An Engineered Bi-Functional Fusion Protein (CD86-Fc-NKG2a) to Reverse NK Cell Tolerance of Malignant Cells

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**Abstract**

As clinical outcomes improve for immunotherapeutic regimens based on PD-1/L1 blockade, the proportion of cancer patients who develop resistance to PD-1/L1 blockade will increase. Some resistance mechanisms, including MHC-I and JAK3 downregulation, reflecting the nature of PD-1/L1 as a T-cell centric checkpoint. Recent clinical success by groups targeting non-T cell immune cells has highlighted the potential of enhancing macrophage or NK cell targeted immunotherapies for the treatment of a wide variety of cancers. Future combinatorial approaches will likely utilize agents targeting multiple different immune cells to effectively arm both the adaptive and the innate immune systems. As a prime example of this, the anti-NKG2a, monoclonal antibody Monalizumab has shown promising preclinical and clinical anti-cancer effects, especially when combined with an ADC-competent antibody [1]. Therefore, targeting non-classical immune checkpoints has the potential to synergize with currently approved cancer therapies. Here we report the generation of a two-sided fusion protein, CD86-Fc-NKG2a, which was designed to provide competitive inhibition of the NK-centric HLA-E checkpoint, which provides co-stimulation to T cells via CD28.

**Methods**

Human and murine variants of CD86-Fc-NKG2a were produced and characterized using a variety of biochemical assays to determine the correct molecular weight, subunit composition and binding affinity, molecular assays to characterize in vitro cell binding, in vitro functional activity, in vitro lysin capabilities; and anti-tumor efficacy in multiple syngeneic model systems.

**Results**

The NKG2a domain bound recombinant and cell-expressed HLA-E with high affinity. The CD86 domain bound recombinant CD8 and CTLA-4, with a higher affinity for CTLA-4. When tested head-to-head in a therapeutic CT26 murine tumor model, mCD86-Fc-NKG2a demonstrated stronger anti-cancer activity compared to anti-NKG2a antibody controls. The combination of the CD86-Fc/NKG2a with an anti-PD1 antibody was effective in controlling tumor growth in immune competent mice. In vitro cell lysis assays, with P815s, have further elucidated the effect of CD86-Fc-NKG2a on NK cell mediated tumor cell death.

**Summary**

- ARCs are a novel class of bi-functional biologics capable of targeting type-I and type-II membrane proteins; and can target all checkpoint molecules, the entire family of TNFR superfamily receptors, and NK stimulatory molecules.

- CD86-Fc-NKG2a can block HLA-E/NKG2a interactions on NK and T cells, while simultaneously delivering a potent co-stimulatory signal through CD28, resulting in enhanced tumor cell killing and antitumor in vivo activity.

**References**