

***Infectious Disease and Immunogenetics Section (IDIS), DTM, CC
Trans-NIH Center for Human Immunology
NIH, BETHESDA, MD***

Francesco M Marincola

*Answers preexist, and it is the question that needs to be discovered
- Jonas Salk*

**Exploring Immune-Mediated Tumor
Destruction in Humans –
Immune Genetic Considerations**

Francesco M Marincola

**SITC Workshop - Focus on the Target: The
Tumor Microenvironment**

October 24-25, 2012

Bethesda North Marriott Hotel & Conference Center
North Bethesda, MD

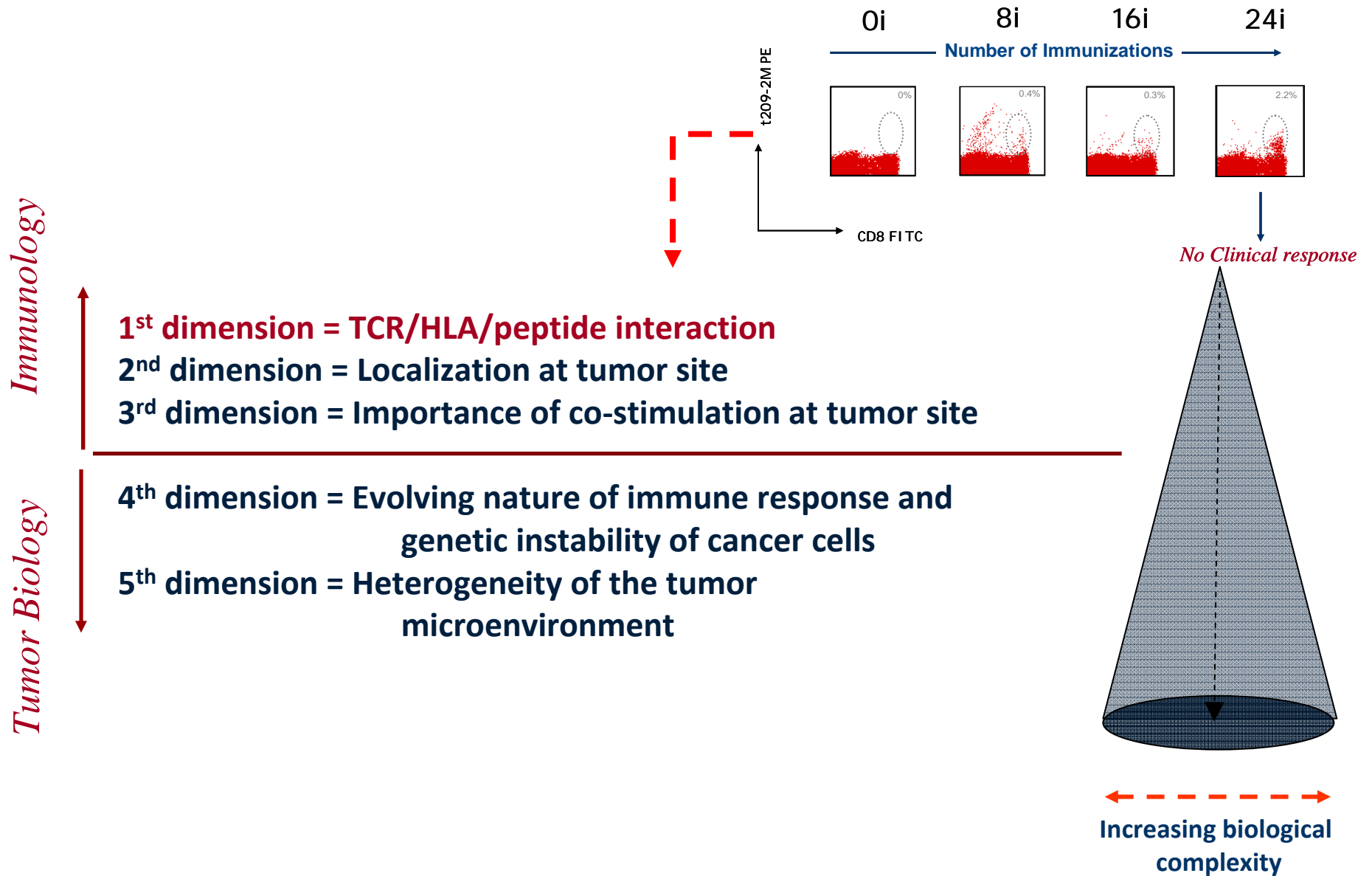


Two questions:

How does (tumor) rejection occur

Why does it occur when it occurs

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



Topics of discussion

How does tumor rejection occur

And the identification of:

The *Immunologic Constant of Rejection*

Why does it occur

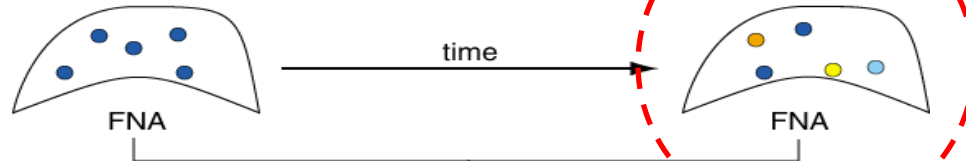
Prospective Molecular Profiling of Melanoma Metastases

Suggests Classifiers of Immune Responsiveness

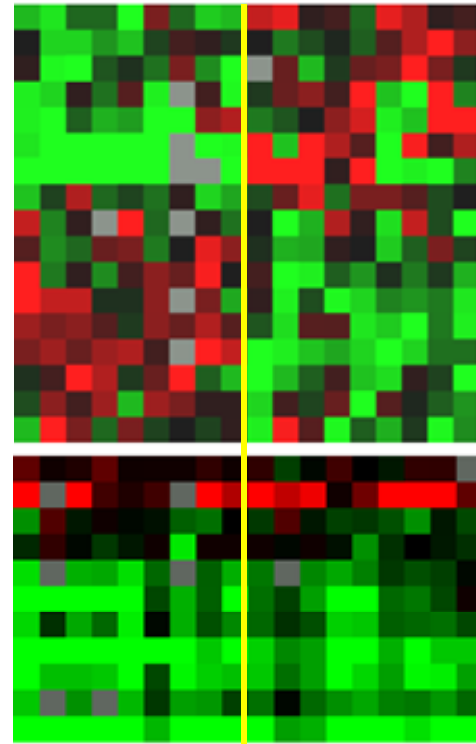
Ena Wang, Lance D. Miller, Galen A. Ohnmacht, Simone Mocellin, Ainhoa Perez-Diez, David Petersen, Yingdong Zhao, Richard Simon, John I. Powell, Esther Asaki, H. Richard Alexander, Paul H. Duray, Meenhard Herlyn, Nicholas P. Restifo, Edison T. Liu, Steven A. Rosenberg, and Francesco M. Marincola¹

Pre-treatment

Following treatment with vaccine + IL-2



P10-a0-cr
P14-a0-cr
P14-b0-cr
P16-a0-cr
P1-a0-cr
P23-a0-cr
P6-a0-cr
P6-b0-cr
P10-a1-cr
P14-a1-cr
P14-b1-cr
P16-a1-cr
P1-a1-cr
P23-a1-cr
P6-a1-cr
P6-b1-cr



IRE1
LOC51128
RBBP8
LMO7
SNTB2
SDF1
COL3A1
CUL5
KIAA0205
HSPC142
ABR
KIAA0264
NUMA1
SERPINA1
SMARCB1
RMD23A
CDR
CD16
CD10
CD14
CD4
CD8 beta
CD8 alpha
CD3 delta
TCR beta locus
CD11A
CD86
CD83

