The Novel Bispecific Innate Cell Engager (ICE®) AFM28 Efficiently Directs Allogeneic NK Cells to CD123+ Leukemic Stem and Progenitor Cells in AML

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BACKGROUND

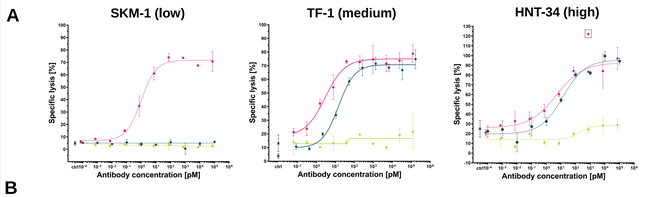
- Acute myeloid leukemia (AML) can be treated with curative intent using high-dose chemotherapy and hematopoietic stem cell transplant[1]; however, substantial medical need remains as many patients are considered ineligible due to age and co-morbidities.[2]
- In treatment-responsive patients, relapse is common due to failure to eradicate residual leukemic stem cells (LSCs)[3], and ultimately prognosis is poor. Novel, alternative therapies are required to treat patients ineligible for standard-of-care therapy, as well as those who relapse or become refractory to frontline therapy due to persistent measurable residual disease (MRD).
- Allogeneic natural killer (NK) cell therapies have shown promising clinical activity in patients with relapsed or refractory (R/R) AML[4], and the activity of these therapies may be enhanced in combination with Innate Cell Engagers (ICE®).[5]
- AFM28 is a CD123/CD16A bispecific ICE® developed to redirect and enhance NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) towards CD123+ AML leukemic blasts and LSCs, which may eradicate MRD in patients with R/R AML.[5]

OBJECTIVE

Evaluate the efficacy of AFM28 to enhance NK cell-mediated ADCC towards CD123+ AML leukemic blasts and LSCs.

RESULTS

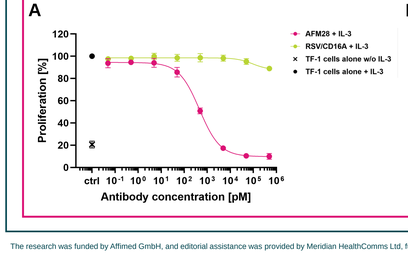
AFM28 induces NK-cell mediated lysis of CD123+ target cells with high efficacy and potency, irrespective of the presence of high-risk driver mutations, as well as CD123 and CD64 expression levels

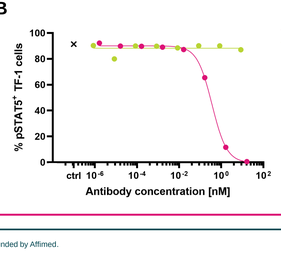


Cell line	Origin/Disease background	Mutational Status (ExPasy Cellosaurus)	CD123 SABC
MOLM-13	Acute myeloid leukemia	FLT3-ITD; MLL-AF9	10610
KG-1	Acute myeloid leukemia	p53 mut	9952
HNT-34	Acute myeloid leukemia (secondary)	Evi1 overexpressed; Ph+ (t9;22)	9297
EOL-1	Chronic eosinophilic leukemia	N/A	7697
NOMO-1	Acute myeloid leukemia	p53 mut; MLL-AF9	5243
THP-1	Acute monocytic leukemia	p53 mut; MLL-AF9	4982
TF-1	Acute erythroid leukemia	N-Ras; p53 mut	4932
SKM-1	Acute myeloid leukemia (secondary)	p53 mut; ASXL-1	2532
OCI-AML3	Acute myeloid leukemia	DNMT3A; NPM1	2380
Kasumi-1	(Childhood) acute myeloid leukemia	RUNX1 (t8;21); p53 mut; KIT; RAD21	1424
OPM-2	Human, multiple myeloma	p53 mut	negative

AFM28 induces lysis of CD123+ tumor cell lines irrespective of CD123 expression level, mutational status, and CD64 expression. Buffy coat-derived allogeneic NK cells were cultured at a 2.5:1 effector:target (E:T) ratio with calcein-labelled leukemic cell lines in the presence of AFM28, Fc-enhanced anti-CD123 IgG1 or a non-targeting control engager (respiratory syncytial virus [RSV]/CD16A). (A) Specific tumor cell lysis of indicated CD123+ tumor cells by NK cells was quantified by calcein release cytotoxicity assay.
(B) Efficacy (E_{max}, left) and potency (EC_{50}, right) of tumor cell lysis are depicted (n=3–5).
(C) Expression of the high-affinity IgG receptor CD64 on AML cell lines completely abolished ADCC induction by an Fc-enhanced anti-CD123 antibody, whereas AFM28 also showed high ADCC efficacy against all tested CD64+ cell lines. Expression of CD32, a low-affinity Fcγ receptor was not associated with reduced ADCC activity of IgG monoclonal antibodies. **Grey bars:** Absolute CD123 quantification on the cell surface of tumor cell lines, measured by flow cytometry. **Magenta and dark green data points:** Specific tumor cell lysis by NK cells was quantified by calcein release cytotoxicity assay.
(C) Quantitative analysis of the median fluorescence intensity (MFI) of CD64 and CD32 relative to the isotype control on indicated cell lines. na, not applicable.

