Designing Precision Cancer Immunotherapies with Streamlined and Accurate CD4 and CD8 T cell Neo-epitopes Analysis Tools

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Abstract

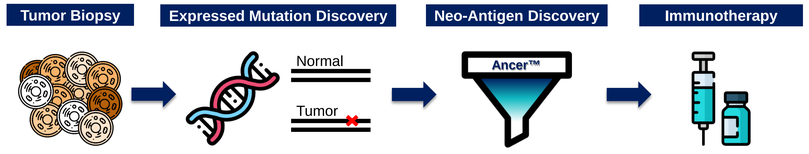
Precision cancer immunotherapy targeting mutations expressed by cancer cells have proven to effectively control the tumor of patients in multiple clinical trials [1,2]. However, the selection of immunogenic T cell neo-epitopes remains challenging and many epitopes selected using traditional methodologies 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 advanced cancer T cell epitope identification and characterization tool, that streamlines the selection of both CD4 and CD8 T cell neo-epitopes from Next Generation Sequencing data. Ancer leverages EpiMatrix and JanusMatrix, state-of-the-art 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.

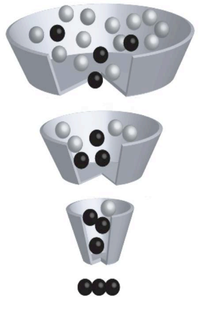
The performance of Ancer was assessed by retrospectively analyzing multiple published datasets. First, we evaluated HLA-Class-I-bound peptides detected by mass spectrometry and published by Abelin et al., Immunity 2017 [5]. Our analysis showed that 95% of eluted peptides were correctly predicted by EpiMatrix to bind to their respective HLA, while only 88% of these sequences were accurately predicted by NetMHCpan. In addition, the majority of eluted peptides were specifically identified by EpiMatrix as strong HLA ligands.
Second, we performed a retrospective analysis of a cancer immunogenicity study published by Strønen et al., Science 2016 where HLA A2-restricted neo-epitopes were validated in T cell assays [6]. Immunogenic sequences had significantly higher binding potentials, as estimated by EpiMatrix, compared to non-immunogenic sequences (p=0.0020). In contrast, no significant differences in predicted binding affinities were observed between these two subsets of sequences using public in silico prediction tools (p=0.0561).
Lastly, Ancer divided immunogenic and non-immunogenic sequences from Strønen et al. with 72% accuracy outperforming the results obtained with public tools (21% accuracy) or with lengthy and costly in vitro characterization techniques (65% accuracy).

These results suggest that higher quality candidate targets are retrieved by Ancer, as compared to other conventional algorithms. CD4 and CD8 neo-epitopes with low Treg activation potential may then be used to support the development of safer and more effective personalized cancer vaccines. Future steps include the design of prospective studies to test the efficacy of Ancer-derived vaccines in the CT26 and GL261 murine cancer models.

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



Ancer™ Platform: the "Answer" to Cancer

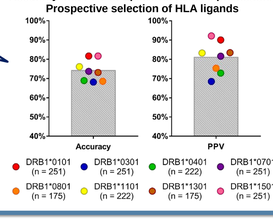


- Triage mutations based on NGS metadata and transcriptomics
- Scan for HLA Matched Epitopes (Patient Class I + II) with EpiMatrix®
- Screen for Humanness and Identify "Non-Self" Neo-epitopes with JanusMatrix™
- Rank and Select Peptide Candidates

Accurate and trusted identification of Class I and Class II epitopes using EpiMatrix®

HLA Class II predictions? Yes we can!

EpiMatrix **Class II HLA** predictions are **74% accurate** when tested in *in vitro* HLA binding assays, with an average observed **PPV of 81%.**
See the Abelin retrospective analysis below for EpiMatrix Class I predictions.

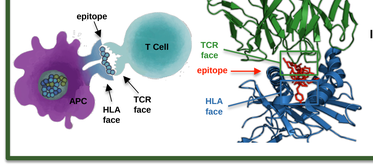


EpiMatrix® and its associated tools are routinely used and trusted by 9 of the top 10 pharmaceutical companies, including:

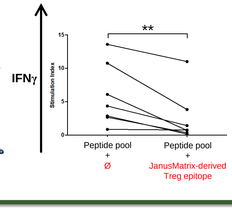
Celgene · CHUGAI · Pfizer · Boehringer Ingelheim · MERCK

Identification and removal of Treg epitopes using JanusMatrix

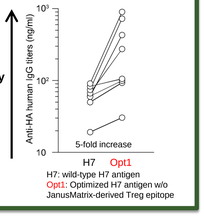
JanusMatrix performs cross-reactivity analyses and can identify regulatory T cell (Treg) epitopes.
Cross-reactive sequences:
- Are predicted to bind to the same MHC allele.
- Share same/similar T cell-facing residues.



Treg epitopes identified with JanusMatrix reduce IFNγ responses observed for peptide pools [7].



Antibody titers are boosted when antigens are optimized with JanusMatrix [4].



