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

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BACKGROUND

- Innate Cell Engager (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 including⁷:
- 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 development⁷
- 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 Redirected Optimized Cell Killing (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

Anti-CD16A Fab domains Fc domain (silenced)

MONOCYTE DIFFERENTIATION AND MACROPHAGE POLARIZATION Anti-EGFR scFv domains Overnight culture CD14 positive selection (MACS) +M-CSF (6 days)

macrophages (M0)

mononuclear cell.

LPS, IFN-γ

IL-4, IL-13

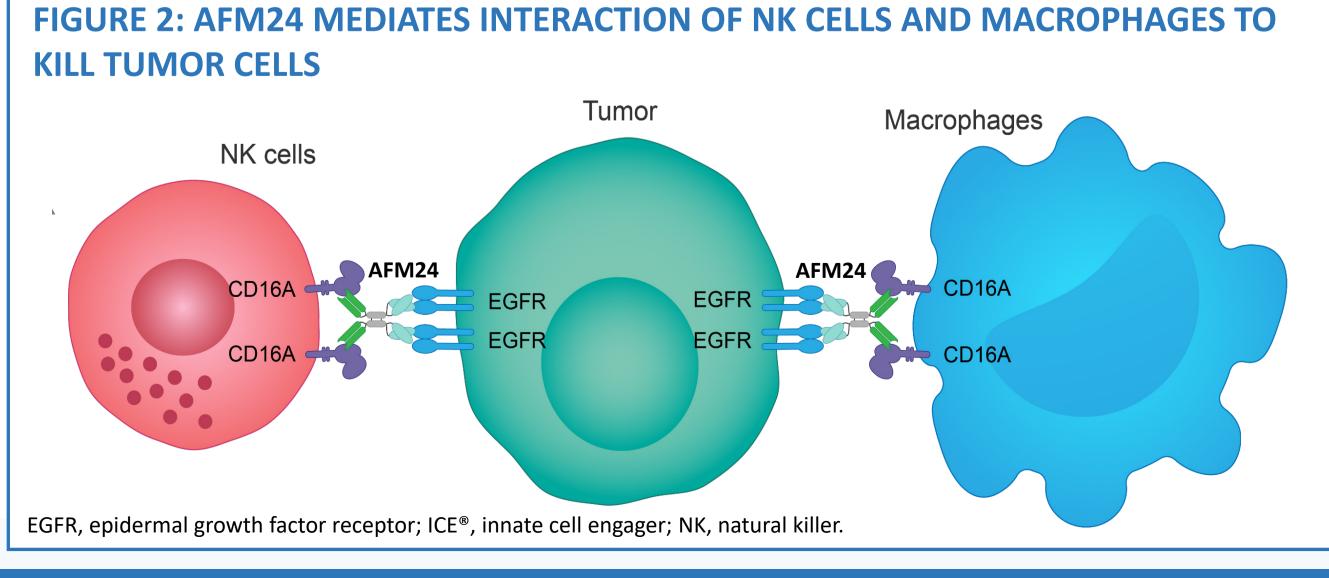
Sketch of PBMC-derived monocyte differentiation into M0 macrophages

