

BCB 731:

Defense Against the Dark Arts



Critic: Genetic basis for
clinical response to CTLA-4
blockade in melanoma

October 25th, 2023



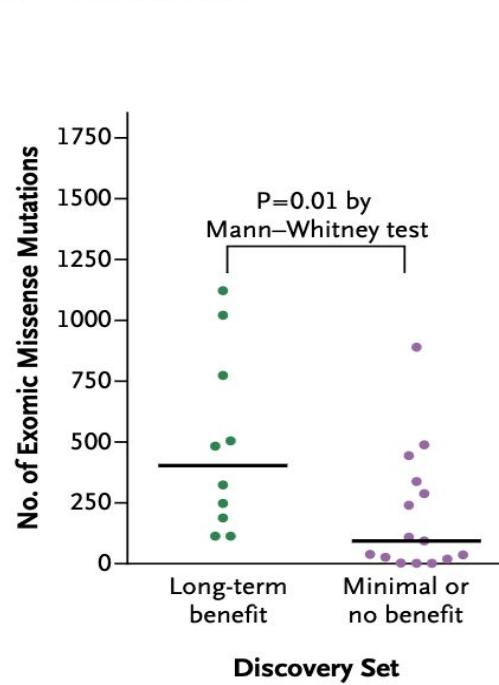
ORIGINAL ARTICLE

Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma

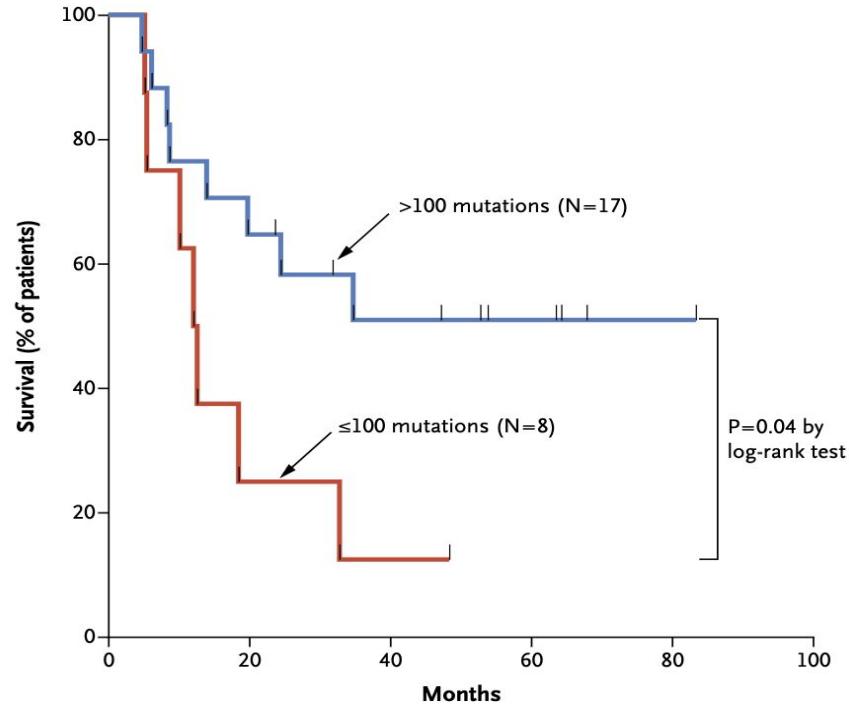
Alexandra Snyder, M.D., Vladimir Makarov, M.D., Taha Merghoub, Ph.D.,
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Kasthuri Kannan, Ph.D., Yanyun Li, M.D., Ph.D., Ceyhan Elipenahli, B.S.,
Cailian Liu, M.D., Christopher T. Harbison, Ph.D., Lisu Wang, M.D.,
Antoni Ribas, M.D., Ph.D., Jedd D. Wolchok, M.D., Ph.D.,
and Timothy A. Chan, M.D., Ph.D.

TMB predicts response to aCTLA-4

A Mutational Load



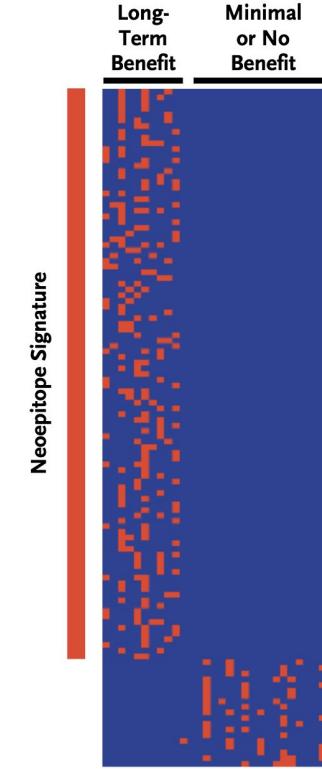
B Survival in Discovery Set



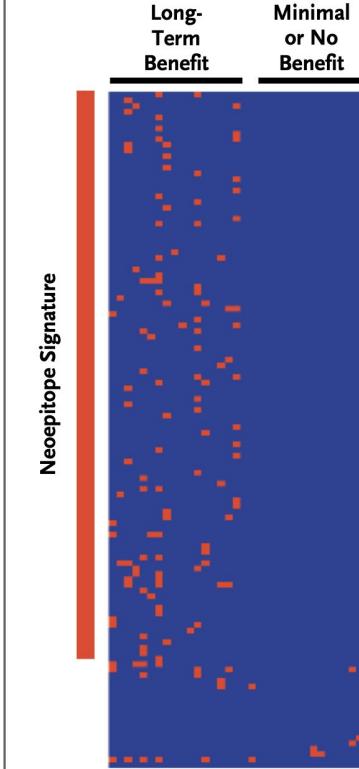
The Tetrapeptide Signature

4mer	Sample	Chr	Position	Gene	Mutation	Amino Acid	WTSeq	MTSeq
AALL								
AALL	CR9699	chrX	14708901	GLRA2	c.G1000A	p.E334K	fvfaallEy	fvfaallKy
AALL	SD1494	chr9	94486131	ROR2	c.C2645T	p.S882F	Smadraall	Fmadraall
AALL	CR0095	chrX	11197491	ARHGAP6	c.G1411A	p.E471K	aallKEflr	aallKKflr
AALL	CR04885	chr19	8389389	KANK3	c.G2326A	p.D776N	Devaallha	Nevaallha
AALL	PR11217	chr19	50209622	CPT1C	c.C1295T	p.S432L	dpaaSiday	dpaalUday
AATA								
AATA	CR9699	chr22	32555082	C22orf42	c.C121T	p.P41S	etvaataPa	etvaataSa
AATA	SD1494	chr20	61528063	DIDO1	c.C1874T	p.S625F	apaaaataaS	apaaaataaF
AATA	CR4880	chrX	24382384	FAM48B1	c.G1507A	p.A503T	aiaaaAaaa	aiaaaTaai
AATA	LSD3484	chr20	44580928	ZNF335	c.C3047T	p.S1016L	saataaSkk	saataaLkk
AFPS								
AFPS	CR9699	chr10	63852764	ARID5B	c.C3542T	p.S1181F	afpsssqlsS	afpsssqlsF
AFPS	SD1494	chr7	12409653	VVDE	c.C2279T	p.P760L	fplfifafps	fplifafps
AFPS	LSD4691	chr3	188202427	LPP	c.241C>T	p.L81F	alpsisgnf	aFpsisgnf
AFPS	PR4092	chr2	237076596	GBX2	c.C19T	p.P7S	aafpPslmm	aafpSslmm
AGAA								
AGAA	CR9699	chr14	94909083	SERPINA11	c.C1129T	p.L377F	teagaasgL	teagaasgF
AGAA	SD1494	chr19	10946865	TMED1	c.G3A	p.M1I	Mmaagaala	Imaagaala
AGAA	LSD3484	chr7	27282896	EVX1	c.G247A	p.E83K	Epqvagaam	Kpqvagaam
ALLK								
ALLK	CR9699	chrX	14708901	GLRA2	c.G1000A	p.E334K	fvfaallEy	fvfaallKy
ALLK	CR0095	chrX	11197491	ARHGAP6	c.G1411A	p.E471K	aallKEflr	aallKKflr
ALLK	CR04885	chrX	122801099	THOC2	c.G1048A	p.E350K	glIEallki	glIKallki
ALLN								
ALLN	CR9699	chr12	29596410	OVCH1	c.C3041T	p.P1014L	wrlvaPlnh	wrlvaLinH
ALLN	SD0346	chr19	11132542	SMARCA4	c.G2758A	p.E920K	ElwallInfl	KlwallInfl
ALLN	CR0095	chr7	142638364	KEL	c.C2174T	p.P725L	gallnPssr	gallnLssr
ALPA								
ALPA	SD0346	chr1	228480244	OBSCN	c.G10624A	p.E3542K	ralparfiE	ralparfiK
ALPA	SD1494	chr10	55587247	PCDH15	c.G4060A	p.A1354T	alpaakpAv	alpaakpTv
ALPA	CR04885	chr14	73958585	C14orf169	c.C863T	p.P288L	alpaaawSl	alpaaawLI
ANLA								
ANLA	CR9699	chr10	115388765	NRAP	c.G2056A	p.D686N	ykaDlawmk	ykaNlawmk
ANLA	CR9306	chr21	30949420	GRIK1	c.C1949T	p.S650F	Sytanlaaf	Fytanlaaf
ANLA	PR4092	chr7	36445925	ANLN	c.C623T	p.T208I	rianlaaTi	rianlaali
ASHL								

