



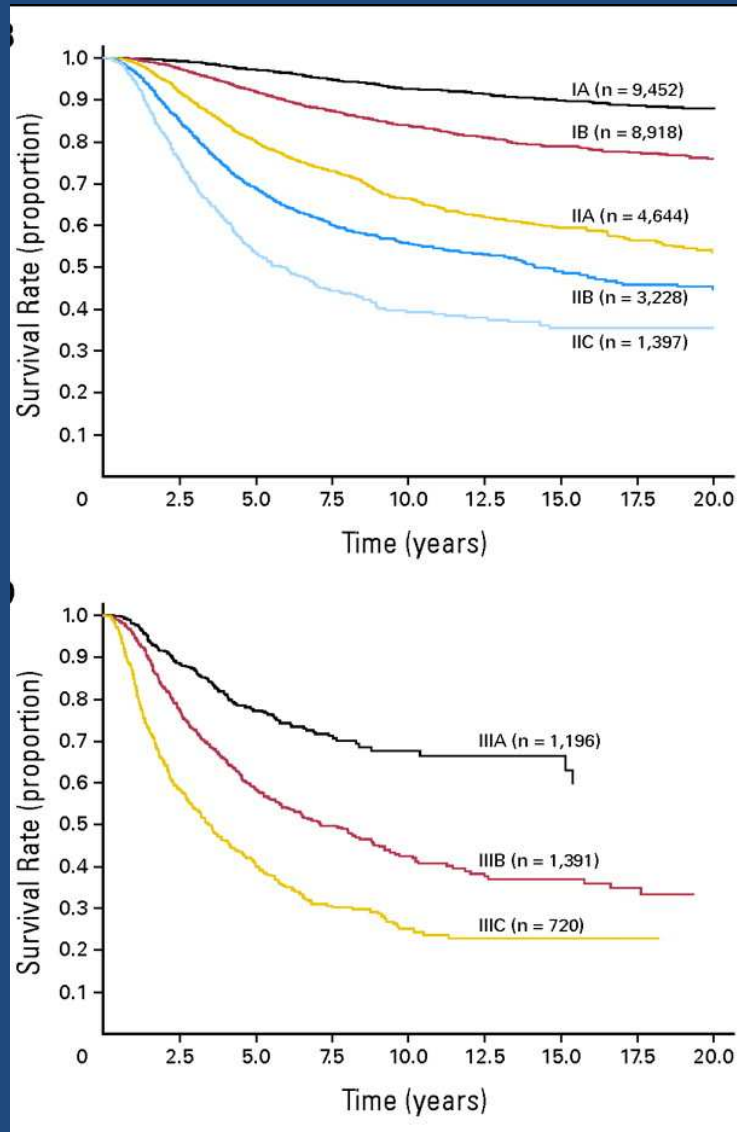
# Dissecting genomic networks of immune surveillance in stage II-III resectable melanoma

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Society for the Immunotherapy of Cancer  
November 9, 2013

# Relevant Disclosures

- *Patent filed for the gene signature*

# The clinical problem of stage II-III resectable melanoma



What do we tell the patient?

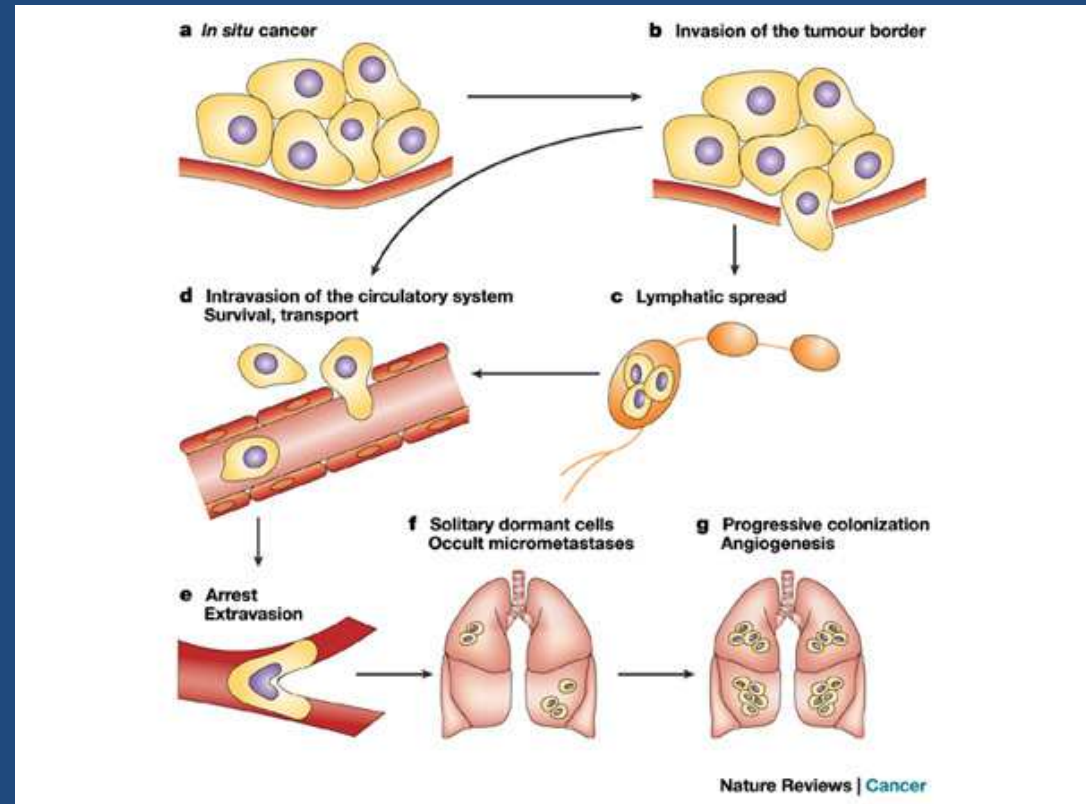
Should she take interferon  
or an experimental  
treatment?

Should she get scans? How  
often?

