Poster No
2786

Pharmacokinetics and *in vitro/in vivo* characterization of high-affinity bispecific EGFR/CD16A NK cell engagers for the treatment of EGFR-expressing tumors

Michael Kluge, Michael Tesar, Uwe Reusch, Stefan Knackmuss, Torsten Haneke, Kristina Ellwanger, Ivica Fucek, Thomas Mueller, Ute Schniegler-Mattox, Martin Treder

Affimed GmbH, Im Neuenheimer Feld 582, 69120 Heidelberg, Germany

Introduction

The epidermal growth factor receptor (EGFR) is a validated target for the treatment of several solid tumor types, and current EGFR-targeting monoclonal antibodies (mAbs) and tyrosine kinase inhibitors (TKIs) function mainly through blocking signal transduction. Treatment with these agents is either dependent on the receptor's mutational status, or activating downstream mutations like K-Ras which may cause treatment resistance in a large number of patients.

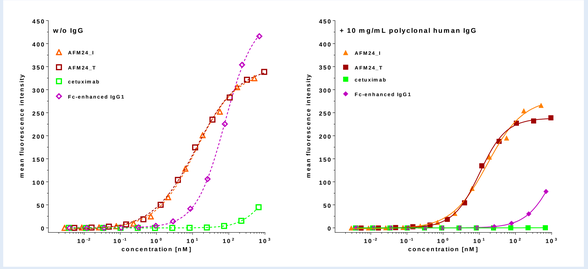
In addition, EGFR-targeting therapies have been associated with side effects such as skin toxicities resulting in treatment interruptions and termination, impacting treatment outcome. Therefore, an approach with significantly reduced skin toxicity would be advantageous.

Consequently, there is a medical need for drugs with a differentiated mode of action (MoA) aimed at reducing or avoiding known limitations of standard of care (SoC). To this end, Affimed has generated tetravalent, bispecific product candidates (AFM24_I and AFM24_T) binding to CD16A and EGFR that offer a differentiated immuno-therapeutic option for the treatment of EGFR-expressing malignancies. This approach might have the potential to widen the therapeutic window, to overcome intrinsic and acquired resistance, and to improve the safety profile observed with current SoC.

Generation of EGFR-targeting tetravalent bispecific NK cell engagers

- Proprietary EGFR and CD16A antibody binding domains were used to construct the NK cell engagers AFM24_T (TandAb) and AFM24_I (IgG backbone) with varying PK properties.
- AFM24_I displaying an IgG–like half-life was engineered with anti-CD16A specificity at the N-terminus (Fab) and anti-EGFR specificity at the C-terminus.

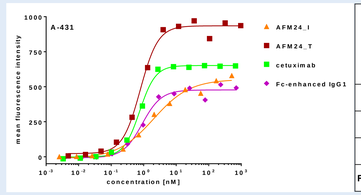
AFM24_I and AFM24_T demonstrate high affinity binding to primary human NK cells, even in presence of competing IgG



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.

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

AFM24_I and AFM24_T display high affinity binding to EGFR$^+$ tumor cells

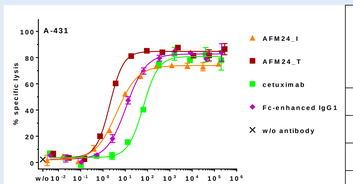


construct	K_D [nM]			
	A-431		HCT-116	
	mean	SD	mean	SD
AFM24_I	1.8	0.02	53.1	3.75
AFM24_T	1.1	0.52	0.3	0.16
cetuximab	0.9	0.17	0.1	0.04
Fc-enhanced IgG1	1.1	0.21	0.8	0.03

Aliquots of A-431 (Ras_{wt}, high EGFR expression) or HCT-116 (mutated Ras, low EGFR expression) cells were incubated with serial dilutions of the indicated antibodies at 37°C. Cell surface-bound AFM24_I and AFM24_T were detected by anti-AFM24 mAb 4-1-1 followed by FITC-conjugated goat anti-mouse IgG. Anti-EGFR IgG (cetuximab, Fc-enhanced (S239D, I332E) IgG1 with AFM24-derived anti-EGFR Fv domains) were detected with FITC-conjugated goat anti-human IgG. Mean fluorescence intensities measured by flow cytometry were used to calculate K_D values by non-linear regression. Mean and SD of two independent experiments are presented.