A Neoepitopes in Discovery Set



B Neoepitopes in Validation Set



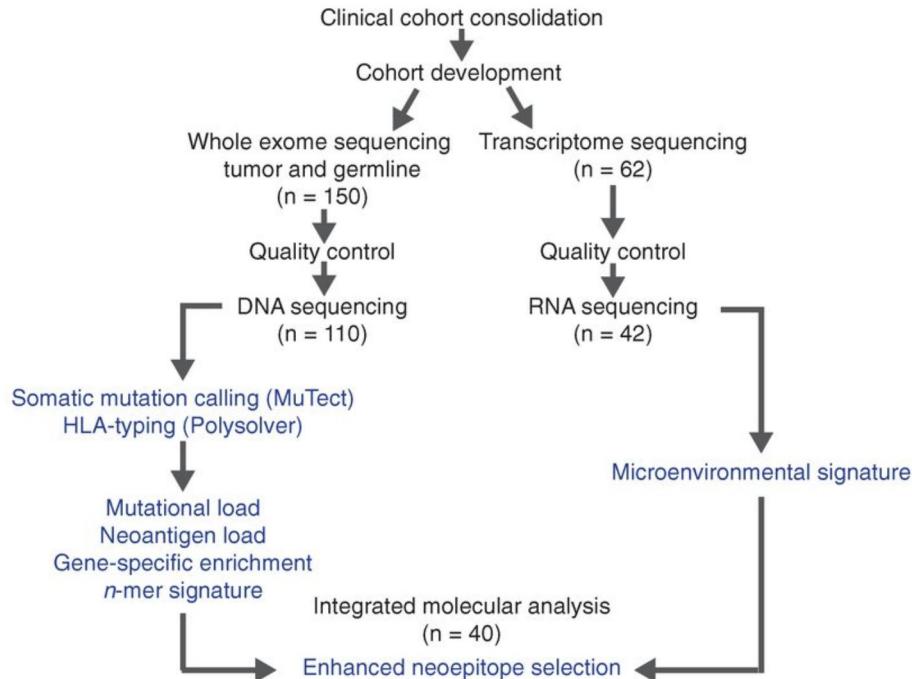
Does it replicate?

New data: Van Allen et al. (2015)

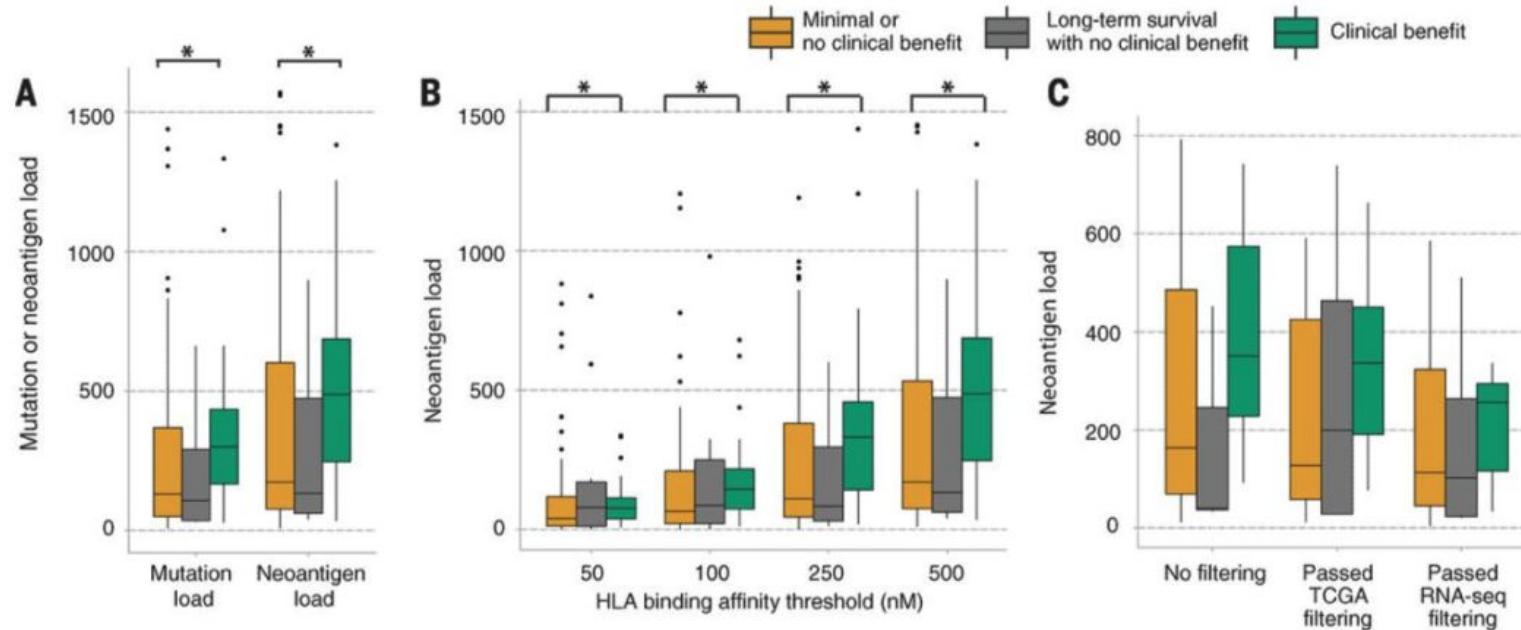
Genomic correlates of response to CTLA-4 blockade in metastatic melanoma

Eliezer M. Van Allen,^{1,2,3*} Diana Miao,^{1,2*} Bastian Schilling,^{4,5*} Sachet A. Shukla,^{1,2} Christian Blank,⁶ Lisa Zimmer,^{4,5} Antje Sucker,^{4,5} Uwe Hillen,^{4,5} Marnix H. Geukes Foppen,⁶ Simone M. Goldinger,⁷ Jochen Utikal,^{5,8,9} Jessica C. Hassel,¹⁰ Benjamin Weide,¹¹ Katharina C. Kaehler,¹² Carmen Loquai,¹³ Peter Mohr,¹⁴ Ralf Gutzmer,¹⁵ Reinhard Dummer,⁷ Stacey Gabriel,² Catherine J. Wu,^{1,2} Dirk Schadendorf,^{4,5†} Levi A. Garraway^{1,2,3†}

