Host-Tumor Interactions

Primer on Tumor Immunology and Biological therapy
Boston, MA
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Preface

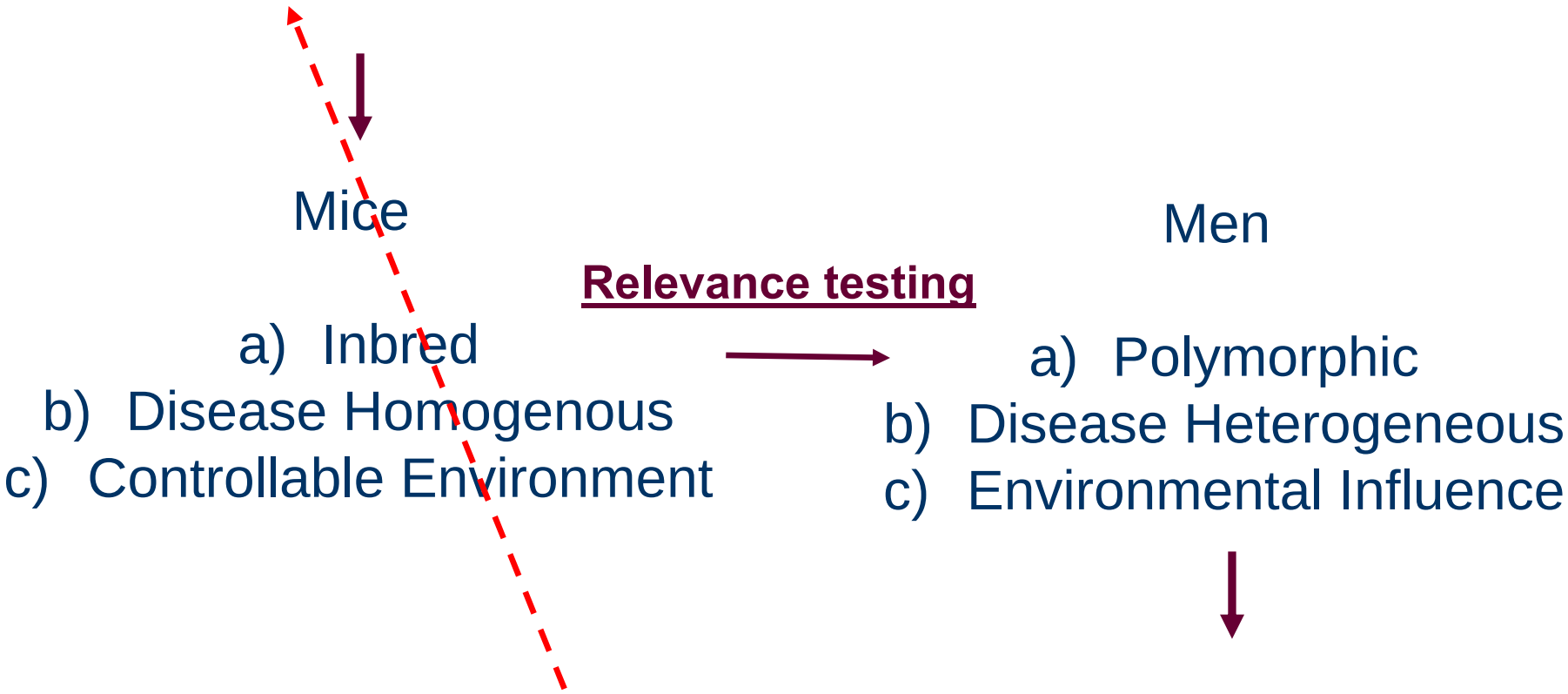
There are three Golden rules that apply to the successful treatment of any disease

Unfortunately we do not know any of them

Anonymous Stanford Professor

A paradigm shift: Evidence Based Research:
Hypothesis *vs* Discovery Driven Research

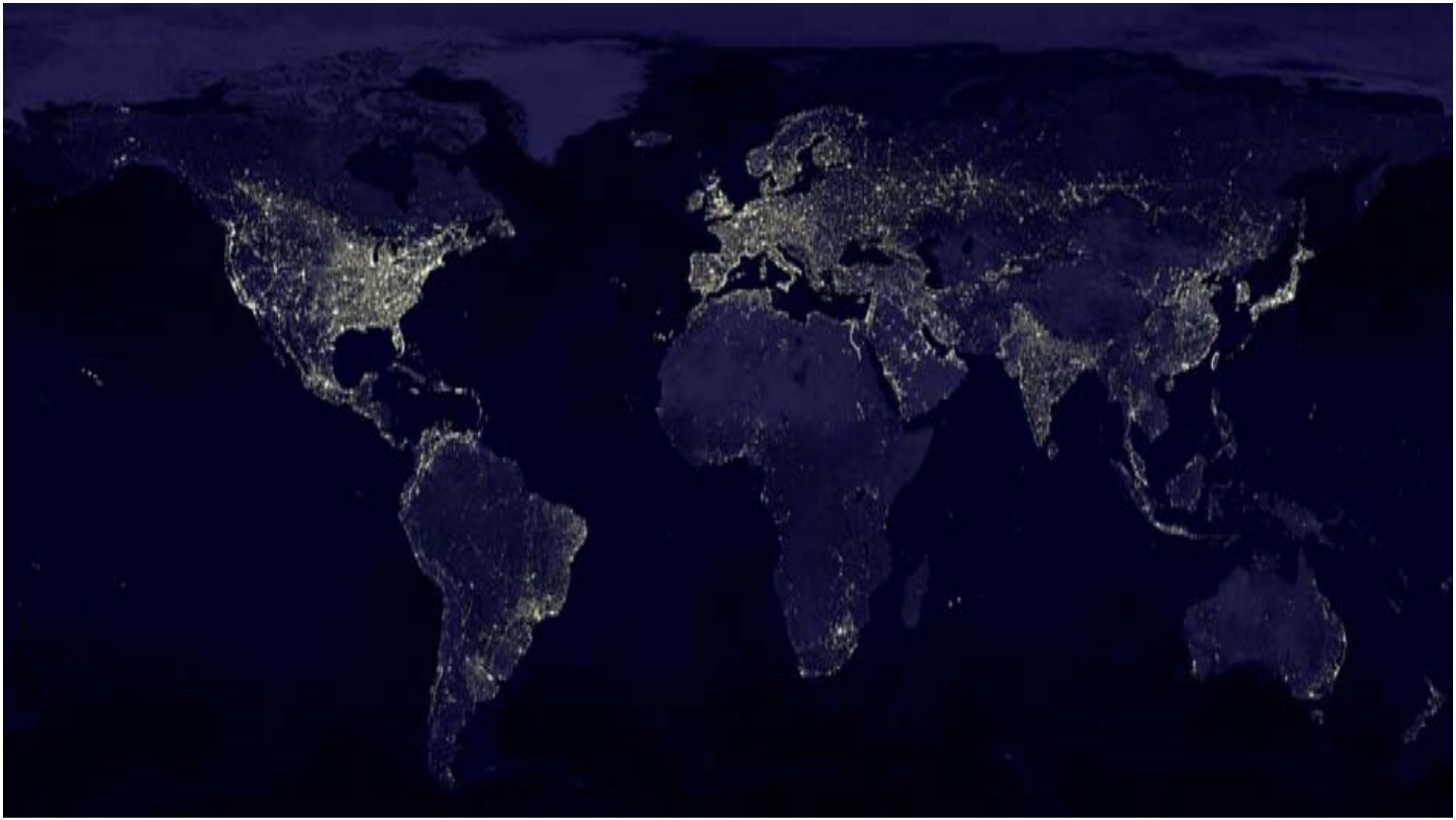
Hypothesis testing most efficient when one variable at the time is analyzed



Hypothesis Generating more realistic in the clinics
Identify **COMMONALITIES** through a **Global Approach**

Marincola J Transl Med 2004; Hörig et al. Nature Med 2005; Littman et al. Clin Sci – 2007

Hypothesis generation in humans requires a global approach



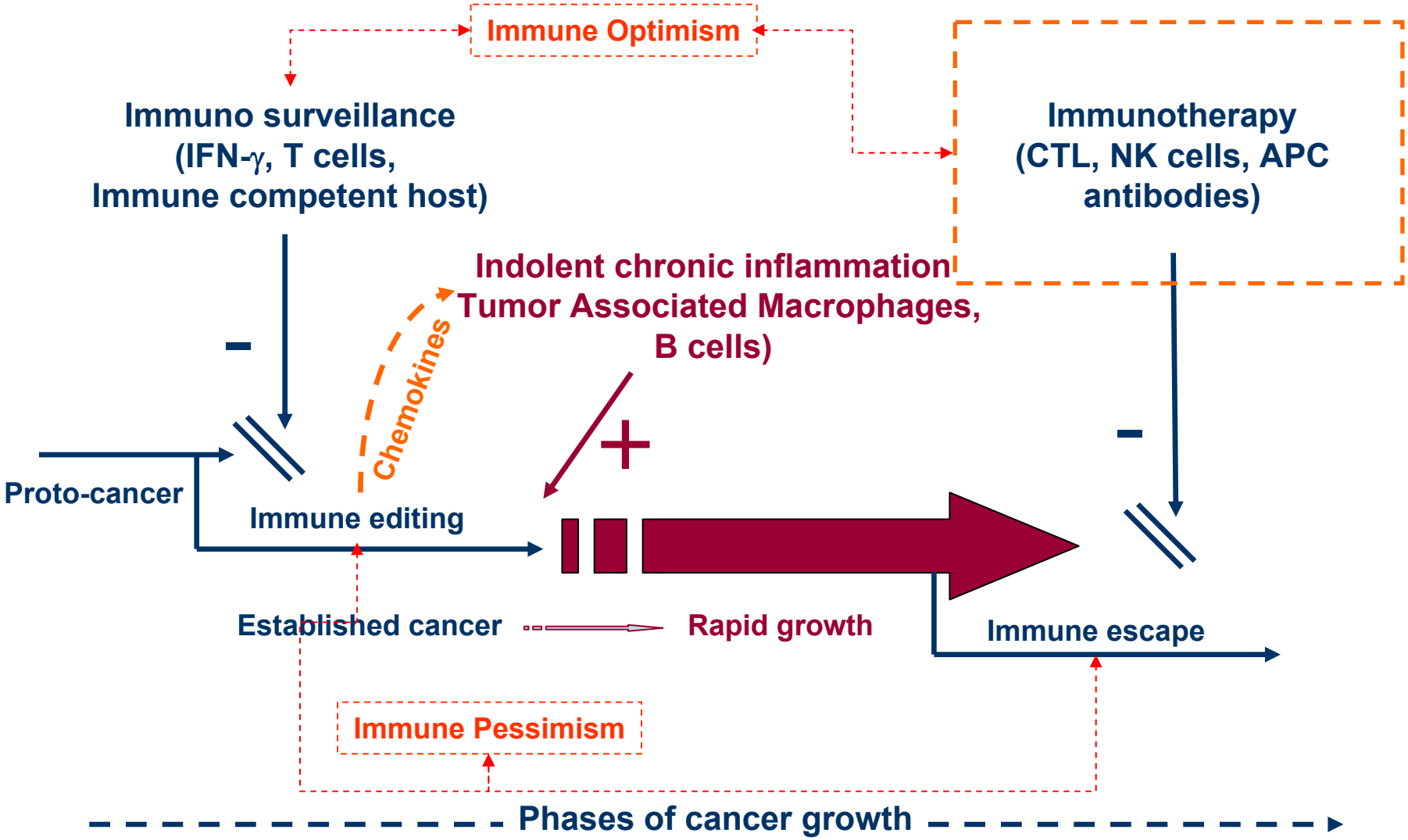
Two Models

- Active specific immunization against melanoma with or without IL-2
- Treatment of basal cell cancer with toll receptor agonists (Imiquimod)

Two Models

- **Active specific immunization against melanoma with or without IL-2**
- **Treatment of basal cell cancer with toll receptor agonists (Imiquimod)**

The role of immunity in its active form generally referred to as "inflammation" at various stages of carcinogenesis and progression



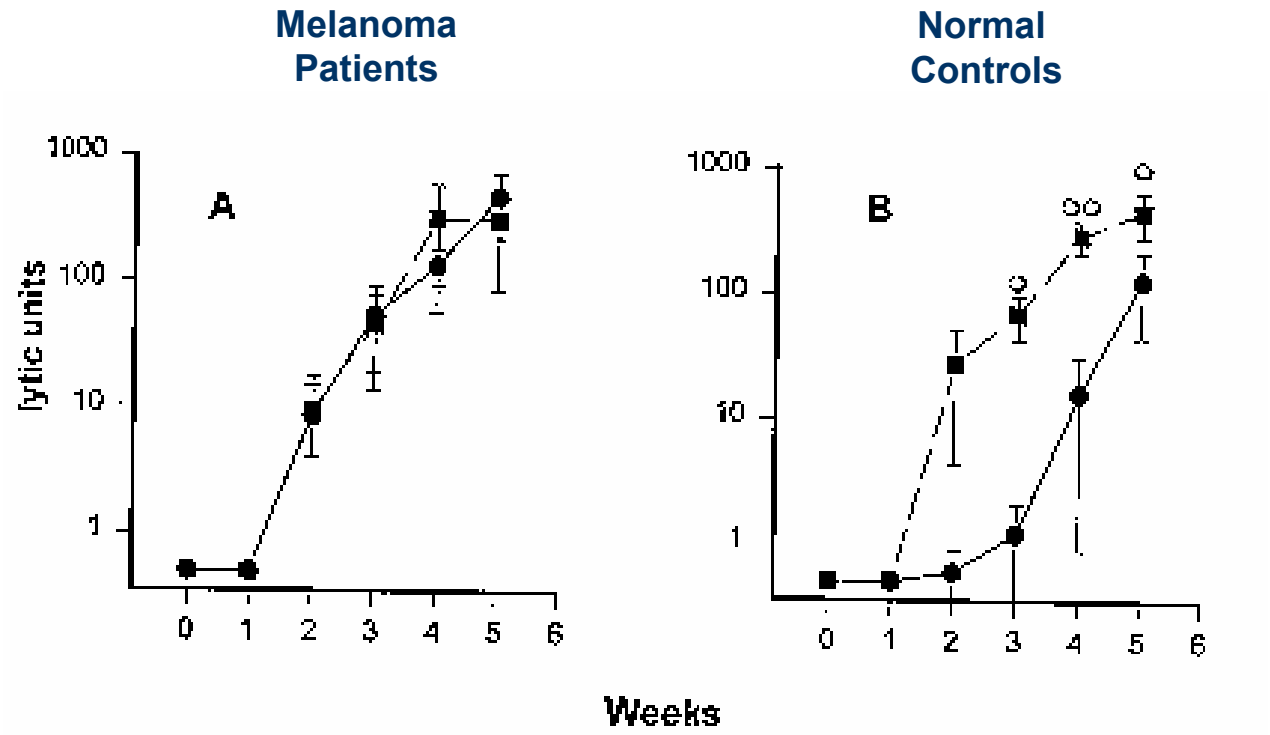
Mantovani, Romero, Palucka and Marincola – The Lancet – in press

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

1st dimension = TCR/HLA/epitope interaction
2nd dimension = Importance of co-stimulation
3rd dimension = Localization at tumor site
4th dimension = Evolving nature of immune response and genetic instability of cancer cells
5th dimension = Heterogeneity of the tumor micro-environment

Is CTL precursor *frequency*
a factor for disease control?

In vitro induction of anti-MART-1 (○) and anti-flu (□) CTL in HLA-A*0201 individuals



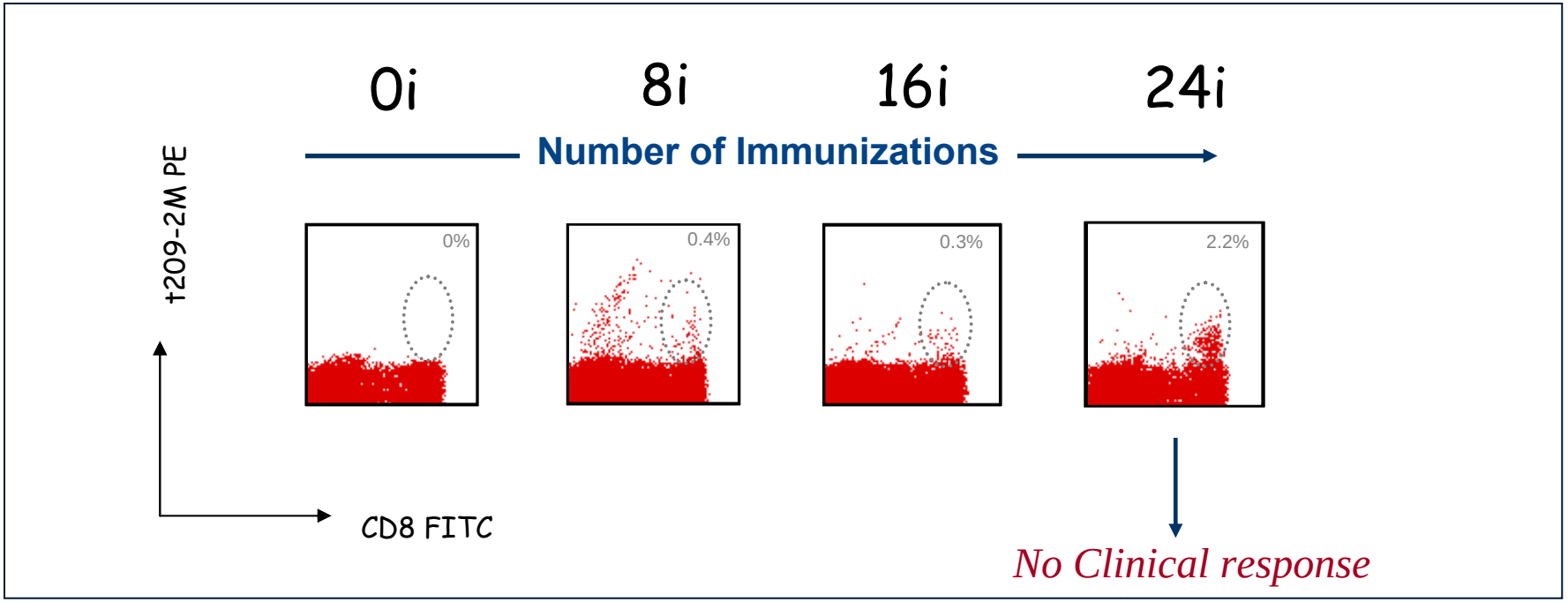
Cytotoxic T Lymphocyte precursor frequency (CTLp*f*):

	Melanoma	Normal
Melan A	1/5,000	1/100,000
Flu M1:	1/1,000	1/1,000

Marincola et al., J. Immunother 1996

The Systemic Response to Anti-Melanoma Vaccines

Model: g209-2M peptide vaccine ± interelukin-2



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

Monsurró et al. J Immunol 2000
Kammula et al. J Natl Canc Inst 2002
Monsurró et al., J Immunol 2002

Is the functional status of CTL a factor for disease control?