IRF-1

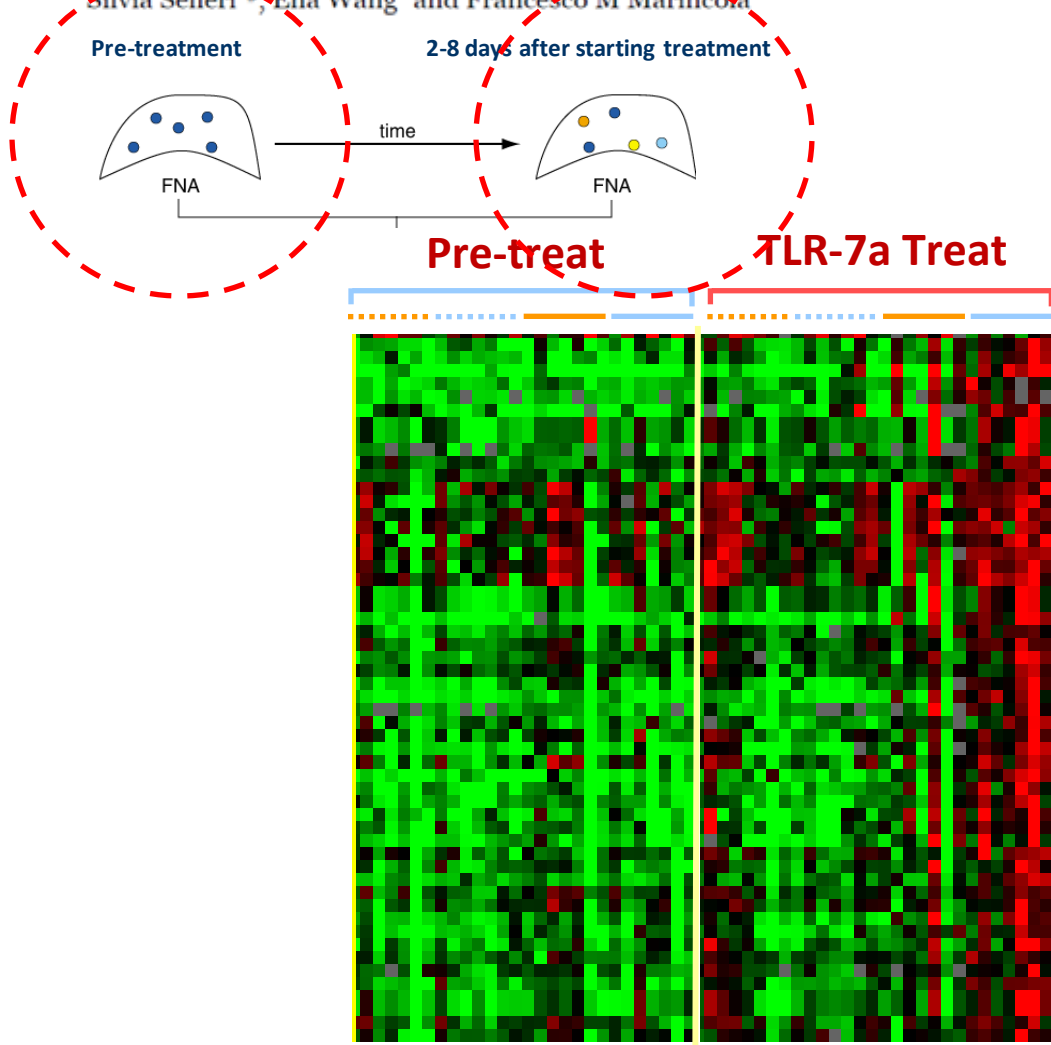
IRF-1

Associated with responsiveness of

- genital warts to Imiquimod
- carcinoid tumors to IFN- α
- CML to IFN- α

Sequential gene profiling of basal cell carcinomas treated with imiquimod in a placebo-controlled study defines the requirements for tissue rejection

Monica C Panelli[†], Mitchell E Stashower[†], Herbert B Slade[†], Kina Smith[†], Christopher Norwood[§], Andrea Abati[¶], Patricia Fetsch[¶], Armando Filie[¶], Shelley-Ann Walters[‡], Calvin Astry[‡], Eleonora Arico[†], Yingdong Zhao[¶], Silvia Selleri^{†*}, Ena Wang[†] and Francesco M Marincola^{†*}



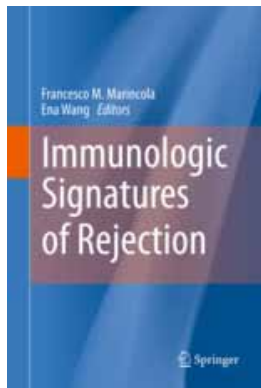
- Placebo X 4 days
- Placebo X 8 days
- Treat X 4 days
- Treat X 8 days

STAT 1/IRF1

Allograft inflammatory factor 1
 IL-15, IL-6
 IL-2/IL15 R β
 IL-15 R α
 IL-2/IL-4/IL-7/IL-9/IL-15 R γ
 HLA Class I, HLA Class II, TAP, LMP

Granzyme A, B, K
 Perforin
 CCL4 (MIP-1b)
 Natural killer-cell transcript 4 (IL-32)
 Natural killer cell gene-5
 Caspase 1, 5, 8

