Memorial Sloan Kettering Cancer Center

Antigens Targeted by Checkpoint Blockade

Alexandra Snyder, M.D.

Assistant Attending

Gynecologic Medical Oncology &

Immunotherapy

November 5th, 2015

The following relationships exist related to this presentation:
Third Rock Ventures, Honorarium, Consultant

Topics for Discussion

- Clinical conundrums and background
- Association of mutation burden with response to checkpoint blockade therapies:
  - Neoantigens
  - Exogenous Insults & Inflammation
- Measurement of anti-neoantigen responses in the peripheral blood
- Future directions
- Lessons and Take Home Messages

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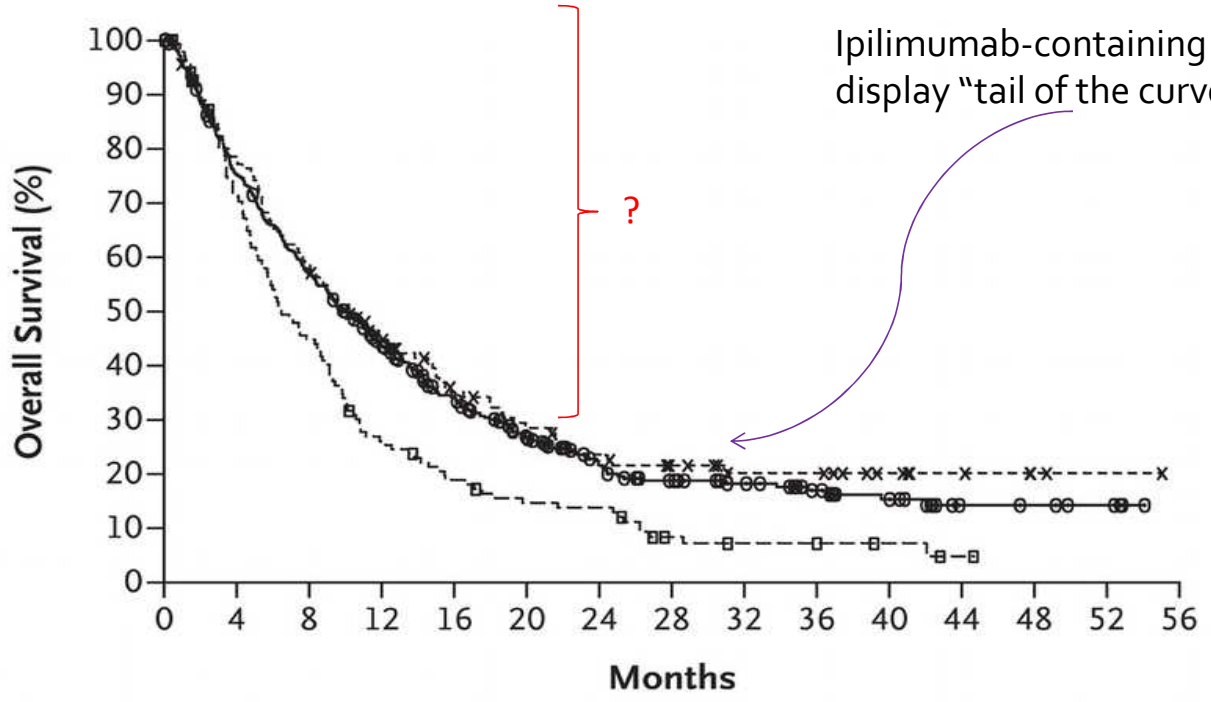
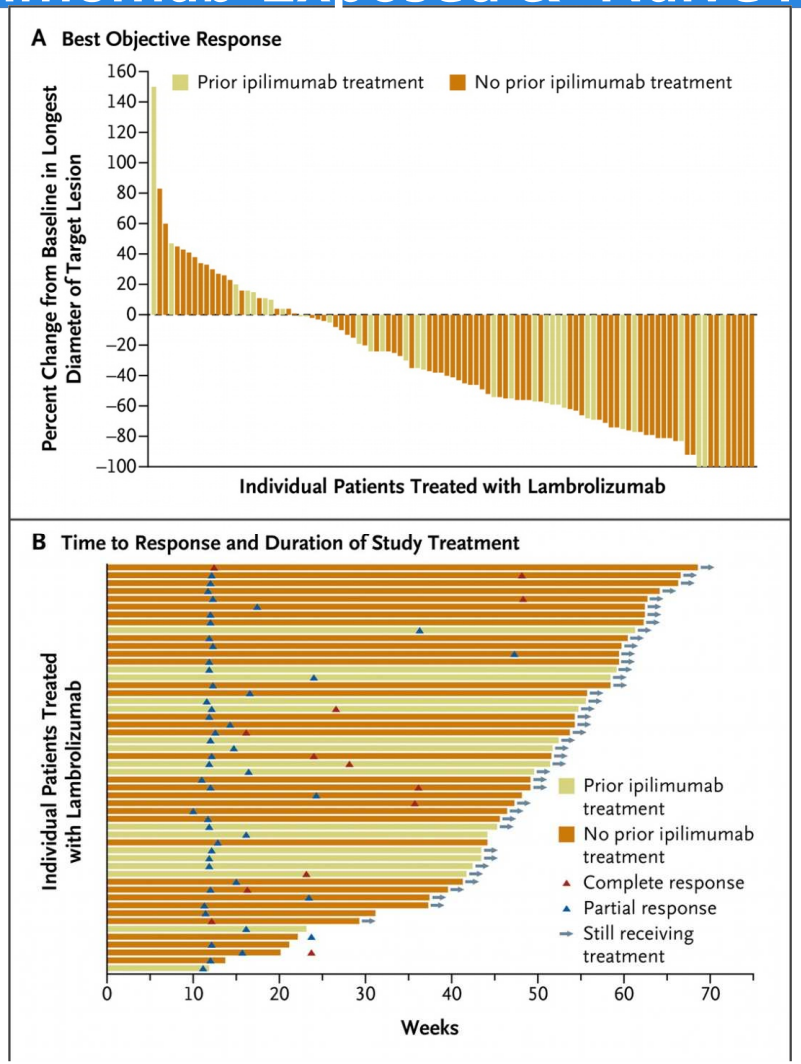
Clinical Conundrum #1: Dramatic Responses, Explosive Disease and Much In Between



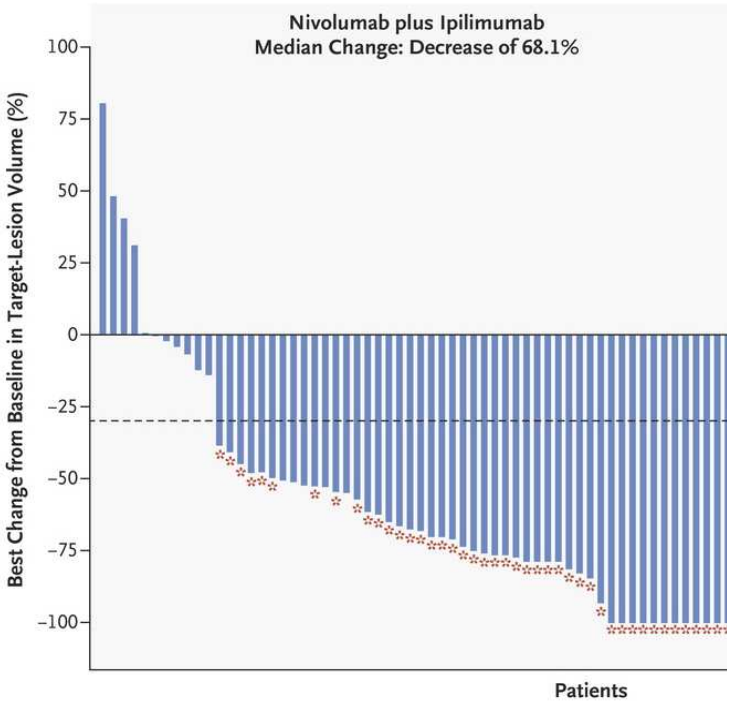
Hodi FS et al. N Engl J Med 2010

Clinical Conundrum #2: Similar Responses to α-PD-1 in Ipilimumab-Exposed & -Naïve Patients



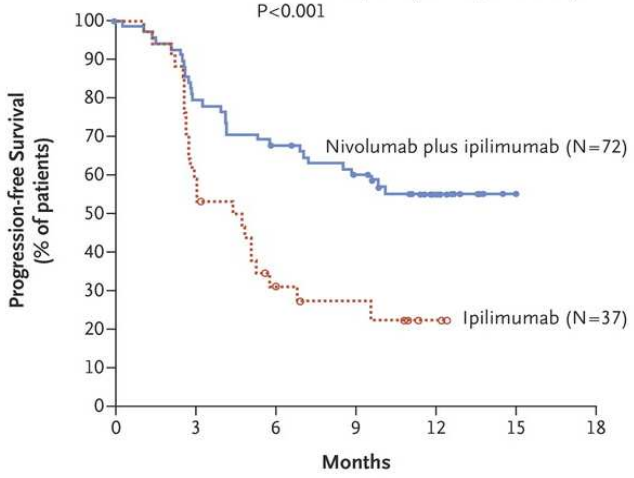
Hamid O et al, NEJM 2013

Clinical Conundrum #3: How to Increase Efficacy through Combination Therapies?



	Death or Disease Progression	Median Progression-free Survival
	no. of patients/total no.	*mo (95% CI)*
Nivolumab plus Ipilimumab	30/72	NR
Ipilimumab	25/37	4.4 (2.8–5.7)

Hazard ratio, 0.40 (95% CI, 0.23–0.68)
P<0.001



No. at Risk							
Nivolumab plus ipilimumab	72	54	45	38	20	1	0
Ipilimumab	37	20	9	6	2	0	0

Postow MA et al. N Engl J Med 2015

Critical Questions for the Field

- Why do certain patients' tumors respond to checkpoint blockade immunotherapies but not others?
- Can we predict who will respond to each agent?
- Ultimate goal: **treat all patients affected by advanced cancer with <u>effective, durable and minimally toxic</u> immunotherapeutic agent(s) ± standard of care agents**

