Ancer-designed CT26 RNA replicon neo-epitope vaccine induces multi-functional CD4 and CD8 T cell responses

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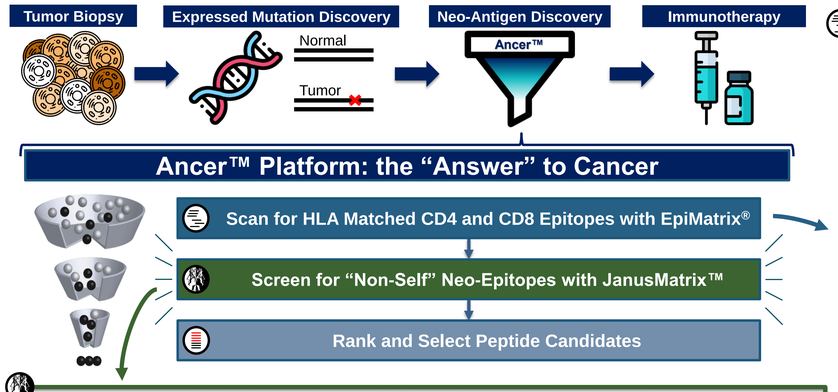
Abstract

Clinical studies showing T cell control of a wide range of malignancies firmly establish the significance of immunotherapeutic approaches that harness T cells to destroy cancer cells. Vaccination is a promising approach that directly activates and expands endogenous T cells capable of recognizing peptide epitopes presented by major histocompatibility complex (MHC) molecules on the surface of cancer cells. The availability of sequencing and informatics tools has made it possible to rapidly identify cancer-specific epitopes composed of mutated sequences that are not found in the patient's normal genome. These sequences, also known as neo-epitopes, are capable of reacting with T cells unaffected by central tolerance to mediate anti-tumor activity.

We developed a cancer T cell neo-epitope identification and characterization tool named Ancer that streamlines the selection of MHC Class I (CD8) and Class II (CD4) T cell neo-epitopes for personalized cancer vaccine design. Ancer leverages EpiMatrix and JanusMatrix, state-of-the-art predictive algorithms that have been extensively validated in prospective vaccine studies for infectious diseases [1,2]. Distinctive features of Ancer over other pipelines are its ability to accurately predict Class II human MHC ligands with EpiMatrix and its 82% positive predictive value, as estimated in previous prospective studies. The application of JanusMatrix to candidate selection allows for the prioritization of neo-epitopes with reduced potential for Treg induction that is responsible for diminished efficacy of current cancer therapies.

From two publicly available mutanomes of the murine colorectal cancer CT26 cell line, we identified Balb/C mouse vaccine antigen candidates using Ancer and ranked them according to tumor expression level, presence of Class I and II neo-epitopes, presence of high-affinity Class I neo-epitopes, and Class I and II neo-epitope count. Genes composed of vaccine candidates assembled head-to-tail were produced and inserted into a self-amplifying, propagation defective alphavirus RNA replicon. Replicons were constructed to evaluate the effects of various epitope concatemer design parameters on immune responses, including junctional immunogenicity, number of epitopes, and MHC I or MHC II targeting motifs. RNA replicons were formulated for lipid-mediated delivery in Balb/C mouse immunizations. A high number of multi-functional CD4 and CD8 T cell responses was generated after one dose with RNA replicons encoding neoantigen cassettes, illustrating the utility of the SGI and Ancer platforms for personalized oncology treatment.

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



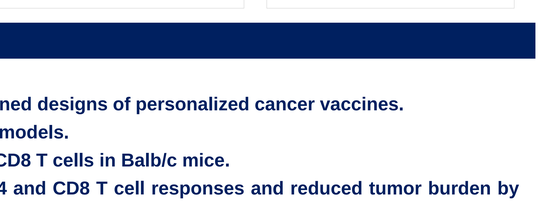
Identification and removal of Treg epitopes using JanusMatrix™

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 [3].



