Computational identification of self-like neoantigens enables the rapid design of immunogenic cancer vaccines

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Abstract

Computationally identified self-like neoantigens reduce immune responses of immunogenic therapies when included in vaccine formulations. Clinical studies have highlighted the potential of precision cancer immunotherapy to effectively control the tumor of patients across cancer indications. However, recent studies showcase the difficulty of establishing robust CD8 and CD4 T cell responses. We hypothesize that poor cancer vaccine performance may be due in part to the inadvertent inclusion of suppressive T cell neo-epitopes in neoantigen vaccines that may be recognized by regulatory T cells (Tregs).

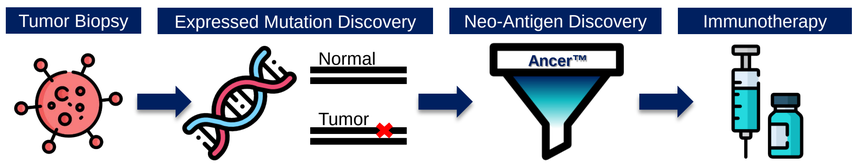
To test this hypothesis, we used the Ancer™ system to identify and select neo-epitopes from the CT26 syngeneic mouse model. Ancer™ leverages EpiMatrix® and JanusMatrix™, state-of-the-art predictive algorithms that have been extensively validated in prospective vaccine studies for infectious diseases [1, 2]. Distinctive features of Ancer™ over other pipelines are its ability to predict CD4 T cell epitopes and to computationally identify tolerated or Treg epitopes.

In a first experiment, optimally selected CT26 neoantigen vaccine candidates encoding CD4 and CD8 neo-epitopes were designed and ranked with Ancer™. Self-like, putative Treg epitopes were removed in this process. Naïve Balb/c mice were immunized subcutaneously with a peptide pool comprised of the 20 highest ranking neoantigens delivered with poly-ICLC (Oncovir). Immunization with Ancer™-derived neoantigens induced strong IFNγ ELISpot responses compared to controls ($p < 0.001$). Flow cytometry confirmed the vaccine stimulated multifunctional CD4+ and CD8+ T cells.

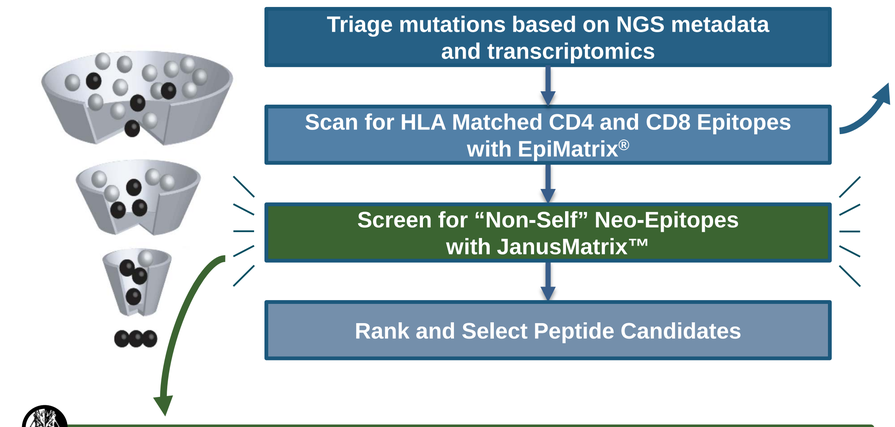
In a follow-on experiment, ten self-like neoantigens, from the same CT26 genome, were selected with Ancer™. These neoantigens may be recognized by Tregs due to their high degree of similarity with self, based on JanusMatrix™. Co-administration of the CT26 self-like neoantigens with our optimally designed neoantigen vaccine in naïve Balb/c mice diminished IFNγ ELISpot responses by 5-fold compared to vaccination without the self-like neoantigens ($p = 0.003$).

While it has been well known that Tregs are present in tumors, these results suggest the possibility that tumor-derived neo-epitopes may be recruiting Tregs to the tumor. More importantly, the inadvertent inclusion of Treg driving neoantigens in vaccine formulations may hinder efforts to induce strong T cell-mediated tumor control. In silico screening of neoantigen sequences using specialized tools offers the possibility of enriching and designing new vaccines with higher quality candidates while minimizing costs and turnaround times. Efforts are ongoing to determine the effect of Ancer™-derived self-like neoantigens on CD4+ and CD8+ T cells and how the inclusion of self-like neoantigens in vaccines affects their efficacy.

