Natural Killer cell Engagers activate innate and adaptive immunity and show synergy with proinflammatory cytokines

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Introduction

- Natural Killer cell Engagers (NKEs) are multifunctional molecules that target activating or inhibitory receptors on the surface of NK cells, bind to tumor associated antigens and engage Fc gamma receptors expressed on effector cells of the immune system.
- NKEs promote tumor cell lysis by redirecting NK cells to their targets, and drive activation and proliferation of NK cells.
- Developed NKE molecules showed enhanced NK mediated cytotoxicity and provided co-stimulatory signal to T cells. Combination of NKEs with proinflammatory cytokines resulted in increased cytotoxicity against tumor cells.

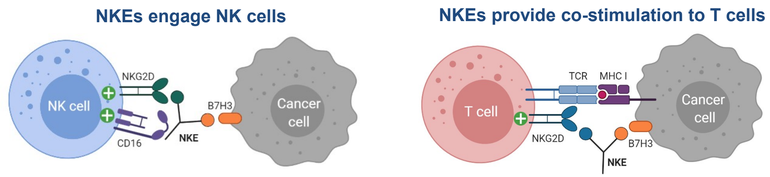

Figure 1. B7H3 x NKG2D NKEs engage NK cells through the simultaneous binding to B7H3, expressed on tumor cells, and activating receptors NKG2D and CD16, expressed on NK cells. Additionally, NKEs provide co-stimulation to T cells via the binding to tumor antigen B7H3 and NKG2D, expressed on T cells.

1. NKEs are designed to engage tumor antigen, NKG2D and FcγR

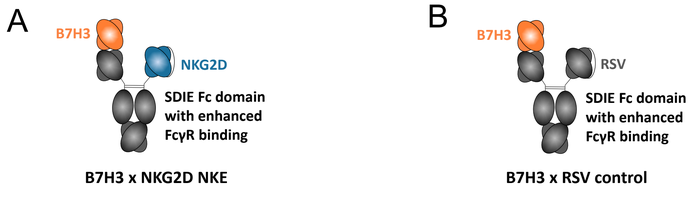

Figure 2. A. Structure of B7H3 x NKG2D NKE bispecific antibody. Modified Fc domain caries S239D/I332E substitutions to enhance FcγR interactions. **B.** Control bispecific antibody B7H3 x RSV, where RSV domain serves as an isotype control.

2. NKG2D engagement enhances FcγR mediated cytotoxicity

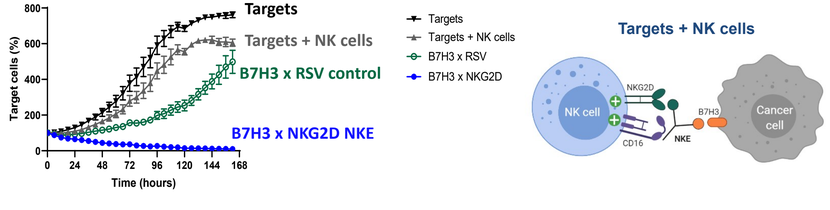

Figure 3. Target cell lysis by NK cells over time in the presence of B7H3 x NKG2D NKE and a control antibody. Resting NK cells were co-cultured with MCF7-RFP tumor cell line and treatments. Tumor cell growth was assessed over time with Incucyte.

3. NKEs augment activation of NK cells

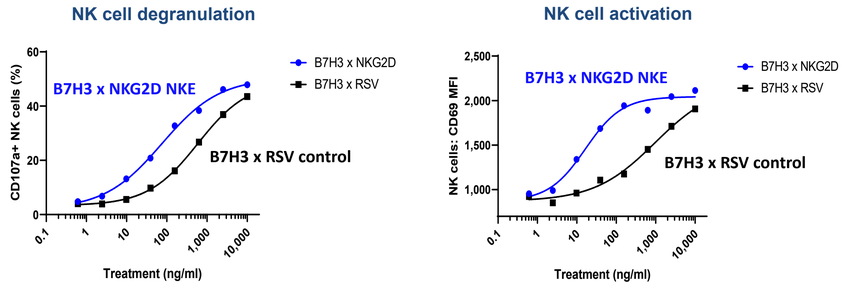

Figure 4. Degranulation and activation of NK cells treated with B7H3 x NKG2D NKE and a control molecule B7H3 x RSV. Resting NK cells were incubated with MCF7 tumor cell line and treatments for 4 hours and assessed on a flow cytometer.

