The CD30/CD16A innate cell engager AFM13 elicits polyfunctional NK cell responses effectively triggering memory (ML) NK cells against CD30+ targets

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Abstract

Natural killer (NK) cells are crucial innate immune effector cells that rapidly recognize and eliminate infected, stressed and malignant cells. One barrier to broadly applying NK cell therapy across many cancer types is inconsistent cancer cell recognition, which may be overcome by immune cell engagers. AFM13 is a bispecific tetrameric antibody based on the ROC3/2 platform characterized by bispecific tetramer binding to CD30 and CD16A, with clinical efficacy in CD30+ malignancies. Additionally, adoptively transferred memory-like (ML) NK cells have demonstrated enhanced anti-tumor activity that may be receptive to AFM13-based targeting to enhance target cell recognition. However, our understanding of NK cell phenotype of functional responses triggered via AFM13 remains incomplete. To address these questions, we analyzed healthy donor-derived conventional (cNK) and ML (IL-12/15/18-induced) NK cell functional responses to CD30+ lymphoma cells treated with AFM13. Primary cNK cells co-incubated with CD13/CD16A+ cNK cells demonstrated increased IFNγ, TNFα, and TNFα, and degranulation, compared to Hut-78 cells or Raji (CD30+) targets + AFM13 as a negative control (p<0.01). ML NK cells also displayed enhanced cytolytic production and killing of CD30+ tumor targets when co-incubated with Hut-78+AFM13 (p<0.01). To define the single-cell specificity of cNK cell responses to AFM13, similar assays were performed using mass cytometry assessing 39 lineage, maturation, activating and inhibitory receptors, and function-relevant NK cell markers. We then used CITRUS to define the NK cell subsets associated with increased IFNγ, MIP1α, CD107a (p<0.05), CITRUS-based clustering divided the NK cell populations into two main groups that associated with increased effector responses. Based on back-gating, we determined that both mature (NGK2A/CD57+) and immature (NGK2A-CD57-) subsets displayed enhanced IFNγ (p<0.05), MIP1α (p<0.001) in the presence of the Hut-78+AFM13 compared to Hut-78+AFM12 (CD19/CD16A bispecific innate immune cell engager, negative control), with mature producing the most IFNγ and MIP1α compared to immature AFM13 triggered NK cells (p<0.05), or NK cells triggered with AFM12 (p<0.001). AFM13 also resulted in increased frequencies of IFNγ/CD107a+MIP1α+ multifunctional cells, in both mature and immature subsets, compared to AFM12 (p<0.05). Finally, within the NK cells stimulated with Hut-78+AFM13, we observed significantly increased CD57, KIR2DL1/2DL3, KIR3DL1 (p<0.01), NKp30, NKp44, and NKp80 (p<0.01) in IFNγ producing cells compared to IFNγ-negative cells. Overall, AFM13 enhanced the magnitude and quality of NK cell responses against lymphoma targets. Collectively, these data indicate that target cell recognition of NK cells can be significantly enhanced by AFM13, yet influenced by inhibitory receptors expression, maturation state, and memory-like differentiation.

Introduction

Natural killer (NK) cells are cytototoxic innate lymphoid cells that display potent effector responses against tumor cells. However, they are frequently deficient or dysfunctional in cancer patients. Strategies to enhance NK cell functionality and tumor targeting are required to fully harness their anti-tumor potential.

AFM13, a first-in-class tetrameric, bispecific innate immune cell engager is characterized by tetrameric binding to CD30 and CD16A (FgRIIIA). AFM13 binding to CD30+ malignancies potentiates NK cell activation resulting in enhanced cytotoxicity and cytokine secretion. What is the contribution of immune cell engagers along with the enhanced functionality of ML NK cells in the overall response to tumor targets? What are the mechanisms underlying this response enhancement?

Methods

AFM13 binding on CD30+ tumors triggers cytokine secretion and degranulation of cNK cells from normal donors. Frequency of IFNγ, TNF and CD107 positive NK cells induced after stimulation with AFM13-pretreated Hut-78 tumor cells. AFM13 triggering does not modify NK cell cytokine secretion or degranulation when Raji cells were used as targets. Bars represent Mean ± SEM. One-way ANOVA. n=3-5 donors. Data of 3 independent experiments. *p<0.05, **p<0.01

Results

Figure 1. AFM13 binding on CD30+ tumors triggers cytokine secretion and degranulation of cNK cells from normal donors. Frequency of IFNγ, TNF and CD107 positive NK cells induced after stimulation with AFM13-pretreated Hut-78 tumor cells. AFM13 triggering does not modify NK cell cytokine secretion or degranulation when Raji cells were used as targets. Bars represent Mean ± SEM. One-way ANOVA. n=3-5 donors. Data of 3 independent experiments. *p<0.05, **p<0.01

Conclusions

- AFM13 significantly enhances NK cell recognition of CD30+ malignancies correlating with increased NK cell activation.
- NK cell expression of inhibitory receptors, maturation state and memory-like differentiation influence AFM13-induced NK cell response against CD30+ targets.
- AFM13 pretreatment of tumor targets potentiates ML NK cell effector functions including cytokine secretion and cytotoxicity.
- AFM13 pretreatment triggers polyfunctional responses in NK cells compared to AFM12 pretreatment.

Mass Cytometry can be successfully applied to evaluate AFM13-triggered functional responses of conventional and ML NK cells at single cell resolution.

The combination of ML NK cells with AFM13 appears to be a promising therapeutic approach for treating CD30+ malignancies.

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