Mutanome-Directed Cancer Immunotherapy Pipeline



Ancer™ Platform: the "Answer" to Cancer

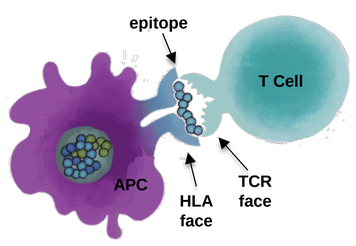


Identification and removal of Treg epitopes using JanusMatrix™

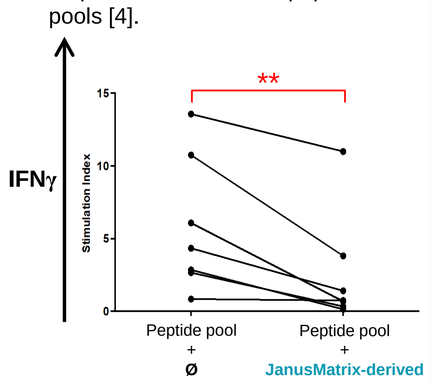
JanusMatrix™ identifies cross-reactive and regulatory T cell (Treg) epitopes.

Cross-reactive sequences:

- Bind to the same MHC allele.
- Share same/similar T cell-facing residues.



Treg epitopes identified with JanusMatrix™ reduce IFNγ responses observed for peptide pools [4].



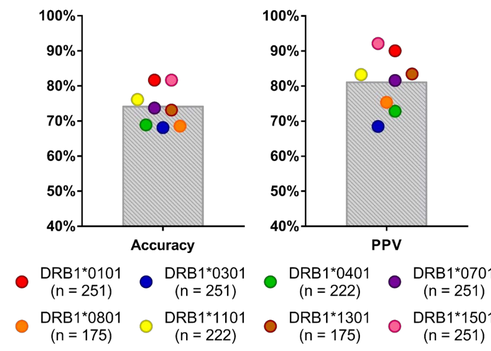
Accurate identification of T cell epitopes using EpiMatrix®

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%.

Accuracy and PPV of EpiMatrix Class II predictions
Prospective selection of HLA ligands

