A Multifaceted Immunomonitoring to Identify Predictive Biomarkers for the Clinical Outcome of Immunotherapy-Treated Melanoma Patients

Immunotherapy Biomarkers: Overcoming the Barriers

NIH, Bethesda, April 1st 2016

Cristina Maccalli, PhD

Sidra Medical and Research Center
Division of Translational Medicine
Doha, Qatar

Biomarkers for Immune checkpoint blockade agents

The hallmark for Immune checkpoint targeting as anti-cancer immunotherapy is the need for biomarkers to monitor treatment, to predict patient's clinical outcome and to optimize the usage of combined therapies;

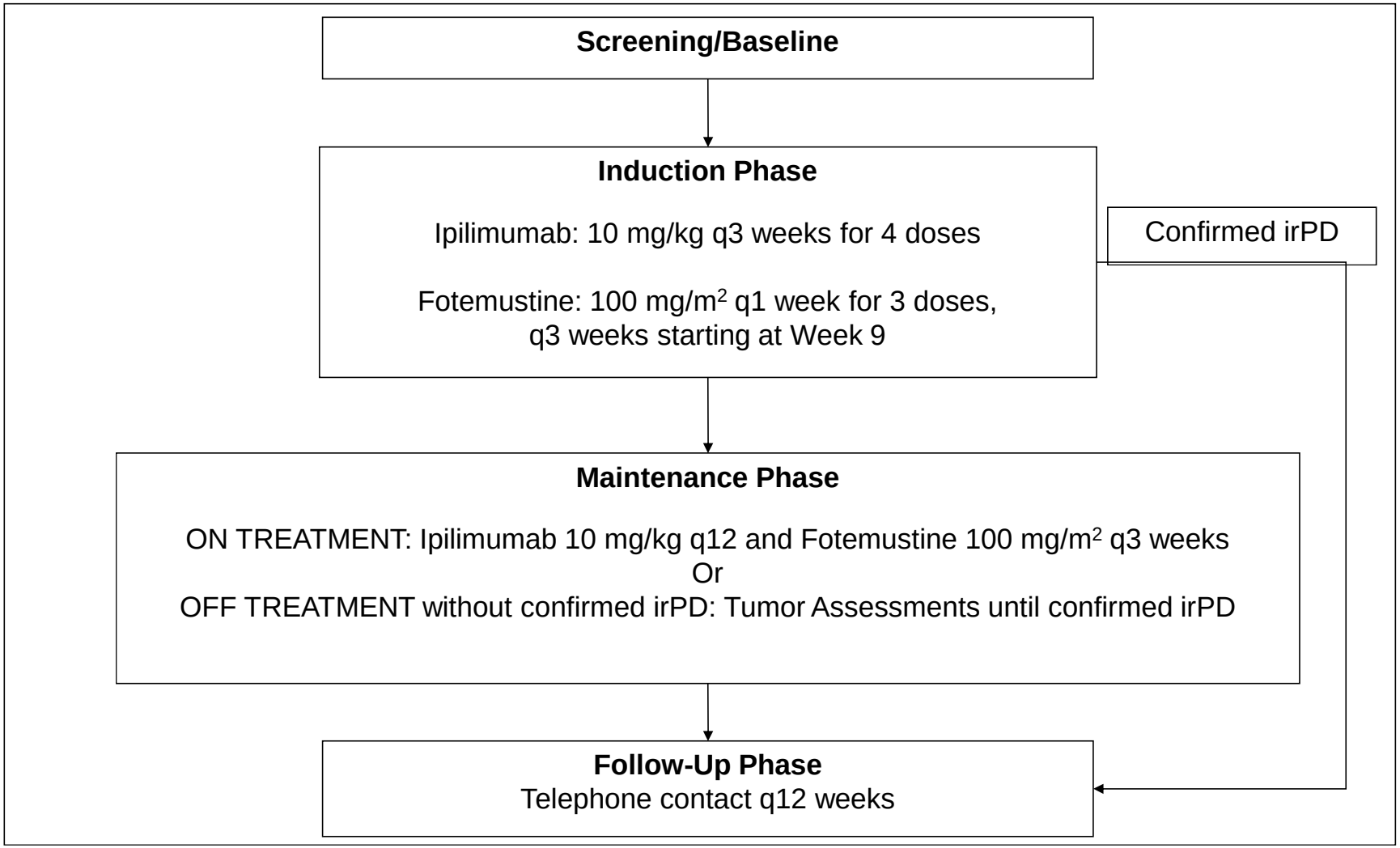
•Several retrospective studies have reported "candidate" biomarkers for cancer immunotherapy;

•However to date no validated and unique biomarker has been shown to accurately predict clinical responses to immunotherapy;

- Immunomonitoring technique(s) or their integrated platforms need:

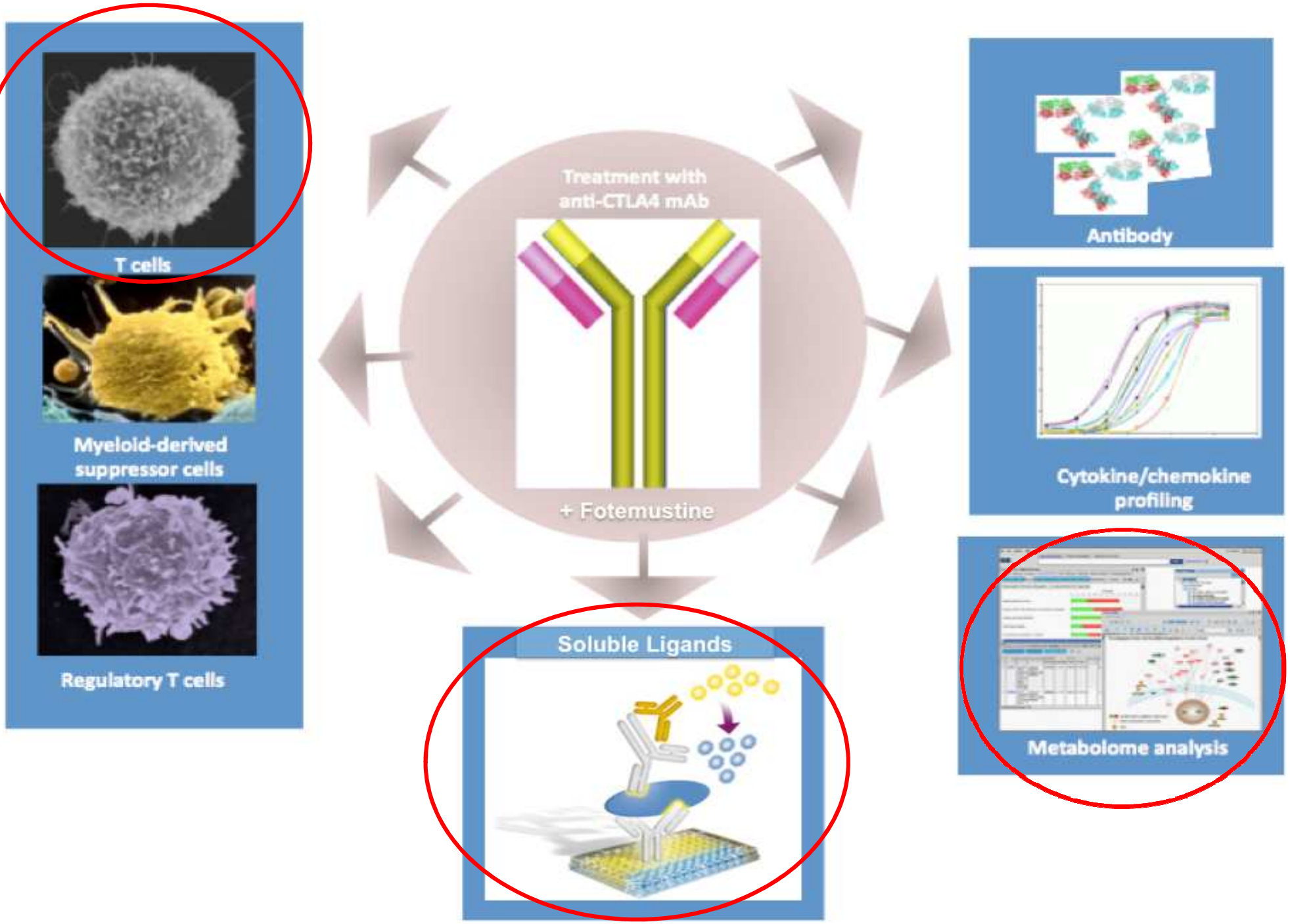
  a. To be harmonized and validated;
  b. To provide univocal results;
  c. To be suitable for application in different labs/cancer centers;
  d. Desirable, to be largely exploited for tumor with different histological origin.

