SITC Winter school, Houston, TX, USA
January 14th, 2020

Immunity and Therapeutic Efficacy

Jérôme GALON

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

Disclosures

Co-founder and chairman of the scientific advisory board:

- *HalioDx*

Collaborative Research Agreement (grants) :

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

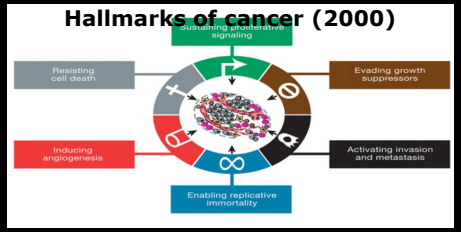
Participation to Scientific Advisory Boards:

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

Consultant :

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

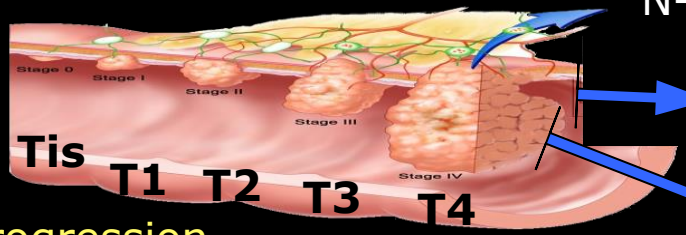
Definition of cancer



Hanahan & Weinberg, *Cell* 2000

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

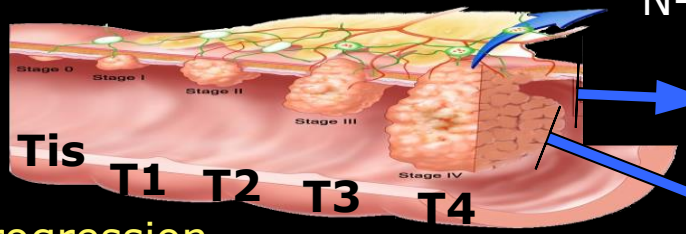
“Hot” Tumor

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…)

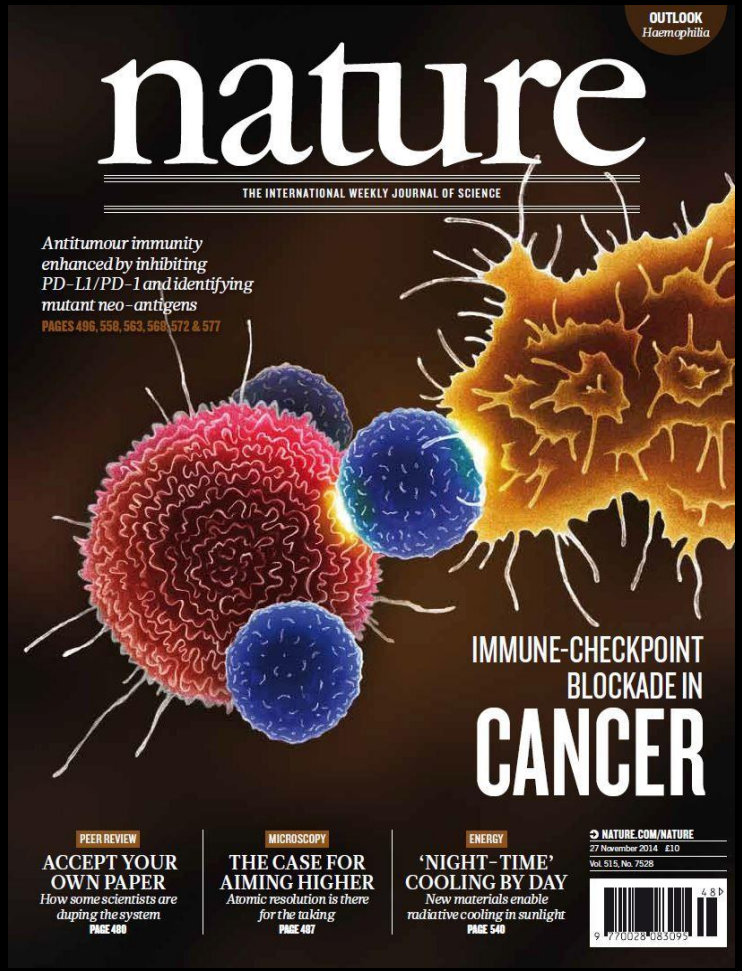
-> Tumor recurrence

-> 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


Science
Breakthrough of the Year
Cancer Immunotherapy
AAAS

2013

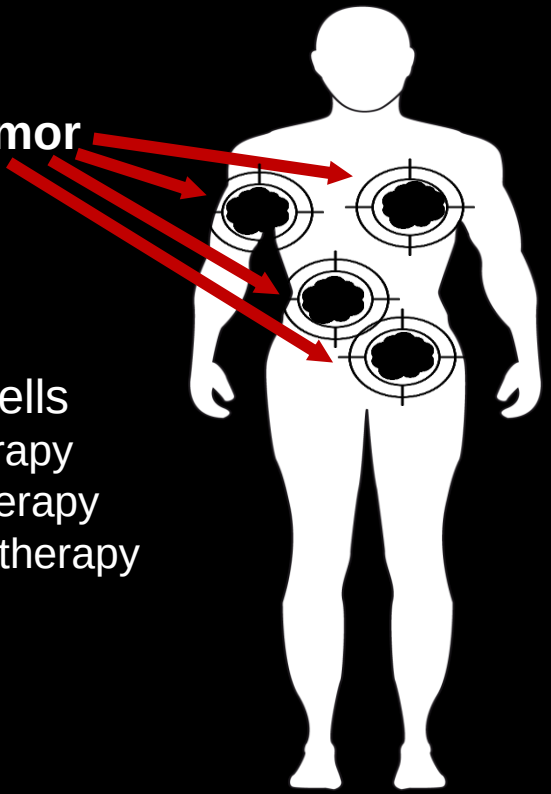

OUTLOOK
Haemophilia
nature
THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE
Antitumour immunity enhanced by inhibiting PD-L1/PD-1 and identifying mutant neo-antigens
PAGES 496, 558, 563, 568-572 & 577
IMMUNE-CHECKPOINT BLOCKADE IN
CANCER
PEER REVIEW
ACCEPT YOUR OWN PAPER
How some scientists are duping the system
PAGE 480
MICROSCOPY
THE CASE FOR AIMING HIGHER
Atomic resolution is there for the taking
PAGE 487
ENERGY
'NIGHT-TIME' COOLING BY DAY
New materials enable radiative cooling in sunlight
PAGE 540
NATURE.COM/NATURE
27 November 2014 . £10
Vol. 515, No. 7528

2014

Cancer Treatment

Conventional Therapy

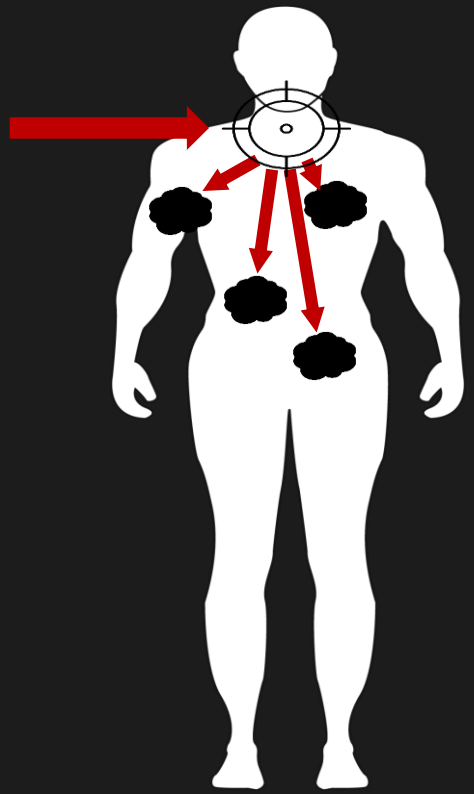
Target: tumor



Kill tumor cells
- ✓ Radiotherapy
- ✓ Chemotherapy
- ✓ Targeted therapy

New Paradigm Immunotherapy

Target: host



Successful immunotherapies unleash natural pre-existing T cells

Co-inhibitory receptors (antagonist)

CTLA4

PD1

PDL1

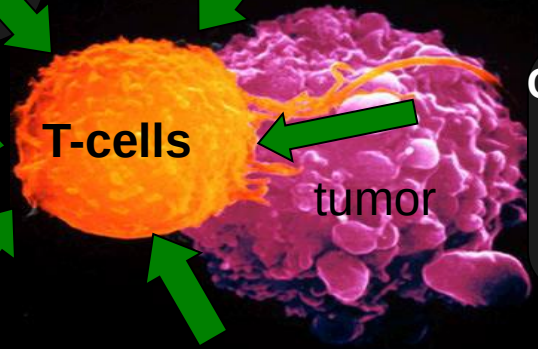
TIM3, LAG3, BTLA, …

Costimulatory receptors (agonist)

CD40

CD137

OX40



Peptide vaccine
Genetic vaccine
DC vaccine

Costimulatory cytokines

IL-2
IFN
IL-15
IL-21

Adoptive Transfert of T cells

Engineered TCR or CAR-T cells

FDA approved

Immunology and cancer: A successful decade

The Renaissance

2001

- ***Demonstration of the immunosurveillance in cancer mouse models***
  - *-> multiple deficient mouse models, >100 studies* 2001, 2007, 2011

2005

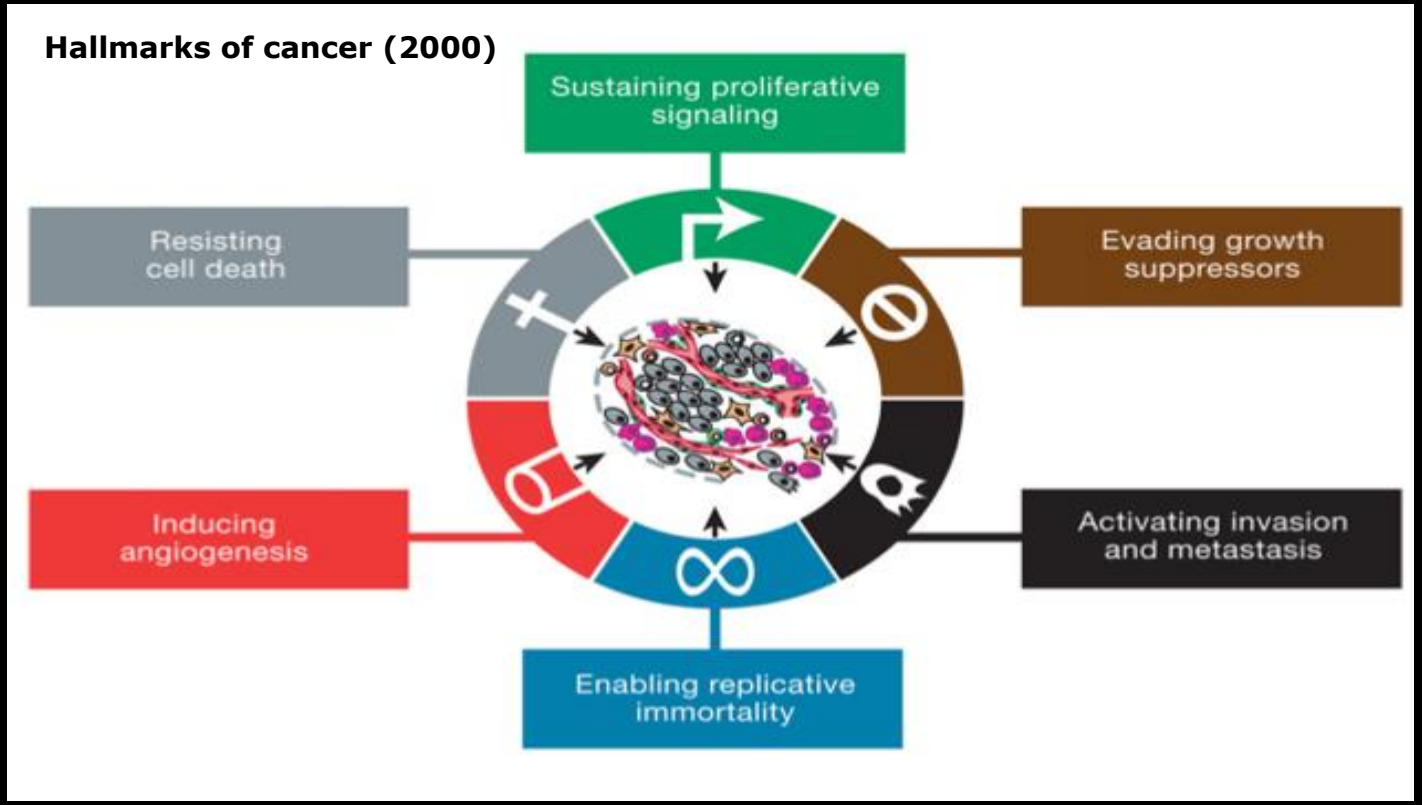
- ***Demonstration of the importance of the immune cells in human cancer***
  - *-> Definition of the immune contexture* 2005, 2006, 2007 ***-> T cells***
  - *-> Definition of the Immunoscore* 2006, 2011, 2014, 2019 *-> Immune > TNM*

sitc

2011

- ***FDA approval (or not yet) of immune T-cell modulators to treat cancer patients***
  - *-> IpilimumAb* (anti-CTLA4, melanoma) 2010, 2011
  - -> Provenge (immune cell-based therapy) 2010, 2011
  - *-> anti-PD1/PDL1…* (melanoma, RCC, NSCLC, Bladder,…) 2013
  - *-> Adoptive Transfert of T cells*
  - *-> CAR-T cells* 2011, 2017

Cancer Hallmarks



Hanahan & Weinberg, *Cell* 2000

Cancer patient

Current cancer classification
Tumor cell characteristics

Immune-based classification
Host immune response

Tumor cell extension and invasion

T-stage

N-stage

M-stage

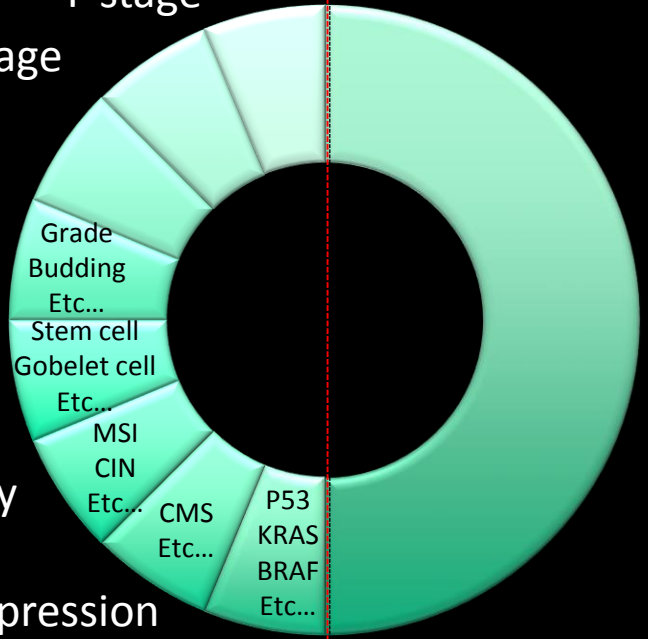
Anatopathology
Tumor Morphology

Tumor cell of origin

Tumor Molecular pathway

Tumor Gene expression

Tumor Mutation status



Currently NONE

Angell & Galon ***Clin Cancer Res***. 2019

Concepts in Immuno-oncology

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

Concept

"***Immune Contexture***" :

- ✓Type
- ✓Quality
- ✓Quantity
- ✓Spatial

- ✓Complexity
- ✓Dynamics

Research purposes

Biomarker

"***Immunoscore***" :

- ✓Digital pathology
- ✓Quantitative
- ✓Location

- ✓Simple
- ✓Powerful

Routine clinical purposes

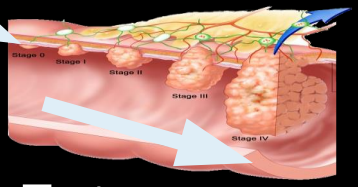
Galon J et al. ***Science*** 2006

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

The Immune landscape and the importance of the immune contexture

Oncogenensis

Pre-cancer lesions



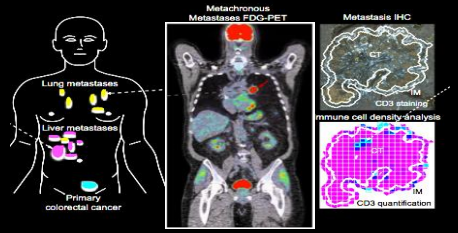
Progression

T-stage

VELIPI+ Dissemination

N+ Invasion

M+



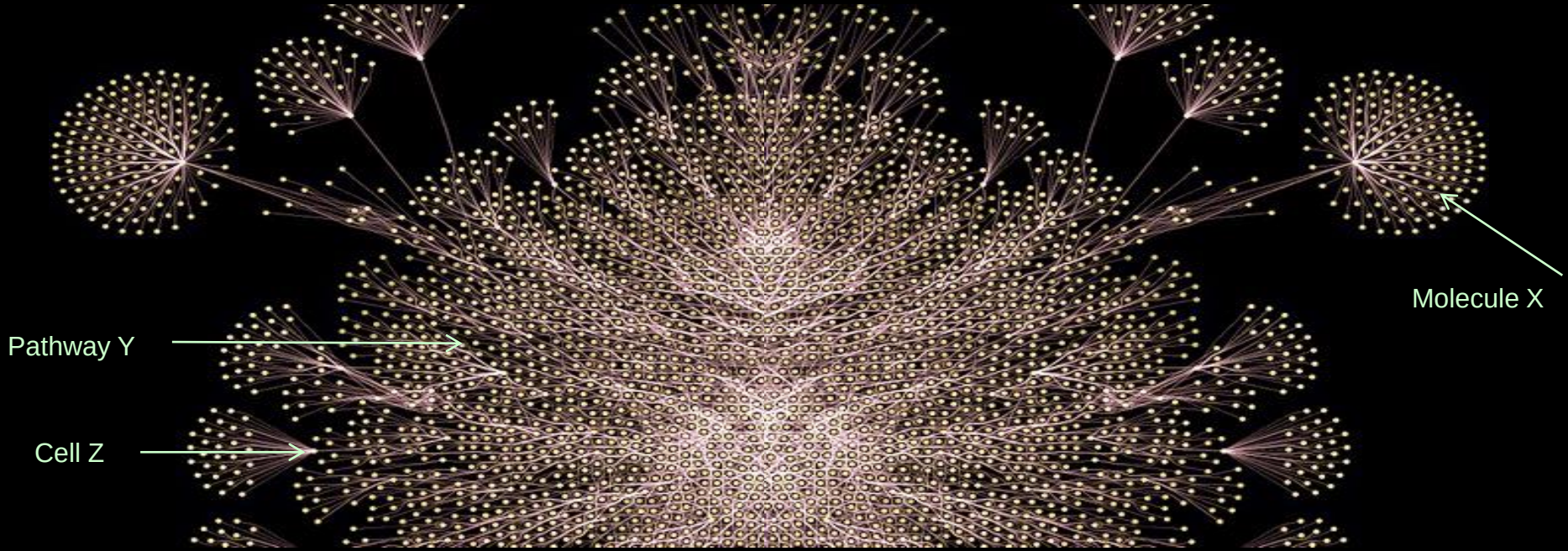
Prognosis

Recurrence

Death

Immunotherapy

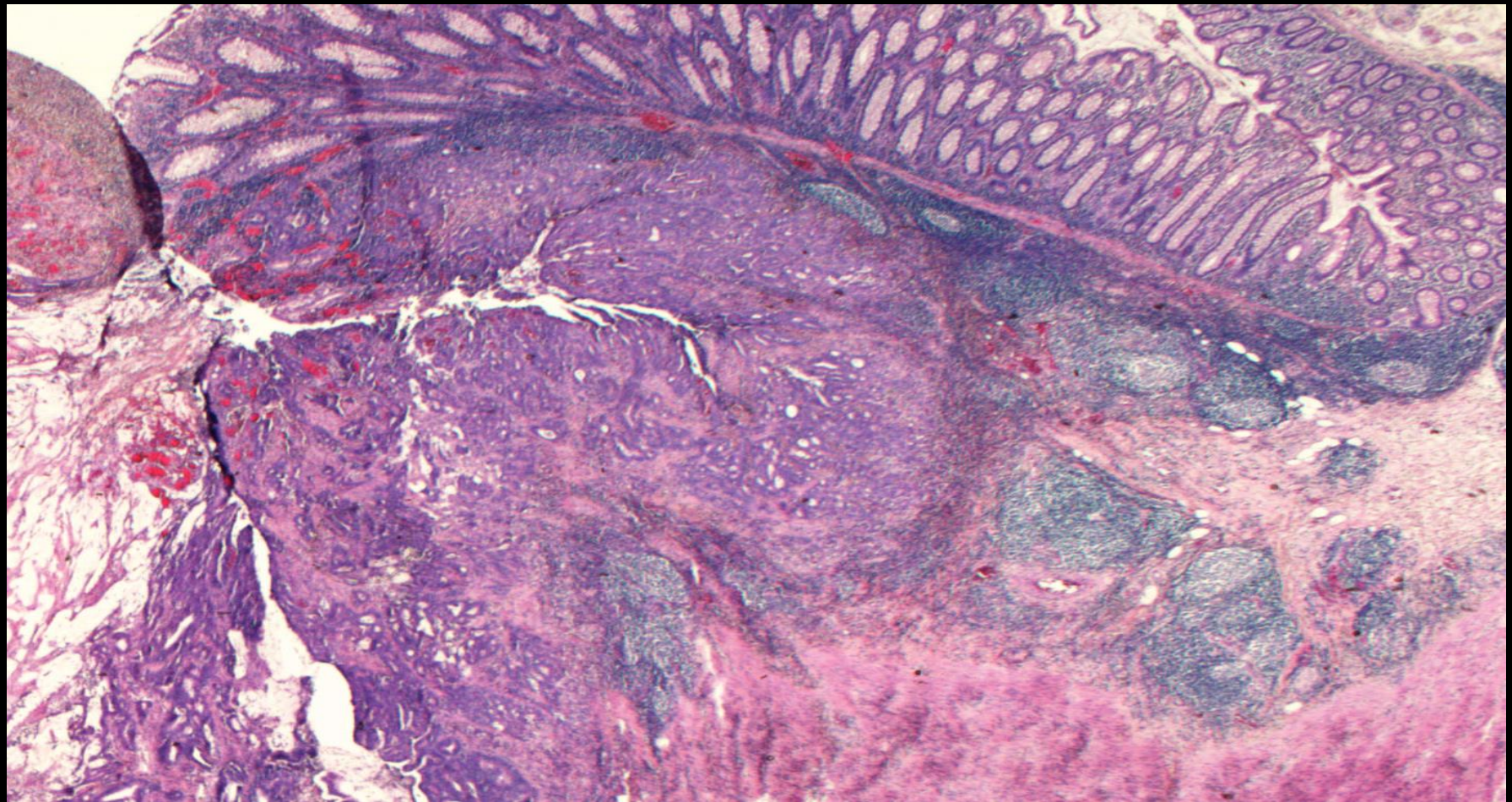
Cancer is one of the most complex biological system of all



