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Integrated approaches for design of precision cancer immunotherapies: Selection of Class I and Class II T cell neo-epitopes and removal of Treg epitopes

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Abstract

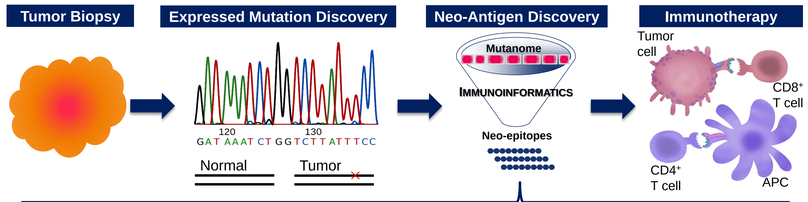
Next-generation sequencing has opened the door to precision cancer therapies targeting mutations expressed by tumor cells. However, most neo-epitopes selected by traditional T cell epitope prediction algorithms prove to be non-immunogenic. Poor predictive performance may partially be due to inclusion of mutated epitopes cross-conserved with self-epitopes recognized by the T cell receptor of 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.

We have developed Ancer, an advanced cancer T cell epitope identification and characterization tool, that streamlines the selection of Class I and Class II T cell neo-epitopes. Ancer leverages EpiMatrix and JanusMatrix, state-of-the-art predictive algorithms that have been extensively validated in prospective vaccine studies for infectious diseases [1-4]. Distinctive features of Ancer are its ability to accurately predict Class II HLA ligands with EpiMatrix and its 82% positive predictive value, as estimated in previous prospective studies. Additionally, the application of JanusMatrix allows for the prioritization of neo-epitopes with reduced potential for Treg induction, that is responsible for diminished efficacy of current cancer therapies.

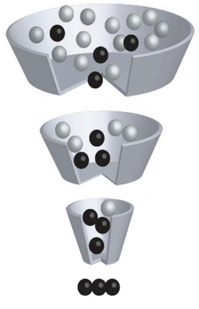
We validated Ancer's predictive accuracy using datasets of HLA-bound peptides detected by mass spectrometry, which are independent of training sequence data used in model development. Analysis of sequences from Abelin *et al.* [5] shows a 95% agreement between Ancer predictions and peptides eluted from common Class I HLAs, while only 88% of these sequences are accurately predicted by NetMHCpan. An additional retrospective analysis of a cancer immunogenicity study [6] demonstrates that Ancer selects immunogenic neo-epitopes with 72% accuracy, as compared to 21% accuracy when using public prediction tools.

These results demonstrate that Ancer may focus epitope candidate selection on higher value sequences than conventional algorithms. Class I and Class II neo-epitopes with low Treg activation potential may then be used to support the development of safer and more effective vaccines.

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



Ancer™ Platform: the "Answer" to Cancer



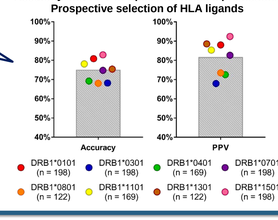
- Scan for HLA Matched Epitopes (Patient Class I + II) with EpiMatrix®
- Screen for Humanness and Identify "Non-Self" Neo-epitopes with JanusMatrix™
- Rank and Select Peptide Candidates
- Design: Commercial-grade Advanced Vaccine Design with VaxCAD™

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

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:

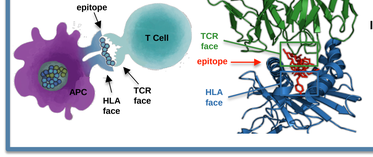
Celgene · CHUGAI · Pfizer · Boehringer Ingelheim · MERCK

Identification and removal of Treg epitopes using JanusMatrix

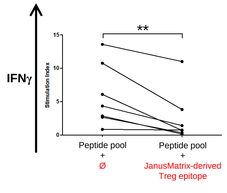
JanusMatrix performs cross-reactivity analyses and can identify regulatory T cell (Treg) epitopes.

Cross-reactive sequences:

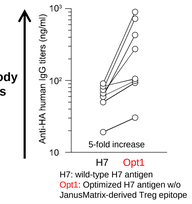
- Are predicted to bind to the same MHC allele.
- Share same/similar T cell-facing residues.



Treg epitopes identified with JanusMatrix reduce IFNγ responses observed for peptide pools [3].