The Immunomonitoring of patients treated with anti-CTLA-4 mAb plus chemotherapy-A multicentered study in the context of the Italian Network for Biotherapy of tumors (NIBIT-M1 study)

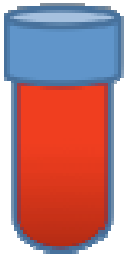


Di Giacomo A.M. et al., 2013; Di Giacomo A.M. et al. 2015

The multifaceted immunomonitoring of NIBIT-M1 study



PBMC



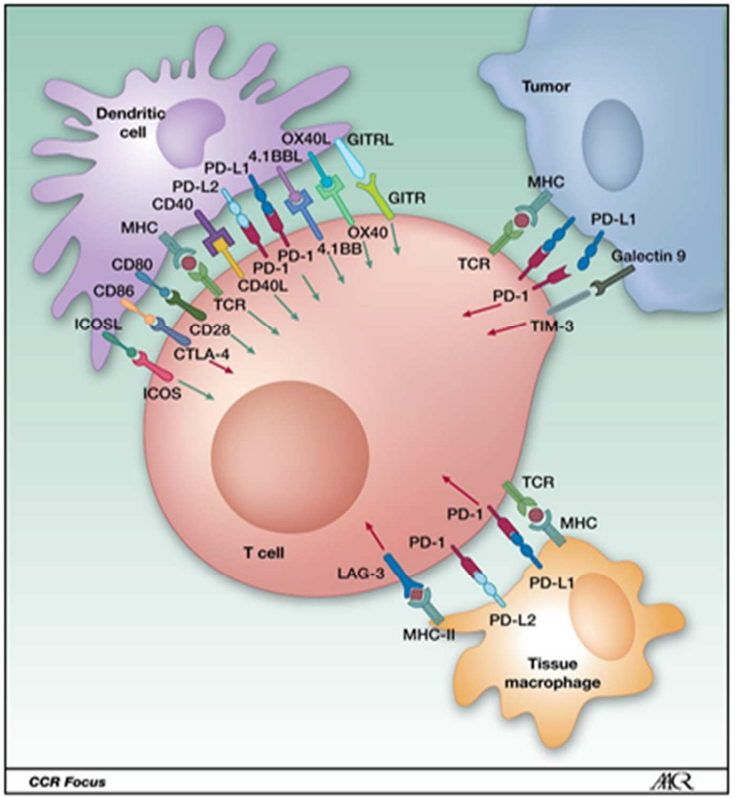
Analysis
of T cell activation

Phenotype analysis:
Multiparametric analysis

To identify TH1, TH2, TH17 subsets (CD3, CD4, CD8, CD45RA, CD45RO, CCR7, CD62L, CCR6, CCR5, CXCR3, IL23R, IL12Ra2, IL-17 etc.) in relation with co-stimulatory or inhibitory molecules (OX40, 4-1BB, NKG2D, ICOS, PD-1, CTLA-4, BTLA etc.) NK and B cells.

.

The complexity of the regulation of T cell mediated responses



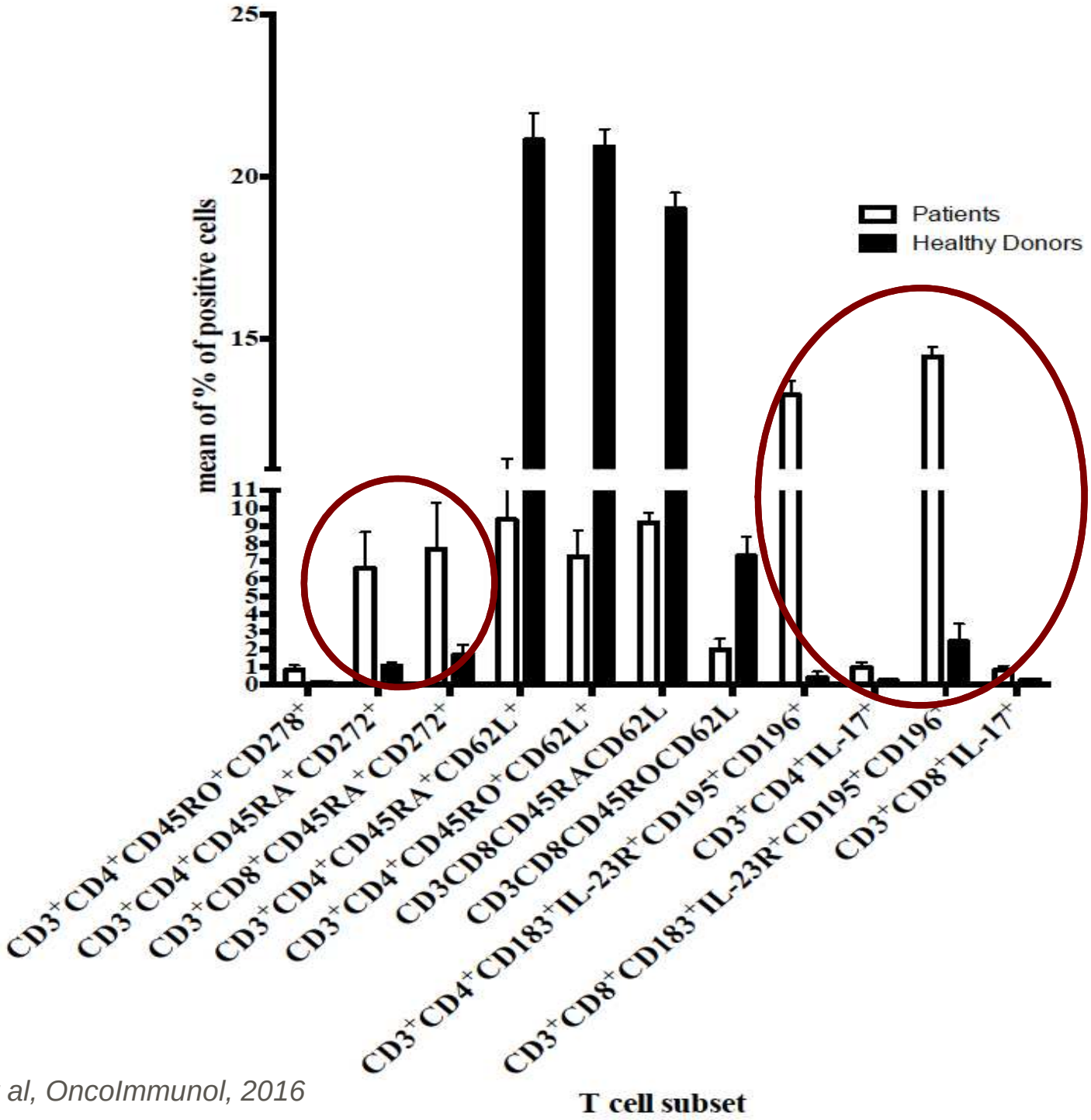
Standardized panels for PBMC analysis

Lambda of Band-Pass Filter	Sample Tube 1	Sample Tube 2	Sample Tube 3	Sample Tube 4	Sample Tube 5	Sample Tube 6	Sample Tube 7	Sample Tube 8	Sample Tube 9	Sample Tube 10	Sample Tube 11
Blank											
488											
530/30	CD134-FITC	CD57-FITC	CD28-FITC	CD62L-FITC	CD28-FITC	CD152-FITC	CD152-FITC	IL23R-FITC	CD152-FITC	IL-12 Rα2-FITC	CD19-FITC
575/20	CD137-PE	CCR7-PE	CCR7-PE	CCR7-PE	CD69-PE	CD278-PE	CD272-PE	CD183-PE	CD183-PE	CD278-PE	CD272-PE
610/20	CD45RA-ECD	CD45RA-ECD	CD45RA-ECD	CD45RA-ECD	CD45RA-ECD	CD45RA-ECD	CD45RA-ECD				
695/40								CD196-PercP-Cy5.5	CD196-PercP-Cy5.5	CD196-PercP-Cy5.5	
780/60	CD45RO-PE-Cy7	CD45RO-PE-Cy7	CD45RO-PE-Cy7	CD45RO-PE-Cy7	CD45RO-PE-Cy7	CD45RO-PE-Cy7	CD45RO-PE-Cy7	CD45RO-PE-Cy7	CD45RO-PE-Cy7	CD45RO-PE-Cy7	
Blank											
660/20	NKG2D-APC	CD56-APC	CD27-APC	CD27-APC	NKG2D-APC	NKG2D-APC	CD279-APC	CD195-APC	CD161-APC	CD279-APC	CD22-APC
780/60	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7	CD4-APC-Cy7
Blank											
450/50	CD8-PB	CD8-PB	CD8-PB	CD8-PB	CD8-PB	CD8-PB	CD8-PB	CD8-PB	CD8-PB	CD8-PB	CD8-PB
510/50	CD3-V500	CD3-V500	CD3-V500	CD3-V500	CD3-V500	CD3-V500	CD3-V500	CD3-V500	CD3-V500	CD3-V500	CD3-V500

