Mesa, Arizona, Feb 18-22, 2019

Topic 4 – Tumor Biology: cellular mechanisms and signaling

…and the magic of pharmacodynamics

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Disclosures

Partner, Consultant or Board Member:

- The TICA Group, San Diego, California
- Kite Pharma, Santa Monica, California
- Nanostring Technologies, Seattle, Washington
- Calidi Biotherapeutics, San Diego, California
- Kiromic, Houston, Texas
- Bayer, Leverkusen, Germany

Topic 4 – Tumor Biology: cellular mechanisms and signaling

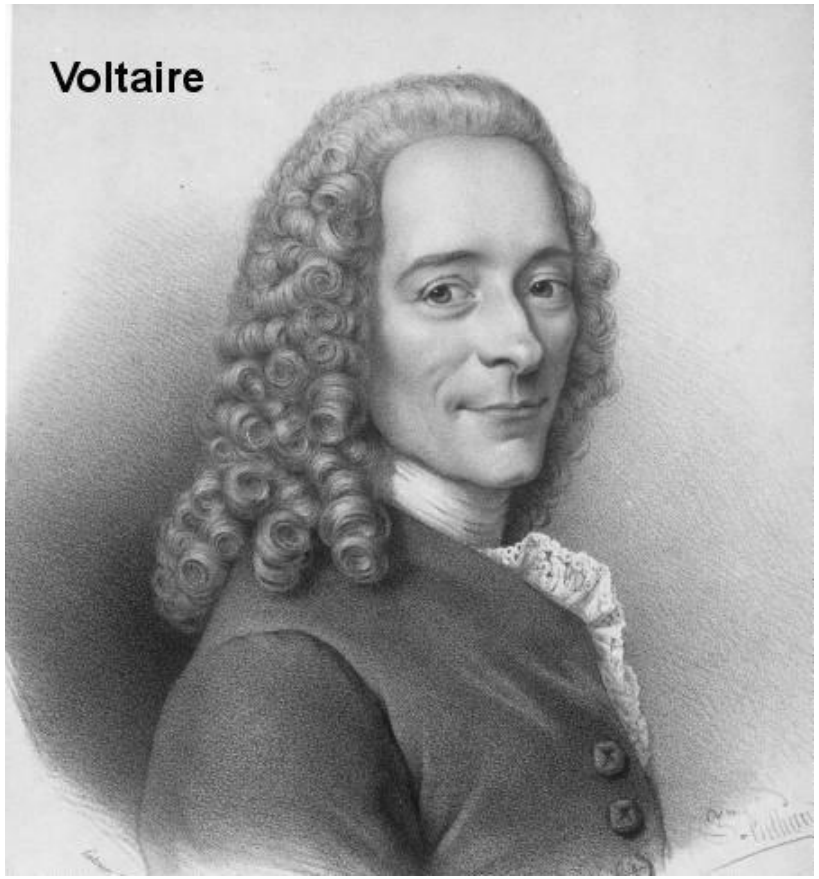
February 18th 2019; 1:00-2:00 pm

- Mechanism of tumor rejection
- Cancer, autoimmunity, allo-recognition, clearance of pathogen and immune-mediated tissue destruction
- The immunologic constant of rejection signature
- The continuum of cancer immune surveillance
- A genetic inference on cancer immune responsiveness
- Cancer Immune landscapes
- The two option choice of cancer evolution
- Mechanisms of cancer immune resistance and the theory of everything
- Semantics of cancer immune resistance
- Basis for combination therapies

There are three golden rules for the successful treatment of any disease…

…Unfortunately we do not know any of them

Anonymous Stanford Professor
Circa 1982



French Philosopher (1694-1778)

“Doctors are men who prescribe medicines of which *they know little*,

…to cure diseases of which *they know less*,

…in human beings of whom *they know nothing*”

- **How** does tumor rejection occur
- **Why** does rejection occur

- **How does tumor rejection occur**

- **Why does rejection occur**

Matrix for sample collection (Umbrella/Basket clinical design)	Baseline		On Treatment		Post Treatment		
	Target Discovery	Prediction	MOA (PK/PD)	Prediction	MOA	Surrogate*	Escape
Germline	Yes	Yes	NA	NA	NA	NA	NA
Product Characteristics	Yes	Yes	Yes	Yes	Yes	??	NA
Peripheral	?	Yes Monitoring!	Yes	Yes Monitoring!	Yes	Yes	??
Draining LNDs	Yes	??	??	??	??	??	??
Tumor Stroma	Yes	Yes	Yes	??	Yes	??	??
Tumor Tissue	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Environmental Factors±	Yes	??	??	Yes	??	??	??
Humanized exp. models	Yes	??	NA	NA	NA	NA	NA

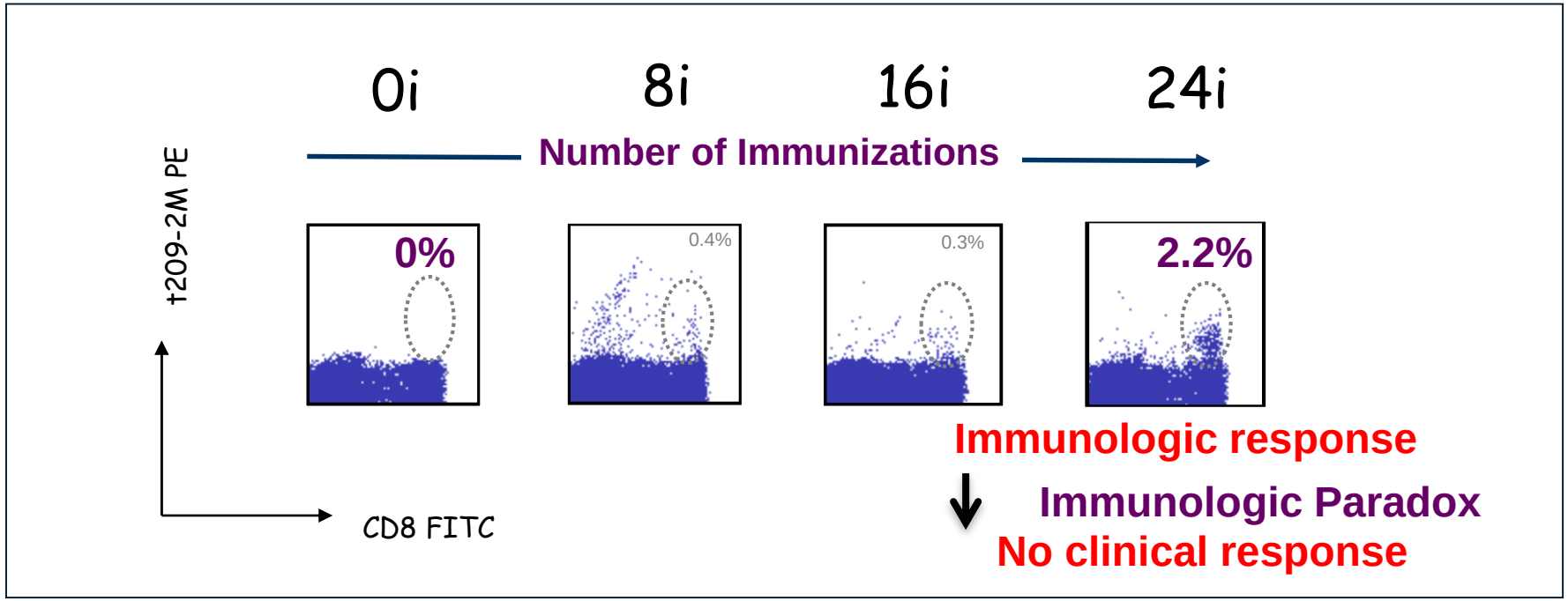
LND = Lymph node; MOA = Mechanism of Action; NA = Not Applicable; PD = Pharmacokinetics; PK = Pharmacodynamics; YES denotes potential usefulness for a given purpose; "??" Signifies unknown or unlikely usefulness, Discovery vs Predictive refers to studies that are meant to enlighten mechanistically the reasons for a given phenomenon (Discovery) rather than only identifying associations (Predictive)

* Surrogate biomarkers of long term benefit; ± Include microbiome, co-morbidities, additional therapies, etc.

Lesson learned from vaccination studies

Cytotoxic T cells can co-exist in the host with their target cells

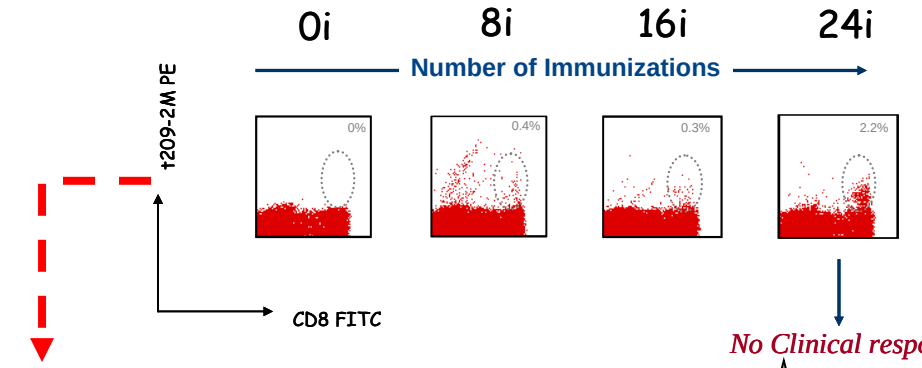
Model: gp100 peptide vaccine ± interleukin-2



Lee et al, J. Immunol. 1999
Kammula et al, J Immunol 1999
Nielsen et al, J Immunol 2000
Bittner et al, Nature 2000

Monsurró et al, J Immunol 2000
Wang E et al, Nature Biotech 2000
Bedognetti et al, J Trans Med 2011
Schwartzentruber et al, NEJM 2011

Multidimensionality of tumor/host interactions in the context of T cell aimed immunization



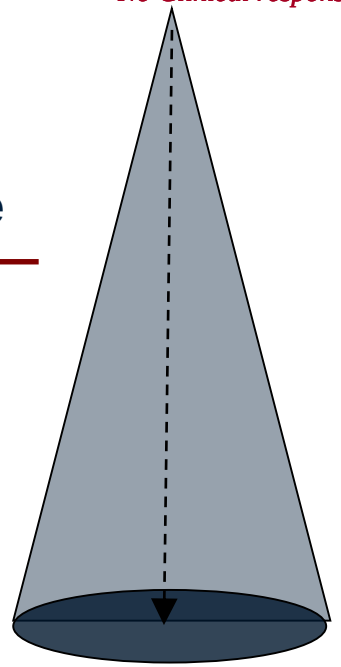
Immunology

1st dimension = TCR/HLA/peptide interaction
2nd dimension = Localization at tumor site
3rd dimension = Importance of co-stimulation at tumor site

Tumor Biology

4th dimension = Evolving nature of immune response and genetic instability of cancer cells
5th dimension = Heterogeneity of the tumor microenvironment

No Clinical response



Increasing biological complexity

Wang E. et al. Trends in Immunol 2000

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Germline	Yes	Yes	NA	NA	NA	NA	NA
Product Characteristics	Yes	Yes	Yes	Yes	Yes	??	NA
Peripheral	?	Yes Monitoring!	Yes	Yes Monitoring!	Yes	Yes	??
Draining LNDs	Yes	??	??	??	??	??	??
Tumor Stroma	Yes	Yes	Yes	??	Yes	??	??
Tumor Tissue	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Environmental Factors±	Yes	??	??	Yes	??	??	??
Humanized exp. models	Yes	??	NA	NA	NA	NA	NA

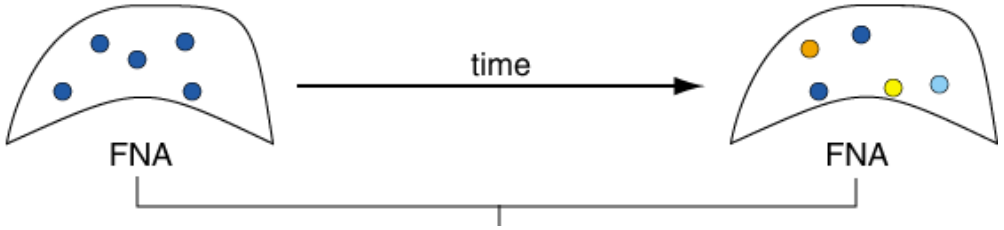
LND = Lymph node; MOA = Mechanism of Action; NA = Not Applicable; PD = Pharmacokinetics; PK = Pharmacodynamics; YES denotes potential usefulness for a given purpose; "??" Signifies unknown or unlikely usefulness, Discovery vs Predictive refers to studies that are meant to enlighten mechanistically the reasons for a given phenomenon (Discovery) rather than only identifying associations (Predictive)

* Surrogate biomarkers of long term benefit; ± Include microbiome, co-morbidities, additional therapies, etc.

Characterizing intra-tumor mechanisms of rejection

Wang E and **Marincola FM** – *A natural history of melanoma: serial gene expression analysis*
Immunol. Today 21(2): 619-23, 2000
Wang E, Miller LD, Ohnmacht GA, Liu ET, and **Marincola FM**. *High-fidelity mRNA amplification for gene profiling*
Nat Biotechnol. 2000 Apr;18(4):457-9.

Pre-treatment FNA **On- or post-treatment FNA**



FNA

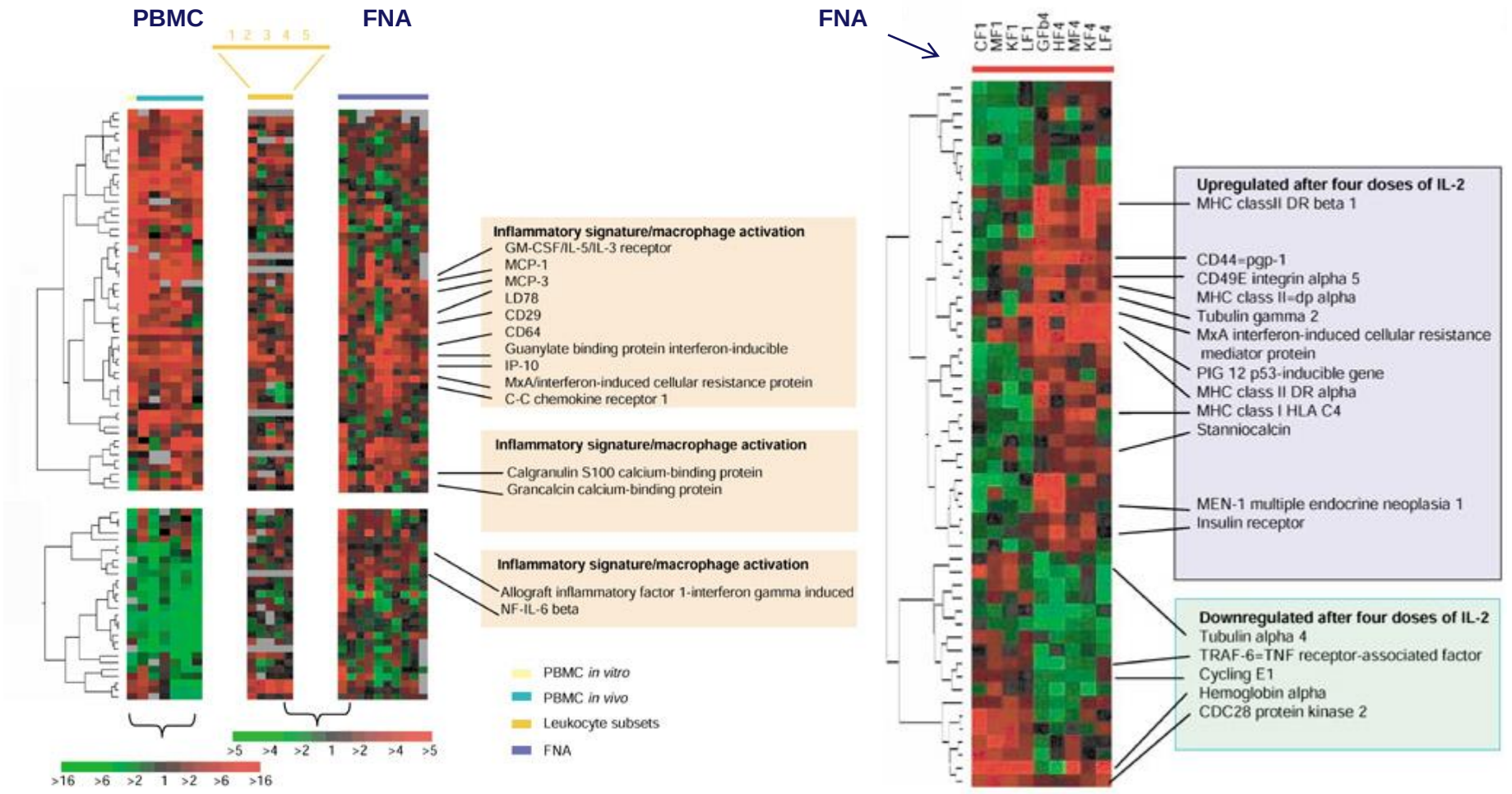
Transcriptome

Research

Gene-expression profiling of the response of peripheral blood mononuclear cells and melanoma metastases to systemic IL-2 administration

Published: 25 June 2002
Genome Biology 2002, 3(7):research0035.1–0035.17

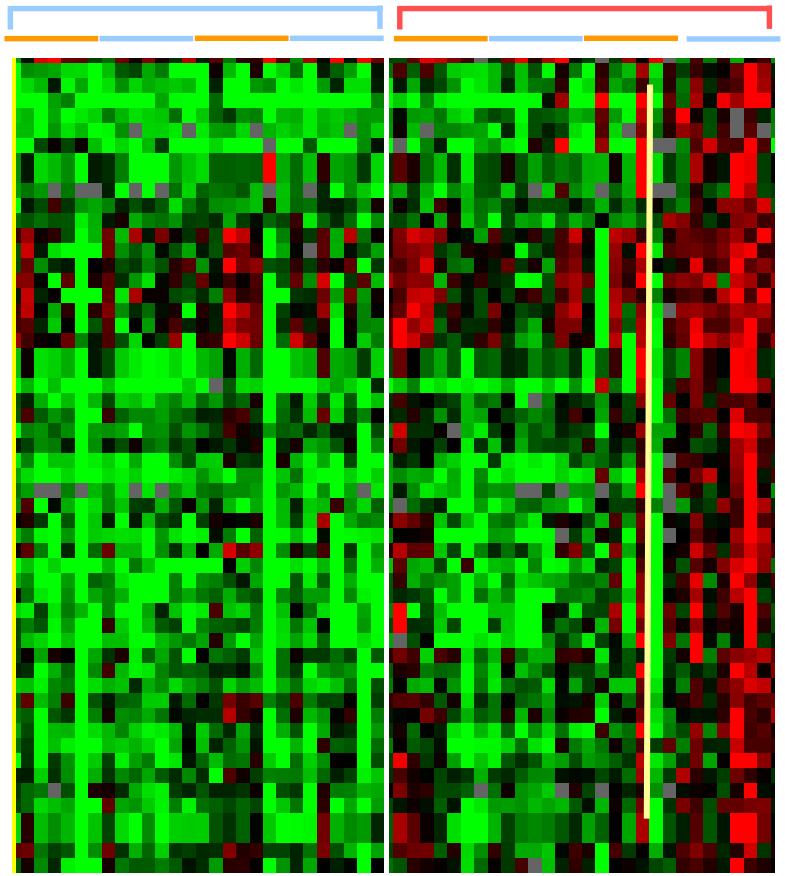
Monica C Panelli*, Ena Wang*, Giao Phan†, Markus Puhlmann†, Lance Miller†, Galen A Ohnmacht†, Harvey G Klein* and Francesco M Marincola*



Imiquimod (TLR-7a)-Basal cell Carcinoma

Pre-treat TLR-7a Treat

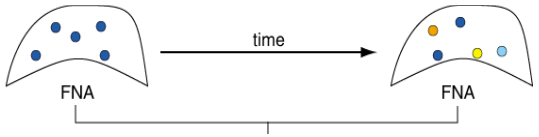
Placebo X 4 days
Placebo X 8 days
Treat X 4 days
Treat X 8 days



