The breakthrough discoveries of checkpoint inhibitors in the field of tumor immunology have driven the clinical success of immunotherapeutics for cancer, despite their beneficial efficacy in only a small portion of patients. This is due in part to immune-evasive mechanisms and the inability of the immune system to recognize tumor antigens as foreign.

As a therapeutic approach to effectively present these tumor antigens in order to elicit an anti-tumor immune response, we previously designed and characterized an allogenic, Gp96-Ig secreting, cell-based vaccine (ImpACT), currently being assessed in a phase II study in non-muscle invasive bladder cancer and a phase Ib study in non-small cell lung cancer – the latter, in combination with the PD-1 antagonist Nivolumab.

We recently characterized a "next-generation" vaccine (ComPACT) that combines the tumor antigen chaperone Gp96-Ig along with the T cell costimulator OX40L-Fc, which are both secreted from the same cell (Fromm et al. Cancer Immunology Research 2016). In preclinical assays, ComPACT is effective at stimulating CD8+ and CD8+ antigen-specific T cell expansion, the programming of a durable memory T cell phenotype, and the elimination of melanoma and colon tumors. This anti-tumor efficacy is enhanced when ComPACT is combined with checkpoint inhibition (anti-PDL1 or anti-PDL1).

To support manufacturing and clinical efforts of both ImpACT and ComPACT, in anticipation of phase III expansion and new trial initiation, we have developed novel potency assays to quantify the biologically active form of Gp96-Ig and the in vitro activity of OX40L-Fc on T cell costimulation.

It has been shown that Gp96 can interact with toll-like receptors (TLRs) and that this interaction results in the activation of the NF-κB pathway. Since Th1 cells express abundant TLR2, we engineered a THP1 cell line to express luciferase that is regulated by NF-κB response elements. Furthermore, we utilized the human T cell line, Jurkat, as host cells in which to also express NF-κB-luciferase, to quantify OX40L-Fc costimulation. Jurkat NF-κB-luciferase cells primed with CD3 and CD28, and subsequently cultured with ComPACT-secreted OX40L-Fc, results in a dose dependent increase in NF-κB (luciferase) expression.

Our current data in both assays shows a correlation with the input of Gp96-Ig and OX40L-Fc, a dose dependent increase in NF-κB activity of OX40L-Fc on T cells. Furthermore, we utilized the human T cell line, Jurkat, as host cells in which to also express NF-κB-luciferase, to quantify OX40L-Fc costimulation. Jurkat NF-κB-luciferase cells primed with CD3 and CD28, and subsequently cultured with ComPACT-secreted OX40L-Fc, results in a dose dependent increase in NF-κB (luciferase) expression.

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