Lorenzo Uccellini

I have no relationships to disclose

Society for Immunotherapy of Cancer

SOCIETY FOR IMMUNOTHERAPY OF CANCER
26TH ANNUAL MEETING & ASSOCIATED PROGRAMS
November 1-6, 2011 • North Bethesda, MD

IRF-5 polymorphism in Melanoma

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NIH, BETHESDA, MD

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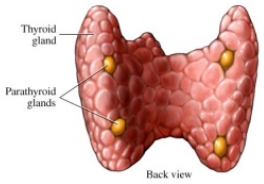
Autoimmunity and immune responsiveness of melanoma

Development of **autoimmunity** during **immunotherapy** (IL-2, IFN-a and anti-CTLA4) has been linked to responsiveness and/or tumor regression in patient with malignant melanoma.

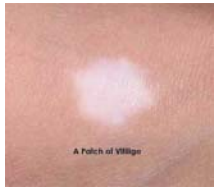
Hypothyroidism

Atkins MB- Kaplan MM, Engl J Med , 1988



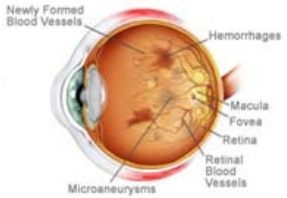
Vitiligo

Rosenberg SA, White DE er Al., 1996 JETI



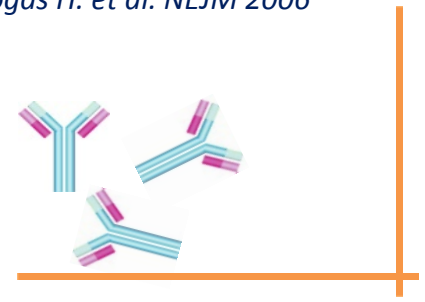
Autoimmune retinopathy

Chan C, O'Day J. Clin Experiment Ophthalmol , 2001



Appearance of antibodies (IFNa-2b)

Gogas H. et al. NEJM 2006

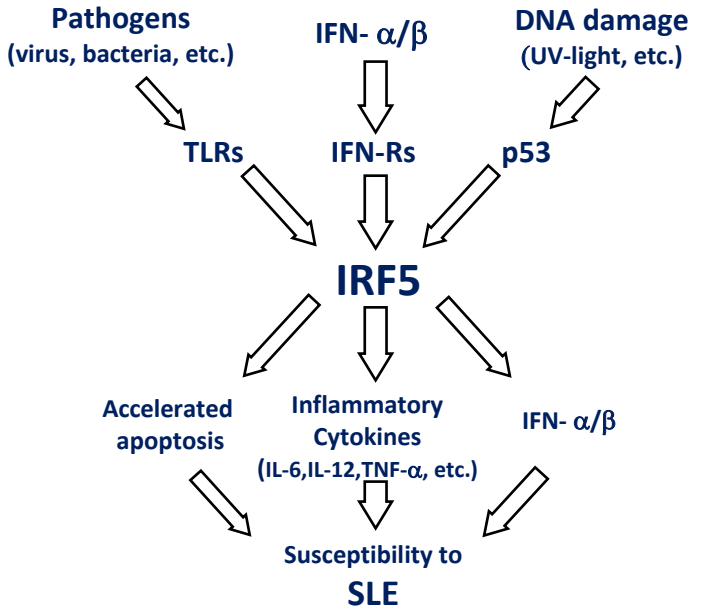


Do predictors of autoimmunity correlate with rejection of melanoma?

Rejection of MM

Autoimmunity and Interferon Regulatory Factor (IRF)-5

Proposed role for IRF-5 signaling in development of SLE

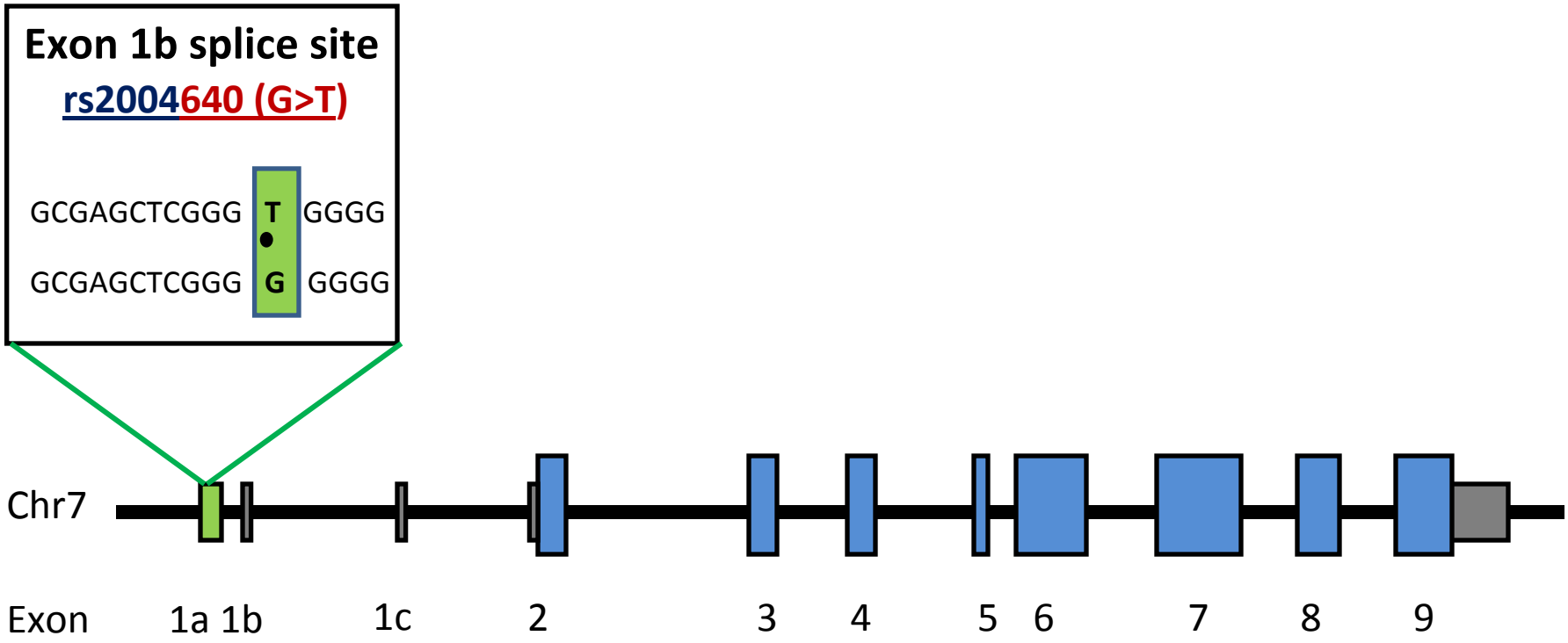


* Kozyrev SV, Alarcon-Riquelme ME. **The genetics and biology of Irf5-mediated signaling in lupus.** Autoimmunity. 2007 Dec;40(8):591-601

IRF-5

- **Strongly and consistently Systemic Lupus Erythematous (SLE)-associated locus**
- **a critical transcription factor in the type I IFN pathway**
- **primarily involved in host defense against viruses and pathogens in general**
- **regulates the expression of IFN-dependent genes, inflammatory cytokines and genes involved in apoptosis**

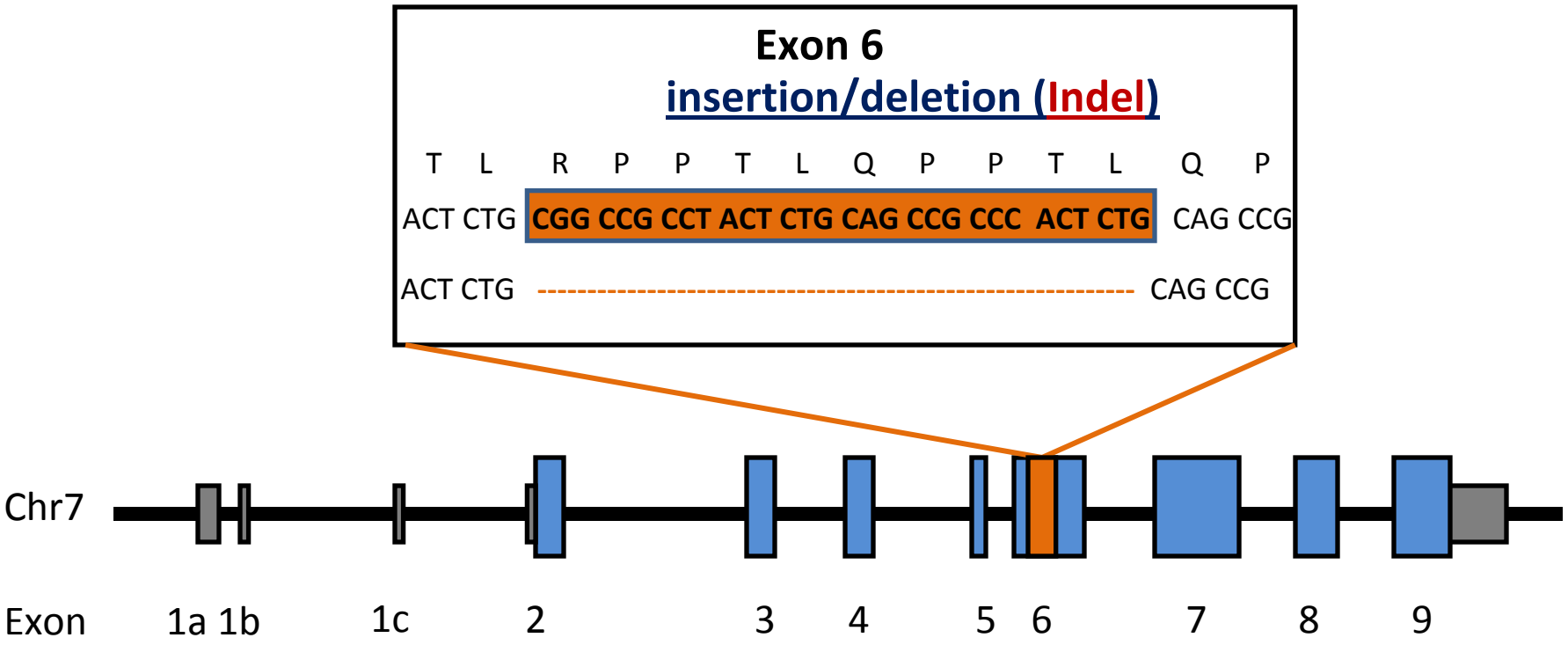
Three functional variants in *IRF5* define risk and protective haplotypes for SLE



T allele of **rs2004640 (G>T)** introduces a donor splice site **leading to the expression of an alternative isoform** (exon 1B transcripts and the reduction of exon 1C–derived transcripts).

