Background: The GITR receptor requires clustering in the plane of the membrane in order to activate downstream signaling events. To provide this clustering, a multimeric form of soluble GITRL was produced by genetically fusing the extracellular domain of GITRL with the body of surfactant protein D, a self-assembling molecule with 4 trimeric arms (Stone et al. J. Virol. 80:1762-72, 2006). The resulting molecule, SP-D-GITRL, is a strong activator of GITR that reverses Treg suppression of the mixed leucocyte reaction in vitro (Stone et al. Clin. Vaccine Immunol. 13:1223-1230, 2006).

Methods: For DNA vaccine studies, mice were vaccinated i.m. with a plasmid for HIV Gag antigen every 2 weeks. Plasmids for multimeric forms of GITR (SP-D-GITRL) or CD40L (SP-D-CD40L) were added to the injections as adjuvants. Two weeks later, T cell and antibody responses were measured. For tumor immunotherapy studies, established A20 lymphoma tumors >4 mm in diameter were injected peritumorally every other day X 5 with plasmids encoding multimeric GITRL or CD40L.

Results: When used as an adjuvant in a DNA vaccine, multimeric GITRL enhanced CD8+ T cell responses, particularly CD8+CD62L+ central memory cells. Multimeric GITR also enhanced CD4+ T cell proliferative responses to the vaccine antigen and was a strong activator for IgG antibody responses. When used for tumor immunotherapy, peritumoral injections of DNA encoding either multimeric GITR or CD40L cured mice of A20 lymphoma tumors.

Conclusions: Multimeric GITR, which has been shown to obviate Treg-mediated immunosuppression in vitro, can be used as an adjuvant for CD4+ T cell, CD8+ T cell, and antibody responses to DNA vaccination. Peritumoral injections of plasmid DNA encoding multimeric GITRL cured mice with A20 lymphoma, a tumor known to be rich in intratumoral Tregs. These data indicate that multimeric GITR has significant potential as a vaccine adjuvant and tumor immunotherapy agent.