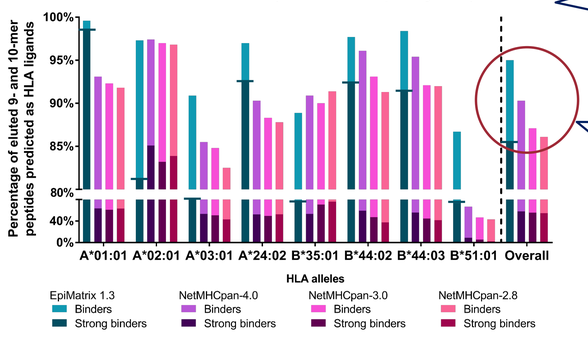
H7: wild-type H7 antigen
Opt1: Optimized H7 antigen w/o JanusMatrix-derived Treg epitope

Retrospective Immunoinformatic Analyses of Published Cancer Datasets

EpiMatrix® analysis of elution datasets from Abelin *et al.* Immunity 2017

- Summary of the Abelin *et al.* study [5]: over 26,000 peptides were eluted across 16 HLA-A or HLA-B monoallelic cell lines.
- 6,284 9-mer and 2,301 10-mer HLA ligands from the Abelin *et al.* dataset were extracted for eight common HLAs (worldwide frequency over 5%) and scored with **EpiMatrix®** and NetMHCpan [8-10].
- **95%** of eluted 9- and 10-mers were predicted to bind to HLA according to **EpiMatrix®** (standard Z-score cutoff of 1.64), while only ~88% of ligands were accurately recalled by NetMHCpan (500nM cutoff).

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



High accuracy of EpiMatrix Class I predictions compared to publicly available prediction tools.

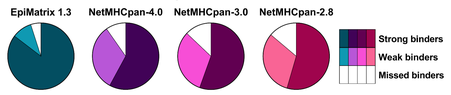
Motifs not captured by NetMHCpan, but already accounted by EpiMatrix after its latest 2015 update?

More candidates are retrieved with EpiMatrix

- The majority of eluted peptides (**85%** of the dataset) are strong **EpiMatrix®** binders (stringent Z-score cutoff of 2.32), while less than 56% of all peptides are high affinity binders (50nM cutoff) based on NetMHCpan predictions.

Predicted binding of 8,585 eluted 9- and 10-mer sequences across 8 HLAs

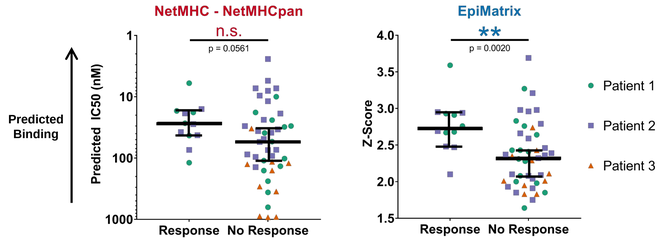
Strong binders are preferred for vaccine formulation. More potential targets are retrieved by **EpiMatrix® which is of importance for patients with low mutational burden**.



Ancer™ Analysis of Mutated Peptides from Strønen *et al.* Science 2016

- Summary of the Strønen *et al.* study [6]: neo-epitopes were identified with NetMHC and NetMHCpan but **few peptides (21%) were immunogenic**. *In vitro* peptide-MHC off-rates separate immunogenic from non-immunogenic peptides with 65% accuracy.
- **Immunogenic peptides have greater binding potentials** than non-immunogenic peptides, **as measured by EpiMatrix®**. No significant difference is observed with public *in silico* tools.

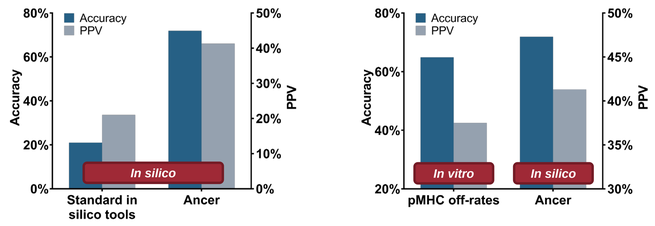
Predicted binding affinities of mutated peptides



Prediction of neo-epitope response with Ancer™ is improved over *in vitro* methods

- **Ancer™**, which uses EpiMatrix® **and JanusMatrix™,** can **differentiate immunogenic and non-immunogenic peptides with 72% accuracy**. PPV of **Ancer™** is twice that of standard *in silico* tools.

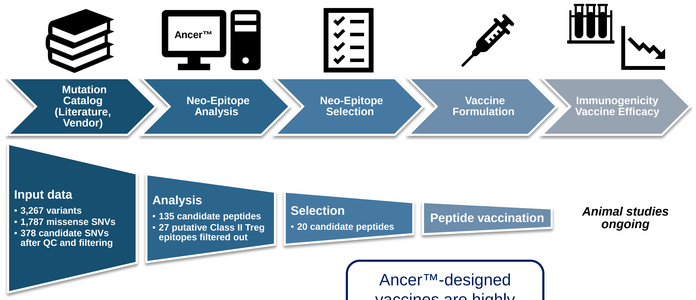
Accuracy and positive predictive value (PPV) of neo-epitope predictors



Ancer™ Prospective Studies

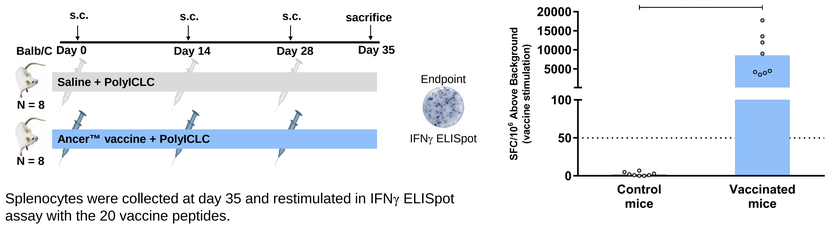
Ancer™ is central to the design of a new prospective murine studies using the CT26 syngeneic mouse model.

CT26
Colon carcinoma



Ancer™-designed vaccines are highly immunogenic

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. Analyses are ongoing to determine the level of $CD8^+$ and $CD4^+$ specific T cell responses.

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.**
- **Sharper definition of neo-epitopes by immunoinformatic analyses may improve epitope selection for mutanome-directed cancer immunotherapy.**
- **Ancer™-derived vaccines are currently being evaluated in prospective studies using the CT26 syngeneic mouse model.**

References

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Acknowledgments

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