AFM28, a Novel Bispecific Innate Cell Engager (ICE®), Designed to Selectively Re-direct NK Cell Lysis to CD123+ Leukemic Cells in Acute Myeloid Leukemia and Myelodysplastic Syndrome

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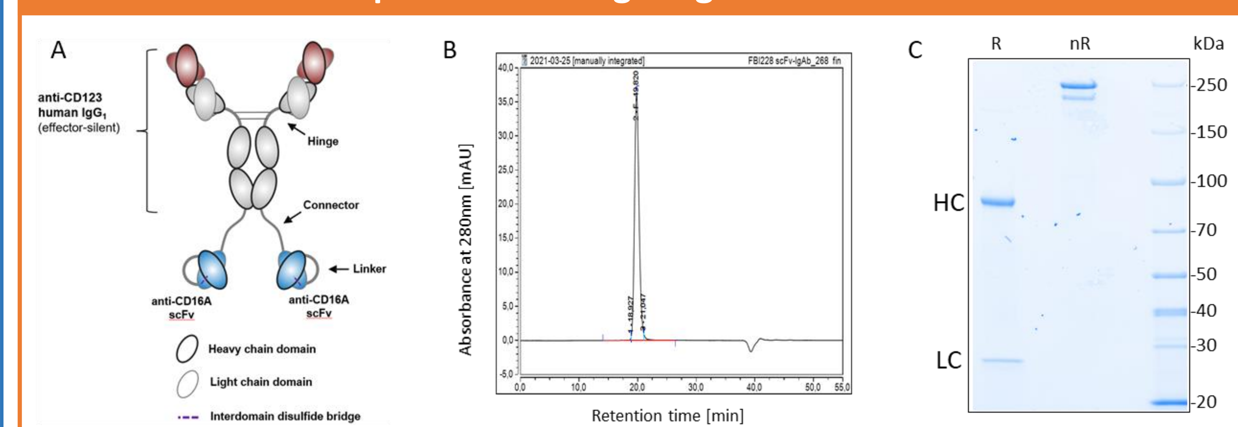
BACKGROUND

- 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]
- Progress in both indications has lagged behind other hematological tumors and novel therapies for patients with relapsed or refractory (R/R) disease and minimal residual disease (MRD) are needed.[1]
- Natural killer (NK) cell-based innate immunotherapy has emerged as a promising treatment option for AML and MDS based on the susceptibility of leukemic blasts for NK cell killing and clinical activity of allogeneic NK cell therapy in R/R disease.[3,4]
- Depletion of leukemic stem cells (LSCs) alongside leukemic blasts is key to eradicate MRD and prevent relapse; therefore, drugs that effectively target both cell types hold promise in achieving long-term remission in patients with AML/MDS.[5]
- The α chain of the interleukin-3 receptor, CD123, is an attractive target for antibody-based immunotherapeutic approaches to target LSCs and leukemic blasts in AML and MDS, because of its almost universal expression on these cell types.[6]
- **I**nnate **C**ell **E**ngager (ICE®) molecules developed from the **R**edirected **O**ptimized **C**ell **K**illing (ROCK®) platform are designed to bivalently engage CD16A+ NK cells and macrophages and induce potent, tumor-directed cytotoxicity via antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP).[2]
- AFM28 is a novel, tetravalent, bispecific ICE® designed to bind CD16A and CD123 and induce potent depletion of LSCs and leukemic blasts in AML and MDS.

OBJECTIVES

- **Preclinical development of AFM28 and investigation of its mechanism of action as a novel treatment modality for patients with AML or high-risk (HR)-MDS.**
- **Testing of safety, tolerability, and pharmacodynamic activity of AFM28 in a preclinical toxicology model in cynomolgus monkeys.**