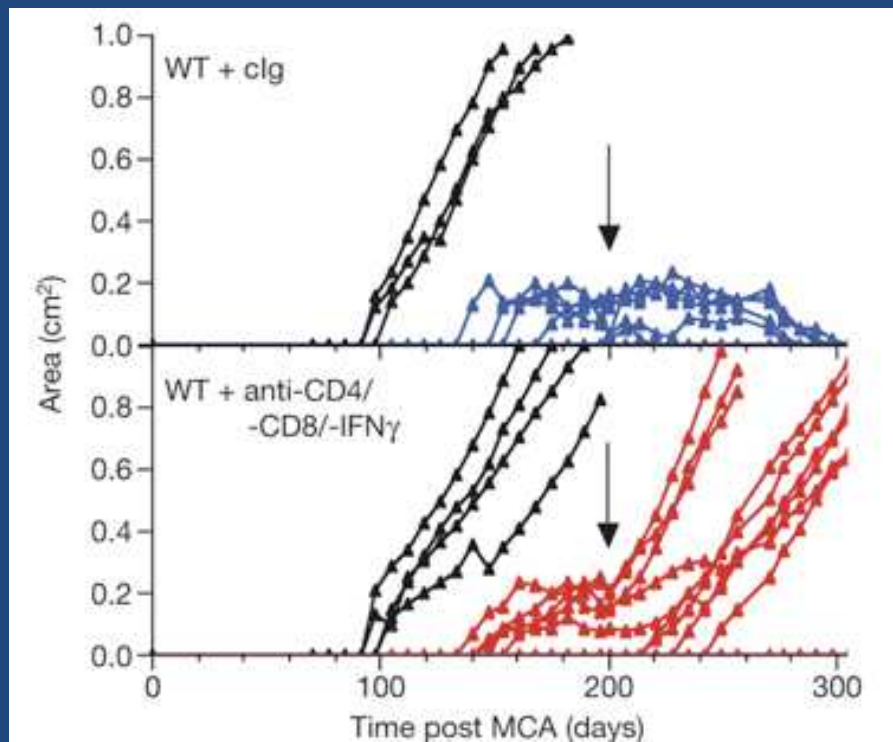
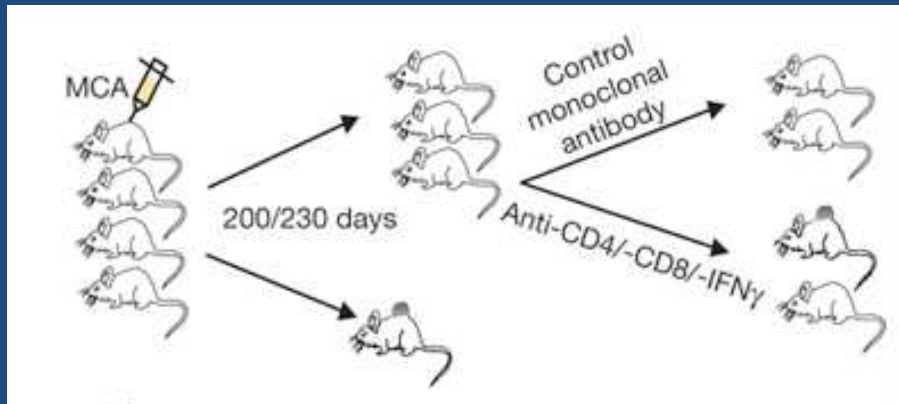
Final version of 2009 AJCC staging for melanoma,  
Balch et al *JCO*

# Has the tumor already spread before it was surgically removed?

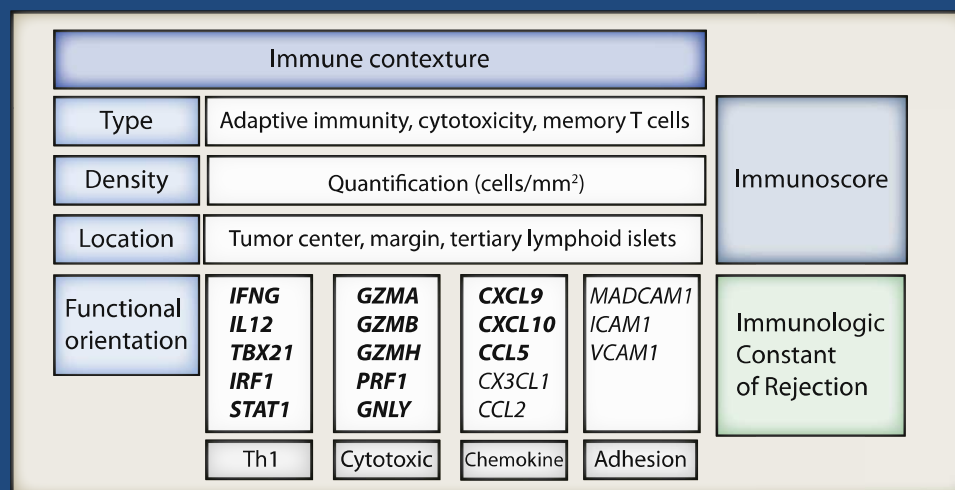
- Tumor intrinsic factors
  - Oncogenes
  - Cell type of origin
  - Location of primary tumor
- Host factors
  - Tumor micro-environment
  - The immune system



# Immune surveillance controls dormant tumors



Koebel et al. Nature;450:903, 2007

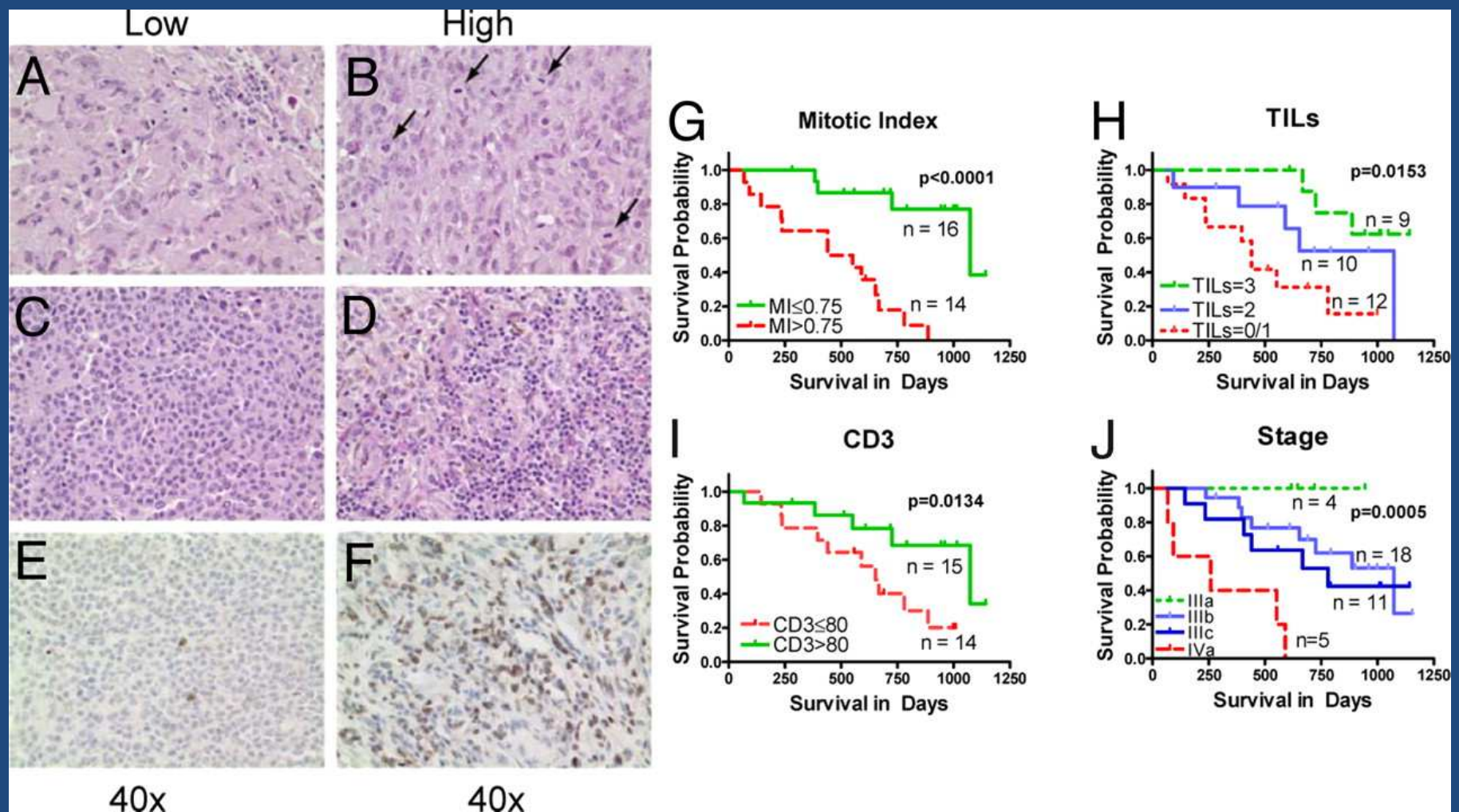


**Table 1. A Summary of the Key Studies and Overlapping Genetic Pathways Observed in Prognostic, Predictive, and Mechanistic Signatures**

