Combinatorial Immunotherapy of Tetravalent Bispecific AFM13 and AB-101 NK Cell Product Confers Tumor Growth Control *In Vivo*.

Jens Pahl[1], José Medina Echeverz[1], Torsten Haneke[1], Lisa Guerrettaz[2], Srinivas Somanchi[2], Heather Raymon[2], Sheena Pinto[1], Peter Flynn[2], Joachim Koch[1]

[1]Affimed GmbH, Im Neuenheimer Feld 582, 69120 Heidelberg, Germany

[2]Artiva Biotherapeutics, Inc., 5505 Morehouse Drive, Suite 100, San Diego, California, 92121, USA

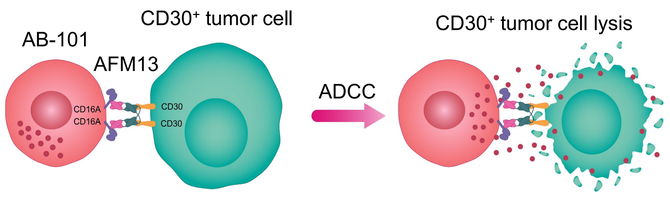
BACKGROUND

- Bispecific Innate Cell Engager (ICE®) molecules bind to CD16A on NK cells and to a tumor cell-surface antigen, inducing NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC)[1]. This enhances the efficacy of allogeneic NK cell immunotherapy [2,3].
- AFM13, a CD30/CD16A ICE® designed to target CD30$^+$ malignancies such as Hodgkin lymphoma (HL), has been demonstrated to mediate ADCC against CD30$^+$ tumor target cells *in vitro* and *in vivo*[4-6].
- In a Phase 1/2 clinical study (NCT04074746), the recommended Phase 2 dose of AFM13 in combination with adoptive NK cell transfer achieved an unprecedented objective response rate of 94%, and a complete response (CR) rate of 71%, in 35 heavily pre-treated patients with CD30$^+$ HL and non-Hodgkin lymphoma (NHL). Of the patients with at least six months follow-up after the initial infusion (n=24), 63% remained in CR for six months or more[7].
- AB-101 is a non-engineered, allogeneic, off-the-shelf, cryopreserved cord blood-derived NK cell product formulated in an infusion-ready medium, currently being tested in a Phase 1/2 trial as monotherapy and in combination with rituximab for relapsed/refractory B cell NHL; preliminary results have demonstrated AB-101 is well tolerated[8].
- AB-101 is optimized for ADCC through pre-selection for the KIR-B haplotype and the natural high-affinity variant of CD16A (158V/V)[8].

OBJECTIVE

To investigate the anti-tumor activity of AFM13 in combination with AB-101 *in vitro* and *in vivo*.

AFM13 + AB-101: Mechanism of Action

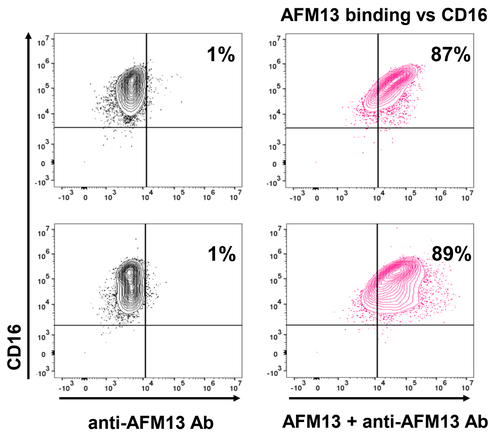


AFM13 acts by binding CD30 on tumor cells and CD16A on AB-101 NK cells, redirecting and potentiating NK cell-mediated lysis of specific tumor cells.
ADCC, antibody-dependent cellular cytotoxicity; NK, natural killer.