Robert R Graham et al., PNAS 2007

Three functional variants in *IRF5* define risk and protective haplotypes for SLE

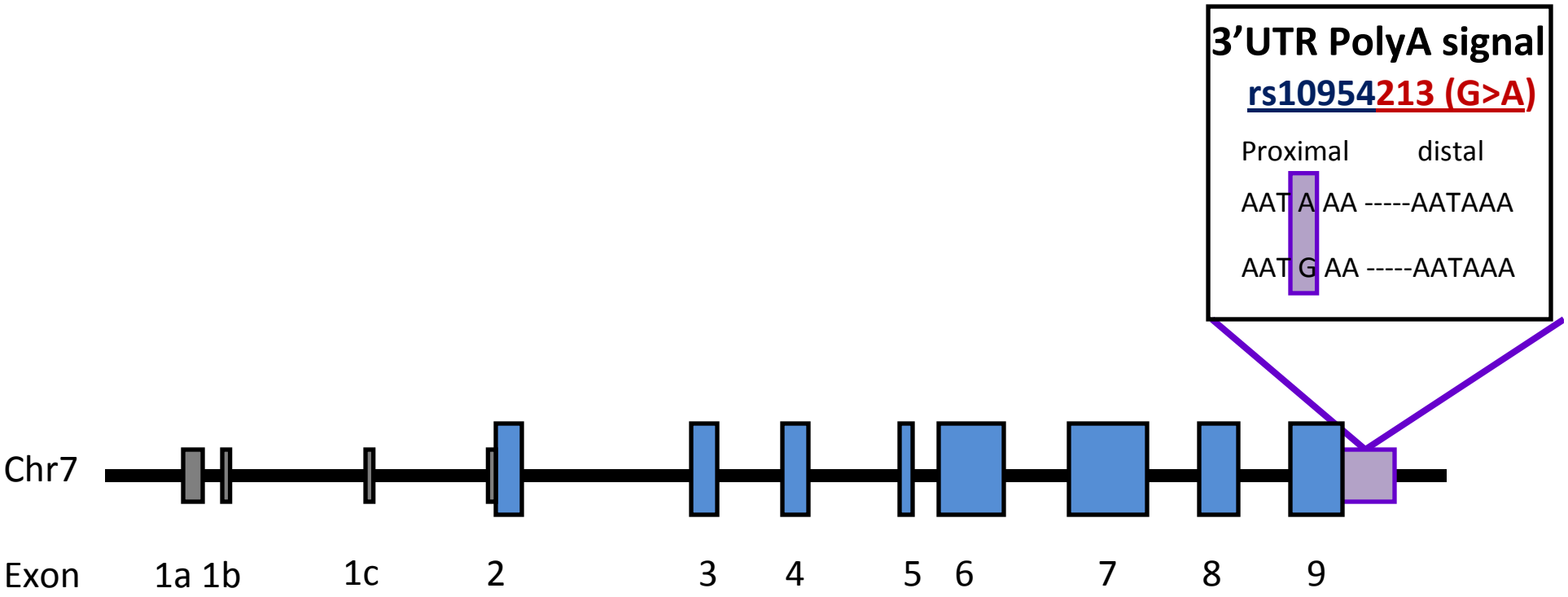


- The presence or absence of the repeats determines the isoforms to be expressed

→ **Leading to differential interactions with co-activator or inhibitor proteins** in specific cells or tissue, **and in turn to the promotion of a particular set of IRF5 targets.**

Robert R Graham et al., PNAS 2007

Three functional variants in *IRF5* define risk and protective haplotypes for SLE



located in the 3-UTR polyadenylation site AAT(G/A)AA.

- G allele disrupts the poly(A) site and thus causing transcription to continue (LONG mRNA).
- A allele predicts mRNA with a short 3-UTR (SHORT mRNA)

⇨ Rs10954**213 (G>A)** could lead to a functional mutation **regulating levels of IRF5 due to increased mRNA stability**

Robert R Graham et al., PNAS 2007

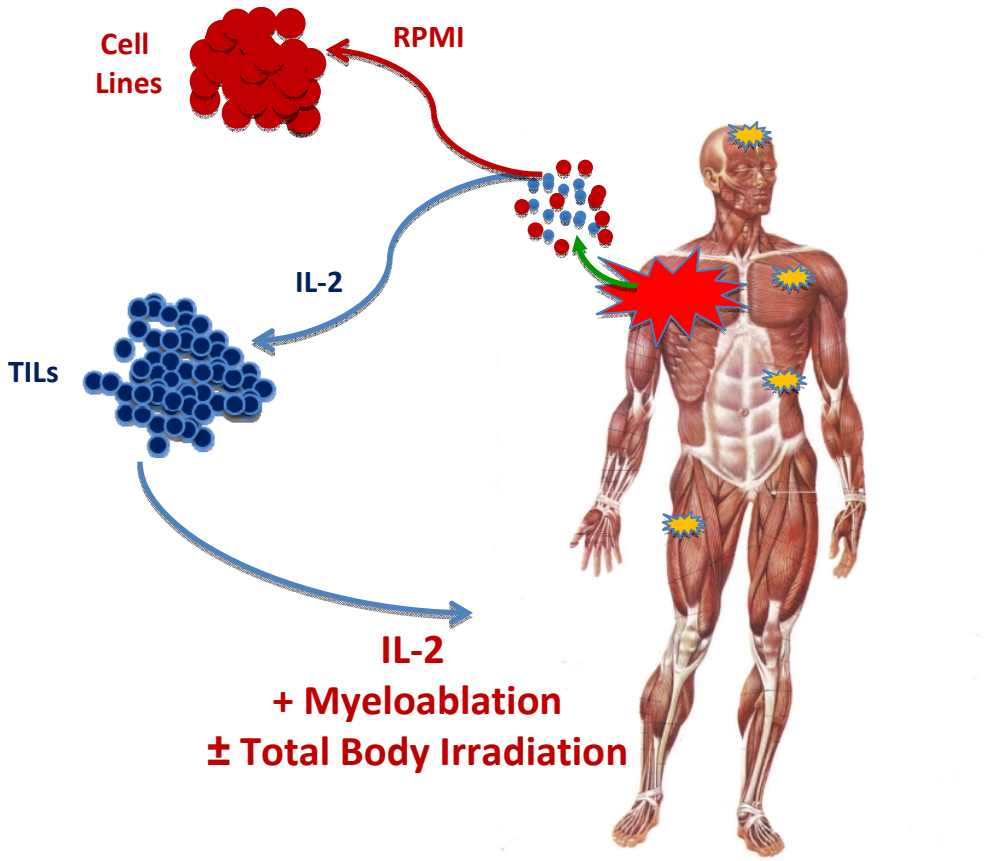
Project overview

SAMPLE STUDIED

142 TILs from patients enrolled in five adoptive cell therapy trials

112 parental melanoma metastases

15 melanoma cell lines derived from the 15 melanoma metastases



Analysis of Tumor Infiltrating Lymphocytes (TIL)

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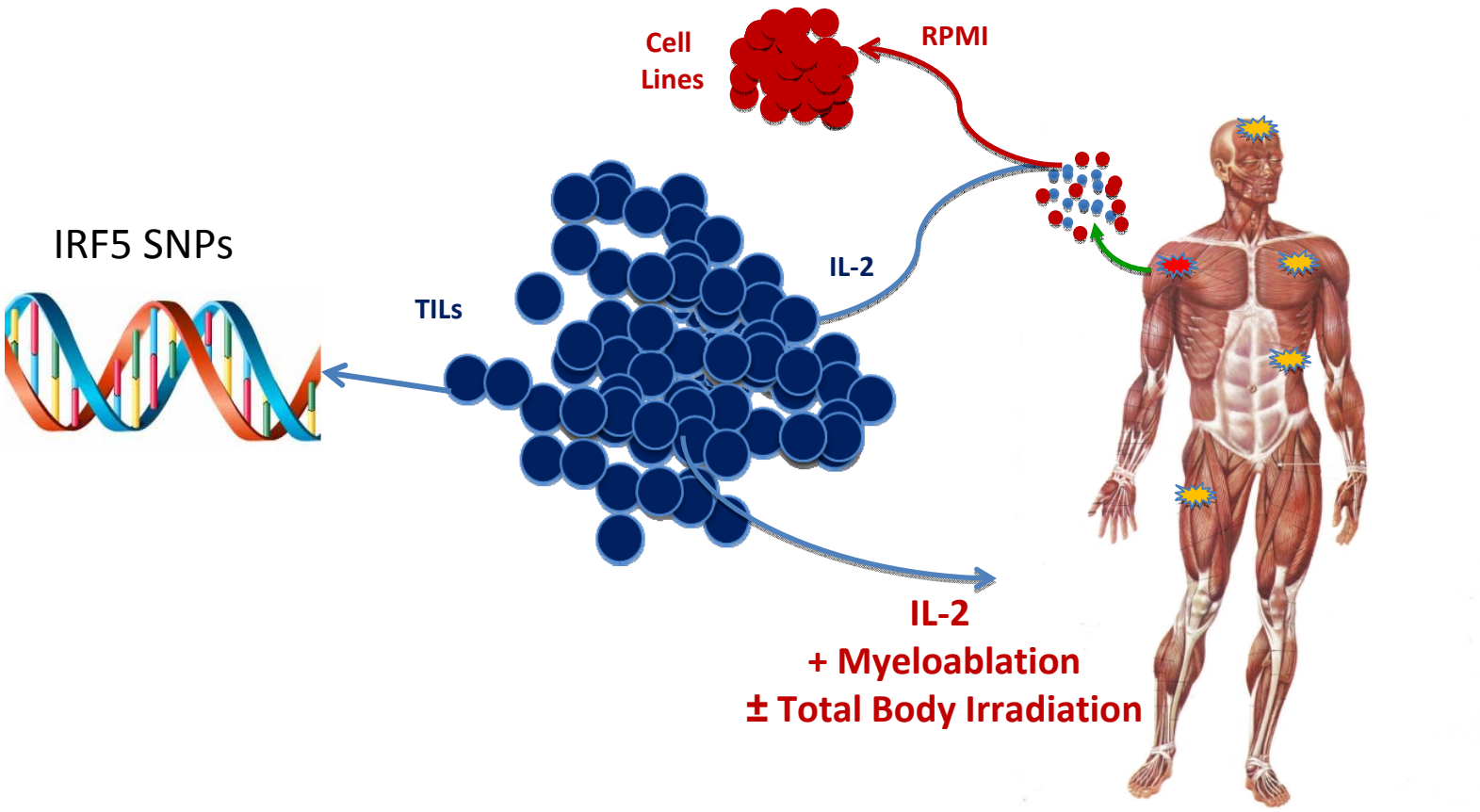
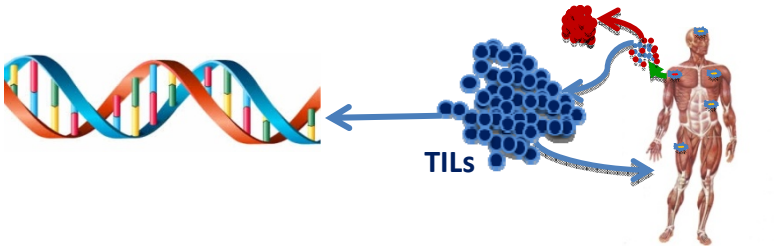


