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AFM24, a bispecific EGFR/CD16A Innate Cell Engager with the potential to overcome resistance to current targeted treatments for EGFR-positive malignancies

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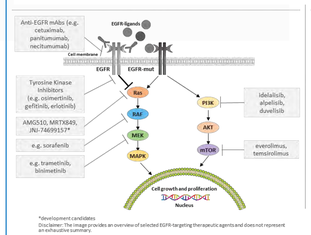
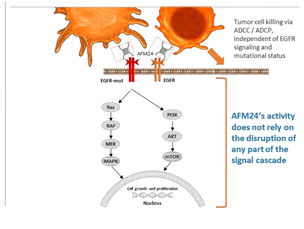
Introduction

- EGFR-expressing solid tumors remain challenging to treat and are a common cause of cancer-related mortality. While there have been advancements, novel mechanisms are needed to address the gaps in approved therapeutic approaches
- Current therapies that target EGFR, such as tyrosine kinase inhibitors (TKIs) or monoclonal antibodies (mAbs), work primarily through the inhibition of EGFR signaling
- Non-desirable side effect profiles which may lead to treatment discontinuation and occurrence of resistances in the EGFR signaling cascade (e.g. EGFR-kinase domain, KRAS, BRAF) limit the use of these treatments to very specific patient populations
- AFM24 represents a distinctive mechanism that engages innate immune cells by recruiting NK cells and macrophages to the site of the tumor for effective and efficient tumor cell killing. The differentiated MOA does not rely on the EGFR signaling pathway for tumor killing
- AFM24 is a bispecific CD16A/EGFR-binding innate cell engager (ICE) that provides this distinctive alternative to treating EGFR expressing solid tumors and by way of its mechanisms holds promise to treat all patient sub-types with a more acceptable safety profile while remaining immune to the challenge of resistance



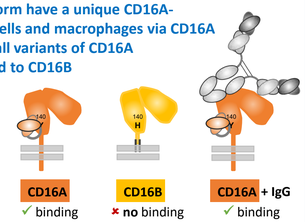
Current EGFR-targeting therapies, such as TKIs and mAbs, disrupt EGFR signaling. Patients may develop resistances.

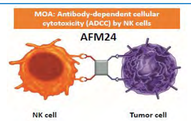


AFM24 activates innate immune cells, which initiate tumor cell killing via ADCC/ADCP. The EGFR signal transduction pathway is not relevant for the AFM24 anti-tumor effect.

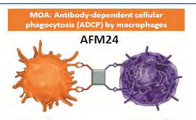
AFM24's potential effectiveness in activating innate immunity via CD16A is not impacted by circulating serum IgG

- All ICEs built on Affimed's ROCK® platform have a unique CD16A-binding paratope, which activates NK cells and macrophages via CD16A
- The CD16A-binding paratope binds to all variants of CD16A with high affinity, while it does not bind to CD16B
- The binding site on CD16A is distinct from the Fc binding site resulting in low IgG competition in ADCC (only 1.3-fold reduction in efficacy in the presence of 10 mg/mL monoclonal anti-RSV IgG1 [palivizumab] for AFM24)





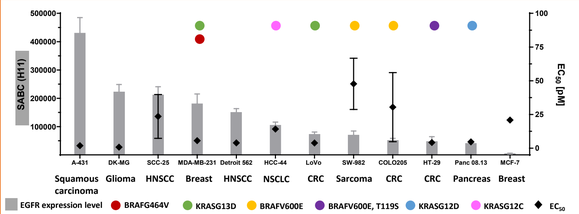
AFM24 has a distinctive MOA, activating innate immune cells which targets solid tumors regardless of mutational status



ADCC – potent tumor cell killing irrespective of EGFR-pathway mutations and EGFR surface density

