Novel Bispecific Innate Cell Engager AFM28 for the Treatment of CD123-Positive Acute Myeloid Leukemia and Myelodysplastic Syndrome

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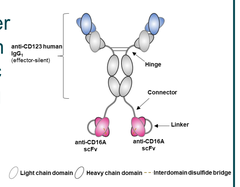
Jana-Julia Siegler[1], Nanni Schmitt[2], Jens Pahl[1], Torsten Haneke[1], Izabela Kozlowska[1], Séverine Sarlang[1], Alexandra Beck[2], Stefan Knackmuss[1], Paulien Ravenstijn[1], Uwe Reusch[1], José Medina-Echeverz[1], Jan Endell[1], Thorsten Ross[1], Daniel Nowak[2] and Christian Merz[1]

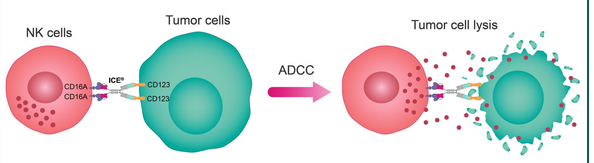
[1]Affimed GmbH, Im Neuenheimer Feld 582, 69120 Heidelberg, Germany
[2]Department of Hematology and Oncology, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany

BACKGROUND

- A significant unmet clinical need remains in patients with high-risk myelodysplastic syndrome (HR-MDS) and relapsed/refractory (R/R) acute myeloid leukemia (AML).[1,2]
- AML development, therapy resistance and relapse are attributed to a population of leukemic stem and progenitor cells (LSCs); removal of both leukemic blasts and LSCs is key in eradicating measurable residual disease (MRD) remaining following frontline treatment and achieving complete remission.[3]
- CD123 represents a promising therapeutic target for R/R AML due to its limited expression in non-malignant tissues yet frequent expression on leukemic blasts and LSCs.[4]
- Antibody-based and cell-based immunotherapies are emerging therapeutic approaches for the treatment of AML and MDS, but other than allogeneic hematopoietic stem cell transplant (allo-HSCT) no curative immunotherapy is currently available.[4-8]
- AFM28 is a novel innate cell engager (ICE®) designed to target CD123, an antigen expressed on both leukemic blasts and LSCs, whilst also binding CD16A on natural killer (NK) cells, thus inducing antibody dependent cellular cytoxicity towards leukemic blasts and LSCs.[9,10]



AFM28 binds to both NK cells and tumor cells and stimulates induction of cytotoxicity[10]



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

OBJECTIVE

To characterize the mechanism of action, anti-tumor activity, safety, tolerability, and pharmacodynamic profile of AFM28 to support future clinical trials in R/R AML.

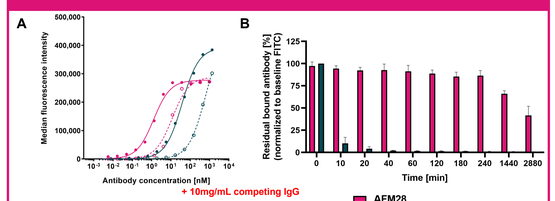
Table 1: Human hematological tumor cell lines utilized in this study.

Cell line	Origin/disease background	Mutational status	CD123 SABC (mean)
MOLM-13	Acute myeloid leukemia	FLT3-ITD; MLL-AF9	10,610
KG-1	Acute myeloid leukemia	p53 mut	9,952
EOL-1	Chronic eosinophilic leukemia	N/A	7,693
NOMO-1	Acute myeloid leukemia	p53 mut; MLL-AF9	5,243
THP-1	Acute monocytic leukemia	p53 mut; MLL-AF9	4,982
TF-1	Acute erythroid leukemia	N-Ras; p53 mut	4,932
OCI-AML3	Acute myeloid leukemia	DNMT3A; NPM1	2,340
Kasumi-1	Childhood acute myeloid leukemia	RUNX1 (t8:21, p53 mut, KIT, RAD21	1,424
OPM-2	Multiple myeloma	p53 mut	96

DNMT3A, DNA methyltransferase 3A; FLT3-ITD, FMS-like tyrosine kinase 3-internal tandem duplication; KIT, KIT proto-oncogene receptor tyrosine kinase; MLL-AF9, mixed lineage leukemia-protein AF9 fusion protein; NPM1, nucleophosmin 1; N-Ras, neuroblastoma RAS viral oncogene homolog; p53 mut, mutant tumor protein p53; RAD21, double-strand-break repair protein RAD21; RUNX1, runt-related transcription factor 1; SABC, specific antibody binding capacity.

