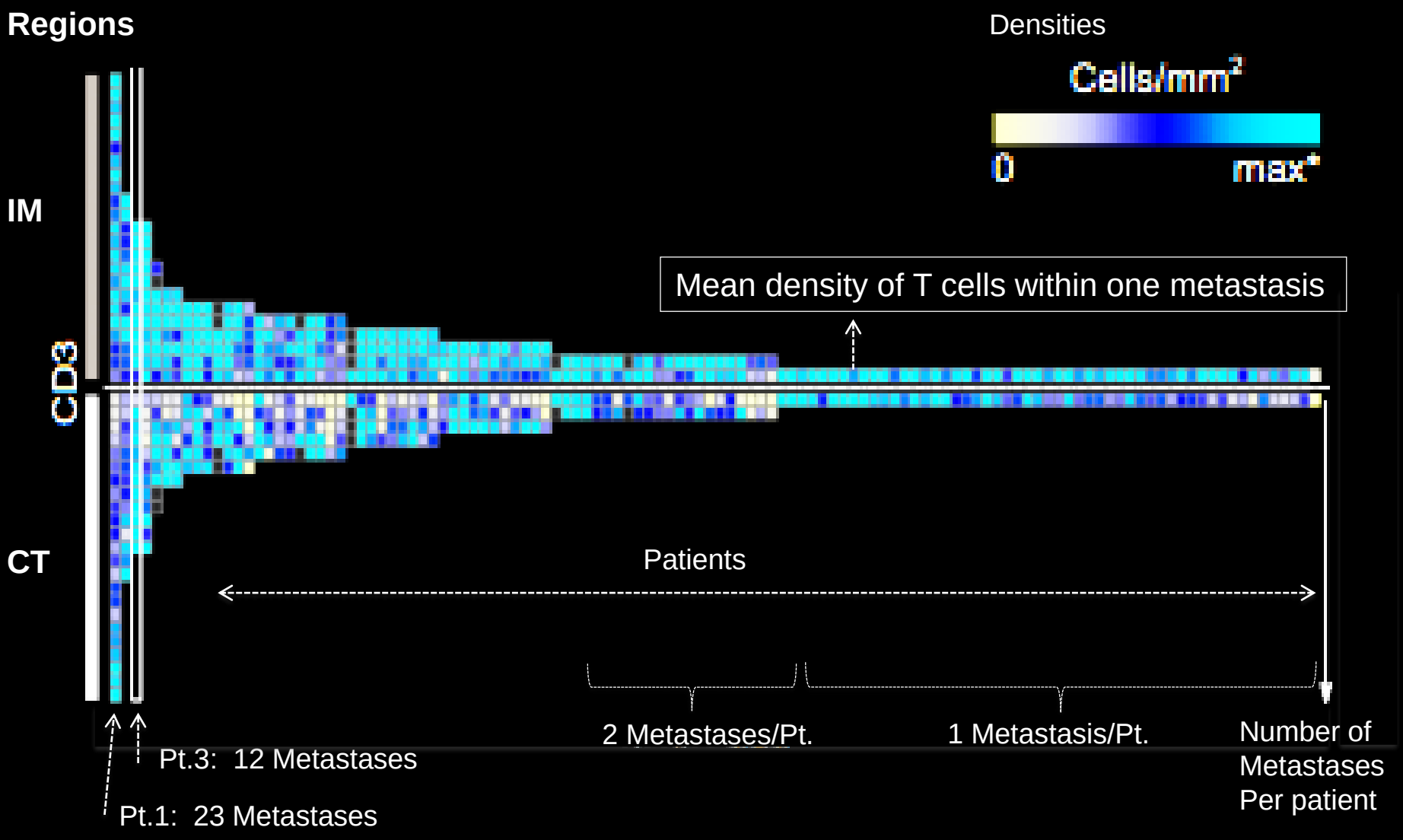
Bernhard Mlecnik et al. submitted

Metastases and primary tumors heterogeneity

- ✓ Cohort of fully resected metastases (synchronous and metachronous)
- ✓ Global densities of lymphocyte subsets were analyzed in the CT and IM of 411 metastases and 69 primary tumors.
- ✓ A total of 37.7 million CD3 T cells were counted on the 411 metastases, with a mean of and 115.000 and 322 million T cells per tumor slide, and metastases respectively.
- ✓ Analysis of Intra-metastasis, inter-metastases, and inter-patients heterogeneity

Bernhard Mlecnik et al. submitted

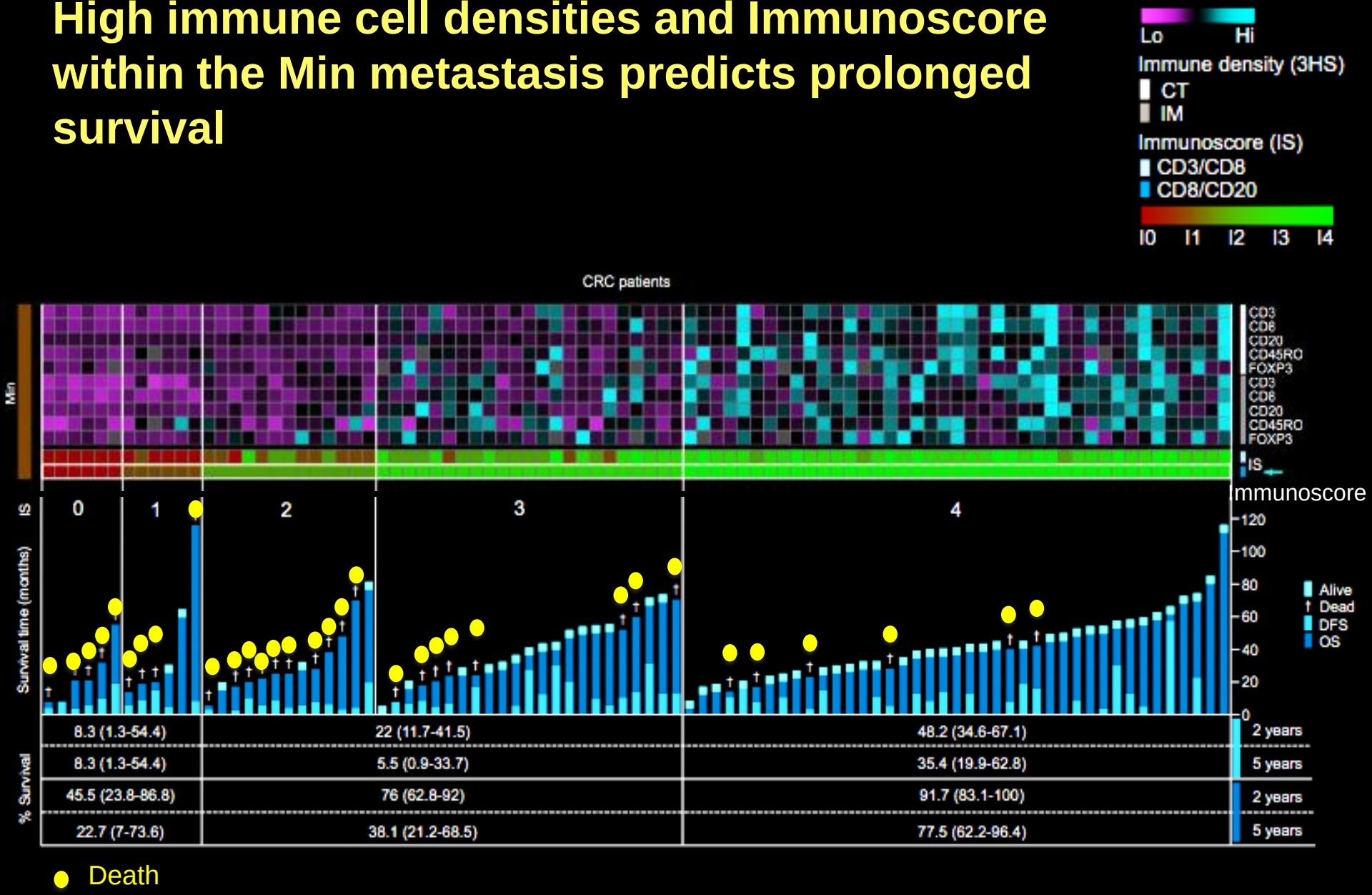
Densities of CD3+ T-cells in CT and IM regions of all metastases from all patients



- Heterogeneity of T-cells across all metastases

Bernhard Mlecnik et al. submitted

High immune cell densities and Immunoscore within the Min metastasis predicts prolonged survival



● Death

Bernhard Mlecnik et al. submitted

Implications for cancer classification and therapies

From the **Immune contexture**

(Complexity of intratumor immune reaction)

To the **Immunoscore**

(A simple and powerfull Immune Test)

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 IN THE CLASSIFICATION OF CANCER

Naples, Italy, Feb 2012
Organizer: **P Ascierto,** J. **Galon,**
Principal investigator: J. **Galon**

Galon J et al. ***J. Transl. Med.*** 2012
Galon J 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)

(23 Centers, 17 countries: >3000 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
- April 2016, USA

Worldwide Immunoscore consortium (PI: J Galon)

Referent Center

Steering Committee

Meetings Organizers

PathForce Image Server

Control slides Center

Clinical data Center

External Statistician

Management Communication

Training Set (TS)

- France
- Germany
- Switzerland
- Sweden
- Czech R
- Japan
- USA
- Canada

Internal Validation Set (IVS)