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

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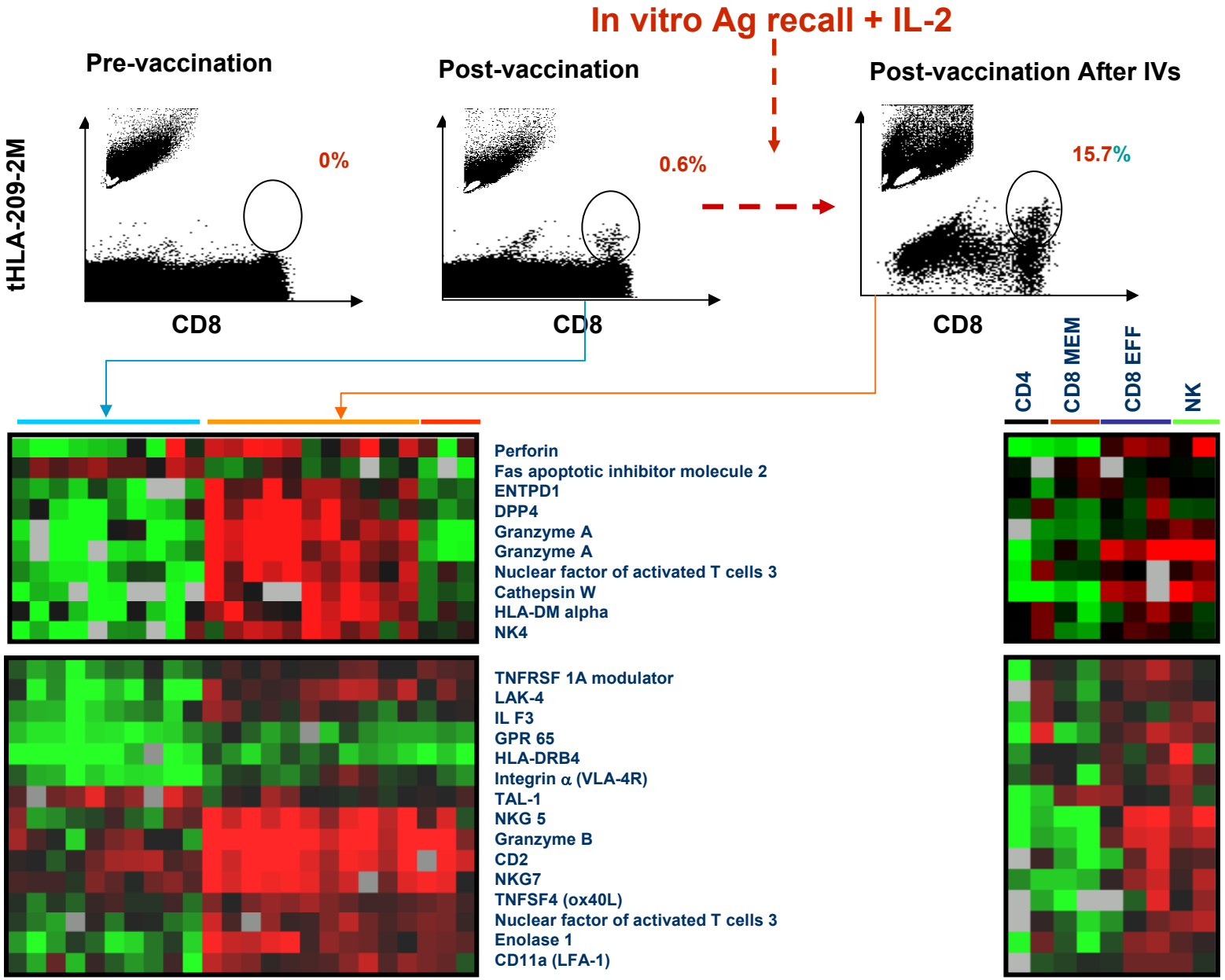
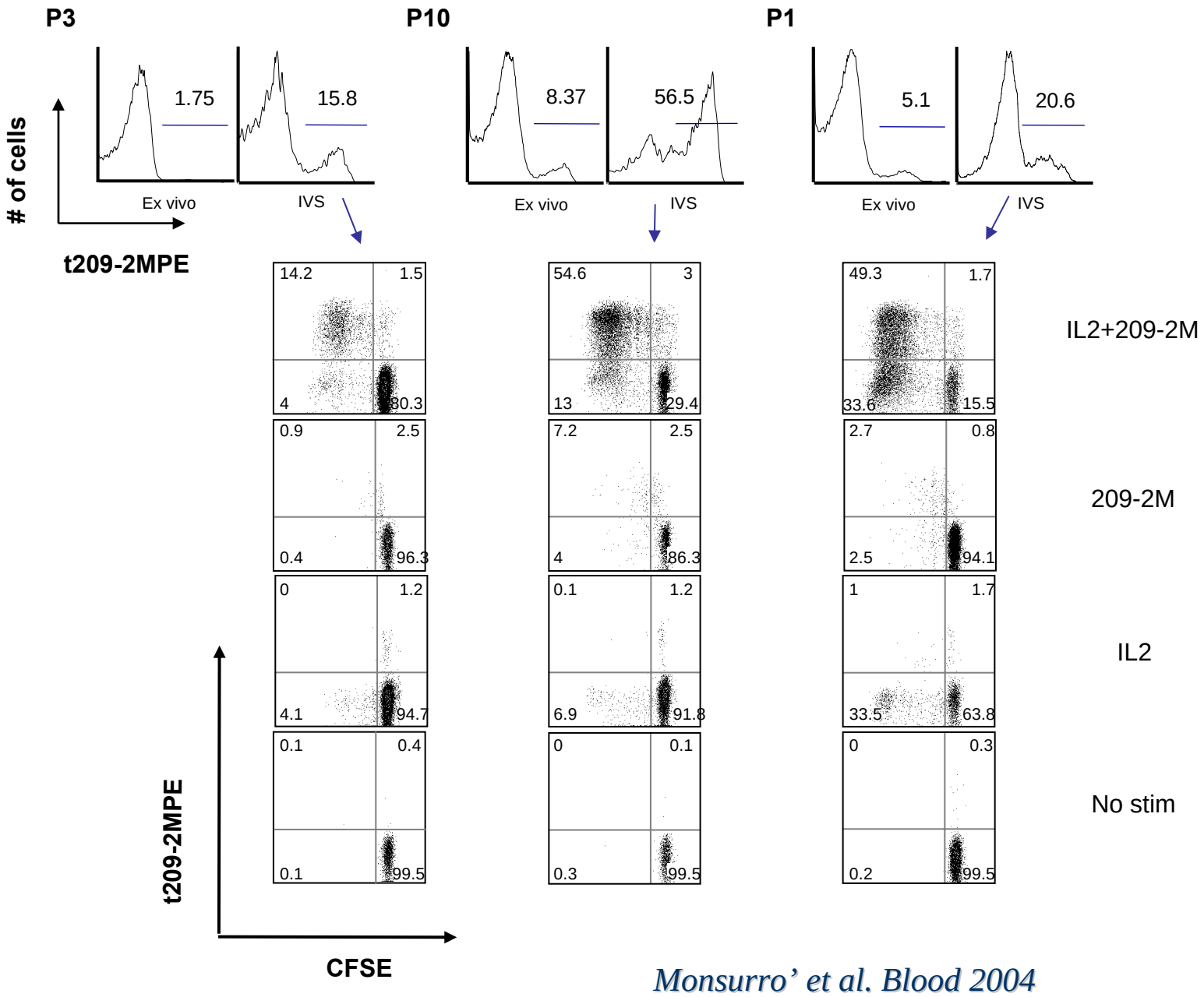
Quiescent phenotype of tumor-specific CD8+ T cells following immunization



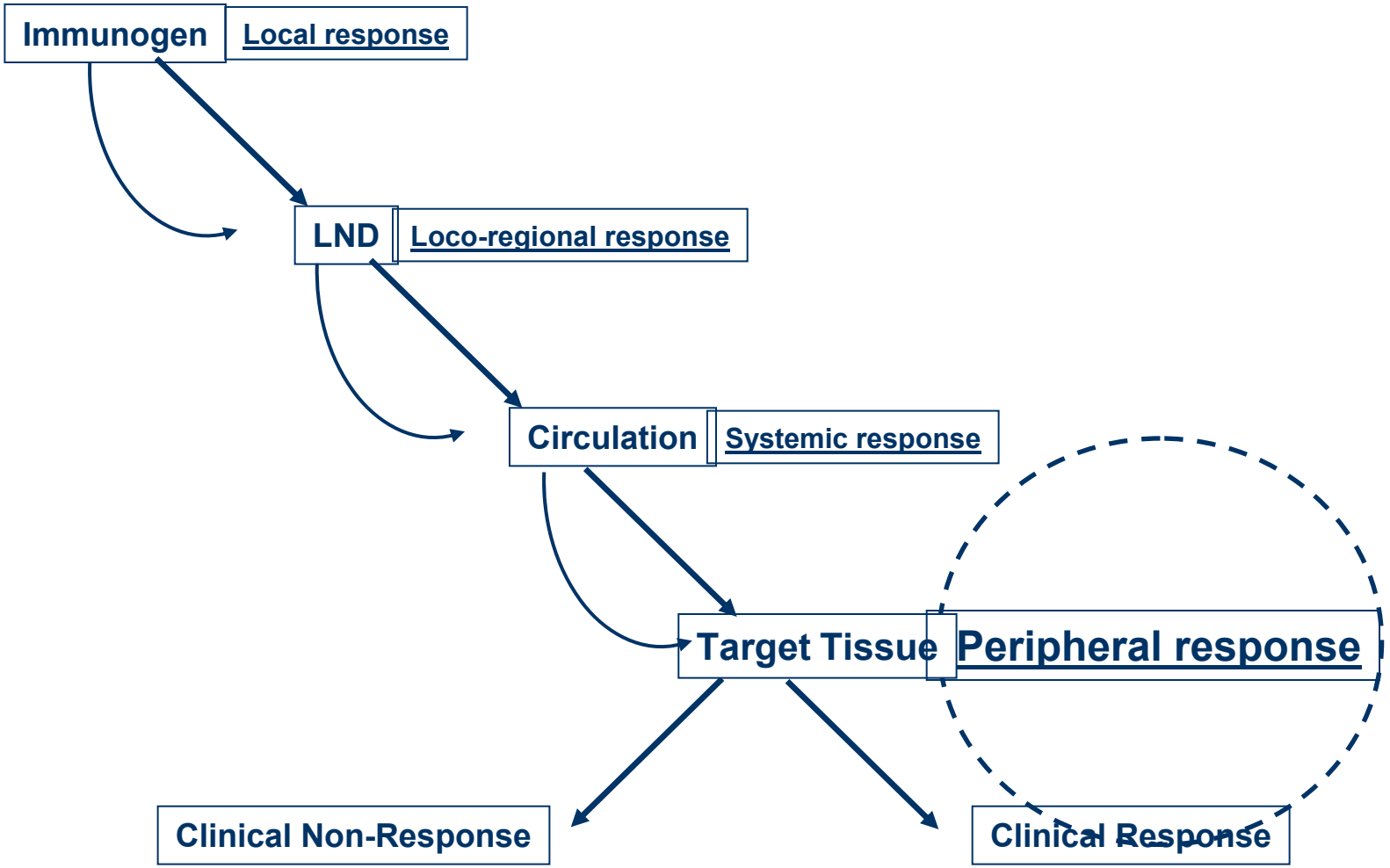
Monsurro' et al. J Immunol 2002

Monsurro' et al. Blood 2004



Monsurro' et al. Blood 2004

Step required for the effective implementation of vaccines



Marincola et al. Trends in Immunol. 2003

Studying the micro-environment

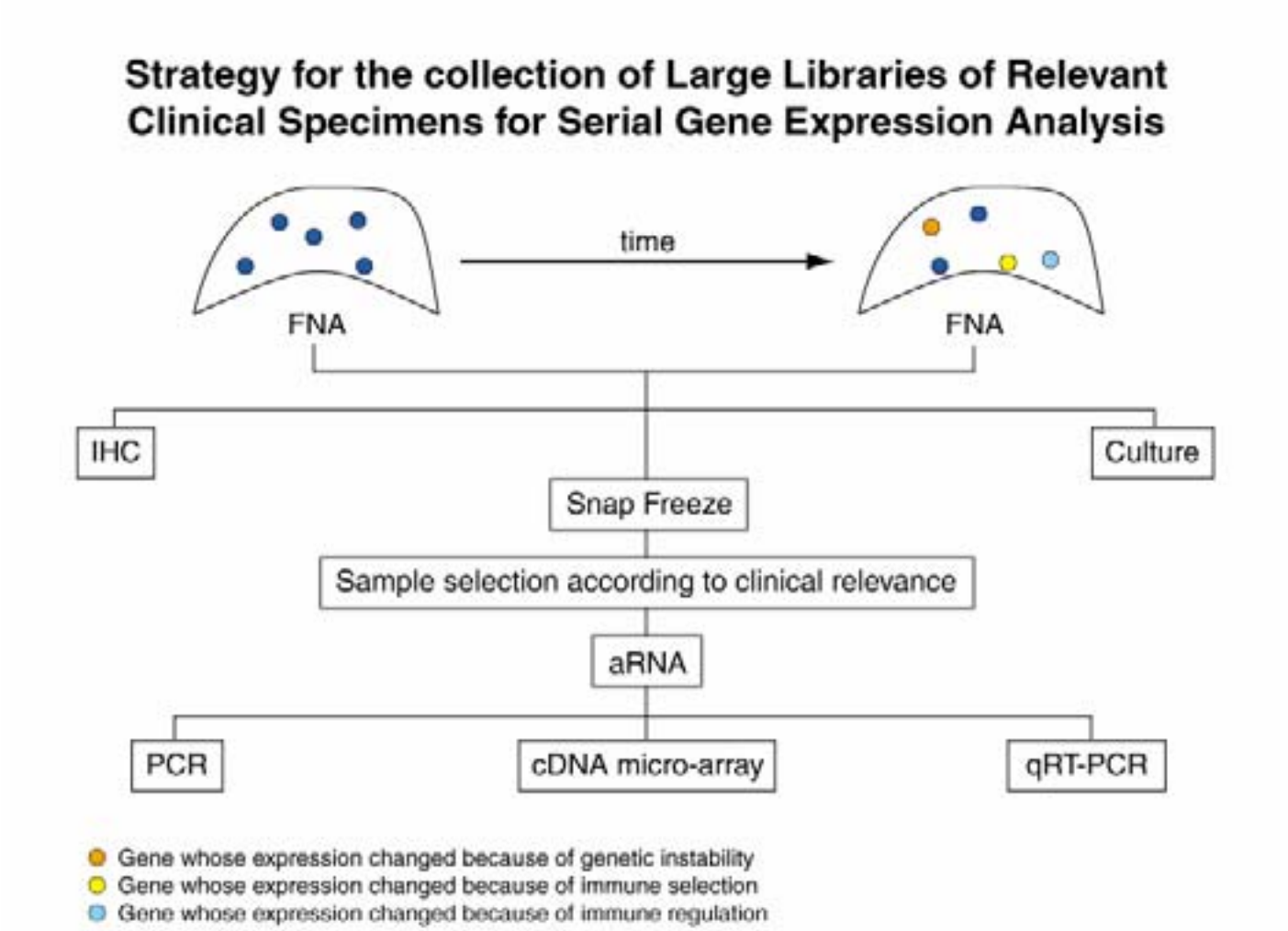
Excisional Biopsies

- Good quantity of material to study
- Do not allow serial sampling of same lesion
- Do not allow prospective assessment of natural history of a given lesion

Fine Needle Aspirates

- Limited quantity of material to study
- They allow serial sampling of same lesion
- They allow prospective follow up of a given lesion

Wang and Marincola, Immunol Today 2000



Wang et al. A Natural History of Melanoma through Serial Gene Profiling. Immunol Today, 2000

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

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Kinetics of IFN-γ and gp-100 expression in melanoma metastases

Treatment: gp100 based vaccine

Question:

Vaccine-elicited T cell may not localize at tumor site.

Results:

Vaccine-elicited T cells

1) localize at tumor site

2) interact with the tumor cells

3) this is not sufficient for tumor rejection

Kammula et al., J. Immunol., 1999

	Tumor Monitoring		
Site	IFN/CD8	Fold Inc	GP100/Actin
R axilla	568		1242
	7586	13.4	1310
L thigh	331		1956
	11865	35.8	3955
R axilla	1187		6186
	7891	6.6	611
L thigh	579		2865
	7788	13.5	894
R thigh	2231		1226
	4452	2.0	235
L chest	1013		1
	5532	5.5	18
L med knee	3247		0
	1649	0.5	1
L lat knee	2865		1
	2131	0.7	1
RL thigh	140		4786
	1585	11.3	2291
RU thigh	466		532
	759	1.6	312
R groin	692		60
	1474	2.1	12

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Tumor Biology

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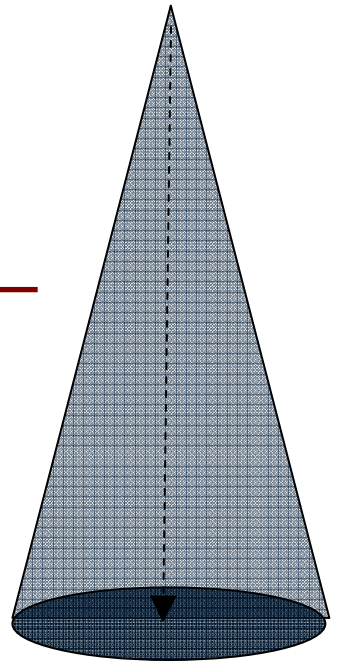
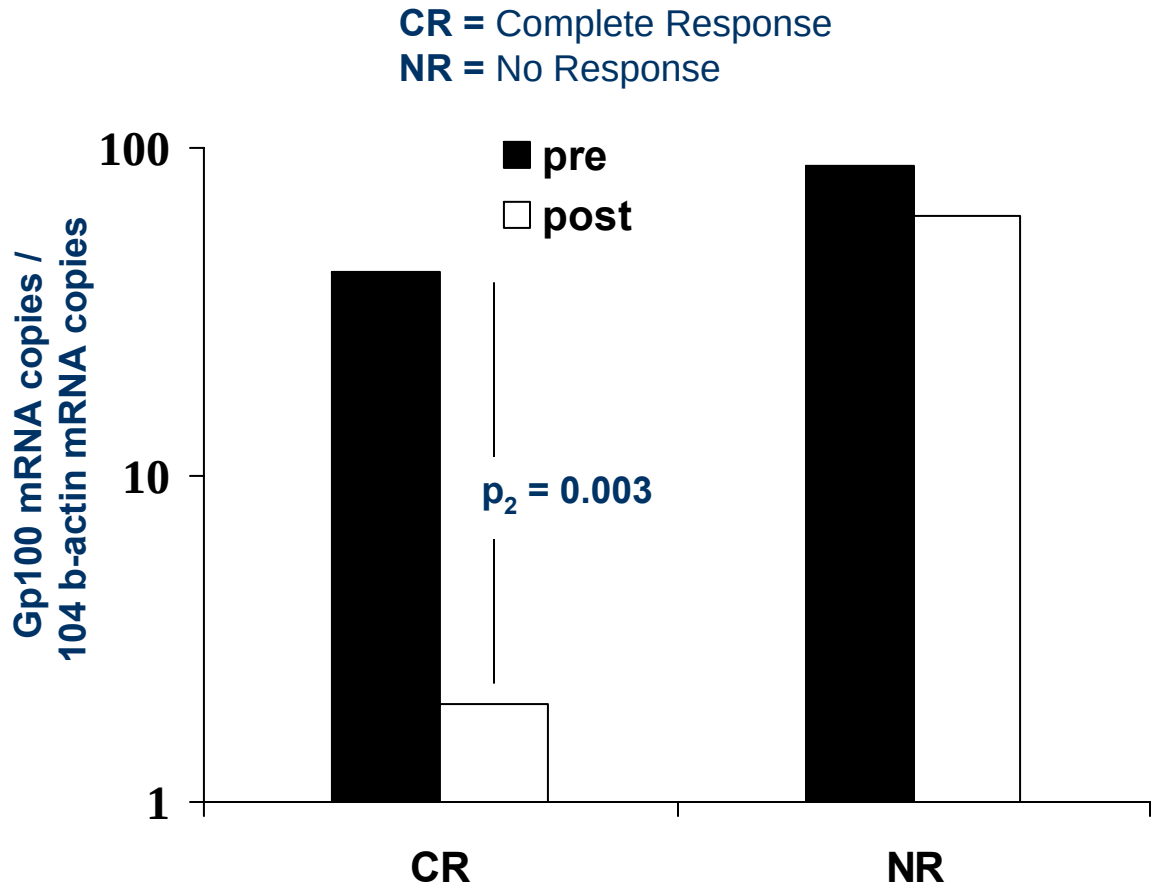


