Cryopreserved CAR-like NK Cells Pre-complexed with the CD30/CD16A Bispecific Innate Cell Engager (ICE®) AFM13 for the Treatment of CD30⁺ Malignancies

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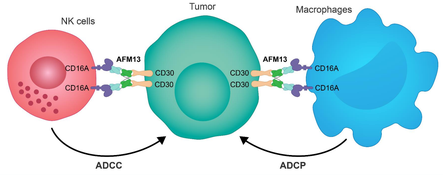
BACKGROUND

- **I**nnate **C**ell **E**ngager (ICE®) constructs developed from the **R**edirected **O**ptimized **C**ell **K**illing (ROCK®) platform are designed to bivalently engage CD16A⁺ natural killer (NK) cells or macrophages to enhance their activity against tumors[1]
- AFM13 is a first-in-class bispecific, tetravalent ICE® designed with two binding sites for CD16A on NK cells or macrophages and two binding sites for the CD30 receptor on target tumor cells, such as Hodgkin lymphoma (HL)[2,3]
- AFM13 is designed to potently activate anti-tumoral responses of NK cells through antibody-dependent cellular cytotoxicity (ADCC), and macrophages via antibody-dependent cellular phagocytosis (ADCP), and has shown promising clinical activity towards CD30⁺ lymphoma cells, including when used in combination with NK cells[3–8]
- The number of NK cells and their anti-tumor activity are often compromised in patients with HL and there is a high unmet medical need for treatment options for patients who relapse or fail to respond to first-line treatment, and for therapies that have fewer side effects compared with existing therapies[9]
- The selective, high-affinity binding of ICE® constructs to CD16A enables stable pre-complexing with NK cells to further enhance ADCC. This differentiating characteristic of ICE® constructs could overcome the limitations of immunosuppression in patients with lymphoma, preferably in the form of a cryopreserved 'off-the-shelf' CAR-like NK cell therapy

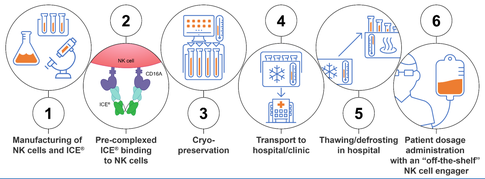
OBJECTIVE

- To assess the feasibility of NK cell pre-complexing using AFM13 to generate a chimeric antigen receptor (CAR)-like NK cell product, and to determine NK cell surface retention and cytotoxic activity
- To evaluate the effects of cryopreservation of pre-complexed NK cells and assess their biological activity after thawing

AFM13 mediates interaction of NK cells and macrophages to kill tumor cells

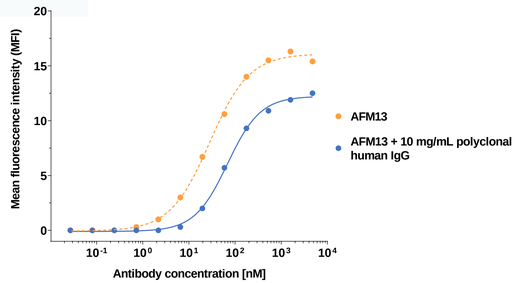


Proposed development path for an 'off-the-shelf' ICE® pre-complexed NK immunotherapy



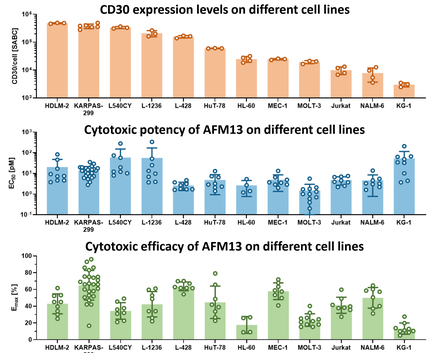
RESULTS

AFM13 had a strong binding affinity for primary human NK cells, even in the presence of IgG



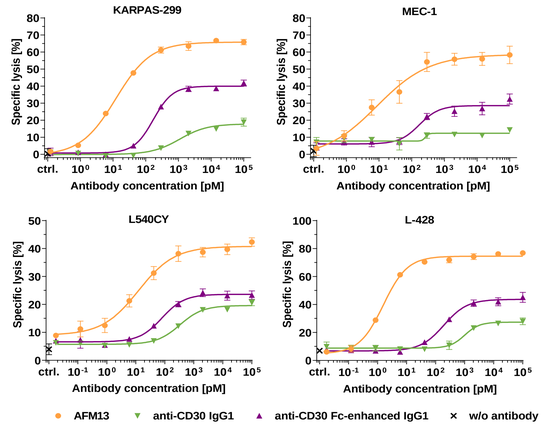
AFM13 was titrated on enriched primary human NK cells at 37°C in the absence or presence of 10 mg/mL polyclonal human IgG. Cell surface bound AFM13 was detected with anti-AFM13 rat mAb clone 7 followed by FITC-conjugated goat anti-rat IgG and flow cytometric analysis. Median fluorescence intensities (MFI) were used to calculate apparent affinities (K_D) by non-linear regression. The mean K_D value on enriched primary human NK cells was 33.2 nM. The affinity was reduced by only a mean factor of 2.6 in the presence of 10 mg/mL polyclonal human IgG, resulting in a mean apparent affinity of 86.8 nM.

