Synergistic combination of Natural Killer cell Engagers (NKEs) with proinflammatory cytokines

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SITC 2022 Abstract #1067

Introduction

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 multicellular responses to promote durable tumor control. We designed tumor antigen specific NK cell engaging antibodies (NKEs) that synergistically activate NK cells via NKG2D 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. Co-engagement of an activating receptor NKG2D with FcγRIIIa enhanced production of IFNγ and increased cytotoxicity towards tumor cells. These activities were further improved in the presence of proinflammatory cytokines, including IL-15. One of the challenges associated with targeting a receptor expressed on effector cells is the potential induction of effector cell fratricide. To address this, we tuned NKG2D affinity 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 and MICB. 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.

Summary

- We designed tumor antigen specific XmAb® NKE molecules targeting the NKG2D pathway with a simultaneous engagement of FcγR via the Fc domain.
- NKG2D-targeting NKEs designed to activate NK and CD8 T cells show potent tumor cell lysis and IFNγ production.
- Induced cytotoxicity and cytokine secretion with XmAb NKEs are further enhanced in the presence of IL15-Fc.
- The hallmark of NKG2D-targeting NKEs is a potent induction of IFNγ production via the NKG2D pathway agonism, independent of FcγR engagement.
- MICA/B-NKEs activate the NKG2D pathway through its native ligands MICA and MICB. MICA/B-targeting NKEs circumvent effector cell fratricide and block MICA and MICB shedding.
- Future directions include evaluation of *in vivo* anti-tumor activity and establishment of safety profile.

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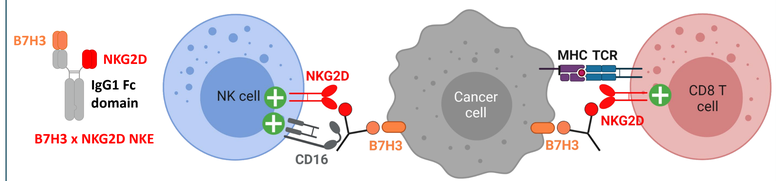
NKG2D-targeting NKEs



NKG2D-NKEs induce NK-mediated anti-tumor cytotoxicity and IFNγ production

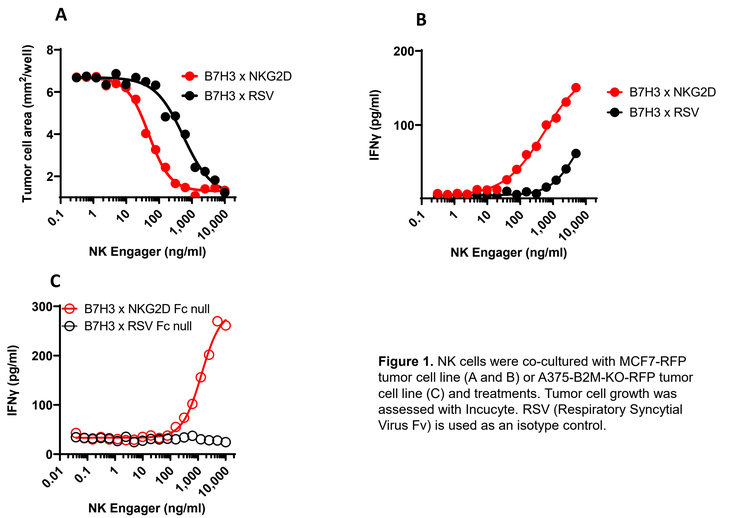


Figure 1. NK cells were co-cultured with MCF7-RFP tumor cell line (A and B) or A375-B2M-KO-RFP tumor cell line (C) and treatments. Tumor cell growth was assessed with Incucyte. RSV (Respiratory Syncytial Virus Fv) is used as an isotype control.

NK cell fratricide depends on NKG2D affinity

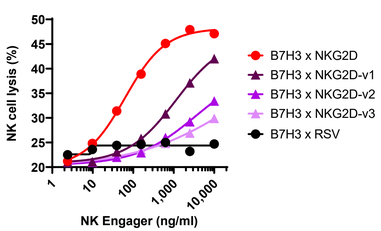


Figure 2. NK cells were co-cultured with treatments for 12 hours. NK cell lysis was assessed via staining with a viability dye, staining was quantified by flow cytometry.

NKG2D-NKEs provide co-stimulation to CD8 T cells

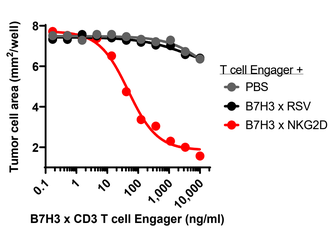
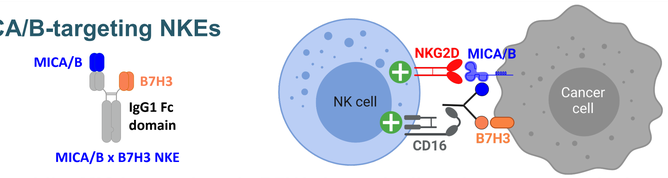


Figure 3. T cells were co-cultured with A431-B2M-KO-RFP tumor cell line and treatments. Tumor cell growth was assessed with Incucyte.

