From infectious disease to personalized cancer vaccines: Regulatory T cell epitopes determine patient outcomes and suppress neoantigen responses

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Abstract

Precision cancer immunotherapy has proven to effectively control the tumor of patients in multiple clinical trials. However, the selection of immunogenic T cell neo-epitopes using traditional methodologies remains a challenging exercise. Poor vaccine performance may partially be due to inclusion of mutated epitopes cross-conserved with self-epitopes recognized by regulatory (Treg), anergic, or deleted T cells. In addition, most cancer vaccine studies focus on the selection of CD8 T cell neo-epitopes while overlooking CD4 T cell neo-epitopes.

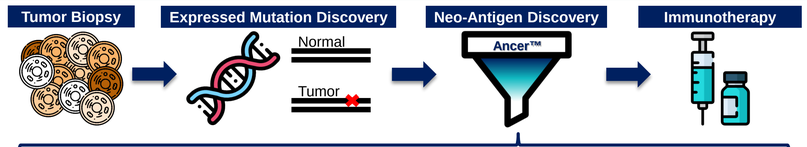
We have developed Ancer, an integrated and streamlined neo-epitope selection pipeline, that accelerates the selection of both CD4 and CD8 T cell neo-epitopes. Ancer leverages EpiMatrix and JanusMatrix, predictive algorithms that have been extensively validated in prospective vaccine studies for infectious diseases [1]. Distinctive features of Ancer are its ability to accurately predict Class II HLA ligands and to identify tolerated or Treg epitopes.

Ancer was evaluated on data from the BLCA bladder cancer cohort hosted at The Cancer Genome Atlas (TCGA) database. An initial analysis was carried out on a representative set of eleven patients to derive the best vaccine candidate sequences from high-quality missense mutations. A median number of 24 [interquartile range: 15-64] candidate sequences were generated for each patient under study. This initial analysis demonstrates the capacity of Ancer to define a sufficient number of candidate sequences for vaccinating bladder cancer patients in a precision immunotherapy setting.

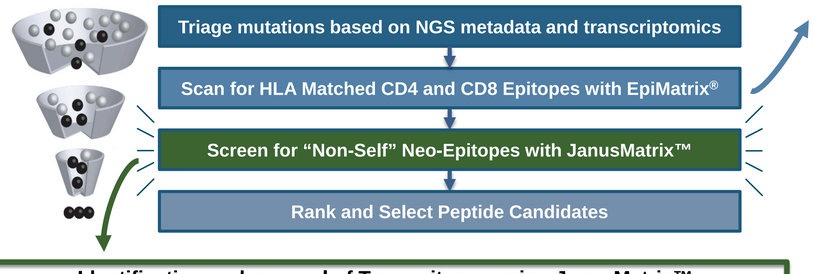
We also assessed Ancer's ability to explain patients' disease free survival (DFS) times. While BLCA patients with DFS greater and lower than 3 months could not be distinguished by their tumor mutational burden (p=0.19), nor by their load of missense mutations (p=0.15), the number of mutations identified as highly distinct from self, based on Ancer, significantly segregated this two patient populations (p=0.02). Similar results were obtained when studying BLCA patients with DFS greater and lower than 6 months. These observations suggest that defining the number of true non-self mutations using Ancer may represent a novel biomarker for more robust anti-tumor immune response and higher likelihood of disease-free survival.

Our preliminary analysis of the BLCA cohort from the TCGA database showcases the value of Ancer in clinical settings. 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 Based on 20 Years of Experience in Epitope Mapping

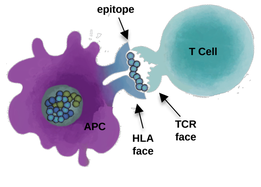


Ancer™ Platform: the "Answer" to Cancer

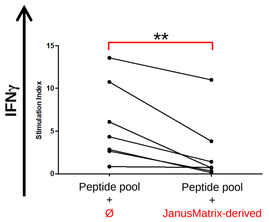


Identification and removal of Treg epitopes using JanusMatrix™

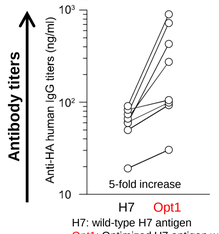
JanusMatrix™ identifies cross-reactive and regulatory T cell (Treg) epitopes.



Treg epitopes can reduce IFNγ ELISpot responses observed for peptide pools [3].



Antigens optimized with JanusMatrix are more immunogenic [4].



