CD16A shedding facilitates repetitive targeting of tumor cells by AFM13-armed NK cells

Poster: 2950

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INTRODUCTION

Antibody dependent cellular cytotoxicity (ADCC) is a powerful mechanism of Natural Killer (NK) cells to kill antibody-opsonized target cells. However, ADCC mediated by conventional antibodies has its limitations in NK cell induced killing of tumor cells commonly being characterized by low tumor antigen expression. Several strategies have been implemented to boost ADCC including shedding inhibition of CD16A, a key ADCC-mediating Fc receptor on the plasma membrane of NK cells (1). However, previous results have shown that genetic engineering of the cleavage site of CD16A leads to decreased serial engagement of target cells (2). Another approach to boost ADCC is provided by tetravalent-bispecific innate cell engagers (ICE®) cross-linking CD16A and tumor antigens triggering anti-tumoral cytotoxicity (3).

In the current study, we have investigated whether ICE® molecules can induce effective ADCC while maintaining the natural function of CD16A with a particular focus on preserved CD16A shedding. To study cytotoxicity and contact dynamics, we have used an in-house developed live-cell microchip screening with single cell resolution (4) together with a micro-contact printing-based assay combined with an ICE® (5,6).

AFM13 Monotherapy

Hodgkin Lymphoma (NCT01221571)	Phase 1	completed
Hodgkin Lymphoma (NCT02321592)	Phase 2	completed
Cutaneous T cell lymphoma (NCT03192202)	Phase 1/2	completed
Peripheral T cell lymphoma (NCT04101331)	Phase 2	Active (not recruiting)

AFM13 + Anti-PD-1

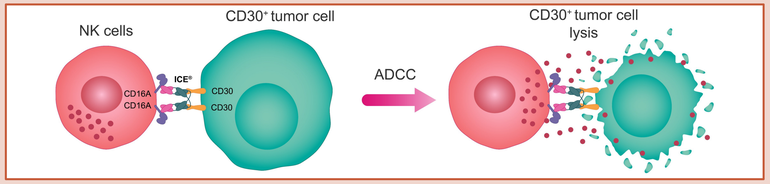
Hodgkin Lymphoma (NCT02665650)	Phase 1	completed

AFM13 + Adoptive NK cells

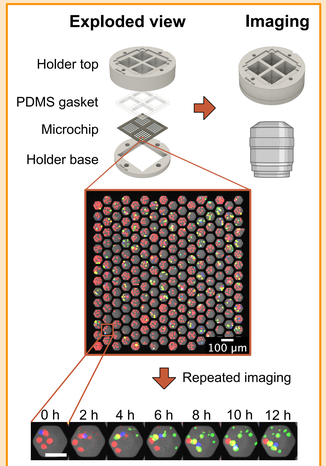
Hodgkin & Non-Hodgkin lymphomas (NCT04074746)	Phase 1/2	recruiting

AFM13 - mode of action and clinical relevance

- AFM13 redirects NKs and macrophages to tumor cells by binding to CD16A on innate immune cells and CD30 on cancer
- Innate immune cells kill tumor cells via ADCC or ADCP
- More than 200 patients have received AFM13, which has demonstrated clinical activity and a differentiated safety profile



1. Microwell technology for monitoring of single cell cytotoxicity



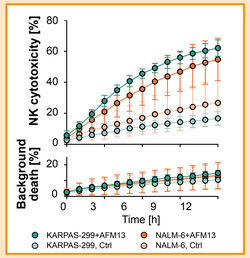
METHODS

Microchips contained arrays of microwells etched in silicon bonded to a glass bottom, allowing time-lapse imaging of thousands of individual NK cells interacting with tumor cells.

Single cell screening with automated or manual analysis was performed in 60 µm wide hexagonal wells distributed into four separated compartments allowing four experimental conditions in parallel.

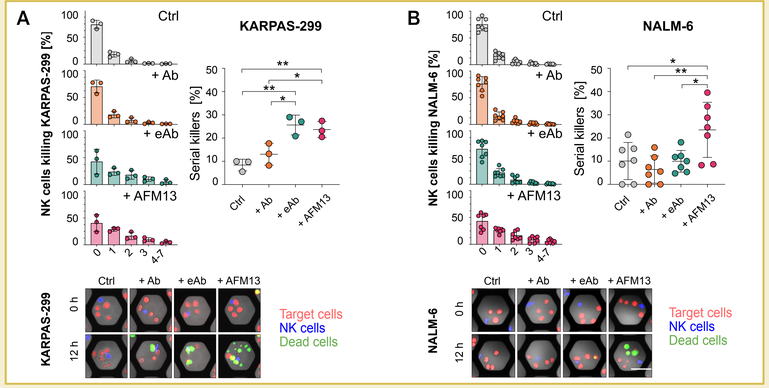
Microchips were assembled in custom-made holders for imaging in standard inverted microscopes.

Cells were labelled with permanent dyes for easy cell detection and a death indicator dye was present during the assay to follow cell death.



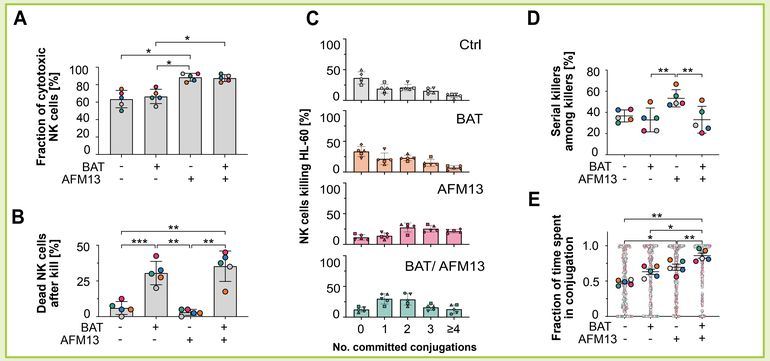
AFM13 increased NK killing efficiency against CD30 positive tumor cells. Buffy-coat derived primary human NK cells were co-cultured with both CD30-high (KARPAS-299) and CD30-low (NALM-6) expressing target cells.
Example of automatic analysis of microwells containing a single NK cell and at least one target cell, showed increased fractions of wells with dead target cells over 12h, imaging every hour. Mean values with SD from 3-5 independent donors are shown.