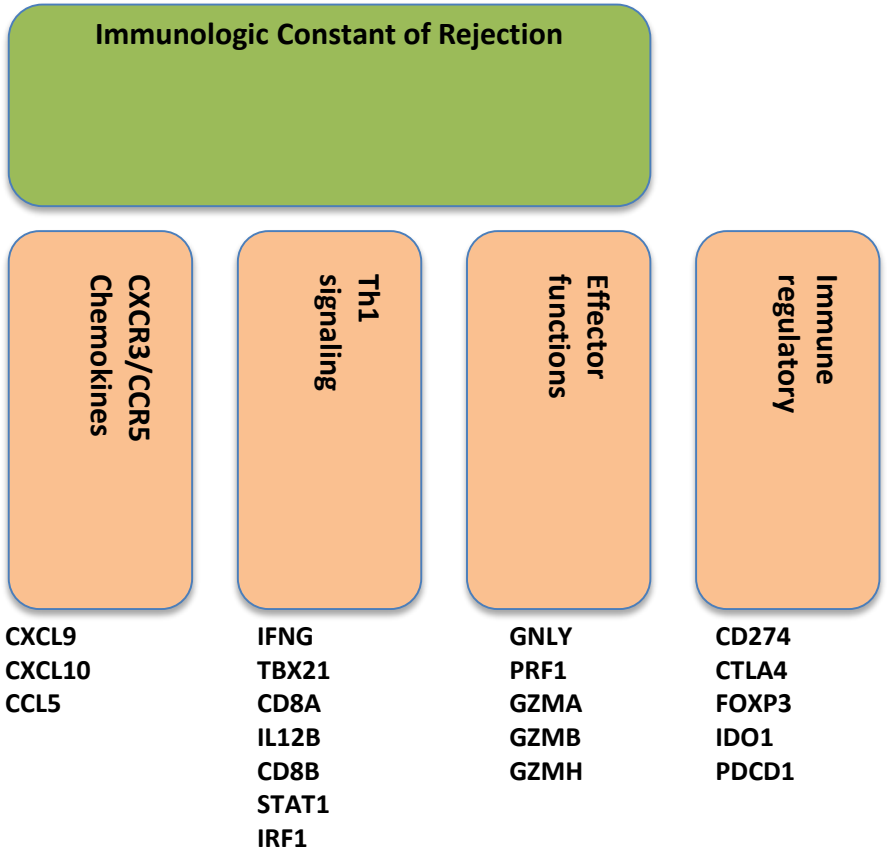
Interferon Stimulated Genes
STAT1 1/IRF1
Allograft inflammatory factor 1
IL-15/IL-2/IL15 R b
IL-15 R a/IL-2/IL-4/IL-7/IL-9/IL-15 R g
IL6

CCR5 Ligands
(CCL4/CCL5)
CXCR3 Ligands
(CXCL9
CXCL10)

Immune Effector Genes (IEG)
Granzyme A, B, K
Perforin



Panelli et al. Genome Biol 2007


Immunologic Constant of Rejection
CXCR3/CCR5 Chemokines
Th1 signaling
Effector functions
Immune regulatory
CXCL9
CXCL10
CCL5
IFNG
TBX21
CD8A
IL12B
CD8B
STAT1
IRF1
GNLY
PRF1
GZMA
GZMB
GZMH
CD274
CTLA4
FOXP3
IDO1
PDCD1

Matrix for sample collection (Umbrella/Basket clinical design)	Baseline		On Treatment		Post Treatment		
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Product Characteristics	Yes	Yes	Yes	Yes	Yes	??	NA
Peripheral	?	Yes Monitoring!	Yes	Yes Monitoring!	Yes	Yes	??
Draining LNDs	Yes	??	??	??	??	??	??
Tumor Stroma	Yes	Yes	Yes	??	Yes	??	??
Tumor Tissue	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Environmental Factors±	Yes	??	??	Yes	??	??	??
Humanized exp. models	Yes	??	NA	NA	NA	NA	NA

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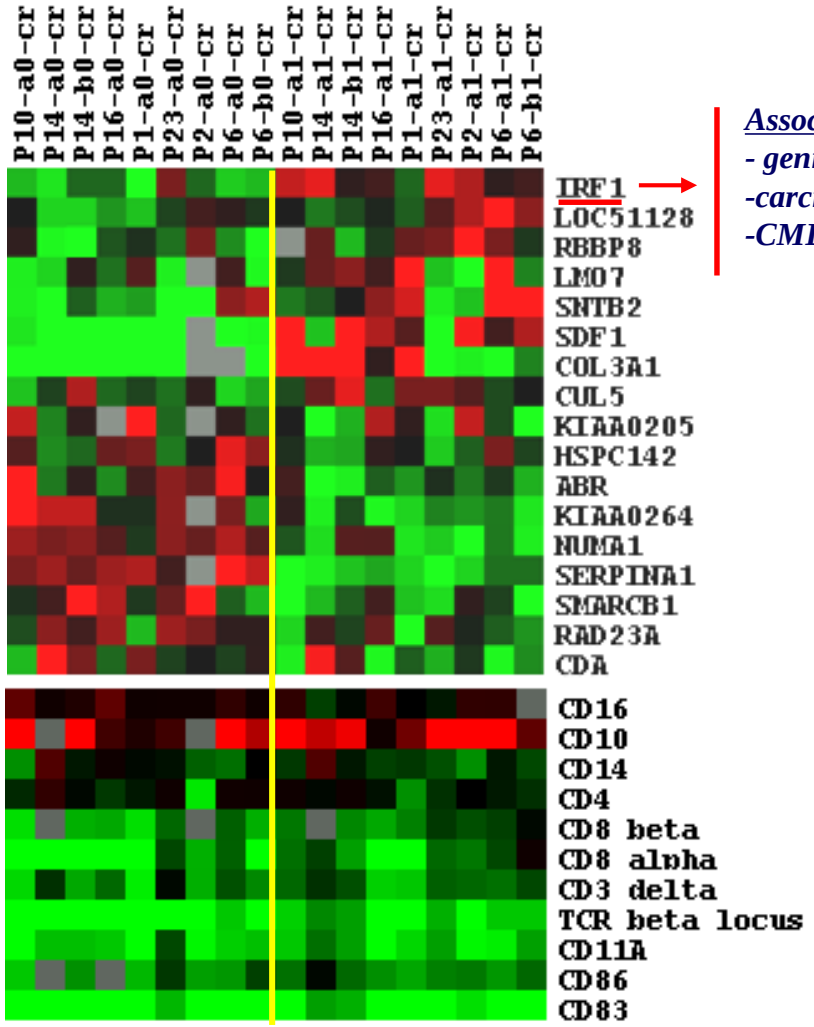
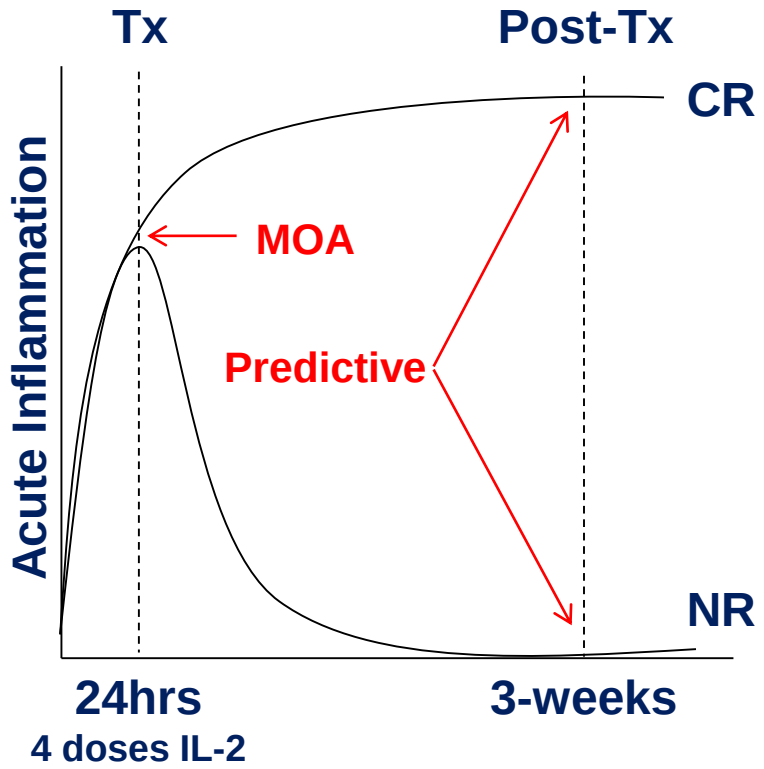
* Surrogate biomarkers of long term benefit; ± Include microbiome, co-morbidities, additional therapies, etc.

Genes exclusively expressed in complete responders (CR) pre- *vs* post-treatment

GP100 vaccine + IL-2



Associated with responsiveness of
- genital warts to Imiquimod
-carcinoid tumors to IFN-α
-CML to IFN-α



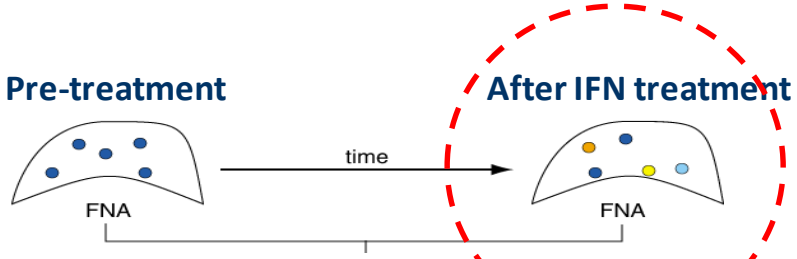
Wang et al. Cancer Res. 2002

uicc
global cancer control

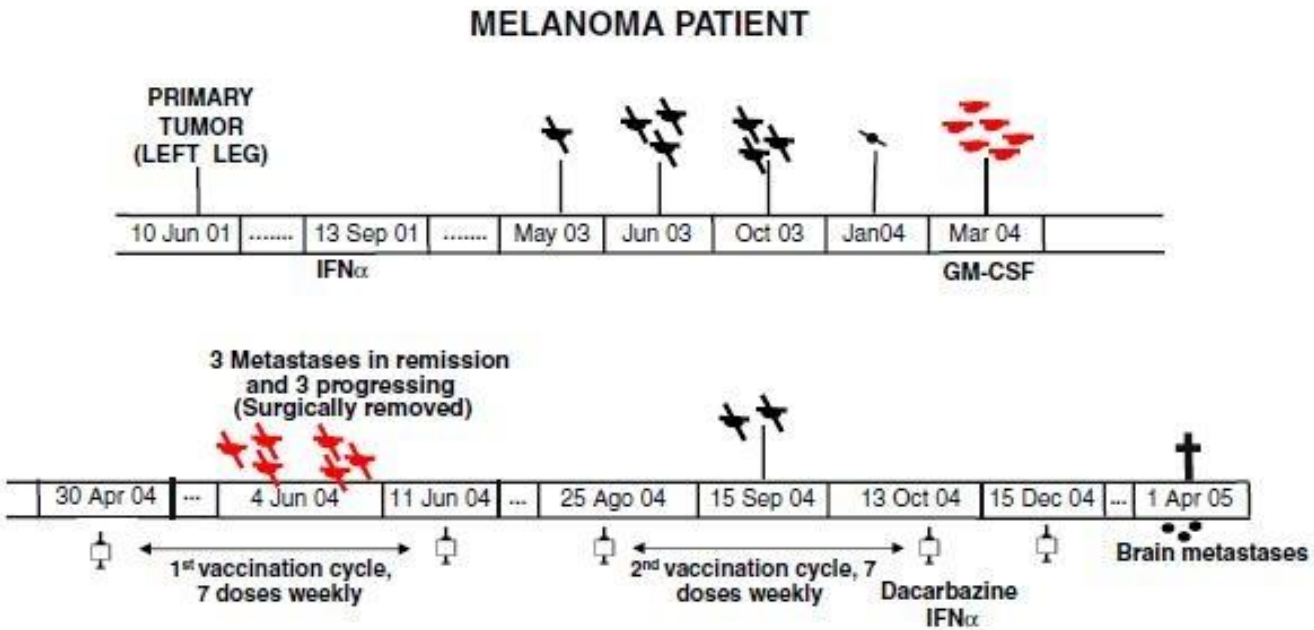
IJC
International Journal of Cancer

Regression of melanoma metastases after immunotherapy is associated with activation of antigen presentation and interferon-mediated rejection genes

Rafael Carretero[1,2], Ena Wang[3], Ana I. Rodriguez[2], Jennifer Reinboth[3,4,5], Maria L. Ascierto[3], Alyson M. Engle[3], Hui Liu[3], Francisco M. Camacho[6], Francesco M. Marincola[3], Federico Garrido[1,2] and Teresa Cabrera[1,2]



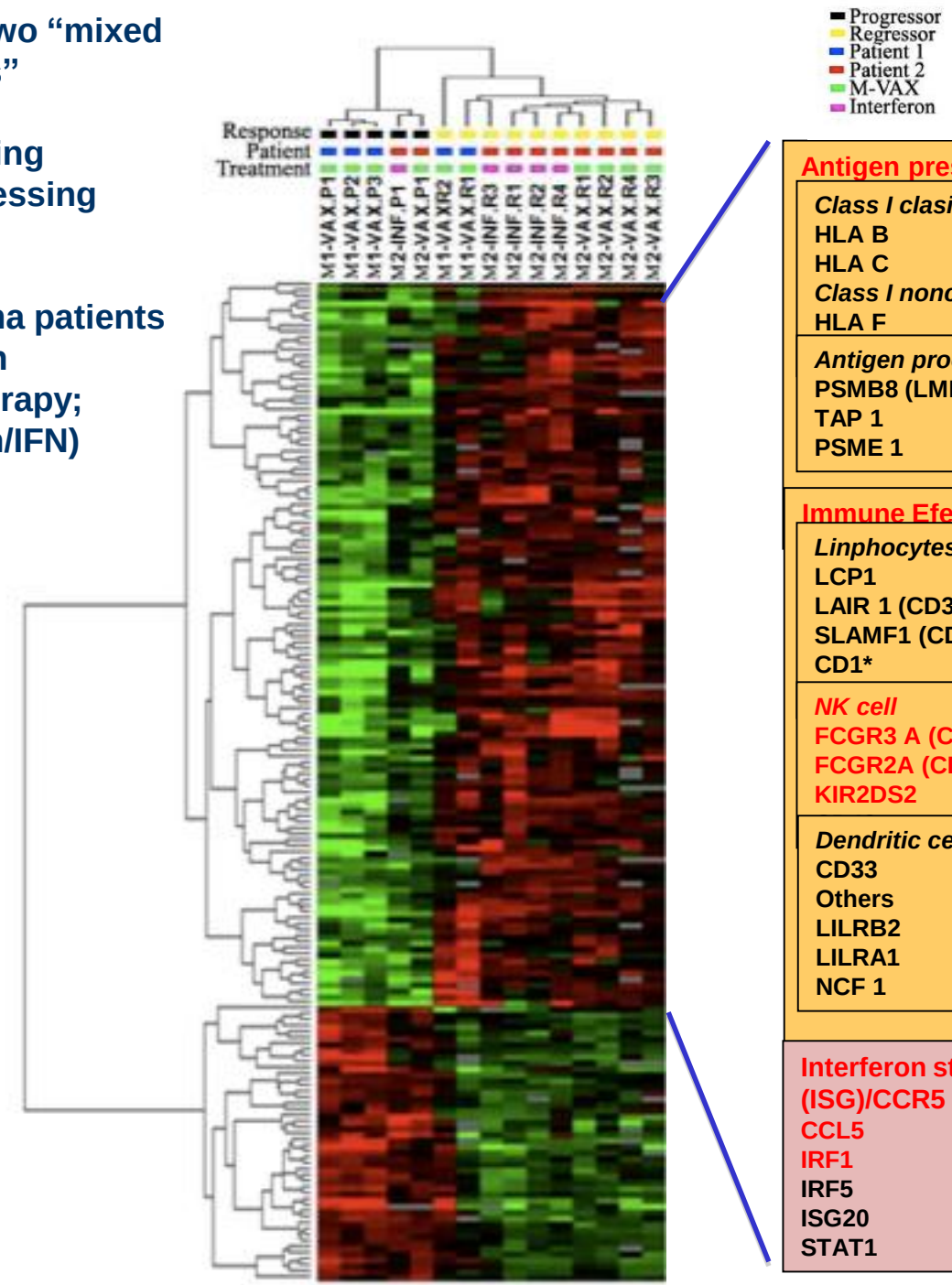
Carretero et al Int J Cancer 2011



Analys of two "mixed responders"

5 Progressing vs 10 Regressing Metastases

(2 melanoma patients treated with Immunotherapy; vaccination/IFN)



Antigen presentation related genes

Class I clasics
HLA B
HLA C
Class I nonclasics
HLA F

HLA class II
HLA DR
HLA DP
HLA DQ
HLA DM

Antigen processing machinery (APM)
PSMB8 (LMP7)
TAP 1
PSME 1
PSMB9 (LMP2)
PSMD2

Immune Efector Factors (IEFs)

Linphocytes
LCP1
LAIR 1 (CD305)
SLAMF1 (CD150)
CD1*

Linphocyte T
GRANZIME A
TCR
CD247
CD2

NK cell
FCGR3 A (CD16a)
FCGR2A (CD32)
KIR2DS2

Complement
C1S
C1QB
C1QA

Dendritic cells
CD33
Others
LILRB2
LILRA1
NCF 1

Linphocyte B
FCGR2A (CD32)
BCL6
immunoglobulins

Interferon stimulated genes (ISG)/CCR5 LIGANDS

CCL5
IRF1
IRF5
ISG20
STAT1

CCL 4
AIF1
GBP2
IFITM1
GBP1

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Product Characteristics	Yes	Yes	Yes	Yes	Yes	??	NA
Peripheral	?	Yes Monitoring!	Yes	Yes Monitoring!	Yes	Yes	??
Draining LNDs	Yes	??	??	??	??	??	??
Tumor Stroma	Yes	Yes	Yes	??	Yes	??	??
Tumor Tissue	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Environmental Factors±	Yes	??	??	Yes	??	??	??
Humanized exp. models	Yes	??	NA	NA	NA	NA	NA

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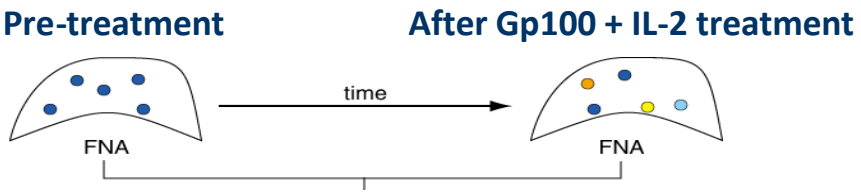
* Surrogate biomarkers of long term benefit; ± Include microbiome, co-morbidities, additional therapies, etc.