AFM28 is a novel bispecific ICE® targeting CD16A and CD123



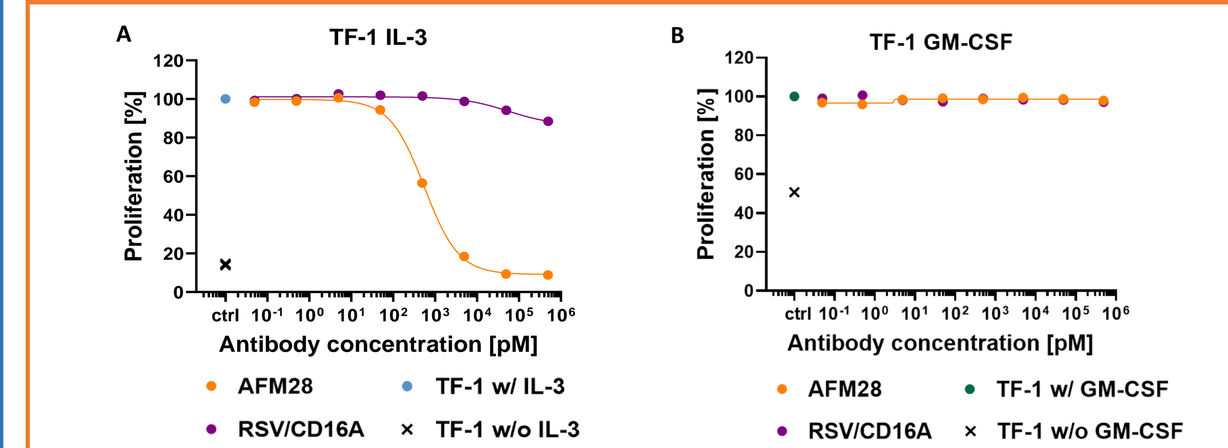
AFM28 selectively binds human CD16A 158V- and F-variants, cynomolgus CD16, and human and cynomolgus CD123 with high affinity

	huCD16A158V	huCD16A158F	huCD16B	cynoCD16	huCD123	cynoCD123
K_{on}	1.4E+06 M-1s-1	1.2E+06 M-1s-1	ND	7.9E+05 M-1s-1	8.5E+05 M-1s-1	1.7E+06 M-1s-1
K_{off}	2.6E-04 s-1	3.2E-04 s-1	ND	3.2E-04 s-1	4.4E-05 s-1	7.1E-05 s-1
K_D	195 pM	271 pM	2.1 ± 1.7 µM	407 pM	52 pM	41.2 pM

A) AFM28 is a tetravalent bispecific IgG1-scFv fusion antibody (scFv-IgAb) specific for human CD123 and human CD16A with effector-silenced Fc region. B) Analytical HPLC demonstrates that AFM28 can be purified as a non-aggregating monomer with high overall purity. C) Reduced (R) vs non-reduced (nR) SDS-PAGE of AFM28 shows purity and integrity, and suggests good producibility. HC, heavy chain; HPLC, high-performance liquid chromatography; LC, light chain; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

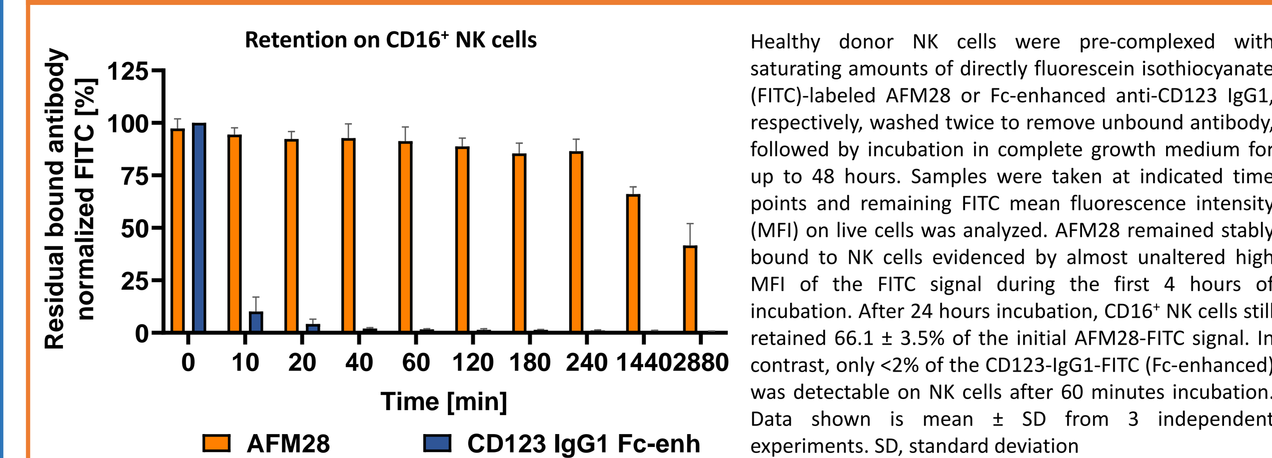
RESULTS

AFM28 specifically inhibits the IL-3-dependent proliferation of TF-1 cells without affecting proliferation induced by GM-CSF