RESULTS

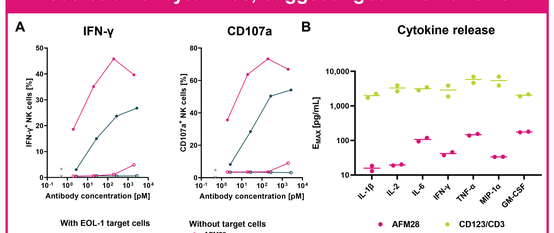
AFM28 binds NK cells with high affinity and exhibits long surface retention



(A) AFM28 binds to NK cells with high affinity in presence and absence of competing IgG. Primary NK cells were incubated with increasing concentrations of AFM28 in presence of 10 mg/mL polyclonal human IgG (Cutaquig). As control, Fc-enhanced anti-CD123 IgG1 was used. NK cell-retained antibodies were detected by flow cytometry. **(B) AFM28 stably binds to NK cell surfaces.** Primary NK cells were pre-loaded with 100 µg/mL AFM28 and incubated at 37ºC for the indicated time periods. Retained antibodies were measured by flow cytometry. Fc-binding Fc-enhanced anti-CD123 IgG1 was used as control. NK cell-retained antibodies were detected by flow cytometry. MFI values at time-point 0 were assumed to be 100%. Data shown is mean ± SD from three independent experiments.

FITC, fluorescein isothiocyanate; IgG1, immunoglobulin 1; MFI, median fluorescence intensity; NK, natural killer; SD, standard deviation.

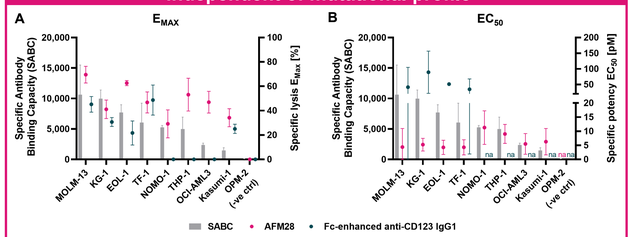
AFM28 induces potent NK cell activation and moderate secretion of cytokines, suggesting low risk of CRS



(A) AFM28 activates potent NK cell degranulation only when CD123⁺ target cells are present. AFM28-mediated IFN-γ expression and degranulation of NK cells in the presence or absence of CD123⁺ EOL-1 target cells (see Table 1) at an E:T ratio of 1:1 was assessed by flow cytometry after 4 hours co-culture. **(B) Only low levels of cytokines are induced by AFM28 in leukocytes suggesting low risk of cytokine-mediated toxicity, e.g., CRS.** Release of cytokines and chemokines from leukocyte cultures in the presence of AFM28 or a CD123/CD3 T-cell engager were measured after 24 hours. Data shown as E_{MAX} (mean ± SEM of two individual donors) of cytokine response with the indicated antibody.

CRS, cytokine release syndrome; E_{MAX}, maximal effect of the antibody; GM-CSF, granulocyte-macrophage colony-stimulating-factor; IFN-γ, interferon gamma; IL-1β, interleukin-1 beta; IL, interleukin; MIP-1α, macrophage inflammatory protein 1 alpha; TNF-α, tumor necrosis factor alpha; SEM, standard error of the mean.

