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Ancer: An Innovative Cancer Neoantigen Prediction System

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Abstract

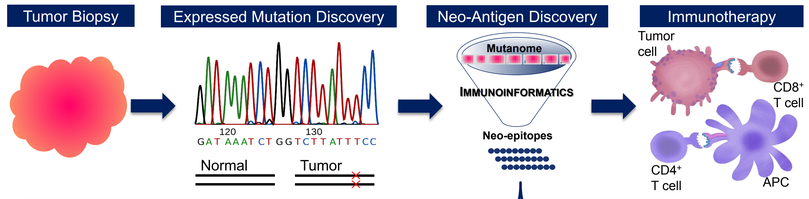
T cell epitopes bearing tumor-specific mutations discovered using next generation sequencing stimulate T cell-mediated processes that lead to tumor regression. Although neoantigen prediction using computational methods rapidly identifies epitope candidates in the mutanome, a large proportion prove to be non-immunogenic. Innovative computational tools validated for infectious disease can be applied to enhance design of personalized cancer immunotherapies by classification of predicted epitopes according to potential for mounting a tumor-specific response.

We developed the JanusMatrix algorithm [Moise et al. 2015 Human Vaccines & Therapeutics 11:9, 2312-2321] that parses query sequences into MHC-facing and T cell receptor (TCR)-facing sequences and screens sequence databases to identify MHC ligands that share TCR faces with host-related proteins. A database of human protein sequences is available to identify tumor-specific epitopes that may reduce anti-tumor activity by sequences that activate regulatory T cells (Tregs) trained in the thymus on self-antigens. Similarly, tumor-specific epitope candidates are screened using databases composed of human commensal- or pathogen-derived sequences to identify epitopes that, respectively, may detrimentally or beneficially cross-react with T cells raised over the course of an individual's immune history.

Analysis of mutanome-derived peptides [Strønen et al. 2016 Science 352(6291), 1337-41] shows that our approach selects immunogenic neo-epitopes with 72% accuracy, compared to 21% when using publicly available tools. In addition, retrospective analyses of cancer vaccine efficacy studies performed in mice [Kreiter et al. 2015 Nature 520, 692-696] show that mutanome-directed vaccines effective at preventing tumor growth contain higher numbers of T cell neo-epitopes with lower self-cross-reactive potential. Likewise, an evaluation of mutanomes derived from non-small cell lung cancer patients [Rizvi et al. 2014 Science 348, 124-128] revealed that improved clinical outcomes were observed in patients with mutanomes enriched in class I MHC neo-epitopes with TCR faces distinct from other self-epitopes.

While retrospective in nature, the suite of tools used for these analyses have been extensively validated in prospective vaccine studies for infectious diseases. Removal of Treg epitopes identified by JanusMatrix has led to the development of a novel H7N9 influenza vaccine scheduled for Phase I clinical trial. These results highlight the benefits of using in silico tools for the selection of high-value neoantigen candidates and how they can improve the design and efficacy of cancer vaccines. Neoantigens with low Treg activation potential may then be used to support development of personalized therapies including vaccination and in vitro expansion of tumor infiltrating lymphocytes for adoptive cell transfer.

Mutanome-Directed Cancer Immunotherapy

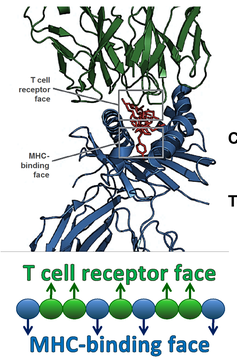


Ancer Platform: the "Answer" to Cancer

From mutanome to vaccine design in 1-2 weeks

- Scan for HLA Matched Epitopes (Patient Class I + II) with EpiMatrix
- Screen for Humanness and Identify "Non-Self" Neo-epitopes with JanusMatrix
- Rank and Select Peptide Candidates
- Design: Commercial-grade Advanced Vaccine Design with VaxCAD