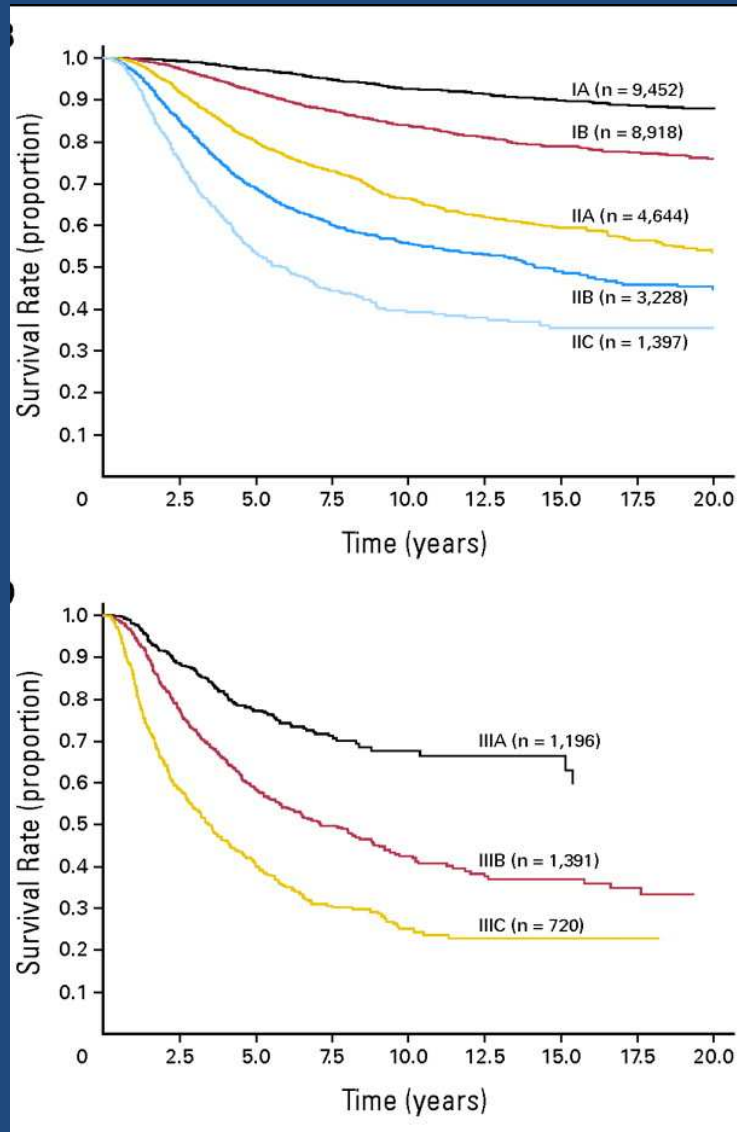
	Th1 STAT1 IRF1/ IFN-γ-SG Pathway	Chemokines CXCR3/ CXCL9-11 Pathway	Cytotoxic CCR5/ CCL3-5 Pathway	Adhesion Granzyme Perforin Granulysin/TIA1/ Caspase Pathway	Adhesion Molecules	References
<b>Cancers</b>						
<b>Prognostic</b>						
Breast	+	+		+	+	Ascierto et al., 2012
	+	+	+		+	Curtis et al., 2012
	+					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
<b>Predictive</b>						

Galon et al: *Immunity* 39(1):  
11 - 26 (2013)

In Melanoma, CD3 count and TILs correlate with improved survival in Stage IIIA-IV C disease. Expression of immune genes as measured by affymetrix also associated with a favorable outcome



# The clinical problem of stage II-III resectable melanoma



What do we tell the patient?

Should she take interferon  
or an experimental  
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Should she get scans? How  
often?

Final version of 2009 AJCC staging for melanoma,  
Balch et al *JCO*

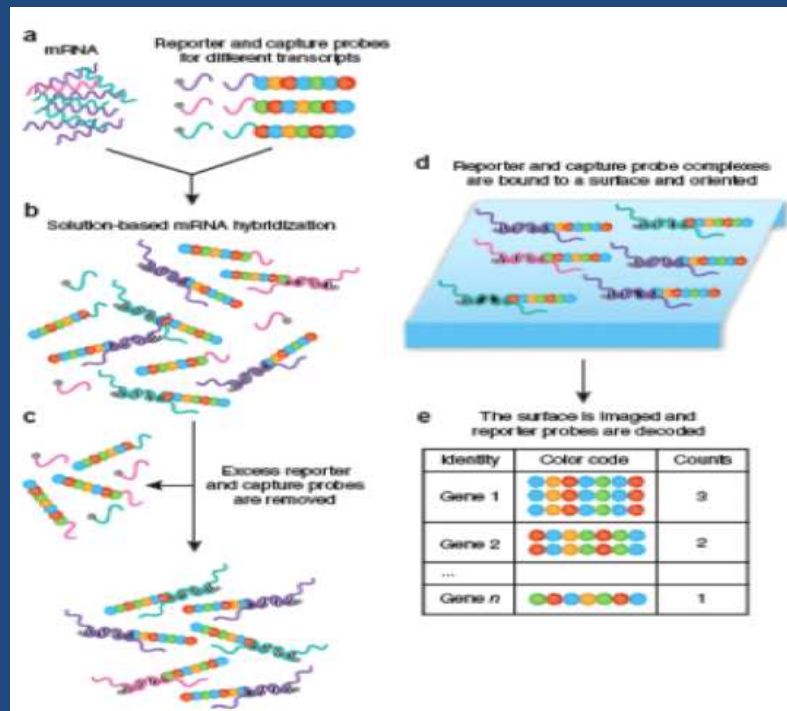
# Using NanoString technology to measure immune mRNA transcripts in FFPE melanomas

Immune gene with proposed impact on melanoma progression:  
294

Immune gene with proposed impact on cancer progression:  
96

Non-immune gene with proposed impact on melanoma progression:  
29

Total number of candidate genes:  
446



# mRNA copy number for 446 genes in a training set of primary tumors from patients with resectable stage II-III melanoma

## Characteristics of the Training Set

### Characteristics Training Set (N=40)

#### Clinical characteristics

Gender	
Male--no.(%)	28 (70)
Female--no.(%)	12 (30)
Age median (range) --no.	67 (29-87)
Location of tumor	
Trunk--no.(%)	24 (60)
Extremity--no.(%)	16 (40)
Stage	
II--no. (%)	18 (45)
III--no. (%)	22 (55)

#### Pathological characteristics

Depth (mm) -- median (range)*	2.7 (1.2 -13)
Ulceration	
Absent--no.(%)	21 (52)
Present--no.(%)	19 (48)
Tumor-infiltrating lymphocytes	
Absent--no.(%)	7 (17)
Non-brisk--no. (%)	28 (70)
Brisk--no.(%)	5 (13)
Mitoses --median (range)	6.5 (0-26)

