# Comprehensive profiling of tumor-immune interaction in anti-PD-1 treated melanoma patients reveals subject-specific tumor escape mechanisms

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# Background

Checkpoint inhibitor therapy has demonstrated meaningful antitumor activity for many patients, though the majority fail to achieve complete response. Thus, it is of particular interest to identify biomarkers and mechanisms that promote positive response to immunotherapy. In the present study, we apply our comprehensive tumor immunogenomics platform (ImmunoID NeXT), integrating data from the tumor, tumor microenvironment and immune system to create a comprehensive biological signature of patient response to therapy.

### Methods

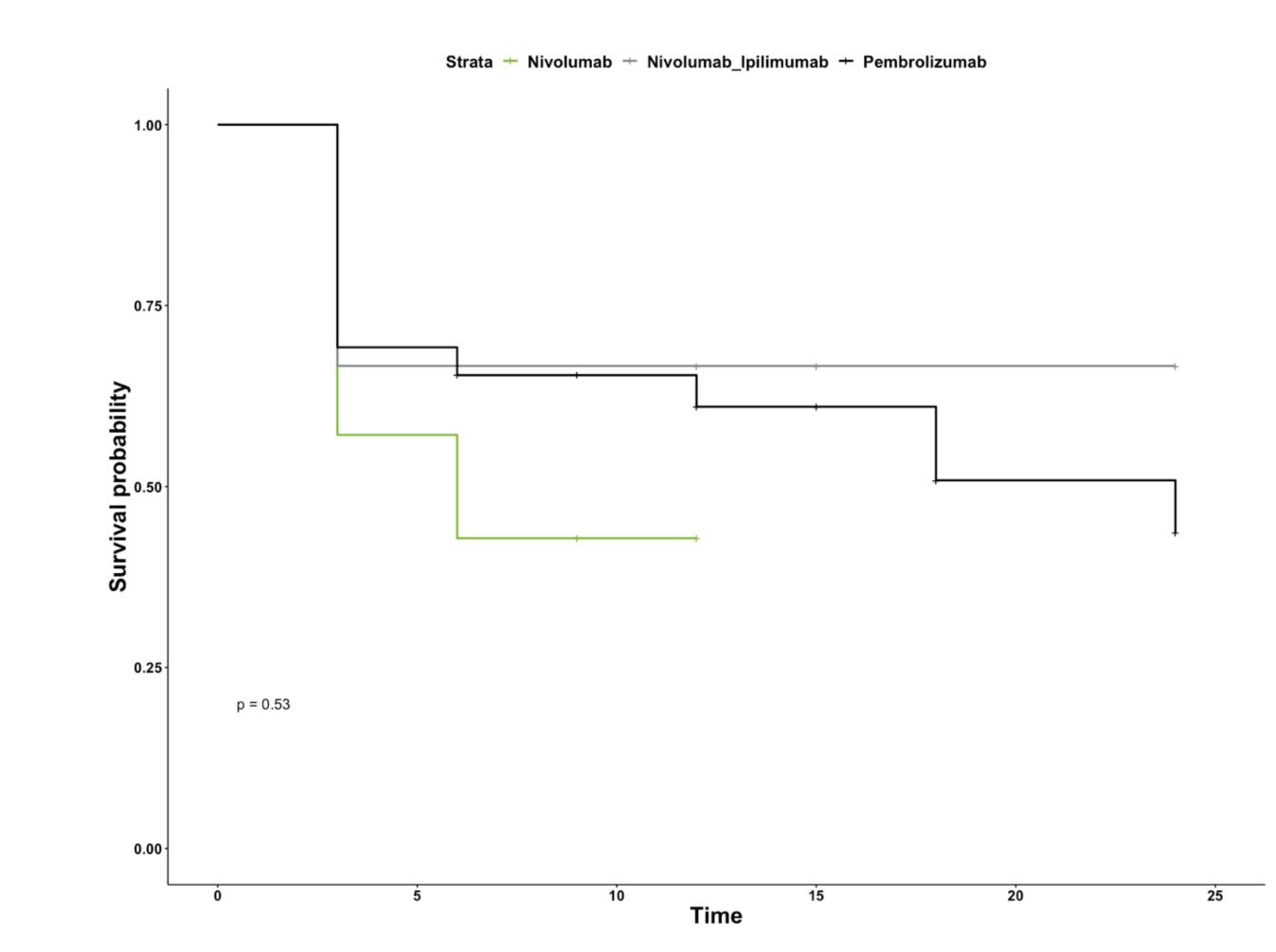
We characterized the immunogenomics of 52 unresectable, stage III/IV melanoma patients who underwent anti-PD-1 therapy to assess factors influencing response. RECIST criteria were used to evaluate tumor response to therapy, with a median follow-up of 12 months. For each patient, a single paired FFPE tumor and normal blood sample was collected and profiled using Personalis' ImmunoID NeXT platform; an augmented exome/transcriptome platform and analysis pipeline, which produces comprehensive tumor mutation information, gene expression quantification, neoantigen characterization, HLA typing and LOH, TCR repertoire profiling and tumor microenvironment profiling. Tumor molecular information was then analyzed together with clinical outcome.