IL-17A was also measured to identify TH17-like cells

C. Maccalli et al, OncoImmunol, 2016

Baseline T cell subset profile of melanoma patients vs. healthy donors



C. Maccalli et al, OncoImmunol, 2016

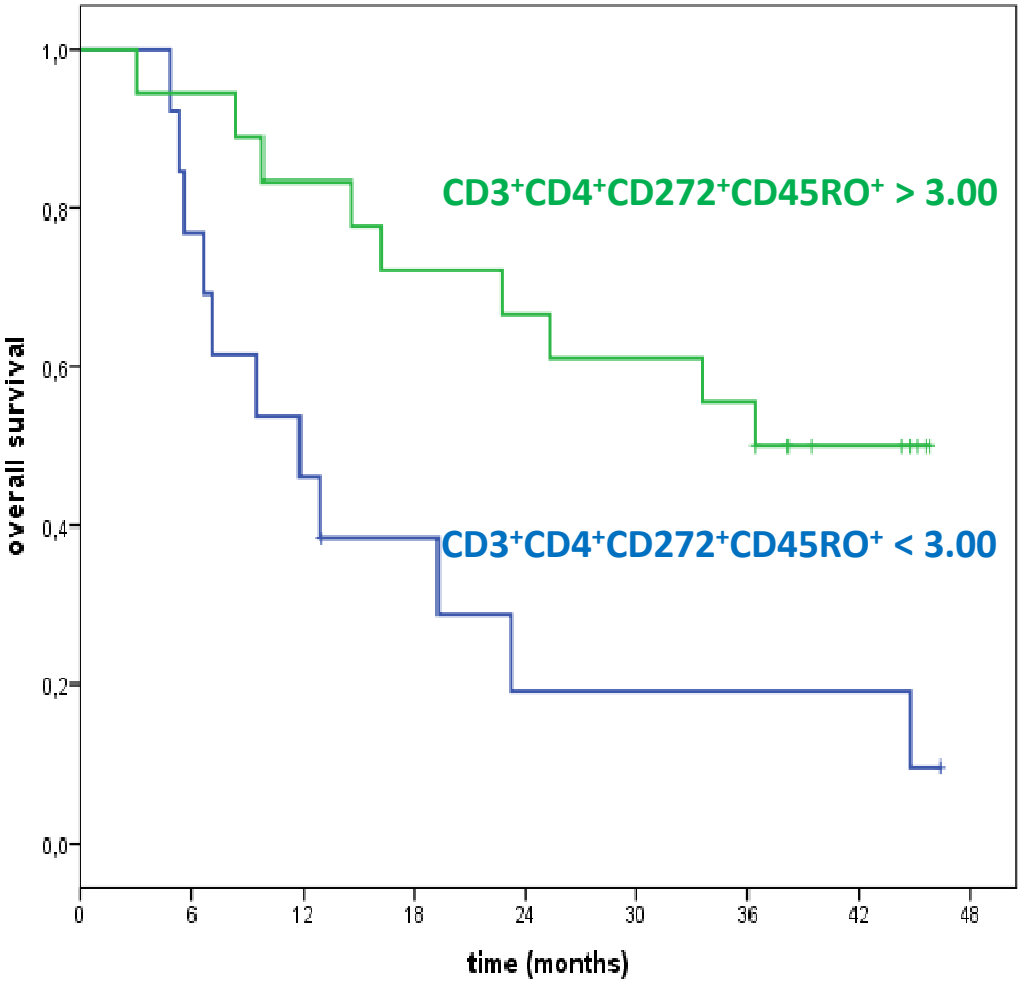
Relationship between the frequency at W12 vs. baseline of circulating T cell subsets and clinical responses of MM patients

T cell subset	DCR	N.	Mean*	St. Error	P
$CD3^+CD45RO^+CD272^+$	Yes	19	258.54	20.97	0.03
	No	12	-24.54	115.63	
$CD3^+CD4^+CD45RO^+BTLA^+$	Yes	19	75.08	23.14	0.05
	No	12	-21.50	26.96	
$CD3^+CD4^+CXCR3^+CCR5^+$ $CCR6^+IL\text{-}23R^+$	Yes	18	95.06	56.69	0.05
	No	12	-25.76	17.49	

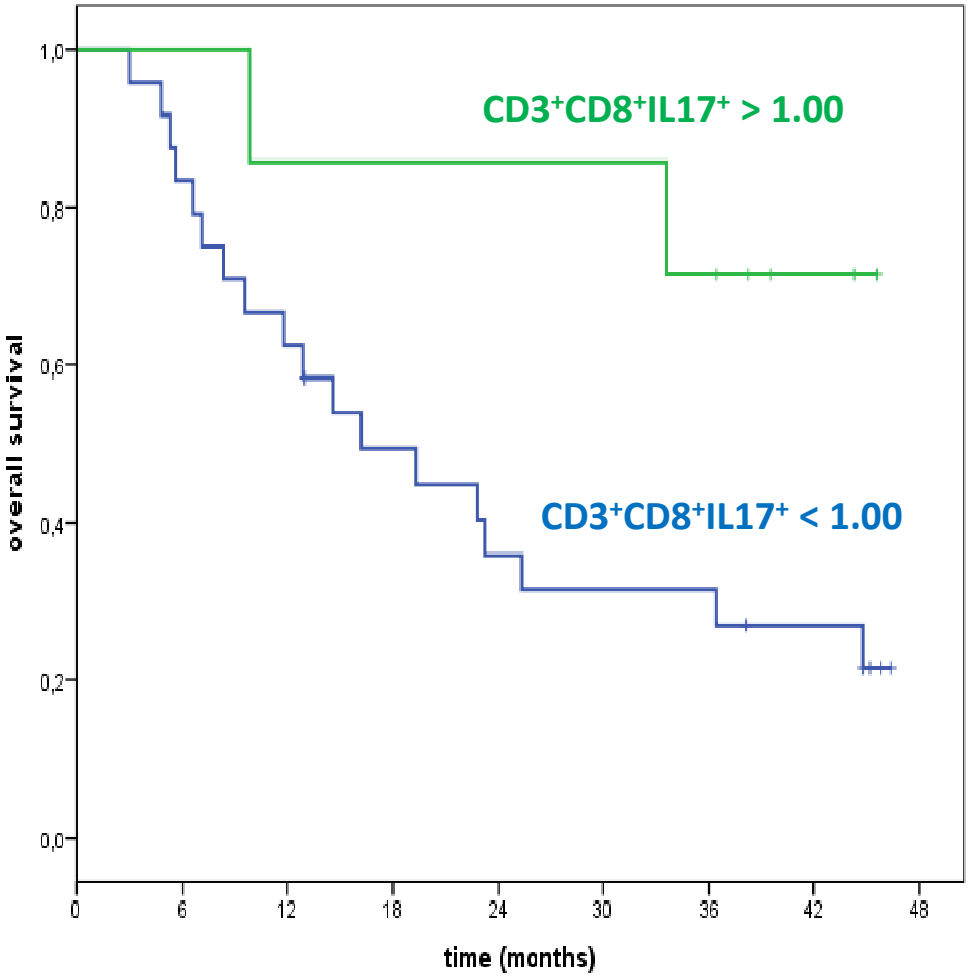
T cell subset	ORR	N.	Mean*	St. Error	P
$CD3^+ CD4^+CD45RO^+ICOS^+$	Yes	14	319.91	101.46	0.05
	No	17	96.05	51.78	
$CD3^+CD45RO^+PD\text{-}1^+$	Yes	14	256.87	101.18	0.05
	No	17	38.96	36.48	
$CD3^+CD8^+CD45RO^+PD\text{-}1^+$	Yes	14	170.57	72.80	0.05
	No	17	21.14	30.78	
$CD3^+CD4^+CD45RO^+BTLA^+$	Yes	14	81.49	31.51	0.05
	No	17	1.62	24,11	
$CD3^+CD8^+CD45RO^+BTLA^+$	Yes	14	67.90	25.59	0.05
	No	17	-4.92	22.79	

C. Maccalli et al, OncoImmunol, 2016

Correlation of the frequency of circulating T cell subsets at baseline with OS of melanoma patients treated with ipi