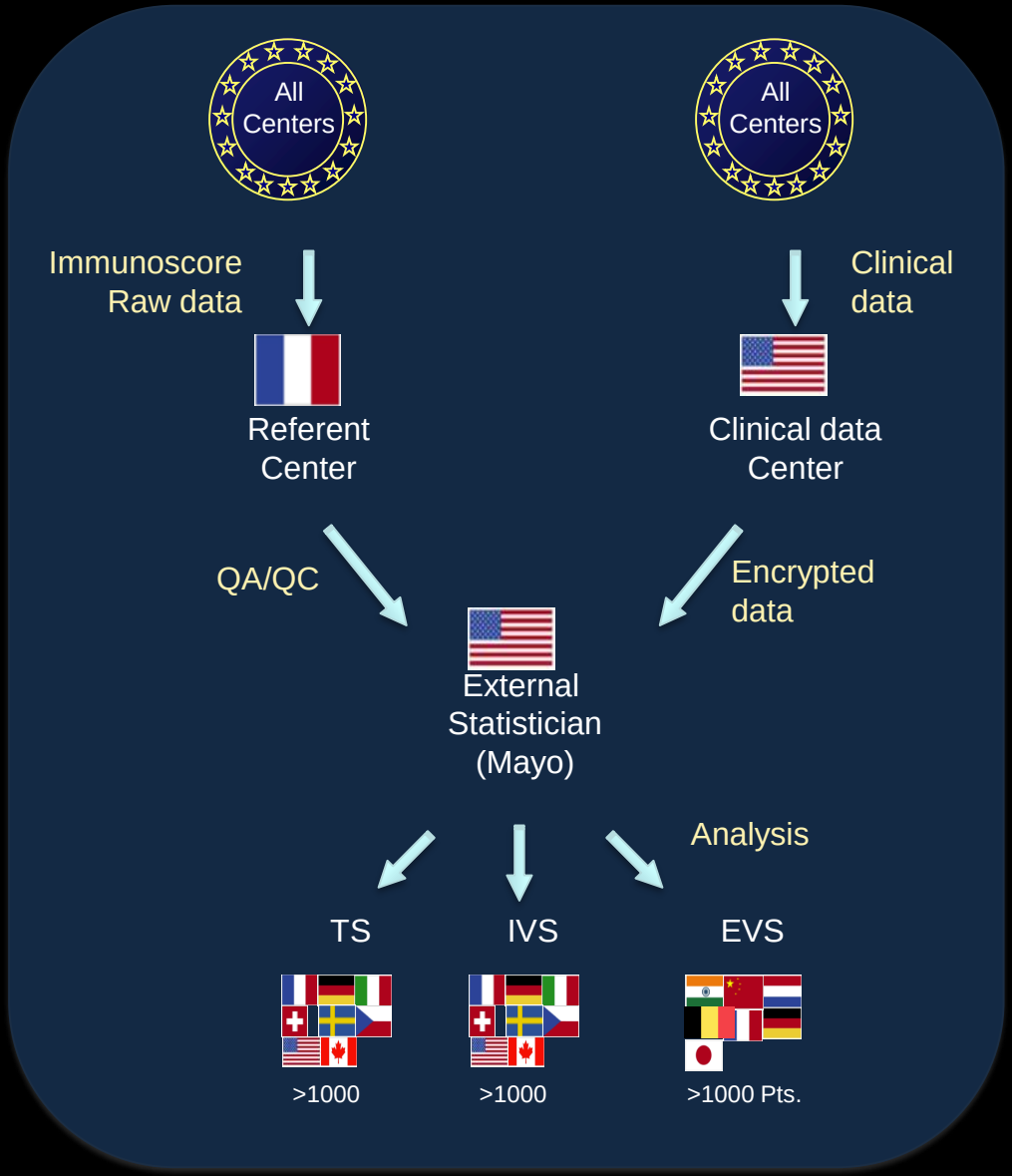
- France
- Germany
- Switzerland
- Sweden
- Czech R
- Japan
- USA
- Canada

External Validation Set (EVS)

- India
- China
- Netherland
- Belgium
- France
- Germany
- Italy
- Switzerland

Worldwide Immunoscore consortium (PI: J Galon)

Study design



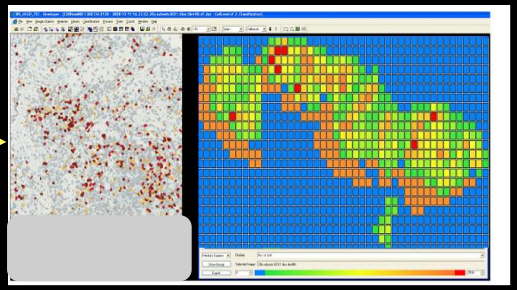
IMMUNOSCORE : METHODS

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

IHC automate

High-resolution scanner

whole slide quantification



Digital pathology

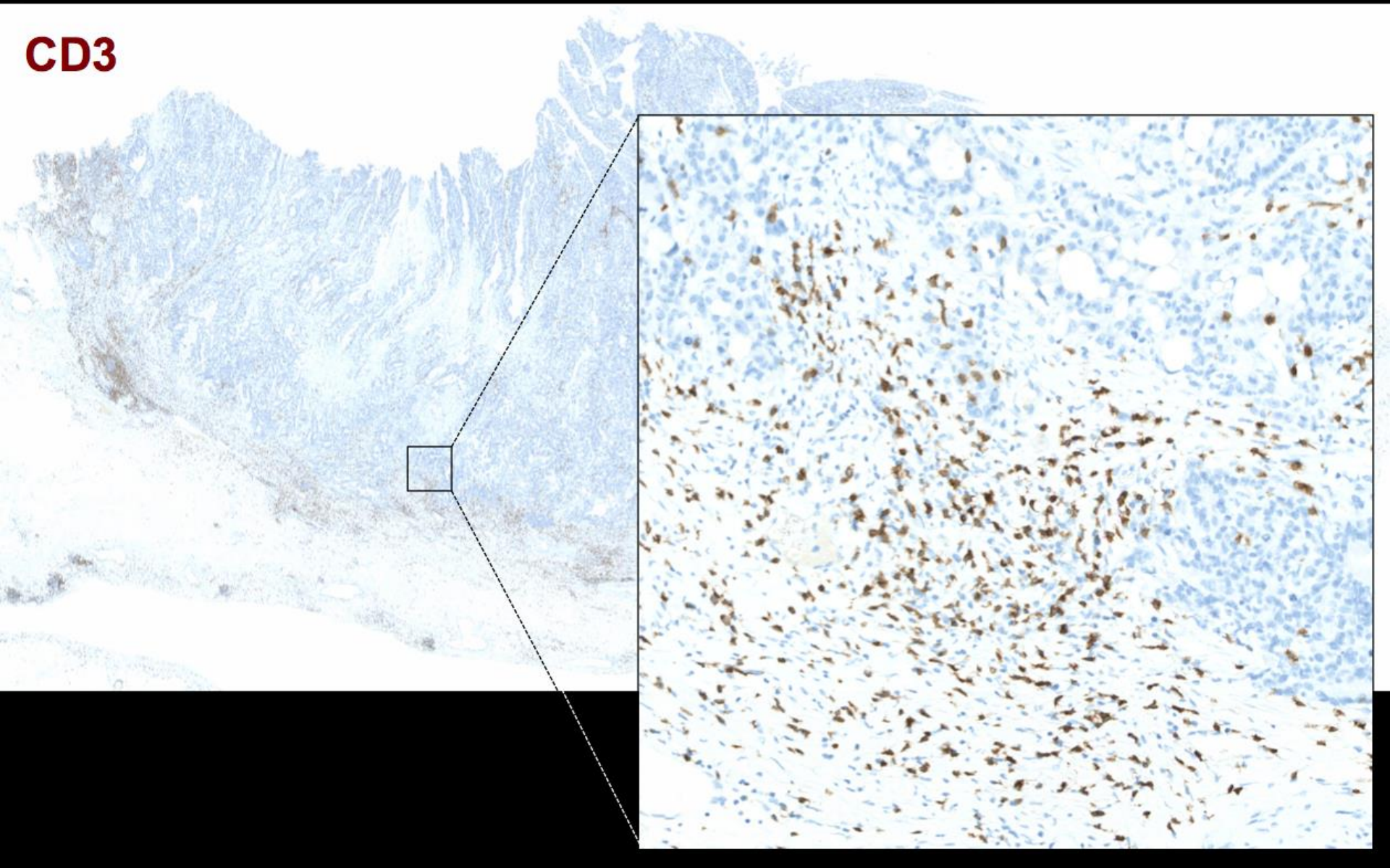
-> Conceptual and technological challenge