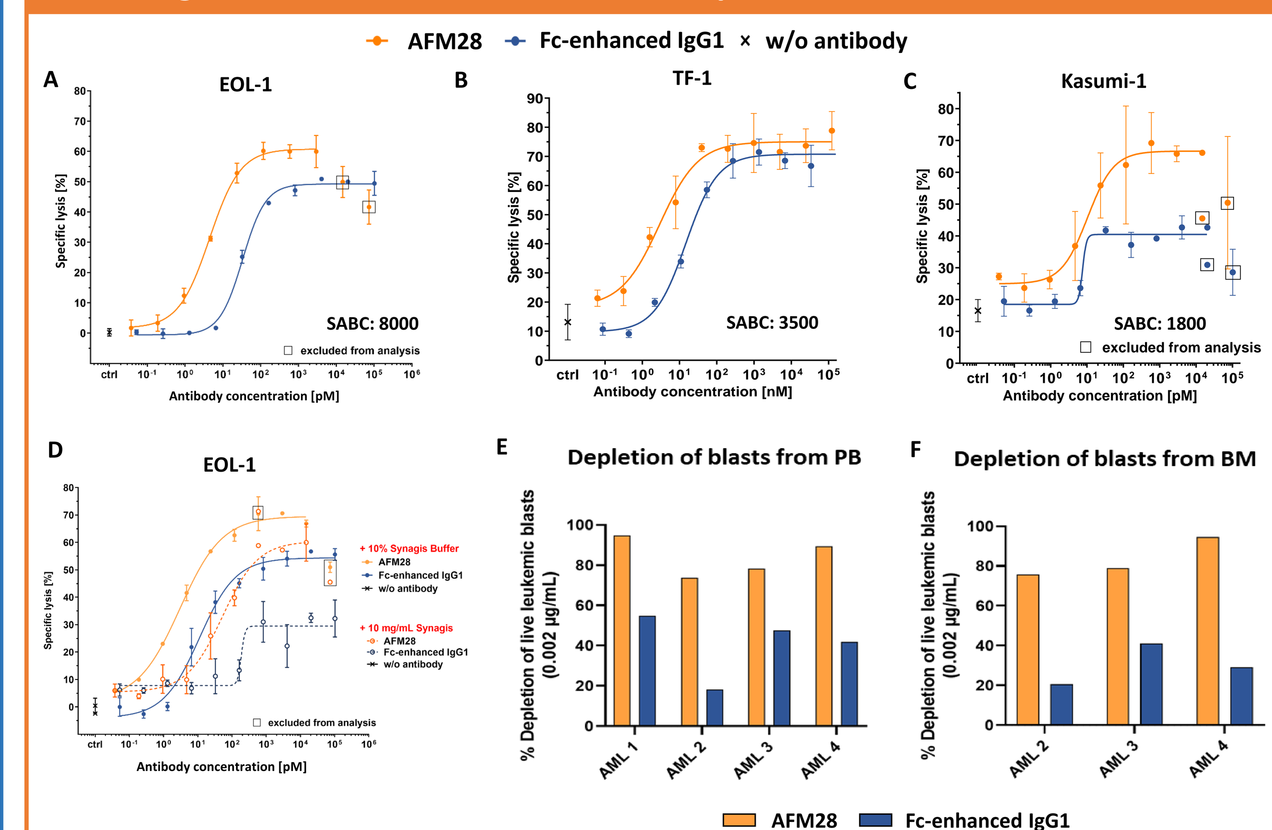
TF-1 cell growth was stimulated with IL-3 or GM-CSF in the presence of titrated AFM28, a non-targeting control engager (RSV/CD16A), or without addition of an antibody, for 72 hours at 37°C. Proliferation activity was determined using CellTiter Glo Assay in three independent experiments; Graph shows representative data from one experiment. E_{max} and IC_{50} = mean ± SD (n=3). A) When cultured in the presence of IL-3, addition of AFM28 resulted in a concentration-dependent decrease in the proliferation of TF-1 cells with an E_{max} of 83 ± 6%. The half-maximal inhibitory concentration (IC_{50}) of AFM28 on the IL-3 dependent proliferation was calculated as 467 ± 60 pM. Proliferation was monitored in the presence of RSV/CD16A and showed no substantial reduction on the proliferation of TF-1 cells cultured with IL-3. B) This inhibitory effect was not seen when TF-1 cells were cultured in the presence of GM-CSF.

