Improved Personalized Vaccine and Adoptive Cell Transfer Immunotherapy Design by Immunoinformatic Analysis of Cancer Neo-epitopes for Regulatory T Cell Activation Potential

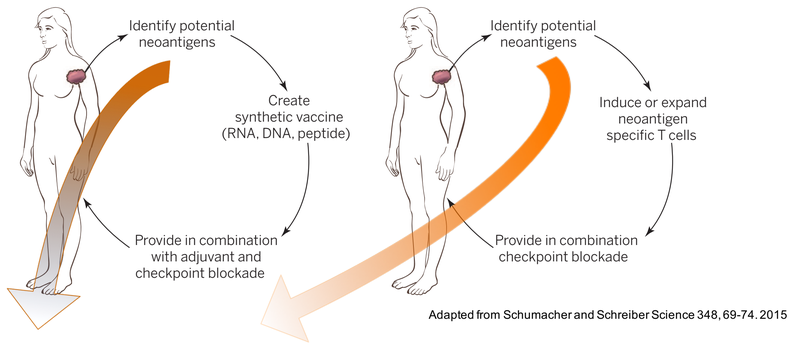
Lenny Moise[1,2], Guilhem Richard[1], Frances Terry[1], Bill Martin[1], Annie De Groot[1,2]

[1] EpiVax, Inc., Providence, RI, United States; [2] Institute for Immunology and Informatics, University of Rhode Island, Providence, RI, United States

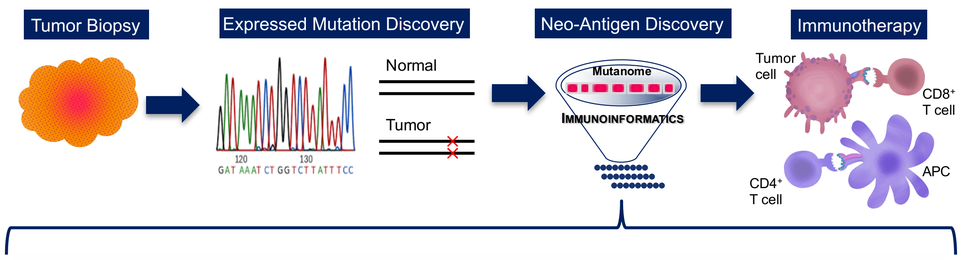
Abstract

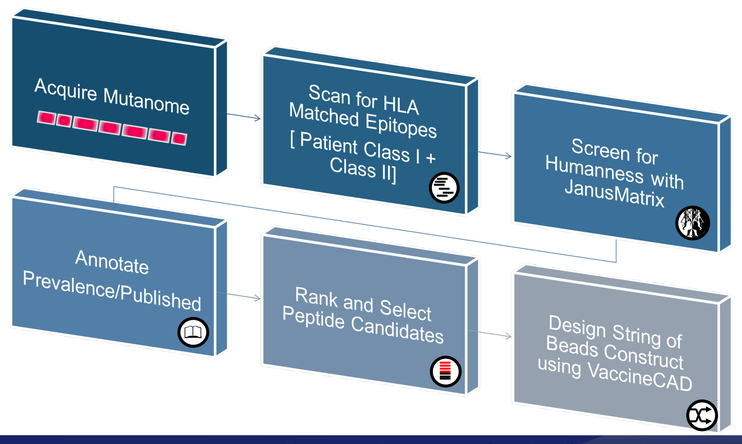
Tumor-specific mutations discovered using whole-exomic sequencing of tumor-normal pairs can be harnessed to identify neo-epitopes capable of stimulating T cell-mediated processes leading to tumor regression. Neo-epitope prediction using computational tools rapidly identifies epitope candidates in the mutanome, but a large proportion of neo-epitopes is not immunogenic. One explanation is that some epitopes, particularly class II major histocompatibility complex (MHC) epitopes, activate regulatory T cells (Tregs) trained in the thymus on self-antigens, which reduces anti-tumor activity. To address this pitfall, we designed the JanusMatrix algorithm to identify tumor-specific sequences capable of Treg activation by parsing candidate sequences into MHC-facing and T cell receptor (TCR)-facing sequences. As epitopes with shared TCR-faces can cross-react with the same T cells, cancer epitopes that share TCR-faces with multiple human sequences may cross-react with thymic-derived Tregs and are thus counter-indicated for immunotherapy. Retrospective analysis of non-small lung cell cancer neo-epitopes showed up to ~30% of neo-epitopes in a single patient exceed the cutoff for significant TCR-face homology with human proteins. Based on prior validation of Treg activating epitope predictions in infectious disease studies, we expect application of JanusMatrix to the neo-epitope discovery pipeline will focus candidate selection on high-value sequences. Neo-epitopes 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