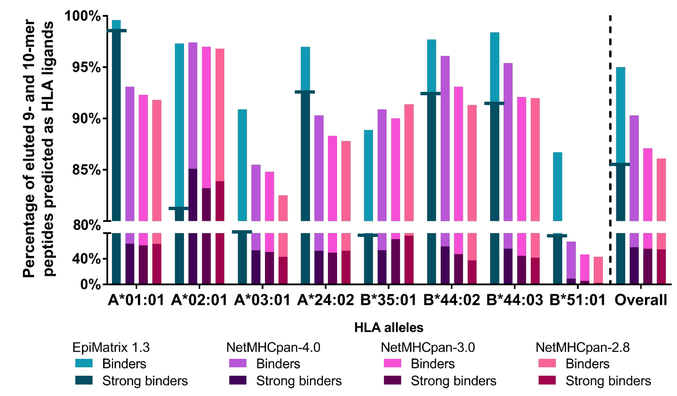


Internal and published data

CD8 T cell epitopes

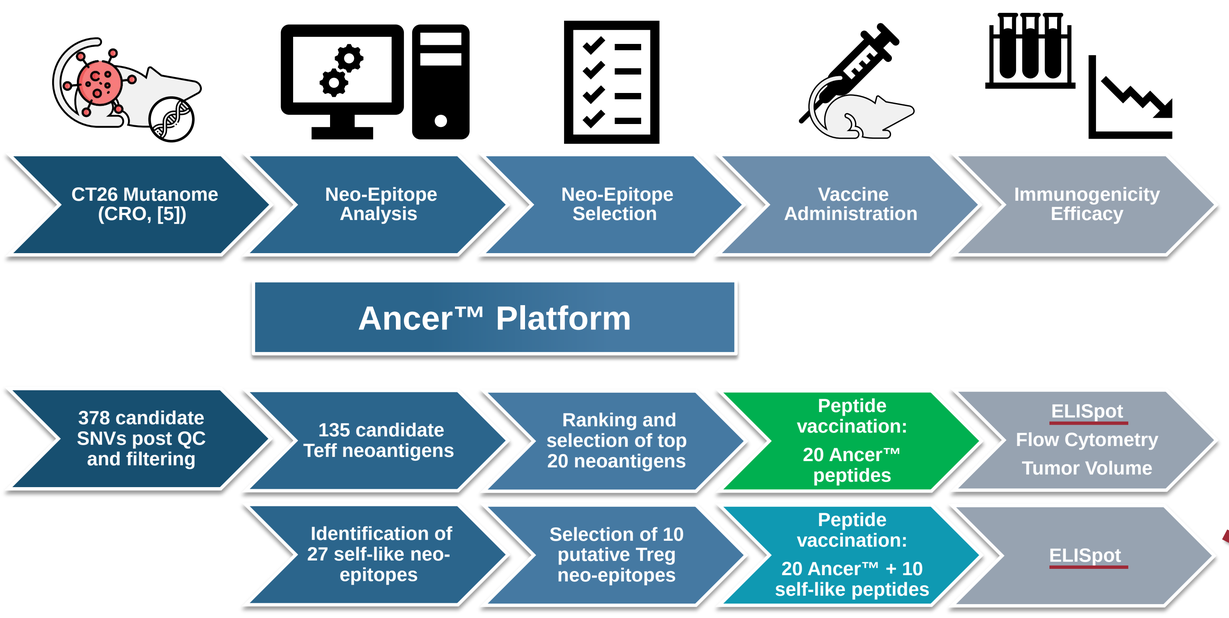
Analysis of eluted peptide dataset [3]: **95%** of eluted 9- and 10-mers were predicted to bind to HLA according to EpiMatrix®, while only ~88% of ligands were accurately recalled by NetMHCpan.

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

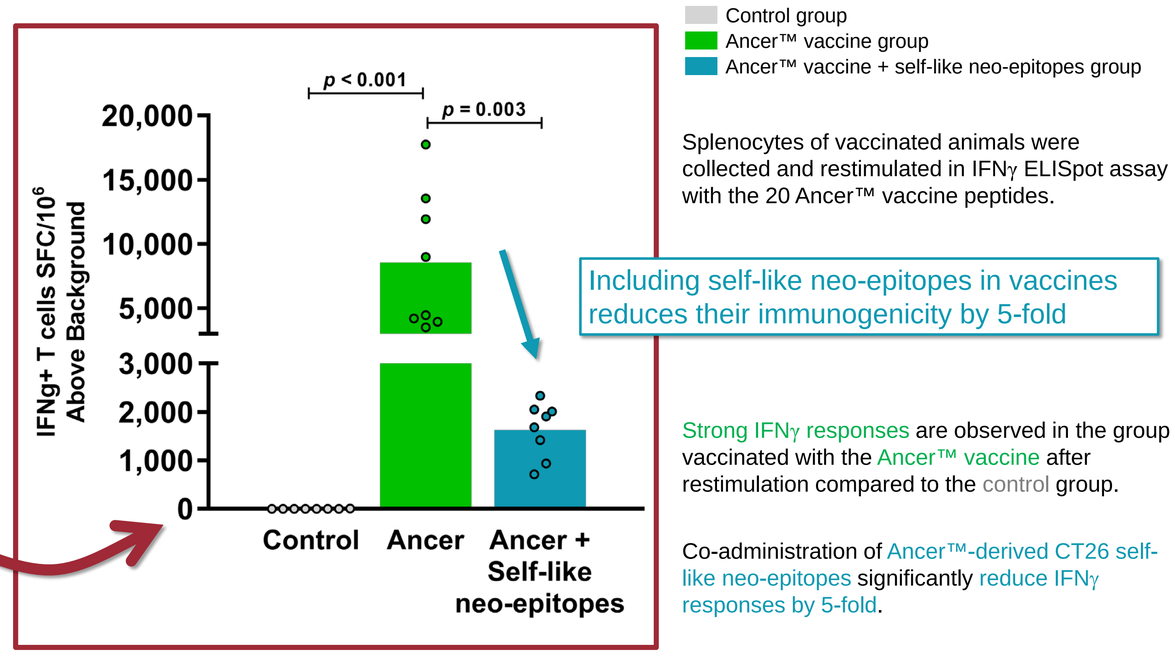


Design of an immunogenic CT26 Ancer™-based vaccine and identification of immunosuppressive CT26 self-like neo-epitopes