AFM28 shows prolonged surface retention on NK cells compared with an Fc-enhanced anti-CD123 IgG1



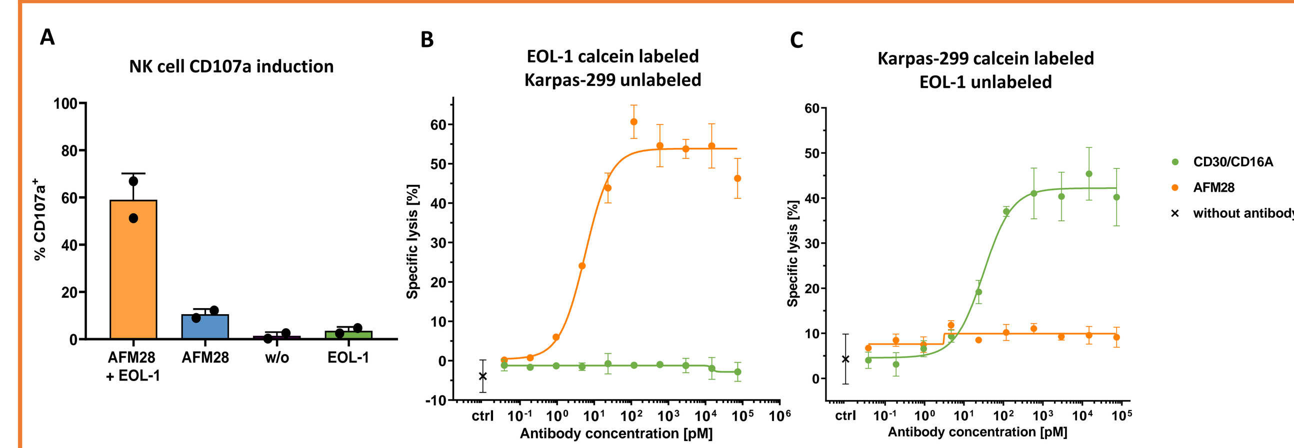
Healthy donor NK cells were pre-complexed with saturating amounts of directly fluorescein isothiocyanate (FITC)-labeled AFM28 or Fc-enhanced anti-CD123 IgG1, respectively, washed twice to remove unbound antibody, followed by incubation in complete growth medium for up to 48 hours. Samples were taken at indicated time points and remaining FITC mean fluorescence intensity (MFI) on live cells was analyzed. AFM28 remained stably bound to NK cells evidenced by almost unaltered high MFI of the FITC signal during the first 4 hours of incubation. After 24 hours incubation, CD16+ NK cells still retained 66.1 ± 3.5% of the initial AFM28-FITC signal. In contrast, only <2% of the CD123-IgG1-FITC (Fc-enhanced) was detectable on NK cells after 60 minutes incubation. Data shown is mean ± SD from 3 independent experiments. SD, standard deviation

AFM28 efficaciously induces NK cell-mediated lysis of CD123+ cells, including cells with low CD123 surface expression and AML blasts