Topics for Discussion

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  - **Neoantigens**
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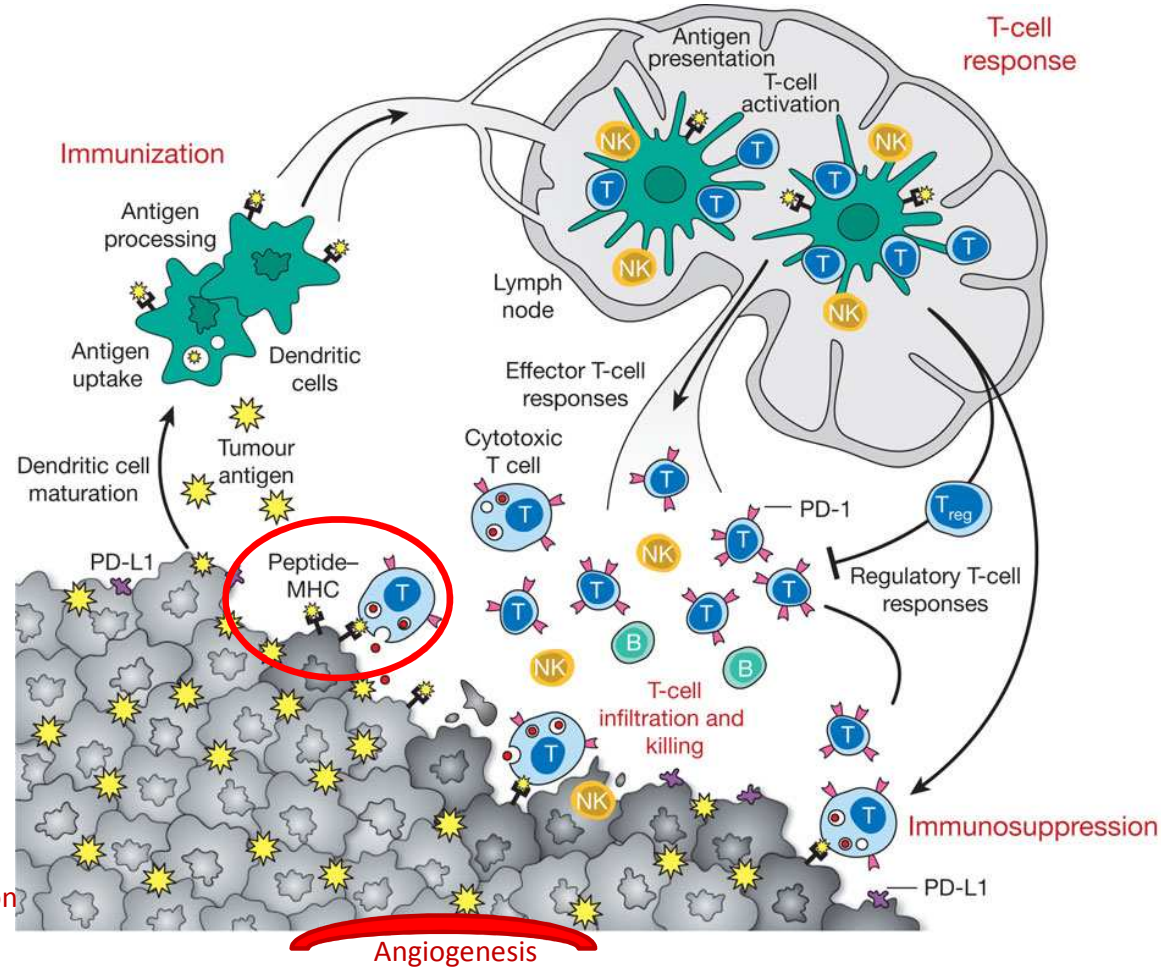
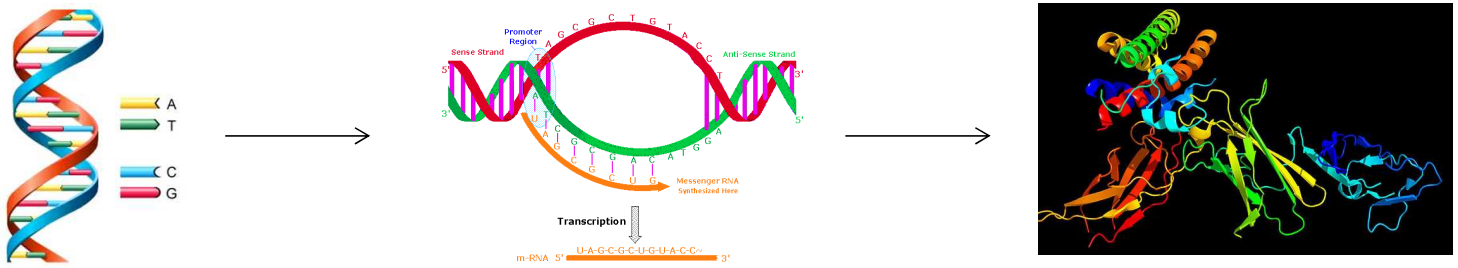
Checkpoint Blockade Success/Failure is Multi-factorial



Mellman et al. Nature 480, 480-489 (2011)
Image modified from Rich Carvajal

How Tumor Genetic Abnormalities Can Generate Neoantigens



DNA **RNA** **PROTEIN**

"Virtually translate" region surrounding mutation:

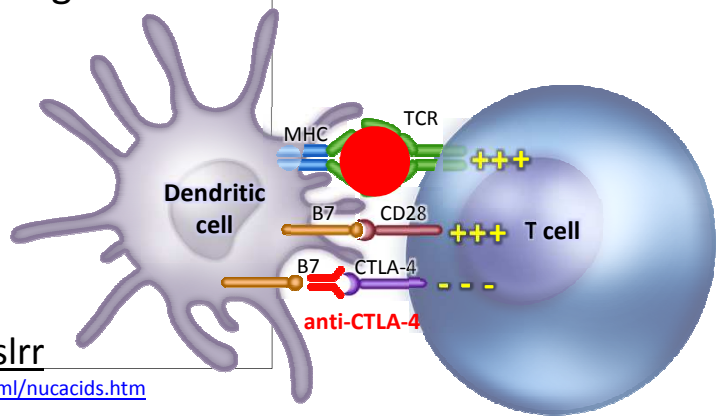
Normal: sldfdsllKeaqrslrr

Mutant: sldfdsllleaqrslrr

↓

Sliding window to find optimal neoantigen:

sldfdsllleaqrslrr
sldfdsllleaqrslrr
sldfdsllleaqrslrr
sldfdsllleaqrslrr
sldfdsllleaqrslrr
sldfdsllleaqrslrr
sldfdsllleaqrslrr
→ sldfdsllleaqrslrr
sldfdsllleaqrslrr



http://www.astrochem.org/sci/Nucleobases.php https://www2.chemistry.msu.edu/faculty/reusch/virttxtjml/nucacids.htm
http://fineartamerica.com/featured/interleukin-12-protein-molecule-dr-tim-evans.html

Precedent for the Importance of Tumor Neoantigens

Gross, L. 1943. Intradermal immunization of C3H mice against a sarcoma that originated in an animal of the same line. *Cancer Res.* 3: 326–33

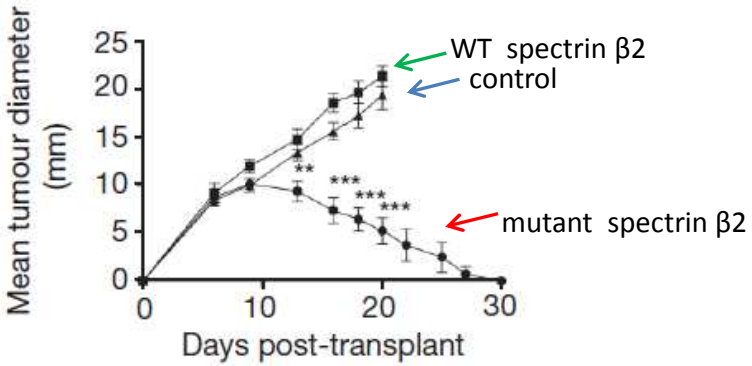
Baldwin, R. W. 1955. Immunity to methylcholanthrene-induced tumors in inbred rats following atrophy and regression of implanted tumors. *Br. J. Cancer* 9: 652–65

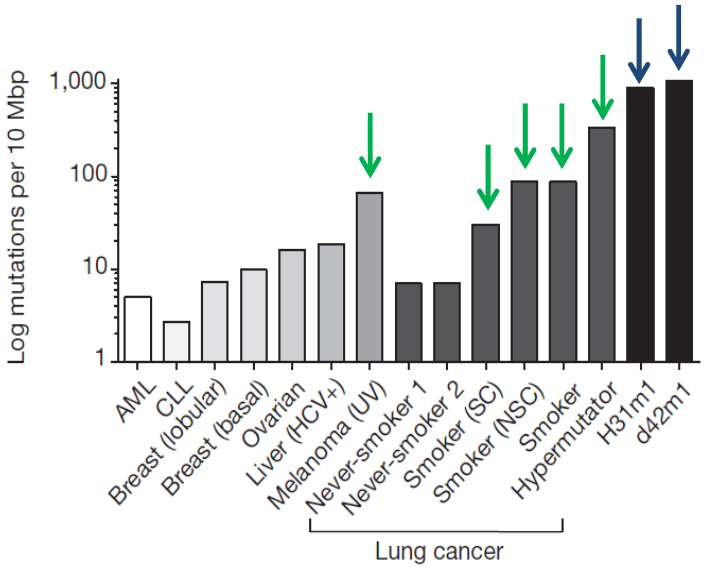
Old, L. J., Boyse, E. A., Clarke, D. A., Carswell, E. A. 1962. Antigenic properties of chemically-induced tumors. *Ann. N.Y. Acad. Sci.* 101: 80–106

Schreiber H Ann Rev Immunol 1988

Mutation and Associated Neoantigen Lead to Tumor Rejection in Mouse Model

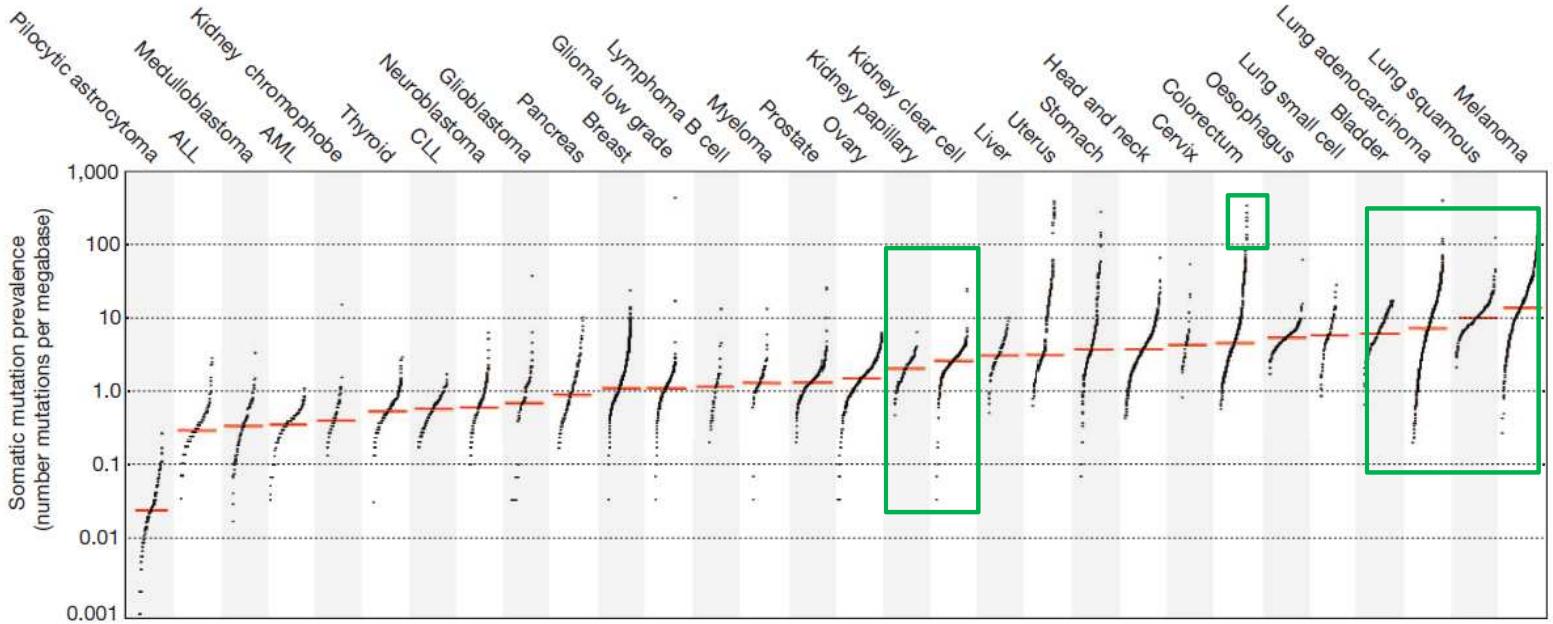
- Carcinogen-induced high mutation burden sarcoma cell line in mice featured highly immunogenic peptides ("rejection antigens")
  - ~1% of mutations constituted "rejectable clones"
  - Mutant spectrin-β2 leads to tumor rejection