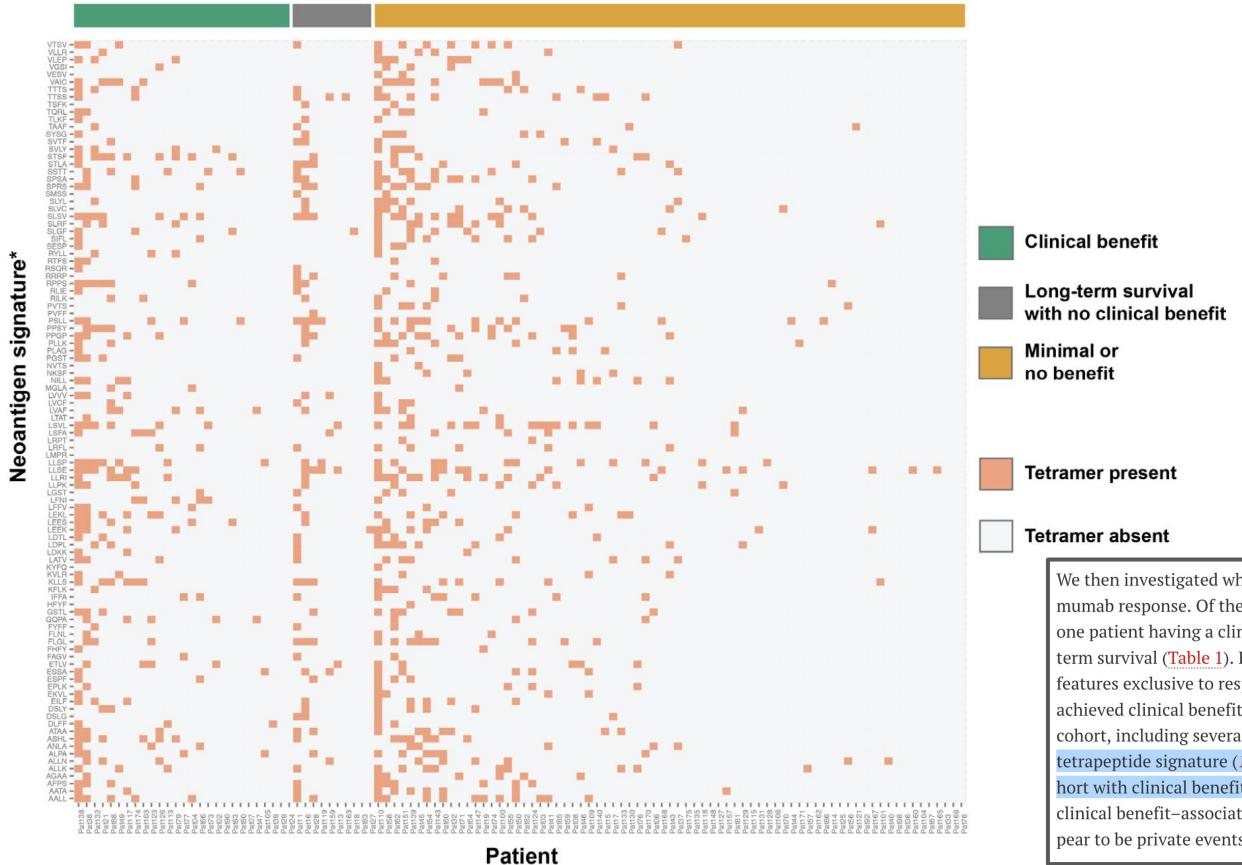
Monoclonal antibodies directed against cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), such as ipilimumab, yield considerable clinical benefit for patients with metastatic melanoma by inhibiting immune checkpoint activity, but clinical predictors of response to these therapies remain incompletely characterized. To investigate the roles of tumor-specific neoantigens and alterations in the tumor microenvironment in the response to ipilimumab, we analyzed whole exomes from pretreatment melanoma tumor biopsies and matching germline tissue samples from 110 patients. For 40 of these patients, we also obtained and analyzed transcriptome data from the pretreatment tumor samples. Overall mutational load, neoantigen load, and expression of cytolytic markers in the immune microenvironment were significantly associated with clinical benefit. However, no recurrent neoantigen peptide sequences predicted responder patient populations. Thus, detailed integrated molecular characterization of large patient cohorts may be needed to identify robust determinants of response and resistance to immune checkpoint inhibitors.



Van Allen: TMB vs. aCTLA-4 response



Van Allen: Tetrapeptide Signature



We then investigated whether any recurrent neoantigens or neoantigen epitopes might predict ipilimumab response. Of the 77,803 unique neoantigens in our cohort, 28 (-0.04%) were found in more than one patient having a clinical benefit but were absent in all patients having no clinical benefit or long-term survival (Table 1). Examination of these recurrent neoantigens did not reveal any shared features or features exclusive to responders. Previously described immunogenic nonamers identified in patients who achieved clinical benefit from immune checkpoint blockade were not observed in any patient within this cohort, including several that have undergone experimental validation (7, 11, 13, 23). Furthermore, a tetrapeptide signature (13) previously associated with ipilimumab response was not enriched in the cohort with clinical benefit relative to that with no clinical benefit (fig. S4) (25). Thus, the vast majority of clinical benefit–associated neoantigens and neoantigen epitopes identified through DNA sequencing appear to be private events without obvious recurrent features.

New data: Wood et al. (2020)

RESEARCH

Open Access

Burden of tumor mutations, neoepitopes, and other variants are weak predictors of cancer immunotherapy response and overall survival

Mary A. Wood^{1,2}, Benjamin R. Weeder^{1,3}, Julianne K. David^{1,3}, Abhinav Nellore^{1,3,4†} and Reid F. Thompson^{1,2,3,5,6,7*}



Abstract

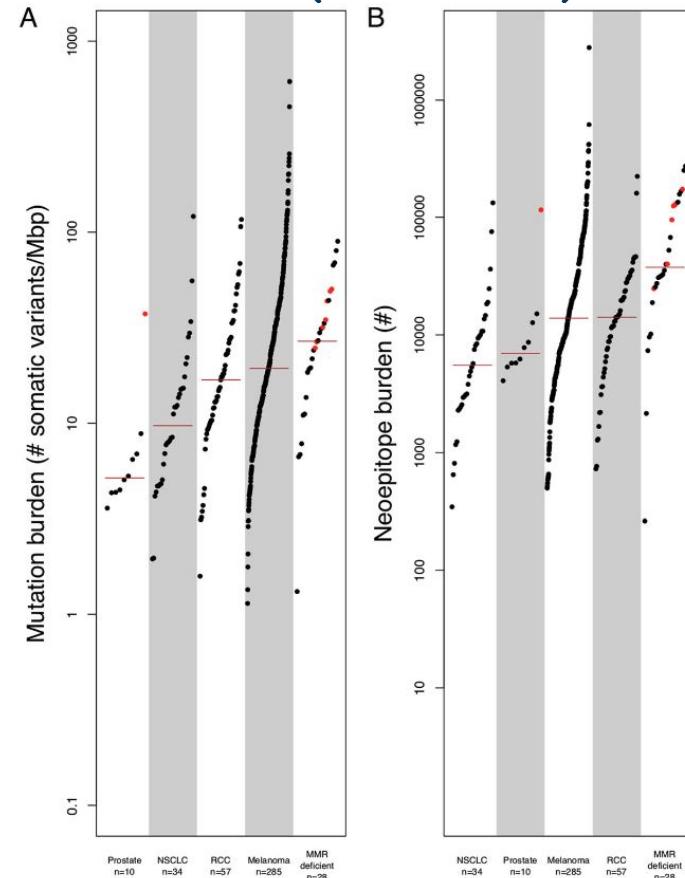
Background: Tumor mutational burden (TMB; the quantity of aberrant nucleotide sequences a given tumor may harbor) has been associated with response to immune checkpoint inhibitor therapy and is gaining broad acceptance as a result. However, TMB harbors intrinsic variability across cancer types, and its assessment and interpretation are poorly standardized.