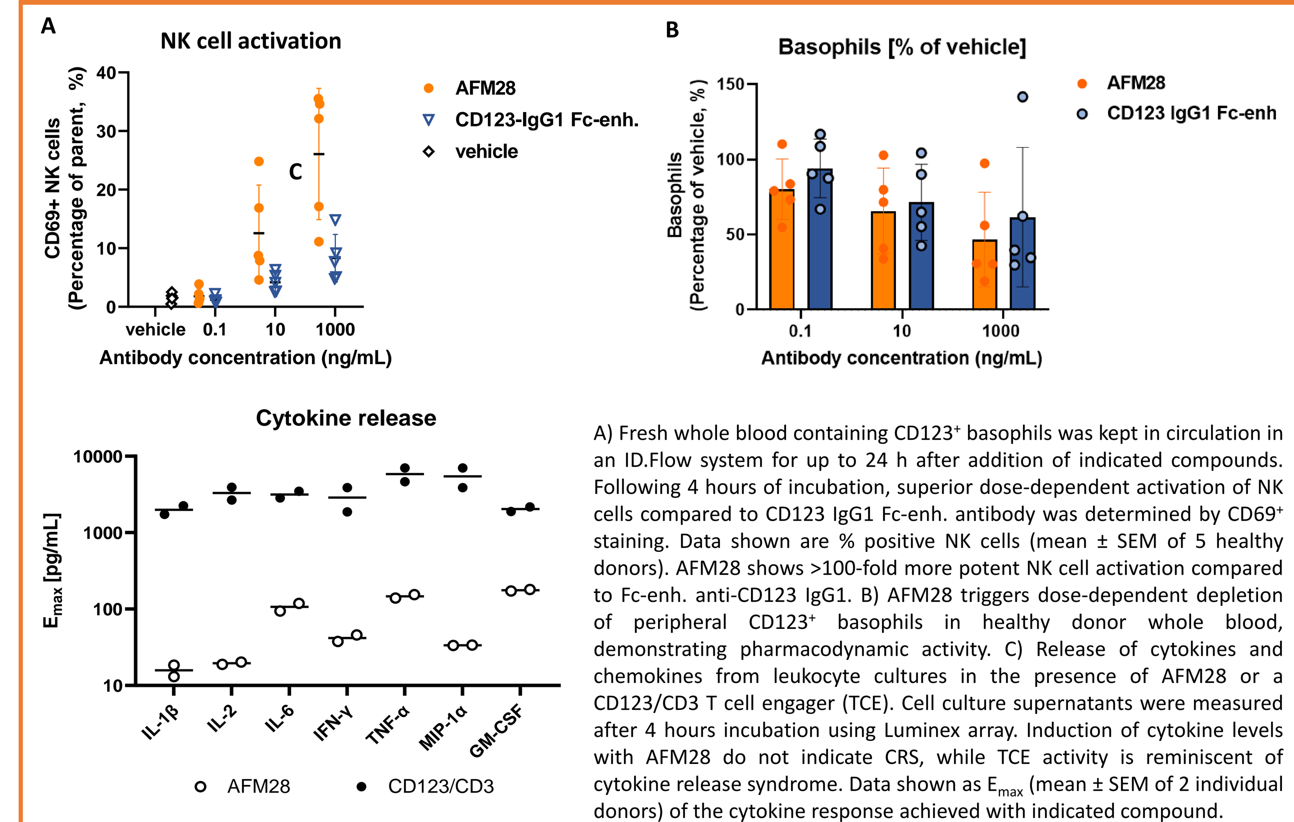
A-C) Cell lines expressing different levels of CD123 were labeled with calcein and used as target cells in ADCC assays comparing AFM28 vs an Fc-enhanced CD123-IgG1. Effector to target (E:T) cell ratio was kept at 2.5:1. AFM28 demonstrated superior potency and efficacy of target cell ADCC, irrespective of CD123 expression, most noticeable on CD123 low-expressing Kasumi-1 cells. D) In the presence of competing human IgG (synagis) AFM28 retained high potency and efficacy (EC_{50} = 3 pM vs. 52 pM with IgG; E_{max} = 67% vs. 55% with IgG), while the Fc-enhanced CD123-IgG1 showed a marked activity decrease (EC_{50} = 11 pM vs. 187 pM with IgG; E_{max} = 58% vs. 22% with IgG). E-F) Primary AML samples from peripheral blood (PB, n=4) and bone marrow (BM, n=3) were subject to ADCC assays using allogeneic NK cells (E:T ratio 1:1) in the presence of 2 ng/mL AFM28 or Fc-enhanced anti-CD123 IgG1. AFM28 induces lysis of >70–95% of PB and BM blasts and thus outcompetes the activity of an Fc-enhanced antibody (max. lysis 54%). AML 2–4 are donor-matched PB and BM samples.