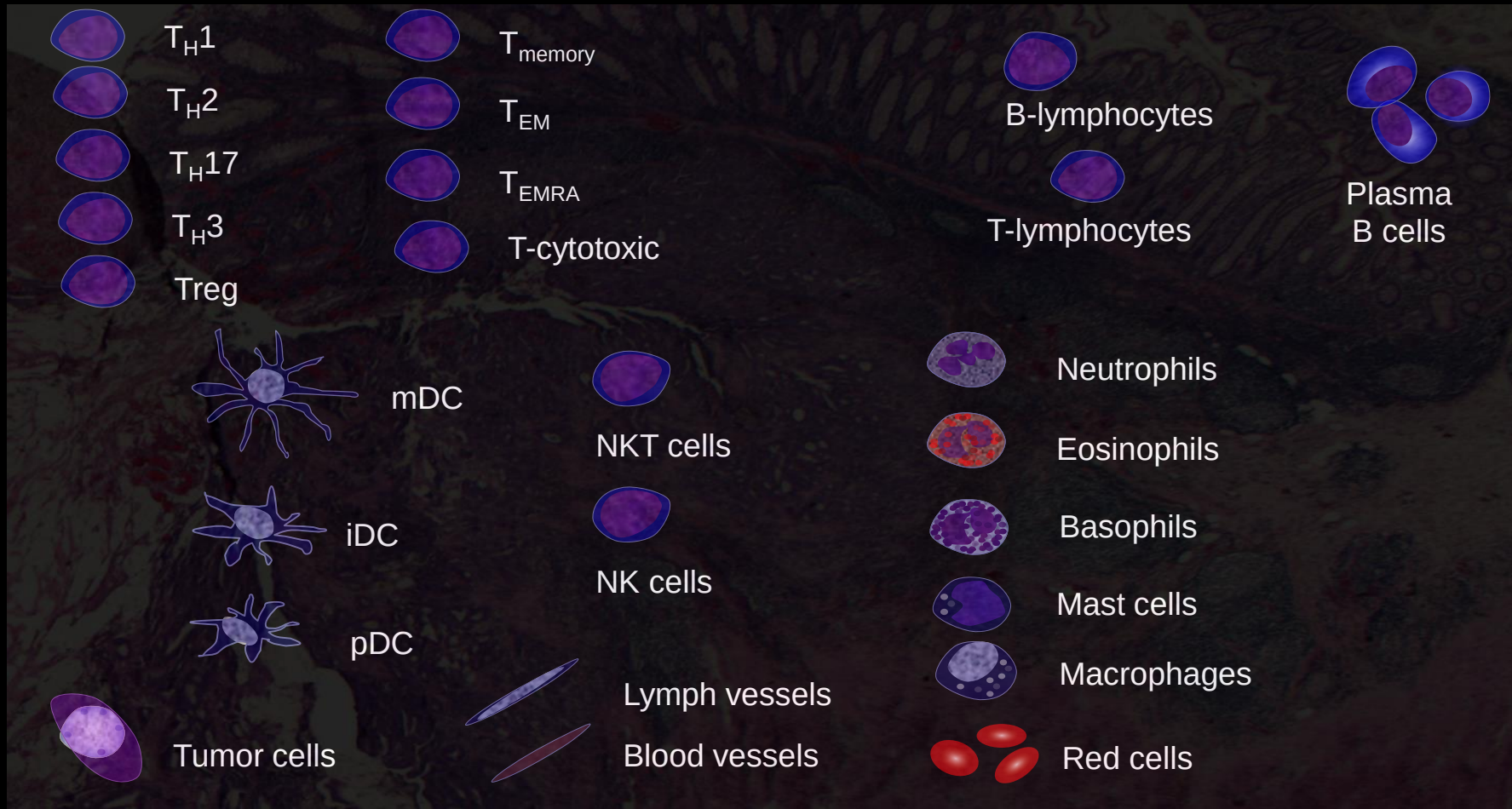
"*The whole is greater than the sum of its parts*", Aristotle

-> Systems biology in human cancer

Tumor microenvironment



Tumor microenvironment



Immunology and Cancer

Rudolf Virchow (1821-1902)
The origin of cancer is at sites of chronic inflammation

Stephen Paget, *Lancet* 1889
Seed & Soil hypothesis: role of microenvironment

Paul Ehrlich, 1909
Immune system may influence the incidence of cancer

Mac-Farlane Burnett, *Brit Med J* 1957
Immunosurveillance concept

Crosstalk between innate and adaptive immune surveillance:
A balance between protumor and antitumor immunity

Inflammation  Immunity

→ Is the immune system important against cancer ?

Published Today, January 14th, 2020

Immunity
Review

CellPress

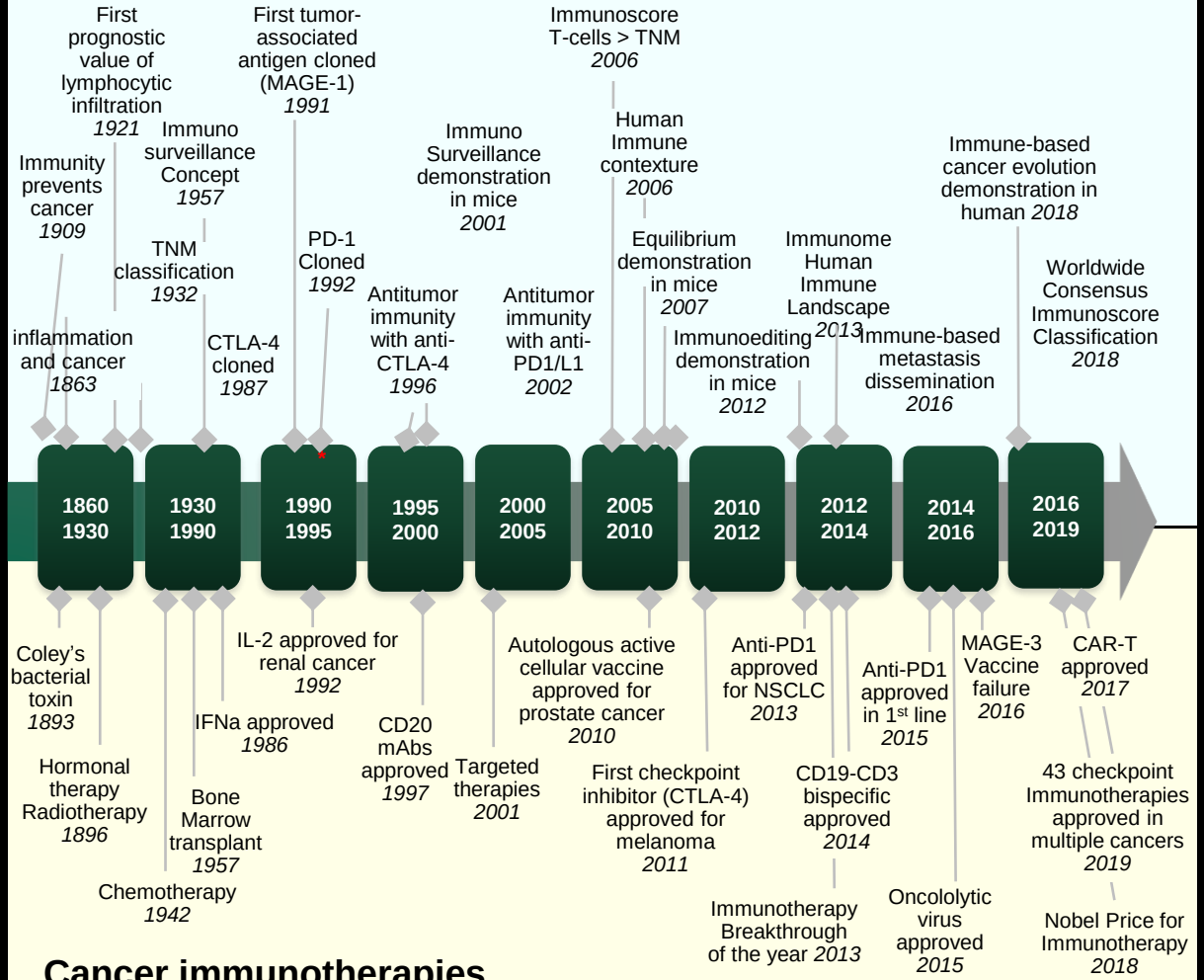
Tumor Immunology and Tumor Evolution: Intertwined Histories

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

Galon and Bruni review the evolution of the field of tumor immunology, how these advances have shaped our understanding of cancer as a disease, and the importance of revising current cancer stratification system to include immune parameters so as to better guide clinical decisions. The cover illustrates the evolution of tumor clones and of the immune contexture during carcinogenesis, tumor progression, and invasion in space and time, with intratumoral T cells depicted in orange and immunotherapy symbolized by blue antibodies. Image credit: Daniela Bruni and Jérôme Galon.

Galon & Bruni ***Immunity*** 2020

Tumor-Immunology

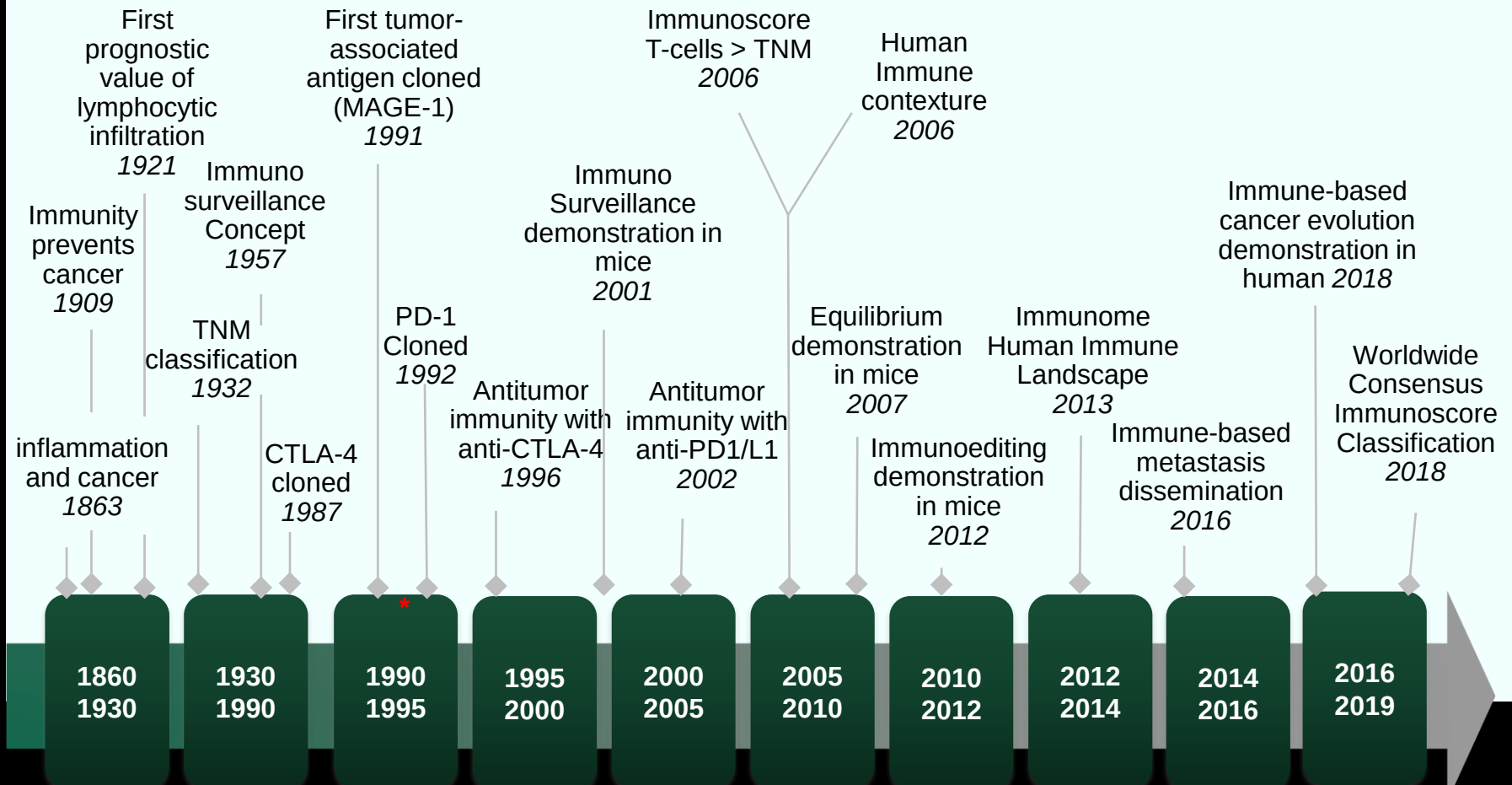


Cancer immunotherapies

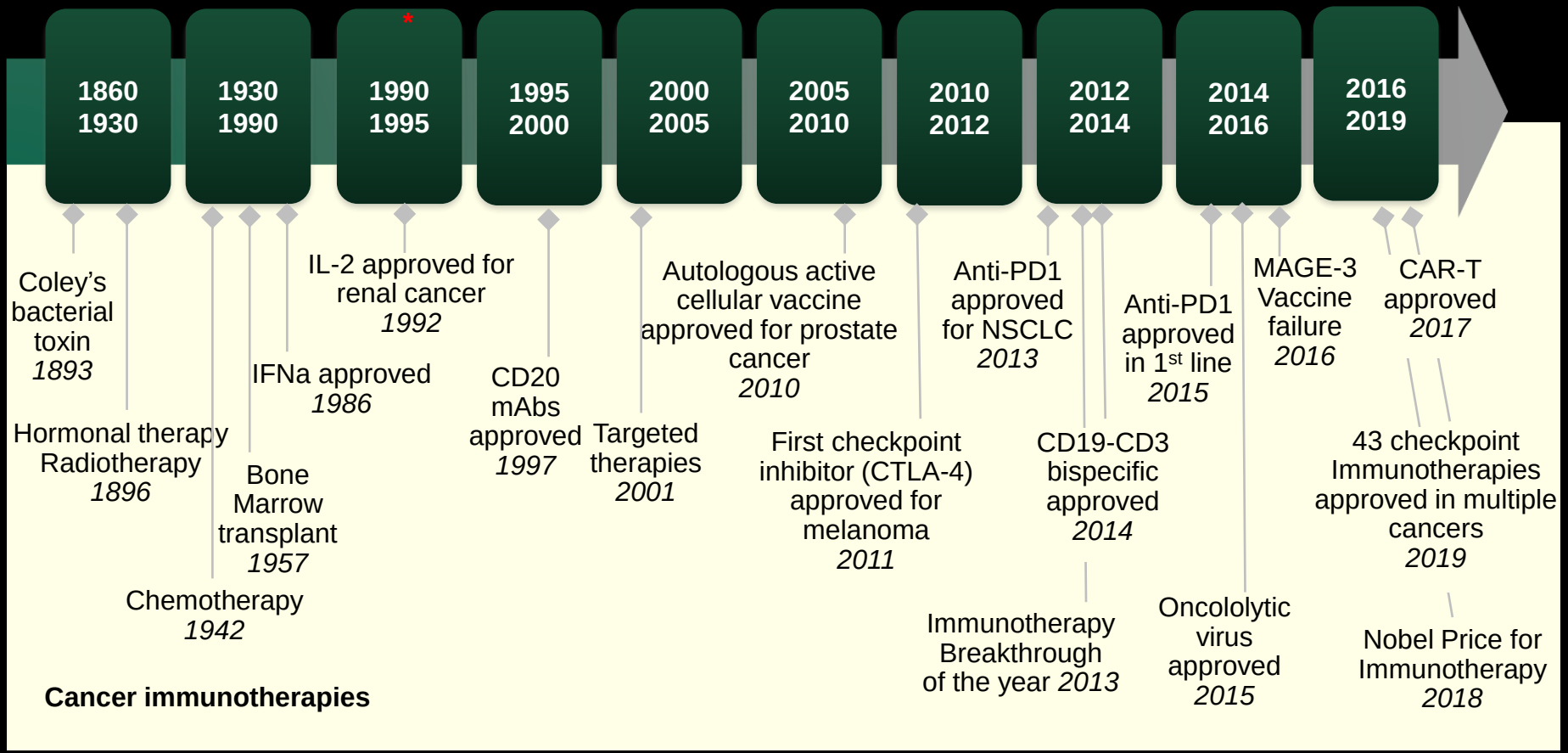
Galon & Bruni
Immunity 2020

Tumor-Immunology

Galon & Bruni ***Immunity*** 2020

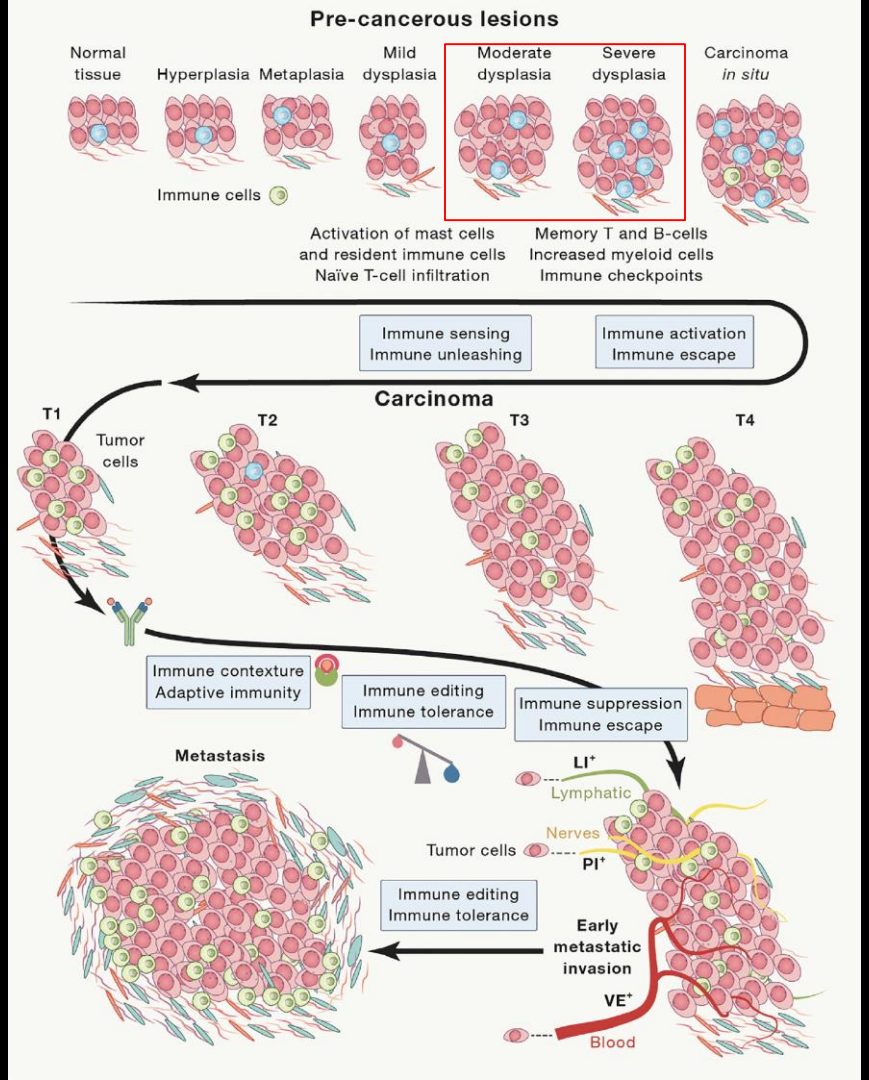


Cancer immunotherapies



Galon & Bruni ***Immunity*** 2020

CANCER EVOLUTION

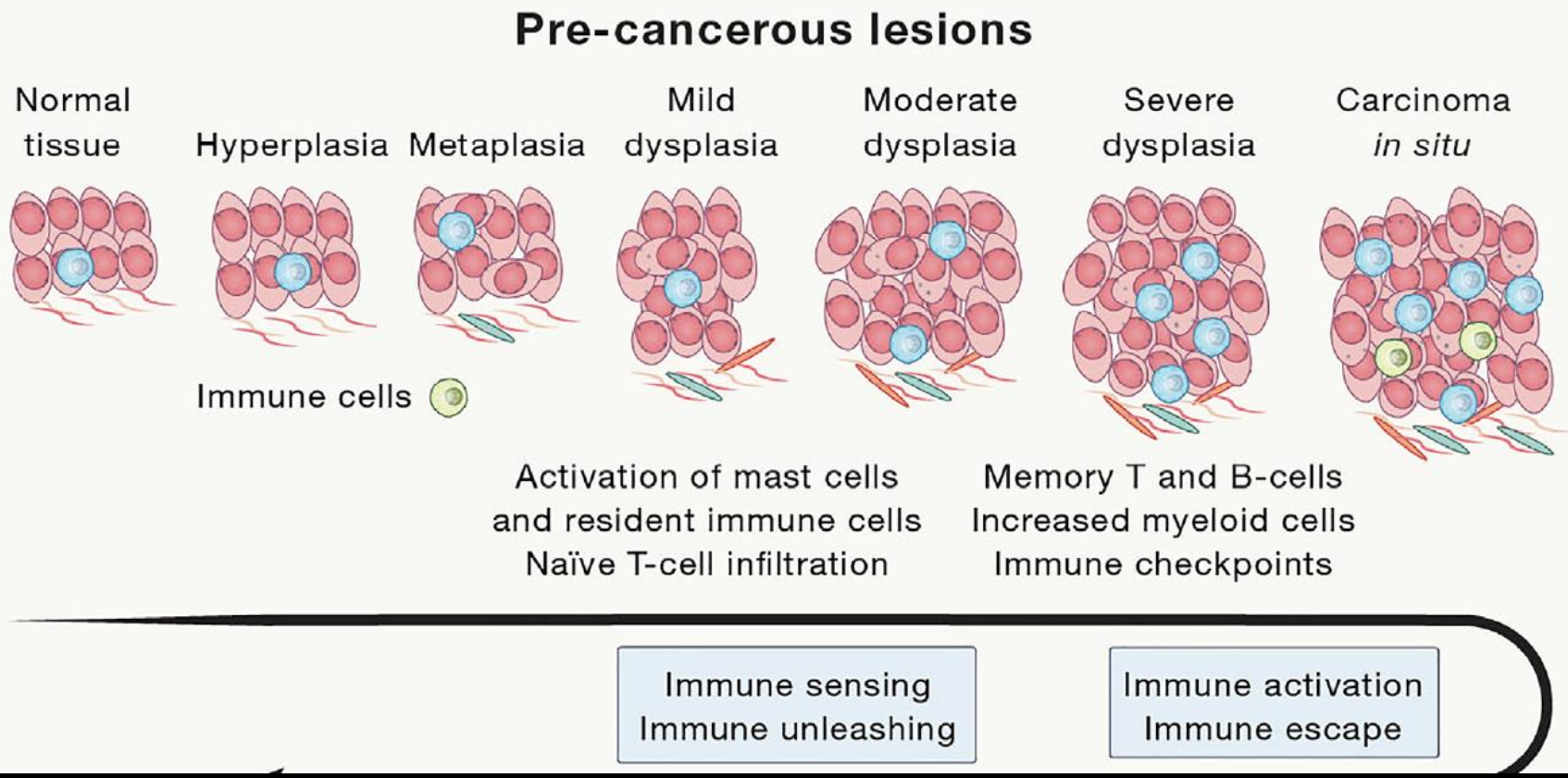


Innate Immunity (hours)
Adaptive immunity (1-3 days)