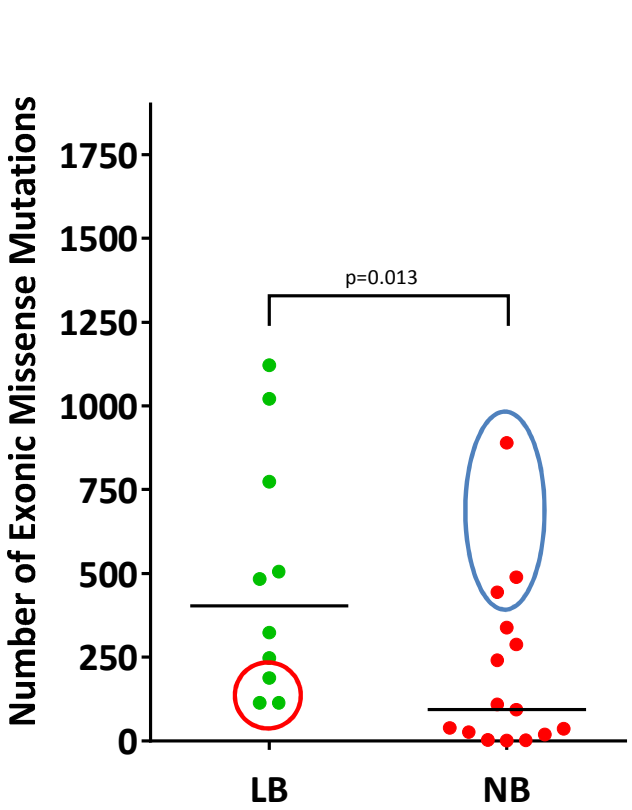
Matsushita et al. Nature 2012

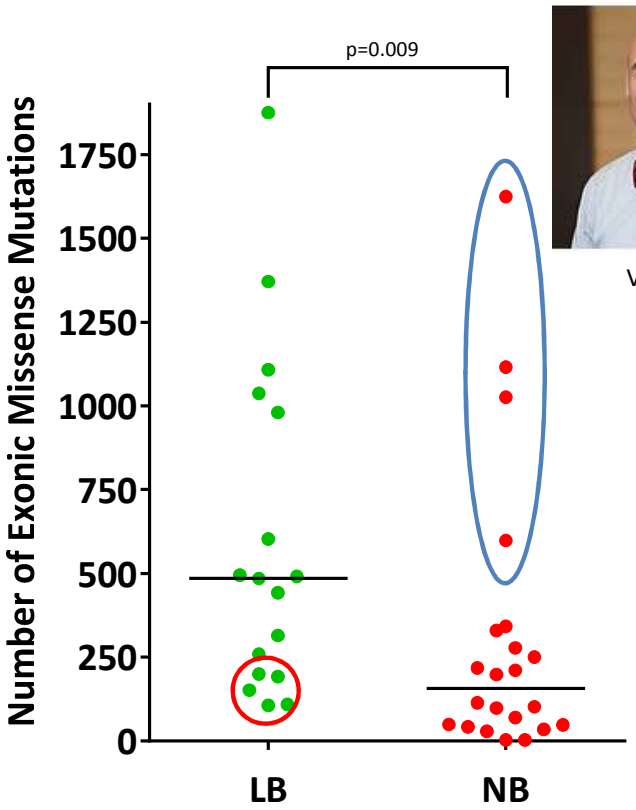
Checkpoint Blockade Effective Primarily in High Mutation-Burden Tumor Types…With Exceptions



Alexandrov et al Nature 2013

Mutational Load Correlates with Benefit in Melanoma Patients Treated with anti-CTLA-4



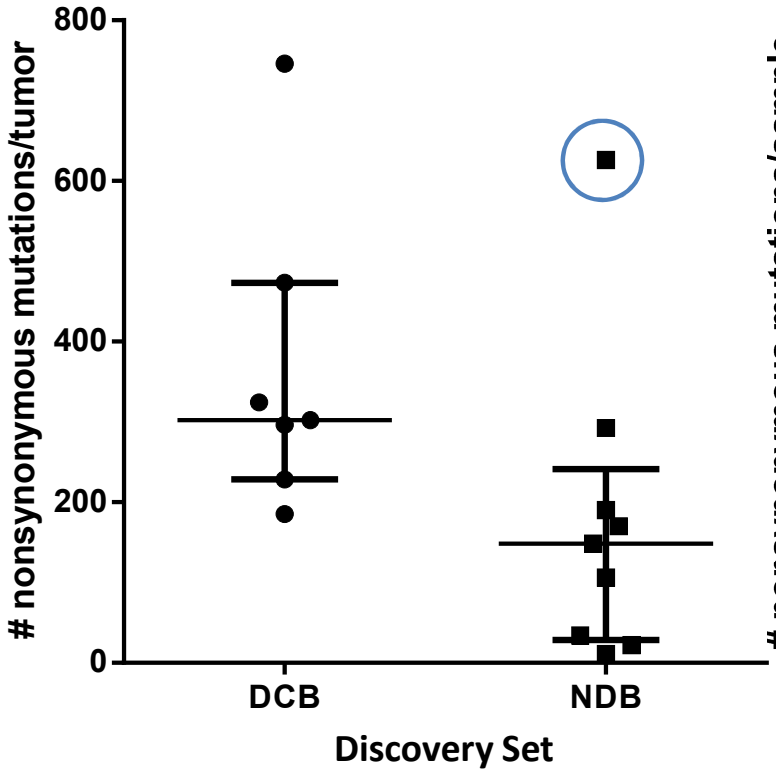


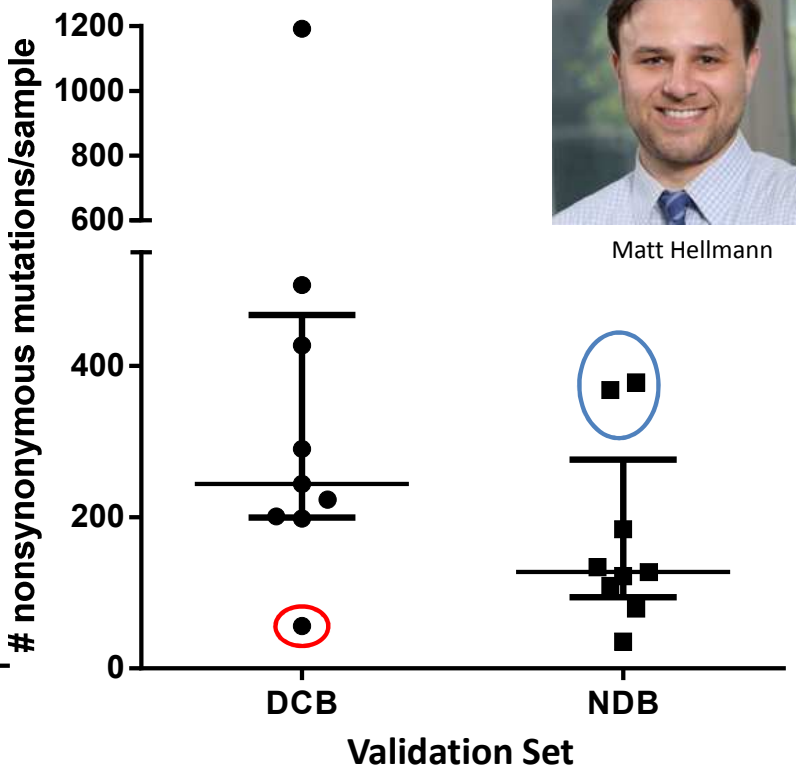
Vlad Makarov

LB, long-term clinical benefit lasting ≥6 months
NB, no durable benefit

Snyder, Makarov, Merghoub, Yuan et al NEJM 2014

Mutational Load Correlates with Clinical Benefit in Lung Cancers/anti-PD1 (pembrolizumab)