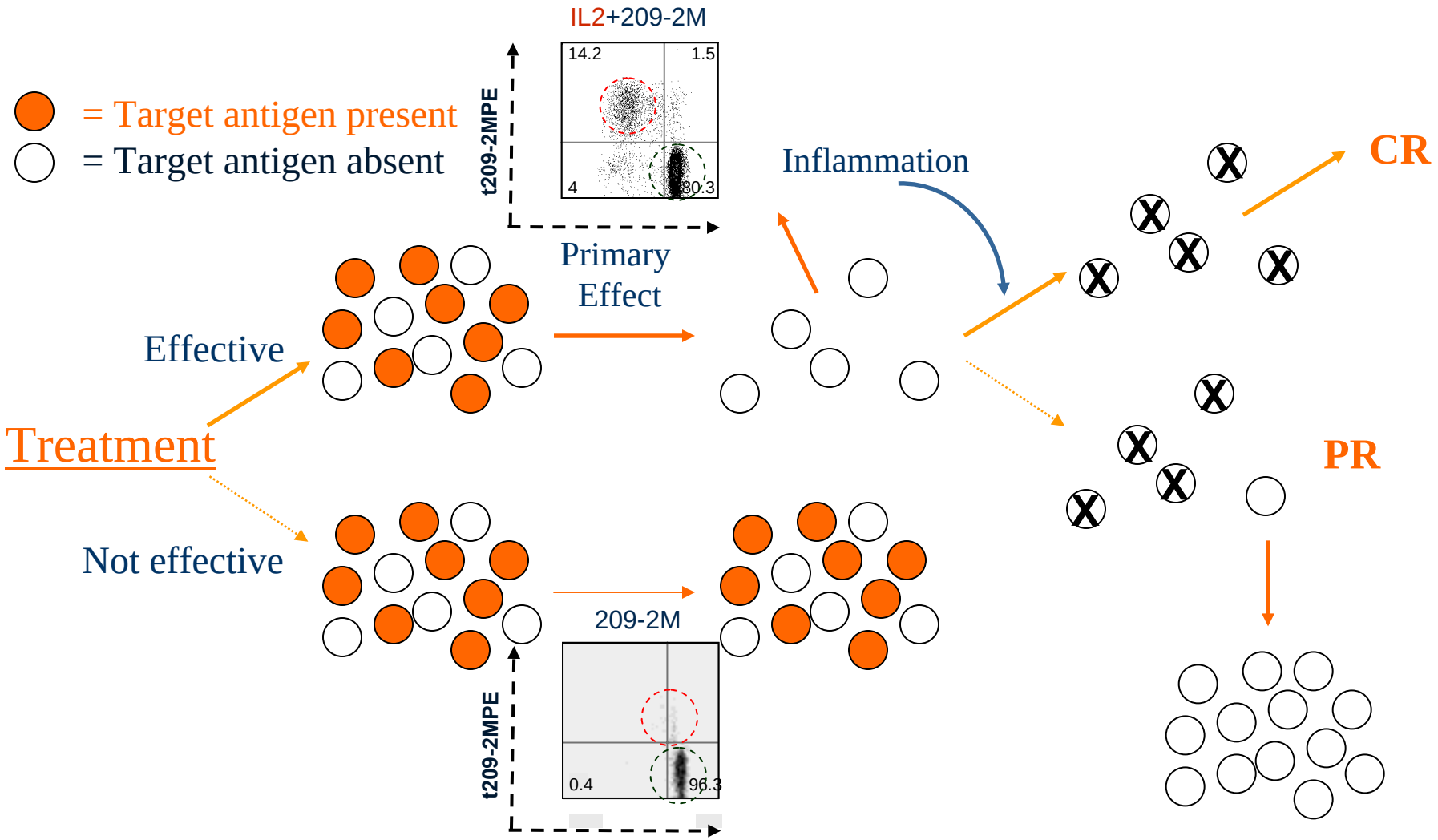
Increasing biological complexity

Tumor variability and evolving with time



Ohnmacht et al. J Immunol. 2001

Proposed hypothesis of how antigen-specific therapy might affect target antigen expression



Marincola et al., Trends in Immunology, 2003

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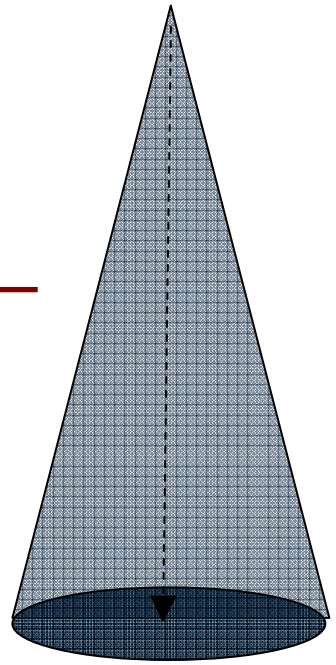
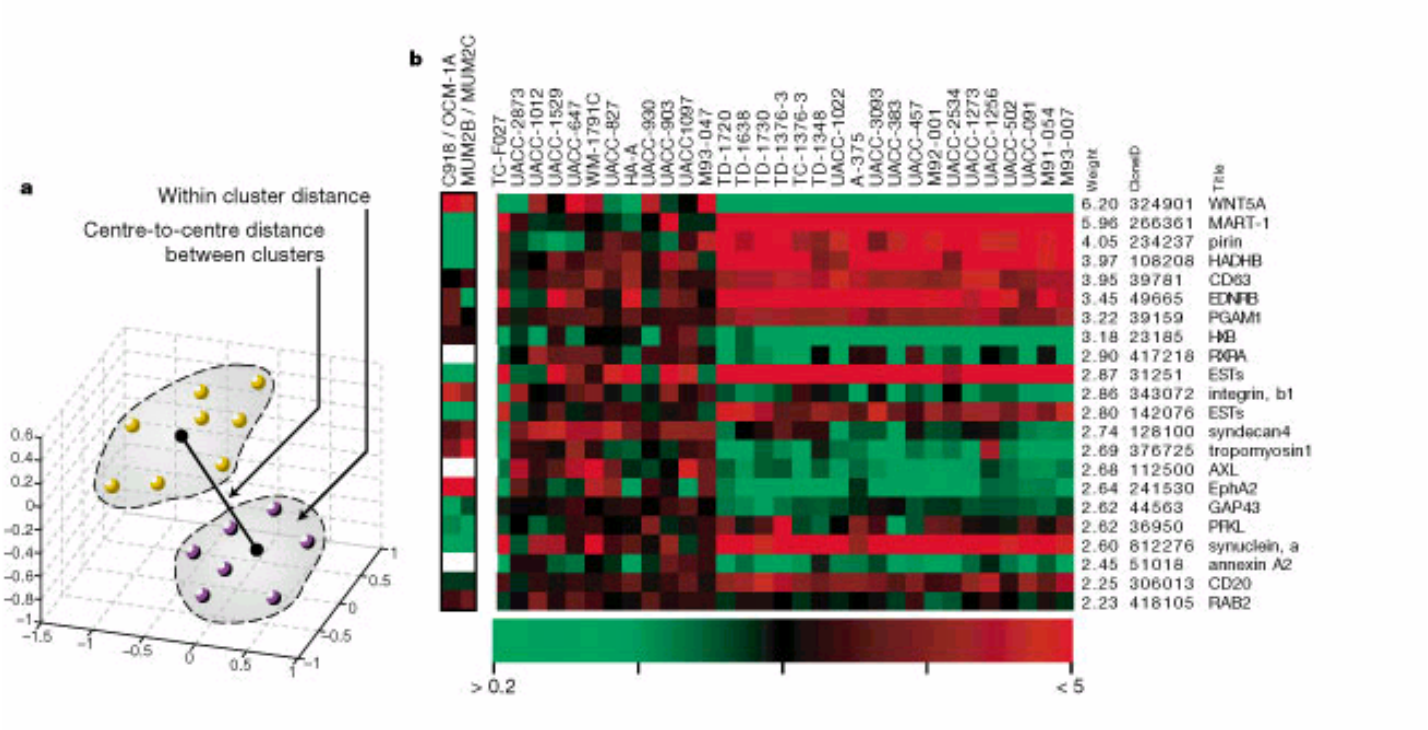


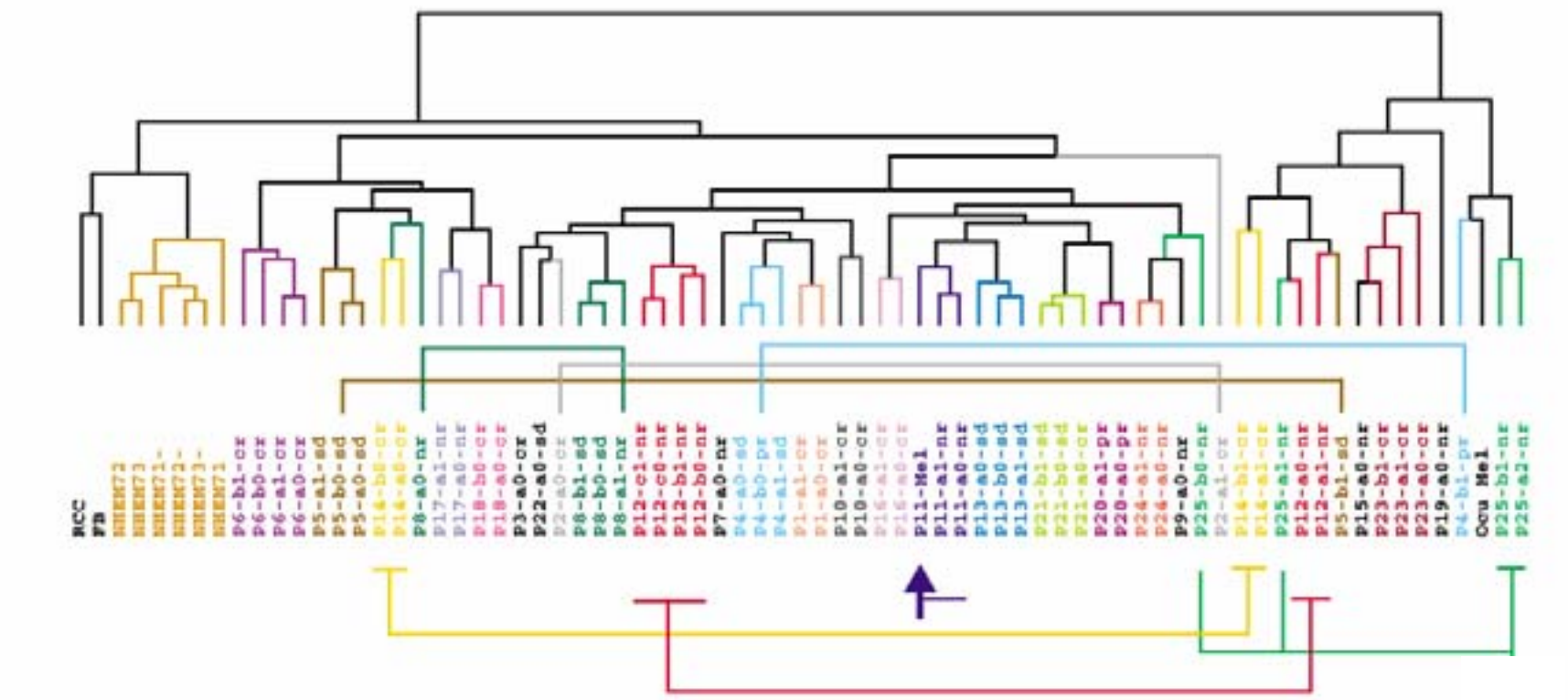
Increasing biological complexity

Identification of two sub-classes of melanoma



Bittner et al, *Nature* 2000.

Unsupervised clustering of melanoma metastases
Sampled by fine needle aspiration (FNA)



Wang et al. Nature Biotech 2000
Wang et al Cancer Res. 2002

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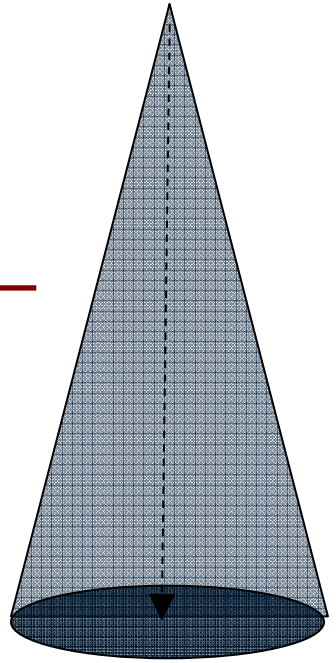
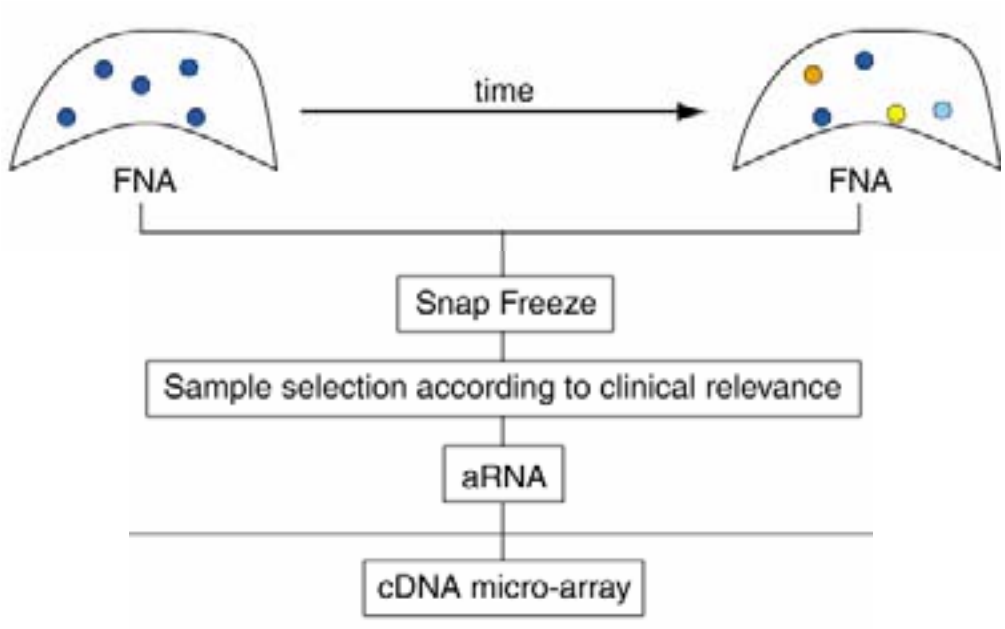


Increasing biological complexity

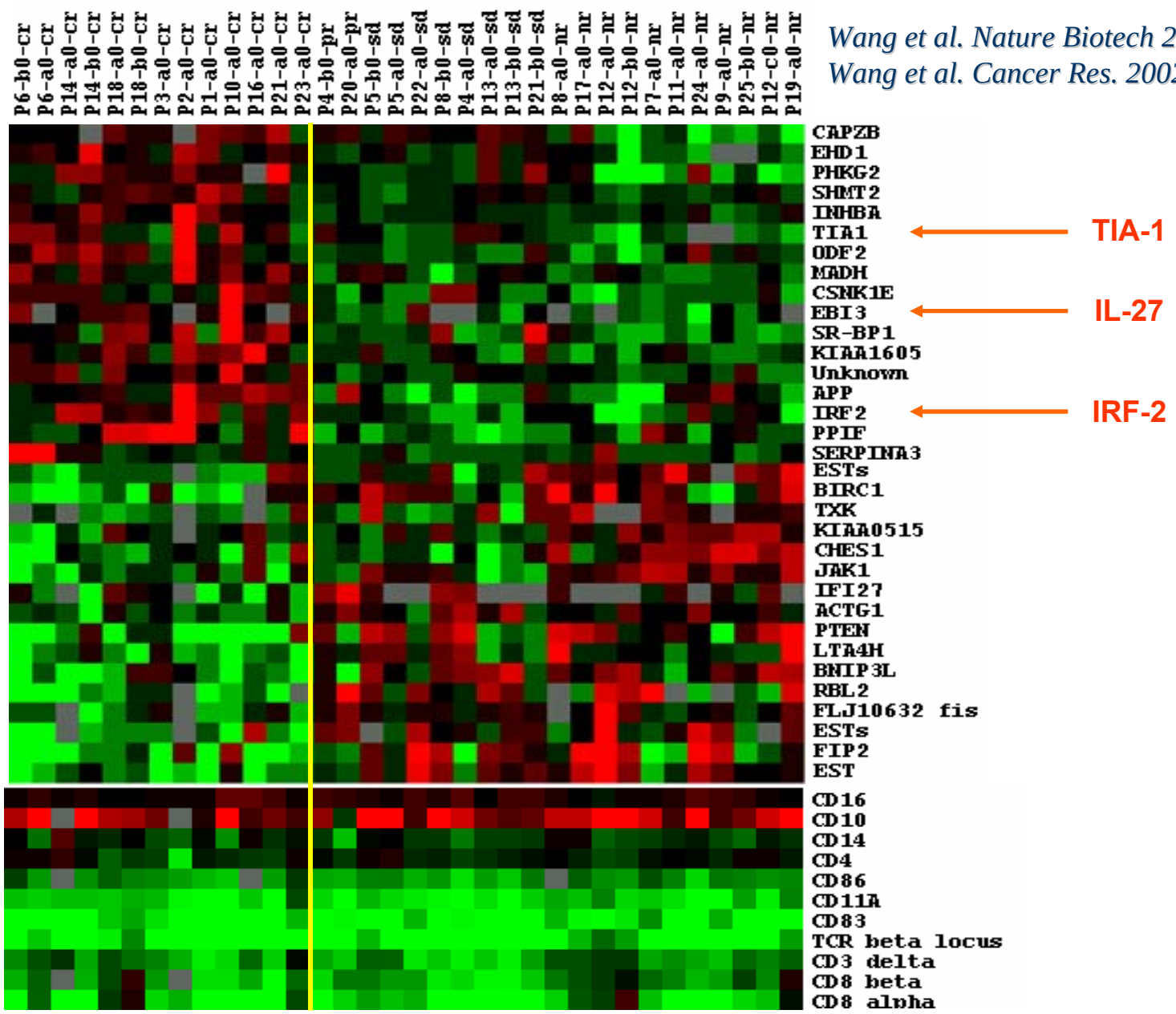
Prediction of response to vaccine plus High-Dose Interleukin-2 Therapy

Pre-treatment FNA



Wang et al. Cancer Res, 2002

Genes differentially expressed pre-treatment in immune responsive metastases

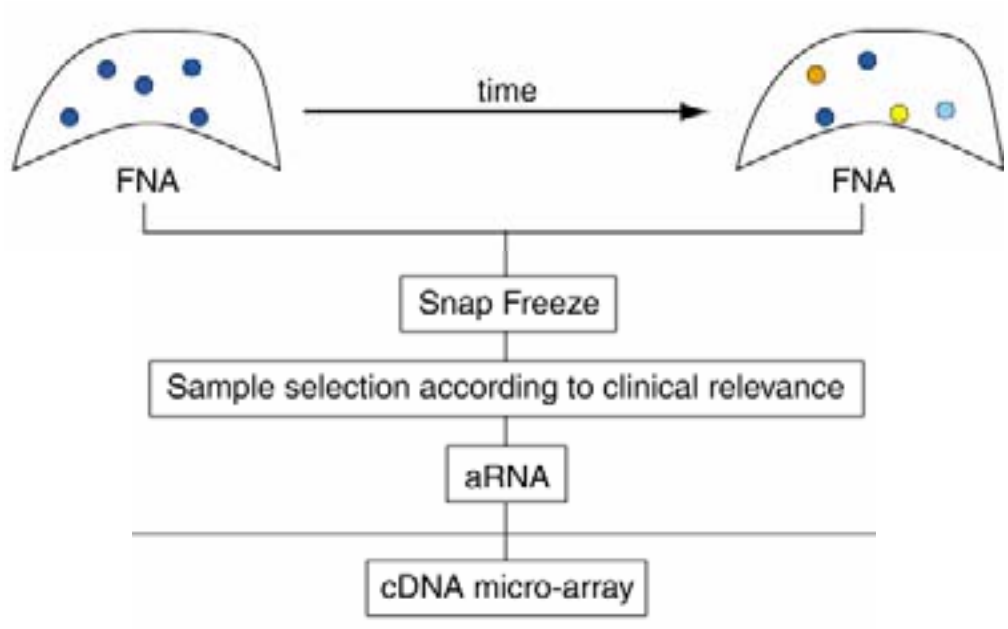


Wang et al. Nature Biotech 2000
Wang et al. Cancer Res. 2002

What is the effect of therapy?