Activation of NK cells by AFM28 is target-dependent and does not induce lysis of CD123− bystander cells



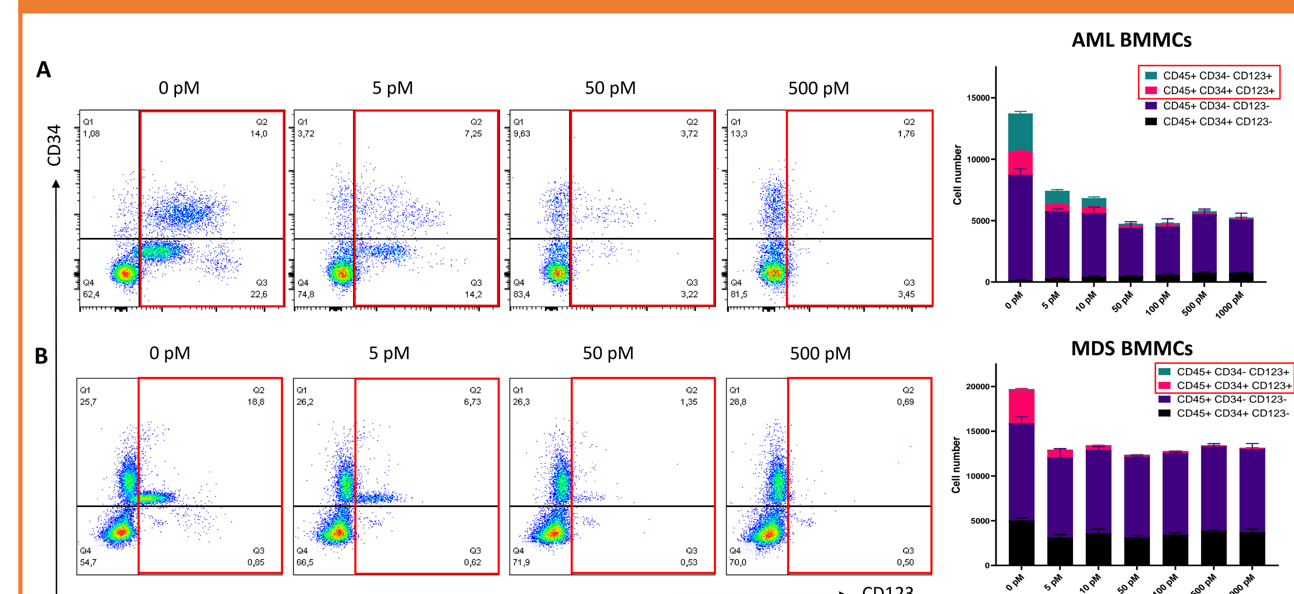
A) Strong CD107a upregulation on NK cells after 4 hours co-culture of NK cells with EOL-1 tumor cells at E:T ratio 1:1 (each 5 × 10^4 cells) by surface staining with anti-CD107a-FITC. EOL-1 cells or AFM28 alone induced minimal NK cell activation. Calcein-release assay showing ADCC using B) CD123+ labeled EOL-1 cells or C) CD123− KARPAS-299 cells each in the presence of same numbers of unlabeled counterpart and healthy donor NK cells (E:T ratio 2.5:1). AFM28 specifically induces lysis of target-positive EOL-1 cells without affecting the viability of CD30+ KARPAS-299 bystander cells, which are only killed in the presence of a CD30/CD16A bispecific ICE® construct.

AFM28 induces potent NK cell activation and moderate secretion of cytokines, suggesting low risk of cytokine release syndrome



A) Fresh whole blood containing CD123+ basophils was kept in circulation in an ID.Flow system for up to 24 h after addition of indicated compounds. Following 4 hours of incubation, superior dose-dependent activation of NK cells compared to CD123 IgG1 Fc-enh. antibody was determined by CD69+ staining. Data shown are % positive NK cells (mean ± SEM of 5 healthy donors). AFM28 shows >100-fold more potent NK cell activation compared to Fc-enh. anti-CD123 IgG1. B) AFM28 triggers dose-dependent depletion of peripheral CD123+ basophils in healthy donor whole blood, demonstrating pharmacodynamic activity. C) Release of cytokines and chemokines from leukocyte cultures in the presence of AFM28 or a CD123/CD3 T cell engager (TCE). Cell culture supernatants were measured after 4 hours incubation using Luminex array. Induction of cytokine levels with AFM28 do not indicate CRS, while TCE activity is reminiscent of cytokine release syndrome. Data shown as E_{max} (mean ± SEM of 2 individual donors) of the cytokine response achieved with indicated compound.

