**Introduction**

Constitutive EGFR activation plays an important role in the pathobiology of various solid cancers, such as colorectal, non-small cell lung or squamous cell carcinomas of the head and neck. Tyrosine kinase inhibitors and monoclonal antibodies (mAbs), which block signal transduction and activation of EGFR, are approved for treatment of such cancers. However, intrinsic or acquired resistance to these treatments has been described for a large number of patients.

Natural killer cells (NK-cells) are important effectors of innate immunity and NK-cell engagers have shown evidence of improved safety in patients compared to T-cell engagers. To specifically enhance the cytotoxic potential of NK-cells, to eliminate EGFR-expressing tumors, we developed various tetrameric bispecific EGFR/CDD16A antibody constructs comprising fully human Fv domains recognizing human and cynomolgus EGFR and CDD1. AFM24 lead candidates recognize a conformational epitope in the extracellular domain of EGFR, extruding from epitopes targeted by other therapeutic mAbs. They show superior cytotoxicity in terms of ADCD and reduced inhibition of EGFR-mediated phosphoregulation compared to cetuximab. Cytotoxicity was demonstrated against several EGFR+ tumor cell lines including examples carrying a Ras mutation, which renders cancer cells less susceptible to inhibitors such as cetuximab or panitumumab. The cetuximab-resistant CRC cell line HCT-116 or the NSCLC cell line A549 (both with Ras mutations) were efficiently killed by AFM24 in vitro. In vivo data in the HCT-116 model indicate anti-tumor activity with cetuximab while AFM24 did not activate NK-cells without target cell binding and does not bind to any other member of the EGFR family.

While binding and cytotoxic efficacy of many therapeutic mAbs are impaired by serum IgG, no substantial change in AFM24's binding affinity to NK-cells was observed in the presence of polyclonal human IgG. In cytotoxicity assays with NK-cells as effectors, the presence of 10 mg/mL polyclonal human IgG had only little inhibitory effect on AFM24 potency and efficacy compared to serum inhibition by IgG of the potency and efficacy of cetuximab.

**AFM24 does not bind to other members of the EGFR family**

**Cytotoxicity of AFM24 against human tumor cell lines with mutated Ras**

**In vitro potency of AFM24 and of cetuximab against three human tumor cell lines**

**Effect of high IgG concentrations on binding and cytotoxicity of AFM24 or cetuximab to NK-cells**

**AFM24 shows no binding to HER3, HER4 and FcReI**

**AFM24 shows specific binding to EGFR**

**A F M 2 4 i s a b l e t a m a n t h u m a n H C T - 1 1 6 ( c o l o n ) a n d A - 5 4 9 ( N S C L C ) tumor cell lines with Ras mutation.**

**AFM24 was able to kill HCT-116 cells with higher efficacy and potency than cetuximab.**

**AFM24 is able to kill human HCT-116 (colon) and A-549 (NSCLC) tumor cell lines with Ras mutation.**

**AFM24 shows higher potency than cetuximab in high EGFR-expressing A-421, DI- MG and in low EGFR-expressing HCT-116 (Ras mutation) cells.**

**Cytotoxicity of AFM24 and of cetuximab in the presence and absence of human IgG (30 mg/mL).**

**In vivo activity of AFM24 in a humanized mouse model against HCT-116 cells with Ras mutation.**

**Treatment schedule in HCT-116 tumor bearing humanized NOG mice. AFM24 or vehicle (both p.o.) injection are indicated by red arrows, cetuximab treatment was given once weekly (orange line not depicted).**

**AFM24 shows higher potency than cetuximab in high EGFR-expressing A-421, DI-MG and in low EGFR-expressing HCT-116 (Ras mutation) cells.**

**Cytotoxicity of AFM24 and of cetuximab in the presence and absence of human IgG was determined in 1x10^6 cells per well at an EGFR:CD20 ratio of 2:1 and 5:1 (in the presence of serial dilutions of AFM24 and cetuximab). Potency (EC50) was determined by non-linear regression/logistic dose-response (individual values mean ± SD).**

**PFU mean humanization rate (ratio of hCD45+ to hCD19+).**

**Tumor growth (T/C ratio) of treated animals relative to control. Mean tumor size of mice in the untreated control group was normalized to the corresponding control group (untreated animals group HCD45-).**

**Percentage of human NK-cells in the peripheral blood of humanized NOG mice. The percentage of human CD3-CD56- from human CD3-CD56+ was determined by flow cytometry.**

**About 4% of nk cells in hCD45+ cell population were initially detected.**

**No effect of cetuximab on tumor growth.**

**Conclusion**

High affinity engagement of NK-cells has the potential to offer synergies with other drugs such as checkpoint modulators to further boost anti-cancer immunity in solid tumors (e.g. lung, colon, head & neck).