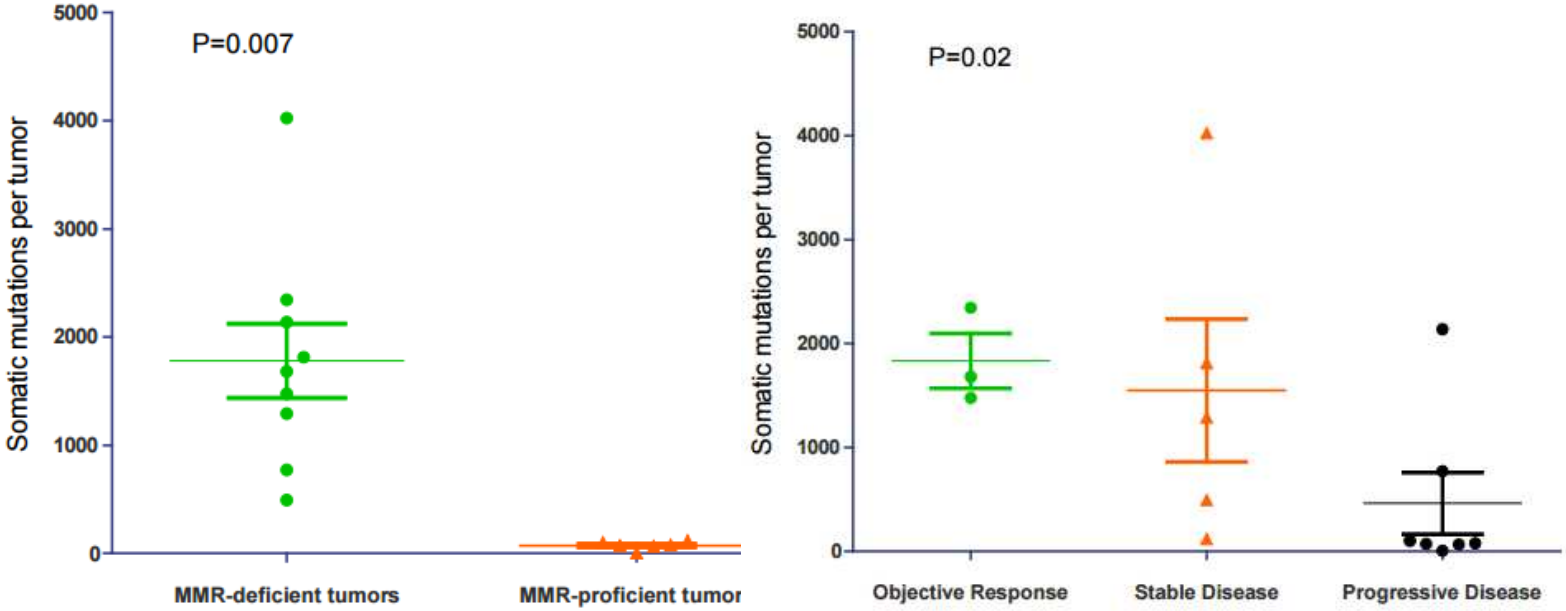


Matt Hellmann

DCB, long-term clinical benefit lasting ≥6 months
NDB, no durable benefit

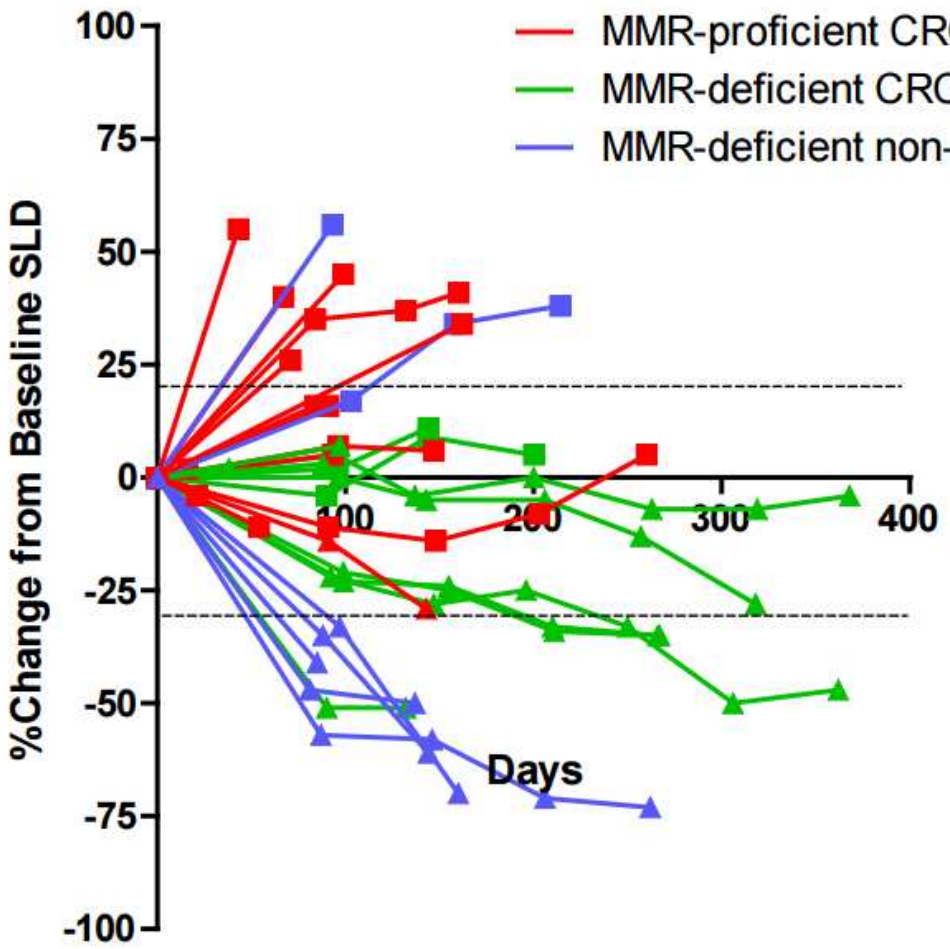
Rizvi, Hellmann, Snyder et al Science 2015

Mutational Load Correlates with Clinical Benefit in MMR-Deficient Cancers/anti-PD1 (pembrolizumab)



Le DT et al NEJM 2015

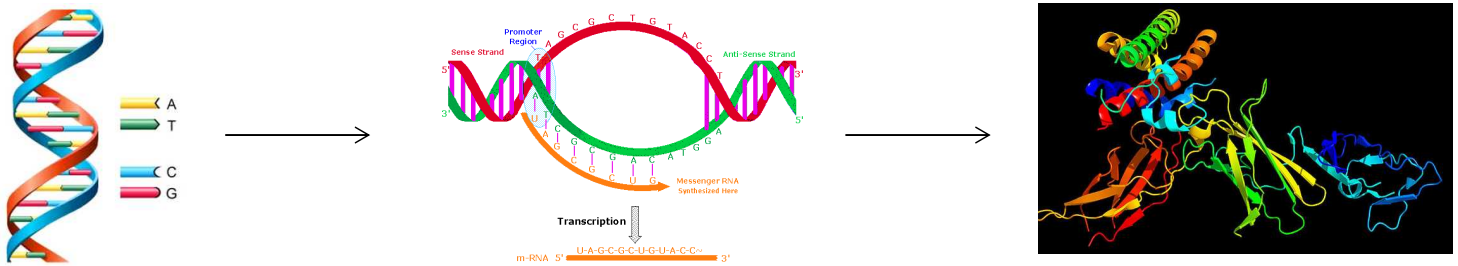
As in Melanoma Study, Exceptions to the Rule



Le DT et al NEJM 2015

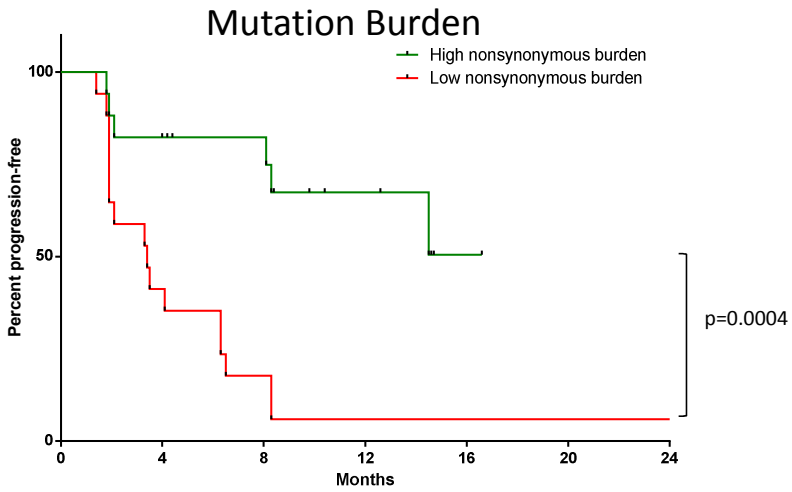
Summary: Mutation Burden

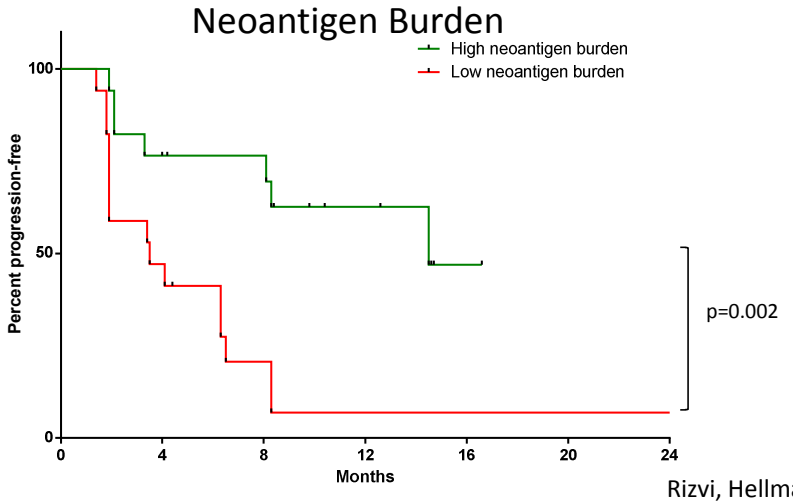
- Elevated mutation burden correlates with, but alone does not predict, clinical benefit from anti-CTLA-4 and anti-PD-1 therapies in melanoma, lung cancers, and MMR-deficient tumors



DNA **RNA** **PROTEIN**

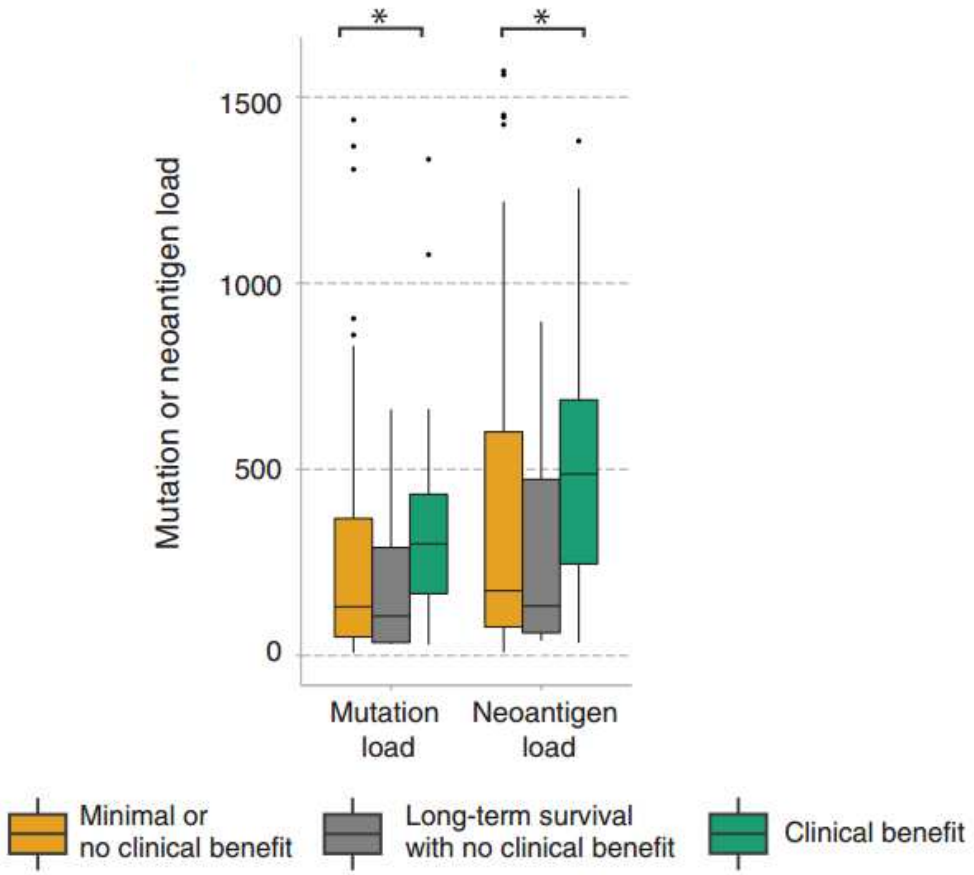
Mutation Burden, Neoantigen Burden Correlate with PFS in Lung Cancers