Sequencing Results

142 TILs from patients enrolled in five adoptive cell therapy trials

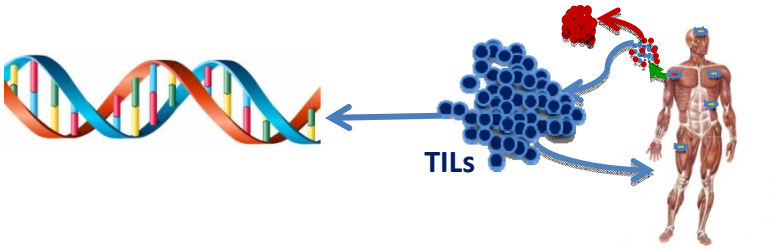


polymorphism	allelic frequencies	Frequencies		two tailed p
		R	NR	
rs10954213	A	0.69	0.49	**0.0015**
	G	0.31	0.51	
rs11770589	A	0.54	0.38	**0.0116**
	G	0.46	0.62	
rs6953165	C	0.97	0.90	**0.025**
	G	0.03	0.10	
rs2004640	C	0.47	0.51	0.55
	G	0.53	0.49	
Indel	ins	0.46	0.62	**0.006**
	del	0.55	0.38	

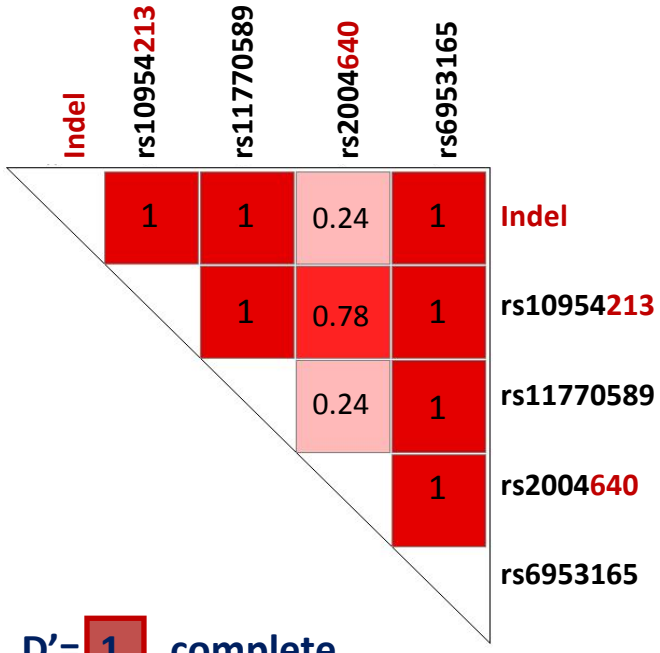
R= Objective Response
NR = No Response

Pair wise pattern of Linkage Disequilibrium across IRF5

142 TILs from patients enrolled in five adoptive cell therapy trials

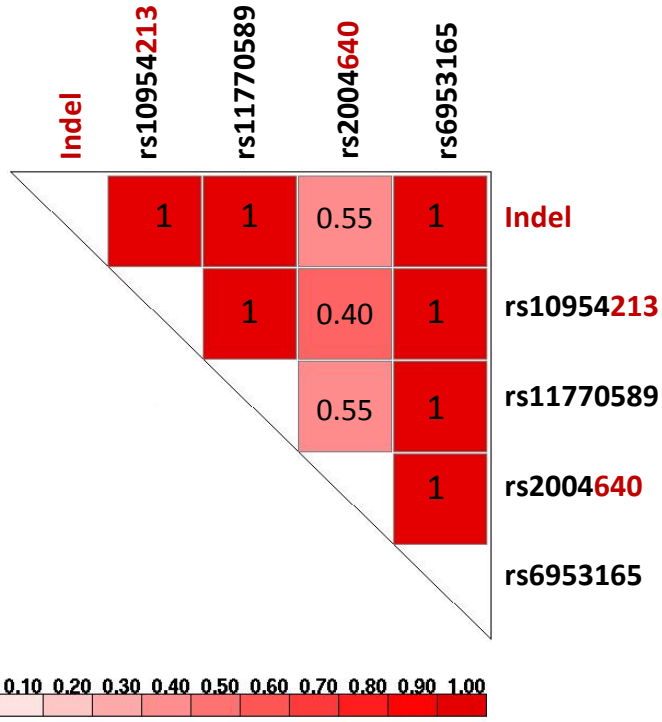


Responders



D'= 1 complete linkage disequilibrium

Non Responders



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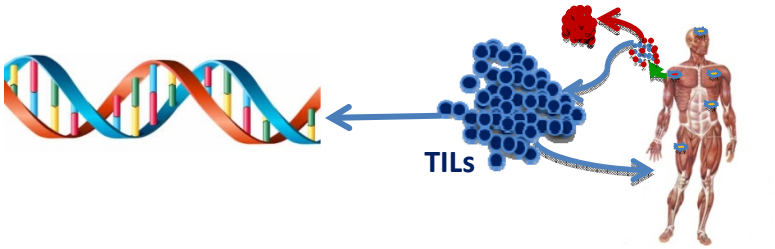

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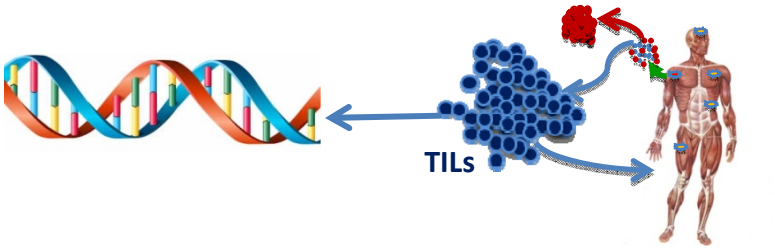
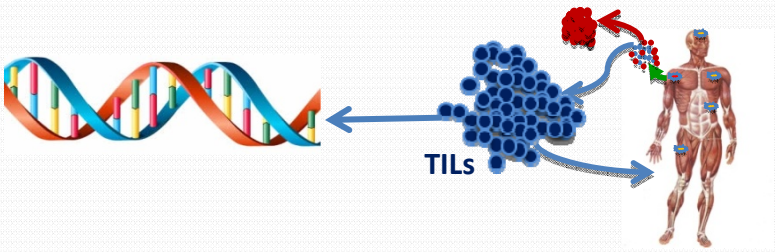
IRF5 rs10954213 AA genotype and A allele are associated to OR in TILs from melanoma patients



Genotype frequency		R	NR	R	NR	X^2	two tailed p
		(N)		Frequency			
rs10954213	AA	33	19	0.47	0.27	10.4	0.007
	AG	30	31	0.43	0.44		
	GG	7	20	0.1	0.29		
Allele frequency		R	NR	R	NR	two tailed p	
		(N)		Frequency			
rs10954213	P(A)=	96	69	0.69	0.49	0.001	
	P(G)=	44	71	0.31	0.51		

IRF5 rs10954213 AA genotype and A allele are associated to OR in TILs from melanoma patients



Presence of A allele predicts response

Genotype frequency		R	NR	R	NR	X^2	two tailed p
		(N)		Frequency			
rs10954213	AA	33	19	0.47	0.27	10.4	0.007
	AG	30	31	0.43	0.44		
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Allele frequency		R	NR	R	NR	two tailed p
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rs10954213	P(A)=	96	69	0.69	0.49	0.001
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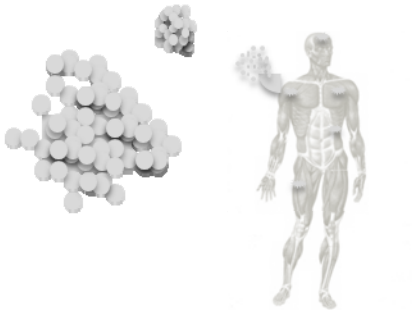
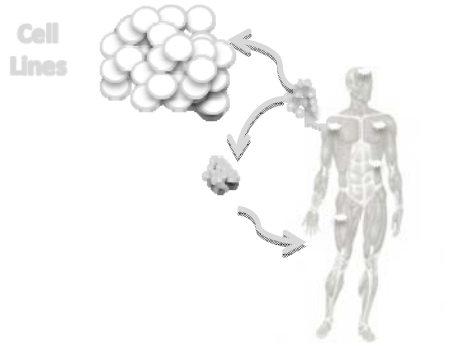
The functional genome of TILs and/or MM belonging to distinct IRF-5 genotypes predicts responsiveness

- TILs



- MM



Gene expression profile of
142 TILs

?

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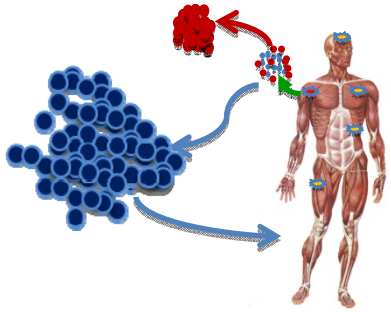
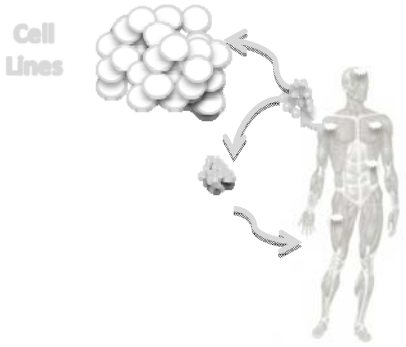


- **MM**



Gene expression profile of
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Analysis of Tumor Infiltrating Lymphocytes (TIL)

