Highly efficient selection of tumor neoantigens improves therapeutic cancer vaccine efficacy

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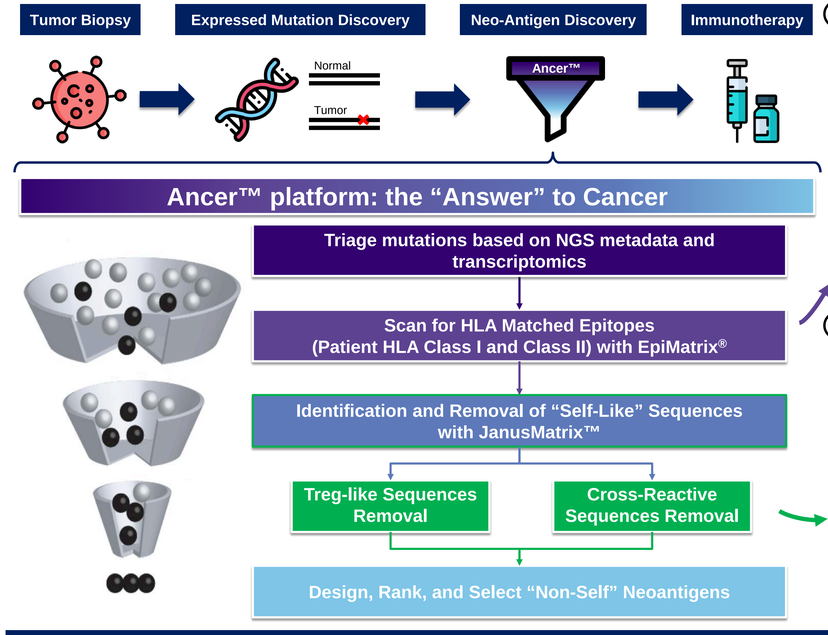
Overview

- **Hypothesis**: Accurately identifying **effector (Teff)** and excluding **regulatory (Treg) neo-epitopes** will help design **more effective personalized cancer vaccines**.
- **Approach**: A CT26 neoantigen-based vaccine was designed with **Ancer™, an advanced neo-epitope screening platform** that combines proprietary machine learning-based **CD8 and CD4** epitope mapping tools with removal of **inhibitory Treg** epitopes.
- **Results**: **63% of neoantigens selected with Ancer™ were immunogenic.**
- Our CT26 vaccine induced **multifunctional CD8+ and CD4+ T cell responses**.
- Our CT26 vaccine (monotherapy) **reduced tumor burden by 45% and 38% compared to vehicle** (poly-ICLC) at days 21 and 25, respectively.
- **Summary**: Ancer™ selects highly immunogenic neoantigens that substantially reduce tumor burden when delivered as monotherapy.

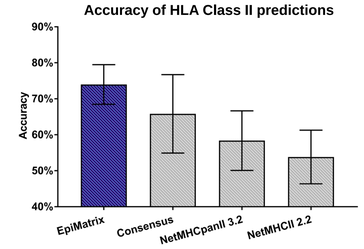
Mutanome-Directed Cancer Immunotherapy Based on 20 Years of Experience in Epitope Mapping



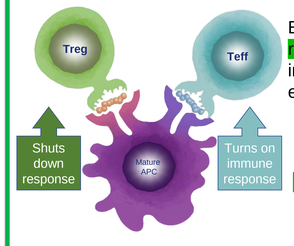
Accurate identification of CD4 and CD8 epitopes using EpiMatrix®

CD4 T cell epitopes. EpiMatrix Class II predictions are **74% accurate** when prospectively tested, while IEDB predictions are 54-66% accurate on the same dataset. EpiMatrix® CD4 predictions and its associated tools are routinely used and trusted by 9 of the top 10 pharmaceutical companies.

Mean accuracy (± SD) of DRB1*0101, DRB1*0301, DRB1*0401, DRB1*0701, DRB1*0802, DRB1*1101, DRB1*1302, and DRB1*1501 predictions. Between 175 and 251 peptides were tested per HLA. Source: peptides prospectively selected by EpiMatrix and tested in *in vitro* HLA binding assays.

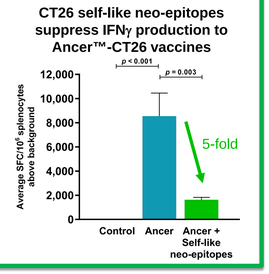


Identification and removal of Treg epitopes using JanusMatrix™

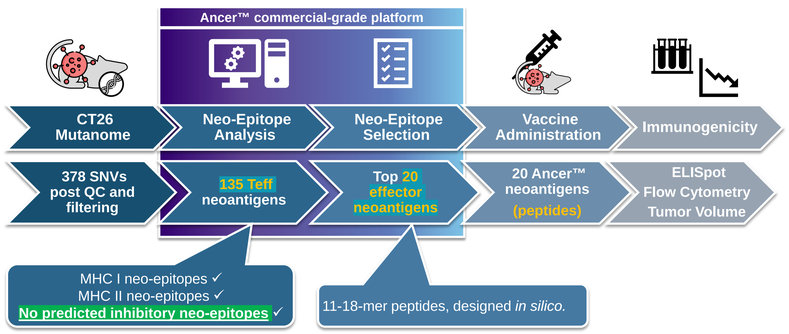


Epitopes can be either effector or regulatory. JanusMatrix™ has identified immunosuppressive (e.g. Treg) T cell epitopes in pathogens [1, 2, 3].