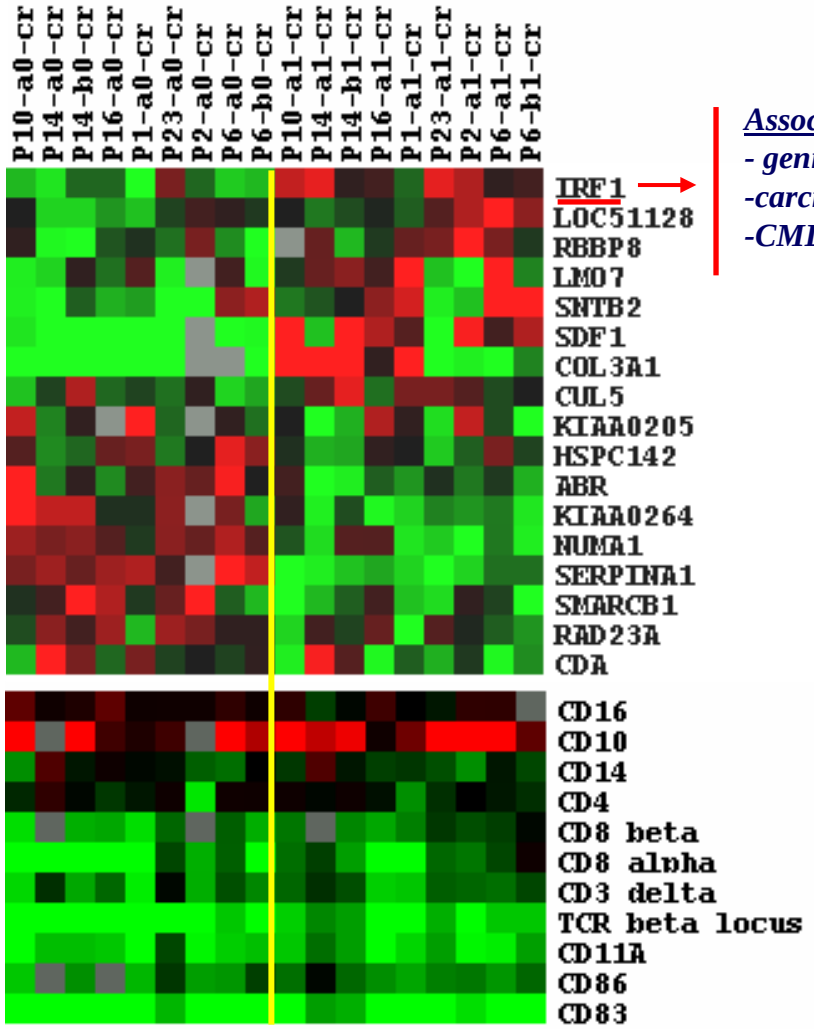
Prediction of response to vaccine plus High-Dose Interleukin-2 Therapy

Pre-treatment FNA **Post-treatment FNA**



Wang et al. Cancer Res, 2002

Genes differentially expressed between pre- *vs* post-treatment CR

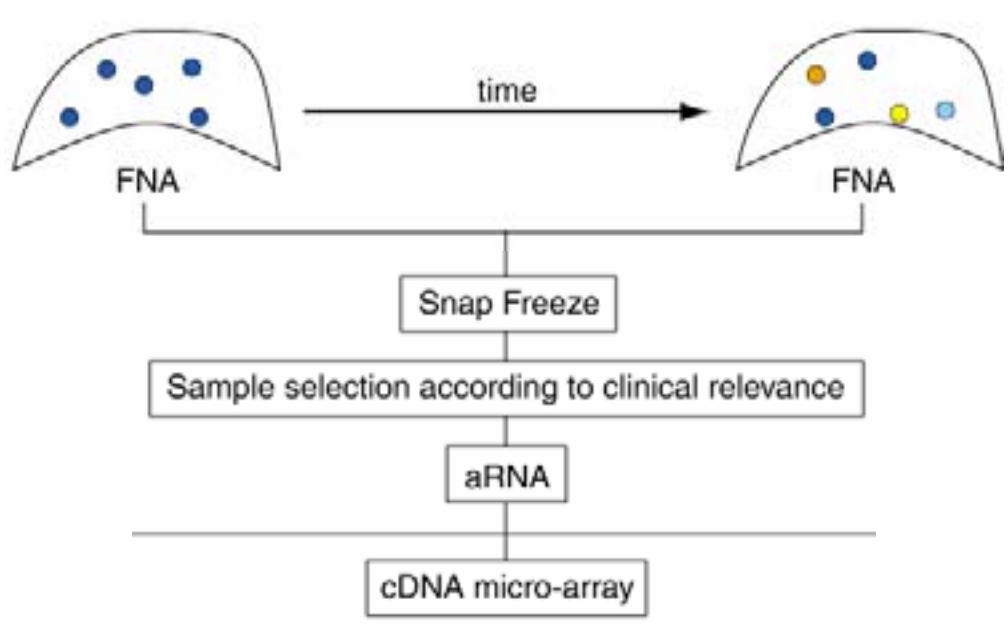


Associated with responsiveness of
- genital warts to Imiquimod
-carcinoid tumors to IFN-α
-CML to IFN-α

Wang et al. Cancer Res. 2002

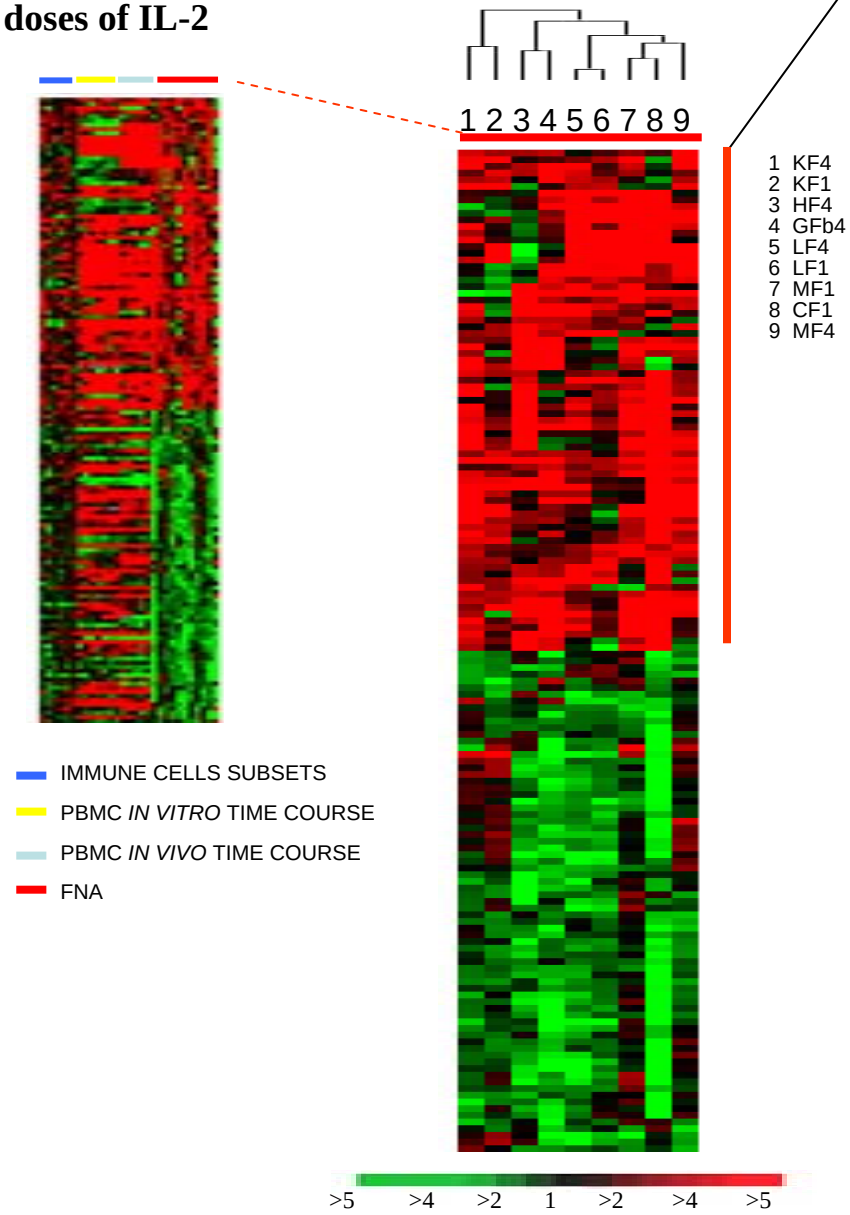
Mechanism(s) of Action of Systemic High-Dose Interleukin-2 Therapy

Pre-treatment **3 hr after 1st and 4th dose**



Panelli et al. Genome Biol, 2002

FNA and the tumor microenvironment: Genes differentially expressed in FNA 3hr post 1 and 4 doses of IL-2



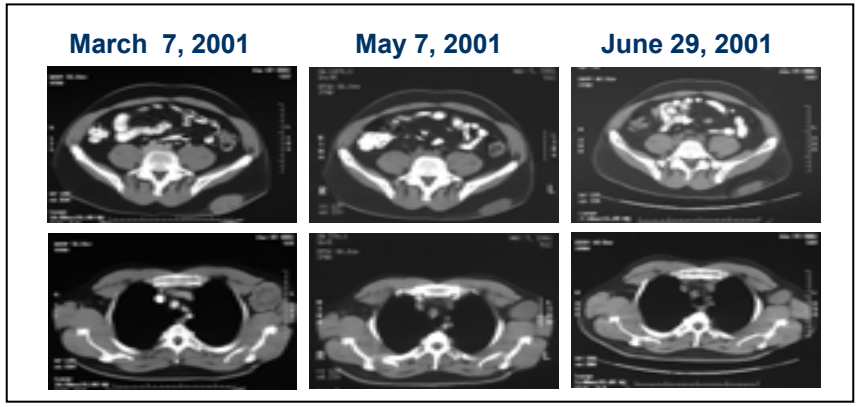
Panelli et al., Genome Biology,2002,3(7):res0035.1-0035.17

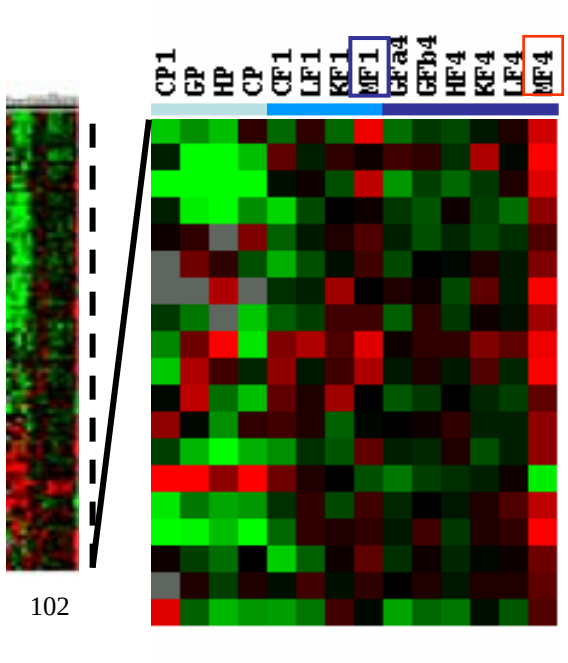
Highest median across experiments: cell surface, adhesion inflammatory proteins

MHC class II DR alpha
MHC class II DR beta
Grancalcin Ca2+ binding protein
Calgranulin Ca2+ binding protein
CD62L L-selectin
CD45
V-CAM-1
CD64
CD29 integrin=beta 1 fibronectin receptor
(Fibronectin 1)
Keratin 10
IL-1 R
IL-1 receptor antagonist
IL-2 R beta chain
TNF-a induced protein 3
TGFβ receptor
Interferon-γ IEF SSP5111upregulated protein
(HSP70)
MxA/interferon induced cellular resistance protein
MxB interferon induced cellular resistance protein
(Interferon-a inducible protein IFI-6-16)
Guanylate binding protein 1 interferon inducible
IRF-1 interferon regulatory factor-1
IFN induced 56KDa protein
IFNγ receptor alpha chain
Nmi=IL-2 and IFN-γ inducible potentiator of STAT
C-C chemokine receptor 1
GRO-1
(MCP-1)
MCP-3
MIP-1beta
MIP-1 alpha
PARC=DC-CK1
Monocyte neutrophils elastase inhibitor
IL-8 chemokine
Human insulin like growth factor
Plasminigen activator urokinase

Genes associated with immune response during IL-2 therapy

Panelli et al Genome Biol 2002

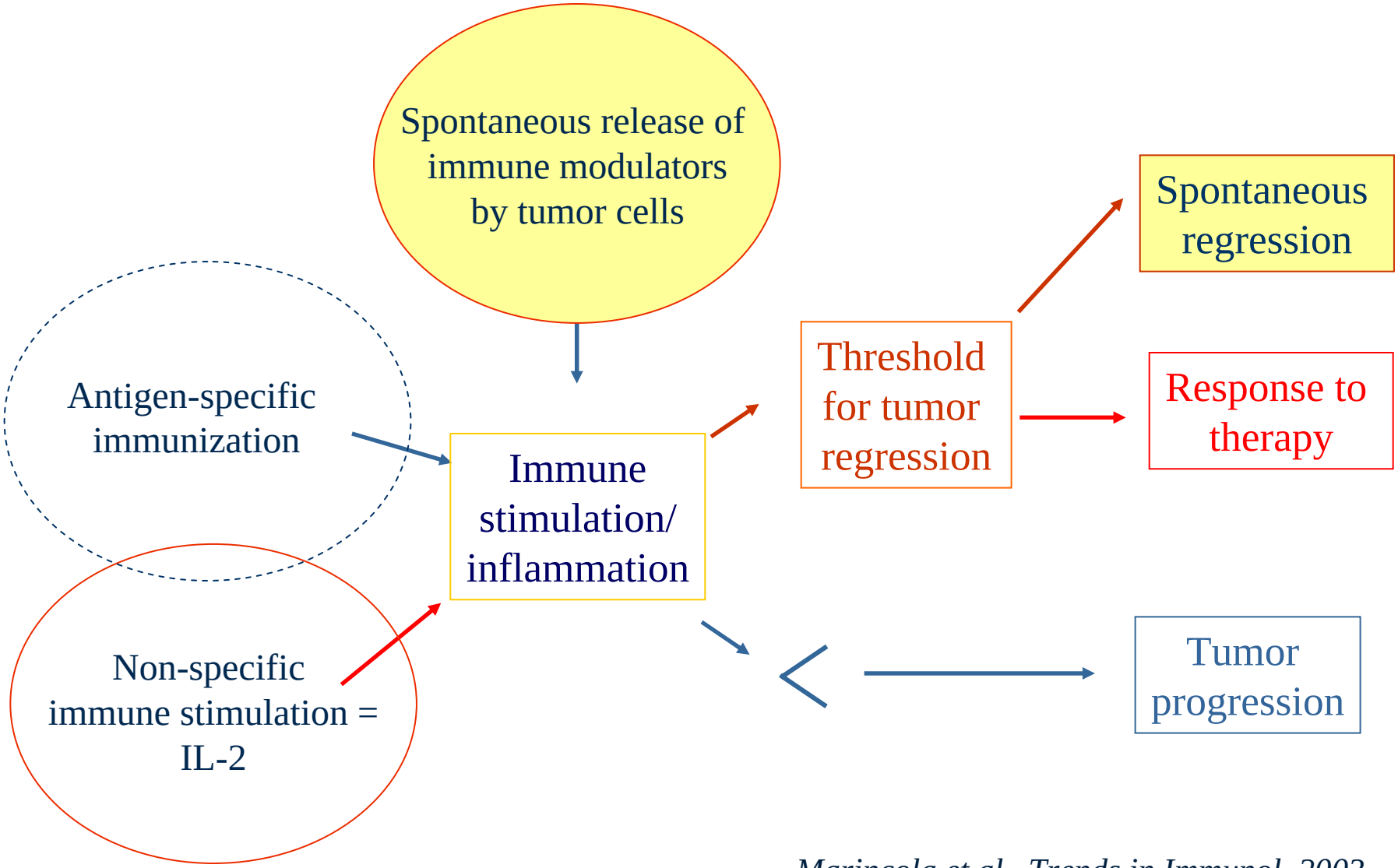




- CR LESSION POST IMMUNIZATION EXPRESSED GENE
- SIMILAR EXPRESSION TO NK EXPOSED TO II2
- UPREGULATED IN ACUTE REJECTION OF KIDNEY TRANSPLANT (PBMC AND RENAL BIOPSY TISSUE *Sorwal, H. Immunol, 2001*)

TIA-1=nucleolysin cytotoxic granule
NK4= natural killer cell protein 4
NKG5=granulysin
EBI3
TCR alpha
DAG kinase
HLA class II region expressed gene KE4
MHC class II DR beta
SERPINB1=Leukocyte elastase inhibitor
MIP-1 delta
FGF-13
STIM1=Stromal interaction molecule 1
VEGF
CD62 P selectin
GALECTIN 1
GALECTIN 1
N-Myc
DAP-1
53BP1=p53 binding protein

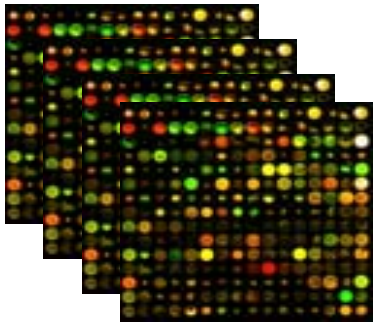
Postulated algorithm of tumor immune responsiveness



Marincola et al., Trends in Immunol. 2003

- **What happens during tissue-specific immune-mediated rejection?**

- **Can a constant mechanism be identified that is necessary and sufficient for the resolution of the pathogenic process (viral clearance, tumor rejection)?**



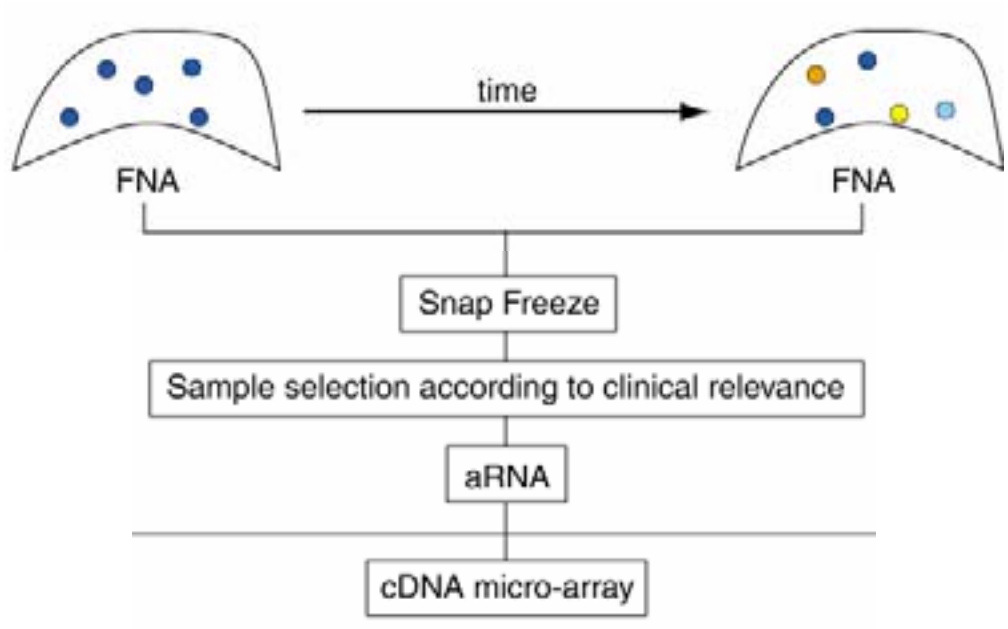
Double Blind Vehicle Control Study To Evaluate Apoptosis in Basal Cell Carcinoma Treated With Aldara ™ (Imiquimod Cream, 5%) Applied Once or Twice a Day

NNMC Bethesda Dermatology Service

Department of Transfusion Medicine Section of Immunogenetics, NIH

Local applications of the TLR-7 agonist Imiquimod for the treatment of Basal Cell Cancer

