AFVT-2101, an Innate Immune-Cell Engager That Selectively Targets FOLR1 Expressing Tumor Cells to Safely Harness Potent Anti-Cancer Responses

Ahmad Trad, Michael Tomaszowski, Josef Caslavsky, Robert Freitag, Keith Haan, Markus Rohrwild, Daniel O’Shamnessy, Tara Siegler, Sudhir Penugonda, Peter Sandy, Eric Gaukel, Daniela Penston, Zoë Johnson.
1Abcheck, Plzen, Czech Republic; 2Roivant Sciences, New York, NY, USA; 3TMDx Consulting LLC, Philadelphia, PA, USA; 4Affimed, Heidelberg, Germany; 5Affivant Sciences GmbH, Basel, Switzerland

Background Innate Cell Engager (ICE®) molecules are designed to bivalently bind CD16A+ natural killer (NK) cells and macrophages and a tumor cell-surface antigen, inducing potent, tumor-directed cytotoxicity via antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). AFVT-2101 (figure 1) is a tetravalent, bispecific ICE® that bridges folate receptor alpha (FOLR1) on tumor cells with innate immune cells, to induce potent and selective targeted tumor cell killing. Herein we describe the structure, mechanism of action and preliminary safety data of AFVT-2101.

Methods Binding of AFVT-2101 to CD16A and human FOLR1 was assessed by ELISA. Binding of AFVT-2101 to NK cells was assessed by flow cytometry in the presence of physiological levels of IgG (10 mg/mL). ADCC was assessed using a 4h calcein release assay with purified NK cells from healthy donors against a panel of tumor cell lines with different expression levels of FOLR1. ADCP was assessed using a flow cytometry-based method with macrophages derived from healthy donor monocytes and target cell lines with different levels of FORL1 expression. Quantification of secreted cytokines by healthy donor PBMC cultures in the presence of AFVT-2101 and target cells was assessed after a 24 h incubation using multiplex cytokine quantification.

Results AFVT-2101 binds to CD16A (both 158V and 158F variants) with an apparent avidity of 0.1 nM and to hFOLR1 with an apparent avidity of 0.5 nM. Physiological levels of competing IgG do not alter binding efficacy. AFVT-2101 induces potent and selective ADCC, even on FOLR1low cells. AFVT-2101 is also shown to induce efficient ADCP in vitro. Further, AFVT-2101 is demonstrated to be more efficacious and potent in both ADCC and ADCP assays than farletuzumab, an Fc competent monoclonal antibody targeting FOLR1 which shares the same VH/VL sequence as AFVT-2101. Co-culture of PBMCs, tumor cells and AFVT-2101 showed concentration-dependent release of pro-inflammatory cytokines (IFNγ, IP-10, TNFα) and minimal off-target cytokine release.

Conclusions We demonstrate that AFVT-2101 selectively and potently kills tumor cells with a range of expression levels of FOLR1 by two complimentary mechanisms: ADCC and ADCP. The high avidity for CD16A imparts increased potency and efficacy compared to an Fc competent, FOLR1-targeting antibody with the same VH/VL sequence. As AFVT-2101 binds selectively to CD16A outside the IgG binding epitope, physiological levels of IgG do not compete for binding. We show that AFVT-2101 induces moderate concentration-dependent pro-inflammatory cytokine release in a target-restricted manner, confirming a potent but safe in vitro profile of AFVT-2101.