AFM28 potently eliminates CD123+ cells from primary AML and HR-MDS bone marrow and spares CD34+/CD123− HSC compartment



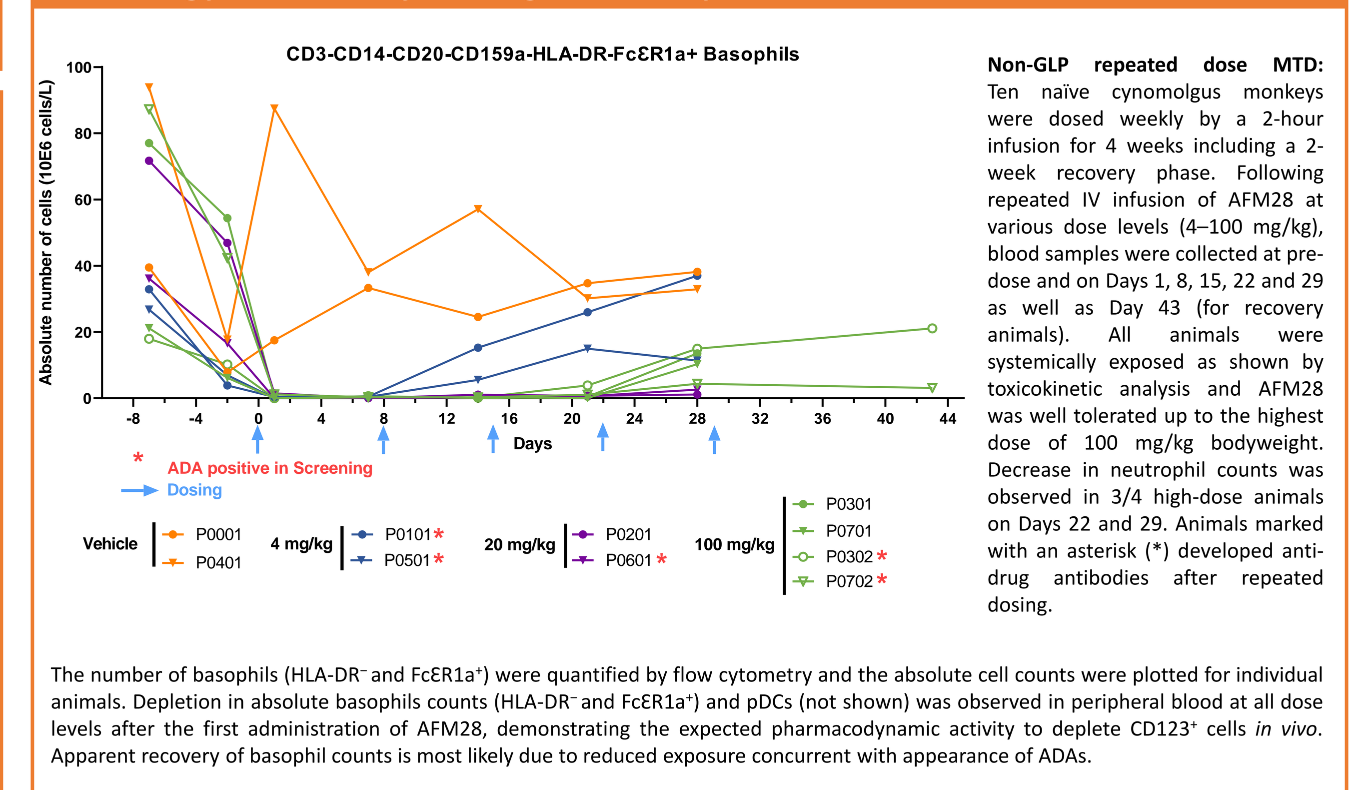
Bone marrow mononuclear cells (BMMC) from newly diagnosed patients with A) AML and B) high-risk MDS were incubated with increasing concentrations of AFM28 (0–1.000 pM) in the presence of allogeneic healthy donor NK cells (E:T ratio 1:1) for 24 hours and analyzed by multi-color flow cytometry on a BD FACSCelesta. The CD45+/CD34+/CD123+ and the CD45+/CD34−/CD123+ cells are very efficiently depleted (red boxes), while the putative hematopoetic stem cells (CD34+/CD123−) compartment and stromal bone marrow (CD45+/CD34−/CD123−) cells remain largely unaffected. An untargeted RSV/CD16A control protein did not show activity against BMMCs (data not shown). Representative scatter plots shown, bar graphs show data acquired from individual assays in triplicates.