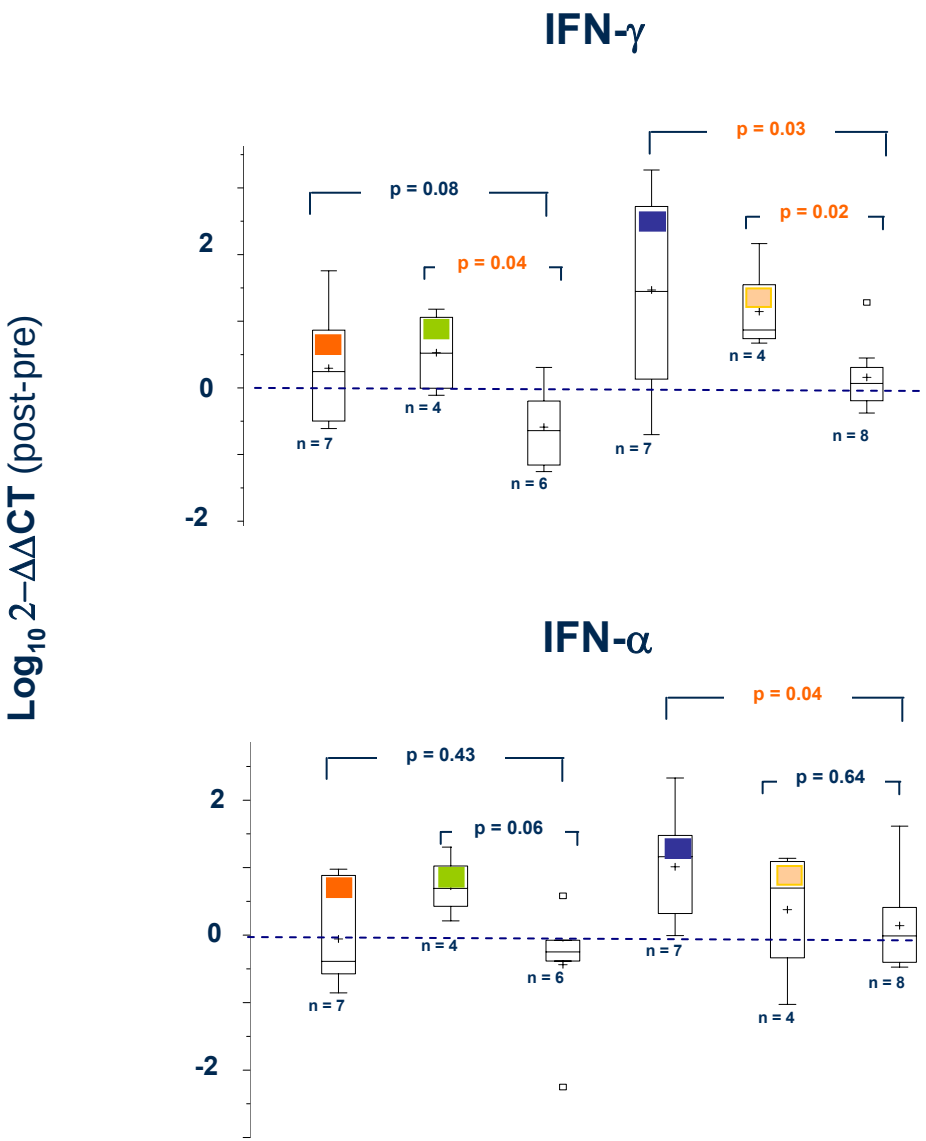
Pre-treatment **2-8 days after starting treatment**

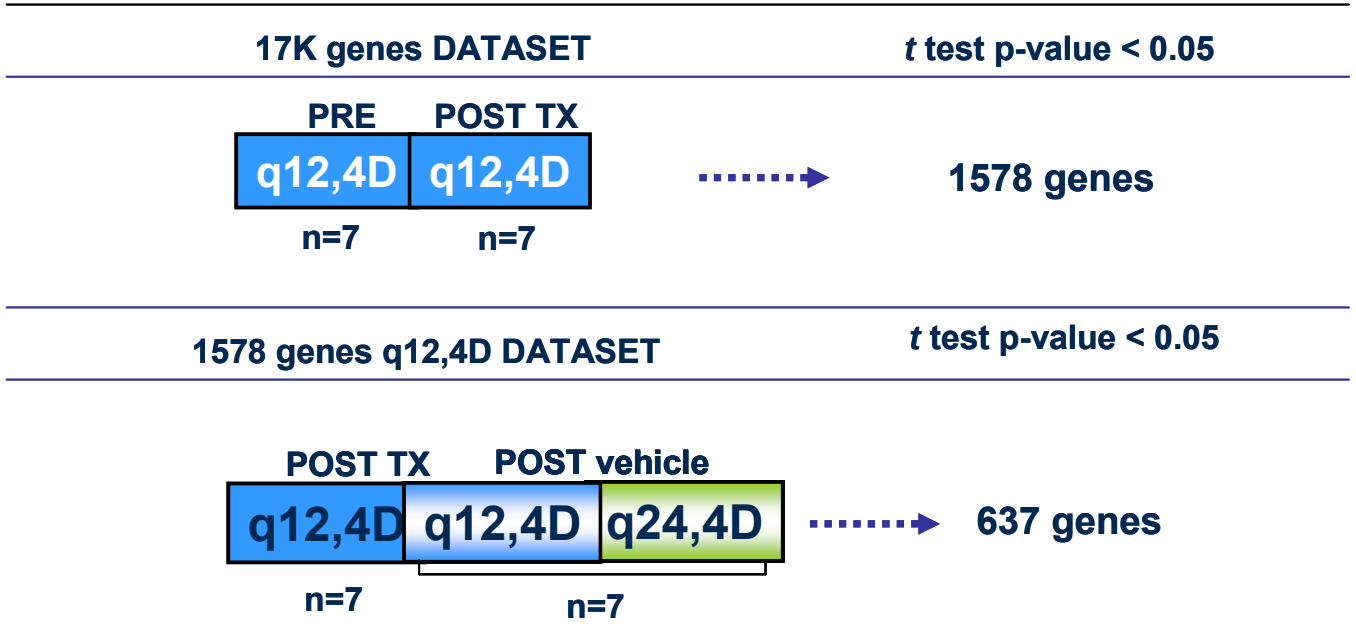


Panelli et al. Genome Biol - 2007

Vehicle-controlled study of the effect of Imiquimod on BCC

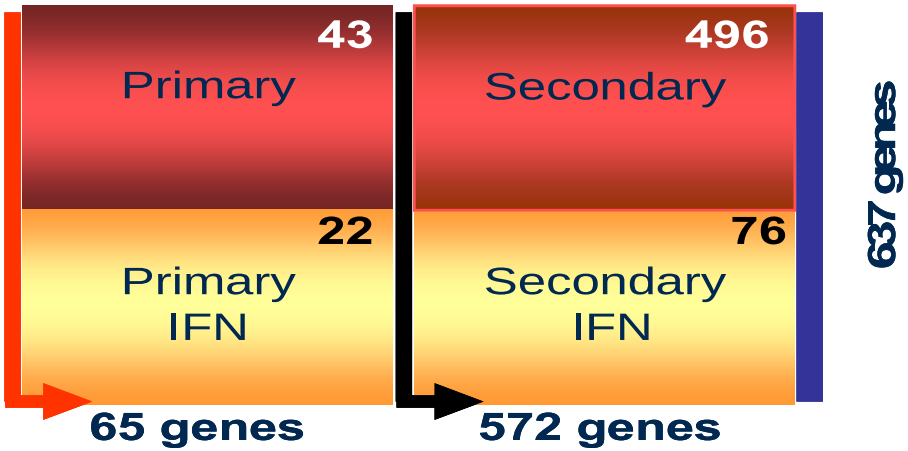
Patient ID	Cohort	Doses Received	EOT → Bx Time Lapse (hrs)	Histology	ΔCD8	ΔCD56	Tumor at EOT
P5	Imiq q12 x 2 days	4	13	Nodular	0	-1	+
P6	Imiq q12 x 2 days	4	14	Undetermined	0	0	+
P17	Imiq q12 x 2 days	4	35	Undetermined	NE	NE	-
P18	Imiq q12 x 2 days	4	33	Nodular	+1	0	+
P30	Imiq q12 x 2 days	4	15	Nodular	0	0	-
P38	Imiq q12 x 2 days	4	17	Nodular	0	0	+
P231	Imiq q12 x 2 days	4	21	Undetermined	+1	0	+
P10	Vehic q12 x 2 days	4	12	Nodular	0	0	+
P23	Vehic q12 x 2 days	2	15	Nodular	+2	0	+
P26	Vehic q12 x 2 days	4	45	Nodular	0	0	+
			Mean±SD = 22.0±11.5				
P1	Imiq q12 x 4 days	8	8	Nodular	0	0	+
P21	Imiq q12 x 4 days	8	41	Nodular	0	+1	+
P22	Imiq q12 x 4 days	8	11	Nodular	+1	0	-
P40	Imiq q12 x 4 days	8	17	Nodular	+1	+1	-
P42	Imiq q12 x 4 days	8	3	Undetermined	+3	0	-
P129	Imiq q12 x 4 days	8	18	Nodular	+1	0	+
P135	Imiq q12 x 4 days	8	21	Superficial	+1	+1	-
P41	Vehic q12 x 4 days	8	27	Nodular	+1	0	+
P134	Vehic q12 x 4 days	8	18	Nodular	+2	0	+
P8	Vehic q12 x 4 days	8	20	Nodular	0	0	+
P20	Vehic q12 x 4 days	8	16	Superficial	0	0	+
			Mean±SD = 18.2±10.0				
P11	Imiq q24 x 4 days	4	26	Nodular	0	+1	+
P28	Imiq q24 x 4 days	3	19	Nodular	NE	NE	+
P112	Imiq q24 x 4 days	4	44	Nodular	0	0	+
P214	Imiq q24 x 4 days	4	51	Nodular	+2	+1	-
P4	Vehic q24 x 4 days	4	13	Superficial	NE	0	-
P13	Vehic q24 x 4 days	4	30	Nodular	0	-1	+
P36	Vehic q24 x 4 days	4	15	Superficial	0	0	+
			Mean±SD = 28.3±14.5				
P233	Imiq q24 x 8 days	8	32	Undetermined	0	+1	+
P132	Imiq q24 x 8 days	8	159	Undetermined	0	NE	-
P24	Imiq q24 x 8 days	8	48	Superficial	+1	0	-
P3	Imiq q24 x 8 days	8	12	Undetermined	-1	0	+
P2	Vehic q24 x 8 days	8	6	Undetermined	0	+2	+
P15	Vehic q24 x 8 days	8	21	Nodular	0	0	+
P27	Vehic q24 x 8 days	8	26	Nodular	NE	NE	+
P137	Vehic q24 x 8 days	8	11	Superficial	0	0	+
			Mean±SD = 39.4±50.2				

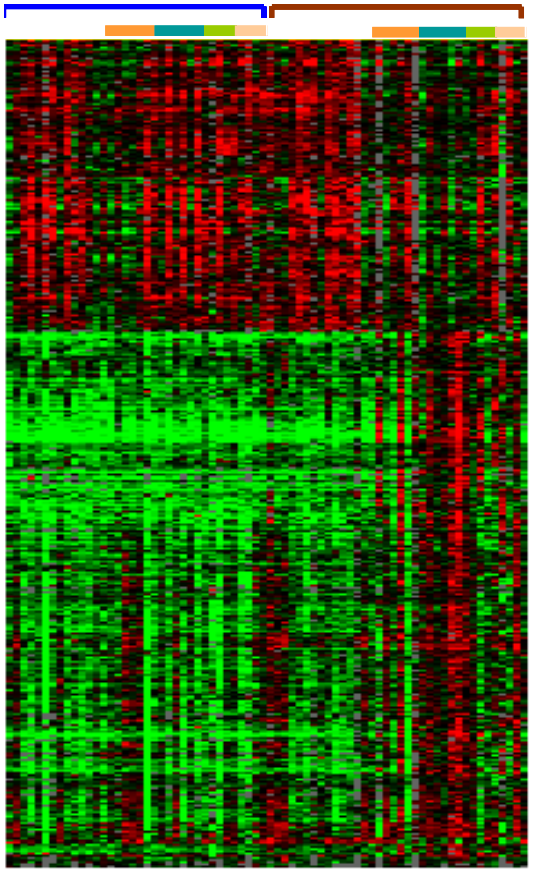

IFN-γ
p = 0.03
p = 0.08
p = 0.02
p = 0.04
2
0
-2
n = 7
n = 4
n = 6
n = 7
n = 4
n = 8
IFN-α
p = 0.04
p = 0.43
p = 0.64
p = 0.06
2
0
-2
n = 7
n = 4
n = 6
n = 7
n = 4
n = 8
Log10 2−ΔΔCT (post-pre)

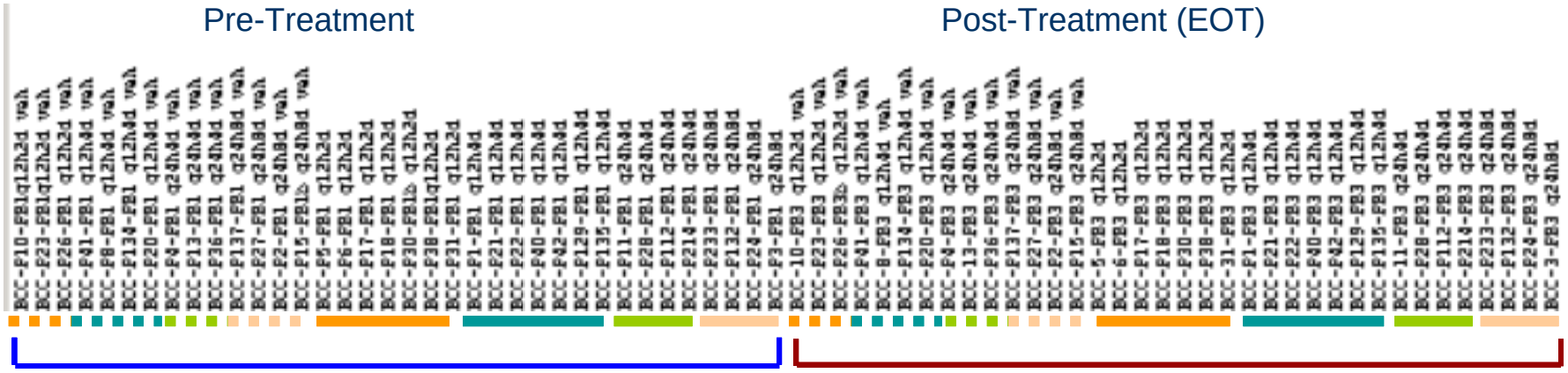


Panelli et al. Genome Biol - 2007

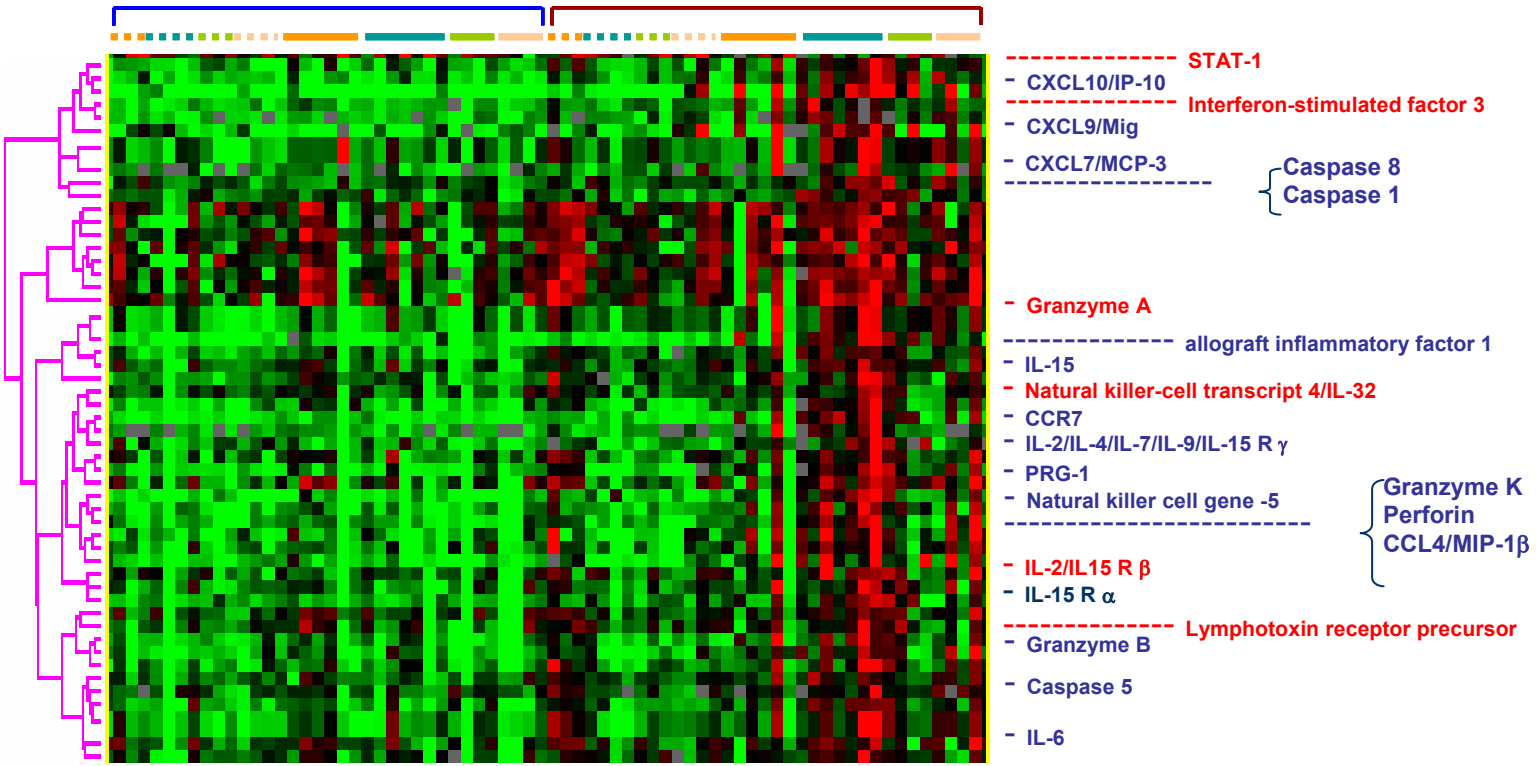
Primary = co-expressed in early and later biopsies
Secondary = expressed in late biopsies only







Imiquimod-specific genes with effector functions: cytokines, cytokine receptors and lytic enzymes



Panelli et al. Genome Biol - 2007

Looking for the immunological constant of rejection (Mantovani et al. *The Lancet* – in press)

	BCC[1]	HCV[9]	HCV[3]	HCV[4]	HCV[5]	IFN-α[6]	IFN-γ[6]	IFMα/β[6]
	TLR-7	Clearence	IFN-Susc	Chronic	Cirrhosis			
Number of genes studied	17,000	7,000	38,500	22,000	13,000	17,000	17,000	17,000
Gene ID								
CXCL10 / IP-10	+	+	+	+		+	+	+
MX1 / MxA	+	+	+	+	+	+		+
OAS1	+	+	+	+		+	+	+
PLSCR1	+	+	+	+		+	+	+
STAT1	+	+	+	+	+	+	+	+
IRF7	+	+	+	+		+		+
C1orf29	+	+	+	+		+		+
CD38 --	+	+	+	+		+	+	+
HLA-DRB1	+	+		+		+	+	+
MX2 / MxB	+	+	+			+		+
PSME2 -- Proteasome	+	+	+				+	+
GBP2	+	+	+				+	+
HLA-F	+	+		+		+	+	+
NCF2 --	+	+				+	+	+
NK4 / IL-32	+	+			+		+	+
IRF-1	+	+					+	+
CD97	+	+					+	+
CCL4 / MIP-1 beta	+	+						
CD5	+	+						
FCER1G --	+	+						
TGB2 / CD18	+	+						
ZAP70	+	+						
CD59	+	+						
TNFRSF7 / CD27	+	+						
CD95 / Fas	+	+						

[1] = Panelli et al. Genome Biol - *in press*

[2] = Bigger et al. J Virol 75: 7059, 2001

[3] = He X-S et al Hepatology 44: 352, 2006

[4] = Bigger et al. J Virol 78: 13779, 2006

[5] = Smith et al. Hepatology 38 (6): 1458, 2003

[6] = Stroncek et al. J Transl Med 3:24, 2005

Conclusions

- Immune mediated tissue destruction whether with beneficial (cancer/viral disease) or pathological (autoimmunity/allograft rejection) effects follows common pathways; among them:
  - Interferon Stimulated Genes (ISGs) appear to be necessary but not sufficient
  - ISGs represent a mixture of genes predominantly induced not only by IFN-α but also by IFN-γ
  - A third group of genes not directly induced by IFNs seems to be more tightly associated with immune-mediated tissue destruction.
  - These genes represent activation of innate and/or adaptive effector functions although, such effector functions appear to be less represented in the context of HCV clearance than in the context of tumor or allograft rejection.

