T cell epitopes bearing tumor-specific mutations discovered using next generation sequencing stimulate T cell-mediated responses 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 influenza 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 (Boegel et al. 2015 Human Vaccines & Immunotherapeutics 11:2312-2321) 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 antitumor 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 343, 124-128) revealed that 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 studies for infectious diseases. Removal of Treg epitopes identified by JanusMatrix led to the development of a novel H7N9 influenza vaccine schedule for Phase I clinical trials. These result highlights the benefit 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 therapeutics including vaccination and in vitro expansion of tumor infiltrating lymphocytes for adoptive cell transfer.

**Results**

- **Summary of the Hansen et al. Science 2016 study.**
  - HLA-A2 restricted mutanome peptides derived from melanoma patients were identified using NetMHC and NetMHCIIscan. Few peptides (2%) with standard in silico tools were immunogenic when tested in T cell assays using PBMCs of healthy donors and patients T.L.C. (Experientially 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 EpitopeNet. (Unpaired t test, p = 0.004).
  - No significant difference is observed between peptides when evaluated with publicly available in silico tools (Unpaired t test, p = 0.10).

- **Ancor can differentiate immunogenic and non-immunogenic peptides with 72% accuracy by using strict neo-epitope definitions and by evaluating their potential for cross-reactivity.**

- **Summary of the Rizvi et al. Science 2015 study.**
  - Tumors from 36 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 Ancor to identify neo-epitopes and to determine their cross-reactivity potential with other human proteins (below).

- **Neon-epitope content, as determined by Ancor, 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 Ancor neon-epitope content, adjusted for both human cross-conservation potential (“low Janus”) and PD-L1 expression.**

**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 high neo-epitope density and low cross-reactivity potential may be indicative of improved clinical outcomes.

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**References**