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# Immunity and therapeutic efficacy Immunoscore

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# Disclosures

Co-founder and chairman of the scientific advisory board:

- *HalioDx*

Collaborative Research Agreement (grants) :

- *Perkin-Elmer, IObiotech, MedImmune, Janssen, Imcheck*

Participation to Scientific Advisory Boards:

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

Consultant :

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

- Immune contexture: prognostic, predictive and mechanistic biomarkers
- Impact of pre-existing immunity on survival: Immunoscore
- Novel concepts in cancer evolution: partial escape mechanisms
- Immune parameters predicting therapeutic efficacy

# Cancer Immunotherapies

Ipilimumab (melanoma)

Multiple checkpoints  
Multiple types of cancers  
With or without predictive biomarkers



**Avant 2010**



**En 2011**

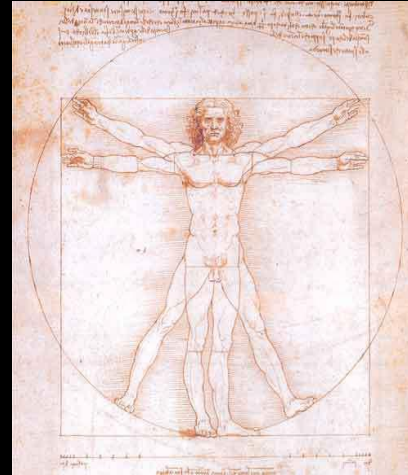


**Depuis 2013**



# Founding principles of immunotherapy

Demonstration of immuno-surveillance <sup>1</sup>



Demonstration of the importance of the pre-existing adaptive immunity (T-cells) <sup>2</sup>

T-cells > TNM

Inhibition of checkpoint receptors targeting T-cells in mouse models <sup>3</sup>

Unleashing the pre-existing immunity via inhibition of checkpoint receptors targeting T-cells in Human cancers <sup>4,5</sup>



Nobel Prizes 2018

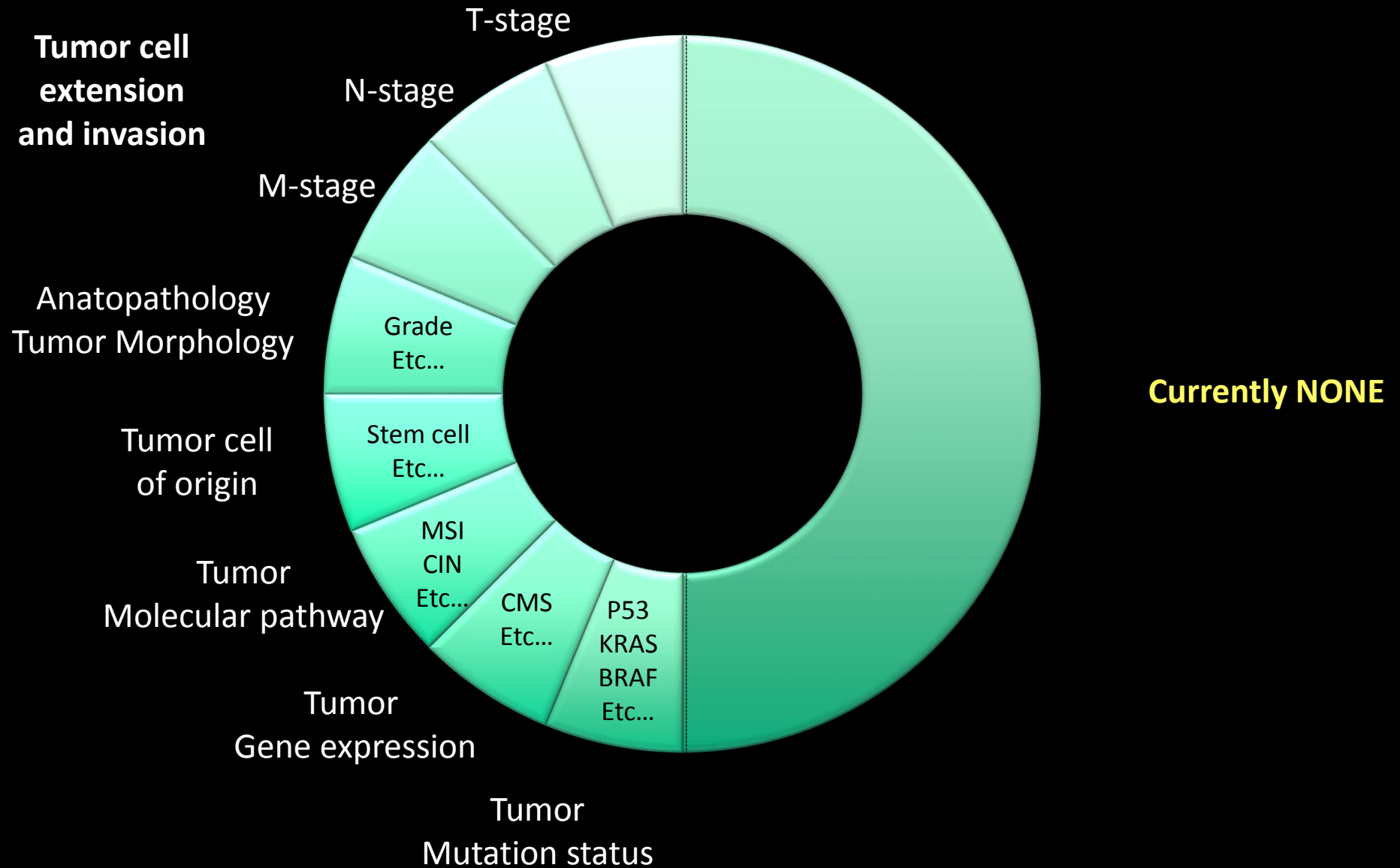
1. Shankaran V, et al. *Nature* 2001
2. Galon J, et al. *Science* 2006

3. Curran MA, et al. *Proc Natl Acad Sci U S A* 2010
4. Hodi S et al. *New Engl J Med* 2010
5. Topalian SL et al. *New Engl J Med* 2012



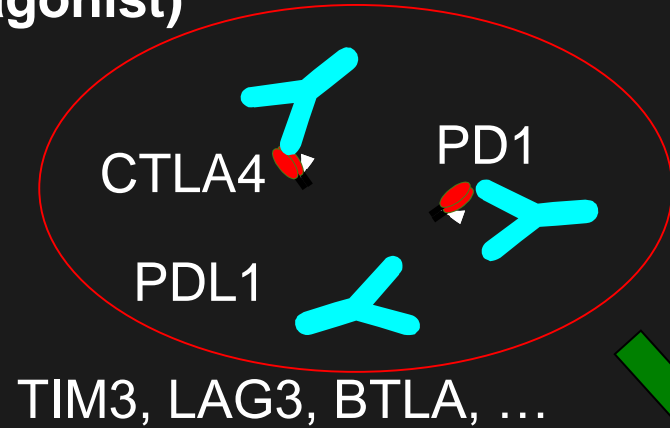
## Immune-based classification

### Host immune response



# Conclusion: Successful immunotherapies unleash natural pre-existing T cells

## Co-inhibitory receptors (antagonist)



## Costimulatory receptors (agonist)



Peptide vaccine  
Genetic vaccine  
DC vaccine

**T-cells**

tumor

## Costimulatory cytokines

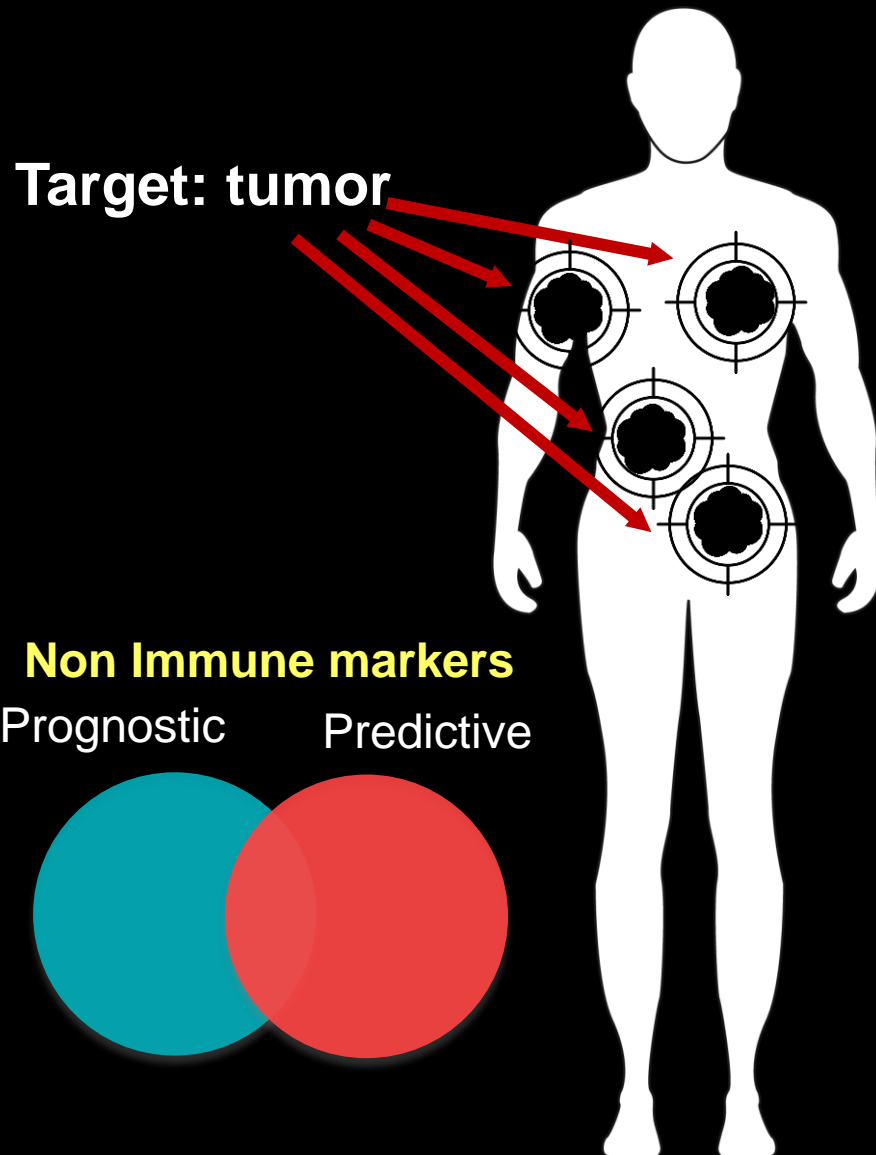
IL-2  
IFN  
IL-15  
IL-21

**Adoptive Transfert of T cells**

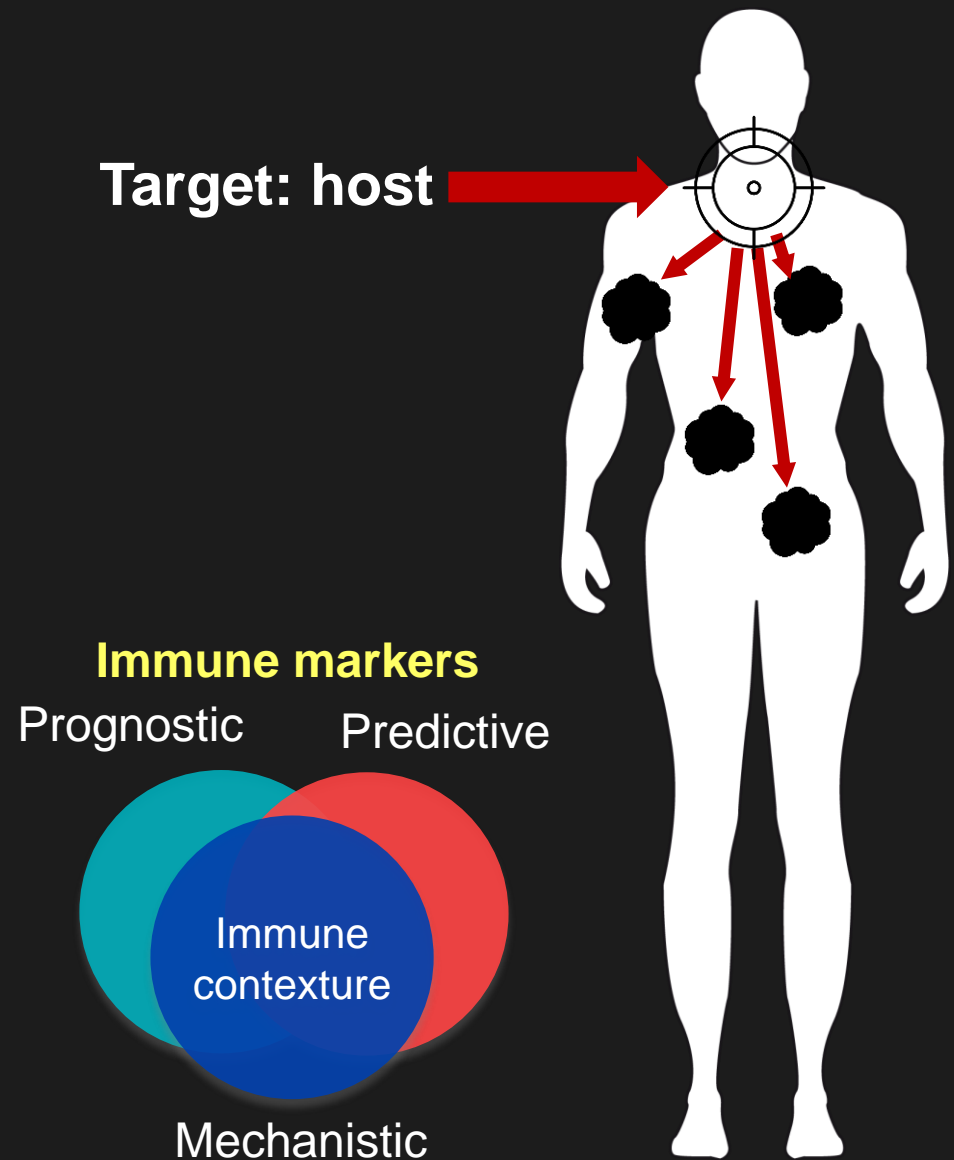
**Engineered TCR or CAR-T cells**

# Cancer Treatment

## Conventional Therapy



## New Paradigm Immunotherapy



# Cancer Treatment

*Immunotherapy*

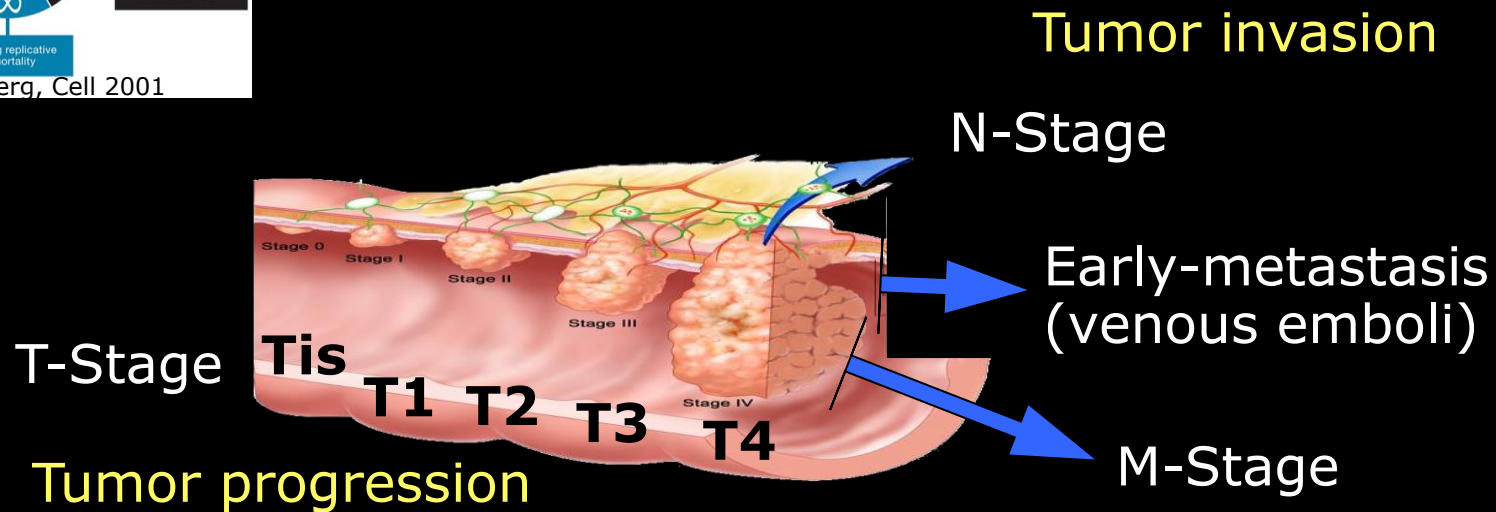
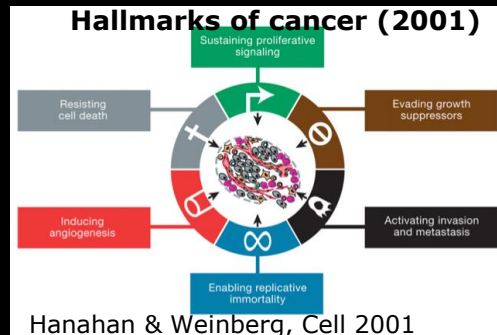
*Chemotherapy*

*Radiotherapy*

*Surgery*



# Definition of cancer



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

## Definition of cancer

“Two quite opposite qualities equally bias our minds: habits and novelty.”

*Jean de la Bruyère, French philosopher (1645-1696)*



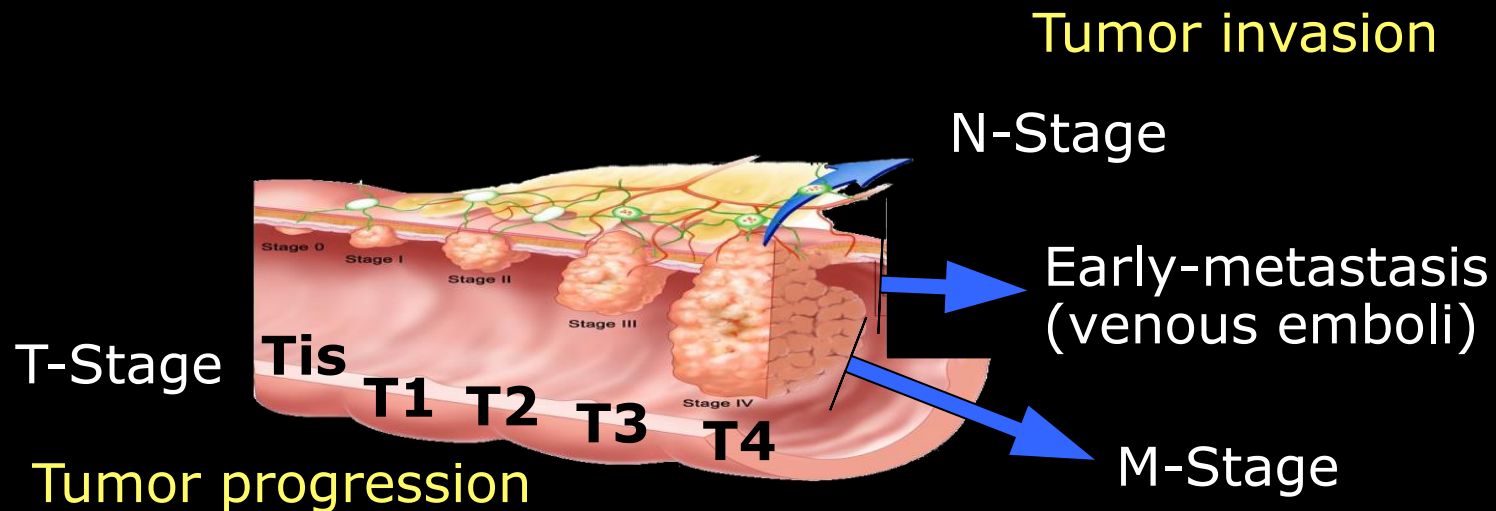


# Novel paradigm

“Hot” Tumor

**Immunoscore**  
**Immune contexture**

“Cold” Tumor

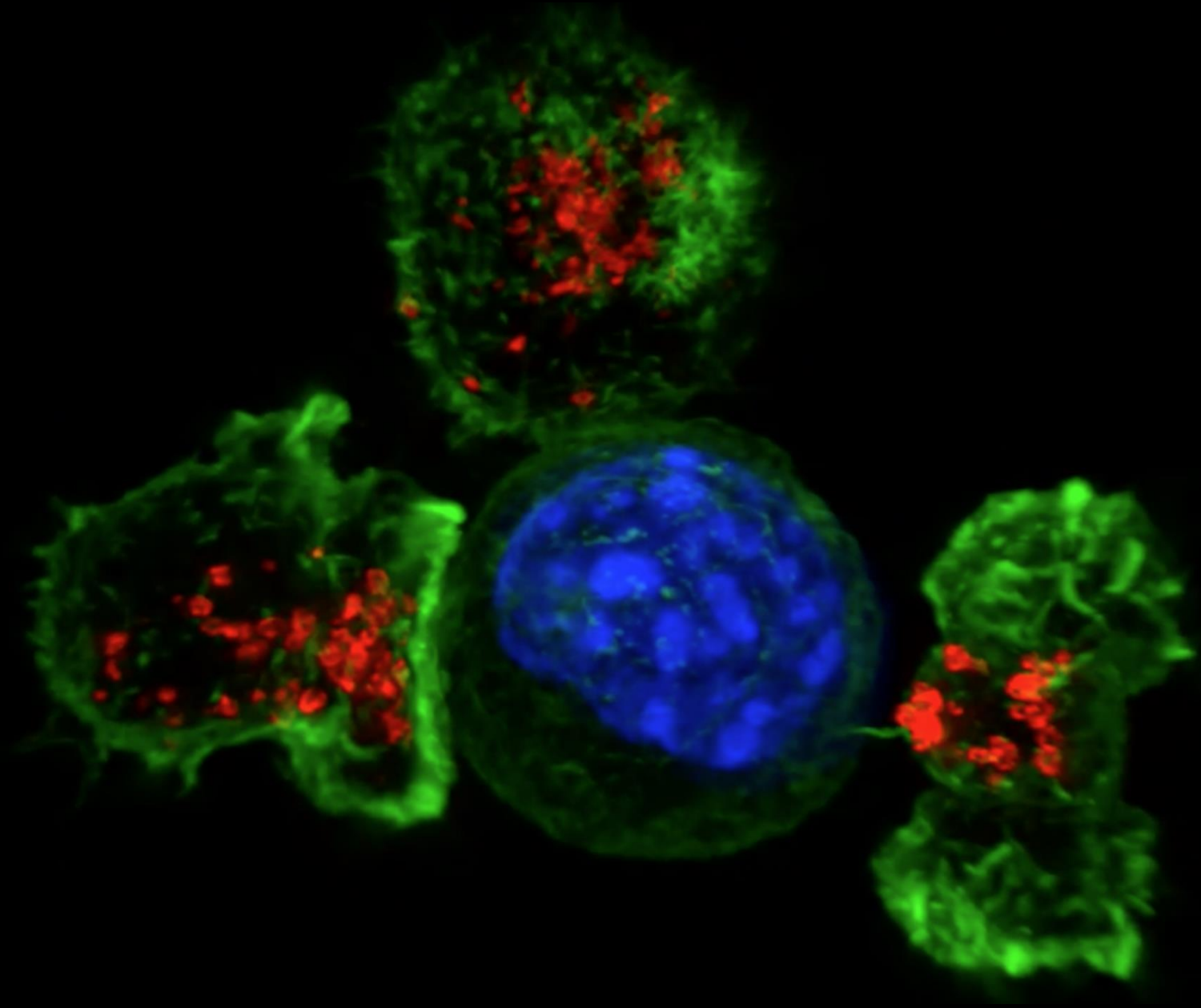


Tumor grade differentiation  
Tumor aggressiveness  
(driver mutations, CIN, MSI, CIMP...)

-> 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

# T-cells attacking tumor cells

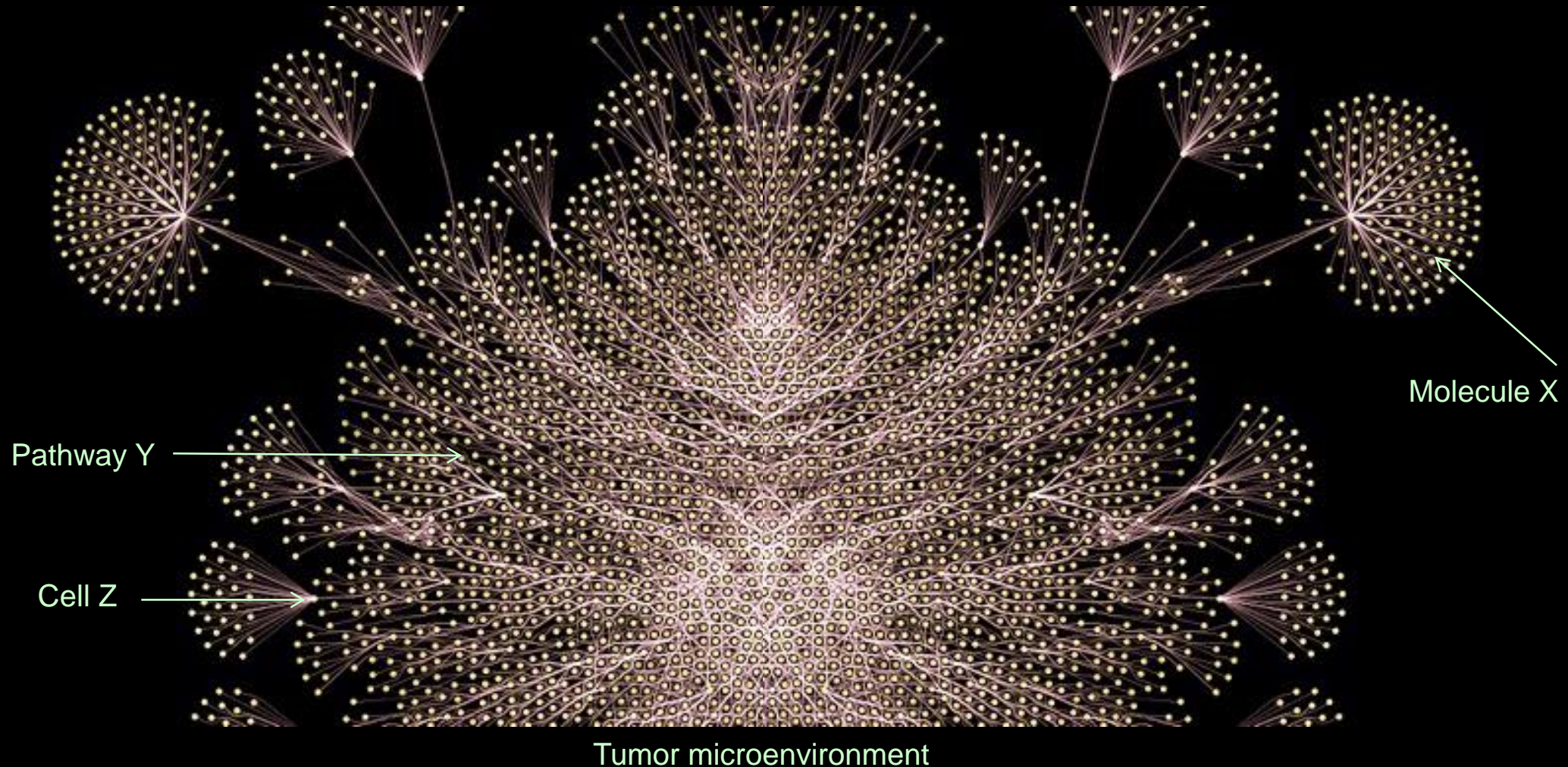


T-cells

Cytotoxic granules (GZM)

Tumor cell

Cancer is one of the most complex biological system of all

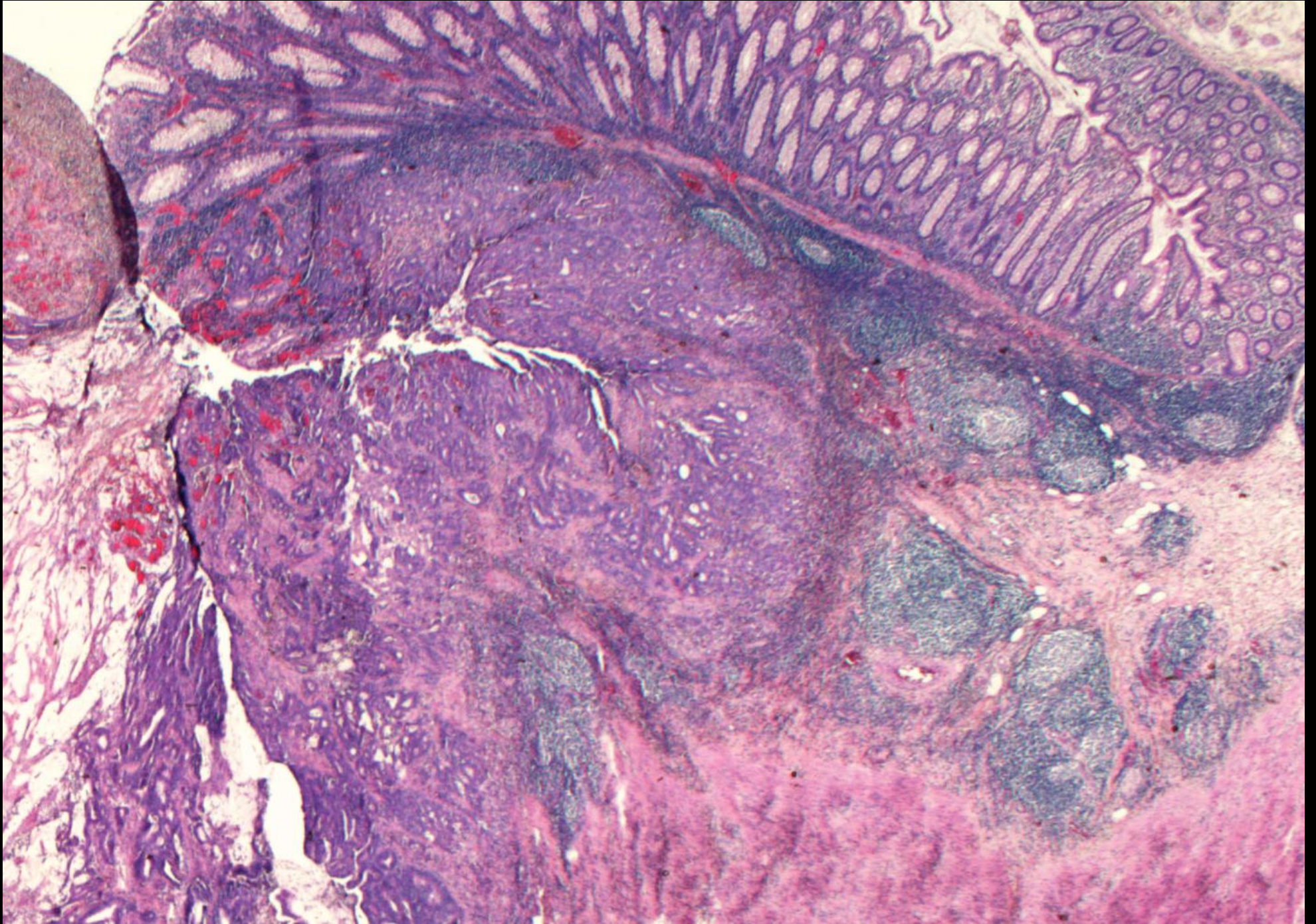


*"The whole is greater than the sum of its parts", Aristotle*

-> Systems biology in human cancer



## Tumor microenvironment





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

## Type, Density, and Location of Immune Cells Within Human Colorectal Tumors Predict Clinical Outcome

Jérôme Galon,<sup>1,\*†</sup> Anne Costes,<sup>1</sup> Fatima Sanchez-Cabo,<sup>2</sup> Amos Kirilovsky,<sup>1</sup> Bernhard Mlecnik,<sup>2</sup> Christine Lagorce-Pagès,<sup>3</sup> Marie Tosolini,<sup>1</sup> Matthieu Camus,<sup>1</sup> Anne Berger,<sup>4</sup> Philippe Wind,<sup>4</sup> Franck Zinzindohoué,<sup>5</sup> Patrick Bruneval,<sup>6</sup> Paul-Henri Cugnenc,<sup>5</sup> Zlatko Trajanoski,<sup>2</sup> Wolf-Herman Fridman,<sup>1,7</sup> Franck Pagès<sup>1,7,†</sup>