#### Results

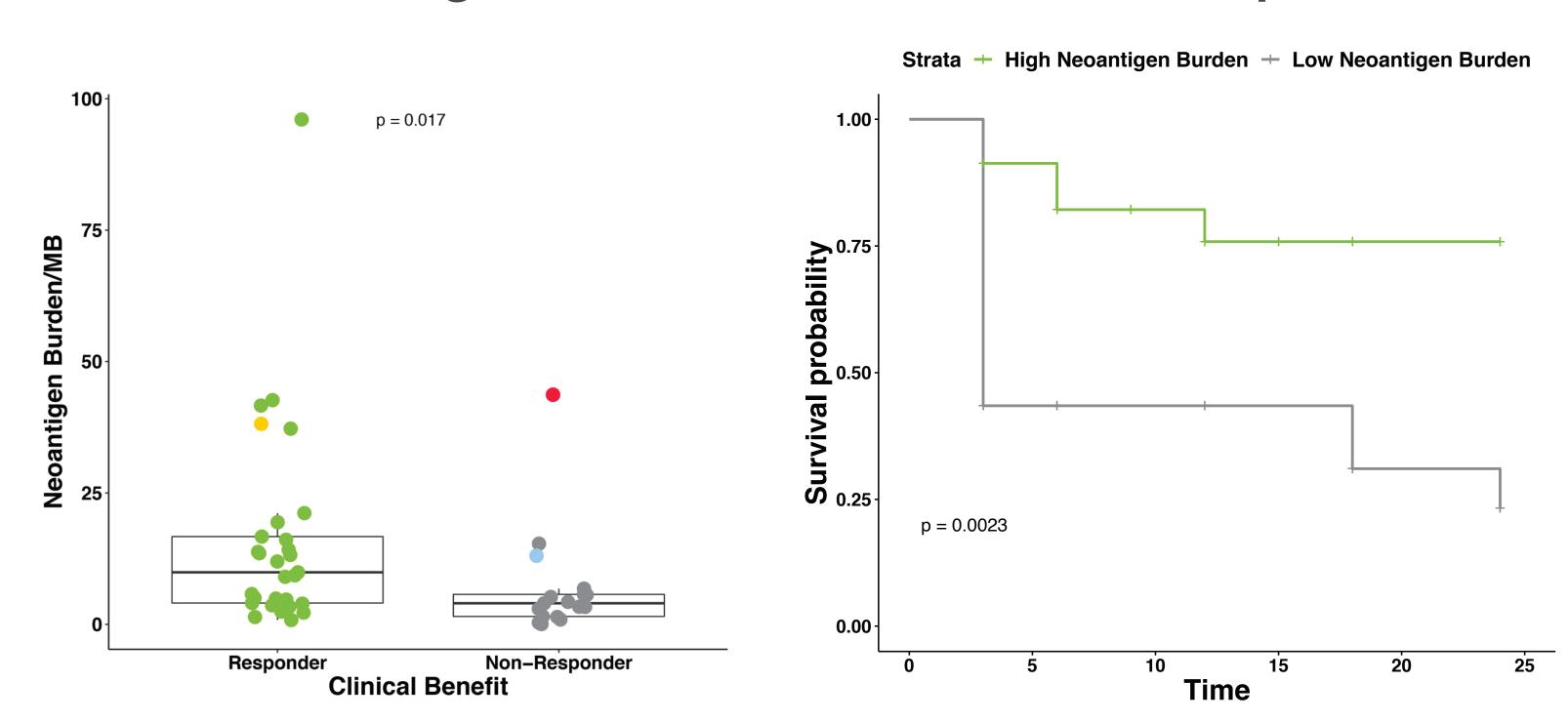
#### Patient demographics

n	Responder (30)	Non-responder (19)	P-value
Age at treatment	70(61-81.75)	67(57-78)	0.59
Disease origin			0.96
acral	2(6.7%)	1(5.3%)	1
extremity	7(23.3%)	5(26.3%)	1
head neck	11(36.7%)	5(26.3%)	0.66
mucosal	1(3.3%)	1(5.3%)	1
trunk	9(30%)	7(36.8%)	0.85
PD1 therapy			0.65
Nivolumab	7(23.3%)	7(36.8%)	0.49
Nivolumab (combo with ipilimumab)	5(16.7%)	2(10.5%)	0.86
Pembrolizumab	18(60%)	10(52.6%)	0.83
Sex			1
Female	9(30%)	6(31.6%)	1
Male	21(70%)	13(68.4%)	1
Stage at treatment			0.42
