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

- Humanized (C57BL/6N-Tg; CD1) nude mice (NOG/CD1-hu-IgG, Taconic) model
- Enhanced NK-cell population by high-dose intravenous injection of a human IL-15 coding plasmid (in-mouse study with TransCre bioliness, Arcasys)
- Subcutaneous inoculation of 3 x 10^6 HCT-116 tumor cells on Day 0 (day 0)
- Treatment started on Day 1 (i.a. administration): AFM24 daily 20 mg/kg for 3 weeks (1 week, twice weekly 50 mg/kg for 4 weeks, similar regimen of vehicle control)
- The presence of HCT-116 was verified during the time course of the study by flow cytometry

Tumor growth (T/C ratio) 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 indicated).

- 50% 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 toxicity studies in cynomolgus monkeys

- AFM24 is fully cross-reactive to cynomolgus monkey EGFR and CD16A.
- Assessment of single (4x dose escalation) and repeated dose toxicity in cynomolgus monkeys to determine the Maximum Tolerated Dose (MTD) upon intravenous administration.

A dosing regimen with short administration intervals of escalating doses (with 4 day wash out periods) was performed (1x i.p. injection, max. 2x). Treatment groups, AFM24 dose levels and number of animals used in the single dose escalation toxicity study are shown.

Treatment groups, AFM24 dose level and number of animals used in the repeated dose toxicity study are shown.

- During the dosing period, clinical signs, body weight, body temperature, hematology, coagulation and blood chemistry were monitored and found to be within the normal range.
- Cytokine levels measured showed a consistently strong group 4 IL-6 response 2-3 p.p. in all treated animals.
- Immunopathology of lymphocytes subsets after each dose level revealed no substantial effect.
- A panel of preselected tissues e.g. visceral 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 toxicity 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 fentanyl 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.

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

- Potency was determined in 4 h calcium-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 (IC50) was determined by non-linear regression/agonist dose-response (individual values, mean ± SD).
- AFM24 shows higher potency than cetuximab in high EGFR-expressing A-431, DE-MG and in low EGFR-expressing, Ras-mutated HCT-116 cell lines.

AFM24 is efficacious against human tumor cell lines with mutated Ras

- 4 h calcium-release assays with NK-cells as effectors (E:T=5:1) at serial dilutions of AFM24 and cetuximab. Similar results were demonstrated for the A-431 (NOG/CD1) cell line not shown.
- AFM24 potently kills human HCT-116 (polar) and A-431 (NOG/CD1) tumor cell lines bearing the Ras mutation.
- AFM24 potently kills HCT-116 cells with higher efficacy and potency than cetuximab.

AFM24 does not bind to other members of the EGFR family

- ELSA to assess AFM24 cross-reactivity to different members of the EGFR receptor family, EGF, HER2, HER3 and HER4. lgG fusion proteins were coated. Detection of AFM24 binding by Vis-Tag anti-His IgG, HRP conjugated.
- AFM24 shows no binding to HER2, HER3 and HER4.
- AFM24 shows specific binding to EGFR.

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 EGF/CD16A NK-cell engaging antibodies with two binding sites for EGF and two binding sites for CD16A. CD16A is an isomeric 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 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 EGF/CD16A antibodies and analyzed their characteristics. Antibodies containing a specific domain showed single digit picomolar or sub-picomolar EC50 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 vivo pharmacology and toxicology studies.

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.

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

- IgG affected the cytotoxicity of AFM4 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.

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

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