- AFM24_I and AFM24_T bind to EGFR$^+$ target cells with similarly high affinity as anti-EGFR IgGs used as comparators.

AFM24_I and AFM24_T demonstrate superior *in vitro* potency versus comparators

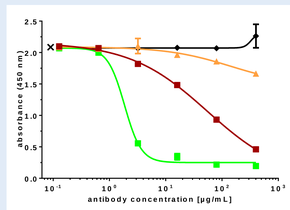


construct	EC_{50} [pM]			
	A-431		HCT-116	
	mean	SD	mean	SD
AFM24_I	2.1	1.87	4.3	2.42
AFM24_T	1.1	0.91	1.5	1.69
cetuximab	24.5	33.90	18.6	17.59
Fc-enhanced IgG1	4.8	5.59	14.1	15.94

Cytotoxic potency was determined in 4 h 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.

- Both AFM24_I and AFM24_T demonstrate superior potency in *in vitro* cytotoxicity assays with target cells expressing Ras_{wt} (A-431, high EGFR expression) or mutant K-Ras (HCT-116, low EGFR expression) compared with all other anti-EGFR antibodies tested.

AFM24_I and AFM24_T induce substantially lower inhibition of EGF-induced EGFR phosphorylation compared to cetuximab



construct	EC_{50} [µg/mL]
irrelevant CD19/CD16A NK cell engager	no
AFM24_I	not applicable
AFM24_T	54.5
cetuximab	1.9

Aliquots of $5x10^4$ A-431 cells were seeded in individual wells of a 96 well micro plate and starved for 4 h before they were incubated for 30 min with the indicated antibodies. After 10 min stimulation with 100 ng/mL EGF cells were lysed, and phosphorylated EGFR was quantified using a phosphotyrosine EGFR ELISA.

- AFM24_I and AFM24_T were designed to show lower inhibition of EGFR phosphorylation with the goal to reduce the side effects (skin toxicity) resulting from the blocking of signal transduction.

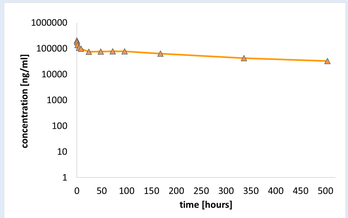
AFM24_T shows no signs of toxicity in cynomolgus monkeys

Two non-GLP toxicology studies were performed in cynomolgus monkeys.

- Study 1: dose escalation study
  Determination of the Maximum Tolerated Dose (MTD) upon i.v. administration (2h infusion). A dosing regimen with a 4 day wash out period of escalating doses (group 1: 0.03, 0.15, 0.75 mg/kg, group 2: 3.75, 18.75, 93.75 mg/kg, n=2 per group) was performed.
- Study 2: 4 weeks repeated dose study
  Toxicity assessment of test item after 28 days repeated dosing (1, 3, 10 and 30 mg/kg; n=2 per group); i.v. administration every other day (q2d x 28d), including an additional high dose recovery group to identify potential delayed toxicity and reversibility.

- All animals were systemically exposed to AFM24_T and terminal half-lives ranged between 18 – 35 hours.
- No skin or tissue toxicity was seen in either study.
- The only finding of note was a substantial IL-6 response 2h post infusion in all animals followed by a rapid decline after 4h.
- AFM24_T showed a favorable safety profile in cynomolgus monkeys.

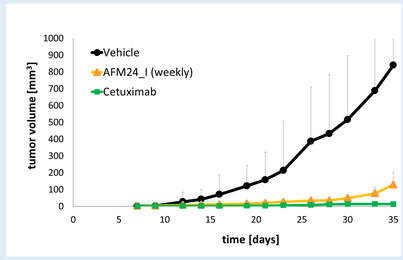
AFM24_I demonstrates IgG-like pharmacokinetics in CD1 mice



Pharmacokinetics of AFM24_I in CD1 mice (serum concentration-time curve): Female mice received a single i.v. injection of 300 µg (10 mg/kg) AFM24_I into the tail vein. Serum concentrations of AFM24_I were determined at the indicated time points by ELISA. Calculation of PK parameters was performed using the PK Solutions, 2.0 software.

