The CD30/CD16A bispecific innate immune cell engager AFM13 elicits heterogeneous single-cell NK cell responses and effectively triggers memory like (ML) NK cells

Washington University in St.Louis School of Medicine

Nancy Marin[1], Michelle Becker-Hapak[1], Joachim Koch[2], Melissa M. Berrien-Elliott[1], Mark Foster[1], Carly Neal[1], Ethan McClain[1], Sweta Desai[1], Julia A. Wagner[1], Timothy Schappe[1], Lynne Marsala[1], Pamela Wong[1], Martin Treder[2], Todd A. Fehniger[1]

[1]Washington University School of Medicine, Saint Louis, MO, [2]Affimed GmbH, Heidelberg, Germany

Abstract

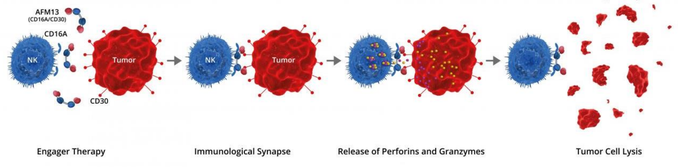
Natural killer (NK) cells are crucial effector cells of the innate immune system capable of rapidly recognizing and eliminating infected, stressed, and malignant cells. NK cells discriminate tumor targets from healthy cells by integrating activating and inhibitory receptor signals. One barrier to broadly applying NK cell immunotherapy across many cancer types is inconsistent cancer cell recognition, which may be overcome by innate cell engagers. AFM13 is a tetravalent, bispecific antibody developed on the ROCK® platform. AFM13 is characterized by bivalent binding to CD30 and CD16A and has demonstrated a favorable safety profile and promising efficacy against CD30+ malignancies in clinical studies as monotherapy and in combination with anti-PD-1. However, our understanding of NK cell functional responses triggered via AFM13 remain incomplete. Moreover, because adoptively transferred memory-like (ML) NK cells have demonstrated enhanced anti-tumor activity (*Romee R et al., Sci Transl Med, 2016*), we expect that they may be receptive to AFM13-based targeting to enhance target cell recognition. To address these questions, we analyzed single-cell conventional (cNK) and ML (IL-12/15/18-induced) NK cell functional responses to NK-resistant CD30+ lymphoma cells +/- AFM13. Primary cNK cells co-incubated with AFM13-treated Hut-78 cells demonstrated increased IFN-γ, TNF, and degranulation, compared to Hut-78 cells or Raji (CD30-) targets + AFM13 as a negative control ($p < 0.05$). To define the single-cell specificity of NK cell responses to AFM13, similar assays were performed using mass cytometry analysis of 39 lineage, maturation, activating and inhibitory receptors, and function-relevant NK cell markers. tSNE-based multidimensional analyses revealed marked distinctions between Hut-78 and AFM13-Hut-78 stimulated cNK cells, due in part to IFN-γ, MIP-1α, CD107a, and CD16. To define the impact of specific NK receptors, SPADE was used to define highly activated IFN-γ+ NK cell sub-populations. In a KIR3DL1+ donor, activation was primarily within the KIR3DL1+ subset, consistent with the lack of its inhibitory ligand on Hut-78 (HLA-Bw4-). In KIR3DL1 negative donors, responding NK cells were enriched in mature KIR2DL2/L3+ CD57+ NK cells that lacked NKG2A. Additional experiments revealed that both control and ML NK cells exhibited increased IFN-γ, degranulation, and cytotoxicity with AFM13 ($P < 0.01$), and AFM13-stimulated ML NK cells exhibited the highest IFN-γ response and killing. Collectively, these data indicate that AFM13 significantly enhanced NK cell target cell recognition. However, NK cell activation is still influenced by inhibitory receptor expression, maturation state, and memory-like differentiation. Thus, these data suggest that the status and repertoire of NK cells in a patient may offer diagnostic potential for therapeutic response, and the combination of ML NK cells with AFM13

Introduction

- Natural killer (NK) cells are cytotoxic innate lymphoid cells that display potent effector responses against tumor cells.
- NK cells are frequently deficient or dysfunctional in cancer patients.
- NK cell activation is regulated by the balance of signals received through a diverse group of activating and inhibitory receptors stochastically expressed on the cell membrane.
- NK cells mediate cytotoxic functions by secreting perforin- and granzyme-containing granules or via death receptor ligands.
- NK cells also communicate and recruit other immune cells by secreting cytokines (e.g. IFN-γ, TNF) and chemokines (e.g. MIP1-α).
- NK cells with memory-like (ML) properties differentiate after a short-term stimulation with IL-12, IL-15 and IL-18 and display enhanced functionality and anti-tumor response.

