The Bispecific Innate Cell Engager AFM28 Induces Potent Anti-Tumor Activity Against AML in a Xenograft Mouse and in a Bone Marrow Niche *in vitro* Model

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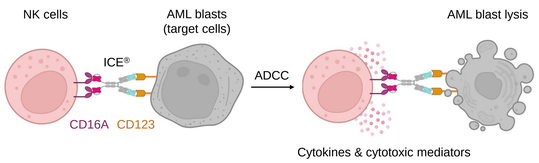
Background

- Depletion of treatment-resistant leukemic stem cells (LSC) is key to reduce relapse-inducing residual disease and promote long-term remissions in patients with acute myeloid leukemia (AML). Therefore, novel therapies are required that enable targeting of not only leukemic blasts but also LSC[1].
- Innate Cell Engager (ICE®) molecules bind to CD16A on natural killer (NK) cells and a tumor cell-surface antigen, redirecting NK cells to tumor cells and stimulating antibody-dependent cellular cytotoxicity (ADCC)[2].
- AFM28 (CD123/CD16A) is a novel ICE® designed to target CD123, an antigen universally expressed on both leukemic blasts and LSCs[3].
- A phase 1 dose escalation study to assess the safety and tolerability of AFM28 monotherapy (NCT05817058) is ongoing.
- AFM28 has previously been shown to induce depletion of primary leukemic cells *in vitro*[3]. The effects of immunosuppressive cellular components of the leukemic bone marrow microenvironment on the efficacy of AFM28 remain unknown.
- While AFM28 showed pharmacodynamic activity in cynomolgus monkeys[3], anti-tumor activity *in vivo* by AFM28 against human CD123+ AML cells remained to be demonstrated.

Objectives

- Assess the preclinical anti-leukemic activity of AFM28 in a disseminated AML xenograft mouse model.
- Evaluate AFM28-mediated targeting of patient-derived primary leukemic cells in a newly engineered Human Organotypic bone Marrow Environment (HOME) model, that closely recapitulates the cellular complexity of the human bone marrow.

AFM28 enables ADCC against AML cells by NK cells



ADCC: Antibody-mediated cellular cytotoxicity, ICE®: Innate cell engager

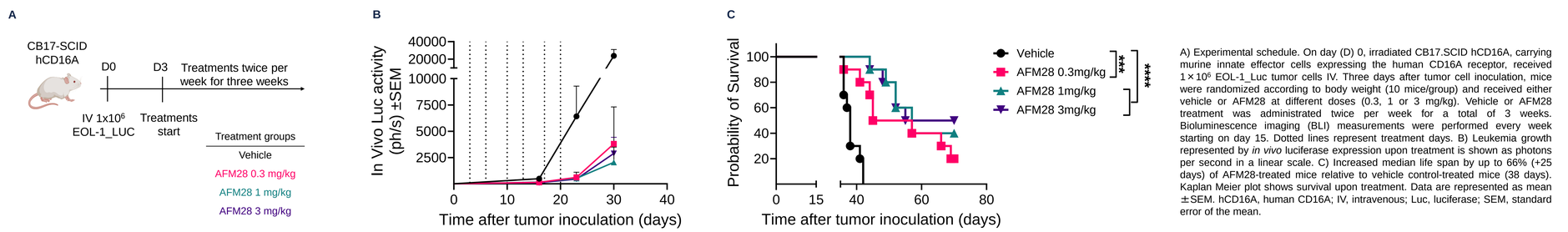
Conclusions

- AFM28 demonstrated significant anti-leukemic activity in both *in vivo* and *ex vivo* settings, including depletion of LSC-like cells.
- The therapeutic effects of AFM28 against leukemic blasts and LSC-like cells were demonstrated in the fully human bone marrow niche model, in which key immune suppressive cellular components of the AML bone marrow niche are present.
- Overall, the preclinical efficacy of AFM28 against leukemic stem-like cells along with its previously shown favorable preclinical safety profile underscores its potential to reduce residual disease and promote long-term remissions for AML patients.
- Building on the proof-of-concept and remarkable response data in lymphoma of the combination of the CD30-targeting ICE® acimtamig/AFM13 with allogenic NK cells[4], combination with highly active allogeneic NK cells is envisioned to further increase AFM28-mediated anti-leukemic efficacy and depth of response in AML patients.