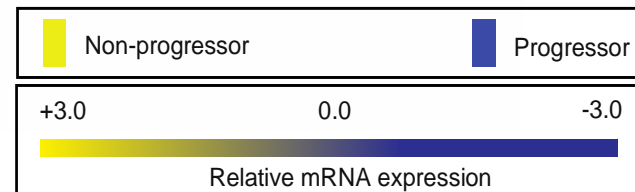
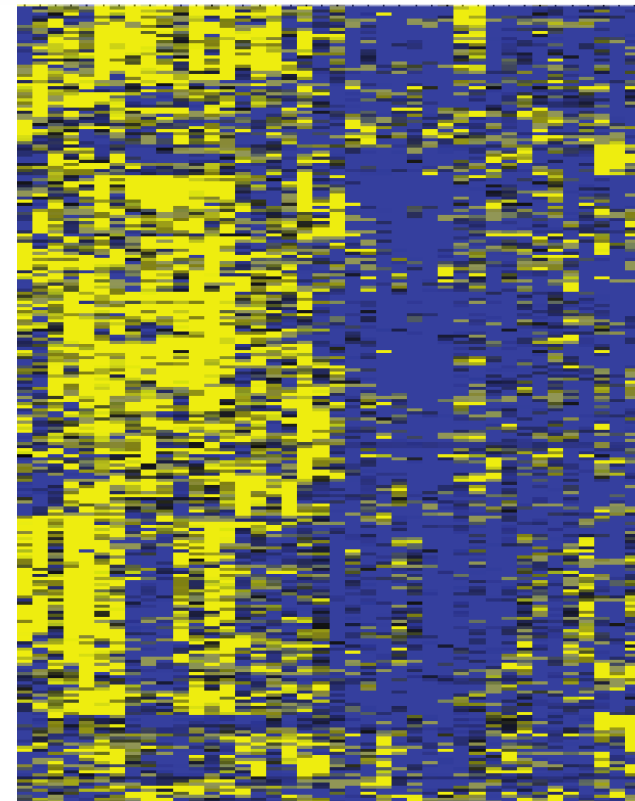
#### Patient outcome

Disease progression-- no. (%)	21 (52)
Died from melanoma-- no.(%)	17 (43)

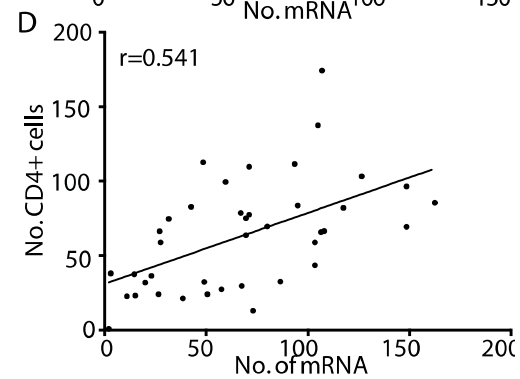
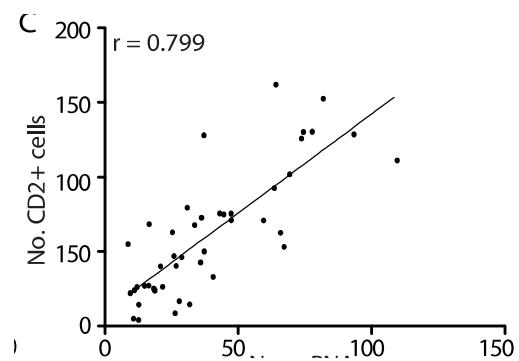
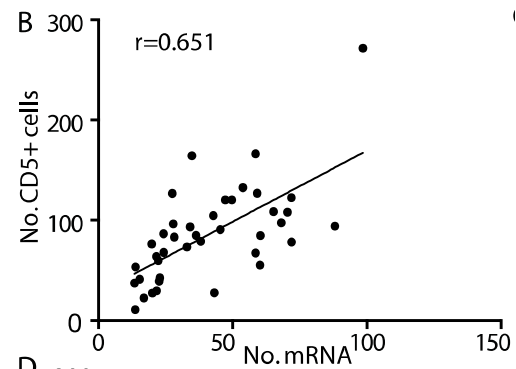
#### Patient Follow-up (months)

Time to death--median (range)	19 (6-81)
Time to censoring-- median(range)	61 (25-130)

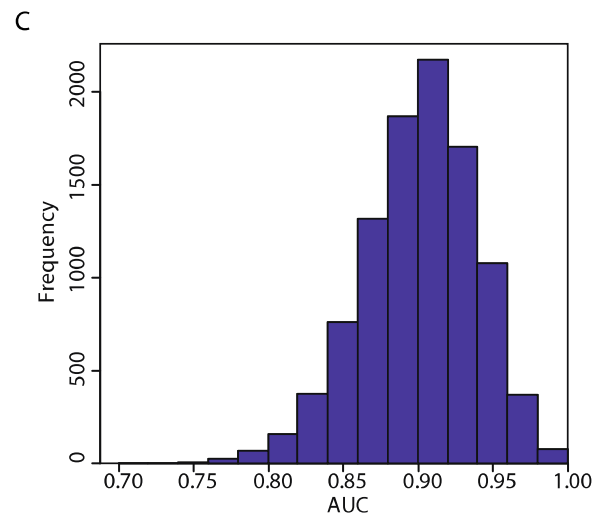
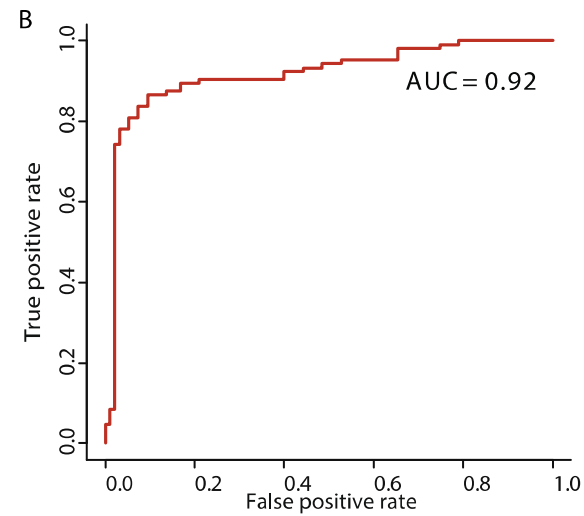
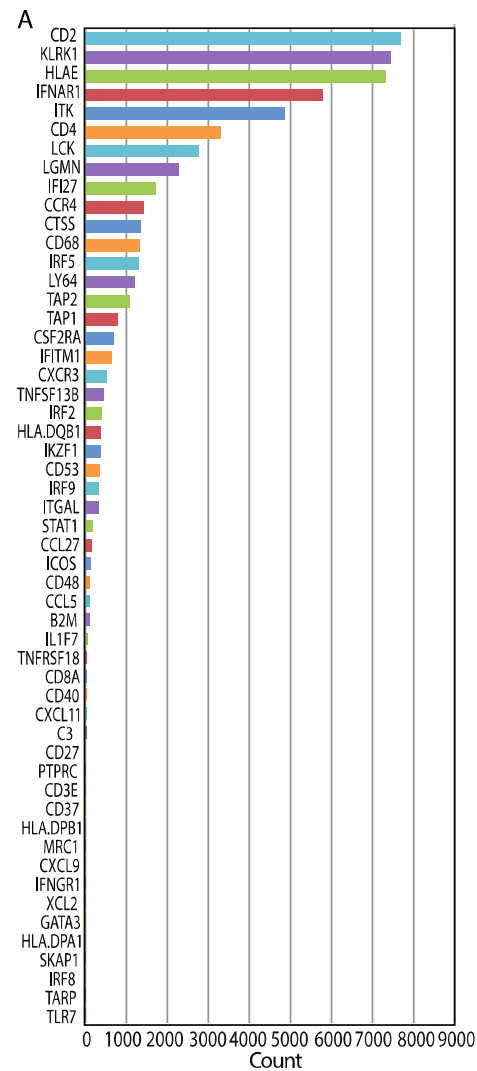
\* Depth is available for 39 patients.