ML NK cells properties	Description
Proliferation	Function passed to progeny after cell division
Long-lived	> 4 months in vivo in mice, >6 weeks in vivo for human
Non-specific = Flexible	Enhanced response to cytokines, activating receptors, or tumor targets
Produce cytokines	Increased INF-γ, TNF, MIP1-α production in response to restimulation
Kill target cells	Increased granzyme B and killing of leukemia targets
Eliminate tumor cells in vivo	Enhanced elimination of murine and human xenograft malignancies in vivo
Rescue "unlicensed" NK cells	Response by both licensed and unlicensed NK cells
Ignore inhibitory KIR	Response by both KIR ligand matched and mismatched NK cells

- AFM13 is a first-in-class tetravalent, bispecific innate immune cell engager characterized by bivalent binding to CD30 and CD16A (FcgRIIIA).
- AFM13 binding to CD30+ malignancies potentiate NK cells activation and results in enhanced cytotoxicity and cytokine and chemokine secretion.
- AFM13 has shown promising efficacy data in CD30+ malignancies both as monotherapy and in combination with anti-PD-1.

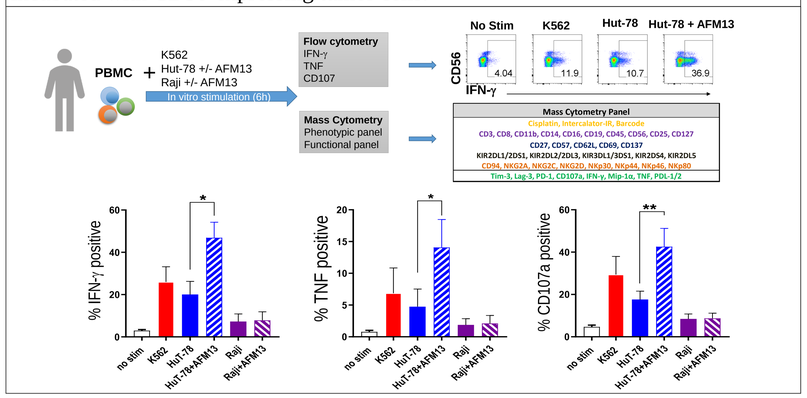


- Based on its favorable safety profile, AFM13 is suitable for combination with alternative approaches to induce NK cells endowed with enhanced ability to recognize and kill tumor targets as potential immunotherapeutic approaches.

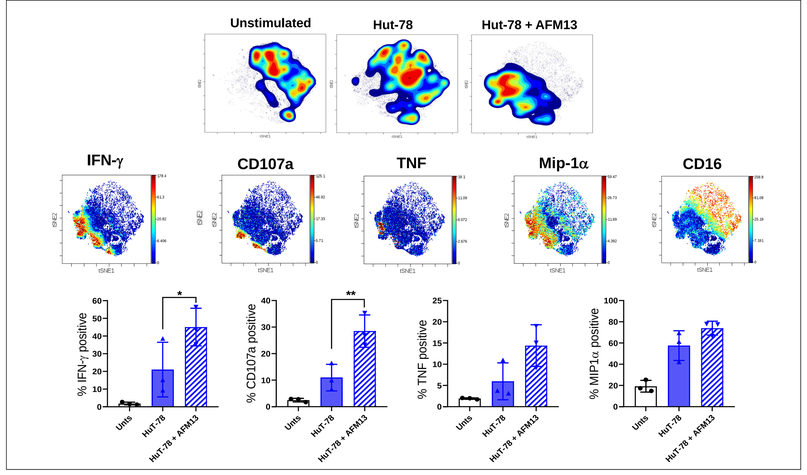
Here, we evaluate the contribution of immune cell engagers along with the enhanced functionality of ML NK cells in the overall response to tumor targets and explore the mechanisms underlying this enhanced response.