IL-3-dependent STAT5 phosphorylation and tumor cell proliferation is inhibited by AFM28, suggesting a secondary mechanism of anti-tumor action, independent of its role in enhancing NK cell-mediated ADCC





AFM28 blocks IL-3R signalling, abrogates STAT5 phosphorylation and inhibits proliferation in TF-1 cells.
(A) TF-1 cell growth was stimulated with IL-3 in the presence of titrated AFM28, RSV/CD16A, or without (w/o) antibody addition, for 72 hours at 37°C. Proliferative activity was assessed using CellTiter-Glo Assay (n=3) and normalized to maximum proliferation signal (100%). Mean ± standard error of the mean (SEM) of triplicates are plotted. (B) Phosphorylated STAT5 was measured in TF-1 cells upon stimulation with IL-3 in the presence of titrated AFM28 or RSV/CD16A. Data points from one representative experiment are shown out of three performed.

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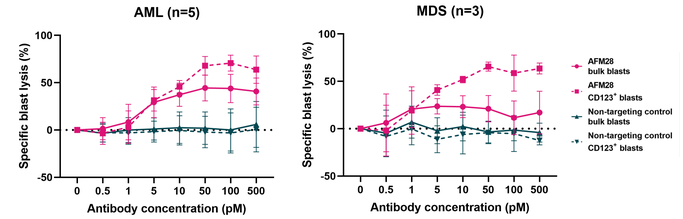
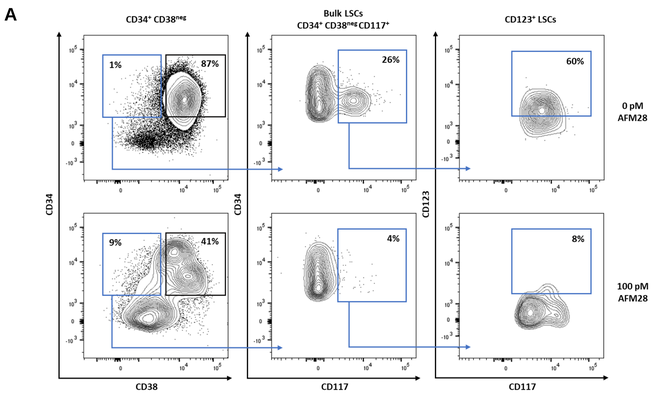
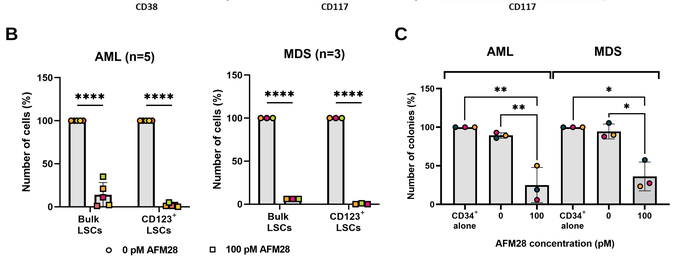
AFM28 efficiently stimulates NK cell-mediated lysis of CD123+ leukemic blasts in bone marrow samples obtained from patients with AML and high-risk myelodysplastic syndrome (HR-MDS)



AFM28 efficiently directs allogeneic NK cells to CD123+ leukemic blasts in AML and HR-MDS patient samples. ADCC assay results from bone marrow mononuclear cell (BMMC) samples isolated from patients with AML (n=5) and HR-MDS (n=3); samples were treated with 0–500 pM of AFM28 or a non-targeting control for 24 hours in the presence of allogeneic NK cells at an E:T ratio of 1:1. Analysis was performed using flow cytometry; bulk blasts were defined as living/CD45+/CD34+/CD38+/CD123$^{+/neg}$ cells, and percentage lysis compared vs. living/CD45+/CD34+/CD38+/CD123+ blasts. Cell counts from samples treated without antibody were set as 0%. Data is represented as mean ± standard deviation (SD).

