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High affinity bispecific EGFR/CD16A antibodies specifically recruit NK-cells to target EGFR-expressing tumors

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Abstract

Introduction:

The epidermal growth factor receptor (EGFR) is an important and established target for the treatment of several solid tumors, including colorectal, head and neck, and lung cancer. EGFR-targeting with tyrosine kinase inhibitors and monoclonal antibodies is dependent on the mutational status of the receptor and downstream pathways which may cause resistance to these treatments. An EGFR-targeting therapy which is effective independent of the mutation status offers a differentiated treatment approach. Natural killer cells (NK-cells) play a central role in the innate immune system, have the capacity to destroy neoplastic cells and can be effectively utilized for effective anti-tumor engagement.

Material and Methods:

To specifically utilize the cytotoxic potential of NK-cells for the elimination of EGFR-expressing cancer cells, we developed tetravalent bispecific EGFR/CD16A NK-cell engaging antibodies with two binding sites for EGFR and two binding sites for CD16A. CD16A is an isoform of CD16 specifically expressed by NK-cells and macrophages but not by neutrophils. The antibodies were generated using proprietary human anti-EGFR and anti-CD16A variable domains and characterized regarding binding, stability, manufacturability, efficacy and safety in a wide range of biophysical and functional assays *in vitro* and *in vivo*.

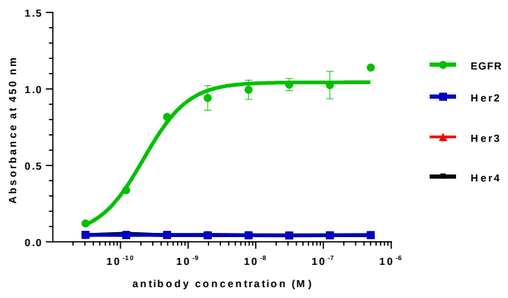
Results and Discussion:

We identified high affinity antibodies recognizing epitopes in the extracellular domain of EGFR, a domain that is not targeted by other therapeutic antibodies. We engineered a set of EGFR/CD16A antibodies and analyzed their characteristics. Antibodies containing a specific domain showed single digit picomolar or sub-picomolar EC_{50} values and were more potent than a control antibody containing the variable domain from cetuximab. In addition, the EGFR/CD16A antibodies demonstrated excellent biophysical properties. In initial studies, the lead candidate AFM24 has shown evidence of a favorable safety profile in *in vivo* pharmacology and toxicology studies.

Conclusions:

In summary, AFM24 is a novel, highly potent drug candidate suitable for the treatment of EGFR-expressing malignancies with the potential to overcome resistance to other EGFR-targeting agents.

AFM24 does not bind to other members of the EGFR family



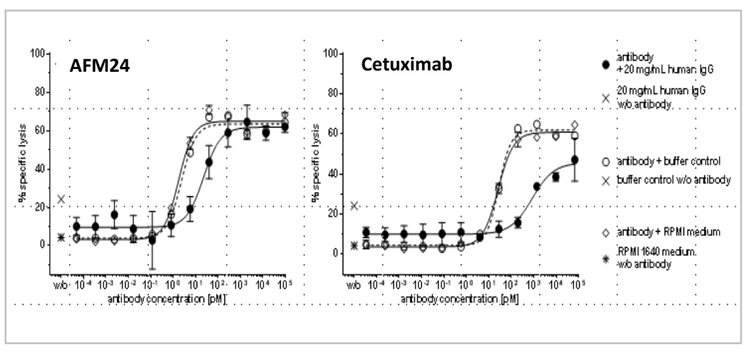
ELISA to assess AFM24 cross-reactivity to different members of the EGF receptor family. EGFR, HER2, HER3 and HER4 Fc fusion proteins were coated. Detection of AFM24 binding by His-tag (anti-Penta His IgG, HRP conjugated).

- AFM24 shows no binding to HER2, HER3 and HER4.
- AFM24 shows specific binding to EGFR.

There is no relevant influence of high IgG concentrations on binding to NK- cells and on cytotoxicity of AFM24

	AFM24 binding to NK-cells K_D (nM) without IgG	AFM24 binding to NK-cells K_D (nM) with IgG
Experiment 1	11.2	15.1
Experiment 2	10.3	12.0
Experiment 3	6.9	7.7
Mean	***9.5***	***11.6***

