Neoantigen Homology and Predicting Response to Immune Checkpoint Blockade in Cancer

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Summary

Immune checkpoint inhibitors are promising cancer treatments for a variety of malignancies, but accurate prediction of clinical response remains an active area of research.

Recent work [1, 2] has revealed that nonsynonymous mutation burden and candidate neoantigen burden significantly correlate with durable clinical benefit to anti-CTLA-4 treated melanomas and anti-PD-1 treated lung cancers.

Using cancer exome and RNAseq data obtained from [1], we further investigated covariates with immune checkpoint inhibitor response.

Methods

- **Patients**
  - The study in [1] consisted of 64 patients treated with an anti-CTLA-4 (ipilimumab or (4) tremelimumab)
  - 44/64 patients were classified as cutaneous melanoma
  - 34/64 samples were taken prior to treatment
- **DNA**
  - DNA samples were obtained for all patients
  - Alignment and post-processing was performed as described in [1], while variants were called using 4 SNV callers (Strelka, MuTect, VarScan, SomaticSniper)
  - Indel calling was performed using Strelka
- **RNA**
  - RNASeq was obtained for 24 patients
  - 9/24 samples were taken prior to treatment
  - Sequence data was aligned using STAR
  - Gene expression quantification was performed using Cufflinks
  - HLA types and HLA expression were found using seq2HLA [4]
- **Mutant Epitope Prediction**
  - Mutant protein sequences were predicted using Varcode *(unpublished)*
  - MHC binding prediction was done using NetMHCcons, restricting to 8-11mers and affinity <= 500nM
- **Pathogen Homology**
  - Predicted neoantigens were aligned with T-cell positive peptides from IEDB of the same length, considering positions 3 through n-1 (n = length)
  - Peptide alignment was scored with the PMBEC matrix [3] and a gap penalty of min(PMBEC). For example, the following entry had a score of 1.4:

```
TP WRAPKK-IE L
QP  RAPIRPIP T
```

SNVs · Indels · Before Treatment · After Treatment

Conclusions

- Mutational burden is correlated with benefit.
- A higher percentage of UV mutations is correlated with mutational burden.
- Predicted neoantigen burden is correlated with benefit.
- Mutational burden increases the number of organisms matched in IEDB.
- Higher HLA expression was correlated with benefit in both pre- and post-treatment samples, independent of mutational burden.

References

1. Snyder, A. *et al.* Genetic Basis for Clinical Response to CTLA-4 Blockade in Melanoma. *N. Engl. J. Med.* (2014). doi:10.1056/NEJMoa1406498
2. Rizvi, N. A. *et al.* Mutational landscape determines sensitivity to PD-1 blockade in non – small cell lung cancer. *Science (80).* 1–10 (2014).
3. Kim, Y., Sidney, J., Pinilla, C., Sette, A. & Peters, B. Derivation of an amino acid similarity matrix for peptide: MHC binding and its application as a Bayesian prior. *BMC Bioinformatics* **10,** 394 (2009).
4. Boegel, S. *et al.* HLA typing from RNA-Seq sequence reads. *Genome Med.* **4,** 102 (2012).

Association Between Response to anti-CTLA-4 Therapy in Melanomas and...

...Mutation Load?

Similar to previous work, mutations per sample was correlated with increased survival (Figure A). However, we saw this was not the case in the samples taken after treatment (Figure B).

