Tetravalent, Bispecific Innate Cell Engager (ICE®) AFM24 Enhances Macrophage Mediated Tumor Cell Phagocytosis

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BACKGROUND

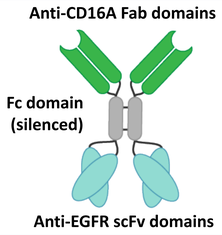
- **I**nnate **C**ell **E**ngager (ICE®) molecules are designed to enhance the activity of innate immune cells against tumors
- ICE® bispecifically engage CD16A+ natural killer (NK) cells and macrophages and tumor antigens
- AFM24 is a tetravalent ICE® which can bind CD16A and epidermal growth factor receptor (EGFR) (Fig. 1)
- EGFR is overexpressed in many solid cancers and can be an indicator of poor prognosis[1,2]
- Clinically used EGFR signaling inhibitors have various limitations including:
  - Toxicities related to the inhibition of EGFR signaling in healthy tissues[3,4]
  - Intrinsic and acquired resistance[5,6]
- AFM24 engages CD16A on NK cells and macrophages with a higher affinity than therapeutic monoclonal antibodies; once engaged, AFM24 can trigger responses against EGFR-expressing cancer cells including[7]:
  - NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC)
  - Macrophage-mediated antibody-dependent cellular phagocytosis (ADCP) (Fig. 2)
- The mode of action of AFM24 can overcome the limitations of current EGFR-targeted therapies by being independent of EGFR activity and avoiding signalling pathway resistance development[7]
- Preclinical and clinical data suggest that ICE® molecules demonstrate promising safety and efficacy as monotherapies as well as in combination with other immunotherapeutic approaches[7,8]

OBJECTIVE

To assess the ability of AFM24 to induce antibody-dependent cellular phagocytosis in solid tumor cell lines expressing wildtype EGFR or EGFR signalling pathway mutations

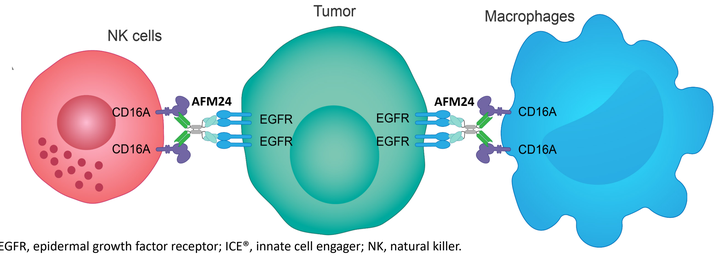
FIGURE 1: AFM24 STRUCTURE

- AFM24 is a prototypic ICE® derived from the **R**edirected **O**ptimized **C**ell **K**illing (ROCK®) antibody platform
- AFM24 is a bispecific, tetravalent EGFR/CD16A IgG1-scFv fusion antibody (scFv-IgAb) with a silenced IgG1 Fc



Fc, fragment crystallizable; Ig, immunoglobulin; scFv, single-chain variable fragment.

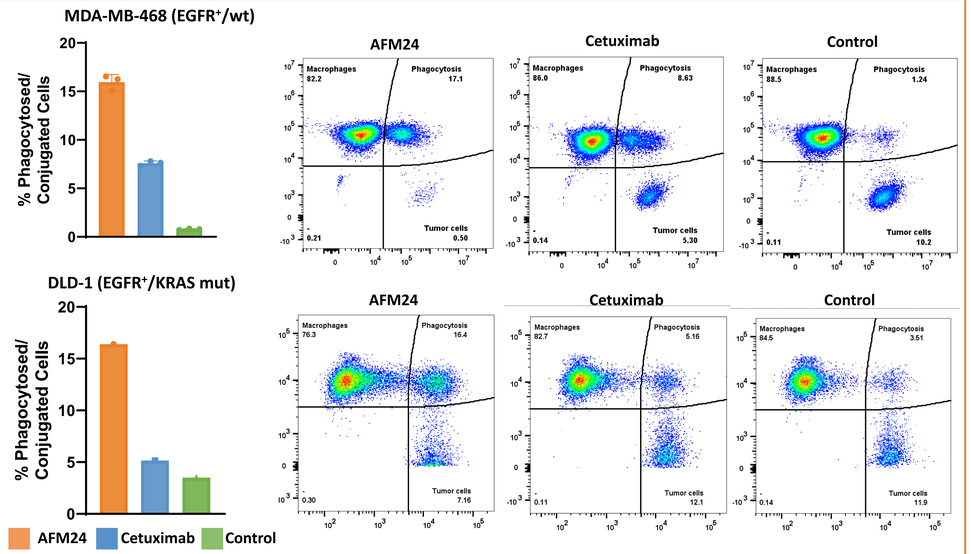
FIGURE 2: AFM24 MEDIATES INTERACTION OF NK CELLS AND MACROPHAGES TO KILL TUMOR CELLS



EGFR, epidermal growth factor receptor; ICE®, innate cell engager; NK, natural killer.