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%.

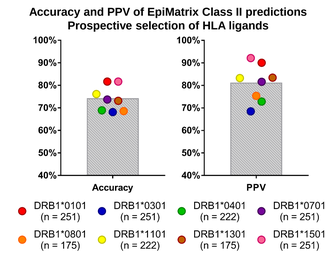
EpiMatrix® CD4 predictions and its associated tools are routinely used and trusted by 9 of the top 10 pharmaceutical companies, including:

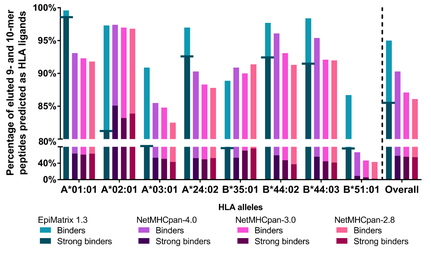
Celgene, CHUGAI, Pfizer, Boehringer Ingelheim, MERCK

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.

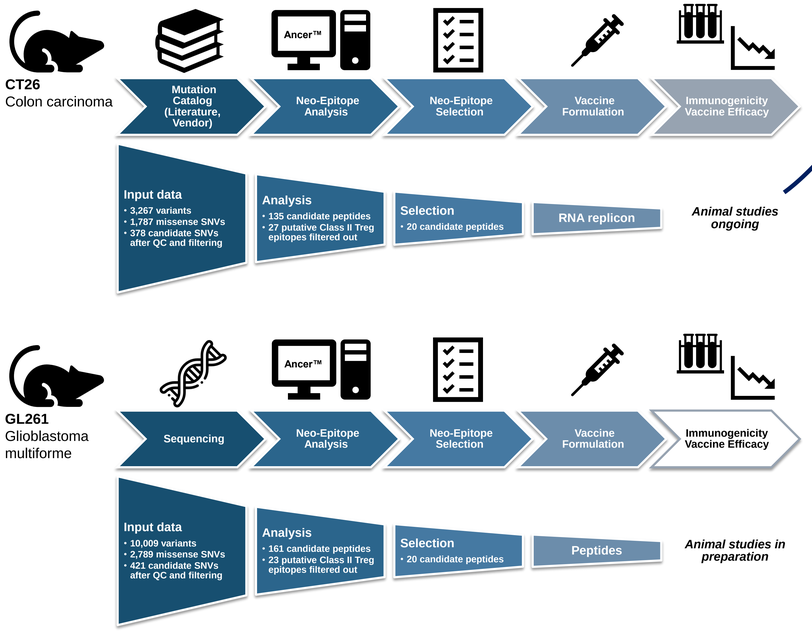
The majority of eluted peptides (85% of the dataset) are strong EpiMatrix® binders, while less than 56% of all peptides are high affinity binders based on NetMHCpan predictions.



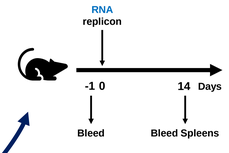


Ancer™ Prospective Studies

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

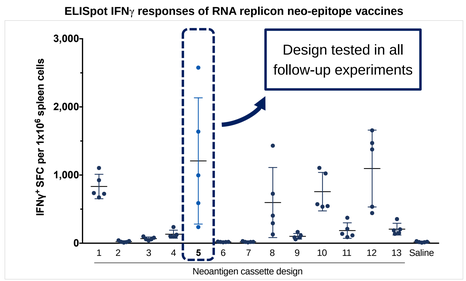


Immunogenicity Studies



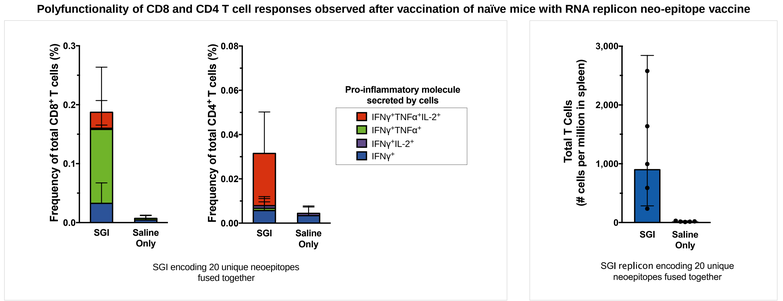
The immunogenicity of multiple vaccine designs was tested in ELISpot assays. Ancer™ designs varied by:

