Abstract #2591

Evidence for selective silencing of MHC-binding neoepitopes to avoid immune surveillance

CONTRIBUTING RESEARCHERS

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BACKGROUND

Overall response rates to immune checkpoint inhibition (ICI) are <50% even in Tumor Mutation Burden (TMB)-high patients (e.g. Checkmate-227), suggesting **other mechanisms of immune escape exist beyond expressing checkpoints**. At least **18% of somatic-specific exonic DNA variants are not expressed** into mRNA (Rabizadeh, 2018), yet the selection criteria for which variants to silence remains unclear. We sought to determine if **immunogenicity of variants factors into their suppression**

METHODS

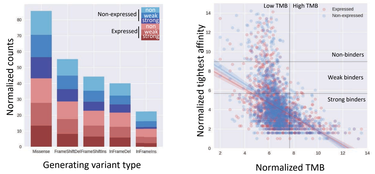
- 1418 clinical cases with paired tumor/normal whole-exome (~150x coverage) and whole-transcriptome (200x10^6 reads) were available from the NantHealth database
- TMB was calculated by counting somatic-specific non-synonymous exonic mutations. High-TMB was defined as >200 exonic mutations as in Rizvi et al 2015
- All possible 9-mer neoepitopes resulting from SNV or INDEL variants were generated and assessed for immunogenicity by NetMHC-4.0. For each variant, the neoepitope with the highest predicted affinity was analyzed further
- Neoepitopes were designated as non-expressed if fewer than 2 RNA reads supported the generating variant
- Immune-cell infiltration was estimated using RNA deconvolution on known immune cell marker genes (Bindea et al. 2013)

RESULTS

Figure 1. Clinical cohort description. Aggregated demographics statistics for 1395 clinical cases with predicted neoepitopes. Cancer types with fewer than 20 cases are grouped as "Other", Unannotated or unknown-primary cases are grouped as "Unreported".

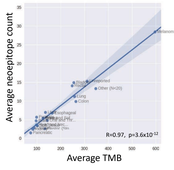
	N	Avg. Age	% Female	Avg. TMB	Avg. #SB
Breast	259	56.1	99.2	126.5	5.3
Colon	137	58.1	55.5	263.6	9.9
Lung	109	63.0	53.2	257.9	11.2
Bone and Soft Tissue Cancers (Including Sarcoma)	107	47.2	45.8	125.6	5.4
Pancreatic	85	63.0	43.5	73.4	1.4
Ovarian	73	59.7	100.0	88.5	2.8
Brain	70	41.9	42.9	96.6	4.7
Prostate	34	63.5	0.0	98.5	5.7
Esophageal	33	64.9	27.3	164.1	6.8
Melanoma	32	63.5	31.3	596.5	28.4
Head and Neck	30	63.8	23.3	97.9	3.7
Gastric (Stomach)	30	58.3	36.7	134.5	2.6
Oral and Throat Cancers (Including Thyroid)	27	63.8	37.0	143.7	4.8
Rectal	27	56.7	29.6	248.3	14.1
Kidney	27	48.6	29.6	83.0	2.4
Liver	25	61.8	32.0	135.3	7.0
Bladder	22	71.3	45.5	255.0	14.9
Soft Tissue	20	32.9	30.0	101.6	3.4
Other (N<20)	129	58.8	57.4	347.5	13.3
Unreported	119	56.4	45.4	311.4	15.3

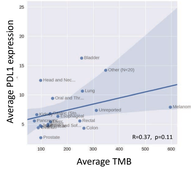
Figure 2. Presence of strong neoepitopes is not exclusively driven by high TMB or variant type. There is little difference in the proportion of predicted binders from disparate variant types or their expression rates (left). High-TMB patients almost all express at least one high-affinity neoepitope (middle, right), however so do the majority of low-TMB patients. Over 90% of patients have a non-expressed neoepitope predicted to be a strong binder.