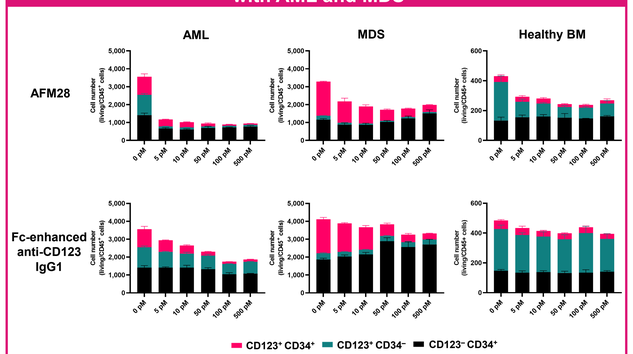
AFM28 anti-tumor activity towards CD123⁺ tumor cell lines is maintained at low CD123 expression levels and occurs independent of mutational profile



AFM28 induces lysis of CD123⁺ cell lines irrespective of CD123 expression level including of cell lines not lysed by an Fc-enhanced anti-CD123 IgG1 control antibody. E_{MAX} **(A)** and EC_{50} **(B)** of tumor cell lysis are depicted (n=3–5). **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. Buffy coat-derived allogeneic NK cells were cultured at a 2.5:1 E:T ratio with calcein-labelled leukemic cell lines in the presence of AFM28 and Fc-enhanced anti-CD123 IgG1.

EC_{50}, concentration of antibody required to give half the maximal effect; E_{MAX}, maximal effect of the antibody; na, not applicable; NK, natural killer; E:T, effector to target.

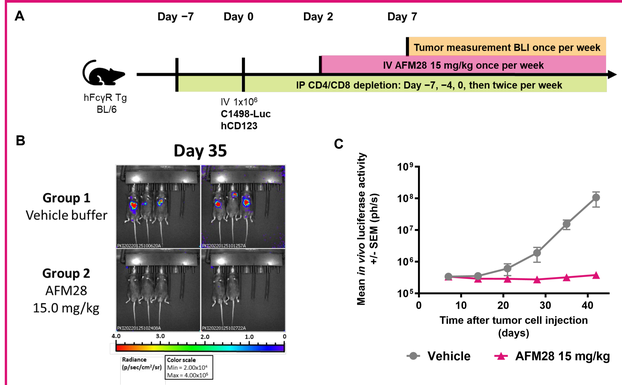
AFM28 induces specific lysis of CD123⁺ leukemic blasts, but not healthy CD123⁻ BM progenitors, in tissue from patients with AML and MDS



AFM28 induces specific NK cell-mediated lysis (ADCC) of primary leukemic cells of AML and MDS, but not of healthy BM-MNC. An ADCC assay was performed using allogeneic NK cells and primary BM-MNC samples from patients with AML and MDS as indicated, at an E:T ratio of 1:1. AFM28 and an Fc-enhanced anti-CD123 IgG1 antibody were added at the indicated concentrations and. Absolute numbers of remaining cells were quantified by flow cytometry after 24 hours culture. Graphs display total cell numbers of CD123⁺CD34⁺, CD123⁺CD34⁻ and CD123⁻CD34⁺ cell populations gated on living and CD45⁺ cells. Representative data from n=4 patient samples.

ADCC, antibody-dependent cellular cytotoxicity; AML, acute myeloid leukemia; BM, bone marrow; E:T, effector to target; MDS, myelodysplastic syndrome; MNC, mononuclear cells; NK, natural killer.

