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

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#P810

Abstract

Background: 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 β2M 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 ADCC competent antibody [1]. Therefore, targeting non-classical innate 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 providing co-stimulation to T cells via CD28.

Methods: Human and mouse 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 lysis 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 CD28 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 PBMCs, have further elucidated the effect of CD86-Fc-NKG2a on NK cell mediated tumor cell death.

Fusion Protein Platform

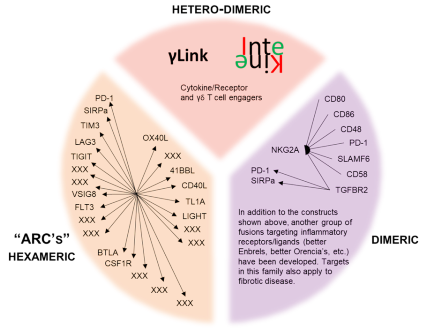

Figure 1. ARC Platform. Agonist Redirected Checkpoints consist of a type I membrane protein extra-cellular domain (ECD), linked to a type II ECD, via a central domain (i.e. Fc). Over 250 unique constructs have been synthesized to date, and include combinations of all checkpoint molecules, the entire family of TNFSF ligands, and NK stimulatory molecules.

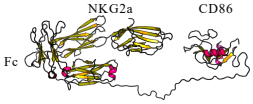

Figure 2. Dimeric ARC Structure. Tertiary structure prediction of the CD86-Fc-NKG2a ARC.

In Vitro Characterization and Function

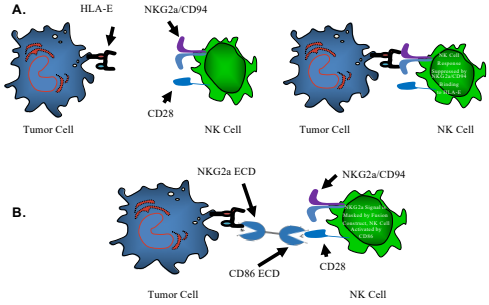

Figure 3. MOA. (A) HLA-E can interact with NKG2a expressed on NK cells or T cells to suppress cytolytic cell activation. (B) CD86-Fc-NKG2a can block the immune suppressive signals and simultaneously provide NK/T cell co-stimulation.

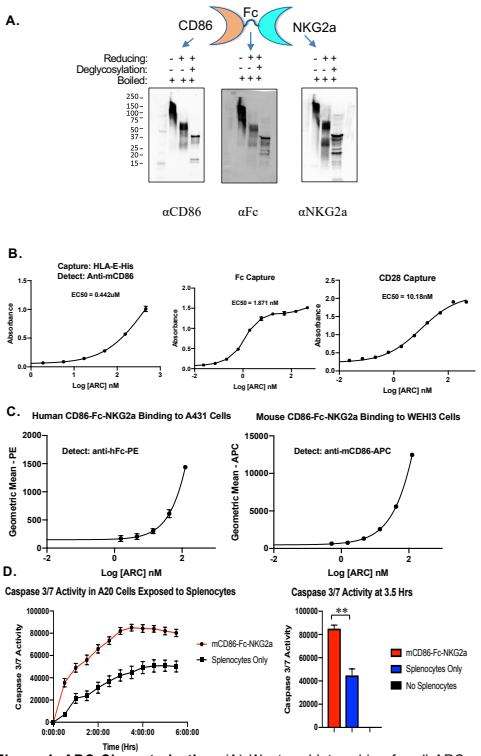

Figure 4. ARC Characterization. (A) Western blot probing for all ARC domains. (B) Representative ELISA binding of HLA-E and CD28. (C) CD86-Fc-NKG2a binds to the cell surface of HLA-E (Qa-1 for mouse) positive tumor cells. (D) CD86-Fc-NKG2a enhances splenocyte mediated killing of murine lymphoma cells in co-culture at 0.34 ug/ml. **p<0.01.

Anti-Tumor Efficacy and Synergy with Checkpoint Blockade

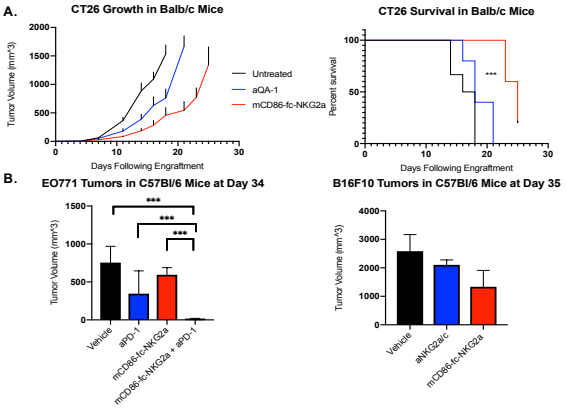

Figure 5. Anti-tumor efficacy (CT26, EO771, B16F10). 3 treatments of ARCs (300 μg each) and/or antibodies (100 μg each) were given on days 7, 10, and 14 following tumor inoculations, when starting tumor volumes were **equivalent**. Monotherapies and combinations with (B) anti-PD1 (RMP1-14) are shown, along with survival.

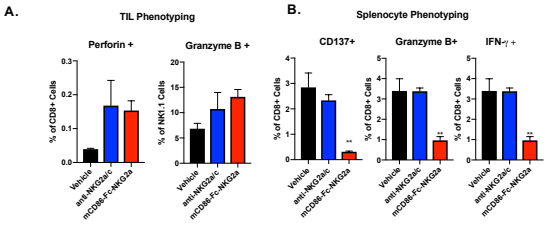

Figure 5. Immune Phenotyping. (A) B16F10 tumors (day 7 post treatment) and Splenocytes (day 7 post treatment) collected from B16F10 bearing mice treated with mCD86-Fc-NKG2a and/antibodies as above, and phenotyped using flow cytometry. **p<0.01

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

1. Andre´ P, Denis C, Soulas C, *et al*. Anti-NKG2A mAb is a Checkpoint Inhibitor that Promotes Anti-tumor Immunity by Unleashing Both T and NK Cells. Cell. 2018 Dec 13; 175(7): 1731-1743.e.13.