Immunoscore

Galon J et al. ***J. Transl. Med.*** 2012
Galon J et al. ***J. Pathol.*** 2014

Immunoscore using whole slide FFPE

Routine whole slide stainings & full image quantification

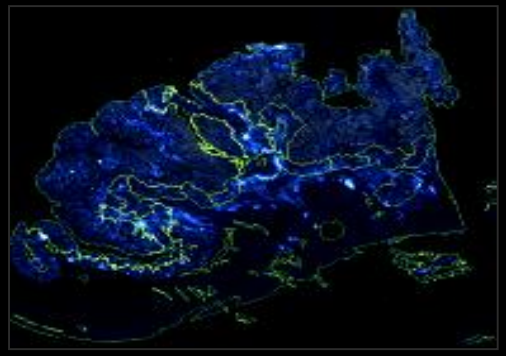


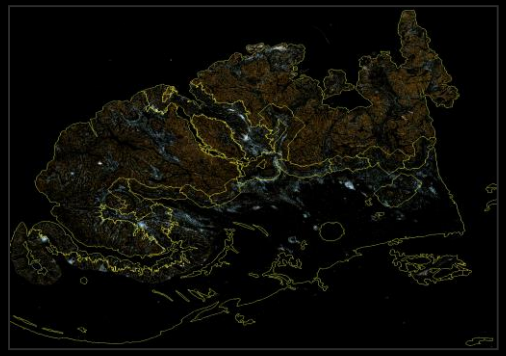
Immunoscore (I) using whole slide FFPE

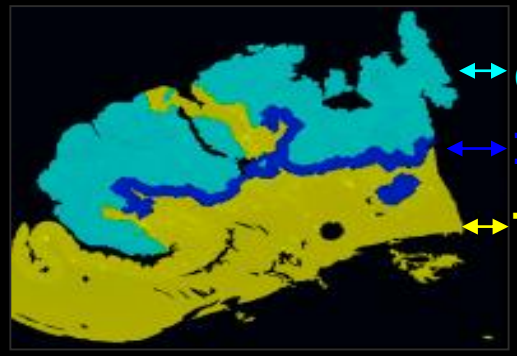
Routine whole slide stainings & precise image quantification

Immunostaining

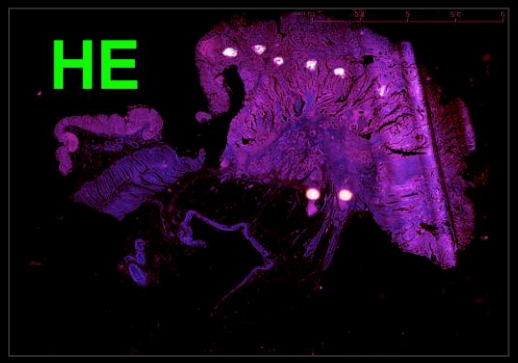
Definition of Tumor Regions

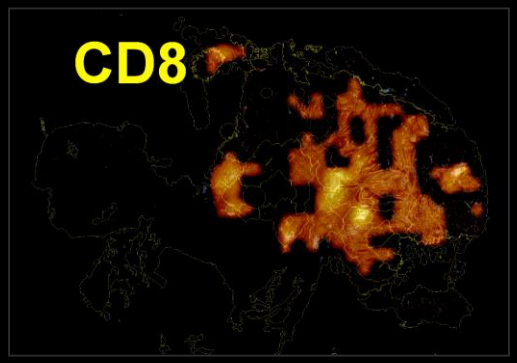


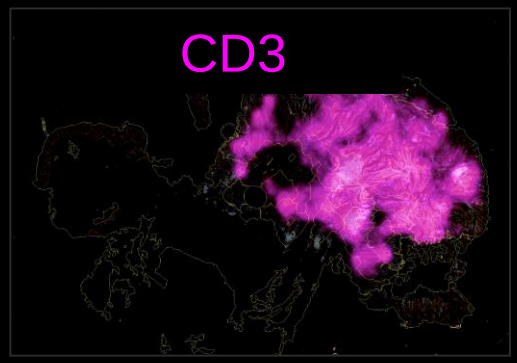




Density plots







→ I

✓ Immunoscore is **Standardized, Objective, Quantitative**

Immunoscore worldwide consortium study

Methods:

- ✓ Statistical analysis plan was pre-defined
- ✓ All statistical analyses performed by external statisticians
- ✓ Primary study endpoint was time-to-recurrence (TTR) for Immunoscore (High/Low)
- ✓ Analyses were performed by Cox models stratified by enrolling center

The main objectives were to demonstrate feasibility, reproducibility, significance and **robustness** of Immunoscore in a Worldwide study

Galon J et al. ***ASCO*** 2016

Patient population and clinical characteristics

Inclusion criteria:

- ✓ Colon cancer
- ✓ Stages I/II/III (T1-T4, N0-N2, M0)
- ✓ No neo-adjuvant treatments
- ✓ Clinical data and follow-up

*** All Multivariate models**

Adjusted for :

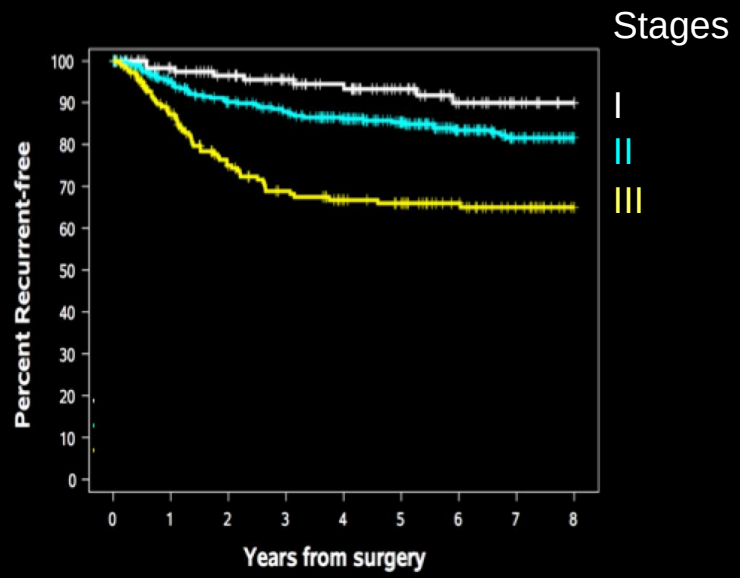
Immunoscore, age, gender, T-Stage, N-Stage, MSI

Stratified by : City Center

3855 patients were quantified for Immunoscore

Exclusion criteria:

- ✓ Rectum cancer
- ✓ Stages IV (M1)
- ✓ Neo-adjuvant treatments
- ✓ Missing Clinical data
- ✓ Missing follow-up
- ✓ Staining intensity <152
- ✓ Missing/incomplete biomarker data

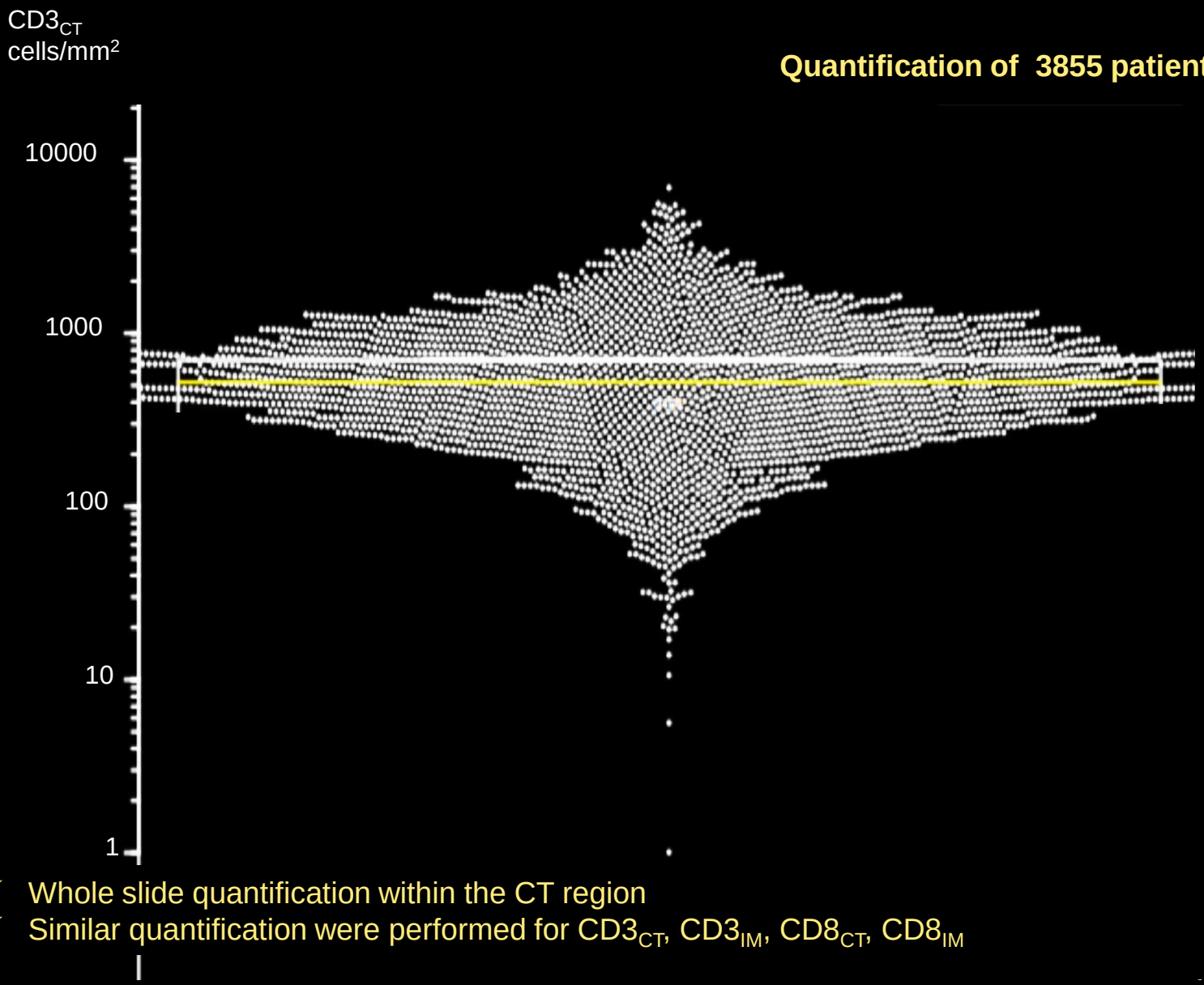


2667 patients were analyzed after QC and exclusion following a pre-defined Statistical analysis workplan