Protocol



Immunogenicity Results – IFNγ ELISpot



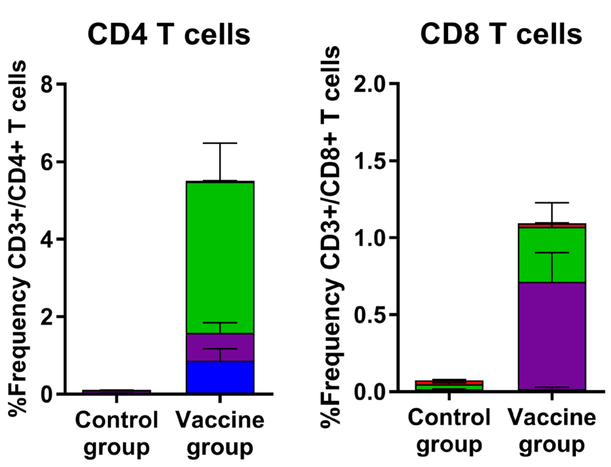
Splenocytes of vaccinated animals were collected and restimulated in IFNγ ELISpot assay with the 20 Ancer™ vaccine peptides.

Including self-like neo-epitopes in vaccines reduces their immunogenicity by 5-fold

Strong IFNγ responses are observed in the group vaccinated with the Ancer™ vaccine after restimulation compared to the control group.

Co-administration of Ancer™-derived CT26 self-like neo-epitopes significantly reduce IFNγ responses by 5-fold.

Immunogenicity Results – Flow Cytometry

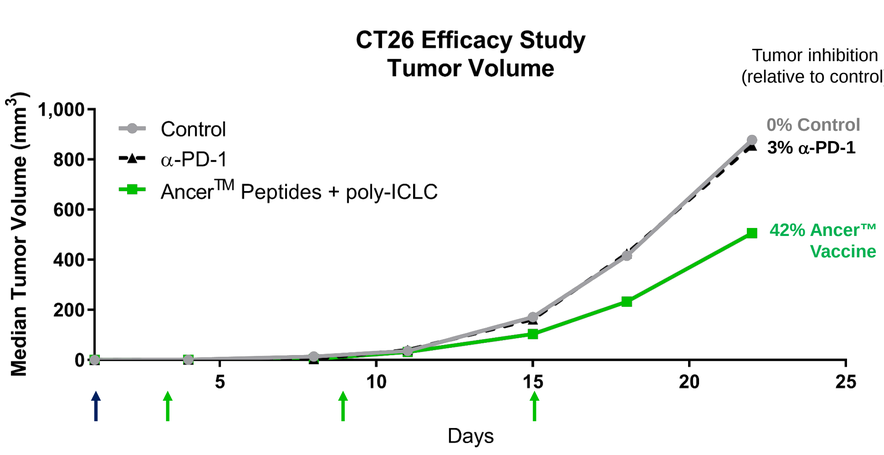


Splenocytes of animals vaccinated with the control and Ancer™ vaccines were collected and evaluated by flow cytometry.

Ancer™ CT26 vaccine generates multifunctional CD4+ and CD8+ T cell responses

Vaccination of Balb/c mice with the Ancer™ vaccine increased the frequency of CD4+ and CD8+ T cells compared to control animals.
The Ancer™ vaccine stimulated multifunctional CD4+ and CD8+ responses.

Efficacy Results – Tumor Volume



Tumor inhibition (relative to control)

N = 12 mice per group

Ancer™ CT26 vaccine reduces tumor burden by 42% at day 22

Ancer™ vaccine reduces median CT26 tumor burden by 42% at day 22 as a monotherapy agent compared to control animals.

Conclusions

- **Not all neo-epitopes are created equal! Some neo-epitopes may suppress immune responses due to their homology with self-sequences.**
- **Using Ancer™, an *in silico* neo-epitope screening platform, we designed a new highly immunogenic CT26 neoantigen-based vaccine enhanced for both CD4 and CD8 T effector content.**
- **Our Ancer™ peptide vaccine induced strong IFNγ ELISpot responses as well as multifunctional CD4+ and CD8+ T cell responses in naïve Balb/c animals.**
- **Co-administration of our Ancer™ vaccine with self-like neo-epitopes, thought to engage with Tregs, reduced the immunogenicity of our vaccine by 5-fold.**

Acknowledgments

We thank our colleagues at EpiVax for contribution to this work.

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References

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