Immunoinformatic Analysis of Pancreatic Cancer Mutanomes: Impact of Regulatory T Cell Neo-Epitopes on Patient Outcome

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Abstract

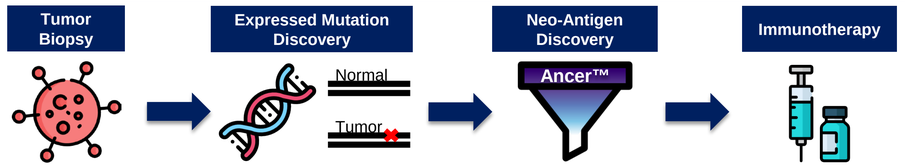
Pancreatic cancer remains one of the deadliest cancers despite immunotherapy breakthroughs. We hypothesized that patients with poor outcomes may have tumors enriched in neo-epitopes activating regulatory T cells (Tregs).

Whole exome sequencing data derived from 13 pancreatic cancer patients with available HLA I and II typing and treated with GVAX, an autologous cancer vaccine engineered to secrete the stimulatory GM-CSF cytokine, was analyzed with Ancer, an *in silico* neo-epitope identification platform. Distinctive features of Ancer are its ability to accurately predict HLA II ligands and to identify tolerated or Treg epitopes. Using Ancer, we estimated the ratio of effector versus regulatory neo-epitope content for each patient.

Median disease-free survival (DFS) time of patients with high effector content (25 months) was over four times greater than in patients whose ratio shifted towards high regulatory content (6 months). Analysis of the same patients using their tumor mutational burden (TMB) did not yield conclusive results. In contrast, patients with high TMB had a similar median DFS time (17 months) than patients with low TMB (14 months).

While no statistically significant conclusions were obtained due to the low number of patients in our study, our results suggest that higher numbers of neo-epitopes that may activate Treg in the tumors are in a trend associated with shorter DFS. Follow-up studies involving larger cohorts of patients are ongoing.

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 HLA Class I and Class II) with EpiMatrix®
- Identification and Removal of "Self-Like" Sequences with JanusMatrix™
  - Treg-like Sequences Removal
  - Cross-Reactive Sequences Removal
- Design, Rank, and Select "Non-Self" Neoantigens

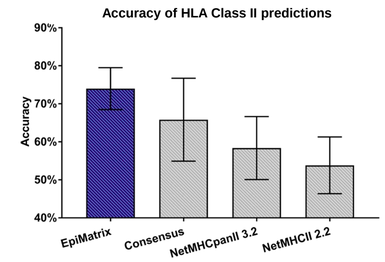
Accurate and identification of CD4 and CD8 epitopes using EpiMatrix®

CD4 T cell epitopes

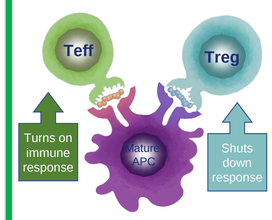
EpiMatrix Class II predictions are **74% accurate** when prospectively tested in *in vitro* HLA binding assays. IEDB predictions are 54-66% accurate when tested against the same set of peptides.

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. Peptides were evaluated on IEDB on November 19th 2018.

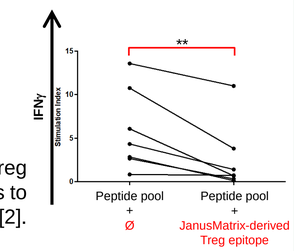


Identification and removal of Treg epitopes using JanusMatrix™

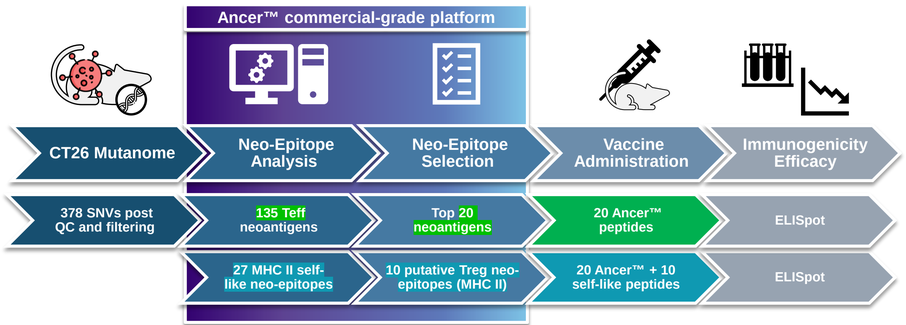


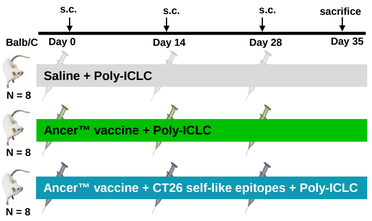
Epitopes can be either effector or regulatory. JanusMatrix™ helps us identify immunosuppressive (e.g. Treg) T cell epitopes [1].

