**Filtering out self-like neoantigens improves immune response to cancer vaccines**

Guilhem Richard1, Bethany Biron1, Christine Boyle1, Matthew Ardito1, Leonard Moise1,2, William Martin1, Gad Berdugo3, Anne S. De Groot1,2

1EpiVax, Inc., Providence, Rhode Island, United States

2University of Rhode Island, Providence, Rhode Island, United States

3EpiVax Oncology, Inc., Providence, Rhode Island, United States

**Computationally identified self-like neoantigens reduce immune responses of immunogenic therapies when included in vaccine formulations.** Clinical studies have highlighted the potential of precision cancer immunotherapy to effectively control the tumor of patients across cancer indications. However, recent studies showcase the difficulty of establishing robust CD8 and CD4 T cell responses. We hypothesize that poor cancer vaccine performance may be due in part to the inadvertent inclusion of suppressive T cell neo-epitopes in neoantigen vaccines that may be recognized by regulatory T cells (Tregs).

To test this hypothesis, we used the Ancer™ system to identify and select neo-epitopes from the CT26 syngeneic mouse model. Ancer™ leverages EpiMatrix® and JanusMatrix™, state-of-the-art predictive algorithms that have been extensively validated in prospective vaccine studies for infectious diseases (Moise et al., Hum. Vaccines Immunother 2015; Wada et al., Sci. Rep. 2017). Distinctive features of Ancer™ over other *in silico* pipelines are its ability to accurately predict CD4 T cell epitopes and to identify tolerated or Treg epitopes.

In a first experiment, optimally selected CT26 neoantigen vaccine candidates were identified with Ancer™ and ranked according to tumor expression level and predicted Class I- and Class II-restricted immunogenicity. Self-like, putative Treg epitopes were removed in this process. Naïve Balb/c mice were immunized subcutaneously with a peptide pool comprised of the 20 highest ranking neoantigens delivered with PolyICLC (Oncovir). Immunization with Ancer™-derived neoantigens induced strong IFNg ELISpot responses compared to controls (p < 0.001). Flow cytometry confirmed the vaccine stimulated multifunctional CD4+ and CD8+ T cells.

In a follow-on experiment, ten self-like neoantigens, from the same CT26 genome, were selected with Ancer™. These neoantigens may be recognized by Tregs due to their high degree of similarity with self, based on JanusMatrix™. Co-administration of the CT26 self-like neoantigens with our optimally designed neoantigen vaccine in naïve Balb/c mice diminished IFNg ELISpot responses by 5-fold compared to vaccination without the self-like neoantigens (p = 0.003).

While it has been well known that Tregs are present in tumors, these results suggest the possibility that tumor-derived neo-epitopes may be recruiting Tregs to the tumor. More importantly, the inadvertent inclusion of Treg driving neoantigens in vaccine formulations may hinder efforts to induce strong T cell-mediated tumor control. *In silico* screening of neoantigen sequences using specialized tools offers the possibility of enriching and designing new vaccines with higher quality candidates. Efforts are ongoing to determine the effect of Ancer™-derived self-like neoantigens on CD4+ and CD8+ T cells and how the inclusion of self-like neoantigens in vaccines affects their efficacy.