JanusMatrix™ has also identified **immunosuppressive neo-epitopes in cancer mutanome** (e.g. in the CT26 colon carcinoma mouse model) [4].



Methods



- The CT26 mouse cell line exome and transcriptome were analyzed with the Ancer™ platform.
- Effector neoantigen sequences were identified and ranked based on their MHC Class I and MHC Class II immunogenicity while avoiding the inclusion of inhibitory sequences.
- Top 20 sequences were synthesized as peptides (Ancer™-CT26 vaccine) and their immunogenicity and efficacy were tested in naïve and CT26 tumor bearing BALB/c mice, respectively.

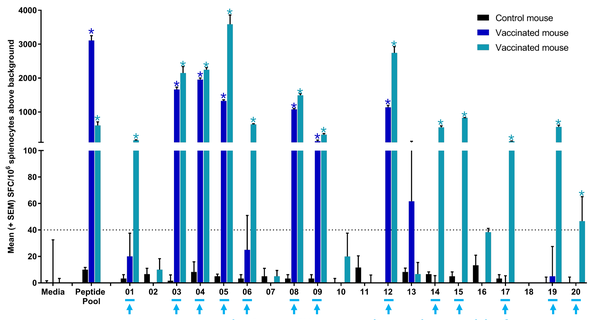
IFNγ ELISpot stimulation: Ancer™-CT26 pool and individual peptides

Flow Cytometry: Ancer™-CT26 pool

Ancer™-selected CT26 neoantigens induce highly immunogenic responses and reduce tumor burden

Immunogenicity

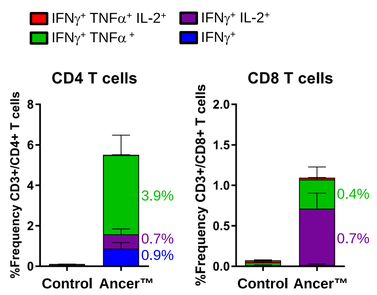
IFNγ ELISpot



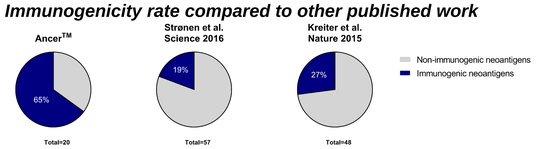
Immunogenic Ancer™ neoantigens in peptide format

** Positive response: SFC ≥ 40, SI ≥ 2, and p-values ≤ 0.05*

Flow Cytometry



Immunogenicity rate compared to other published work



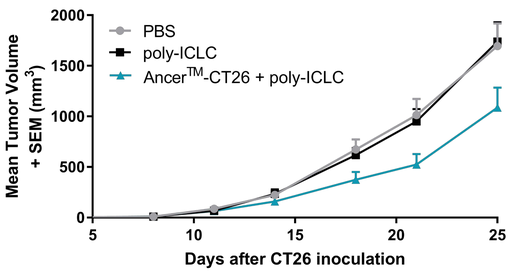
High rate of immunogenicity

Multifunctional CD4+/CD8+ response

- The Ancer™-CT26 vaccine generated T cell responses, as measured by IFNγ production, against **13 of 20 (63%) neoantigens** targeted by the vaccine.
- Ancer™-CT26 generates both CD4+ and CD8+ **multifunctional T cell responses**, as measured by IFNγ, TNFα, and IL2 production.

Efficacy

Tumor Burden



Tumor reduction	Day 21	Day 25
Ancer™ vs PBS	48%	36%
Ancer™ vs poly-ICLC	45%	38%

Significant reduction of tumor burden

- Ancer™-CT26 peptide vaccination with poly-ICLC **controls tumor growth** as a monotherapy in the implantable CT26 murine tumor model.

Conclusions

- **EpiVax's immunogenicity screening tools (EpiMatrix® and JanusMatrix™) are integrated into the Ancer™ platform for streamlined designs of personalized cancer vaccines. Analysis of the MHC- and TCR-facing residues of T cell epitopes by JanusMatrix™ enables prediction of epitope phenotype.**
- **The CT26 mutanome was analyzed with Ancer™ in order to design a CT26 neoantigen-based vaccine (Ancer™-CT26).**
- **68% of sequences that compose Ancer™-CT26 induced IFNγ production in naïve animals. Ancer™-CT26 induced both multifunctional CD8+ and CD4+ T cell responses.**
- **Ancer™-CT26 reduced tumor burden up to 45% compared to vehicle (poly-ICLC) when tested in an efficacy study with CT26 tumor bearing animals.**
- **Ancer™ selects neoantigens that can drive immunogenic and effector responses.**

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) Liu R. et al., H7N9 T-cell epitopes that mimic human sequences are less immunogenic and may induce Treg-mediated tolerance, Hum Vaccin Immunother. 2015 11:9, 2241-2252
3) 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
4) Richard G. et al., Filtering out self-like neoantigens improves immune response to cancer vaccines. Proceedings: AACR Annual Meeting 2019; March 29-April 3, 2019; Atlanta, GA

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