Novel Bispecific Innate Cell Engager AFM28 in Combination with Allogeneic NK Cells for the Treatment of CD123+ Acute Myeloid Leukemia and Myelodysplastic Syndrome

Poster 64

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BACKGROUND

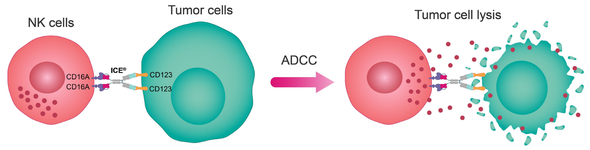
- Acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) are common hematological malignancies characterized by clonal expansion of myeloid progenitors (blasts) in the bone marrow and peripheral blood.[1]
- Removal of both leukemic blasts and leukemic stem cells (LSCs) is key to eradicate minimal residual disease (MRD) and prevent relapse, therefore novel therapies are required that target both of these cell types.[2]
- Adoptive transfer of allogeneic NK cells has emerged as a promising investigational therapy to address the unmet need in AML and high-risk MDS.[3]
- The efficacy of allogeneic natural killer (NK) cell immunotherapies can be enhanced by tumor-targeting bispecific antibodies that redirect NK cells to tumors, and enhance NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC).[4,5]
- Innate Cell Engager (ICE®) molecules bind both to CD16A on NK cells and a tumor cell-surface antigen, redirecting NK cells to tumor cells and stimulating ADCC.[6]
- AFM28 (CD123/CD16A) is a novel ICE® designed to target CD123, an antigen universally expressed on both leukemic blasts and LSCs.[7]
- Initial studies have shown effective anti-tumor activity of AFM28, and a favorable safety profile in cynomolgus toxicology models.[7]
- Combination of AFM28 with allogeneic NK cells, as a pre-complexed product or co-administered, may represent a novel treatment modality by enhancing ADCC towards both leukemic blasts and LSCs expressing CD123.

OBJECTIVE

To investigate the anti-leukemic activity of AFM28 both when pre-complexed and co-administered with allogeneic NK cells.

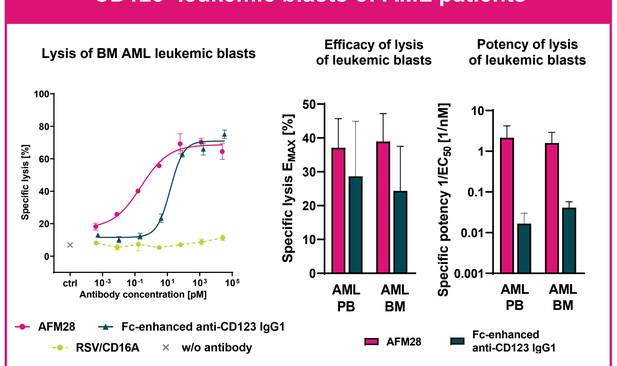
RESULTS

AFM28 binds to both NK cells and tumor cells and stimulates induction of ADCC



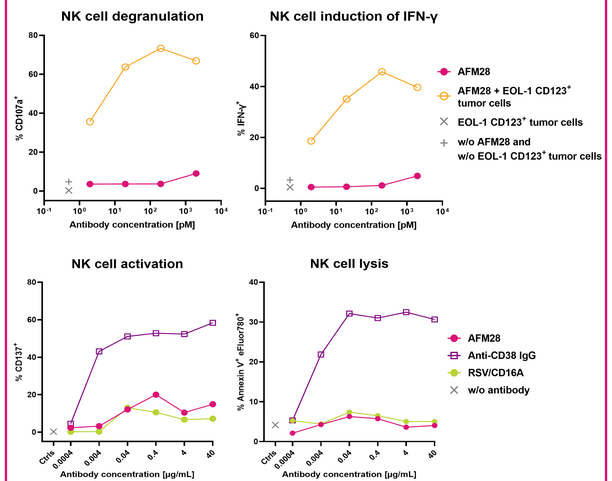
ADCC, antibody-dependent cellular cytotoxicity; ICE®, innate cell engager

