Molecular Profiling of Anti-PD-1 Treated Melanoma Patients Reveals Importance of Assessing Neoepitope Burden and Tumor Escape Mechanisms for Clinical Treatment

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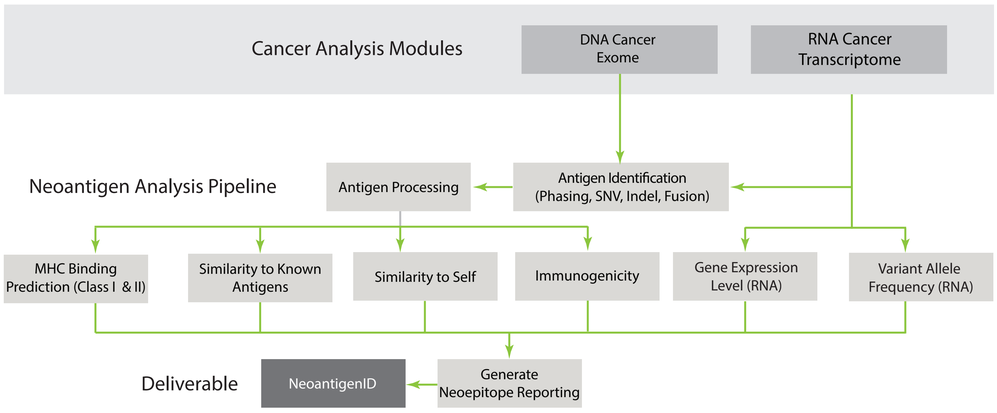
Background

Despite the remarkable response of some melanoma patients to checkpoint inhibitor therapy, significant numbers of patients do not achieve complete response. It is of great interest to identify biomarkers and mechanisms that influence immunotherapy effectiveness. Here we apply our comprehensive tumor immuno-genomics platform (ImmunoID NeXT) to identify potential biomarkers of response to checkpoint blockade therapy related to both the tumor and tumor microenvironment.

Methods

We characterized the immuno-genomics of tumors from 19 stage III/IV melanoma patients who have received anti-PD-1 treatments to assess potential factors influencing response. Tumor responses to the therapy were evaluated using RECIST criteria with a median follow-up of 12 months. Immuno-genomic profiling was performed using Personalis' ImmunoID NeXT platform: an augmented exome/transcriptome platform and analysis pipeline. Analyses included assessment of tumor mutations, neoepitope characterization, HLA typing, gene expression quantification, and tumor microenvironment profiling. The molecular information of the tumors was then analyzed together with their corresponding clinical response.

Neoepitope analytics engine



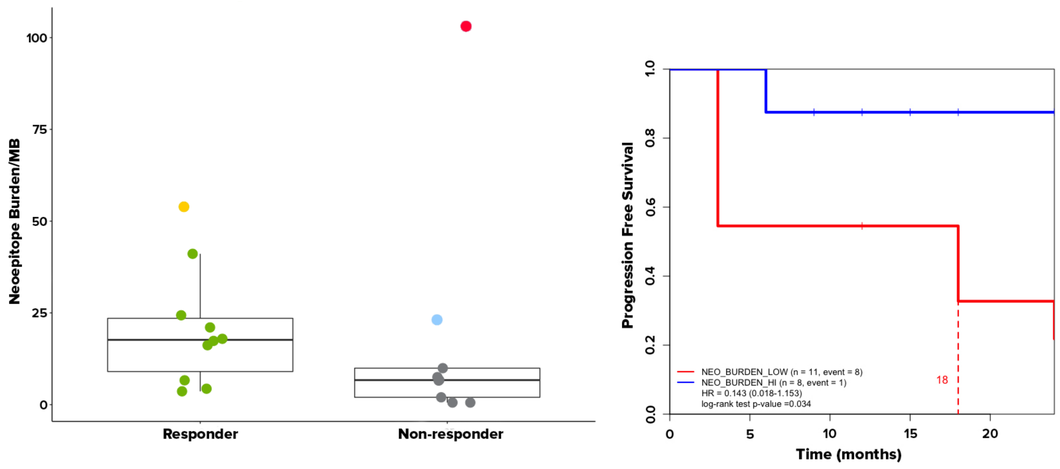
Within our neoepitope pipeline, variants that are detected by our DNA and RNA cancer analysis pipelines are processed for antigen identification, including SNVs, indels, and fusion events. Importantly, both in-frame and out-of-frame events are analyzed by peptide, allowing for detection of a wealth of candidate neoepitopes. Our pipeline includes assessment of important immunologic components including HLA prediction, MHC binding (class I and II), immunogenicity, similarity to self, and similarity to known antigens. Additionally, peptides are evaluated for variant allele frequency in both the RNA and DNA of the tumor sample and gene expression level is considered.