142 TILs from patients enrolled in five adoptive cell therapy trials

112 parental melanoma metastases

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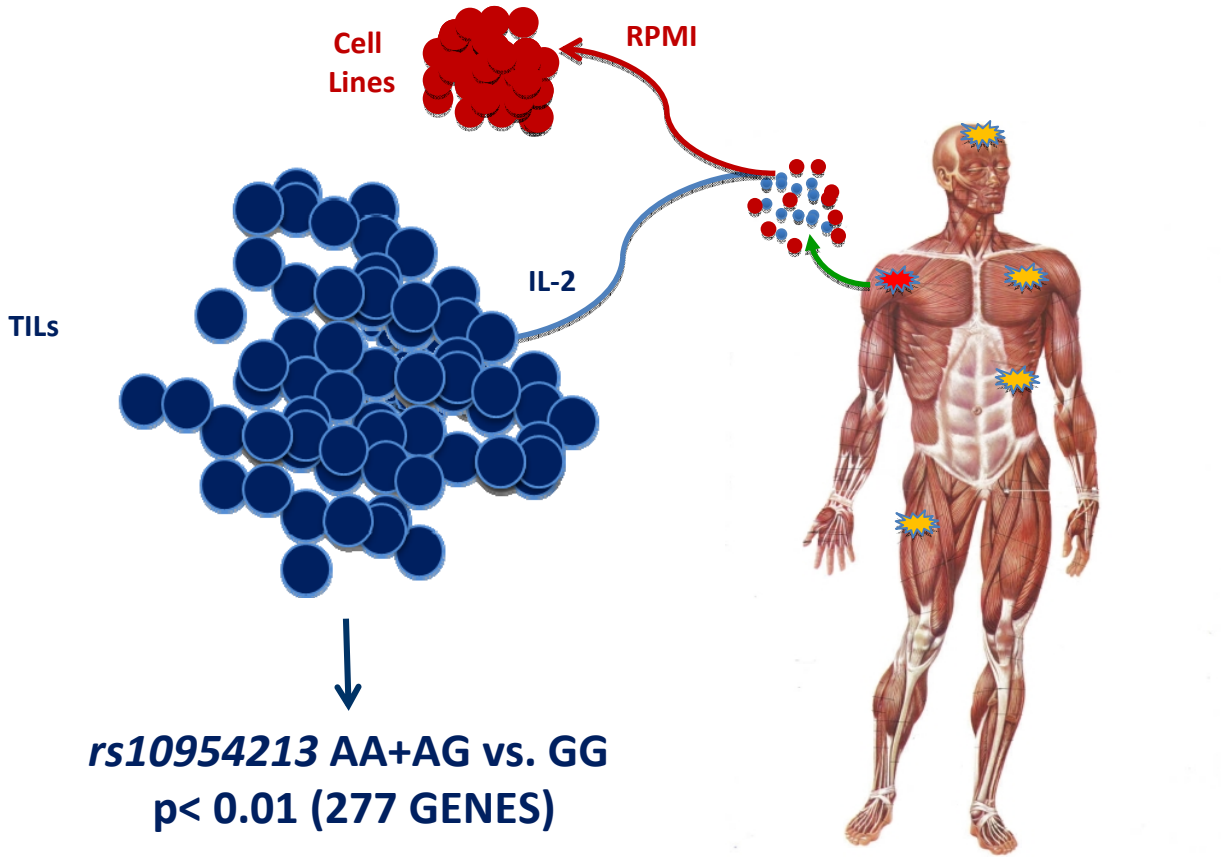
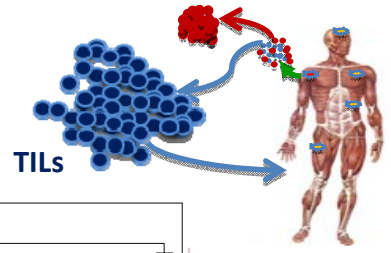
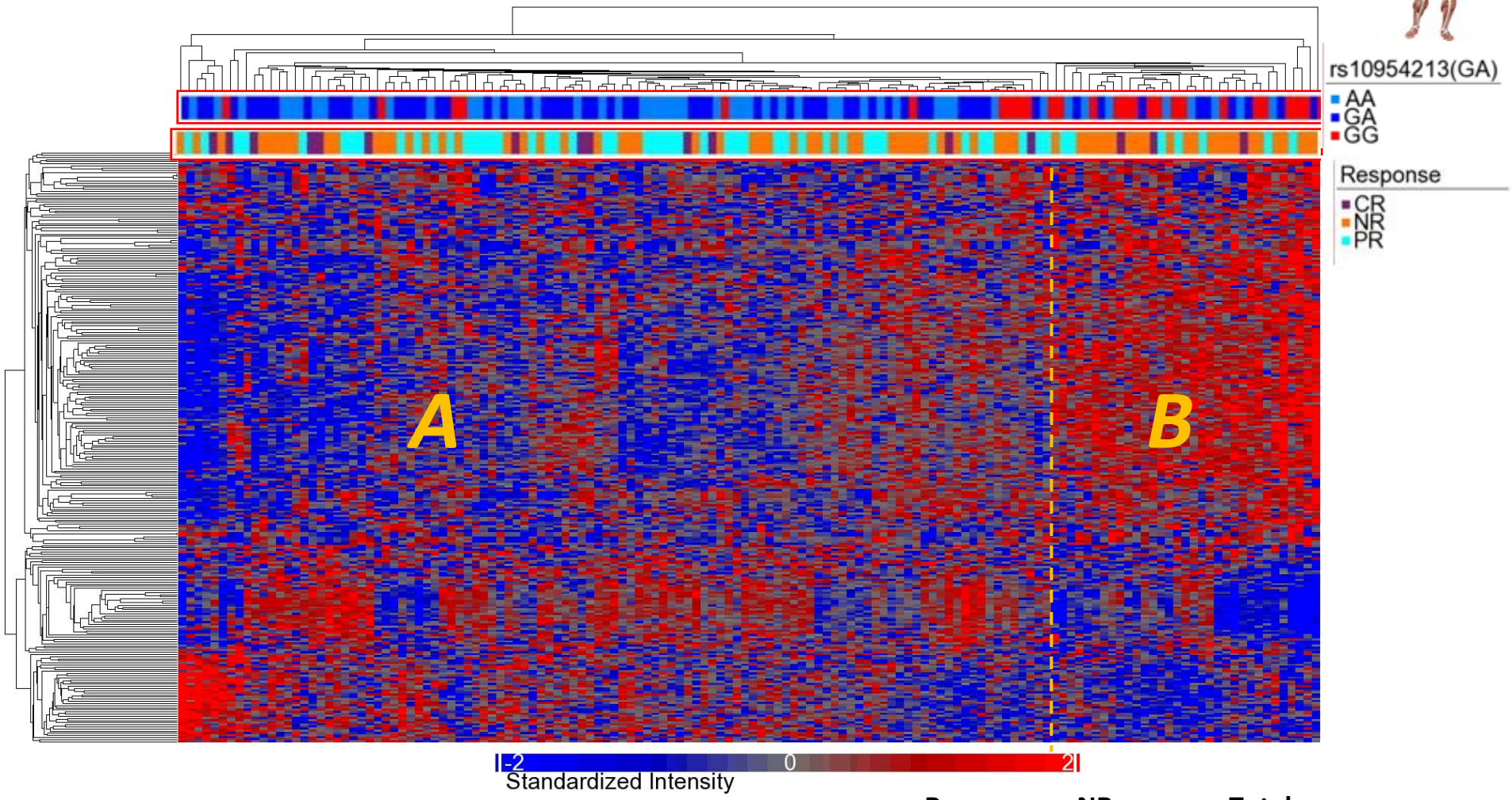


Tumor Infiltrating lymphocytes (TILs):

Cluster built on *rs10954213* AA+AG vs. GG

p< 0.01 (277 Genes)





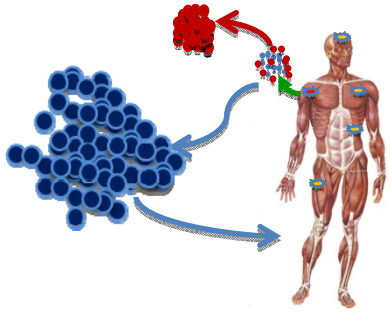
Fisher's exact test :

Two-tailed p value < 0.017

	R	NR	Total
Group A	61	48	109
Group B	10	23	33
Total	71	71	142

The functional genome of TILs and/or MM belonging to distinct IRF-5 genotypes predicts responsiveness

- **TILs**



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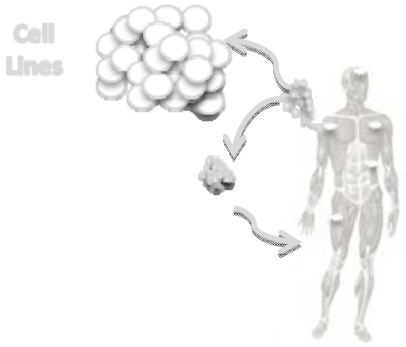

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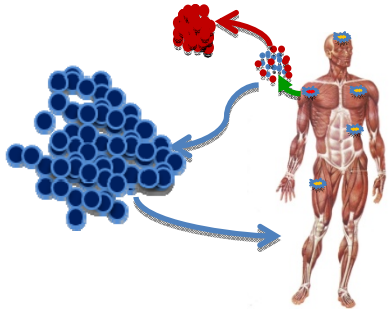
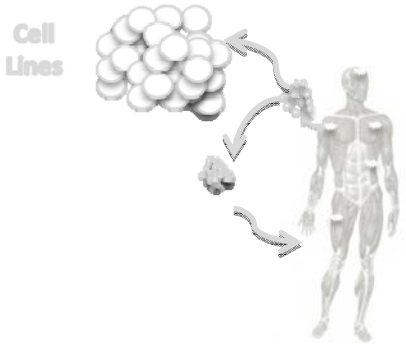


- **MM**



Gene expression profile of
142 TILs

borderline differences
in prediction of Response

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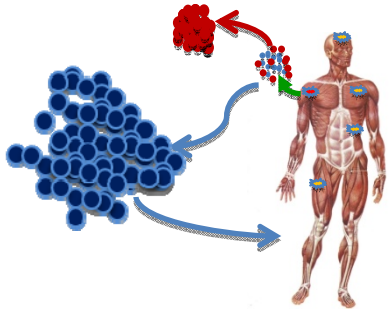
- **TILs**



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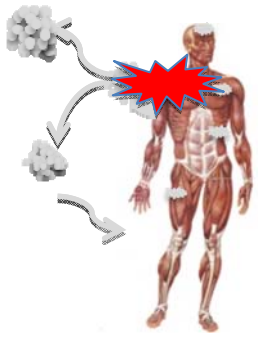

Gene expression profile of
112 pre-treatment melanoma metastasis

?