Figure A: Extended survival in patients with > median mutations and samples taken before treatment (log-rank test p=0.01)

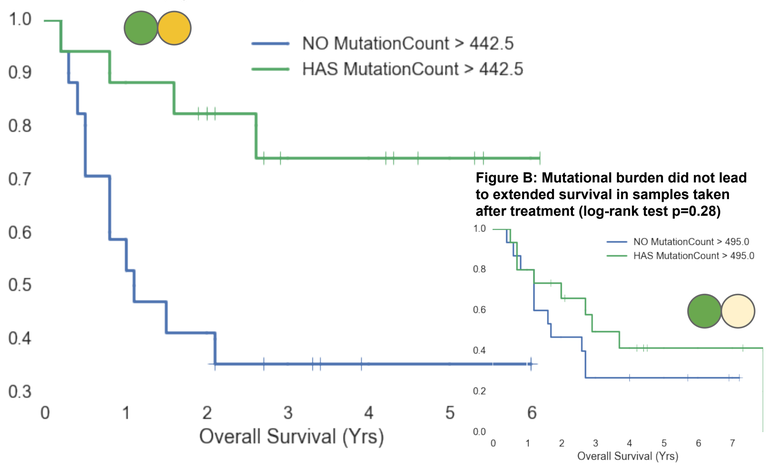


Figure B: Mutational burden did not lead to extended survival in samples taken after treatment (log-rank test p=0.28)

...Proportion of UV Mutations?

Mutations in high mutation burden samples were mostly C>T or G>A mutations, signatures of UV damage. Patients with higher ratios of these mutations showed increased survival (Figure C), however this was correlated with mutational burden (Figure D).

Figure C: Extended survival in patients where >50% of the mutations were C>T or G>A

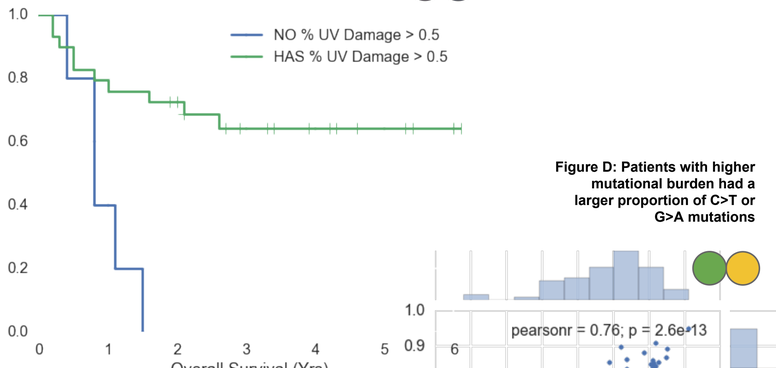


Figure D: Patients with higher mutational burden had a larger proportion of C>T or G>A mutations

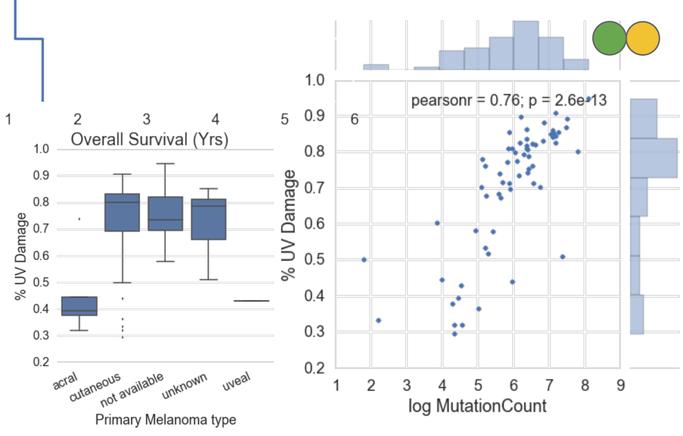
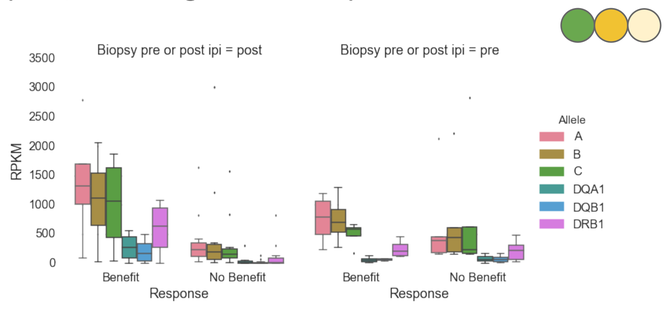