Methods: Using a standardized approach, we quantify the robustness of TMB as a metric and its potential as a predictor of immunotherapy response and survival among a diverse cohort of cancer patients. We also explore the additive predictive potential of RNA-derived variants and neoepitope burden, incorporating several novel metrics of immunogenic potential.

Results: We find that TMB is a partial predictor of immunotherapy response in melanoma and non-small cell lung cancer, but not renal cell carcinoma. We find that TMB is predictive of overall survival in melanoma patients receiving immunotherapy, but not in an immunotherapy-naïve population. We also find that it is an unstable metric with potentially problematic repercussions for clinical cohort classification. We finally note minimal additional predictive benefit to assessing neoepitope burden or its bulk derivatives, including RNA-derived sources of neoepitopes.

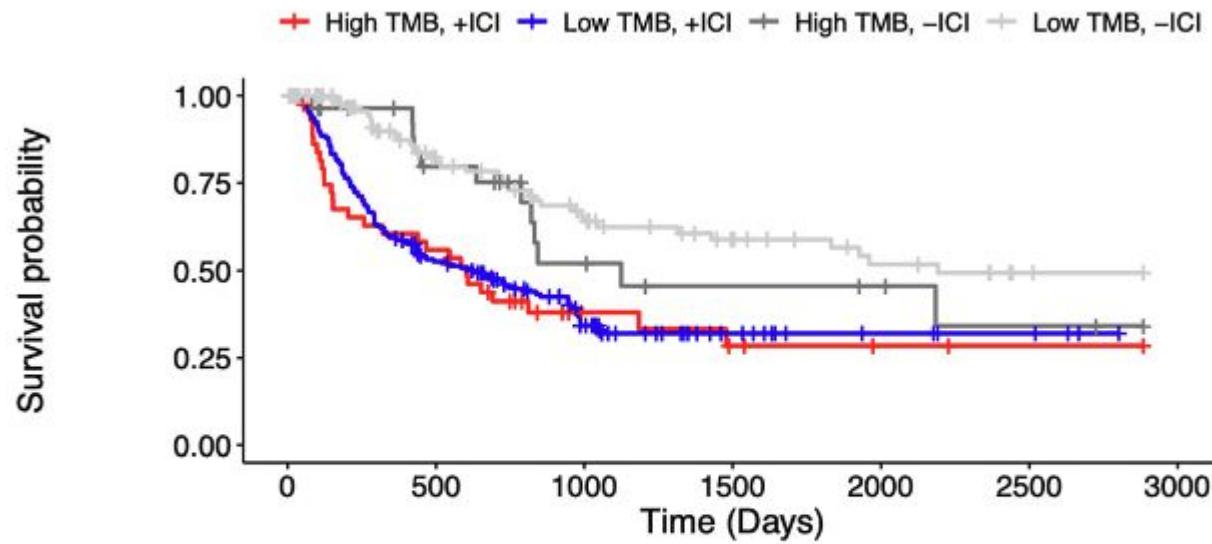
Conclusions: We find sufficient cause to suggest that the predictive clinical value of TMB should not be overstated or oversimplified. While it is readily quantified, TMB is at best a limited surrogate biomarker of immunotherapy response. The data do not support isolated use of TMB in renal cell carcinoma.

Keywords: Tumor mutational burden, TMB, Neoepitopes, Neoepitope burden, Neoantigens, Splice junctions, Retained introns, Tumor variant burden, Immunotherapy response



Wood: TMB vs. aPD-1/aCTLA-4

A)



Number at risk

High TMB, +ICI	44	24	8	5	3	1	0
Low TMB, +ICI	174	86	38	15	7	4	0
High TMB, -ICI	29	18	9	6	5	3	0
Low TMB, -ICI	115	62	42	30	22	16	0

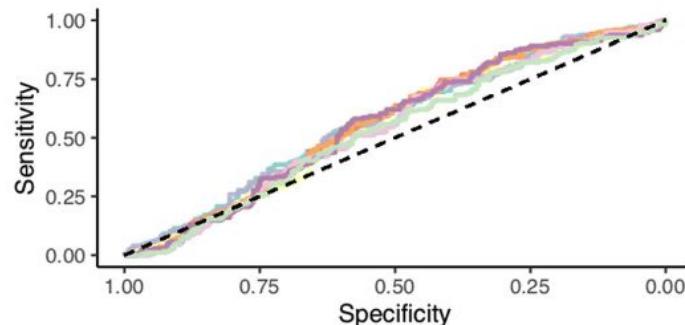
Wood: splitting aCTLA-4 vs. aPD-1

Table 1 Immunotherapy (aPD1 and aCTLA4) response probability based on logistic regression models of tumor mutational burden (TMB), neoepitope burden (Neoepitopes), and combined tumor DNA- and RNA- variant burden (TVB) for melanoma, non-small cell lung cancer (NSCLC), and renal cell carcinoma (RCC). *P* values are reported on a per-model basis without correction for multiple comparisons per cancer type

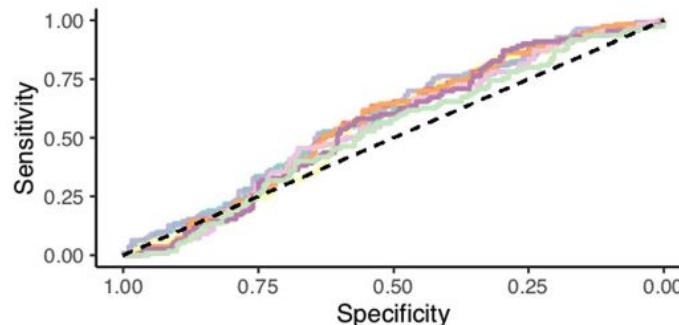
Cancer type & Metric	aPD1			aCTLA4			<i>p</i> -value
	N	Response Probability (25th %ile)	Response Probability (75th %ile)	N	Response Probability (25th %ile)	Response Probability (75th %ile)	
Melanoma							
TMB	50	0.456	0.507	195	0.298	0.342	0.267
Neoepitopes	50	0.470	0.507	195	0.302	0.334	0.378
TVB	27	0.367	0.490	61	0.307	0.424	0.079
NSCLC							
TMB	33	0.291	0.578				0.034
Neoepitopes	33	0.345	0.583				0.053
RCC							
TMB	50	0.656	0.666				0.894
Neoepitopes	50	0.662	0.66				0.973
TVB	17	0.641	0.538				0.600

Wood: Different measures of TMB

All Cancers



Melanoma



AUC by Burden and Cancer Type

Cancer type	N	TMB1	TMB2	TMB1*HLA	NB	M	A	T	M*A	M*T	A*T	M*A*T
All	431	0.572	0.555	0.571	0.568	0.547	0.567	0.563	0.547	0.534	0.563	0.534
Melanoma	302	0.582	0.560	0.587	0.572	0.550	0.572	0.558	0.550	0.529	0.558	0.529
RCC	57	0.477	0.533	0.509	0.549	0.563	0.556	0.552	0.563	0.459	0.552	0.459
NSCLC	34	0.726	0.722	0.736	0.760	0.740	0.760	0.677	0.740	0.694	0.677	0.694

Same data: Nathanson et al (2017)

Research Article

Cancer
Immunology
Research

Somatic Mutations and Neoepitope Homology in Melanomas Treated with CTLA-4 Blockade

Tavi Nathanson¹, Arun Ahuja¹, Alexander Rubinstein¹, Bulent Arman Aksoy¹, Matthew D. Hellmann^{2,3}, Diana Miao^{4,5}, Eliezer Van Allen^{4,5}, Taha Merghoub^{2,3,6}, Jedd D. Wolchok^{2,3,6}, Alexandra Snyder^{2,3}, and Jeff Hammerbacher¹

Abstract

Immune checkpoint inhibitors are promising treatments for patients with a variety of malignancies. Toward understanding the determinants of response to immune checkpoint inhibitors, it was previously demonstrated that the presence of somatic mutations is associated with benefit from checkpoint inhibition. A hypothesis was posited that neoantigen homology to pathogens may in part explain the link between somatic mutations and response. To further examine this hypothesis, we reanalyzed cancer exome data obtained from our previously published study of 64 melanoma patients treated with CTLA-4 blockade and a new dataset of RNA-Seq data from 24 of these patients. We found that the ability to accurately predict patient benefit did not increase as the analysis

narrowed from somatic mutation burden, to inclusion of only those mutations predicted to be MHC class I neoantigens, to only including those neoantigens that were expressed or that had homology to pathogens. The only association between somatic mutation burden and response was found when examining samples obtained prior to treatment. Neoantigen and expressed neoantigen burden were also associated with response, but neither was more predictive than somatic mutation burden. Neither the previously described tetrapeptide signature nor an updated method to evaluate neoepitope homology to pathogens was more predictive than mutation burden. *Cancer Immunol Res*; 5(1); 84–91. ©2016 AACR.