unresectable III	14(46.7%)	7(36.8%)	0.70
M1a	1(3.3%)	1(5.3%)	1
M1b	3(10%)	O(O%)	0.42
M1c	12(40%)	11(36.8%)	0.70



Complete demographics data were available for 49 of the 52 patients enrolled. Patient responses, as defined by standard RECIST criteria, were classified as either responder or non-responder. Few patients (n = 5) had a partial response to therapy. Analysis of progression free survival (PFS) revealed no significant variation between treatment groups.

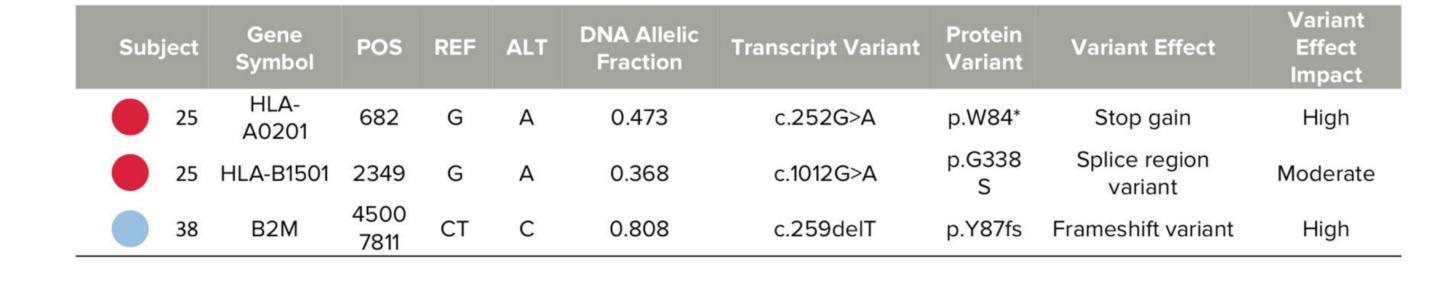
#### Elevated neoantigen burden is associated with improved anti-PD1 response

