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AFM24 is a novel, highly potent, tetravalent bispecific EGFR/CD16A-targeting Innate Cell Engager (ICE®) designed for the treatment of EGFR-positive malignancies

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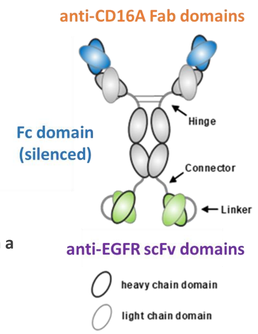
INTRODUCTION

- AFM24 is a tetravalent bispecific ICE® binding CD16A and epidermal growth factor receptor (EGFR) [Figure 1]
- AFM24 engages CD16A (FcγRIIIa) on natural killer (NK) cells and macrophages with a much higher affinity than monoclonal antibodies and triggers NK cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC) and macrophage-mediated antibody-dependent cellular phagocytosis (ADCP) responses directed at EGFR-expressing cancer cells
- EGFR is frequently overexpressed in a broad range of solid tumors, including colorectal cancer (CRC), head and neck squamous cell carcinoma, and non-small-cell lung carcinoma (NSCLC)[1]
- EGFR overexpression in tumors is a strong prognostic factor associated with reduced recurrence-free or overall survival[2]
- Clinically approved EGFR signaling inhibitors have limitations such as:
  - i. specific toxicities related to the inhibition of EGFR signaling in healthy tissues, particularly skin and gastrointestinal linings[3,4]
  - ii. intrinsic and unavoidable acquired resistance[5,6]
- Mouse xenograft models allow for screening of I-O regimens and can provide guidance towards promising therapeutic combinations, such as with NK cell products
- The unique MOA of AFM24 and its favorable preclinical safety profile[7] promise to overcome the limitations of existing EGFR-targeted therapies and to provide additional therapeutic options to patients with EGFR-expressing tumors who do not respond to these therapies

Figure 1: AFM24 structure



AFM24 is a prototypic ICE® derived from the redirected optimized cell killing (ROCK®) antibody platform

AFM24 is a tetravalent EGFR/CD16A-specific IgG1-scFv fusion antibody (scFv-IgAb) with a silenced IgG1 Fc

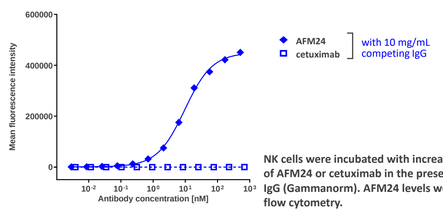
Fc, fragment crystallizable; Ig, immunoglobulin; scFv, single-chain variable fragment

References

1. Yano S, et al. Anticancer Res 2003,23:3639-50
2. Nicholson RI, et al. Eur J Cancer 2001,37(Suppl 4):S9-15
3. Yin X, et al. Clin Transl Sci 2020
4. Lacouture ME, et al. Clin Colorectal Cancer 2018,17:85-96
5. Nagano T, et al. Cells 2018,7:212
6. Cai WQ, et al. Front Oncol 2020,10:1249
7. Wingert S, et al. 2021 (in submission)

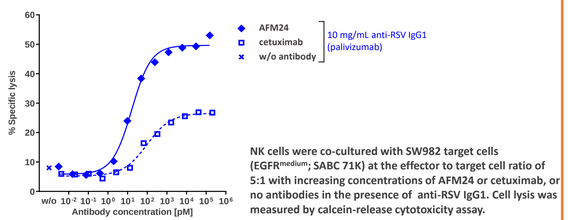
RESULTS

AFM24 exhibits high binding capacity to enriched primary human NK cells in the presence of competing IgG



NK cells were incubated with increasing concentrations of AFM24 or cetuximab in the presence of polyclonal IgG (Gammanorm). AFM24 levels were measured by flow cytometry.

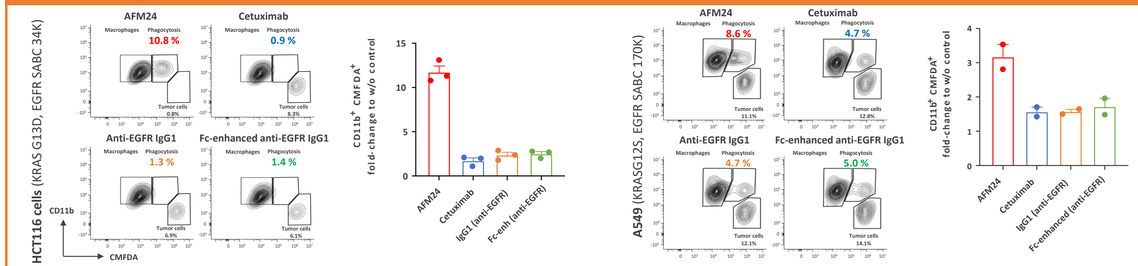
AFM24 exhibits high NK cell-mediated ADCC activity against EGFR-expressing tumor cells in the presence of competing IgG



NK cells were co-cultured with SW982 target cells ($EGFR^{medium}$; SABC 71K) at the effector to target cell ratio of 5:1 with increasing concentrations of AFM24 or cetuximab, or no antibodies in the presence of anti-RSV IgG1. Cell lysis was measured by calcein-release cytotoxicity assay.