Rizvi, Hellmann, Snyder et al Science 2015

Mutation Burden, Neoantigen Burden Confirmed to Correlate with Outcome in Melanoma/Ipilimumab



Van Allen E et al Science 2015

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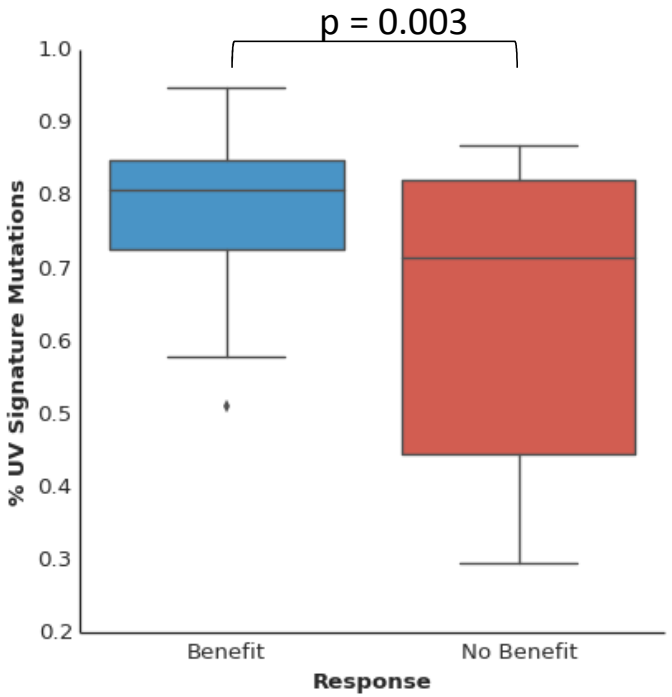
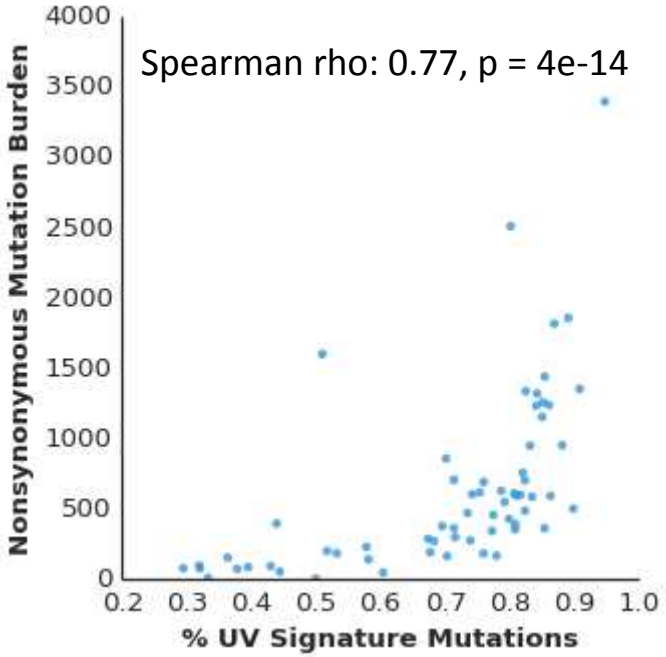
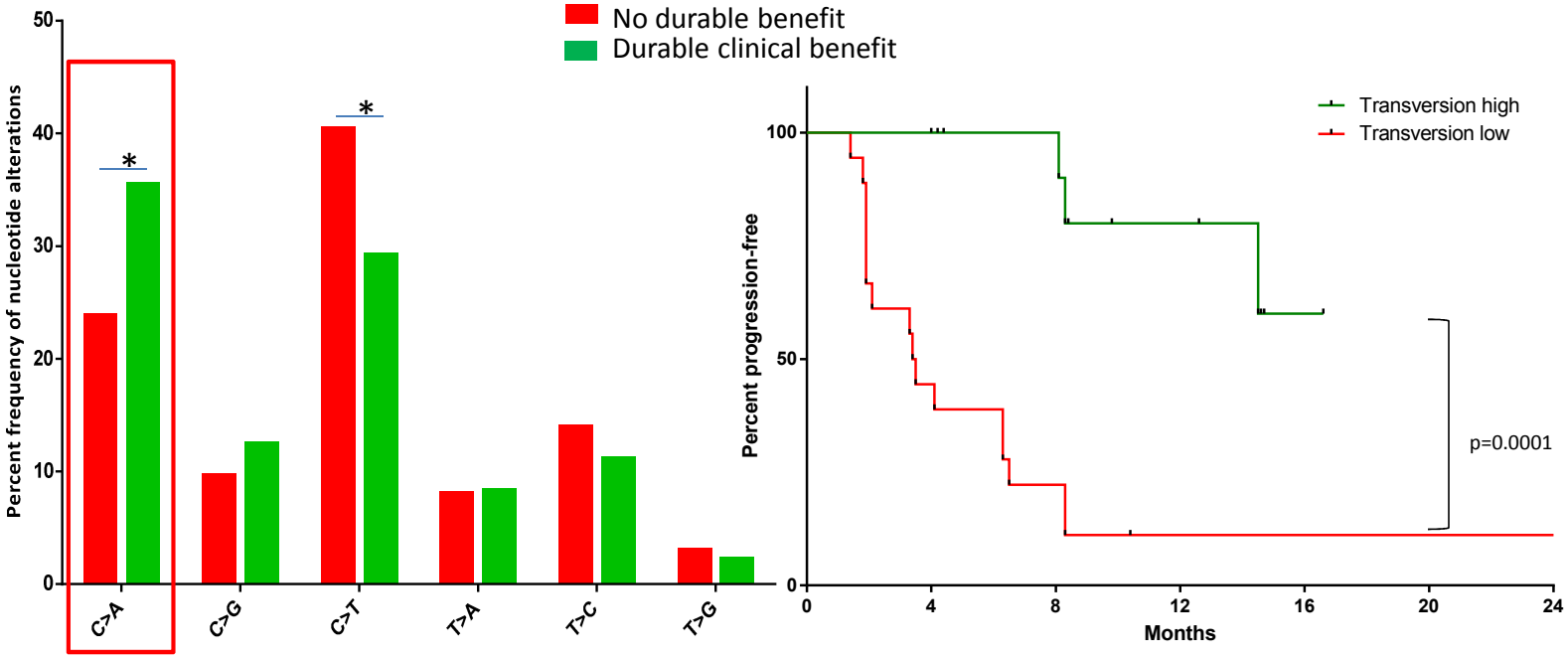
Evidence of Ultraviolet-Induced DNA Damage Correlates with Mutation Burden & Benefit in Melanoma/α-CTLA-4





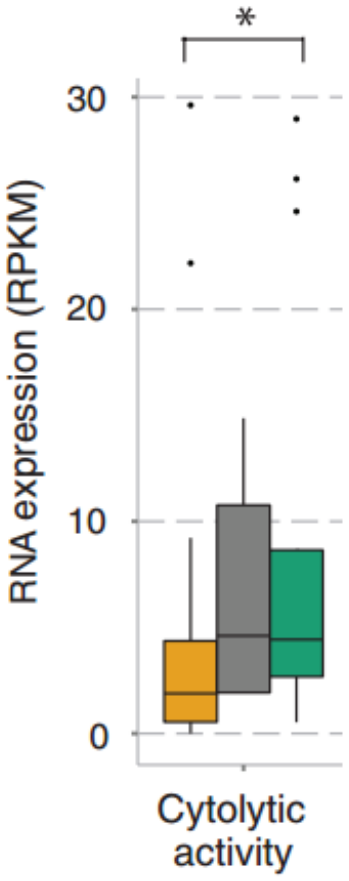
Hammerbacher Lab

Evidence of Smoking-Induced DNA Damage Correlates with Outcome in Lung Cancers



Rizvi, Hellmann, Snyder et al Science 2015

Inflammation Associated with Response to Ipilimumab in Melanoma



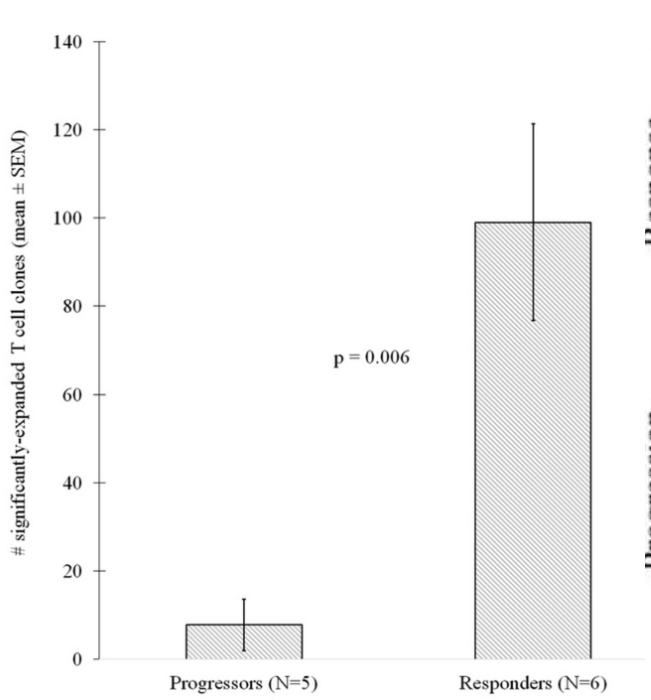
	# members	# members in signal	Signal strength
HALLMARK_INTERFERON_GAMMA_RESPONSE	171	111	76%
HALLMARK_INTERFERON_ALPHA_RESPONSE	86	58	80%
HALLMARK_ALLOGRAFT_REJECTION	137	55	43%
HALLMARK_INFLAMMATORY_RESPONSE	133	62	54%
HALLMARK_COMPLEMENT	141	58	48%
HALLMARK_IL6_JAK_STAT3_SIGNALING	65	30	54%
HALLMARK_XENOBIOTIC_METABOLISM	132	37	32%
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	44	22	61%
HALLMARK_TNFA_SIGNALING_VIA_NFKB	175	54	36%
HALLMARK_P53_PATHWAY	170	36	23%
HALLMARK_GLYCOLYSIS	166	56	40%
HALLMARK_APOPTOSIS	142	46	39%
HALLMARK_PROTEIN_SECRETION	90	37	52%
HALLMARK_BILE_ACID_METABOLISM	68	21	35%
HALLMARK_UV_RESPONSE_UP	126	42	40%
HALLMARK_PEROXISOME	81	32	49%
HALLMARK_IL2_STAT5_SIGNALING	158	49	37%
HALLMARK_ESTROGEN_RESPONSE_LATE	133	27	22%
HALLMARK_ADIPOGENESIS	177	57	38%
HALLMARK_MTORC1_SIGNALING	196	52	31%
HALLMARK_ANDROGEN_RESPONSE	86	19	24%