Cancer evolution (decades)

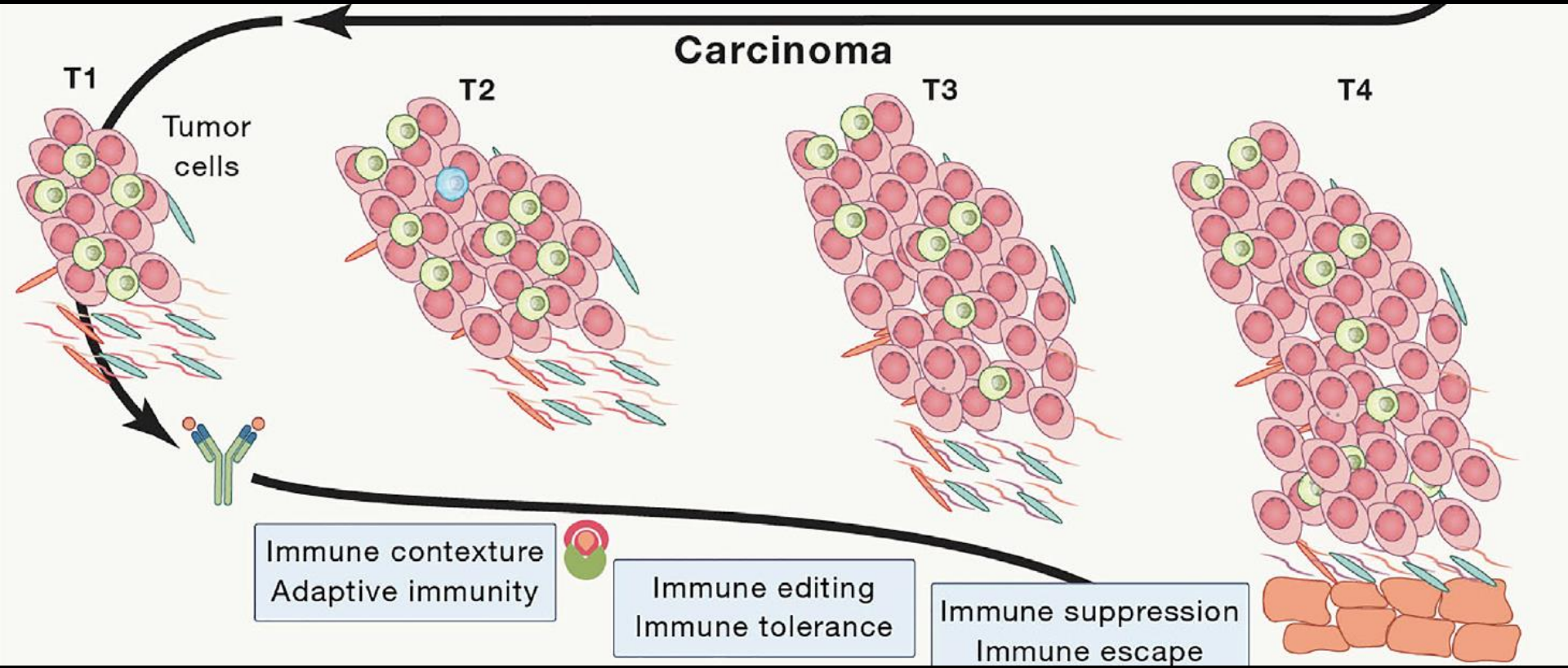
Galon & Bruni ***Immunity*** 2020

CANCER EVOLUTION



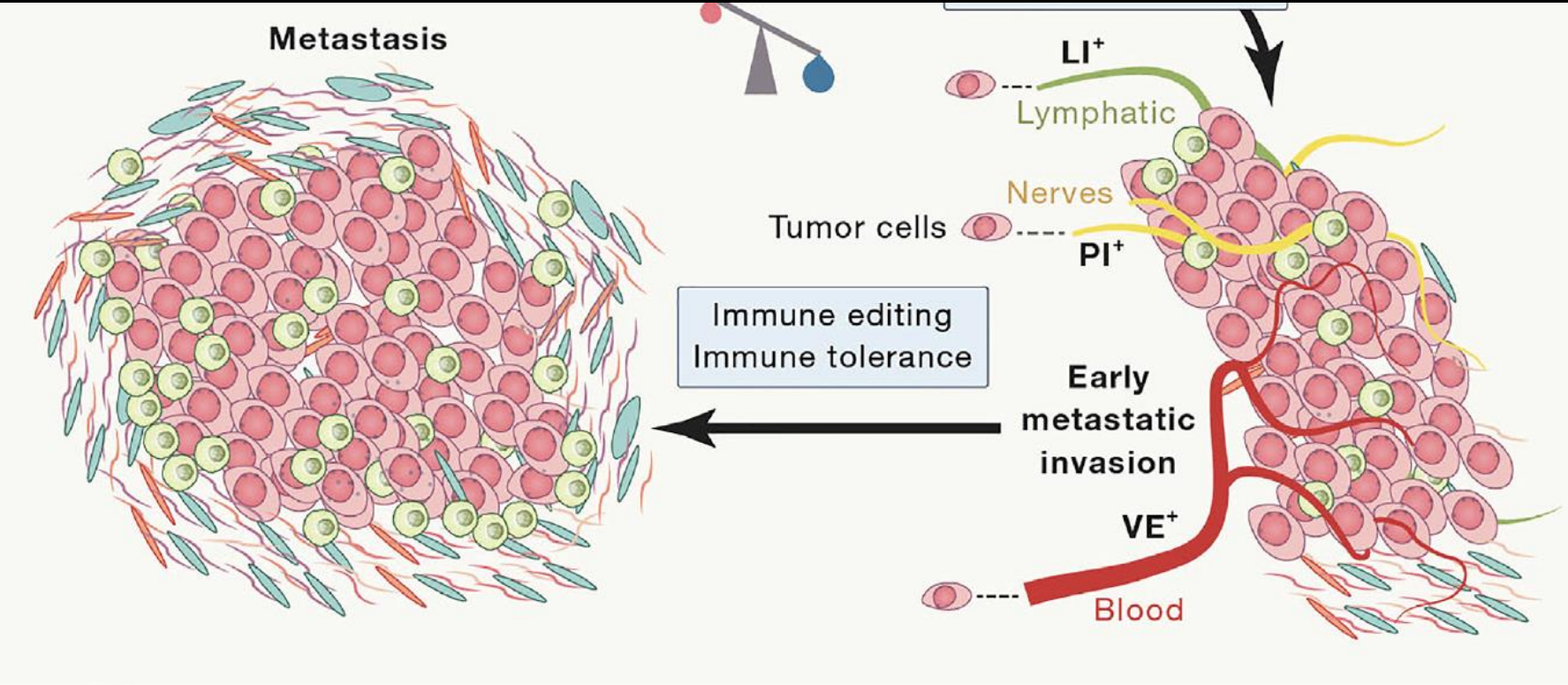
Galon & Bruni ***Immunity*** 2020

CANCER EVOLUTION



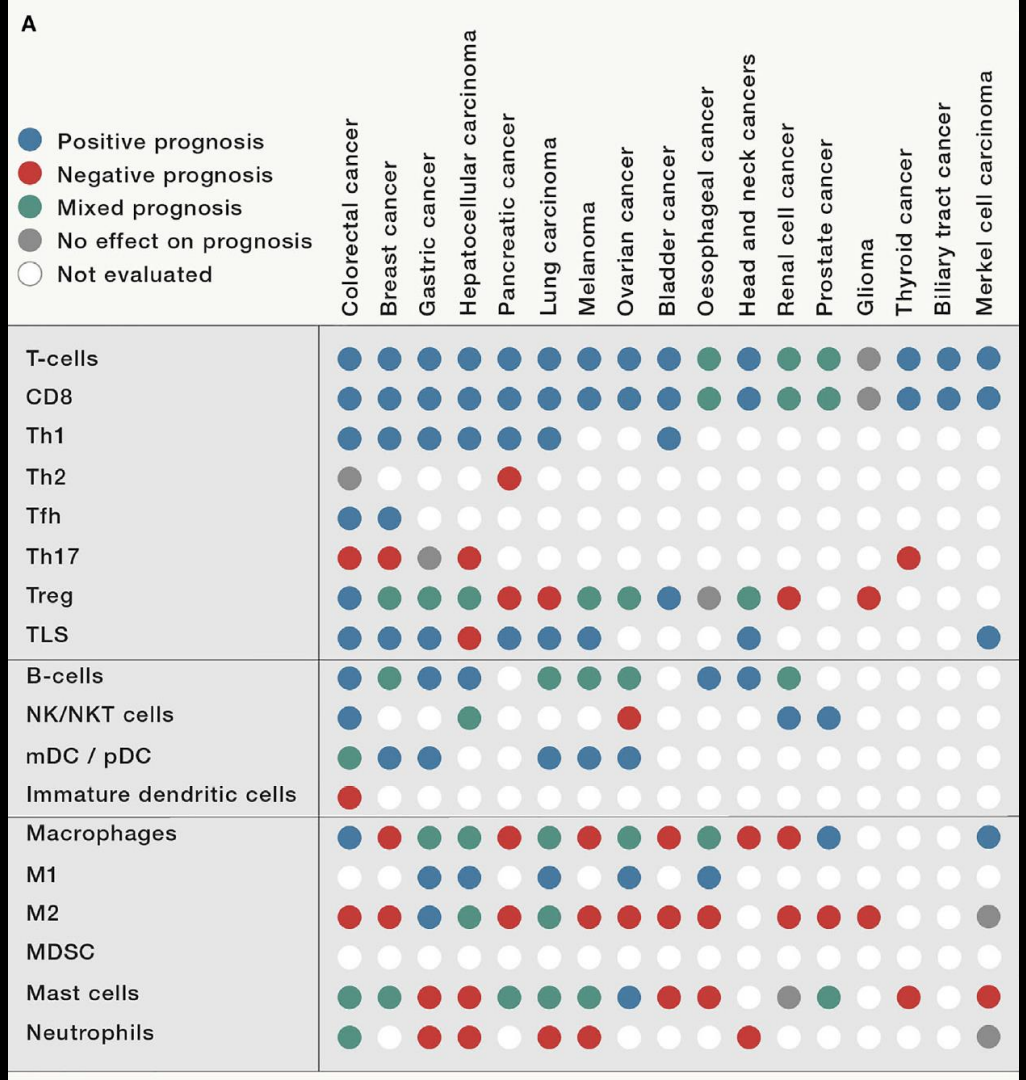
Galon & Bruni ***Immunity*** 2020

CANCER EVOLUTION

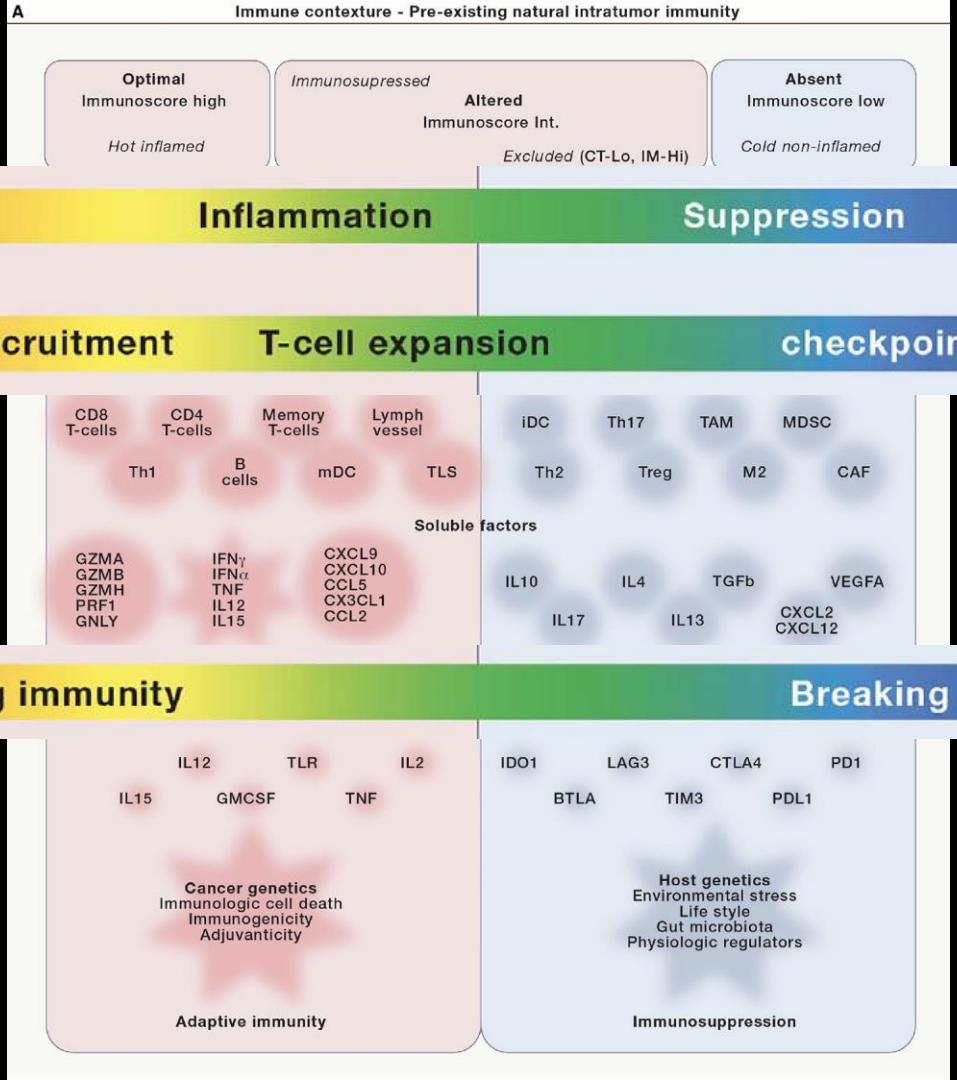


Galon & Bruni ***Immunity*** 2020

Prognostic impact of immune cells



Galon & Bruni ***Immunity*** 2020



Galon & Bruni ***Immunity*** 2020

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

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

A Novel Paradigm for Cancer

Science
AAAS

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

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

29 SEPTEMBER 2006 VOL 313 SCIENCE www.sciencemag.org

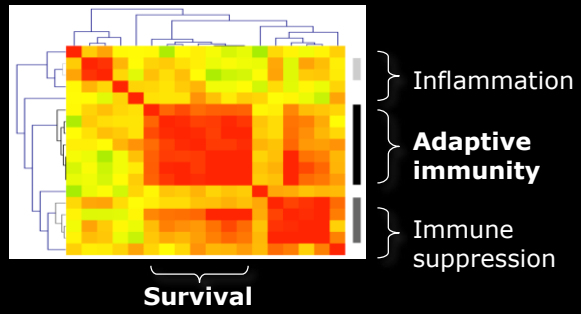
The foundation a new concept

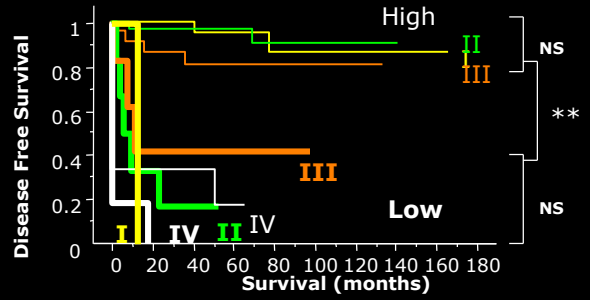
↓

Immune contexture

- ✓ Gene expression profiling
- ✓ Qualitative immune signature

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





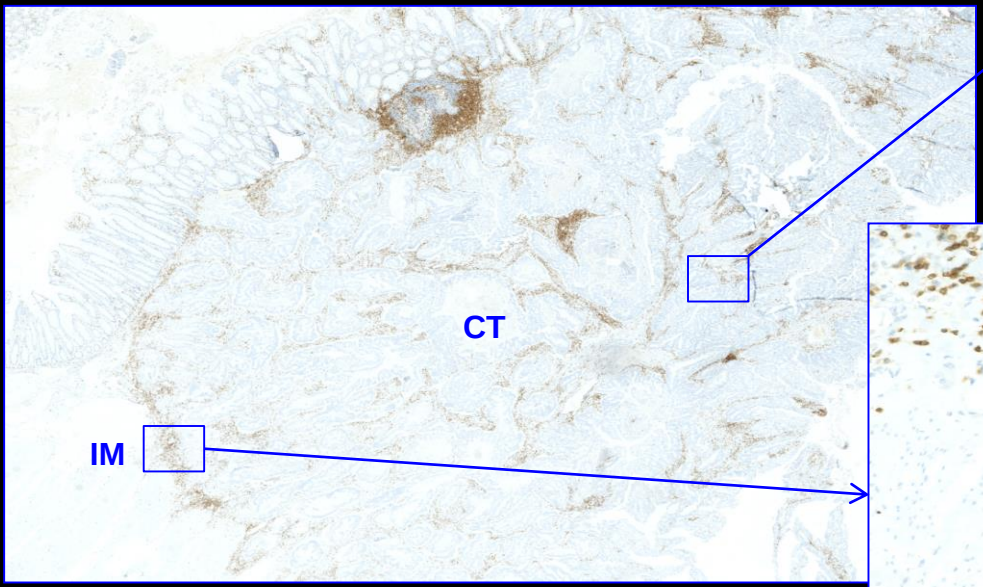
Optimized Immunosign

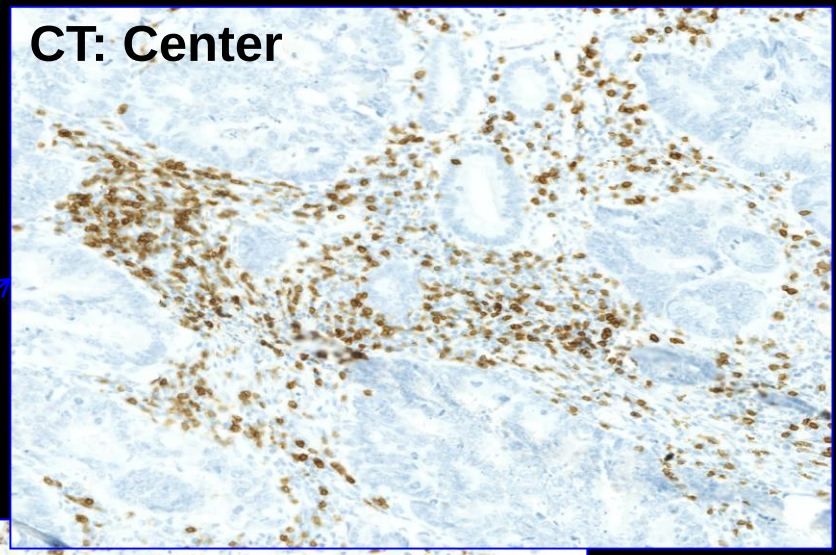
Quality

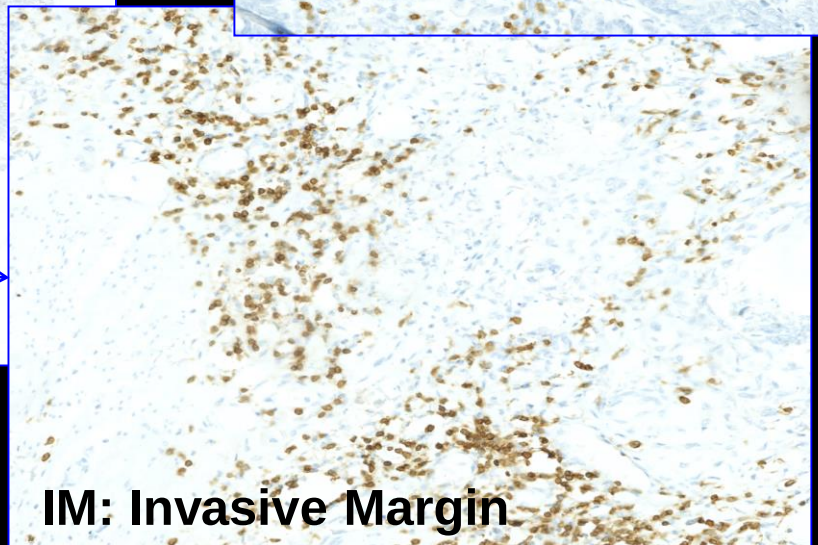
Type/Density/Location

Galon J et al. *Science* 2006

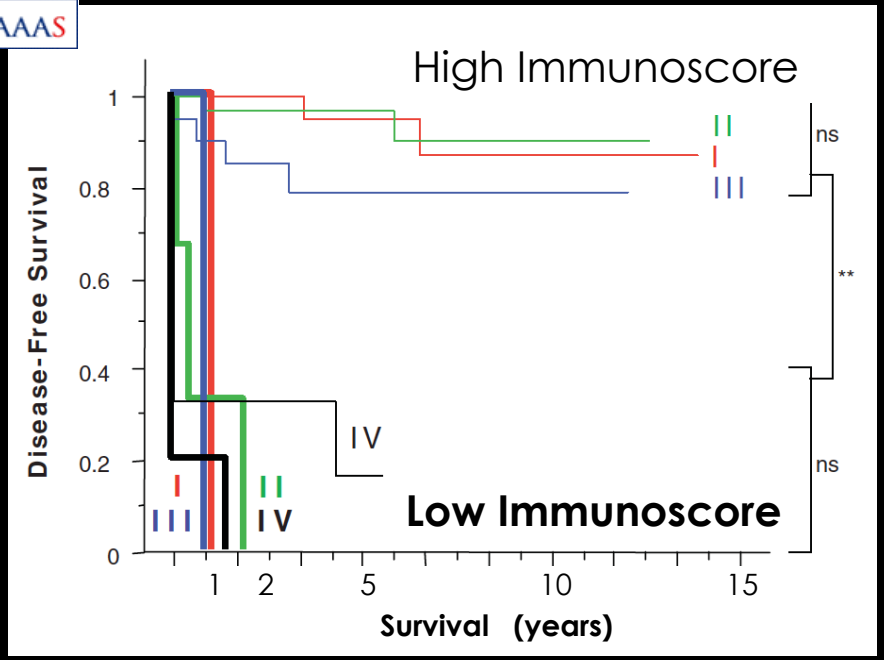
Digital quantification of immune cells infiltrating tumors: *Immunoscore*