- Number of encoded neo-epitopes.
- Neo-epitope ordering.
- Spacers.
- MHC I/MHC II trafficking sequence.

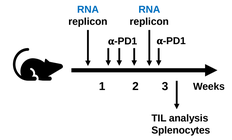


The best design (Design 5) was used to evaluate the polyfunctionality of the observed immune response (bottom). This design was also employed in follow-up efficacy studies (right panel).

Polyfunctionality of CD8 and CD4 T cell responses observed after vaccination of naïve mice with RNA replicon neo-epitope vaccine



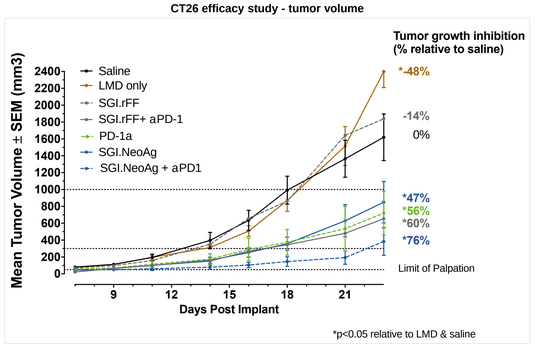
Efficacy Studies



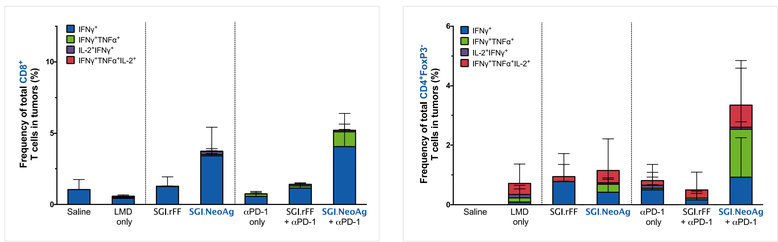
The efficacy of an RNA replicon vaccine encoding Ancer™-selected neo-epitopes was evaluated in CT26 tumor-bearing mice.

The efficacy of the vaccine was evaluated in conjunction with an anti-PD-1 antibody.

Polyfunctionality of the T cell response was evaluated in TILs.



Polyfunctionality of CD8 and CD4 T cell responses observed after vaccination of CT26-bearing mice with RNA replicon neo-epitope vaccine



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.**
- **Ancer™-designed vaccines are currently being evaluated in prospective studies using the CT26 and GL261 syngeneic mouse models.**
- **A single dose of RNA replicon encoding Ancer™-derived CT26 neo-epitopes generated high levels of polyfunctional CD4 and CD8 T cells in Balb/c mice.**
- **Vaccination of CT26-bearing mice with an RNA replicon encoding an Ancer™-designed vaccine produced polyfunctional CD4 and CD8 T cell responses and reduced tumor burden by 47% and by 76% when combined with an anti-PD-1 antibody.**

References and Acknowledgments

1) Moise L. et al., iVAX: An integrated toolkit for the selection and optimization of antigens and the design of epitope-driven vaccines. Hum Vaccin Immunother. 2015;11(9):2312-21.

2) Wada Y. et al., A humanized mouse model identifies key amino acids for low immunogenicity of H7N9 vaccines. Sci Rep. 2017 Apr 28;7(1):1283

3) Liu R. et al., H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance, Human Vaccines & Immunotherapeutics. 2015 11:9, 2241-2252

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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