Analysis of parental tumor

142 TILs from patients enrolled in five adoptive cell therapy trials

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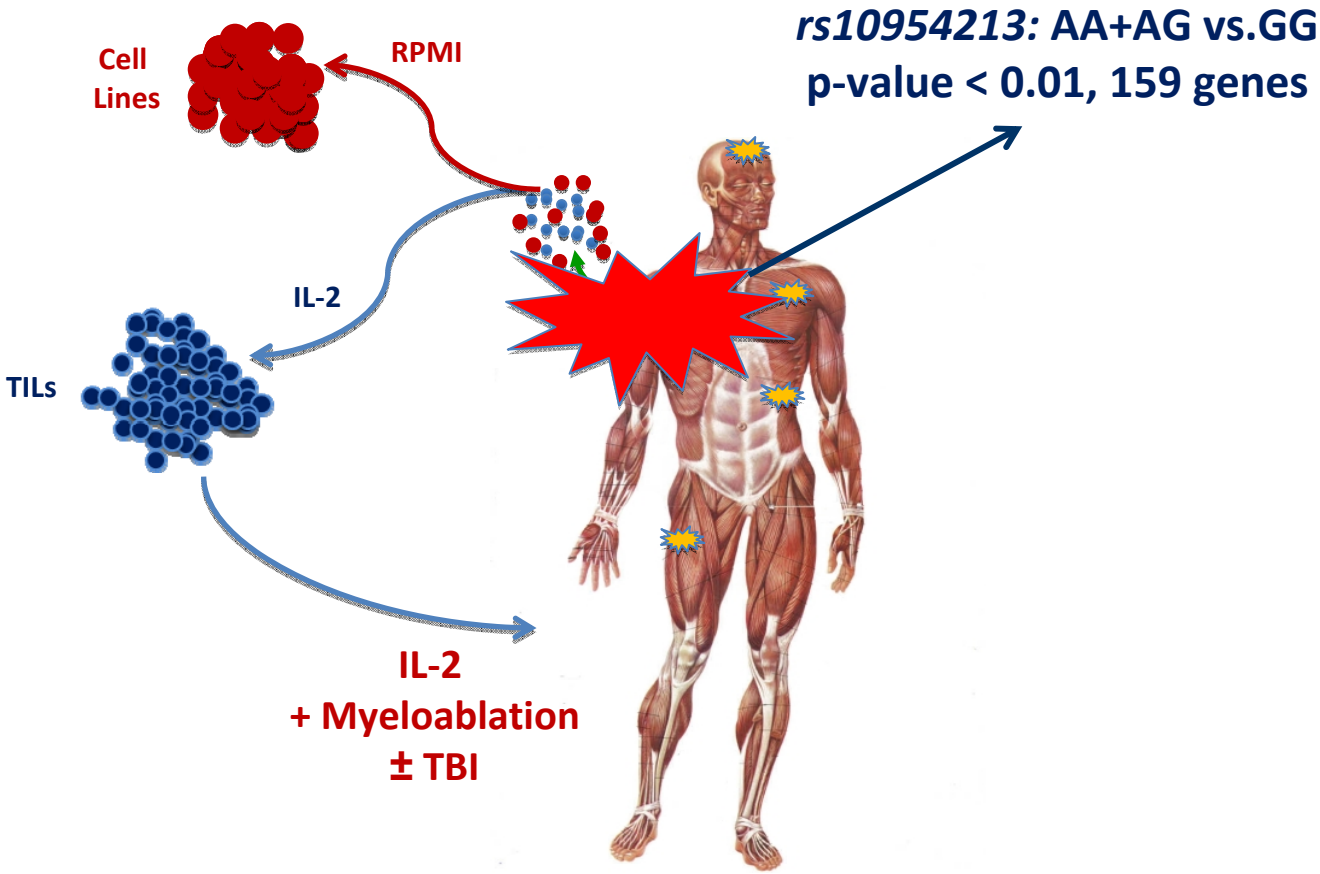
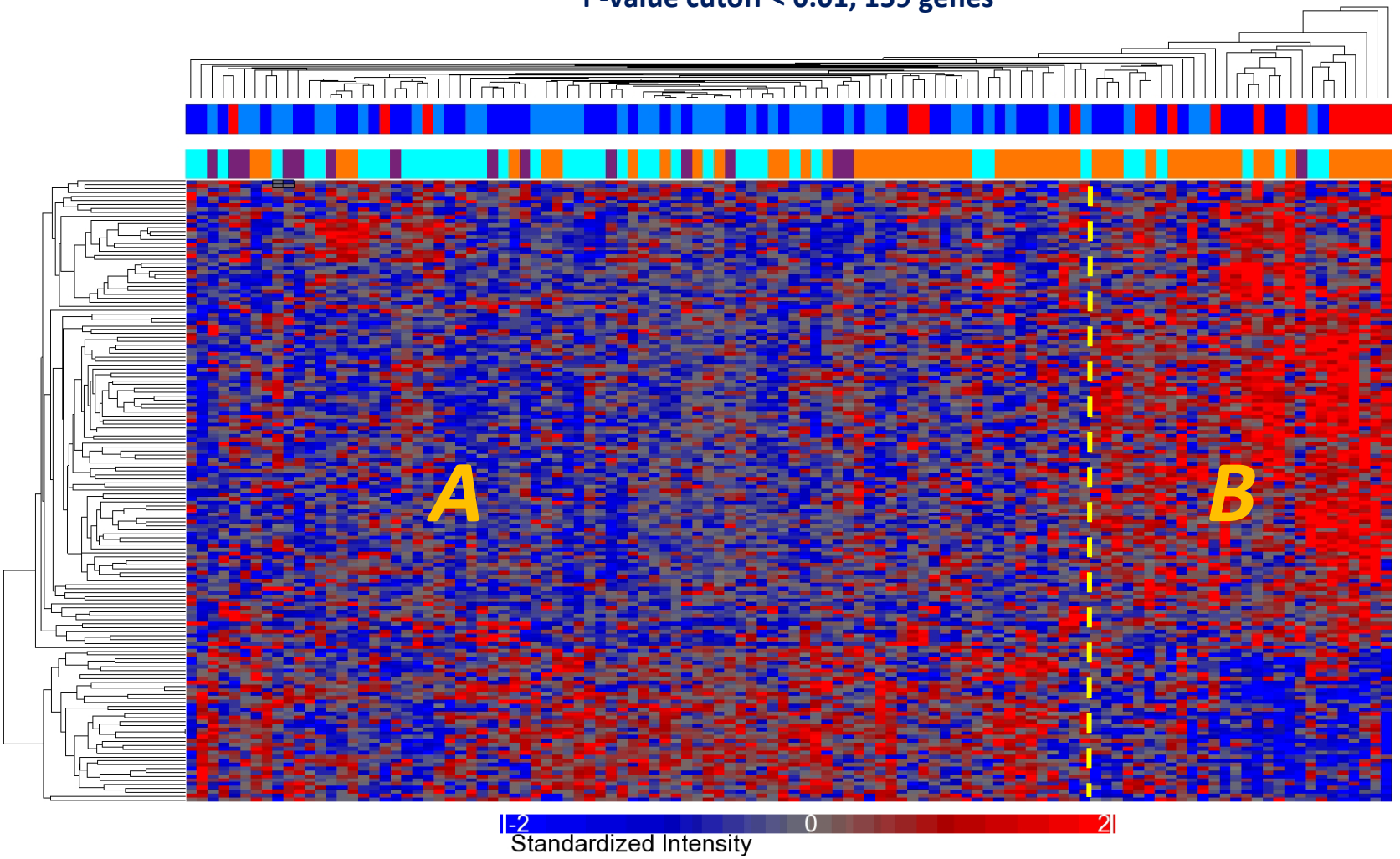
15 melanoma cell lines derived from the 15 melanoma metastases



Parental Tumors

Cluster built on *rs10954213:* AA+AG vs.GG among 112 melanoma metastases

P-value cutoff < 0.01, 159 genes



	R	NR	Total
Group A	50	33	83
Group B	8	21	29
Total	58	54	112

Fisher's exact test Two-tailed p value < 0.0027

Parental Tumors

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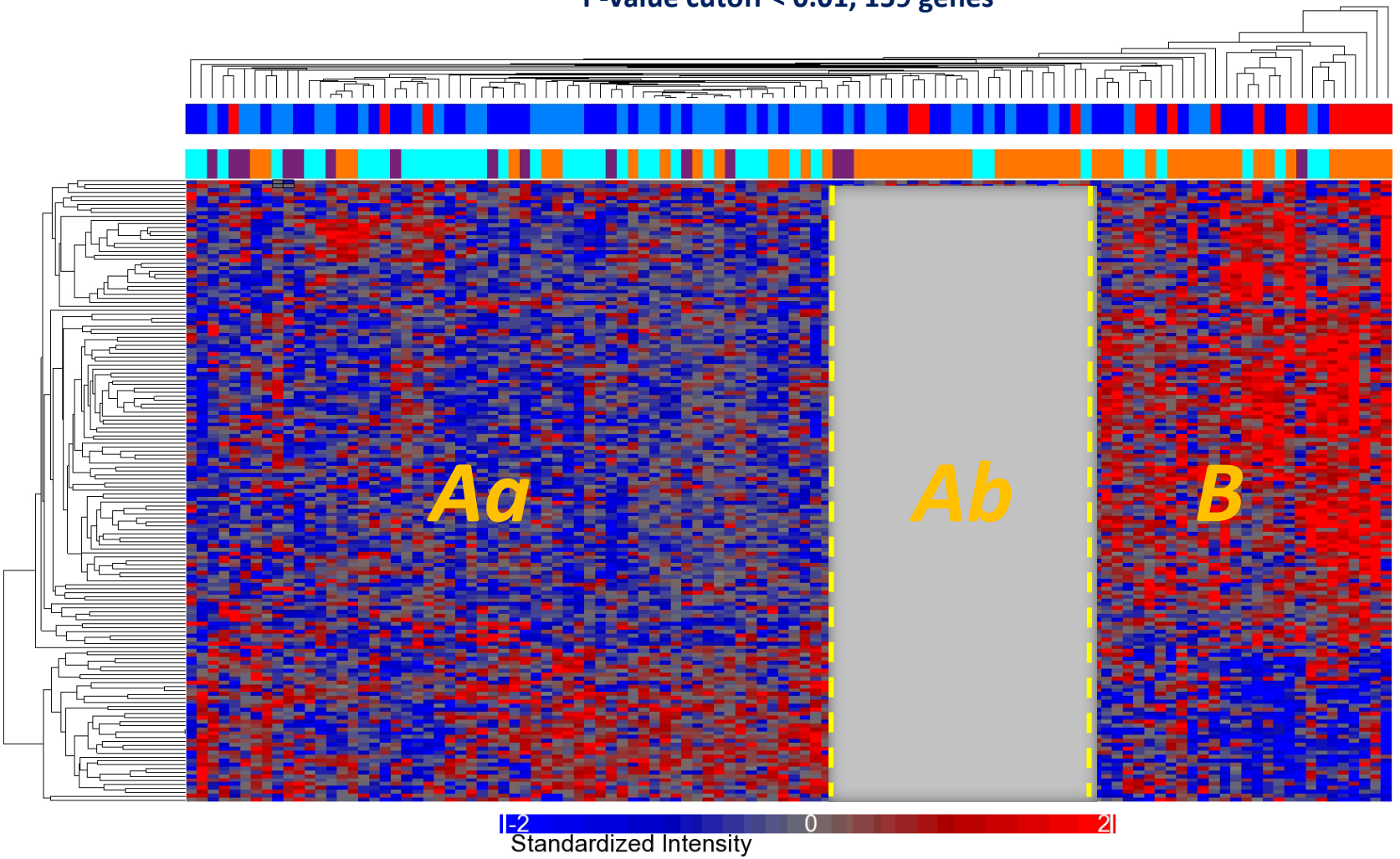


	R	NR	Total
Group A	50	33	83
Group B	8	21	29
Total	58	54	112

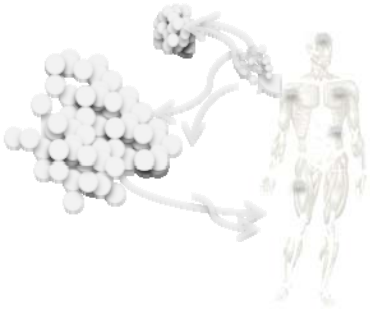
Fisher's exact test Two-tailed p value < 0.0027

	R	NR	Total
Group Aa	45	15	60
Group B	8	21	29
Total	53	54	89

Fisher's exact test Two-tailed p value < 0.00003

The functional genome of TILs and/or MM belonging to distinct IRF-5 genotypes predicts responsiveness