Results

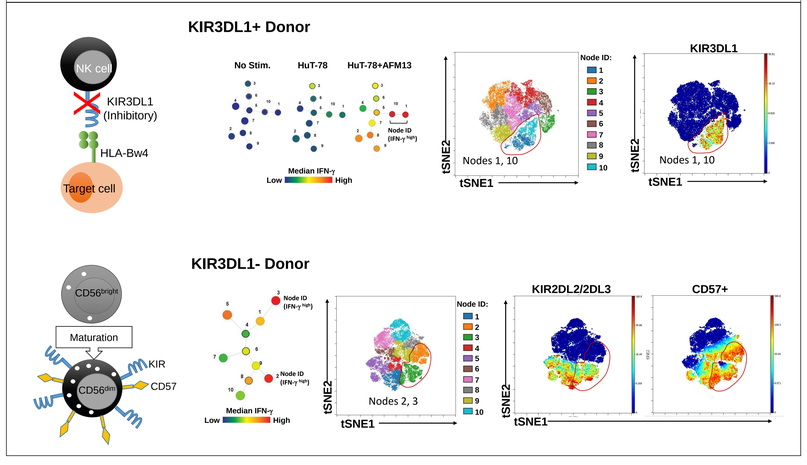
1. AFM13 triggers IFN-γ, TNF and degranulation of conventional (cNK) cells co-incubated with CD30-expressing tumor cells.



2. Functional mass cytometry reveals that AFM13 profoundly alters cNK cell viSNE maps via activation, cytokine secretion and reveals single cell heterogeneity.

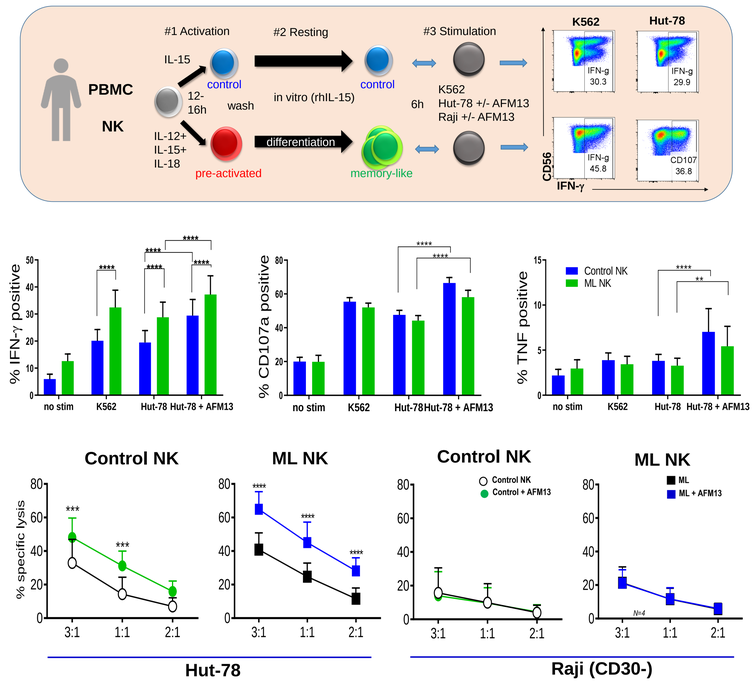


3. Enhanced AFM13 mediated IFN-γ response correlates with NK maturation status and absence of KIR-KIR ligand mediated inhibition.



Results

4. ML NK display enhanced cytokine response and superior killing of CD30-expressing tumor cells. AFM13 significantly enhanced ML NK cell functionality.



Conclusions

- AFM13 significantly enhances NK cell recognition of CD30+ malignancies correlating with superior NK cell activation.
- ML NK cells exhibit improved tumor recognition and enhanced functionality against tumor cells.
- AFM13 pretreatment of tumor targets potentiates ML NK cell effector functions including cytokine secretion and cytotoxicity.
- NK cells expression of inhibitory receptors, maturation state and memory-like differentiation influence AFM13 mediated NK cell response against CD30+ targets
- Superior induction of IFN-γ, TNF and MIP1-α by AFM13 in conventional and ML NK cells leads to consider potential bystander effects in the cellular composition and leukocytes recruitment into the tumor microenvironment.
- 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.

Ongoing Work and Future Directions

- Explore AFM13 binding to CD16+ monocytes and whether this binding affects their activation and function.
- Evaluate how AFM13 binding in NK cells modifies phenotype and function of surrounding immune cells including T cells, monocytes and even NK cells.
- Characterize the immune profile of cytokines and chemokines secreted by conventional and ML NK cells upon co-incubation with AFM13-coated or non-coated target cells. Evaluate whether they can differentially induce migration of specific immune cell subsets.
- Evaluate efficacy of AFM13 to induced superior NK cell responses using CD30+ primary tumors as target cells.

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