29 SEPTEMBER 2006 VOL 313 SCIENCE www.sciencemag.org

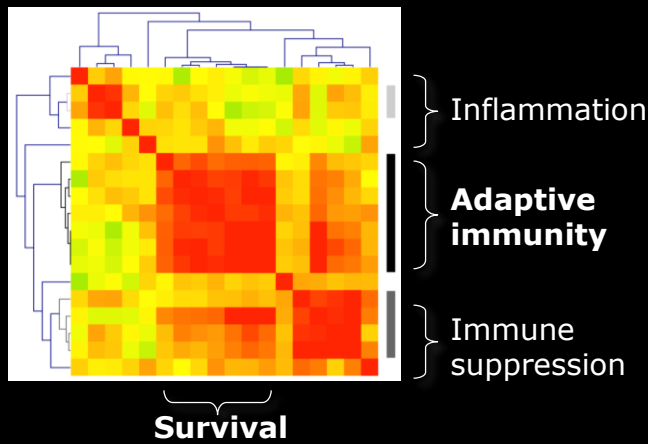
The foundation a new concept



**Immune contexture**

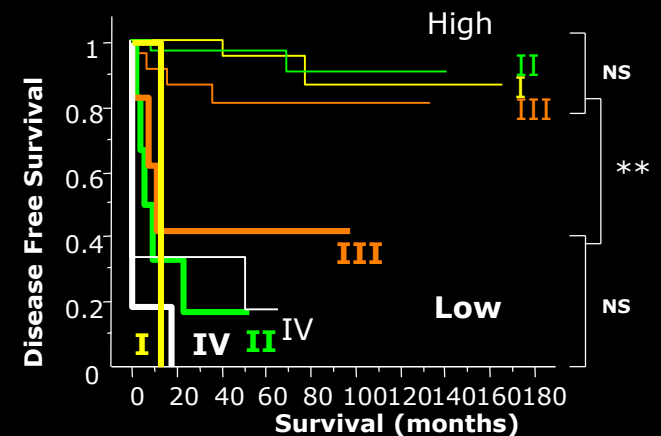
- ✓ Gene expression profiling
- ✓ Qualitative immune signature

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



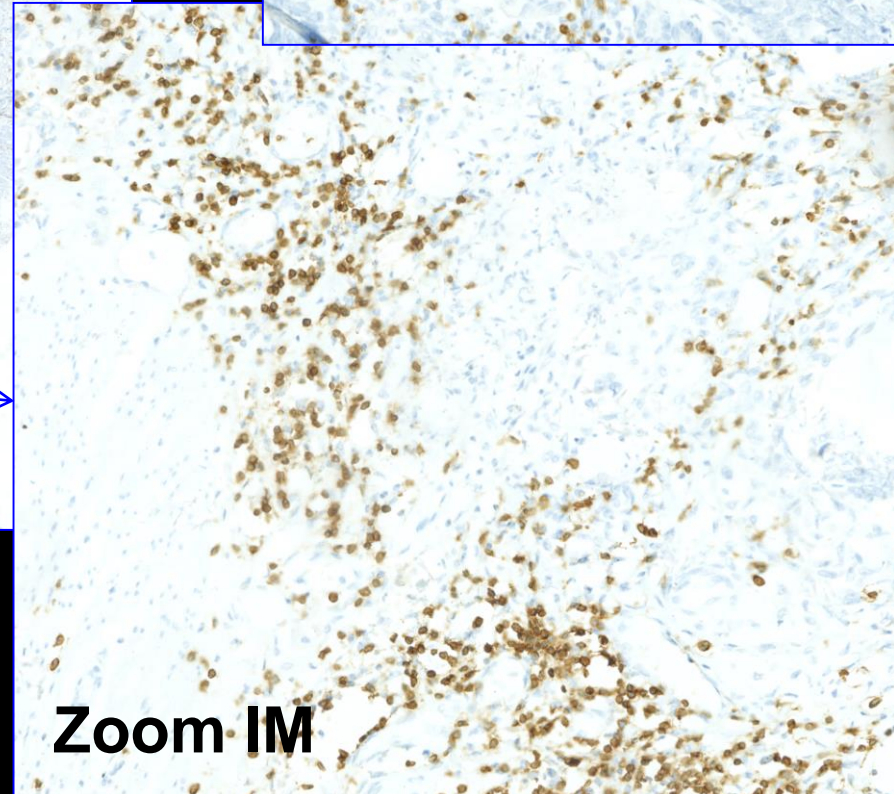
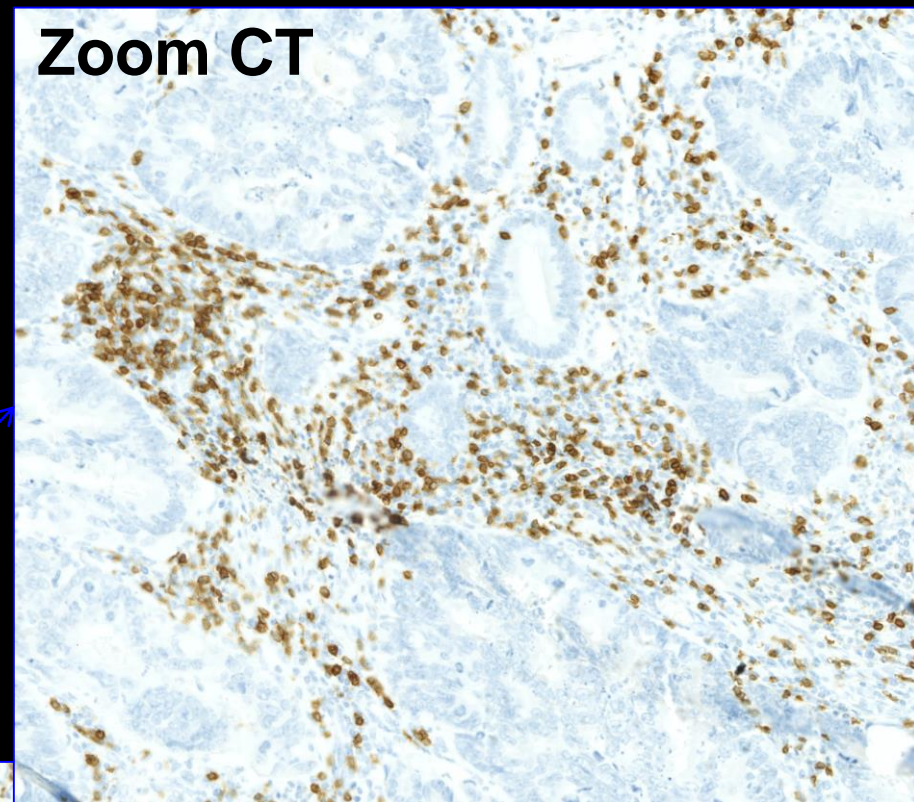
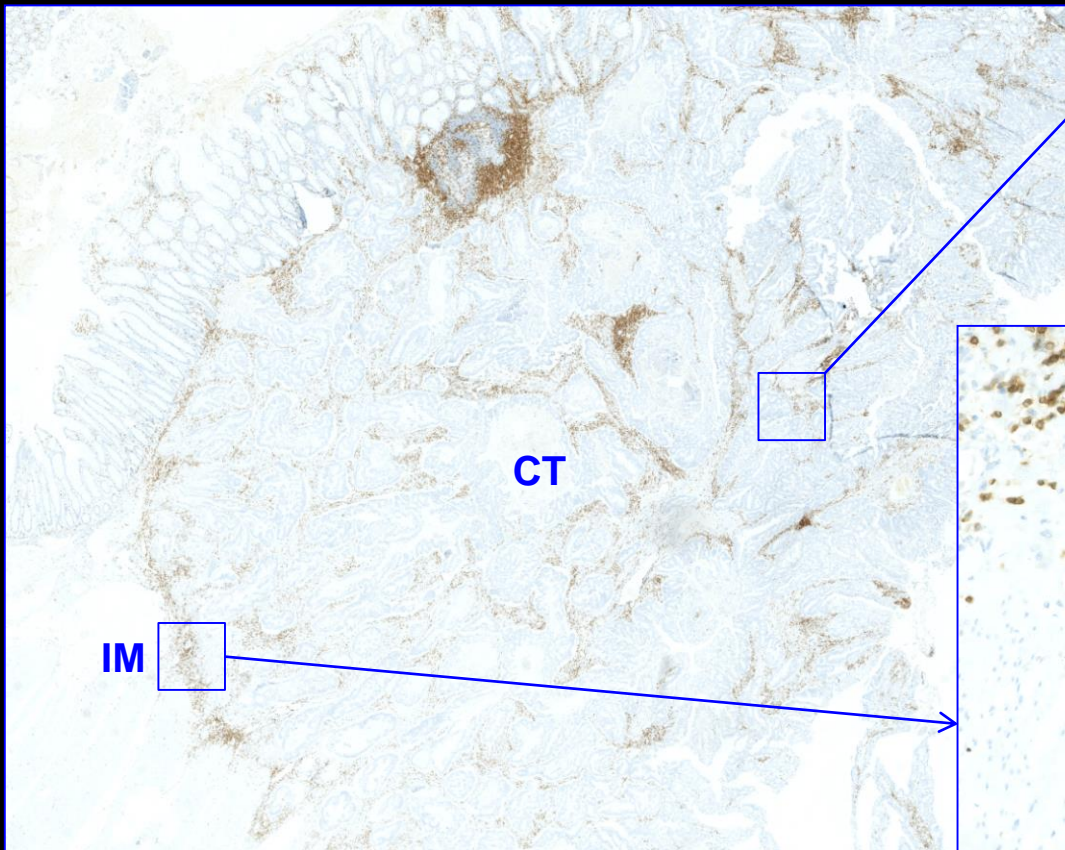
Optimized  
Immunosign

**Quality**



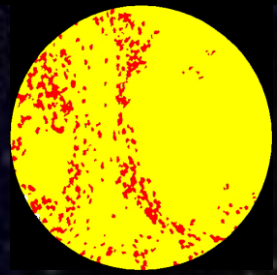
**Type/Density/Location**

# Digital quantification of immune cells infiltrating tumors: Immunoscore





# IHC images : Past - Present - Future

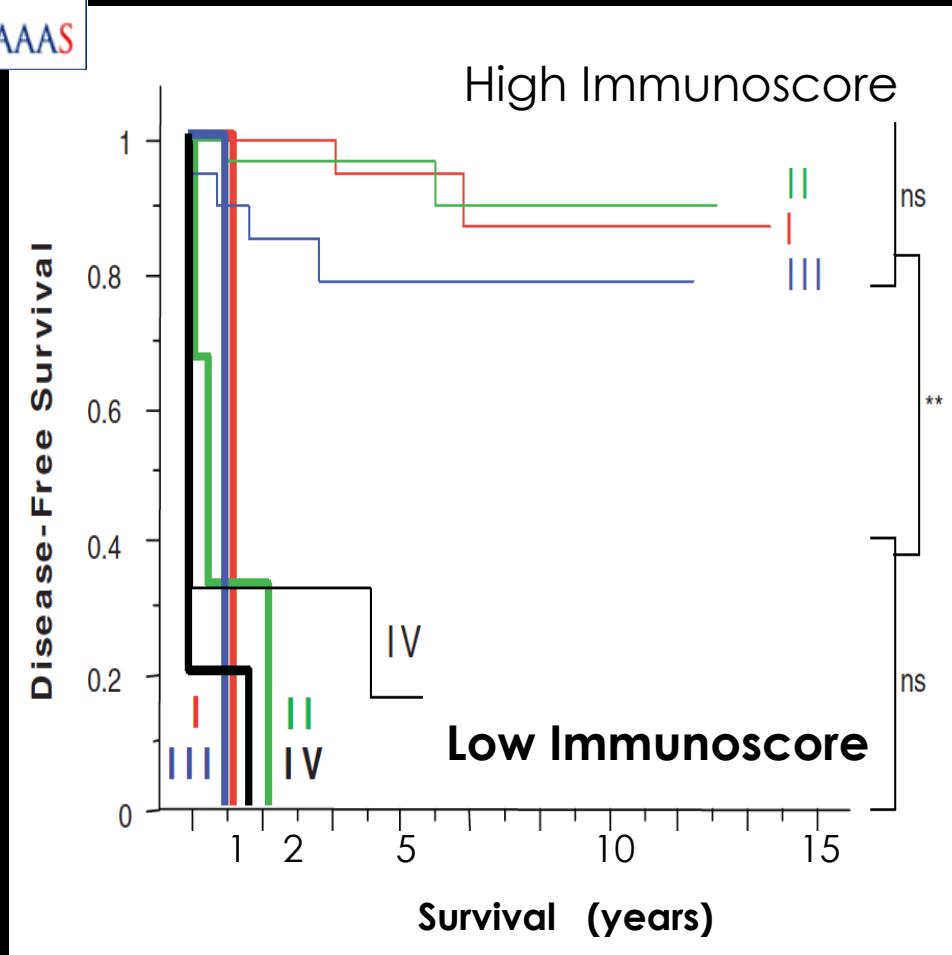


What really means IHC photos at the time of surgery ?

*"The distinction between past, present, and future is only a stubbornly persistent illusion"*  
A. Einstein

- ✓ An image of the past ? -> Reflecting the accumulation of memory T-cells from initial priming
- ✓ An image of the present ? -> ongoing proliferation of T-cells, *in situ* Effector T-cells,  $T_{EM}$
- ✓ An image of the future ? -> Predicting long-term memory and survival of patients

# Immunoscore: a novel paradigm for cancer



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

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

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

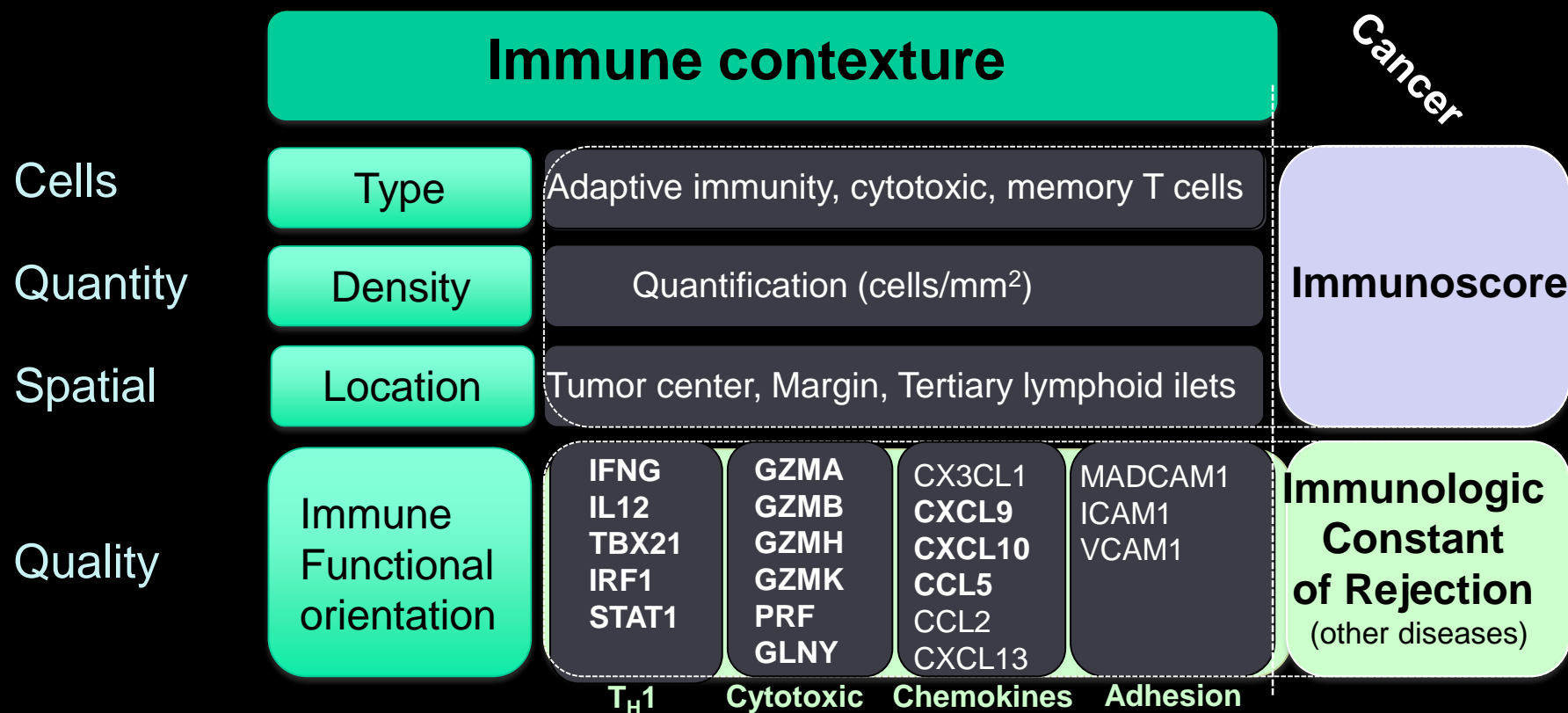
# A Novel Paradigm for Cancer

<i>Parameter</i>	<i>HR</i>	<i>P</i> value
• T-stage	1.2	0.25
• N-stage	1.4	0.15
• Differentiation	1.1	0.84
• <b>Immunoscore</b>	1.9	0.00001

***“Immune Contexture” :***

- ✓ **Type**
- ✓ **Density**
- ✓ **Location**
- ✓ Immune **functional** orientation

# 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

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

## Major immune categories of tumors

2

### Absent

Immunoscore Low  
Non-Inflamed  
COLD

### Optimal

Immunoscore High  
Inflamed  
HOT

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

3

### Absent

Immunoscore Low  
Non-Inflamed  
COLD

### Altered

Immunoscore Int.

### Optimal

Immunoscore High  
Inflamed  
HOT

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

4

### Absent

Immunoscore Low  
Non-Inflamed  
COLD

### Altered

Immunoscore Int.  
*Exclusion*

### Altered

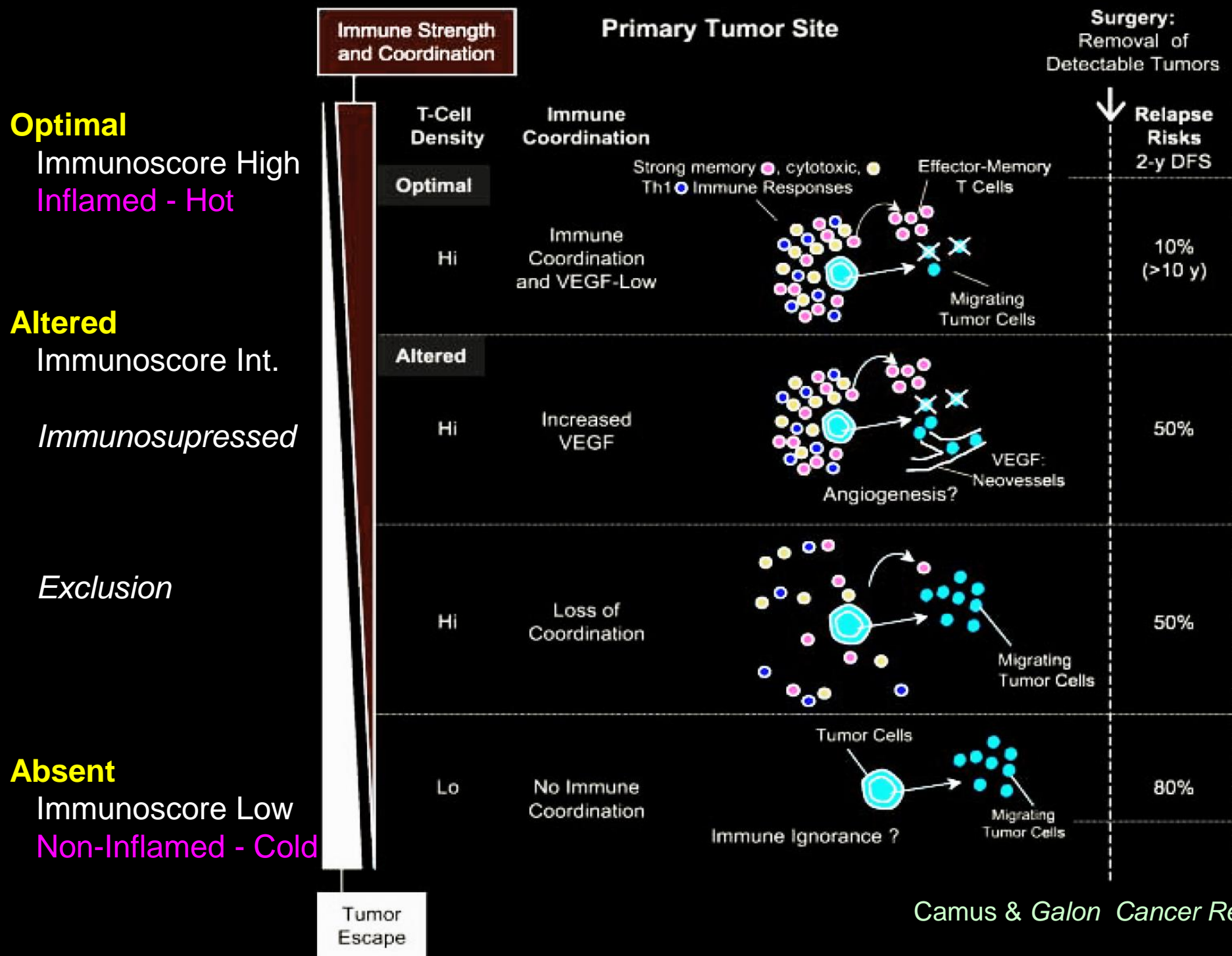
Immunoscore Int.  
*Immuno  
supressed*

### Optimal

Immunoscore High  
Inflamed  
HOT

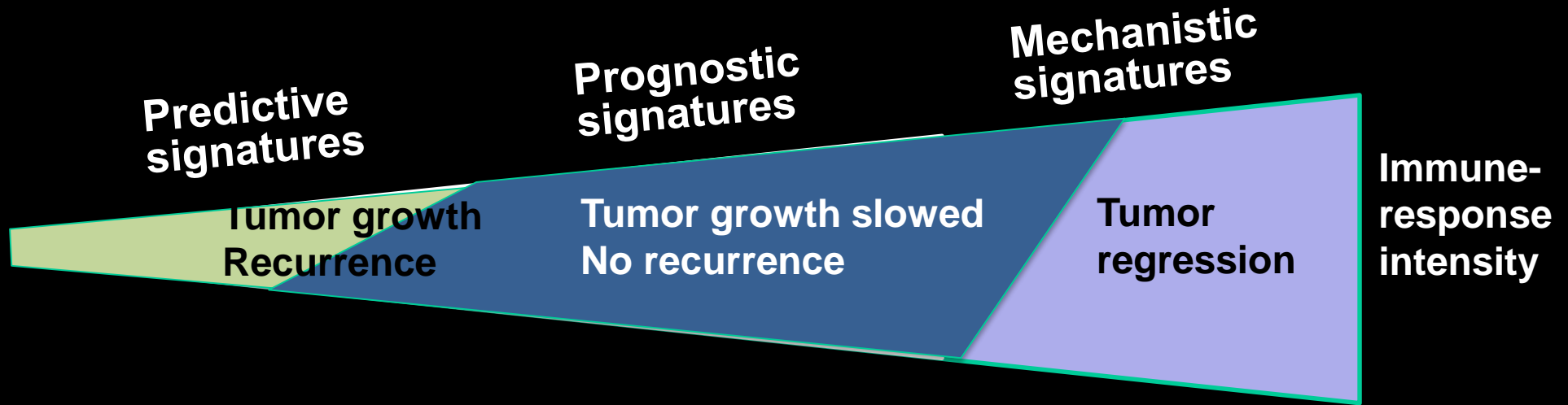
*Camus & Galon Cancer Res 2009*

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





# The continuum of cancer immunosurveillance: predictive, prognostic and mechanistic signatures



Immune signatures are detectable during :

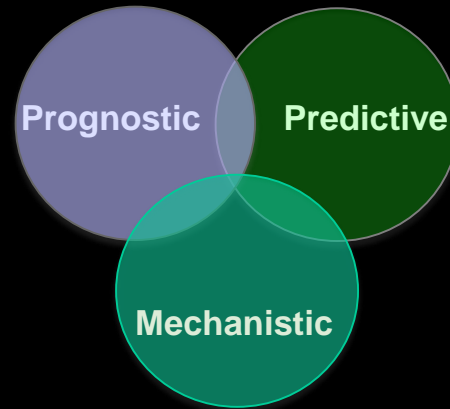
- ✓ Naturally occurring intratumor immune response in long survivors
- ✓ Regression of cancer following immunotherapy
- ✓ Allograft rejection
- ✓ Graft versus host disease
- ✓ Flares of autoimmunity
- ✓ Destruction of virally infected cells





# The overlap between prognostic, predictive and mechanistic immune signatures

NON-Immune signatures



IMMUNE signatures

Prognostic

Predictive

Immune  
contexture

Immunoscore  
Th1  
Cytotoxicity  
Chemokines  
Cytokines  
Adhesion

Mechanistic



# Immunoscore in early-stage (I/II) colorectal cancer

JOURNAL OF  
CLINICAL  
ONCOLOGY

Official Journal of the  
American Society of Clinical Oncology

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

## In Situ Cytotoxic and Memory T Cells Predict Outcome in Patients With Early-Stage Colorectal Cancer

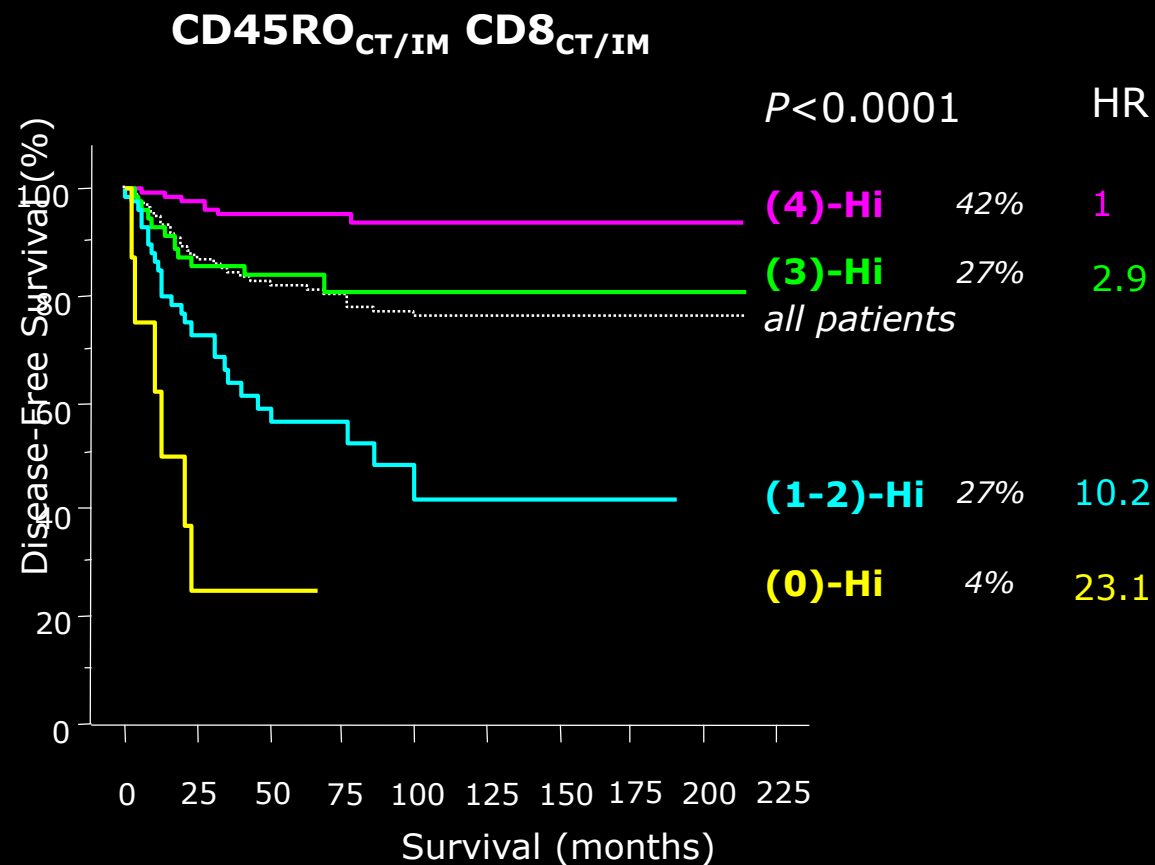
*Franck Pagès, Amos Kirilovsky, Bernhard Mlecnik, Martin Asslaber, Marie Tosolini, Gabriela Bindea, Christine Lagorce, Philippe Wind, Florence Marliot, Patrick Bruneval, Kurt Zatloukal, Zlatko Trajanoski, Anne Berger, Wolf-Herman Fridman, and Jérôme Galon*