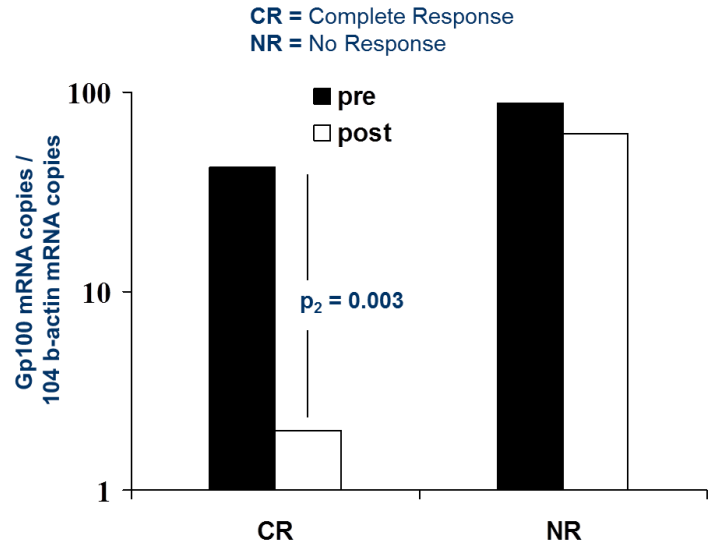
The Journal of Immunology

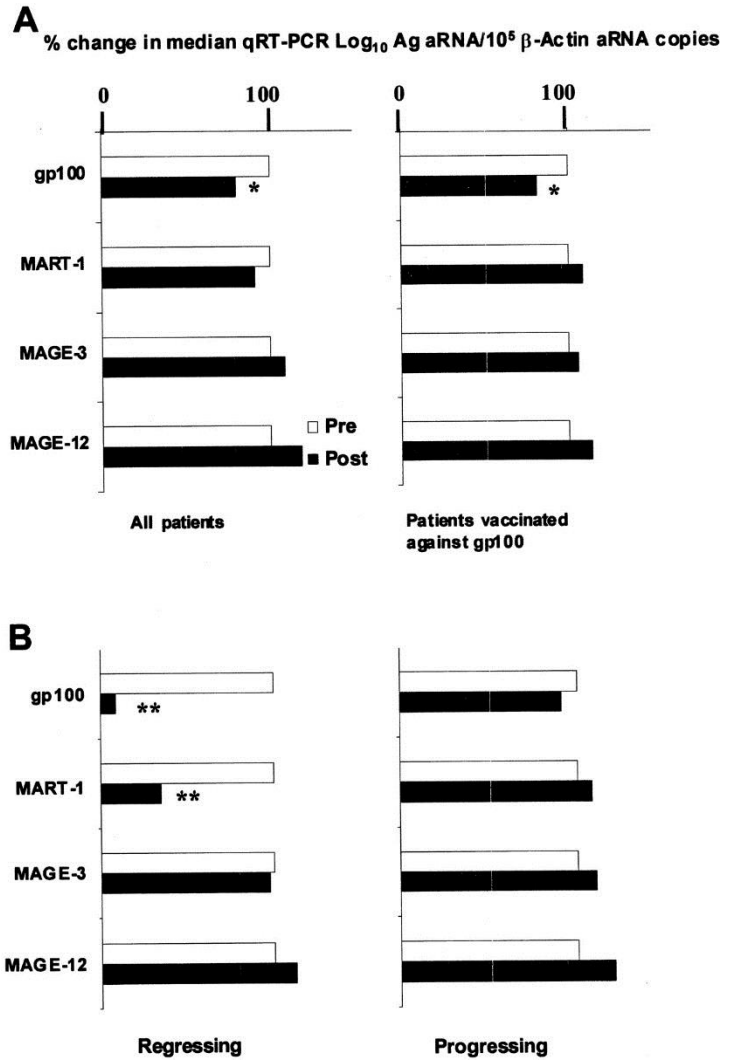
Short-Term Kinetics of Tumor Antigen Expression in Response to Vaccination

Galen A. Ohnmacht, Ena Wang, Simone Mocellin, Andrea Abati, Armando Filie, Patricia Fetsch, Adam I. Riker, Udai S. Kammula, Steven A. Rosenberg and Francesco M. Marincola

J Immunol August 1, 2001, 167 (3) 1809-1820; DOI: https://doi.org/10.4049/jimmunol.167.3.1809

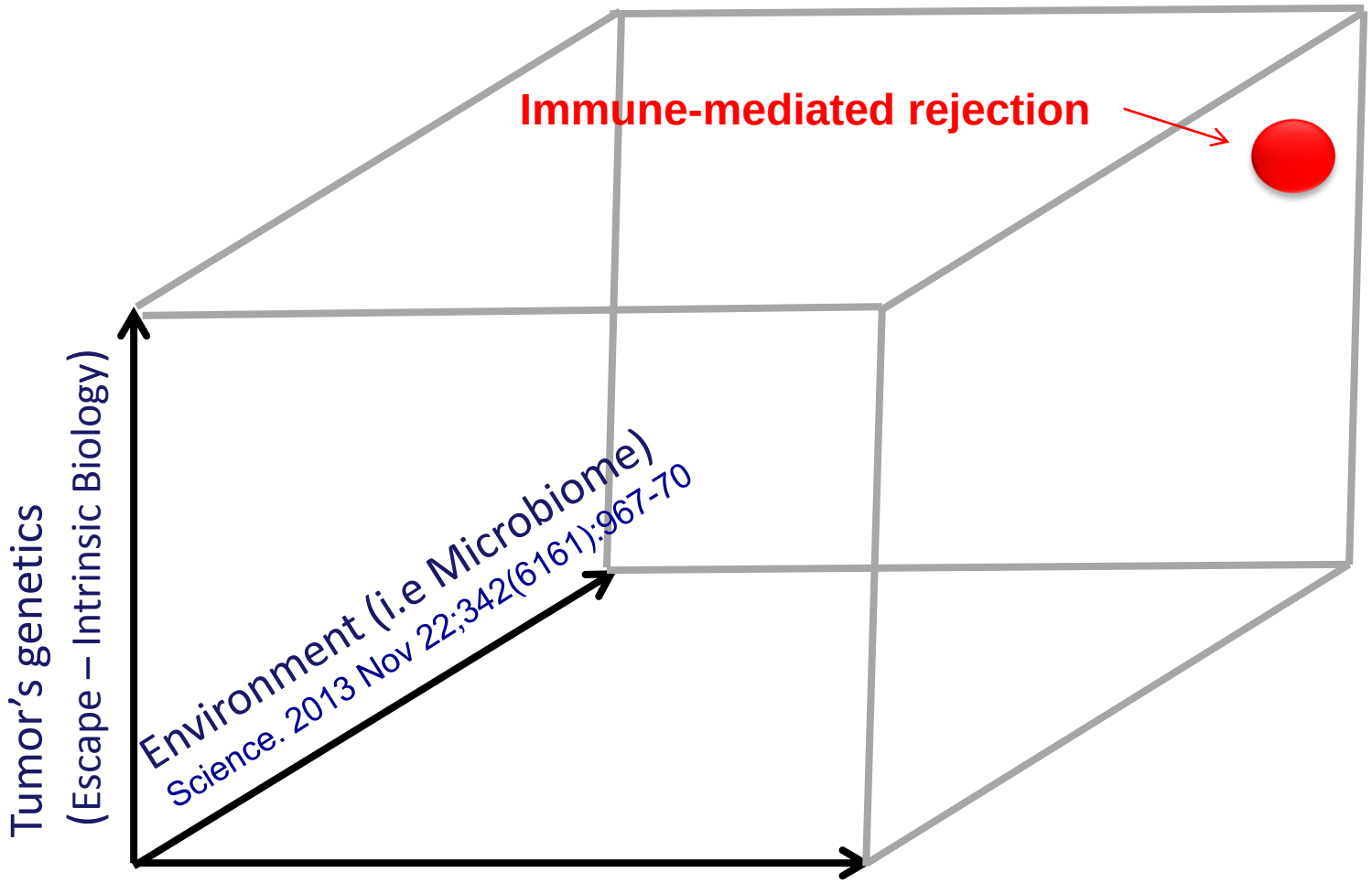






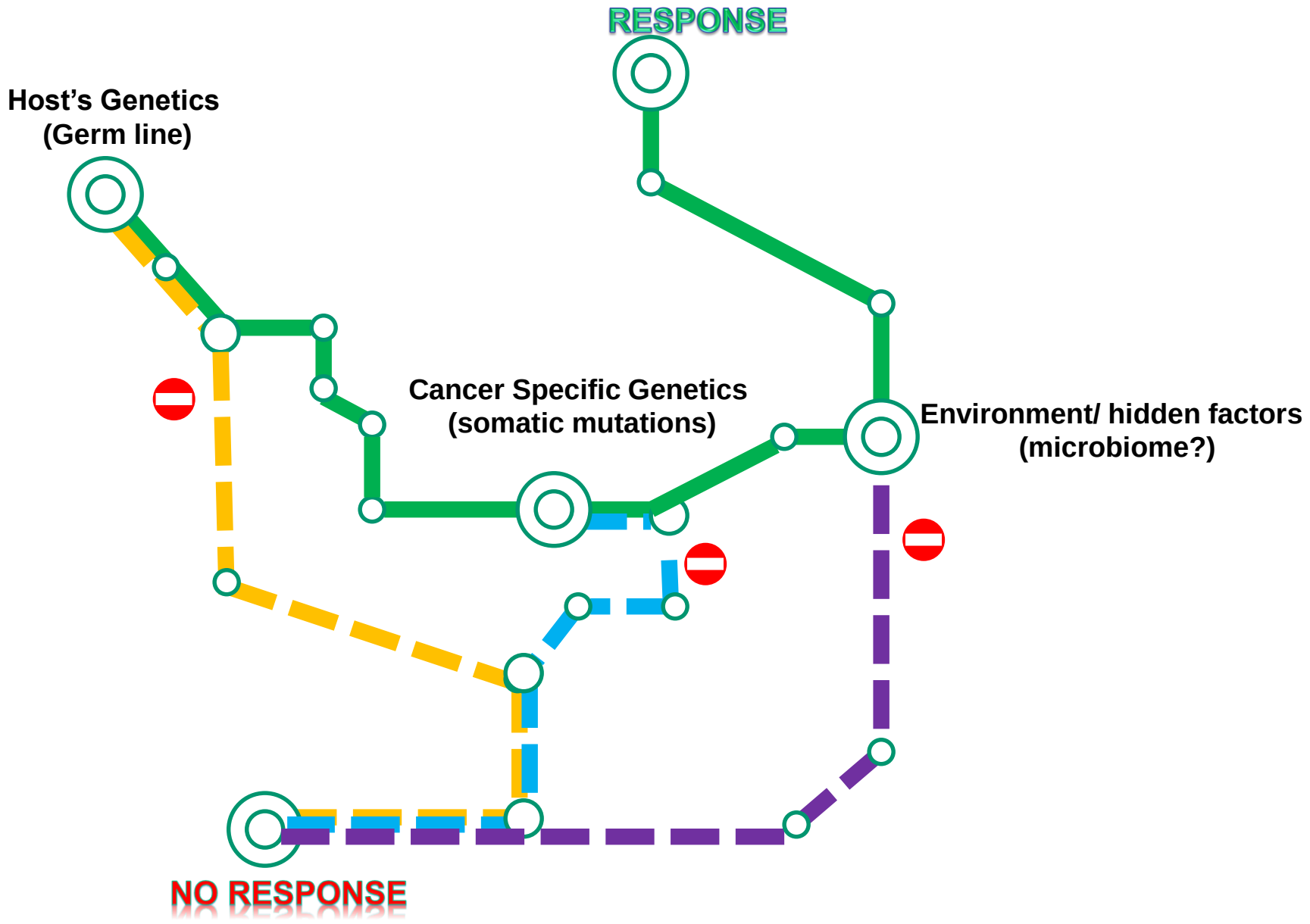
- How does tumor rejection occur
- **Why does rejection occur**

Factors influencing immune responsiveness
…and the theory of everything



A genetic inference on cancer immune responsiveness

Wang E, Uccellini L, Marincola FM - Oncoimmunology, 2012



A comprehensive view of cancer immune responsiveness: A synopsis from the SITC workshop

J Immunother. Cancer – Submitted

Davide Bedognetti1†, Michele Ceccarelli2, Lorenzo Galluzzi3,4,5, Rongze Lu2†, Karolina Palucka6, Josue Samayoa2†, Stefani Spranger7†, Sarah Warren8†, Kwok-Kin Wong9, Elad Ziv10, Diego Chowell11, Lisa M. Coussens12, Daniel D. De Carvalho13, David G. DeNardo14, Jérôme Galon15, Howard L. Kaufman16, Tomas Kirchhoff17, Michael T. Lotze18, Jason J. Luke19, Andy J. Minn20, Katerina Politi21, Leonard D. Shultz22, Richard Simon23, Vésteinn Thórsson24, Joanne B. Weidhaas25, Maria Libera Ascierto26, Paolo Antonio Ascierto27, James M. Barnes2, Valentin Barsan28, Praveen K. Bommareddy29, Adrian Bot30, Sarah E. Church8, Gennaro Ciliberto31, Andrea De Maria32, Dobrin Draganov33, Winson S. Ho34, Heather M. McGee35, Anne Monette36, Joseph F. Murphy37, Paola Nisticò31, Wungki Park11, Maulik Patel2, Michael Quigley38, Laszlo Radvanyi39, Harry Raftopoulos40, Nils-Petter Rudqvist3, Alexandra Snyder41, Randy F. Sweis19, Sara Valpione42, Lisa H. Butterfield18, Mary L. Disis43, Bernard A. Fox44, Alessandra Cesano8, Francesco M. Marincola45*
on behalf of the Society for Immunotherapy of Cancer (SITC) Cancer Immune Responsiveness Task Force and Working Groups.

Matrix for sample collection (Umbrella/Basket clinical design)	**Baseline**		**On Treatment**		**Post Treatment**		
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Tumor Stroma	Yes	Yes	Yes	??	Yes	??	??
Tumor Tissue	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Environmental Factors±	Yes	??	??	Yes	??	??	??
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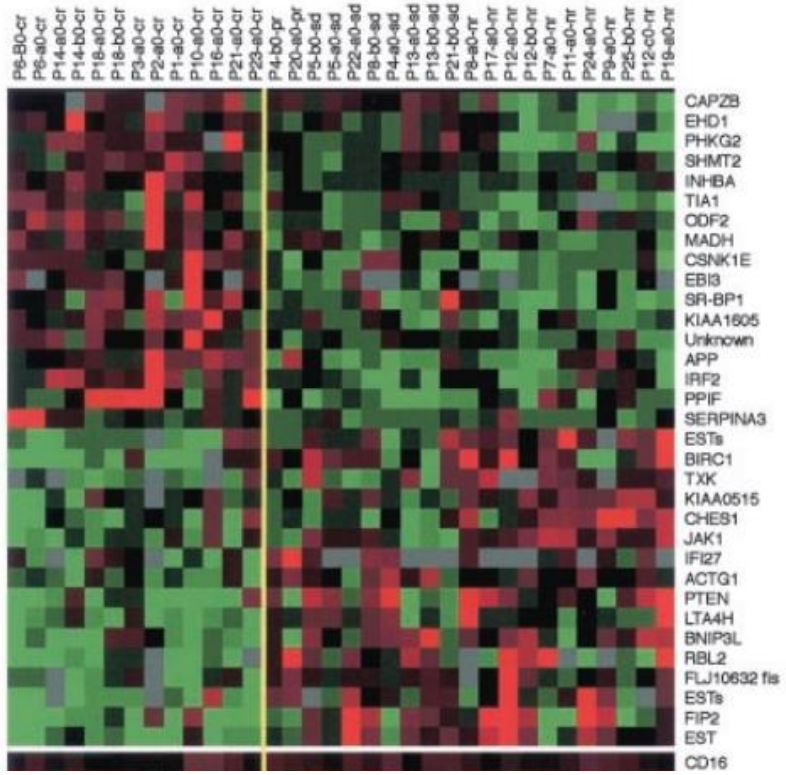
CANCER RESEARCH 62, 3581-3586, 2002

Prospective Molecular Profiling of Melanoma Metastases Suggests Classifiers of Immune Responsiveness

Ena Wang, Lance D. Miller, Galen A. Ohnmacht, Simone Mocellin, Ainhoa Perez-Diez, David Petersen, Yingdong Zhao, Richard Simon, John I. Powell, Esther Asaki, H. Richard Alexander, Paul H. Duray, Meenhard Herlyn, Nicholas P. Restifo, Edison T. Liu, Steven A. Rosenberg, and Francesco M. Marincola[1]



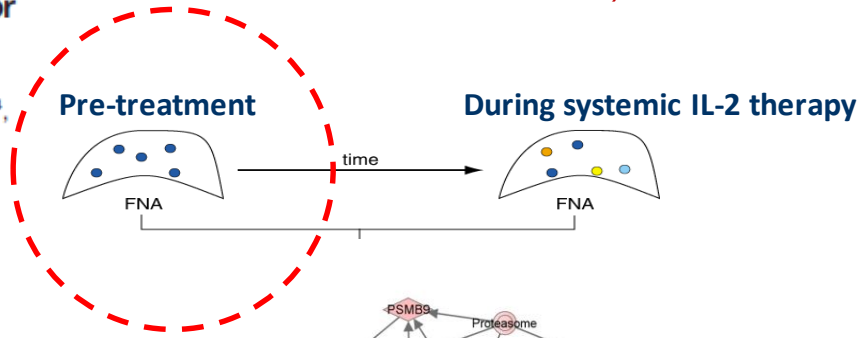
come. Ranking of gene expression data from pretreatment samples identified ~30 genes predictive of clinical response ($P < 0.001$). Analysis of their annotations denoted that approximately half of them were related to T-cell regulation, suggesting that immune responsiveness might be predetermined by a tumor microenvironment conducive to immune recognition.

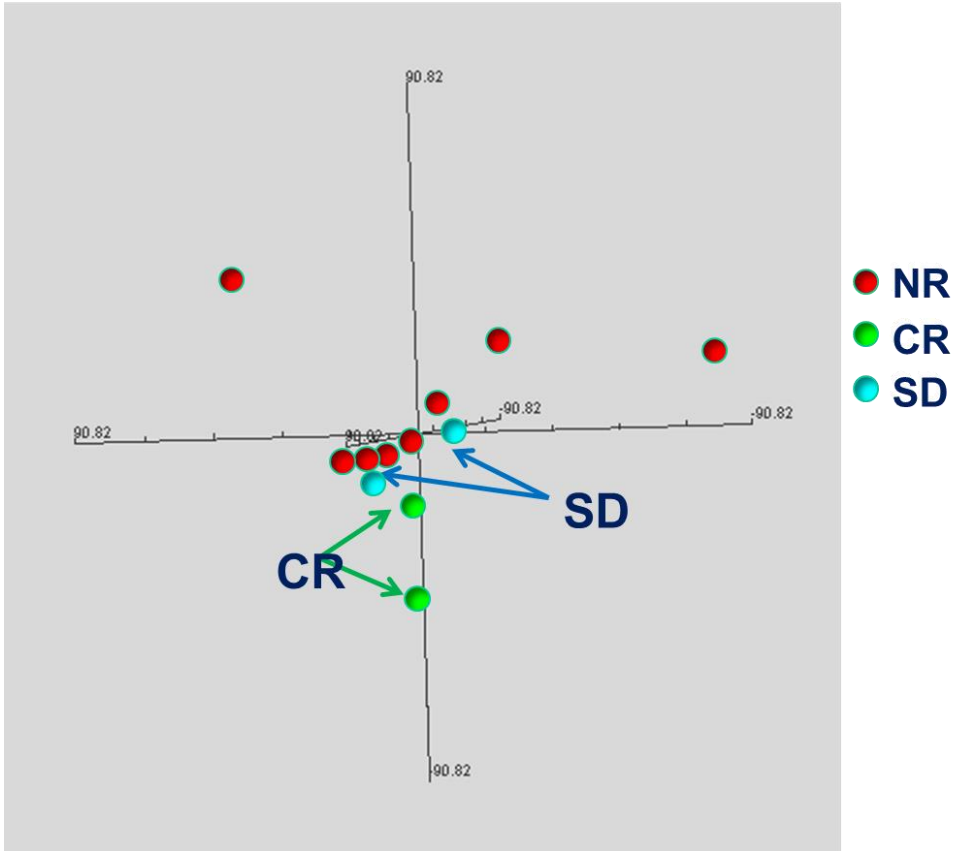
Clinical Cancer Research

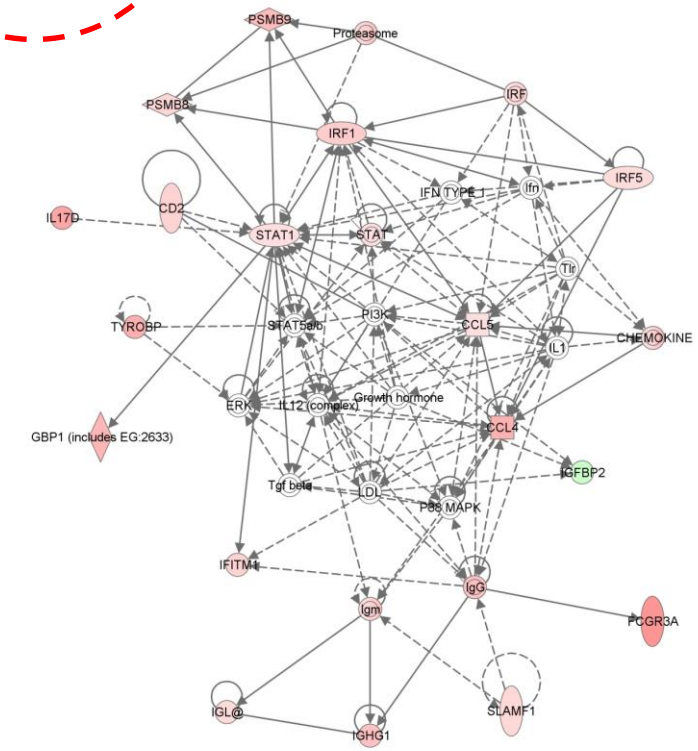
Molecular Insights on the Peripheral and Intratumoral Effects of Systemic High-Dose rIL-2 (Aldesleukin) Administration for the Treatment of Metastatic Melanoma