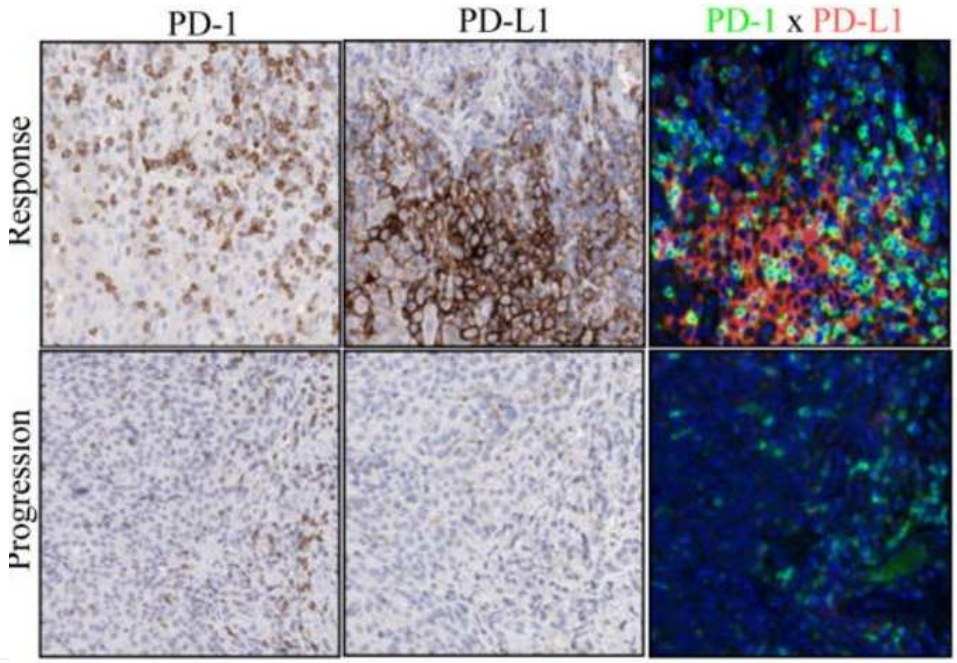
Van Allen et al, Science 2015
Arman Aksoy, Hammerbacher Lab

T Cell Infiltrate Associated with Response to Pembrolizumab in Melanoma

Pre-treatment samples obtained from responding melanomas showed:

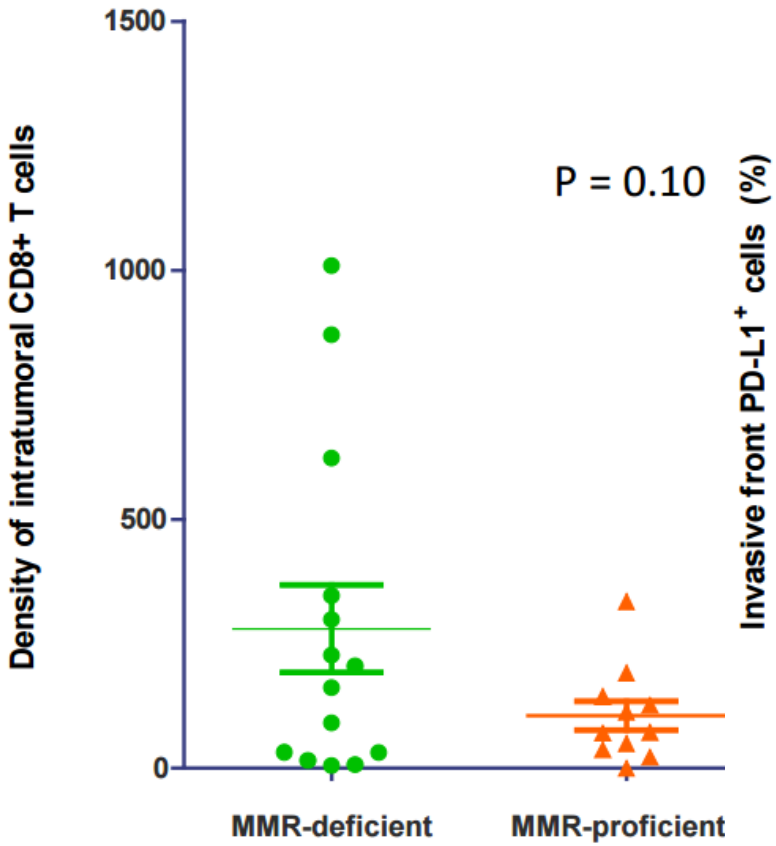
- Higher numbers of CD8, PD-1 and PD-L1-expressing cells at the invasive tumor margin and inside tumors
- Close proximity between PD-1 and PD-L1
- More clonal TCR repertoire and clonal expansion upon treatment

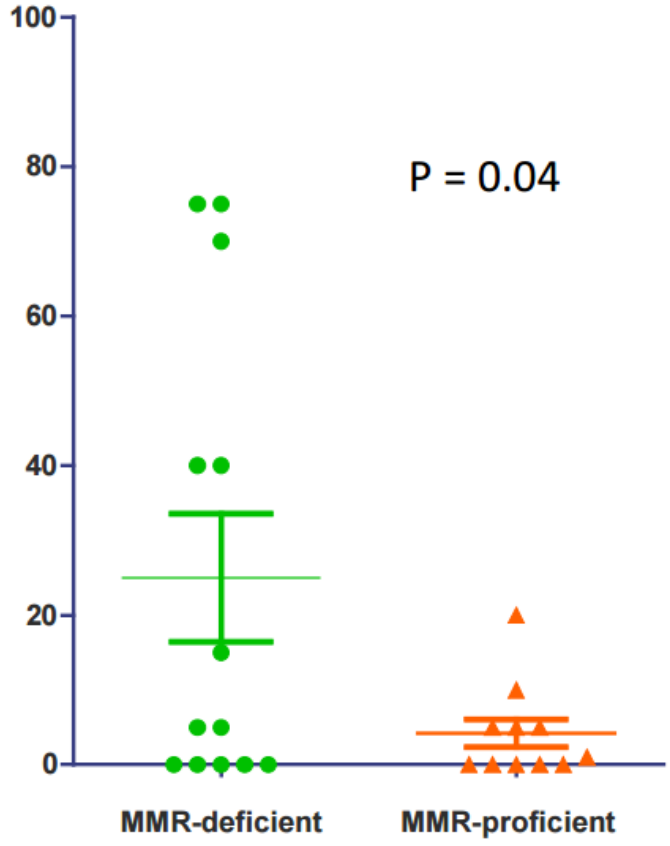




Tumeh et al Nature 2014

T Cell Infiltrate Associated with Response to Pembrolizumab in MMR-deficient Cancers





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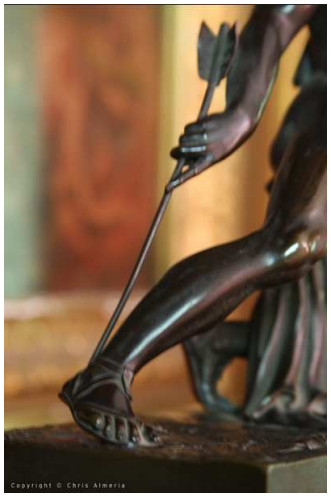
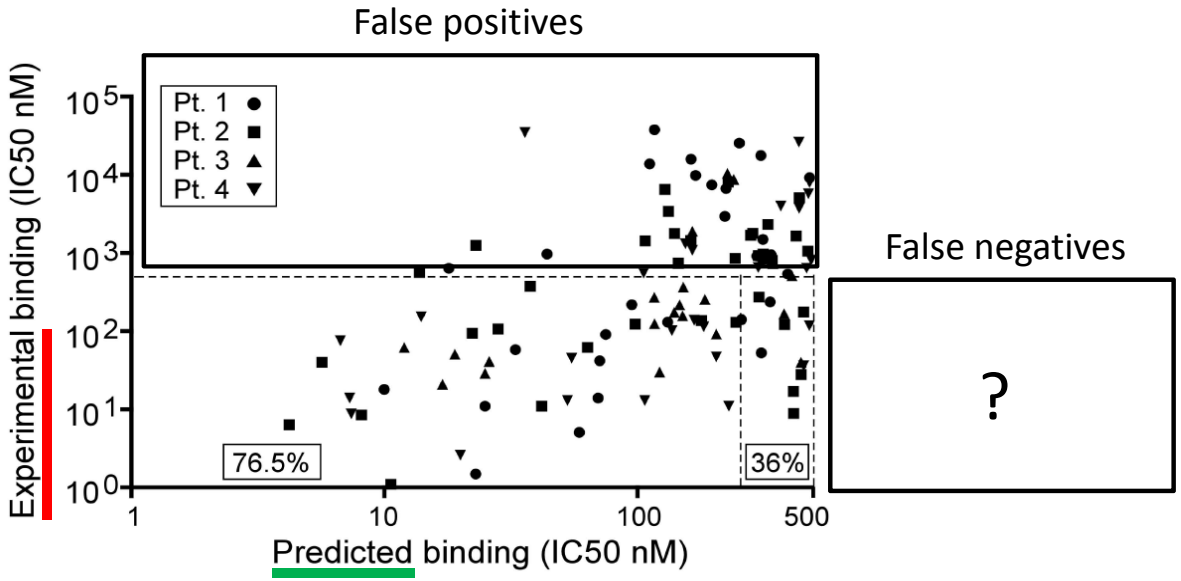
Neoantigens May Represent Tumor's "Achilles heel"



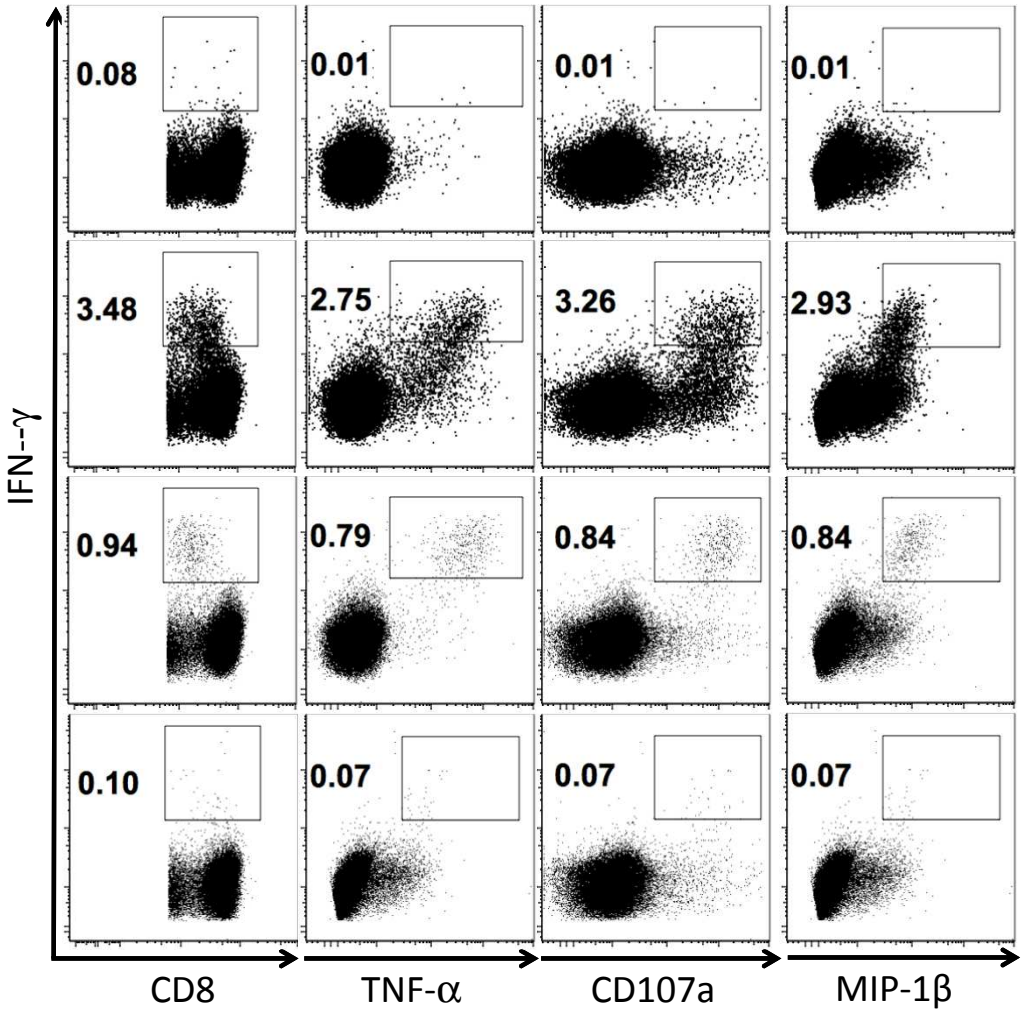
Trials & Tribulations of Neoantigen Identification in Patients

- Validation of neoantigen prediction in 4 patients with CLL



Rajasagi et al Blood 2014.