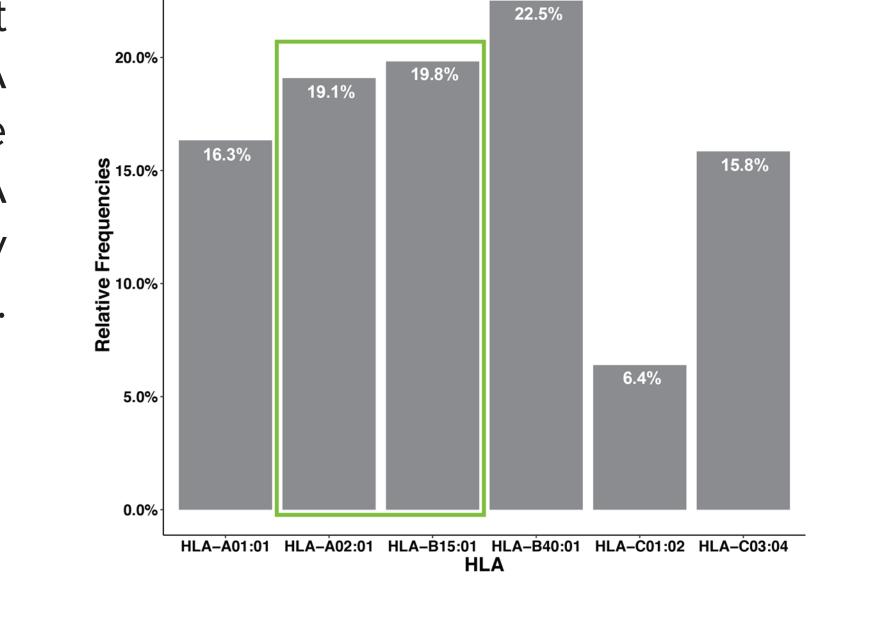


Higher neoantigen burden is significantly associated with improved response to anti-PD1 therapy, with three notable outliers (yellow: Inova 021; red: Inova 025; blue: Inova 38). A cutoff value of 12.5 neoantigens per MB was used to dichotomize samples into high and low neoantigen burden. We observe significantly longer PFS in patients with high neoantigen burden (P-value 0.0023).

#### Some non-responders with high neoantigen burden have damaging APM mutations

HLA class I is necessary for antigen presentation to the immune system, marking cells for destruction. We identified two distinct somatic HLA mutations in patient 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-responding patient, 38, we detected a frameshift variant in B2M, which potentially prevents proper HLA class I folding and antigen presentation. These tumor escape mechanisms may explain the observed high neoantigen burden, yet lack of response to therapy.





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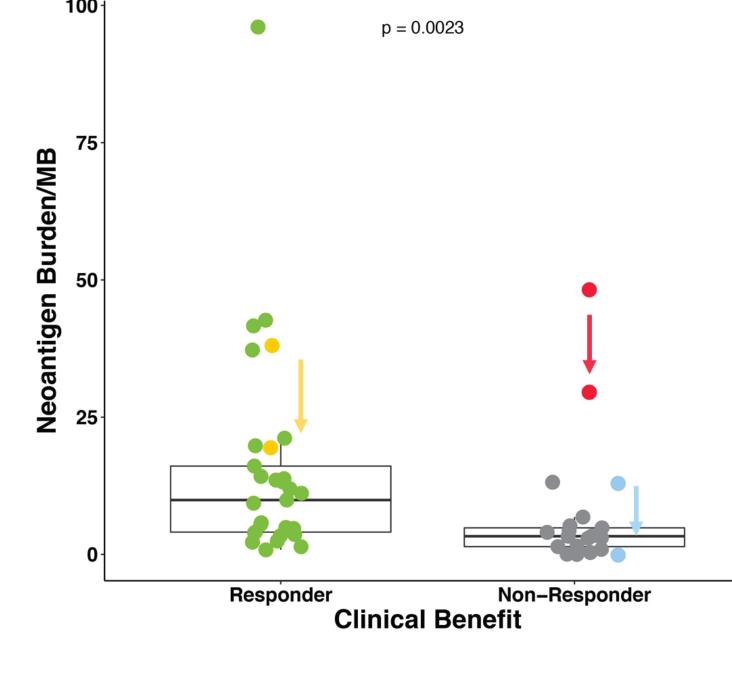
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HLA-A