2. AFM13 boosts serial killing on CD30 low targets



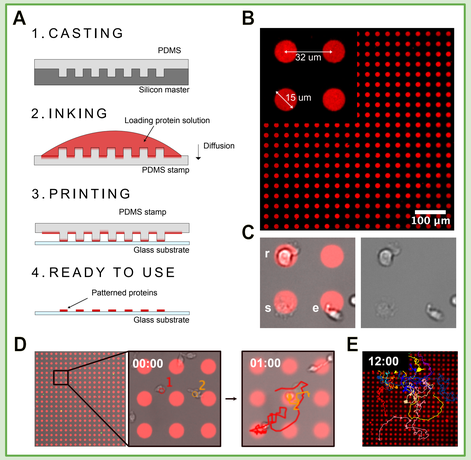
AFM13 increased NK serial killing. AFM13 and Fc-enhanced anti-CD30 antibody (eAb) triggered higher ADCC and NK serial killing compared to monoclonal anti-CD30 antibody (Ab) and control (Ctrl) in CD30-high expressing KARPAS-299 cells (A). However, AFM13 triggered stronger NK serial killing towards CD30-low expressing NALM-6 cells compared to eAb (B). Scale bar 50 µm. Only microwells with E:T ratios 1:≥4, were used to calculate percentages of serial killers. Serial killers were NK cells that killed ≥ 3 target cells over the 12h assay. Percentage of serial killers was calculated among cytotoxic NK cells. Graphs display mean value with SD from 3-7 independent donors. One-way ANOVA were used for statistical analysis of serial killers. Significant values are indicated with ∗(P≤0.05) and ∗∗ (P≤0.01).

3. Shedding inhibitor Batimastat reduced AFM13 induced NK serial killing



Co-treatment with the CD16 shedding inhibitor Batimastat (BAT) did not alter the fraction of cytotoxic NK cells against CD30 intermediate expressing target HL-60. Fraction of NK cells that performed at least one kill (A) was not affected by adding BAT, but NK cells that died during the assay after killing was greatly increased (B). Serial killing abilities were enhanced in the AFM13 condition compared to double stimulation (BAT+AFM13) (C-D), which led to NK cells getting stuck to target cells, shown by the increase of fraction of time spent in conjugation (E). Graphs show mean values with SD from 5 independent donors. Statistics was analyzed by multiple comparison one-way anova and significant values are indicated with ∗(P≤0.05), ∗∗ (P≤0.01) and ∗∗∗(P≤0.001).

4. Micro-contact printing of AFM13



Creating an immobile target

Shedding inhibition with Batimastat increased conjugation times and induced clumping in NK-target cell co-cultures. However, NK cells could still move around dragging targets along with them.

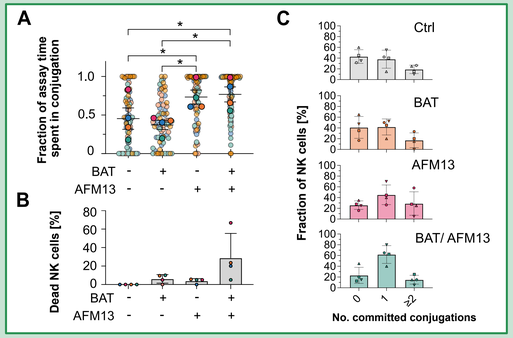
To investigate if migratory abilities and synapse termination were effected by shedding inhibition we applied micro-contact printing for making arrays of artificial immune synapses.

Prints consist of a mixture of fluorescently labeled BSA, anti-LFA-1 and +/-AFM13.

METHODS

A poly-dimethylsiloxane (PDMS) stamp inked with protein solution was used to deposit proteins in a specific pattern (A). Full field of view with inset (10x objective) showing dimensions of the prints used (B). NK cells were seeded onto the prints and monitored over time for 12 h (C-E). Both timing and conjugational behavior was scored. Here we show an example of round (r), spread (s) and elongated (e) NK cell morphologies on prints. NK cells were tracked to investigate the migrational behavior (D-E). Example of tracking of 2 NK cells for the first hour (D) and 10 individual NK cells tracked for 12 h (E).

5. AFM13 enhances synapse formation of NK cells



NK cells interacted more with prints containing AFM13.
NK cells spent more time on prints when AFM13 was present. Addition of the CD16 shedding inhibitor Batimastat (BAT) did not change the fraction of assay time spent in conjugation (A). However, more NK cells died when Batimastat was added to NK cells interacting with the AFM13 prints (B). AFM13 also increased the total number of conjugations per NK cell. Multiple conjugations of NK cells to AFM13 prints were tendentially reduced by BAT (C). Graphs show mean and SD of four individual experiments. Friedman test for multiple comparisons was used for statistical analysis in A. Significant values are indicated with ∗(P≤0.05).

CONCLUSIONS

- Treatment with AFM13 increased the populations of cytotoxic NK cells.
- AFM13 boosted NK cell serial killing against CD30 low target cells.
- Shedding inhibitor Batimastat induced tumor dependent NK cell death and blocked repetitive targeting of tumor cells.

Based on our data we hypothesize that CD16A shedding facilitates ADCC potential of NK cells by allowing migration to distantly located tumor cells and serial killing.

Contact information

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