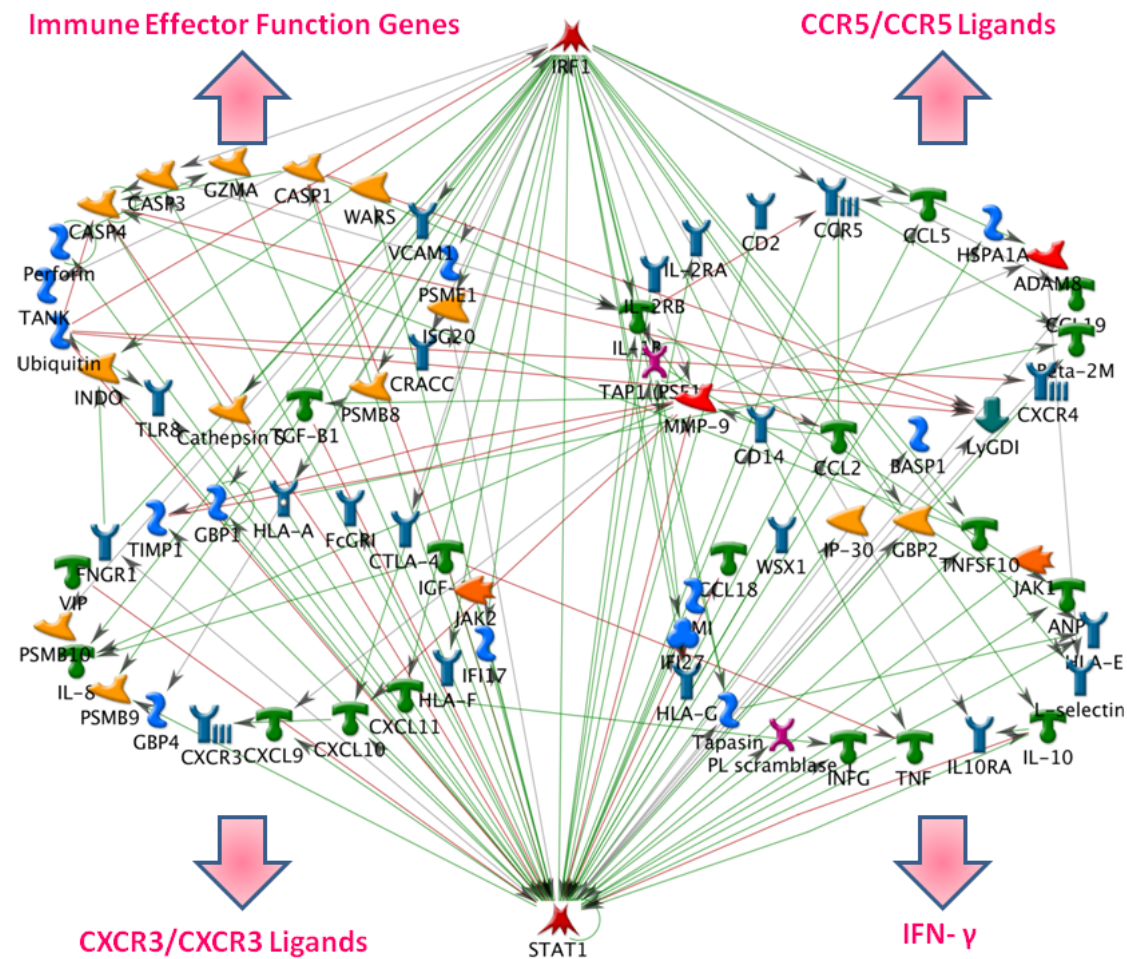
CXCL10/IP-10
 CXCL9/Mig
 CCL5



	Stat-1 IRF-1/IRF5 T-bet ⁺ IFN-g IL-15	GNLY GZM TIA	CXCL-9 CXCL-10 CXCL-11 CXCR3	CCL5 CCR5	References
<u>Cancer prognosis</u>					
Colorectal <i>hu</i> CA	+	+	+	+	(Camus et al. 2009;Galon et al. 2006;Pages et al. 2005)
Lung <i>hu</i> CA	+	+	+	+	(Dieu-Nosjean et al. 2008)
Melanoma <i>hu</i> Xeno	n.t.	n.t.			(Harlin et al. 2009)
Ovarian <i>hu</i> CA Xeno	+	+			(Benencia et al. 2005)
<u>Tumor rejection</u>					
Mastocytoma <i>mus</i>	+	+	+	+	(Shanker et al. 2007)
Breast <i>hu</i> CA Xeno	+	+		+	(Worschech et al. 2009)
BCC <i>hu</i> CA				+	(Panelli et al. 2006)
<u>Allo-transplant rejection</u>					
Kidney <i>hu</i>	n.t.	n.t.	+	n.t.	(Reeve et al. 2009;Saint-Mezard et al. 2009;Sarwal et al. 2003)
Heart <i>hu</i>	n.t.	+	+	+	(Karason et al. 2006)
Islet <i>pig</i>	+	n.t.	+	+	(Hardstedt et al. 2005)
Liver <i>rat</i>					(Hama et al. 2009)
GVHD	+	+	+	n.t.	(Imanguli et al. 2009)
<u>HCV viral clearance</u>					
<i>Chimp</i>	+	+	+		(Bigger, Brasky, & Lanford 2001;Nanda et al. 2008)
<i>Hu</i>	+		+		(Asselah et al. 2008;Feld et al. 2007;He et al. 2006)
<u>Acute cardiovascular events</u>					
(<i>hu</i>)	+		+		(Okamoto et al. 2008;Zhao et al. 2002)
<u>COPD</u>					
Interface Dermatitis	+		+		(Costa et al. 2008)
Villitis	+		+	+	(Wenzel & Tuting 2008)
					(Kim et al. 2009)

Gene expression profiling in acute allograft rejection: challenging the immunologic constant of rejection hypothesis

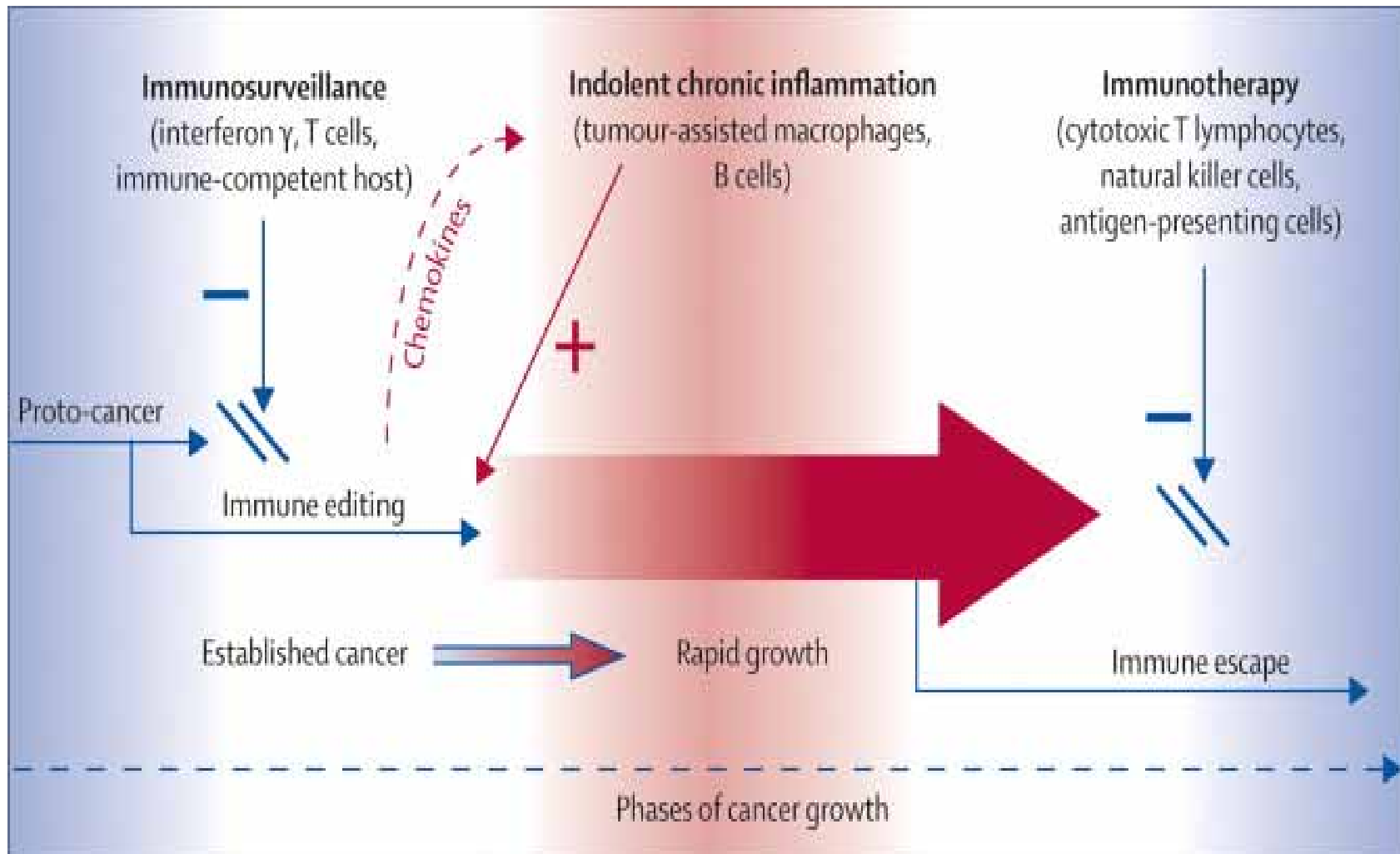
Tara L Spivey^{1,2,3}, Lorenzo Uccellini^{1,4}, Maria Libera Ascierio^{1,5,6}, Gabriele Zoppoli^{5,7}, Valeria De Giorgi¹, Lucia Gemma Delogu⁸, Alyson M Engle¹, Jaime M Thomas¹, Ena Wang¹, Francesco M Marincola^{1*} and Davide Bedognetti^{1,5,9*}



Tumour immunity: effector response to tumour and role of the microenvironment

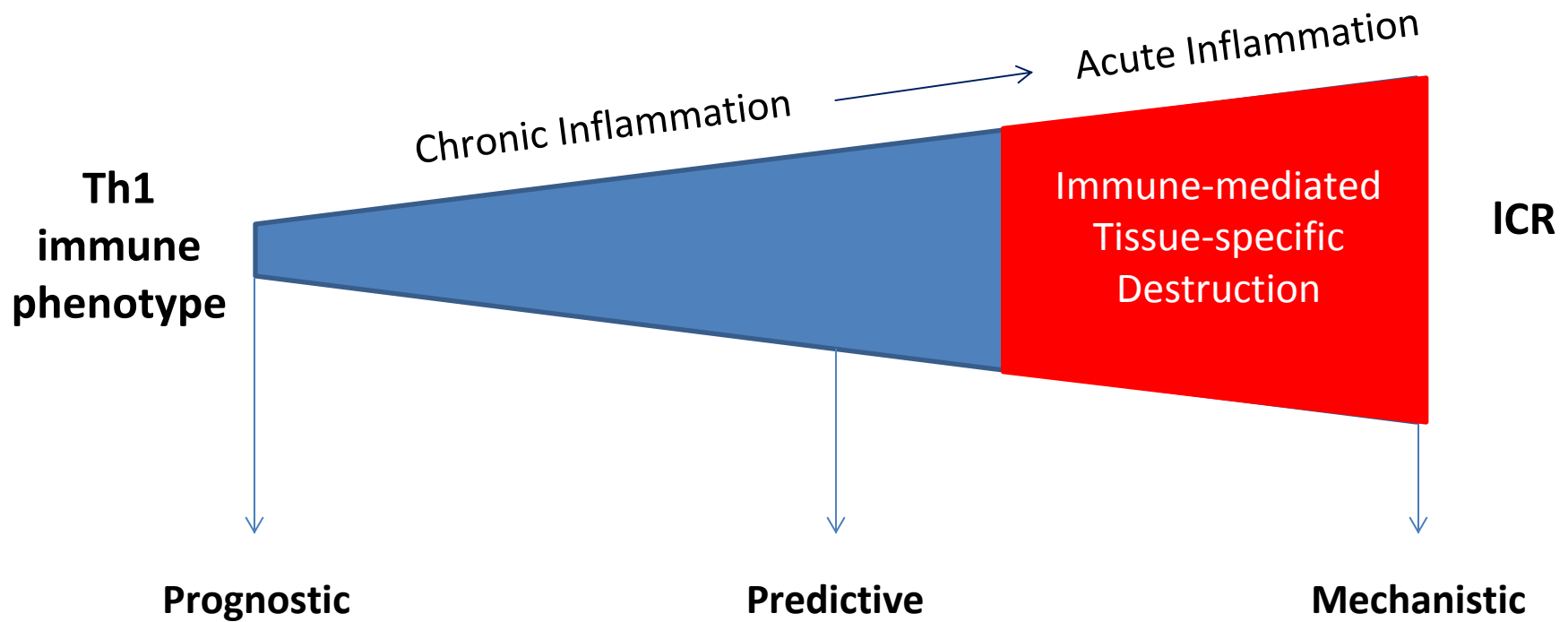
Alberto Mantovani, Pedro Romero, A Karolina Palucka, Francesco M Marincola