**J Clin Oncol.** 27, 5944-51 (2009)

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

Evaluation in the Center (CT) and the Invasive margin (IM) of the tumor

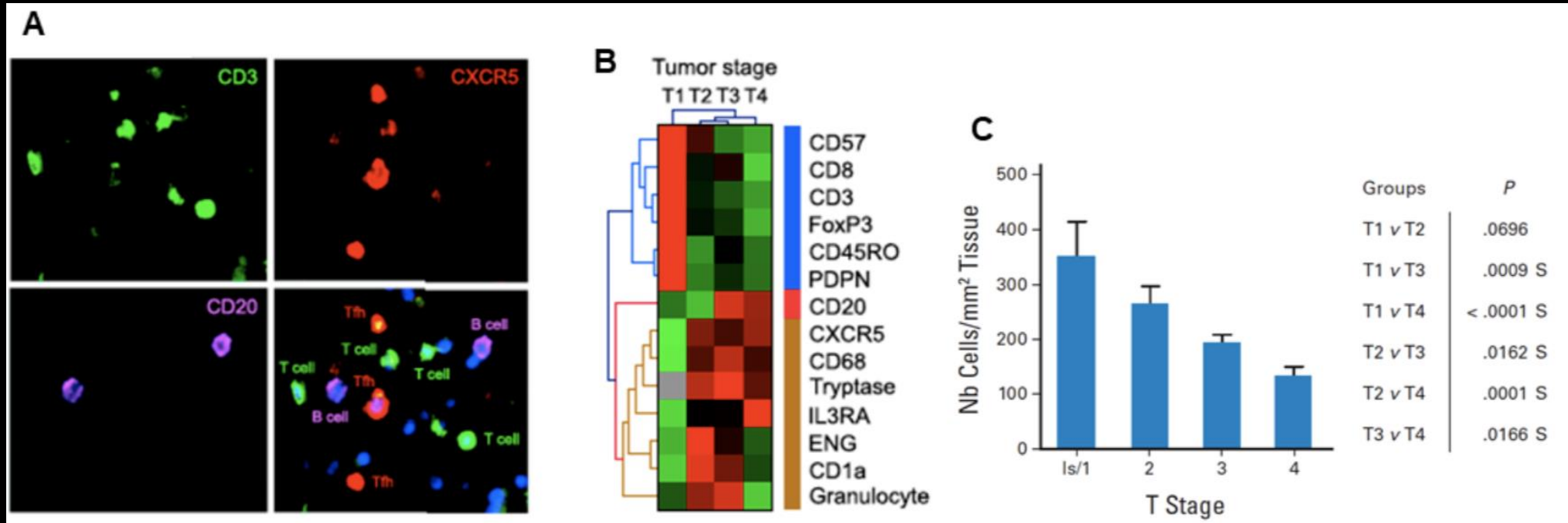
Cohort 1= 411 patients, cohort 2= 188 patients



## COX multivariate analysis

Parameter	HR	P value
• T-stage	1.2	0.41
• Perforation	5.5	0.003
• Immune pattern	0.3	<0.00001

# Adaptive immunity decreases with tumor progression



Bindea G. et al. *Immunity* 2013

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

# “TNM staging: T is for T cell and M is for Memory”



Editorial: Broussard et al. JCO 2011

## Multivariate Analysis

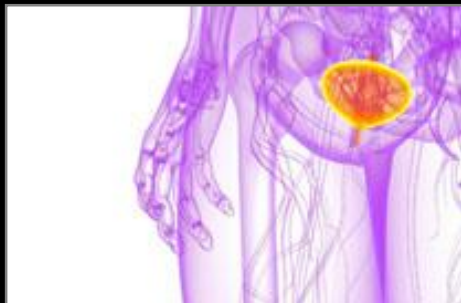
Cox Analysis	DFS		OS		DSS	
	HR	P-value	HR	P-value	HR	P-value
AJCC/UICC-TNM	1.38	0.09 ns	1.18	0.29 ns	1.43	0.10 ns
Immunoscore	0.64	<0.0001	0.71	<0.0001	0.63	<0.0001

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

# Impacting all cancers

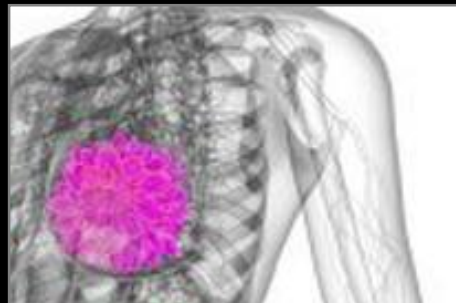
Bladder cancer



Brain cancer



Breast cancer



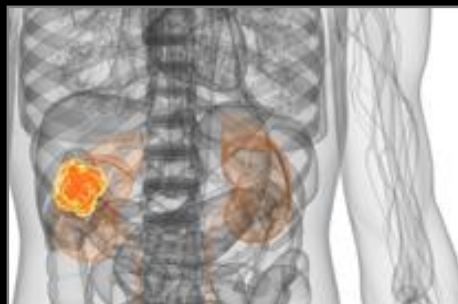
Cervical cancer



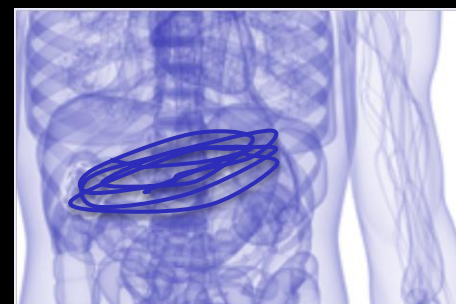
Colorectal cancer



Kidney cancer



Liver cancer



Lung cancer



Melanoma cancer



Ovarian cancer



Pancreatic cancer



Prostate cancer





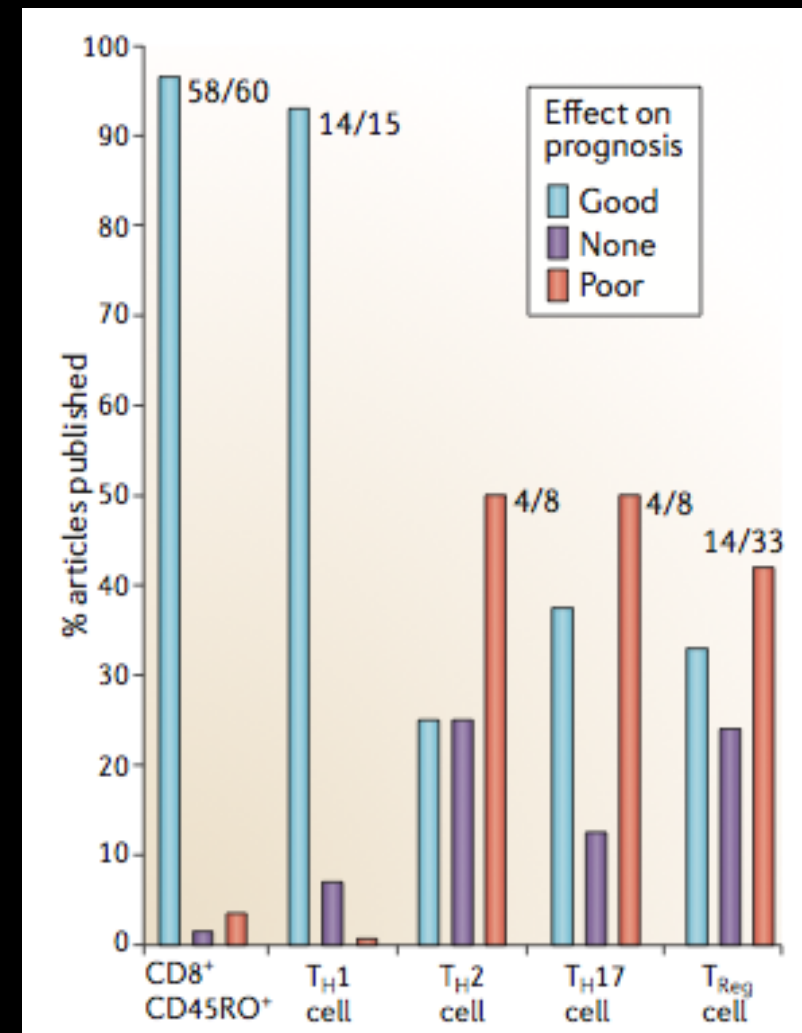
# Meta-analysis of 124 published articles studying the impact of cytotoxic T cells, memory T cells, and T-helper subpopulations with regards to prognosis of patients with cancer (20 cancer types analyzed)

## NATURE REVIEWS | CANCER

Table 1 | The association of immune cell infiltrates with prognosis

Cells	CD8 <sup>+</sup> CD45RO <sup>+</sup> T cells	T <sub>H</sub> 1 cells
Melanoma	Good <sup>109-106</sup>	
Head and neck cancers	Good <sup>110,109,110</sup>	
Breast cancer	Good <sup>111-114</sup>	* Good <sup>115,116</sup> * None <sup>117</sup>
Bladder cancer	Good <sup>118,119</sup>	
Ovarian cancer	Good <sup>120-122</sup>	Good <sup>123,124</sup>
Oesophageal cancer	Good <sup>126,127</sup>	Good <sup>128</sup>
Colorectal cancer	Good <sup>125,126,127,129,130-148</sup>	Good <sup>135,136,79</sup>
Renal cell carcinoma	* Good <sup>175</sup> * Poor <sup>175</sup>	Good <sup>171</sup>
Prostatic adenocarcinoma	Good <sup>151-153</sup>	
Lung carcinoma	* Good <sup>173,154-157</sup> * None <sup>158</sup>	Good <sup>13</sup>
Pancreatic cancer	Good <sup>165</sup>	
Cervical cancer		Good <sup>166</sup>
Anal squamous cell carcinoma		
Brain cancer		
Hepatocellular carcinoma	* Good <sup>167,168</sup> * Poor <sup>170</sup>	Good <sup>169</sup>
Gastric cancer		Good <sup>171</sup>
Medulloblastoma		Good <sup>173</sup>
Merkel cell carcinoma	Good <sup>174</sup>	
Urothelial cell carcinoma	Good <sup>179</sup>	
Follicular lymphoma and Hodgkin's lymphoma		

Fridman WH, Pagès F, Sautès-Fridman C, **Galon J\***  
The immune contexture in human tumours:  
impact on clinical outcome  
*Nat Rev Cancer* 2012





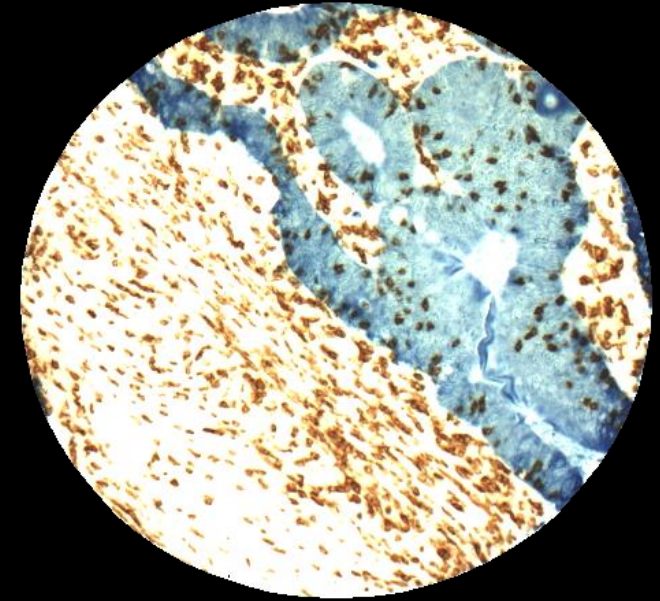
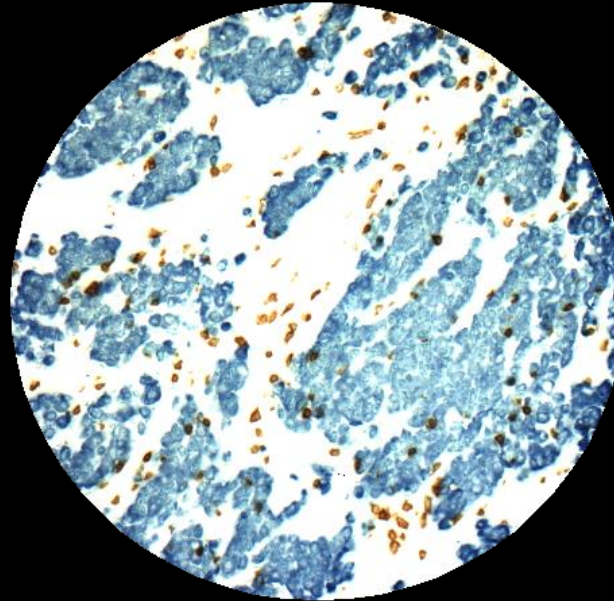
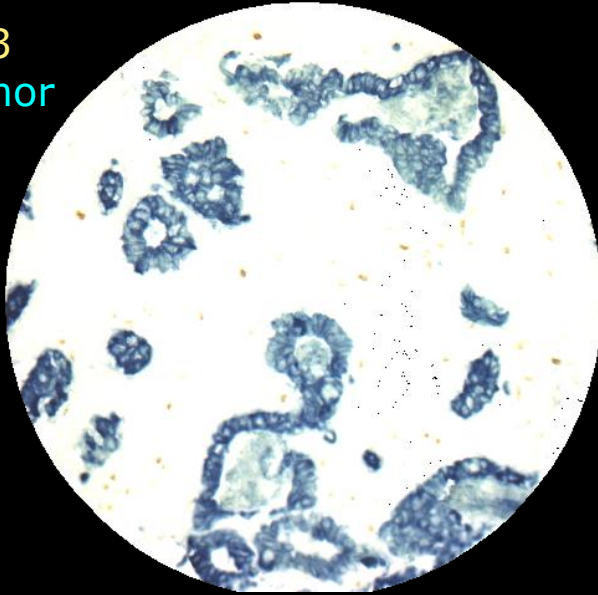
# Is the quantification of the pre-existing immunity with Immunoscore clinically relevant ?

Patient 1 (weak)

Patient 2 (moderate)

Patient 3 (strong)

CD3  
Tumor



**Immunoscore I 0**

**I 2**

**I 4**

CD3/CD8  
Center/Margin

Median OS < 2 years  
(death)

4.9 years

> 15 years

“All that is simple is wrong,  
But all that is not is useless”

*Paul Valéry, French poet and philosopher (1871-1945)*



# 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			
	Cribriiform comedo-type				
Host immune response	Immunoscore	CD3+ T cells	CD8+ T cells	Density	Location (CT, IM)

# 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

# Immunoscore Steering Committee



Bernard Fox,  
SITC Past-President  
Tumor-Immunologist



Jérôme Galon,  
SITC Board Member  
Tumor-Immunologist



Francesco Marincola,  
SITC Past-President  
Tumor-Immunologist



Paolo Ascierto,  
SITC Board Member  
Medical oncologist

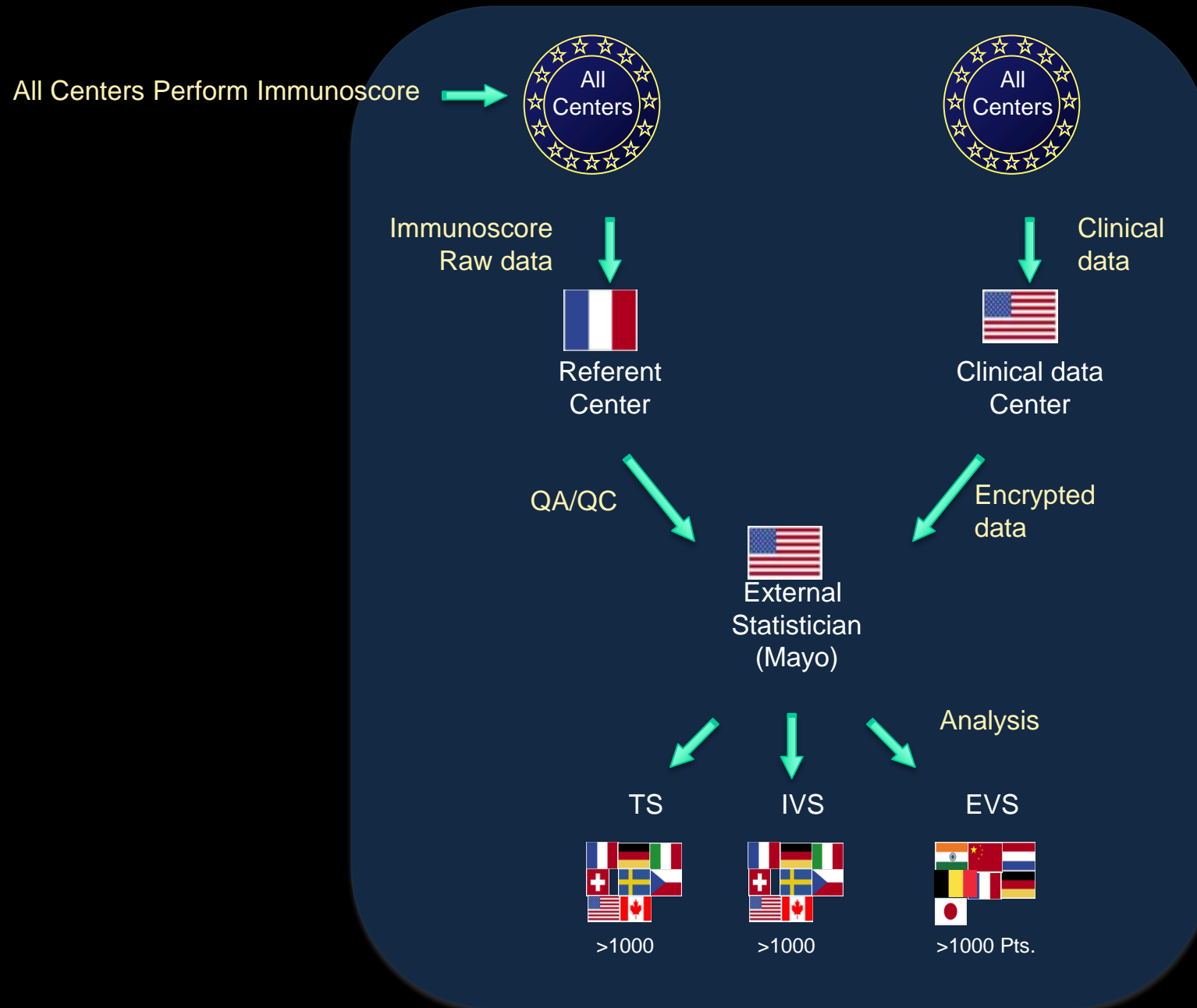


Carlo Bifulco,  
SITC Member  
Pathologist



# Worldwide Immunoscore consortium (PI: J Galon)

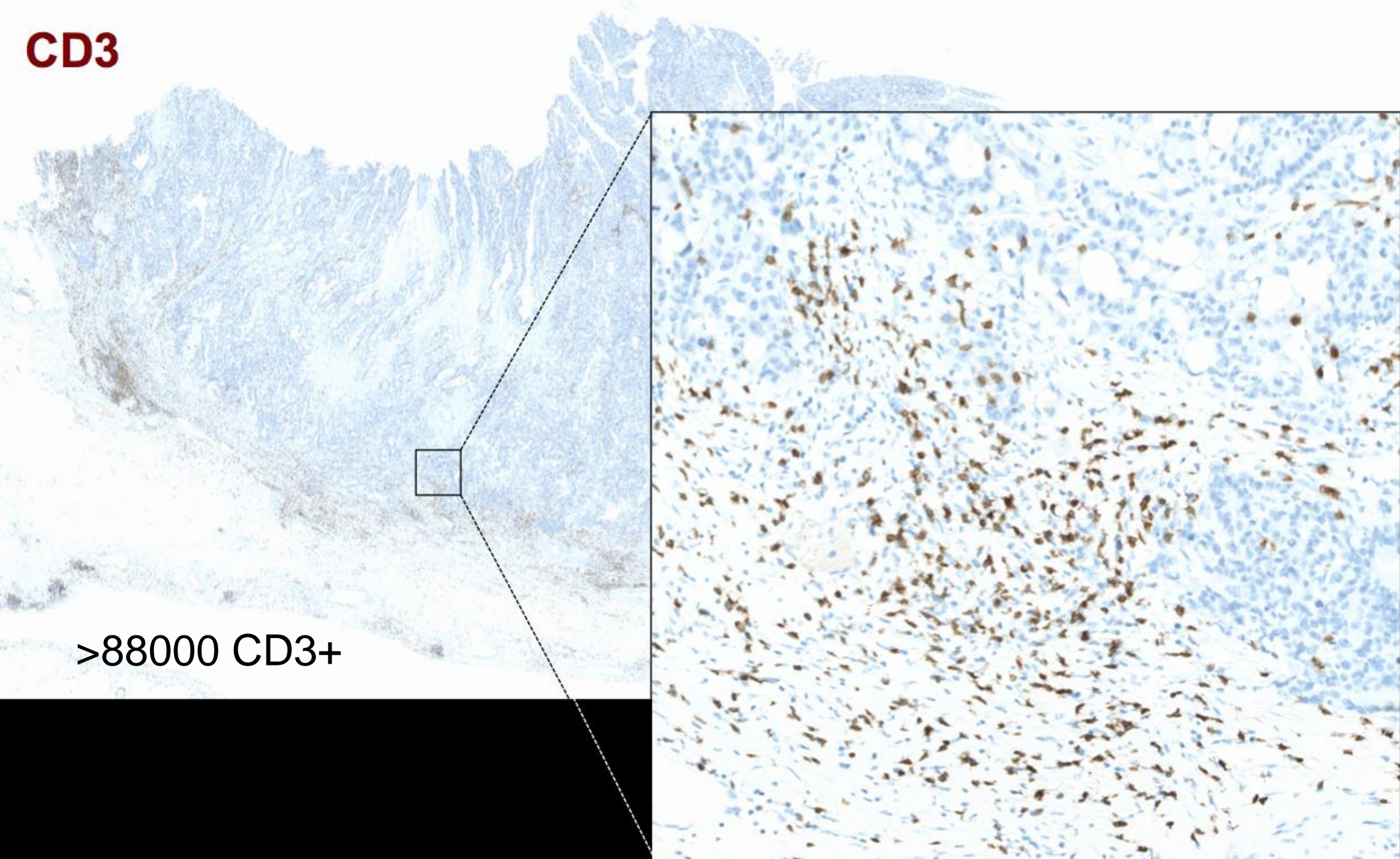
## Study design



# Immunoscore using whole slide FFPE

Routine whole slide stainings & full image quantification

**CD3**



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

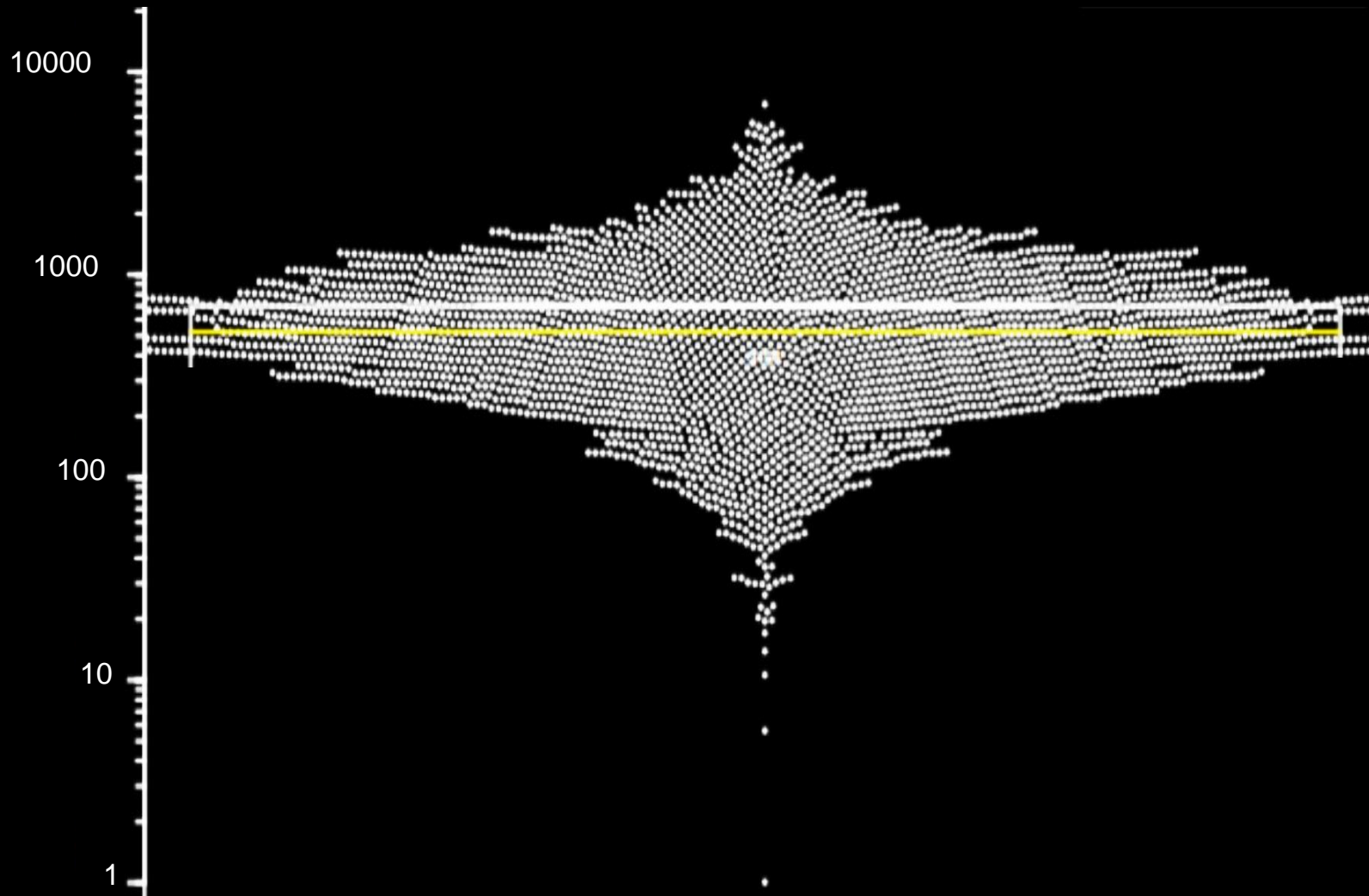
Franck Pagès, Bernhard Mlecnik, Florence Marliot, Gabriela Bindea, Fang-Shu Ou, Carlo Bifulco, Alessandro Lugli, Inti Zlobec, Tilman T Rau, Martin D Berger, Iris D Nagtegaal, Elisa Vink-Börger, Arndt Hartmann, Carol Geppert, Julie Kolwelter, Susanne Merkel, Robert Grützmann, Marc Van den Eynde, Anne 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 Petruzela, 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 Muşină, Dragos-Viorel Scripcariu, Boryana Popivanova, Mingli Xu, Tomonobu Fujita, Shoichi Hazama, Nobuaki Suzuki, Hiroaki Nagano, Kiyotaka Okuno, Toshihiko Torigoe, Noriyuki Sato, Tomohisa Furuhashi, 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



## Densities of CD3<sub>CT</sub> (cells/mm<sup>2</sup>) within tumors

CD3<sub>CT</sub>  
cells/mm<sup>2</sup>

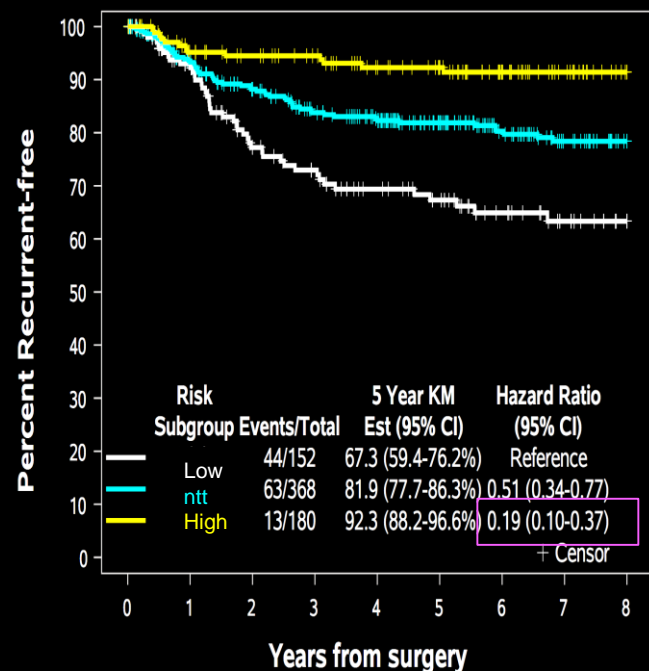
Quantification of 3855 patients



- ✓ Whole slide quantification within the CT region
- ✓ Similar quantification were performed for CD3<sub>CT</sub>, CD3<sub>IM</sub>, CD8<sub>CT</sub>, CD8<sub>IM</sub>

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

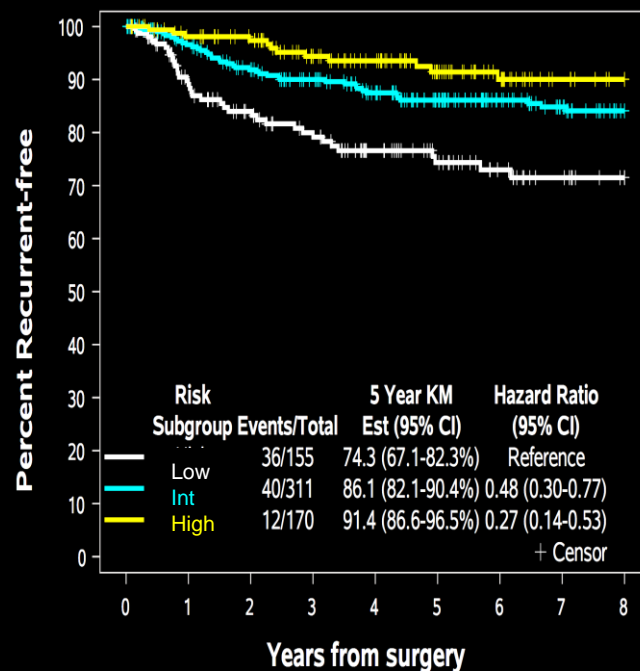
TS



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

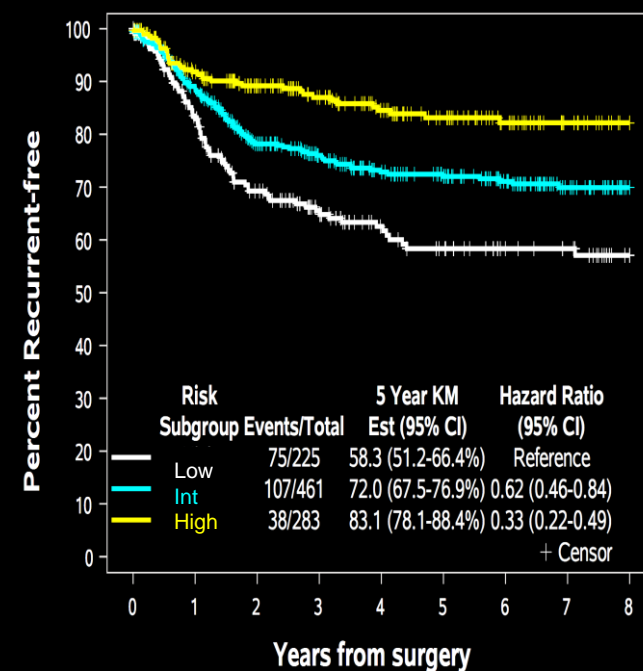
IVS



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

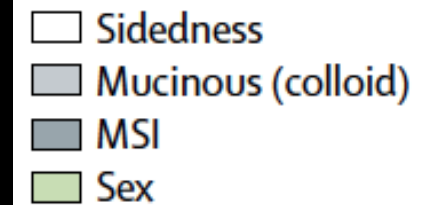
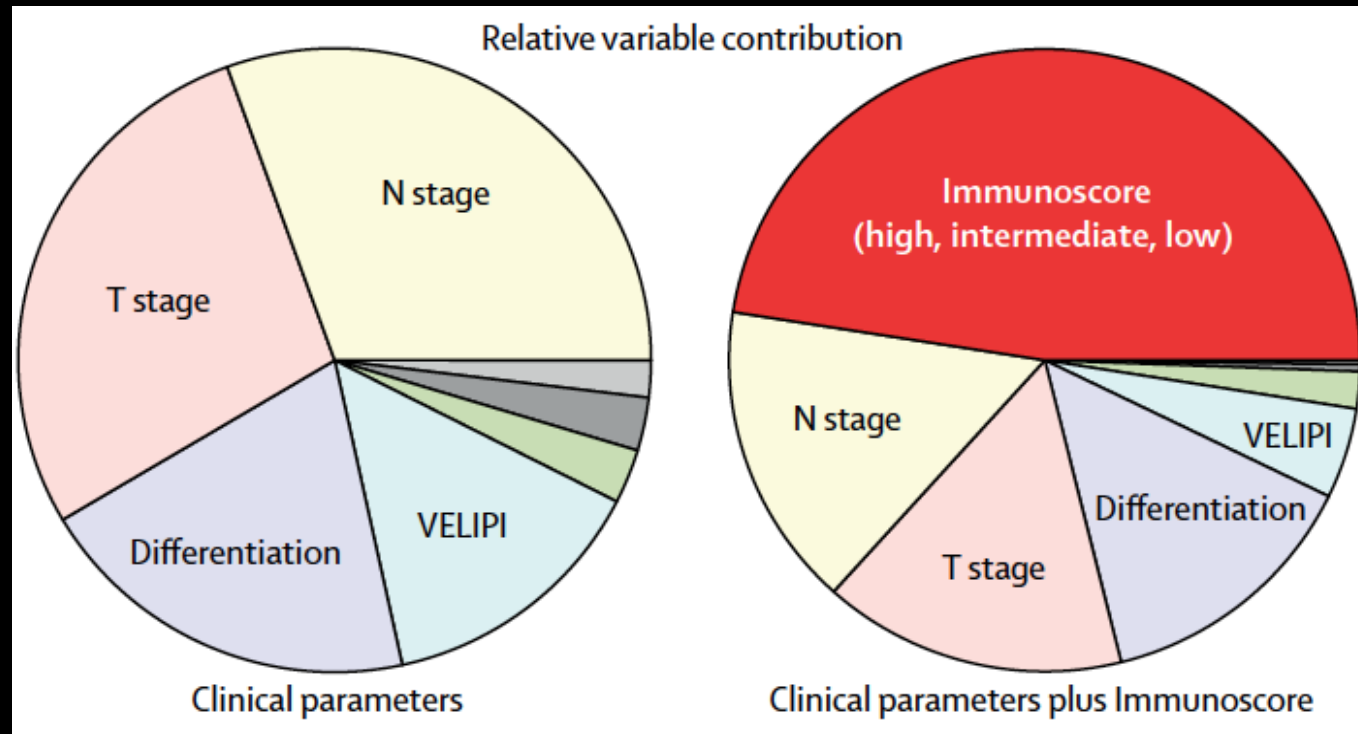
$P < 0.0001$   
 HR (0-2)= 0.33  
 C-index= 0.60

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.

