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

# 5710

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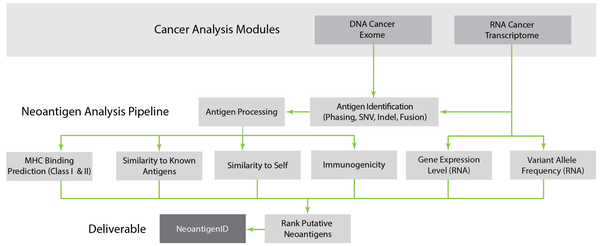
## Introduction

Despite the remarkable response of some melanoma patients to checkpoint inhibitor therapy, significant numbers of patients do not achieve complete response. To understand this differential response, there is an increasing interest in identifying biomarkers and mechanisms that influence immunotherapy effectiveness. In this study, we characterize the immuno-genomics of tumors from a series of melanoma patients that have received anti-PD-1 checkpoint inhibitors to assess potential factors influencing response.

## Methods

To better understand mechanisms of anti-PD-1 response, we sequenced and genomically profiled tumors from 19 stage III and IV melanoma patients where response was evaluated using RECIST criteria. Of the 19 patients, there were 8 complete responders (CR), 2 partial responders (PR), and 9 progressive disease (PD) patients. Immuno-genomic profiling was performed using Personalis' ACE ImmunoID platform, an augmented exome/transcriptome platform and analysis pipeline that allows for assessment of tumor mutations, neoantigens, HLA typing, gene expression quantification, tumor micro-environment, and tumor escape mechanisms. The molecular information for each of the 19 melanoma patient samples was then analyzed together with the corresponding clinical response to anti-PD-1 therapy.

### Developing a Neoantigen Analytics Engine



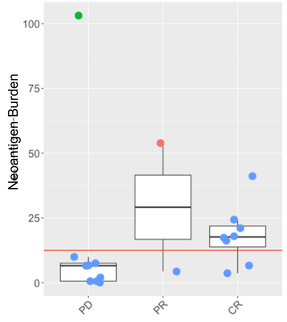

Within our neoantigen pipeline, variants that are detected by our DNA and RNA cancer analysis pipelines are processed for antigen identification, including SNVs, indels, and fusion events. Importantly, both in-frame and out-of-frame events are accurately considered by transcript, allowing for detection of a wealth of candidate neoantigens. 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.

### Therapeutic Response Was Determined By RECIST Criteria

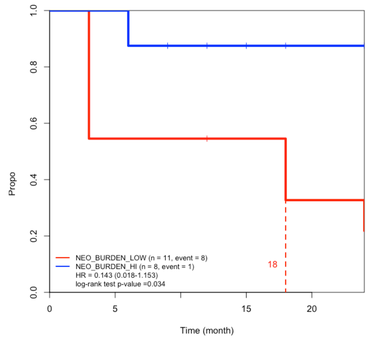
| n | PD (9) | PR (2) | CR (8) | P-value |
|---|---|---|---|---|
| **Age-at-treatment** | 66(57-80) | 77.5(69.25-85.75) | 76.5(55.75-79.25) | 1 |
| **Disease origin** | | | | 0.5789 |
| acral | 2(22.2%) | 0(0%) | 0(0%) | 0.2889 |
| extremity | 3(33.3%) | 0(0%) | 1(12.5%) | 0.427 |
| head neck | 3(33.3%) | 1(50%) | 4(50%) | 0.7635 |
| mucosal | 0(0%) | 0(0%) | 0(0%) | NaN |
| trunk | 1(11.1%) | 1(50%) | 3(37.5%) | 0.3383 |
| **pd1_therapy** | | | | 0.1001 |
| nivolumab | 0(0%) | 0(0%) | 1(12.5%) | 0.484 |
| Nivolumab (in combination with ipilimumab) | 0(0%) | 1(50%) | 1(12.5%) | 0.1108 |
| pembrolizumab | 9(100%) | 1(50%) | 6(75%) | 0.1382 |
| **sex** | | | | 0.3354 |
| F | 1(11.1%) | 1(50%) | 3(37.5%) | 0.3383 |
| M | 8(88.9%) | 1(50%) | 5(62.5%) | 0.3383 |
| **stage-at-treatment** | | | | 0.1329 |
| unresectable III | 6(66.7%) | 0(0%) | 2(25%) | 0.0982 |
| M1a | 0(0%) | 0(0%) | 0(0%) | NaN |
| M1b | 0(0%) | 0(0%) | 2(25%) | 0.2151 |
| M1c | 3(33.3%) | 2(100%) | 4(50%) | 0.2281 |

Patient responses, as defined by RECIST criteria, were preferentially defined as either progressive disease or complete responder. Few patients had a partial response to therapy.

### Higher neoantigen burden is associated with better anti-PD1 response

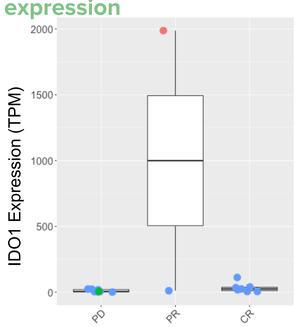



Higher neoantigen burden is associated with better response to anti-PD1 therapy with two strong exceptions (green: Inova_025; red: Inova_021). Red horizontal line indicates the cutoff value (12.5 neopeptide per MB) used to dichotomize samples into neoantigen burden high and neoantigen burden low.




We observe significantly longer progression free survival in patients with high neoantigen burden (P-value 0.034). Only a single progressive disease individual had high mutational burden (as defined by 12.5 neopeptides per MB).

### Partial response neoantigen burden outlier shows very high IDO1 expression




IDO1 is a key and rate limiting enzyme responsible for the degradation of the amino acid tryptophan, which is cleaved by IDO1 to produce immuno-suppressive kynurenines. It has been observed that high IDO1 expression can lead to multiple immuno-suppressive actions, including T-cell anergy, apoptosis, and increased proliferations of immunosuppressive Tregs. This immuno-suppressive activity has been independently observed multiple times in numerous tumor types, leading to 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.

### Partial progressive disease neoantigen burden outlier has two likely damaging somatic HLA class I mutations

| Gene Symbol | POS | REF | ALT | DNA Allelic Fraction | Transcript Variant | Protein Variant | Variant Effect | Variant Effect Impact |
|---|---|---|---|---|---|---|---|---|
| HLA-A0201 | 682 | G | A | 0.473 | c.252G>A | p.W84* | Stop gain | HIGH |
| HLA-B1501 | 2349 | G | A | 0.368 | c.1012G>A | p.G338S | Splice region variant | MODERATE |

HLA class I genes are a major members of the antigen presentation machinery. They are necessary for presenting epitopes (including neo-epitopes) in tumor cells to the immune system, marking the cells for destruction. Damaging somatic HLA mutations are widely accepted as highly impactful tumor escape mechanisms. We identified two distinct somatic HLA mutations in our progressive disease neoantigen burden outlier (Inova_25), which likely play a large role in the patients lack of response to anti_PD-1 therapy.

## Conclusion

We observed a strong correlation between response to anti-PD-1 therapy in melanoma patients and neoantigen burden when tumor escape mechanisms are considered. In our patients, we saw highly suggestive resistance mechanisms that involve perturbations to elements of the antigen presenting machinery and checkpoint blockade. 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 observe both how well the neoantigen burden holds to anti-PD-1 response and to identify additional mechanisms for immune evasion.

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