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AFVT-2101:
a FRα × CD16A bispecific innate cell engager for the treatment of solid tumors

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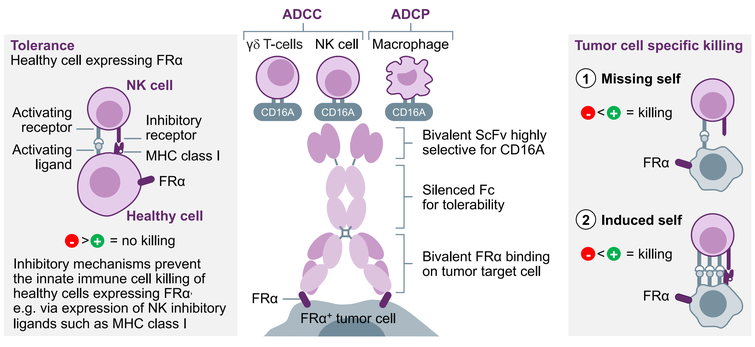
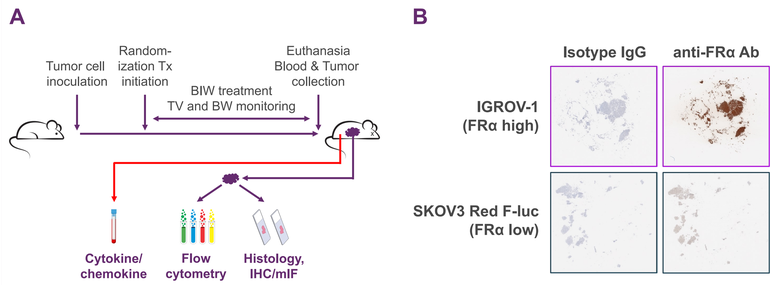
Introduction

- T-cell engagers display meaningful, but limited, clinical activity often associated with toxicity. Recent literature suggests that activation of natural killer (NK) and other innate cells may provide a safer alternative for immunotherapy1.
- AFVT-2101 is a novel bispecific Innate Cell Engager (ICE®)2,3 designed to induce tumor killing by high avidity binding to CD16A on innate immune cells (NK cells, monocytes and macrophages) and to folate receptor alpha (FRα), a tumor-associated antigen with upregulated expression on certain solid tumor cells.
- We report here on the preclinical development of AFVT-2101, showing its selective antitumor activity in antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) *in vitro* assays.
- To evaluate the *in vivo* antitumor efficacy of AFVT-2101, a novel humanized CD16A knock-in mouse strain (CB17-SCID-hCD16A) was employed in which the mouse Fcgr4 gene was replaced by human FCGR3A exons 1~4 and regulatory region.
- AFVT-2101 exhibited dose-dependent tumor growth inhibition in implanted IGROV-1 (FRα high) and SKOV3 Red F-luc (FRα low) tumors and led to the reduction in frequency of tumor-associated macrophages (TAMs).
- These data demonstrate that AFVT-2101 efficiently kills tumor cells through activation of innate immune cells and should be considered as a novel therapeutic approach for the treatment of FRα-expressing solid tumors.

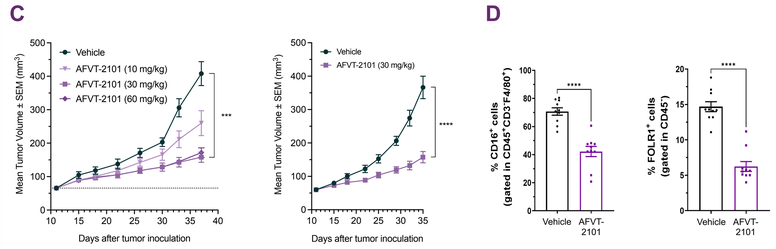
AFVT-2101 (201 kDa) and its proposed MOA