# Confirming of results using Immunohistochemistry



# Defining a 53 immune gene panel predictive of non-progression



## The 53-immune gene panel correlates closely with overall survival in training set (N=40)

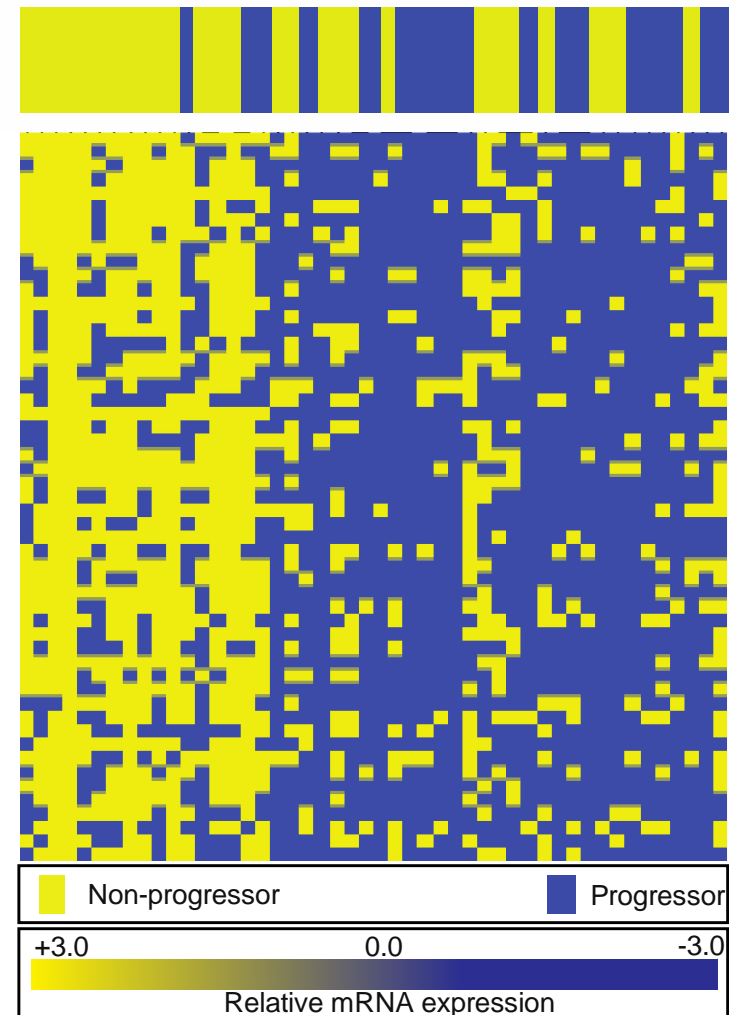
Variable	DF	Chi-square	Pr > Chi <sup>2</sup>
PRED SCOR	1	26.1776796	< 0.0001
depth	1	4.140359603	0.042
mitotic rate	1	2.853251262	0.091
age	1	6.265897259	0.012
TILS	1	7.325302867	0.007
location	1	5.249929277	0.022
stage II vs III	1	3.226333623	0.072
gender	1	0.263269823	0.608
ulceration	1	3.534401681	0.060

# Confirming results from the training set in a second independent set of melanoma patients with stage II-III resectable melanoma

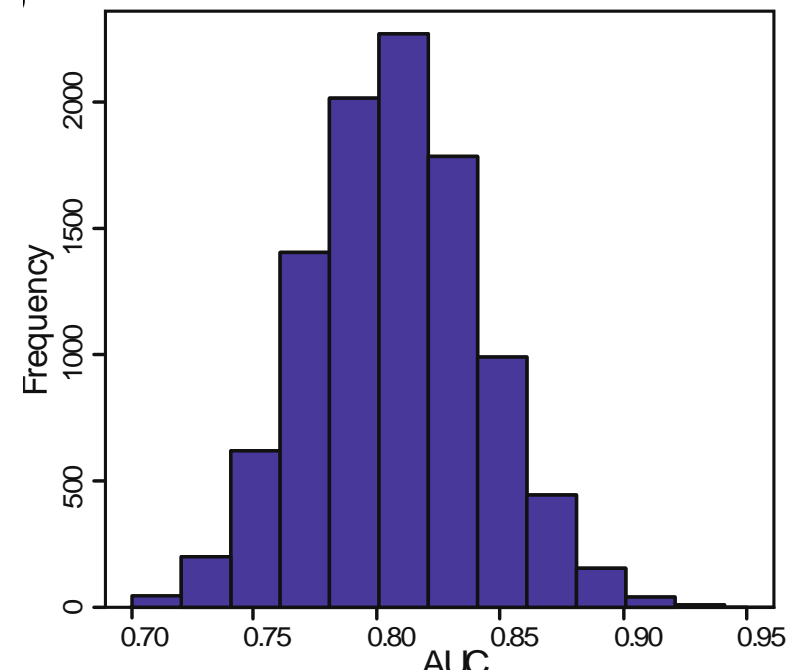
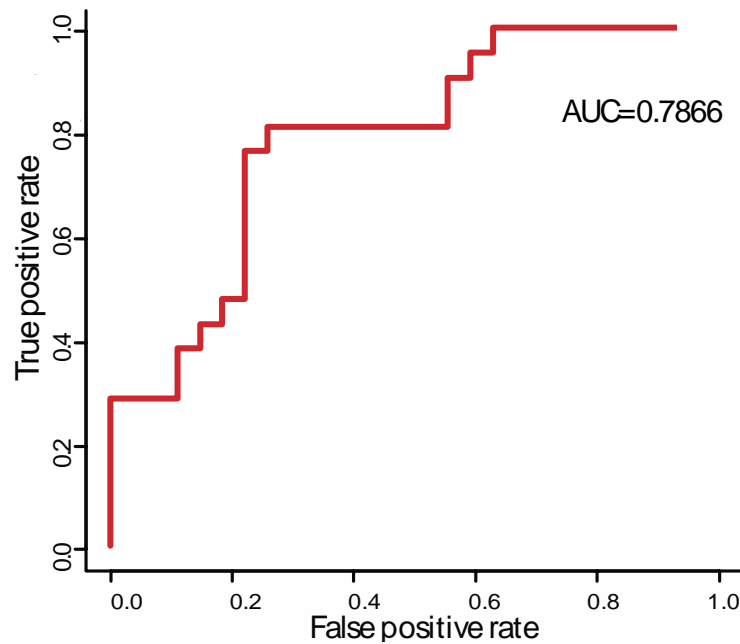
## Characteristics of the Test Set

Characteristics	Test set (N=48)
<b>Gender</b>	
Male -- no.(%)	26 (54)
Female -- no.(%)	22 (46)
<b>Age</b>	
Median (range) --no.	65 (27-90)
<b>Location of tumor</b>	
Trunk -- no.(%)	25 (52)
Extremity -- no.(%)	23 (48)
<b>Pathological characteristics</b>	
Depth (mm) -- median (range)	3.47 (1-30)
Ulceration	
Absent -- no.(%)	20 (42)
Present -- no.(%)	28 (58)
Tumor-infiltrating lymphocytes*	
Absent -- no.(%)	1 (2)
Non-brisk -- no. (%)	38 (79)
Brisk -- no.(%)	7 (15)
Mitoses -- median (range)*	3 (0-20)
Stage	
II -- no. (%)	25 (52)
III -- no. (%)	23 (48)
<b>Patient outcome</b>	
Disease progression- no.(%)	22 (46)
Died from melanoma -no.(%)	18 (45)
<b>Patient follow-up (months)</b>	
Time to death -- median (range)	36 (25-158)
Time to censoring -- median(range)	46 (26-146)

\* TILs and mitotic rate is available for 46 patients.