Allele 2

# **HLA** loss of heterozygosity

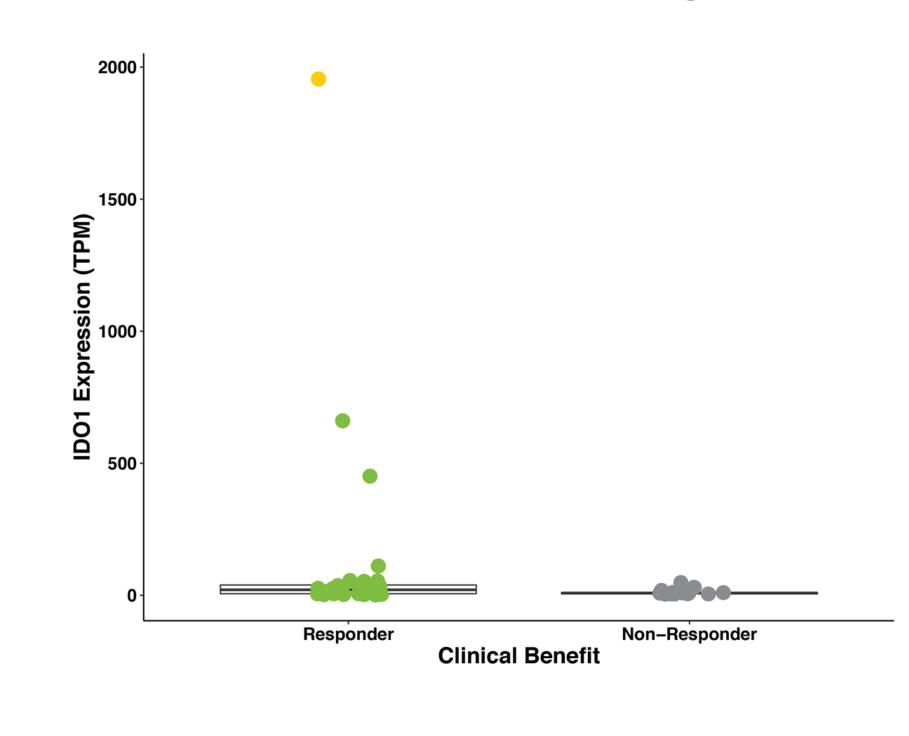
HLA function can also be impaired via loss of heterozygosity (LOH) at the HLA locus, reducing the immune system's ability to recognize tumor antigens. HLA LOH is increasingly recognized as a major tumor escape mechanism, particularly for immuno-oncology. To better understand the role of HLA-LOH in response to therapy we developed our own proprietary HLA LOH detection platform, DASH. Using DASH, HLA-LOH of at least 1 MHC class I allele was observed in 17% of study participants, with patient 21 highlighted at right. Here allele-specific loss was detected in HLA- A, B and C, likely resulting in a reduced capacity for antigen presentation in that patient.



#### Adjusted neoantigen burden

Melanoma is categorized as having one of the highest mutational burdens of any cancer. For PD-1 inhibition to be effective, immunogenic-neoantigens generated from these somatic mutations must be able to trigger anti-tumor immune responses. HLA-LOH and damaging APM mutations may facilitate immune evasion through reduced capacity for antigen presentation. As such, we believe a corrected neoantigen burden calculation, which accounts for escape by removing neoantigens which are predicted to present on the affected alleles. By applying this adjustment, we are able to more effectively separate responders to checkpoint blockade therapy those who do not respond.

#### Partial responder neoantigen burden outlier shows high IDO1 expression



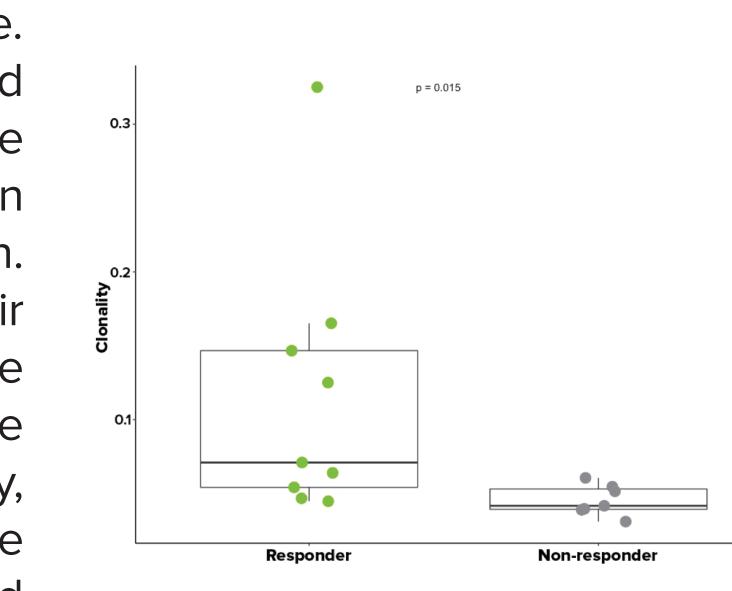
IDO1 is a key rate limiting enzyme responsible for the degradation of tryptophan, which is cleaved by IDO1 to produce immuno-suppressive kynurenines. High IDO1 expression, as observed in patient 21, can lead to multiple immuno-suppressive actions, including T-cell anergy, apoptosis, and increased proliferations 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.