Lancet 2008; 371: 771–83



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

J Galon and FM Marincola – in preparation



How does tumor rejection occur

Why does rejection occur

Wang E et al. Ann NY Acad Sci – in press

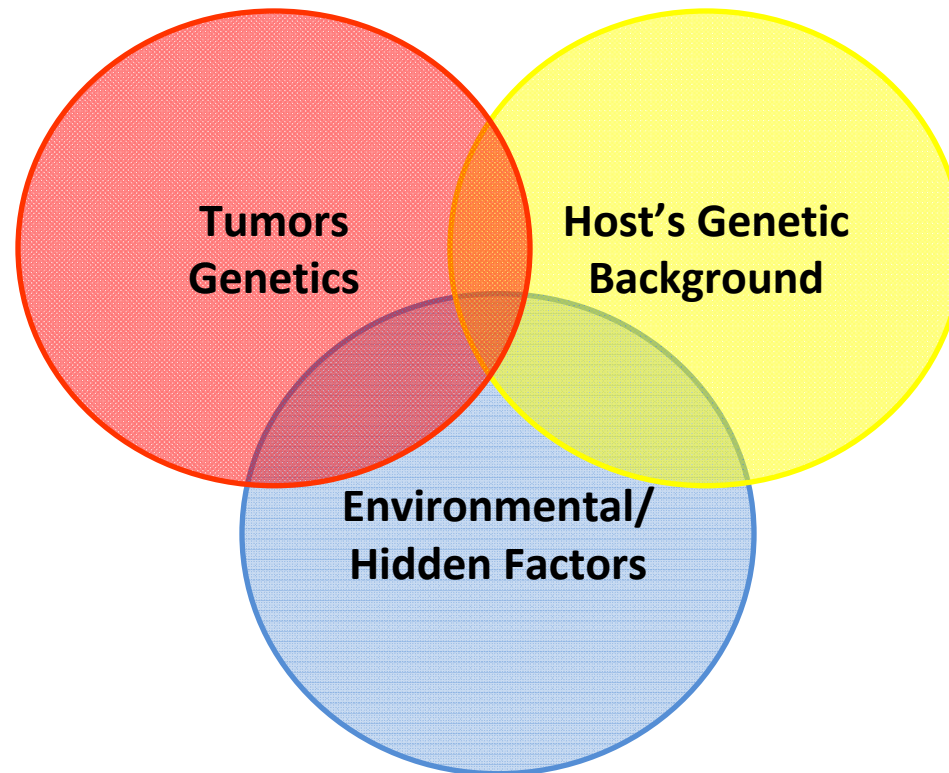
Factors limiting the study of tumor immune responsiveness

Multifactorial Phenomenon

Limited power of clinical trials

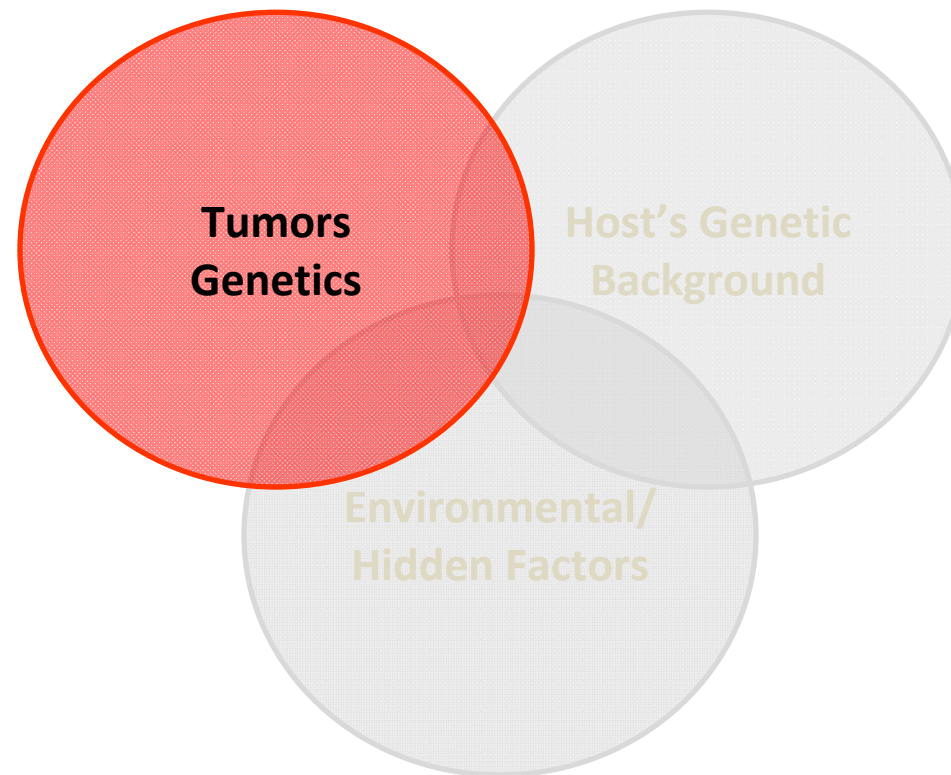
Univariate class comparison

Classes of factors influencing immune responsiveness



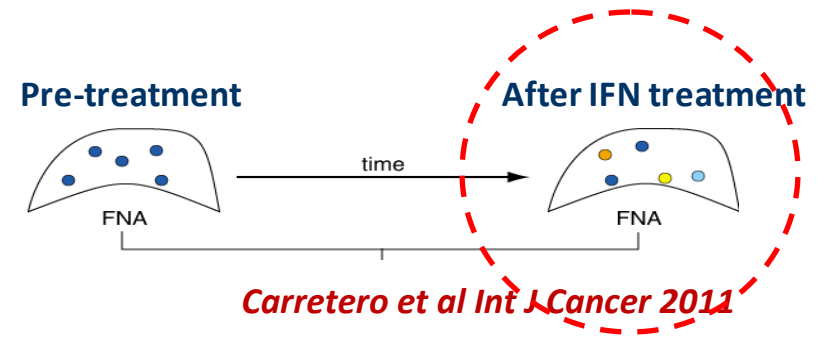
A genetic inference on cancer immune responsiveness
Ena Wang, Lorenzo Uccellini and Francesco M. Marincola

Classes of factors influencing immune responsiveness

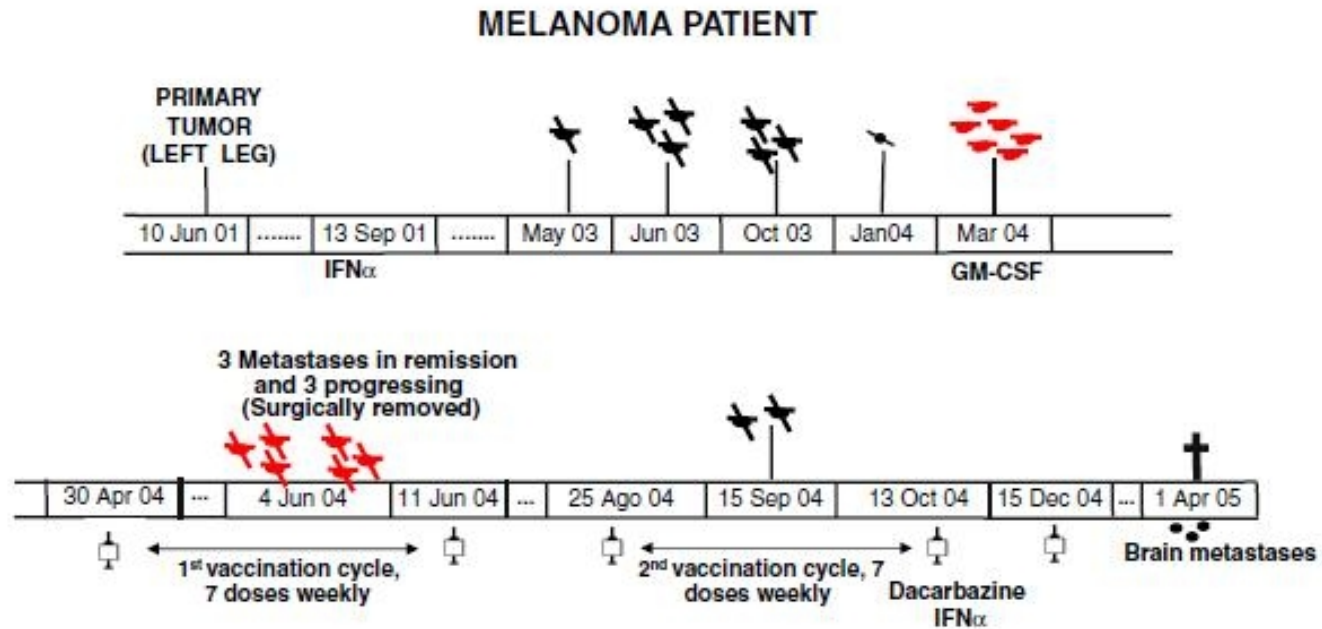


Regression of melanoma metastases after immunotherapy is associated with activation of antigen presentation and interferon-mediated rejection genes