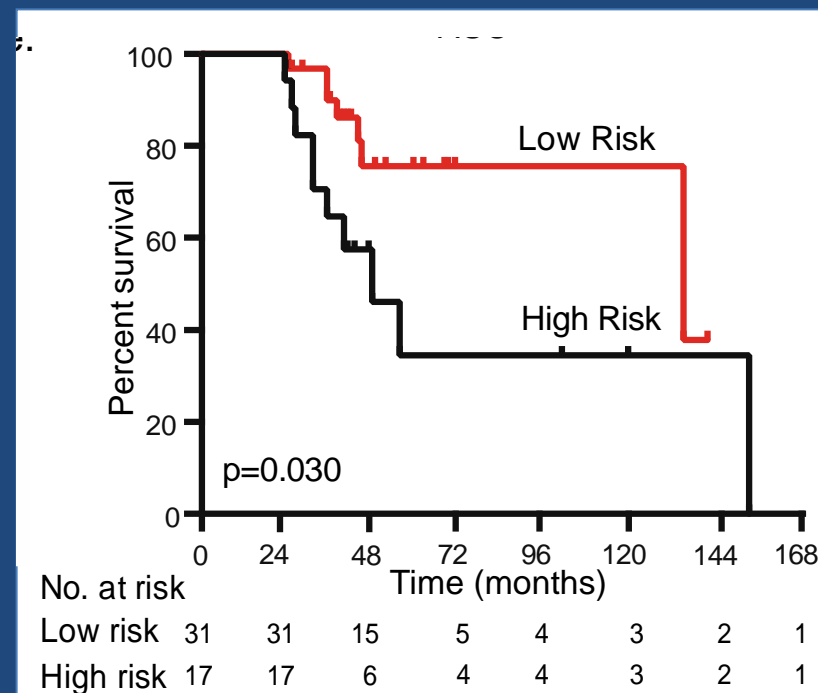


# Confirming predictive power of the 53-gene signature in an independent patient set



## 53-gene signature is correlates with overall survival in an independent patient set (N=48)

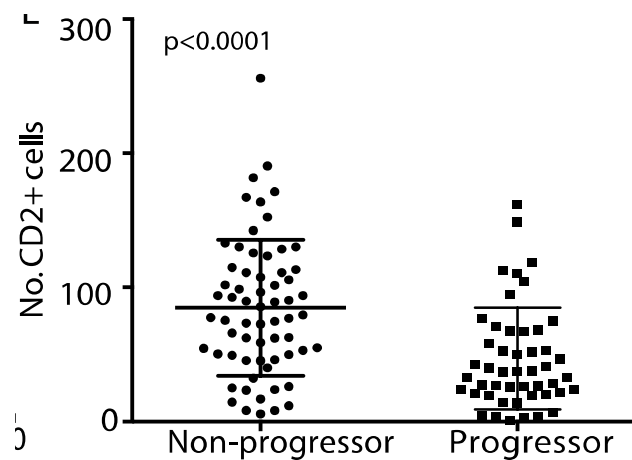
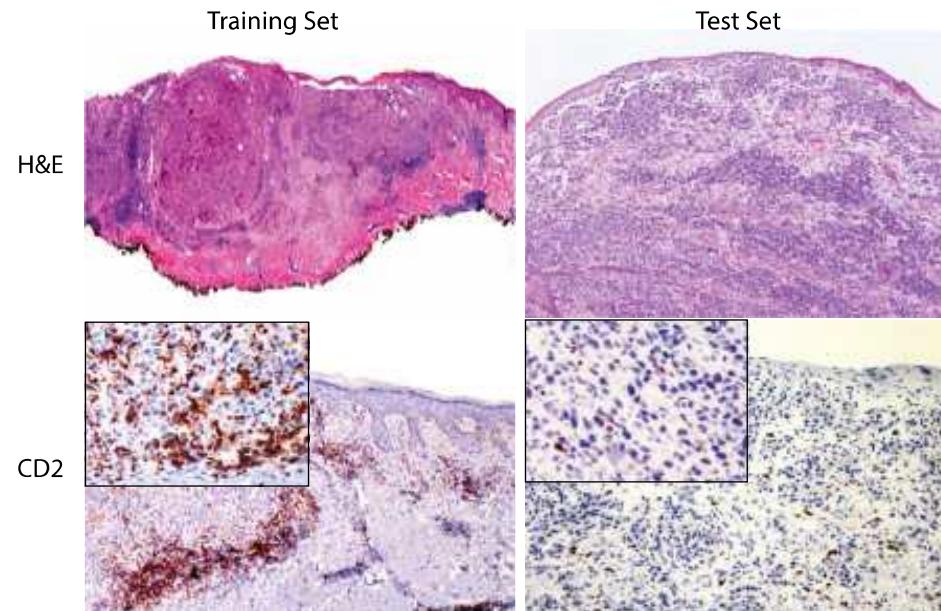
Variable	DF	Chi-square	Pr > Chi <sup>2</sup>
PRED SCOR	1	5.095	0.024
PRED SCORE+ ULC	2	7.977	0.019



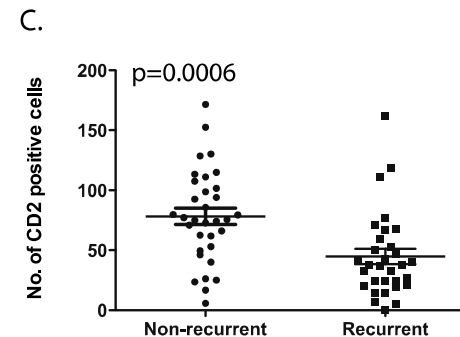
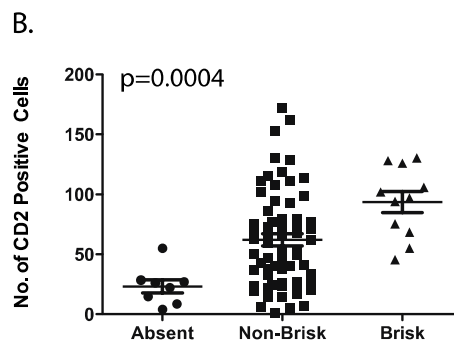
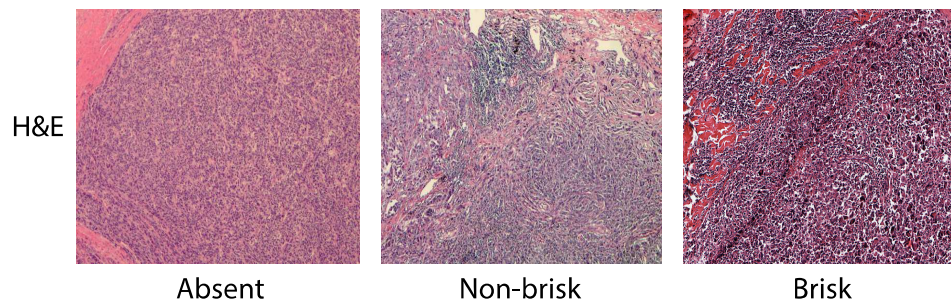
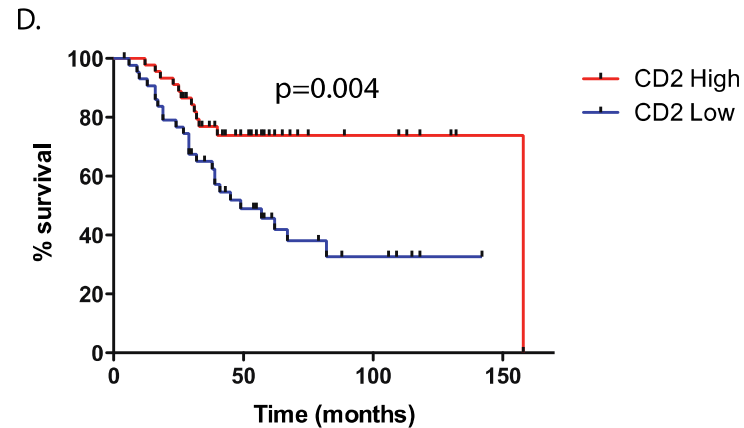
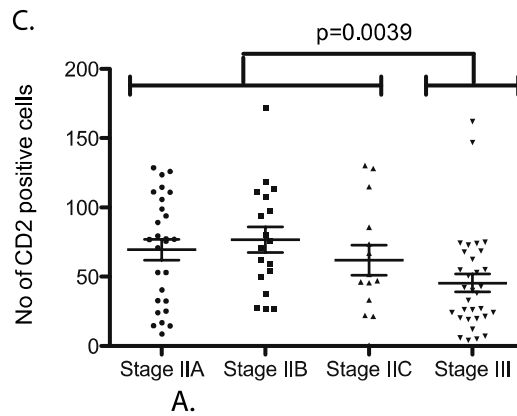
High risk= ulcerated and unfavorable gene score.  
median survival 39 months