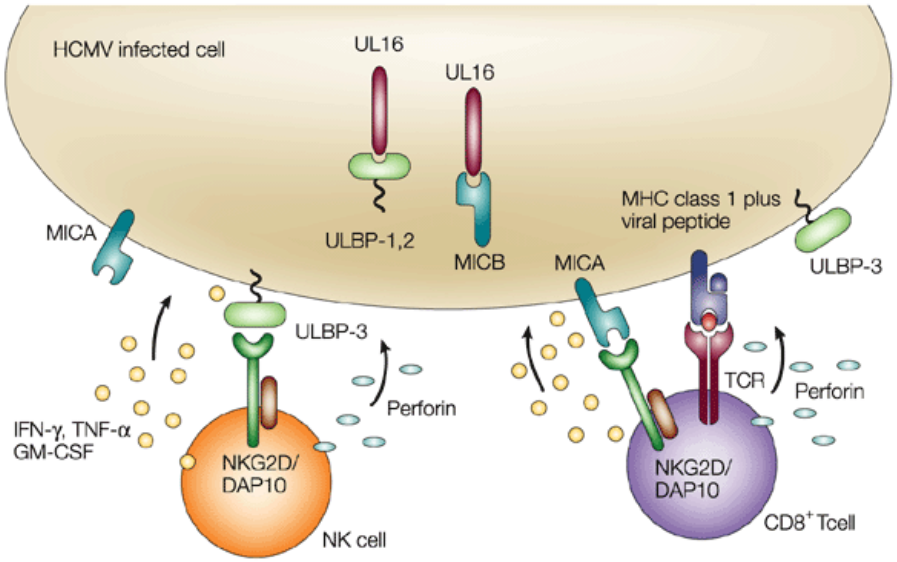
P=0.009



P=0.04; similar for $CD3^+CD4^+IL17^+$

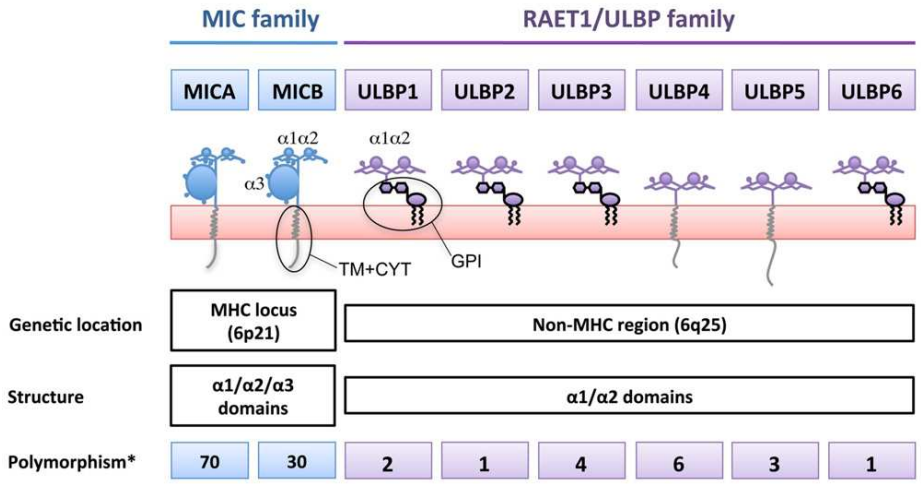
C. Maccalli et al, OncoImmunol, 2016

NKG2D Ligands



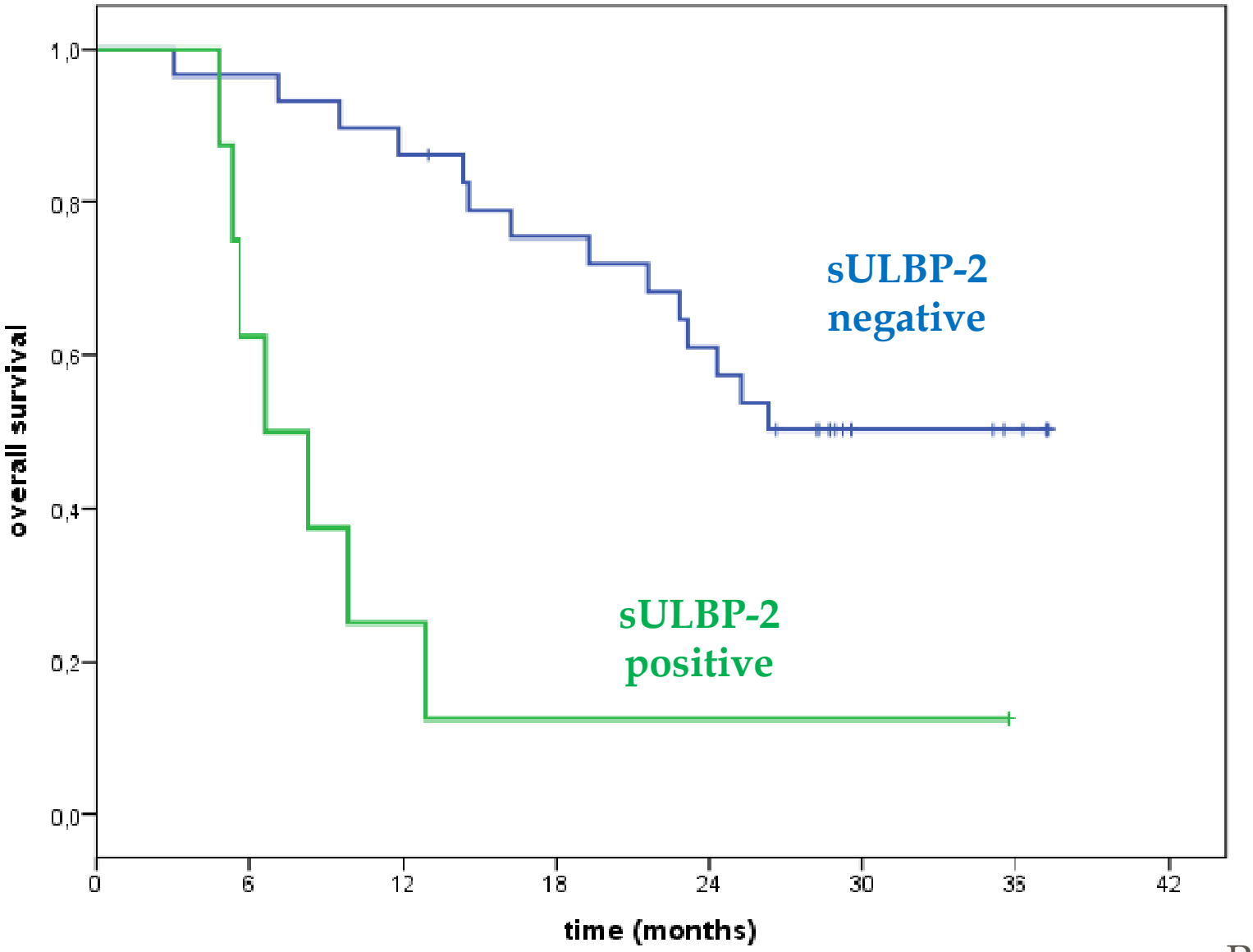
Nature Reviews | Immunology

The soluble forms of these molecules have been reported to play a negative immunomodulatory activity on T and NK cells. Moreover, a prognostic value for these molecules have been found in some tumor types (e.g. CRC, breast cancer, melanoma, leukemia).



Relationship between baseline ULBP-2 detection in the serum and OS for MM patients from NIBIT-M1 study