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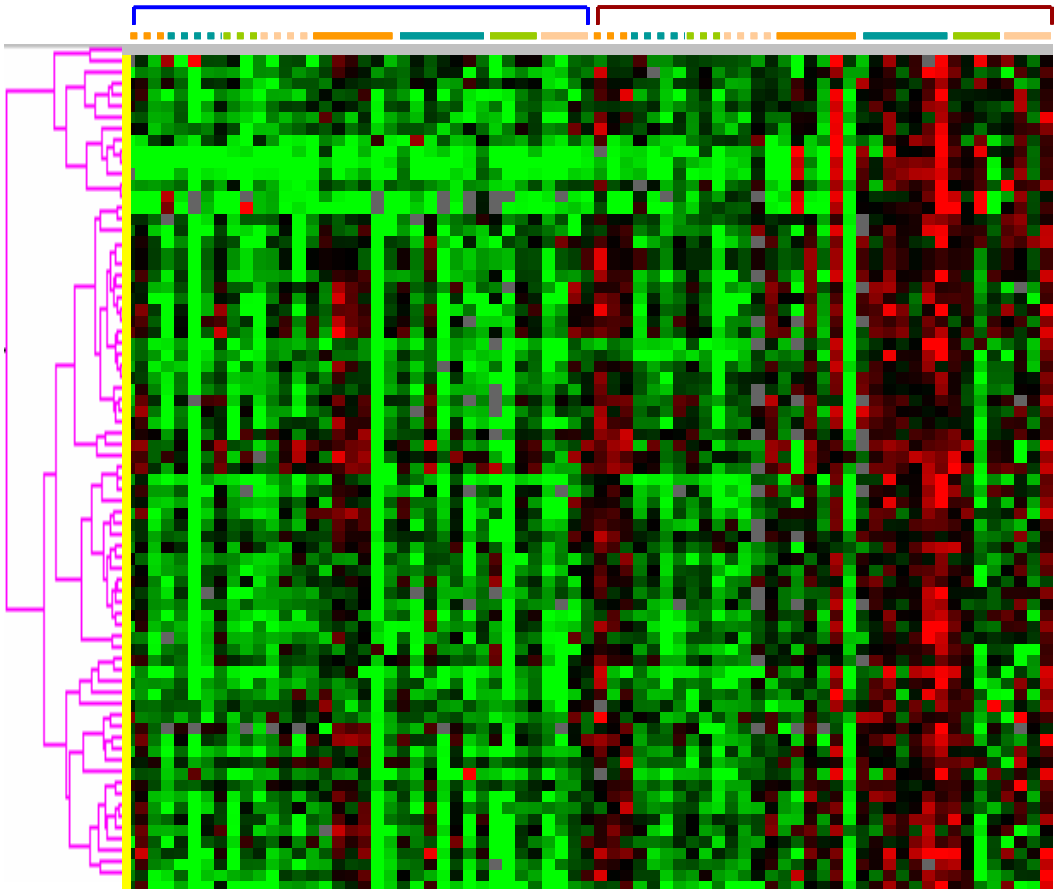
Imiquimod-specific genes with effector functions: Receptors and associated molecules



- JAK-2
- CD68

---------- Macrophage stimulating 1

- CD64

---------- HLA-G

---------- CD2

--------- KLRC3

- CD59

- CD4

---------- TNF receptor

- ZAP-70
- T cell immune-regulator 1
- insulin-like growth factor 1 receptor

- CD8

---------- CD5

---------- CD62L

- T-cell receptor

- CD3 Zeta
- Minor histocompatibility antigen HA-1
- Cathepsin W
- CD69

Response rate after peptide-based vaccinations against melanoma

Treatment:	Responses	Patients	%
MART-1_{27-35} in IFA	0	20	0
g209-2M in IFA	0	11	0
g209-2M in IFA + IL-2 (720,000 IU/Kg TID)	13	31	42
IL-2 alone for treatment of metastatic melanoma			17

(Rosenberg et al. Nature Med,1998*)*

Conclusion:

Enhanced T cell responses induced by vaccination have no independent effect on clinical outcome

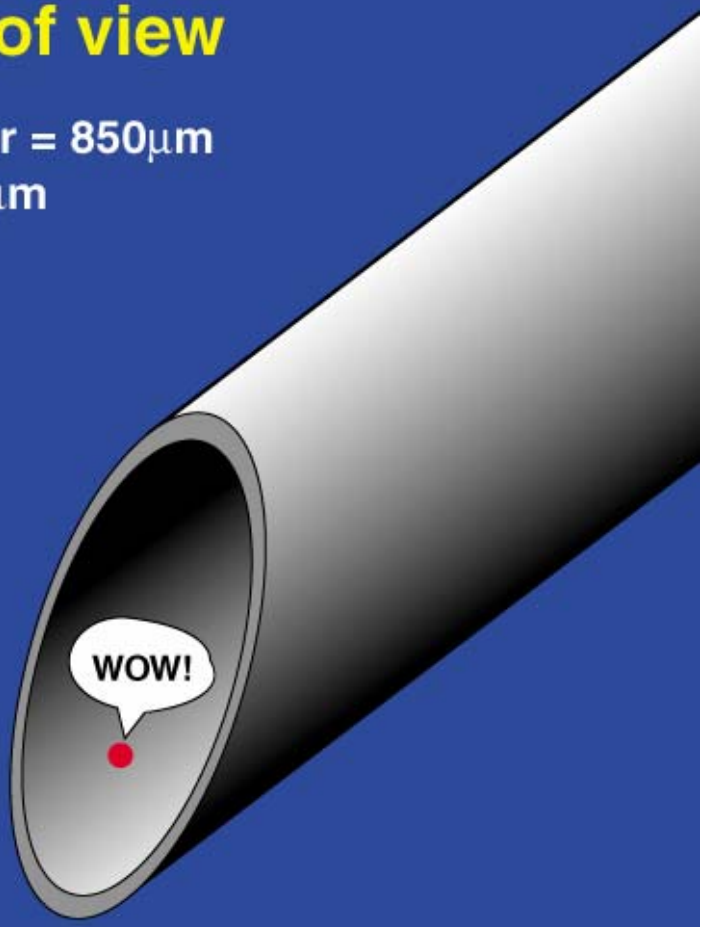
What is the mechanism of immune rejection?

Transferable anti-cancer innate immunity in spontaneous regression/complete resistance mice – *Hicks AM et al: Proc Natl Acad Sci 103(20): 7753-8, 2006*

Spontaneous anti-cancer resistance is mediated through a rapid infiltration of leukocyte mostly of the innate immunity including natural killer cells, neutrophils, and macrophages that are required for the destruction of cancer through rapid cytolysis

A 23 gauge needle seen from a cell point of view

23 Gauge Needle Inner Diameter = 850μm
Average Cell Diameter = 10-20 μm



Material Obtainable:
1 - 100 x 10^5 cells
(median = 10^6)
.1 to 10 μg total RNA
(median = 2 μg)

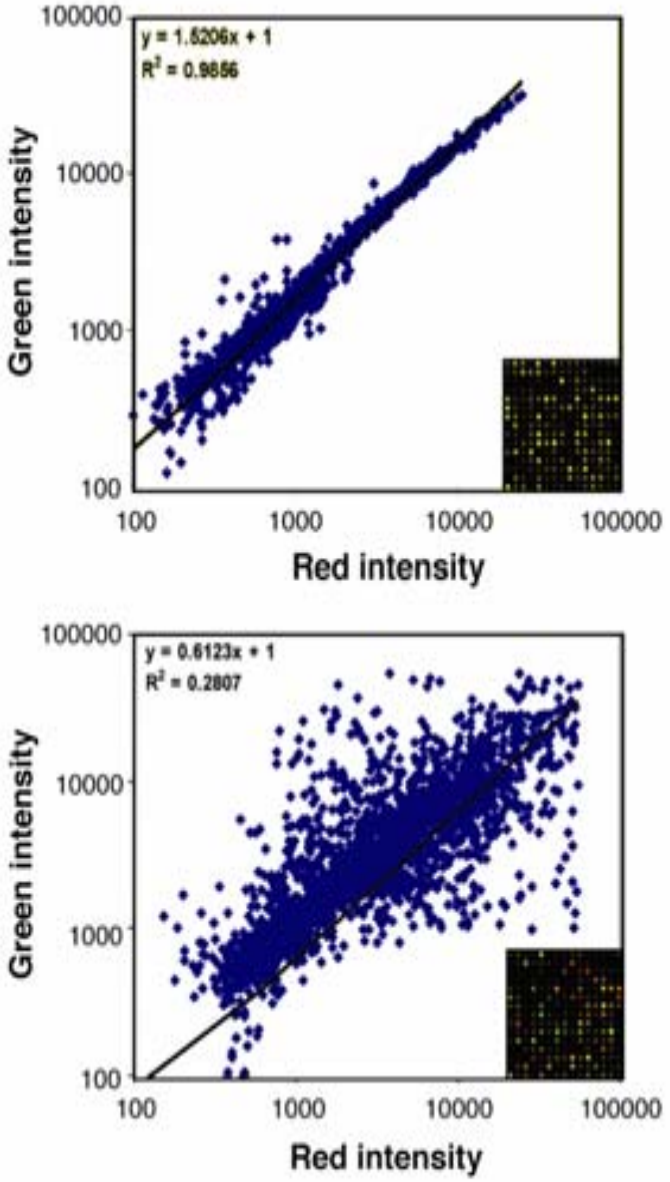
Wang et al. Cancer J Sci Am, 2001

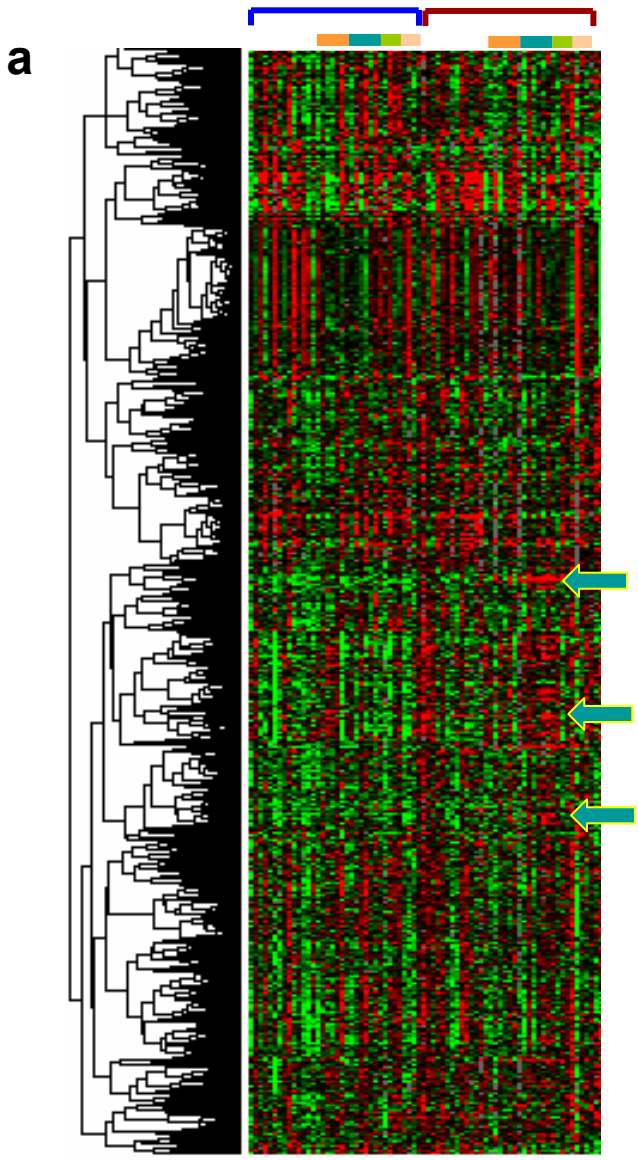
High-fidelity mRNA amplification for gene profiling
Wang et al. Nature Biotech. 2000
Wang et al J Trans Med 2006

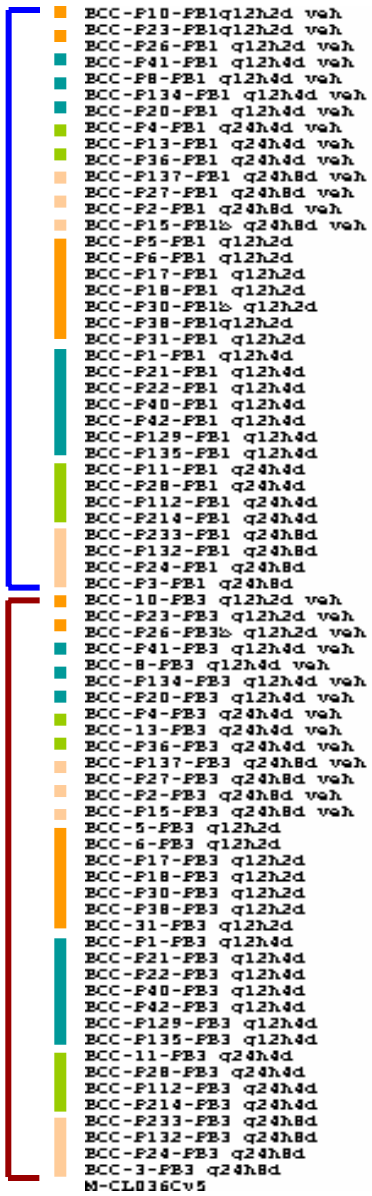
Source T-RNA	Round of Amplification	aRNA generated (μg)		aRNA used for hybridization (μg)	
		A375	ML-1	A375	ML-1
3μg	one	17.2	16.3	3	3
1μg	one	12.4	13.7	3	3
0.5μg	one	4.7	6.1	3	3
0.25μg	one	3.4	4.0	3	3
0.125μg	one	3.1	3.0	3	3
0.062μg	one	2.0	2.0	2.0	2.0
0.031μg	one	1.3	2.0	1.3	2.0
0.031μg	two	32.4	42.0	3	3
0.010μg	two	20.4	22.6	3	3

A375: Melanoma cell line
ML-1: Lymphoid cell line

Figure 2







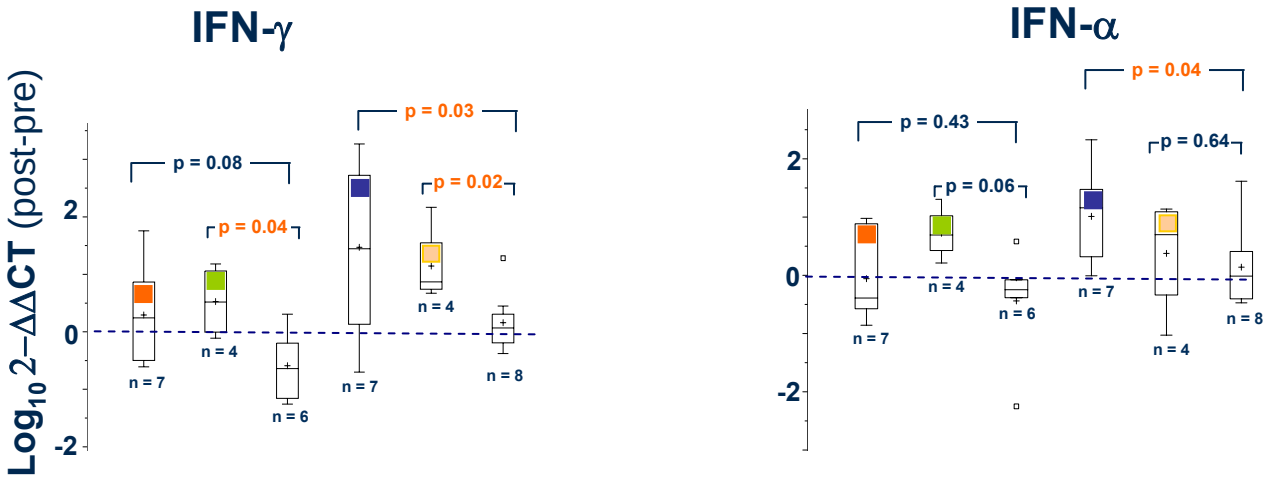
Pre therapy (Px-PB1)

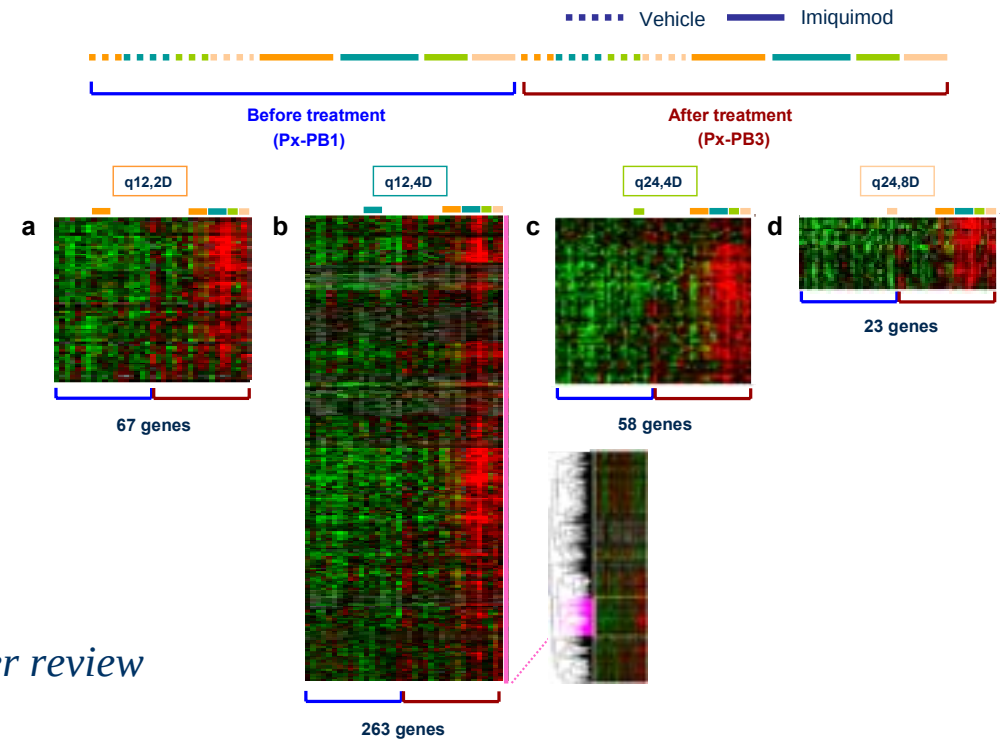
Post therapy (Px-PB3)

