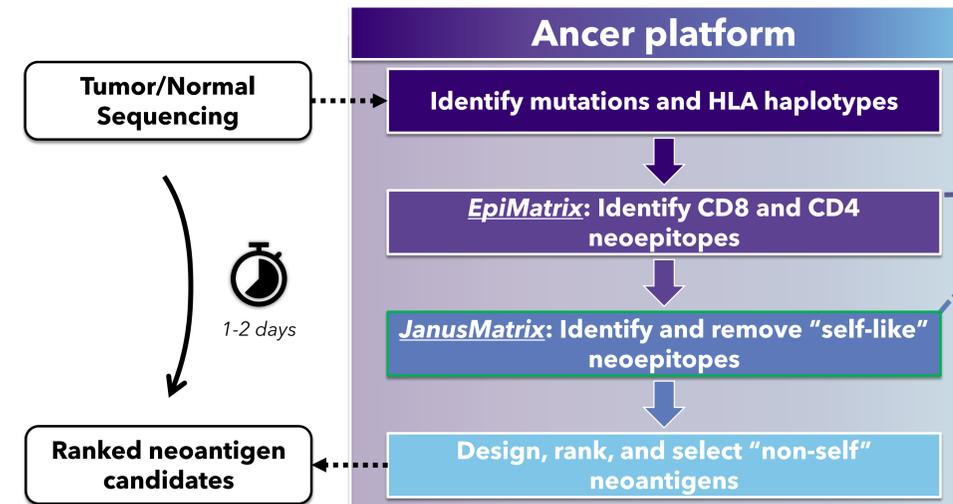


Overview

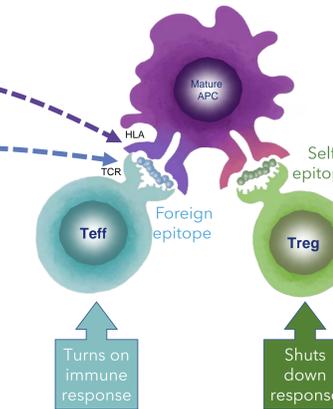
- Hypothesis:** Tumor clones surviving checkpoint inhibition therapy harbor mutations more prone to immune avoidance.
- Approach:** Tumors from melanoma patients collected **before and after** nivolumab immunotherapy (n=41) were analyzed with **Ancer, an advanced neoepitope screening platform** that combines proprietary machine learning-based **CD8 and CD4** epitope mapping tools with removal of **inhibitory Treg** epitopes [1,2].
- Results:** **Mutations gained after nivolumab therapy are less immunogenic and more tolerogenic** compared to mutations found prior to therapy.
- Response to therapy is associated with Ancer results.**
- Summary:** Our Ancer analysis suggests that nivolumab therapy affects the immunogenicity and tolerance profiles of newly generated mutations in a manner that is **consistent with the concepts of immunoediting and immune camouflaging**.

Background - Mutanome-Directed Cancer Immunotherapy Based on 25 Years of Experience in Epitope Mapping

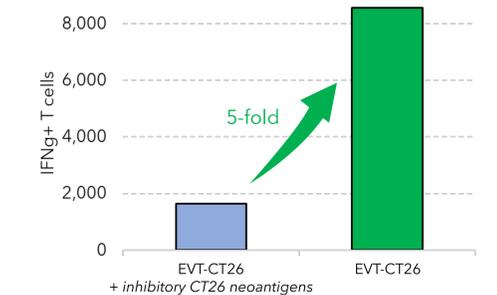


HLA binding predictions. *EpiMatrix* Class I and Class II predictions are respectively **95% and 74% accurate** [1].

Foreign vs self epitopes: *JanusMatrix* identifies **inhibitory (self-like) epitopes** *in silico*. Validation studies with pathogens [3, 4, 5] and cancer mutanomes [6].

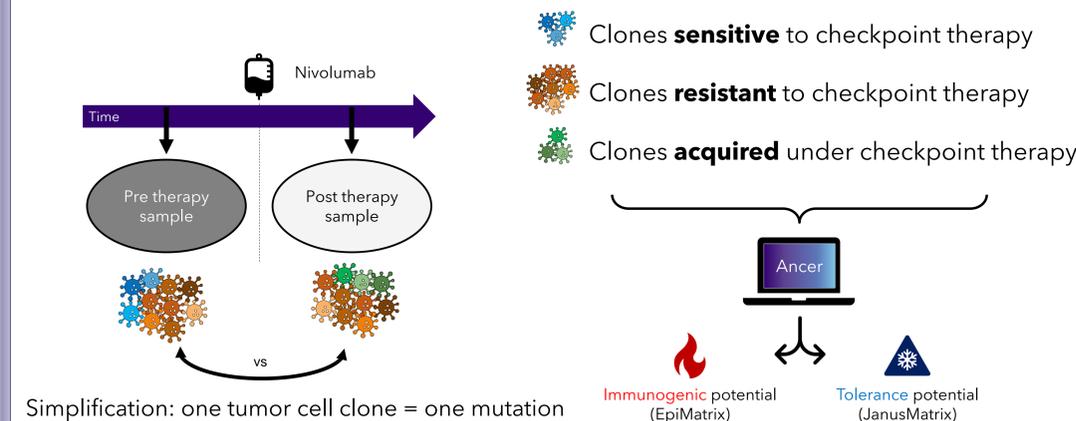


CT26 self-like neoepitopes (**identified in silico with JanusMatrix**) suppress IFN γ production to EVT-CT26 vaccine [6]