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



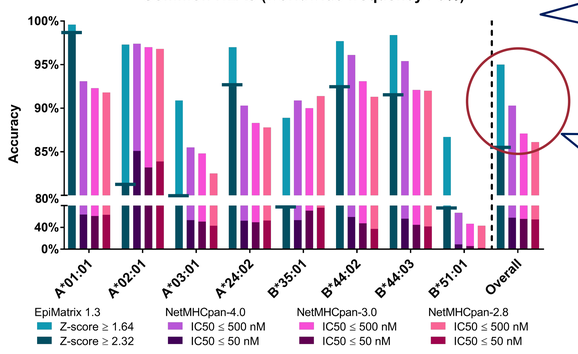
H7: wild-type H7 antigen
Opt1: Optimized H7 antigen w/o JanusMatrix-derived Treg epitope

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 [7-9].
- **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 (9- and 10-mers)
Common HLAs (worldwide frequency >5%)



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

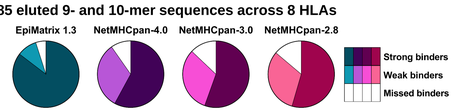
Motifs not captured by NetMHCpan, but already accounted by EpiMatrix after its latest 2015 update?

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

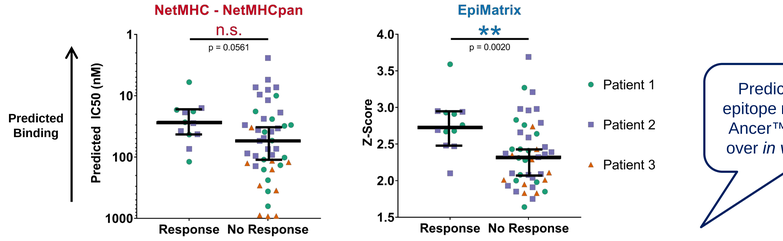
Strong binders are preferred for vaccine formulation. More potential targets are retrieved by **EpiMatrix® which is of importance for patients with low mutational burden**.



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.

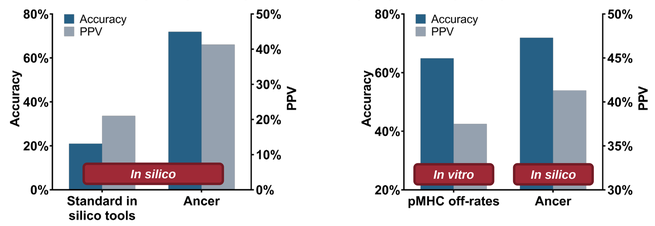
Predicted binding affinities of mutated peptides



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

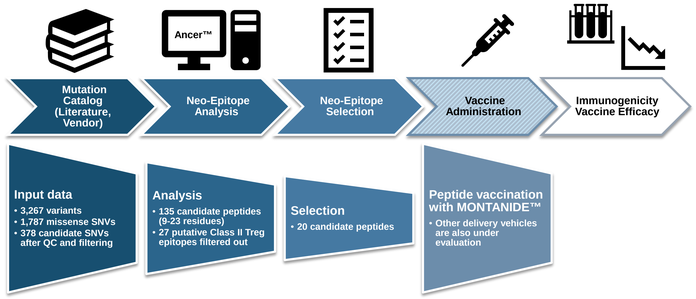


New prospective studies involving Ancer™

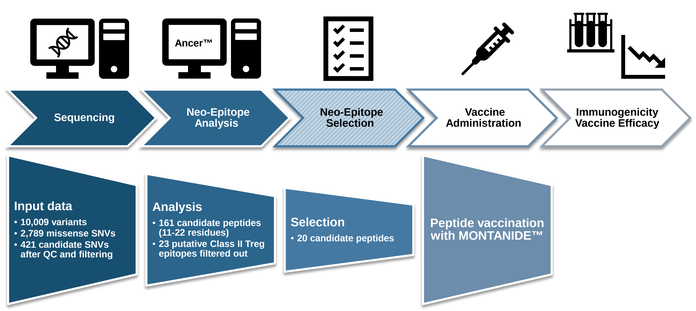
Ancer™ Prospective Studies

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

CT26
Colon carcinoma



GL261
Glioblastoma multiforme



Conclusions

- **Analysis of the MHC- and TCR-facing residues of T cell epitopes by JanusMatrix™ enables prediction of epitope phenotype.**
- **Epitopes that share a TCR-face with numerous human sequences may activate regulatory T cells, as seen for pathogen-derived epitopes.**
- **Sharper definition of neo-epitopes by immunoinformatic analyses may improve epitope selection for mutanome-directed cancer immunotherapy.**
- **EpiVax's immunogenicity screening tools (EpiMatrix® and JanusMatrix™) are integrated into the Ancer™ platform for streamlined designs of personalized cancer vaccines.**

References

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Acknowledgments

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