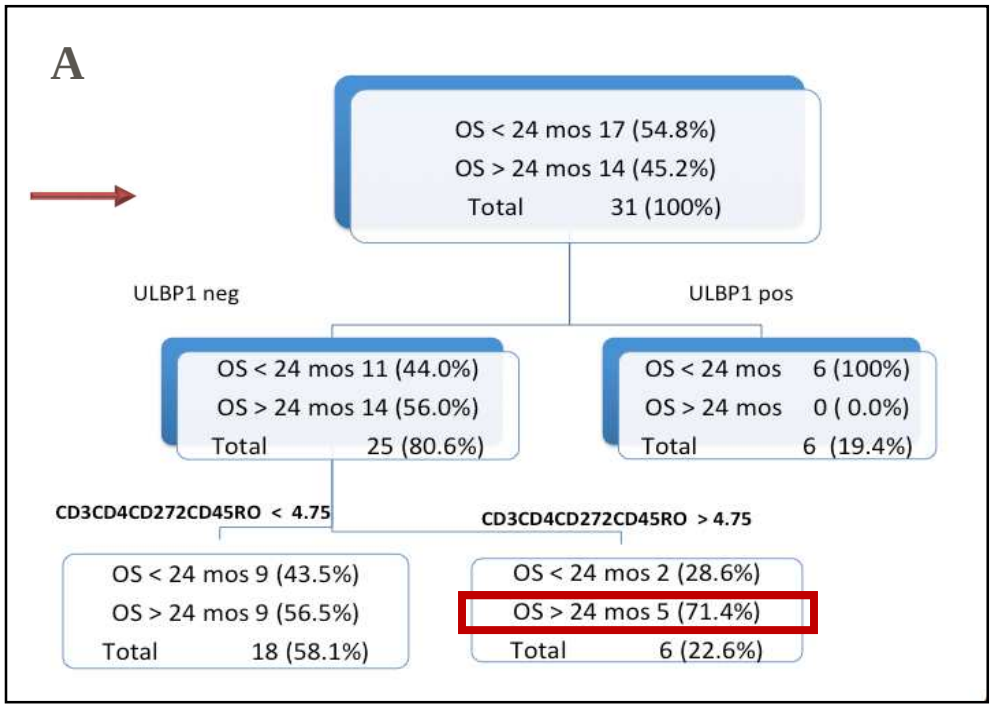
ULBP-2

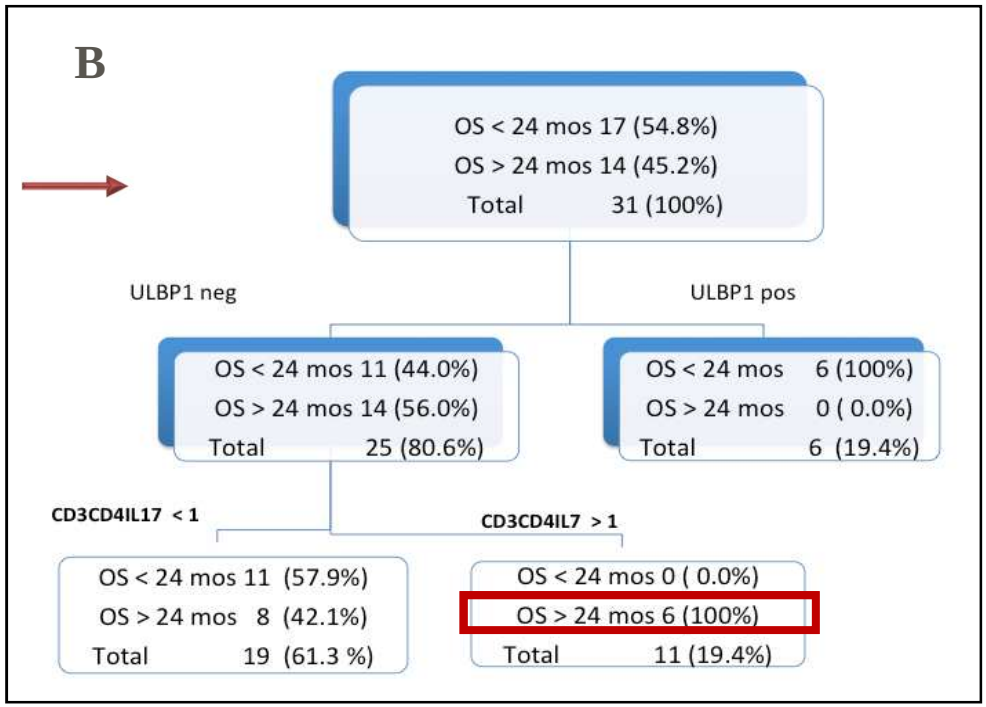


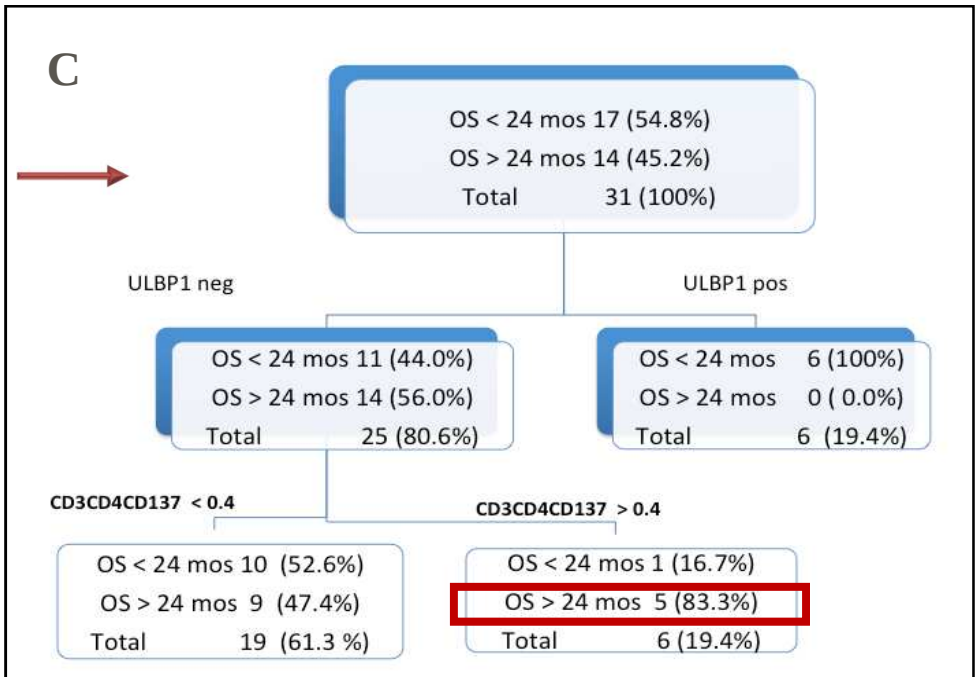
P<0.0001

C. Maccalli et al, OncoImmunol, 2016

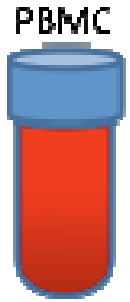
Classification and Regression Trees
CART







C. Maccalli et al, OncoImmunol, 2016

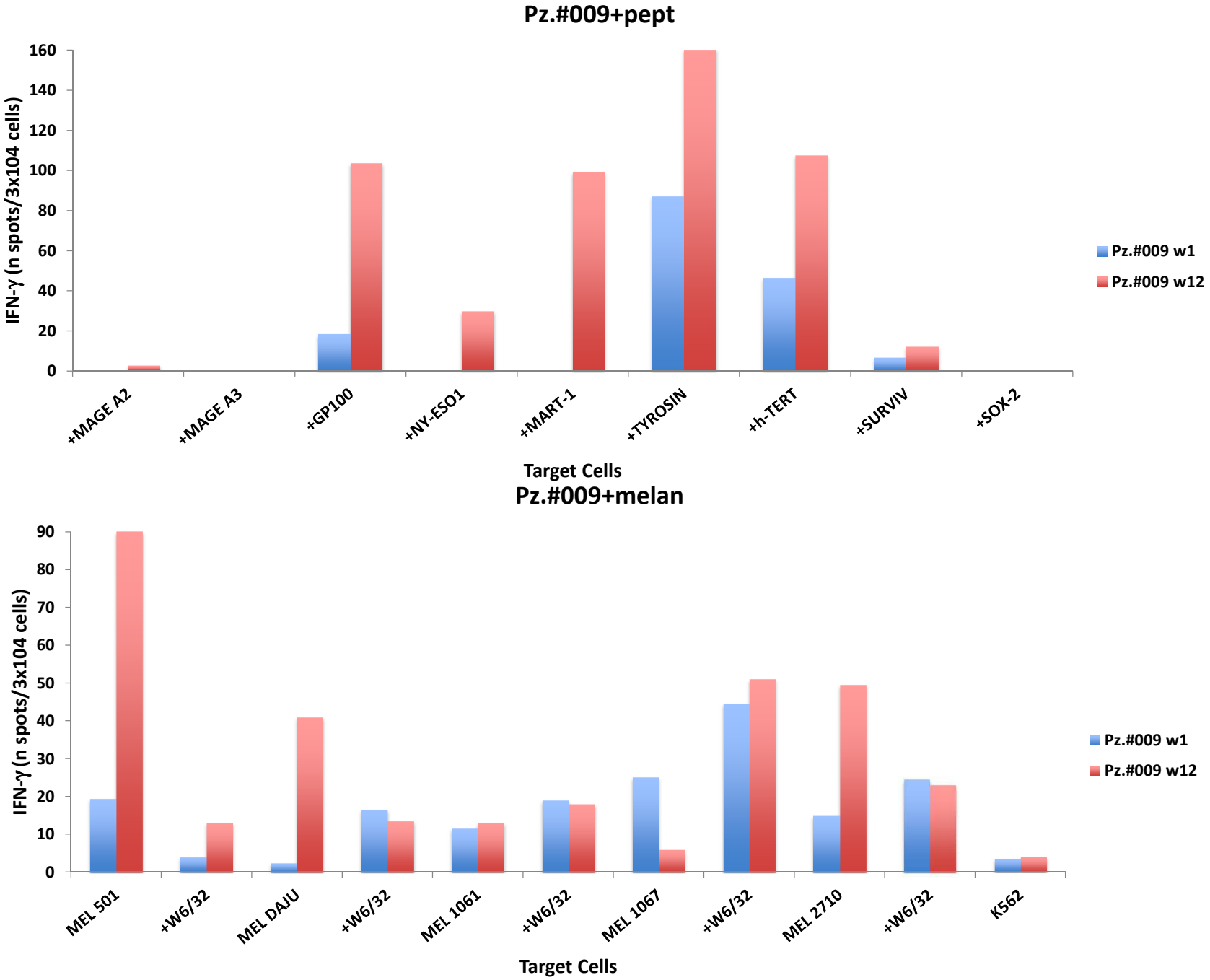


Analysis
of T cell activation

Functional activity of T cells:

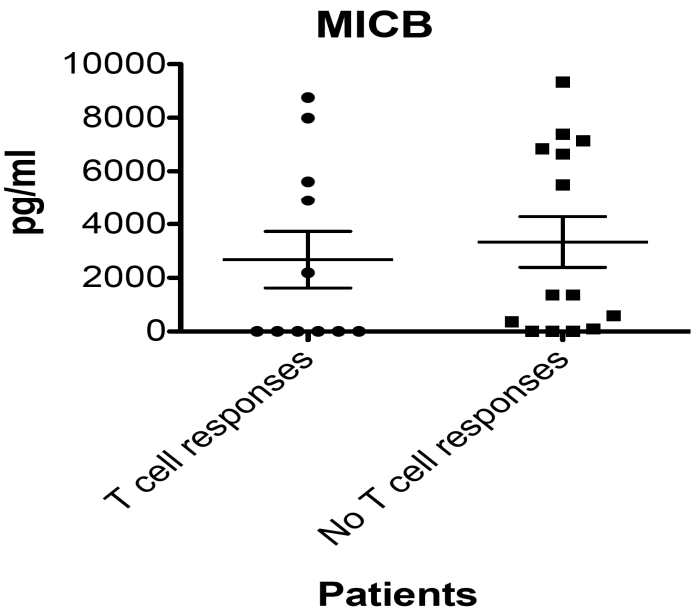
TH1-type (IFN-g release; ELISPOT) response toward Melanoma Associated Antigens (MAAs; HLA-A*0201/*0101/*0301-restricted epitopes from MAGE-A2, MAGE-A3, NY-ESO-1, MART-1, Gp100, Tyr, SVV-1, h-TERT, SOX-2) and allogeneic HLA-matched or unmatched tumor cell lines.

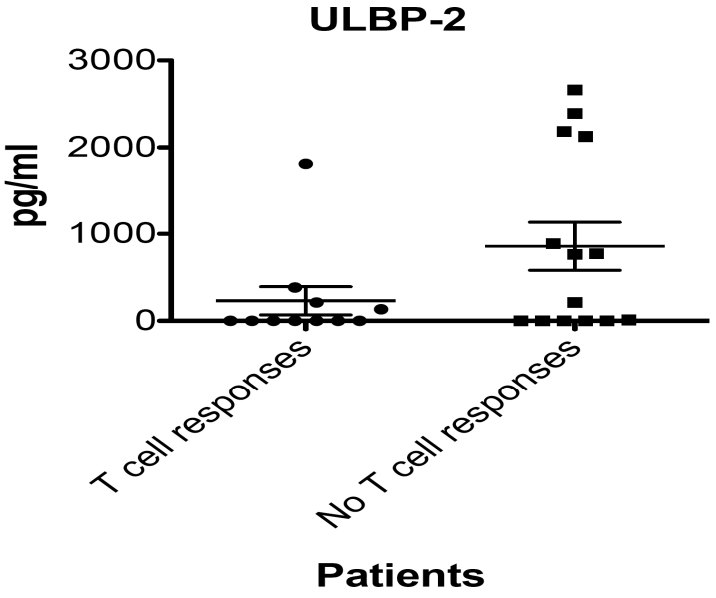
Antigen-specific T cell responses in fresh PBMCs from IPI plus treated patients