# Relative variable contribution to risk

Chi squared proportion ( $\chi^2$ ) test for clinical parameters

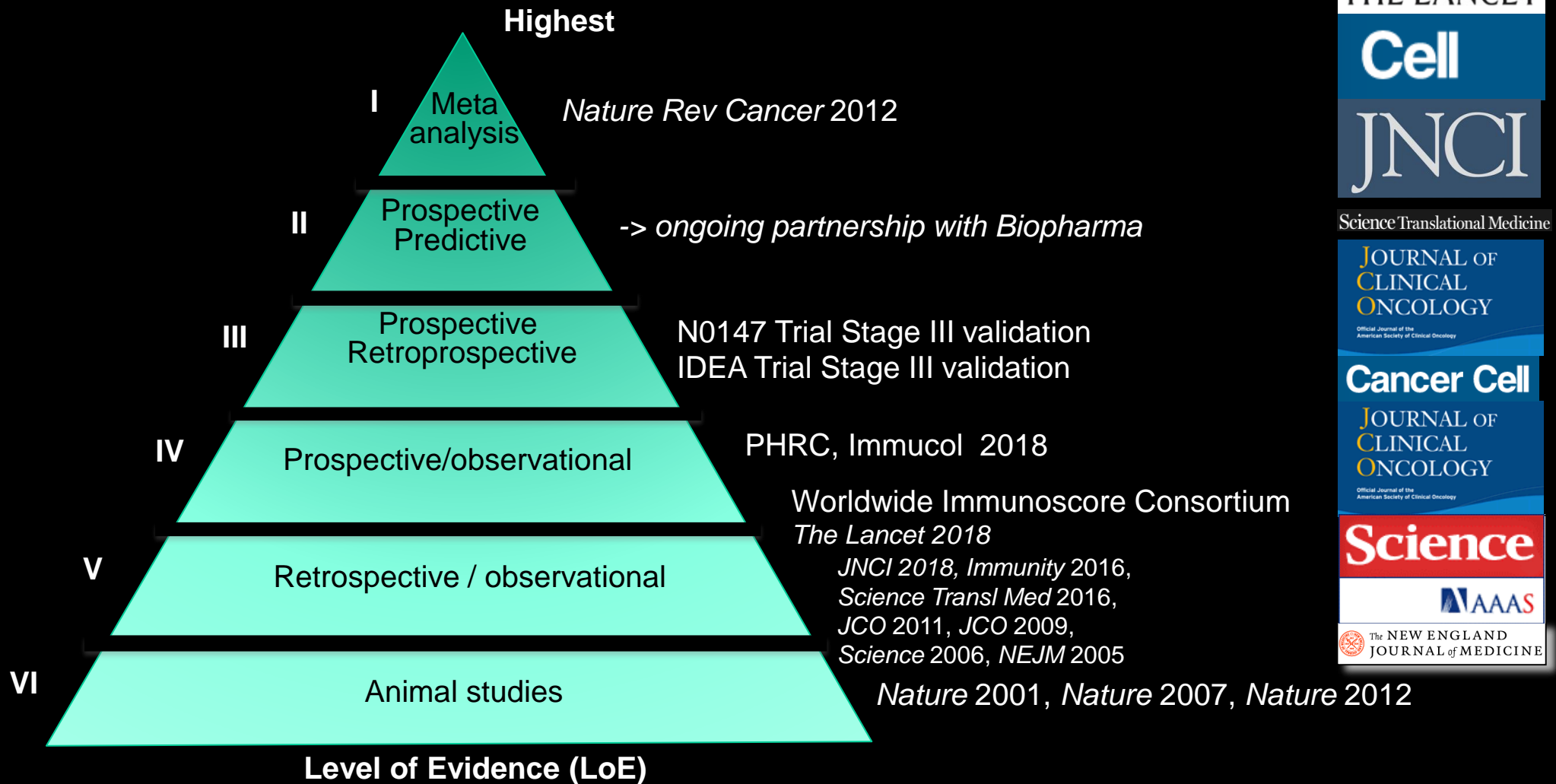


## All patients

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)



# Validation of Immunoscore : Level of Evidence



**Immunoscore is validated at multiple level of evidence (LoE)**

# 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: I-TNM

# Cancer classification

*Many*

Tumor cell characteristics

*None (until today)*

Host immune characteristics

**Tumor cell  
extension  
and  
invasion**

T N M

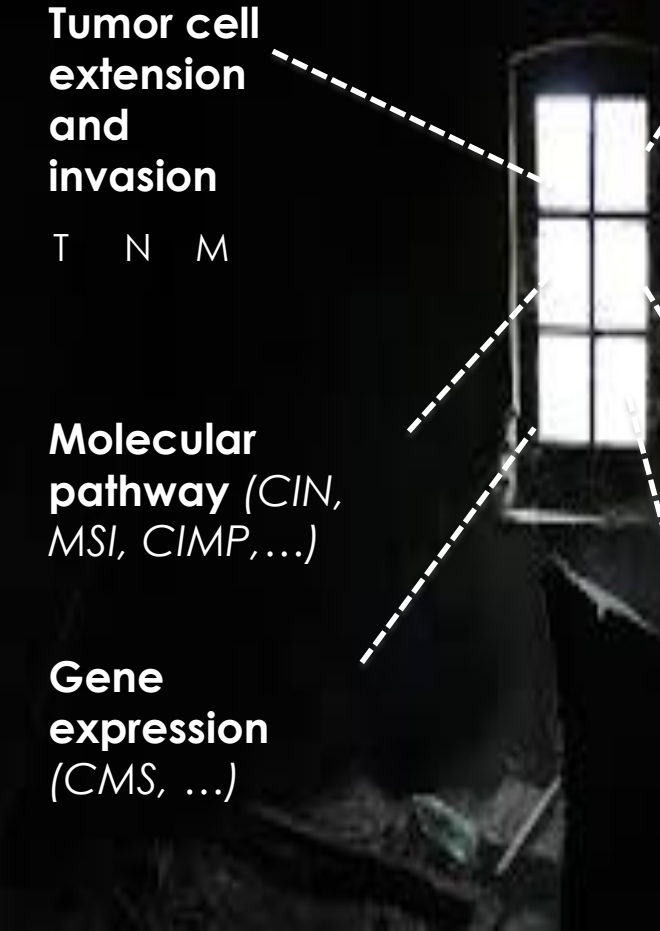
**Molecular  
pathway** (CIN,  
MSI, CIMP,...)

**Gene  
expression**  
(CMS, ...)

**Morphology**  
(medullary,  
mucinous,  
serrated, ...)

**Cell of origin**  
(Stem-like,  
goblet, ...)

**Mutation status**  
(APC, TP53,  
BRAF, KRAS, ...)



# Cancer classification

Many

Tumor cell characteristics

**Tumor cell extension and invasion**

T N M

**Molecular pathway** (CIN, MSI, CIMP,...)

**Gene expression** (CMS, ...)

**Morphology** (medullary, mucinous, serrated, ...)

**Cell of origin** (Stem-like, goblet, ...)

**Mutation status** (APC, TP53, BRAF, KRAS, ...)

None (until today)

Host immune characteristics

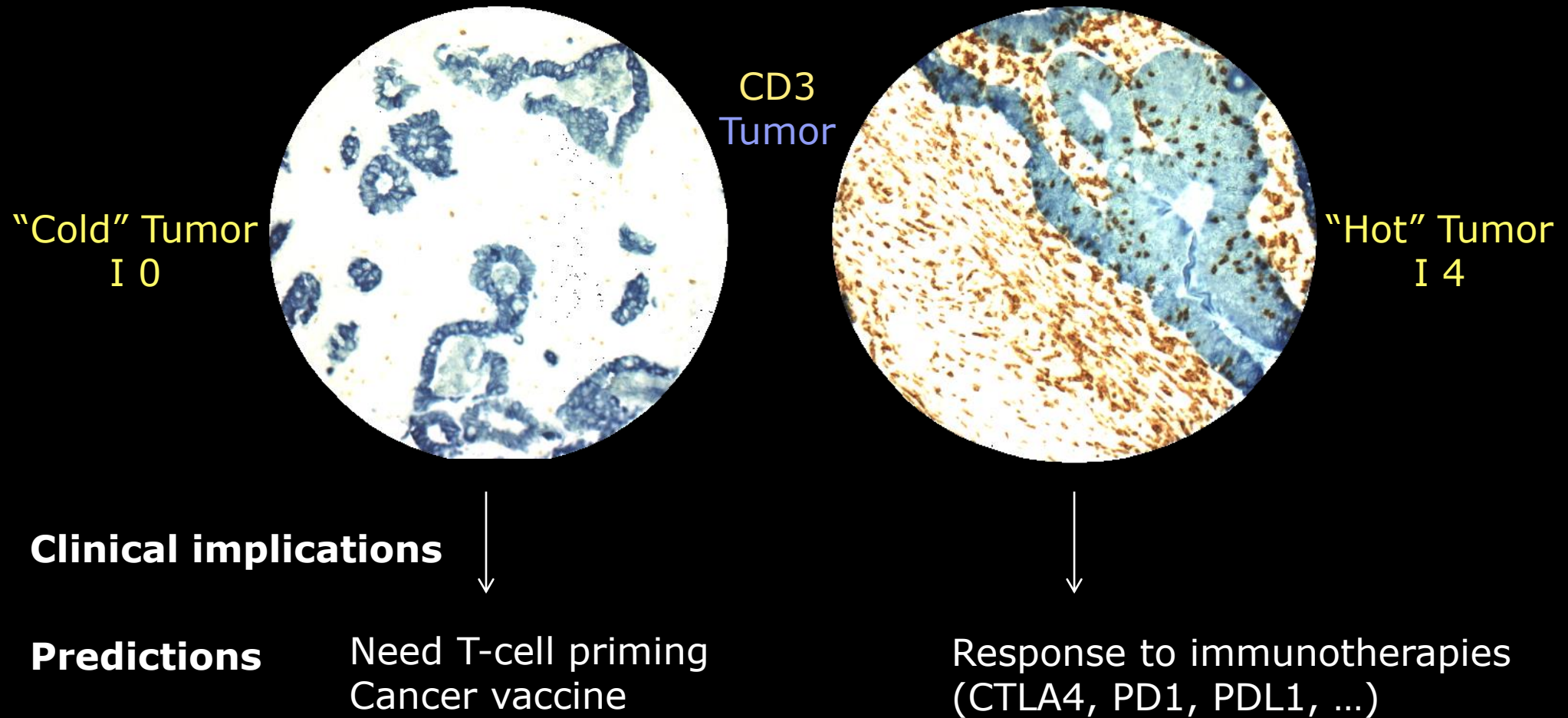
**Immunoscore, Microenvironment,**

...



# Deciphering the tumor immune microenvironment: Clinical implications

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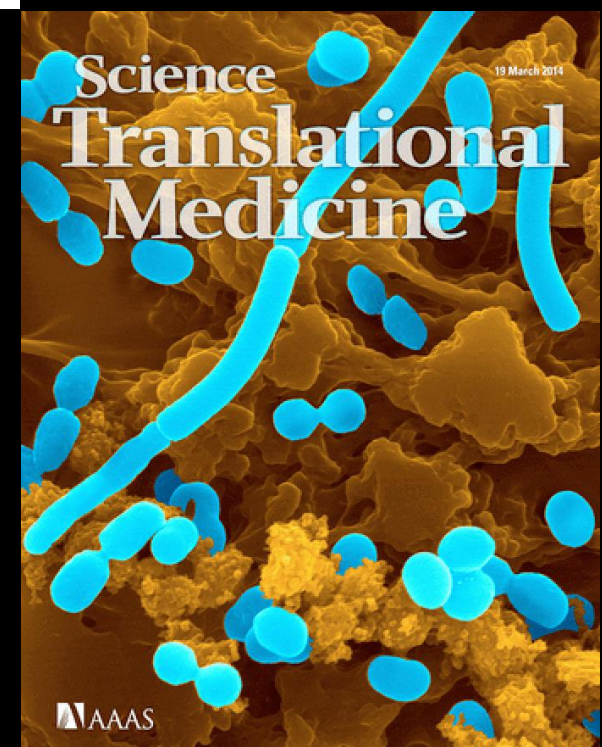
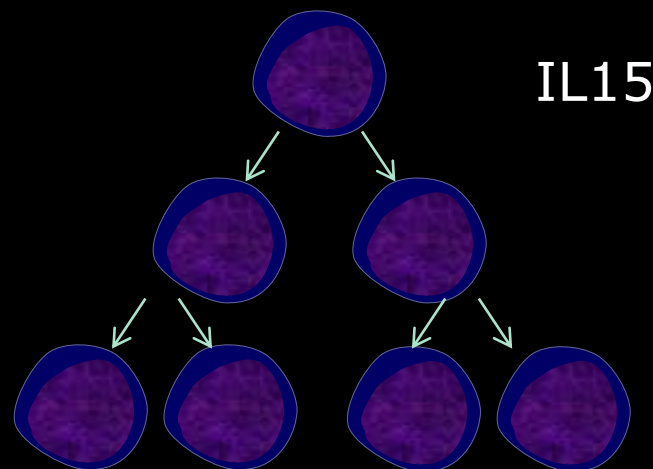
But it is not as simple since biology is complex and is not dichotomized in good & bad

## CANCER

# Functional Network Pipeline Reveals Genetic Determinants Associated with in Situ Lymphocyte Proliferation and Survival of Cancer Patients

Bernhard Mlecnik,<sup>1,2,3\*</sup> Gabriela Bindea,<sup>1,2,3\*</sup> Helen K. Angell,<sup>1,2,3</sup> Maria Stella Sasso,<sup>1,2,3</sup> Anna C. Obenauf,<sup>4</sup> Tessa Fredriksen,<sup>1,2,3</sup> Lucie Lafontaine,<sup>1,2,3</sup> Amelie M. Bilocq,<sup>1,2,3</sup> Amos Kirilovsky,<sup>1,2,3</sup> Marie Tosolini,<sup>1,2,3</sup> Maximilian Waldner,<sup>1,2,3,5</sup> Anne Berger,<sup>6</sup> Wolf Herman Fridman,<sup>2,3,7</sup> Arash Rafii,<sup>8</sup> Viia Valge-Archer,<sup>9</sup> Franck Pagès,<sup>1,2,3,10</sup> Michael R. Speicher,<sup>4</sup> Jérôme Galon<sup>1,2,3†</sup>

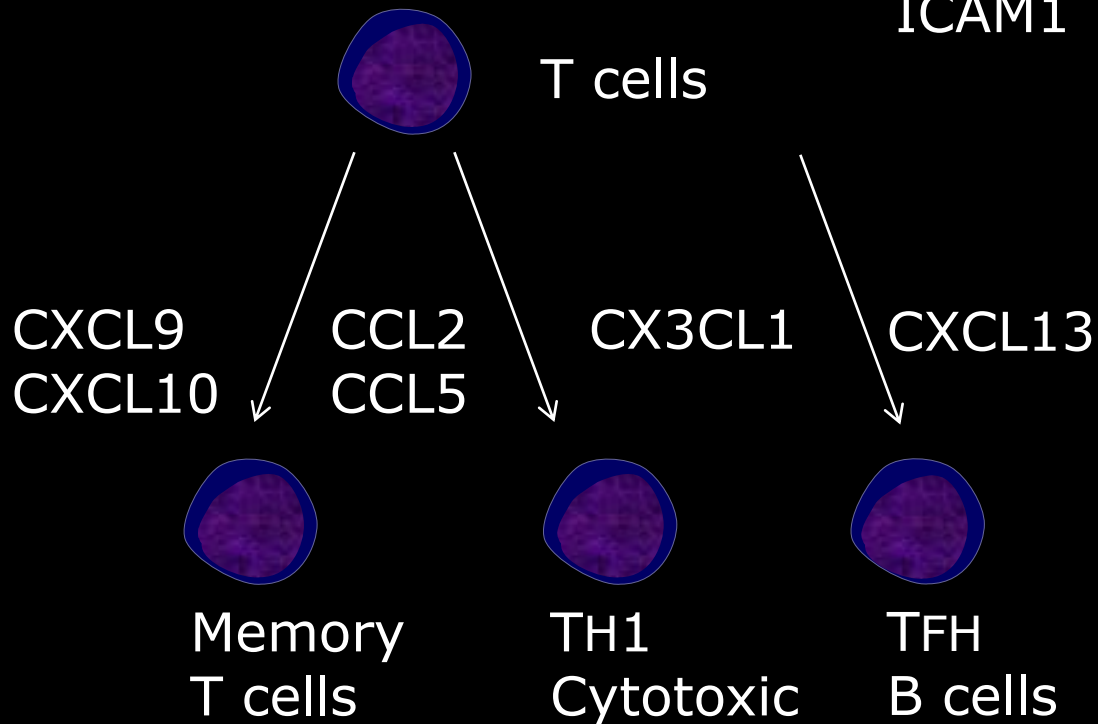
## IL15-dependent Local lymphocyte proliferation





# Mechanisms associated with T cells infiltration

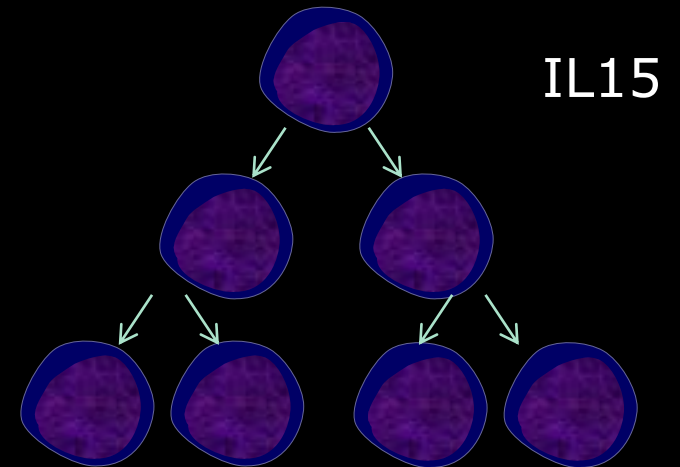
## Attraction



## Adhesion

MADCAM1  
VCAM1  
ICAM1

## Local lymphocyte proliferation



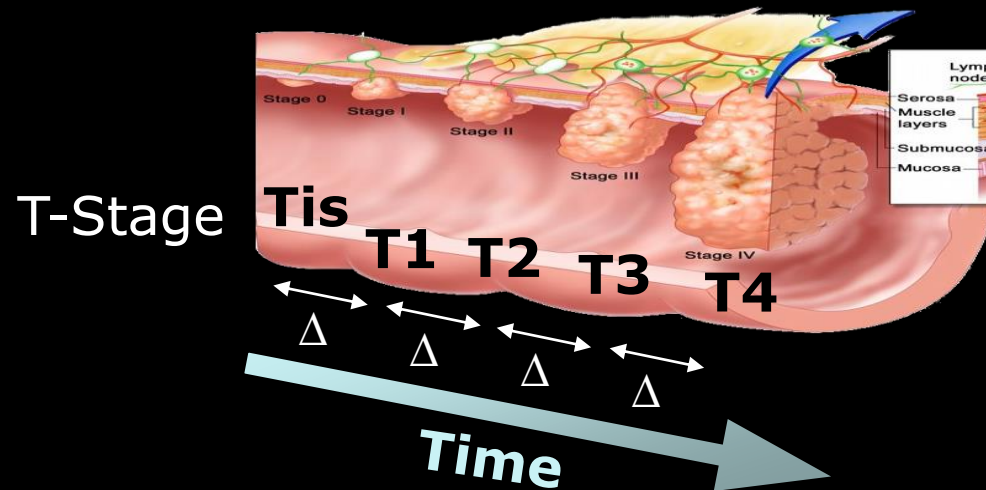
Mlecnik et al. *Gastroenterology* 2010

Mlecnik et al. *Science Transl Med* 2014

Bindea et al. *Immunity* 2013

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

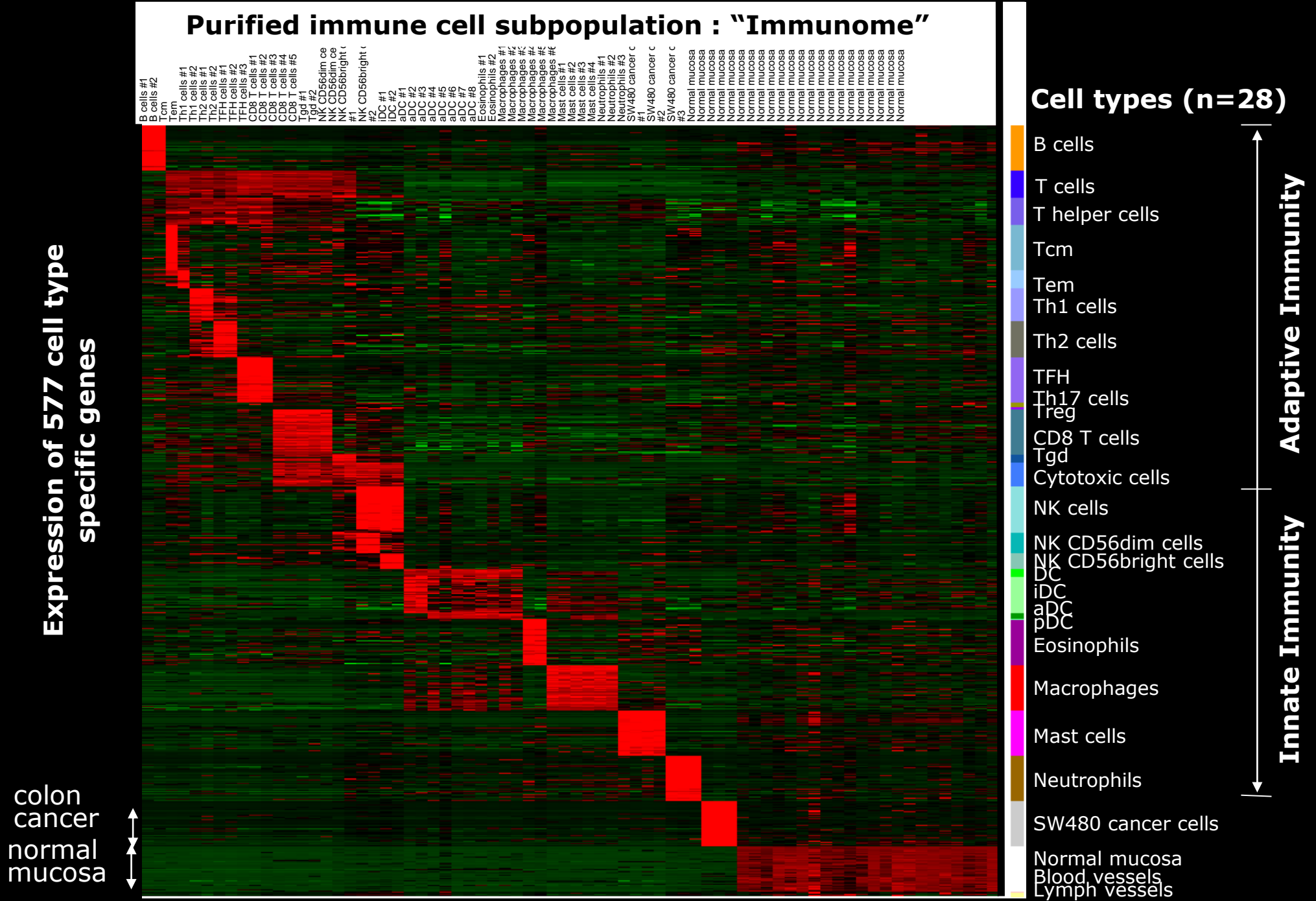
- Evolution of the tumor microenvironment with tumor progression?
- Immune escape mechanisms in human tumors?



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

Bindea G et al. *Immunity*, 2013

# "Immunome" of purified immune cell subpopulations



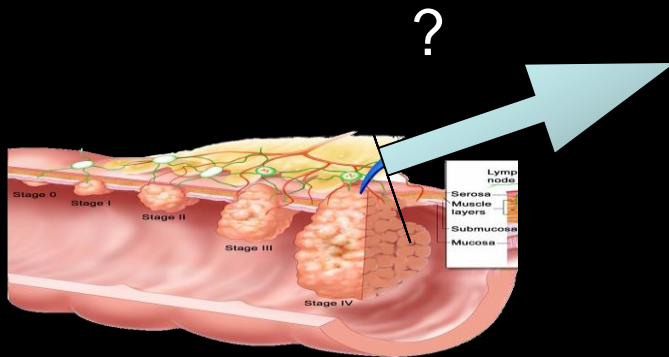






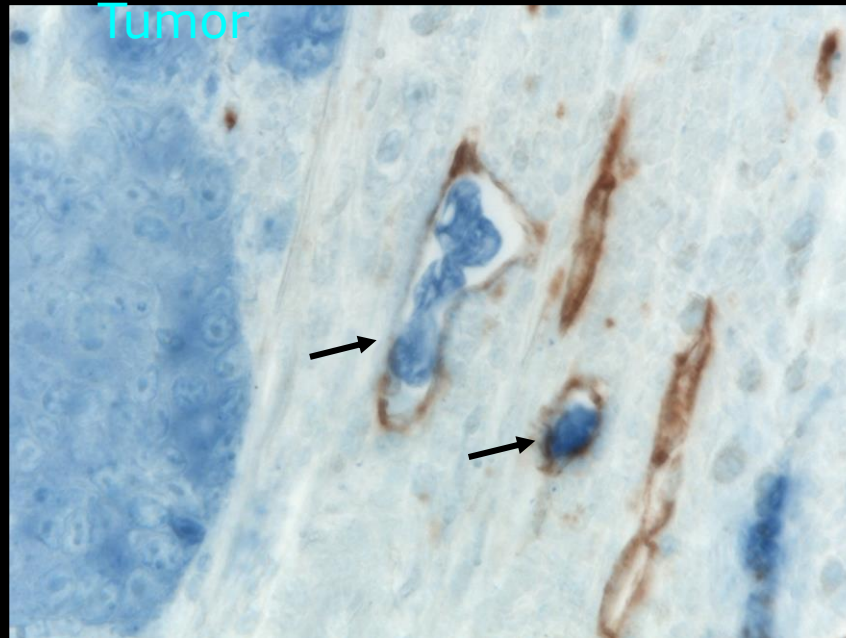
# What are the mechanisms of early-metastatic dissemination ?