RSV, respiratory syncytial virus; SABC, standard antibody binding capacity, as measure for EGFR cell surface expression density

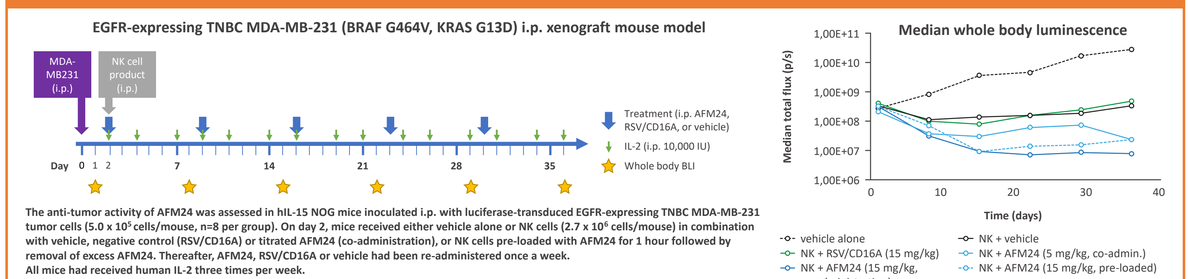
AFM24 induces superior macrophage-mediated ADCP against tumor cells with KRAS mutations and medium or high EGFR expression levels



M-CSF-differentiated monocyte-derived macrophages (CD11b+) were co-cultured for 4 hours with CMFDA-loaded $EGFR^{medium}$ HCT116 or $EGFR^{high}$ A549 tumor cells at the effector to target ratio of 5:1 with saturating concentrations (>10 µg/mL) of AFM24, cetuximab, anti-EGFR IgG1 or Fc-enhanced anti-EGFR IgG1. Phagocytosis was quantified as a percentage of CMFDA+ CD11b+ macrophages by flow cytometry.

CMFDA, 5-chloromethylfluorescein diacetate; KRAS, Kirsten rat sarcoma viral oncogene homolog; M-CSF, macrophage colony-stimulating factor

Adoptive transfer of NK cells in combination with AFM24 induces an AFM24 dose-dependent tumor growth regression in vivo



The anti-tumor activity of AFM24 was assessed in hIL-15 NOG mice inoculated i.p. with luciferase-transduced EGFR-expressing TNBC MDA-MB-231 tumor cells (5.0 x 10^5 cells/mouse, n=8 per group). On day 2, mice received either vehicle alone or NK cells (2.7 x 10^6 cells/mouse) in combination with vehicle, negative control (RSV/CD16A) or titrated AFM24 (co-administration), or NK cells pre-loaded with AFM24 for 1 hour followed by removal of excess AFM24. Thereafter, AFM24, RSV/CD16A or vehicle had been re-administered once a week. All mice had received human IL-2 three times per week.

BLI, bioluminescence imaging; hIL-15, human interleukin-15; IL-2, interleukin-2; i.p., intraperitoneally; TNBC, triple negative breast cancer

AFM24-101 phase 1/2a study (NCT04259450)

In the US and EU countries, patients with EGFR-expressing tumors are being actively recruited to an ongoing, first-in-human, open-label phase 1/2a study investigating the safety and efficacy of AFM24 monotherapy

Phase 1: MTD and RP2D → RP2D → *Phase 2a: efficacy and safety*

R/R patients with EGFR-expressing solid tumors
- All tumor types; CRC and NSCLC are anticipated to be the most common
- ≥ 1 prior line of therapy

Bayesian logistic regression model-based design
- Dose escalation in 5 cohorts (weekly IV dosing)
- *Cycle 1*: DLT observation period of 4 weeks
- *Cycle 2 and beyond*: tumor assessment
- Until MTD or RP2D is determined (25-30 patients)

Positive staining for EGFR in > 1% of tumor cells

Dose expansion phase using the MTD/RP2D

Stratification according to tumor type

Aim:
- Collect preliminary evidence of efficacy
- Confirm safety of AFM24 as a monotherapy

DLT, dose-limiting toxicity; MTD, maximal tolerated dose; RP2D, recommended phase 2a dose; MSS, microsatellite stable; R/R, relapsed/refractory; wt, wildtype

Key requirements for ICE®	AFM24 properties
To engage innate immune cells for antigen-dependent targeting of tumor cells	✓ Bispecific tetravalent scFv-IgAb construct targeting EGFR-expressing tumor cells and engaging CD16A+ innate immune cells
MOA independent of *RAS/RAF* mutation status in tumor cells	✓ MOA (ADCC and ADCP) underpins the activity against tumor cells with *KRAS*/*BRAF* mutations and a range of EGFR expression levels
MOA independent of CD16A allelic variants	✓ CD16A low-affinity 158F/F and high-affinity 158V/V variants induce high ADCC response
Minimal competition with physiological levels of human serum IgG	✓ High ADCC response in the presence of 10 mg/mL human IgG
Favorable safety profile	✓ Favorable toxicity profile in cynomolgus monkeys as a result of retained EGFR activity in tissues

CONCLUSIONS

- AFM24 with its unique MOA has the potential to change the treatment paradigm for patients with various EGFR-expressing solid tumors
- AFM24 induces high ADCC also in the presence of IgG1
- AFM24 induces a prominent ADCP response against tumor cells with *KRAS* mutations and medium or high EGFR levels
- AFM24, in combination with adoptive NK cells, leads to dose-dependent tumor regression in a mouse xenograft model
- AFM24 may overcome the limitations of currently approved EGFR signaling inhibitors due to its distinct MOA that is independent of EGFR signaling and related treatment-resistant mutations
- AFM24 shows a favorable preclinical safety profile due to its minimal impact on the EGFR activity-dependent functions in healthy tissues
- AFM24 is currently being investigated in a phase 1/2a study in patients with different EGFR-expressing tumors

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