with M-CSF for 6 days. Polarization of M0 macrophages into M1 (with

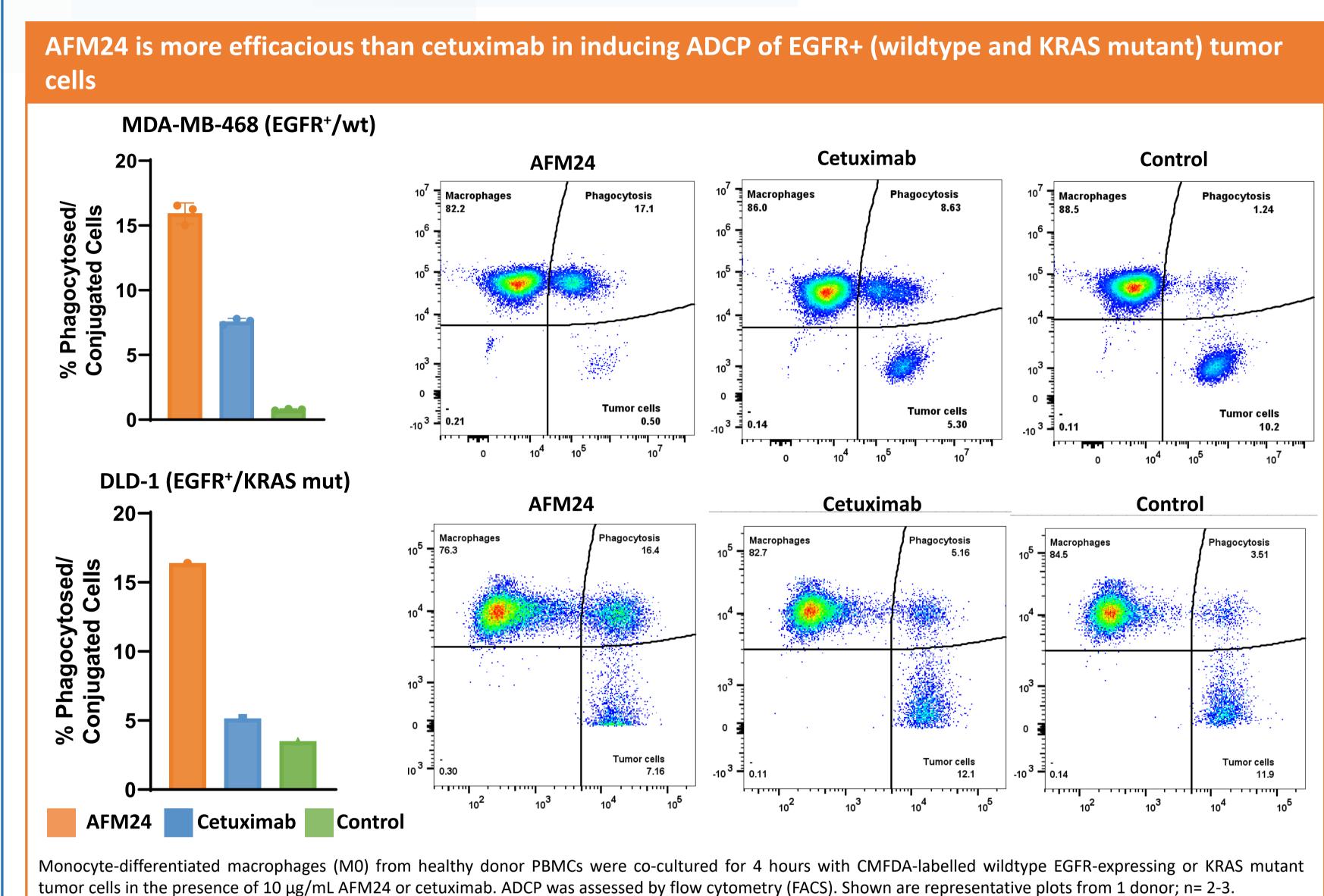
LPS and IFN-v) and M2 (with IL-4 and IL-13) macrophages over 24 h.

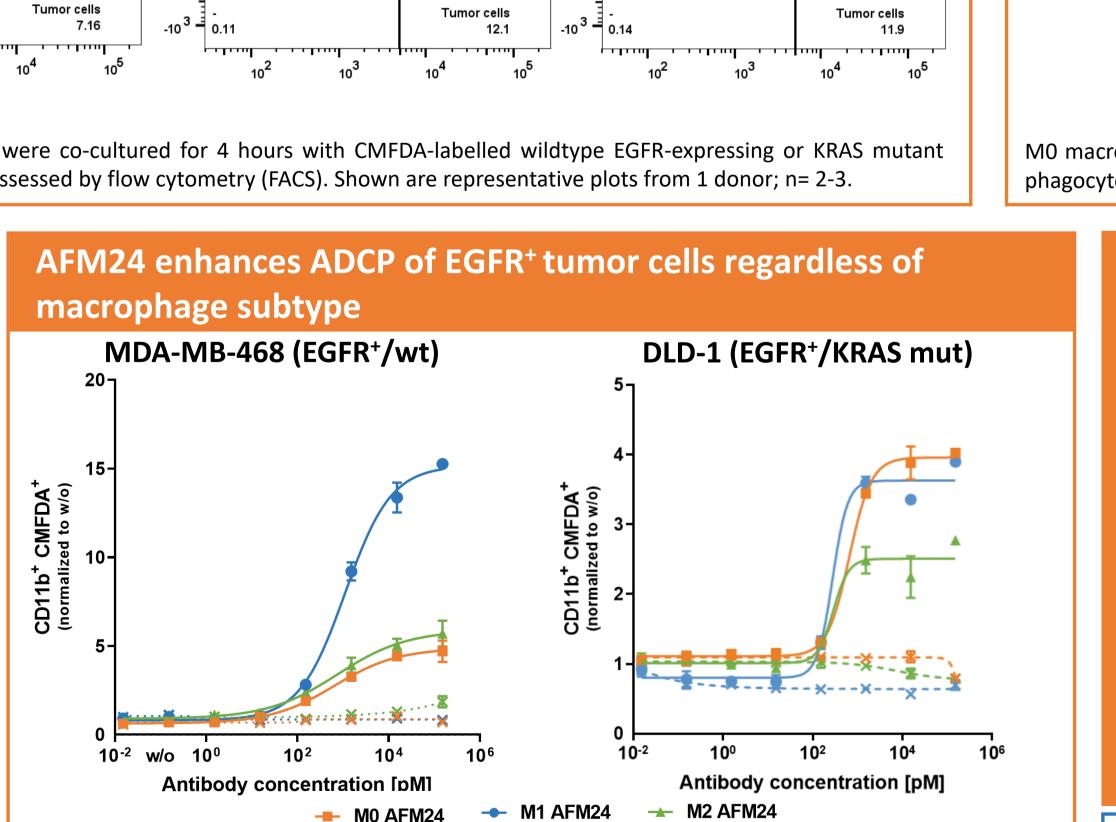
IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; M-CSF