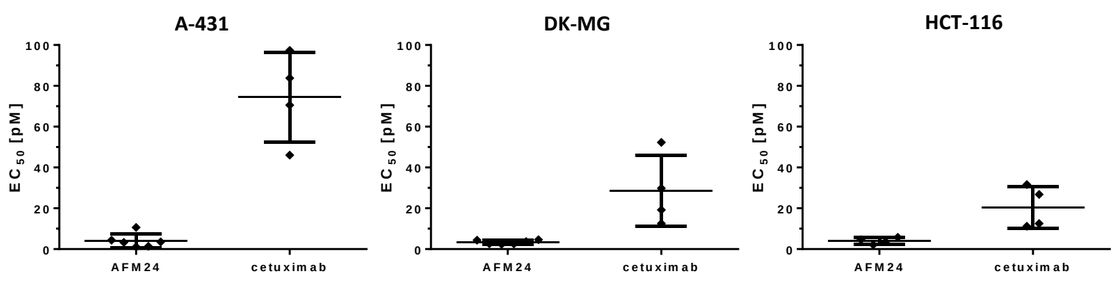
AFM24 binding to NK-cells in the presence and absence of human IgG (20 mg/mL).



Cytotoxicity of AFM24 and of cetuximab in the presence and absence of human IgG was determined in 4 h calcein-release assays on A-431 target cells with NK-cells as effector cells (E:T=5:1) in the presence or absence of polyclonal human IgG (20 mg/mL). As a control, cells were cultured in medium alone or the corresponding buffer control.

- IgG affected the cytotoxicity of AFM24 much less than that of cetuximab.
- A slight decrease in potency, but not of efficacy was seen for AFM24.
- In contrast, potency and efficacy of cetuximab were decreased significantly in the presence of IgG.

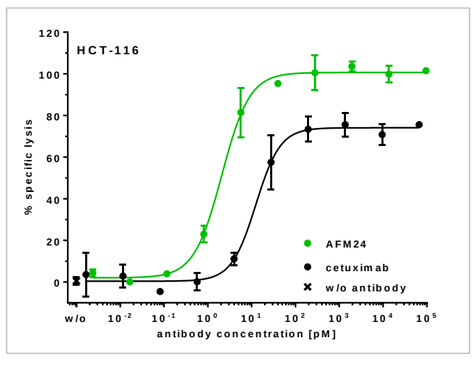
AFM24 shows higher *in vitro* potency than cetuximab against human tumor cell lines



Potency was determined in 4 h calcein-release cytotoxicity assays with human NK-cells as effector cells at an E:T ratio of 5:1 in the presence of serial dilutions of AFM24 and cetuximab. Potency (EC_{50}) was determined by non-linear regression/sigmoidal dose-response (individual values, mean and SD).

- AFM24 shows higher potency than cetuximab in high EGFR-expressing A-431, DK-MG and in low EGFR-expressing, Ras-mutated HCT-116 cells.

AFM24 is efficacious against human tumor cell lines with mutated Ras

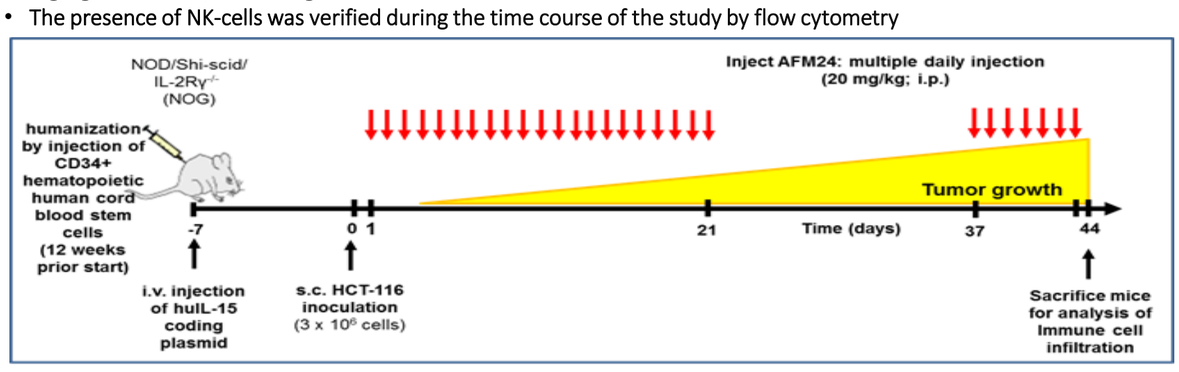


4 h cytotoxicity assays with NK-cells as effectors (E:T=5:1) at serial dilutions of AFM24 and cetuximab. Similar results were demonstrated for the A-549 NSCLC cell line (data not shown).

- AFM24 potently kills human HCT-116 (colon) and A-549 (NSCLC) tumor cell lines bearing the Ras mutation.
- AFM24 potently kills HCT-116 cells with higher efficacy and potency than cetuximab.