- AFM24's in vitro potency EC_{50} ranged from 0.7 ± 0.4 pM to 47.7 ± 19.0 pM
  - No correlation between potency and EGFR expression level (SABC) observed (Spearman coefficient, r= -0.3326, p=0.3158)
- AFM24's efficacy E_{max} ranged from 92.9 ± 19.3% to 21.2% (not shown)
  - No correlation between efficacy and SABC (Spearman correlation, r= 0.3636, p=0.2731) observed
- AFM24 induces ADCC in KRAS/BRAF mutated cell lines to a comparable extent as in non-mutated, which indicatively proves the hypothesis of the novel mechanism not relying on EGFR signaling
- While AFM24 potently induces ADCC in MCF-7 cells with a low EGFR-expression, it does not induce ADCC in EGFR-negative cell lines such as KARPAS 299 (not shown)

Figure 1: AFM24-induced ADCC potency in cells with various EGFR densities and mutations



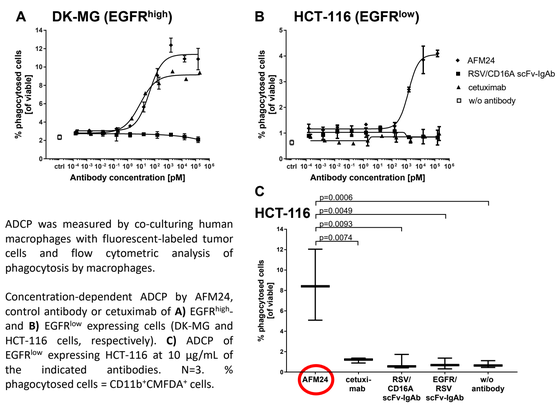
AFM24-mediated ADCC by NK cells on various tumor cell lines *in vitro*

KRAS/BRAF mutated cell lines are marked by colored dots. Specific antibody-binding capacity (SABC, grey bars) was determined using QIFIKIT® and anti-EGFR mAb H11. Mean and SD of ≥3 independent measurements are plotted as bars. EC_{50} of AFM24 was determined by titration of AFM24 in 4 h calcein-release cytotoxicity assays with the indicated EGFR+ tumor cell lines and primary human NK cells as effector cells at an E:T ratio of 5:1. Mean EC_{50} values and SD of independent measurements are plotted.

ADCP – potent and differentiated activity compared to mAbs towards low EGFR expressing target cell lines

- *In vitro*, AFM24 binds to human macrophages and efficiently induces phagocytosis of EGFR-expressing tumor cells through ADCP
- ADCP is dependent on the presence of both EGFR and CD16 binding domains
  - Control antibodies anti-RSV/CD16A and anti-EGFR/RSV do not induce ADCP
- ADCP is effective in cells with high or low EGFR density (Fig. A and B) and downstream mutations (Fig. B and C)
- The HCT-116 cell line bears the mutation KRAS-G13D, resulting in constitutively active RAS/RAF/MAPK signaling. ADCP mediated by AFM24 was not affected by this mutation

Figure 2: AFM24-induced ADCP in EGFR-expressing tumor cells by human macrophages



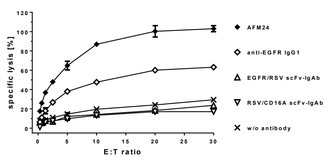
ADCP was measured by co-culturing human macrophages with fluorescent-labeled tumor cells and flow cytometric analysis of phagocytosis by macrophages.

Concentration-dependent ADCP by AFM24, control antibody or cetuximab of **A)** $EGFR^{high}$- and **B)** $EGFR^{low}$ expressing cells (DK-MG and HCT-116 cells, respectively). **C)** ADCP of $EGFR^{low}$ expressing HCT-116 at 10 µg/mL of the indicated antibodies. N=3. % phagocytosed cells = $CD11b^+CMFDA^+$ cells.