VELIPI: Venous Emboli, Lymphatic Invasion, Perineural Invasion



Lymphatic emboli /

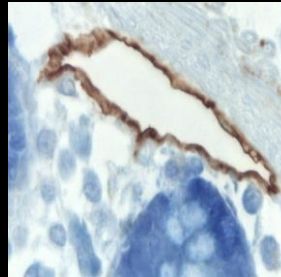
Tumor



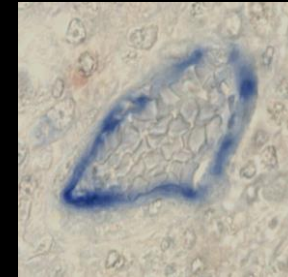
## Questions

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

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



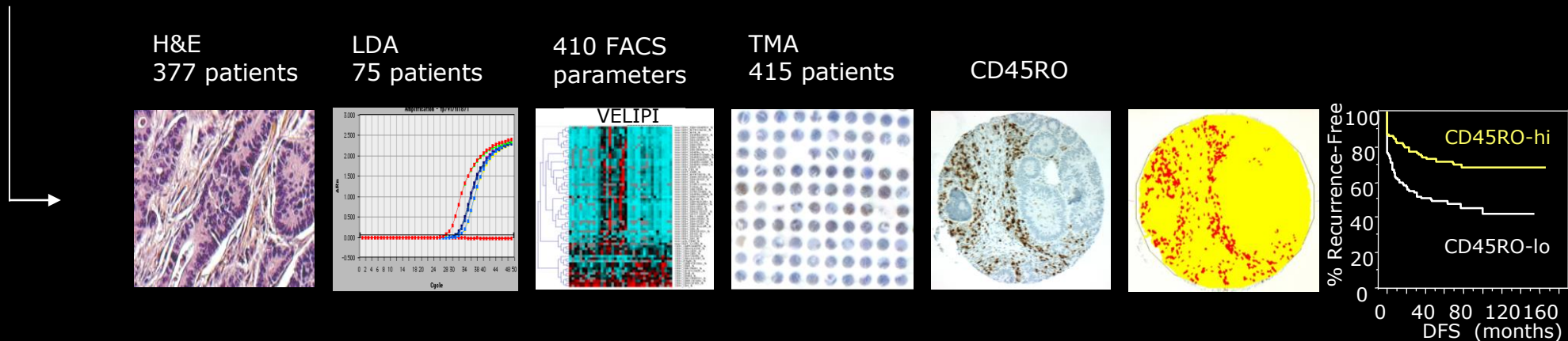
Lymphatic  
vessels  
(PDPN)



blood  
vessels  
(Endoglin)

-> Global analysis of tumor microenvironment

**VELIPI** is prognostic  
**959** colorectal cancer patients



Pagès F et al. *New Engl J Med* 2005

ORIGINAL ARTICLE

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

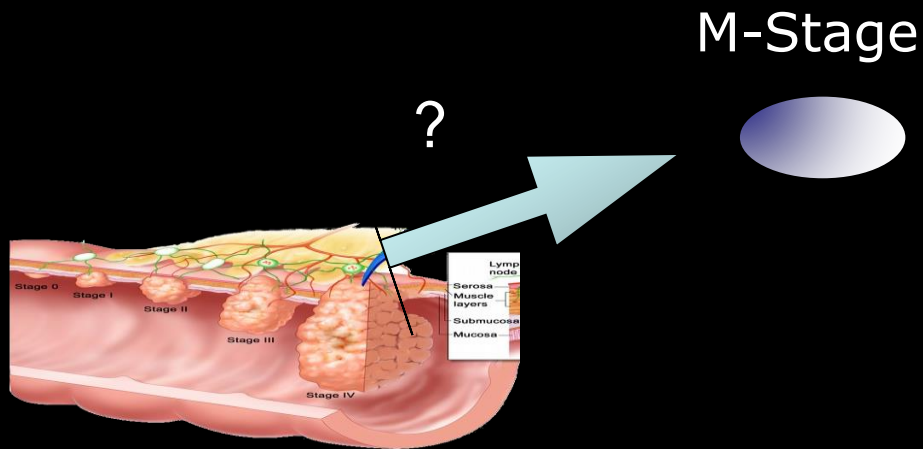
Franck Pagès, M.D., Ph.D., Anne Berger, M.D., Ph.D., Matthieu Camus, M.Sc.,  
Fatima Sanchez-Cabo, Ph.D., Anne Costes, B.S., Robert Molidor, Ph.D.,  
Bernhard Mlecnik, M.Sc., Amos Kirilovsky, M.Sc., Malin Nilsson, B.S.,  
Diane Damotte, M.D., Ph.D., Tchao Meatchi, M.D., Patrick Bruneval, M.D., Ph.D.,  
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<sub>EM</sub> correlate with the absence of early-metastatic invasion, and improved clinical outcome in colorectal carcinoma.

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

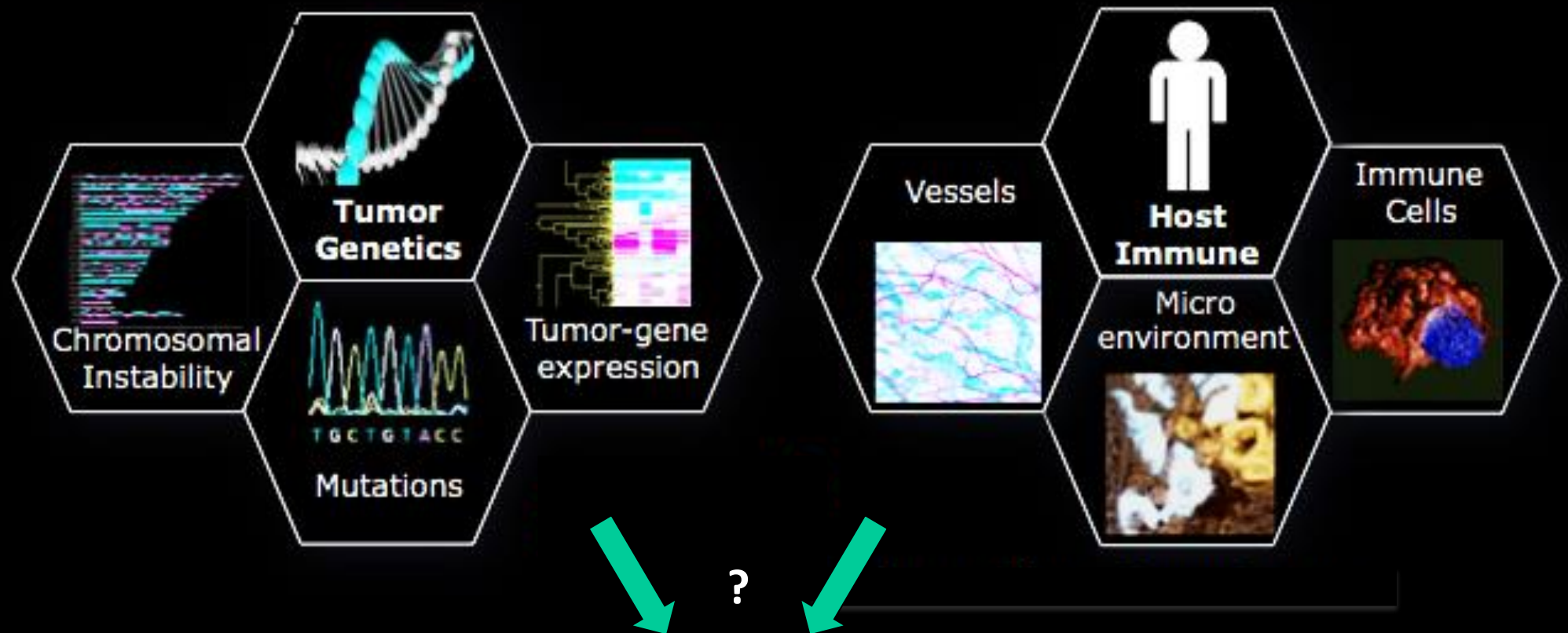
Pagès F & Galon J. **N Engl J Med.** 2006

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





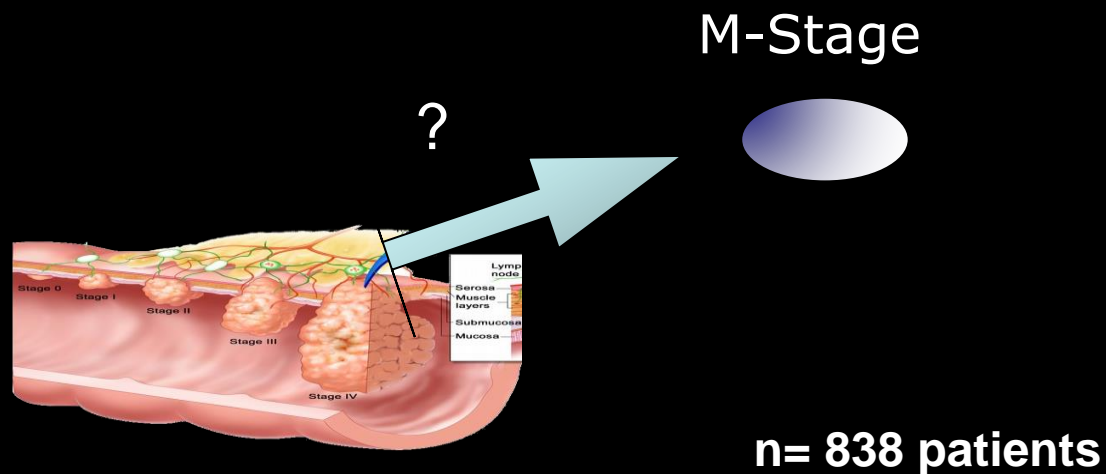
# What drives metastasis?



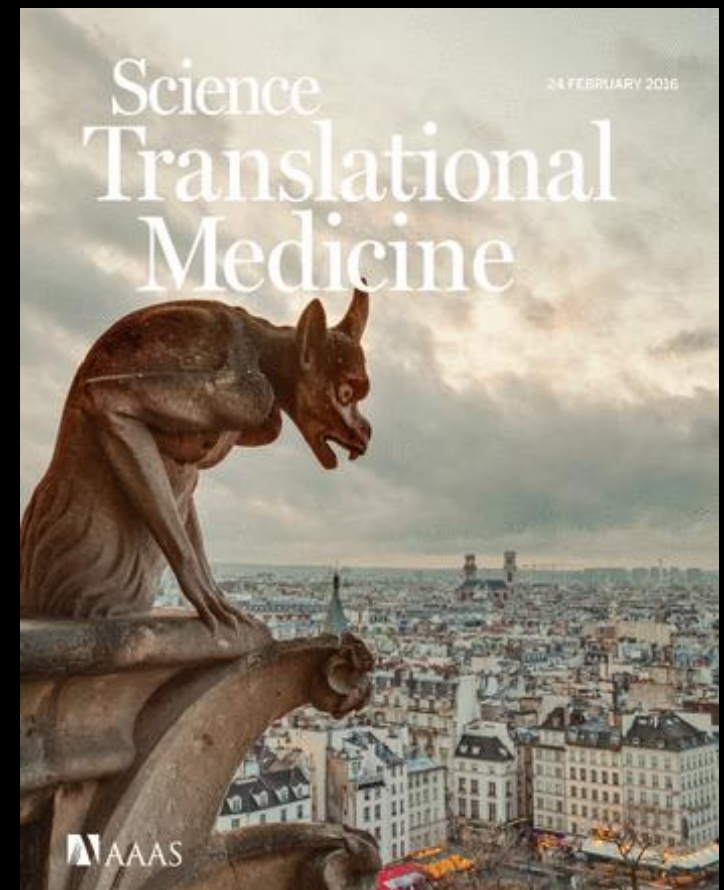
## CANCER

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

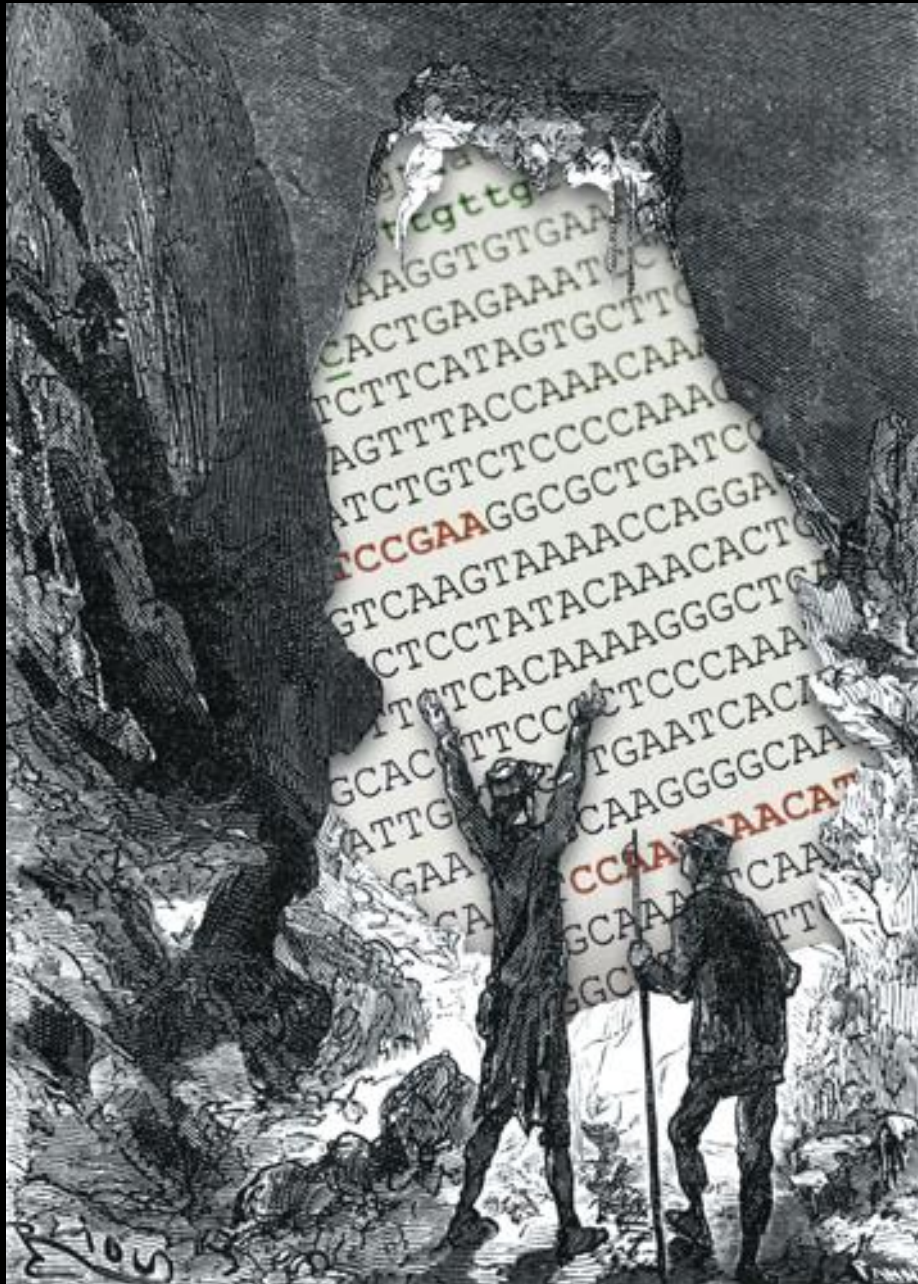
Bernhard Mlecnik,<sup>1,2,3\*</sup> Gabriela Bindea,<sup>1,2,3\*</sup> Amos Kirilovsky,<sup>1,2,3\*</sup> Helen K. Angell,<sup>1,2,3,4</sup> Anna C. Obenauf,<sup>5</sup> Marie Tosolini,<sup>1,2,3</sup> Sarah E. Church,<sup>1,2,3</sup> Pauline Maby,<sup>1,2,3</sup> Angela Vasaturo,<sup>1,2,3</sup> Mihaela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



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.



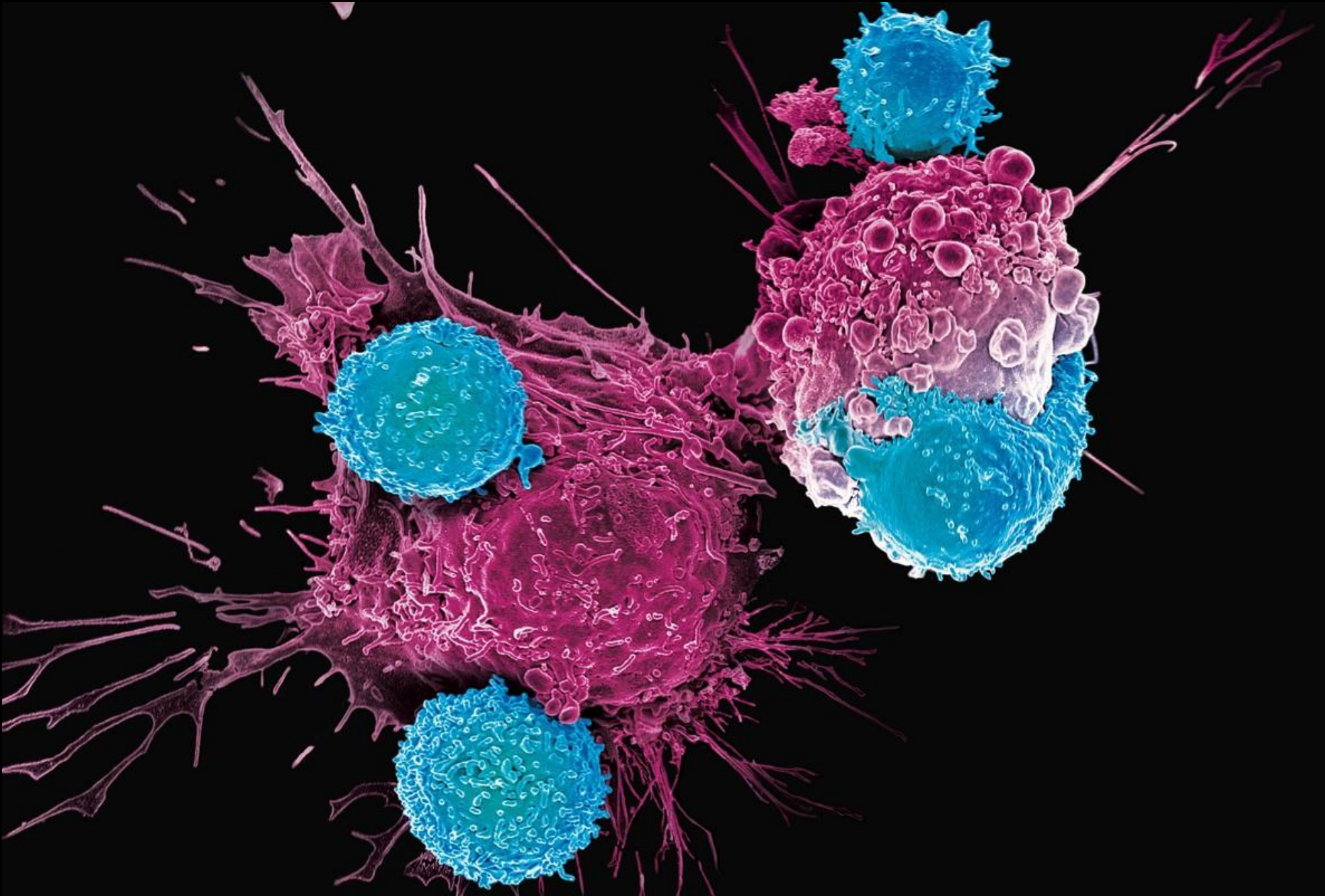
Even if important, cancer is not only about DNA



Adapted from  
"Twenty Thousand Leagues Under the Sea"  
Jules Verne 1869



# Human immune T-cells fighting cancer cells





**Deep-sequencing revealed multiple genotype defect in tumor cells**



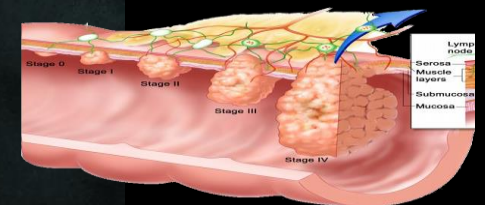


# What is the mechanistic impact of DNA-mismatch repair deficiency on the immune response?

hotspot → DNA polymerase slippage

A A A A A A A

DNA-mismatch  
Repair deficiency  
(pMMR, dMMR (MSI) patients)



# What are the mechanistic relationships between tumor genotype and Immunoscore ?

CellPress

Immunity  
Article

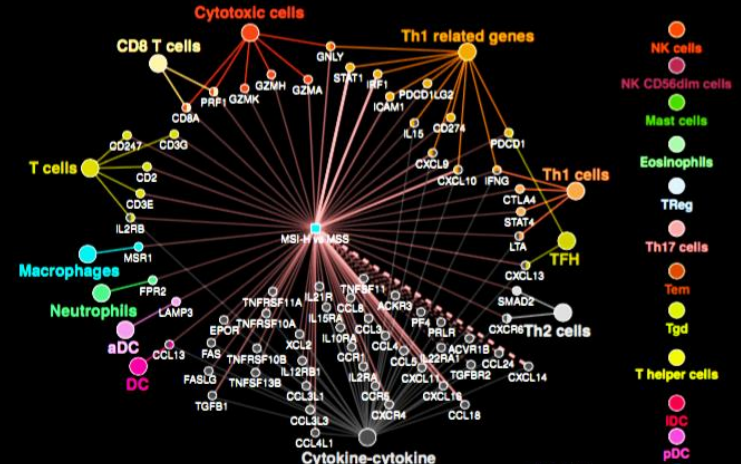
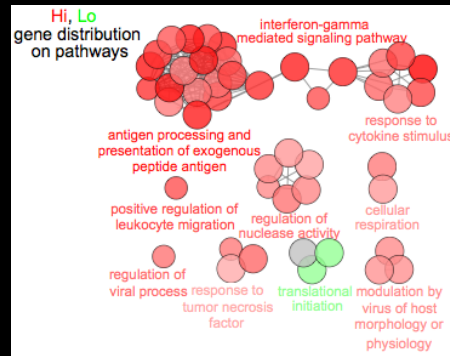
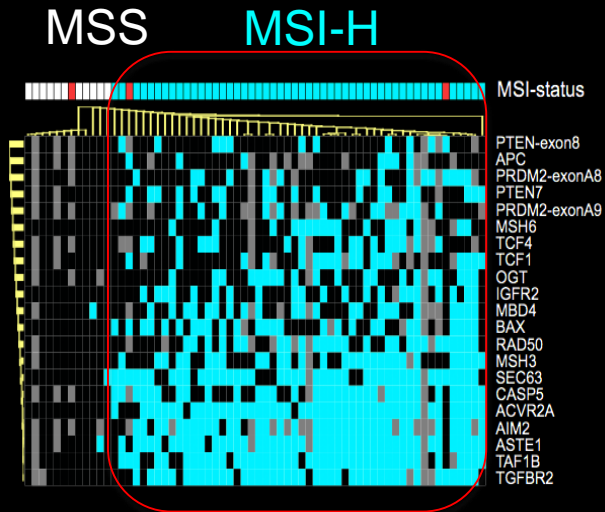
## Integrative Analyses of Colorectal Cancer Show Immunoscore Is a Stronger Predictor of Patient Survival Than Microsatellite Instability

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

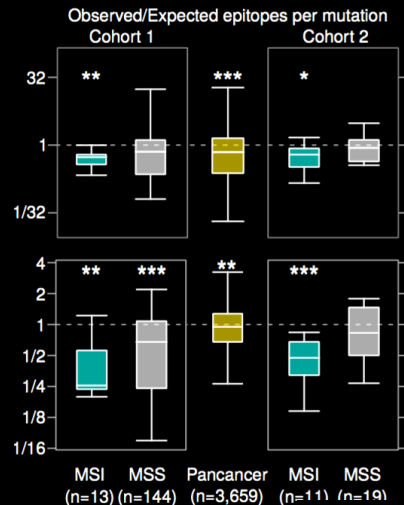
TCGA CRC cohort: n= 270 patients

Inserm cohort: n= 689 patients

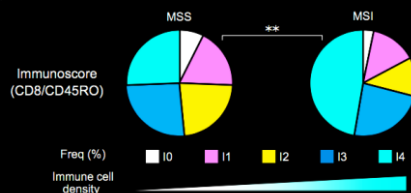
# Mechanistic impact of DNA-mismatch repair deficiency



Increased frequency of  
Frameshift mutations



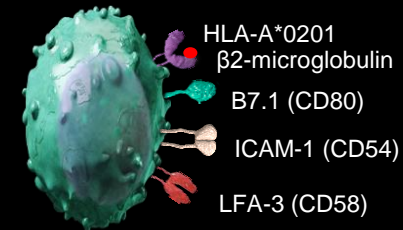
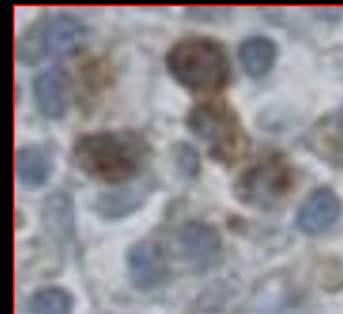
Genetic evidence  
of Immunoediting



Increased frequency of High-Immunoscore

Increased Proliferating T-cells, Th1, cytotoxic T-cells

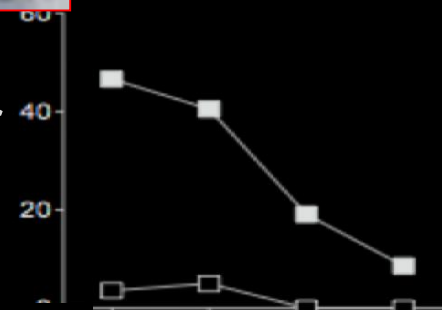
Anti-TGFB2mutFS Specific T-cells



CRC cells

<sup>51</sup>Cr

Anti-tumor T-cell killing

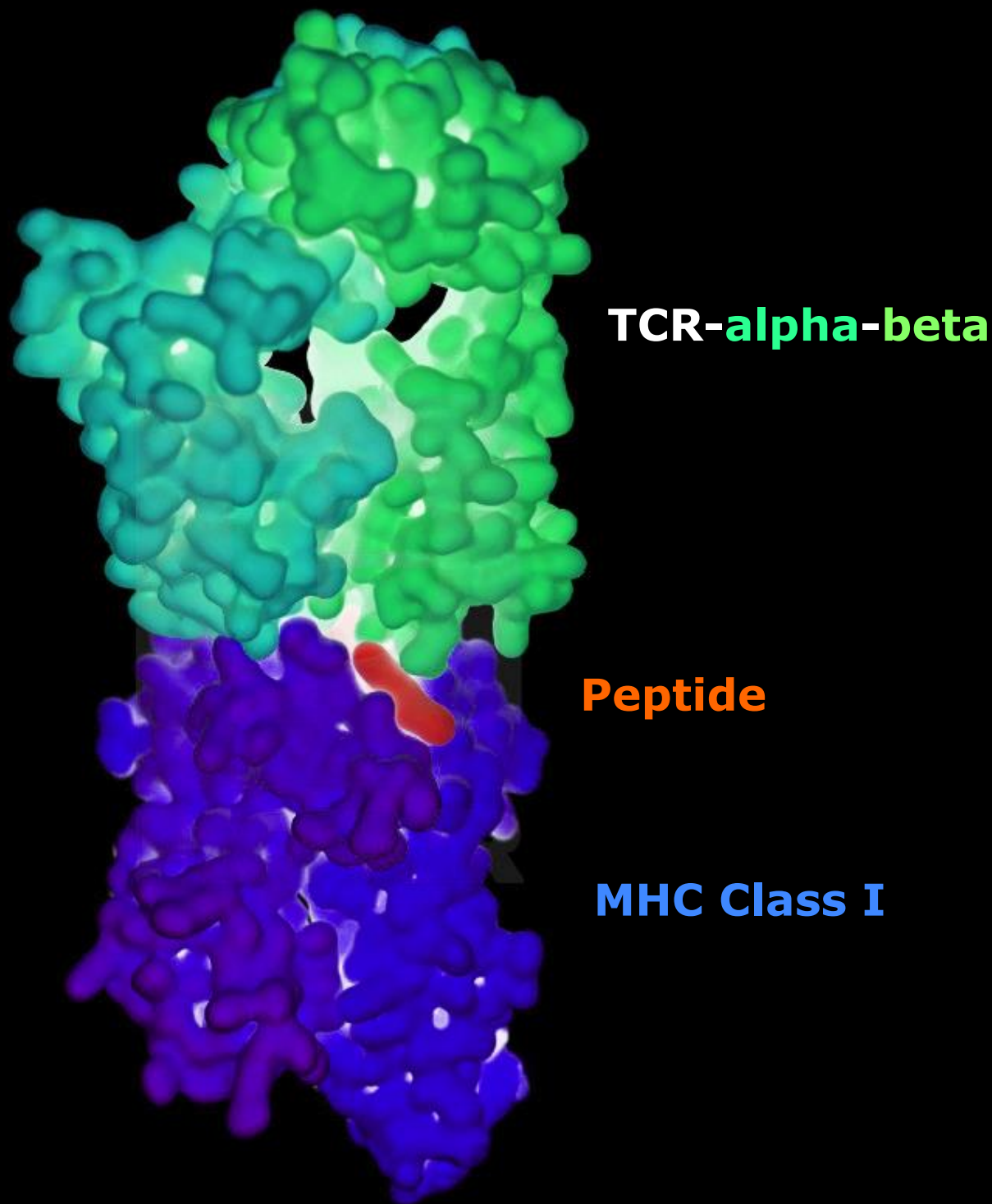


Mlecnik et al. *Immunity* 2016



# MHC-Peptide-TCR

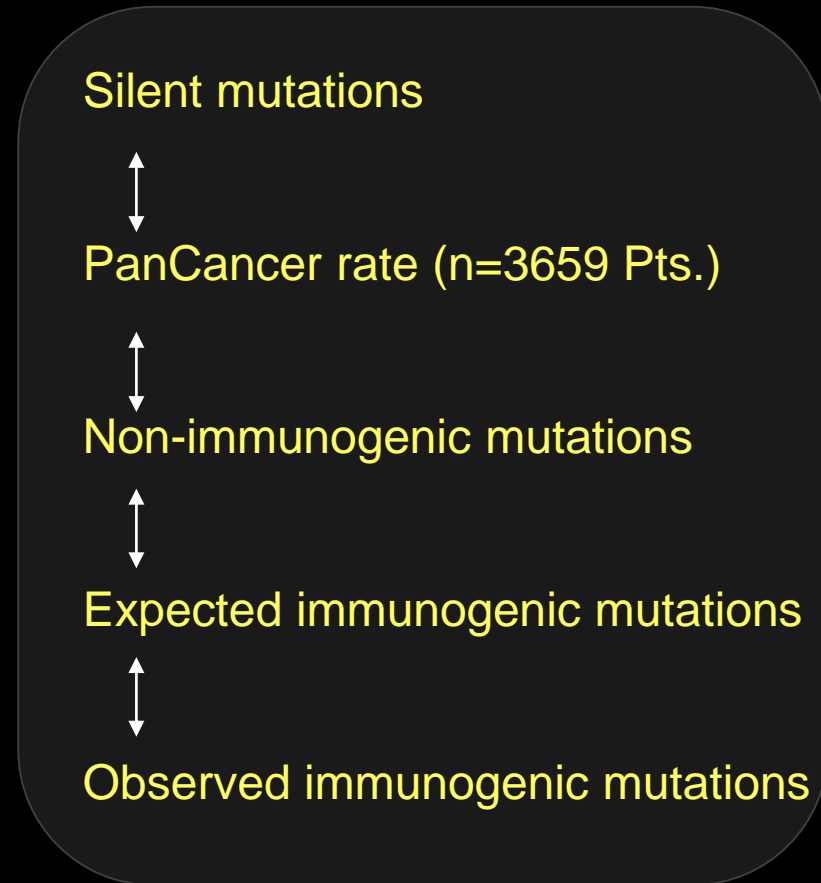
- ✓ ExomeSeq
- ✓ RNAseq
- ✓ Mutations detection
- ✓ Variant calling
- ✓ HLA haplotypes prediction
- ✓ Epitopes prediction
- ✓ HLA / TCR peptide binding prediction
- ✓ Immunogenicity scores