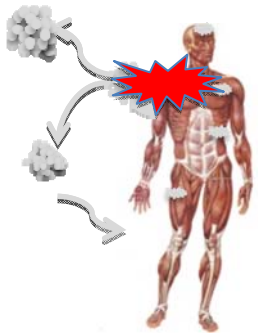
- TILs



Gene expression profile of
142 TILs

borderline differences
in prediction of Response

- **MM**



Gene expression profile of
112 pre-treatment melanoma metastasis

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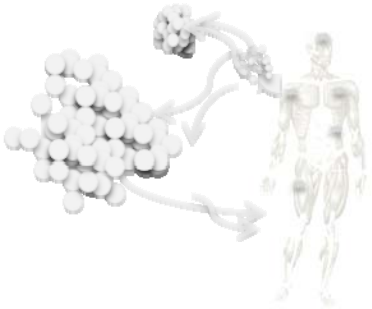

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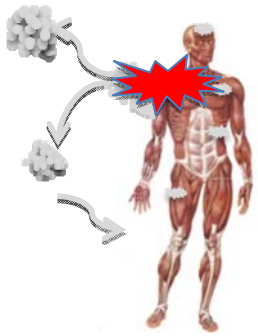

Gene expression profile of
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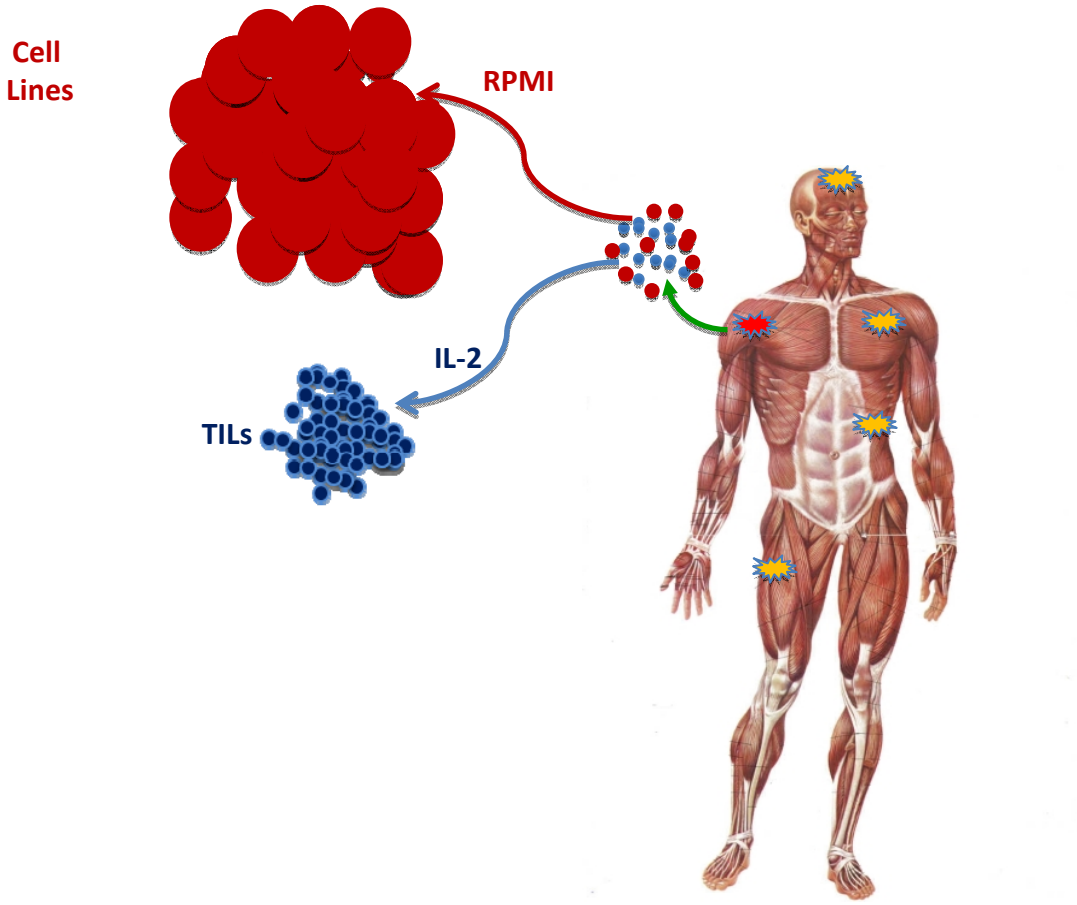
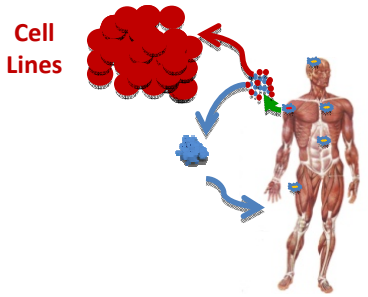
Is a stronger predictor of Response
compared with TILs

Analysis of melanoma cell lines

to test the weight of the IRF5 genotype on the intrinsic biology of cancer cells independent of microenvironment influences



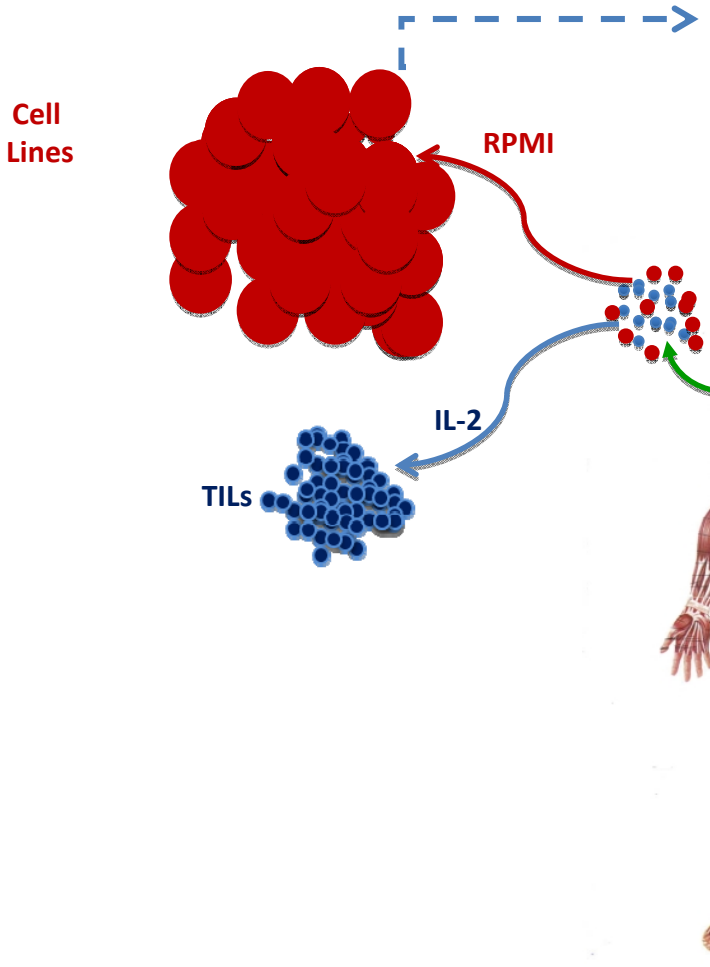
IRF5 *rs10954213* (G>A) in cell lines vs. TILs



Cell lines ID		rs10954213 (G>A)	
		Melanoma cell lines	Germline
Coded TRi_120	3104	AA	AA
Coded TRi_064	2458	AA	AA
Coded TRi_121	3107	AA	AA
Coded TRi_030	2155	AA	AA
Coded TRi_077	2744	AA	AA
Coded TRi_048	2492	AA	AA
Coded TRi_047	2448	AA	AA
Coded TRi_032	2224	AG	AG
Coded TRi_062	2523	AG	AG
Coded TRi_013	2035	AG	AG
Coded TRi_040	2427	AG	AG
Coded TRi_016	2075	AG	AG
Coded TRi_109	3025	-/G **(LOH)**	AG
Coded TRi_005	1866	GG	GG
Coded_Tri_088	2805	GG	GG

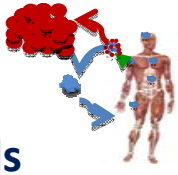
Analysis of melanoma cell lines

to test the weight of the IRF5 genotype on the intrinsic biology of cancer cells independent of microenvironment influences



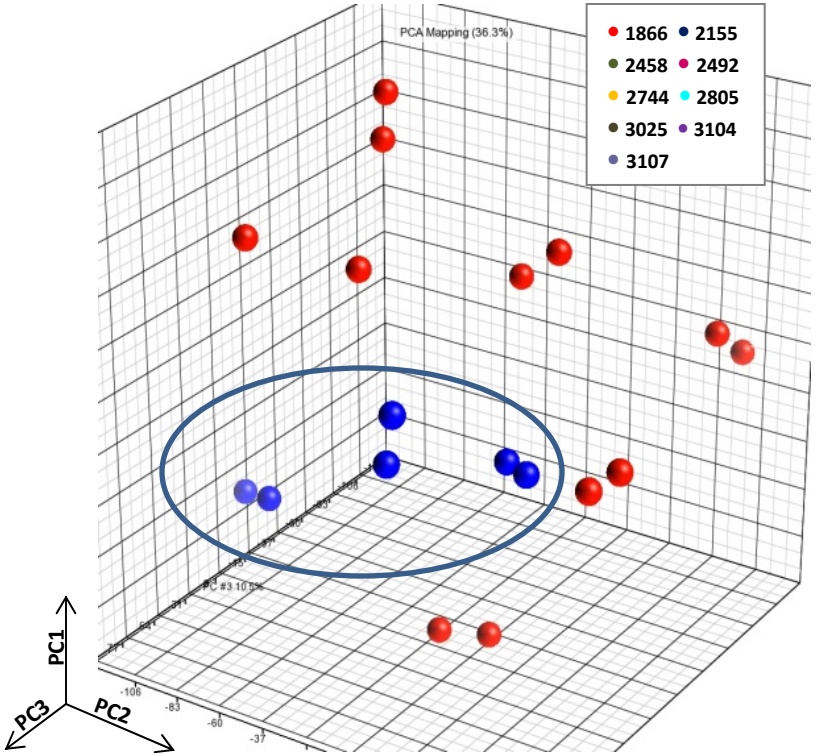
t test	IFN-α vs no Tx	AA vs GG		
		IFN+	IFN-	IFN +/-
p<0.001	118	47	50	255
p<0.05	1151	1570	1395	3489

Analysis of melanoma cell lines



to test the weight of the IRF5 genotype on the intrinsic biology of cancer cells independent of microenvironment influences