Identification of Anti-Neoantigen T Cell Response in the Peripheral Blood in Melanoma/Ipilimumab Using ICS



No-stimulation

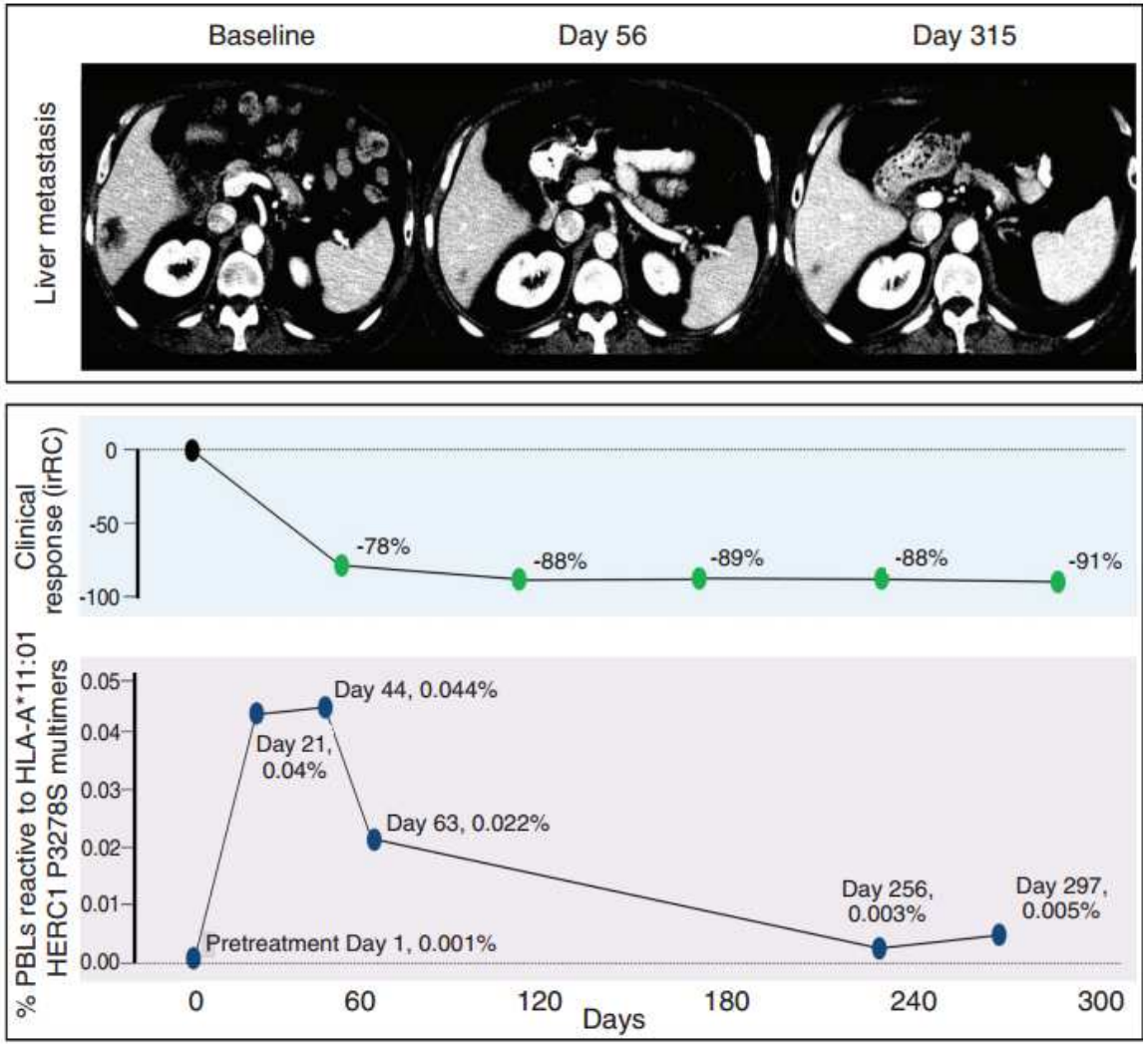
SEB

Mutant peptide
TESPFEQHI

Wild type peptide
TKSPFEQHI

Jianda Yuan, Phil Wong, Teresa Rasalan

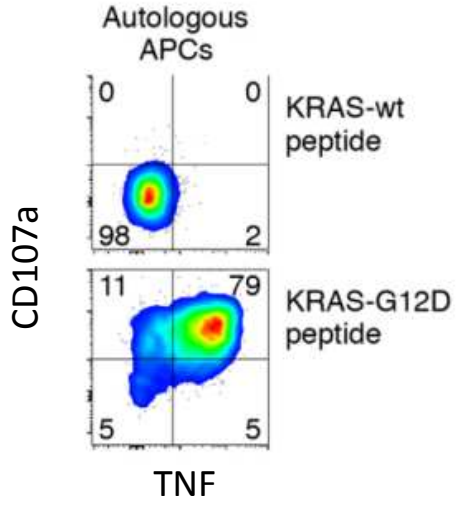
Neoantigen-Specific Response in Peripheral Blood of NSCLC/Pembrolizumab Patient Using Tetramers



Pia Kvistborg
Ton Schumacher

Identification of Neoantigen-Specific TILs using Tandem Mini-Gene Approach in GI Cancers

- Transfect autologous APC with tandem mini-gene RNA encoding mutation flanked by wild type sequence
- Identified KRAS G12D HLA-C*08:02-restriced neoantigen
- Immunogenic mutations occurred in genes with wide range of expression
- Mutation-reactive T cells ranged from 0.009-1.25% of TIL



Tran et al Science 2015.

Summary: Neoantigens

- Elevated predicted neoantigen burden correlates with mutation burden and with clinical benefit from anti-CTLA-4 and anti-PD-1 therapies in melanoma and lung cancers
- Neoantigen prediction remains an area of focus for improvement
- Neoantigen-specific T cell responses in the peripheral blood can be measured using ICS, tetramer staining and other approaches

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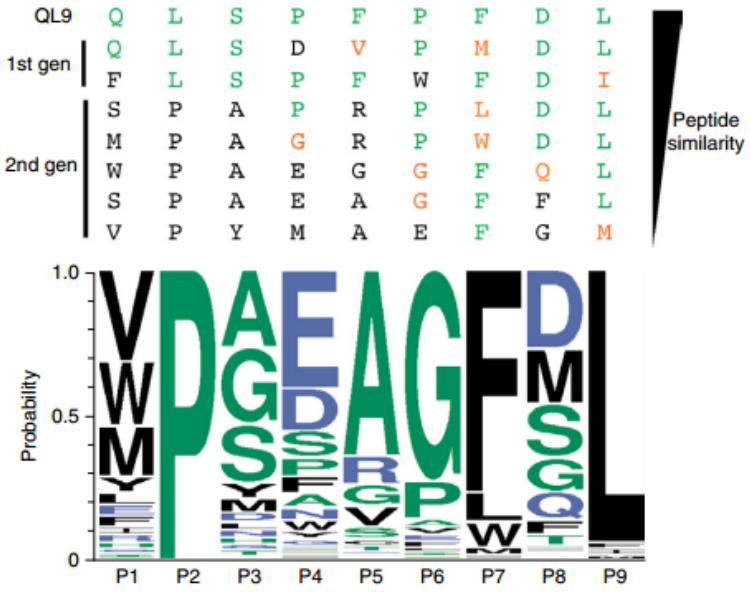
Can We Predict What Neoantigens T Cells Will Recognize?

MAGE A3 peptide: EVDPIGHLY
Titin peptide: ESDPIVAQY

Cameron et al Sci Transl Med 2013



Adams et al Nat Immunol 2015.

Tool for Antigen Comparison

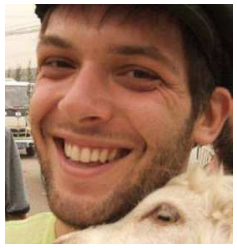
- High-throughput, automated bioinformatic technique to compare amino acid stretches, including neoantigens to pathogens & neoantigens to neoantigens
- Create an **open-source** tool to determine which peptides to test *in vitro*