Geoffrey R. Weiss[1], William W. Grosh[1], Kimberly A. Chianese-Bullock[2], Yingdong Zhao[3], Hui Liu[4], Craig L. Slingluff Jr[2], Francesco M. Marincola[4], and Ena Wang[4]

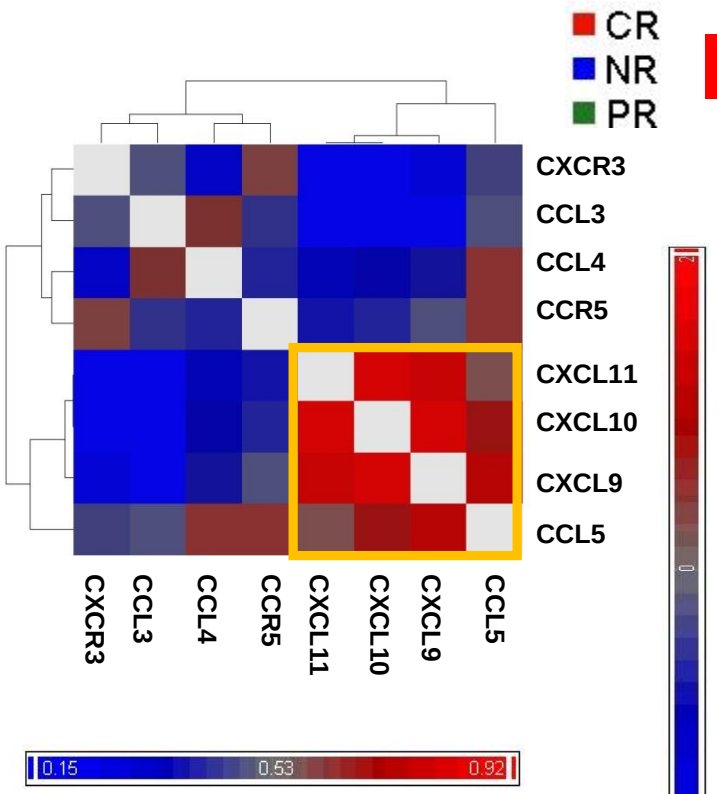
Weiss G R et al. Clin Cancer Res 2011;17:7440-7450



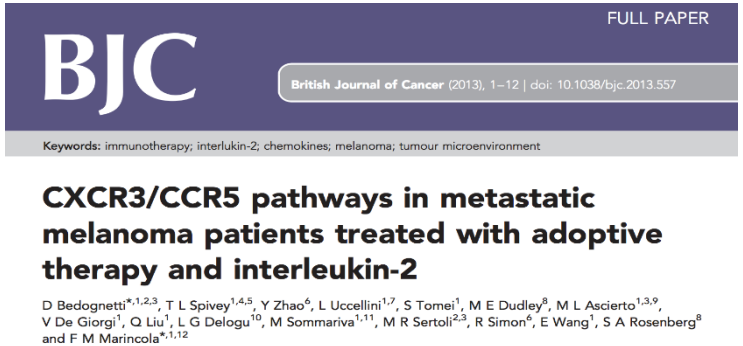




Adoptive therapy + IL-2 (113 pre-treatment melanoma lesions)



113 pre-treatment melanoma biopsies


FULL PAPER

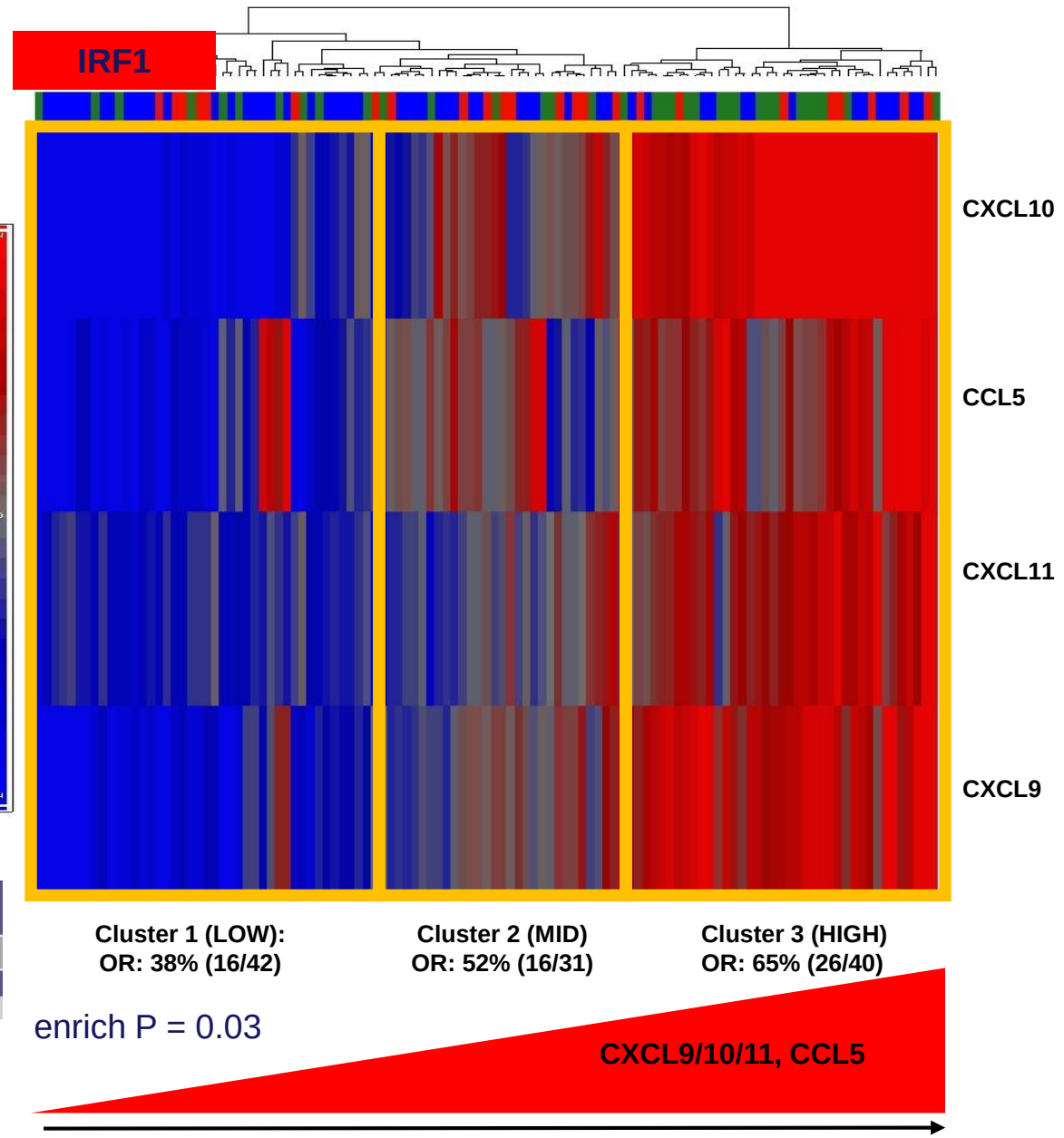
BJC

British Journal of Cancer (2013), 1–12 | doi: 10.1038/bjc.2013.557

Keywords: immunotherapy; interlukin-2; chemokines; melanoma; tumour microenvironment

CXCR3/CCR5 pathways in metastatic melanoma patients treated with adoptive therapy and interleukin-2

D Bedognetti[*,1,2,3], T L Spivey[1,4,5], Y Zhao[6], L Uccellini[1,7], S Tomei[1], M E Dudley[8], M L Ascierto[1,3,9], V De Giorgi[1], Q Liu[1], L G Delogu[10], M Sommariva[1,11], M R Sertoli[2,3], R Simon[6], E Wang[1], S A Rosenberg[8] and F M Marincola[*,1,12]



Cluster 1 (LOW): OR: 38% (16/42)

Cluster 2 (MID) OR: 52% (16/31)

Cluster 3 (HIGH) OR: 65% (26/40)

enrich P = 0.03

CXCL9/10/11, CCL5

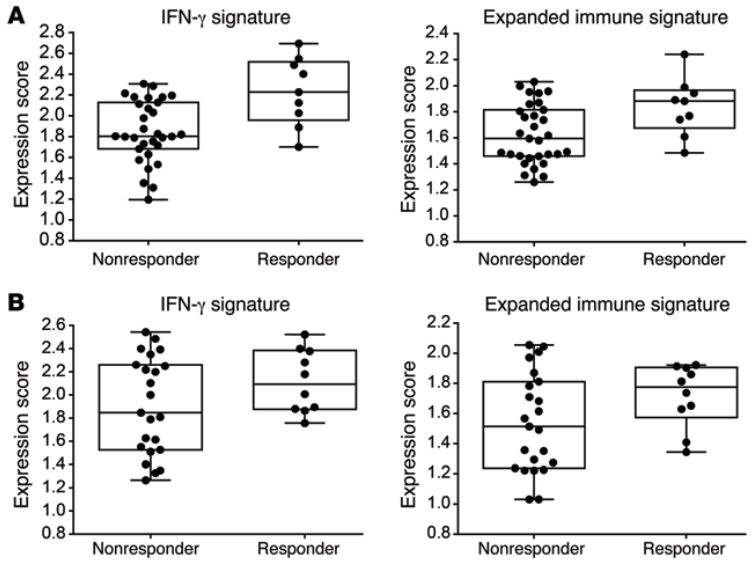
OR Rate: Cluster 1 < Cluster 2 < Cluster 3

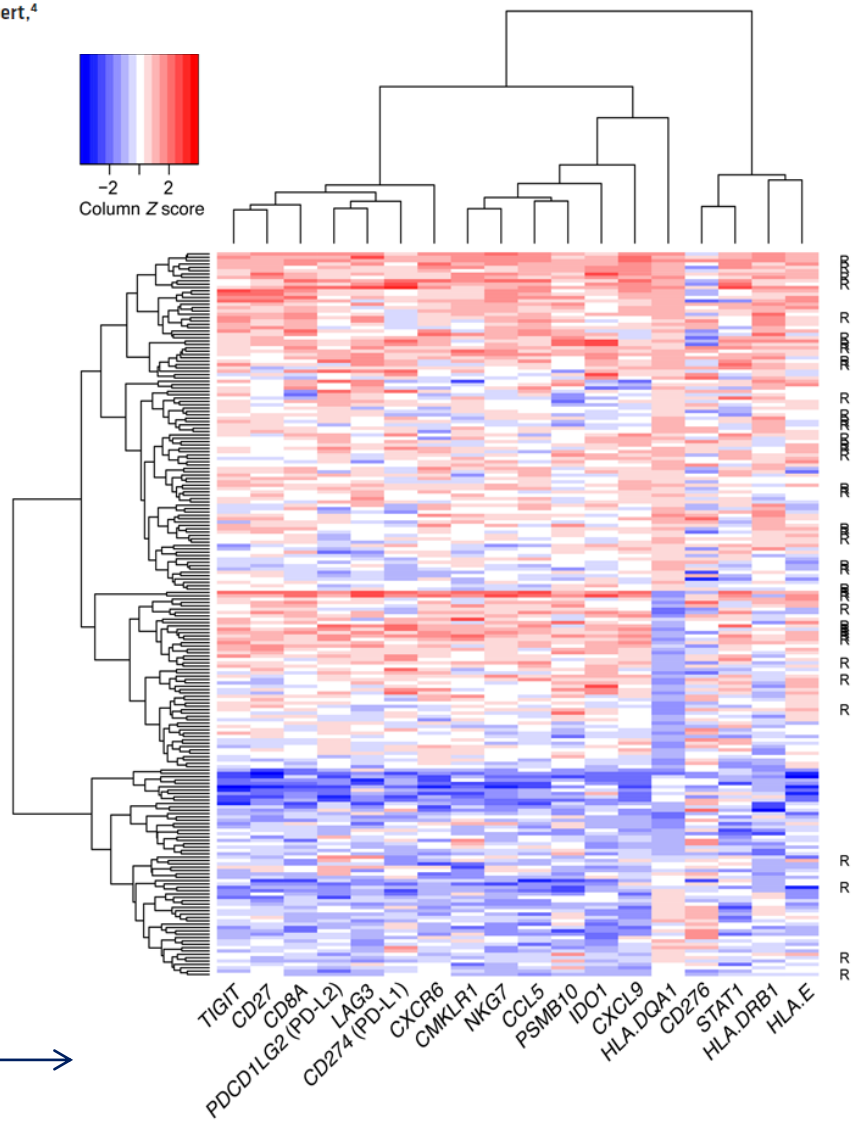
The Journal of Clinical Investigation RESEARCH ARTICLE

J Clin Invest. 2017 Aug 1;127(8):2930-2940..

IFN-γ–related mRNA profile predicts clinical response to PD-1 blockade

Mark Ayers,[1] Jared Lunceford,[1] Michael Nebozhyn,[1] Erin Murphy,[1] Andrey Loboda,[1] David R. Kaufman,[1] Andrew Albright,[1] Jonathan D. Cheng,[1] S. Peter Kang,[1] Veena Shankaran,[2] Sarina A. Piha-Paul,[3] Jennifer Yearley,[1] Tanguy Y. Seiwert,[4] Antoni Ribas,[5] and Terrill K. McClanahan[1]





Tumor Inflammation Signature

IFN-γ responsive genes: CD27, **STAT1**, **IDO1**, HLA-E, NKG7
Antigen Presentation: HLA-DQA1, HLA-DRB1, PSMB10, CMKLR1
Chemokines genes: **CCL5, CXCL9**, CXCR6
Cytotoxic activity: **CD8**
Adaptive Immune Resistance: TIGIT, LAG3, **CD274**, CD276, **PDCD1LG2**

Tumor Inflammation Signature is Predictive in Combination Immune Checkpoint Blockade

Tumor Inflammation Signature - 18 gene biomarker classifier of peripherally suppressed adaptive immune responses in tumor (Ayers 2017 JCI)

OpACIN Trial – neo-/adjuvant ipilimumab + nivolumab in stage III melanoma

Gene expression profiling shows elevated TIS score correlates with durable remission

Tumor Inflammation Signature

IFN-γ responsive genes: CD27, **STAT1**, **IDO1**, HLA-E, NKG7
Antigen Presentation: HLA-DQA1, HLA-DRB1, PSMB10, CMKLR1
Chemokines genes: **CCL5, CXCL9**, CXCR6
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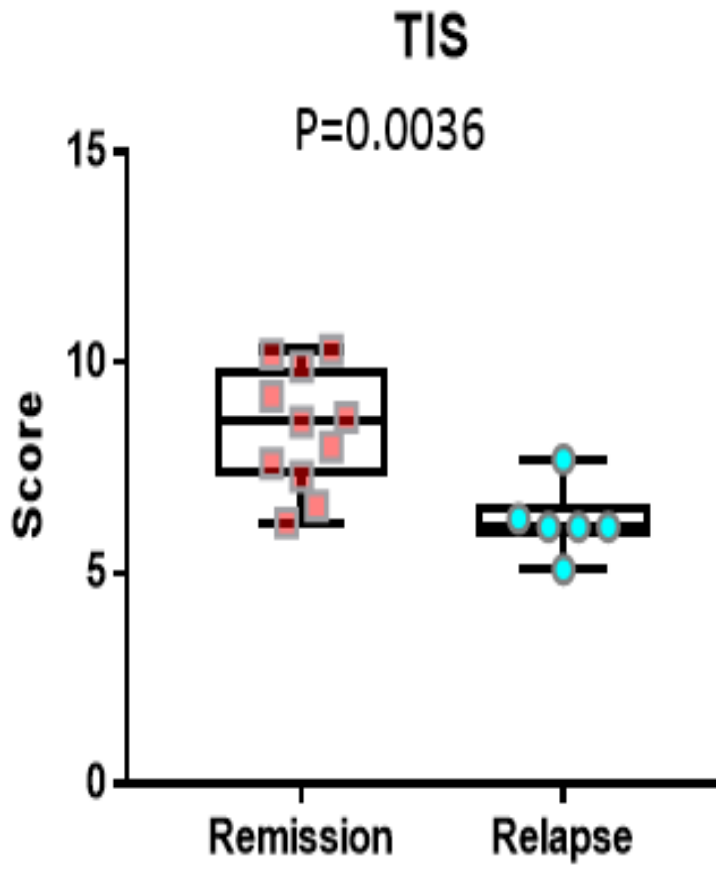
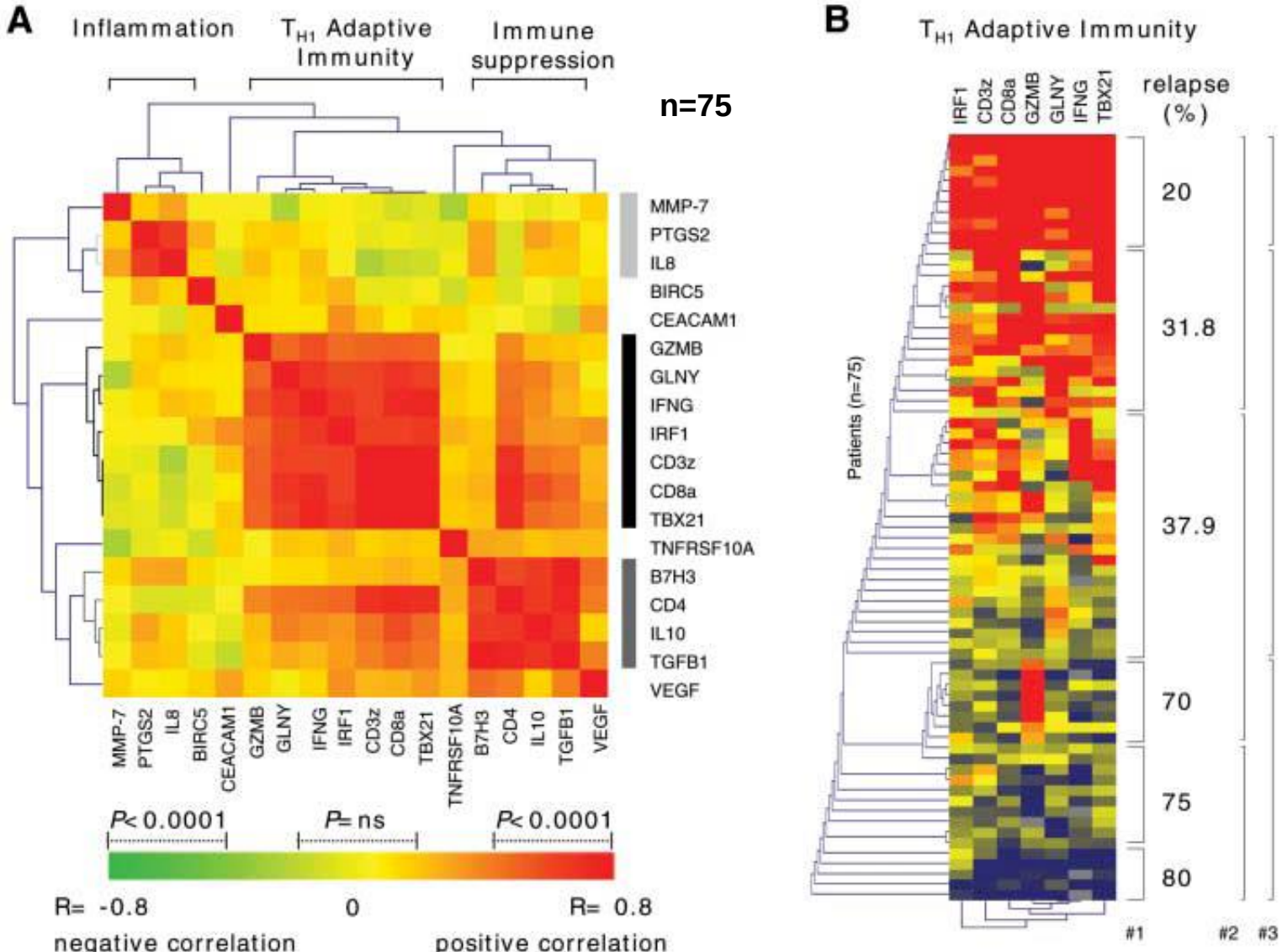