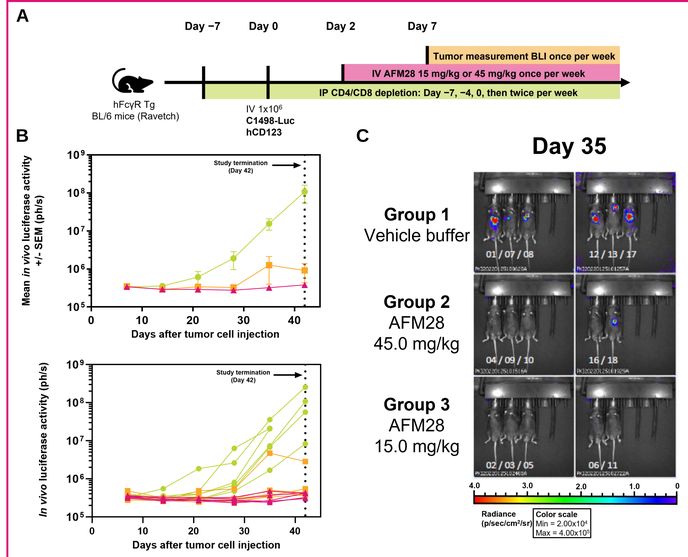
AFM28 induces efficient depletion of LSCs and leukemic progenitors in bone marrow samples obtained from patients with AML and HR-MDS





AFM28 efficiently directs allogeneic NK cells to CD123+ leukemic stem and progenitor cells in AML and MDS patient samples.
(A) Representative dot plot of patient-derived AML samples showing LSC lysis following treatment with 100 pM AFM28.
(B) LSC ADCC assay results of patient BMMC samples from AML (n=5) and MDS (n=3) treated with vehicle or 100 pM of AFM28 for 24 hours in the presence of allogeneic NK cells at an E:T ratio of 1:1. Bulk LSCs were defined as living/CD45+/CD34+/CD38^{neg}/CD117+/CD123$^{+/neg}$ cells. Cell counts from the 0 pM AFM28 condition for bulk and CD123+ LSCs were set to baseline.
(C) Colony formation assay using CD34+ cells derived from BMMC samples obtained from patients with AML (n=3) or MDS (n=3) and treated with 0, 10 (not shown), 100, or 1000 (not shown) pM of AFM28 for 24 hours in the presence of allogeneic NK cells at an E:T ratio of 1:1. Colony count of the "CD34+ alone" condition was normalized to 100%. Data is represented as mean ± SD, analysed using one-way and two-way ANOVA. * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001, **** p ≤ 0.0001.

AFM28 inhibits tumor outgrowth in a murine model of AML



- Group 1: Vehicle buffer PBS (10 ml/kg), i.v., once weekly on Days 2, 9, 16, and 23 (n=6/4)
- Group 2: AFM28 (45 mg/kg), i.v., once weekly on Days 2, 9, 16, and 23 (n=5/5)
- Group 3: AFM28 (15 mg/kg), i.v., once weekly on Days 2, 9, 16, and 23 (n=5/5)

AFM28 efficacy in C1498-Luc hCD123 mouse model.
(A) Male huFcγR-C57BL6 mice were treated with anti-mCD4 and anti-mCD8 antibodies to facilitate T cell depletion starting on Day −7 before tumor cell implantation. On Day 0, mice were implanted intravenously with C1498-Luc hCD123 cells and were treated with two doses of either 15 or 45 mg/kg AFM28 once weekly.
(B) *In vivo* luciferase activity was used to measure tumor growth and was assessed once weekly by bioluminescence (BLI) imaging. Data are given both as means ± SEM (top) and as individual data points (bottom).
(C) Representative BLI images taken on Day 35. (Photons/second; presented as semi-log plot).

CONCLUSIONS

- **AFM28 induces NK cell-mediated lysis of CD123+ tumor cells with high efficacy and potency.**
- **AFM28-mediated ADCC activity *in vitro* is not affected by high-risk mutational status or low CD123 expression and, in contrast to Fc-enhanced monoclonal antibodies, activity is unaffected by CD64 expression.**
- **AFM28 can inhibit tumor cell proliferation through blocking of IL-3-induced STAT5 phosphorylation downstream of CD123, thus exhibiting a secondary mechanism of action in addition to induction of NK cell-mediated ADCC.**
- **In *ex vivo* AML and MDS patient BMMC samples, AFM28 demonstrated efficient depletion of both CD123+ leukemic blasts and, importantly LSCs.**
- **Potent anti-tumor efficacy of AFM28 was exhibited in a murine AML model.**

Leveraging the innate immune system by combining AFM28 with allogeneic NK cells may offer the potential to induce long-term remissions in a broad patient population in R/R AML and HR-MDS.

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