Disease status explained by neo-epitopes with low potential for activating regulatory T cells: an application of precision cancer immunotherapy design tools to bladder cancer

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Abstract

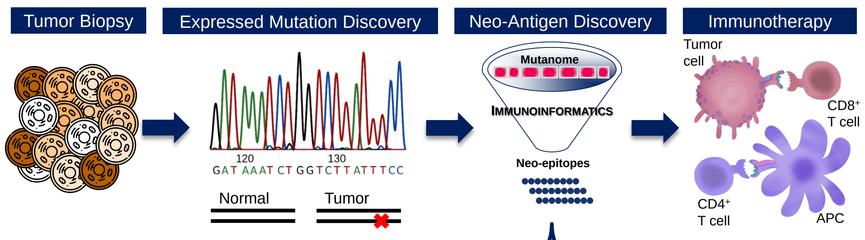
Precision cancer immunotherapy targeting mutations expressed by cancer cells has proven to effectively control the tumor of patients in multiple clinical trials [1,2]. However, the selection of immunogenic T cell neo-epitopes remains challenging and many epitopes selected using traditional methodologies fail to induce effector T cell responses. Poor performance may partially be due to inclusion of mutated epitopes cross-conserved with self-epitopes recognized by regulatory (Treg), anergic, or deleted T cells. Vaccination with self-epitopes can lead to weak effector responses, active immune suppression, and toxicity due to immune-mediated adverse effects. In addition, most cancer vaccine studies focus on the selection of CD8 T cell neo-epitopes due to an apparent lack of robust and accurate CD4 T cell epitope prediction tools.

We have developed Ancer, an integrated and streamlined neo-epitope selection pipeline, that accelerates the selection of both CD4 and CD8 T cell neo-epitopes from NGS data. Ancer leverages EpiMatrix and JanusMatrix, predictive algorithms that have been extensively validated in prospective vaccine studies for infectious diseases [3,4]. Distinctive features of Ancer are its ability to accurately predict Class II HLA ligands, or CD4 epitopes, with EpiMatrix, and to identify tolerated or Treg epitopes with JanusMatrix. In addition, screening candidate sequences with JanusMatrix enables to the removal of neo-epitopes that may trigger off-target events, which have in some cases abruptly halted the development of promising cancer therapies.

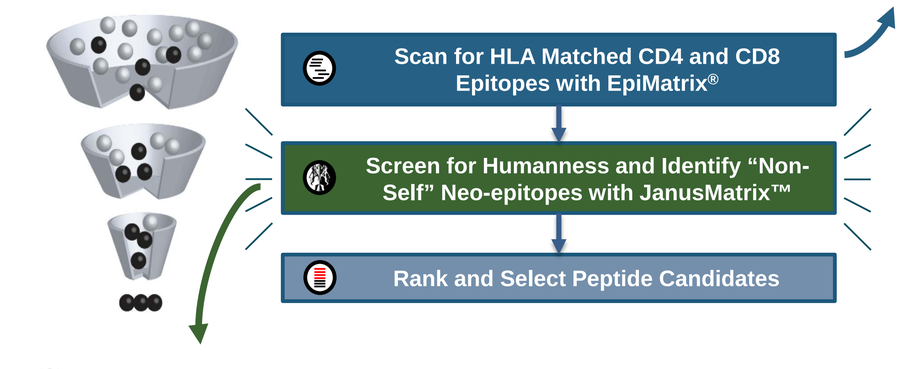
Ancer was applied to NGS data derived from the BLCA bladder cancer cohort from The Cancer Genome Atlas (TCGA) database. On average, 55 out of 204 missense mutations in bladder cancer patients' tumors met Ancer's quality control standards, in an initial analysis carried out for a representative set of 11 patients. This subset of high-quality missense variants was then screened using Ancer settings defined by the unique HLA of each patient, to derive the best vaccine candidate sequences encompassing these mutations. A median number of 24 [interquartile range: 15-64] candidate sequences were generated for each patient under study. The time required to select sequences for all of the patients in this study was less than two days. This initial analysis of eleven BLCA bladder cancer cohort patients demonstrates the capacity of Ancer to define a sufficient number of candidate sequences for vaccinating bladder cancer patients in a precision immunotherapy setting.
We also assessed Ancer's ability to predict patient outcomes on a larger subset of 58 individuals. While the disease-free status of BLCA patients could not be explained by their tumor mutational burden (AUC=0.55, p-value=0.13), nor by their load of missense mutations (AUC=0.54, p-value=0.17), the number of neo-epitopes highly different from self, as defined by Ancer, significantly segregated disease-free patients from patients who recurred or progressed (AUC=0.68, p-value=0.02). These results suggest that defining the number of true neo-epitopes using Ancer may represent a novel biomarker for more robust anti-tumor immune response and higher likelihood of disease-free survival.