Immunoscore in Colon Cancer
Statistical Analysis Plan — Society for Immunotherapy of Cancer/Mayo Clinic

Galon J et al.
ASCO 2016

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



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

Biomarker characteristics : Results

- ✓ More than 352,000,000 CD3+ T cells were counted by all Centers

	Number of CD3+ T cells / slide	Whole slide density of CD3+ (cells / mm^2)	Whole slide density of CD8+ (cells / mm^2)
Center (CT)	64,537 ± 80,962	685 ± 1297	239 ± 534
Margin (IM)	23,643 ± 23,524	1174 ± 1985	436 ± 832
Total	88,180		

Distribution of Immunoscore across all Centers

- ✓ High Immunoscore: 26%
- ✓ Int. Immunoscore: 49%
- ✓ Low Immunoscore: 25%

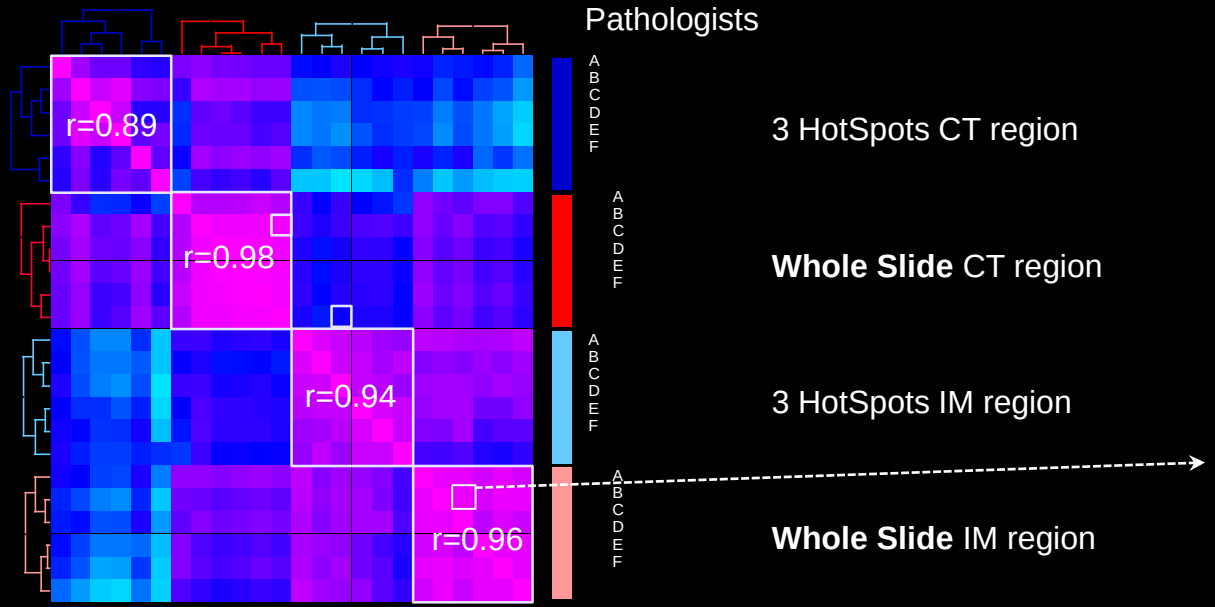
Galon J et al. ***ASCO*** 2016

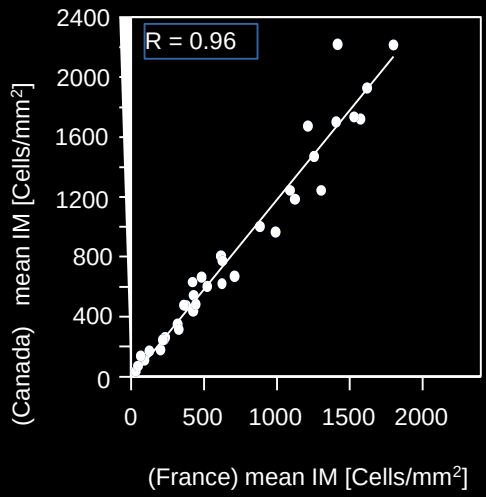
High reproducibility of Immunoscore

Correlation

Pearson's r 0.0 0.5 0.8 1.0

Correlation Matrix



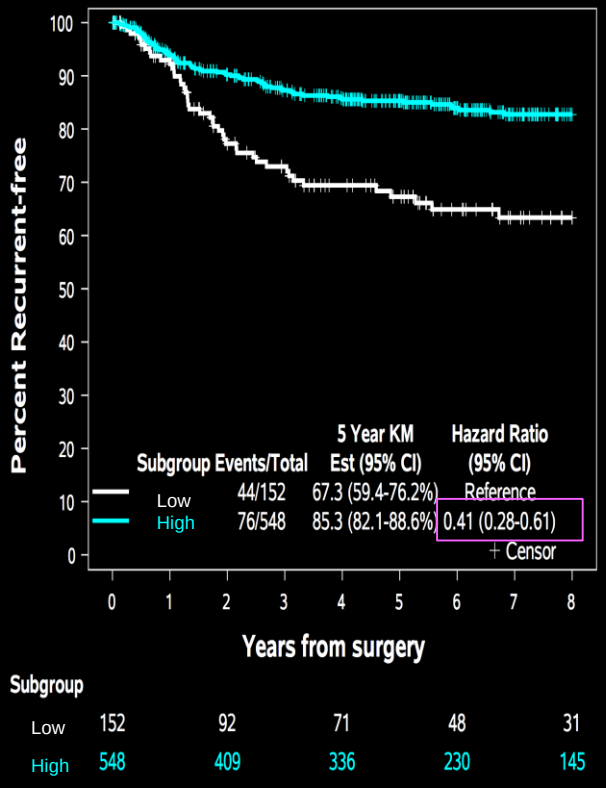


- ✓ Whole slide quantification shows the best correlation and reproducibility
- ✓ Immunoscore is **quantitative, reproducible and robust**

Galon J et al. ***ASCO*** 2016

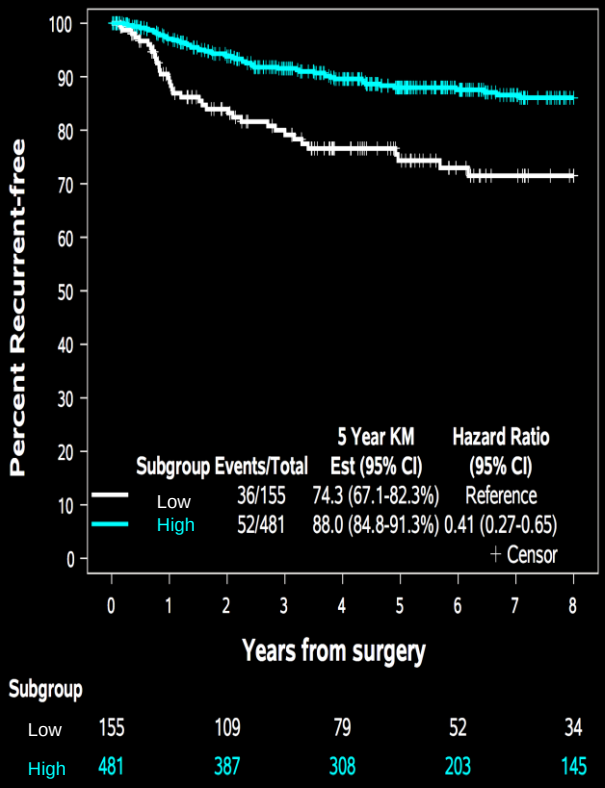
Primary Objective: Time to recurrence (TTR) for Immunoscore (High/Low)

TS



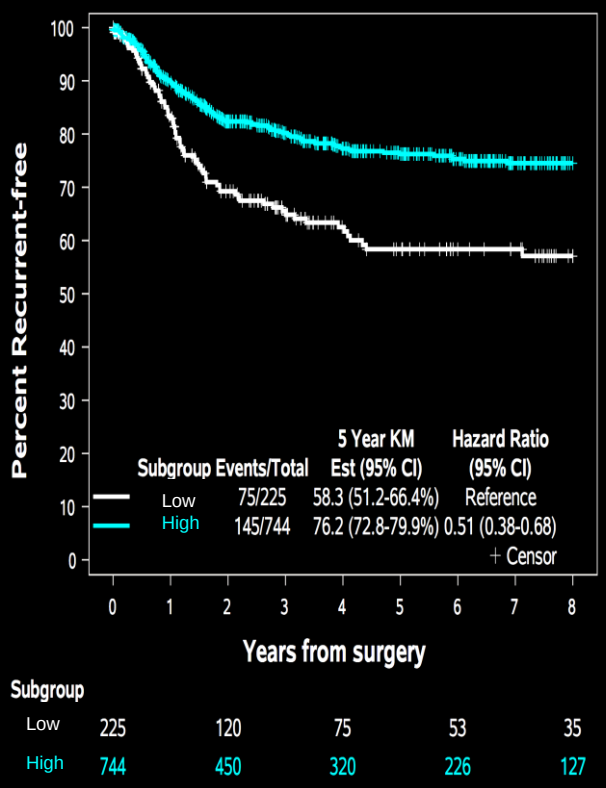
***P*< 0.0001**
HR = 0.41
C-index = 0.60

