

PRX303 is an IL-2 Proaerolysin Fusion Protein Toxin that Selectively Targets and Kills FOXP3 Regulatory T Cells: Potential Role as a Vaccine Adjuvant

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The goal of cancer vaccination is the induction or amplification of functionally active, tumor antigen-specific immune cells capable of eradicating tumor cells. A recent summary of 1306 cancer vaccine treatments for patients with solid tumors reported an overall objective response rate of 3.3%, emphasizing the need for new vaccine approaches. Although CD4⁺ T cells can provide help to enhance CD8-mediated responses, a subset of these cells, the CD25⁺CD4⁺ T regulatory (Treg) cells, possesses the ability to suppress T cell responses and regulate tolerance to self proteins. In mice, Tregs can inhibit the ability to vaccinate against tumor antigen(s) (Ag). If Tregs are reduced before vaccination, effective antitumor responses can be achieved with self Ag vaccination and recruitment of high-avidity tumor Ag specific CD8⁺ effector T cells. To achieve selective depletion of Tregs in vivo, a fusion protein, PRX303, was generated by fusing the cytokine IL-2 to a modified form of the potent bacterial toxin Proaerolysin (PA). PA is a pore-forming toxin that contains a cell binding, a channel forming, and an inhibitory domains. PA binds to GPI-anchored proteins expressed on the surface of mammalian cells. Cleavage of the C-terminal inhibitory domain by furin proteases results in rapid membrane insertion, heptamerization and formation of stable pores in the cell membrane that produce loss of membrane integrity and lytic cell death. PA is lytic to cells in vitro with an IC₅₀ of 1-10 pM but has no therapeutic index in vivo. To redirect and selectively target PA's potent cytotoxicity, a point mutation was first introduced into the GPI anchor binding domain to generate a toxin that is ~3-4 orders of magnitude less toxic than PA. Subsequently, PRX303 was generated by fusing this modified PA to IL-2 via a urokinase sensitive linker to produce a protoxin that is toxic to IL-2 receptor expressing T cells at sub nM concentrations, but is inactive against IL-2 receptor negative T cells at a dose up to 100 nM. While a single IV dose of 0.2 micrograms of PA is 100% lethal, a dose of 15 micrograms of PRX303 produces no toxicity. A single dose of PRX303 reduced levels of Tregs by 50% at 24 h post treatment. Significant augmentation of immune response to both a Vaccinia virus and *Listeria monocytogenes* vaccine was also observed following a single IV injection. PRX303 also decreased Treg count by 50% in mice bearing 4T1 mouse breast cancer xenografts and three consecutive doses of 10 micrograms of PRX303 were well-tolerated and markedly reduced Tregs in tumor draining lymph nodes. These preliminary results suggest that PRX303 may be effective as a vaccine adjuvant through its ability to selectively kill Tregs. Further studies are underway to evaluate the ability of PRX303 to augment antitumor response and prolong survival following vaccination with a variety of cell-based vaccines.