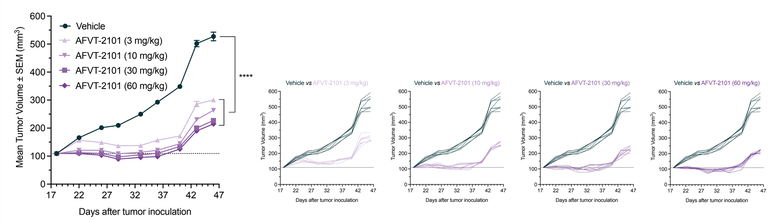
AFVT-2101 inhibits tumor growth *in vivo* with concomitant reduction of tumor-associated macrophages (TAMs)



IGROV-1 (FRα high)



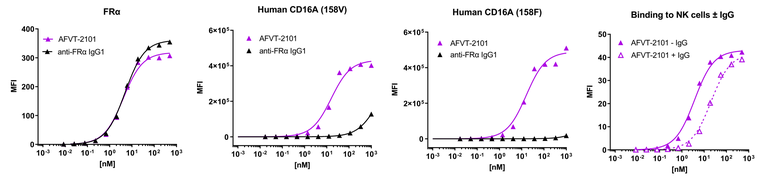
SKOV3 Red F-luc (FRα low)



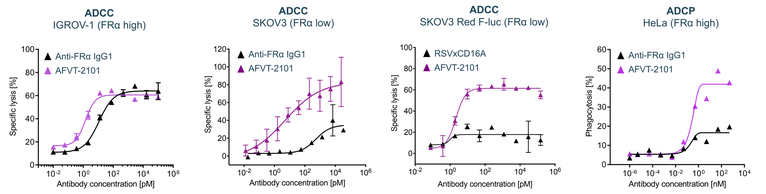
(A) Schematic of experimental design. (B) FRα expression in cell pellets detected by IHC. 5 x 106 of IGROV-1 (C) or SKOV3 Red F-luc (E) cells were subcutaneously injected into the right hind limbs of CB17-SCID-hCD16A mice on Day 0. Animals were treated IV, BIW, with either vehicle or AFVT-2101 as indicated. Two independent experiments are shown for IGROV-1. (D) *Ex vivo* flow cytometry analysis performed on excised IGROV-1 tumors collected at time of euthanasia. CD45+CD3+F4/80+ population represents TAMs.

*0.01<P<0.05; **0.001<P< 0.01; ***0.0001<P<0.001; ****P< 0.0001

AFVT-2101 binds to FRα and CD16A with high avidity and induces tumor cell killing via ADCC and ADCP



AFVT-2101 binding to FRα on IGROV-1 cells and to CD16A (158V and 158F) on transfected CHO cells was assessed by flow cytometry. AFVT-2101 binds to NK cells with high avidity which is slightly reduced (~5 fold) in the presence of physiological levels of IgG (10 mg/mL).



Representative ADCC and ADCP results. ADCC was performed using a 4 h calcein-release assay with NK cells purified from healthy donors. ADCP was performed using flow cytometry with macrophages derived from healthy donor monocytes.

Summary

- AFVT-2101 bound FRa and CD16A with high avidity.
- AFVT-2101 induced potent and target-restricted tumor cell killing *in vitro* in tumor cell lines with different FRα expression levels.
- AFVT-2101 showed increased potency and efficacy compared to an Fc-competent, FRα-targeting antibody with the same VH/VL sequence *in vitro*.
- AFVT-2101 inhibited tumor growth *in vivo* in models with high (IGROV-1) and low (SKOV3 Red F-luc) FRα expression levels.
- Ex vivo flow cytometry analyses on AFVT-2101-treated animals showed 1) a reduction of FRα+ tumor cells and 2) a reduction of the frequency of TAMs.
- AFVT-2101 is expected to enter phase I clinical trials in Q2 2023.

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Abbreviations
ADCC: antibody-dependent cellular cytotoxicity; ADCP: antibody-dependent cellular phagocytosis; BIW: twice weekly; BW: body weight; Fc: fragment, crystallizable; FRα: folate receptor alpha; F-luc: Firefly luciferase; ICE: innate cell engager; IgG: immunoglobulin G; IHC: immunohistochemistry; IV: intravenous; MHC: major histocompatibility complex; MOA: mechanism of action; NK: natural killer; SCID: severe combined immune deficiency; TAM: tumor-associated macrophage; TV: tumor volume; Tx: therapy

Acknowledgements
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References
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