IVS



***P*< 0.0001**
HR = 0.41
C-index = 0.60

EVS



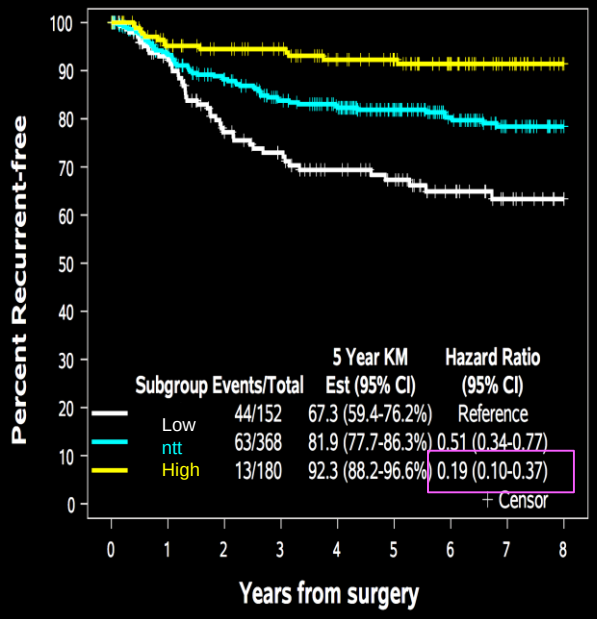
***P*< 0.0001**
HR = 0.51
C-index = 0.56

Primary objective is reached

Immunoscore predicted time to recurrence on Training Set (TS), and on 2 independent validation sets (IVS and EVS), blinded to clinical outcome.

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

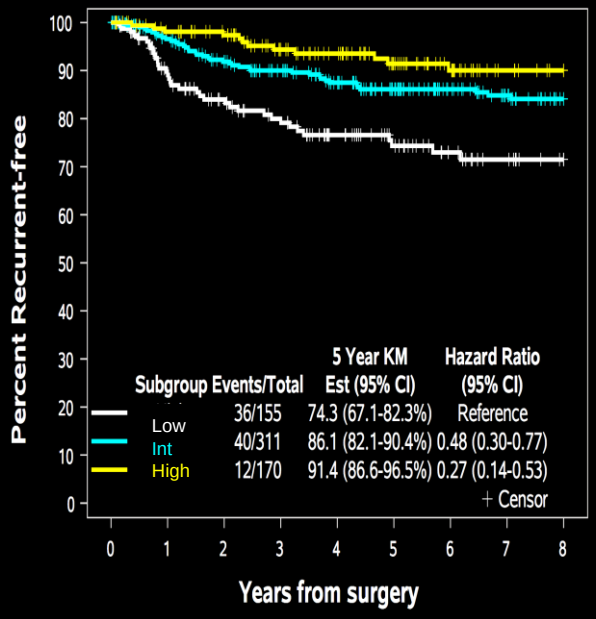
TS



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

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

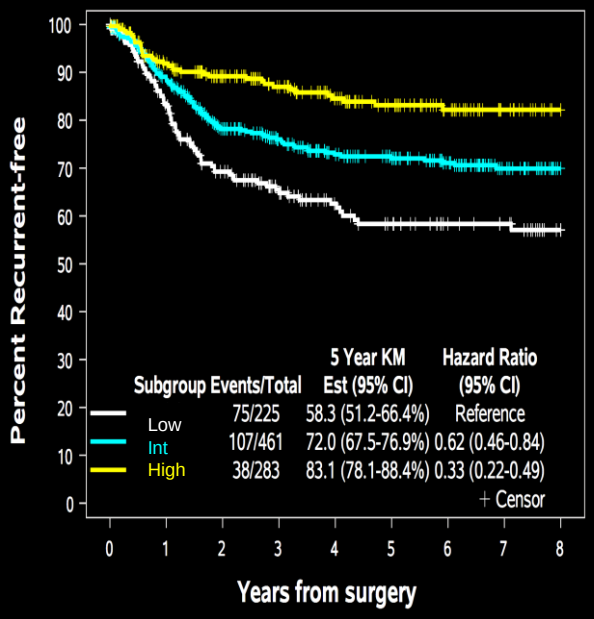
IVS



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

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

EVS



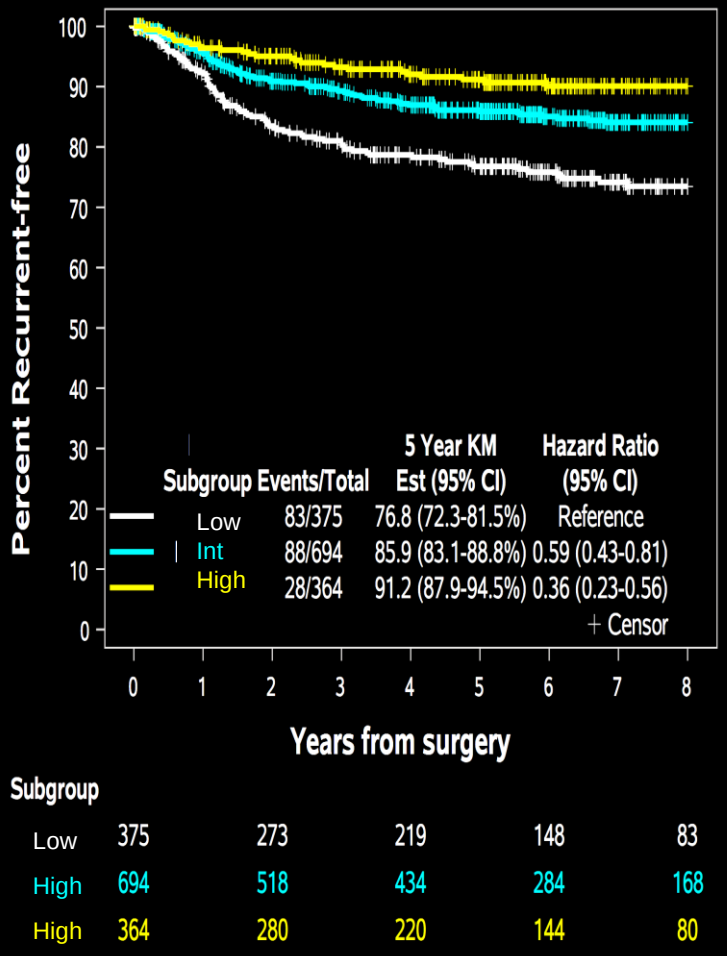
Subgroup					
Low	225	120	75	53	35
High	461	268	191	142	76
High	283	182	129	84	51

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

Secondary objective is reached

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

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



Stage II patients (n=1433)

***P*< 0.0001**

HR(0-2)= 0.36
C-index= 0.65 (0.54-0.75) *

Objective is reached
Immunoscore predicted time to recurrence in Stage II colon cancer

Multivariate anlayses for Immunoscore (2, 3, or 5 groups)

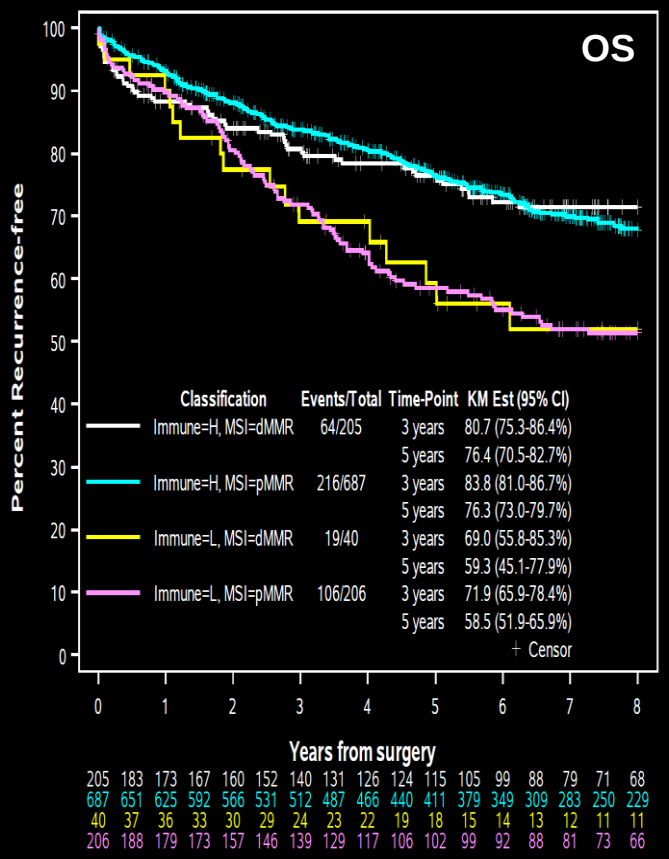
Multivariate Analysis for Time to Recurrence (TTR)