4. Loss of MHC I increases target cell sensitivity to NKEs

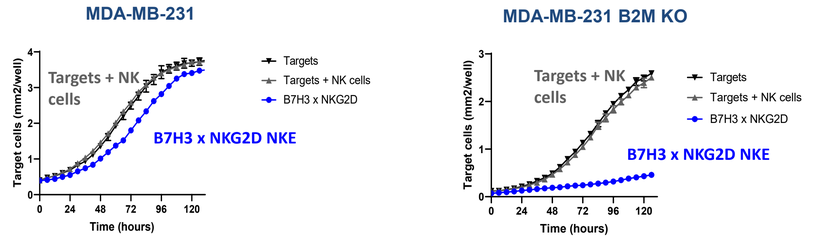

Figure 5. Target cell lysis by NK cells over time in the presence of B7H3 x NKG2D NKE. Resting NK cells were co-cultured with parental MDA-MB-231 cell line or MDA-MD-231 beta-2 microglobulin KO cell line. Tumor cell growth was assessed over time with Incucyte.

5. NKEs provide co-stimulation to T cells

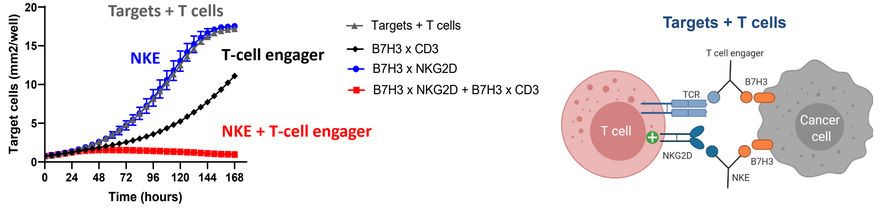

Figure 6. Target cell lysis mediated by T cells in the presence of B7H3 x NKG2D NKE and T-cell engager B7H3 x CD3. T cells were co-cultured with MCF7-RFP tumor cell line and treatments. Tumor cell growth was assessed over time with Incucyte.

6. IL15 and IL12 cytokines enhance NKE activity

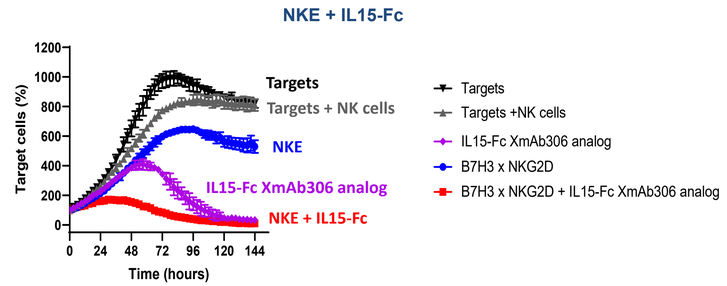

Figure 7. Target cell lysis by NK cells over time in the presence of B7H3 x NKG2D NKE and IL15-Fc. Resting NK cells were co-cultured with OVCAR8-NIR tumor cell line and treatments. Tumor cell growth was assessed over time with Incucyte.

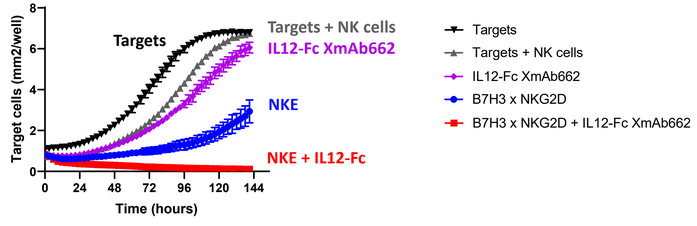

Figure 8. Target cell lysis by NK cells over time in the presence of B7H3 x NKG2D NKE and IL12-Fc. Resting NK cells were co-cultured with MCF7-RFP tumor cell line and treatments. Tumor cell growth was assessed over time with Incucyte.

Summary

- XmAb® bispecific NKE molecules B7H3 x NKG2D activate NK cells, enhance NK cell mediated lysis of tumor cells and provide co-stimulation to T cells.
- B7H3 x NKG2D NKEs show synergistic cytotoxicity in combination with IL15-Fc XmAb306 analog and IL12-Fc XmAb662. This observation opens an opportunity for NK Engagers to be combined with cytokine therapies.

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