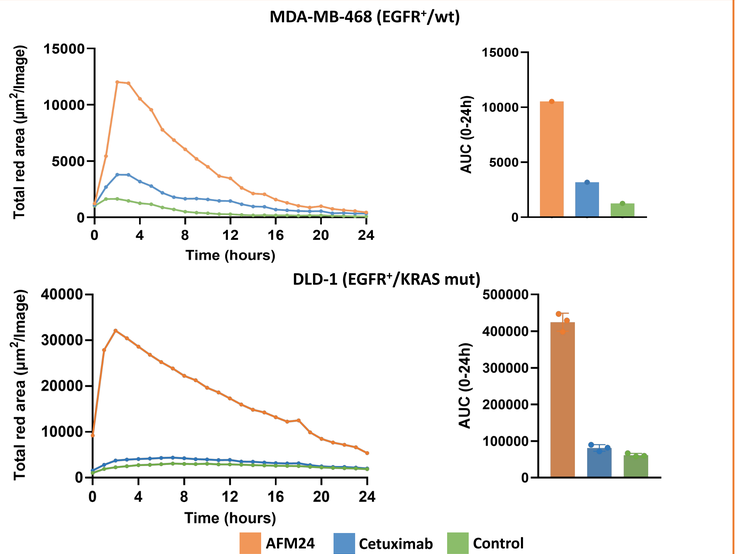
RESULTS

AFM24 is more efficacious than cetuximab in inducing ADCP of EGFR+ (wildtype and KRAS mutant) tumor cells



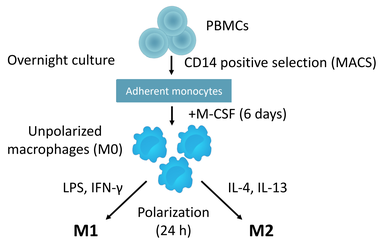
Monocyte-differentiated macrophages (M0) from healthy donor PBMCs were co-cultured for 4 hours with CMFDA-labelled wildtype EGFR-expressing or KRAS mutant tumor cells in the presence of 10 µg/mL AFM24 or cetuximab. ADCP was assessed by flow cytometry (FACS). Shown are representative plots from 1 donor; n= 2-3.

AFM24 is superior to cetuximab at inducing phagocytosis of EGFR+ (wildtype and KRAS mutant) cells in live cell imaging analysis over 24 hours



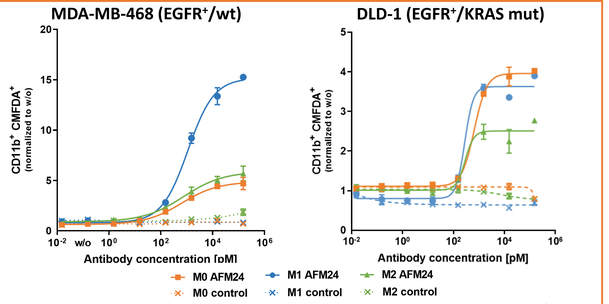
M0 macrophages were co-cultured with pHRodo™-labelled tumor cells in the presence of 10 µg/mL AFM24 or cetuximab and phagocytosis was assessed by live cell-imaging analysis (IncuCyte®) over 24 hours. Data shown represent 1 donor; n= 2-3.

MONOCYTE DIFFERENTIATION AND MACROPHAGE POLARIZATION



Sketch of PBMC-derived monocyte differentiation into M0 macrophages with M-CSF for 6 days. Polarization of M0 macrophages into M1 (with LPS and IFN-γ) and M2 (with IL-4 and IL-13) macrophages over 24 h. IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; M-CSF, macrophage colony-stimulating factor; PBMC, peripheral blood mononuclear cell.

AFM24 enhances ADCP of EGFR+ tumor cells regardless of macrophage subtype



M0, M1 or M2 macrophages were co-cultured with CMFDA-labelled tumor cells in the presence of increasing concentrations of AFM24. ADCP was assessed by flow cytometry. Shown are representative plots from 1 donor; n= 2-4.

CONCLUSIONS

- AFM24 enhances macrophage-mediated ADCP of various EGFR expressing tumor cell lines, irrespective of the EGFR signaling pathway; this has been confirmed using two independent methods over 24 h kinetics
- AFM24 can induce ADCP mediated by various macrophage subtypes
- This mechanism of action may be instrumental to the efficacy of AFM24, especially in macrophage-rich tumors
- AFM24 is superior to cetuximab in induction of ADCP
- AFM24 is being investigated in a Phase 1/2 clinical study in subjects with EGFR+ tumors and may offer an alternative therapeutic option, particularly for patients with resistance to conventional EGFR-targeting agents

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