6646 Genes ratio abs=2
% present = 90

Imiquimod
Vehicle

Panelli et al. under review



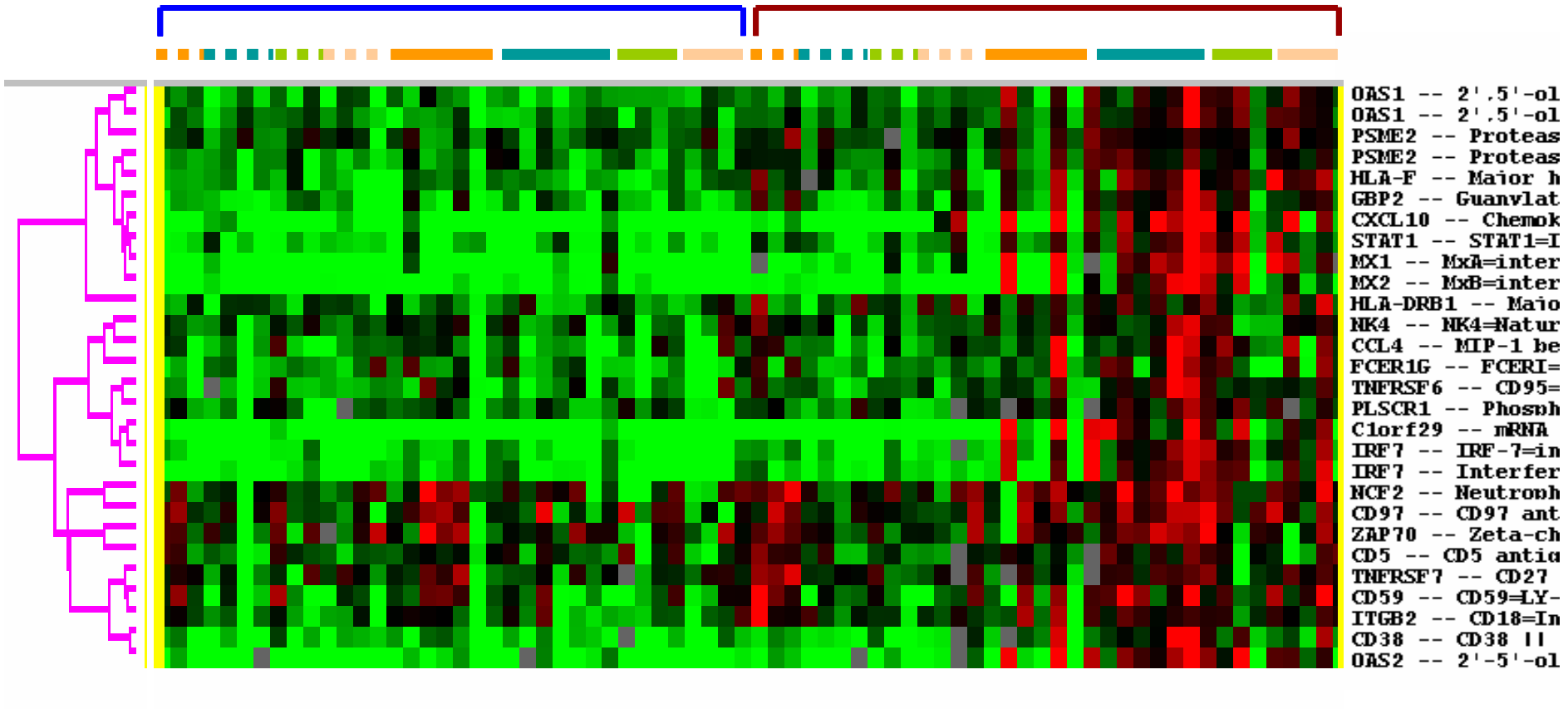


Panelli et al – under review

Proposed Studies

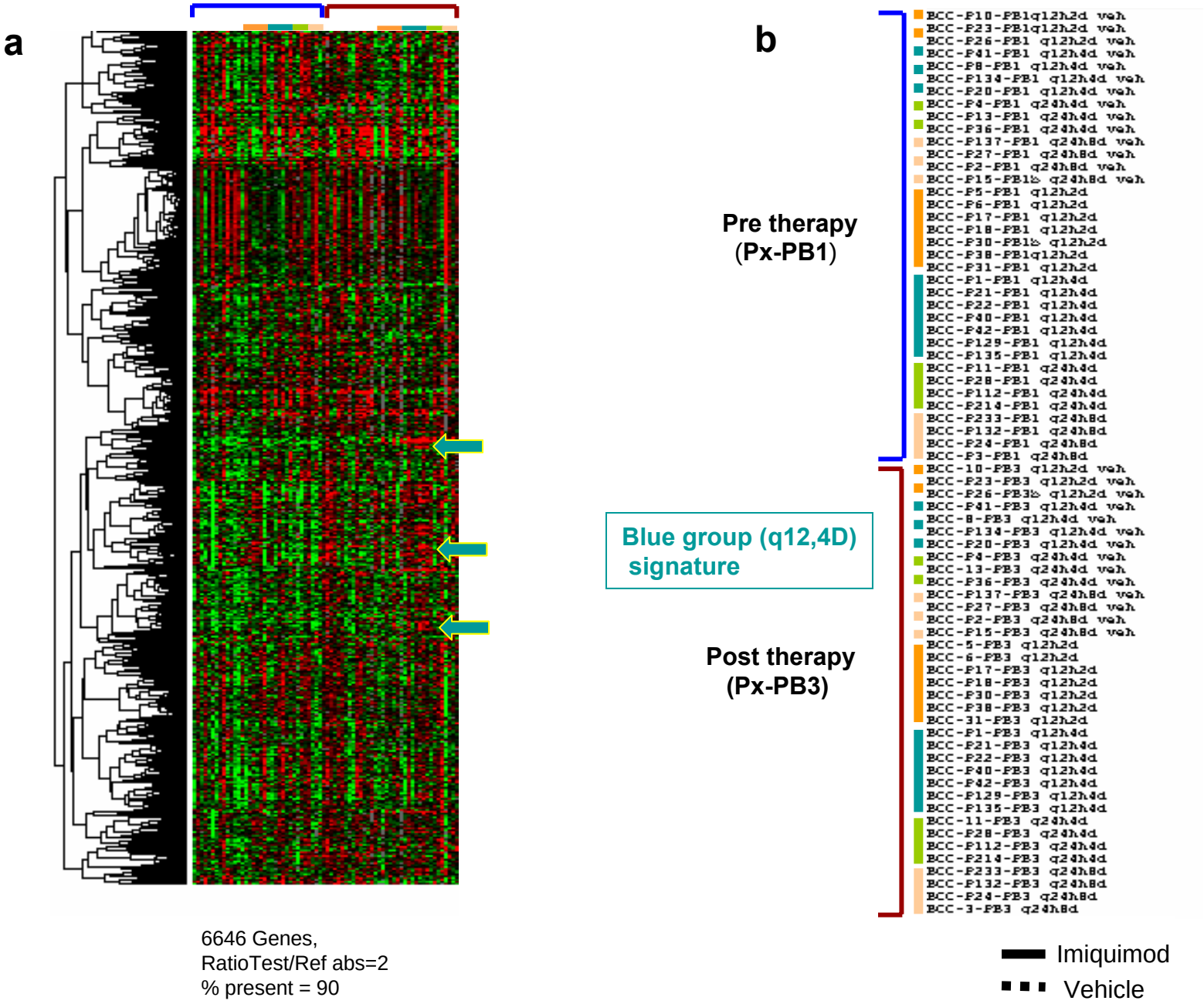
- Transcriptional comparison of PBMC of individuals with HVC viremia, individuals who cleared the viremia and normal donors
- Comparison of CD8 and CD4 T cell responses between individuals with progressive compared to stable liver disease

Genes commonly up-regulated in Imiquimod-treated BCC and in liver of chimps with resolving hepatitis C virus infection



Supervised analysis of all BCC samples

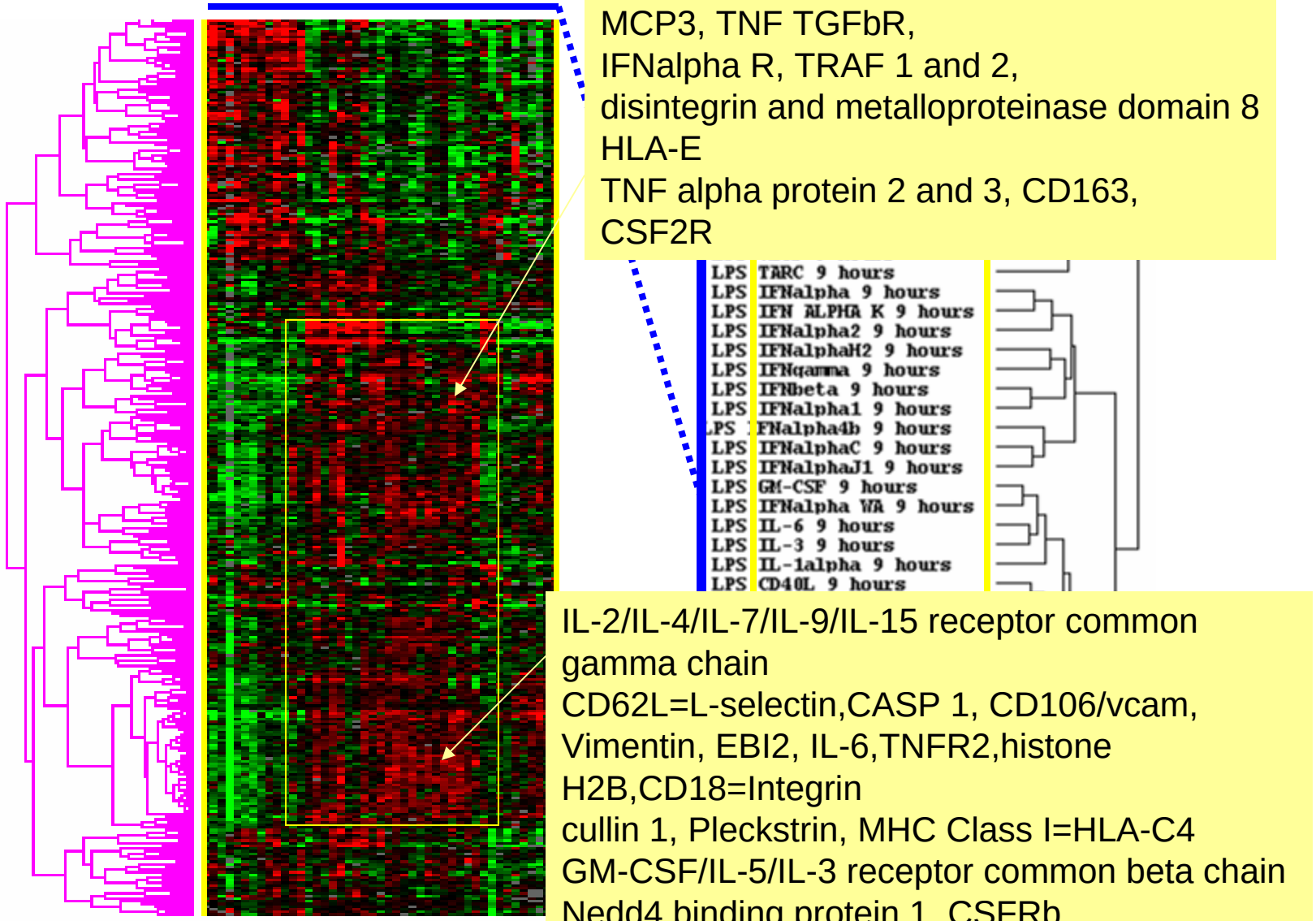
Figure 3



6646 Genes,
RatioTest/Ref abs=2
% present = 90

A

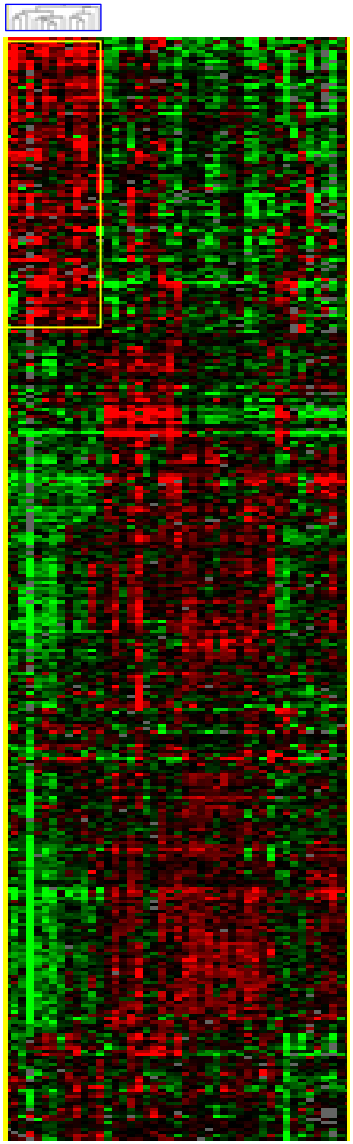
Dissecting Imiquimod-associated signatures

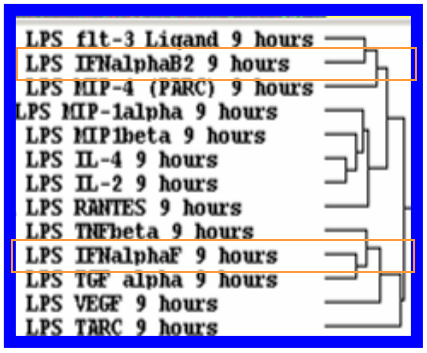


360 genes

MCP3, TNF TGFbR,
IFNalpha R, TRAF 1 and 2,
disintegrin and metalloproteinase domain 8
HLA-E
TNF alpha protein 2 and 3, CD163,
CSF2R

IL-2/IL-4/IL-7/IL-9/IL-15 receptor common gamma chain
CD62L=L-selectin,CASP 1, CD106/vcam,
Vimentin, EBI2, IL-6,TNFR2,histone H2B,CD18=Integrin
cullin 1, Pleckstrin, MHC Class I=HLA-C4
GM-CSF/IL-5/IL-3 receptor common beta chain
Nedd4 binding protein 1, CSFRb





CD5, CD2, Lymphotoxin beta,TCR beta, CD4, CD95 FAS, MHC class I, Granzyme B, NKG5, MHC class II DQ, IL-18, CD27, allograft inflammatory factor 1, CD14, CD69, CD38, Lactotransferrin, Perforin 1, KLRC1, formyl peptide eceptor/FPRL2, Tapasin/NGF17,Cathepsin W

Role of CD4+CD25^{high} regulatory T cells

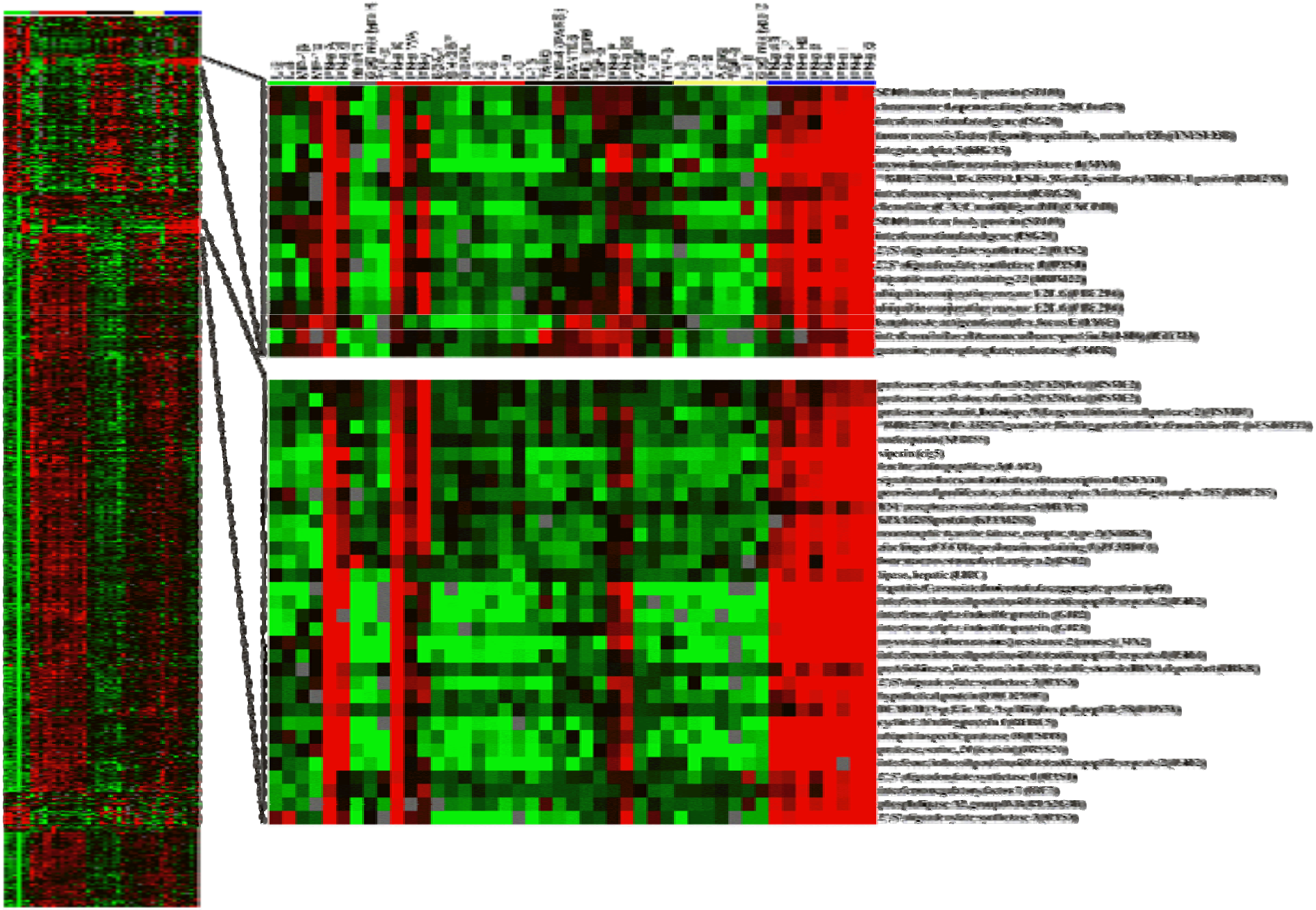
- CD4+CD25high T cells frequency is higher in melanoma and renal cell cancer patients than non tumor bearing individuals, after tumor regression in response to rIL-2 therapy their frequency reverts to normal (*Desana CG et al. J Clin Oncol 24: 1169, 2006)*
- Foxp3+CD4+CD25+ T cells frequency is higher in HCV infected chimps compared to unaffected chimps *(Manigold T et al. Blood 107: 4424, 2006)*
- Foxp3+CD4+CD25+ T cells frequency is similar in infected chimps compared to those who cleared the infection *(Manigold T et al. Blood 107: 4424, 2006)*

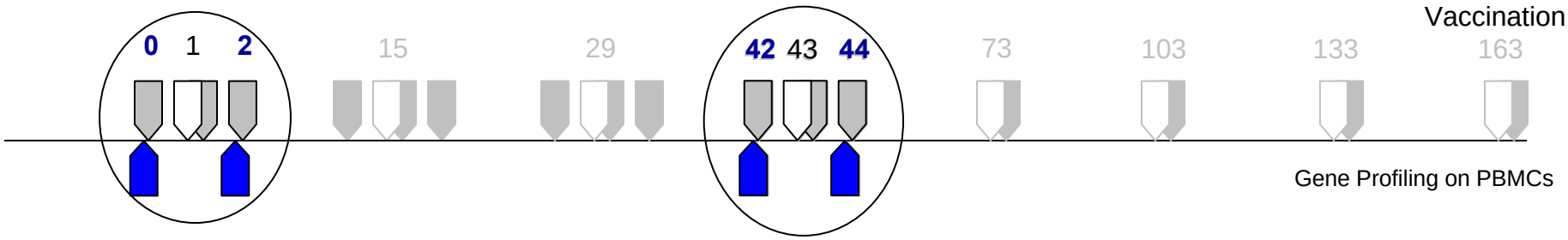
Conclusion

The frequency of regulatory T cells is higher in chronic inflammatory conditions and persists after clearing of the pathogenic condition in HCV but not in cancer

What about IFN-α?

