FG-3165 IS A NOVEL GALECTIN-9 NEUTRALIZING ANTIBODY THAT INHIBITS GALECTIN-9-MEDIATED DIMERIZATION OF TIM-3 AND GALECTIN-9-INDUCED APOPTOSIS OF CD4+ AND CD8+ T CELLS

Background
Galectin-9 (Gal-9) is a β-galactoside binding lectin that contains two conserved carbohydrate-recognition domains. Gal-9 is produced by a variety of cell types and binds to cell-surface receptors on immune cells to promote an immunosuppressive phenotype within the tumor microenvironment. One well characterized Gal-9 receptor that is thought to play a key role in mediating the effects of Gal-9 on T cells, is T-cell immunoglobulin mucin-3 (TIM-3). Here we describe the in vitro characterization of FG-3165, a humanized monoclonal anti-Gal-9 antibody that is being developed for the treatment of solid tumors.

Methods
The K_D of FG-3165 for recombinant human Gal-9 was determined using a solution equilibrium assay based on a method described previously. Effects of Gal-9 +/- FG-3165 on TIM-3 dimerization on the cell surface were assessed using a PathHunter® TIM-3 dimerization assay from Eurofins-DiscoverX. For assessment of T cell apoptosis, human CD4+ or CD8+ T cells were activated with CD3/CD28 beads and treated with 1 μg/mL human Gal-9 +/- FG-3165 for 48 hours. Cells were then stained with annexin V and propidium iodide (PI) and analyzed by flow cytometry. RNAseq was also performed to evaluate the transcriptional changes occurring in activated CD8+ T cells treated with Gal-9 +/- FG-3165 for 3 hours and 6 hours.

Results
The observed K_D of FG-3165 for human Gal-9 was 0.4 ± 0.2 nM (n = 25). Dimerization of TIM-3 was shown to occur upon treatment of cells with Gal-9 and FG-3165 inhibited this dimerization with an IC50 of ~25 nM. As reported by others, Gal-9 induced apoptosis of both CD4+ and CD8+ T cells. FG-3165 inhibited Gal-9-mediated apoptosis with IC50s of 6.1 nM and 1.3 nM for CD4+ and CD8+ T cells, respectively. RNA-seq analysis revealed that an early transcriptional response to Gal-9 in CD8+ T cells, including regulation of interleukin, proliferation and apoptosis signaling genes, was normalized by FG-3165.

Conclusions
FG-3165 binds with sub-nanomolar affinity to human Gal-9, preventing Gal-9-induced dimerization of TIM-3, and inhibiting apoptosis of both cytotoxic T cells and T helper cells. These data indicate that neutralization of Gal-9 by FG-3165 may overcome an important immunosuppressive mechanism in the tumor microenvironment, positioning FG-3165 as a promising candidate for the treatment of solid tumors.

REFERENCE

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