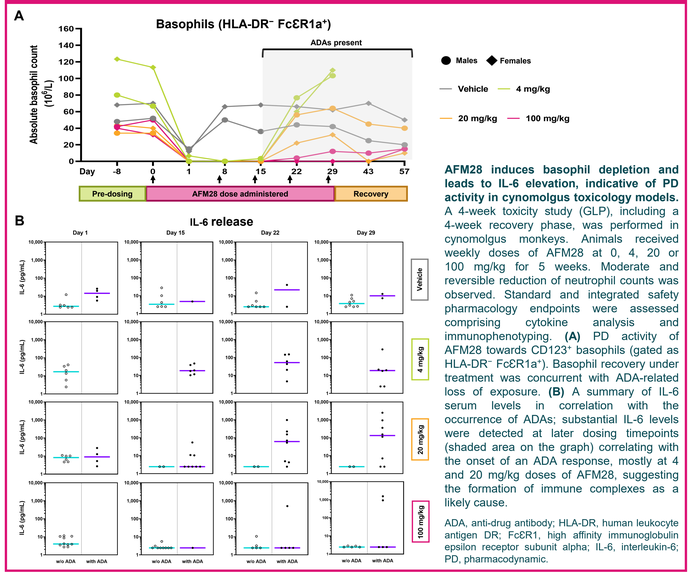
AFM28 inhibits tumor growth in an FcγR Tg BL/6 murine model of AML



AFM28 can prevent outgrowth of C1498-Luc-hCD123 tumor cells in a humanized murine model for AML. (A) Study Design: hFcγR Tg BL/6 mice were treated with anti-mCD4 and anti-mCD8 antibodies to induce T-cell depletion starting on Day −7 and Day −4 before tumor cell implantation, then twice weekly for the duration of the study. On Day 0, mice were implanted intravenously with C1498-Luc-hCD123 tumor cells and treated on Day 2 intravenously either with vehicle (n=6) or with 15 mg/kg AFM28 (n=5). From Day 7 onwards, luciferase activity was monitored using an IVIS® Lumina III bioluminescence imaging system (Perkin Elmer) with a CCD-camera. **(B) Bioluminescence results at Day 35**. All groups were monitored until Day 42 and compared to the corresponding vehicle control. **(C) *In vivo* luciferase activity over time.** Data are depicted as mean ± SEM.

BLI, bioluminescent imaging; hCD123, human CD123; IP, intraperitoneal; IV, intravenous; ph/s, photons per second; SEM, standard error of the mean; Tg, transgenic.

AFM28 exhibits limited IL-6 release and shows pharmacodynamic activity in cynomolgus monkeys



AFM28 induces basophil depletion and leads to IL-6 elevation, indicative of PD activity in cynomolgus toxicology models. A 4-week toxicity study (GLP), including a 4-week recovery phase, was performed in cynomolgus monkeys. Animals received weekly doses of AFM28 at 0, 4, 20 or 100 mg/kg for 5 weeks. Moderate and reversible reduction of neutrophil counts was observed. Standard and integrated safety pharmacology endpoints were assessed comprising cytokine analysis and immunophenotyping. **(A)** PD activity of AFM28 towards CD123⁺ basophils (gated as HLA-DR⁻ FcεR1a⁺). Basophil recovery under treatment was concurrent with ADA-related loss of exposure. **(B)** A summary of IL-6 serum levels in correlation with the occurrence of ADAs; substantial IL-6 levels were detected at later dosing timepoints (shaded area on the graph) correlating with the onset of an ADA response, mostly at 4 and 20 mg/kg doses of AFM28, suggesting the formation of immune complexes as a likely cause.

ADA, anti-drug antibody; HLA-DR, human leukocyte antigen DR; FcεR1, high affinity immunoglobulin epsilon receptor subunit alpha; IL-6, interleukin-6; PD, pharmacodynamic.

CONCLUSIONS

- These preclinical data demonstrate that AFM28 induces potent, effective, and specific anti-tumor activity towards CD123⁺ cells without impacting CD123⁻ healthy bone marrow progenitors.
- AFM28 induces NK cell-mediated ADCC also towards cells expressing low levels of CD123, irrespective of mutational status, suggesting potential for deep and broad anti-tumor responses.
- *In vivo* studies in a murine model of AML and in cynomolgus toxicology models suggest anti-tumor efficacy, and predicted pharmacodynamic activity with a well-tolerated safety profile and low risk of cytokine release syndrome, respectively.
- Clinical development will start with a first-in-human Phase 1 trial of AFM28 monotherapy in adult patients with R/R AML starting in 2022.
- Beyond monotherapy, development of AFM28 is also planned in combination with allogeneic NK cell therapy.

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