H7: wild-type H7 antigen
Opt1: Optimized H7 antigen w/o JanusMatrix-derived Treg epitope

Accurate and identification of CD4 and CD8 epitopes using EpiMatrix®

CD4 T cell epitopes

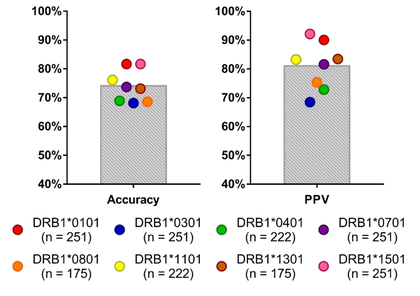
The predictive accuracy of EpiMatrix® is routinely evaluated by testing predicted HLA ligands in *in vitro* HLA binding assays.

EpiMatrix Class II HLA predictions are 74% accurate when tested in *in vitro* HLA binding assays, with an average observed PPV of 81%.

EpiMatrix® CD4 predictions and its associated tools are routinely used and trusted by 9 of the top 10 pharmaceutical companies, including:

Celgene, CHUGAI, Pfizer, Boehringer Ingelheim, MERCK

Accuracy and PPV of EpiMatrix Class II predictions
Prospective selection of HLA ligands

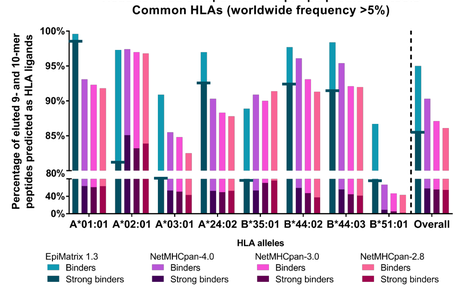


CD8 T cell epitopes

Analysis of eluted peptide dataset [2]: **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.

The majority of eluted peptides (85% of the dataset) are strong EpiMatrix® binders, while less than 56% of all peptides are high affinity binders based on NetMHCpan predictions.

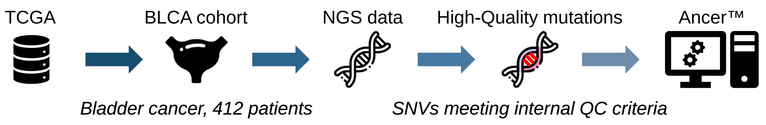
Head-to-head comparison of epitope prediction tools
Common HLAs (worldwide frequency >5%)



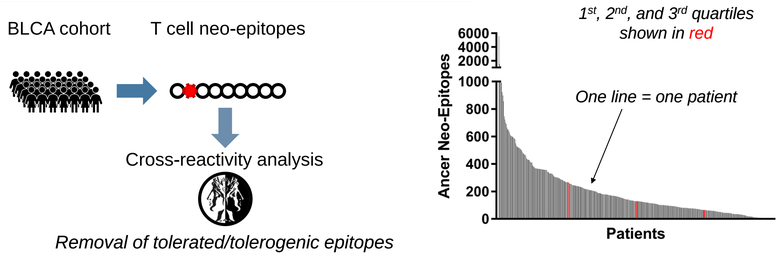
Ancer™ Retrospective Study of the TCGA Database: Bladder Cancer (BLCA) cohort

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

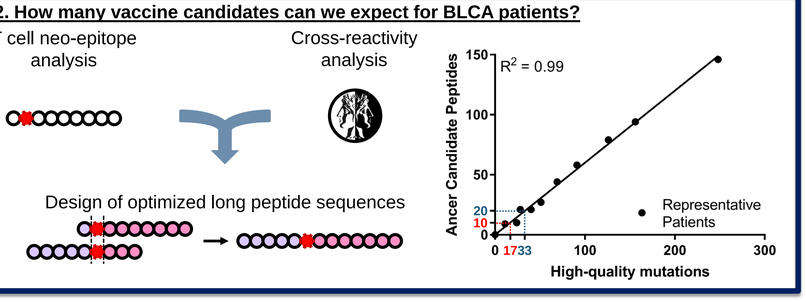
Input data:



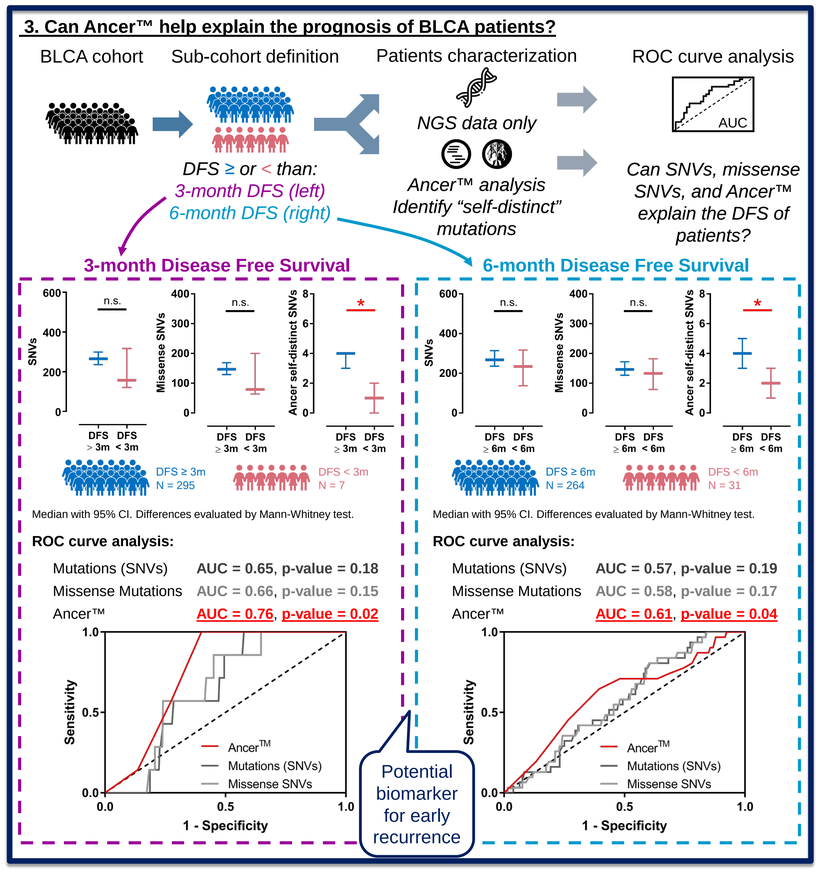
1. What is the neo-epitope landscape of BLCA patients?



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

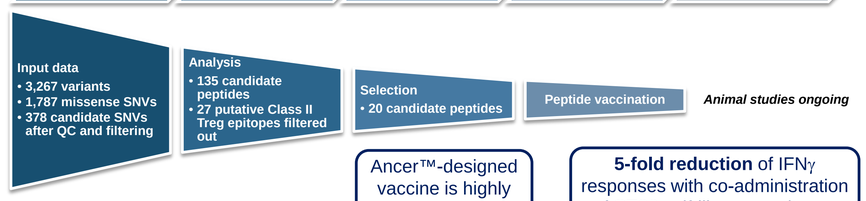


3. Can Ancer™ help explain the prognosis of BLCA patients?



Ancer™ Prospective CT26 Studies

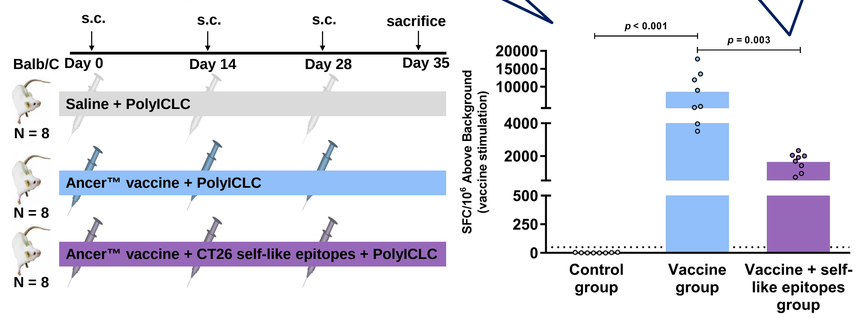
Ancer™ is central to the design of prospective studies using the CT26 syngeneic mouse models.



Ancer™-designed vaccine is highly immunogenic

5-fold reduction of IFNγ responses with co-administration of CT26 self-like neo-epitopes

Preliminary immunogenicity results:



Splenocytes were collected at day 35 and restimulated in IFNγ ELISpot assay with the 20 vaccine peptides.

Strong IFNγ responses are observed in the vaccinated group after restimulation compared to the control group. Co-administration of Ancer™-derived CT26 self-like neo-epitopes identified with JanusMatrix™ significantly reduce IFNγ responses by 5-fold.

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, results reveal that Ancer™ may predict likelihood of disease-free survival. Follow-up analyses are planned where HLA Class II will be considered.**
- **Ancer™-derived vaccines are highly immunogenic in a CT26 mouse model. Our results showcase that inclusion of Treg neo-epitopes in cancer vaccines can downregulate immune responses.**

References and Acknowledgments

1) Moise L. et al., Hum Vaccin Immunother. 2015;11(9):2312-21.
2) Abelin J. et al., Immunity. 2017 46, 315–326
3) Wada Y. et al., Sci Rep. 2017 Apr 28;7(1):1283
4) Liu R. et al., Hum Vaccin Immunother. 2015 11:9, 2241-2252

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

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