macrophage colony-stimulating factor; PBMC, peripheral blood



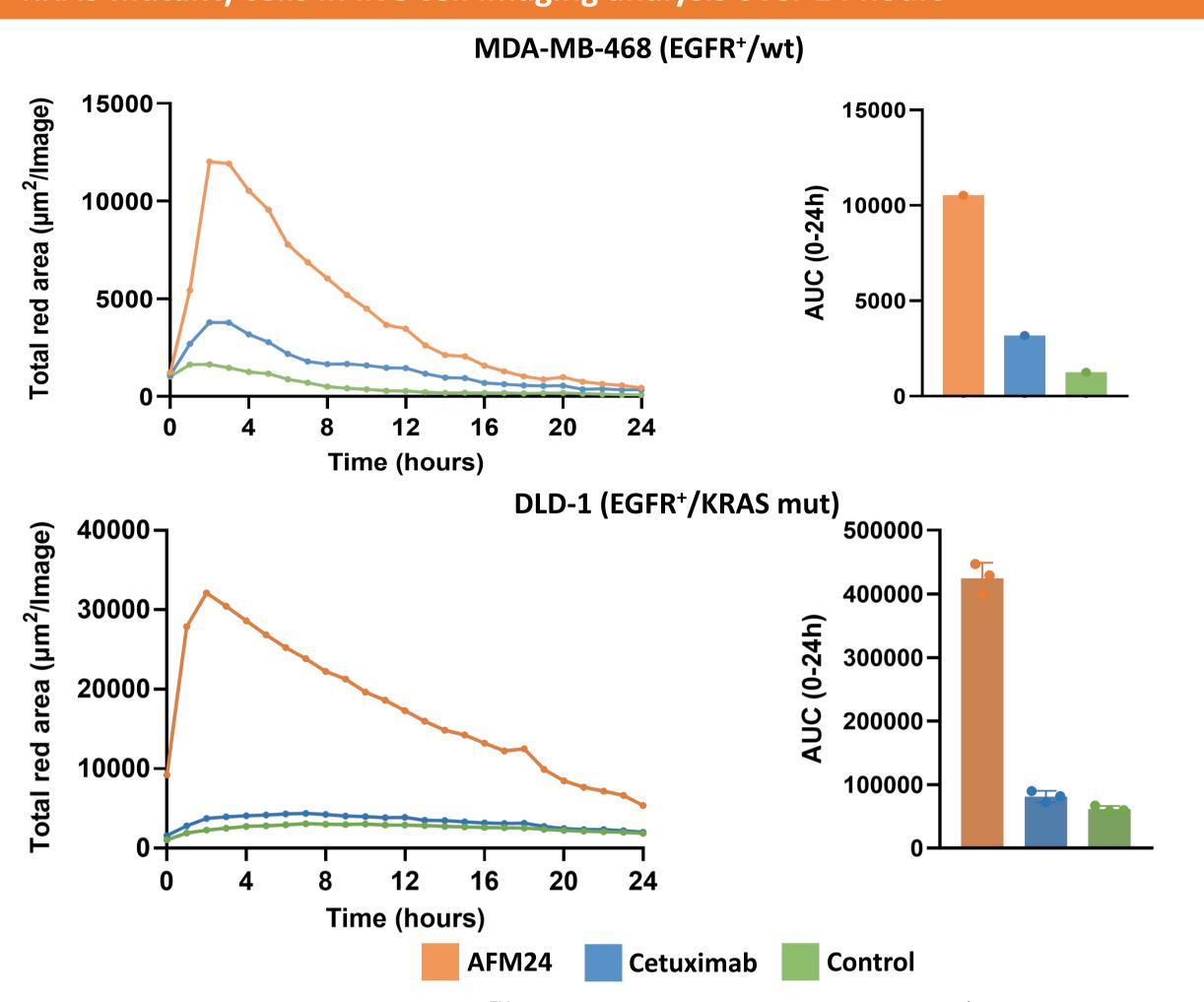
RESULTS





increasing concentrations of AFM24. ADCP was assessed by flow cytometry. Shown are representative plots from 1 donor; n = 2-4.

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 pHRodoTM-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.

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

REFERENCES

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