Nathanson: “Discordant Lesions”

Table 1.

Cohort summary

Patient samples

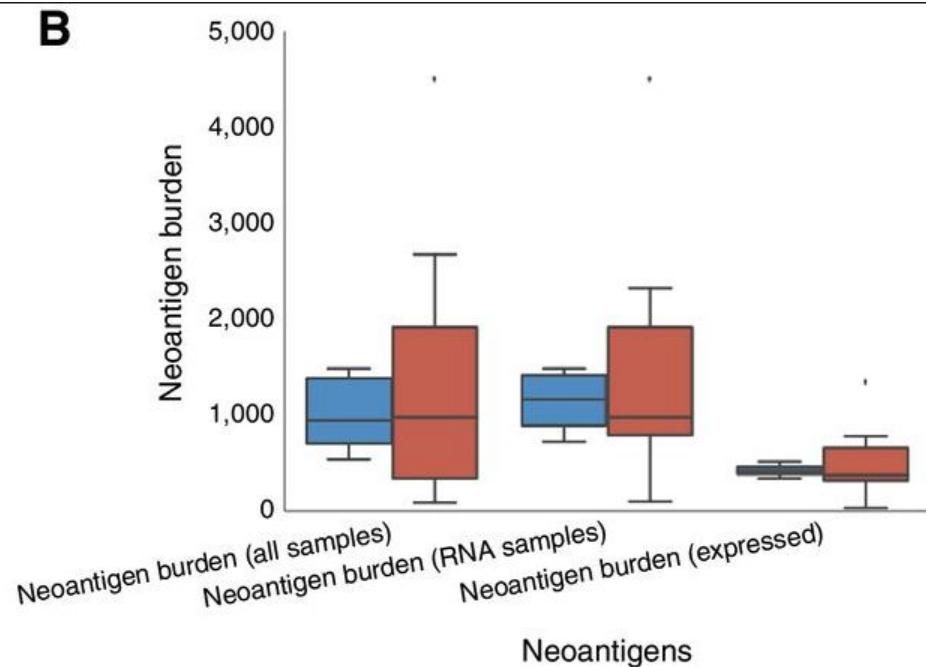
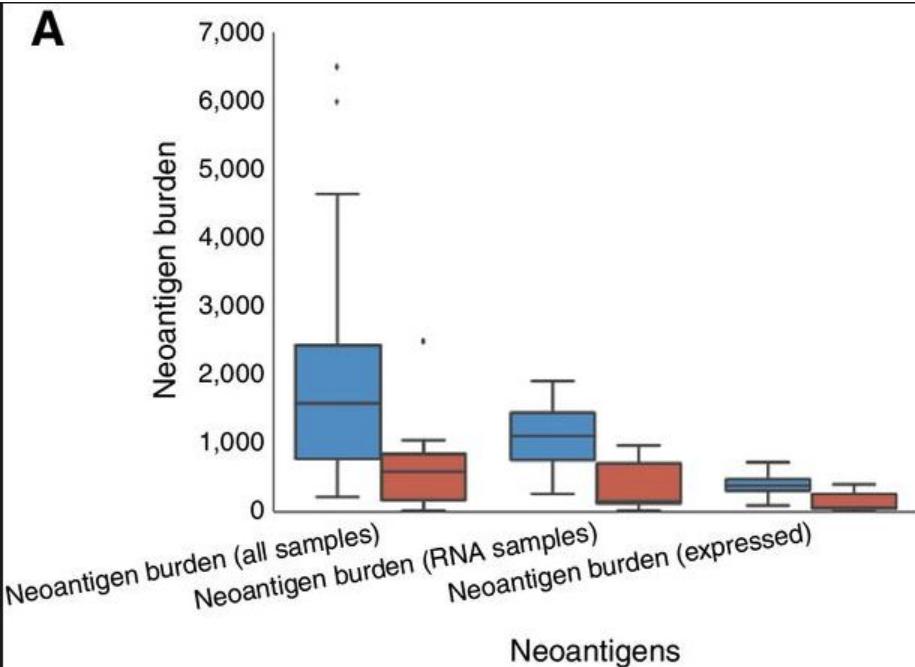
All analyzed samples were collected in accordance with local Internal Review Board policies as described in ref. 8 and summarized in Table 1. Thirty-four patients had tumor samples collected prior to initiating CTLA-4 blockade, and 30 patients had samples collected after initiating CTLA-4 blockade. Clinical benefit was defined as progression-free survival lasting for greater than 24 weeks after initiation of therapy (Online Data File 1). Nine discordant lesions were present, where overall patient benefit did not match individual tumor progression. See Table 1 for details about this patient cohort.

Group	Benefit	No benefit	Discordant
<i>N</i>	27	28	9
% Cutaneous	20/27	19/28	5/9
OS	3.7 (1.6–7.3)	0.8 (0.2–2.7)	4 (1.7–7.9)
Age	65 (33–81)	58.5 (18–79)	68 (40–90)
Mutations	611 (165–3,394)	321 (6–1,816)	549 (93–1,336)
Neoantigens	1,388 (209–6,502)	714.5 (3–4,510)	1,048 (197–2,584)

NOTE: Features of tumors from patients with clinical benefit, no benefit, or in which a discordant lesion was resected.

Abbreviation: OS, overall survival.

Nathanson: TMB vs. CTLA-4 response



Nathanson: Tetrapeptide Signature

presence did not outperform mutation burden (Online Data File 10). In addition to directly replicating the approach and data we used previously, we performed a similar analysis using only pretreatment, nondiscordant samples as well as updated variant calls. The presence of tetrapeptide signature tetrapeptides achieved an AUC of 0.50 (95% CI, 0.50–0.50), compared with an updated mutation burden baseline of 0.85 (95% CI, 0.69–0.98). Using counts of signature peptides did not outperform mutation burden here either (Online Data File 10).

Some red flags

Small heterogeneous sample

- 64 patients
- 12 clinical trials (!!)
- Treatments:
 - ipilimumab {3, 10} mg/kg
 - +/- dacarbazine
 - +/- vemurafenib
 - tremelimumab {10, 15}mg/kg

Patient Data

Charts were reviewed independently by two investigators to assign the clinical subgroup and other parameters for discovery and validation sets. Overall survival was calculated as the difference between date of death or censure and first dose of anti-CTLA4 therapy (ipilimumab in the discovery set or ipilimumab or tremelimumab in the validation set). All patients in the discovery set had stage IV melanoma and were treated between 2006 and 2012; samples were collected between 2007 and 2012. Patients in the validation set were treated from 2006 to 2013, and samples were collected between 2005 and 2013. Patients were treated either with commercial ipilimumab (Yervoy) or on one of the following clinical trials, including NCT00796991, NCT00495066, NCT00920907, NCT00324155, NCT00162123, NCT0140045 and NCT00289640, NCT00495066, NCT00636168, NCT01515189, NCT00086489, NCT00471887. Patients received varied doses and regimens of ipilimumab, at 3 or 10mg/kg, and 2 patients were co-treated with dacarbazine or vemurafenib (see Table S2 in the Supplementary Appendix). Four patients in the validation set were treated with tremelimumab at a dose of 10mg/kg x6 (1 patient) or 15mg/kg x4 (3 patients).