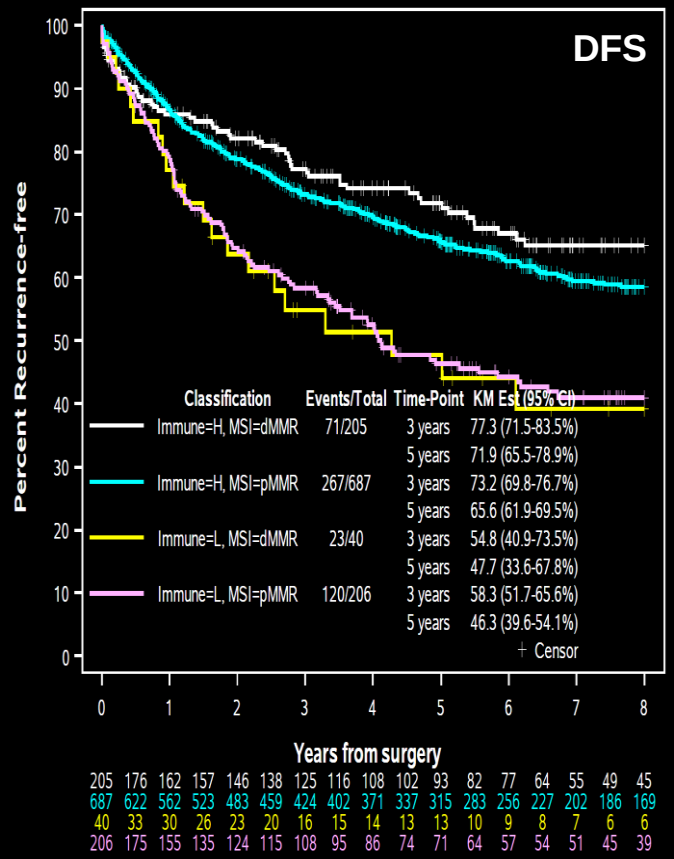
All patients

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)

- ✓ Immunoscore (2, 3, or 5 groups) is significant in multivariate analyses in TTR
- ✓ Similar results are found for DFS and OS

Secondary Objective: Immunoscore & MSI





Immunoscore High & MSI
Immunoscore High & MSS

Immunoscore Low & MSI
Immunoscore Low & MSS

Multivariate Model: Immunoscore
***P*< 0.0001**
HR (95% CI)= 0.62 (0.50-0.77) *

Multivariate Model: Immunoscore
***P*< 0.0001**
HR (95% CI)= 0.57 (0.47-0.70) *

N = 1326 patients

Secondary objective is reached

✓ Immunoscore is significant in multivariate analyses in OS, DFS, TTR (including MSI, T-stage, N-stage, Age, Gender)

Conclusions:

✓The primary endpoint of the Worldwide pre-specified Immunoscore study was reached
✓Time to recurrence (TTR) was significantly longer in patient's stages I/II/III with High-Immunoscore
✓Low-Immunoscore identified a subgroup of patients with high-risk stage II colon cancer
✓Immunoscore is significant in multivariate analysis in all cohorts, TS, IVS and EVS
✓Immunoscore predicts TTR, DFS and OS

Perspective:

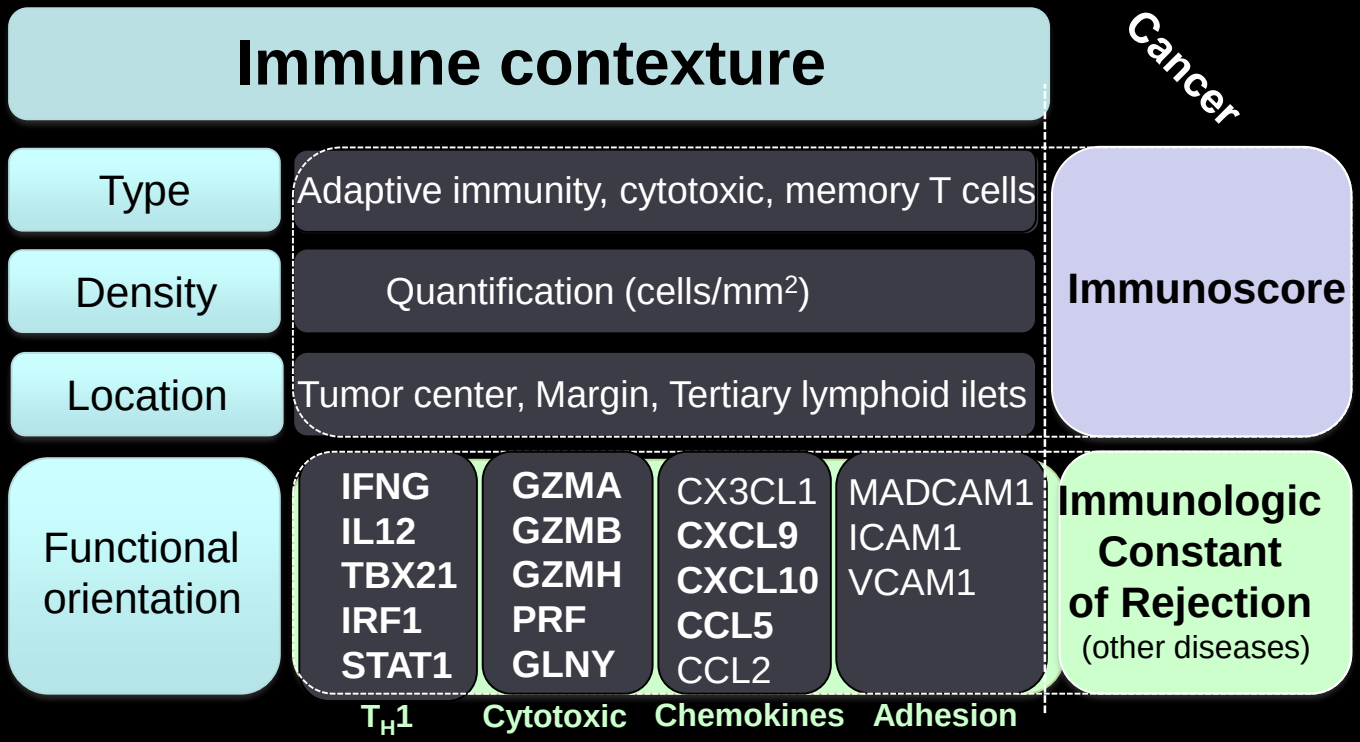
- ✓ The results of this international consortium may result in the implementation of the Immunoscore as a new component for the classification of cancer, designated TNM-I (TNM-Immune)
- ✓ This will represent the first standardized immune-based assay for the classification of cancer
- ✓ In the era of immunotherapy, it is becoming essential to start classifying cancer patients based on immune parameters

Galon J et al. ***ASCO*** 2016

Need to take into account the pre-existing immunity for efficient cancer treatment:

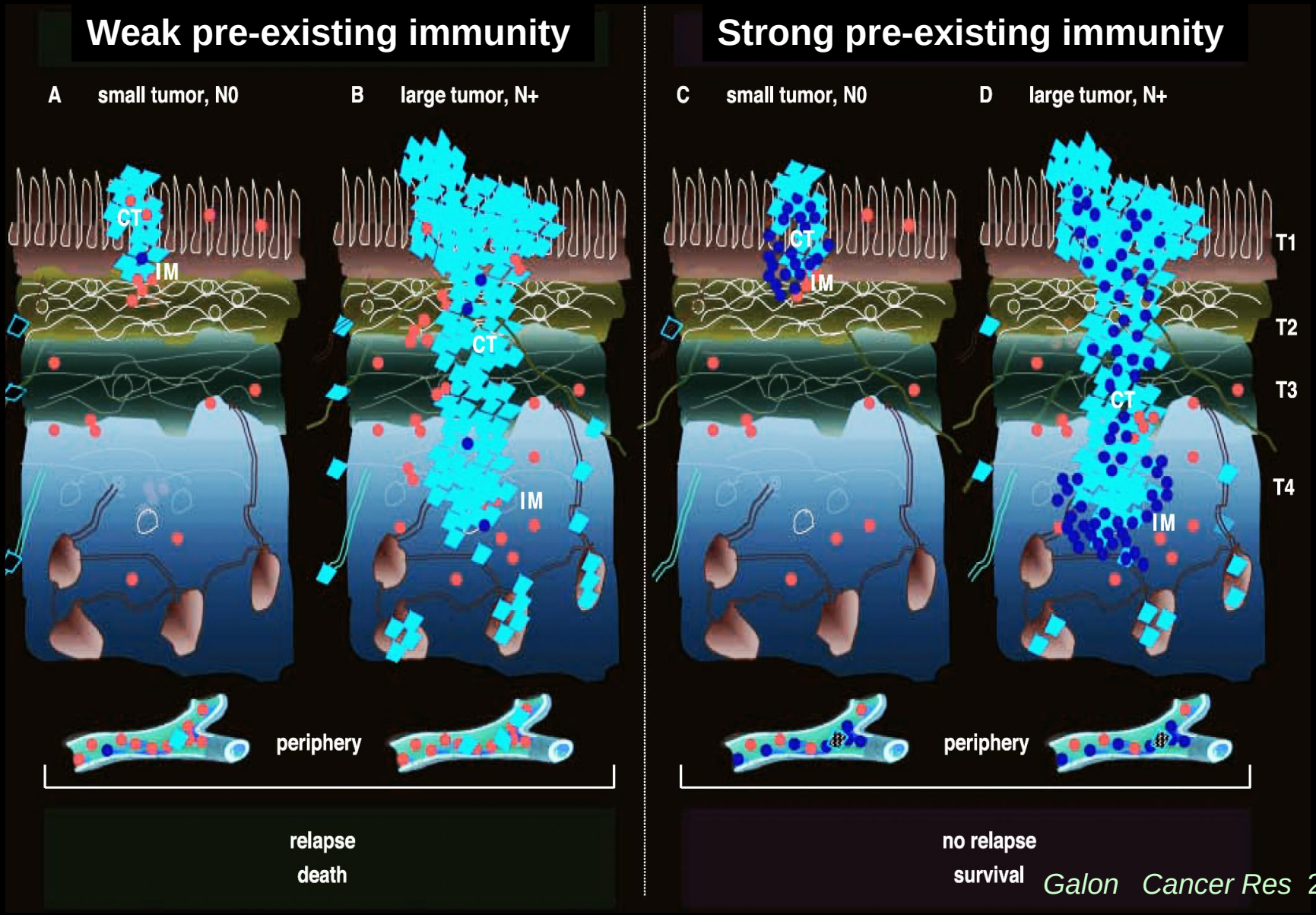
Towards an immune precision medicine

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



Galon J et al. ***Immunity*** 2013

Definition of the Immune contexture



Galon Cancer Res 2007

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

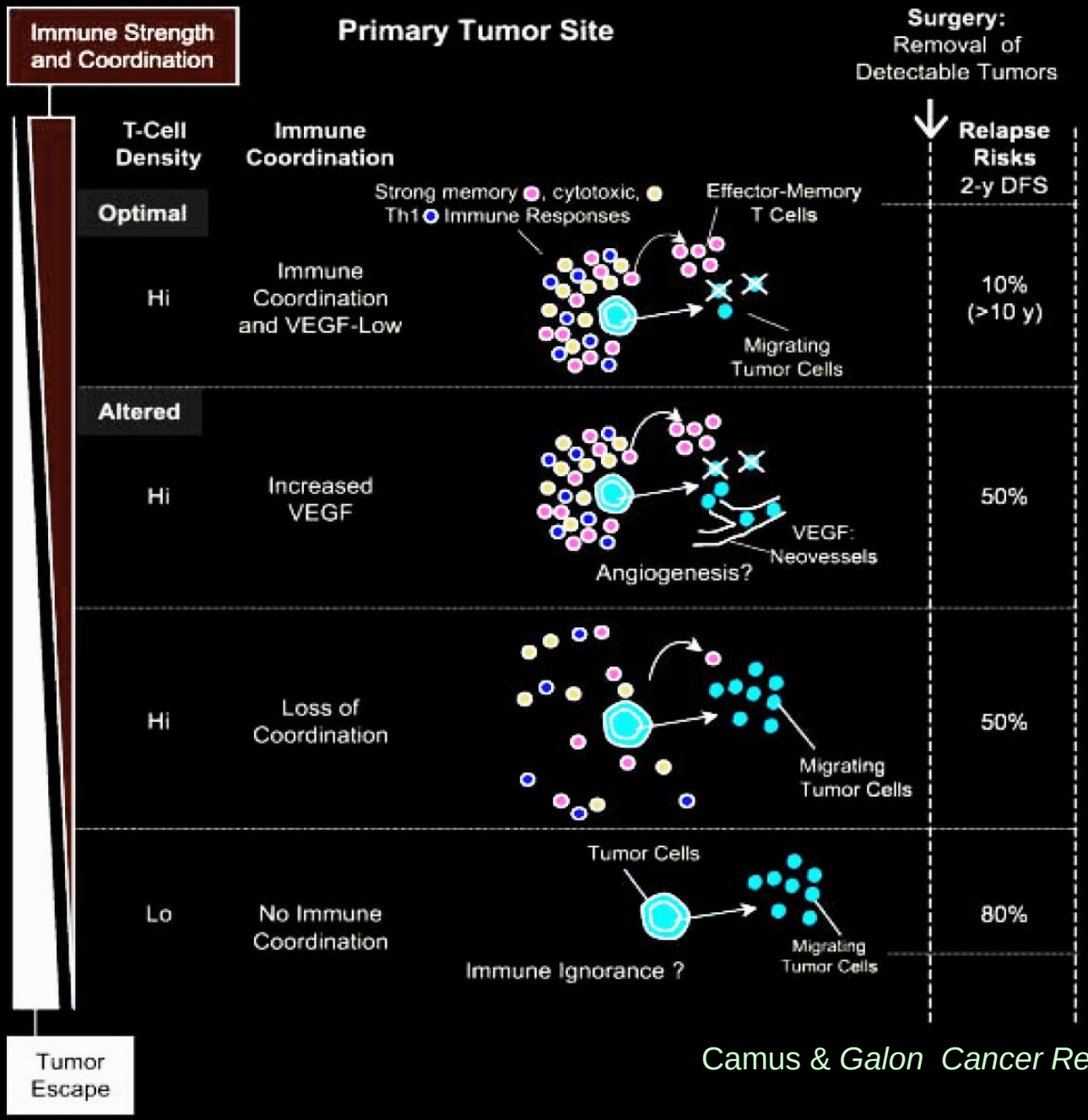
Optimal
Immunoscore High
Inflamed

Altered
Immunoscore Int.