EA Rozeman…CU Blank
SITC 2017 #P99

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†]

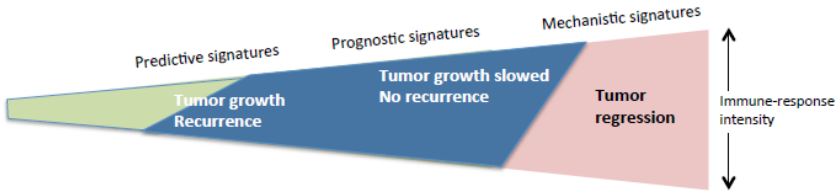


Immunity
Review

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,*]

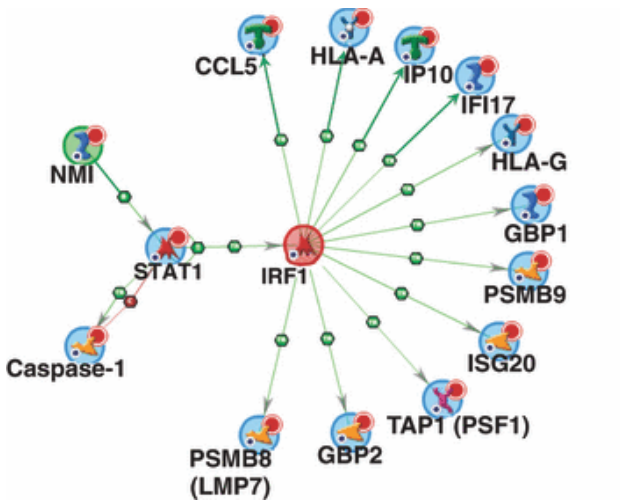
	Th1	Chemokines		Cytotoxic	Adhesion	
	STAT-1 IRF-1/ IFN-γ-SG Pathway	CXCR3/ CXCL9-11 Pathway	CCR5/ CCL3-5 Pathway	Granzyme Perforin Granulysin/T IA-1/CASPs Pathway	Adhesion Molecules	References
Prognostic						
Breast	+	+		+	+	Ascierto et al., 2012
	+	+	+		+	Curtis et al., 2012
	+					Desmedt et al., 2008
Ovarian	+		+	+	+	Leffers et al., 2010
	+	+				Zhang et al., 2003
		+				Verhaak et al, 2013
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
Predictive						
Breast (Chemo)		+				Denkert et al., 2010
	+					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., 201
Lung	+	+	+	+		Ulloa-Montoya et al., 201
Mechanistics						
Melanoma (IL-2/ vaccine/anti-CTLA-4)	+	+	+	+	+	Panelli et al., 2002
	+					Wang et al., 2002
	+		+			Weiss et al., 2011
	+		+	+		Carretero et al., 2012
	+	+	+	+	+	Ji et al., 2012
Basal cell carcinoma (Imiquimod)	+	+	+	+		Panelli et al., 2007



Analysis of independent microarray datasets of renal biopsies identifies a robust transcript signature of acute allograft rejection

Pierre Saint-Mezard [1], Céline C. Berthier [2], Hai Zhang [1†], Alexandre Hertig [3], Sergio Kaiser [1], Martin Schumacher [1], Grazyna Wieczorek [1], Marc Bigaud [1], Jeanne Kehren [1], Eric Rondeau [3], Friedrich Raulf [1] and Hans-Peter Marti [2,3]*

ESOT 2009

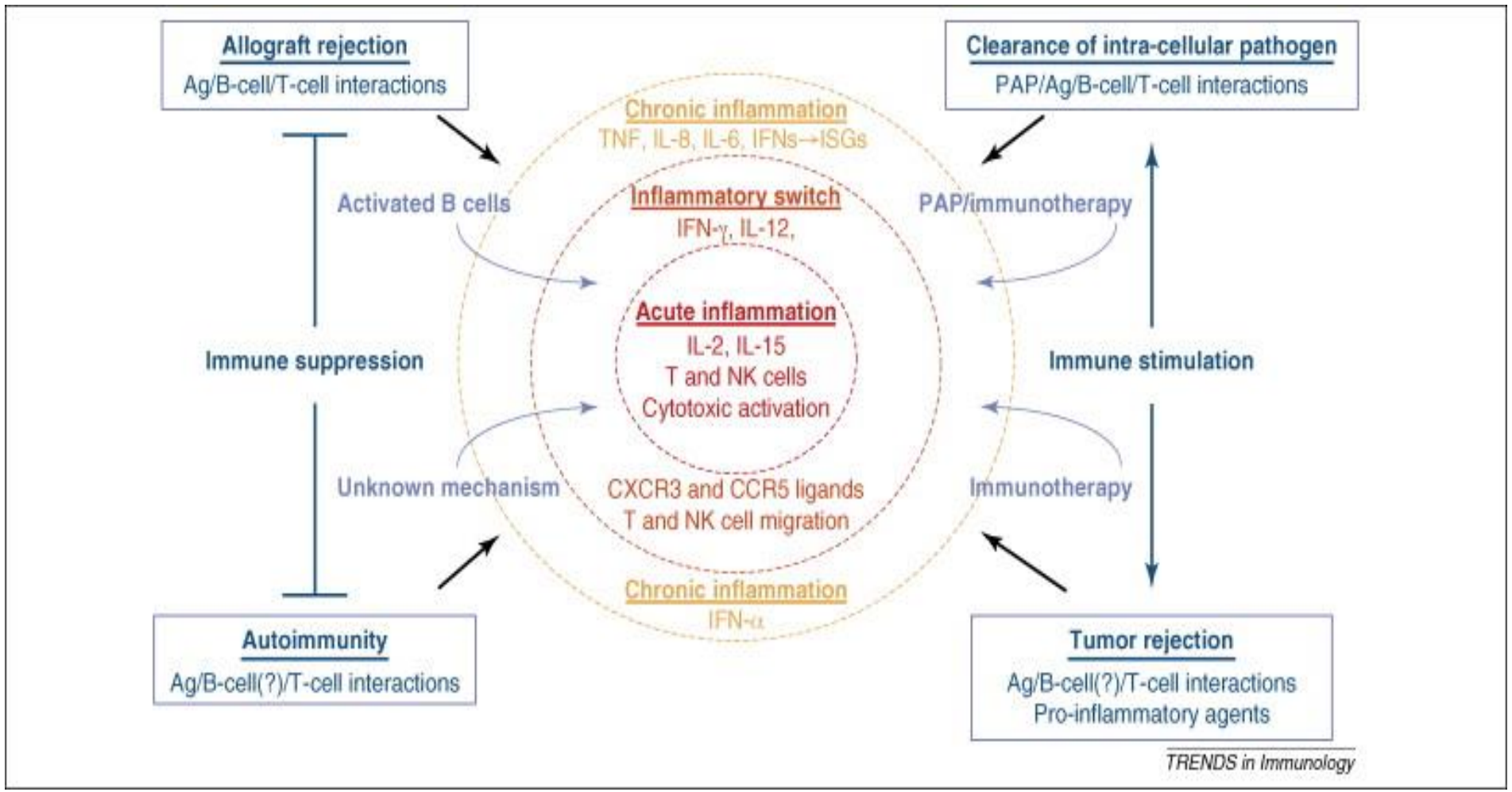


Opinion

The immunologic constant of rejection

Ena Wang[1,2], Andrea Worschech[1,2,3] and Francesco M. Marincola[1,2]

"Immune-mediated tumor rejection is just a facet of autoimmunity"



A comprehensive view of cancer immune responsiveness: A synopsis from the SITC workshop

J Immunother. Cancer – Submitted

Davide Bedognetti1†, Michele Ceccarelli2, Lorenzo Galluzzi3,4,5, Rongze Lu2†, Karolina Palucka6, Josue Samayoa2†, Stefani Spranger7†, Sarah Warren8†, Kwok-Kin Wong9, Elad Ziv10, Diego Chowell11, Lisa M. Coussens12, Daniel D. De Carvalho13, David G. DeNardo14, Jérôme Galon15, Howard L. Kaufman16, Tomas Kirchhoff17, Michael T. Lotze18, Jason J. Luke19, Andy J. Minn20, Katerina Politi21, Leonard D. Shultz22, Richard Simon23, Vésteinn Thórsson24, Joanne B. Weidhaas25, Maria Libera Ascierto26, Paolo Antonio Ascierto27, James M. Barnes2, Valentin Barsan28, Praveen K. Bommareddy29, Adrian Bot30, Sarah E. Church8, Gennaro Ciliberto31, Andrea De Maria32, Dobrin Draganov33, Winson S. Ho34, Heather M. McGee35, Anne Monette36, Joseph F. Murphy37, Paola Nisticò31, Wungki Park11, Maulik Patel2, Michael Quigley38, Laszlo Radvanyi39, Harry Raftopoulos40, Nils-Petter Rudqvist3, Alexandra Snyder41, Randy F. Sweis19, Sara Valpione42, Lisa H. Butterfield18, Mary L. Disis43, Bernard A. Fox44, Alessandra Cesano8, Francesco M. Marincola45*
on behalf of the Society for Immunotherapy of Cancer (SITC) Cancer Immune Responsiveness Task Force and Working Groups.

Matrix for sample collection (Umbrella/Basket clinical design)	Baseline		On Treatment		Post Treatment		
	Target Discovery	Prediction	MOA (PK/PD)	Prediction	MOA	Surrogate*	Escape
Germline	Yes	Yes	NA	NA	NA	NA	NA
Product Characteristics	Yes	Yes	Yes	Yes	Yes	??	NA
Peripheral	?	Yes Monitoring!	Yes	Yes Monitoring!	Yes	Yes	??
Draining LNDs	Yes	??	??	??	??	??	??
Tumor Stroma	Yes	Yes	Yes	??	Yes	??	??
Tumor Tissue	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Environmental Factors±	Yes	??	??	Yes	??	??	??
Humanized exp. models	Yes	??	NA	NA	NA	NA	NA

LND = Lymph node; MOA = Mechanism of Action; NA = Not Applicable; PD = Pharmacokinetics; PK = Pharmacodynamics; YES denotes potential usefulness for a given purpose; "??" Signifies unknown or unlikely usefulness, Discovery vs Predictive refers to studies that are meant to enlighten mechanistically the reasons for a given phenomenon (Discovery) rather than only identifying associations (Predictive)

* Surrogate biomarkers of long term benefit; ± Include microbiome, co-morbidities, additional therapies, etc.

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2017, VOL. 6, NO. 2, e1253654 (19 pages)
http://dx.doi.org/10.1080/2162402X.2016.1253654

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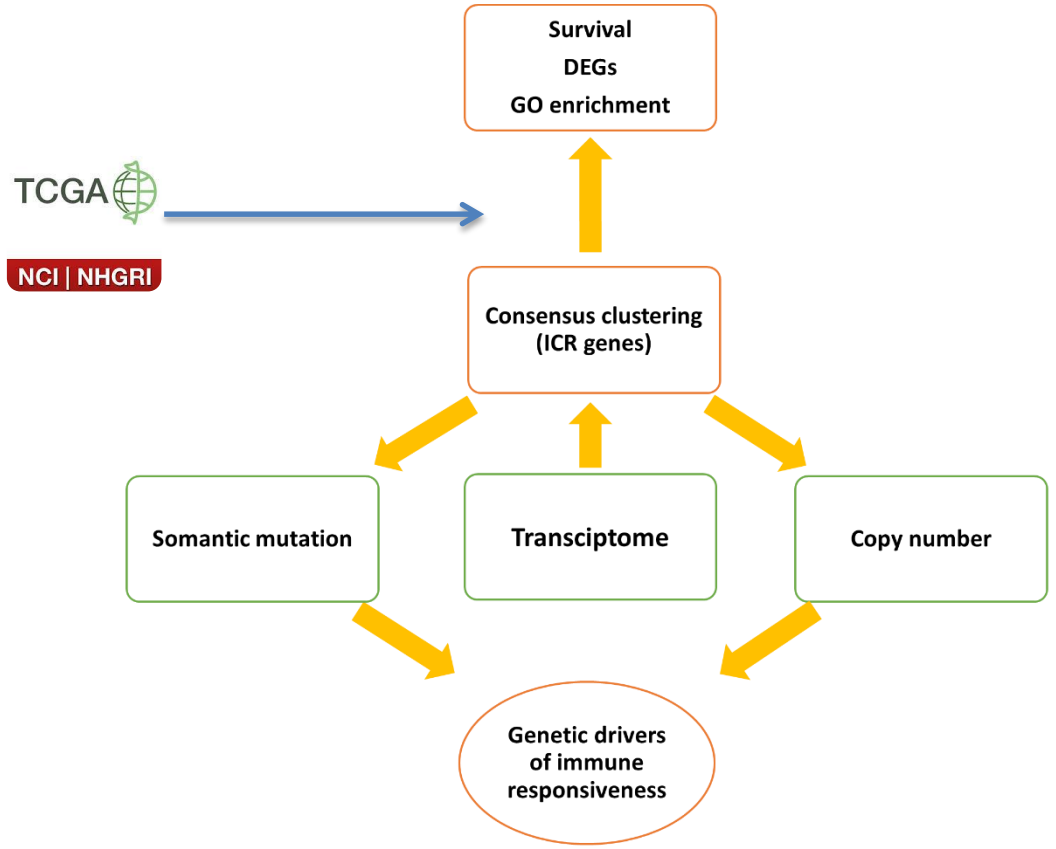
ORIGINAL RESEARCH

OPEN ACCESS

Identification of genetic determinants of breast cancer immune phenotypes by integrative genome-scale analysis

Wouter Hendrickx[a,*], Ines Simeone[b,c,*], Samreen Anjum[b], Younes Mokrab[d], François Bertucci[e,f,g], Pascal Finetti[e], Giuseppe Curigliano[h], Barbara Seliger[i], Luigi Cerulo[c,j], Sara Tomei[k], Lucia Gemma Delogu[l], Cristina Maccalli[a], Ena Wang[k], Lance D. Miller[m], Francesco M. Marincola[n], Michele Ceccarelli[b,#], and Davide Bedognetti[a,#]

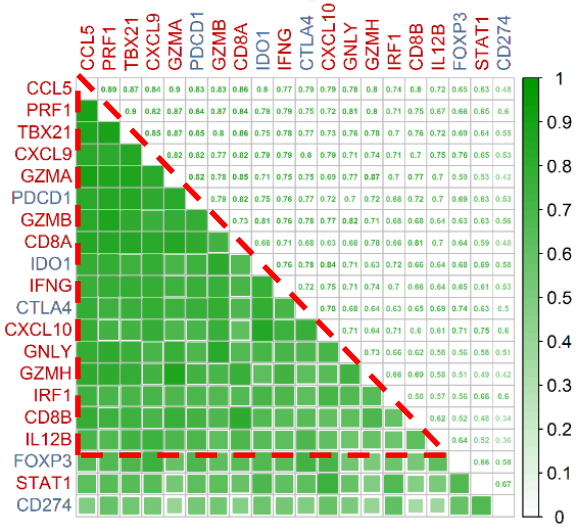


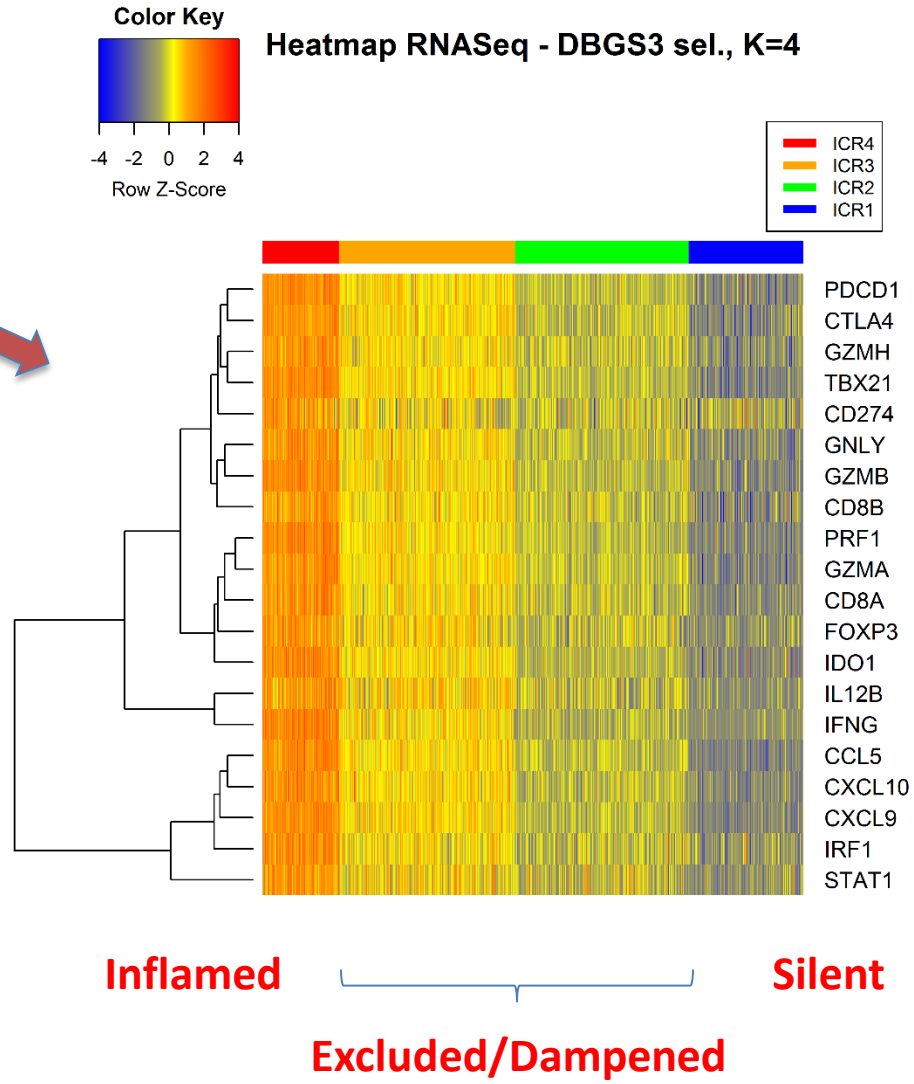
Functional pipeline

ICR based Consensus Clustering

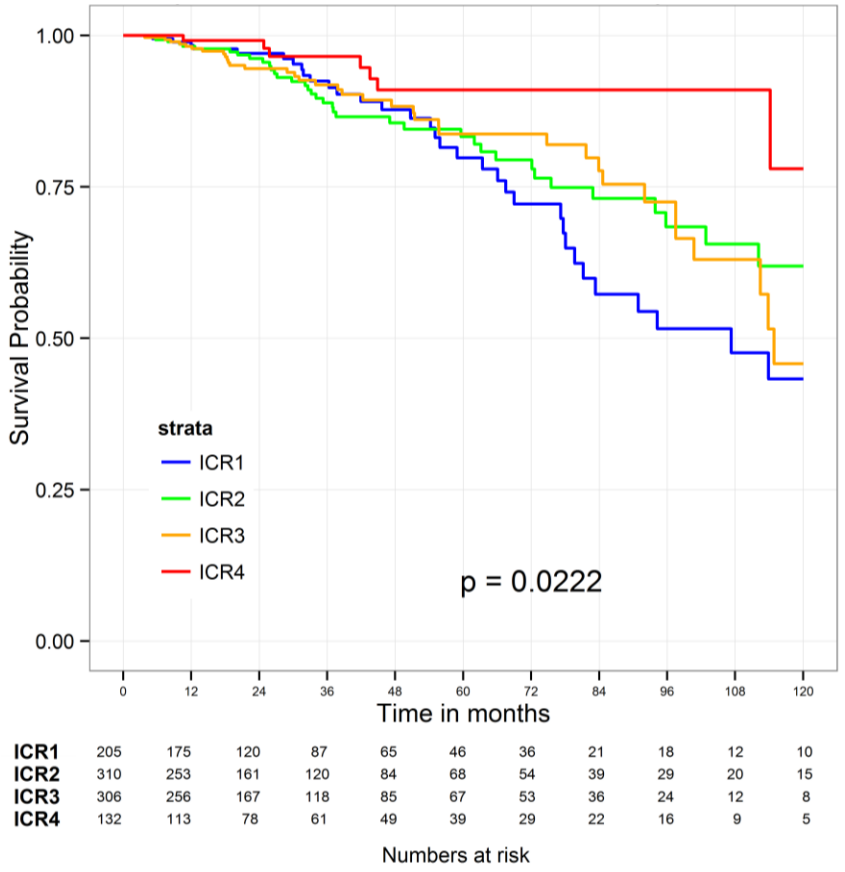
Immunologic Constant of Rejection

CXCR3/CCR5 Chemokines	Th1 signaling	Effector functions	Immune regulatory
CXCL9	IFNG	GNLY	CD274
CXCL10	TBX21	PRF1	CTLA4
CCL5	CD8A	GZMA	FOXP3
	IL12B	GZMB	IDO1
	CD8B	GZMH	PDCD1
	STAT1		
	IRF1		





