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

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Introduction

The epidermal growth factor receptor (EGFR) is a validated target for the treatment of several solid tumor types, and currently 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 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 tetavalent, 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.

AFM24_I and AFM24_T display high affinity binding to EGFR

AFM24_I and AFM24_T display high affinity 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

Both AFM24_I and AFM24_T demonstrate superior potency in in vitro cytotoxicity assays with target cells expressing Ras (K-Ras, high EGFR expression) or mutant (C-terminus of EGFR mutant) compared with all other anti-EGFR antibodies tested.

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

AFM24_I and AFM24_T show no signs of toxicity in cynomolgus monkeys

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

Study 1: Determination of the Maximum Tolerated Dose (MTD) upon i.v. administration (2h) Intubation, a dosing schedule with a 4 day wash out period of escalating doses (group 1: 0.05, 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-week repeated dose study

Toxicity assessment was done after 28 days (dosing: 1, 3, 10 and 30 mg/kg; n=2 per group; i.v. administration every other day (i.d. x 28), including an additional high dose incremental group to identify potential delayed toxicity and/or reversion.

AFM24_I and AFM24_T showed a favorable safety profile in cynomolgus monkeys.

Summary

• AFM24_I and AFM24_T are differentiated from cetuximab by their immunotherapeutic engagement (AFM24_I and AFM24_T) via inhibition of EGFR signaling (cetuximab).

• Both NK cell engagers are 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 finding might be beneficial in reducing this major limiting side effect of anti-EGFR mAbs and TKIs.

• Two pilot toxicology studies of AFM24_I confirmed a favorable safety profile.

• The t1/2 of AFM24_I and AFM24_T is effective and convenient dosing similar to classical IgG-type therapeutic antibodies.

• In vitro studies demonstrate strong dose-dependent tumor growth inhibition in vitro using a high EGFR-expressing cell line.

Conclusion and outlook

• AFM24_I and AFM24_T are novel, highly potent and differentiated tetavalent 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 aP1 antibody pembrolizumab.