Abstract
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Redirected Optimized Cell Killing: ROCK - A next generation immune cell engager platform

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Introduction

We develop first-in-class tetravalent multi-specific CD16A cell-engaging antibodies based on our recently introduced ROCK platform. These CD16A-specific immune cell engagers are differentiated from classical or Fc-enhanced mAbs by their high-affinity binding and long cell surface retention times, resulting in superior potency and efficacy in cytotoxicity assays. Their binding affinity to immune cells is also substantially less prone to impairment by competing serum IgG.

ROCK – Redirected Optimized Cell Killing

- Unique modular platform built on extensive drug development expertise and long-standing engineering know-how to redirect immune cell cytotoxicity to a specific target via CD16A
- Multivalent and multi-specific antibody formats with:
  - Variable pharmacokinetic (PK) profiles
  - High affinity for tumor target and effector cell
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  - Opportunity for long cell surface retention time
  - Lack of serum IgG competition due to unique CD16A specific epitope
- Enables generation of product candidates tailored to different indications and settings
- Combines properties essential for the design and development of novel, potent and manufacturable custom immune cell engagers
- Also suitable for T cell engagement

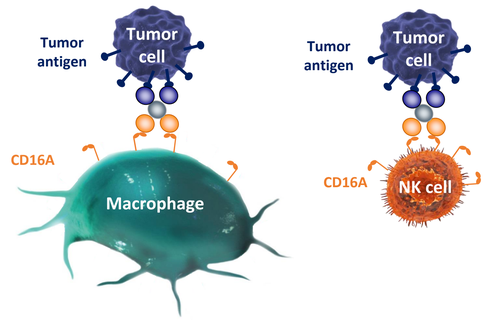
AFM26 (BCMA/CD16A) to treat multiple myeloma

- **Medical need:** Elimination of minimal residual disease (MRD) post ASCT, avoiding relapse
- **Targeting BCMA**
  - BCMA is a promising target based on early clinical data (CAR-T and ADCs)
  - Low expression of BCMA is a significant hurdle to eliminate malignant cells
  - NK cells are the first population of lymphocytes to recover post ASCT (opportunity for AFM26)

AFM24 (EGFR/CD16A) to treat EGFR+ solid tumors

- **Medical need:** Widen therapeutic window and address resistant patient population
- **Targeting EGFR**
  - EGFR as validated target in solid tumors, however, resistance (KRas) and side effects negatively impact benefit to patients (e.g. skin toxicity)
  - EGFR-binding domain was selected to minimize inhibition of EGFR-mediated signal transduction
    → potentially lowering the risk of side effects
  - NK cell-retargeting introduces a novel, potent effector function → addressing EGFR resistance

ROCK innate immune cell platform



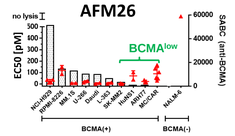
Data on engagers designed to induce Antibody-Dependent Cellular Phagocytosis (ADCP) on macrophages can be found here:
Poster 1111, ASH conference 2018: December 1, 18:15-20:15, Hall GH

AFM13 (CD30/CD16A) to treat r/r HL and CD30+ lymphoma

- **Phase 1: Safety and clinical activity in heavily pre-treated HL patients**
  - Dose escalation study: 0.01 – 7.0 mg/kg
  - No MTD reached, favorable safety profile determined
  - Tumor shrinkage in 62% (8/13) and PRs in 23% (3/13) of patients at doses of ≥1.5 mg/kg
- **Phase 2a: IST in r/r HL (GHSG, ongoing)**
  - Favorable safety profile confirmed
  - ORR of 29% (2/7) in patients failing standard treatments including B.V. and who were anti-PD1 naïve
- **Phase 1b: Combination with pembrolizumab in r/r HL (ongoing)**
  - Recruitment completed into dose expansion cohort; total of 24 patients evaluable in highest AFM13 dose cohort
  - Preliminary efficacy data for 24 patients treated at highest dose level of AFM13
    - ORR of 88% (21/24) and CR rates of 42% (10/24) and 46% (11/24) by local and independent assessments
  - Well-tolerated with most common AE being IRRs that are mostly mild to moderate in nature and manageable with standard of care measures
- **Phase 1b/2a: IST in r/r CD30+ lymphoma (Columbia University, ongoing)**
  - Total of 9 patients treated to date
  - Preliminary efficacy data:
    - 8 patients treated in 3 dose cohorts: 50% ORR including 1 CR (13%) and 3 PRs (38%)

Targeting BCMA to treat multiple myeloma with AFM26 (BCMA/CD16A)

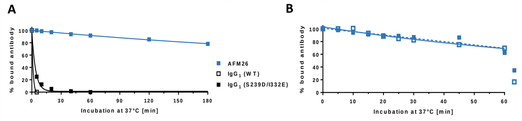
AFM26 shows potent killing of BCMA^{low} cells in vitro



Summary of EC50 values (red triangles, left axis) of AFM26-induced target cell lysis in in vitro cytotoxicity assays using primary human NK cells and the indicated target cells (4h calcein-release assay, E:T ratio 5:1). Data are means ± SD. Right axis (grey bars): Specific antibody binding capacity (SABC) of anti-BCMA ANC3B1; selected SABC: HuNS1: 1438; ARH77: 1183; MC/CAR: 449

Potency of AFM26-induced target cell lysis *in vitro*

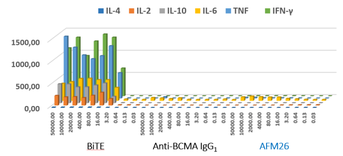
AFM26 shows significantly longer cell surface retention times than mAbs even in presence of competing polyclonal IgG



A) AFM26, anti-BCMA IgG$_1$, and Fc-enhanced anti-BCMA IgG$_1$ (S239D/I332E) were added to primary human NK cells at 100 µg/ml, 400 µg/ml and 400 µg/ml, respectively, before removal of unbound antibody by washing and incubation in 10 ml PBS (2% FCS, 0.1% NaN_3) at 37°C. Remaining surface bound antibody was quantified by detection with BCMA-His/anti-His-FITC and flow cytometry. B) AFM26 NK cell surface retention in presence and absence of 10 mg/ml polyclonal IgG (Gammanorm, Octapharma). Remaining surface bound antibody was detected as in A).