Sample ID
rs10954213 IRF5 genotype **IFN-α treatment**

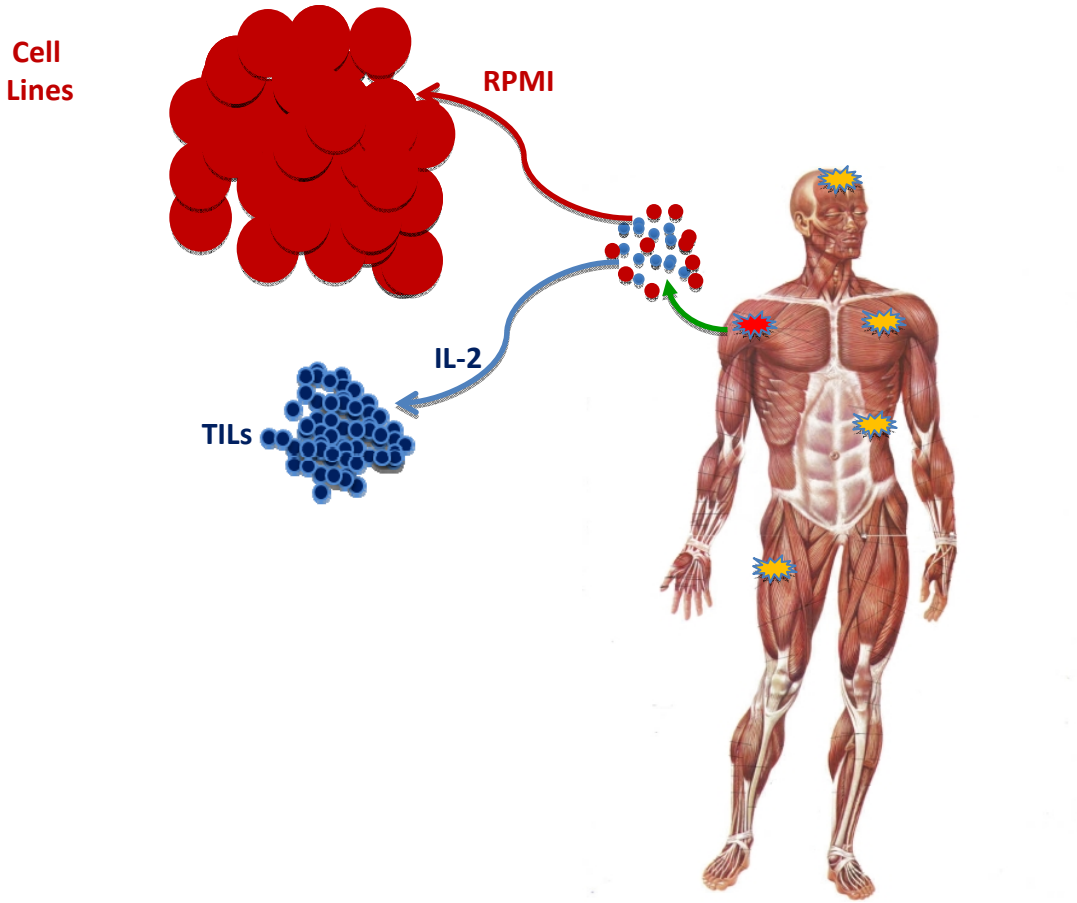


t test	IFN-α vs no Tx	AA vs GG		
		IFN+	IFN-	IFN +/-
p<0.001	118	47	50	**255**
p<0.05	1151	1570	1395	**3489**

signature of 255 genes that differentiate the two cell line genotypes independently of the IFN treatment

Analysis of melanoma cell lines

to test the weight of the IRF5 genotype on the intrinsic biology of cancer cells independent of microenvironment influences

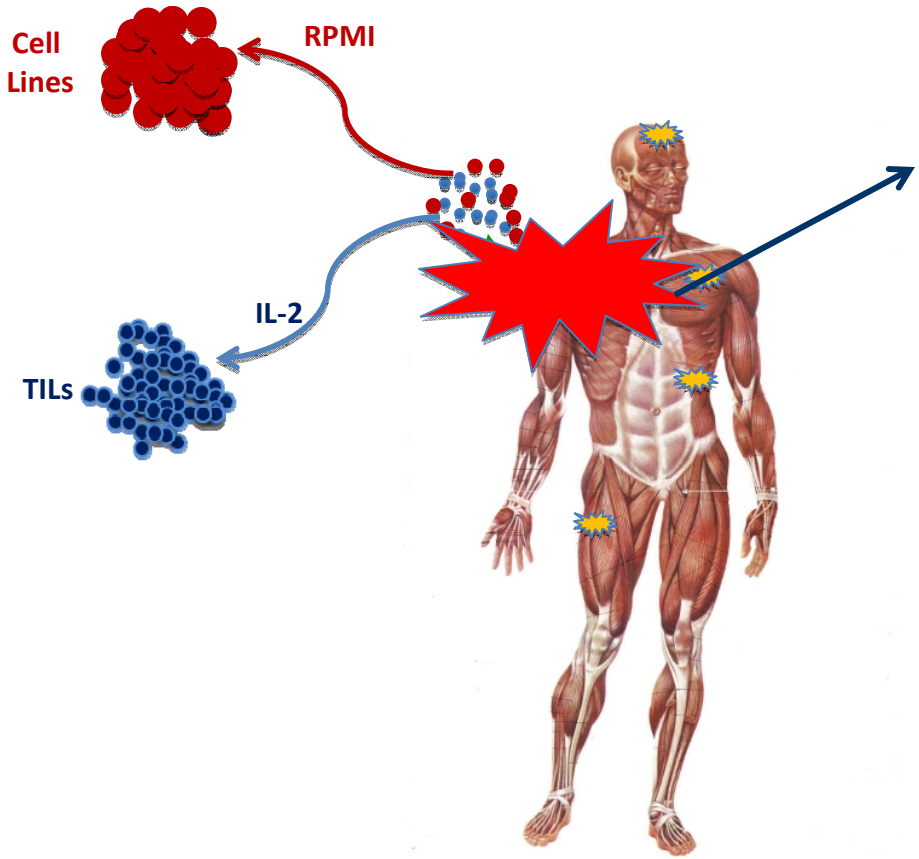


Analysis of Parental Tumors

142 TILs from patients enrolled in five adoptive cell therapy trials

112 parental melanoma metastases

15 melanoma cell lines derived from the 15 melanoma metastases

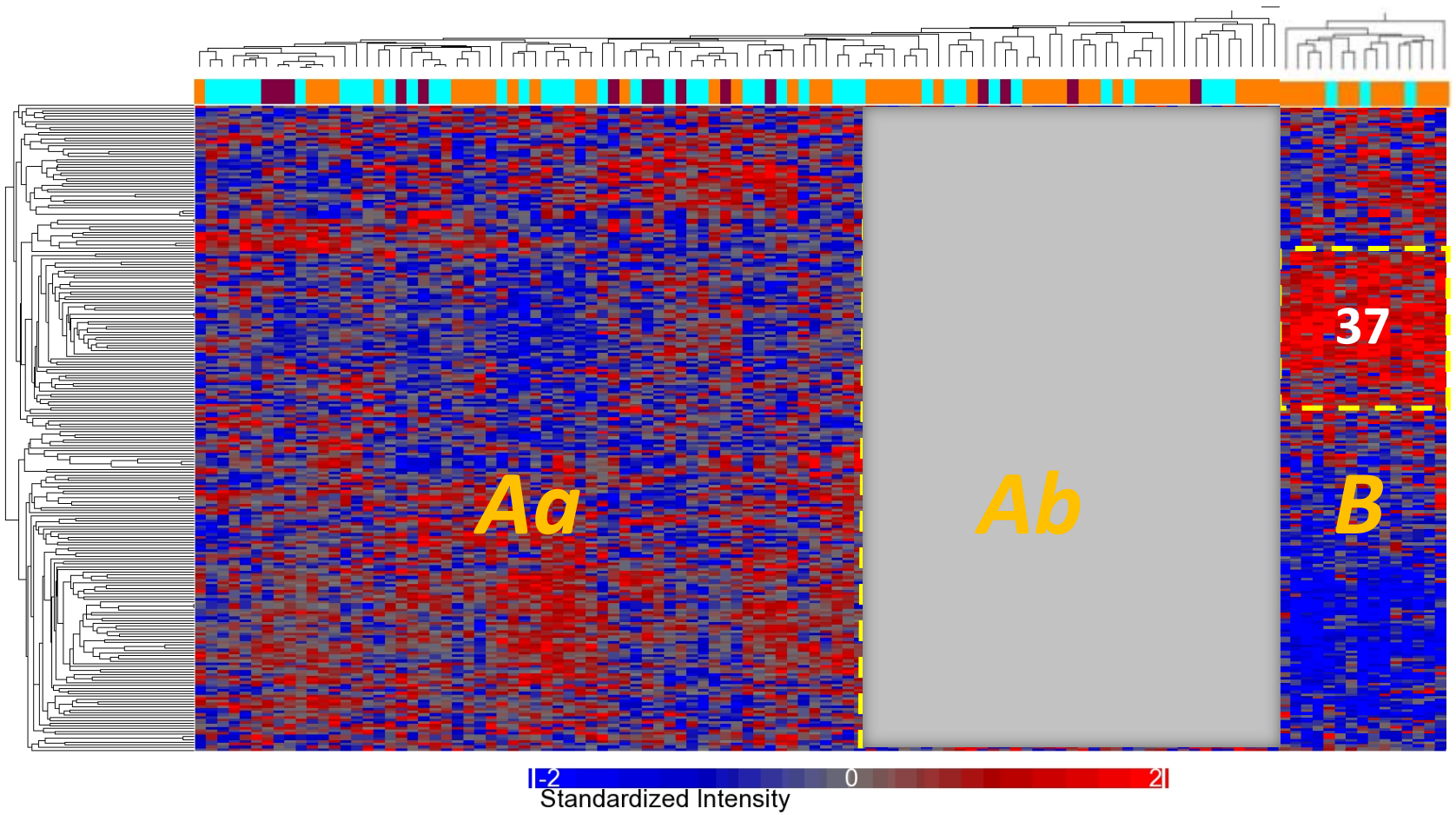


rs10954213: **AA+AG vs.GG**
p-value < 0.001, 236 genes

Cluster of Tumors based on cell lines

comparing AA vs GG at p < 0.001

37 genes



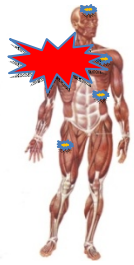
Fisher test *Aa* vs *B* p_2-value < 0.00001