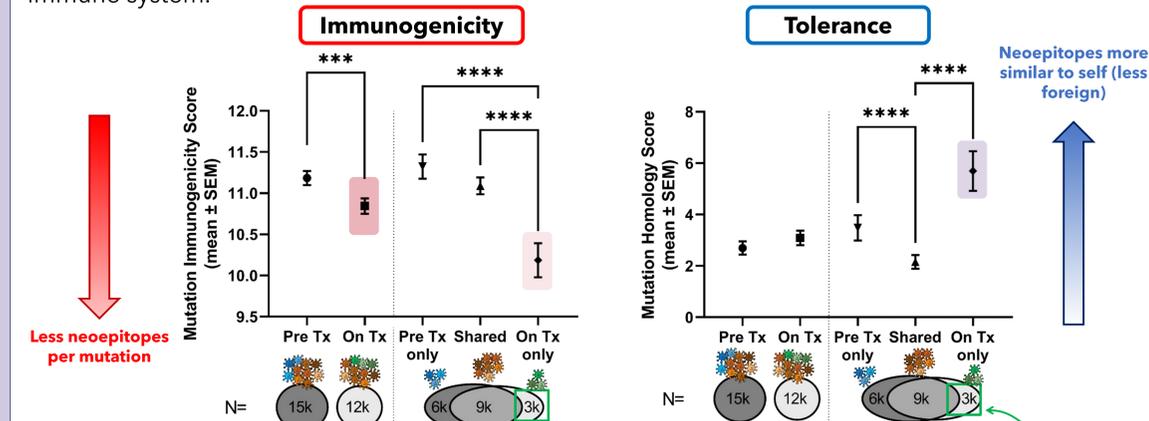
Approach - Ancer analysis of melanoma samples

- Forty-one pairs of mutanomes collected before (*Pre*) and while on (*Post*) nivolumab therapy were retrieved from [7].
- Pre and Post mutanomes were compared to identify deleted, maintained, and newly acquired mutations.
- Immunogenic and tolerance potentials were calculated for all mutations with Ancer.



Results - Checkpoint therapy affects tumor immune profile

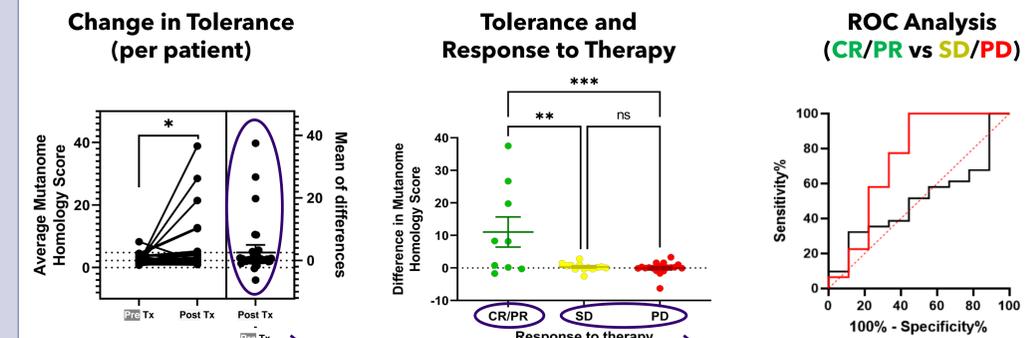
- Mutations are less immunogenic (less neoepitopes) in tumors Tx collected after nivolumab therapy
 - Mutations acquired after nivolumab therapy have low immunogenicity
 - Mutations acquired after nivolumab therapy are likely to be tolerated due to high homology with self antigens
- Tumors respond to immunotherapy by reducing their immunogenicity and by avoiding the immune system.



N = 41 paired Pre/On Tx samples. Mutations grouped across all patients. Pre Tx vs On Tx: Mann-Whitney test. Pre Tx only vs Shared vs On Tx only: ANOVA (Kruskal-Wallis, multiple comparisons)

Results - Ancer analysis is associated with response to therapy

- Tumors increase their tolerance potential (JanusMatrix homology scores) after nivolumab therapy.
- Change in tolerance is associated with response to therapy.



N = 41 paired Pre/On Tx samples. Samples paired by patient. Pre Tx vs Post Tx: paired t test. CR/PR vs SD vs PD: ANOVA (multiple comparisons). CR: Complete Response, PR: Partial Response, SD: Stable Disease, PD: Progressive Disease

Summary and Conclusions

- This study demonstrates **the utility of immunogenicity screening tools in the Ancer** platform for streamlined designs of personalized cancer vaccines.
- Our Ancer analysis suggests tumors reduce their immunogenicity (less neoepitopes) and increase their tolerance potentials (mutations more likely to be tolerated) in response to nivolumab therapy. **Mutations acquired after immunotherapy are more "stealth"** than mutations found prior to therapy.
- Ancer can be employed to **identify novel biomarkers** associated with clinical responses. Tumor neoepitopes become more similar to self in patients that respond to nivolumab therapy.
- These results highlight that **identifying relevant mutations for precision immunotherapy (e.g. personalized vaccines) will become more difficult** once patients are treated with a checkpoint inhibitor. Specialized tools, such as JanusMatrix are needed to correctly and quickly identify immunogenic mutations.

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