“Discordant lesions”

- Definition of “progression” vs. “benefit” getting very subjective
- Assumes that each lesion has independent TMB-dependent reasons for growing
- ...was this a predetermined choice?

lesion resected in order to render them disease-free. One progressing lesion (CR7623) was sequenced in the training set. In the validation set, 8 tumors represent the non-

responding lesions from patients who otherwise had long-term benefit. These include CRNR4941, LSDNR1650, CRNR2472, LSDNR1120, CRNR0244, LSDNR9298, LSDNR3086 and PR03803. All tumors that progressed undergo molecular analysis as “no benefit” tumors.

Patients not randomly selected!

CCR Drug Updates

Clinical
Cancer
Research

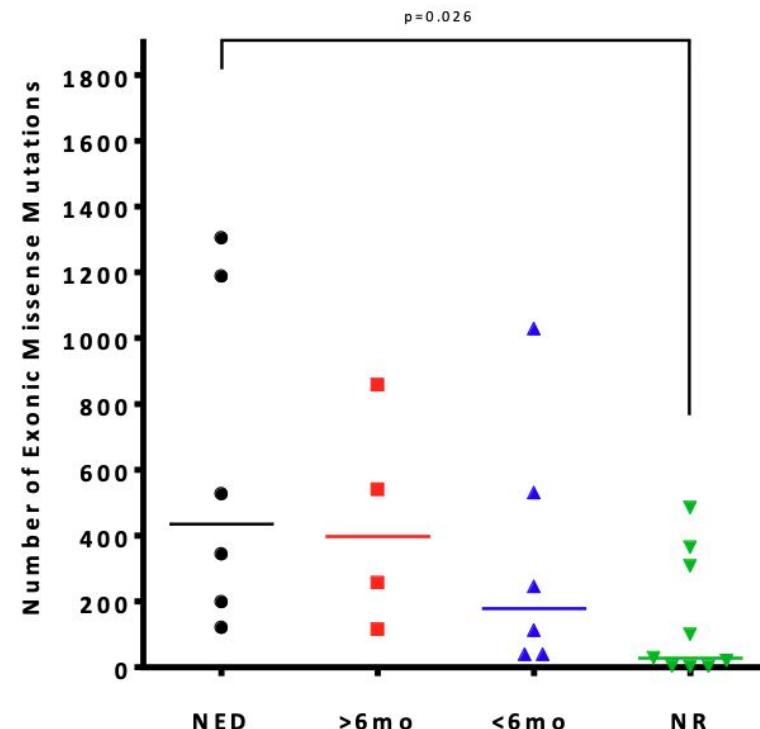
Ipilimumab: An Anti-CTLA-4 Antibody for Metastatic Melanoma

Evan J. Lipson and Charles G. Drake

Abstract

Ipilimumab (MDX-010, Yervoy; Bristol-Myers Squibb), a fully human monoclonal antibody against CTL antigen 4 (CTLA-4), was recently approved by the U.S. Food and Drug Administration (FDA) for the treatment of metastatic melanoma. In both early- and late-phase trials, ipilimumab has shown consistent activity against melanoma. For example, in a randomized phase III trial that enrolled patients with previously treated metastatic disease, ipilimumab, with or without a peptide vaccine, improved overall survival: Median overall survival was 10.1 and 10.0 months in the ipilimumab and ipilimumab plus vaccine arms, respectively, versus 6.4 months in the vaccine-alone group (hazard ratio, 0.68; $P \leq 0.003$). Serious (grade 3–5) immune-related adverse events occurred in 10% to 15% of patients. Thus, although it provides a clear survival benefit, ipilimumab administration requires careful patient monitoring and sometimes necessitates treatment with immune-suppressive therapy. Here, we review the mechanism of action, preclinical data, and multiple clinical trials that led to FDA approval of ipilimumab for metastatic melanoma. *Clin Cancer Res.* 17(22): 6958–69. © 2011 AACR.

every 12 weeks. The primary endpoint of this study was efficacy. The best overall response rates were 0%, 4.2%, and 11.1% in the 0.3-mg/kg, 3-mg/kg, and 10-mg/kg groups, respectively. Survival data were encouraging, with 30% of patients in the 10-mg/kg cohort alive at 2 years, as opposed to 18% in the 0.3-mg/kg cohort. IrAEs were again noted in 0%, 5%, and 18% of patients in the 3 dose cohorts. These findings supported the efficacy of MDX-010 and reinforced the every-3-weeks dosing regimen that was subsequently adapted for phase III investigation.



Implausible T-cell cross-reactivity

Article | Published: 17 September 2018

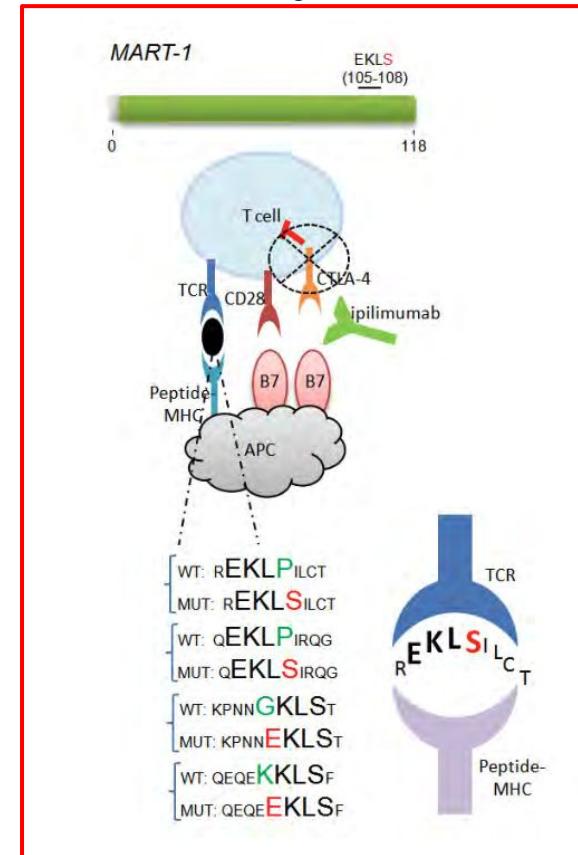
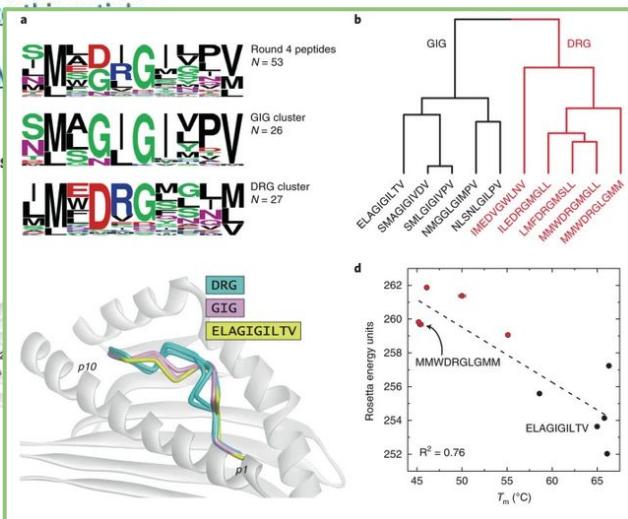
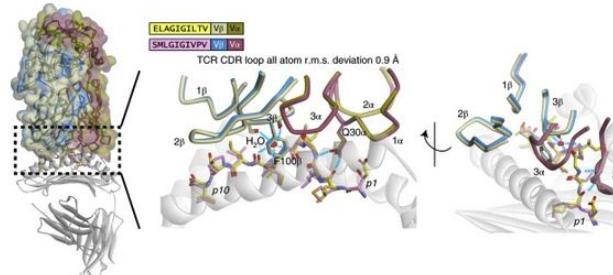
T cell receptor cross-reactivity expanded by dramatic peptide–MHC adaptability