References

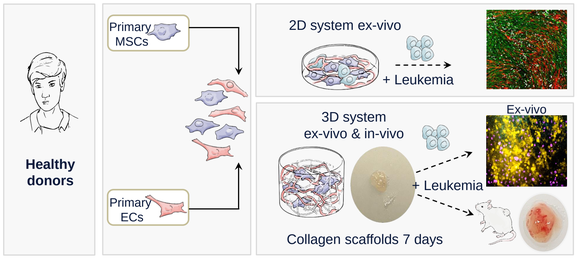
1. Hanekamp D et al. Int J Hematol 2017;105:549–57; 2. Ellwanger K et al. MAbs 2019;11(5):899–918; 3. Schmitt N et al. ASH. 2022;138:3344; 4.Nieto et al. Blood (2023) 142 (Supplement 1): 774.

AFM28 induces tumor control and prolongs survival in a huCD16A+ transgenic xenograft mouse model



A) Experimental schedule. On day (D) 0, irradiated CB17.SCID hCD16A, carrying murine innate effector cells expressing the human CD16A receptor, received 1×10^6 EOL-1_Luc tumor cells IV. Three days after tumor cell inoculation, mice were randomized according to body weight (10 mice/group) and received either vehicle or AFM28 at different doses (0.3, 1 or 3 mg/kg). Vehicle or AFM28 treatment was administrated twice per week for a total of 3 weeks. Bioluminescence imaging (BLI) measurements were performed every week starting on day 15. Dotted lines represent treatment days. B) Leukemia growth represented by *in vivo* luciferase expression upon treatment is shown as photons per second in a linear scale. C) Increased median life span by up to 66% (+25 days) of AFM28-treated mice relative to vehicle control-treated mice (38 days). Kaplan Meier plot shows survival upon treatment. Data are represented as mean ±SEM. hCD16A, human CD16A; IV, intravenous; Luc, luciferase; SEM, standard error of the mean.

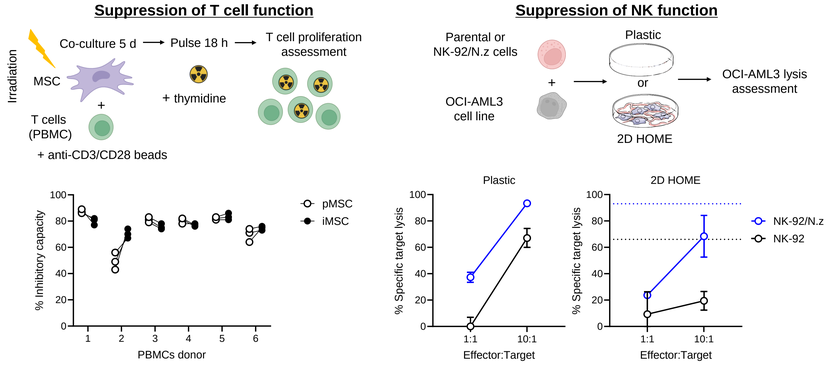
Human Organotypic Marrow Environment (HOME)



MSCs: Mesenchymal stromal cells, ECs: Endothelial cells

Bone marrow (BM) donation aspiration filters were processed, to remove the hematopoietic cells and isolate high purity BM stromal cells (MSCs) and BM endothelial cells (ECs) in a reproducible and robust manner. MSCs and ECs were co-cultured at a defined ratio, to initiate the formation of vessel-like structures on plastic (2D) or collagen scaffolds (3D).

Immunsuppressive effect of the non-hematopoietic marrow compartment



Irradiated mesenchymal stromal cells (MSCs) were co-cultured with allogeneic PBMCs in the presence of stimulating anti-CD3/CD28 antibodies for 5 days. Radiolabeled 3H-thymidine was added for the last 18 hours of culture. Cells were harvested and incorporation was determined in a beta counter. pMSC: parental MSC, iMSC: immortalized MSC.

Parental NK-92 cells or NK-92/N.z (NKG2D CAR-expressing NK-92 cells) were co-cultured with NKG2D ligand –expressing OCI-AML3 cells (AML cell line) at 1:1 and 10:1 effector to target ratio for 4 h. The cytotoxic effect was assessed on plastic and 2D HOMEs by flow cytometry. Data are represented as mean ±SD.