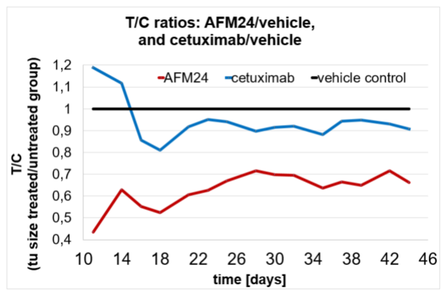
AFM24 shows efficacy against Ras-mutated, cetuximab-resistant HCT-116 cells in a humanized mouse model

- Humanized (human CD34+ stem cells) NOG mouse (NOD/Shi-scid/IL-2Rγnull, Taconic) model
- Enhanced NK-cell population by hydrodynamic injection of a human IL-15 coding plasmid (in-life study with TransCure bioServices, Archamps)
- Subcutaneous inoculation of 3 x 10^6 HCT-116 tumor cells on Day 0 into the flank
- Treatment start at Day 1 (i.p. administration): AFM24 daily 20 mg/kg for 3 weeks + 1 week, cetuximab twice weekly 50 mg/kg for 6 weeks, similar regiment of vehicle control
- The presence of NK-cells was verified during the time course of the study by flow cytometry



Treatment schedule in HCT-116 tumor-bearing humanized NOG mice. AFM24 or vehicle (i.p. injections) are indicated by red arrows, cetuximab was applied twice weekly (arrows not depicted).

- A 50% mean humanization rate (ratio of hCD45+ to mCD45+) was obtained.



Tumor growth (T/C ratios) of treated animals relative to control animals. Mean tumor sizes in AFM24 and cetuximab treatment groups were normalized to the corresponding vehicle control group animals (group sizes: n=5).

- 100% tumor take rate.
- Approximately 40% inhibition in initial tumor outgrowth in the AFM24-treated group.
- Delay in tumor growth in AFM24-treated mice.
- No effect of cetuximab on tumor growth.

AFM24 shows no skin toxicity and a favorable safety profile in toxicology studies in cynomolgus monkeys

- AFM24 is fully cross-reactive to cynomolgus monkey EGFR and CD16A.
- Assessment of single (by dose escalation) and repeated dose toxicity in cynomolgus monkeys to determine the Maximum Tolerated Dose (MTD) upon intravenous administration.

Group number	Dose escalation Dose level (mg/kg)	Number of animals per group (n)
1	0, 0.03, 0.15, 0.75	2
2	0, 3.75, 18.75, 93.75	2

A dosing regimen with short administration intervals of escalating doses (with 4 day wash out period) was performed (chair infusion, max. 2 h). Treatment groups, AFM24 dose levels and number of animals used in the single dose escalation toxicology study are shown.

Group number	Dose level (mg/kg)	Number of animals per group (n)
1	1	2
2	3	2
3	10	2
4	30	2 + 2 recovery

Treatment groups, AFM24 dose levels and number of animals used in the repeated dose (every other day for 28 days) toxicology study are shown.

- During the dosing period, clinical signs, body weight, body temperature, hematology, coagulation and blood chemistry were monitored and found to be in the normal range.
- Cytokine level measurements showed a consistently strong IL-6 response 2 h post infusion in all treated animals.
- Immunophenotyping of lymphocyte subsets after each dose level revealed no substantial effect.
- A panel of preselected tissues e.g. vital organs, skin, injection site were subjected to histopathology. There were no test item-related macroscopic or microscopic changes.
- No evidence of skin toxicity was seen in the single dose escalation study, and, most importantly also not in the repeated dose toxicology study.
- In the repeated dose study recovery animals, no signs of a delayed toxicity were observed.
- AFM24 shows a well-differentiated safety profile (no skin toxicity) compared to that described for other anti-EGFR antibodies and for tyrosine kinase inhibitors.

Summary

Compared to monoclonal antibodies, AFM24 demonstrates:

- Very high affinity binding to both, CD16A on NK-cells and EGFR on tumor cells.
- Superior NK-cell-mediated cytotoxicity against tumor cell lines with high and low EGFR expression.
- Highly potent killing of tumor cells bearing mutated Ras *in vitro* and *in vivo.*
- No substantial interference by polyclonal IgG on binding or efficacy.
- A well-differentiated safety profile from other anti-EGFR antibodies and tyrosine kinase inhibitors.

Conclusions and Outlook

- High affinity redirection of NK-cells to EGFR+ tumor cells offers a novel mode of action that may overcome intrinsic or acquired resistance which is described in a substantial number of patients.
- AFM24 exhibits a favorable side effect profile representing an important improvement over other EGFR-targeting molecules.
- High affinity engagement of NK-cells and the safety profile of AFM24 have the potential to offer synergies with other drugs such as checkpoint modulators to further boost anti-cancer immunity in solid tumors.

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