Rafael Carretero^{1,2}, Ena Wang¹, Ana L. Rodriguez², Jennifer Reinboth^{3,4,5}, Maria L. Asciero¹, Alyson M. Engle³, Hui Liu³, Francisco M. Camacho⁶, Francesco M. Marincola¹, Federico Garrido^{1,2} and Teresa Cabrera^{1,2}



The phenomenon of the mixed response

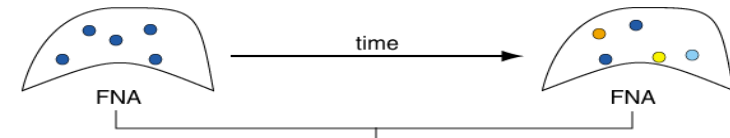


Regression of melanoma metastases after immunotherapy is associated with activation of antigen presentation and interferon-mediated rejection genes

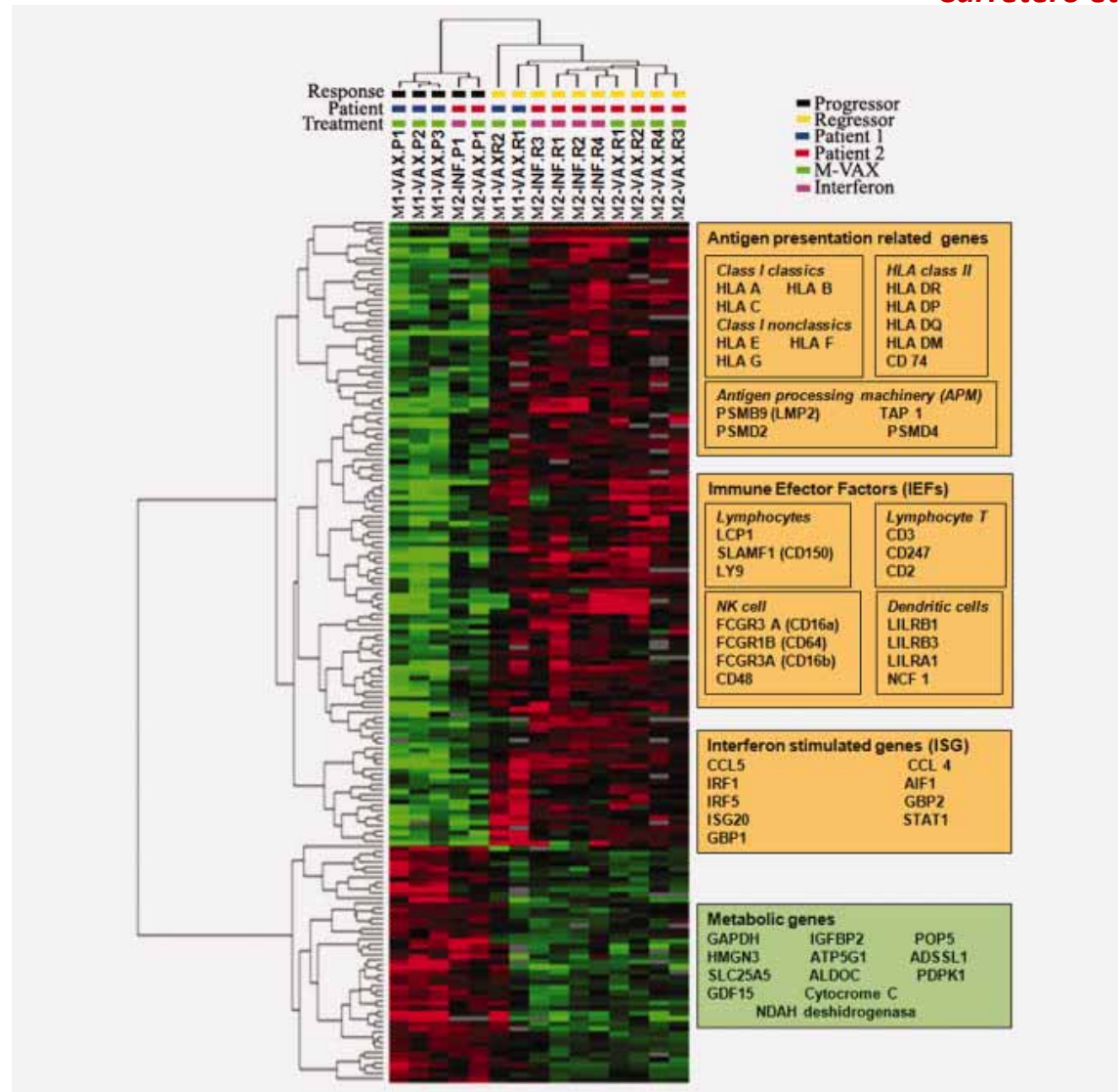
Rafael Carretero^{1,3}, Ena Wang¹, Ana L. Rodriguez², Jennifer Reinboth^{3,4,5}, Maria L. Ascierto¹, Alyson M. Engle³, Hui Liu³, Francisco M. Camacho⁶, Francesco M. Marincola³, Federico Garrido^{1,3} and Teresa Cabrera^{1,2}

Pre-treatment

After IFN treatment

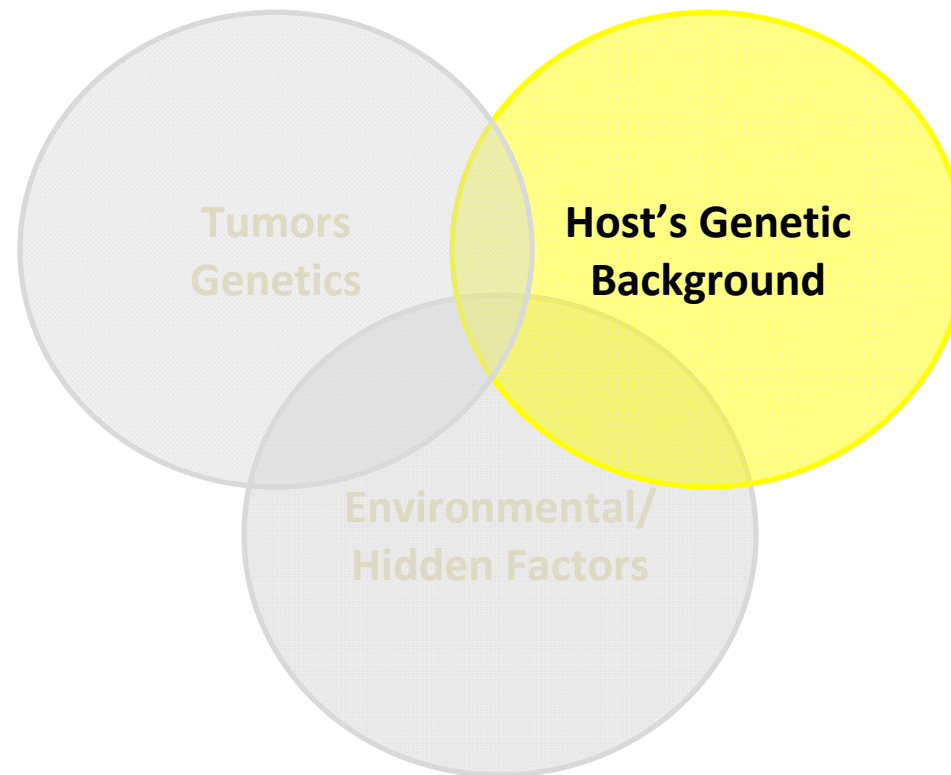


Carretero et al Int J Cancer 2011



A genetic inference on cancer immune responsiveness
Ena Wang, Lorenzo Uccellini and Francesco M. Marincola