MICA/B-targeting NKEs



High avidity MICA targeting via B7H3 dramatically enhances target lysis and IFNγ production

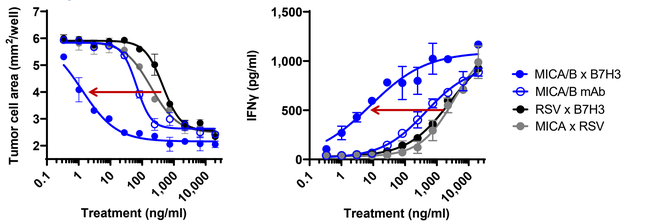


Figure 4. NK cells were co-cultured with A375-B2M-KO-RFP tumor cell line and treatments. Tumor cell growth was assessed with Incucyte. RSV (Respiratory Syncytial Virus Fv) is used as an isotype control.

MICA/B-NKEs signal via NKG2D receptor

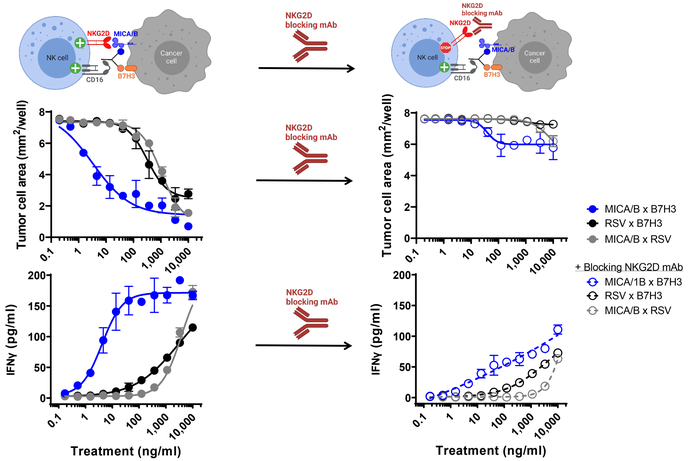


Figure 5. NK cells were co-cultured with A375-B2M-KO-RFP tumor cell line and treatments with or without an NKG2D antibody that blocks MICA binding to the receptor. Tumor cell growth was assessed with Incucyte. RSV (Respiratory Syncytial Virus Fv) is used as an isotype control.

MICA/B-NKEs block MICA shedding

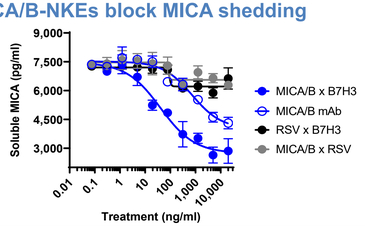


Figure 6. A375-B2M-KO-RFP tumor cell line was cultured in the presence of MICA/B-NKEs or control antibodies. Soluble MICA was measured in the supernatant 24 hours later.

Synergistic combination of NKEs with IL-15

IL15-Fc enhances NKG2D-targeting NKE cytotoxicity and IFNγ production

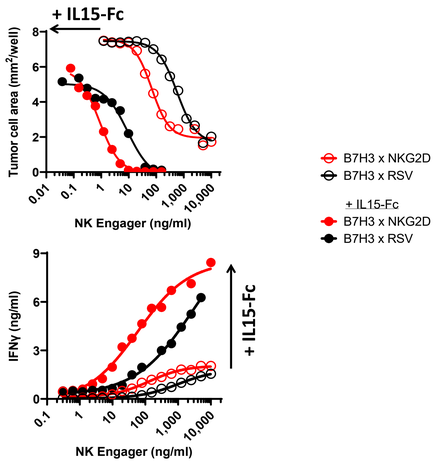


Figure 7. NK cells were co-cultured with A375-RFP tumor cell line and treatments in combination with IL15-Fc. Tumor cell growth was assessed with Incucyte. RSV (Respiratory Syncytial Virus Fv) is used as an isotype control.

IL15-Fc enhances MICA/B-targeting NKE cytotoxicity and IFNγ production

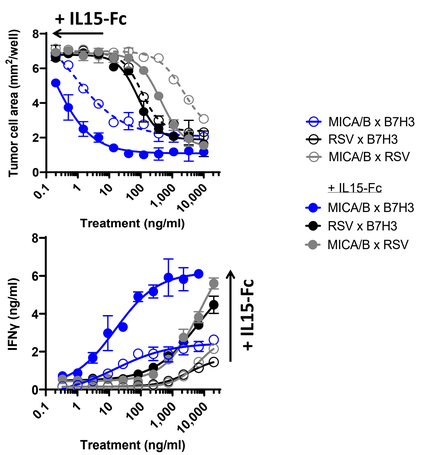


Figure 8. NK cells were co-cultured with A375-RFP tumor cell line and treatments in combination with IL15-Fc. Tumor cell growth was assessed with Incucyte. RSV (Respiratory Syncytial Virus Fv) is used as an isotype control.