AML patient characteristics

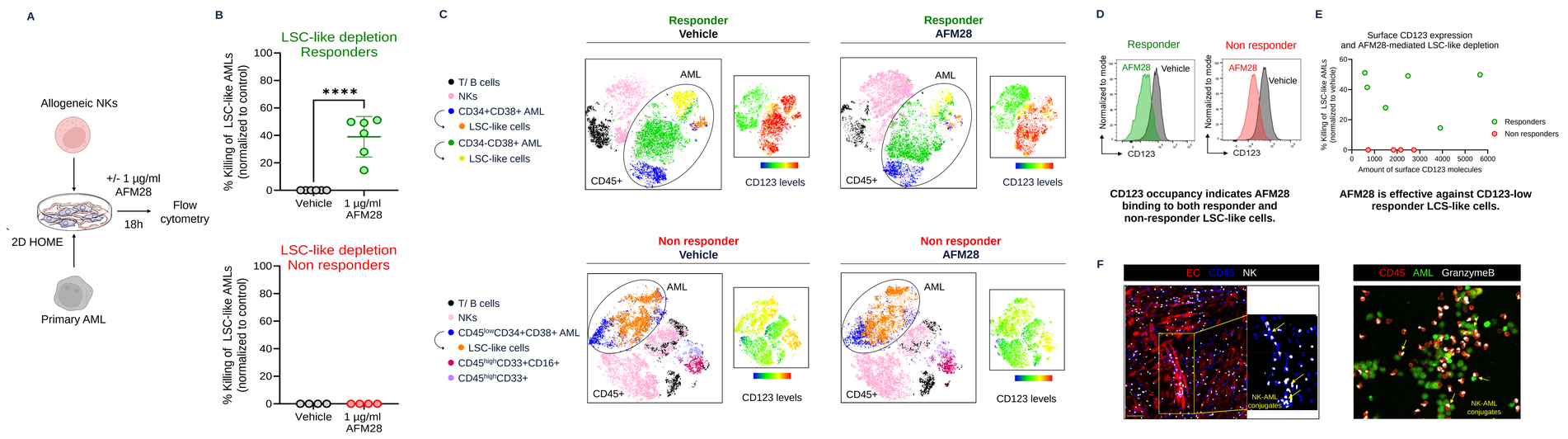
	Relapsed/refractory AML	Mutated genes	CD123 molecules per LSC-like cell
Responders	AML-1	*FLT3/NPM1*	677
	AML-2	*FLT3 AML, TET2, RUNX1, PTPN11*	5660
	AML-3	*screening failure*	3904
	AML-5	*GATA2,Kras,Nras, SF3B1*	2478
	AML-8	*CEBPA*	574
	AML-9	*FLT3I, FLT3R (0.73)*	1491
Non responders	AML-4	*BCOR, EZH2, RUNX1*	2742
	AML-6	*n.d.*	723
	AML-10	*n.d.*	1838
	AML-11	*MLL, FLT3I, FLT3R (0.24)*	2162

Characteristics of cryopreserved AML patient samples and quantification of CD123 surface molecules on LSC-like cells measured by flow cytometry.

Donor	Sex	Age	Blood group
#1	F	61	A+
#2	F	70	0+
#3	M	65	A+
#4	M	65	B+
#5	F	58	0+
#6	M	71	B+
#7	M	63	A+
#8	M	60	A+

Characteristics of PBMCs obtained from >55 years-old healthy donors, reflecting the AML patient age group. NK cells were enriched from PBMCs by negative isolation.

AFM28 enables depletion of AML LSC-like cells in 2D HOMEs mediated by allogeneic NK cells



A) Experimental setup. Cryopreserved relapsed/refractory AML samples were co-cultured at 1:1 ratio with healthy, allogeneic NK cells (donors >55 years old) on 2D HOMEs and treated for 18 h with vehicle or AFM28 (1 µg/ml). B) Quantification of the depletion of AML LSC-like cells (as defined by CD11b, CD64 and GPR56 and/or IL1RAP expression Samples were subdivided in responders (upper panel) and non-responders (lower panel). Data are represented as mean ±SD. C) Representative t-SNE visualization of the CD45+ population in treated AML samples (vehicle or 1 µg/ml AFM28) harvested from the 2D HOME cultures with cell type assignment and corresponding CD123 expression levels on remaining cells. D) Representative histograms of CD123 receptor occupancy in responders and non-responders. E) Correlation of absolute CD123 molecules and AFM28-mediated depletion of LSC-like cells. F) Exemplary immunofluorescence stainings of allogeneic NK/ AML co-cultures on 2D HOMES.