Classes of factors influencing immune responsiveness



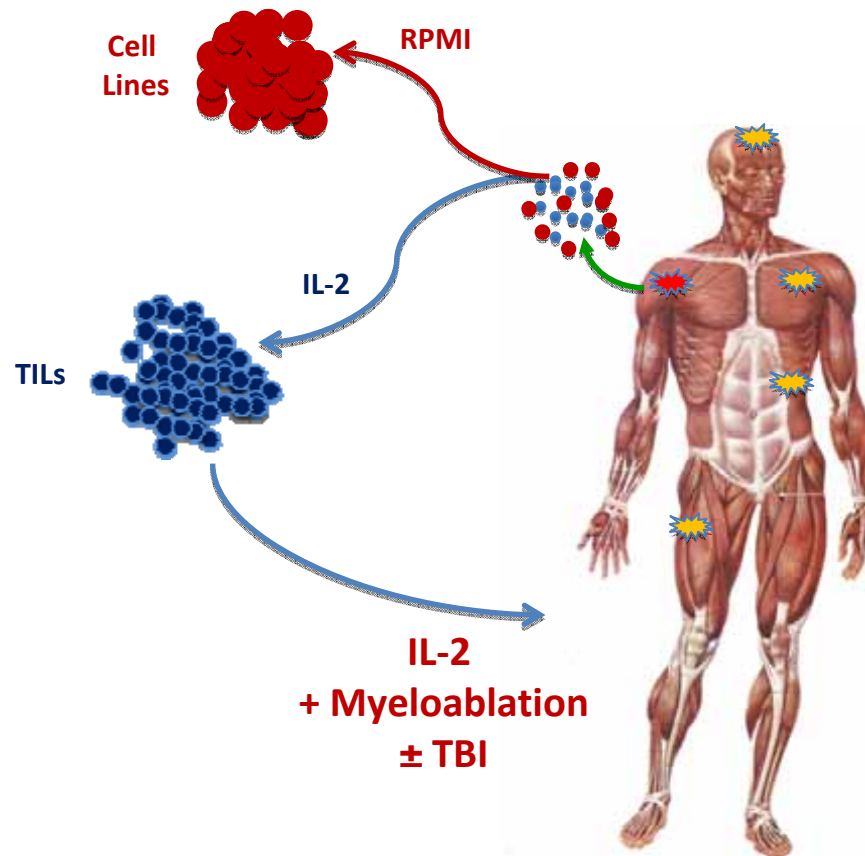
Project overview

SAMPLE STUDIED

142 TILs from patients enrolled in five adoptive cell therapy trials

113 parental melanoma metastases

15 melanoma cell lines derived from the 15 melanoma metastases



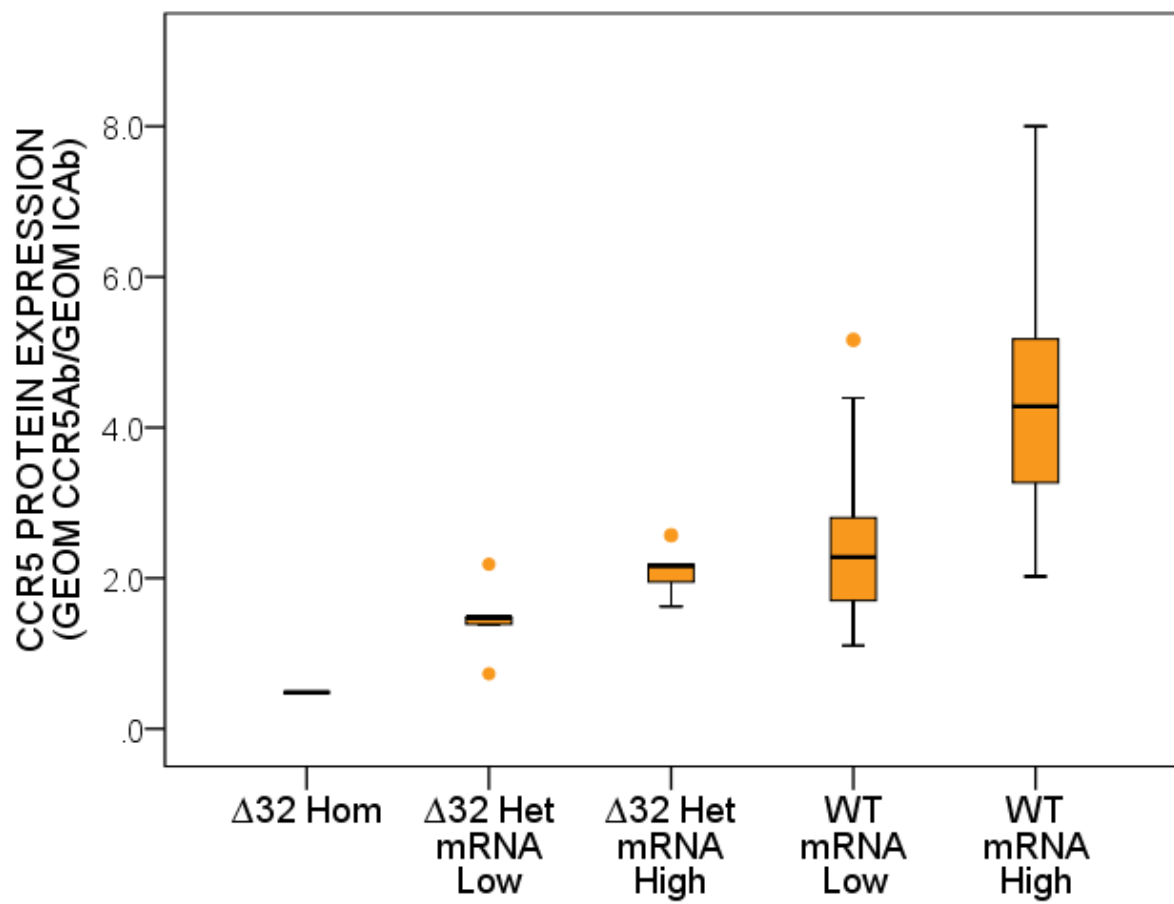


	Stat-1 IRF-1/RF5 T-bet+ IFN-g IL-15	GNLY GZM TIA	CXCL-9 CXCL-10 CXCL-11 CXCR3	CCL5 CCR5	References
Cancer prognosis					
Colorectal <i>hu</i> CA	+	+	+	+	(Camus et al. 2009;Galon et al. 2006;Pages et al. 2005)
Lung <i>hu</i> CA	+	+	+	+	(Dieu-Nosjean et al. 2008)
Melanoma <i>hu</i> Xeno	n.t.	n.t.			(Harlin et al. 2009)
Ovarian <i>hu</i> CA Xeno	+	+			(Benencia et al. 2005)
Tumor rejection					
Mastocytoma <i>mus</i>	+	+	+	+	(Shanker et al. 2007)
Breast <i>hu</i> CA Xeno	+	+		+	(Worschech et al. 2009)
BCC <i>hu</i> CA				+	(Panelli et al. 2006)
Allo-transplant rejection					
Kidney <i>hu</i>	+	+	+	+	(Reeve et al. 2009;Saint-Mezard et al. 2009;Sarwal et al. 2003)
Heart <i>hu</i>	n.t.	n.t.	+	n.t.	(Karason et al. 2006)
Islet <i>pig</i>	n.t.	+	+	+	(Hardstedt et al. 2005)
Liver <i>rat</i>	+	n.t.	+	+	(Hama et al. 2009)
GVHD	+	+	+	n.t.	(Imanguli et al. 2009)
HCV viral clearance					
Chimp	+	+	+		(Bigger, Brasky, & Lanford 2001;Nanda et al. 2008)
Hu	+		+		(Asselah et al. 2008;Feld et al. 2007;He et al. 2006)
Acute cardiovascular events					
(<i>hu</i>)	+		+		(Okamoto et al. 2008;Zhao et al. 2002)
COPD					
Interface Dermatitis	+		+		(Costa et al. 2008)
Villitis	+		+	+	(Wenzel & Tuting 2008)
					(Kim et al. 2009)

