Background: Immunologically “cold” tumors, which lack tumor infiltrating lymphocytes (TILs), are minimally responsive to immune checkpoint blockade (ICB). VAX014 is a novel clinical stage targeted oncolytic agent based on recombinant bacterial minicells and previous preclinical work demonstrated intratumoral (i.t.) administration of VAX014 increased TILs and led to effective lymphocyte-dependent clearance of immunologically cold tumors. 1,2 When combined with systemic ICB against PD-1 (αPD-1) and CTLA-4 (αCTLA-4) (tripartite combination), this treatment effect was enhanced and systemically expanded to control or eliminate distal noninjected tumors in most mice. Here, we more deeply explored the underlying immune mechanisms in injected and noninjected tumors following VAX014 treatment alone and in combination with systemic ICB by evaluating tumor immunotranscriptomes, TIL populations/phenotypes, and the potential development of protective immunologic memory.

Methods: The syngeneic B16F10 model (immune desert phenotype) was used for these studies. Initial changes in immunotranscriptomes of injected tumors were evaluated 24 hours following i.t. treatment with VAX014 in mice bearing a single intradermal (i.d.) tumor. A bilateral variation of the i.d. B16F10 model was then used to systematically evaluate changes in immune gene signatures in both injected and noninjected tumors after addition of systemic αCTLA-4, and again after the further addition of αPD-1. Noninjected tumors from the same series of combination treatment groups were then evaluated by flow cytometry to assess and compare changes in TIL populations/phenotypes. Finally, mice surviving tripartite combination treatment were rechallenged with a second round of i.d. B16F10 tumors to evaluate protective long-term immunologic memory.

Results: Immunotranscriptome analysis of injected tumors following i.t. treatment of VAX014 resulted in upregulation of multiple immune gene networks, including Type I/II interferon, dendritic cell function/activation, Natural Killer cell function/activation, antigen processing/presentation, and T cell lymphotaxis and effector function. In distal noninjected tumors, the dual combination of VAX014 with αCTLA-4 resulted in slower tumor growth, upregulation of the same pathways, but also indicated upregulation of Pdcd1 (PD-1). Addition of αPD-1 to the treatment regimen led to clearance of noninjected tumors, coinciding with an increased cytotoxic cell gene signature and increased cytotoxic and effector memory CD8+ TILs. Finally, mice exhibiting complete response following tripartite combination treatment could limit tumor growth or completely protect against B16F10 tumor rechallenge.

Conclusions: VAX014 stimulated rapid local immune activation and lymphocyte-mediated clearance of injected tumors following i.t. administration. When combined with systemic ICB blockade, this treatment effect was systemically expanded to control or eliminate distal noninjected tumors while promoting protective antitumor immunologic memory.

References:

Ethics Approval: All animal studies were conducted in accordance with protocols approved by the Institutional Animal Welfare and Use Committee at San Diego State University.