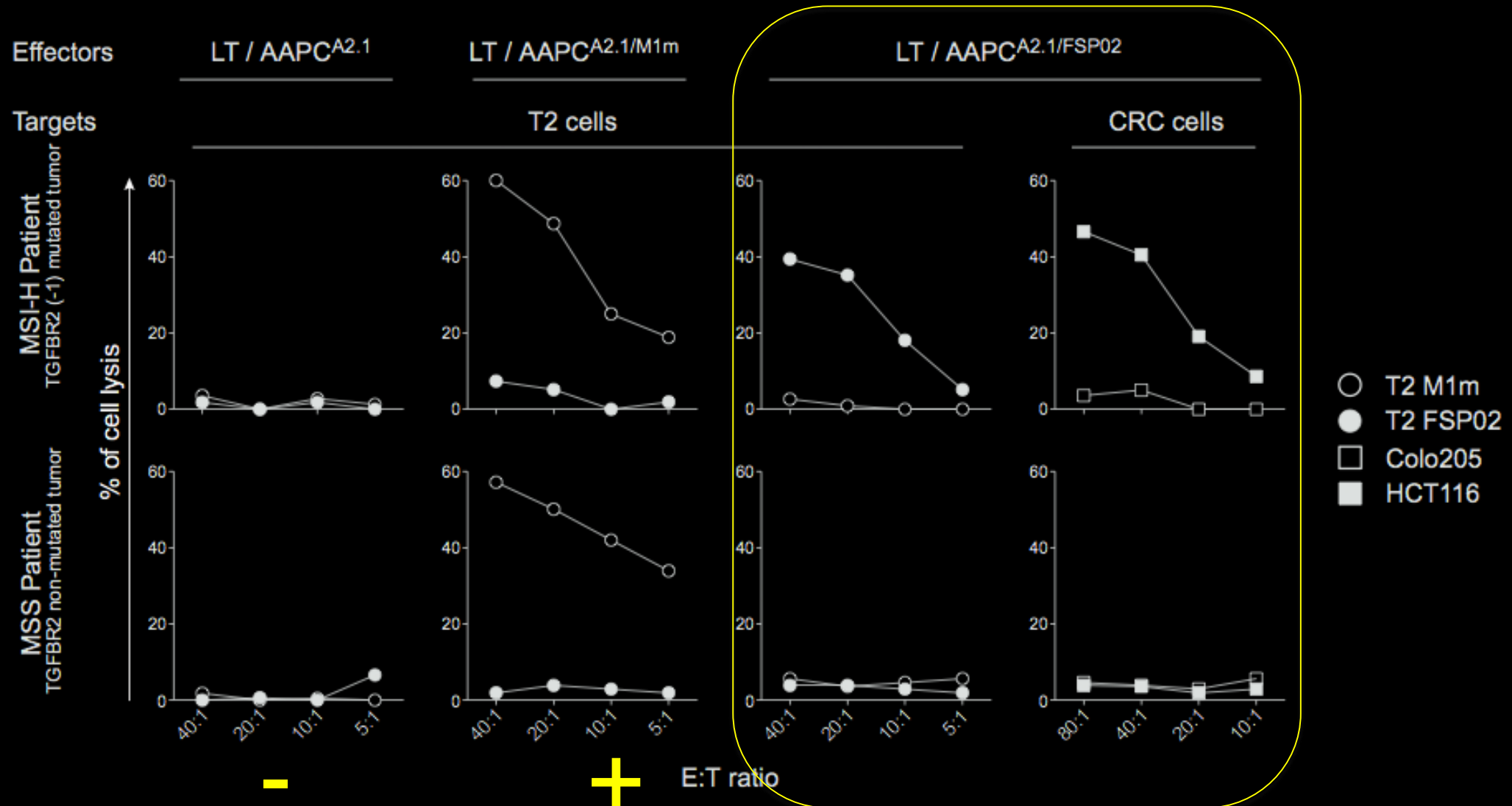
# Observed compared to expected frameshift and missense epitopes (immunogenic mutations) using ExomeSeq data

Genetic analysis of missense and frameshift immunogenic mutations (epitopes)



**Demonstration of Immunoediting (misense and frameshift) with Genetic evidence**

# MSI-H patients with TGFR2 FSmut have anti-TGFR2-FSmut T-cells able to kill APC<sup>A2.1/FSmutP2</sup> cells



**Anti-Tumor specific T-cells are frequent:  
MSI-H patients with different FSmut have  
anti-FSmut T-cells able to kill APC<sup>A2.1/FSmutP2</sup> cells**

**FSmut**

**TGFBR2**

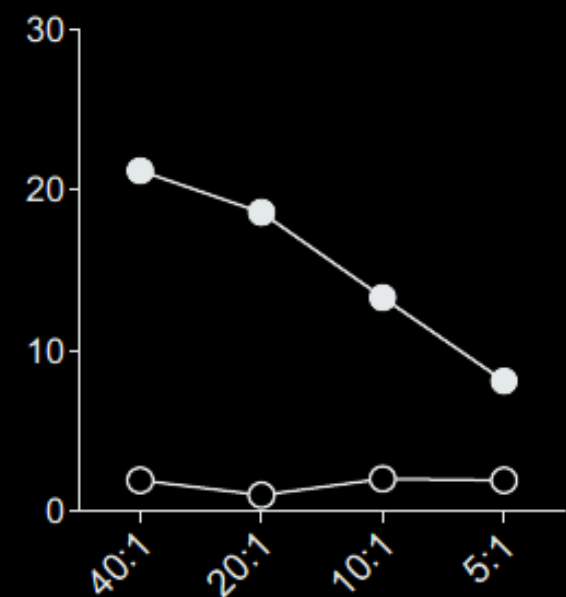
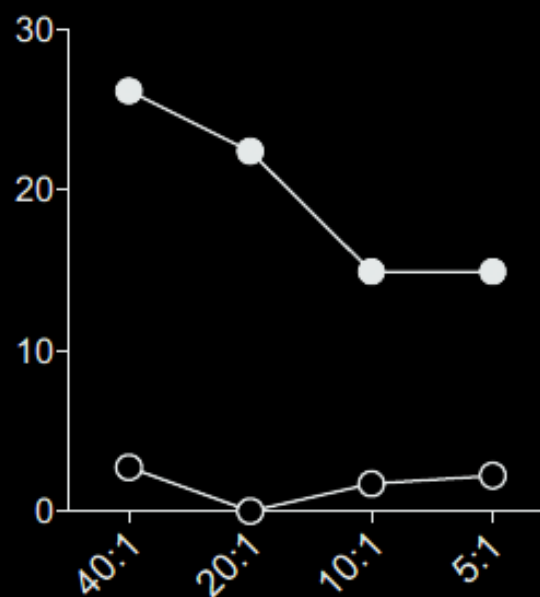
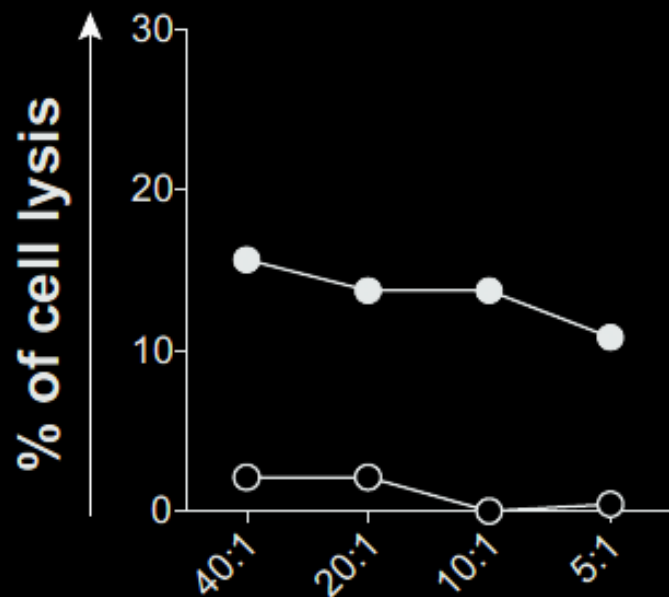
**TAF1B**

**ASTE1**

**AAPC<sup>A2.1/FSP02</sup>**

**AAPC<sup>A2.1/FSP27</sup>**

**AAPC<sup>A2.1/FSP30</sup>**

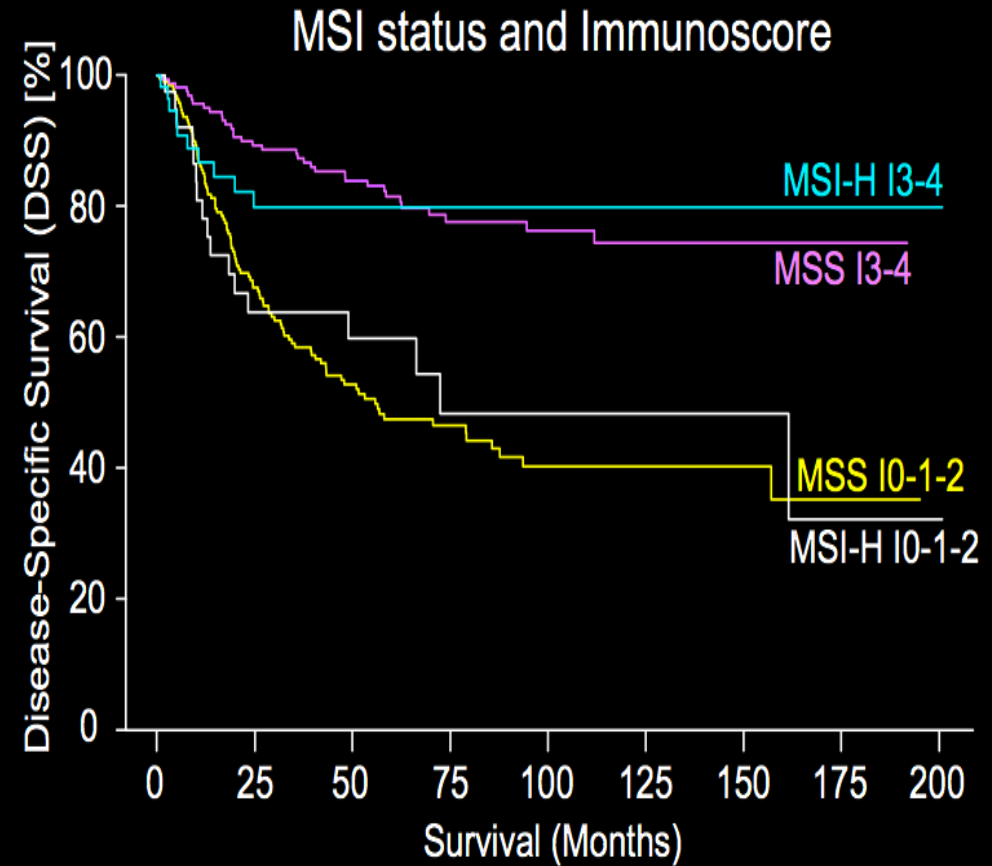
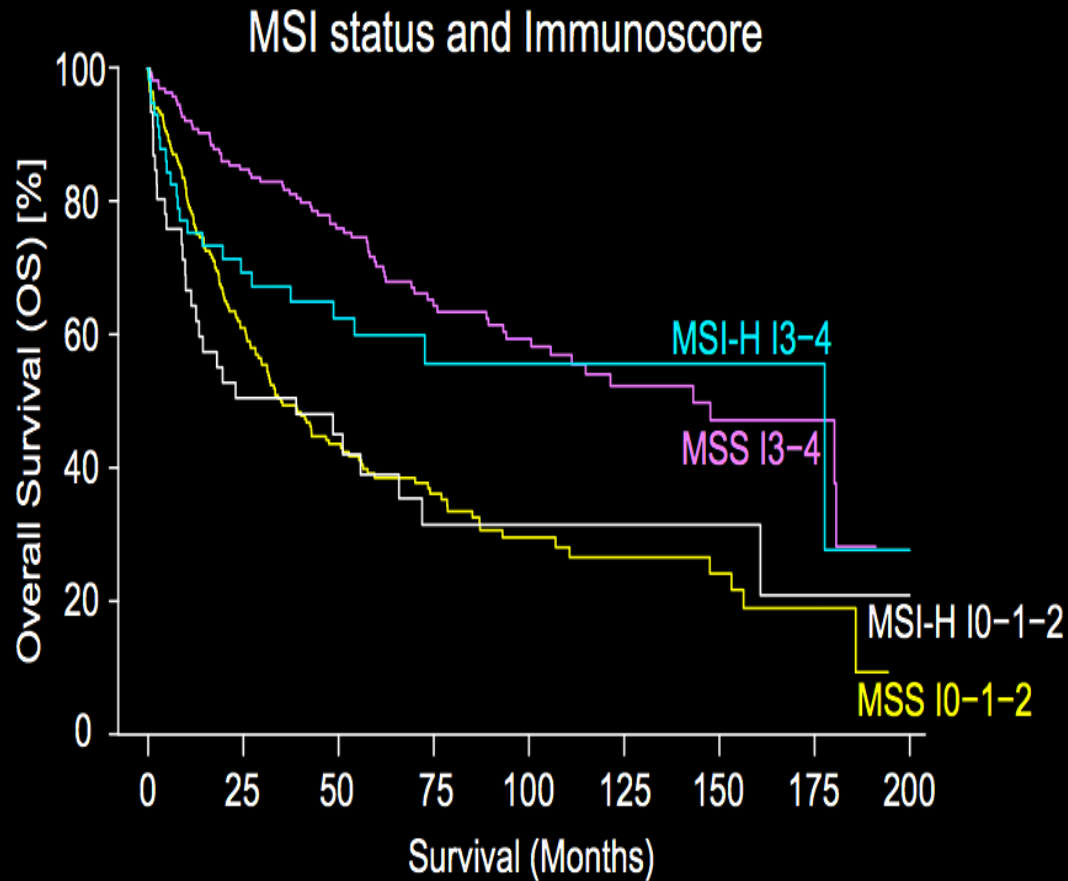


**E:T ratio**

- T2-relevant FSP
- T2-irrelevant peptide

**High-prevalence (3 out of 3 tested) of anti-FSmut tumor specific T-cells in MSI**

# 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 *

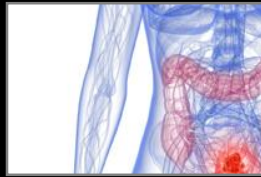


Is there an immune escape at the metastatic stage ?

# Metastasis analysis

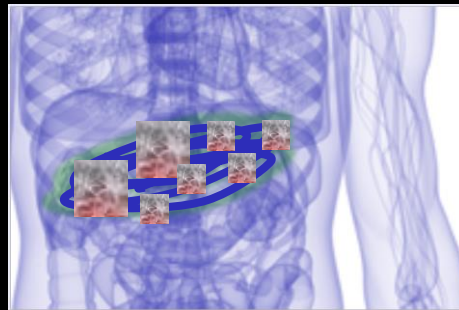
One primary tumor

Colorectal cancer



Multiple metastatic sites

Liver Metastasis



Lung Metastasis



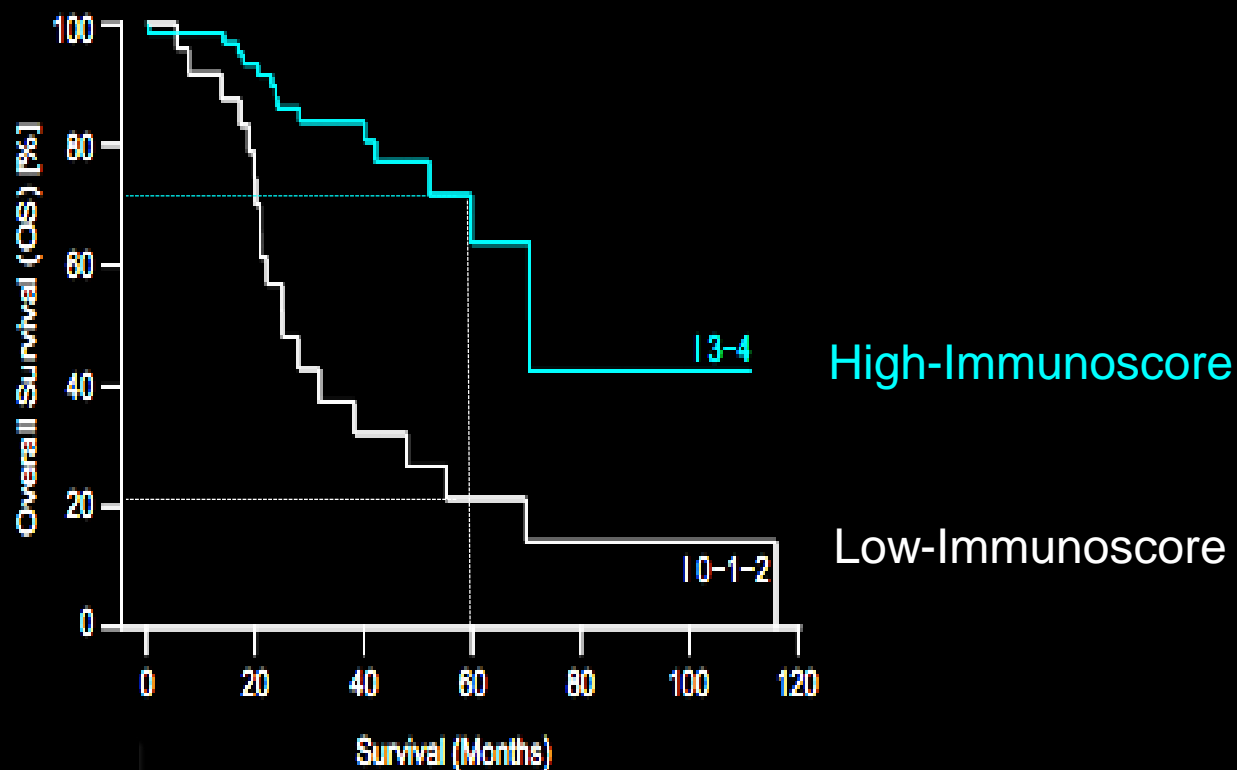
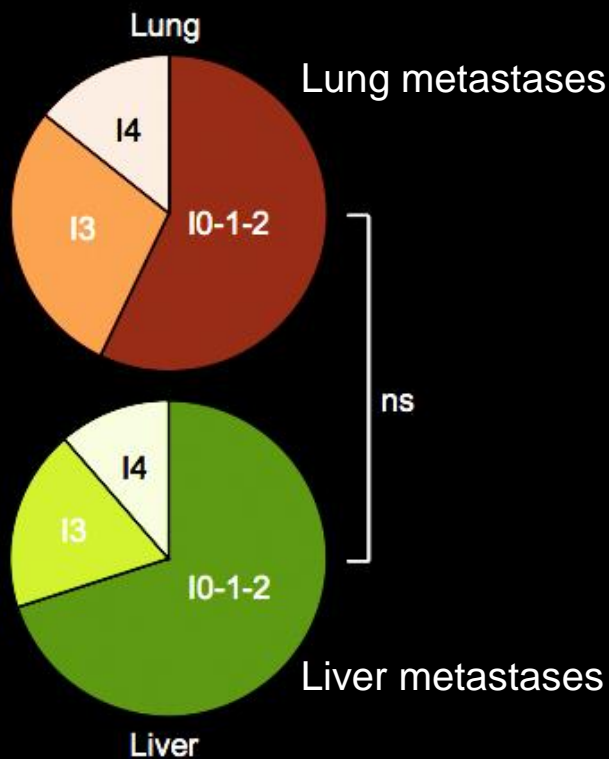
N=603 metastases

➤ Immunoscore within multiple metastases at different sites

Mlecnik et al. *JNCI* 2018

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

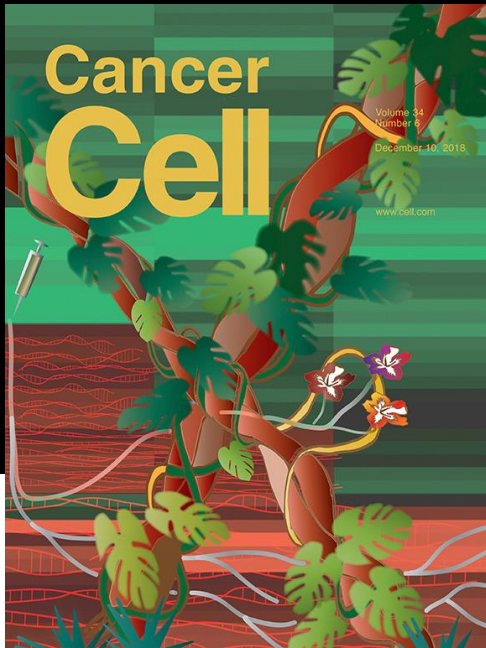
# High-Immunoscore within metastasis predicts prolonged survival



JNCI

Journal of the National Cancer Institute

# Metastasis analysis



CellPress

Cancer Cell  
**Article**

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

Marc Van den Eynde,<sup>1,2,9</sup> Bernhard Mlecnik,<sup>2,3,9</sup> Gabriela Bindea,<sup>2,9</sup> Tessa Fredriksen,<sup>2</sup> Sarah E. Church,<sup>2</sup> Lucie Lafontaine,<sup>2</sup> Nacilla Haicheur,<sup>4</sup> Florence Marliot,<sup>2,4</sup> Mihaela Angelova,<sup>2</sup> Angela Vasaturo,<sup>2</sup> Daniela Bruni,<sup>2</sup> Anne Jouret-Mourin,<sup>1</sup> Pamela Baldin,<sup>1</sup> Nicolas Huyghe,<sup>1</sup> Karin Haustermans,<sup>5,6</sup> Annelies Debucquoy,<sup>5</sup> Eric Van Cutsem,<sup>7</sup> Jean-Francois Gigot,<sup>1</sup> Catherine Hubert,<sup>1</sup> Alex Kartheuser,<sup>1</sup> Christophe Remue,<sup>1</sup> Daniel Léonard,<sup>1</sup> Viia Valge-Archer,<sup>8</sup> Franck Pagès,<sup>2,4</sup> Jean-Pascal Machiels,<sup>1</sup> and Jérôme Galon<sup>2,10,\*</sup>

- Immunoscore within multiple metastases at different sites

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

# Biological variability and biomarker accuracy

Whole slide  
Metastasis IHC



ROC analysis

sensitivity

-> Real positive (High)

-> **False** positive (High)

1 – specificity

-> Real negative (Low)

-> **False** negative (Low)

Biopsy

PDL1>50%

PDL1>50%

PDL1<50%

PDL1<50%

Whole slide  
Metastasis

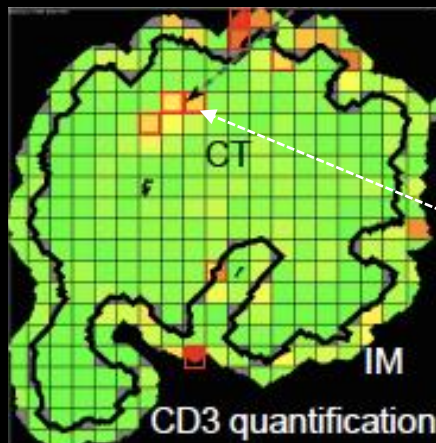
PDL1>50%

PDL1<50%

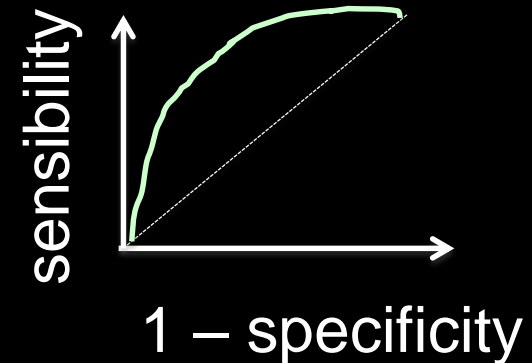
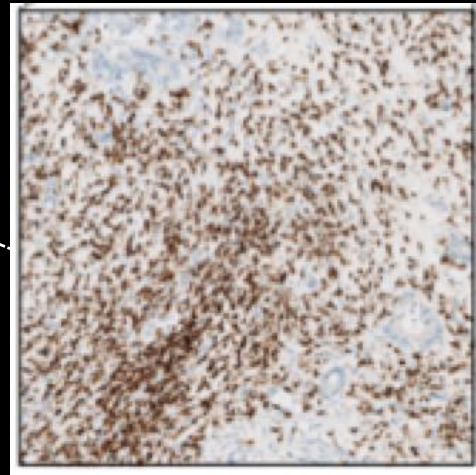
PDL1<50%

PDL1>50%

All non-overlapping biopsies



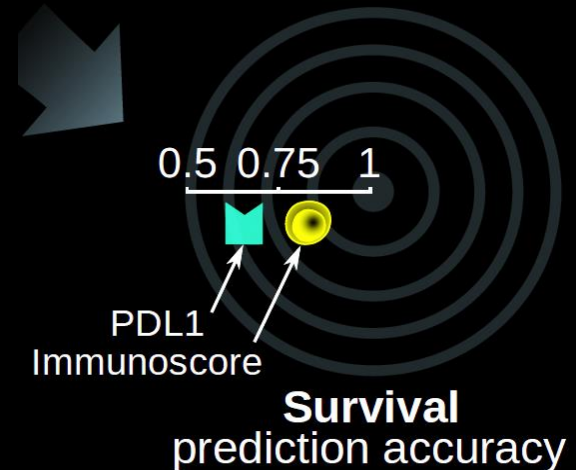
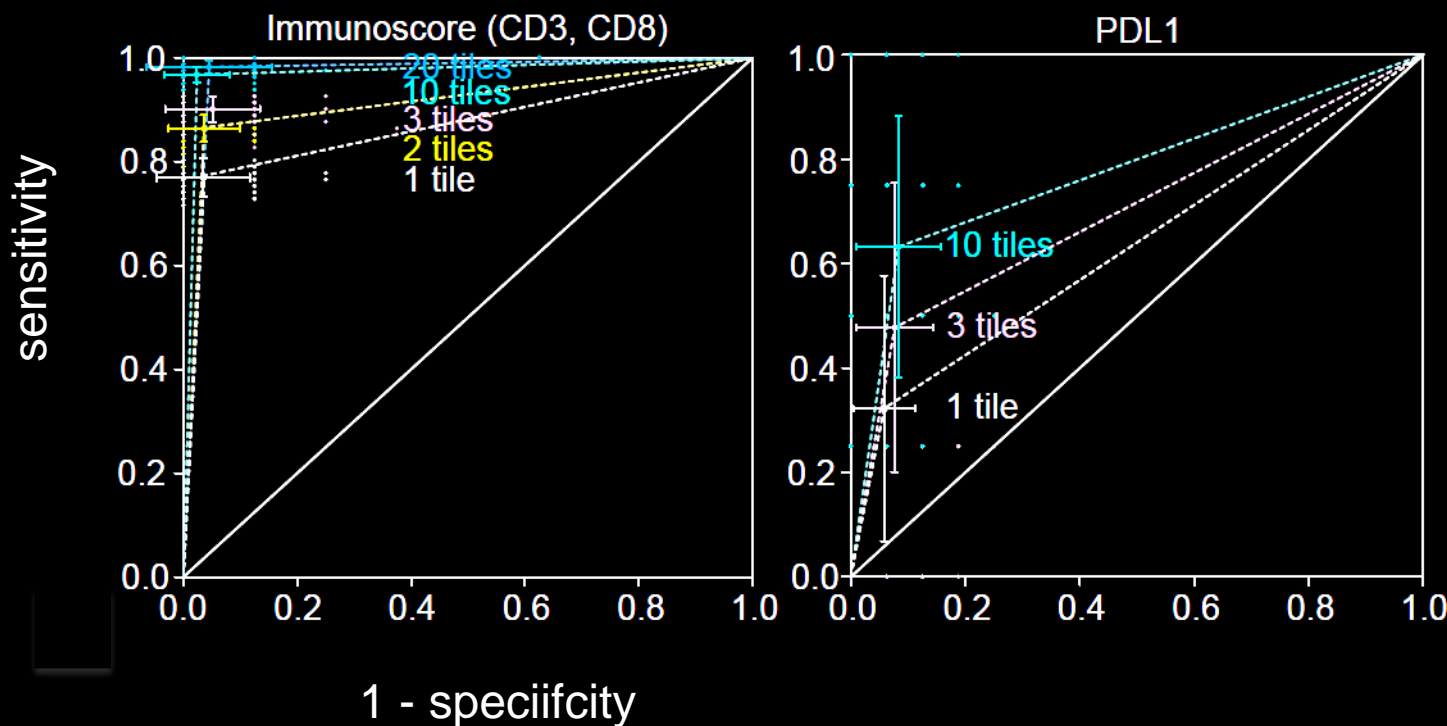
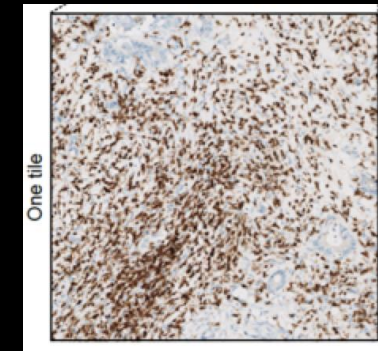
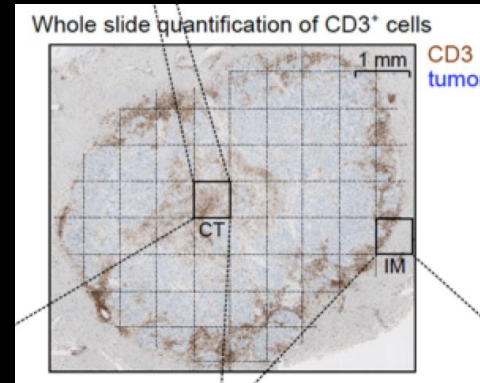
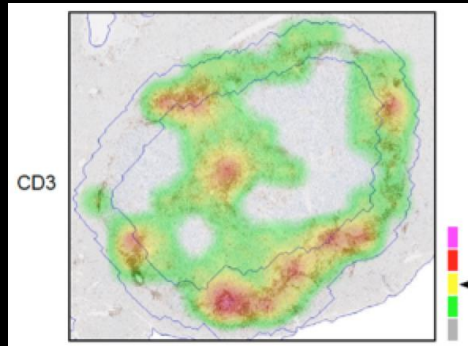
Biopsy - tile



✓ Marker heterogeneity and biomarker accuracy evaluation



# ROC curves illustrating the predictive value of 1, 2, 3, 10 biopsies compared to whole slide metastasis evaluation



✓ *Immunoscore Biopsy* is more reliable than PD-L1 expression

What drives metastasis ?