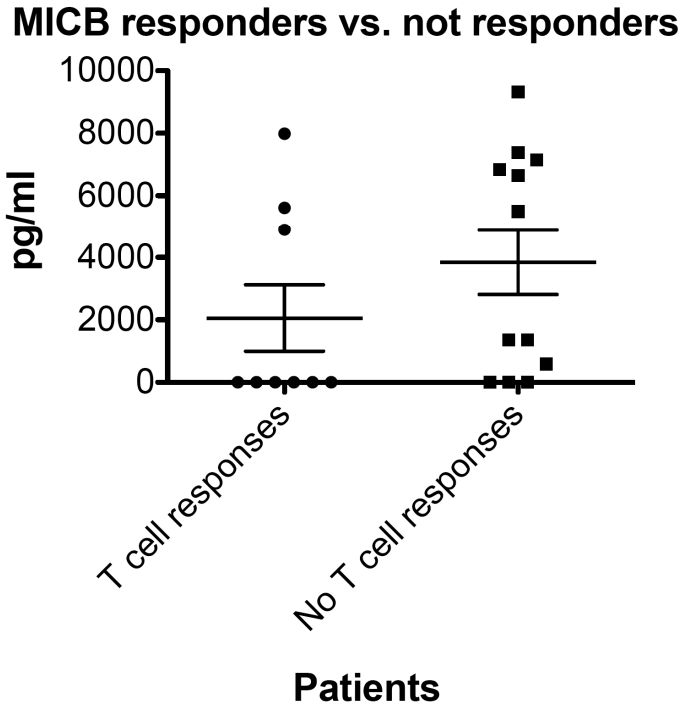


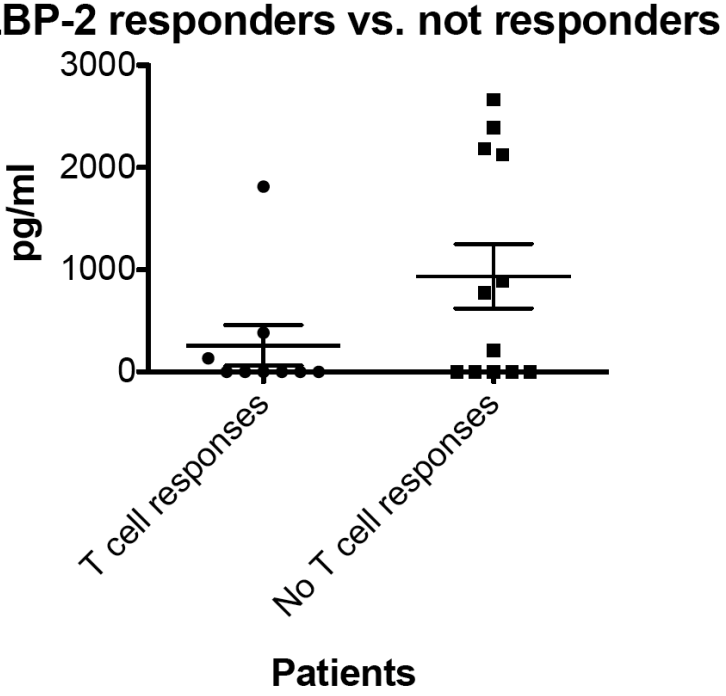
C. Maccalli et al, OncoImmunol, 2016

Correlation of soluble NKG2DLs with T cell and clinical responses in MM patients treated with IPI









C. Maccalli et al, unpublished

Conclusions I

1. A multifaceted immunomonitoring platform allowed to identify candidate biomarkers either predictive or associated with clinical outcome of melanoma patients treated with immune checkpoint blockade agents.

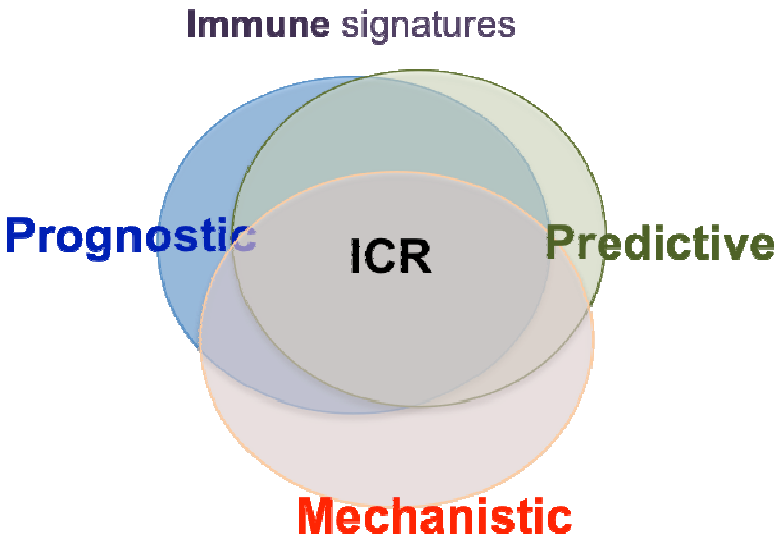
2. The detection of soluble immunoregulatory molecules (sNKG2DLs) can be associated with T cell subset population analysis and/or T cell mediated immune responses directed to tumor antigen and/or melanoma cell lines in order to identify a ***functional immunological signature*** predictive for the clinical outcome to immunotherapy.

3. This platform needs to be optimized and standardized in large cohort of patients undergoing immunotherapy treatments.

4. Will be worthy and feasible to combine it with the screening of T cell mediated neoantigen reactivity?

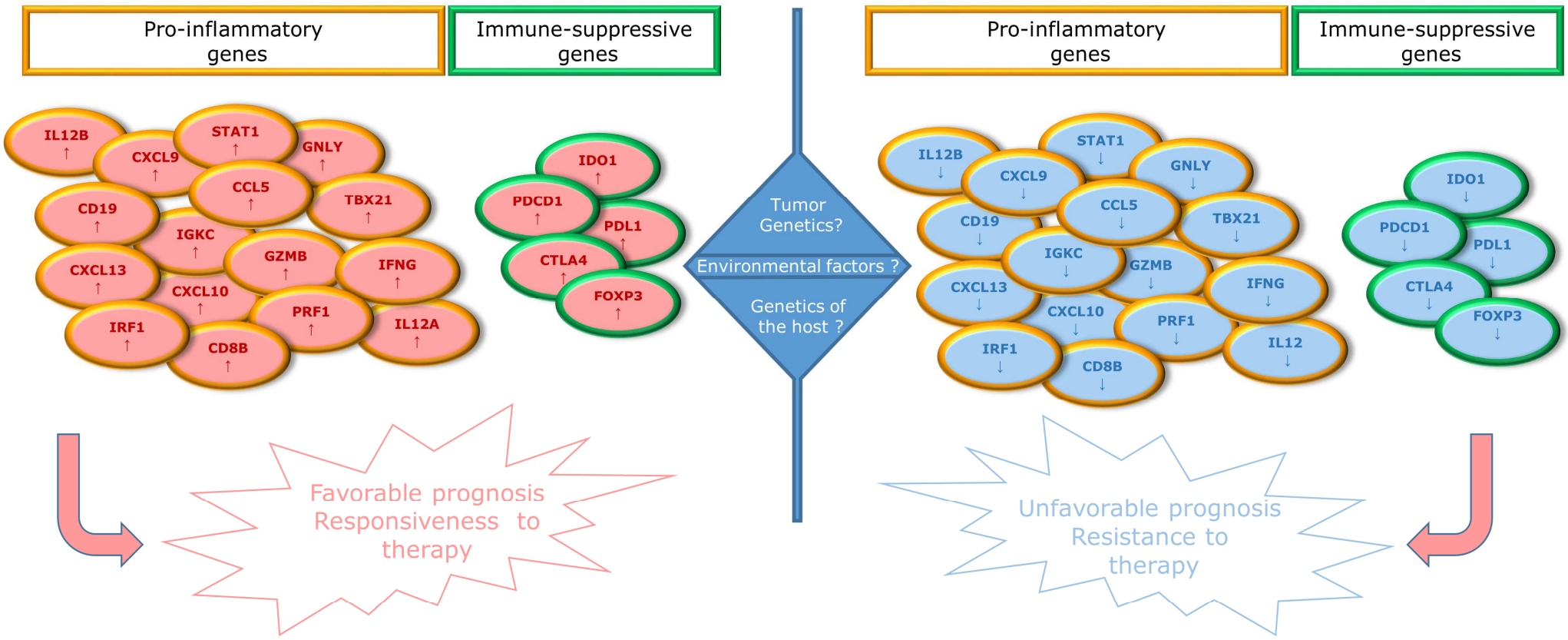
How the identification of immunotherapy biomarker can be further developed?