Immunoscore: a novel paradigm for cancer



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

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

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

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

A Novel Paradigm for Cancer

Multivariate Cox Analysis

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

"***Immune Contexture***" :

Cells -> ✓**Type**

Quantity -> ✓**Density**

Spatial -> ✓**Location**

-> Immunoscore

Quality -> ✓Immune **functional** orientation

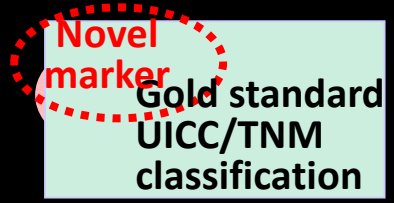
-> Immunosign

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

Assessment of a novel marker for prognosis

multivariate analysis (COX)

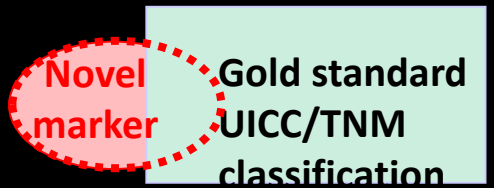
Not good marker



P: ns

No improvement for prediction

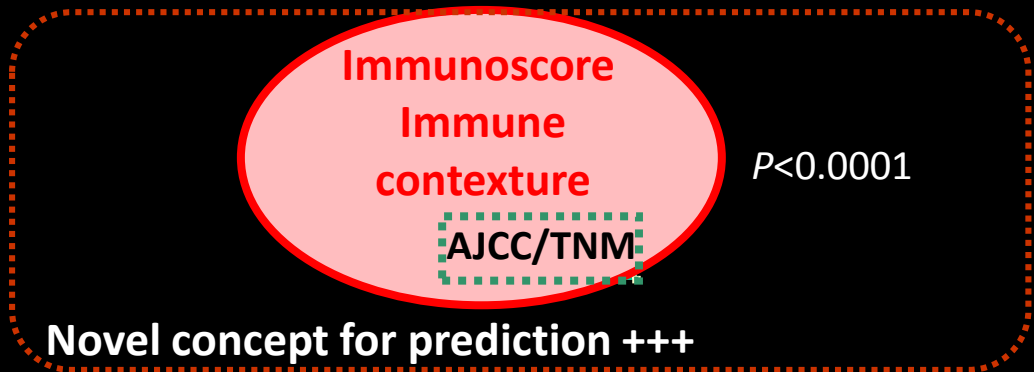
Good marker



P<0.05

Better accuracy for prediction +

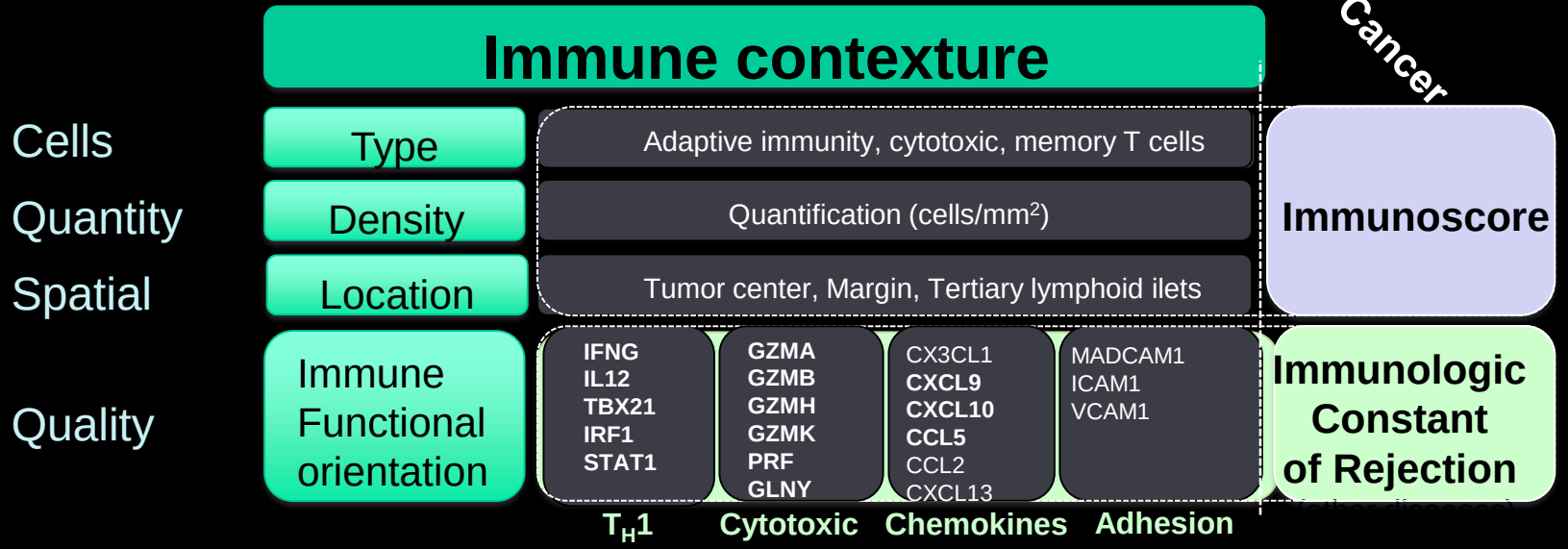
Novel concept



P<0.0001

Novel concept for prediction +++

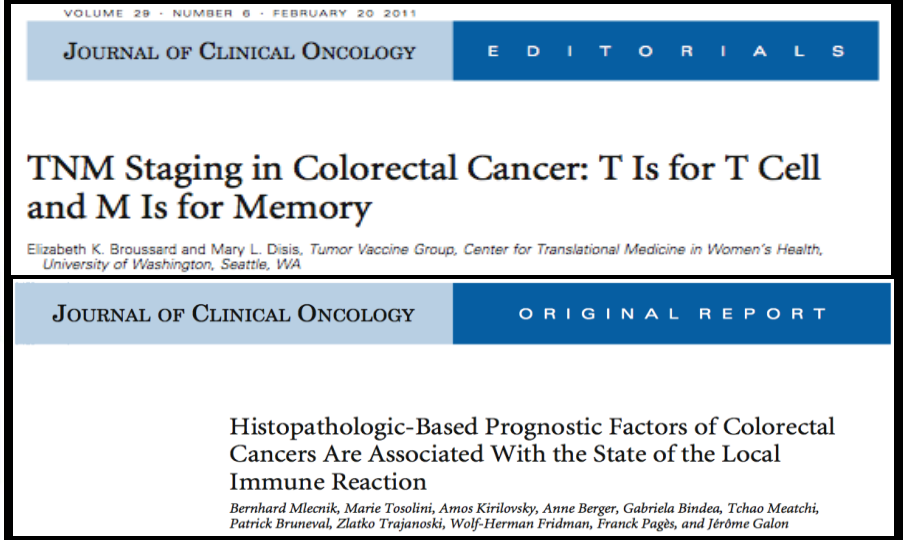
The overlap between the immunologic constant of rejection, the immune contexture and the Immunoscore



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

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



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

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

Editorial: Broussard et al. *JCO* 2011

Multivariate Analysis

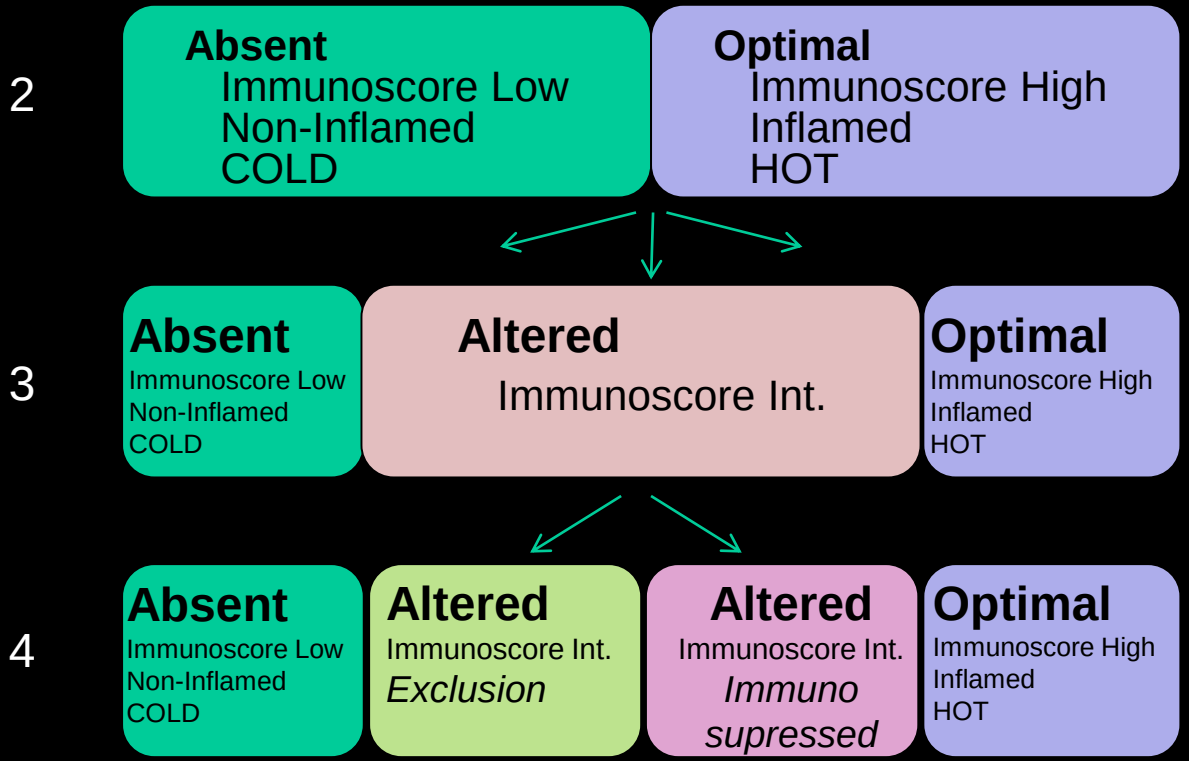
Cox Analysis	DFS		OS		DSS	
	HR	*P*-value	HR	*P*-value	HR	*P*-value
AJCC/UICC-TNM	1.38	0.09 ns	1.18	0.29 ns	1.43	0.10 ns
Immunoscore	0.64	<0.0001	0.71	<0.0001	0.63	<0.0001

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

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

Major immune categories of tumors



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

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

Camus & *Galon Cancer Res* 2009

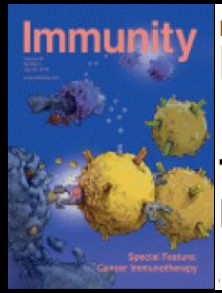
Immunity
Review

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 continuum of cancer immunosurveillance

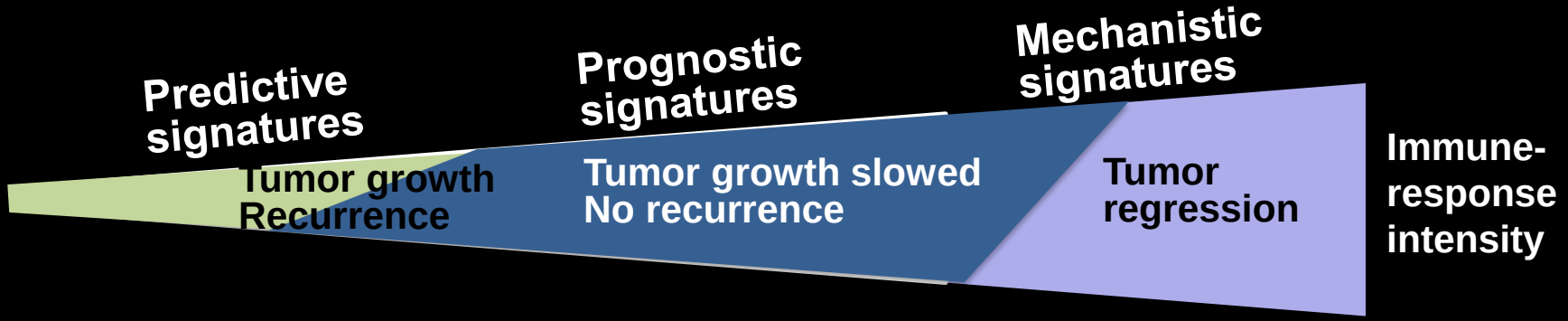


Immunity
Review

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

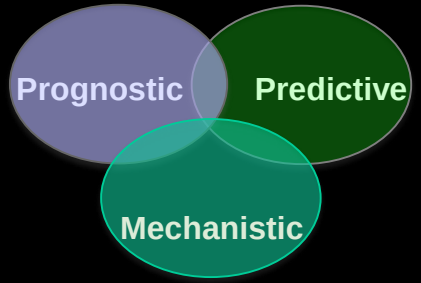
The overlap between prognostic, predictive and mechanistic immune signatures

- **Prognostic signatures**: better disease-free and overall survival
- **Predictive signatures**: increased likelihood to respond to therapy
- **Mechanistic signatures**: cancers studied during treatments that subsequently undergo complete regression

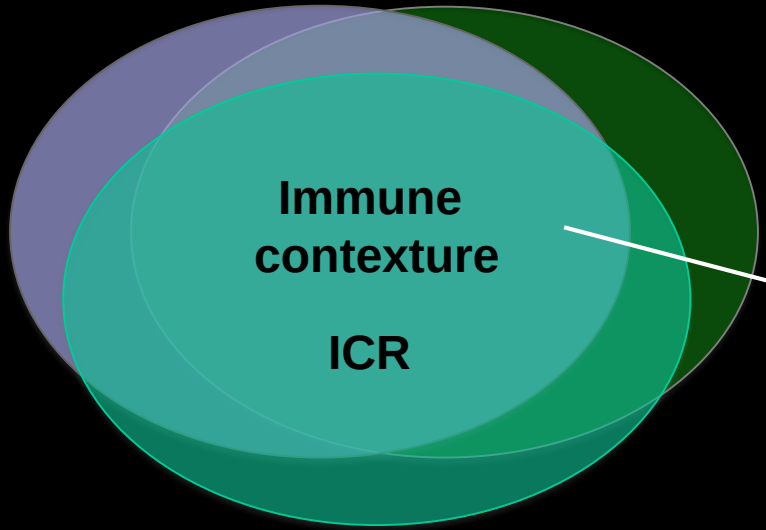
Galon J et al. ***Immunity*** 2013

The overlap between prognostic, predictive and mechanistic immune signatures

NON-Immune signatures



IMMUNE signatures



Immunoscore
Th1
Cytotoxicity
Chemokines
Cytokines
Adhesion

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

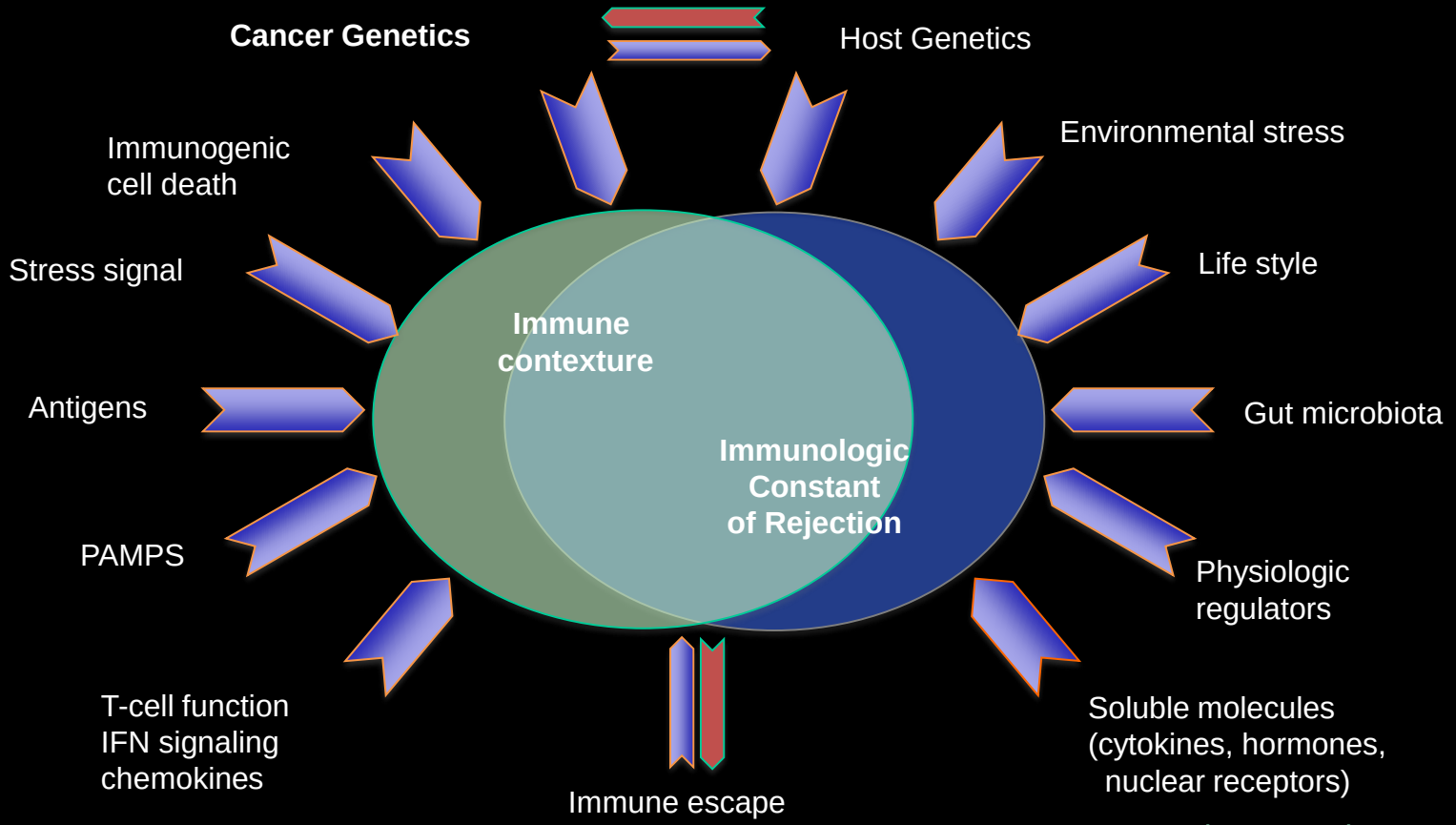
The overlap between prognostic, predictive and mechanistic immune signatures