Our analysis of the BLCA cohort from the TCGA database showcases the value of Ancer in clinical settings. Ancer can be used to identify high-value candidate sequences for inclusion in personalized therapies while removing potentially tolerated or tolerogenic self-epitopes from consideration. Our next step will be to investigate whether Ancer-defined neo-epitope load will serve as a biomarker for prognosis and response to therapy in the full BLCA cohort.

Mutanome-Directed Cancer Immunotherapy Pipeline



Ancer™ Platform: the "Answer" to Cancer

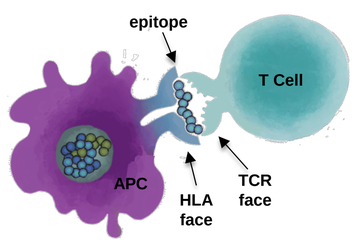


Identification and removal of Treg epitopes using JanusMatrix™

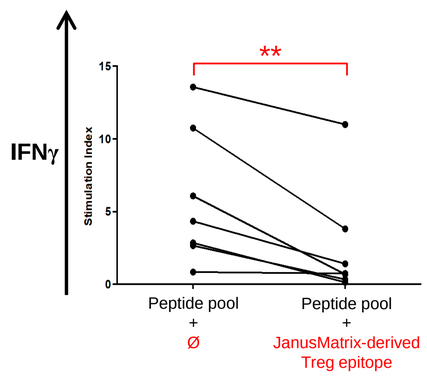
JanusMatrix identifies cross-reactive and regulatory T cell (Treg) epitopes.

Cross-reactive sequences:

- Bind to the same MHC allele.
- Share same/similar T cell-facing residues.



Treg epitopes identified with JanusMatrix reduce IFNγ responses observed for peptide pools [6].

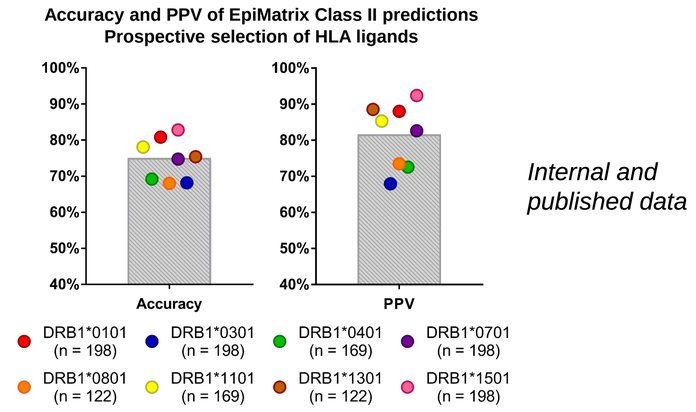


Accurate identification of T cell epitopes using EpiMatrix®

CD4 T cell epitopes

The predictive accuracy of EpiMatrix is routinely evaluated by testing predicted HLA ligands in *in vitro* HLA binding assays.

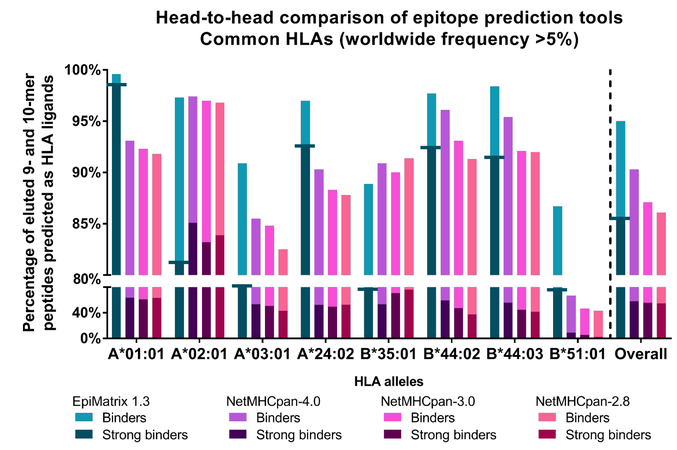
EpiMatrix Class II HLA predictions are 75% accurate when tested in *in vitro* HLA binding assays, with an average observed PPV of 82%.