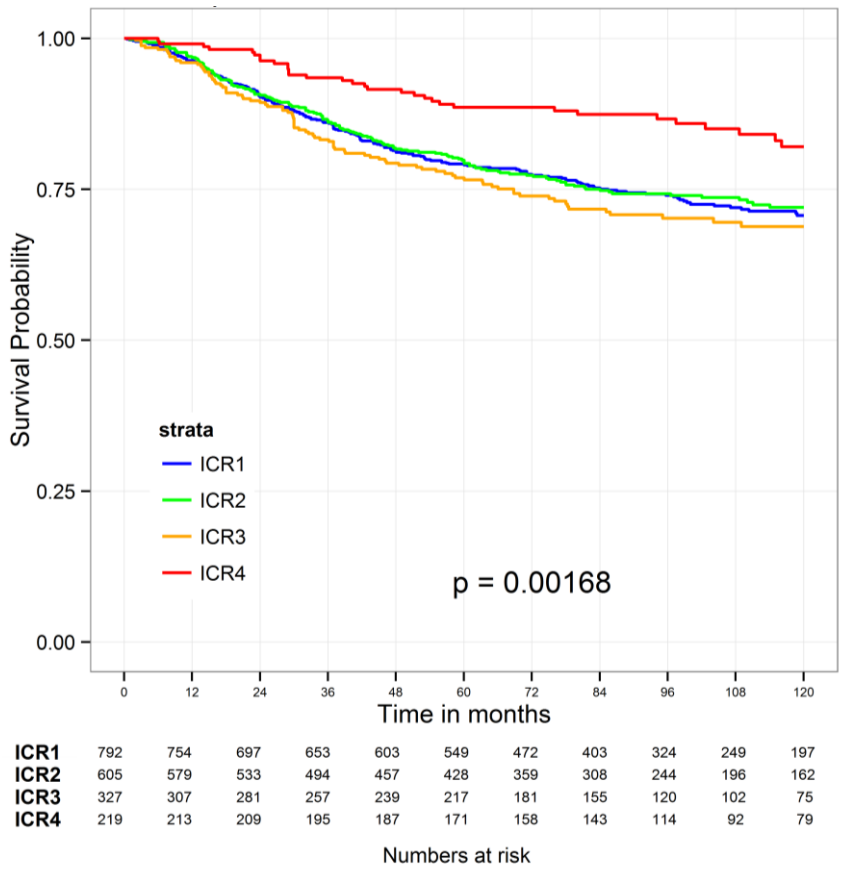
Survival Analysis – TCGA (N=953)



ICR1	205	175	120	87	65	46	36	21	18	12	10
ICR2	310	253	161	120	84	68	54	39	29	20	15
ICR3	306	256	167	118	85	67	53	36	24	12	8
ICR4	132	113	78	61	49	39	29	22	16	9	5

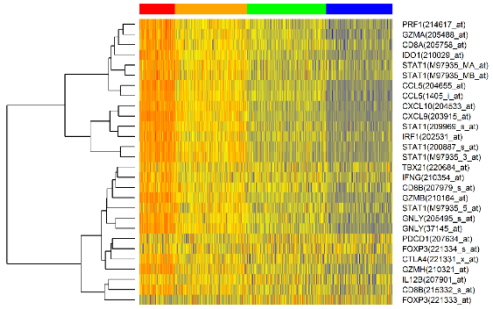
Numbers at risk

Validation (LM dataset, N=1943)



ICR1	792	754	697	653	603	549	472	403	324	249	197
ICR2	605	579	533	494	457	428	359	308	244	196	162
ICR3	327	307	281	257	239	217	181	155	120	102	75
ICR4	219	213	209	195	187	171	158	143	114	92	79

Numbers at risk



Published OnlineFirst April 28, 2016; DOI: 10.1158/2326-6066.CIR-15-0149

Research Article

Cancer Immunology Research

Immunogenic Subtypes of Breast Cancer Delineated by Gene Classifiers of Immune Responsiveness

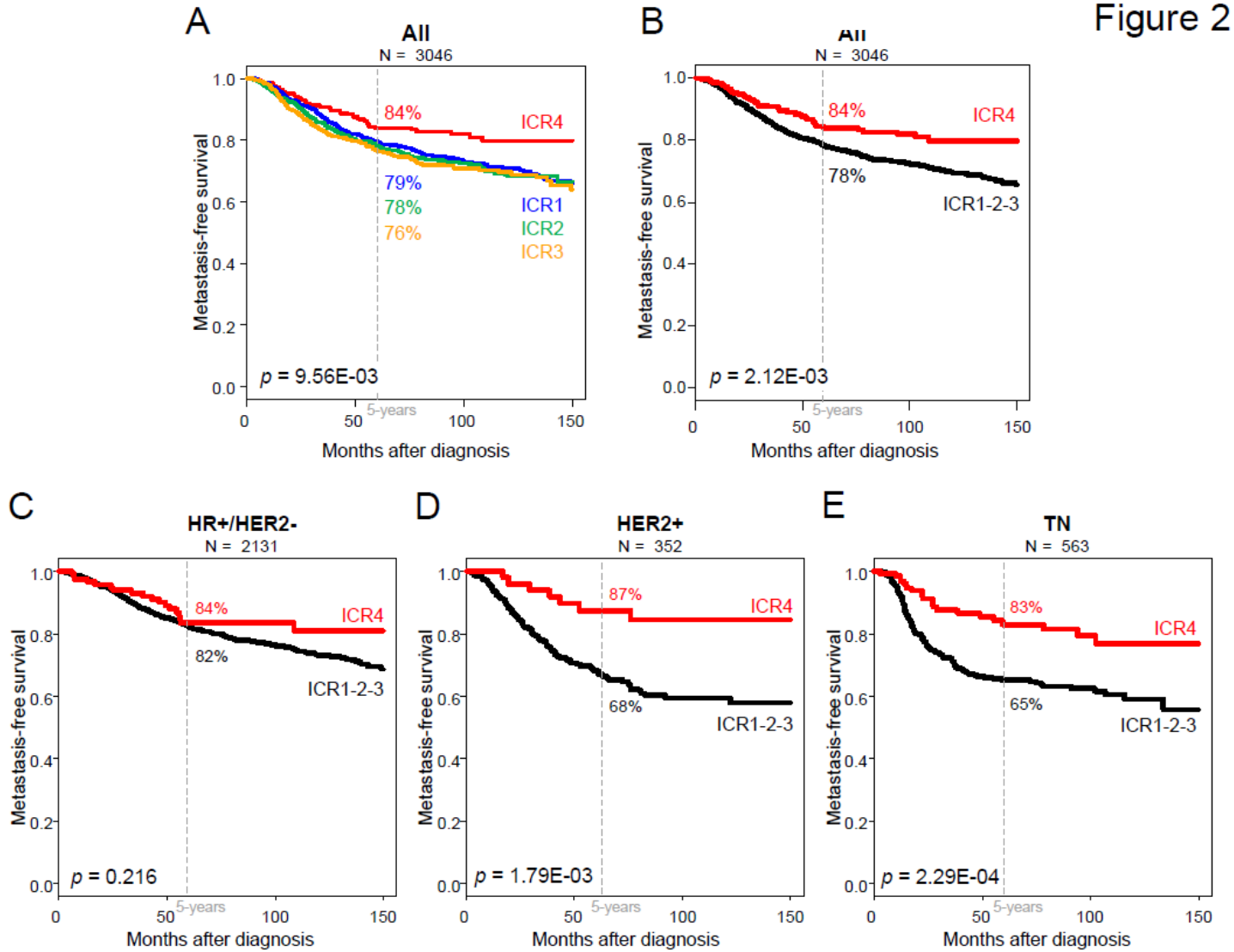
Lance D. Miller[1,2], Jeff A. Chou[3], Michael A. Black[4], Cristin Print[5], Julia Chifman[1], Angela Alistar[2,6], Thomas Putti[7], Xiaobo Zhou[8], Davide Bedognetti[9], Wouter Hendrickx[9], Ashok Pullikuth[1], Jonathan Rennhack[10], Eran R. Andrechek[10], Sandra Demaria[11], Ena Wang[9], and Francesco M. Marincola[12]

Br J Cancer. 2018 Nov;119(11):1383-1391. doi: 10.1038/s41416-018-0309-1. Epub 2018 Oct 24.

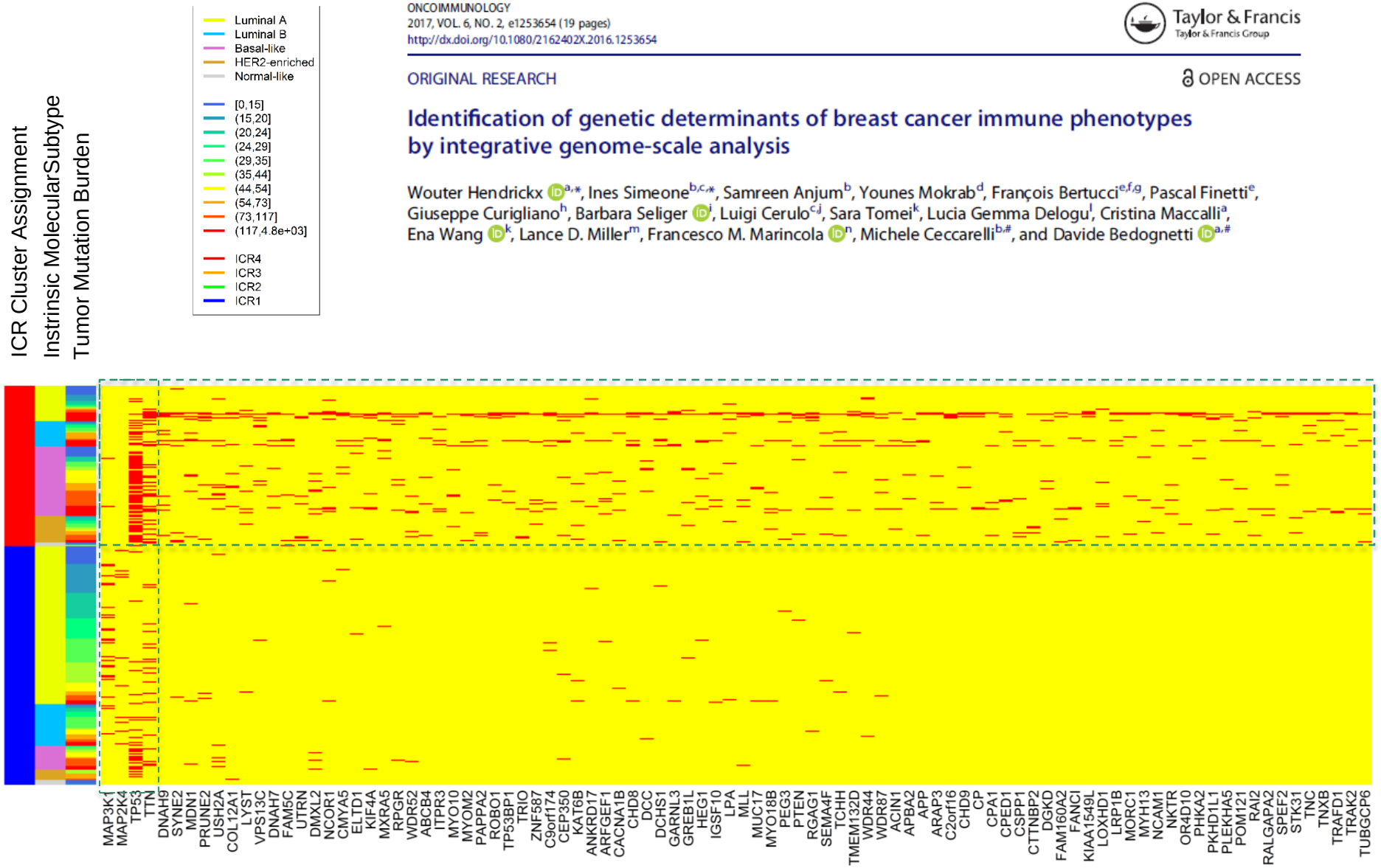
The immunologic constant of rejection classification refines the prognostic value of conventional prognostic signatures in breast cancer.

Bertucci F[1,2,3], Finetti P[4], Simeone I[5], Hendrickx W[6], Wang E[6], Marincola FM[6,7], Viens P, Mamessier E, Ceccarelli M[8,9], Birnbaum D, Bedognetti D.

Figure 2



Driver genes (Chisqr < 0.05)



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ORIGINAL RESEARCH

OPEN ACCESS

Identification of genetic determinants of breast cancer immune phenotypes by integrative genome-scale analysis

Wouter Hendrickx[a,*], Ines Simeone[b,c,*], Samreen Anjum[b], Younes Mokrab[d], François Bertucci[e,f,g], Pascal Finetti[e], Giuseppe Curigliano[h], Barbara Seliger[i], Luigi Cerulo[c,j], Sara Tomei[k], Lucia Gemma Delogu[l], Cristina Maccalli[a], Ena Wang[k], Lance D. Miller[m], Francesco M. Marincola[n], Michele Ceccarelli[b,#], and Davide Bedognetti[a,#]

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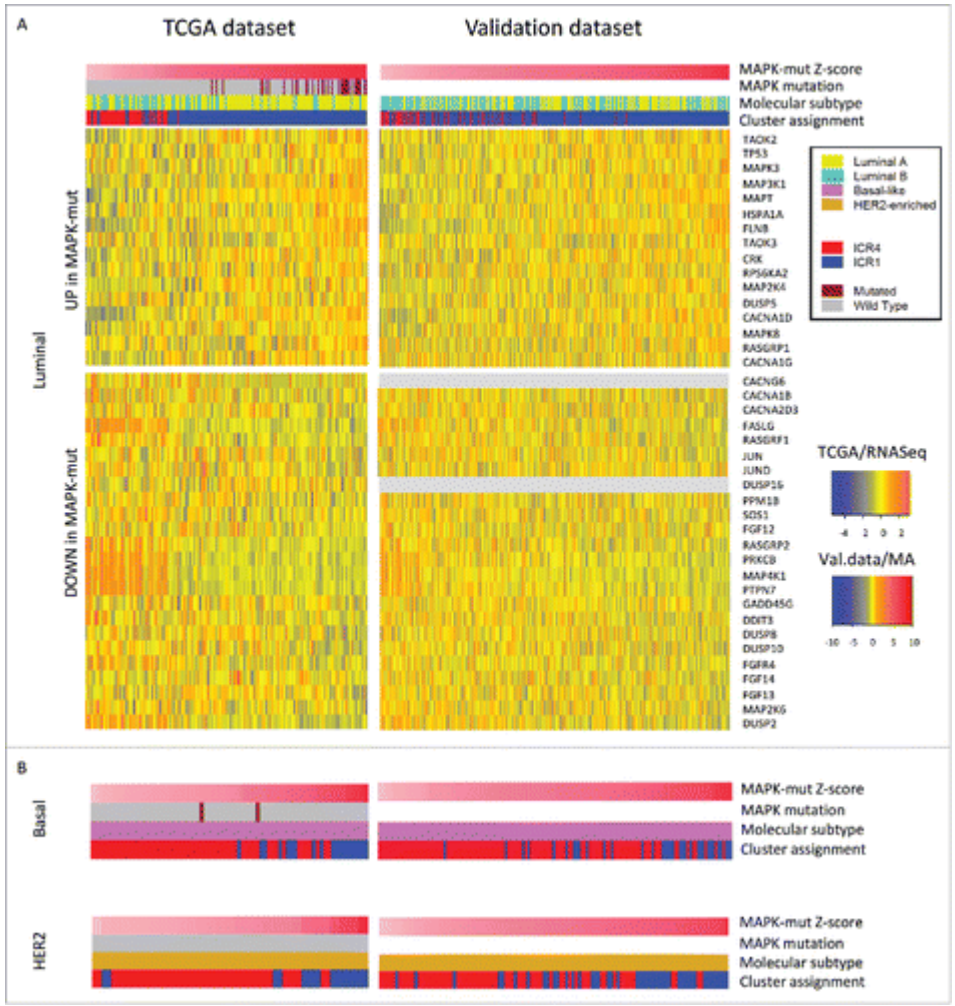
ORIGINAL RESEARCH

OPEN ACCESS

Identification of genetic determinants of breast cancer immune phenotypes by integrative genome-scale analysis

Wouter Hendrickx[a,*], Ines Simeone[b,c,*], Samreen Anjum[b], Younes Mokrab[d], François Bertucci[e,f,g], Pascal Finetti[e], Giuseppe Curigliano[h], Barbara Seliger[i], Luigi Cerulo[c,j], Sara Tomei[k], Lucia Gemma Delogu[l], Cristina Maccalli[a], Ena Wang[k], Lance D. Miller[m], Francesco M. Marincola[n], Michele Ceccarelli[b,#], and Davide Bedognetti[a,#]

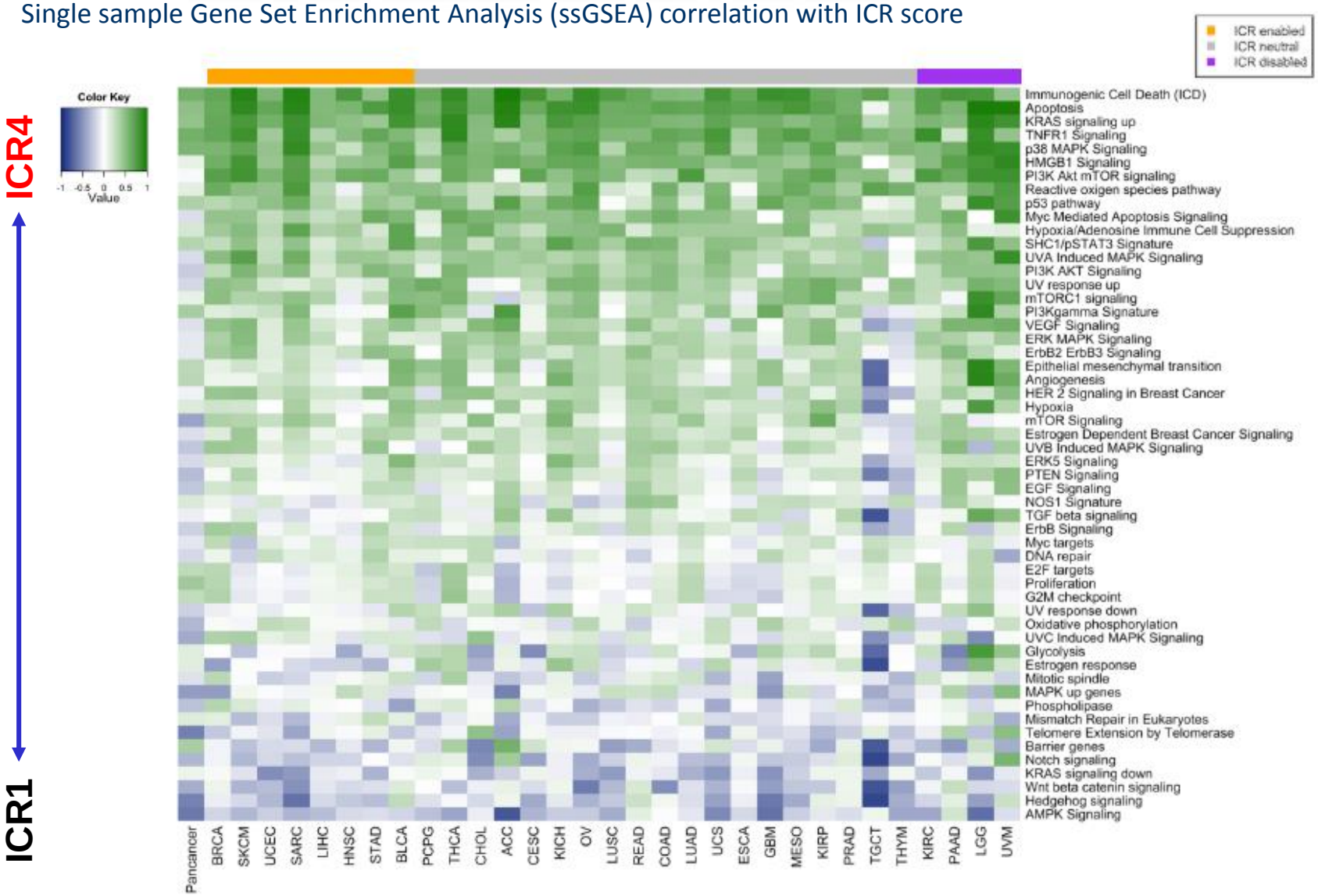


The MAPK-mutation score can segregate different immune phenotypes of breast cancer within intrinsic molecular subtypes. (A) Left panel: MAPK-pathway genes differentially expressed between *MAP3K1* or *MAP2K4* mutated (MAPK-mut) and wild-type TCGA Luminal samples are used to segregate ICR1–ICR4 TCGA Luminal samples (*N* = 206). Right panel: MAPK-mut transcripts defined in the TCGA dataset are used to segregate ICR1–ICR4 Luminal samples of the validation dataset (*N* = 428). (B) The same transcripts are used to segregate ICR1–ICR4 Basal-like and HER2-enriched samples in the TCGA cohort (*N* = 74 and *N* = 29, respectively) and in the validation dataset (*N* = 140 and *N* = 109, respectively). Samples are ordered by MAPK-mut score, which is the average ranking of the samples in upregulated and downregulated *Z*-scores (see *Materials and Methods* section for detail). The TCGA heatmaps are based on the TCGA samples for which mutational data were available.