- Maximum serum concentrations of AFM24_I are observed at the first blood sampling post-dose (i.e. 5 min).
- Serum concentrations decline in a bi-exponential manner with a terminal $t_{1/2}$ of approximately 14 days.
- Systemic exposure, as measured by $AUC_{0-\infty}$, is $4.4x10^7$ (ng/mL)*h.
- AFM24_I demonstrates PK properties similar to standard mAbs.

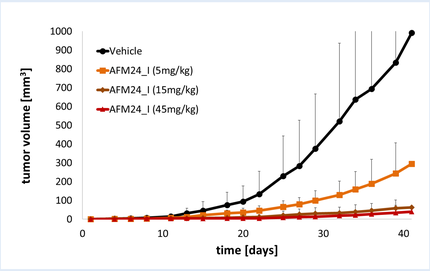
Despite reduced inhibition of EGFR signaling, AFM24_I shows anti-tumor efficacy similar to cetuximab in the A431 tumor model (responsive to inhibition of EGFR signaling)



A-431 human epidermoid carcinoma cells were inoculated (s.c. $1x10^6$ cells/mouse) at day 0. The animals received vehicle, AFM24_I (15 mg/kg, i.v.) or cetuximab (5 mg/kg i.v.). AFM24_I and cetuximab were injected on days 1, 8, 15 and 22. Group size was n= 5.

- AFM24_I and cetuximab both demonstrate potent tumor growth inhibition when dosed repeatedly once weekly in the EGFR$^+$ A-431 target cell line (high EGFR expression).

AFM24_I demonstrates dose-dependent anti-tumor efficacy in a humanized mouse model



A-431 human epidermoid carcinoma cells were inoculated (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.

- Tumor growth inhibition was significant compared to the vehicle group from day 11 for the two high dose groups treated with 45 and 15 mg/kg (p< 0.001) and from day 25 for the low dose group (p<0.05) treated with 5 mg/kg.

Summary

- AFM24_I and AFM24_T are differentiated from cetuximab by their immunotherapeutic MoA: NK cell engagement (AFM24_I and AFM24_T) vs. inhibition of EGFR signaling (cetuximab).
- Both NK cell engagers are also differentiated from mAbs and Fc-enhanced mAbs by binding with high affinity to NK cells and showing virtually no IgG competition at physiological IgG levels.
- AFM24_I and AFM24_T demonstrate superior cytotoxicity of target cells irrespective of their Ras mutational status; mutated Ras is a negative predictive biomarker for marketed EGFR-targeting mAbs, and patients bearing this mutation cannot be treated with these antibodies.
- Both AFM24_I and AFM24_T showed less inhibition of EGFR signaling compared to cetuximab. As skin toxicity is associated with inhibition of EGFR signal transduction, this may potentially be beneficial in reducing this major limiting side effect of anti-EGFR mAbs and TKIs.
- Two pilot toxicology studies of AFM24_T confirmed a favorable safety profile.
- The $t_{1/2}$ of AFM24_I (~14 d) enables effective and convenient dosing similar to classical IgG-type therapeutic antibodies.
- AFM24_I demonstrates strong dose-dependent tumor growth inhibition *in vivo* using a high EGFR-expressing cell line.

Conclusion and outlook

- AFM24_I and AFM24_T are novel, highly potent and differentiated tetravalent bispecific NK cell engagers designed to overcome limitations of standard of care in EGFR$^+$ malignancies.
- Both candidates were engineered for reduced inhibition of EGFR phosphorylation to improve the safety profile of current EGFR+ targeting agents.
- Both candidates show a PK enabling effective and convenient dosing and widening of the therapeutic window.
- IND-enabling studies are ongoing and both candidates are being explored in combination with immune activating agents, based on encouraging data for Affimed's lead NK cell engager AFM13 indicating clinical synergy with the aPD-1 antibody pembrolizumab.