AFM24 induces substantially higher ADCC-mediated cell killing than conventional IgG1

- AFM24 was able to induce tumor cell lysis at an E:T ratio as low as 0.3:1
- AFM24 shows substantially better ADCC efficacy over all E:T ratios compared to the IgG1 with AFM24-derived anti-EGFR Fv domains
- Binding of the bispecific control anti-RSV/CD16A scFv-IgAb to CD16A alone does not enhance the natural cytotoxicity of NK cells towards tumor target cells, indicating that EGFR-binding is essential for AFM24's functionality

Figure 3: AFM24-induced ADCC at low effector-to-target ratios

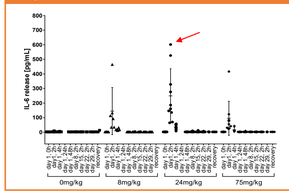


Lysis of Panc 08.13 target cells was determined in a 4 h calcein-release assays with primary human NK cells as effector cells at increasing E:T ratios in the presence of 5 µg/mL AFM24, IgG1 (with AFM24-derived anti-EGFR Fv domains), EGFR/RSV and RSV/CD16A control scFv-IgAbs, or without antibody.

Good tolerance in cynomolgus monkeys

- By limiting EGFR signal inhibition, AFM24 has been designed to have an improved safety profile
- The safety profile was determined in a 28-day toxicity study (18F, 18M) with repeated weekly i.v. infusion (vehicle, 8, 24 and 75 mg/kg/week five doses in total), followed by a 28-day recovery phase
- Anti-drug antibodies were found in 10/26 animals; only 2 animals receiving 24 mg/kg AFM24 revealed a strong anti-drug response effecting exposure
- Increases in AFM24 exposure (mean C_{max} and $AUC_{0\text{-}168h}$) were proportional to the AFM24 dose level between 8 and 75 mg/kg/week
- In summary, AFM24 was well tolerated up to the highest dose level (75 mg/kg) and no skin or organ toxicity was observed

Figure 4: Transient IL-6 release after first AFM24 administration



IL-6 serum levels in cynomolgus monkeys at three dose levels and the vehicle control. Time points indicate hours after start of infusion.

Transient IL-6 release after AFM24 administration, with a highest observed level of 602 pg/mL (arrow).
IL-6 levels returned to baseline within 24 h.

Actively recruiting (NCT04259450) in US and EU

- Patients with EGFR-positive tumors, regardless of mutational status, are currently being recruited to our ongoing, open-label Phase 1/2a study with AFM24 monotherapy

AFM24-101 *Phase 1:*
Determine MTD and RP2D

R/R patients known to be EGFR-positive
- Any tumor; any line of treatment
- Anticipated majority of patients: CRC and NSCLC

Bayesian logistic regression model-based design
- Escalation in 5 cohorts (weekly IV)
- Cycle 1: 4 weeks DLT observation period
- Cycle 2 and beyond: tumor assessment
- until determination of MTD or RP2D of approx. 25-30 pts

RP2D

AFM24-101 *Phase 2:*
Collect preliminary efficacy data and confirm safety

- CRC: w/ RAS mutations
- CRC: w/o RAS mutations

→ CRC regardless of mutational status

- NSCLC: w/ EGFR, ALK, BRAF mutations (post TKI)
- NSCLC: w/o mutations

→ NSCLC regardless of mutational status

Conclusions

- AFM24 is a novel innate cell engager (ICE) that harnesses the innate immune system to induce potent tumor cell killing via ADCC and ADCP
- Due to its distinctive MOA AFM24 is potentially eligible for treatment of all EGFR-positive tumors, regardless of EGFR-pathway mutations and EGFR receptor density
  - EGFR is used as a docking site only, whereas AFM24's cytotoxicity is independent of EGFR functionality and the downstream signal transduction
- AFM24 is well tolerated with no off-target toxicity in cynomolgus monkey
- A broad set of patients with hard-to-treat EGFR-expressing cancers may benefit from AFM24

ADCC, antibody-dependent cell-mediated cytotoxicity; **ADCP**, antibody-dependent cell-mediated phagocytosis; **DLT**, dose-limiting toxicity; EC_{50}, half maximal effective concentration; E_{max}, maximal observed efficacy; **E:T ratio**, effector-to-target ratio; **MOA**, mechanism of action; **MTD**, maximal tolerated dose; **PBMC**, peripheral blood mononuclear cells; **RP2D**, the recommended Phase 2a dose; **SABC**, standard antibody binding capacity, as measure for EGFR cell surface expression density

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