In silico-derived influenza (H7N9) Treg epitope reduces IFNγ responses to effector peptides [2].

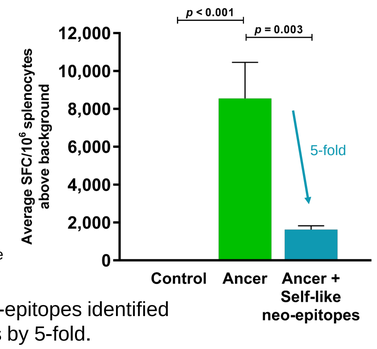


Immunosuppression of IFNγ responses by CT26 self-like T cell neo-epitopes





Prime + boost x2, bi-weekly. Subcutaneous injections.
Splenocytes collected at Day 35 and restimulated in IFNγ ELISpot assay with the 20 vaccine peptides.

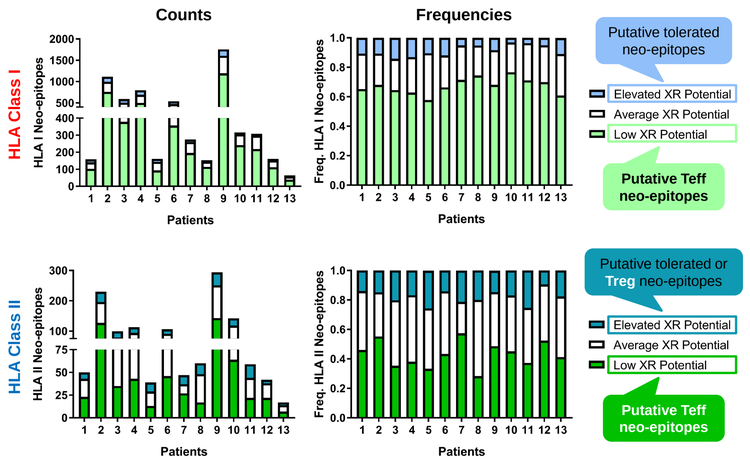


Co-administration of Ancer™-derived CT26 self-like neo-epitopes identified with JanusMatrix™ significantly reduces IFNγ responses by 5-fold.

Poor outcome in pancreatic cancer patients with higher proportions of regulatory T cell neo-epitopes

We used Ancer™ to analyze the mutanome of 13 GVAX-treated pancreatic cancer patients.

Neo-epitope composition:



Number and frequency of Class I and Class II neo-epitopes with low, average, or high cross-reactivity (XR) potential, as determined by JanusMatrix™, across pancreatic cancer patients.

Do patients with more Treg neo-epitopes have a worse outcome?

Is the ratio of MHC Class II effector (Teff) vs regulatory (Treg) neo-epitopes found in the tumor associated with patient outcomes?

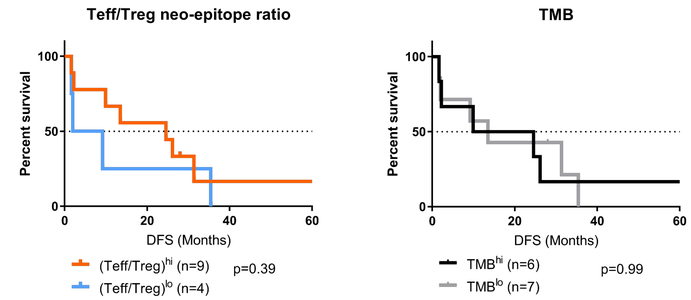
$$\text{Teff/Treg Ratio} = \frac{\text{Frequency HLA II "Teff" neo-epitopes}}{\text{Frequency HLA II "Treg" neo-epitopes}}$$

n = 13

Ratio shifted towards Teff: $(\text{Teff/Treg})^{hi}$ n = 9

Ratio shifted towards Treg: $(\text{Teff/Treg})^{lo}$ n = 4

- Do $(\text{Teff/Treg})^{hi}$ patients have a higher disease free survival (DFS) time than $(\text{Teff/Treg})^{lo}$ patients?
- How does the ratio of Teff/Treg neo-epitopes compare to a TMB predictor?



Median DFS $(\text{Teff/Treg})^{hi}$: 25 mo.
Median DFS $(\text{Teff/Treg})^{lo}$: 6 mo. x 4.2

Median DFS TMB^{hi}: 17 mo.
Median DFS TMB^{lo}: 14 mo. x 1.2

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.**
- **The ratio of effector vs regulatory T cell neo-epitopes encoded by the tumor may help us understand patient outcomes. Our results suggest that higher proportions of neo-epitopes that may activate Treg in the tumors are in a trend associated with shorter DFS.**
- **Our prospective CT26 mouse study showcases that inclusion of Treg neo-epitopes in cancer vaccines can downregulate immune responses.**

References and Acknowledgments

1) Moise L. et al., Hum Vaccin Immunother. 2015;11(9):2312-21.
2) Liu R. et al., Hum Vaccin Immunother. 2015 11:9, 2241-2252

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