IMMUNE signatures

	Immune contexture					
	STAT-1 IRF-1/IFN-γ-SG Pathway	CXCR3/ CXCL9-11 Pathway	CCR5/ CCL3-5 Pathway	Granzyme Perforin Granulisin/ TIA-1/CASPs Pathway	Adhesion Molecules	References
Prognostic						
Breast		+		+	+	Ascierto et al., 2012
	+	+	+			Curtis et al., 2012
Ovarian	+		+	+		Leffers et al., 2010
	+	+			+	Zhang et al., 2003
Melanoma	+	+	+			Messina et al., 2012
	+	+				Mann et al., 2013
Colorectal	+	+	+	+	+	Mlecnik et al., 2010
	+			+		Galon et al., 2006
	+			+		Pagès et al., 2005
	+		+	+		Tosolini et al., 2011
		+				Jiang et al., 2010
Lung			+			Moran et al., 2002
Hepatocellular	+	+	+		+	Chew et al., 2012
	+		+	+	+	Chew et al., 2010
Predictive						
Breast (Chemo)		+				Denkert et al., 2010
	+	+				Desmedt et al., 2008
	+	+				Teschendorff et al., 2007
	+	+				Ignatiadis et al., 2012
Melanoma (IL-2/ vaccine/ adoptive therapy/anti-CTLA-4)	+					Wang et al., 2002
	+		+		+	Weiss et al., 2011
	+	+				Gajewski et al., 2010
		+	+			Bedognetti et al., 2012
	+	+	+	+		Ji et al., 2012
	+	+	+	+		Ulloa-Montoya et al., 2013
Lung	+	+	+	+		Ulloa-Montoya et al., 2013
Mechanistic						
Melanoma (IL-2/ vaccine/anti-CTLA-4)		+	+	+	+	Panelli et al., 2002
	+					Wang et al., 2002
	+		+		+	Weiss et al., 2011
				+		Aarntzen et al., 2012
	+	+	+	+		Ji et al., 2012
Basal Cell (Imiquimod)	+	+	+			Panelli et al., 2007

Galon J et al.
Immunity 2013

Mechanisms associated with proper immune contexture and immunologic constant of rejection



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

Memory T cells: Remember to stay alive

Persistence of Memory,
Dali S. 1931

Persistence of memory T cells at the tumor site plays a role in preventing tumor recurrence

Correlation analyses may not reflect a direct activity

However, several arguments support this hypothesis

- Mouse models of immunosurveillance
- Many adaptive immune genes are associated with prognosis
- Signs of T cell activation, proliferation, maturation, cytotoxicity
- Tumor antigens recognized by T lymphocytes
- Specific CTLs efficiently lyzed colon carcinoma cells

Attest a cytotoxic lymphocyte priming of sufficient quality
Attest a general process extending behond primary tumor

-> Persistence of Memory in the periphery

Camus M, & Galon J.
Memory T-cell responses and survival in human cancer: remember to stay alive. **Adv Exp Med Biol.** 2010

Memory T cells

Disintegration of the Persistence of Memory,
Dali S. 1952

-> “Disintegration of the Persistence of Memory”

-> Absence of Memory T cells generated *in situ*

-> Absence of Memory T cells in the periphery

-> Tumor recurrence

- hallmarks of tumor cells
- absence of danger signal
- immunosuppression

-> • absence of appropriate T cells (chemokines)

- absence of cytotoxicity

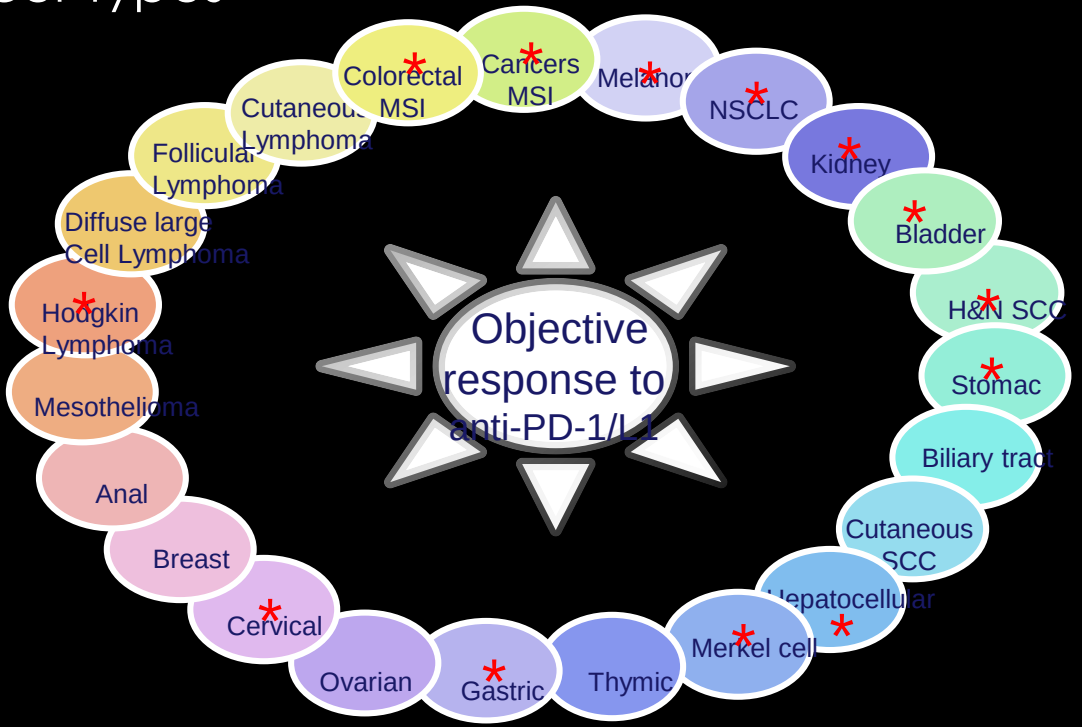
-> • incorrect orientation (T_H1, T_H2, T_H3, T_H17, TAM…)

-> • absence of immune coordination

- lethargic memory (without CD4 and CD8)
- T-Reg
- coinhibition
- APC / presentation
- loss of MHC molecules
- *etc* …

Objective responses to anti-PD-1/L1

Multiple cancer types



Modified from Marabelle A, et al.
Oncoimmunology, 2015

* *FDA approved*

Approved Immunotherapies

Melanoma

Ipilimumab monotherapy

Pembrolizumab 2nd line

Nivolumab 2nd line

T-VEC oncolytic V

Nivo/Ipi Combo

Ipilimumab adjuvant

Nivolumab 1st line

Pembro 1st line

Nivolumab *biomarker*

Pembro All cancers *biomarker MSI-H*

Multiple immunotherapies In multiple cancers

2011 | 2012 | 2013 | 2014 | 2015 | 2016 | 2017 | 2018

Other cancers

Immunotherapy: Breakthrough of the year

Nivo NSCLC squamous 2nd line

Pembro NSCLC Squamous 2nd line *biomarker*

Nivo NSCLC Non-squamous 2nd line *biomarker*

Nivo RCC 2nd line

Nivo CHL 4th line

Pembro SCCHN 2nd line

Atezo Bladder 2nd line *biomarker*

Atezo NSCLC 2nd line

Pembro NSCLC 1st line *biomarker*

Nivo SCCHN 2nd line

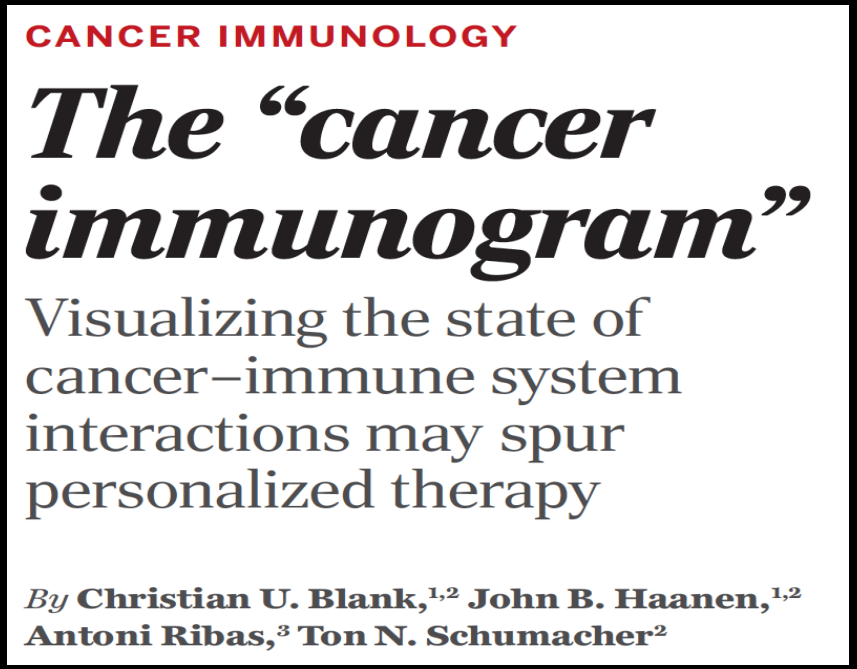
Nivo Bladder 2nd line

Durva Bladder *Biomarker comp*

Avelu Merkel 2nd line

Atezo Bladder 1st line *Biomarker comp*

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,[1,2] John B. Haanen,[1,2] Antoni Ribas,[3] Ton N. Schumacher[2]**

Blank et al. *Science* 2016

Review about all published biomarkers of response to immunotherapy

Predictive markers to immunotherapies: the cancer Immunogram

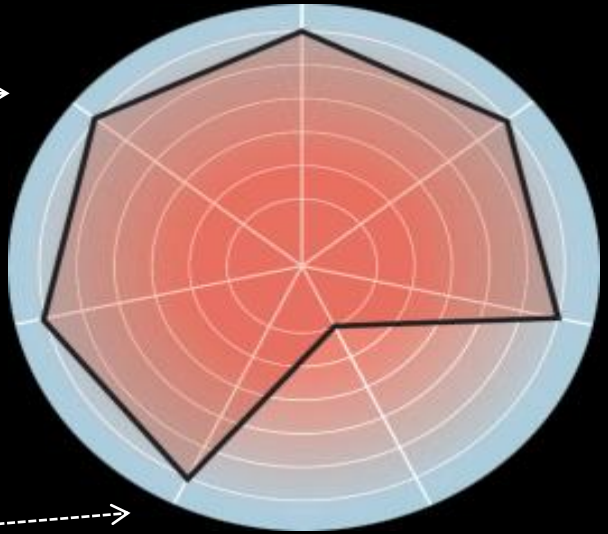
Peripheral

Peripheral immune status
Lymphocyte count

Abscence of inhibitory tumor metabolism
LDH, glucose

Abscence of soluble inhibitors
IL-6, CRP

Tumor foreignness
*Mutational load, MSI **



Intra-tumoral

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

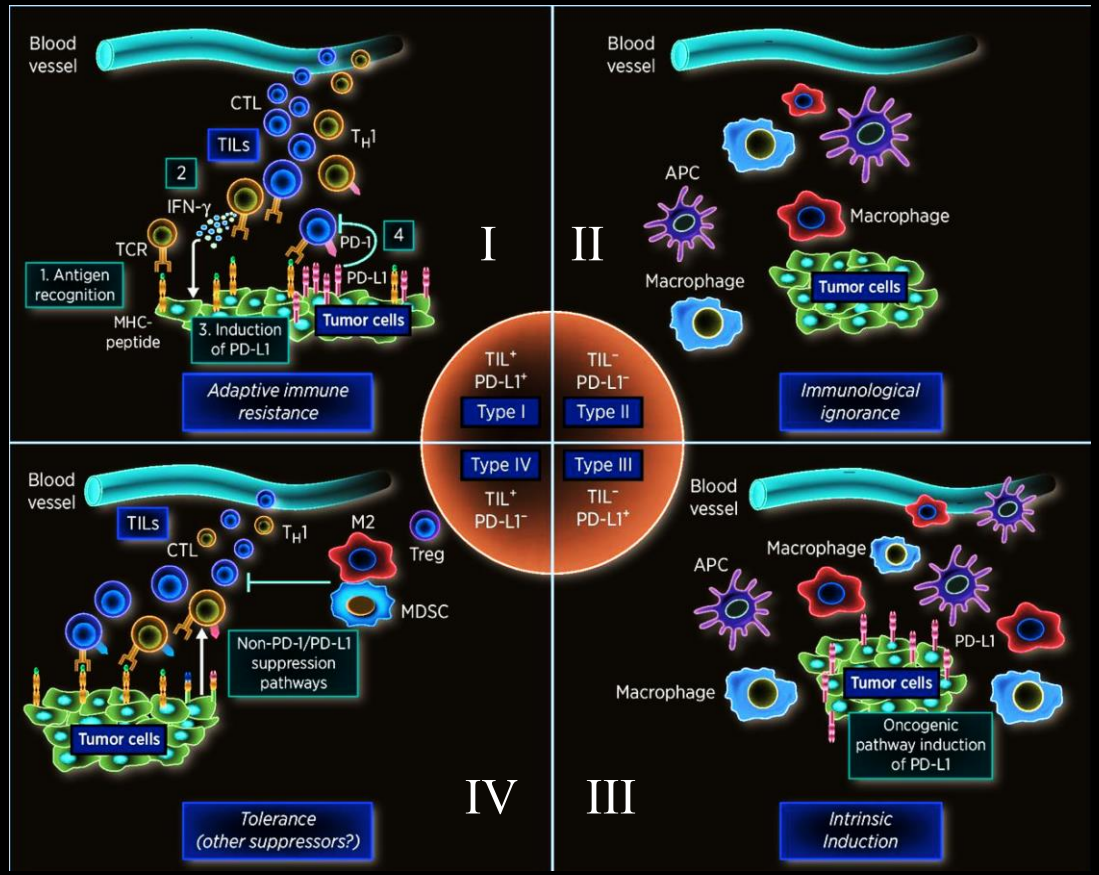
Immune cell infiltration
Immunoscore

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

Predictive immune biomarkers for immunotherapy response in melanoma

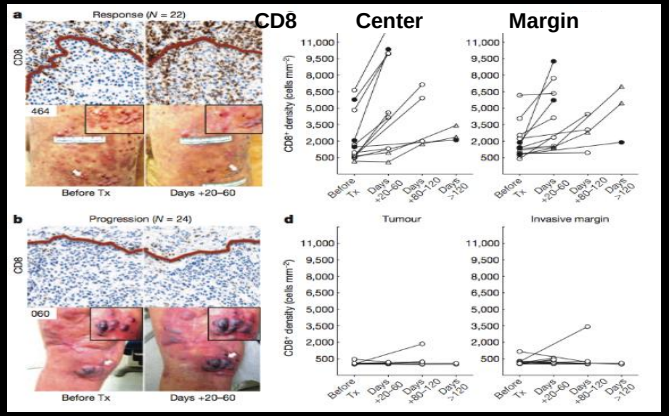
n = 46 meta. melanoma biopsies from pts treated with an anti-PD-1

Selection of best predictors of response to anti-PD1 (stepwise procedure)

CD8+ density in IM best predictive marker:

$CD8_{IM}$ ($p<0.0001$) > **$CD8_{CT}$** ($p<0.0001$) > **PD1** ($p<0.001$) > **PDL1** ($p<0.01$) > CD4 ns

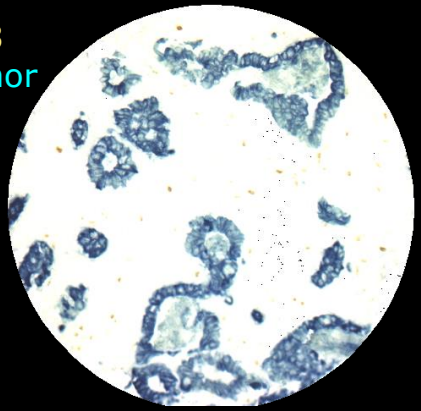
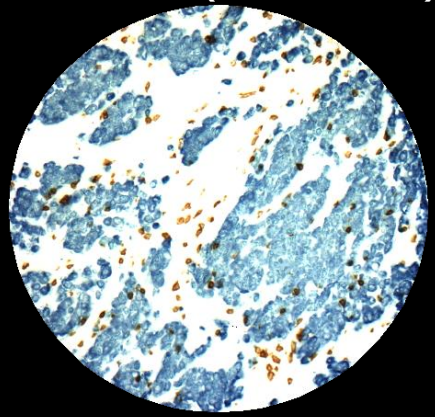
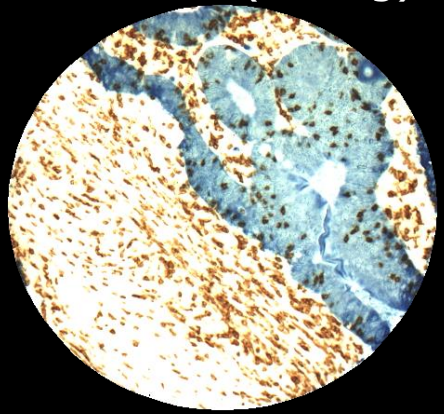
Validation on an independent cohort:



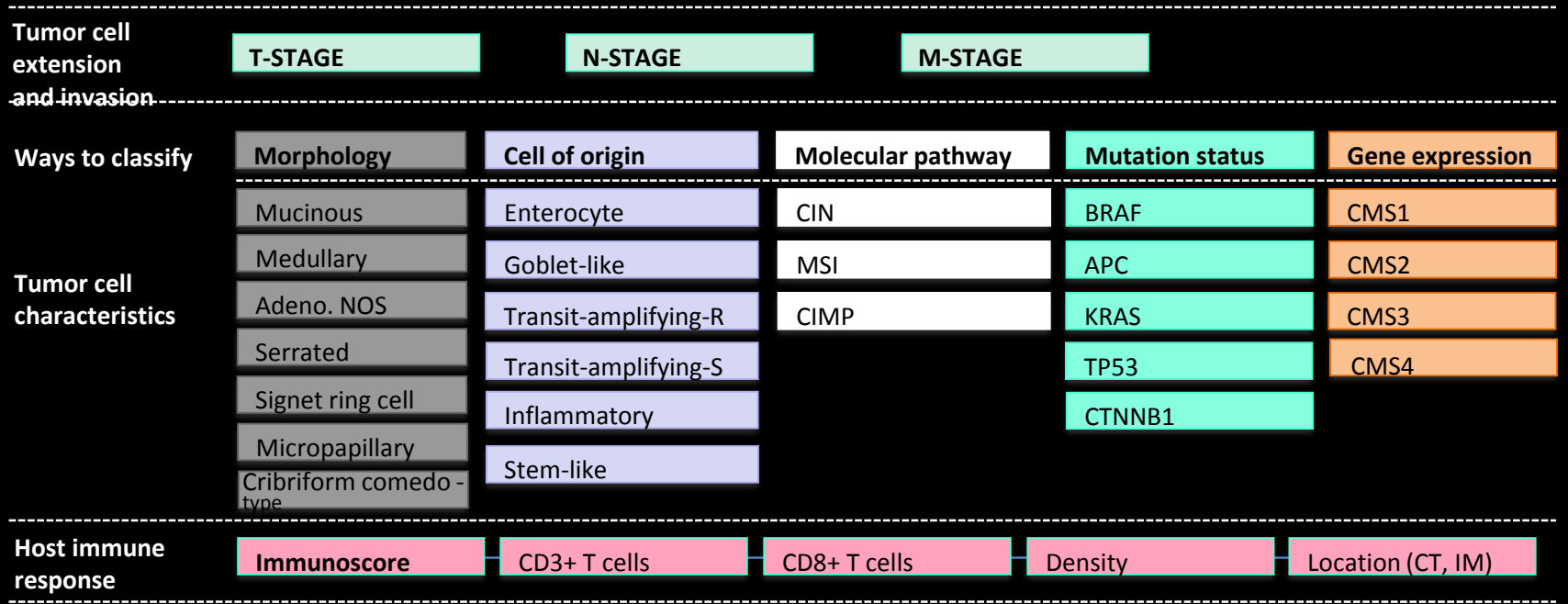
Patient ID	CD8+ Density, Before Tx (Invasive Margin)	Predicted Probability of Response (Logistic Model)	Blinded Prediction	True Clinical Response (RECIST 1.1)
IGR - A	58	0.35	Progression	Progression
IGR - B	159	0.37	Progression	Progression
IGR - C	329	0.40	Progression	Progression
IGR - D	341	0.41	Progression	Progression
IGR - E	2120	0.75	Response	Stable
IGR - F	5466	0.98	Response	Progression
IGR - G	2211	0.76	Response	Response
IGR - H	3810	0.92	Response	Response
IGR - I	4294	0.95	Response	Response
IGR - J	4948	0.97	Response	Response
IGR - K	5565	0.98	Response	Response
IGR - L	6004	0.99	Response	Response
IGR - M	5951	0.99	Response	Complete Response
IGR - N	7230	0.99	Response	Complete Response
IGR - O	6320	0.99	Response	Complete Response

Tumeh et al. ***Nature*** 2014

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

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

Colorectal cancer classifications



Galon et al. ***J Pathol***. 2014

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

World Immunotherapy Council inaugural meeting (Feb 2012)

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

Worldwide Immunoscore consortium (PI: J Galon)

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

Assay harmonization

Immunoscore meetings :