Conditional activation of immune-related signatures and prognostic significance: a pan-cancer analysis (in preparation)

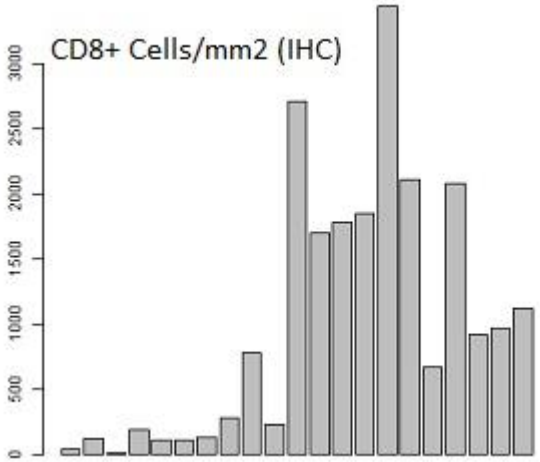
Jessica Roelands, Wouter Hendrickx, Raghvendra Mall, Mohamad Saad, Kyle Halliwill, Gabriele Zoppoli, Giuseppe Curigliano, Darawan Rinchai, Julie Decock, Lucia G Delogu, Lotfi Chouchane, Ena Wang, Peter Kuppen, Pascal Finetti, Francois Bertucci, Lance D Miller, Jerome Galon, Francesco M Marincola, Michele Ceccarelli, Davide Bedognetti

RNA-seq data of samples from a total of **9,282 patients across 31 cancer** from The Tumor Genome Atlas (TCGA).
Single sample Gene Set Enrichment Analysis (ssGSEA) correlation with ICR score

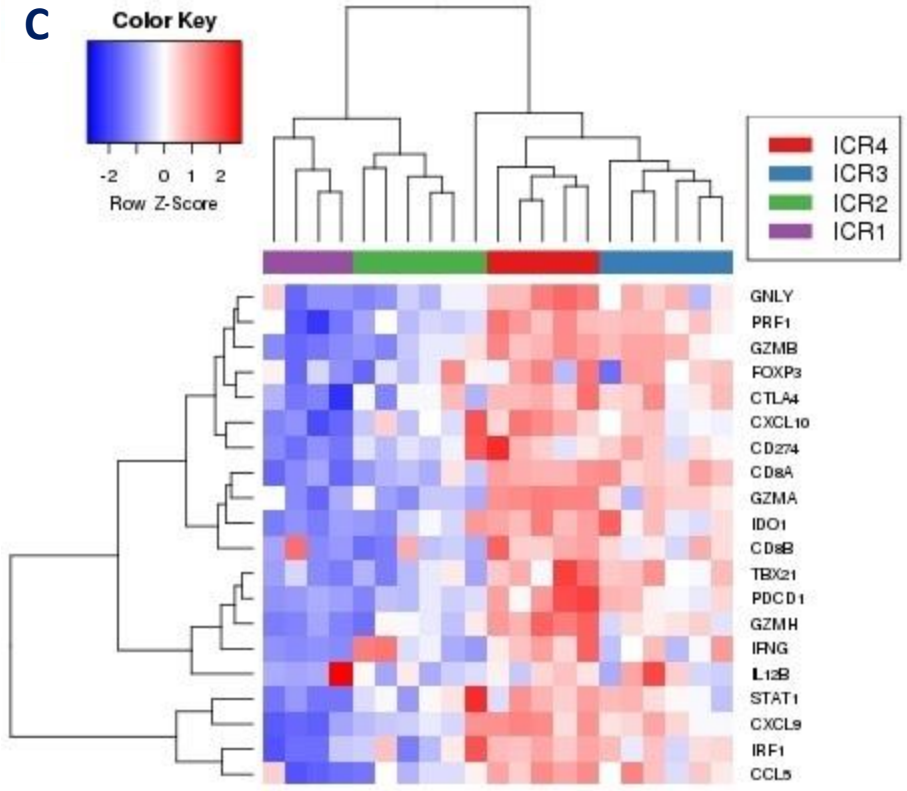


Three fundamental cancer immune landscapes (IHC samples from Dr. Sara Pai, Mas Gen., Harvard U.)

A

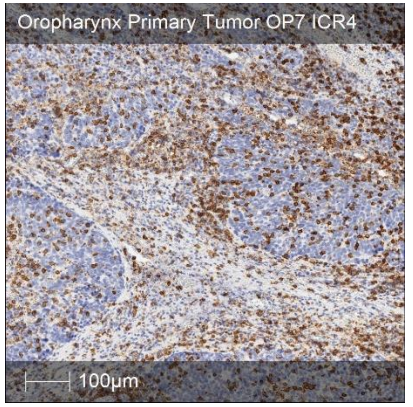


C

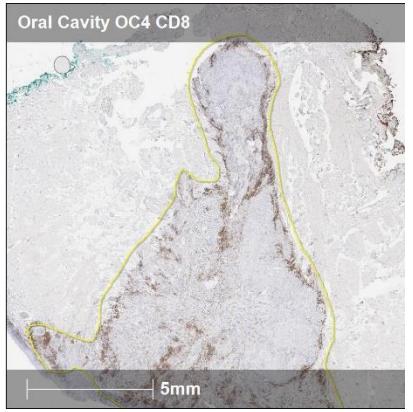


B

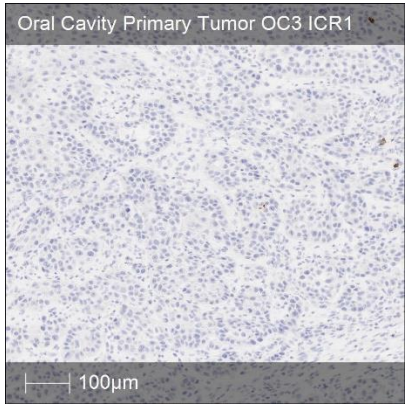
Inflamed



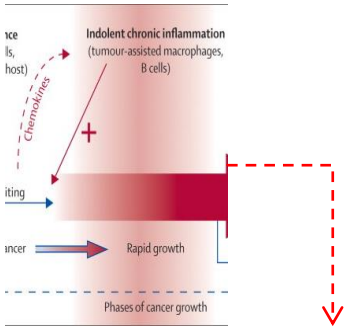
Stromal Excluded

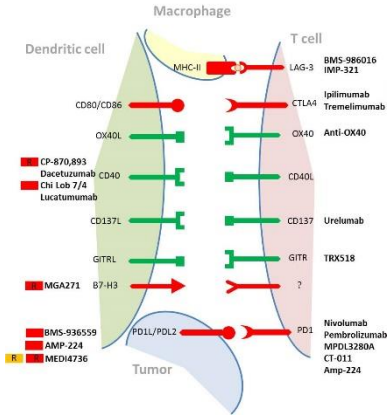


Immune Desert



The immune landscape of cancer





"Sloppy" Tumors

Immune Active

- Growth Factors
- Pro-inflammatory Factors (CytDNA*/STING/pTNK1/IRF3/IFN)
- Chemokines (i.e. Batf3+IRF8/CD103,CD141 DCs)

Immunogenicity: metagenome
Adjuvancy: Immunogenic cell death

Excluded Tumors

Immune Exclusion:
Interference Hypothesis

↑ β-catenin/↓batf3 DCs ⊣ CCL4/IFN-β

- Growth Factors

Pro-inflammatory Factors

"Clean/Silent" Tumors

No Inflammation
Immune Ignorance
Epigenetic Silencing

Distinct hypotheses/mechanisms:

Immune stimulatory
SCFN11
RxTx (suppressed by Trex1)
Chemotx and Necroptosis
HMGB1/TLR4/MyD88
CALR/LRP1
Extracellular ATP ⟷ P2RY2/P2RX7
Microbiota

Immune suppressive/proliferative
PI3Kγ
TIM-3
MAP Kinase Activation
AIM2, IL1β/IL18-driven Pyroptosis
CD73-driven ATP degradation
IL-23/IL-17 axis/pSTAT3
iNOS
Mesenchymal transition
Efferocytosis (MERTK)
Hypoxia/Adenosine

Epithelial Barriers and Stromal Components:
Dystonin → Immune depletion (x)
Tight Junction
Desmosomal Proteins } Mixed Expression Immune genes (w)
Cancer-associated Fibroblasts, Secretome, Matrix deposition and remodeling

Fibrotic Mechanical Barries
TGF-β driven fibro-genesis

Vascular Fidelity
VEGF ⊣ VCAM, ICAM

Immune Oncology, Immune Responsiveness and the Theory of Everything

Tolga Turan, Deepti Kannan, Maulik Patel, Matt J. Barnes, Sonia G. Tanlimco, RongZe Lu, Kyle Halliwill, Douglas E. Kline, Wouter Hendrickx, Alessandra Cesano, Lisa H. Butterfield, Howard L. Kaufman, Thomas J Hudson, Davide Bedognetti, Francesco Marincola, Josue Samayoa

J Immun Cancer – 2018

Top models to explain immune resistance	
WNT/beta Catenin Hypothesis	Not associated with prognosis
MAPK Hypothesis	Associated with poor survival
Th17 AxiS (Psoriatic Signature/pSTAT3 Activ	Associated with poor survival
Th2 Signatures	No association with survival
PI3Kγ Signature	Associated with poor response to checkpoint inhibitors
NOS1 Signature	Low likelihood to respond to TIL therapy
SGK1 Signature	
Barrier Molecules	
Type 1 (Group W)	Associated with poor survival
Type 2 (Groups x and y)	(Not associated with prognosis)
Type 3 - Endotelin Receptor B)	(association with prognosis controversial)
Mesenchymal Transition	IPRES (Innate α-PD1 immune resistance) signature
TAM receptor tyrosine kinases (TAMs)	
Tolerogenic DCs (TolDCs)	
Hypoxia/Adenosine Immune Cell Suppressic	Signature including CD73 associated with poor prognosis
Stromal cell suppressive mechanisms	
TREX1 (clearance of Cytosolic DNA/indirect inhibitor of STING	
Checkpoint Cluster	

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https://doi.org/10.1186/s40425-018-0355-5

Journal for ImmunoTherapy of Cancer

HYPOTHESIS Open Access

Immune oncology, immune responsiveness and the theory of everything

Tolga Turan[1], Deepti Kannan[1], Maulik Patel[2], J. Matthew Barnes[1], Sonia G. Tanlimco[1], Rongze Lu[1], Kyle Halliwill[1], Sarah Kongpachith[1], Douglas E. Kline[3], Wouter Hendrickx[4], Alessandra Cesano[5], Lisa H. Butterfield[6], Howard L. Kaufman[7], Thomas J. Hudson[1], Davide Bedognetti[4], Francesco Marincola[1] and Josue Samayoa[1*]

Major hypotheses explaining immune resistance	
WNT/beta Catenin Hypothesis	Not associated with prognosis
MAPK Hypothesis	Associated with poor survival
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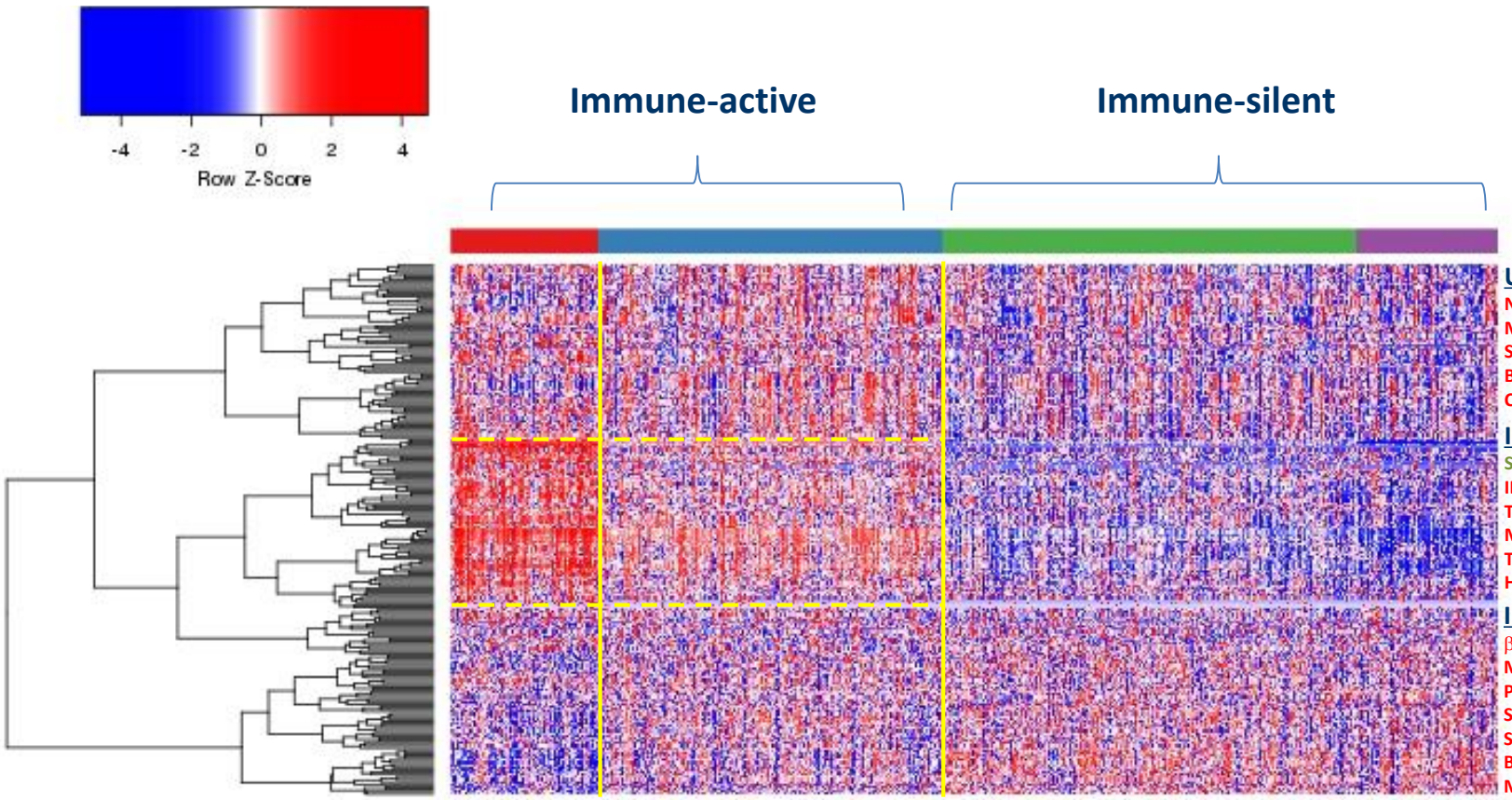

Ubiquitous:
NOS
Mesenchymal Transition
SHC1/STAT3
Barrier Molecules
Checkpoints: B7-H3, B7-H4

Immune enriched:
Sting/Immunogenic Cell Death
IL23/Th17 axis
Th2 Signatures
Most Checkpoints
TAM receptors
Hypoxia

Immune depleted:
β-catenin
MAPK
PI3Kγ
SGK1
SHC1/STAT3
Barrier Molecules
Mesenchymal Transition

Turan et al. Journal for ImmunoTherapy of Cancer (2018) 6:50
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Journal for ImmunoTherapy of Cancer