JanusMatrix Discovers Potential Treg Neo-Epitopes

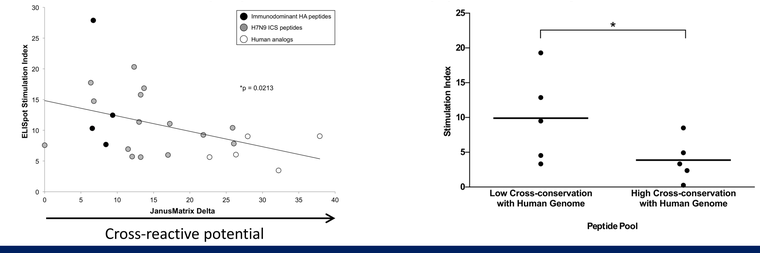


JanusMatrix separates the amino acid sequence of T cell epitopes into TCR-facing residues (epitope) and HLA binding cleft-facing residues (agretope), then compares the TCR face to other putative T cell epitopes.

Cross-reactive peptides:
- Are predicted to bind the same MHC allele.
- Share same/similar T cell-facing residues.

TCR cross-reactivity prediction:
- Given a protein or peptide, T cell epitopes are identified based on MHC contacts using EpiMatrix.
- JanusMatrix searches for potentially cross-reactive TCR by screening TCR-facing residues against a preloaded, EpiMatrix-processed reference database.
- Peptides with high cross-reactive potential are associated with reduced INFγ secretion in PBMCs of healthy donors (Liu *et al.* Human Vaccines & Immunotherapeutics 2015).

Human IFNγ responses to cross-conserved peptides identified by JanusMatrix

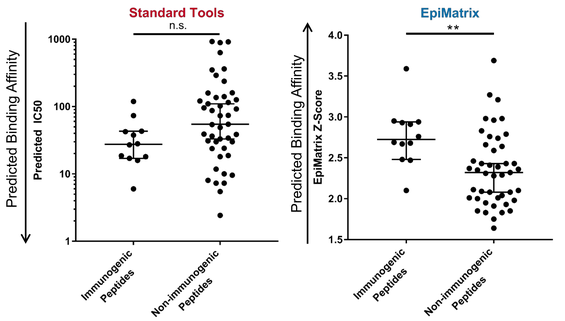


Retrospective Immunoinformatic Analyses of Published Cancer Datasets

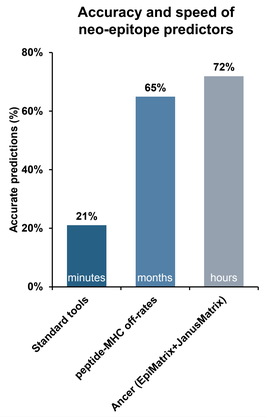
Ancer Analysis of Mutated Peptides from Strønen et al. Science 2016

- Summary of the Strønen *et al.* Science 2016 study:
  HLA-A2-restricted mutated peptides derived from melanoma patients were identified using NetMHC and NetMHCpan. **Few peptides (21%) selected with standard in silico tools were immunogenic** when tested in T cell assays using PBMCs of healthy donors and patients' TILs. Experimentally determined peptide-MHC off-rates can be used to discriminate immunogenic from non-immunogenic peptides with 65% accuracy.
- **Immunogenic peptides have significantly greater binding potentials** than non-immunogenic peptides, **as measured by EpiMatrix** (Unpaired t test, **p = 0.0053**). No significant difference is observed between peptides when evaluated with publicly available in silico tools (Unpaired t test, **p = 0.1064**).
- **Ancer can differentiate immunogenic and non-immunogenic peptides with 72% accuracy** by using strict neo-epitope definitions and by evaluating their potential for cross-reactivity.

