Integrating CD8 and CD4 effector neo-epitope content with regulatory T cell epitope exclusion is a superior prognostic biomarker for bladder cancer patients compared to their tumor mutation burden

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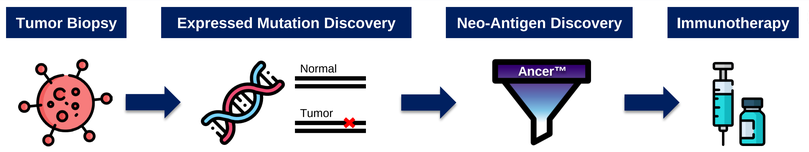
Overview

- **Hypothesis**: Accurately defining effector (Teff) and excluding **regulatory (Treg) neo-epitopes** will help **identify patients with improved prognosis**.
- **Approach**: TCGA bladder mutanomes (n=412) were analyzed 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**: **Improved stratification of patients is obtained with Ancer™** compared to public epitope prediction tools or TMB.
- Stratification is improved when predicting, with Ancer™, **CD4 neo-epitopes and excluding putative Treg neo-epitopes**.
- **Summary**: While defining CD8 neo-epitope burden enhanced associations with survival**,** the inclusion of CD4 Teff neo-epitope burden helped identify long-term survivors.
- Ancer™ may represent a novel tool for defining new **prognostic or predictive biomarkers.**

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



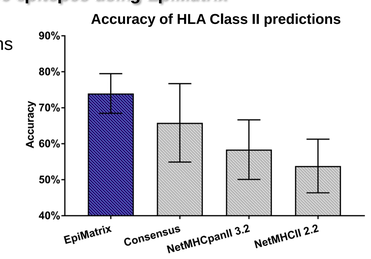
Ancer™ platform: the "Answer" to Cancer

- 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

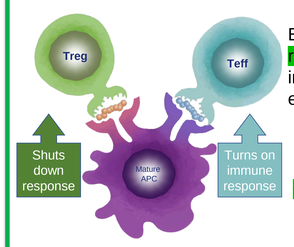
Accurate identification of CD4 and CD8 epitopes using EpiMatrix®

CD4 T cell epitopes. EpiMatrix Class II predictions are **74% accurate** when prospectively tested, while predictions using public tools 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.

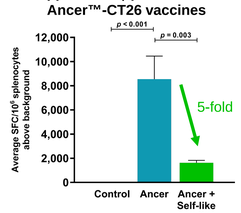


Identification and removal of Treg epitopes using JanusMatrix™

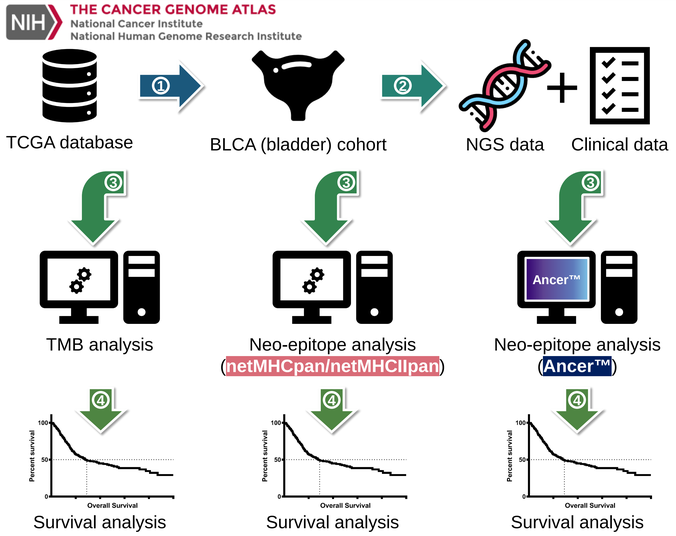


Epitopes can be either effector or regulatory. JanusMatrix™ has identified immunosuppressive (e.g. Treg) T cell epitopes in pathogens [1, 2, 3].

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



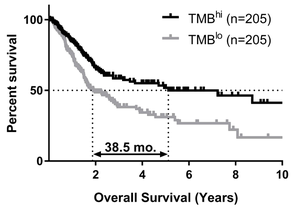
Methods – TCGA analysis of bladder cancer data



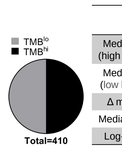
- HLA types inferred for each patient based on three HLA typing algorithms.
- NGS data analyzed with two pipelines (public tools vs Ancer™) to evaluate CD8 and CD4 neo-epitope burdens. High/low burdens are defined by medians.
- Survival analyses (Kaplan-Meier) used to compare overall survival (OS) of patients with high/low mutational or neo-epitope burdens.
- High/low burden categories used to calculate PPV and NPV at various OS cutoffs.