The Immunological Constant Rejection



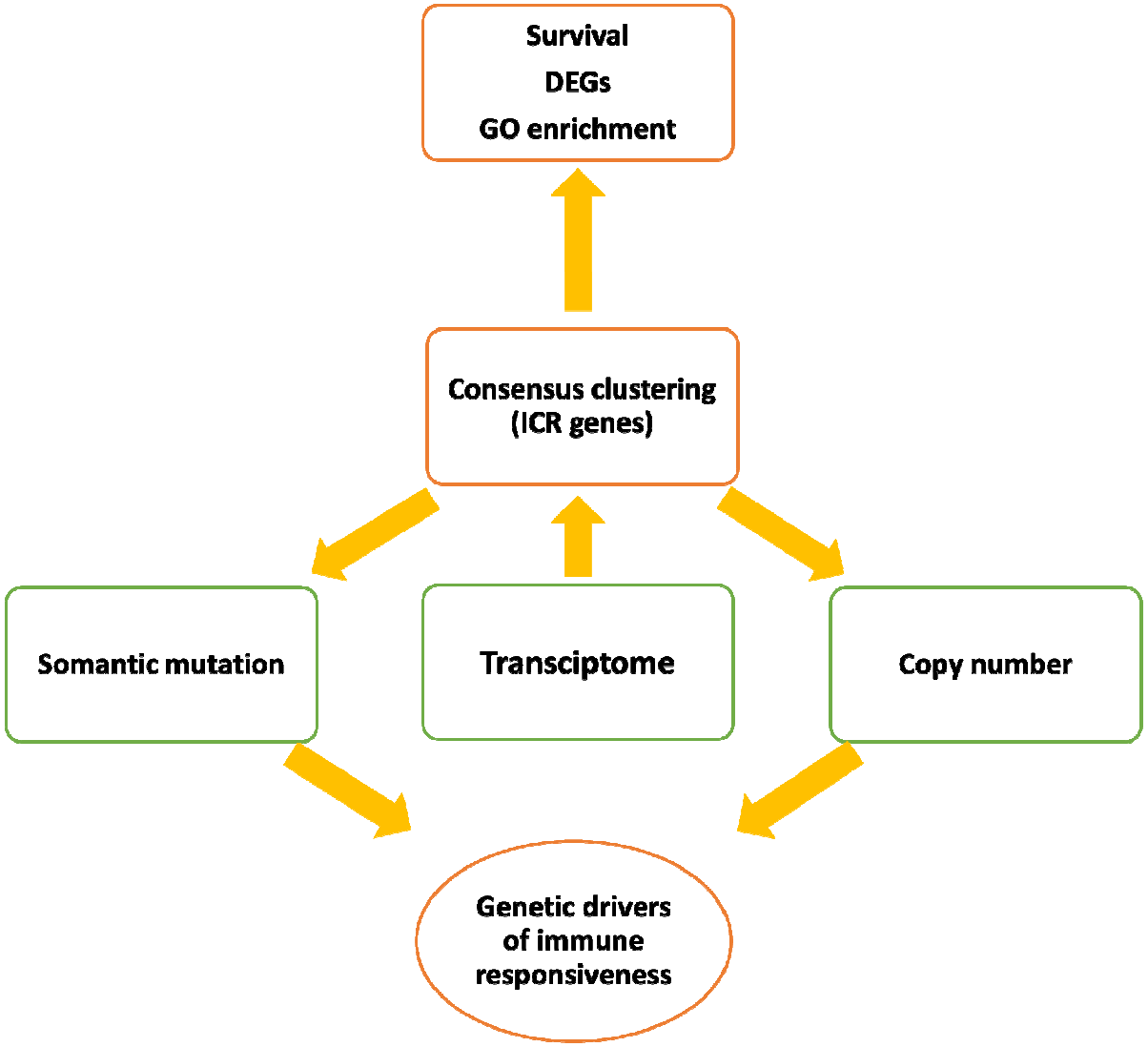
Galon, Angell, Bedognetti, Marincola, Immunity 2013

Two opposite cancer immune phenotypes



Bedognetti, Hendrickx,& Miller. Curr Op Onc, 2015

Genetic drivers of immune responsiveness in Breast Cancer (TCGA)