https://github.com/hammerlab/topiary


Tavi Nathanson


Arun Ahuja

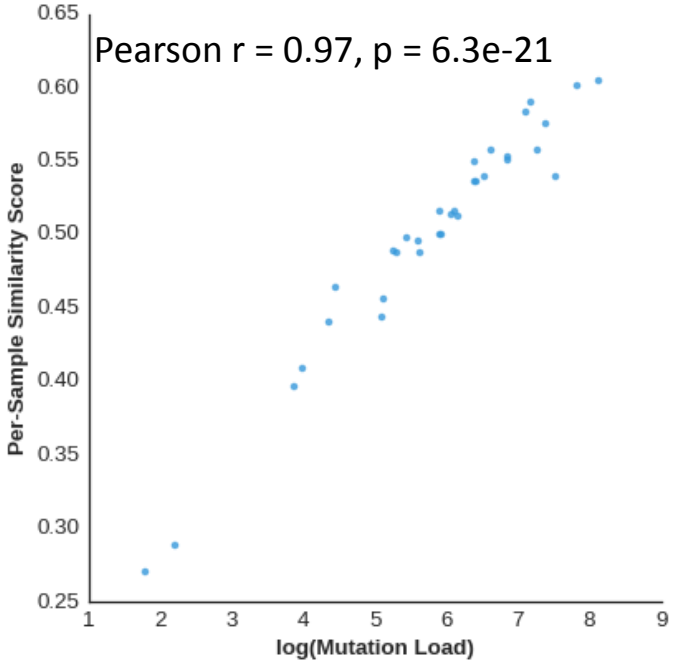

Arman Aksoy


Alex Rubinsteyn

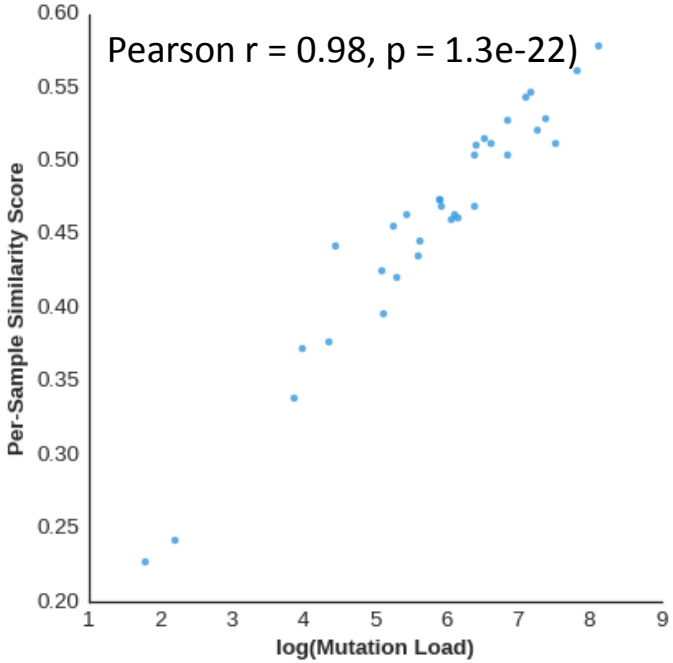

Jeff Hammerbacher

Similarity Correlates With, but Does Not Outperform Mutation Burden

Tumor-Antigen (IEDB) Similarity Score

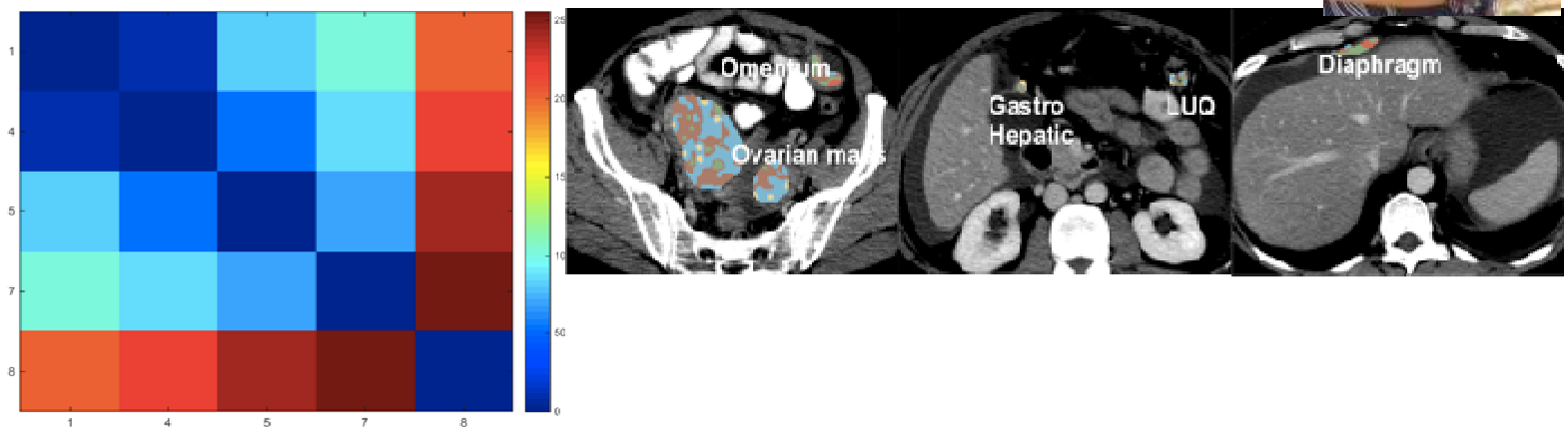


Tumor-Tumor Similarity Score

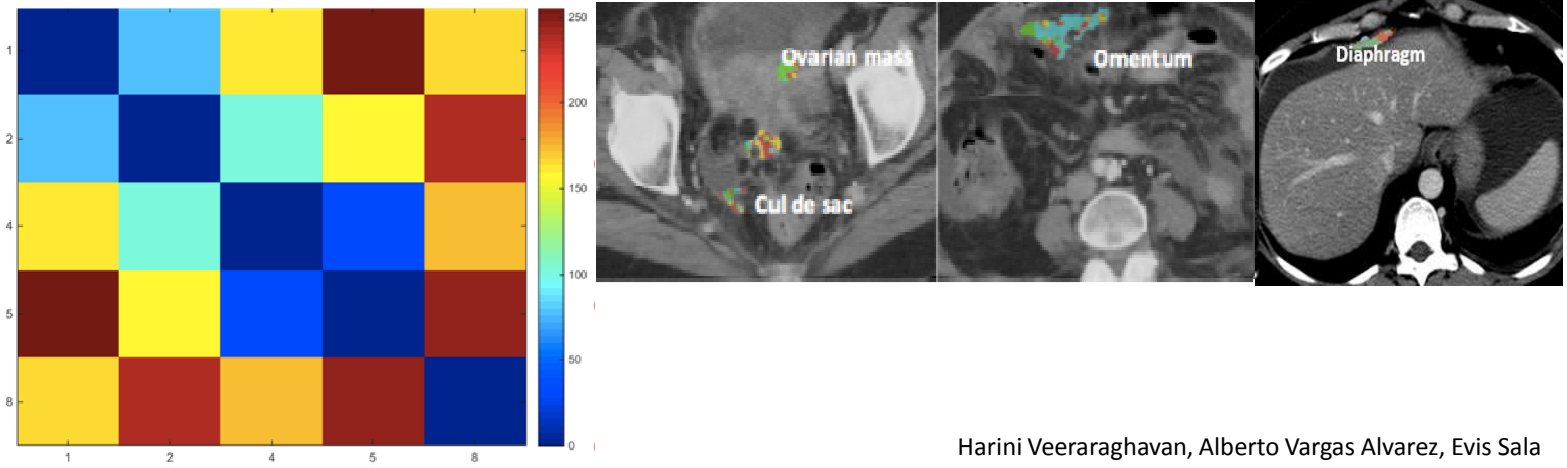


Radiomics: Texture & Tumor Heterogeneity

#1 : Mesenchymal ovarian cancer, complete resection, 69mo survival

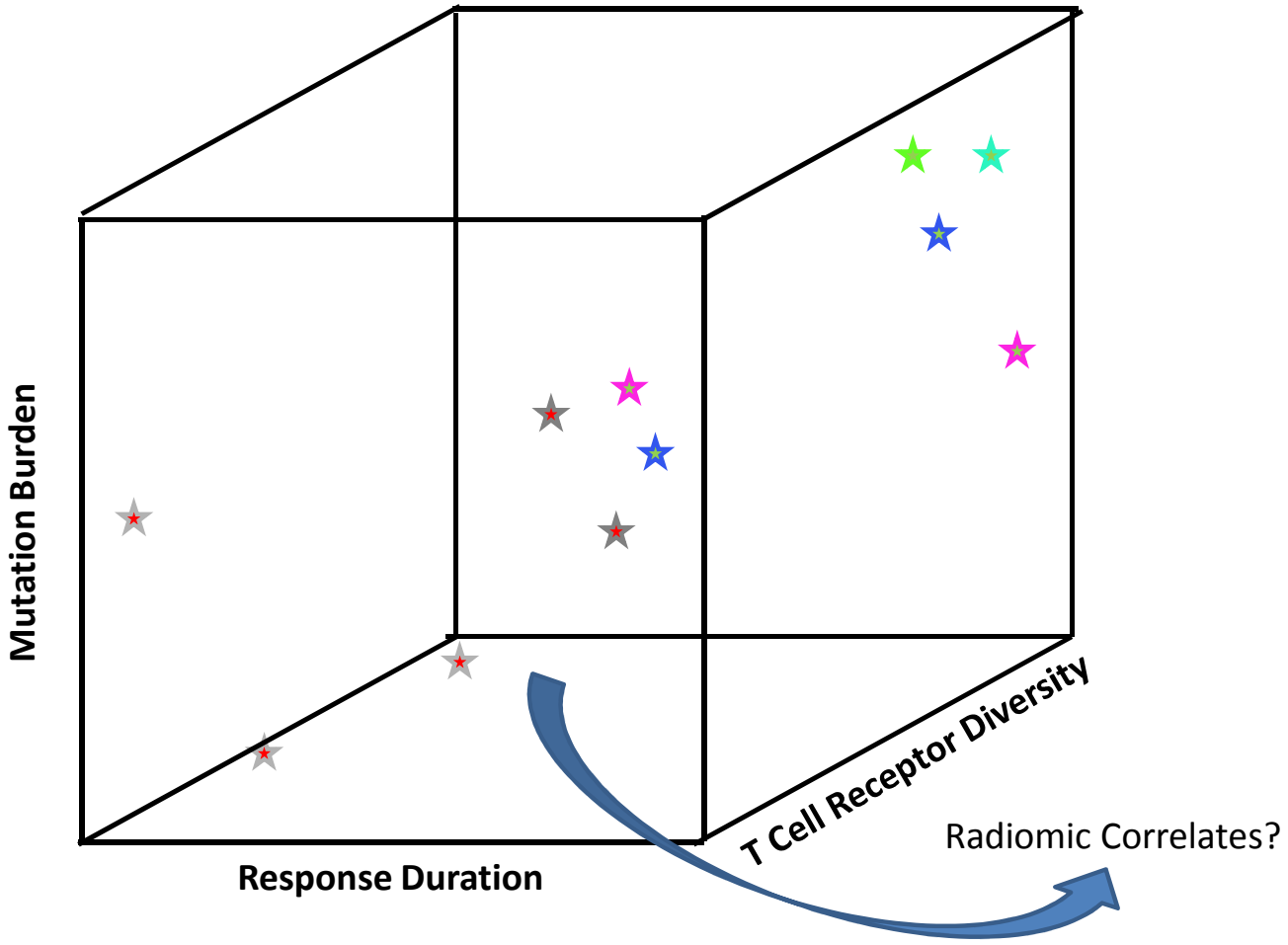


#2 : Mesenchymal ovarian cancer, suboptimal debulking, 10mo survival



Harini Veeraraghavan, Alberto Vargas Alvarez, Evis Sala

Data Integration: Mutations/Neoantigens, Inflammation, TCR Diversity and Radiomics



Lessons and Take Home Messages

Key Points:

- In melanoma, lung cancers and MSI-H cancers treated with checkpoint blockade, genetic correlates of clinical benefit include:
  - elevated mutational load
  - elevated neoantigen load
  - evidence of DNA damage (melanoma and lung)

Potential Impact on the Field:

- Along with expression/IHC data, these genetic data could be used to stratify patients in trials
- Upcoming data from dual-blockade-treated patients will further elucidate these concepts

Lessons Learned:

- Neoantigen-specific T cells can be identified in the peripheral blood of patients treated with anti-CTLA-4 and anti-PD-1 therapies
- Generation of automated tool to compare peptides to apply to *in vitro* studies

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