Timothy P. Riley, Lance M. Hellman, Marvin H. Gee, Juan L. Mendoza, Jesus A. Alonso, Kendra C. Foley, Michael I. Nishimura, Craig W. Vander Kooi, K. Christopher Garcia & Brian M. Baker 

Nature Chemical Biology 14, 934–942 (2018) | Cite as

7174 Accesses | 55 Citations | 100 Altmetric | 

Fig. 4: DMF5 recognizes the GIG peptides with common structural solutions

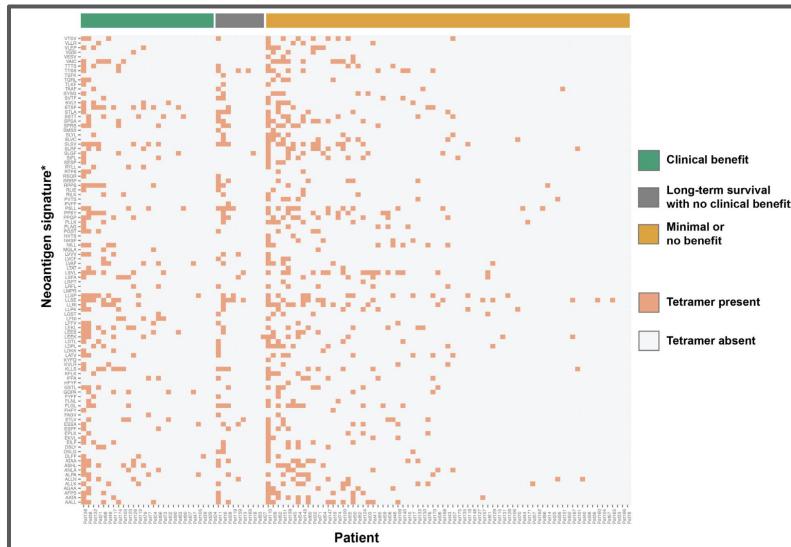


Weird pathogens!

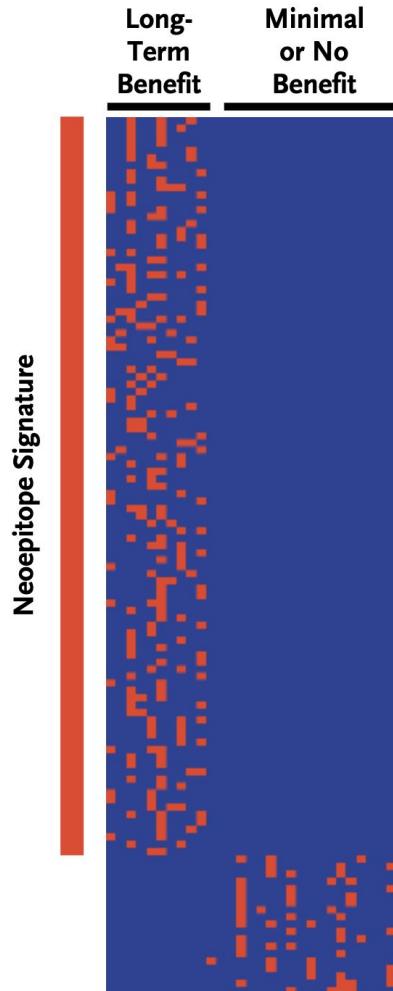
Pathogen	LB	NB
Adeno-associated virus-2		
Adenovirus		
Aspergillus fumigatus		
Bacillus anthracis		
BK polyomavirus		
Bordetella pertussis		
Borrelia burgdorferi		
Burkholderia pseudomallei		
C. diphtheriae		
C. tetani		
C. trachomatis		
Candida albicans		
Coxackievirus B4		
Dengue virus		
Francisella tularensis		
Group A Strep		
H. pylori		
Hantaan virus		
Hepatitis B virus		
Hepatitis C virus		
Hepatitis E virus		
Human herpesvirus 1		
Human herpesvirus 2		
Human herpesvirus 3		
Human herpesvirus 4		
Human herpesvirus 5		
Human herpesvirus 6		
Human herpesvirus 8		
Human papillomavirus 16		
Human papillomavirus 33		
Human papillomavirus 6		
Human papillomavirus 1a		
HTLV-1		
Influenza A		
Japanese encephalitis virus		
JC polyomavirus		
Lymphocytic choriomeningitis virus		
M. bovis		
M. leprae		
Major prion protein		
Measles virus		
Modified Vaccinia Ankara virus		

M. Tuberculosis		
Mupapillomavirus		
Mupapillomavirus 1		
P. falciparum		
P. vivax		
Porphyromonas gingivalis		
Rift Valley Fever virus		
Rubella virus		
SARS coronavirus		
Sin nombre virus		
Streptococcus pyogenes		
Streptococcus mutans		
Tick-borne encephalitis virus		
Torque teno virus		
Toxoplasma gondii		
Trypanosoma cruzi		
Vaccinia virus		
Variola minor		
West Nile virus		
Yellow fever virus		
Yersinia enterocolitica		

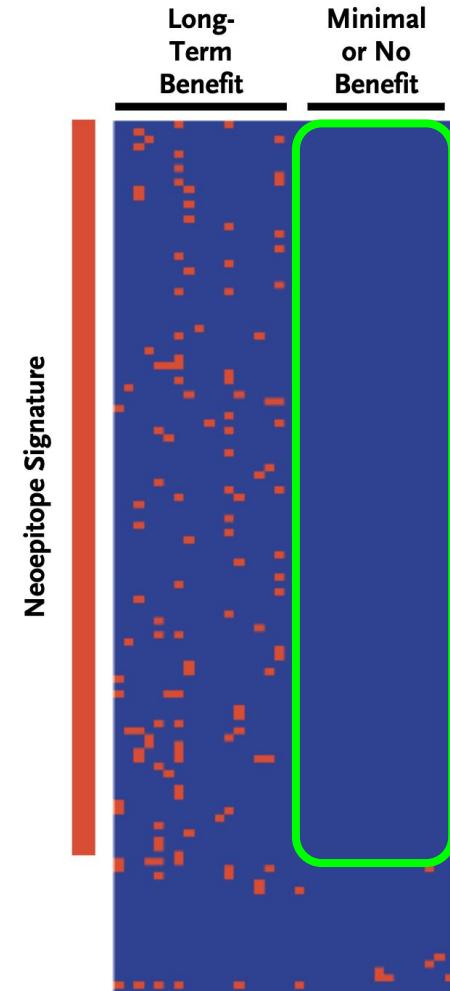
Suspiciously perfect predictive features



A Neoepitopes in Discovery Set



B Neoepitopes in Validation Set



What happened?

The “correction”

We are writing to provide clarification and correction to our article “Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma” (Dec. 4, 2014, issue).¹ Along with the publication, additional information on the data and methods that we used were posted in an online Supplementary Appendix, available with the full text of the article at NEJM.org. Some readers were confused by our incomplete description of part of the data analysis and our use of the term “validation set.” We acknowledge that our use of “validation set” was not appropriate in the context of the search for a neoantigen signature, since information from both data sets was used to derive the results. Here, we provide additional explanation and changes to the original article. The Supplementary Appendix has also been updated.

With respect to the neoantigen signature and its validation, the neoantigen signature we generated was not a comprehensive, all-inclusive signature that could subsequently be applied to any data set without further learning or iteration. We identified a group of neoantigen peptides with recurrent, shared, and nonrandom features that were found only among patients who had a response in the discovery set. We then used this group of peptides to evaluate the responders in the validation set and found them to be significantly enriched in frequency, as compared with peptides that were not in the group (Figure 3 of the original article).