RESULTS

Saturation of CD16 by AFM13 on AB-101 after cryopreservation

AFM13 binding was detected on approximately 90% of AB-101

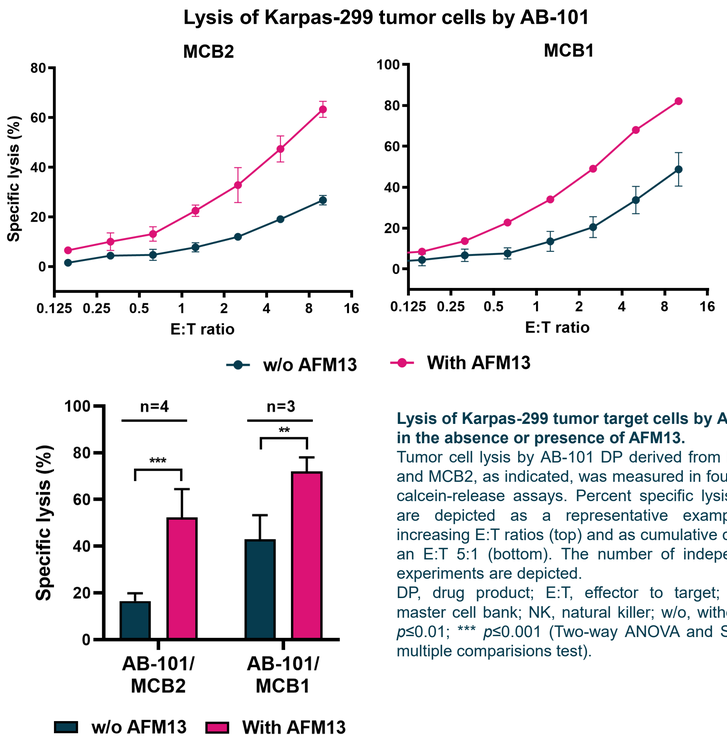


Homogenous binding of AFM13 to approximately 90% of AB-101. AB-101 was subjected to saturating concentrations of AFM13 immediately after thaw and AFM13 was detected with anti-AFM13 antibodies relative to CD16 expression levels. Representative data of AB-101 DP derived from MCB2 (upper row) and MCB1 (lower row), depicting the percentage of viable CD56$^+$ CD16$^+$ cells and viable CD56$^+$ AFM13$^+$ cells
Ab, antibody; DP, drug product; MCB, master cell bank.

RESULTS

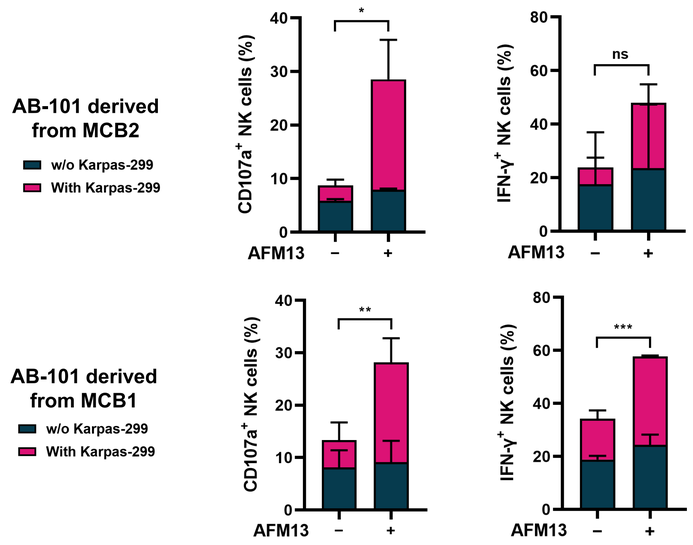
Combination with AFM13 enhanced the cytotoxic activity of AB-101 after cryopreservation

The addition of AFM13 specifically increased the cytotoxic activity of AB-101 towards CD30$^+$ tumor cells