	Low TMB	High TMB
No binding neoepitopes expressed	62 (5.3%)	0 (0%)
>1 one weak binder expressed	1066 (91.9%)	235 (100%)
>1 strong binder expressed	887 (76.5%)	231 (98.3%)
Total	1160 (83.2%)	235 (16.8%)

Figure 3. TMB does not drive checkpoint expression. TMB is highly correlated with neoantigen load when aggregating on a tissue level (left). However TMB and PDL1 expression appear to be independent, both when aggregated on the tissue level (middle) and when observing individual patients (right), as has been previously reported (Goodman, 2017)





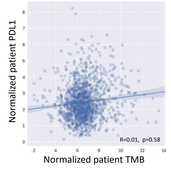
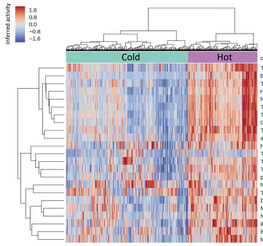


Figure 4. Immune-deconvolution significantly differentiates expression of multiple checkpoints. Inferred activity of immune cell types clusters tumors into two subgroups; Hot and Cold (left). These subgroups have highly significant differential expression of 7 key immunoregulatory genes (right).



gene	t	p	adj. p
FOXP3	31.6	8.37E-166	5.86E-165
CTLA4	29.2	6.06E-147	2.12E-146
PDL2	21.2	1.18E-86	2.75E-86
PDL1	20.2	7.72E-80	1.35E-79
LAG3	19.8	3.19E-77	4.47E-77
TIM3	18.3	2.31E-67	2.69E-67
IDO	12.0	1.31E-31	1.31E-31

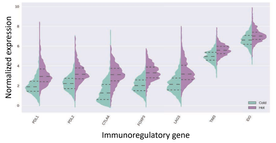
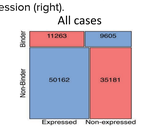


Figure 5. Evidence for systematic silencing of strong neoepitopes. Mosaic plots showing significant enrichment for silencing strong-binding neoepitopes across all patients (left), and especially in patients with active immunity but low checkpoint expression (right).



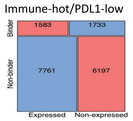


Figure 6. Proposed immune-evasion mechanism. Enrichment of silencing in immune-activated low-PDL1 patients suggests neoepitope modulation as an alternative to checkpoint expression to evade immune surveillance.

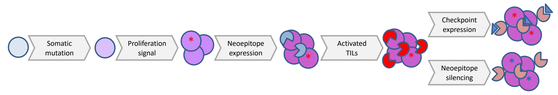
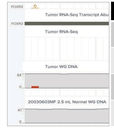


Figure 7. Patient case study. Renal medullary carcinoma with a very low TMB (0.9mts/Mb) yet is detected as immune-hot. Expression and binding characteristics are suggestive of selective neoepitope silencing.

Variant 1	
Gene	*PRPF40A*
Classification	Pathogenic
Predicted binding affinity	2024.0
RNA support (alt/total)	26/107



Variant 2	
Gene	*FOXR2*
Classification	Benign
Predicted binding affinity	37.0
RNA support (alt/total)	0/0

KEY FINDINGS

- A total of 147,015 potential neoepitopes were identified from 1,395/1,418 patients (98.4%).
- While high-TMB patients almost all expressed at least one high-affinity neoepitope, strong binders were not exclusively expressed in this group; 80% of all patients (1,116/1,395) expressed at least one high-affinity neoepitope.
- Across all cases a small but significant enrichment was observed for silencing neoepitopes that are predicted to bind strongly to MHC1 (OR = 1.21, p = 1.8x10^{-36})
- Silencing of potential neoepitopes was most prominent in 19% of patients with high inferred immune infiltration but low PDL1 expression (N = 261, OR = 1.37, p = 2.0x10^{-16})
- TMB and neoantigen load are highly similar biomarkers. TMB and PDL1 expression are independent.

CONCLUSIONS:

We observe **significant preferential silencing of MHC binding neoepitopes**. Specifically, **when tumor infiltrating immune cells are activated**, silencing neoepitopes may be an alternative to checkpoint expression for avoiding an immune cascade. Patients with TILs and silenced neoepitopes may benefit from epigenetic priming therapy prior to ICI therapy.

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