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

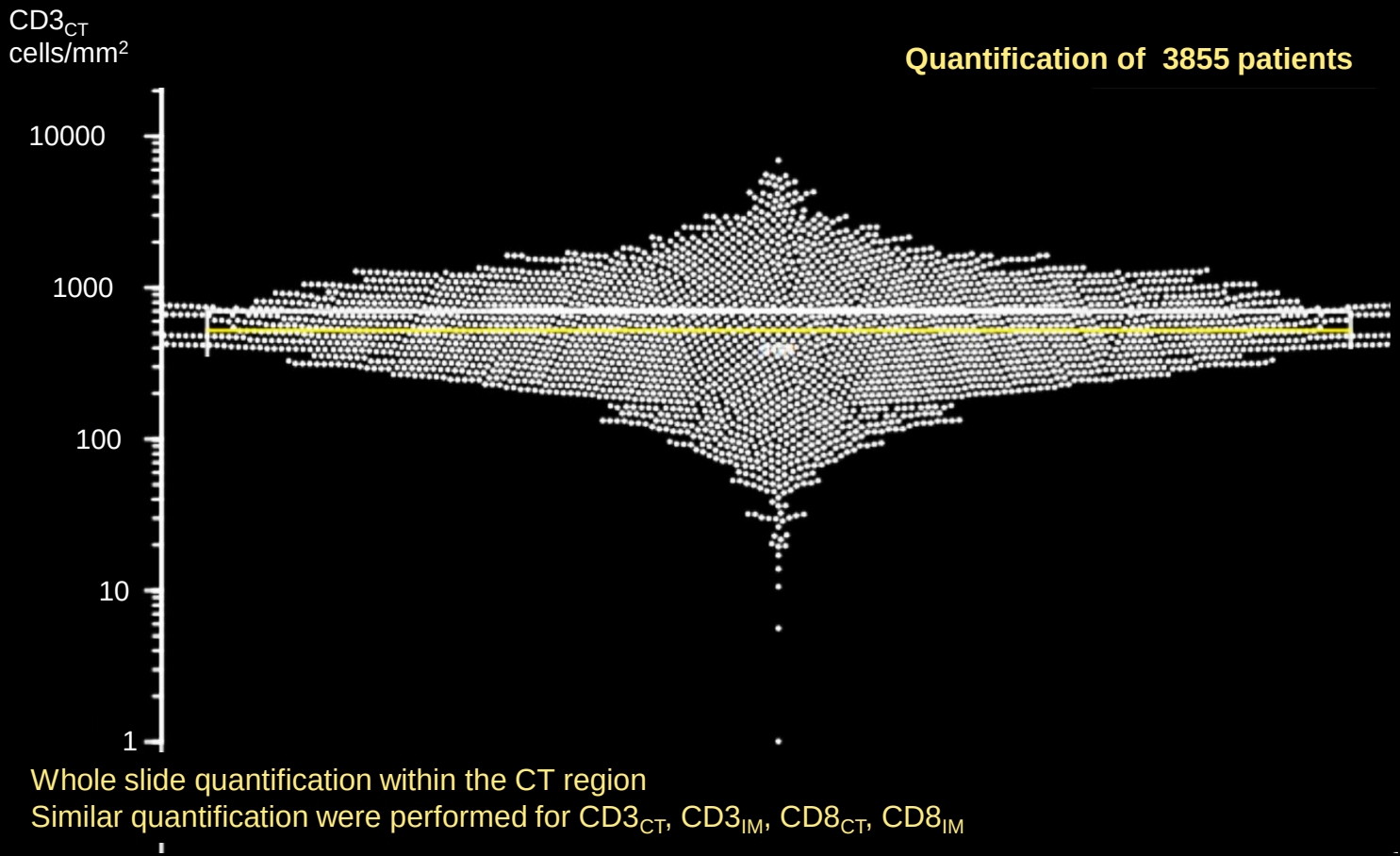
THE LANCET

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

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

Pages et al. *The Lancet* 2018

Densities of $CD3_{CT}$ (cells/mm²) within tumors

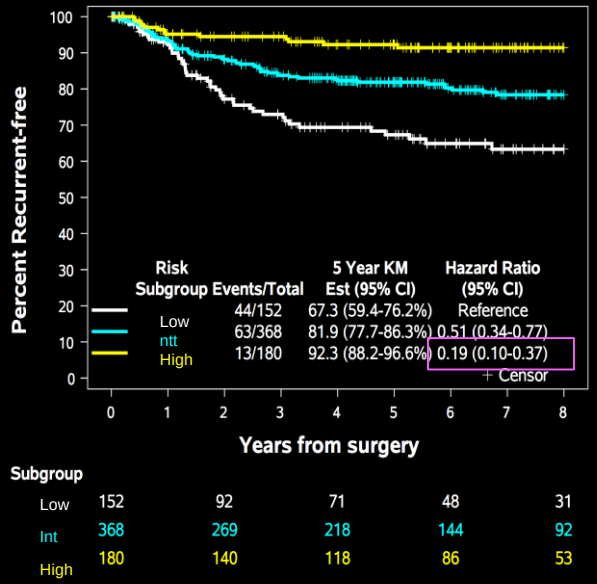


Quantification of 3855 patients

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

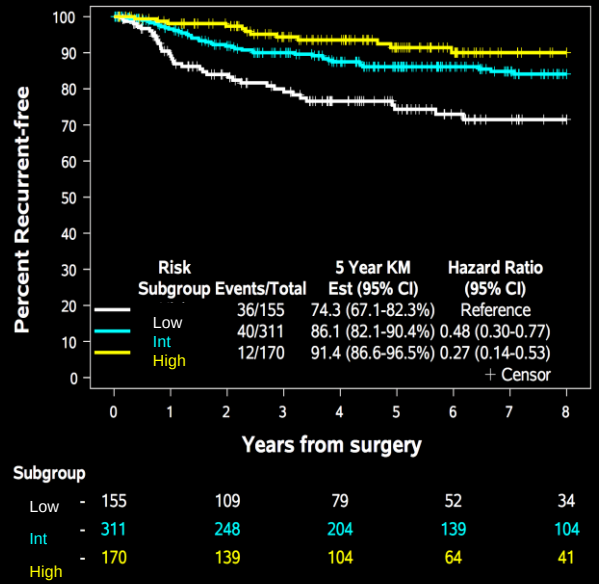
Time to recurrence for Immunoscore (High/Int/Low)

TS



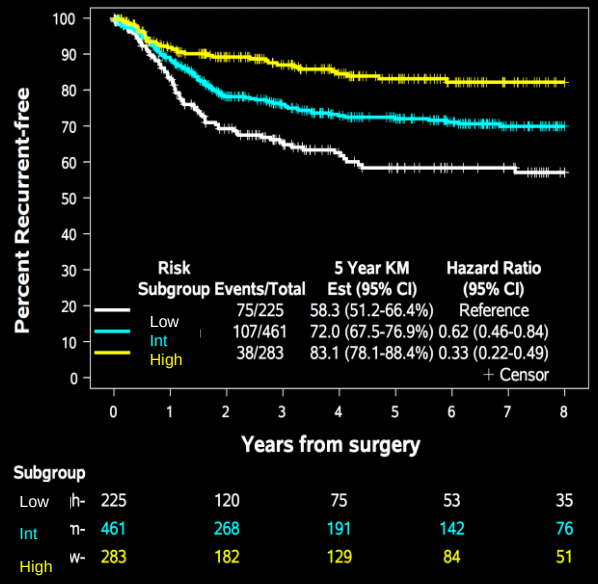
***P*< 0.0001**
HR (0-2)= 0.19
C-index= 0.64

IVS



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

EVS



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

Primary and Secondary objectives are reached

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

Multivariate anlayses for Immunoscore

Multivariate Overall Survival (OS) analysis stratifed by center

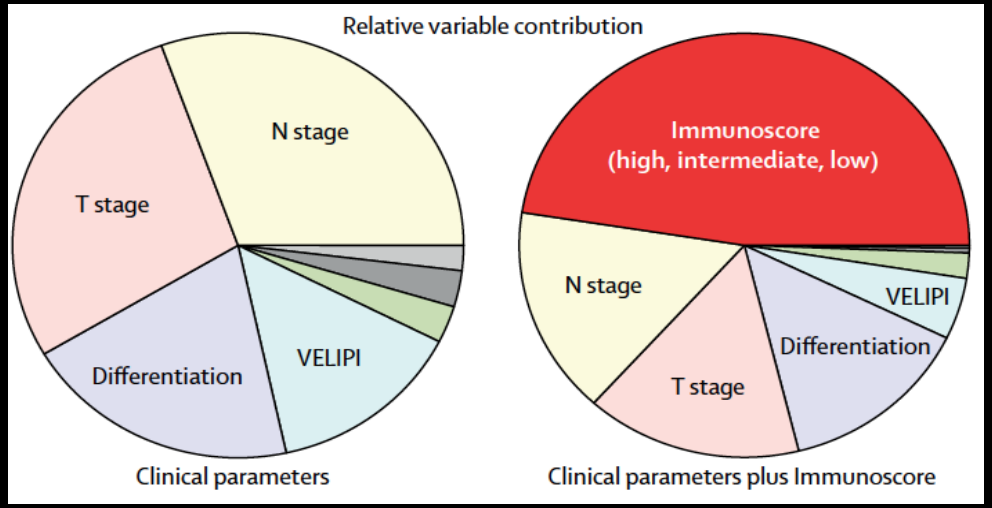
Individual Parameters	Hazard ratio (95%CI)	P-value
Gender Female *vs* Male	0.90 (0.72-1.12)	0.34
T Stage T2 *vs* T1	1.49 (0.62-3.57)	0.37
T Stage T3 *vs* T1	1.91 (0.84-4.38)	0.12
T Stage T4 *vs* T1	**2.36 (1.01-5.55)**	**0.0484**
N Stage N1 *vs* N0	1.16 (0.89-1.52)	0.28
N Stage N2 *vs* N0	**1.58 (1.15-2.17)**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

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

The Lancet 2018

Relative variable contribution to risk

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



Cox Multivariate

All patients

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

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

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

irAEs: immune-related Adverse Effects.

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

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

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

Immunoscore in locally advanced colon cancer

Stage III

Immunity and chemotherapeutic Efficacy

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

3 *vs* 6 months of chemotherapy

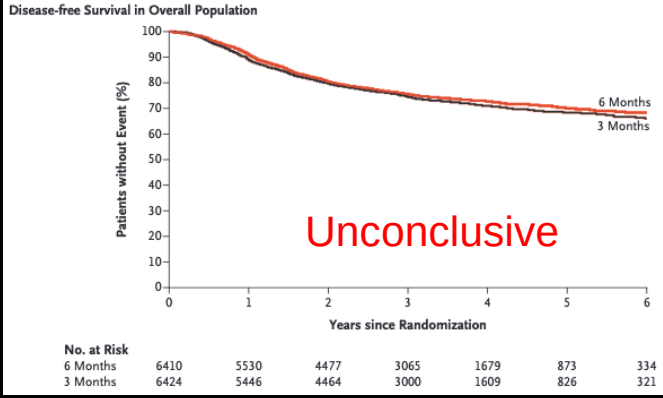
The NEW ENGLAND JOURNAL of MEDICINE

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

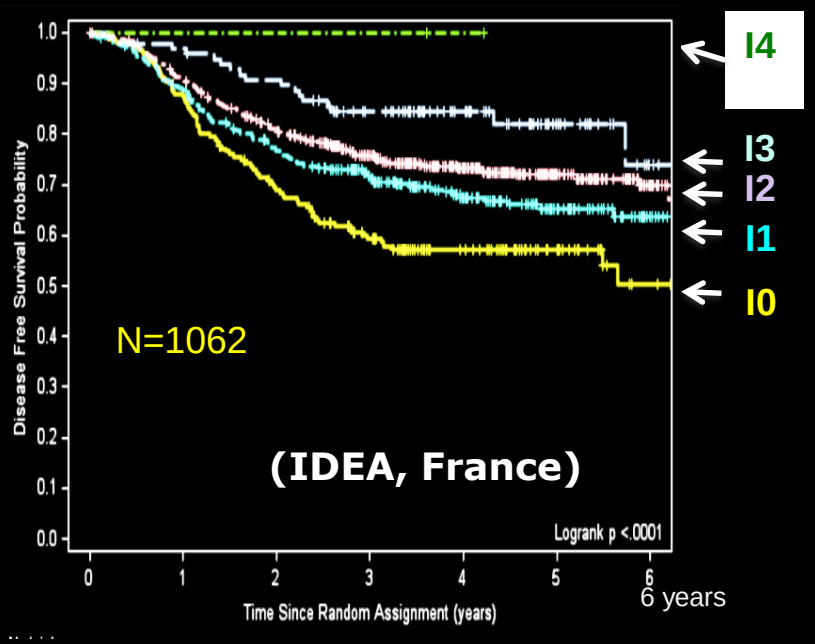
2018

Duration of Adjuvant Chemotherapy for Stage III Colon Cancer

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



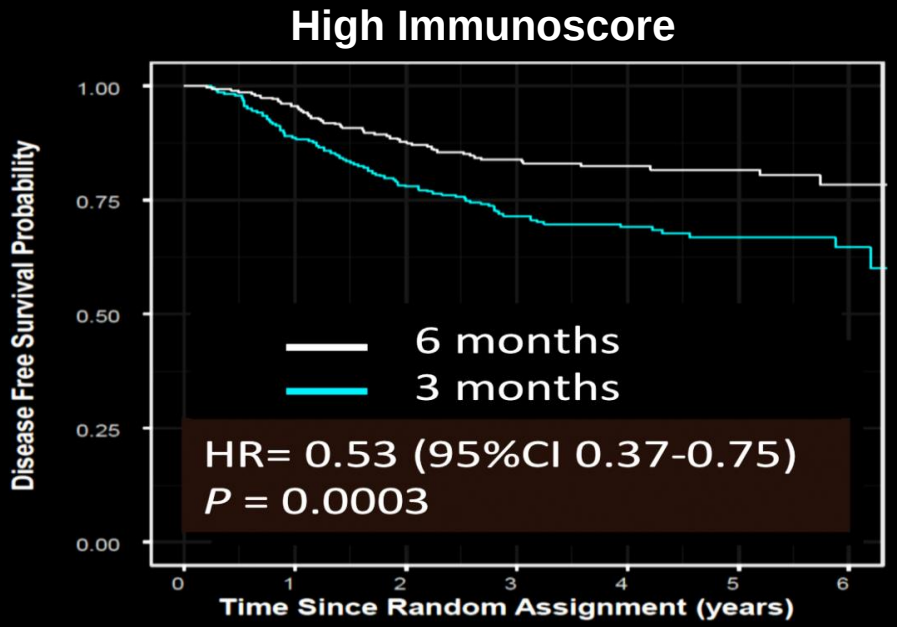
Immunoscore

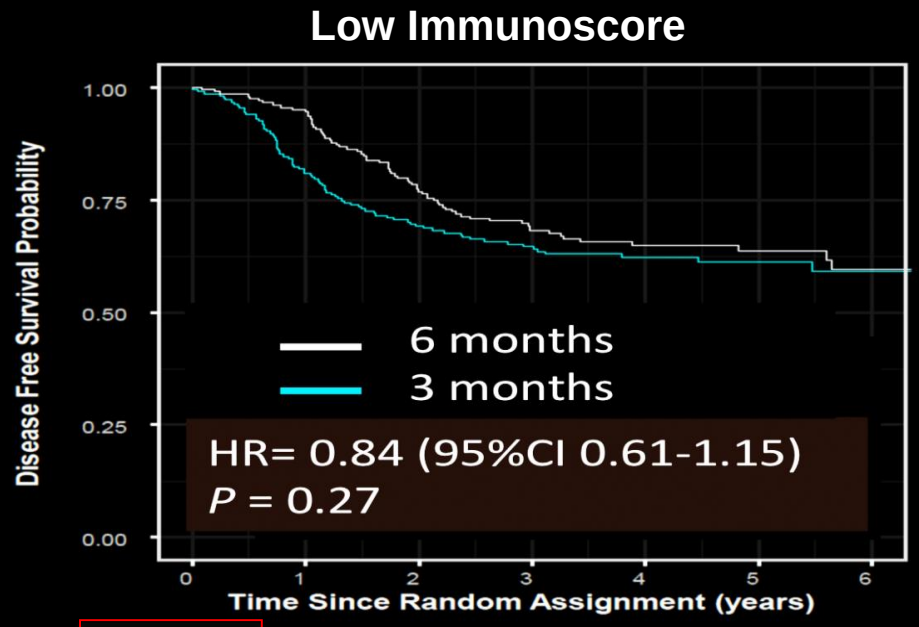


Clinical Utility (1): Immunoscore for defines patients at high-risk and NO risk in Stage III

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

All Stage III treated with FOLFOX





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

ASCO 2019

CAR-T cells

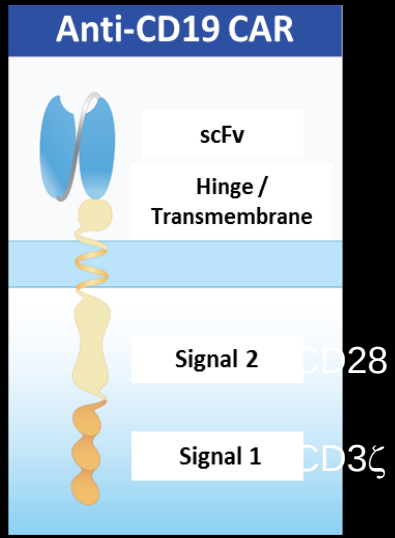
The NEW ENGLAND JOURNAL of MEDICINE

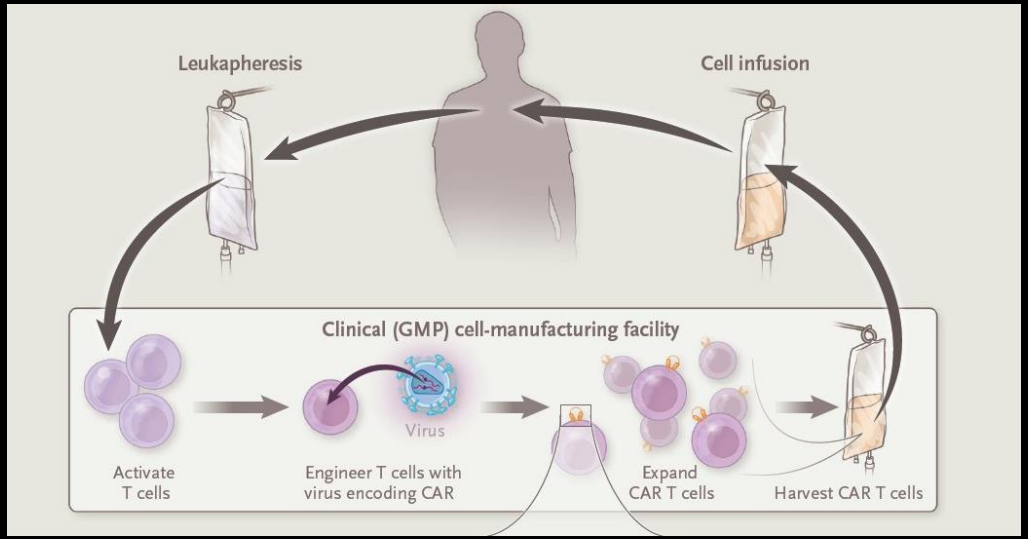
ORIGINAL ARTICLE

Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma

S.S. Neelapu, F.L. Locke, N.L. Bartlett, L.J. Lekakis, D.B. Miklos, C.A. Jacobson, I. Braunschweig, O.O. Oluwole, T. Siddiqi, Y. Lin, J.M. Timmerman, P.J. Stiff, J.W. Friedberg, I.W. Flinn, A. Goy, B.T. Hill, M.R. Smith, A. Deol, U. Farooq, P. McSweeney, J. Munoz, I. Avivi, J.E. Castro, J.R. Westin, J.C. Chavez, A. Ghobadi, K.V. Komanduri, R. Levy, E.D. Jacobsen, T.E. Witzig, P. Reagan, A. Bot, J. Rossi, L. Navale, Y. Jiang, J. Aycock, M. Elias, D. Chang, J. Wiezorek, and W.Y. Go

CAR-T Design and Product Manufacturing





Adapted from Tran et al, NEJM 2017

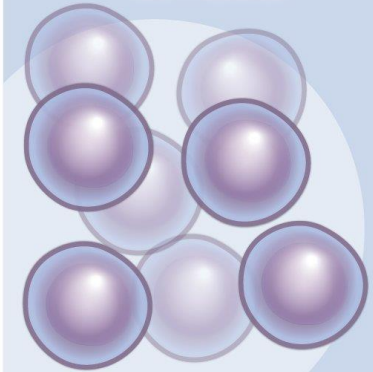
The CAR-T was approved by the US FDA and European Commission for the treatment of adult patients with relapsed/refractory large B cell lymphoma after ≥ 2 lines of systemic therapy

ZUMA-1 Trial: Clinical Outcomes

CAR T-Cell Therapy for Refractory Large B-Cell Lymphoma

MULTICENTER, PHASE 2 CLINICAL TRIAL

CAR T-cell Therapy
N=101



82% Objective response

54% Complete response

(20% Objective response in historical controls)

52% Overall survival at 18 months

96 Patients Had grade ≥3 adverse events:

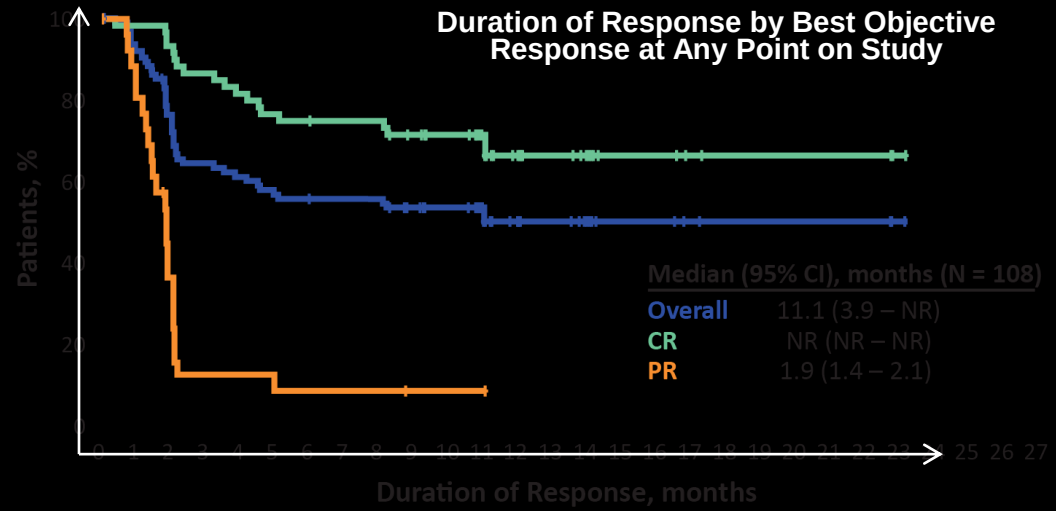
13 Patients Had cytokine release syndrome (including 2 deaths)

28 Patients Had neurologic events

The NEW ENGLAND JOURNAL of MEDICINE

Neelapu et al. 2017

ZUMA-1 Trial: Long-Term Follow Up



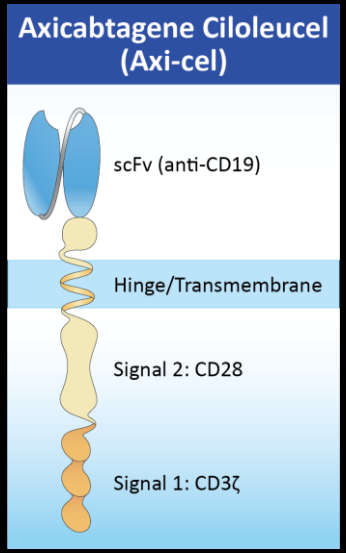
Overall	89	82	67	56	53	49	48	47	47	42	38	31	19	16	12	6	6	4	3	3	3	3	1	0
CR	63	61	58	53	50	47	46	45	45	41	37	30	19	16	12	6	6	4	3	3	3	3	1	0
PR	26	21	9	3	3	2	2	2	2	1	1	1	0											

CR, complete response; PR, partial response; NR, not reached; CI, confidence interval.

