**TETRAVALENT, BISPECIFIC INNATE CELL ENGAGER
AFM24 ENHANCES MACROPHAGE MEDIATED TUMOR
CELL PHAGOCYTOSIS**

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Background Enabling innate immunity holds promise to provide a treatment option for patients suffering from various kinds of malignancies. Innate cell engagers (ICE[®]) derived from the ROCK[®] (redirected optimized cell killing) platform have demonstrated to induce antibody dependent cellular cytotoxicity (ADCC) and phagocytosis (ADCP) via bivalent targeting of a unique epitope on CD16A of NK cells and macrophages, respectively. Previously published preclinical and clinical data of ICE[®] molecules show promising efficacy and safety as monotherapy, as a combination therapy with immuno-oncology drugs such as PD-1/PD-L1, and in combination with adoptive NK cell transfer. AFM24 is a tetravalent bispecific epidermal growth factor (EGFR)- and CD16A-binding ICE[®] for enhanced targeting and killing of EGFR+ tumor cells currently in clinical development. In contrast to approved EGFR-targeting antibodies, AFM24 does not inhibit the signaling pathway of the EGFR but utilizes this receptor merely as an "anchor" to direct NK cells and macrophages to attack tumor cells via ADCC and ADCP.

Methods ADCP assays were performed with monocyte-differentiated macrophages from healthy donor PBMCs. Target tumor cells were labelled and co-cultures with macrophages, AFM24 and control antibodies. FACS analysis and live-cell imaging (Incucyte[®]) were used to measure ADCP events.

Results We show that AFM24 enhances macrophage mediated tumor cell phagocytosis i.e., ADCP of tumor cell lines with varying levels of EGFR expression and irrespective of EGFR signaling pathway mutations. The ability of AFM24 to enhance ADCP was further demonstrated in patient-derived xenograft cell lines from various EGFR+ tumor indications. Assays with myeloid-derived suppressor cells, natural killer cells and other immune modulators were designed to address the activity of our ICE[®] in the context of the suppressive nature of the tumor microenvironment.

Conclusions We report the ability of our ICE[®] to enhance ADCP, which might be instrumental to their efficacy, especially in tumors enriched with macrophages. In addition, due to its novel mechanism of action, AFM24 may overcome limitations of existing EGFR-targeting agents, such as dose limiting toxicity, and/or intrinsic or acquired resistance of the tumor. Consequently, AFM24 may be a potential future treatment option for a wide spectrum of patients including those that do not respond or are resistant to current EGFR-directed therapies that inhibit the signaling pathway.

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