What are the metastatic escape mechanisms ?

A Novel theory of cancer evolution ?

Angelova M. et al. *CELL* 2018

# 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

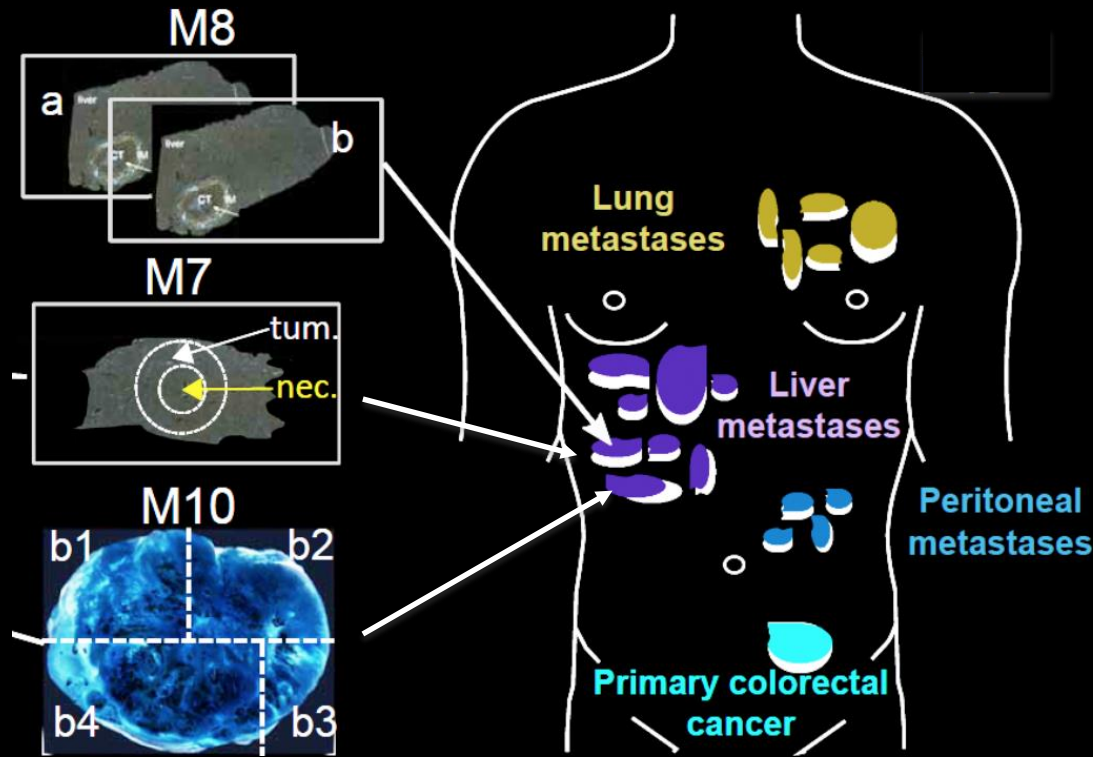
# Evolution of Metastases in Space and Time under Immune Selection

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

Angelova M. *et al.* **Cell** 2018  
Published October 18<sup>th</sup> 2018



# What drives metastasis?



Primary tumors

↓  
Synchronous metastases

↓  
Metachronous metastases

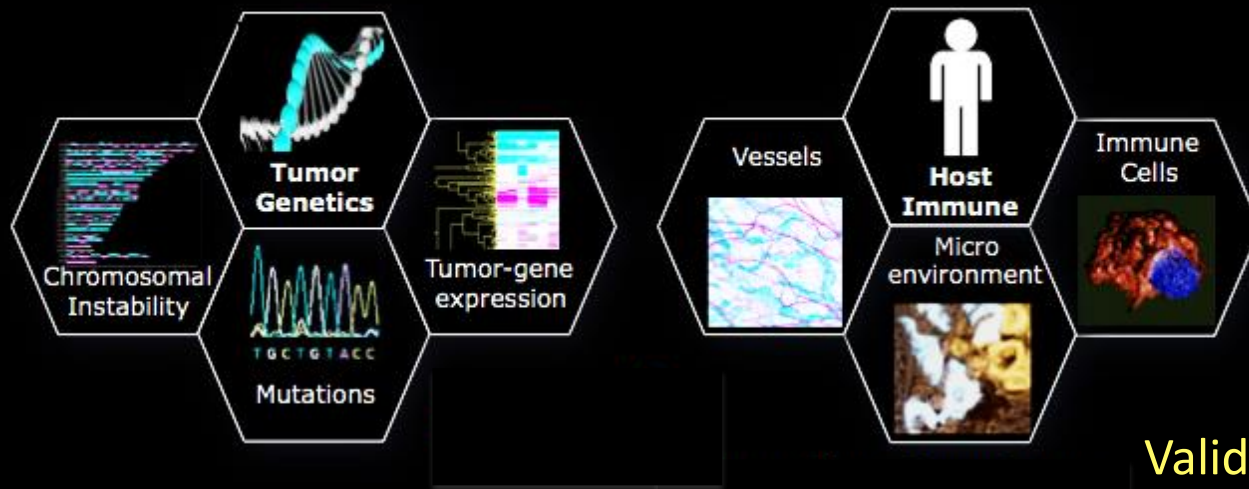
↓  
Metachronous metastases

↓  
Metachronous metastases

↓  
Follow-up

↓  
> 11 years

Multi-Omics technologies



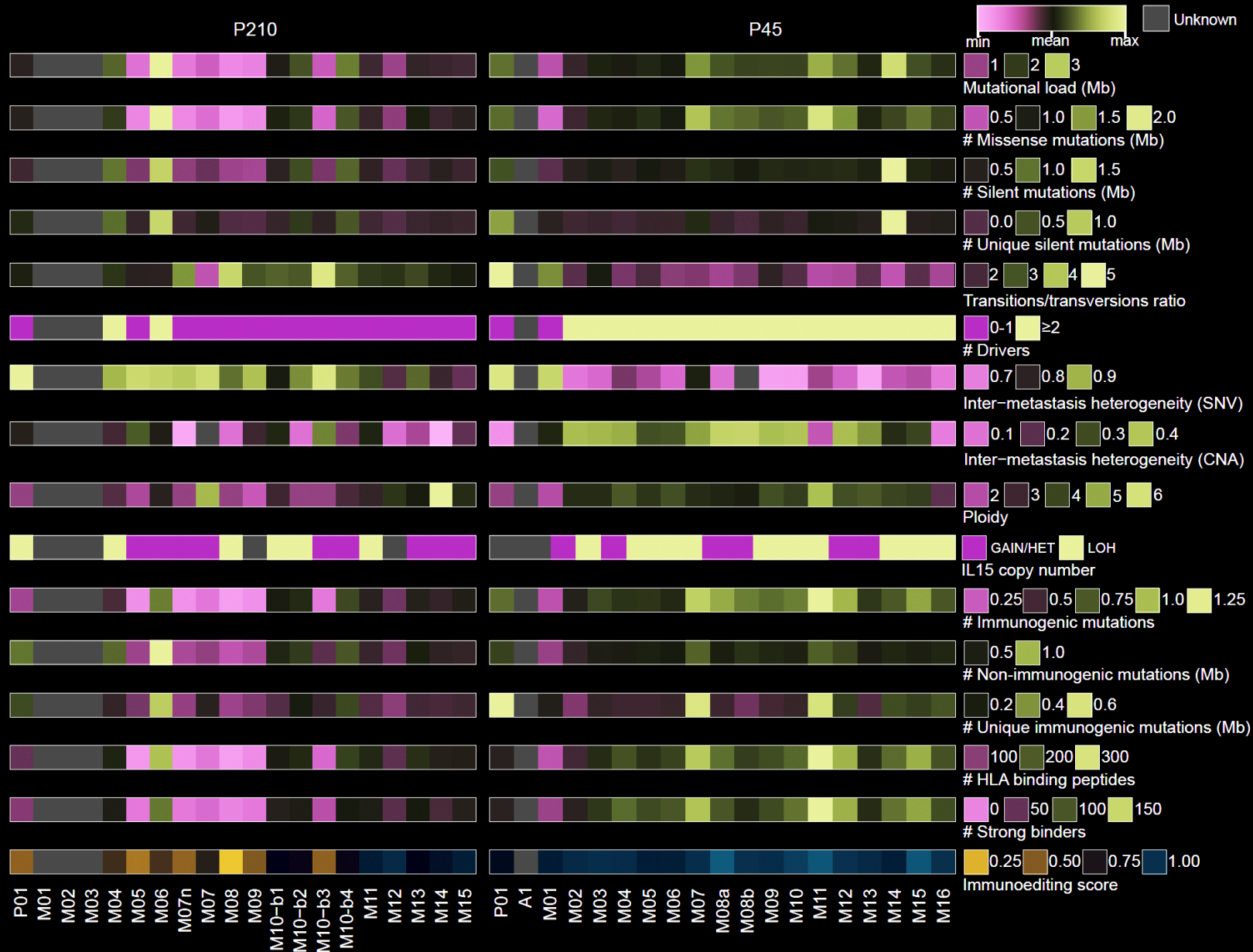
Validation cohort N=132



# Deep analysis of genomics defect in tumor cells



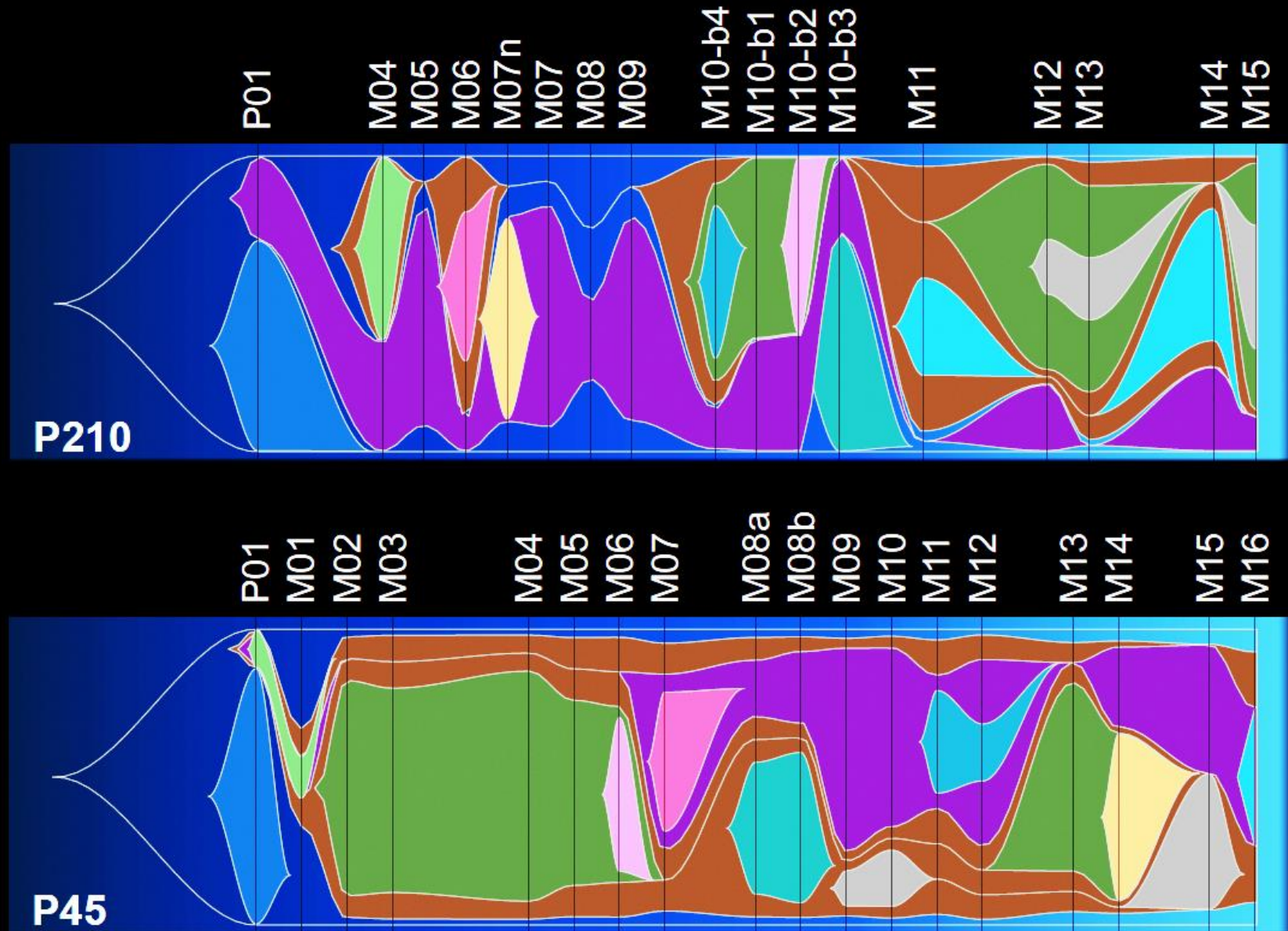
# Genomics of primary tumors and metastases



✓ Highly heterogeneous genomic patterns between metastases

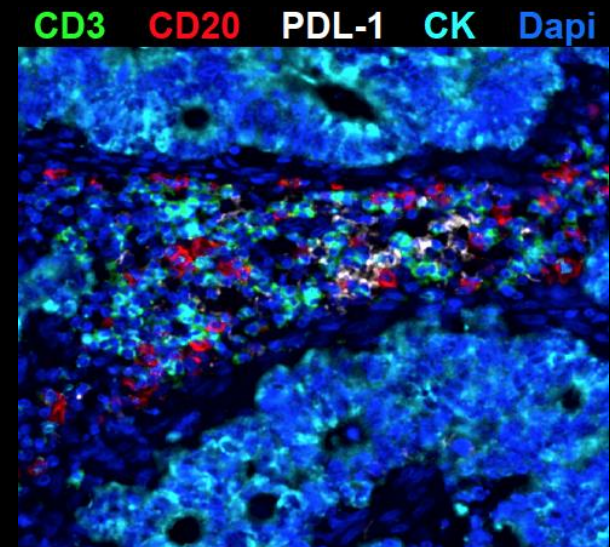
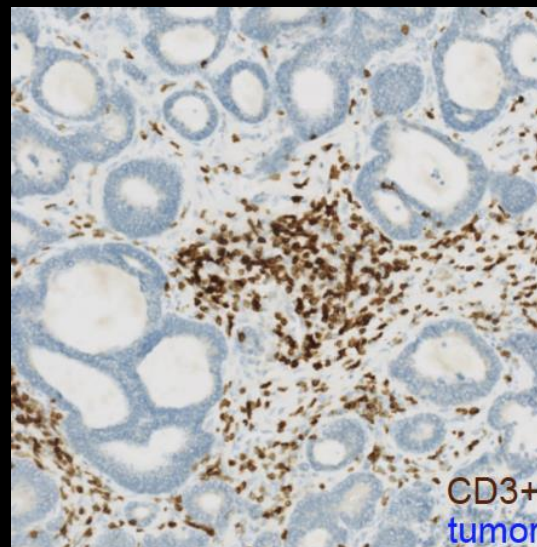
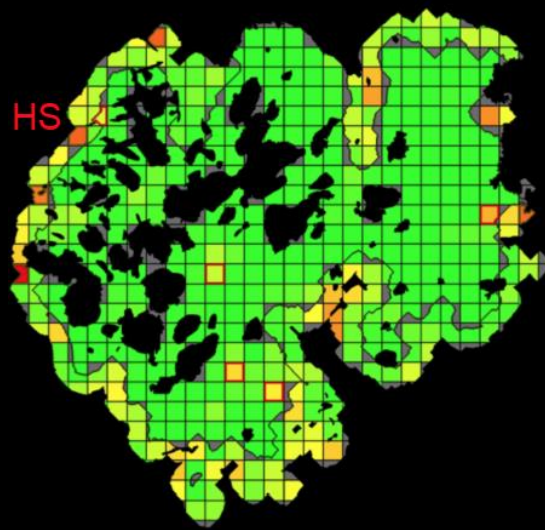
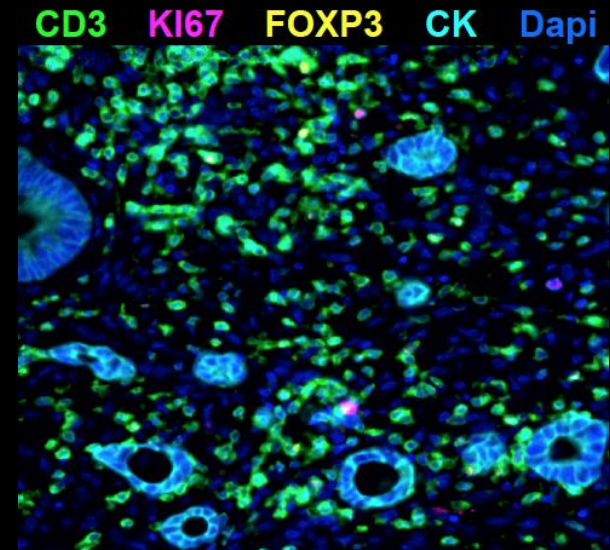
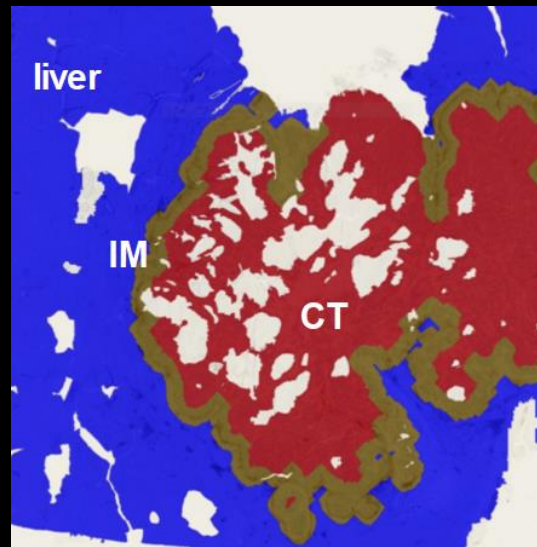
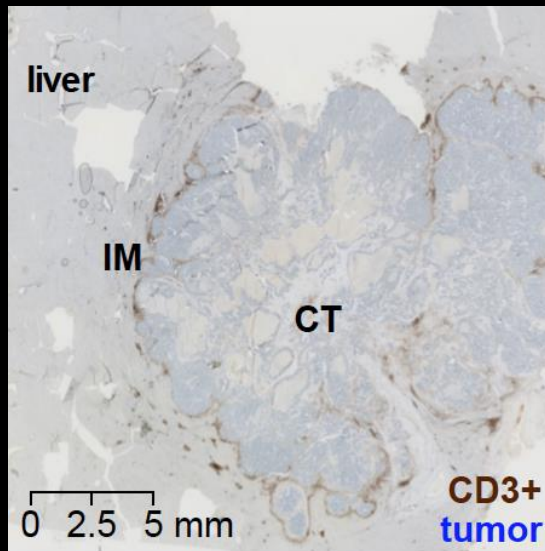


# Evolvogram of tumor clones



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

# What drives metastasis?



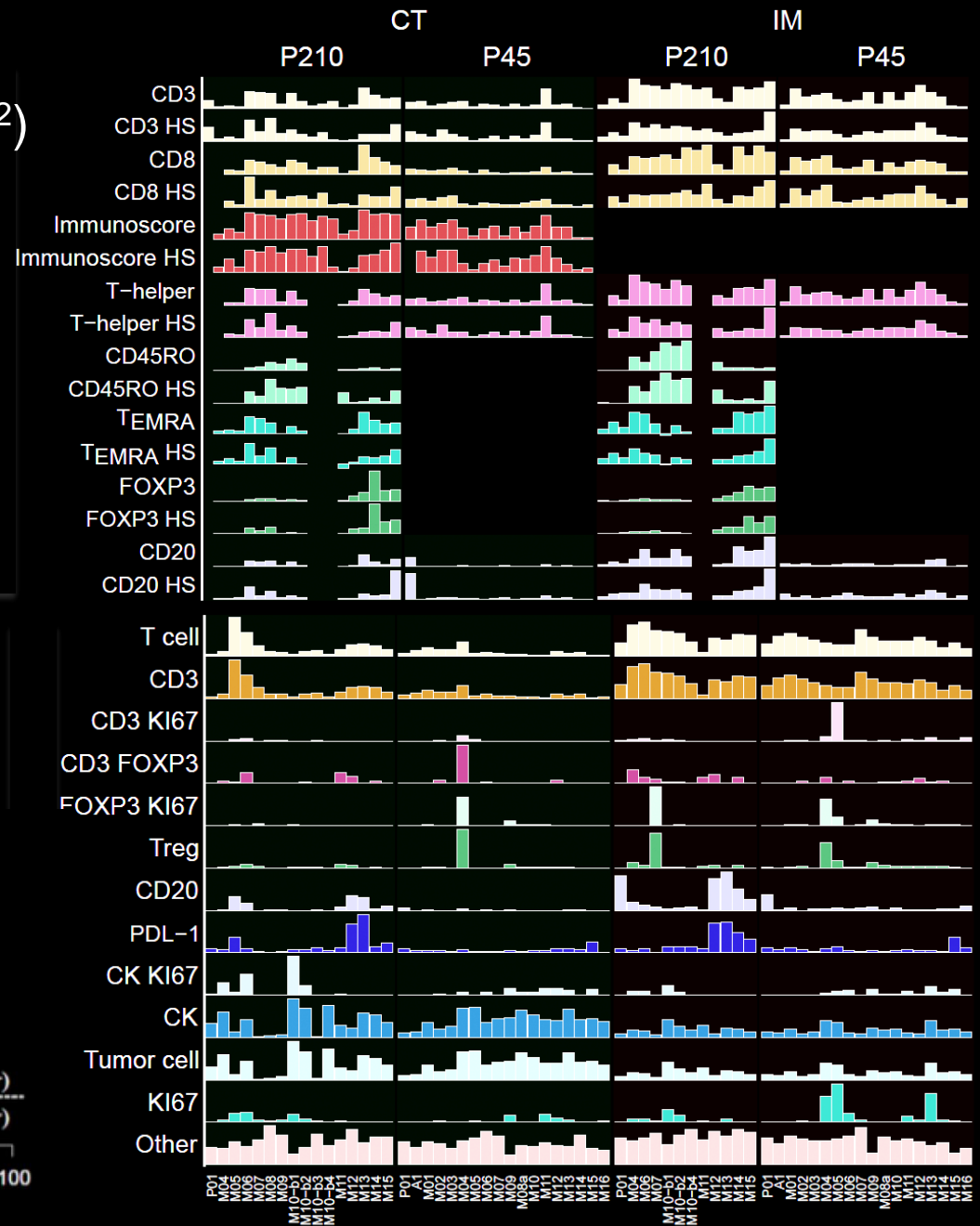
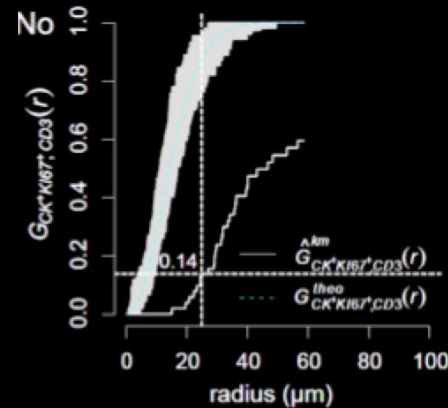
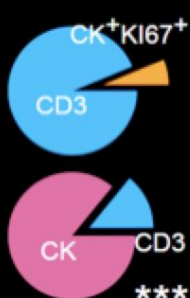
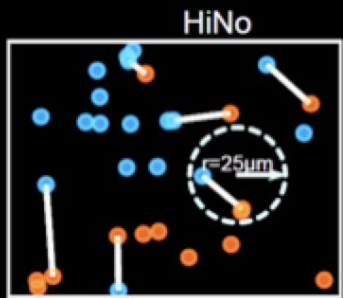
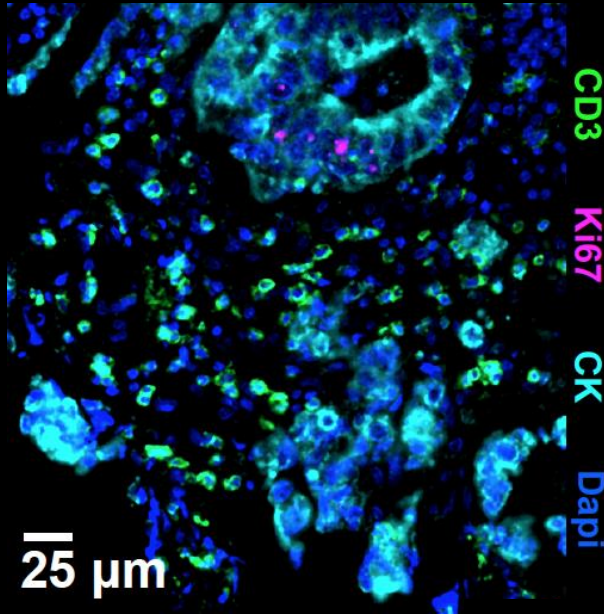
- ✓ Immunohistochemistry / Digital pathology quantification
- ✓ Multispectral imaging



# Immune microenvironment

Immune cell densities (cells/mm<sup>2</sup>)