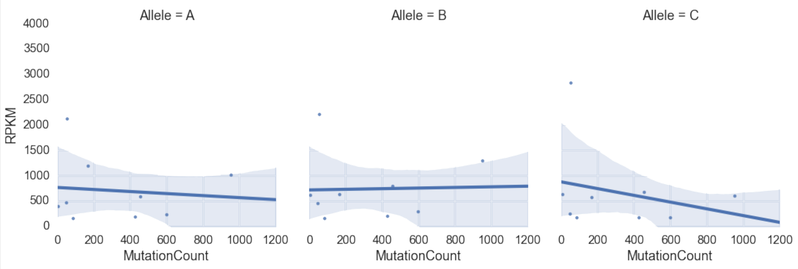
Figure E: C>T or G>A mutations were most prevalent in the cutaneous and unclassified melanomas

...Expression of Immune Activity?

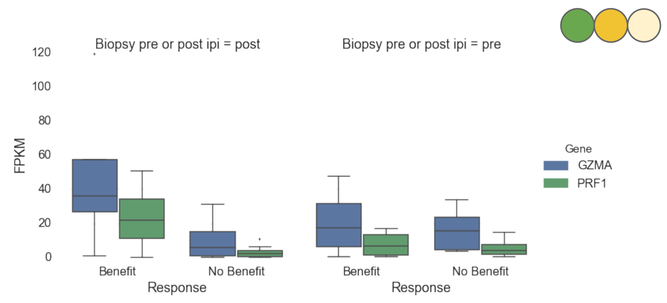
We found HLA expression to be higher in responding samples, including those sampled before treatment.



However, unlike other covariates mentioned, HLA expression in these samples was **not** associated with mutation load.



In samples taken post-treatment, granzyme A and perforin 1 expression was higher in responders.



...Predicted Neoantigen Count?

We computed predicted epitopes based on MHC binding strength for each nonsynonymous SNV and indel.

Figure F: Extended survival in patients with > median predicted neoantigens, with samples taken before treatment

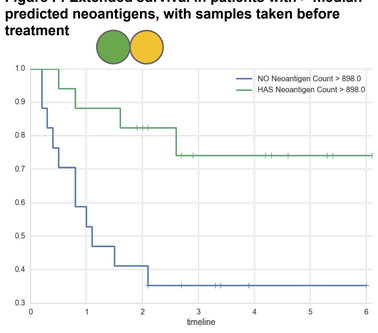
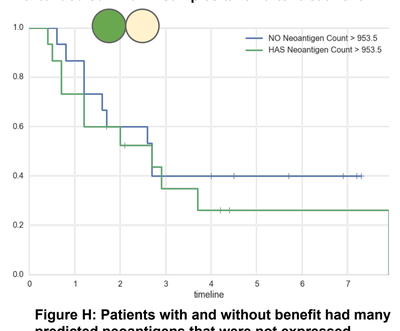


Figure G: Predicted neoantigen count did not lead to extended survival in samples taken after treatment



The number of predicted neoantigens was correlated with increased survival and clinical benefit. This held after we removed antigens that were not expressed in the tumor.

Figure H: Patients with and without benefit had many predicted neoantigens that were not expressed

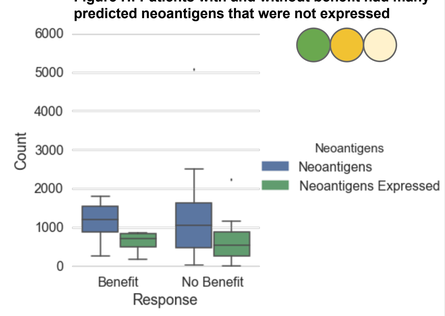


Figure I: Predicted neoantigen burden was strongly correlated with mutational burden

