AFVT-2101: an innate immune-cell engager that selectively targets FRα expressing tumor cells to safely harness potent anti-cancer responses

1212

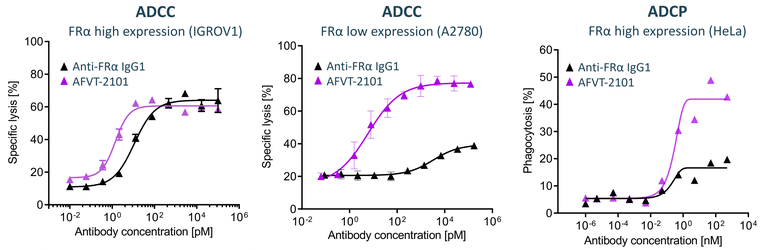
Ahmad Trad[1], Michael Tomaszowski[1], Josef Caslavsky[1], Robert Freitag[2], Sudhir Penugonda[2], Keith Haan[2], Markus Rohrwild[3], Daniel O'Shannessy[4], Jana Siegler[5], Peter Sandy[3], Eric Gaukel[2], Uwe Reusch[5], Daniela Penston[5], Zoë Johnson[3]

[1]AbCheck, Plzen, Czech Republic; [2]Roivant, New York, NY, USA; [3]Affivant, Basel, Switzerland; [4]TMDx Consulting LLC, Philadelphia, PA, USA; [5]Affimed, Heidelberg, Germany

Introduction

- Folate receptor α (FRα), a 38–40 kDa molecule, is a well characterized member of the folate receptor (FR) family with high affinity for folates
- FRα exhibits limited normal tissue distribution, with measurable expression restricted to the apical surfaces of a few epithelia, predominantly in the lung, kidney, and choroid plexus, but is overexpressed in a spectrum of solid tumors, including ovarian cancer and breast cancer[1]
- Several approaches to target FRα have been investigated including monoclonal antibodies, antibody-drug conjugates and T cell activating strategies (i.e. bispecific antibodies and CAR-T cells)
- 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)[2,3]
- AFVT-2101 represents a novel approach to treating FRα expressing tumors by engaging the innate immune response for safe and effective tumor cell killing

AFVT-2101 induces tumor cell killing via ADCC and ADCP



Potency is unaffected by level of FRα expression across multiple cancer types

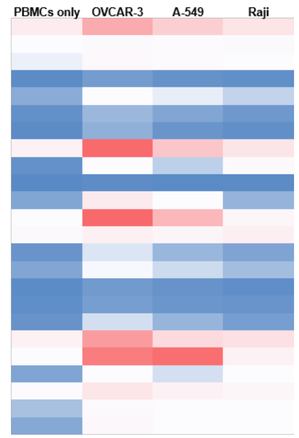
Cell Line	Cancer	AFVT-2101 EC_{50} (pM)	AFVT-2101 Fold-Improvement in Potency vs. FRα targeting IgG1 (ADCC) (1 / 10 / 100 / 1000)	FRα Expression
NCI-H2110	NSCLC	0.833		116,216
HCC-78	NSCLC	24.5		96,969
T47-D	BC	0.545		51,099
HCC1954	BC	13.8		41,543
OVCAR-3	OC	1.11		36,094
HCC-15	LC	49.8		27,520
SKOV-3	OC	4.45		25,244
HCC-827	NSCLC	2.8		24,267
SK-BR-3	BC	3.61		4,570
HCC1187	BC	4.49		4,189
HCC-44	NSCLC	18.3		2,323
MDA-MB-231	TNBC	6.52		1,976
A2780	OC	5.51		1,662
KARPAS-299	ALCL	NC		None
Raji	BCL	NC	No specific cell lysis observed for either AFVT-2101 or FRα targeting IgG	None

Enhanced efficacy & potency demonstrated in:

(i) **ADCC** assay using a 4h- calcein release assay with purified NK cells from healthy donors

(ii) **ADCP** assay using a flow cytometry-based method with macrophages derived from healthy donor monocytes

AFVT-2101: target restricted cytokine release *in vitro*



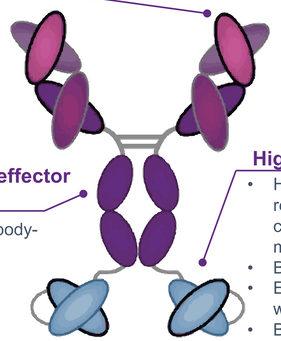
Human PBMC cultures with or without target cells + 100µg/mL AFVT-2101