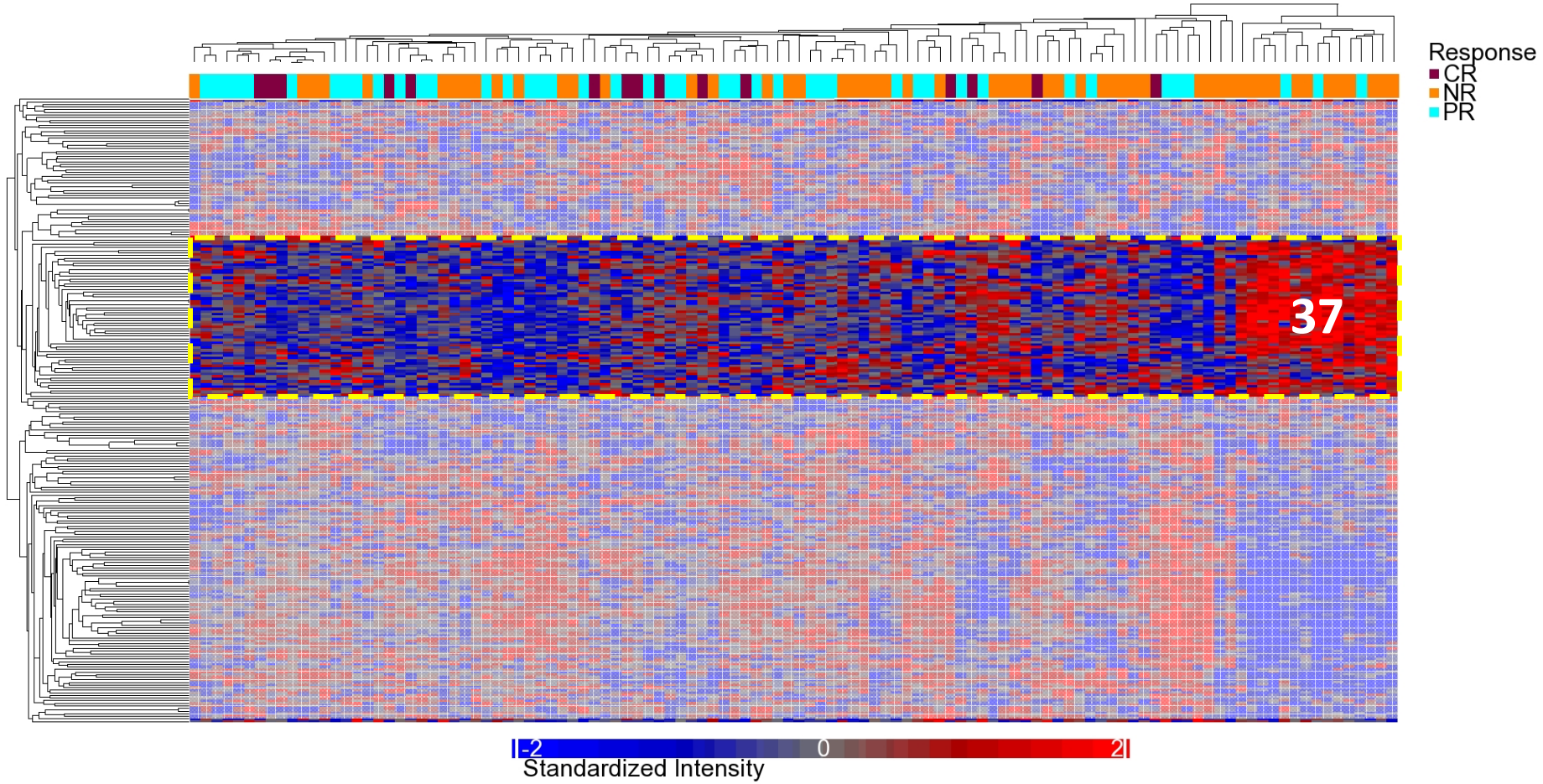
	R	NR	Total
Group Aa	41	19	60
Group B	3	15	29
Total	44	34	89

Cluster of Tumors based on cell lines

comparing AA vs GG at p < 0.001

37 genes

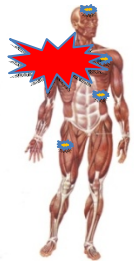


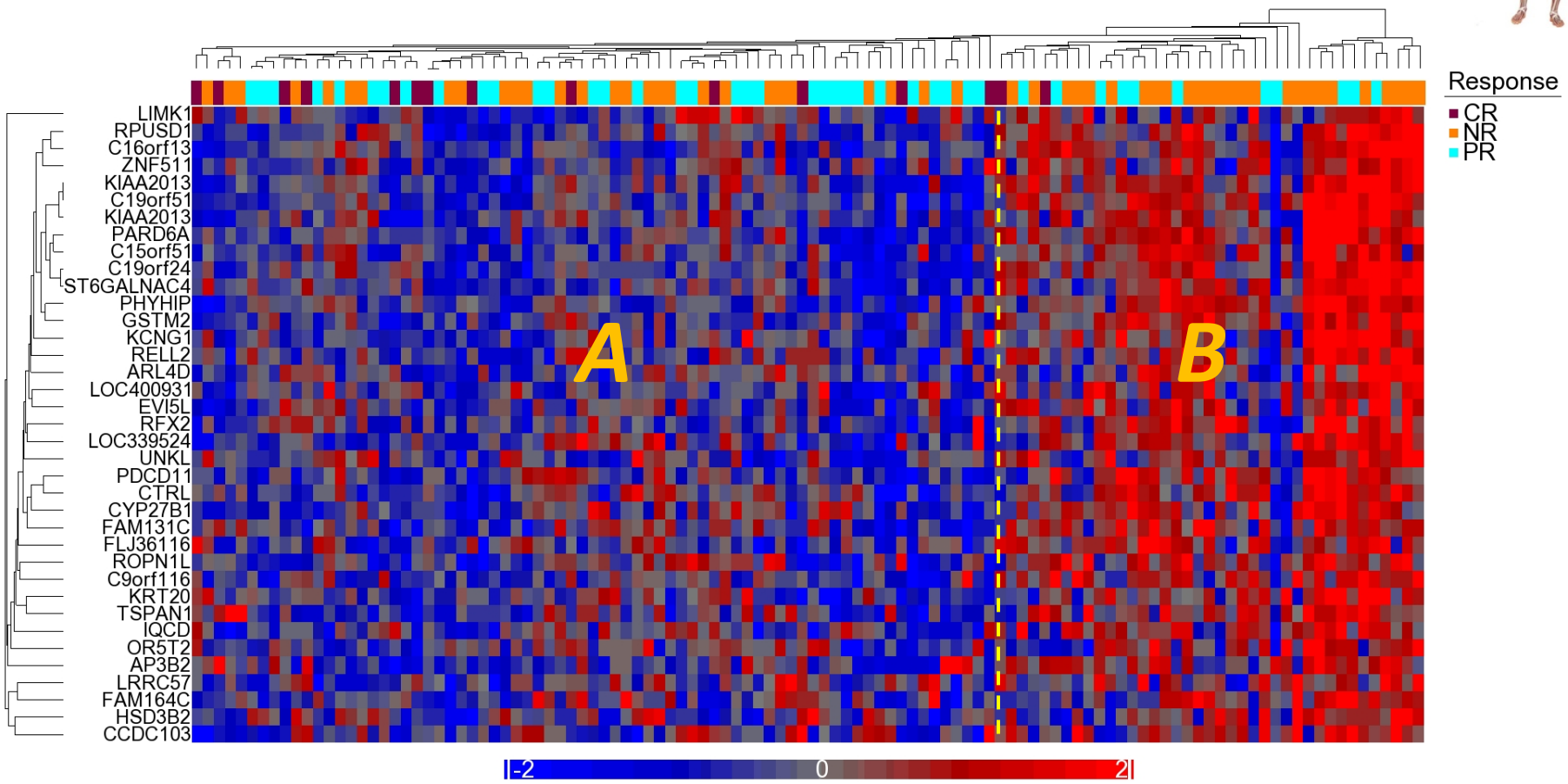


Cluster of Tumors based on cell lines

comparing AA vs GG at p < 0.001

37 Predictors of No Response





Fisher's test A vs B p_2-value is < 0.005

	R	NR	Total
Group A	45	28	73
Group B	13	26	39
Total	58	54	112

Summary

1. **Polymorphism of IRF-5 appears to be a predictor of immune responsiveness of melanoma metastases to adoptive therapy with TIL**

2. **The rs10954213 G allele, which is protective against SLE, is the most predictive of non responsiveness suggesting a correlation between autoimmunity and melanoma immune responsiveness.**

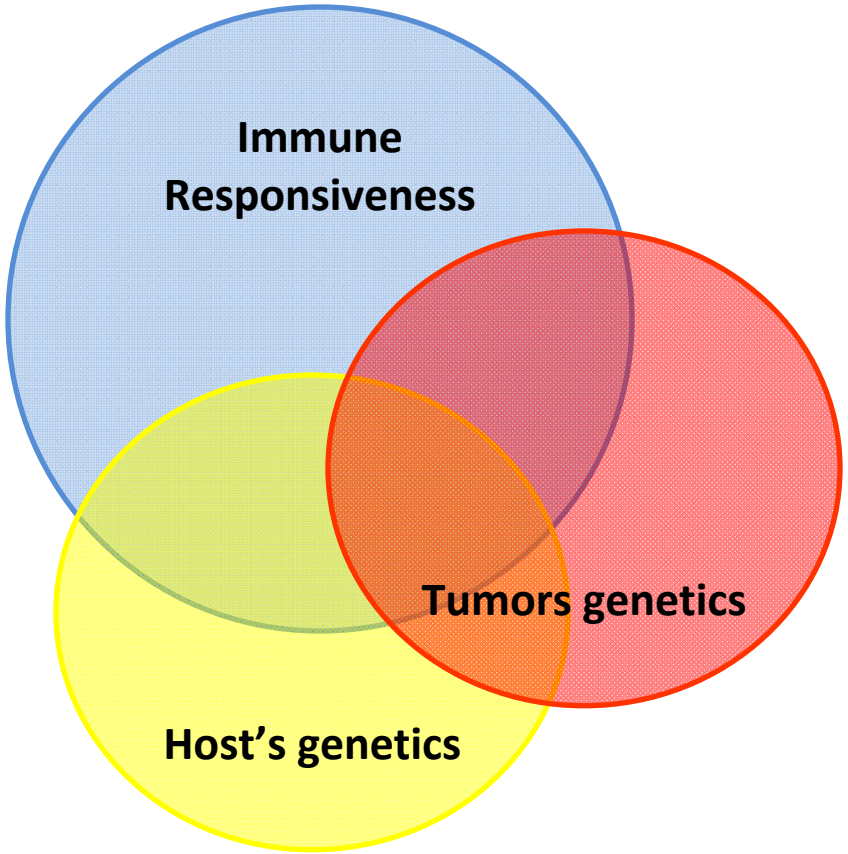
3. **The expression profile of TIL classified according to AA vs GG IRF5 *rs10954213* (G>A) appears to be a borderline predictor of immune responsiveness**

4. **The expression profile of pre-treatment melanoma metastases classified according to AA vs GG IRF5 *rs10954213* (G>A) appears to be a stronger predictor of immune responsiveness compared with TILs**

5. **Comparison of melanoma cell lines classified according to the AA vs GG IRF5 *rs10954213* (G>A) highlights a signature of genes that differentiates the two genotypes independently of micro environmental influences**

6. **The signatures differentiating the two cell line genotypes *in vitro* could predict of the responsiveness of melanoma metastases *in vivo* suggesting that immune responsiveness is at least in part genetically determined.**

CONCLUSIONS



Thus, it appears that immune responsiveness is at least in part dependent on the genetic background of the host which affects the biology of cancer cells primarily and secondarily the immune responsiveness of tumors

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