Immune oncology, immune responsiveness and the theory of everything

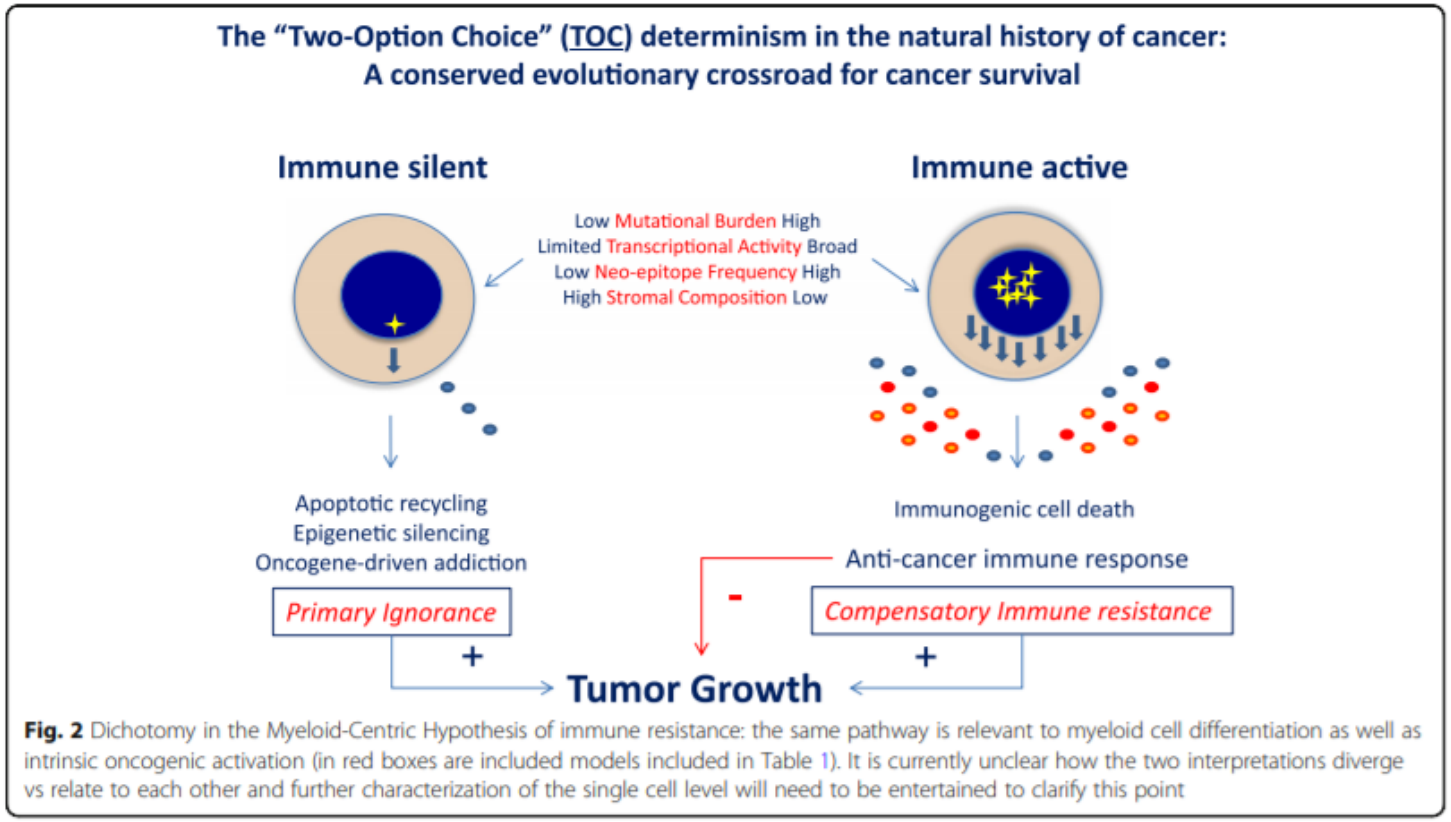


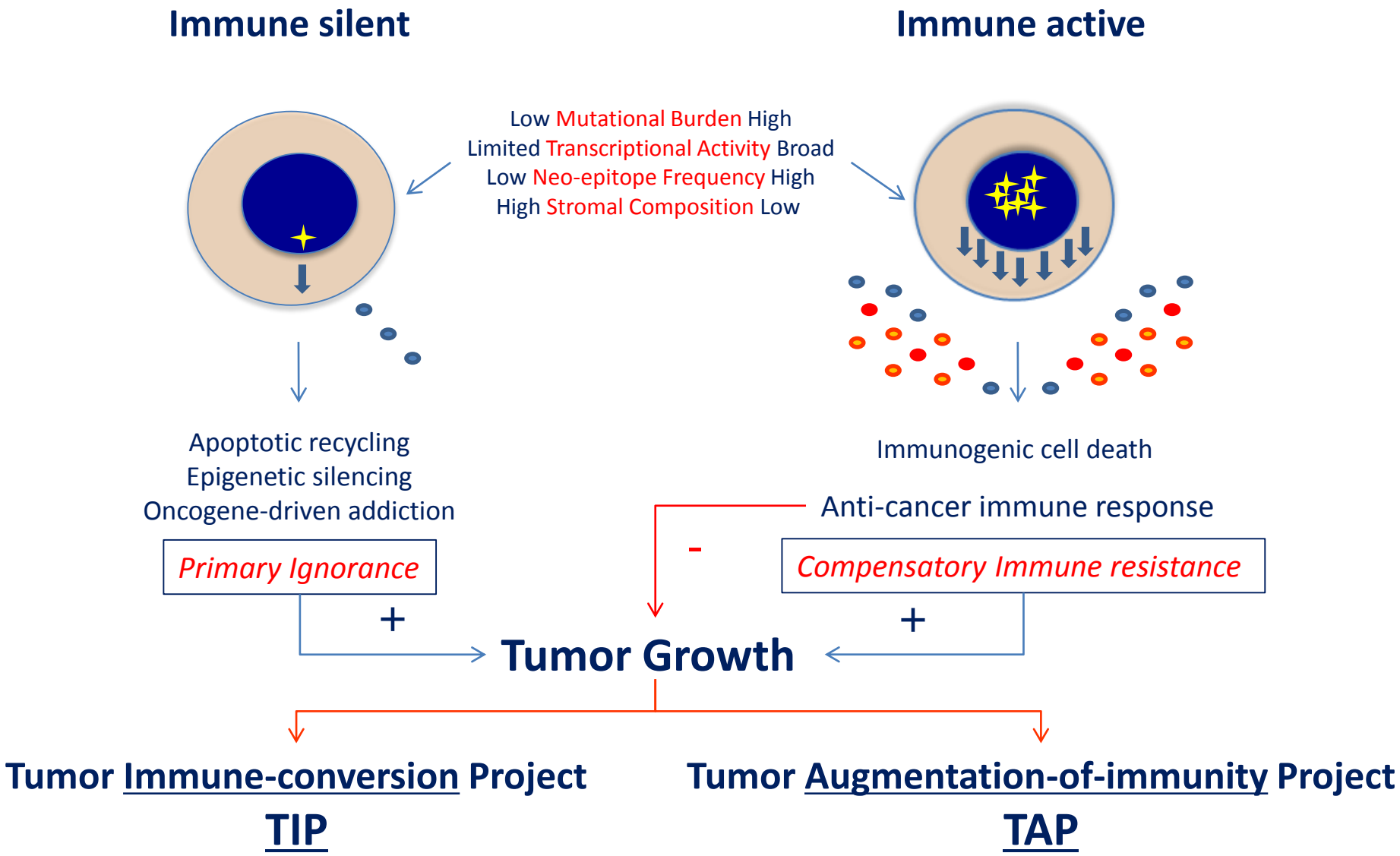
Fig. 2 Dichotomy in the Myeloid-Centric Hypothesis of immune resistance: the same pathway is relevant to myeloid cell differentiation as well as intrinsic oncogenic activation (in red boxes are included models included in Table 1). It is currently unclear how the two interpretations diverge vs relate to each other and further characterization of the single cell level will need to be entertained to clarify this point

Table 1 Principal models related to immune responsiveness

	Immune Landscape[a]	References
WNT/βCatenin	Silent (0.03)	[38, 39]
MAPK Hypothesis	Silent (0.001)	[10]
Immunogenic Cell Death	Active (< 0.001)	[19], [20, 21]
Regulatory T cells	Active (< 0.001)	[24, 25]
IL23-Th17 Axis	Active (< 0.001)	[26, 41–44]
Myeloid Suppressor Cells	Active (< 0.001)	[50]
PI3K-γ Signature	Active (< 0.01)	[52–55, 63]
IDO/NOS Signature	Active (< 0.01)	[51, 81, 82]
SGK1 Signature	Ubiquitous	[56, 57]
Shc1 signature	Ubiquitous	[62]
Barrier Molecules	Ubiquitous	[27, 28]
Mesenchymal Transition	Ubiquitous	[29, 30, 83]
Cancer-Associated Fibroblasts	Ubiquitous	[31–35, 84]
TAM receptor tyrosine kinases	Ubiquitous	[47, 58–60, 85]
Hypoxia/Adenosine suppression	Ubiquitous	[48, 49]
TREX1clearence of Cytosolic DNA	NA	[86, 87]
Checkpoint Cluster	Active (< 0.001)	[22, 23]
oncogene addicted tumors	Silent	[11, 68]
Epigenetic Regulation	Ubiquitous	[12, 88–90]

[a]Distinct models have been assigned to either the Silent or the Active Landscape according to the results of the survey shown in Fig. 1. Ubiquitous refers to models that are not significantly associated with either immune landscape

The "Two-Option Choice" (TOC) determinism in the natural history of cancer: A conserved evolutionary crossroad for cancer survival



A comprehensive view of cancer immune responsiveness: A synopsis from the SITC workshop
J Immunother. Cancer – Submitted

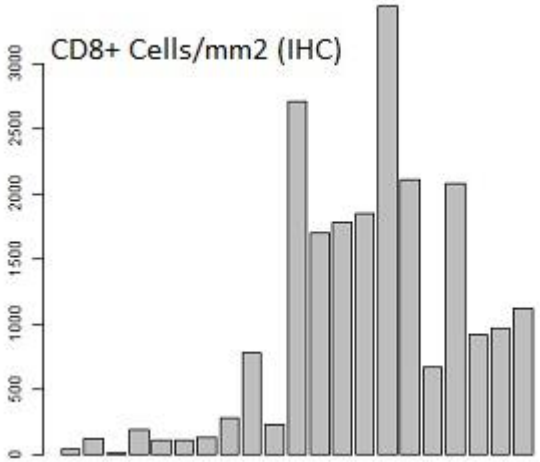
Davide Bedognetti1†, Michele Ceccarelli2, Lorenzo Galluzzi3,4,5, Rongze Lu2†, Karolina Palucka6, Josue Samayoa2†, Stefani Spranger7†, Sarah Warren8†, Kwok-Kin Wong9, Elad Ziv10, Diego Chowell11, Lisa M. Coussens12, Daniel D. De Carvalho13, David G. DeNardo14, Jérôme Galon15, Howard L. Kaufman16, Tomas Kirchhoff17, Michael T. Lotze18, Jason J. Luke19, Andy J. Minn20, Katerina Politi21, Leonard D. Shultz22, Richard Simon23, Vésteinn Thórsson24, Joanne B. Weidhaas25, Maria Libera Ascierto26, Paolo Antonio Ascierto27, James M. Barnes2, Valentin Barsan28, Praveen K. Bommareddy29, Adrian Bot30, Sarah E. Church8, Gennaro Ciliberto31, Andrea De Maria32, Dobrin Draganov33, Winson S. Ho34, Heather M. McGee35, Anne Monette36, Joseph F. Murphy37, Paola Nisticò31, Wungki Park11, Maulik Patel2, Michael Quigley38, Laszlo Radvanyi39, Harry Raftopoulos40, Nils-Petter Rudqvist3, Alexandra Snyder41, Randy F. Sweis19, Sara Valpione42, Lisa H. Butterfield18, Mary L. Disis43, Bernard A. Fox44, Alessandra Cesano8, Francesco M. Marincola45*
on behalf of the Society for Immunotherapy of Cancer (SITC) Cancer Immune Responsiveness Task Force and Working Groups.

1. Primary IR: Lack of response at initial treatment
    a) Primary Ignorance
    b) Compensatory IR
    c) Circumstantial IR (due to factors extrinsic to cancer cell and host biology)
        a) Product fitness in ACT
        b) Environmental and behavioral factors (microbiome, nutritional status, exposure to pathogens)
        c) Co-morbidities and non-cancer-related therapies
        d) Pharmacokinetics, pharmacodynamics and pharmacogenomics determinants of early limiting toxicity

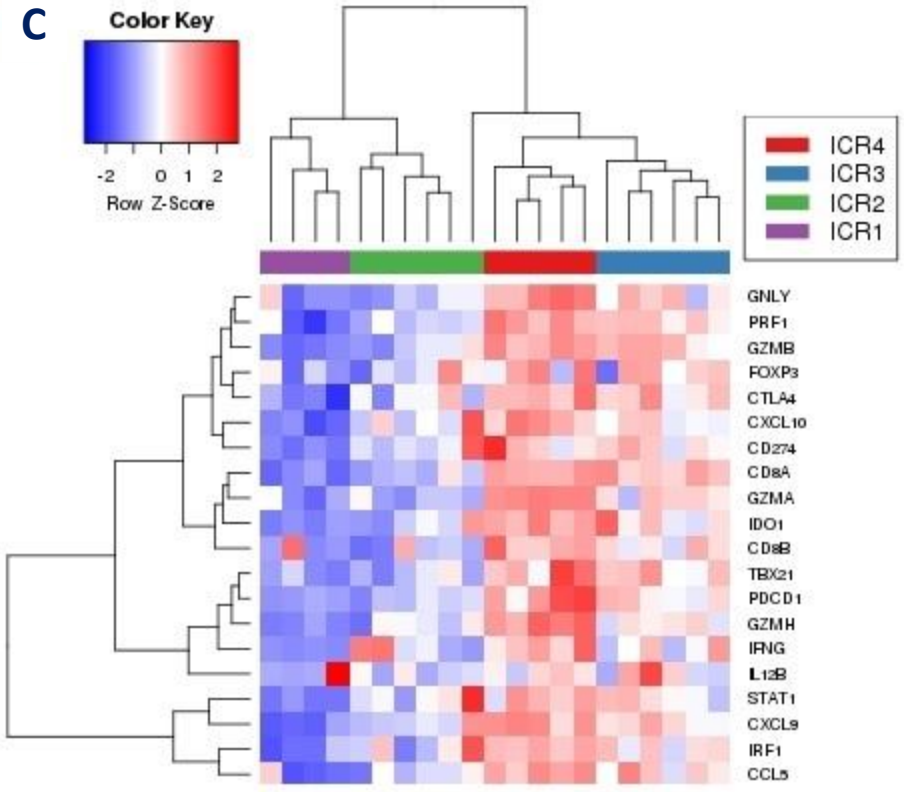
2. Secondary (acquired) IR: Relapse after initial response generally due to escape mechanisms

Three fundamental cancer immune landscapes (IHC samples from Dr. Sara Pai, Mas Gen., Harvard U. Boston

A

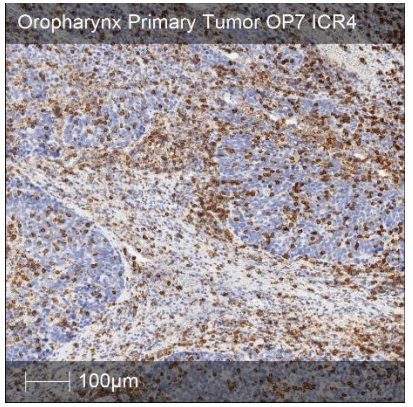


C

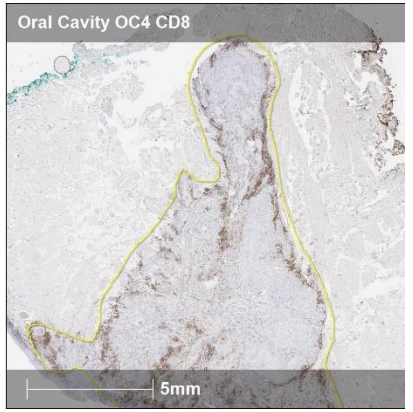


B

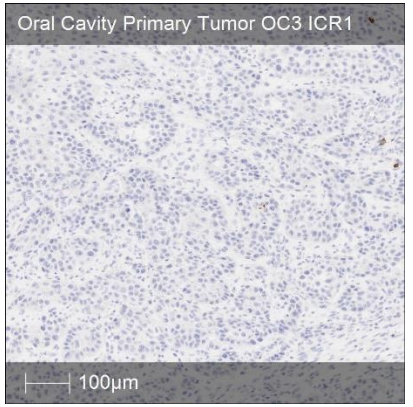
Inflamed



Stromal Excluded

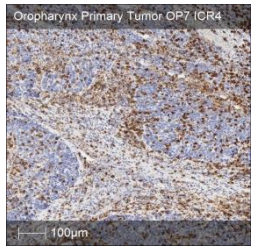


Immune Desert

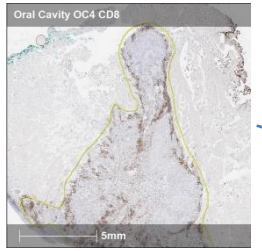


The Paradox of Immune Exclusion –
Cesano A and Marincola FM, in preparation

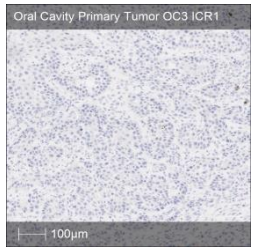
Inflamed

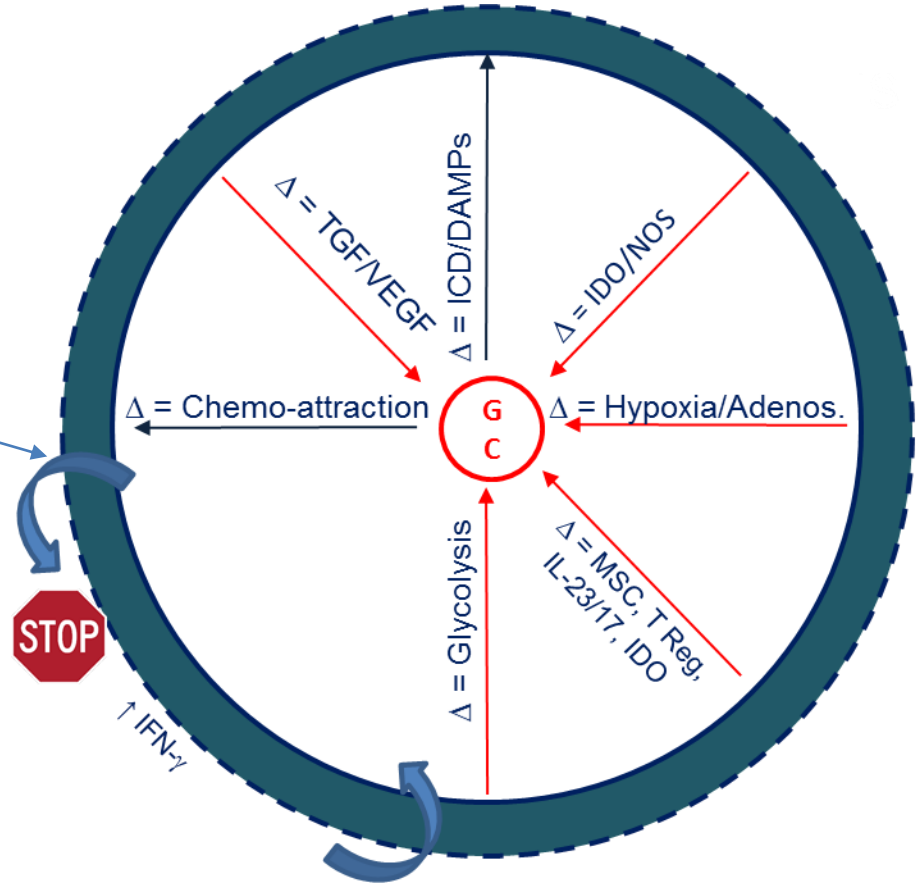


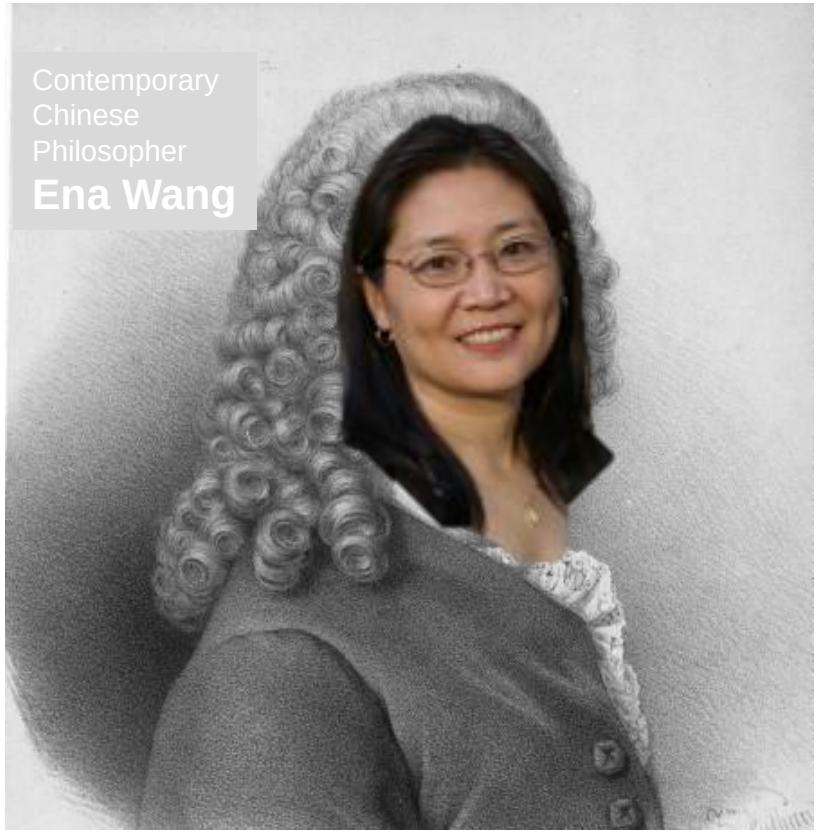
Stromal Excluded



Immune Desert







"Doctors are men who prescribe medicines of which they know *pharmacogenomics*,

…to cure diseases of which they know *functional genomics*,

…in human beings of whom they know *the whole genome*"

Precision/Personalized Medicine!

In conclusion…

- ***I hope you liked my talk:***
- ***“Before criticizing anybody, one should walk for at least a mile in that person shoes…***
- ***…Therefore, if the person does not take the criticism well…you are a mile away and he has no shoes!”***

Transcriptomic profiles conducive to immune-mediated tumor rejection in human breast cancer skin metastases treated with Imiquimod

Mariya Rozenblit[1], Wouter Hendrickx[2], Adriana Heguy[3,4], Luis Chiriboga[3], Cynthia Loomis[3], Karina Ray[3], Farbod Darvishian[3], Mikala Egeblad[5], Sandra Demaria [6], Francesco Marincola[7], Davide Bedognetti*[2], Sylvia Adams*[8]