AFM13 induced lysis of CD30⁺ tumor cell lines, irrespective of their origin or CD30 cell surface expression levels



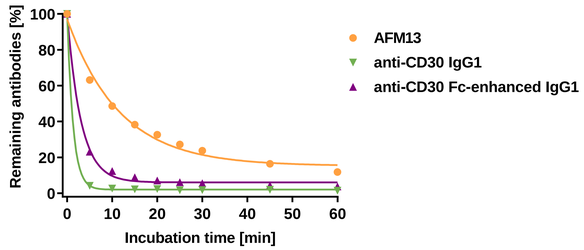
CD30 cell surface expression levels were determined on the indicated tumor cell lines using anti-CD30 mAb HRS-4 and QIFIKIT. Individual specific antibody binding capacity (SABC)/cell and mean and SD from independent experiments are plotted. Potency (EC_{50}) and efficacy (E_{max}) of AFM13 were determined in 3-hour calcein-release cytotoxicity assays with enriched primary human NK cells as effector cells at an effector to target (E:T) ratio of 5:1 in the presence of serial dilutions of AFM13. EC_{50} and E_{max} values were determined by non-linear regression/sigmoidal dose-response analysis in multiple independent experiments and plotted together with mean and SD. There was no correlation between CD30 density on tumor cells and the cytotoxic potency or efficacy of AFM13. E:T, effector cell to target cell ratio; SD, standard deviation.

AFM13 exhibited higher potency and efficacy than anti-CD30 IgG1 and Fc-enhanced anti-CD30 IgG1 on various cell lines



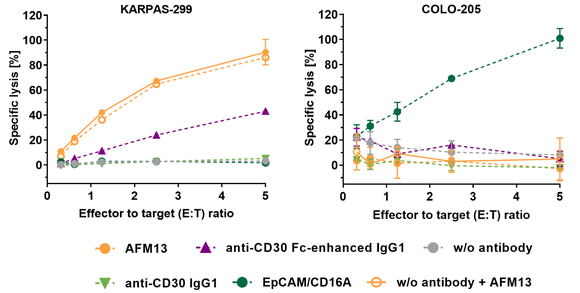
The potency and efficacy of AFM13 was determined using a 3-hour calcein-release cytotoxicity assay across cell lines of various origin expressing different levels of CD30: KARPAS-299 (ALCL), MEC-1 (B-CLL), L540CY and L-428 (HL) target cells were enriched with primary human NK cells as effector cells at an E:T ratio of 5:1 in the presence of serial dilutions of AFM13, anti-CD30 IgG1, or anti-CD30 Fc-enhanced IgG1. Mean and SD of duplicate lysis values were plotted. ALCL, anaplastic large cell lymphoma; B-CLL, B-cell chronic lymphocytic leukemia.

AFM13 exhibited a substantial longer retention on the surface of pre-complexed NK cells than IgG1 or Fc-enhanced IgG1



Aliquots of enriched primary human NK cells were pre-complexed with biotinylated AFM13, anti-CD30 IgG1, or anti-CD30 Fc-enhanced IgG1 on ice. After removal of unbound antibodies, aliquots were either directly stained with DyLight488-conjugated streptavidin on ice and analyzed by flow cytometry, or incubated for the indicated periods of time at 37°C before retained antibodies were quantified by flow cytometry. For normalization, median fluorescence intensity values determined at timepoint 0 were set to 100%. Of the initially bound AFM13, 11.8% was retained after 60 minutes of incubation at 37°C; in contrast, only 1.4% of IgG1 and 3.8% of Fc-enhanced IgG1 were retained after 60 minutes of incubation.

Cryopreserved NK cells pre-complexed with AFM13 showed high efficacy in specific lysis of CD30⁺ KARPAS-299 target cells, but did not induce lysis of CD30⁻ COLO-205 cells



NK cells were pre-complexed with 10 µg/mL of the indicated antibodies and frozen at −80°C. As a control, aliquots of the same NK cells were not pre-complexed (w/o antibody) but were still subjected to one freeze/thaw cycle. Thawed NK cells were washed and directly used as effector cells at the indicated E:T ratios. For the cytotoxicity assay, AFM13 was freshly added to NK cells that were not pre-complexed at a concentration of 10 µg/mL on calcein-labeled CD30⁺/EpCAM⁻ KARPAS-299 or CD30⁻/EpCAM⁺ COLO-205 target cells. AFM13 pre-complexed NK cells showed the same efficacy as NK cells that were not pre-complexed but combined with freshly added AFM13 (w/o antibody + AFM13). NK cells pre-complexed with Fc-enhanced anti-CD30 IgG1 induced lysis of KARPAS-299 target cells with substantial lower efficacy, and NK cells pre-complexed with IgG1 anti-CD30 induced no lysis at all. Mean and SD of duplicate lysis values from one experiment were plotted.

CONCLUSIONS

- Adoptive NK cells stably pre-complexed with bispecific, CD16A-selective ICE®, such as AFM13, could be a novel, CAR-like NK cell immunotherapeutic for patients with HL
- AFM13 pre-complexed NK cells showed comparable efficacy to NK cells that were not pre-complexed but combined with AFM13
- AFM13 pre-complexed NK cells showed enhanced cell lysis compared to NK cells pre-complexed with an Fc-enhanced antibody
- The activity of AFM13 pre-complexed NK cells remained unaffected after cryopreservation and showed promise as an off-the-shelf immunotherapy for the effective depletion of CD30⁺ tumor cells

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