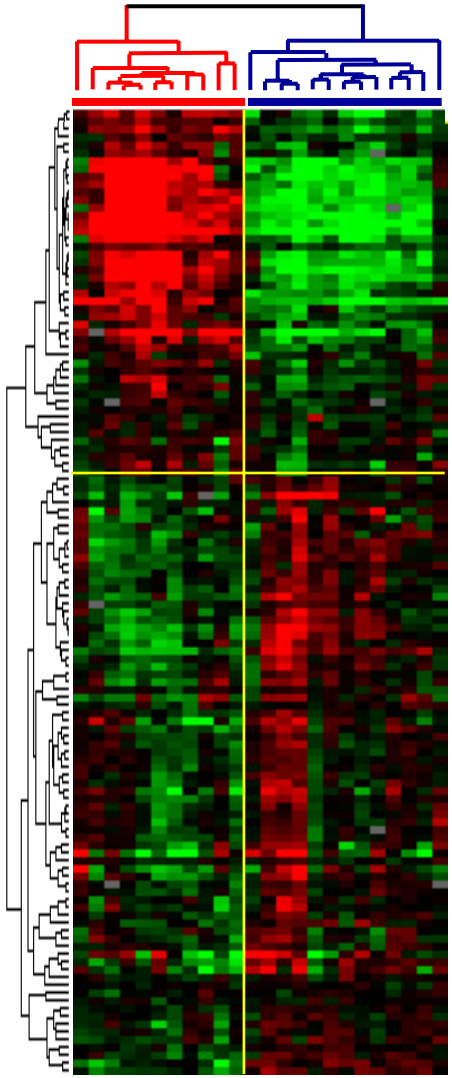
Delayed polarization of mononuclear phagocyte transcriptional program by type I interferon isoforms – *Stroncek et al. J Transl Med 3: 24, 2005*





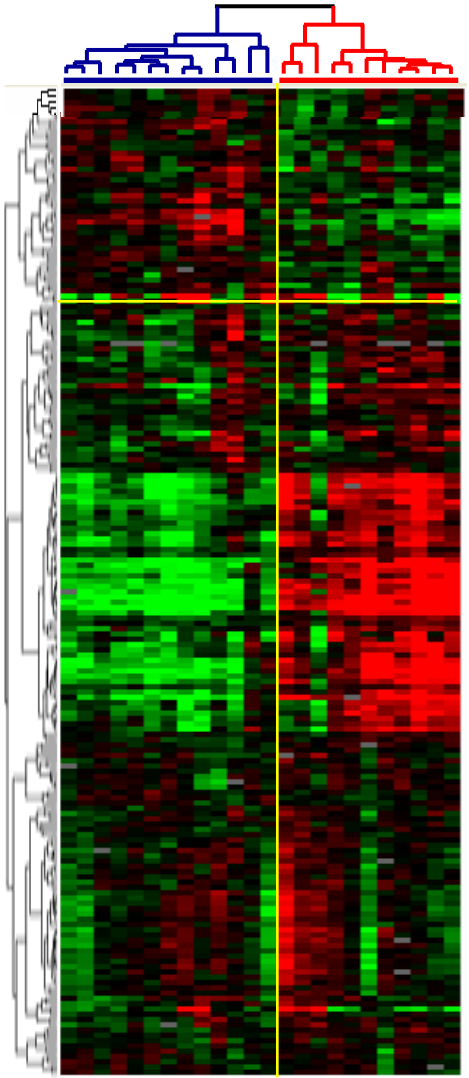
T0-T2=124 differentially expressed genes

T42-T44=187 differentially expressed genes

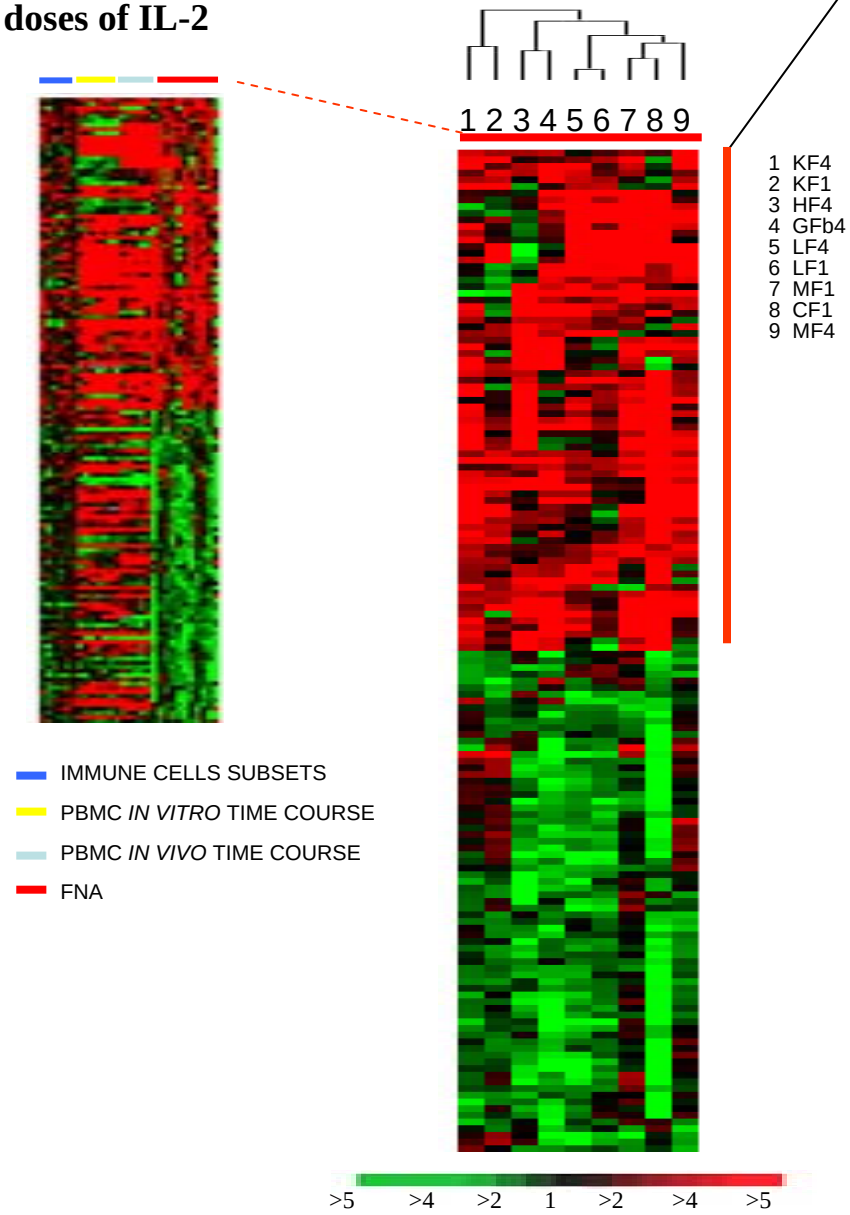


Pre IFN-pep Vacc. (T0,T42)

Post IFN-pep Vacc. (T2, T44)



FNA and the tumor microenvironment: Genes differentially expressed in FNA 3hr post 1 and 4 doses of IL-2

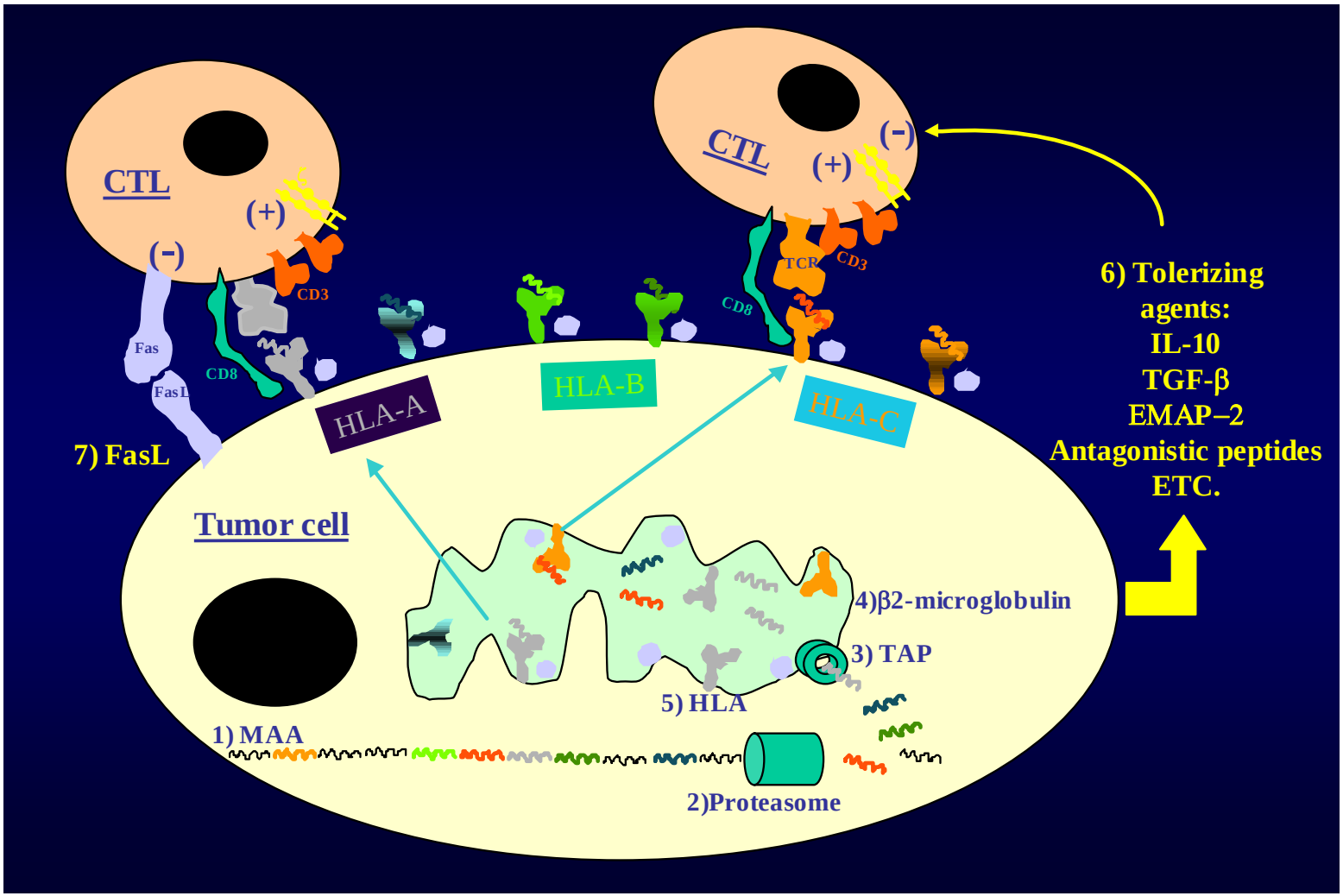


Panelli et al., Genome Biology,2002,3(7):res0035.1-0035.17

Highest median across experiments: cell surface, adhesion inflammatory proteins

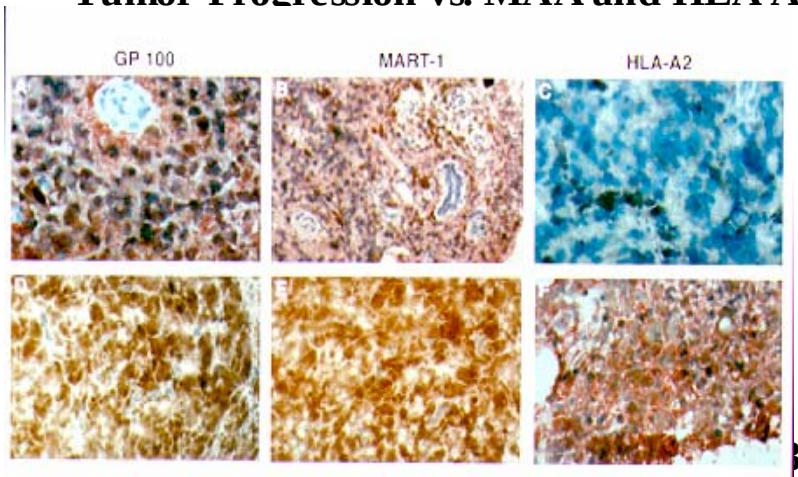
MHC class II DR alpha
MHC class II DR beta
Grancalcin Ca2+ binding protein
Calgranulin Ca2+ binding protein
CD62L L-selectin
CD45
V-CAM-1
CD64
CD29 integrin=beta 1 fibronectin receptor
(Fibronectin 1)
Keratin 10
IL-1 R
IL-1 receptor antagonist
IL-2 R beta chain
TNF-a induced protein 3
TGFβ receptor
Interferon-γ IEF SSP5111upregulated protein
(HSP70)
MxA/interferon induced cellular resistance protein
MxB interferon induced cellular resistance protein
(Interferon-a inducible protein IFI-6-16)
Guanylate binding protein 1 interferon inducible
IRF-1 interferon regulatory factor-1
IFN induced 56KDa protein
IFNγ receptor alpha chain
Nmi=IL-2 and IFN-γ inducible potentiator of STAT
C-C chemokine receptor 1
GRO-1
(MCP-1)
MCP-3
MIP-1beta
MIP-1 alpha
PARC=DC-CK1
Monocyte neutrophils elastase inhibitor
IL-8 chemokine
Human insulin like growth factor
Plasminigen activator urokinase

The Model

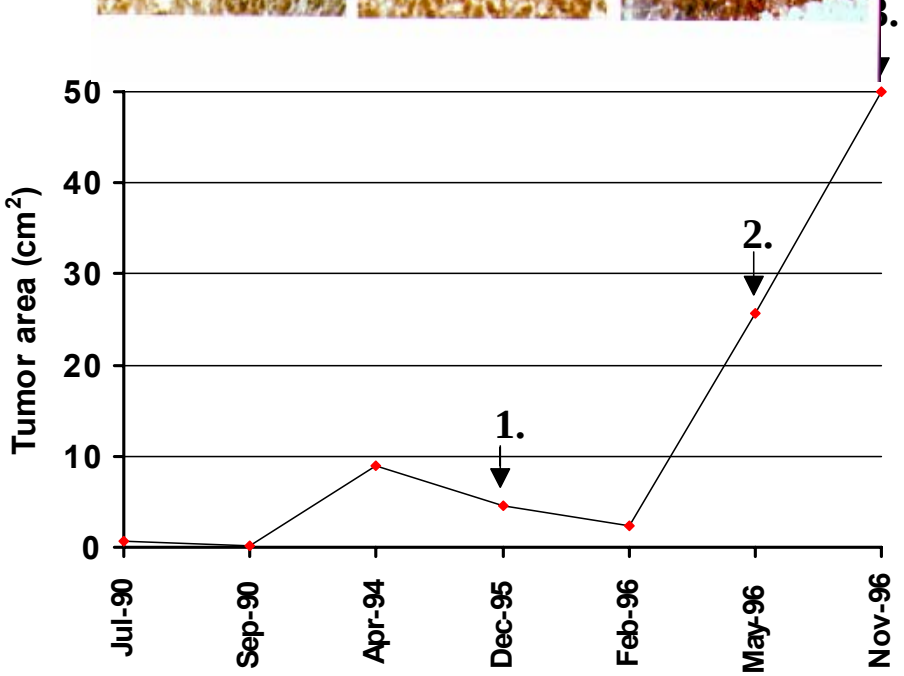


Marincola et al., Adv. Immunol. 2000

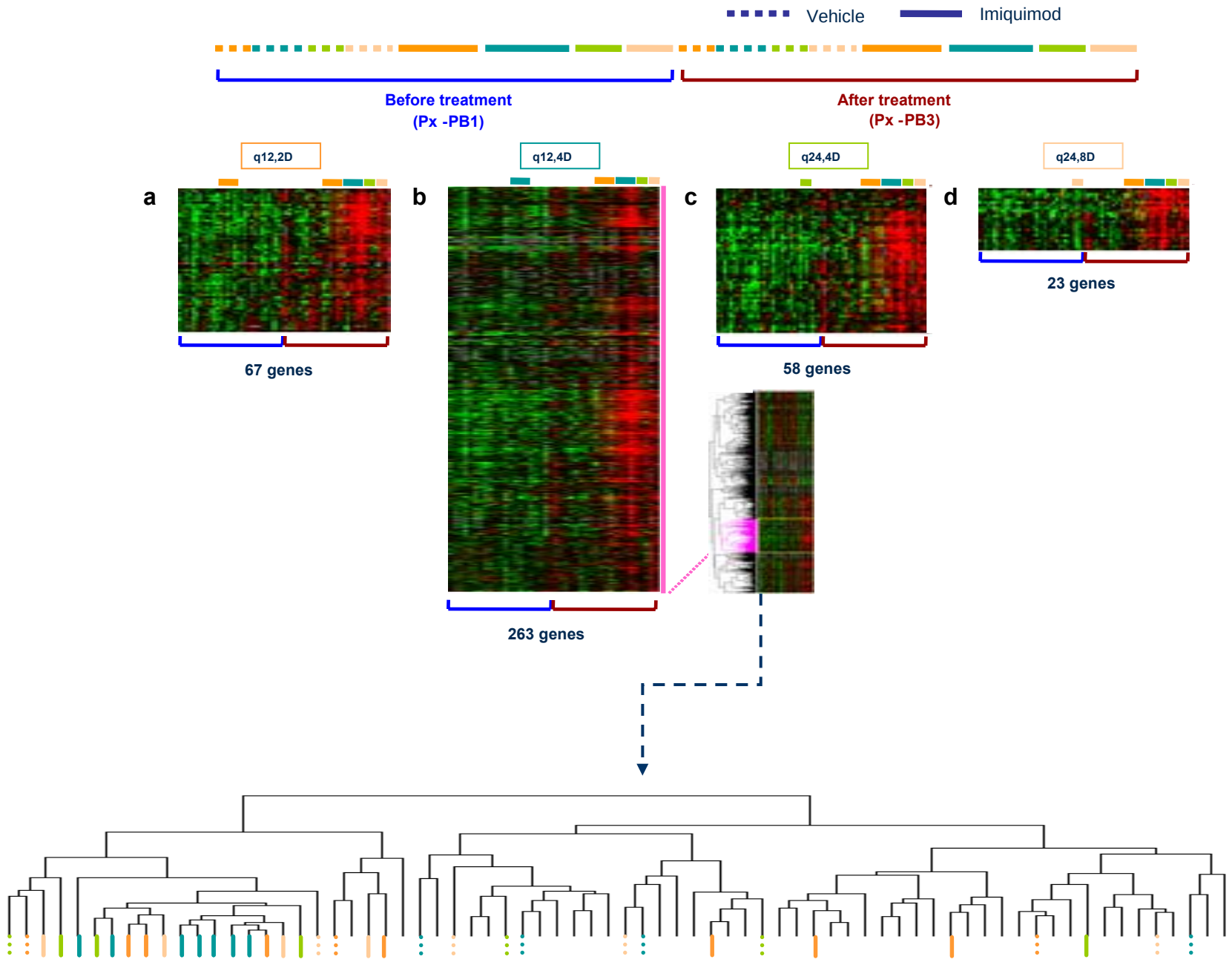
Tumor Progression vs. MAA and HLA A2 Expression for patient CC



	BIOPSIES		
	HMB-45	MART-1	HLA-A2
1.	> 75%, 4+	25-50%, 3+	Positive
2.	> 75%, 3+	< 25%, 2+	Negative
	> 75%, 3+	50-75%, 3+	Negative
3.	> 75%, 2+	> 75%, 1+	Negative
	Negative	Negative	Positive



Cormier et al., 1999



Panelli et al. Genome Biol - 2007

Looking for the immunological constant of rejection

	BCC[1]	Allo-RX[2]	HCV[3]	HCV(4)	IL-2 Tx[4]	RA (5)	Chron's[6]
			Cirrhosis	Clearence			
Number of genes studied	17,000	12,000	13,000	7,000	6,000	171	1
Gene ID							
Natural killer-cell transcript 4/IL-32	+	+	+	+	+	+	+
TCR	+	+		+	+		
HLA-Class II	+	+	+	+	+		
HLA-Class I	+	+	+				
STAT-1	+	+	+	+			
Granzyme-A	+	+					
IL-2/IL-15Rβ	+	+					
Lymphotoxin receptor precurson	+	+					
Interferon-stimulated factor 3	+	+					
Macrophage stimulating 1	+	+					
CD59	+	+		+			
CD54	+		+				
TNF receptor	+	+		+			
Insulin-like growth factor 1	+	+					
CXCL9/MIG	+		+				
Catepsin S	+		+				
DUSP-5	+		+				

[1] = Panelli et al submitted

[2] = Sarw al et al. NEJM; 349 (2): 125 2003

[3] = Smith et al. Hepatology 38 (6): 1458, 2003 and He X-S et al Hepatology 44: 352, 2006

[4] Bigger et al. J Virol 75: 7059, 2001

[5] = Panelli et al. Genome Biol 3 (7), 2002

(6) = Cagnard et al. Eur Cytokine Netw 16(4): 289-292, 2005