Low risk= all others,  
median survival 149 months

# CD2 staining by IHC is also predictive of non-progression

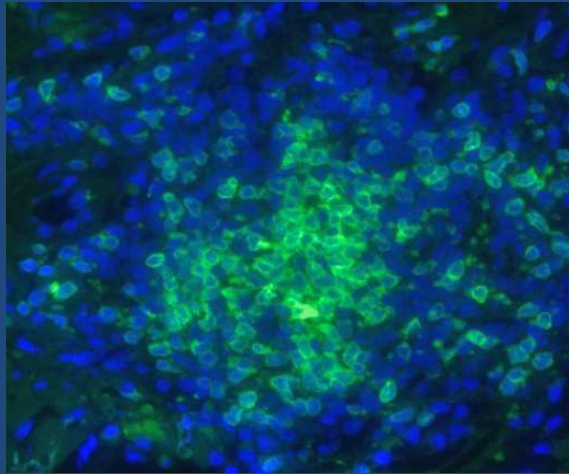


# CD2 staining by IHC is also predicts prolonged survival and correlates with stage, and adds to predictive power of TILs

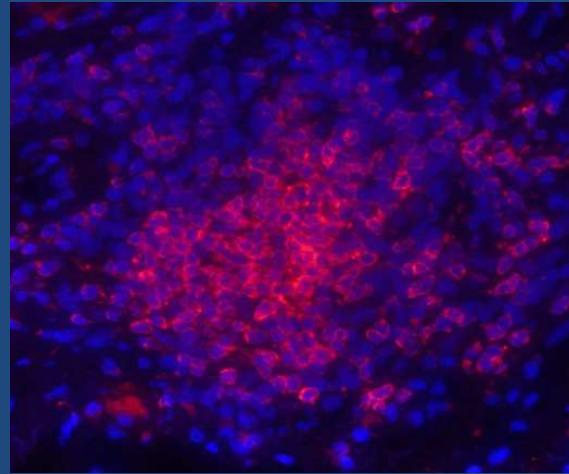


# What type of cells in the tumor express CD2?

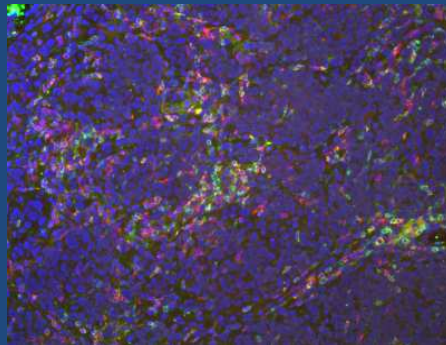
Anti-CD3



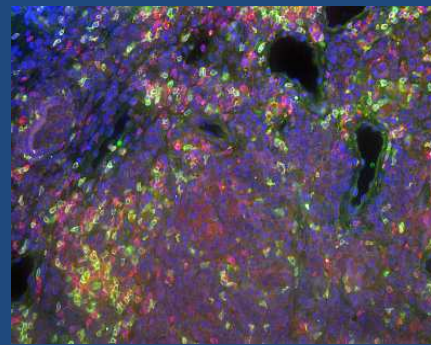
Anti-CD2



CD2/CD4



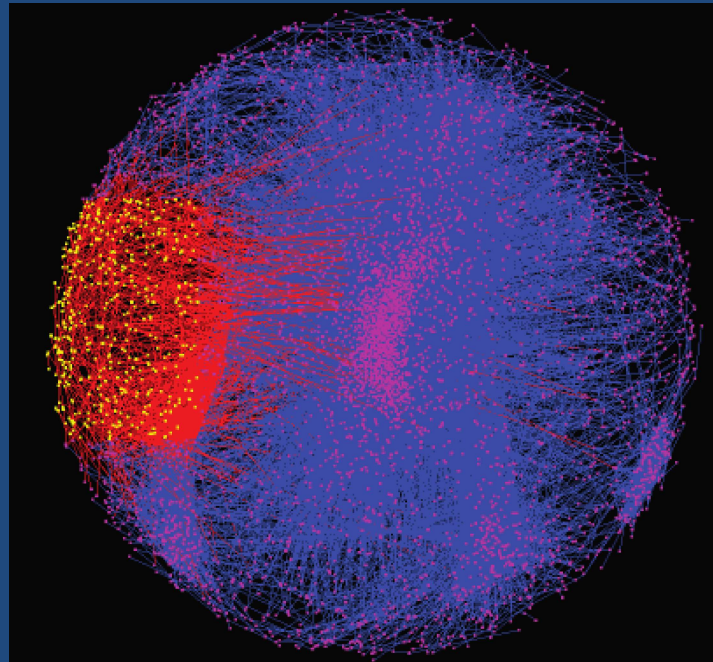
CD2/CD8



# Screen of primary melanoma expression data available in GEO identifies subnetwork of 758 gene module with immune function containing 42 out of the 53-gene immune panel

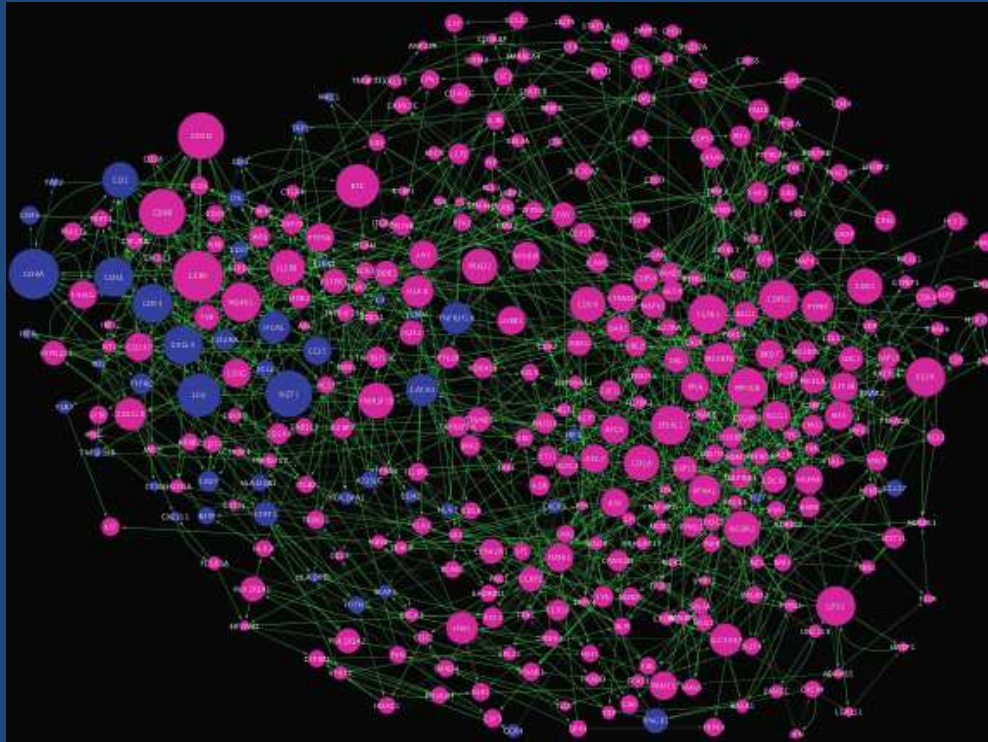
Table S3. Gene expression data from primary melanoma tumors GEO (GSE15605).