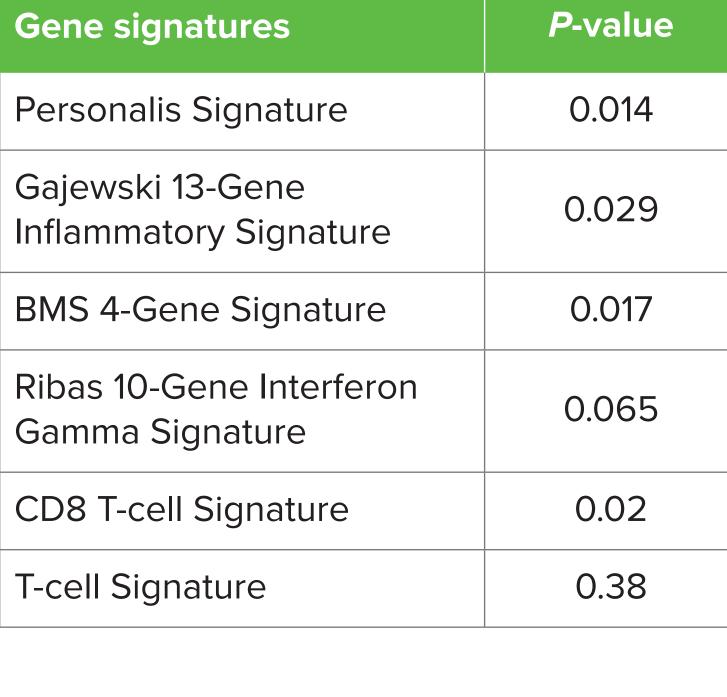
#### Gene signatures are associated with patient response to therapy

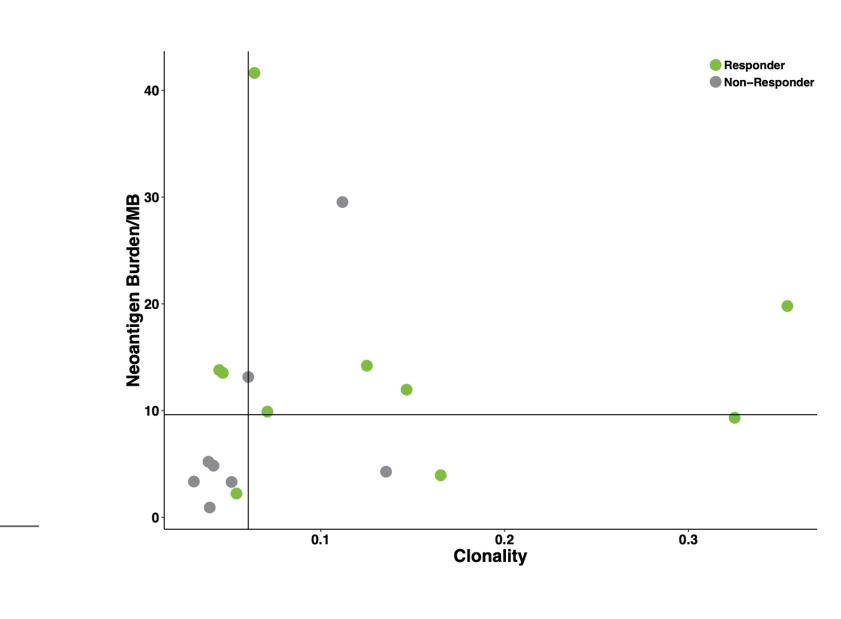
Tumor immune infiltration and inflammatory activity were investigated using established gene signatures, revealing several inflammatory signatures which were significantly associated with response to therapy. These findings were used to form a novel, combination data-derived/a priori knowledge-based gene signature, which was significantly associated with therapeutic response. Taken together these findings suggest that baseline differential immune activation, as captured by gene expression-based signatures, may be an effective marker for predicting response to therapy.



T cell infiltration and clonality in patients prior to checkpoint blockade therapy has been associatedwithapositivetherapeuticresponse. When our TCR sequencing platform is applied to a subset of the patient cohort, we observe significantlyhigherclonalityinresponderswhen compared to the non-responder population. By plotting a patient's TCRB data with their corrected neoantigen burden, we are able to capture both sides of the tumor-immune interaction. While these data are preliminary, they suggest that a combined tumor/immune approach to biomarker discovery may yield positive results.







## Conclusions

In summary, our comprehensive cancer immunogenomic analyses demonstrate that genomic and immune profiling of pretreatment patient samples can identify biomarkers and resistance mechanisms to immune checkpoint blockade, suggesting the potential efficacy of these as an integrated biomarker to optimize anti-PD-1 therapy patient selection.