Patient characteristics

n	Responder (10)	Non-responder (9)	P-value
Age at treatment	70.7(46-94)	67.2(55-83)	0.77
Disease origin			0.21
acral	0(0%)	2(22.2%)	0.41
extremity	1(10%)	3(33.3%)	0.49
head neck	6(60%)	2(22.2%)	0.23
mucosal	0(0%)	0(0%)	-
trunk	3(30%)	2(22.2%)	1
PD1 therapy			0.72
Nivolumab	1(10%)	1(11.1%)	1
Nivolumab (in combination with ipilimumab)	2(20%)	0(0%)	0.50
Pembrolizumab	7(70%)	8(88.9%)	0.66
Sex			0.30
Female	4(40%)	1(11.1%)	0.36
Male	6(60%)	8(88.9%)	0.36
Stage at treatment			0.65
unresectable III	3(30%)	4(44.4%)	0.86
M1a	0(0%)	0(0%)	-
M1b	2(20%)	0(0%)	0.50
M1c	5(50%)	5(55.6%)	1

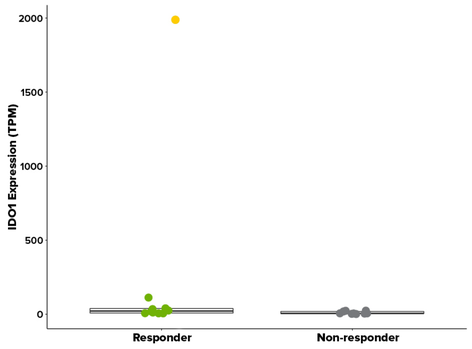
Patient responses, as defined by RECIST criteria, were classified as either responder or non responsder . Few patients (n = 2) had a partial response to therapy.

Higher neoepitope burden is associated with improved anti-PD1 response



Higher neoepitope burden is associated with improved response to anti-PD1 therapy, with three exceptions (yellow: Inova 021; red: Inova 025; blue: Inova 38). A cutoff value of 12.5 neoepitopes per MB was used to dichotomize samples into high and low neoepitope burden. We observe significantly longer PFS in patients with high neoepitope burden (P-value 0.03).

Partial response neoepitope burden outlier shows high IDO1 expression



IDO1 is a key rate limiting enzyme responsible for the degradation of tryptophan, which is cleaved by IDO1 to produce immuno-suppressive kynurenines. High IDO1 expression, as observed in subject 21, can lead to multiple immuno-suppressive actions, including T-cell anergy, apoptosis, and increased proliferations of Tregs. This immuno-suppressive activity has been independently observed multiple times in numerous tumor types, leading to the development of companion therapies targeted at reducing IDO1 levels. Anti-IDO1 therapy, in combination with anti-PD1 treatment, could prove to offer improved outcomes for cancer patients with high IDO1 expression levels.

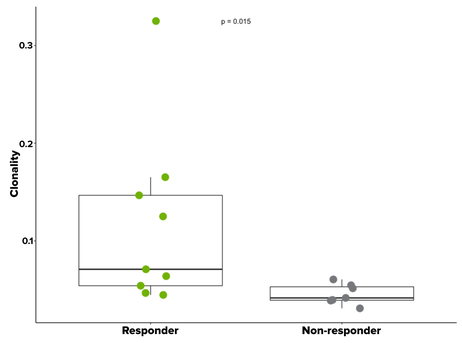
Non-responders with high neoepitope burden have deleterious mutations

Subject	Gene Symbol	POS	REF	ALT	DNA Allelic Fraction	Transcript Variant	Protein Variant	Variant Effect	Variant Effect Impact
25	HLA-A0201	682	G	A	0.473	c.252G>A	p.W84*	Stop gain	High
25	HLA-B1501	2349	G	A	0.368	c.1012G>A	p.G338S	Splice region variant	Moderate
38	B2M	4500 7811	CT	C	0.808	c.259delT	p.Y87fs	Frameshift variant	High

HLA class I is necessary for presenting epitopes (including neo-epitopes) in tumor cells to the immune system, marking the cells for destruction. Damaging HLA mutations can be highly impactful tumor escape mechanisms. We identified two distinct somatic HLA mutations in one non-responder with high neoepitope burden (Inova 25). These mutations likely lead to the loss of surface expression of of HLA-A and possible mis-folding of HLA-B . In a second non-responder (Inova 38) we detected a frameshift variant in the B2M gene, which potentially prevents proper HLA class I folding and antigen presentation for the majority of class I receptors. These tumor escape mechanism may explain the observed high neoepitope burden yet lack of checkpoint blockade response.

TCR clonality correlates with response

T cell infiltration and clonality in patients prior to checkpoint blockade therapy has been associated with a positive therapeutic response. Personalis has recently added TCR beta sequencing to our ImmunoID NeXT platform. When our TCR sequencing tool is applied to a subset of the patient cohort, we observe significantly higher clonality in responders when compared to the non-responder population. These data suggest that higher pretreatment clonality may be predictive of patient response to checkpoint therapy.



Conclusions

While we observed the expected association between neoepitope burden and response to checkpoint blockade therapy, we also identified potential resistance mechanisms in patients that involve perturbations to antigen presenting machinery and high expression of non-targeted checkpoint genes. This highlights the potential importance of broad immuno-genomic profiling of patients that are candidates for receiving immunotherapy. We are continuing to increase our cohort size to identify additional mechanisms for immune evasion.

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