Locke et al, ASCO 2018

CAR-T cell therapy

CAR Design and Schematic Representation of ZUMA-1 Trial



Phase 1

Refractory DLBCL/PMBCL/TFL
(cohort of n = 7)

Phase 2

Cohort 1
Refractory DLBCL
(n = 77)

Cohort 2
Refractory PMBCL/TFL
(n = 24)

Of 101 patients in Phase 2 ZUMA-1 with refractory large B cell lymphoma treated with axi-cel :
ORR, 83%
CR, 58%
Ongoing responses: 39% including 37% CR

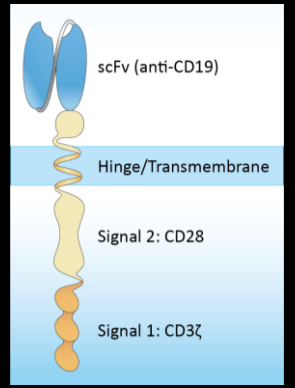
CRS and NE were mostly reversible (n = 108 from Phases 1 & 2):
Grade ≥ 3 CRS, 11%
Grade ≥ 3 NE, 32%
4 Grade 5 AEs (2 axi-cel related and 2 unrelated)

Axi-Cel Maintained Ongoing Responses at Median Follow-Up of 27.1 Months

AE, adverse event; axi-cel, axicabtagene ciloleucel; CR, complete response; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; DLBCL, diffuse large B cell lymphoma; NE, neurologic event; NHL, non-Hodgkin lymphoma; ORR, objective response rate; PMBCL, primary mediastinal B cell lymphoma; TFL, transformed follicular lymphoma.

Improval of CAR-T cell therapy

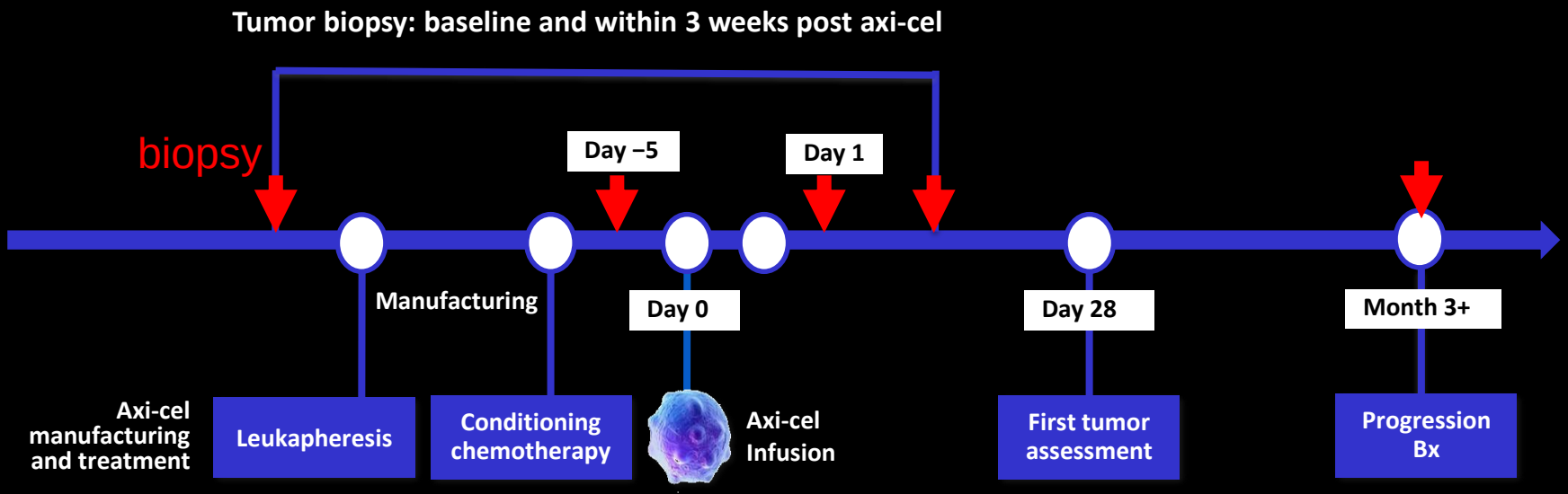
- ✓ Improved CAR constructs
- ✓ Get better intracellular CAR signalling (1st, 2nd, 3rd generation CARs)
- ✓ Get better target (especially for solid tumors)
- ✓ Get dual targets, inducible CAR, killing-construct CAR
- ✓ Select subtypes of T-cells for infusion



But again,

- ✓ Ignoring the fact that a cancer is not tumor cells in a test-tube
- ✓ and that adoptive CAR-T cells are not working alone, but within a patient

Tumor microenvironment analysis: Zuma 1 - Protocol and Timing of Paired Biopsies



axi-cel, axicabtagene ciloleucel.

ZUMA-1 clinical trial Translational Biomarkers analysis

- *What are the changes in TME Post-CAR-T?*
- *Which patients are responding to CAR-T?*
- *What are the mechanisms of relapse?*
- *Can we predict toxicities?*

TME: Tumor MicroEnvironment

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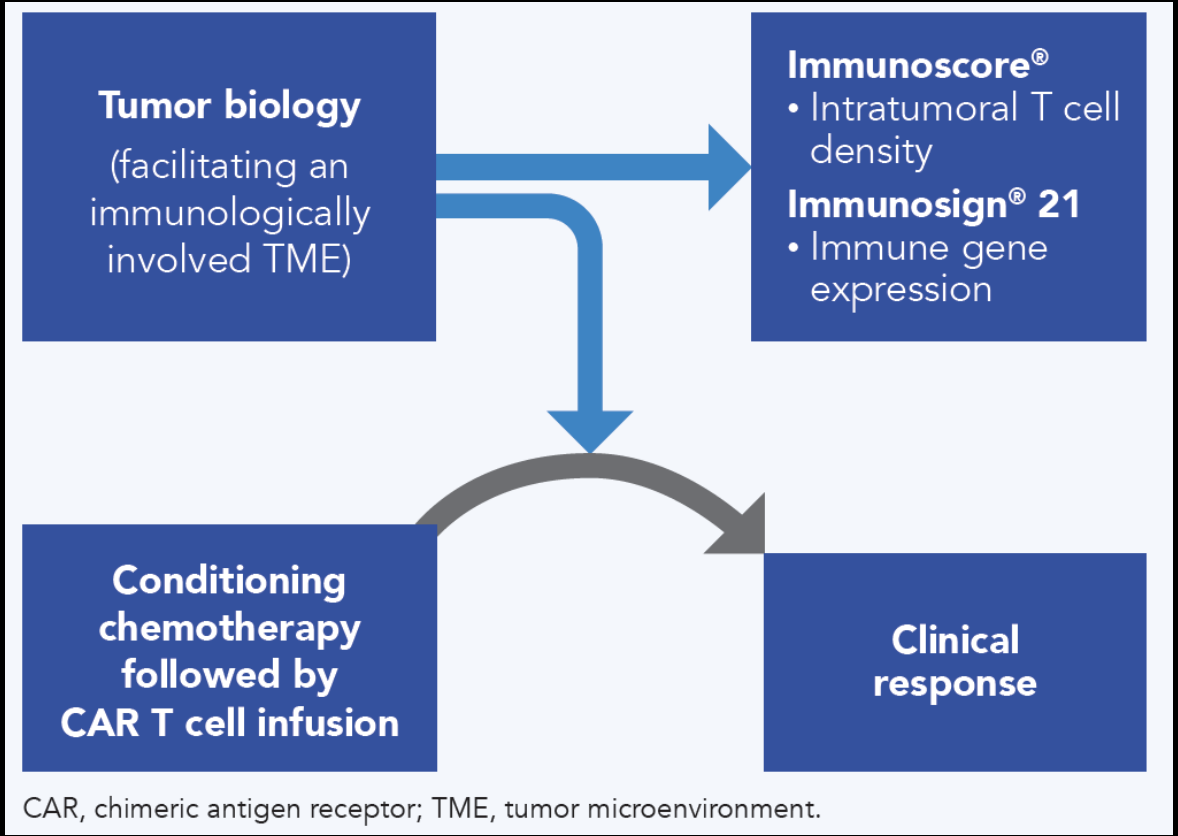
CONCLUSIONS

- ✓ Pre-existing T cell-involved features of the TME (High Immunoscore, High Immunosign) may be associated with a response to CAR-T
- ✓ Factors intrinsic to tumor biology may influence CAR T cell efficacy through the immune microenvironment (Pre-treatment TME enriched in T cell and innate immune-related genes)
- ✓ CAR-T could overcome an unfavorable TME (low Immunoscore) in a subset of patients
- ✓ CAR T cell treatment is associated with rapid and profound changes in the TME
  - Increase of immune checkpoints, IFN-related genes and chemokines
  - Elevation of IL-15 and PD-L1 gene expression in CR and PR
- ✓ These results support anti-CD19 CAR T cell treatment optimizations designed to overcome an immune-detrimental TME

Model Linking Tumor Biology Features With TME and Response to CAR T Cell Therapy



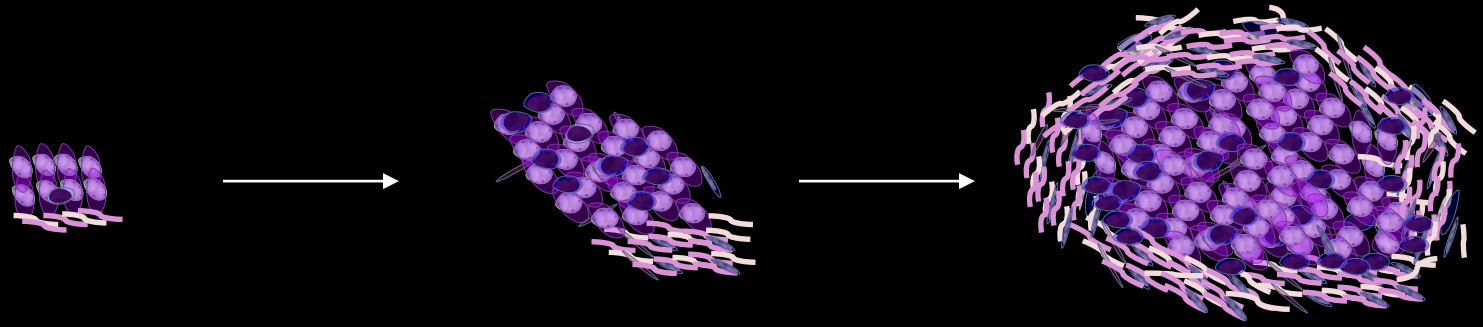
CAR, chimeric antigen receptor; TME, tumor microenvironment.

The continuum of cancer immunosurveillance

Pre-cancerous lesions

Primary Carcinoma

Metastasis



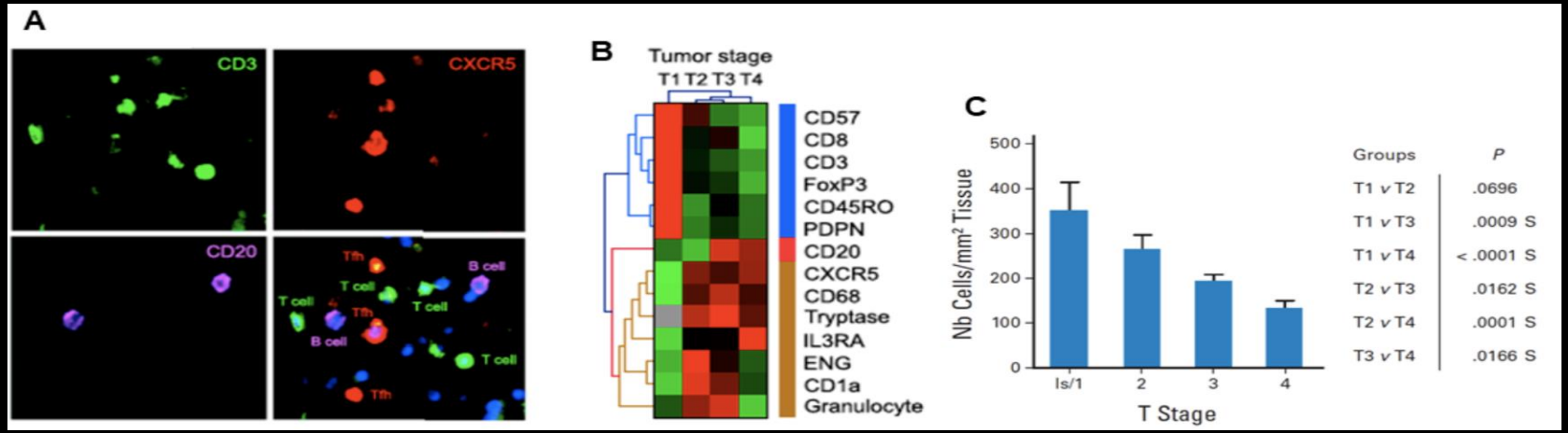
Mascaux C. … Galon J.
Nature 2019

Pagès F. … Galon J.
Lancet 2018

Van den Eynde. … Galon J.
Cancer Cell 2018

Angelova M. … Galon J.
Cell 2018

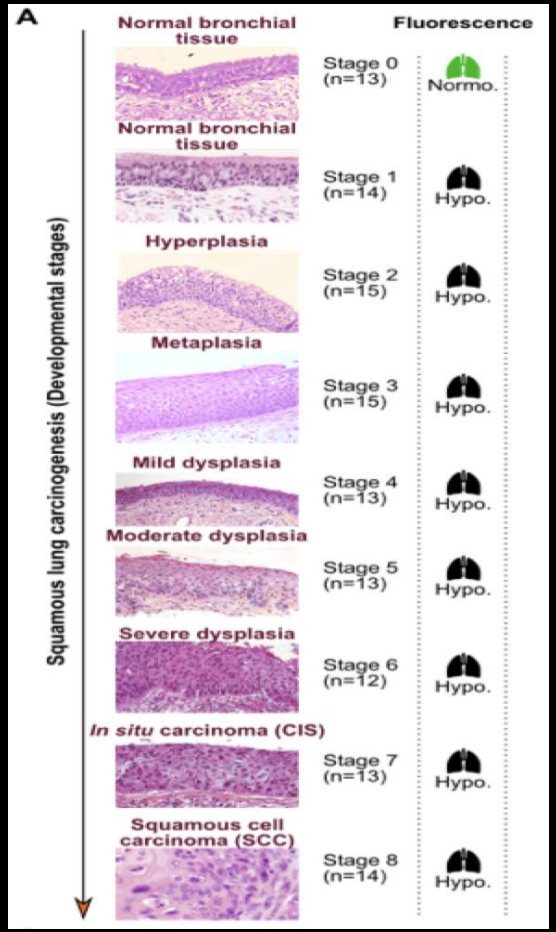
Adaptive immunity decreases with tumor progression



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

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

Oncogenesis of lung squamous cell carcinoma



- ✓ Analysis of 122 pre-cancer lesions across 9 developmental stages

Mascaux C et al. ***Nature*** *2019*

Main gene expression patterns across 9 developmental stages

Ascending

Ascending from High-Grade

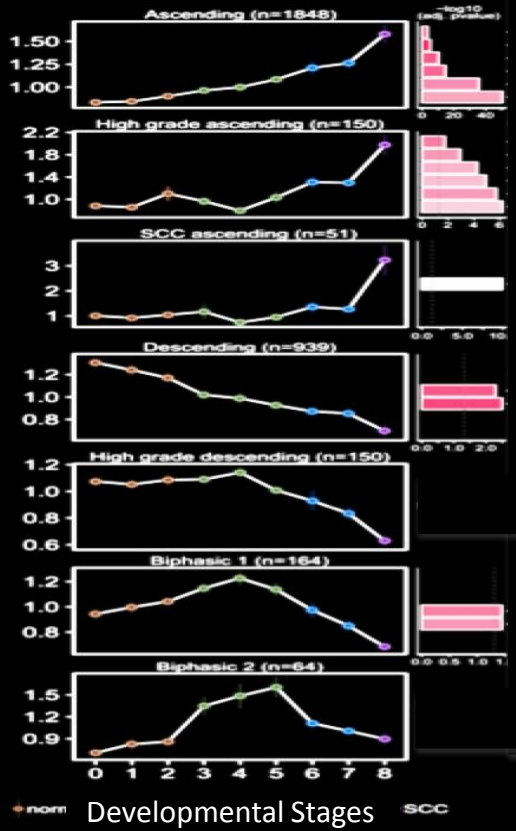
SCC Ascending

Descending

Descending from High-Grade

Biphasic 1

Biphasic 2



Proliferation

Immune

Develomental (EMT)

DNA damage

Heterogeneous

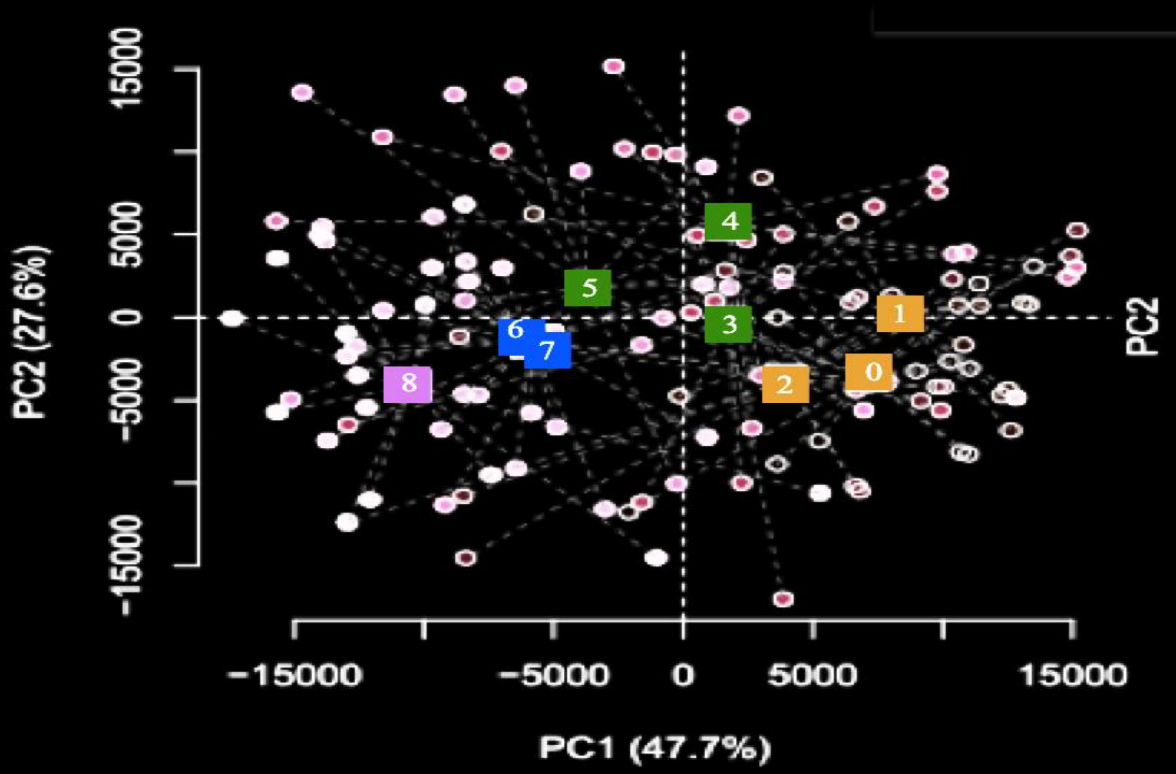
Metabolism

Heterogeneous

Immune functions mostly associated with genes ascending from high-Grade

Mascaux C et al. ***Nature*** *2019*

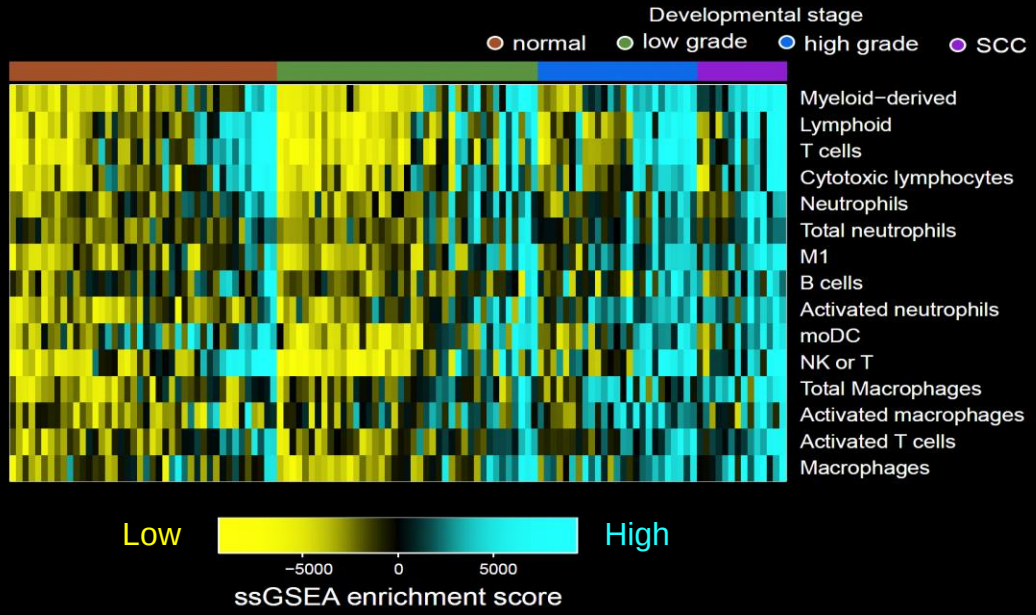
Principal components evolution of the 9 developmental stages