CCR5 DNA – LEVEL: $\Delta 32$ mutation

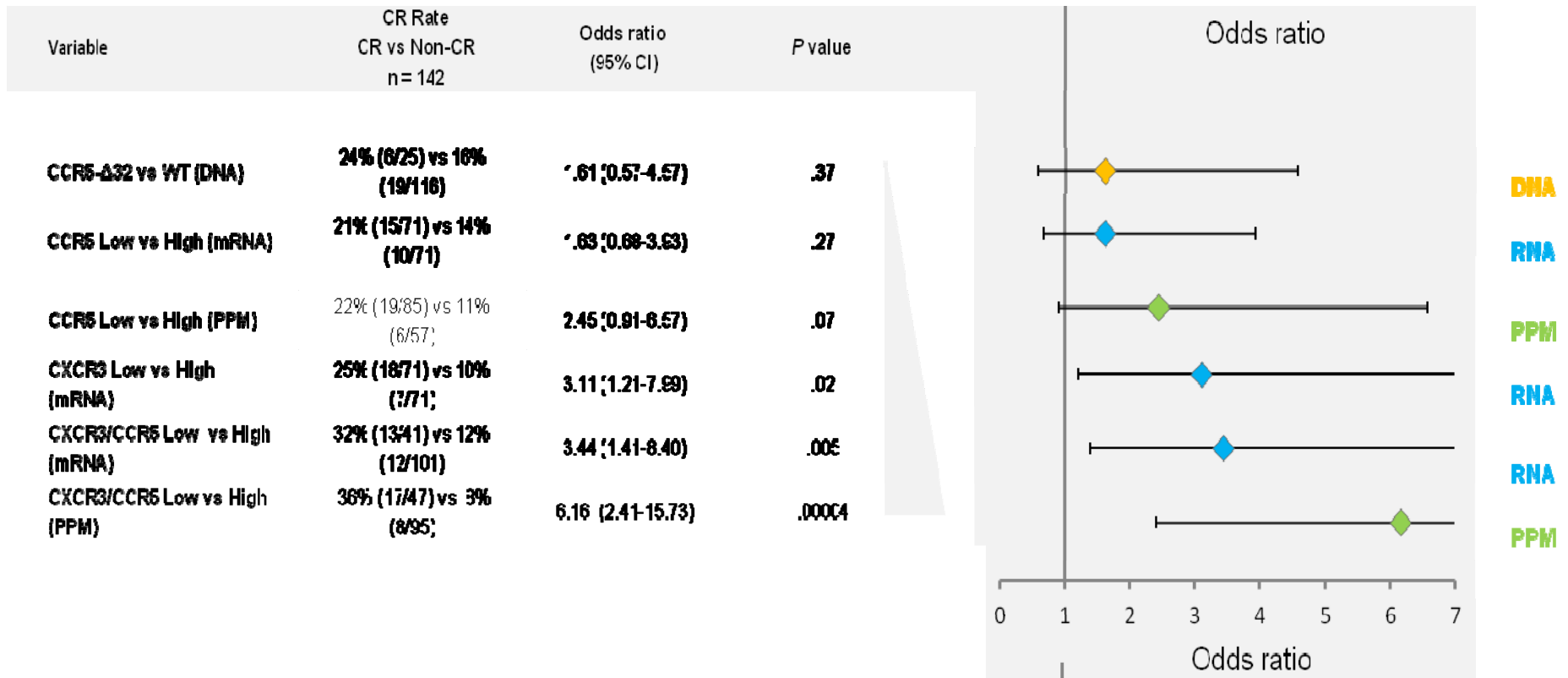
PCR and Sequencing

Allele	Frequency	Expected*
$\Delta 32$ Heterozygous	17% (24/141)	~ 10-15%
$\Delta 32$ Homozygous (N=1)	0.7% (1/141)	~ 1%
Wild type (n=116)	82% (118/141)	~ 85-90%

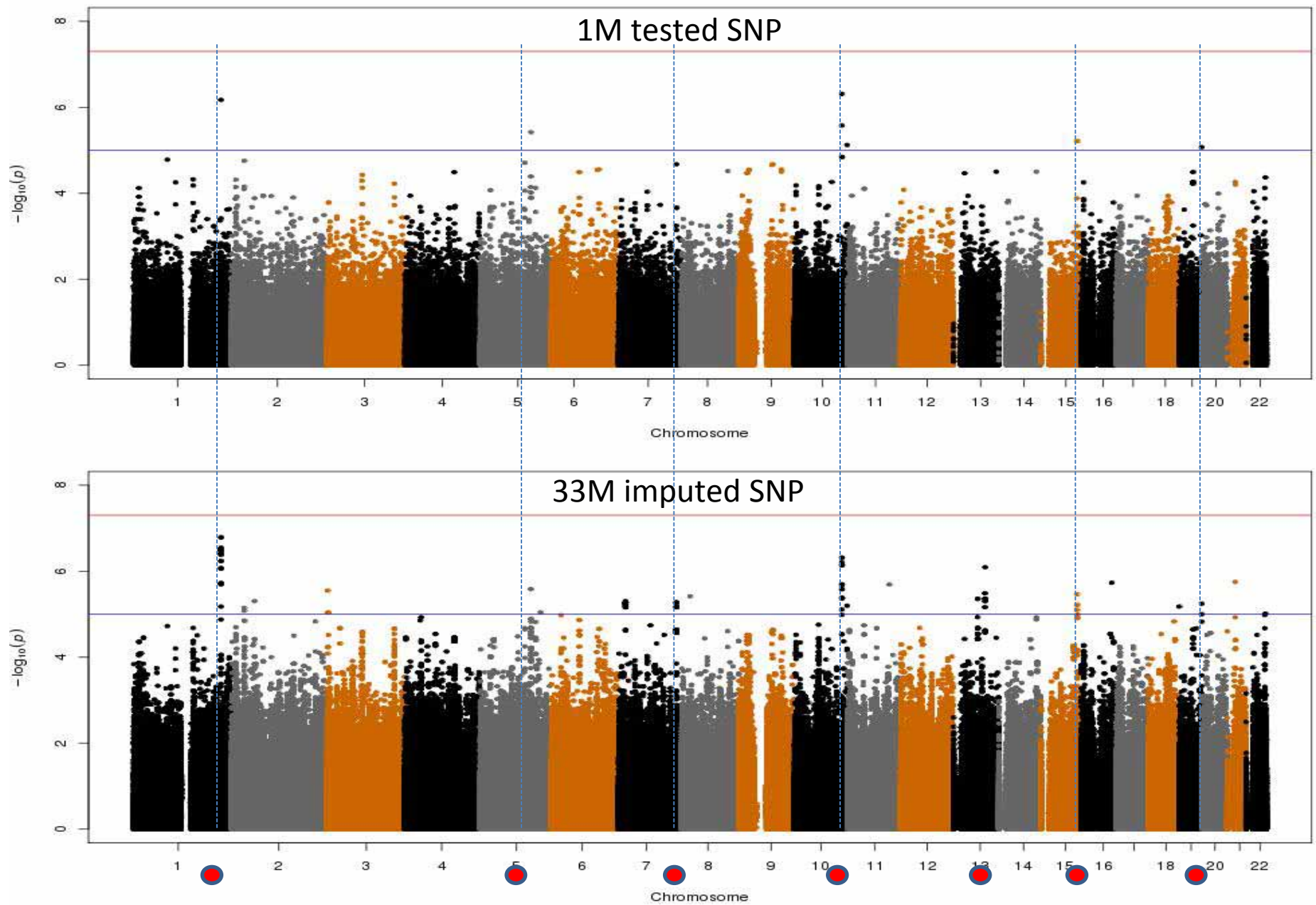


A

Complete Response (CR vs Non-CR)