In the article, we did not use “validation set” in the conventional way that the term is typically used in biomarker studies — namely, as an entirely independent data set in which findings from the discovery set are either confirmed or refuted. Rather, the term was carried over from the initial mutational-load analysis into the peptide-signature studies. In contrast to a formal biomarker analysis, our study design focused on defining a recurrent genetic footprint that occurred in a nonrandom fashion.

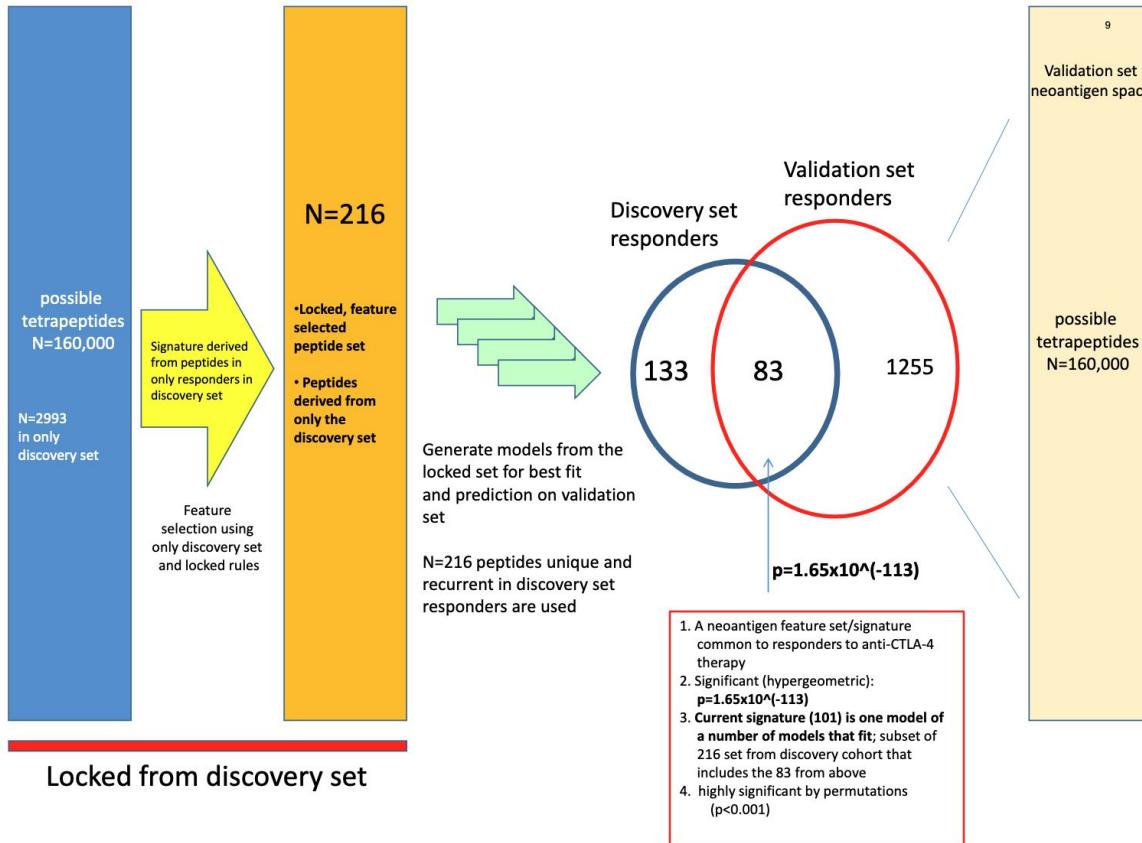
To clarify this difference, the following changes have been made to our article online. In the fourth paragraph of the Somatic Neoepitopes in Responding Tumors and Efficacy of CTLA-4 Blockade subsection of Results (page 2193), the paragraph beginning “We used the discovery set” now continues “to generate a peptide signature from the candidate neoepitopes. This analysis initially pooled the aforementioned discovery and validation sets to remove low-frequency tetrapeptides in the combined exomes. Subsequent analysis is restricted to post-filtering peptides (see the Methods section in the Supplementary Appendix).”

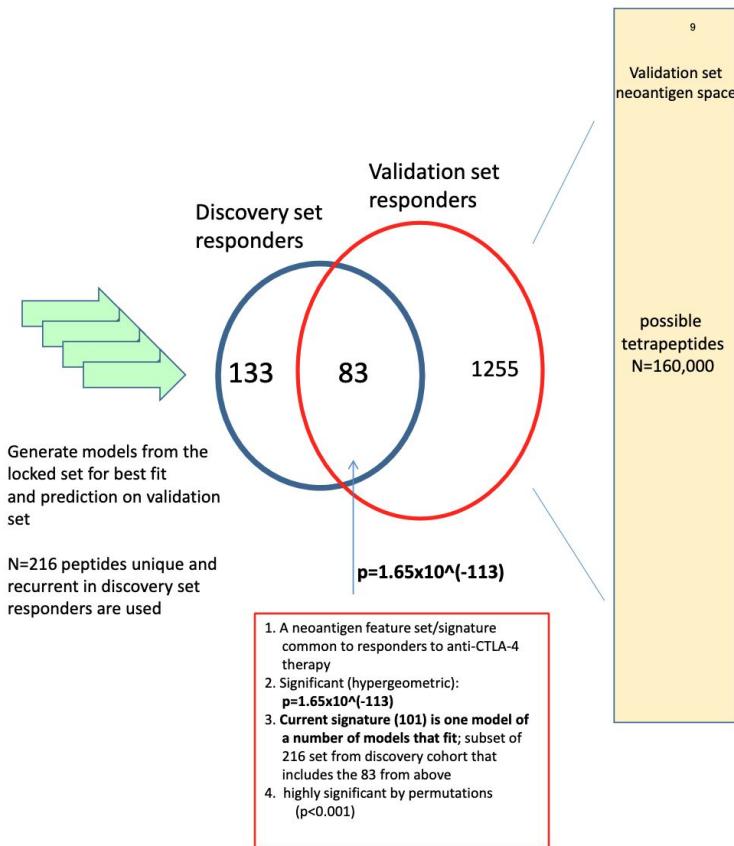
In the third paragraph of the same subsection (page 2193), beginning “Using only peptides,” the second sentence now reads, “Using the methods described in the Methods section in the Supplementary Appendix, we identified shared, consensus sequences.” The second paragraph of the Discussion (page 2197) now ends as follows: “and will require further prospective study before use as a definitive biomarker.” In the sixth paragraph of the Results subsection mentioned above (page 2195), the first sentence now reads, “Presence of the neoepitope signature peptides correlated strongly with survival in both the discovery set and the validation set ($P<0.001$ and $P<0.002$, respectively, by the log-rank test) (Figure 3C and 3D).”

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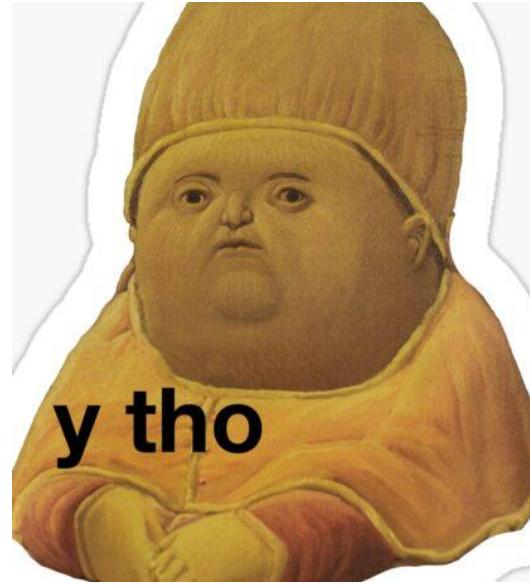
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The real tetrapeptide algorithm





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p=1.65x10⁻¹¹³

 *Fin*