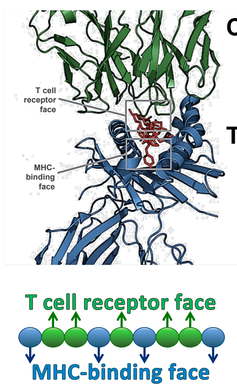
Adapted from Schumacher and Schreiber Science 348, 69-74. 2015





JanusMatrix Discovers Potential Treg 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.

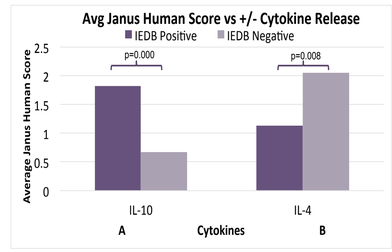


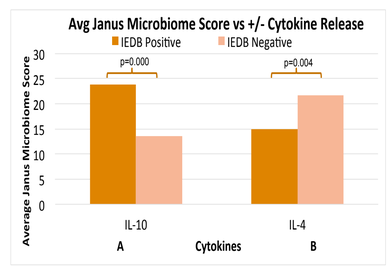
Cross-reactive peptides:

- 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 (P1, P4, P6, P9) using EpiMatrix.
- JanusMatrix searches for potentially cross-reactive TCR by screening TCR-facing residues (P2, P3, P5, P7, P8) against a preloaded, EpiMatrix-processed reference databases.



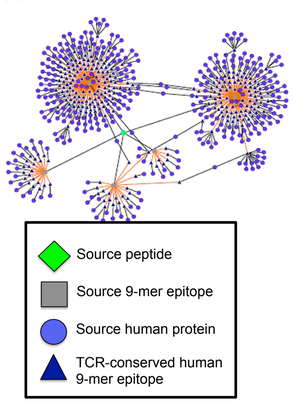


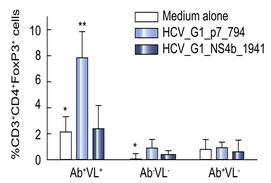
A) Peptides documented as IL-10-positive in IEDB have significantly higher potential for Human Genome (HG, above) and Human Microbiome (HM, below) cross-reactivity as measured by Janus Matrix.

B) Peptides documented as IL-4-positive in IEDB have significantly lower potential for HG and HM cross-reactivity as measured by Janus Matrix.

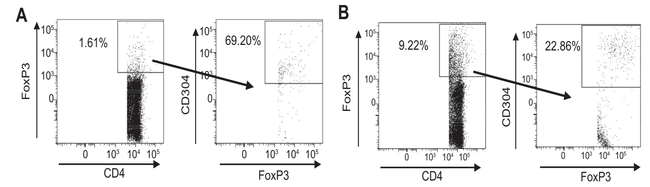
Predicted Treg-activating HCV sequence possesses TCR faces shared by numerous human proteins

Epitope networks are shown, illustrating the abundance of TCR faces one HCV peptide shares with the human genome as determined by JanusMatrix analysis. The HCV source peptide is represented by a green diamond, its constituent 9-mer epitopes by gray squares, their cross-conserved partners in the human genome by blue triangles, and the source human proteins by light purple circles