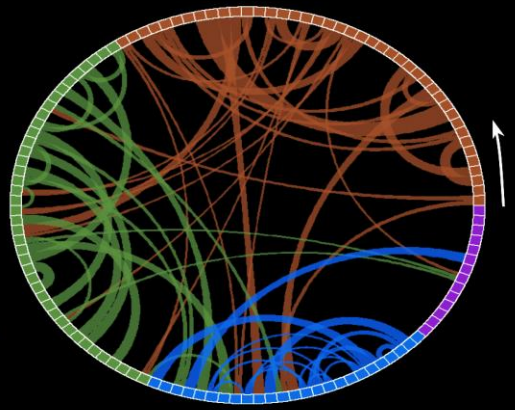


- Normal
- Low Grade dysplasia
- High Grade dysplasia
- Squamous cell carcinoma (SCC)

Mascaux C et al. ***Nature*** *2019*

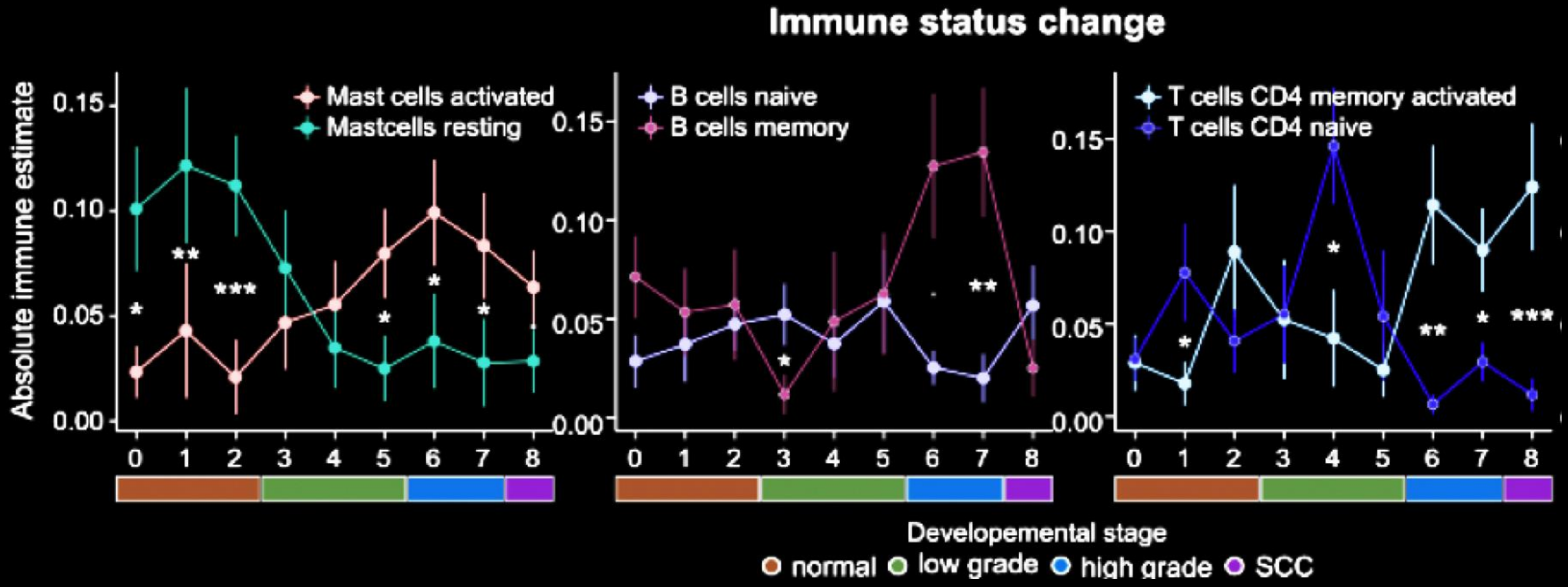
Immune cell infiltration across the main 4 developmental stages





Mascaux C et al. ***Nature*** *2019*

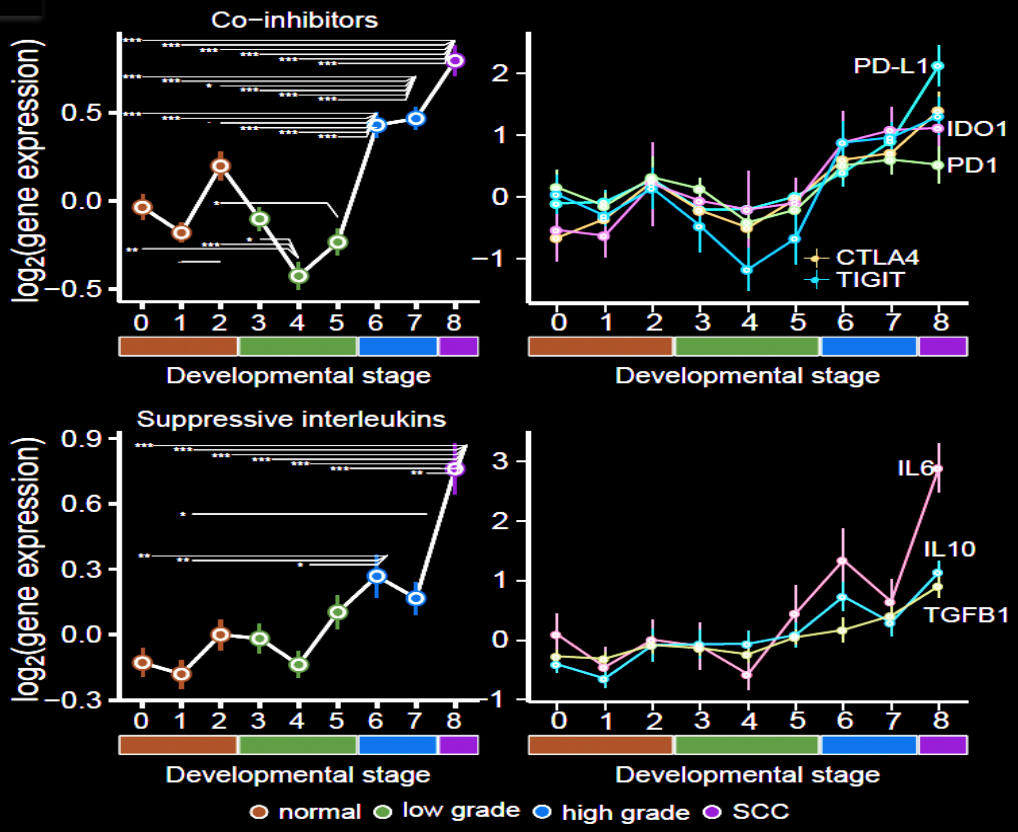
Immune activation across developmental stages



- ✓ Early Immune activation in Low-Grade dysplasia (Immune sensing)
- ✓ Adaptive immune activation and memory in High-Grade dysplasia

Mascaux C et al. ***Nature*** *2019*

Immune escape mechanisms in pre-cancer lesions



✓ Decreased expression of co-inhibitors in Low-Grade

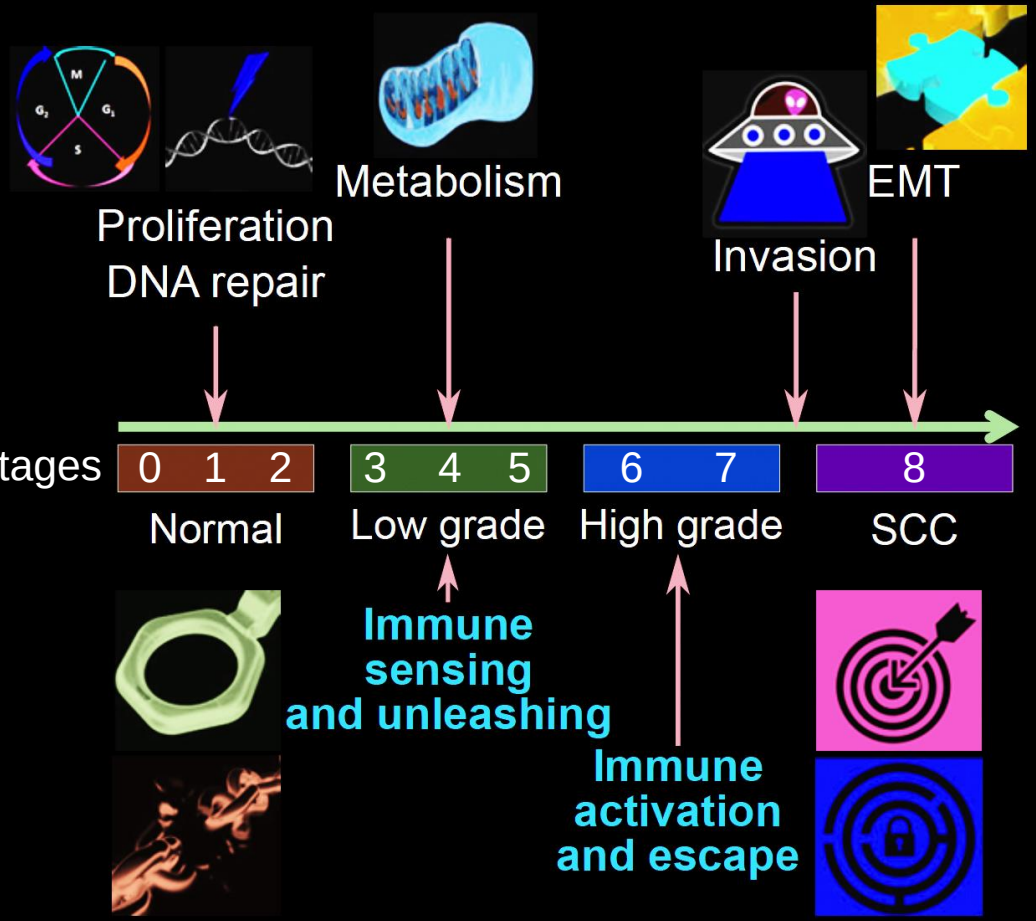
✓ Increased expression of co-inhibitors in High-Grade

✓ Increased expression of suppressive cytokines in High-Grade

-> Immune evasion before tumor invasion (SCC)

Mascaux C et al. ***Nature*** *2019*

Pre-Neoplastic / Pre-Cancer Lesion evolution



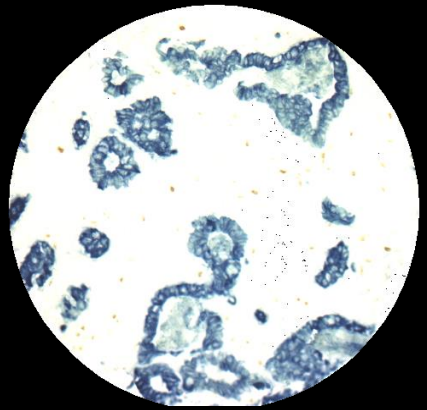
Epithelial cells
Stroma
Tumor cells

Immune microenvironment

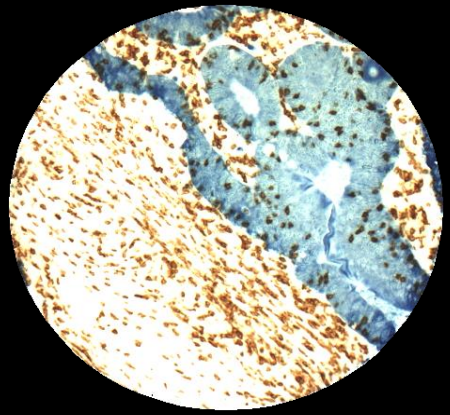
Mascaux C. … Galon J. ***Nature*** 2019

Deciphering the tumor immune microenvironment: Clinical implications

"Cold" Tumor
I 0



CD3
Tumor



"Hot" Tumor
I 4

Clinical implications

Predictions

Need T-cell priming
Cancer vaccine

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

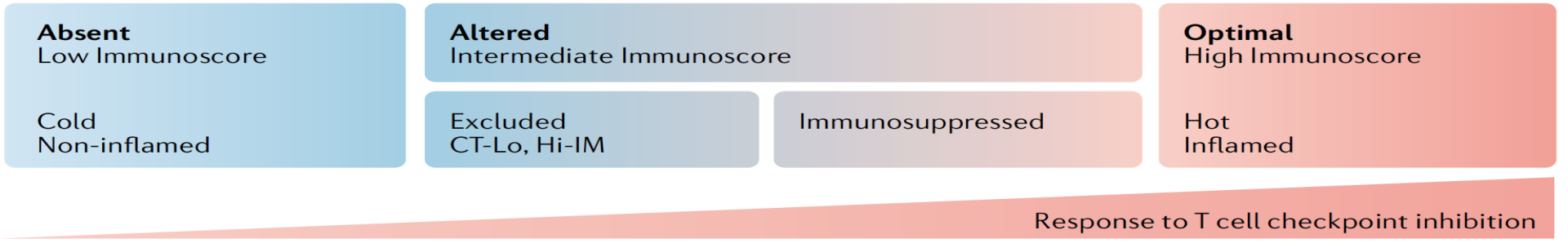
But it is not as simple since biology is complex and is not dichotomized in good & bad

NATURE REVIEWS | DRUG DISCOVERY

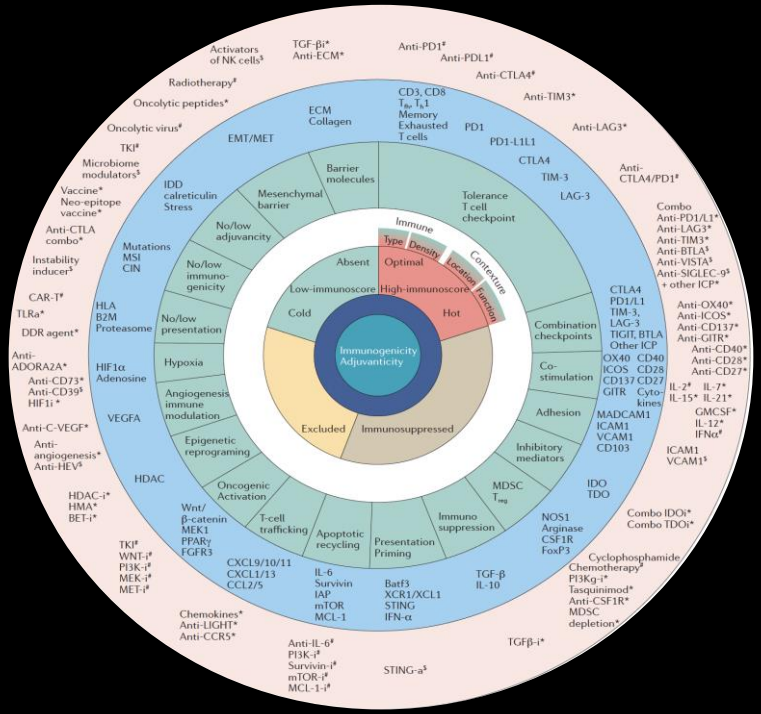
Approaches to treat immune hot, altered and cold tumours with combination immunotherapies

Jérôme Galon * *and Daniela Bruni*

2019

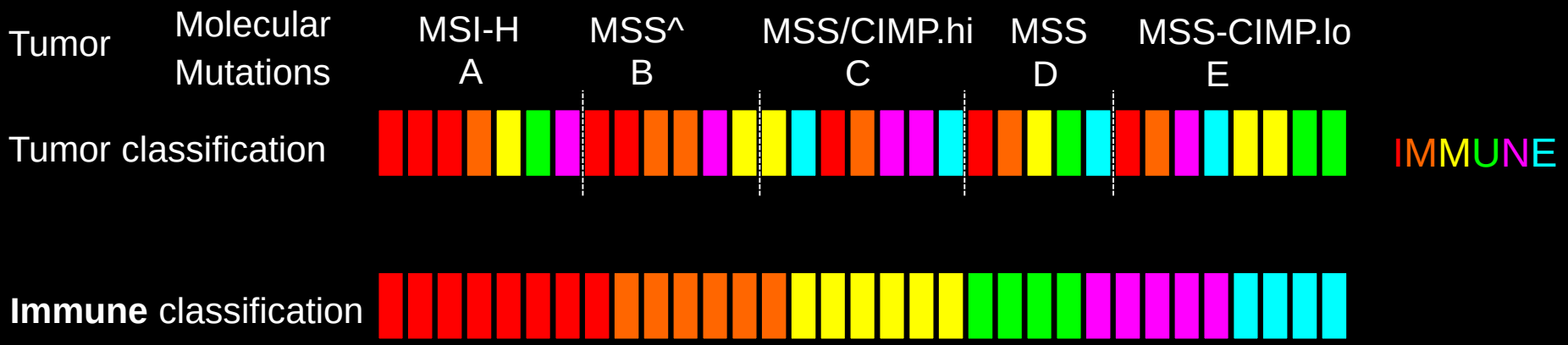


Treating hot, altered and cold immune tumors with immunotherapy



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

Stratification of cancer based on the immune status



-> Importance of having standardized immune Assays

Galon lab.

INSERM, CRC, Paris, France

Franck Pagès
Tessa Fredriksen
Florence Marliot
Lucie Lafontaine
Stéphanie Mauger
Amélie Bilocq
Bénédicte Buttard
Amos Kirilovsky
Marie Tosolini
Maximilian Waldner
Sarah Church
Pauline Maby
Helen Angell
Mihaela Angelova
Angela Vasaturo
Bernhard Mlecnik
Gabriela Bindea
Daniela Bruni

Institute for Bioinformatics, Innsbruck, Austria

Pornpimol Charaoetong
Zlatko Trajanoski

LabEx Immuno-oncology

Kroemer G, Zitvogel L, Tartour E, Sautès-*Fridman* C, Fridman H, *Zucman*-Rossi J,

Institut Curie, Paris, France

Hervé Brisse
Sylvie Bonvalot

University Clinic, Erlangen, Germany

Christopher Becker

Institute for Genetics, Graz, Austria

Anna Obenauf
Michael Speicher

Rouen University, France

Jean Baptiste Latouche

Dpt. of General and Digestive Surgery, HEGP, Paris, France

Anne Berger

Dpt. of Pathology, HEGP, Paris, France

Tchao Meatchi
Christine Lagorce
Patrick Bruneval

CHU Strasbourg, France

Celine Mascaux

Kite Pharma, Gilead

Adrian Bot, John Rossi

Clinic St Luc, Bruxelle,

Marc Van den Eynde

Thanks Worldwide Consortium Centers

Galon lab.
INSERM, Cordeliers Research Center, Paris, France
Franck Pagès, Tessa Fredriksen, Florence Marliot, Lucie Lafontaine, Bénédicte Buttard, Sarah Church, Pauline Maby, Helen Angell, Mihaela Angelova, Angela Vasaturo, Bernhard Mlecnik, Gabriela Bindea

Dpts. of Pathology *, Surgery $, Immunology #, HEGP, Paris, France
*Christine Lagorce *, Patrick Bruneval *, Anne Berger $, Franck Pagès #, Florence Marliot #, Nacilla Haicheur #*

Department of Pathology, Providence Portland Medical Center, Portland, OR, USA
Carlo Bifulco

Laboratory of Molecular and Tumor Immunology, Earle A. Chiles Research Institute, Robert W. Franz Cancer Center, Portland, OR, USA
Bernard Fox

Princess Margaret Hospital, University Health Network, Department of Pathology , Toronto, ON, Canada
Pamela S. Ohashi, Michael Roehrl, Prashant Bavi, Sara Hafezi-Bakhtiari, Bradly G. Wouters, Linh Nguyen

Department of Pathology and Oncology, Istituto Nazionale per lo Studio e la Cura dei Tumori, "Fondazione G.Pascale" Naples-Italy
Paolo A Ascierto, Gerardo Botti, Fabiana Tatangelo, Paolo Delrio, Gennaro Cilberto

Humanitas Clinical and Research Center, Rozzano, Milan, Italy
Fabio Grizzi, Luigi Laghi

Institute of Pathology, University of Bern, Bern, Switzerland
Alessandro Lugli, Inti Zlobec, Tilman Rau

Research Branch, Sidra Medical and Research Centre, Doha, Qatar
Francesco M. Marincola

Institut Roi Albert II, Cliniques universitaires St-Luc, Université Catholique de Louvain, Brussels, Belgium
Marc Van den Eynde, Jean-Pierre Machiels

Department of Pathology, University of Erlangen, Erlangen, Germany
Arndt Hartmann, Tilman Rau, Carol Geppert

Pathology Department, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands
Iris D. Nagtegaal, Elisa Vink-Borger

Department of Oncology-Pathology, Karolinska Institutet, Karolinska University, Stockholm, Sweden
Giuseppe V. Masucci, Emilia K. Andersson

Department of Oncology, Medical School and general hospital, Prague, Czech Republic
Eva Zavadova, Michal Vocka

Institute for Cancer Research, Center of Translational medicine, Xi'an Jiaotong university, Xian, China
Yili Wang

The Gujarat Cancer & Research Institute, Asarwa, Ahmedabad, India
Prabhu S. Patel, Shilin N. Shukla, Hemangini H. Vora, Birva Shah, Jayendrakumar B. Patel, Kruti N. Rajvik, Shashank J. Pandya

Institute for Advanced Medical Research, Keio University School of Medicine, Tokyo, Japan
Yutaka Kawakami, Shoichi Hazama, Kiyotaka Okuno, Kyogo Itoh, Boryana Papivanova

Department of Pathology, Sapporo Medical University School of Medicine, Sapporo, Japan
Toshihiko Torigoe, Noriyuki Sato