AFM28 exhibits antibody-like pharmacokinetic profiles in mice and cynomolgus monkeys

Species	Dose [mg/kg]	$t_{1/2}$ [h]	C_{max} [µg/mL]	AUC [h·µg/mL]	CL [mL/h·kg]
CD1 mice	100	288	139.36	34,913.79	0.010
Cynomolgus monkey	4	29*	91.25	4,565.50	0.85
	20	47*	474.65	29,328.00	0.5
	100	67*	3,132.93	188,386.25	0.47

CD1-swiss mice received a single intravenous (IV) infusion of AFM28 (100 mg/kg) and pharmacokinetic samples were collected. Serum $t_{1/2}$ was calculated to 12 days (288 h) and lies within historic expectations. Toxicokinetic data in cynomolgus monkeys is derived from a repeated dose-range finding study with weekly IV infusions at the indicated dose levels. Serum half-lives were calculated after the first dose. Results likely underestimate the true $t_{1/2}$ since ß-elimination phase may not be fully reached at the end of the first dosing interval (indicated by asterisk). Exposure as measured by C_{max} and AUC increases slightly more than dose proportional. AUC, area under the curve; CL, clearance; C_{max}, maximum concentration; $t_{1/2}$, half-life.

AFM28 induces rapid complete depletion of CD123+ basophils in a preclinical toxicology model in cynomolgus monkeys



Non-GLP repeated dose MTD: Ten naïve cynomolgus monkeys were dosed weekly by a 2-hour infusion for 4 weeks including a 2-week recovery phase. Following repeated IV infusion of AFM28 at various dose levels (4–100 mg/kg), blood samples were collected at pre-dose and on Days 1, 8, 15, 22 and 29 as well as Day 43 (for recovery animals). All animals were systemically exposed as shown by toxicokinetic analysis and AFM28 was well tolerated up to the highest dose of 100 mg/kg bodyweight. Decrease in neutrophil counts was observed in 3/4 high-dose animals on Days 22 and 29. Animals marked with an asterisk (*) developed anti-drug antibodies after repeated dosing.

The number of basophils (HLA-DR− and FcεR1a+) were quantified by flow cytometry and the absolute cell counts were plotted for individual animals. Depletion in absolute basophils counts (HLA-DR− and FcεR1a+) and pDCs (not shown) was observed in peripheral blood at all dose levels after the first administration of AFM28, demonstrating the expected pharmacodynamic activity to deplete CD123+ cells *in vivo*. Apparent recovery of basophil counts is most likely due to reduced exposure concurrent with appearance of ADAs.

CONCLUSIONS

- **AFM28 potently depletes primary CD123+ tumor cells via NK cell-mediated ADCC and promises to effectively target both leukemic blasts and LSCs.**
- **High-binding affinity, potent NK-cell activation and target cell lysis with low risk of CRS suggest AFM28 is superior to previously developed Fc-enhanced anti-CD123 IgG and T cell-based therapies.**
- **In a cynomolgus toxicology model AFM28 was well-tolerated and demonstrated the anticipated pharmacodynamic activity.**
- **AFM28 may hold particular promise when used in combination with allogeneic NK cell therapy due to high-binding affinity and potent engagement of NK cells with long-lasting surface retention.**
- **These data suggest AFM28 has potential as a novel treatment modality in AML and MDS and warrants investigation in a first-in-human study.**

REFERENCES

1. Saultz JN and Garzon R. J Clin Med 2016;5(3):33; 2. Ellwanger K et al. MAbs 2019;11(5):899–918; 3. Kapoor S et al. Cancers 2021;13(19):5026; 4. Kaweme NM and Zhou F. Front Immunol 2021;12:683381; 5. Hanekamp D et al. Int J Hematol 2017;105:549–57; 6. Testa U et al. Cancers (Basel) 2019;11(9):1356.

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