SYNERGISTIC COMBINATION OF NATURAL KILLER CELL ENGAGERS (NKES) WITH PROINFLAMMATORY CYTOKINES

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**Background**
While promoting antigen-dependent T cell responses has proven successful in a subset of patients and in certain tumors, the emergence of resistance and tumor heterogeneity with respect to MHC class I expression suggests additional approaches be considered. Expanding the anti-tumor response to include natural killer (NK) cell activation could broaden the clinical impact against tumors with heterogenous MHC I status. Our aim was to develop a new format capable of stimulating both the innate and adaptive arms of immunity, to induce direct tumor elimination while also eliciting multacellular responses to promote durable tumor control. NK cells’ intrinsic anti-tumor activity and low toxicity profile make them an attractive effector cell population for immunotherapy.

We designed tumor antigen targeted NK cell engaging antibodies (NKEs) that synergistically activate NK cells via NKG2D or NKp46 agonism with simultaneous engagement of Fc gamma receptors via the Fc domain. In addition to stimulating NK cells, NKG2D targeting NKEs also provide co-stimulation to CD8 T cells.

**Methods**
Expanding on Xencor’s XmAb protein engineering platform, we developed tumor-targeted NKE molecules agonizing multiple activation pathways including NKG2D or NKp46, together with FcγRIIIa engagement through the intrinsic Fc domain interaction. Functional activity of these novel NKE molecules was evaluated through assessment of anti-tumor cytotoxicity, production of proinflammatory cytokines, and activation of NK and T cells.

**Results**
Co-engagement of activating receptors, NKG2D or NKp46, with FcγRIIIa enhanced production of IFNγ and increased cytotoxicity towards tumor cells. These activities were further enhanced in the presence of proinflammatory cytokines, including IL-15. One of the challenges associated with targeting multiple receptors expressed on effector cells is a potential induction of effector cell fratricide. To address this, we tuned NKG2D and FcγR affinities to balance the desired anti-tumor activity with the off-target activity against effector cells. In a parallel approach, we developed NKEs targeting NKG2D ligands MICA/B. MICA/B NKEs activate effector cells by co-targeting a tumor associated antigen and MICA/B on the surface of target cells, facilitating their interaction with NKG2D on effector cells. MICA/B NKEs showed potent anti-tumor activity in vitro and induced production of IFNγ by effector cells.

**Conclusions**
XmAb NKE molecules designed to activate NK and T cells show potent tumor cell lysis and cytokine production activities that are further enhanced in the presence of proinflammatory cytokines. Future directions include evaluation of in vivo activity and establishment of safety profile.