AFM28 stimulates NK cells to induce lysis of CD123+ leukemic blasts of AML patients



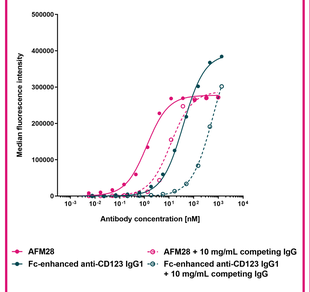
AFM28 induced ADCC against both PB and BM CD123+ leukemic blasts with greater efficacy than an Fc-enhanced anti-CD123 IgG1. Buffy coat-derived allogeneic NK cells were cultured at a 2.5:1 E:T ratio with calcein-labelled leukemic blasts in the presence of AFM28; Fc-enhanced anti-CD123 IgG1; a bispecific, non-CD123-targeting negative control antibody (RSV/CD16A); and without antibody addition. Release of calcein after 4-hour co-culture was used to quantify specifc tumor cell lysis by NK cells. Representative data and cumulative data, showing mean maximal efficacy and specific potency of lysis of leukemic blasts derived from matched PB and BM AML samples of three patients. BM, bone marrow; EC_{50}, concentration of drug required to give half the maximal effect; E_{MAX}, maximal effect of the drug; E:T, effector to target; PB, peripheral blood; RSV, respiratory syncytial virus targeted by NIST RM8671; w/o, without.

AFM28-mediated NK cell activation is CD123+ target cell-dependent and does not induce NK cell fratricide



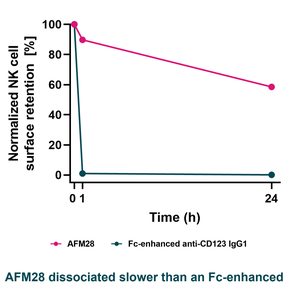
AFM28 triggered potent NK cell degranulation, IFN-γ expression and activation only when CD123+ target cells were also present. Low levels of NK cell fratricide were induced by AFM28. Surface expression of CD107a (NK cell degranulation marker) and intracellular IFN-γ expression was assessed by flow cytometry after 4-hour culture with AFM28, or without antibody addition (w/o) in the absence or presence of CD123+ EOL-1 target cells at an E:T ratio of 1:1. CD137 expression (NK cell activation marker) and NK cell lysis was assessed following 24-hour culture of primary NK cells in the presence of AFM28, RSV/CD16A, an anti-CD38 IgG1 (positive control for NK fratricide), or without an antibody. Lysed NK cells were identified as those stained double-positive for Annexin V and an eFluor780 cell death marker. Ctrls, controls; IFN-γ, interferon gamma; w/o, without.

AFM28 binds NK cells with high affinity in the presence of IgG



High affinity binding of AFM28 was retained in the presence of competing IgG and was higher than that of a comparator Fc-enhanced anti-CD123 IgG1. Primary NK cells were incubated with increasing concentrations of AFM28 or Fc-enhanced anti-CD123 IgG1 in the presence or absence of 10 mg/mL polyclonal human IgG (Cutaquig) at 37°C. MFI of bound antibodies was determined by flow cytometry using an anti-CD123 idiotype mAb and tertiary goat anti-mouse IgG. MFI, median fluorescence intensity. mAb, monoclonal antibody.

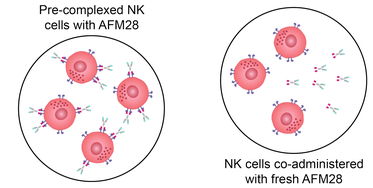
AFM28 exhibits prolonged retention on NK cells



