Application of precision cancer immunotherapy design tools to bladder cancer: Non-self-like neo-epitopes as a prognostic biomarker

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Abstract

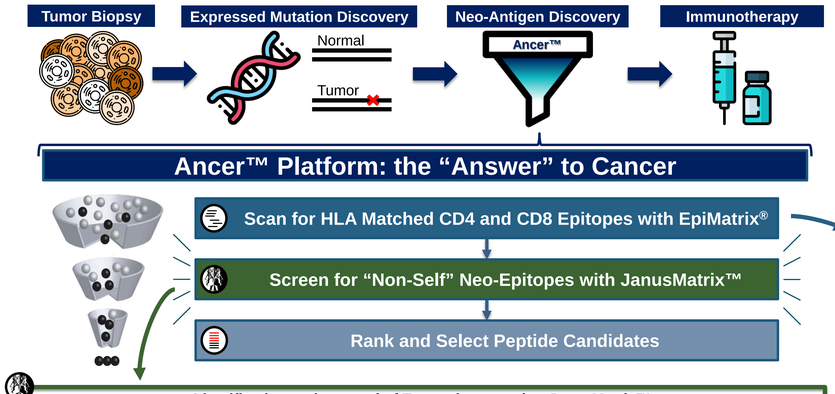
Precision cancer immunotherapy targeting mutations expressed by cancer cells has proven to effectively control the tumor of patients in multiple clinical trials [1,2]. However, the selection of immunogenic T cell neo-epitopes remains challenging and many epitopes selected using traditional methodologies 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, 88 out of 202 missense mutations in bladder cancer patients' tumors met Ancer's quality control standards. 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 a representative set of eleven patients. 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 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 (AUC = 0.65, p = 0.18), nor by their load of missense mutations (AUC = 0.66, p = 0.15), the number mutations identified as highly distinct from self, based on Ancer, significantly segregated this two patient populations (AUC = 0.76, 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 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 Class II neo-epitope load will serve as a biomarker for prognosis and response to therapy in the BLCA cohort.

Mutanome-Directed Cancer Immunotherapy Based on 20 Years of Experience in Epitope Mapping



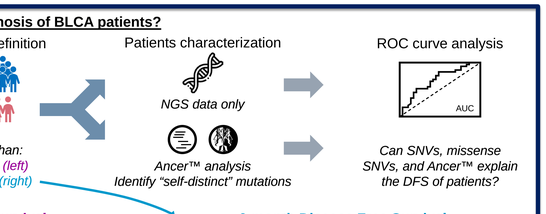
Identification and removal of Treg epitopes using JanusMatrix™

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 (JMX) reduce IFNγ responses observed for peptide pools [6].



Accurate and identification of CD4 and CD8 epitopes using EpiMatrix®

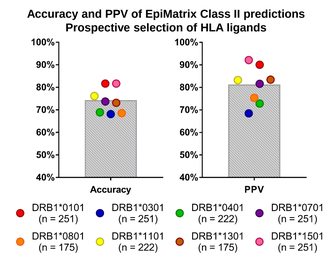
CD4 T cell epitopes

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:

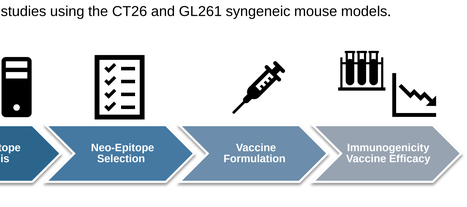
Celgene, CHUGAI, Pfizer, Boehringer Ingelheim, MERCK



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.

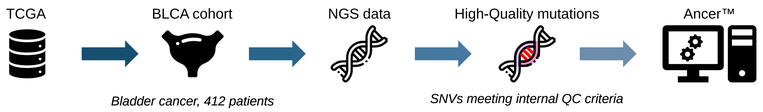
The majority of eluted peptides (85% of the dataset) are strong EpiMatrix® binders, while less than 56% of all peptides are high affinity binders based on NetMHCpan predictions.



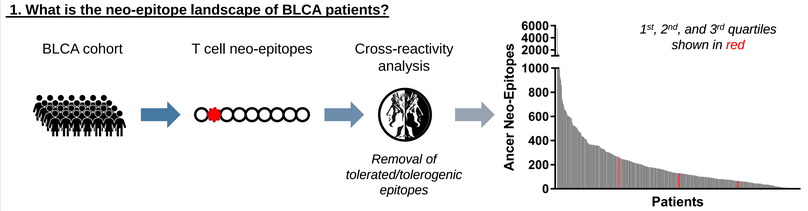
Ancer™ Retrospective Study of the TCGA Database: Bladder Cancer (BLCA) cohort

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

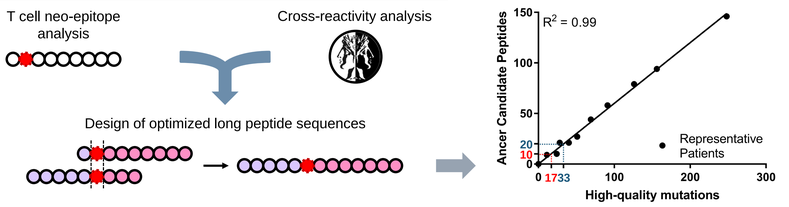
Input data:



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?

Median with 95% CI. Differences evaluated by Mann-Whitney test.

Median with 95% CI. Differences evaluated by Mann-Whitney test.

Ancer™ Prospective Studies

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

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™ may predict likelihood of disease-free survival. Follow-up analyses are planned where HLA Class II will be considered.**
- **Ancer™-derived vaccines are currently being evaluated in prospective studies using the CT26 and GL261 syngeneic mouse models.**

References

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5) Abelin J. et al., Mass Spectrometry Profiling of HLA-Associated Peptidomes in Mono-allelic Cells Enables More Accurate Epitope Prediction Resource Mass Spectrometry Profiling of HLA-Associated Peptidomes in Mono-allelic Cells Enables More Accurate Epitope Prediction. Immunity. 2017 46, 315–326
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Acknowledgments

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