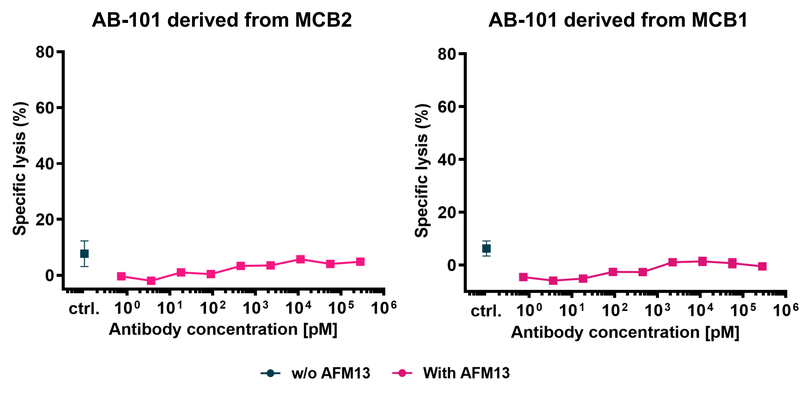
Lysis of Karpas-299 tumor target cells by AB-101 in the absence or presence of AFM13.
Tumor cell lysis by AB-101 DP derived from MCB1 and MCB2, as indicated, was measured in four-hour calcein-release assays. Percent specific lysis data are depicted as a representative example at increasing E:T ratios (top) and as cumulative data at an E:T 5:1 (bottom). The number of independent experiments are depicted.
DP, drug product; E:T, effector to target; MCB, master cell bank; NK, natural killer; w/o, without; ** $p \leq 0.01$; *** $p \leq 0.001$ (Two-way ANOVA and Sidak's multiple comparisions test).

The addition of AFM13 specifically increased the degranulation and IFN-γ production by AB-101



Percentage of CD107a expression (marker for NK cell degranulation) and intracellular IFN-γ expression of AB-101, with or without Karpas-299 cells with or without AFM13.
Values were measured after four hours co-culture; data are depicted as cumulative data from two independent experiments.
MCB, master cell bank; ns, non-significant; w/o, without; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (Two-way ANOVA and Tukey's multiple comparisions test).

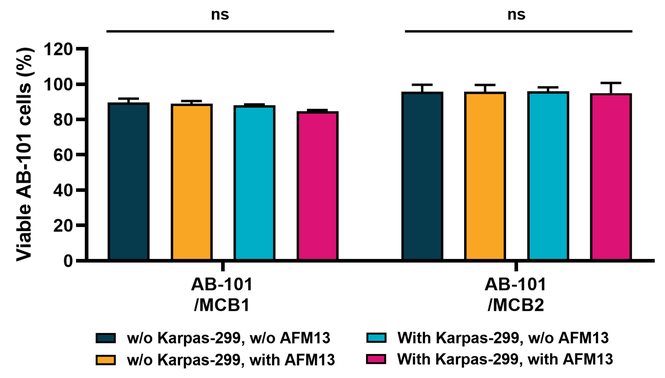
AFM13 does not induce fratricide of AB-101 after cryopreservation



Evaluation of AB-101 fratricide in the presence of AFM13. Lysis of AB-101 after cryopreservation, referred to as fratricide, in the presence of increasing concentrations of AFM13 was measured in four-hour calcein-release assays. Representative data of AB-101 DP from two (MCB1) or three (MCB2) independent experiments are shown.
DP, drug product; MCB, master cell bank; w/o, without.