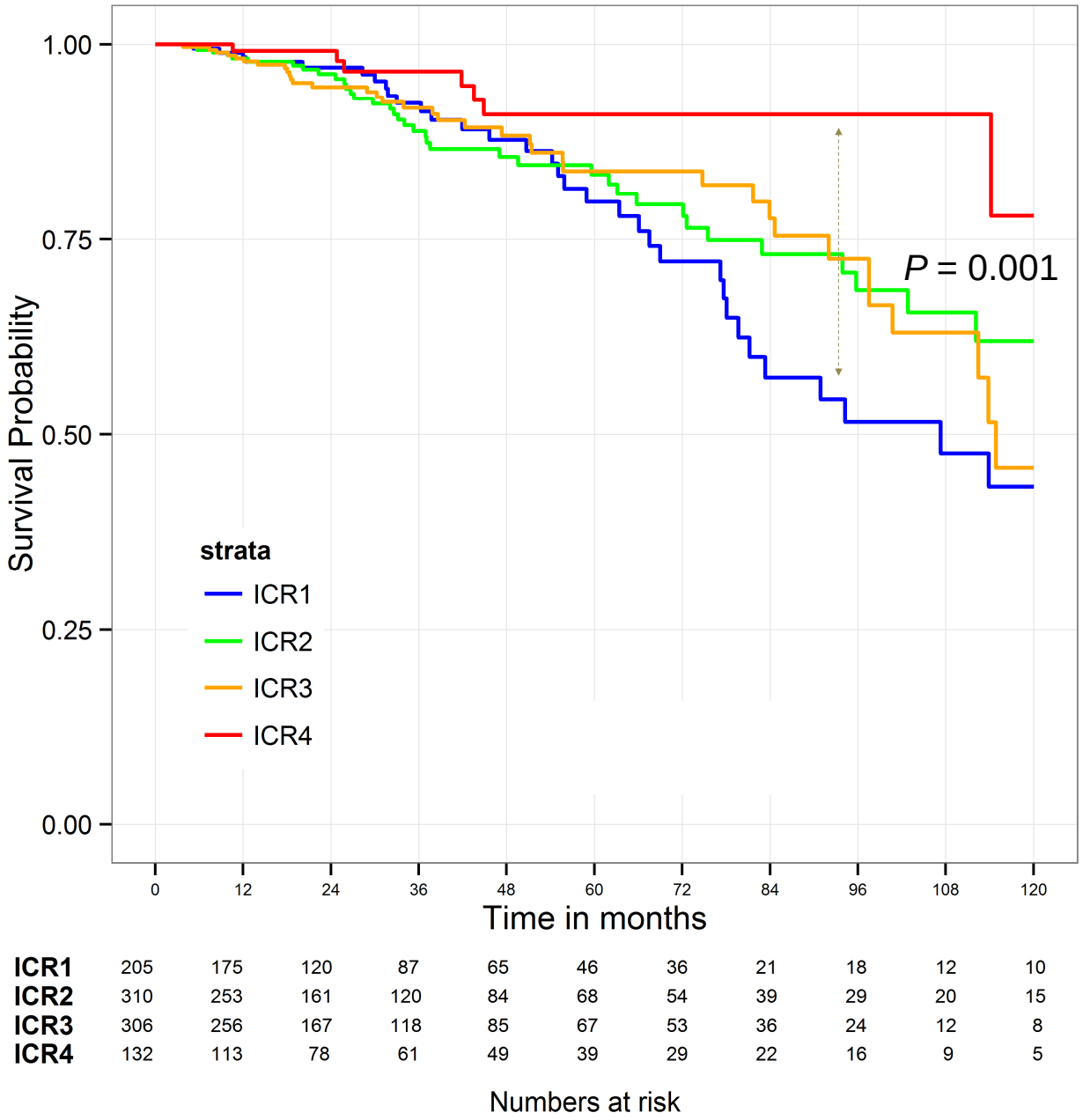


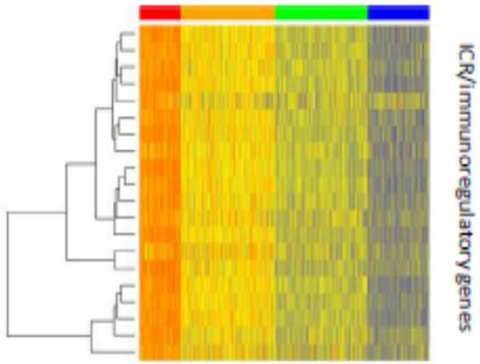
TCGA

NCI | NHGRI

Functional pipeline

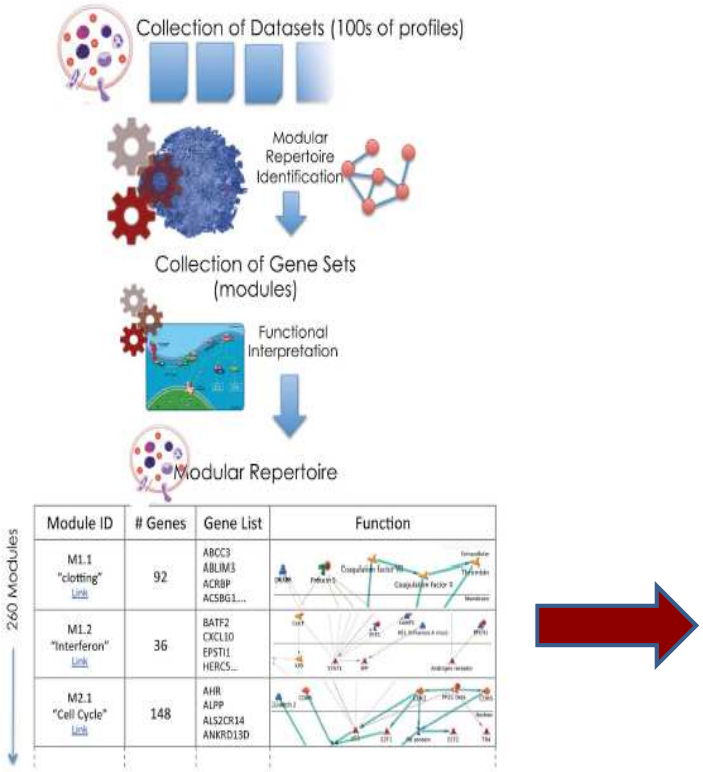
Survival Analysis – TCGA

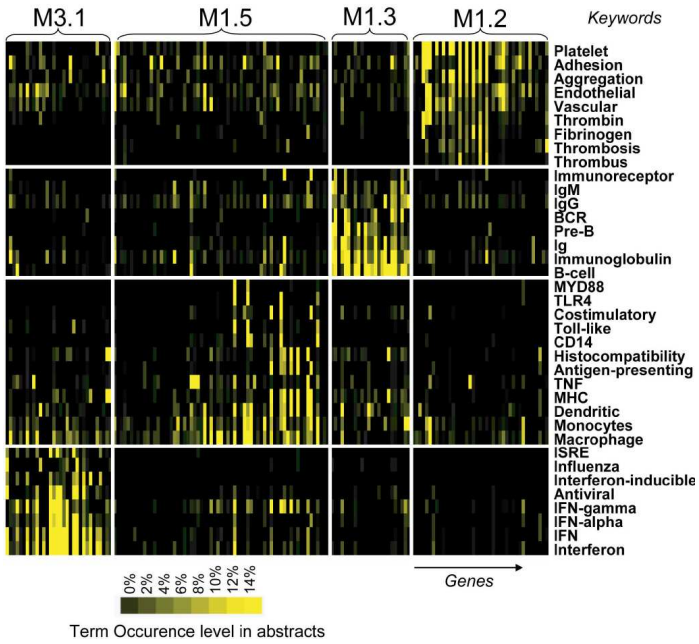


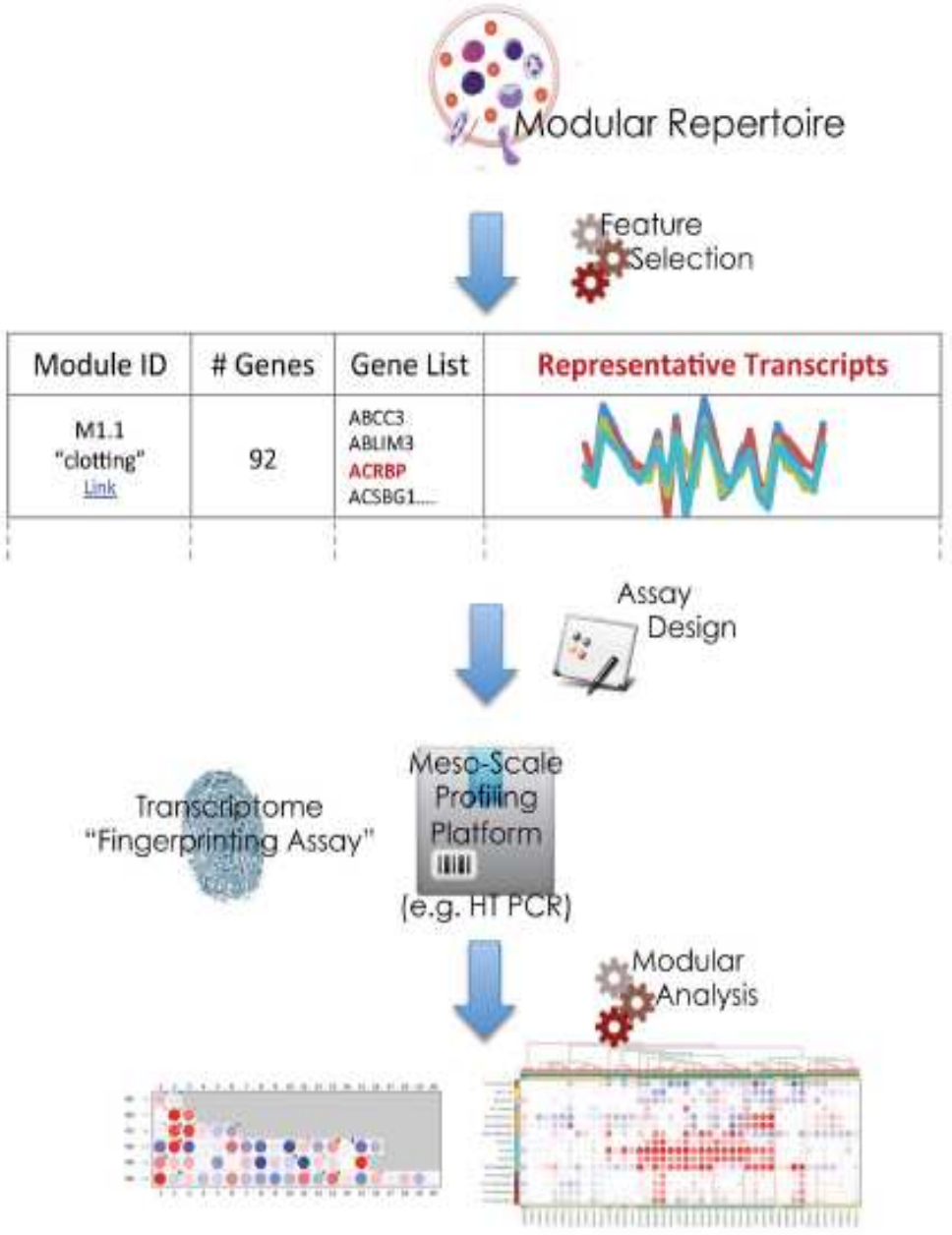


Bedognetti and Ceccarelli, unpublished

Modular Repertoire Identification and Transcriptome Fingerprinting Assay

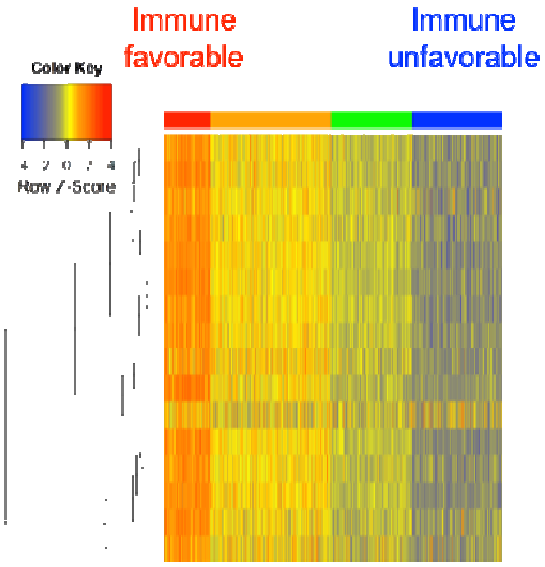




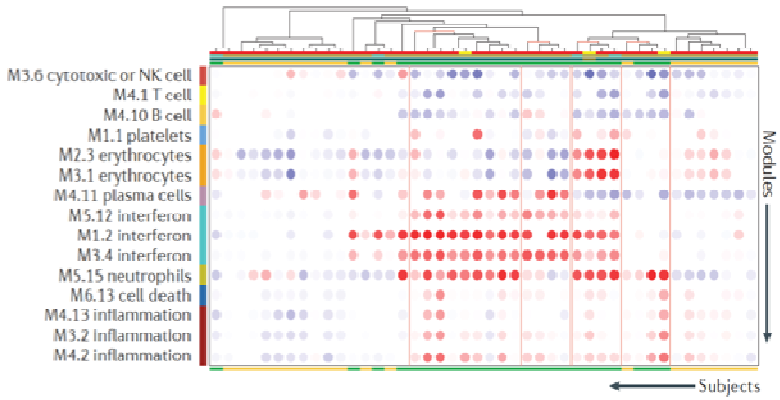


Chaussabel and Baldwin, Nature Rev Immunol, 2014

Tumor transcriptome



Peripheral blood transcriptome



?

Clinical Outcome

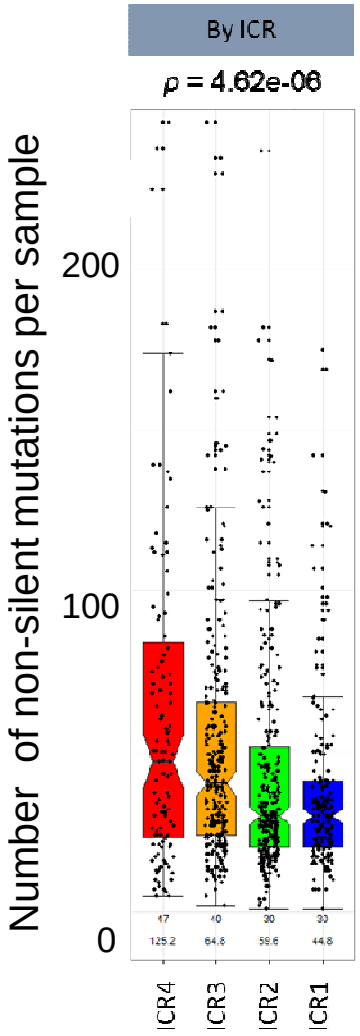
Right panels by Chaussabel and Baldwin, Nature Rev Immunol, 2014

The contemporary analysis of tumor and peripheral blood could define whether i) a specific tumor immunophenotype is characterized by a distinct peripheral blood gene signatures and ii) to correlate those signatures with clinical outcome.

These gene signatures might be correlated with the cell subset phenotype analysis in the peripheral blood.

Mutational Burden vs Breast Cancer ICR Immune Phenotypes

P



Bedognetti and Ceccarelli, unpublished

**Non Silent Mutations*

Conclusion II

By using consensus clustering analysis based on RNA-seq data of ICR genes in >1000 breast cancer samples , 4 major immunophenotypes (ICR1, ICR2, ICR3, and ICR4) could be identified.

Patients bearing the immune-favorable phenotype (ICR4) experienced prolonged survival

Genetic variables associated with the different immunophenotypes could be identified.

The contemporary analysis of tumor and peripheral blood could define whether i) a specific tumor immunophenotype is characterized by a distinct peripheral blood gene signatures and ii) to correlate those signatures with clinical outcome.
The correlation of gene signatures with the cell subset phenotype analysis in the peripheral blood would provide a detailed characterization of specific immunophenotype associated with patients' clinical outcome.

Acknowledgments I

NIBIT
Network Italiano per la Bioterapia dei Tumori

Acknowledgments II

Tumor Biology, Immunology and Therapy,
Sidra Medical Research Center, Doha Qatar
Davide Bedognetti
Wouter Hendrickx

Computer Science and Engineering, QCRI, Doha, Qatar
Michele Ceccarelli

Research Branch, Sidra Medical and Research Center, Doha, Qatar
Francesco Marincola
Ena Wang