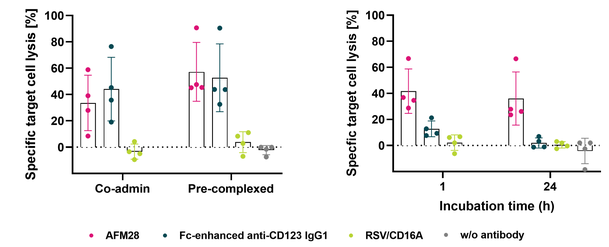
AFM28 dissociated slower than an Fc-enhanced anti-CD123 IgG1 monoclonal antibody from NK cells, suggesting prolonged retention to CD16A. Primary NK cells were incubated with 100 µg/mL AFM28 or Fc-enhanced anti-CD123 IgG1 on ice to allow the formation of NK cell/antibody complexes. Pre-complexed NK cells were then washed and incubated at 37°C for 1 and 24 hours in an excess volume of medium to allow dissociation of bound antibody and prevent re-association. NK cell-retained antibodies were detected by flow cytometry using an anti-CD123 idiotype mAb and tertiary goat anti-mouse IgG. MFI values at time-point 0 were assumed to be 100%.

AFM28 can be pre-complexed or co-administered with NK cells

AFM28 can be incubated with NK cells to form complexes prior to administration to tumor cells, or co-administered fresh with NK cells to tumor cells.



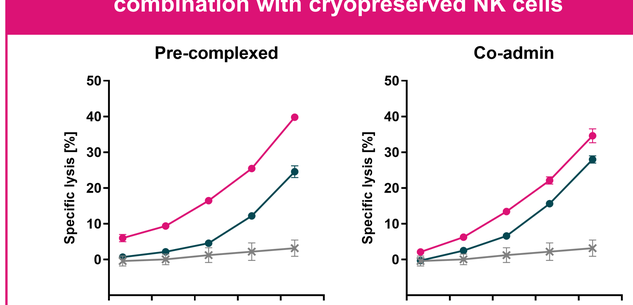
AFM28 induces ADCC at comparable levels both when pre-complexed or co-administered with NK cells



NK cells pre-complexed or co-administered with AFM28 both exhibited efficacious lysis of CD123+ tumor cells (left). Lysis of calcein-labelled EOL-1 CD123+ tumor cells after co-culture with primary NK cells that were either pre-complexed with the indicated antibodies and washed (pre-complexed), or freshly combined with the indicated antibodies (co-administered).

ADCC activity of AFM28 pre-complexed NK cells was fully maintained for more than 24 hours after pre-complexing (right). Lysis of calcein-labelled EOL-1 CD123+ tumor cells after co-culture with primary NK cells that were pre-complexed with the indicated antibodies. Prior to the cytotoxicity assay, pre-complexed NK cells had been incubated at 37°C for 1 and 24 hours in an excess volume of medium to allow dissociation of bound antibody and prevent re-association.

AFM28 can induce targeted tumor cell lysis (via ADCC) in combination with cryopreserved NK cells



AFM28 can efficiently induce ADCC against CD123+ tumor cells when pre-complexed or co-administered with cryopreserved NK cells. Cryopreserved primary NK cells pre-complexed **(left)** or co-administered **(right)** with AFM28 or Fc-enhanced anti-CD123 IgG1 (at concentrations providing maximal efficacy) were cultured at increasing E:T ratios with calcein-labelled CD123+ MOLM-13 tumor cells. Anti-tumor efficacy of cryopreserved NK cells which were neither pre-complexed nor co-administered with AFM28 was also assessed (w/o antibody).

CONCLUSIONS

- AFM28 is a novel ICE® specific to CD16A on NK cells, and CD123 on AML and MDS leukemic cells.
- AFM28-mediated activation of NK cells induces ADCC towards primary leukemic blasts from peripheral blood and bone marrow of patients with AML and requires the presence of CD123+ tumor target cells.
- AFM28 stimulates ADCC both when pre-complexed and when co-administered with NK cells.
- AFM28 binds with high affinity to NK cells, even in the presence of competing IgG, and exhibits greater cell surface retention than conventional monoclonal antibodies, including Fc-enhanced IgG1.
- Feasibility of cryopreserving AFM28 pre-complexed NK cells whilst maintaining anti-tumor activity suggests promise for an off-the-shelf therapy targeting leukemic blasts and LSCs in patients with AML and MDS.

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