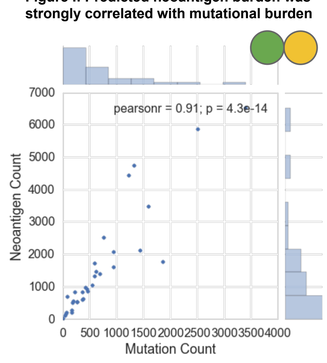
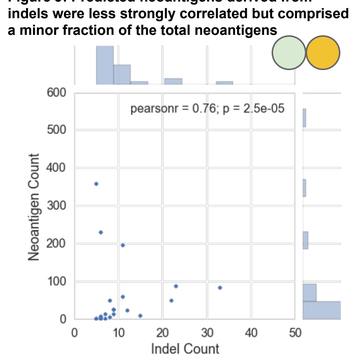


Figure J: Predicted neoantigens derived from indels were less strongly correlated but comprised a minor fraction of the total neoantigens



...Neoantigen Overlap with Pathogens?

There are some differences in the set of pathogens matched by responding samples as compared with non-responding samples. Mutational burden was correlated with the number of pathogens patched.

FIgure K: Predicted neoantigens were aligned to known reactive epitopes in IEDB. Samples, which are before treatment, are ordered by mutation burden. Each cell indicates the number of IEDB entries that a given sample matches.

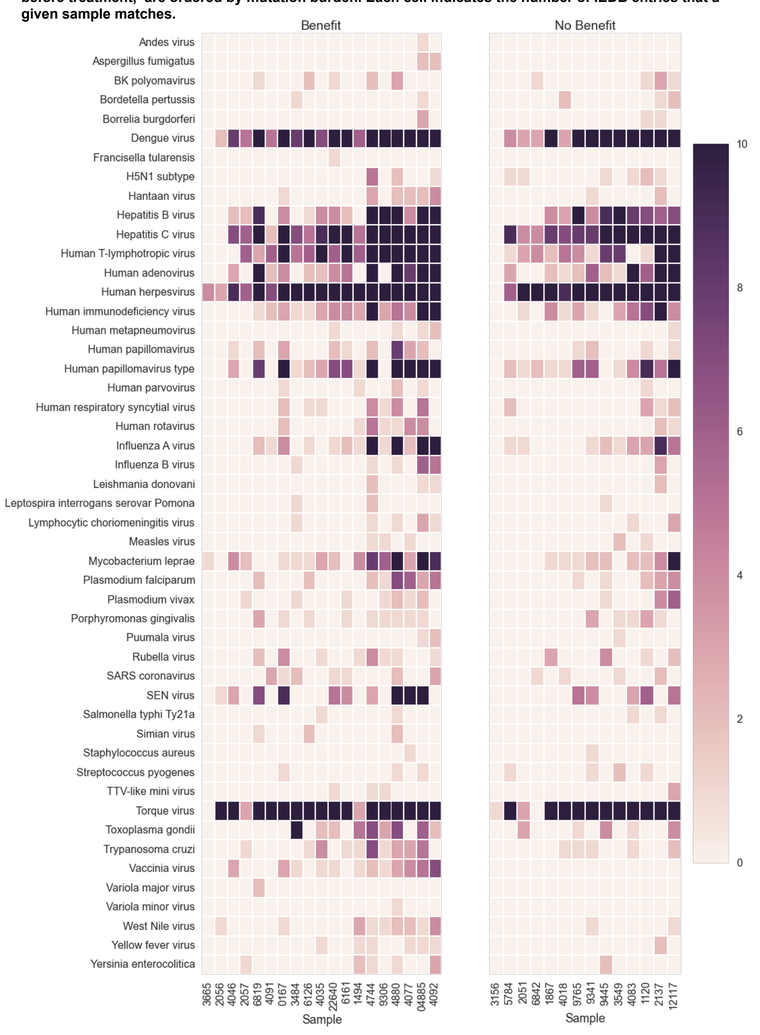


FIgure L: Patients with more mutations matched more unique organisms in IEDB.