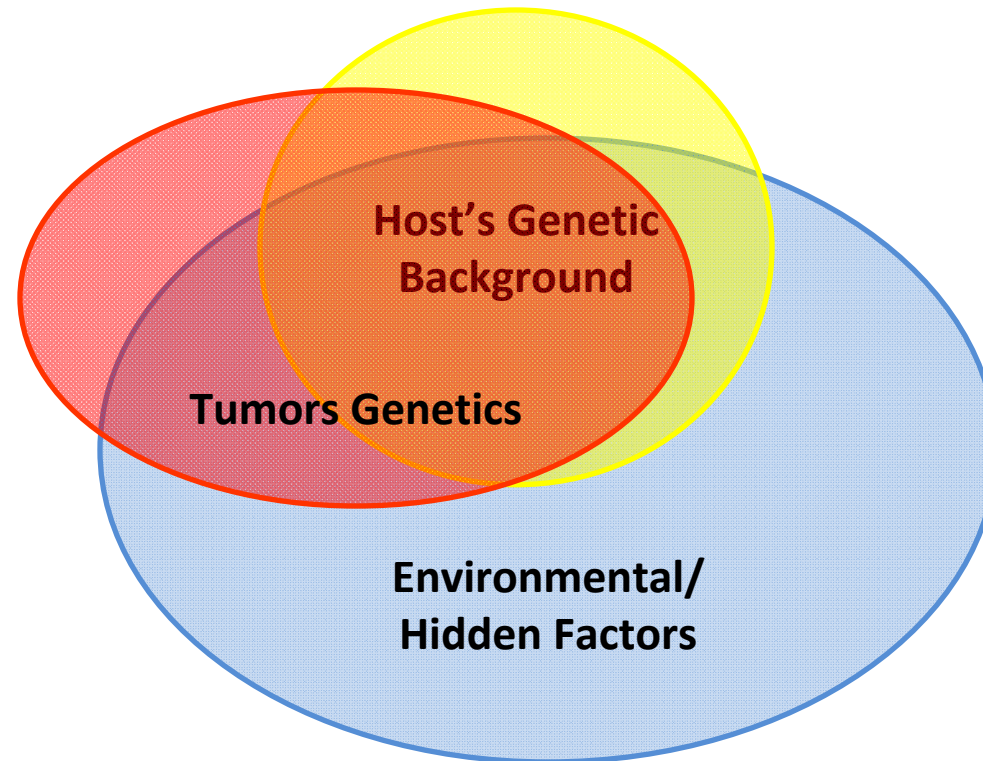


Genome-wide scan for allele frequency differences between CR and PD

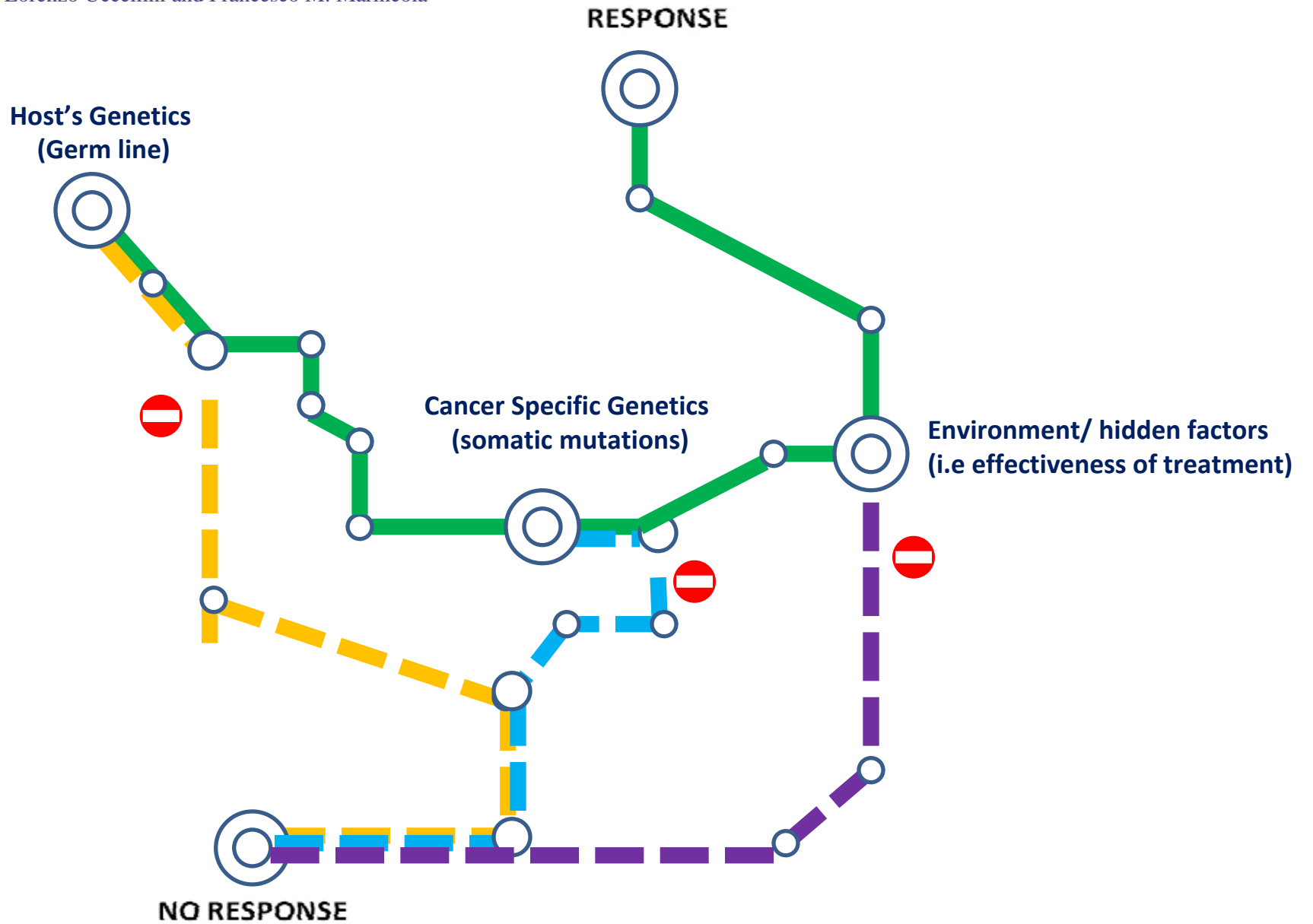


A genetic inference on cancer immune responsiveness

Ena Wang, Lorenzo Uccellini and Francesco M. Marincola

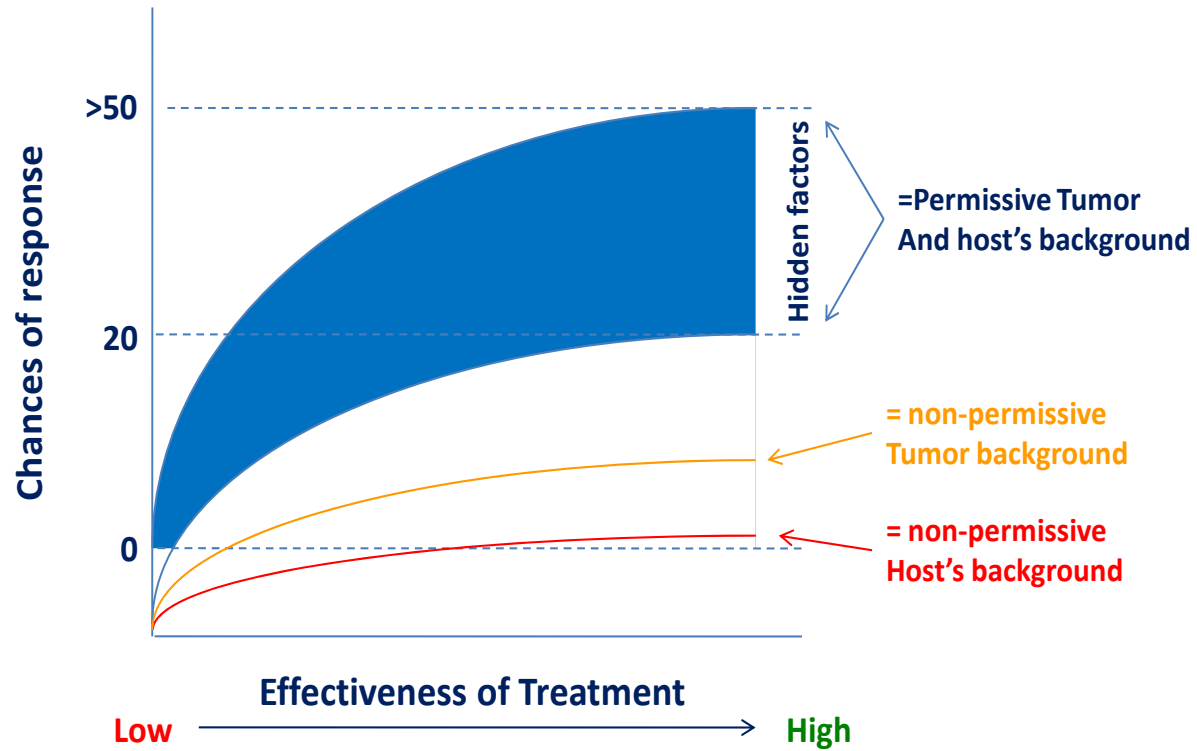


A genetic inference on cancer immune responsiveness
Ena Wang, Lorenzo Uccellini and Francesco M. Marincola



A genetic inference on cancer immune responsiveness

Ena Wang, Lorenzo Uccellini and Francesco M. Marincola



Future strategy

Factors limiting the study of tumor immune responsiveness

A recipe for the identification of the algorithm governing tumor rejection

Multifactorial Phenomenon

Collect samples for germ line and somatic analyses

Collect samples obtained from patients receiving conceptually similar treatments

Prioritize analyses according to question

Limited power of clinical trials

Use univariate class comparison for enrichment

Combine results from different platforms

Univariate class comparison

Apply functional analysis to identify prevalent biological themes

Apply combinatorial approaches

Validate on independent data set

Mark O. Hatfield
Clinical Research Center