NK cell surface retention of AFM26 *in vitro*

AFM26 may offer superior safety compared with T cell engagement

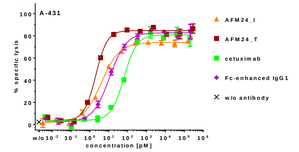


Cytokine release by human PBMCs induced by increasing concentrations of BCMA-targeting BiTE, anti-BCMA IgG$_1$ and AFM26 in presence of NCI-H929 target cells was quantified in supernatant following 24h incubation (E:T ratio 50:1).

In vitro cytokine release induced by AFM26 and comparators

Targeting EGFR to treat EGFR+ solid tumors with AFM24 (EGFR/CD16A)

AFM24_T and AFM24_I demonstrate superior in vitro potency versus comparators



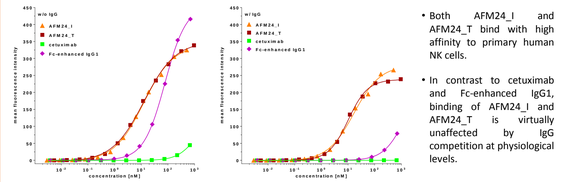
construct	EC$_{50}$ [pM] A-431 mean	A-431 SD	HCT-116 mean	HCT-116 SD
AFM24_I	2.1	1.87	4.3	2.42
AFM24_T	1.1	0.91	1.5	1.69
cetuximab	24.5	33.90	18.6	17.59
Fc-enhanced IgG1	4.8	5.59	14.1	15.94

Cytotoxic potency was determined in 4h calcein-release cytotoxicity assays on A-431 (Ras_{wt}, high EGFR expression) or HCT-116 (mutated Ras, low EGFR expression) target cells with human NK cells as effector cells at an E:T ratio of 5:1 in the presence of serial dilutions of the indicated antibodies. Potency (EC$_{50}$) was determined by non-linear regression/sigmoidal dose-response. Mean and SD of three independent experiments are presented in the table.

Potency and efficacy of AFM24_T and AFM24_I *in vitro*

Targeting EGFR to treat EGFR+ solid tumors with AFM24 (EGFR/CD16A) – continued

AFM24_I and AFM24_T demonstrate high affinity binding to primary human NK cells, even in presence of competing IgG

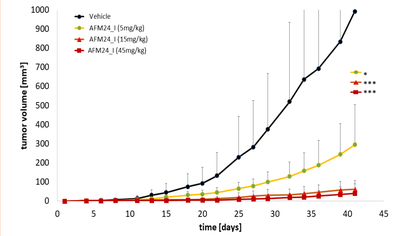


- Both AFM24_I and AFM24_T bind with high affinity to primary human NK cells.
- In contrast to cetuximab and Fc-enhanced IgG1, binding of AFM24_I and AFM24_T is virtually unaffected by IgG competition at physiological levels.

Primary human NK cells were incubated with serial dilutions of the indicated antibodies in the presence or absence of polyclonal human IgG at 37°C. Cell surface-bound AFM24_I and AFM24_T were detected by anti-AFM24 mAb 62-1-1 followed by FITC-conjugated goat anti-mouse IgG. Biotinylated anti-EGFR IgG (cetuximab, Fc-enhanced (S239D, I332E) IgG1 with AFM24-derived anti-EGFR Fv domains) were detected with AlexaFluor488-conjugated Streptavidin.

NK cell surface binding of AFM24_T and AFM24_I *in vitro*

Dose-dependent anti-tumor efficacy of AFM24_I in a humanized mouse model



NOG mice (n=7 per group) humanized with CD34$^+$ human cord blood stem cells were inoculated with A-431 human epidermoid carcinoma cells (s.c. 1x10^6 cells/mouse) on day 0. The animals received vehicle or three different doses of AFM24_I once a week (5 mg/kg, 15 mg/kg or 45 mg/kg i.v.) on days 1, 7, 14, and 21. Data were tested for significance using a non-parametric Mann-Whitney U test (p*<0.05, p**<0.01, p***<0.001 vs. vehicle).

Tumor growth inhibition of AFM24_I *in vivo*

Key conclusions – ROCK platform

- **Differentiated (high affinity and specificity to CD16A) and versatile, enabling generation of antibodies with unique features; designed to address medical need in different indications, including multiple myeloma (AFM26) and solid tumors (AFM24) .**
- **Allows for specific engagement of innate immunity (NK cells and macrophages) w/o competition of polyclonal antibodies in circulation.**
- **Enables killing of target cells with very low target expression (<1000 copies/cell).**
- **AFM13, the most advanced ROCK platform-based development candidate appears to be well tolerated and effective (based on early phase clinical studies in patients with r/r HL and CD30+ lymphomas).**

_T: 4 variable domains: 2 CD16 binding domains and 2 tumor targeting domains. **_I:** IgG backbone and 4 variable domains: 2 CD16 binding domains and 2 tumor targeting domains

Disclosure: All authors are employees of Affimed GmbH.