Predicted binding affinities of mutated peptides

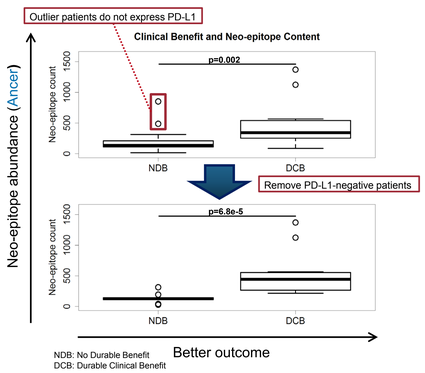


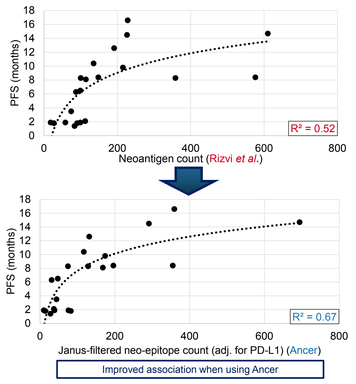
Accuracy and speed of neo-epitope predictors



Ancer Analysis of Mutanomes from Rizvi *et al.* Science 2015

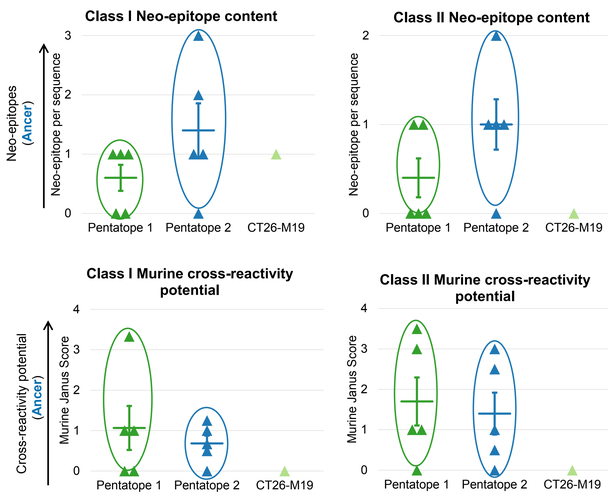
- Summary of the Rizvi *et al.* Science 2015 study:
  Tumors from 34 anti-PD-1 treated patients with non-small cell lung cancer were sampled and sequenced to identify tumor-specific mutations. Tumor mutation burden was found to be associated with clinical response.
- Mutanomes were analyzed using Ancer to identify neo-epitopes and to determine their cross-reactivity potential with other human proteins (below).
- **Neo-epitope content**, as determined by Ancer, **was significantly associated with clinical benefit**. Association was improved when removing patients not expressing PD-L1 for whom the anti-PD-1 treatment may not be suitable.
- **Progression-free survival (PFS) of patients** expressing some level of PD-L1 **was best estimated by** Ancer's **neo-epitope content**, adjusted for both Human cross-conservation potential ("low Janus") and PD-L1 expression.

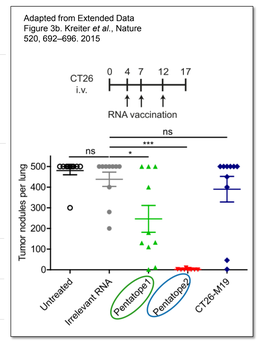




Ancer Analysis of Cancer Vaccines from Kreiter *et al.* Nature 2015

- Summary of the Kreiter *et al.* Nature 2015 study:
  Pentatopes were constructed from mutated sequences derived from colon carcinoma model CT26. **Pentatope2 showed greater antitumor activity** compared to Pentatope1 in BALB/c mice inoculated with CT26 tumor cells (right).
- Pentatope2 increased anti-tumor activity may be explained by a **higher number of Class I and II neo-epitopes** and overall **lower potential for cross-reactivity**, as identified by Ancer (left).





Conclusions

- **Sequence analysis of the MHC- and TCR-facing residues of T cell epitopes enables prediction of epitope phenotype.**
- **Epitopes that share a TCR-face with numerous human sequences may activate Tregs.**
- **Sharper definition of neo-antigens by immunoinformatic analysis may improve epitope selection for mutanome-directed cancer immunotherapy.**
- ***In silico* analyses of cancer vaccines suggest that high neo-epitope density and low cross-reactivity potential may be indicative of improved clinical outcomes.**

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