Internal and published data

CD8 T cell epitopes

Analysis of eluted peptide dataset [5]: **95%** of eluted 9- and 10-mers were predicted to bind to HLA according to EpiMatrix®, while only ~88% of ligands were accurately recalled by NetMHCpan.



Ancer™ Retrospective Study of the TCGA Database

Ancer™ was used to analyze bladder cancer NGS data from the TCGA database and to answer the following questions.

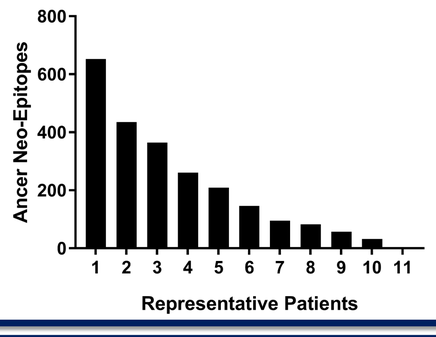
Input data:

1. What is the neo-epitope landscape of BLCA patients?

Representative subset of 11 patients → T cell neo-epitopes → Cross-reactivity analysis

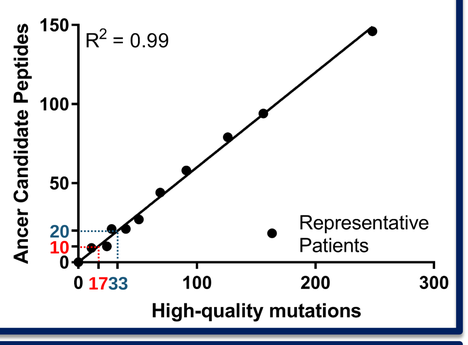
Patients selected throughout the range of high-quality mutations

Removal of tolerated/tolerogenic epitopes



2. How many vaccine candidates can we expect for BLCA patients?

T cell neo-epitope analysis + Cross-reactivity analysis → Design of optimized long peptide sequences



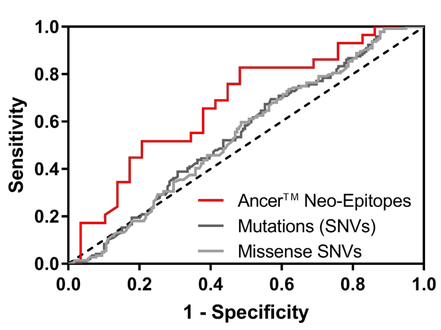
3. Can Ancer™ help explain the disease status of BLCA patients?

Subset of 58 patients → Disease Status → ROC curve analysis

Ongoing analysis

Disease Free vs Recurred/Progressed

Can SNVs, missense SNVs, and Ancer neo-epitope explain patient's disease status?



Mutations (SNVs): **AUC = 0.55**, **p-value = 0.13**

Missense Mutations: **AUC = 0.54**, **p-value = 0.17**

Ancer™ neo-epitopes: **AUC = 0.68**, **p-value = 0.02**

Ancer™ Prospective Studies

Ancer™ is central to the design of two prospective studies using the CT26 and GL261 syngeneic mouse models.

CT26

Colon carcinoma

Input data
- 3,267 variants
- 1,787 missense SNVs
- 378 candidate SNVs after QC and filtering

Analysis
- 135 candidate peptides
- 27 putative Class II Treg epitopes filtered out

Selection
- 20 candidate peptides

Peptide vaccination

Animal studies ongoing

GL261

Glioblastoma multiforme

Input data
- 10,009 variants
- 2,789 missense SNVs
- 421 candidate SNVs after QC and filtering

Analysis
- 161 candidate peptides
- 23 putative Class II Treg epitopes filtered out

Selection
- 20 candidate peptides

Peptide vaccination

Vaccine in preparation

Conclusions

- **Analysis of the MHC- and TCR-facing residues of T cell epitopes by JanusMatrix™ enables prediction of epitope phenotype.**
- **EpiVax's immunogenicity screening tools (EpiMatrix® and JanusMatrix™) are integrated into the Ancer™ platform for streamlined designs of personalized cancer vaccines.**
- **While only HLA Class I alleles were available for TCGA bladder cancer patients, preliminary results reveal that Ancer™-derived neo-epitopes may predict likelihood of disease-free survival. Follow-up analyses are planned where both HLA Class I and Class II will be considered.**
- **Ancer™-derived vaccines are currently being evaluated in prospective studies using the CT26 and GL261 syngeneic mouse models.**

Acknowledgments

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The results shown here are in part based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/.

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For questions regarding in silico antigen screening and vaccine design, please contact: Katie Porter at 401-272-2123, ext. 115; or at info@epivax.com www.epivax.com