- Heat map of the relative expression level of 24 cytokines in the supernatant after 24h incubation
- PBMCs were cultured with or without target cells in presence of 100ug/mL AFVT-2101
- OVCAR-3 & A-549 are FRα positive cell lines (high and low levels respectively)
- Raji cells do not express FRα
- Color code:
  - blue: low expression (< 100 pg/mL);
  - white: moderate expression (> 100 pg/mL);
  - red: high expression (> 1000 pg/mL)

***In vitro* cytokine release profile is consistent with an active immune engager, but with no red flags indicating a risk for cytokine release syndrome in patients**

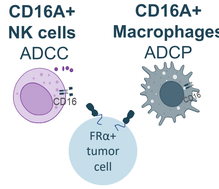
AFVT-2101 (201 kDa)

FRα Fab´ binding domains
- Bivalent TAA engagement
- Confers strong avidity
- Apparent avidity for FRα ~0.05 nM



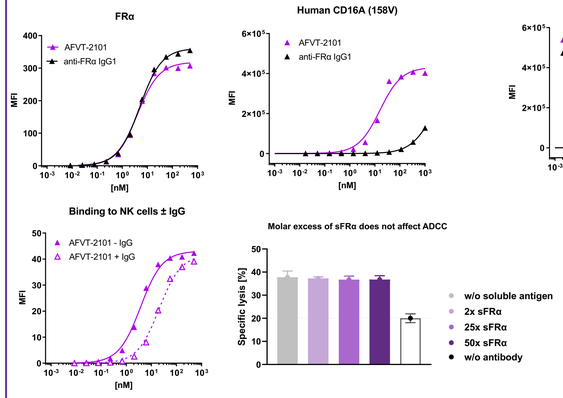
Engineered to reduce Fc effector function
- Retains FcRn binding for antibody-like PK properties

heavy chain domain light chain domain



High affinity CD16A binding
- High avidity (~0.1 nM) construct resulting in improved ADCC compared to a FRα targeting IgG1 monoclonal
- Bivalent, selective CD16A binding
- Engineered to avoid competition with circulating serum IgG
- Binds with equipotency to CD16A 158 V and 158F
- Engineered to reduce ADA reactivity

High avidity binding of AFVT-2101 to FRα and CD16A



Binding to FRα expressing IGROV1 cells and CD16A (158V), CD16A (158F) on transfected CHO cells was assessed by flow cytometry. AFVT-2101 binding to NK cells is unaffected by addition of IgG (10 mg/mL). sFRα does not inhibit the ADCC activity of AFVT-2101

Summary

- AFVT-2101 selectively and potently kills tumor cells with a range of expression levels of FRα by two complimentary mechanisms: ADCC and ADCP
- The high avidity for CD16A imparts increased potency and efficacy compared to an Fc competent, FRα-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 and therefore AFVT-2101 is expected to maintain high potency *in vivo*
- 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
- AFVT-2101 represents a novel approach to treating FRα expressing tumors including HGSOC, TNBC and NSCLC
- AFVT-2101 is in IND-enabling studies and is expected to enter Phase I clinical trials in 2023

Abbreviations

FRα: folate receptor alpha; HGSOC: High-grade serous ovarian carcinoma; TNBC: Triple-negative breast cancer; NSCLC: non small-cell lung cancer; CAR-T cells: Chimeric antigen receptor T cells; ICE: innate cell engager; NK cell: Natural killer cell; ADCC: antibody dependent cellular cytotoxicity; ADCP: antibody dependent cellular phagocytosis; Fab´: Fragment antigen binding, FcRn: neonatal Fc receptor; Fc: fragment, crystallizable

References

1. Nawaz & Kipreos "Emerging roles for folate receptor FOLR1 in signaling and cancer". Trends in Endocrinology & Metabolism (2022) Vol 33, p 159-174
2. Ellwanger, Reusch, Fucek et al "Redirected optimized cell killing (ROCK®): A highly versatile multispecific fit-for-purpose antibody platform for engaging innate immunity". MABS (2019) Vol 11 p 899-918
3. Wingert, Reusch, Knackmuss et al "Preclinical evaluation of AFM24, a novel CD16A-specific innate immune cell engager targeting EGFR-positive tumors". MABS (2021) Vol 13 e1950264

SITC Annual Meeting | November 2022 | Boston, MA, USA