Immunosupressed

Exclusion

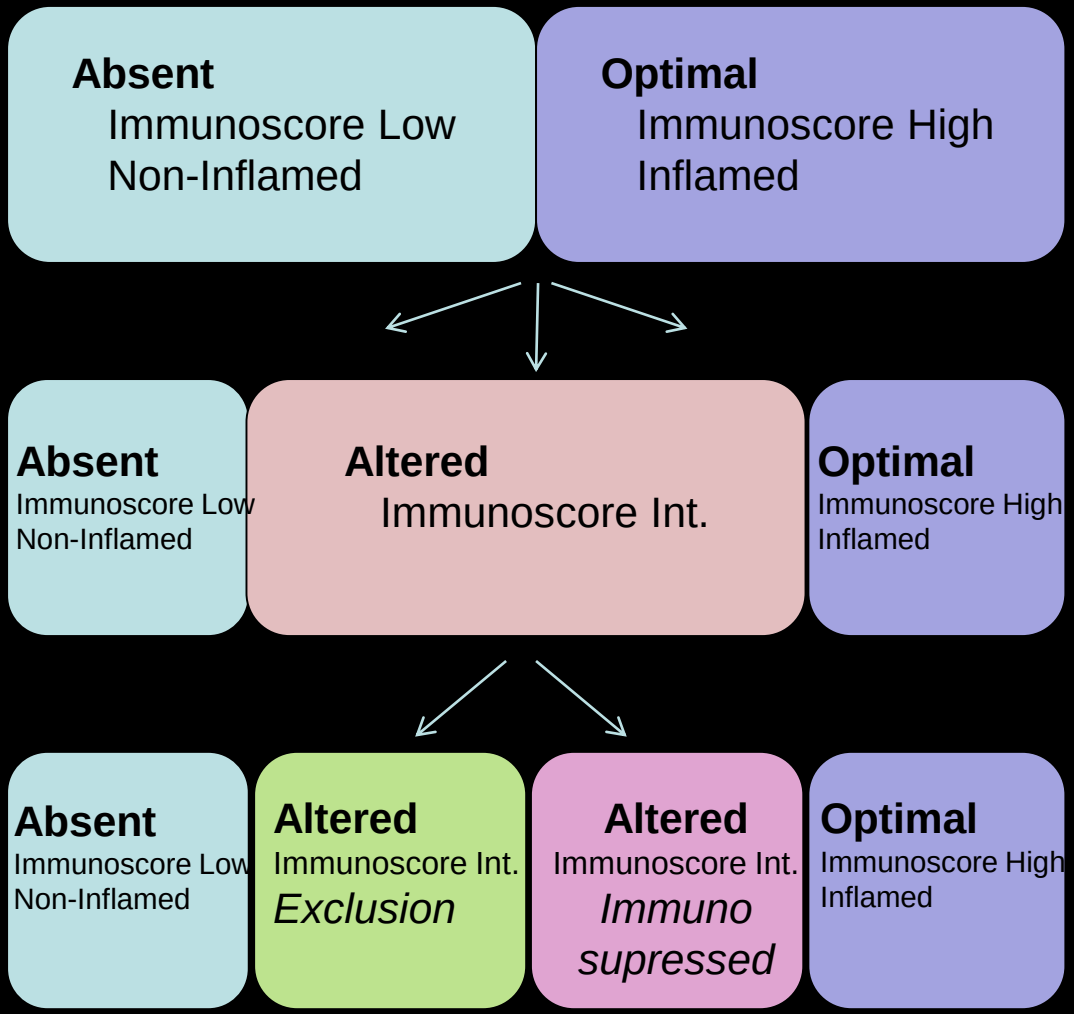
Absent
Immunoscore Low
Non-Inflamed



Camus & *Galon* *Cancer Res* 2009

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

Major immune subgroups



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

Camus & *Galon Cancer Res* 2009

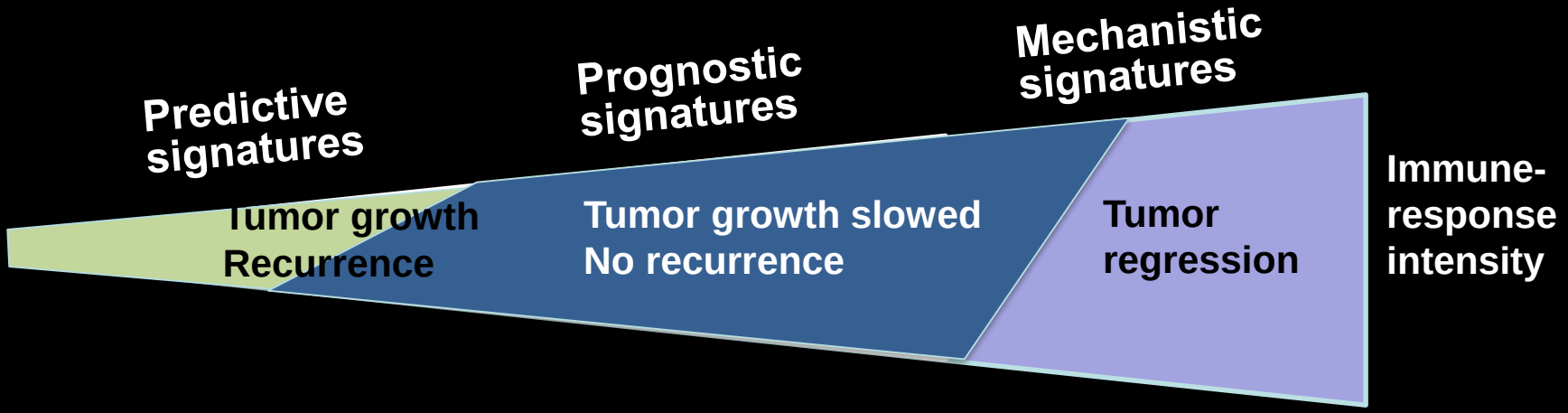
Camus & *Galon Cancer Res* 2009

The continuum of cancer immunosurveillance

Immunity
Review
Cell PRESS

The Continuum of Cancer Immunosurveillance: Prognostic, Predictive, and Mechanistic Signatures

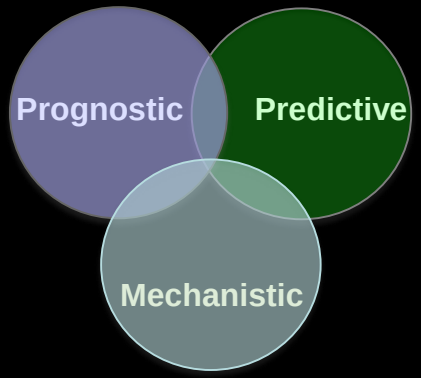
Jérôme Galon,[1,2,3,*] Helen K. Angell,[1,2,3] Davide Bedognetti,[4] and Francesco M. Marincola[4,5,*]



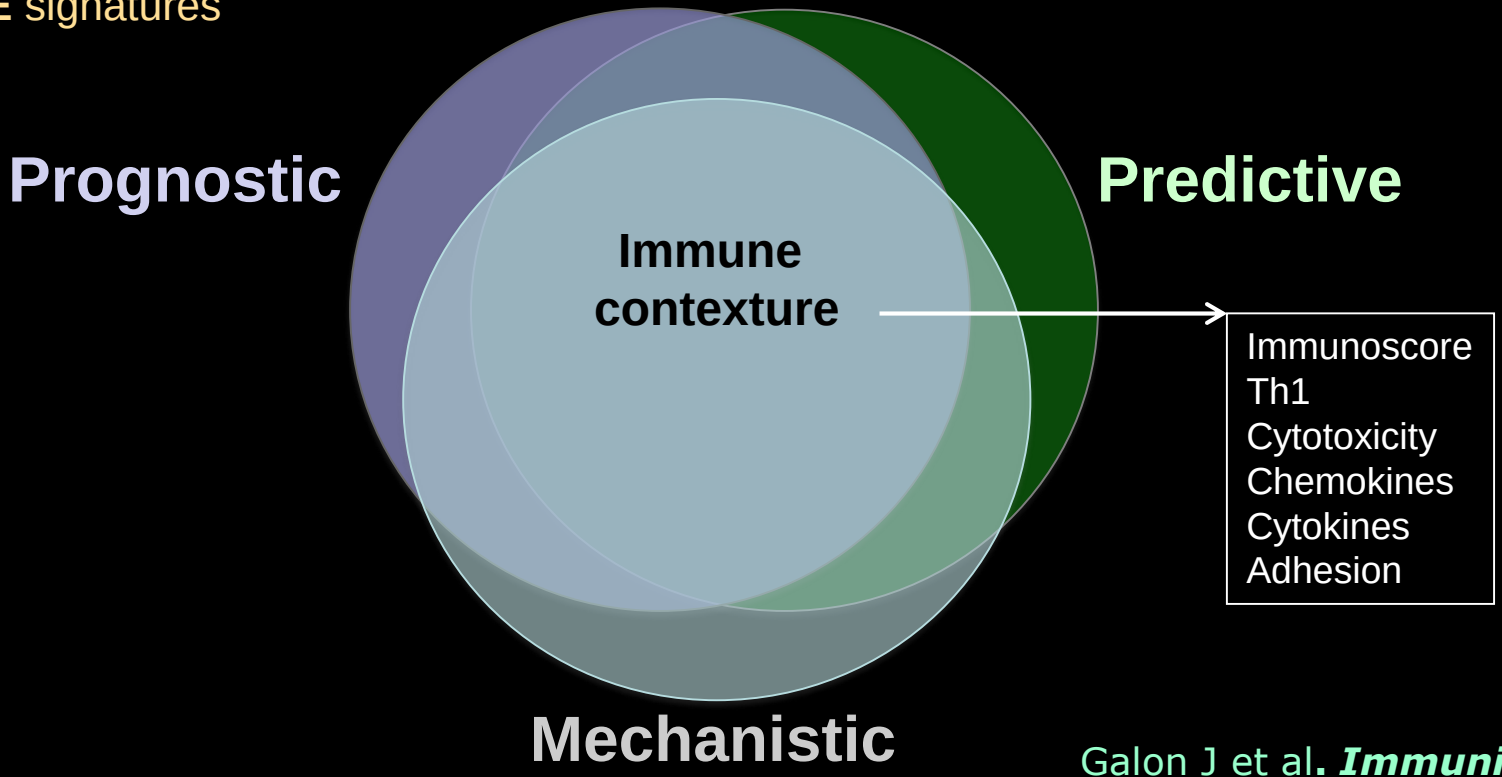
Galon J et al. ***Immunity*** 2013

The overlap between prognostic, predictive and mechanistic immune signatures

NON-Immune signatures



IMMUNE signatures



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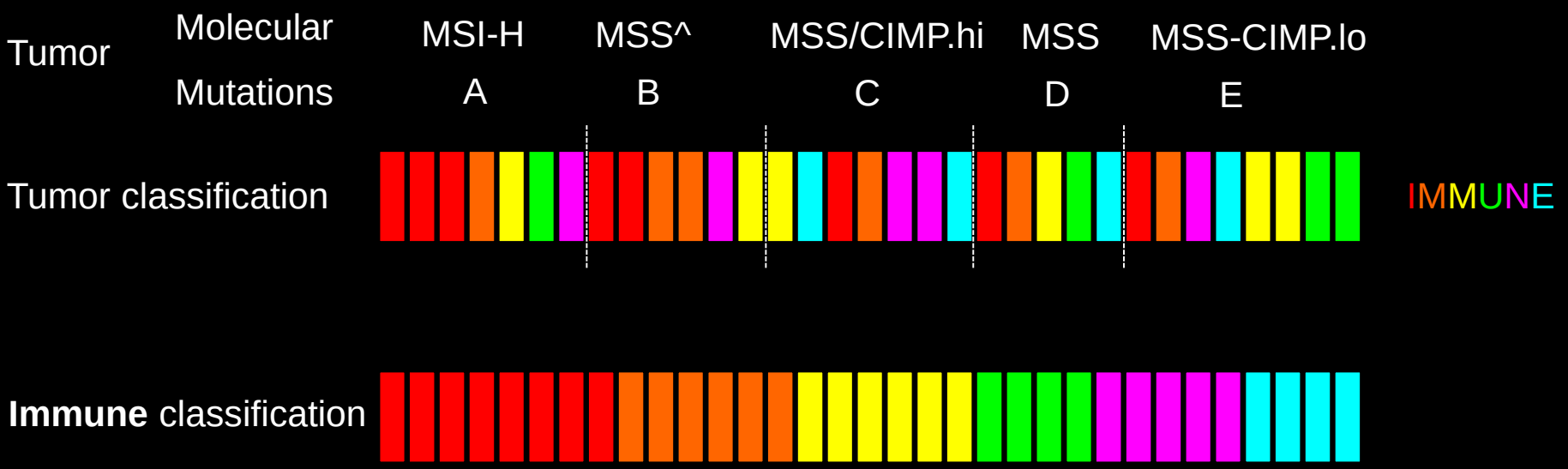
Stratification of cancer based on the immune status



-> Importance of having standardized immune Assays

Impacting all cancers

Invitation to collaborate on the evaluation of the role of Immunoscore on multiple cancers, in particular (among others)

- ✓ Melanoma
- ✓ Cervical
- ✓ Uterine
- ✓ Liver
- ✓ Pancreas
- ✓ Endometrial
- ✓ Renal
- ✓ Thyroid
- ✓ Glioma
- ✓ Glioblastoma

- ✓ Large retrospective cohorts (at all stages) with access to FFPE blocks
- ✓ Available clinical database
- ✓ 5 years follow-up

Outcome

1) Review and articles on Immunoscore in different cancer types
2) Enhancing the topic of immune infiltrates as markers for tumor prognosis and/or response to therapy
3) Bridging continents: Stimulate international collaborations between research and clinical investigators worldwide

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Definiens, PathForce, MedImmune

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