Stratification of TCGA bladder cancer patients is better achieved with Ancer™

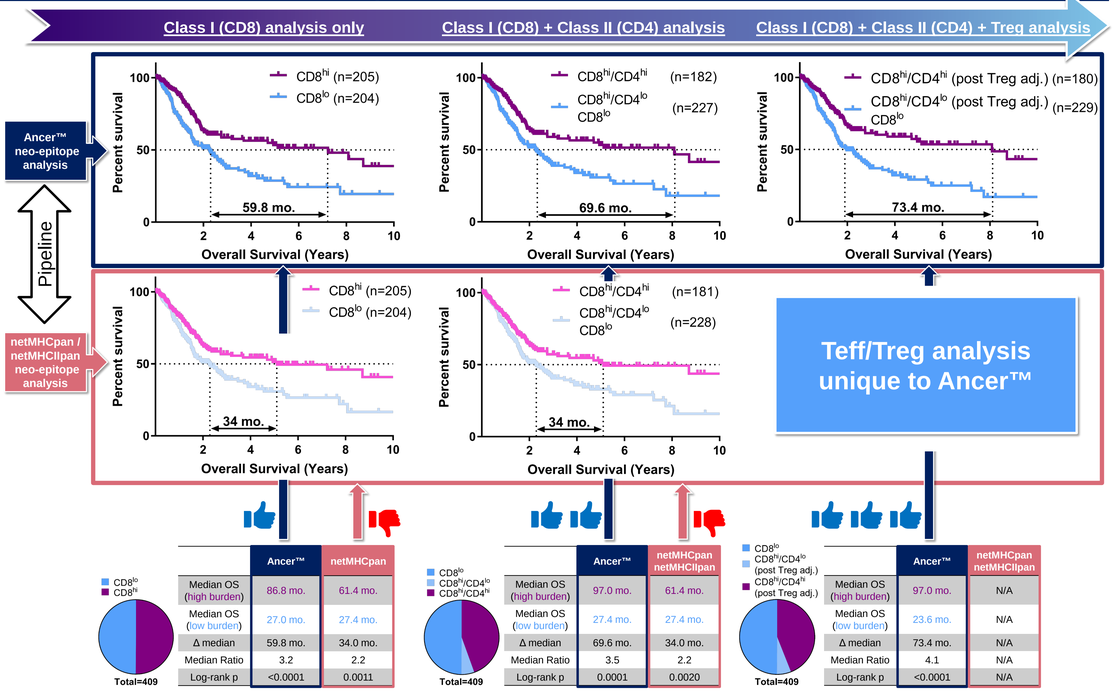
- OS is significantly higher in patients with high TMB (as expected).



- **Can a neo-epitope analysis further enhance patient stratification?**
- We tested this hypothesis by analyzing patients' mutanomes with Ancer™ and with netMHCpan/ netMHCIIpan.
- **Improved stratification is obtained after analyzing mutanomes with Ancer™.**



	TMB
Median OS (high burden)	61.4 mo.
Median OS (low burden)	22.9 mo.
Δ median	38.5 mo.
Median Ratio	2.7
Log-rank p	0.0001



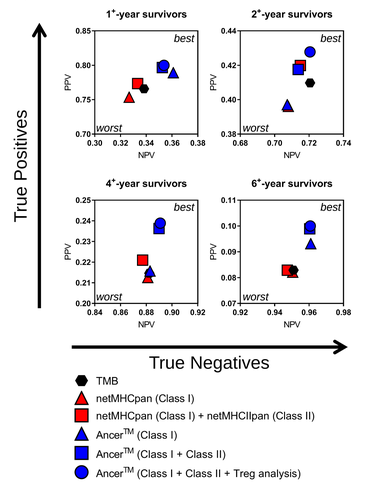
	Ancer™	netMHCpan
Median OS (high burden)	86.8 mo.	61.4 mo.
Median OS (low burden)	27.0 mo.	27.4 mo.
Δ median	59.8 mo.	34.0 mo.
Median Ratio	3.2	2.2
Log-rank p	<0.0001	0.0011

	Ancer™	netMHCpan netMHCIIpan
Median OS (high burden)	97.0 mo.	61.4 mo.
Median OS (low burden)	27.4 mo.	27.4 mo.
Δ median	69.6 mo.	34.0 mo.
Median Ratio	3.5	2.2
Log-rank p	0.0001	0.0020

	Ancer™	netMHCpan netMHCIIpan
Median OS (high burden)	97.0 mo.	N/A
Median OS (low burden)	23.6 mo.	N/A
Δ median	73.4 mo.	N/A
Median Ratio	4.1	N/A
Log-rank p	<0.0001	N/A

Survivorship predictions

- **Hypothesis**: Patients with "high" (mutational or neo-epitope) burden survive more than X years. We tested X = 1, 2, 4, or 6 years.
- This hypothesis can be tested by comparing predicted and observed survival statuses.



- **Ancer™ consistently predicts the survival status of bladder cancer patients at a higher PPV and NPV than other predictors.**

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.**
- **Improved stratification of TCGA bladder cancer patients was obtained with Ancer™ compared to public *in silico* tools or traditional TMB analyses. These results highlight the importance of identifying neo-epitopes with high-quality epitope prediction tools and of evaluating their phenotype (effector or regulatory) using specialized homology tools.**
- **Ancer™ may help understand patients' survival based on an in-depth analysis of their mutanome, including an evaluation of their CD8 and CD4 effector neo-epitope contents.**
- **Follow-up studies include multivariate analyses of overall survivals including additional co-factors and extension of this analysis to other TCGA cohorts.**

References

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

Acknowledgments

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