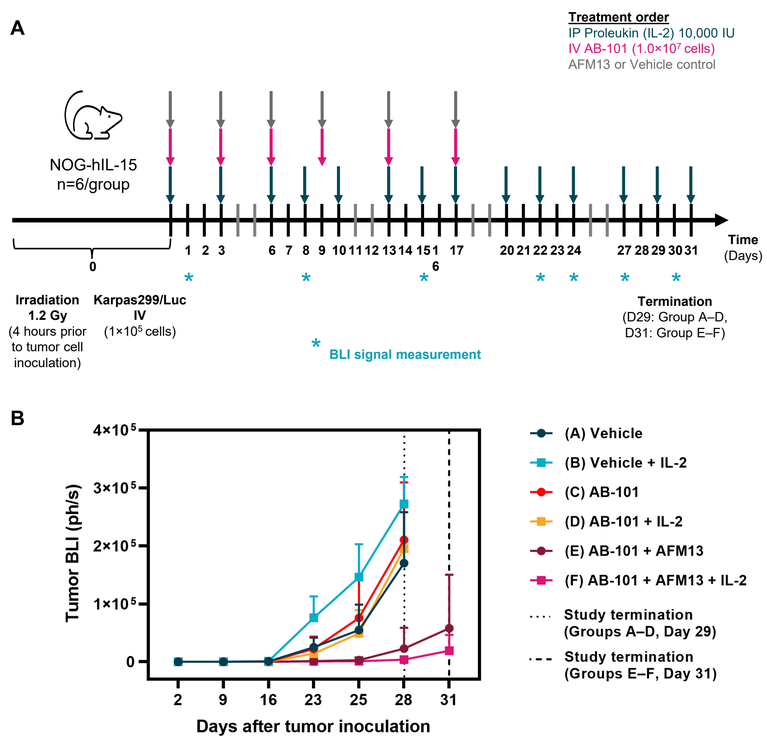
RESULTS

Viability of AB-101 was maintained upon exposure to AFM13 with/without CD30$^+$ tumor cells



Viability of AB-101 after cryopreservation. Viability was determined as the percentage of AB-101 negative for the eFluor780 dead cell marker after four hours co-culture with or without Karpas-299 target cells at a 1:1 E:T cell ratio, with or without AFM13. Cumulative data of AB-101 DP from two (MCB1) or three (MCB2) independent experiments are shown.
DP, drug product; E:T, effector to target; MCB, master cell bank; w/o, without; ns, non-significant (Two-way ANOVA and Sidak's multiple comparisions test).

AB-101 co-administered with AFM13 conferred tumor growth control *in vivo*



(A) Experimental design. Tumor growth control was determined in a hematologic (Karpas-299/Luc CD30$^+$ T-cell lymphoma) NOG-hIL-15 mouse xenograft model by whole BLI. NOG-hIL-15 mice were sub-lethally irradiated (1.2 Gy) on Day 0 and four hours later received an IV infusion of 1×10^5 Karpas-299/Luc tumor cells. Immediately following tumor inoculation, animals were administered IV with AB-101 alone (1×10^7 cells) or AB-101 followed immediately by AFM13 (10 mg/kg) via alternating tail veins. Treatment with AB-101 and AFM13 continued every third day for a total of six doses. IL-2 (10,000 IU) was administered by IP injection as indicated. Tumor cell administration resulted in disseminated lymphoma engraftment in mice.
(B) *In vivo* Karpas-299/Luc tumor growth upon treatment. BLI measurements were taken at the indicated timepoints. Mean BLI values ± SD shown. Comparison of groups over time (Days 1–27) was statistically significant ($p<0.0001$, mixed effects REML model).
BLI, bioluminescence imaging; D, day; IP, intraperitoneal; IU, international unit; IV, intravenous; Luc, luciferase; NK, natural killer; ph/s, photon per second; REML, restricted maximum likelihood; SD, standard deviation.

CONCLUSIONS

- The combination of AFM13 with AB-101 demonstrated synergistic anti-tumor activity towards CD30-expressing tumor cells
- Building on the clinical data with fresh cord blood-derived stimulated/expanded NK cells combined with AFM13 (NCT04074746)[7], co-administration of AFM13 with cryopreserved AB-101 offers a promising, highly scalable off-the-shelf treatment for patients with CD30$^+$ malignancies
- IND status for the combination of AFM13 with AB-101 has been granted by the FDA for initiation of a Phase 2, open-label, multi-center, multi-cohort study (NCT05883449, LuminICE-203)

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For more information, please contact Jens Pahl at J.Pahl@affimed.com.

REFERENCES
1. Pinto S *et al. Trends Immunol.* 2022, 43:932-946; 2. Carlsten M *et al. Front Immunol.* 2019, 10:2357; 3. Gauthier M *et al. Crit Rev Oncol Hematol.* 2021, 160:103261; 4. Reusch U *et al. MAbs.* 2014, 6:728–739; 5. Kerbauy L *et al. Clin Cancer Res.* 2021, 27:3744–3756; 6. Pahl J *et al. Cancer Immunol Res.* 2018, 6: 517–527; 7. Nieto Y *et al. Blood* 2022, 140:415–416; 8. Khanal *et al.* Poster presentation at the American Society for Clinical Oncology, June 3–6, 2023, Chicago, IL, USA.