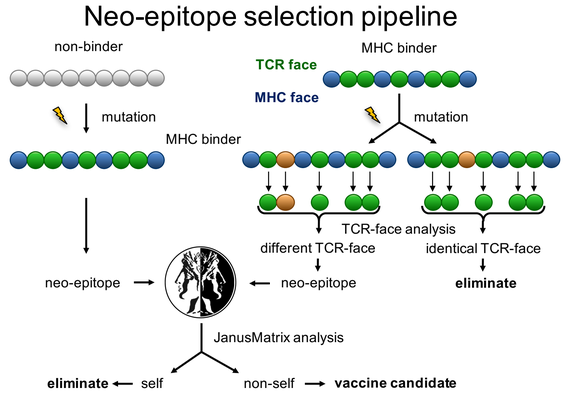
- HCV_G1_p7_794 expands Tregs in chronic HCV-infected individuals but not in those who clear infection or are not infected.
- Its human analog expands Tregs in uninfected and chronic infected subjects, while a control peptide HCV_G1_NS4b_194, does not for any group.



- $CD3^+CD4^+FoxP3^+$ cells from chronic HCV-infected individuals, cultured in the absence of HCV_G1_p7_794 express CD304 (neuropilin), indicative of nTreg cells.
- The vast majority of $CD3^+CD4^+FoxP3^+$ cells, cultured in the presence of HCV_G1_p7_794, were $CD304^-$ characteristic of iTreg cells.

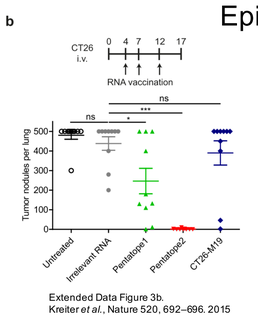
Cancer Vaccine Epitope Selection

Neo-epitope selection pipeline

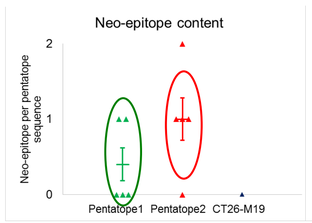


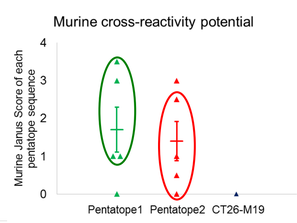
- EpiMatrix identifies neo-epitopes by analyzing the binding potential of normal and mutated peptides.
- JanusMatrix further down selects candidate epitopes based on their potential to cross-react with self-proteins.

EpiMatrix analysis of cancer vaccine efficacy



Extended Data Figure 3b.
Kreiter *et al.*, Nature 520, 692–696. 2015





- Pentatopes were constructed from mutated sequences derived from colon carcinoma model CT26 (study performed by Kreiter *et al.*).
- Pentatope2 showed greater antitumor activity compared to Pentatope1 in BALB/c mice inoculated with CT26 tumor cells.
- EpiMatrix identified a higher number of neo-epitopes in Pentatope2 compared to Pentatope1. Sequences from Pentatope2 also have a decreased potential to cross-react with other murine proteins, as identified by JanusMatrix.

Conclusions

- **Sequence analysis of the two faces 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.**

References

- Moise L, Gutierrez A, Kibria F, Martin R, Tassone R, Liu R, Terry F, Martin B, De Groot AS. 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.
- Moise L, Gutierrez AH, Bailey-Kellogg C, Terry F, Leng Q, Abdel Hady KM, VerBerkmoes NC, Sztein MB, Losikoff PT, Martin WD, Rothman AL, De Groot AS. The two-faced T cell epitope: examining the host-microbe interface with JanusMatrix. Hum Vaccin Immunother. 2013 Jul;9(7):1577-86.
- Losikoff PT, Mishra S, Terry F, Gutierrez A, Ardito MT, Fast L, Nevola M, Martin WD, Bailey-Kellogg C, De Groot AS, Gregory SH. HCV epitope, homologous to multiple human protein sequences, induces a regulatory T cell response in infected patients. J Hepatol. 2015 Jan;62(1):48-55.
- Kreiter S, Vormehr M, van de Roemer N, Diken M, Löwer M, Diekmann J, Boegel S, Schrörs B, Vascotto F, Castle JC, Tadmor AD, Schoenberger SP, Huber C, Türeci Ö, Sahin U. Mutant MHC class II epitopes drive therapeutic immune responses to cancer. Nature. 2015 Apr 30;520(7549):692-6.