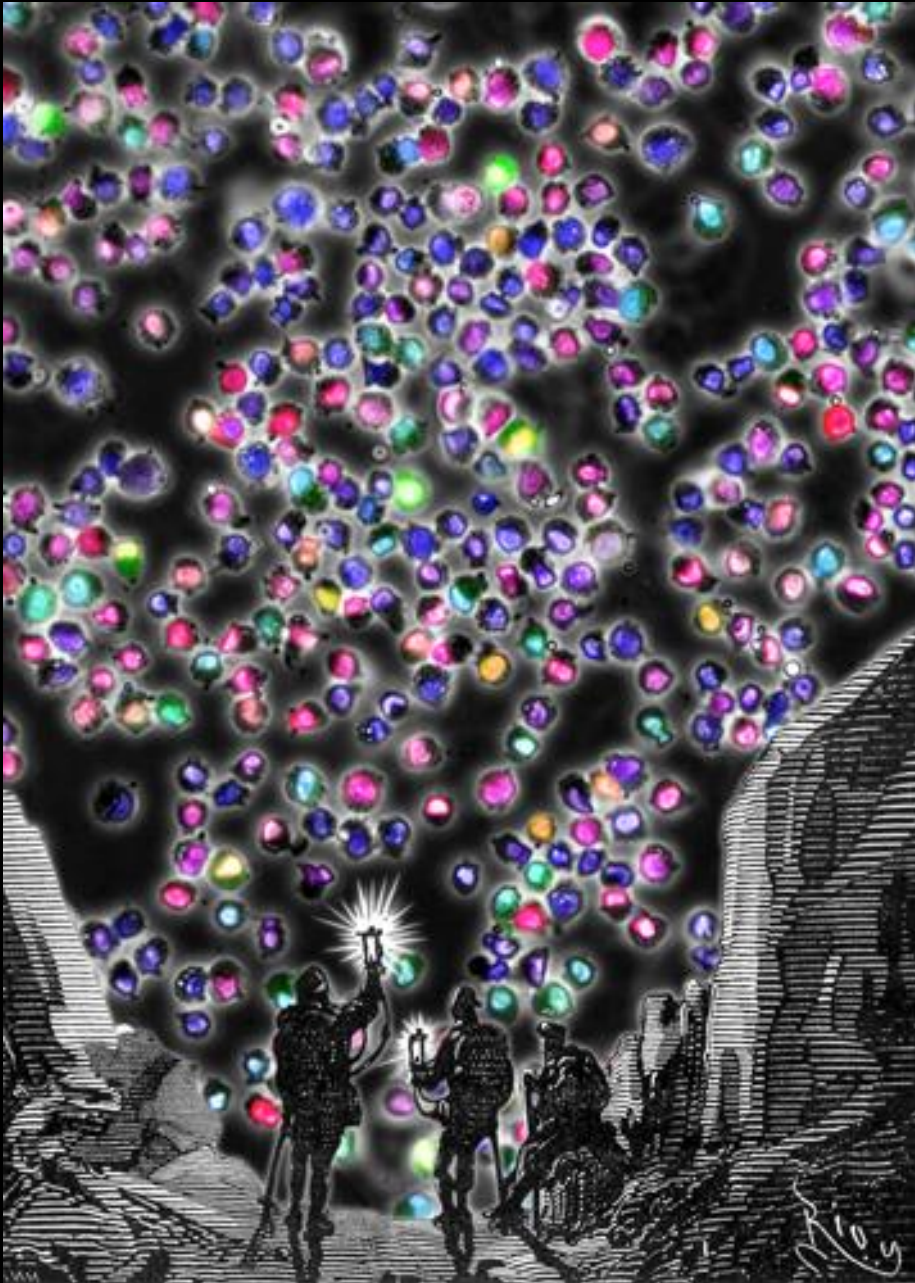
Spatial profiling



✓ Immunomics patterns and immune cell infiltration within metastases

Angelova M. et al. *CELL* 2018

# Tumor cells within a tumor are highly heterogeneous



Tumor Immunoediting

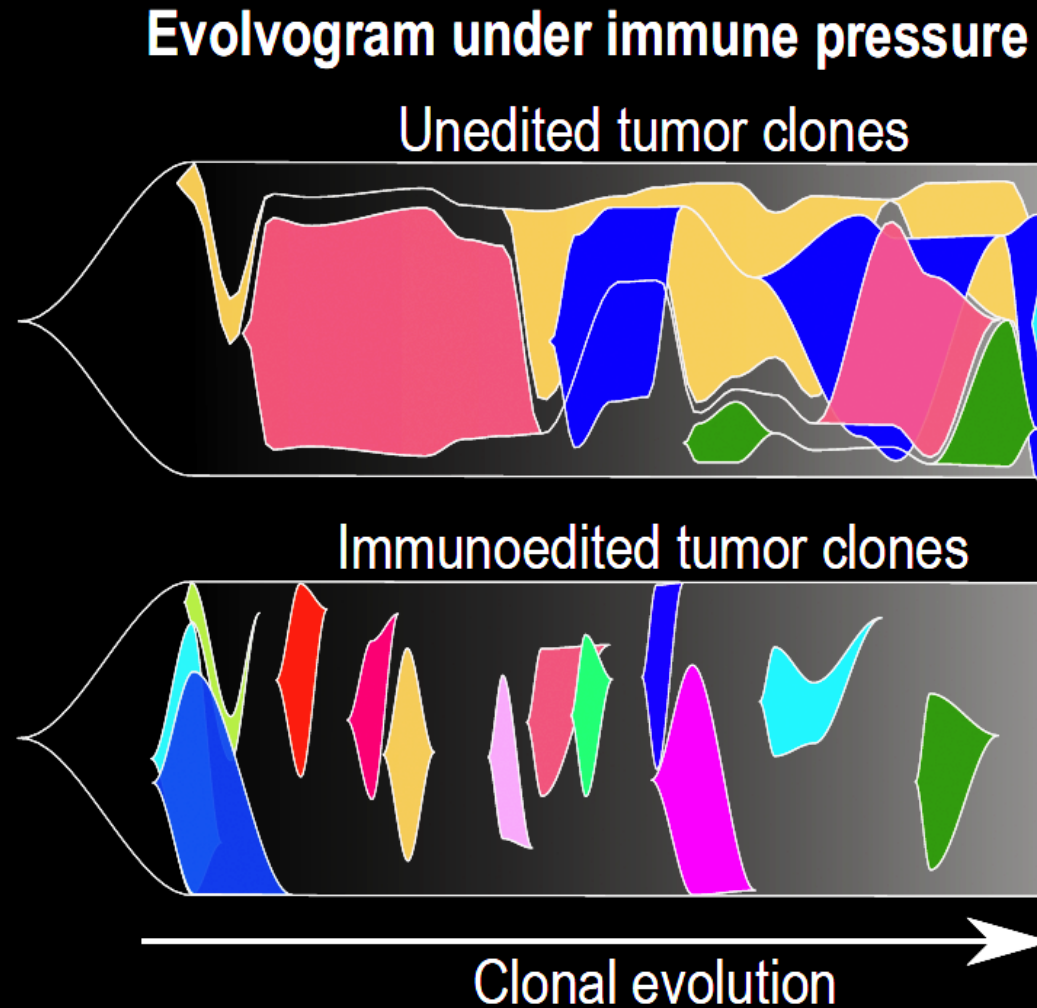


Tumor progression

Immunoscore

Adapted from  
"Twenty Thousand Leagues Under the Sea"  
Jules Verne 1869

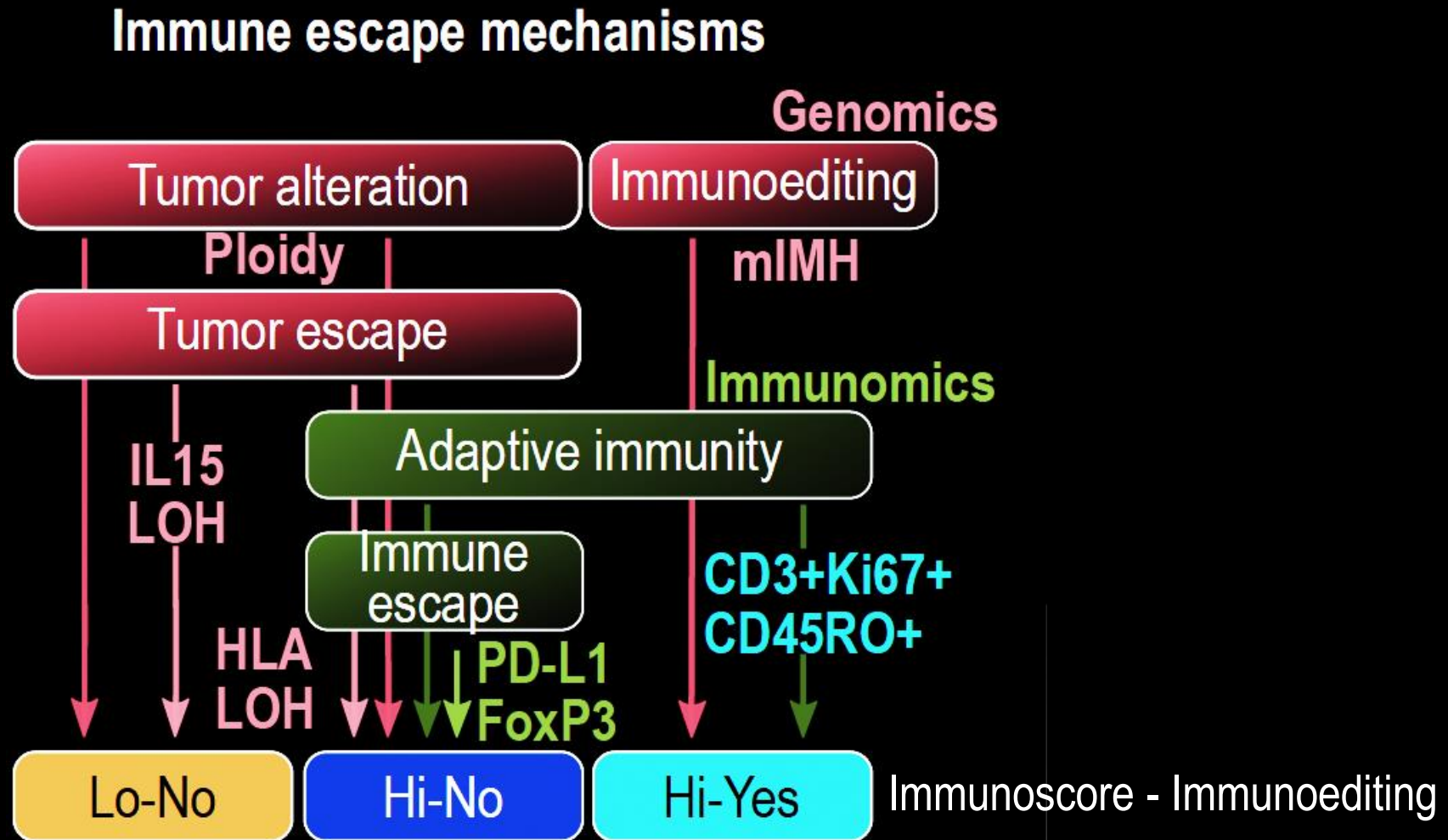
# What drives metastasis? Conclusions



- Evolution of tumor clones is linked to the intra-metastatic immune contexture.
- Non-recurrent clones are immunoedited. Progressing clones are immune privileged.
- Clonal evolution is dependent upon Immunoediting



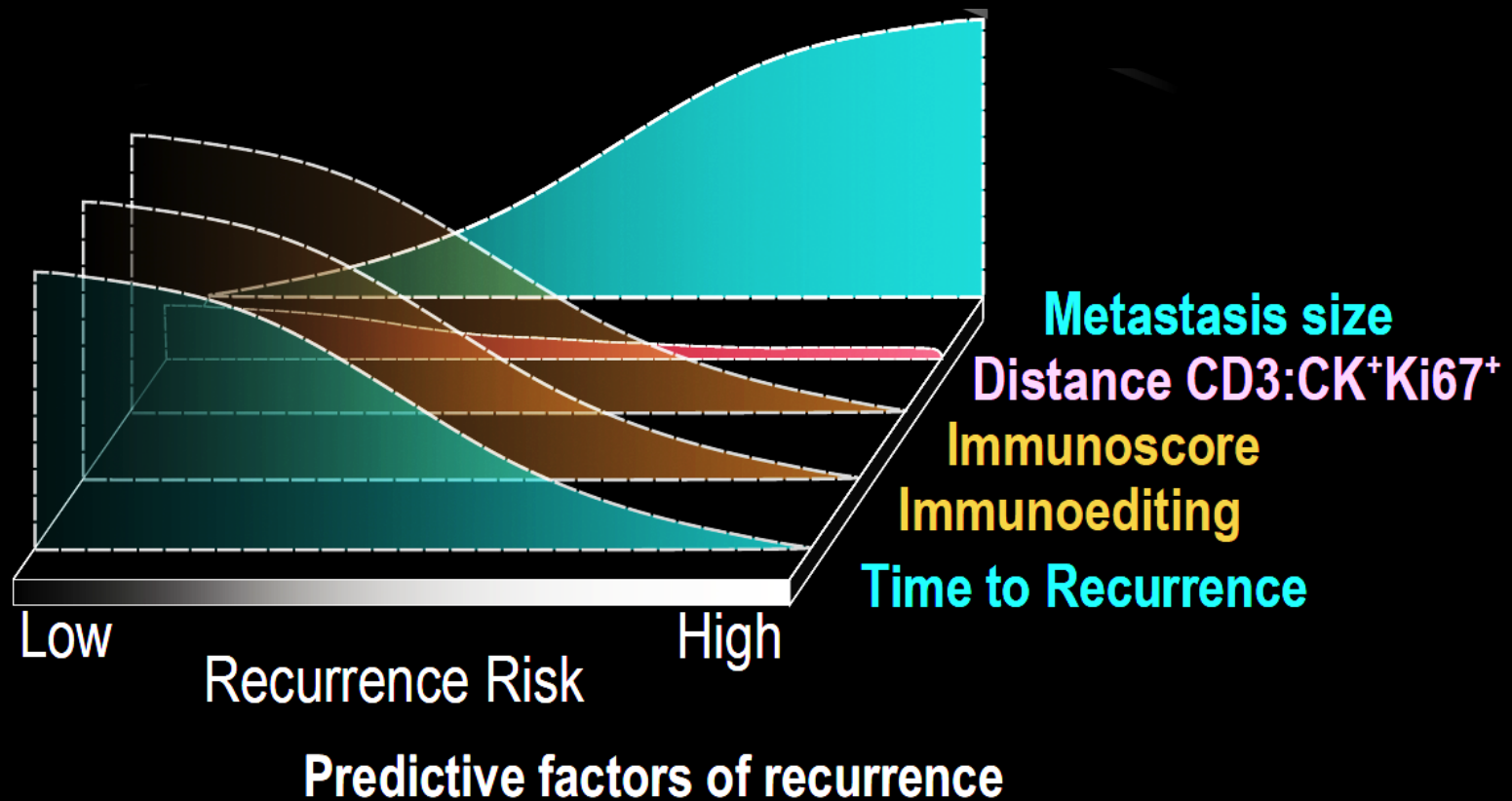
# What drives metastasis? Conclusions



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



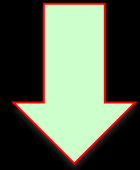
# What drives metastasis? Conclusions



- 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.

# 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.

# Immunogram of response to immunotherapy

CANCER IMMUNOLOGY

## *The “cancer immunogram”*

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

*By* **Christian U. Blank,<sup>1,2</sup> John B. Haanen,<sup>1,2</sup> Antoni Ribas,<sup>3</sup> Ton N. Schumacher<sup>2</sup>**

Blank et al. *Science* 2016

Review about all published biomarkers of response to immunotherapy

# Predictive markers to immunotherapies: the cancer Immunogram

## Peripheral

Peripheral immune status  
*Lymphocyte count*

Absence of inhibitory  
tumor metabolism  
*LDH, glucose*

Absence of soluble  
inhibitors  
*IL-6, CRP*

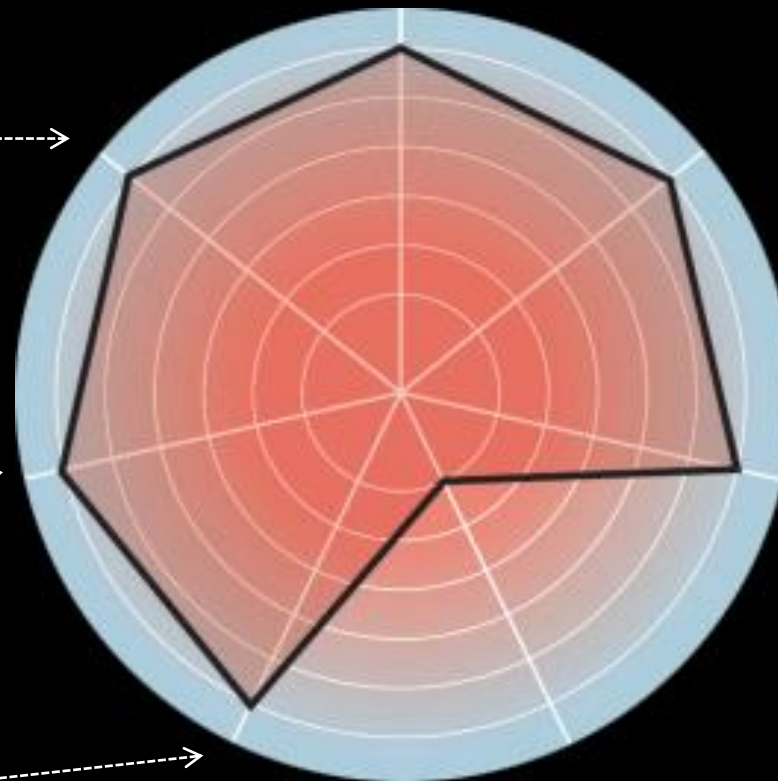
## Intra-tumoral

Tumor foreignness  
*Mutational load, MSI \**

Tumor sensitivity to immune  
effectors  
*IFNG, MHC, cytokines,  
chemokines,*

Immune cell infiltration  
*Immunoscore*

Absence of checkpoint  
*PD-L1 \**

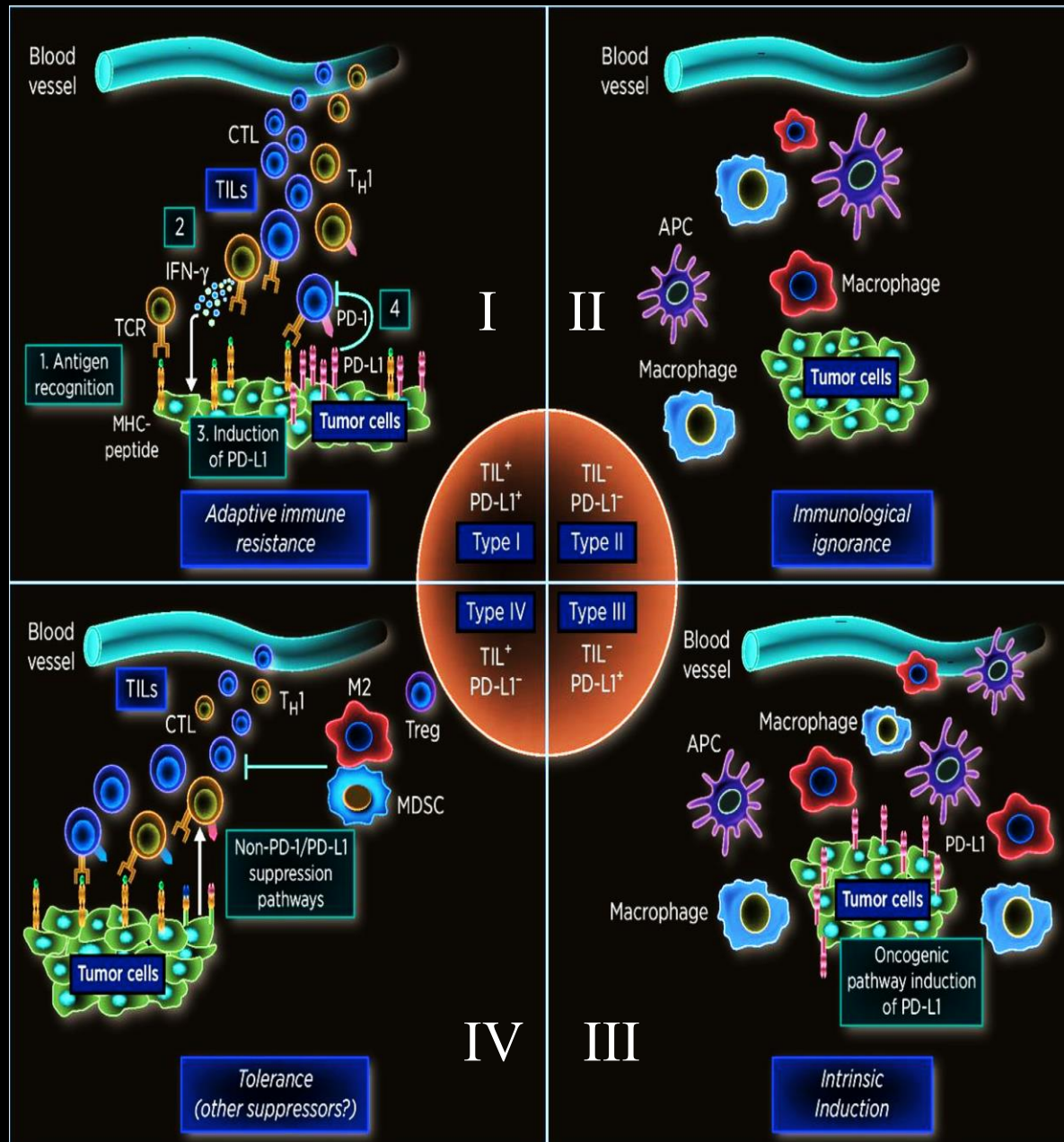


\* FDA approved

Adapted from Blank C et al. "The cancer immunogram" Science 2016



# Classifying cancers based on cytotoxic T-cells & PDL-1 expression



## Types


- I: CD8+PDL1+
- II: CD8-PDL1-
- III: CD8-PDL1+
- IV: CD8+PDL1-

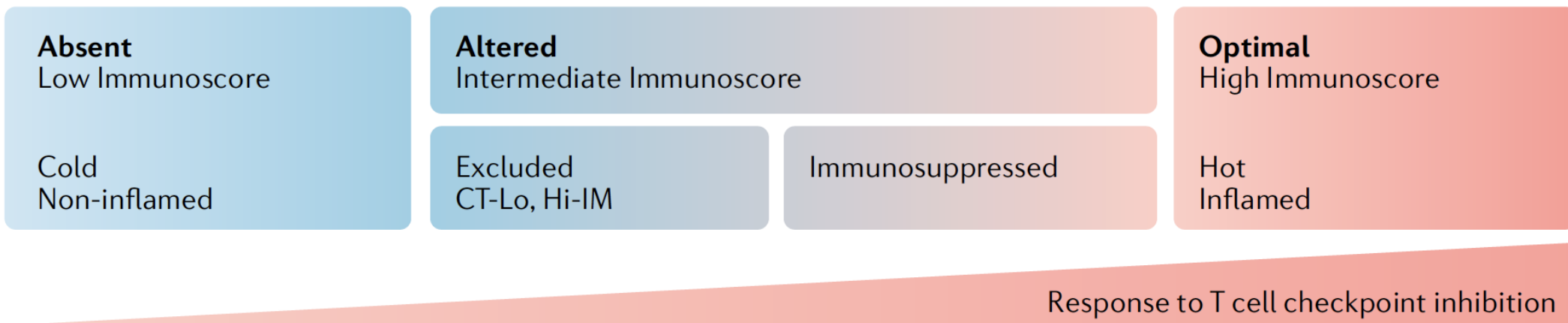
- I: Adaptive resistance
- II: Immune ignorance
- III: Intrinsic induction
- IV: Tolerance (other suppressors)

Teng MW, Ngiow SF, Ribas A, Smyth MJ.  
Cancer Res. 2015

Teng MW, Galon J, Fridman WH, Smyth MJ.  
J Clin Invest. 2015

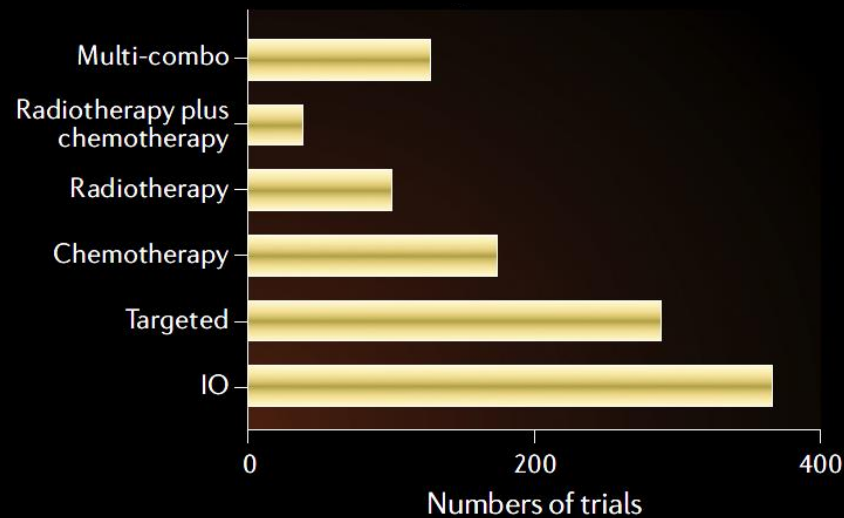
# Approaches to treat immune hot, altered and cold tumours with combination immunotherapies

Jérôme Galon \* and Daniela Bruni

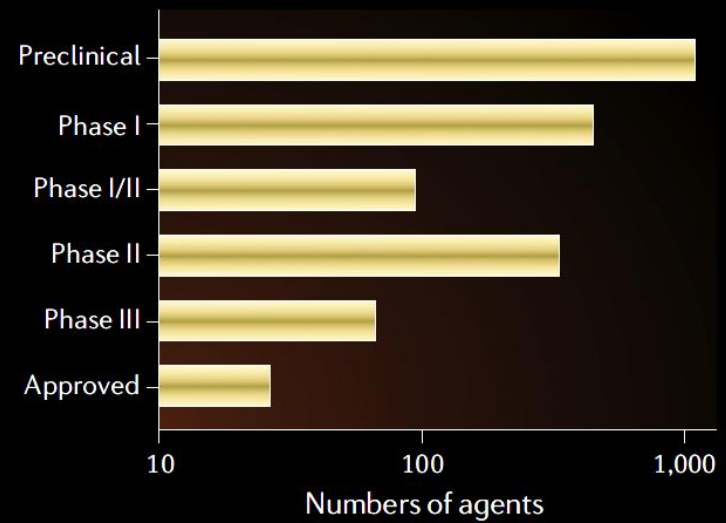


# Immunotherapy clinical trials 2019

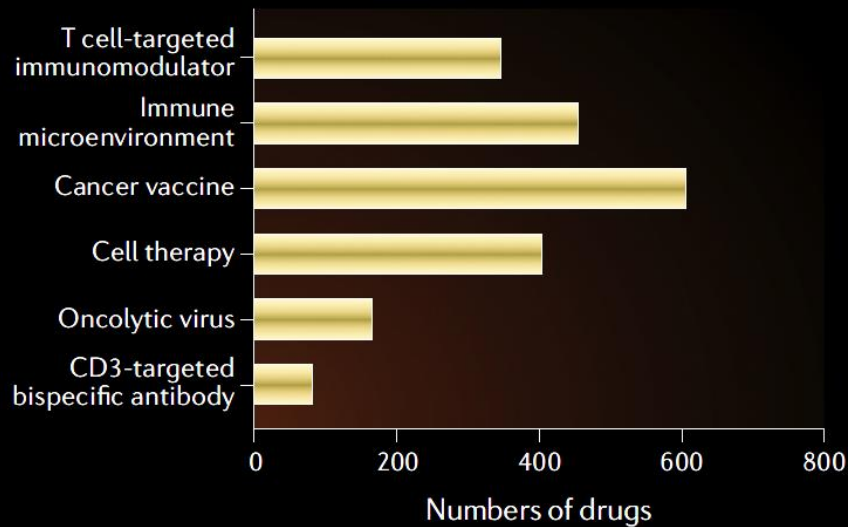
## Number of trials in combination with aPD1/L1



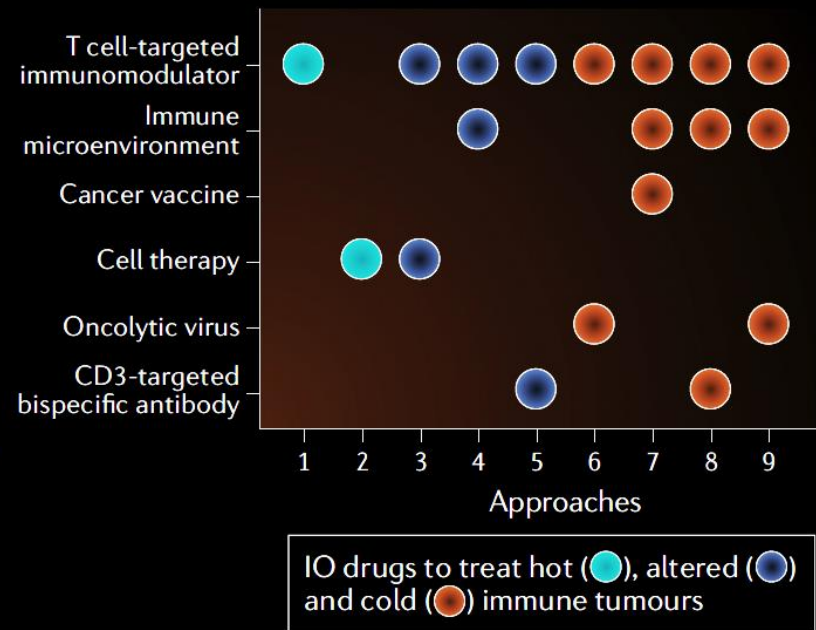
## Number of IO agents



## Number of IO drugs

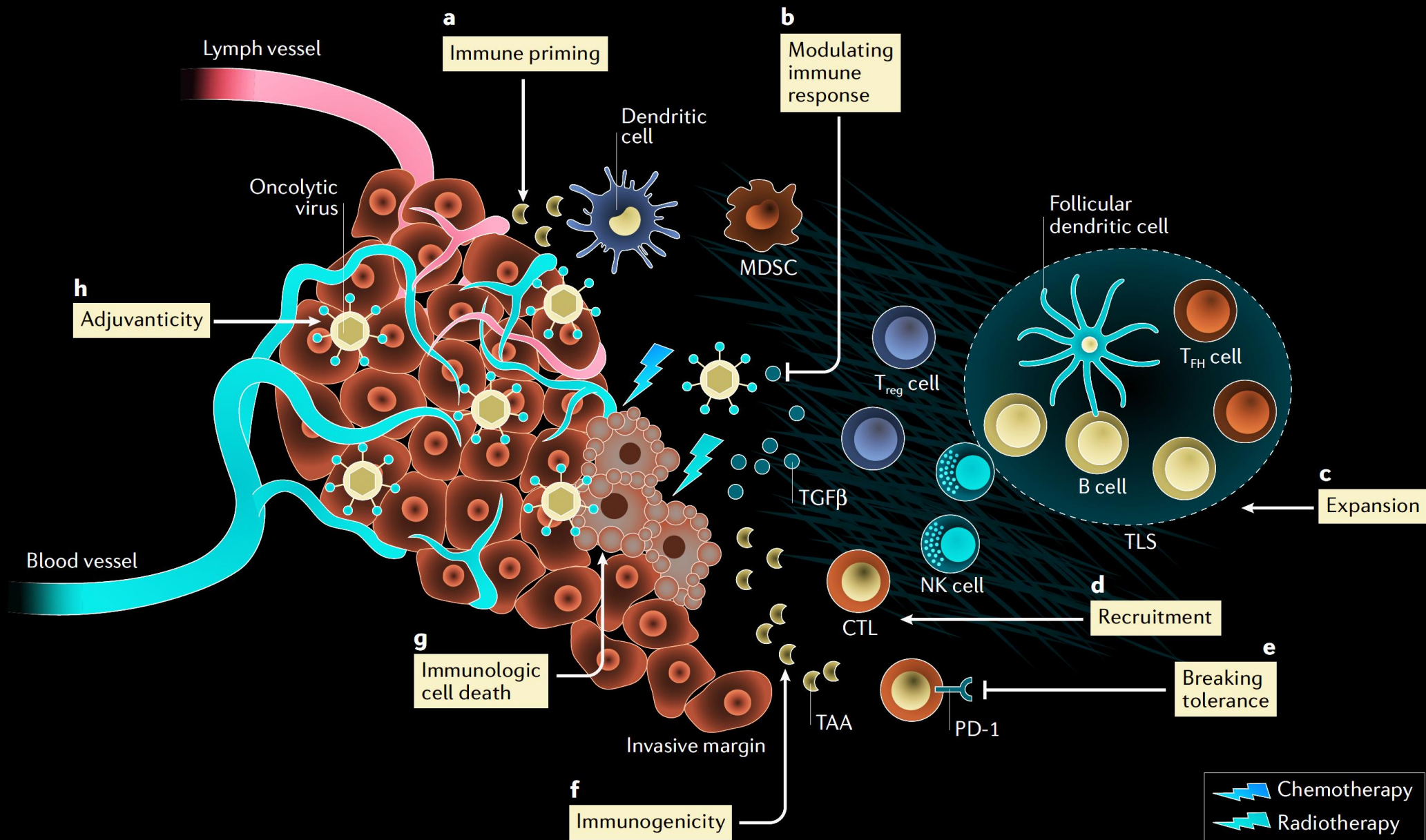


## Major treatment combination approaches





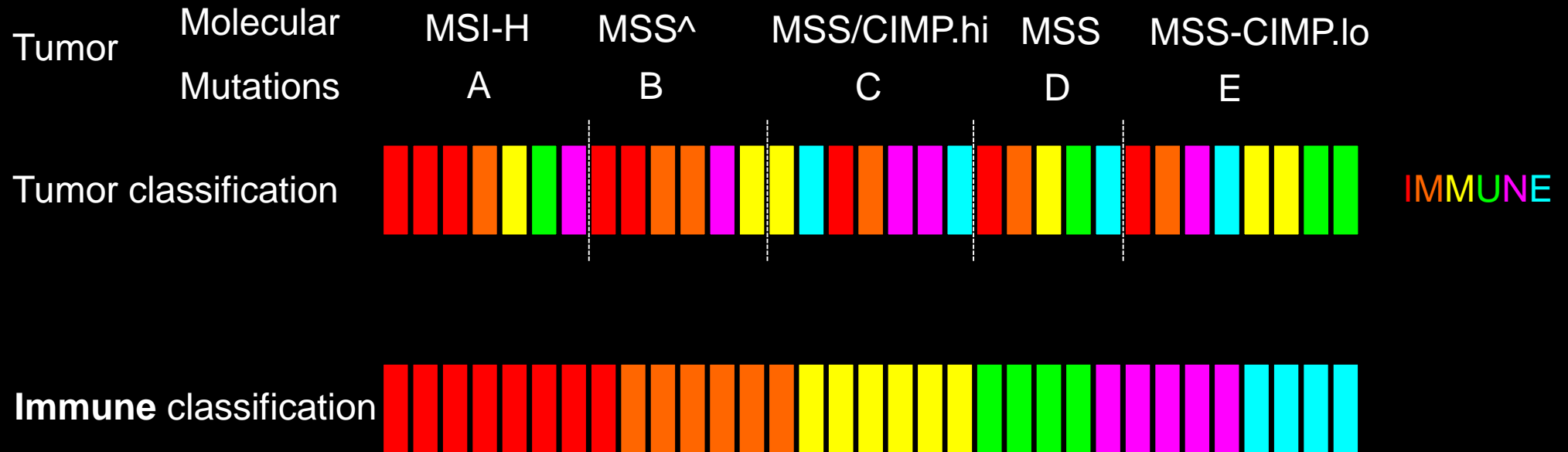
# NATURE REVIEWS | DRUG DISCOVERY



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



# Stratification of cancer based on the immune status



-> Importance of having standardized immune Assays

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*Amélie Bilocq*

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*Amos Kirilovsky*

*Marie Tosolini*

*Maximilian Waldner*

*Sarah Church*

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*Helen Angell*

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## Thanks (2) Worldwide Consortium Centers



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