**Characterization of the Response of Human T cells to an Agonistic Anti-CD27 mAb**

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**CD27 Background**

Member of tumor necrosis super family of receptors (TNFRSF7). It is constitutively expressed on T cells, B cells and a subset of NK cells. It plays a key role in T cell activation, survival, proliferation and cytokines upon interaction with ligand CD27L (CD70). Monoclonal antibodies to the CD27 molecule have been shown to be useful in effectively modulating immune responses including antitumor immunity in preclinical models (Roberts, CI et al. 2010 journal of immunotherapy; French RR et al. 2007 Blood; Keller AM et al. Immunity 2008).

**CDX 1127 (Human anti-CD27 mAb, 1FS)**

Anti-CD27 mAb 1FS is a fully human antibody previously described in preclinical models with anti-tumor, proliferation and cytokine inducing anti-tumor properties (Vitale et al. 2012 Clin. Cancer Res.). In this study we analyze the in vitro activation of human T cells with 1FS in the context of TCR signaling and present data that support a consistent pattern of immune regulation at the mRNA, protein and cellular level. CDX 1127 is currently in Phase I trials as monotherapy to treat lymphoma and solid tumors.

**Expression Profiling with Whole Genome Microarrays**

Gene expression microarray analysis reveal modulation of signaling, protein kinases, growth and dependence & kinetics 441 genes markers consistent with activated pattern of immune regulation at the mRNA level. Oligonucleotide microarray analysis of CDX 1127 Phase I trial subjects to demonstrate in vivo T cell activation.

**Activated T subset analysis following TCR/CD27 costimulation**

Multi-cytokine analysis of CD27 activated T cells with cytokine multiplex Luminex bead arrays

**Summary & Conclusions**

- Anti-CD27 mAb, 1FS can provide co-stimulatory signals to human T cells in a TCR-dependent manner. Concomitant TCR signaling is required for 1FS induced effects. Removal of TCR signaling abolishes any subsequent signaling by 1FS.
- The T cell cytokine response to 1FS and 1FS has a dominant Th1-like pro-inflammatory signature (IFNγ, IL-2, TNFα) accompanied by a delayed Th2 cytokine production, IL-4, IL-13, suggesting a regulatory role for the related TNFR1, 1-4-BB (CDS17) to limit overt Th1 activity.
- T Cells proliferating in response to stimulation with OKT3 and 1FS are IFNγ and TNFα producing CD4+ and CD8+ T cells. Proliferating T cells also express markers consistent with activated phenotype.
- Subset analysis of OKT3 and 1FS responding cells further point to effector memory IFNy-CD4 and CD8 T cells.
- Gene expression microarray analysis reveal modulation of signaling, protein kinases, growth and cytokine-chemokine pathways.
- These in vitro characterizations will be used to guide the biomarker analysis of CDX 1127 Phase I trial subjects to demonstrate in vivo T cell activation.