GEO sample	Phenotype
GSM390224	Primary_melanoma MEL101
GSM390225	Primary_melanoma MEL128
GSM390226	Primary_melanoma MEL131
GSM390227	Primary_melanoma MEL135
GSM390228	Primary_melanoma MEL142
GSM390229	Primary_melanoma MEL145
GSM390230	Primary_melanoma MEL157
GSM390231	Primary_melanoma MEL173
GSM390232	Primary_melanoma MEL176
GSM390233	Primary_melanoma MEL185
GSM390234	Primary_melanoma MEL190
GSM390235	Primary_melanoma MEL197
GSM390236	Primary_melanoma MEL209
GSM390237	Primary_melanoma MEL213
GSM390238	Primary_melanoma MEL233
GSM390239	Primary_melanoma MEL236
GSM390240	Primary_melanoma MEL243
GSM390241	Primary_melanoma MEL244
GSM390242	Primary_melanoma MEL250
GSM390243	Primary_melanoma MEL257
GSM390244	Primary_melanoma MEL258
GSM390245	Primary_melanoma MEL272
GSM390246	Primary_melanoma MEL275
GSM390247	Primary_melanoma MEL276
GSM390248	Primary_melanoma MEL280
GSM390249	Primary_melanoma MEL282
GSM390250	Primary_melanoma MEL283
GSM390251	Primary_melanoma MEL287
GSM390252	Primary_melanoma MEL290
GSM390253	Primary_melanoma MEL294
GSM390254	Primary_melanoma MEL298
GSM390255	Primary_melanoma MEL307
GSM390256	Primary_melanoma MEL310
GSM390257	Primary_melanoma MEL326
GSM390258	Primary_melanoma MEL339
GSM390259	Primary_melanoma MEL340
GSM390260	Primary_melanoma MEL356
GSM390261	Primary_melanoma MEL362

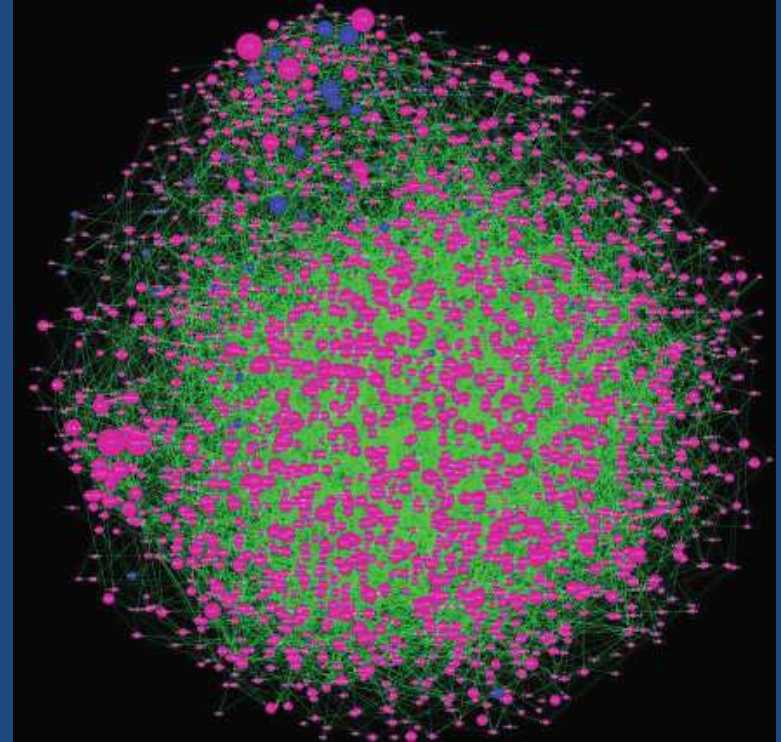


# Baysian network analysis defines gene networks surrounding the 53 genes within the GEO network

B



C



D

	Genes (Nodes)	Interactions (Edges)	Density	Average Local CC	Local CCSE	Average Local CC P-value	Global CC	Global CC P-value
53-gene panel network	377	1187	8.37E-3	0.0756	8.34E-3	0	0.0811	0
446-gene panel network	2259	10014	1.96E-3	0.0355	1.57E-3	0	0.0397	0
Fold Change			4.26	2.13			2.04	

# Key hub genes within defined immune signaling pathways identified as potential key regulators

**Table S6.** Relative mRNA counts for top 25 hub genes comparing progressors and non-progressors in the training set.

Gene Symbol	Out-Degree	Log2 Fold Change	T-Statistic	P Value	FDR
CCR5	12	-0.5754	1.9699	0.0593	0.1598
CD8A	12	-0.9392	2.5076	0.0182	0.1021
CD3D	11	NT*	NT	NT	NT
CD8B	11	NT	NT	NT	NT
IKZF1	11	-0.5553	2.3094	0.0265	0.1044
BTK	10	-0.4292	2.3713	0.0234	0.1044
LCK	10	-0.9079	2.9279	0.0063	0.1021
CD3E	9	-0.6031	2.192	0.0357	0.1205
CD53	9	-0.5731	2.425	0.0222	0.1044
COPS2	9	NT	NT	NT	NT
CXCL9	9	-0.7667	1.9471	0.0592	0.1589
EGFR	9	NT	NT	NT	NT
EPS8L1	9	NT	NT	NT	NT
FGFR3	9	NT	NT	NT	NT
GPS1	9	NT	NT	NT	NT
MS4A1	9	NT	NT	NT	NT
PPP3CB	9	NT	NT	NT	NT
CD1A	8	-0.1511	0.5498	0.5857	0.6981
CD2	8	-0.9678	3.4058	0.0020	0.0852
CD58	8	-0.0554	0.3335	0.7406	0.8211
CD81	8	NT	NT	NT	NT
IL2RB	8	NT	NT	NT	NT
NCOR2	8	NT	NT	NT	NT
PRKD2	8	NT	NT	NT	NT
TNFRSF1B	8	NT	NT	NT	NT

\* Not tested. Data is only available for those hub genes selected as part of the 446 gene panel.

## Conclusions

- 53 immune gene panel identified by hypothesis driven approach is predictive of non-progression and prolonged survival in two independent cohorts of melanoma patients with resectable stage II-III melanoma
- These 53-genes have high overlap with a co-expression network identified using unbiased methods using GEO data
- Key nodes in a Bayesian network using the GEO expression data identify Th1 processes, TCR and BCR activation, and CD2 as critical modulatory pathways
- Protein levels of CD2 also correlated with non-progression and prolonged survival
- The 53-gene panel should be studied prospectively in larger studies
- Nodes in the Bayesian network are potential therapeutic targets

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  - Tisch Cancer Center- Nina Bhardwaj, Steven Burakoff, Philip Friedlander, Miriam Merad, William Oh, Shanthi Sivendran
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- Geisinger Health Systems- Tammie Ferringer, David Hall, Meera Sivendran
- Johns Hopkins- Elizabeth Jaffee
- New York University- Iman Osman
- Memorial Sloan Kettering- Alan Houghton, Jedd Wolchok
- Baylor Insititute for Immunology Research- Karolina Palucka