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Preclinical Safety & Pharmacokinetics of AFVT-2101, a Tetravalent FRα x CD16A Bispecific Innate Cell Engager for the Treatment of Solid Tumors

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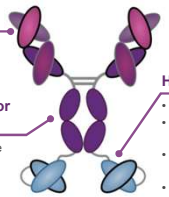
AFVT-2101

- AFVT-2101 is a novel bispecific Innate Cell Engager (ICE®) designed to bivalently bind CD16A+ innate immune cells and a tumor cell-surface antigen, folate receptor alpha (FRα), inducing potent, tumor-directed cytotoxicity via antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP)1,2
- AFVT-2101 is being developed for the treatment of FRα+ solid tumor indications including ovarian cancer, non-small cell lung cancer, triple-negative breast cancer, and clear cell renal cell carcinoma
- A comprehensive package of IND-enabling studies has been completed for AFVT-2101, generating robust preclinical safety and pharmacokinetic data alongside proof-of-concept pharmacology models
- With a clean safety profile in nonhuman primates (NHPs) and pharmacokinetics (PK) consistent with the drug-like properties of the molecule, AFVT-2101 is strongly positioned for first-in-human trials

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



High affinity CD16A binding
- Bivalent, selective CD16A binding
- Engineered to avoid competition with circulating serum IgG
- Binds with equipotency to CD16A 158V and 158F
- Engineered to reduce ADA reactivity

AFVT-2101 Toxicokinetics in Nonhuman Primates

AFVT-2101 Mean NHP Serum Toxicokinetics Following 2-hour IV Infusion Administration

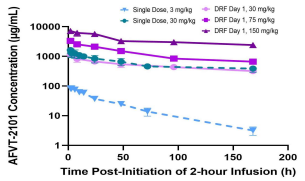
Study	Dose[a] (mg/kg/dose)	Sex[b]	Day (Total Dose)[c]	C_{max}[d] (µg/mL)	Dose Norm. C_{max}[e]	Accum. Ratio, C_{max}	$AUC_{0\text{-}168}$[g] (h*µg/mL)	Dose Norm. $AUC_{0\text{-}168}$[h]	Accum. Ratio, $AUC_{0\text{-}168}$[i]	ADA[j]
Non-GLP Single Dose	3	3M	1 (Single)	85.1	28.4	-	3426	1142	-	NA
	30	3M	1 (Single)	1624	54.1	-	96953	3232	-	NA
Non-GLP DRF Repeat Dose	30	3F	1 (First)	1120	37.3	-	83600	2787	-	2/3 (66.7%)
			15 (Third)	1560	52	1.39	147000	4900	1.76	
	75	3F	1 (First)	3320	44.3	-	209000	2787	-	3/3 (100%)
			15 (Third)	4840	64.5	1.46	387000	5160	1.85	
	150	3F	1 (First)	7190	47.9	-	592000	3947	-	3/3 (100%)
			15 (Third)	13000	86.7	1.81	1050000	7000	1.77	
GLP 28-Day Repeat Dose	30	3M/3F	1 (First)	916	30.5	-	73700	2457	-	1/6 (16.7%)
			22 (Fourth)	1330	44.3	1.45	135000	4500	1.83	
	75	5M/5F	1 (First)	1880	25.1	-	173000	2307	-	6/10 (60%)
			22 (Fourth)	3390	45.2	1.8	324000	4320	1.87	
	150	5M/5F	1 (First)	3810	25.4	-	311000	2073	-	5/10 (50%)
			22 (Fourth)	6040	40.3	1.59	609000	4060	1.96	

[a]AFVT-2101 administered via 2-hour duration IV infusion. [b]Mean data presented; non-GLP studies were single sex, combined-sex data presented for 28-day study as no significant differences were seen between males and females (<2-fold difference). [c]Day of dose administration and overall total dose; repeat dose studies were administered once-weekly. [d]Peak measured AFVT-2101 serum concentration following 2-hour IV infusion. [e]Dose normalized peak concentration: C_{max} divided by nominal dose. [f]Accumulation ratio for C_{max} in repeat dose studies: Day 15 (or Day 22) C_{max} divided by Day 1 C_{max}. [g]Area under the AFVT-2101 concentration-time curve from 0 to 168 hours from the beginning of the 2-hour IV infusion. [h]Dose normalized AUC: $AUC_{0\text{-}168}$ divided by nominal dose. [i]Accumulation ratio for AUC in repeat dose studies: Day 15 (or Day 22) $AUC_{0\text{-}168}$ divided by Day 1 $AUC_{0\text{-}168}$. [j]Animals per group developing treatment-emergent anti-AFVT-2101 antibodies.

- AFVT-2101 possesses an IgG-like pharmacokinetic profile in nonhuman primates, with half-life ranging from 6.5-12.6 days, and clearance ~0.01% of hepatic blood flow
- Nonlinear PK observed <30 mg/kg; consistent with target-mediated drug disposition (TMDD)
- C_{max} and AUC increased in a generally dose-proportional manner from 30 to 150 mg/kg
- No impact of treatment-emergent ADA on PK – all animals included in data analysis

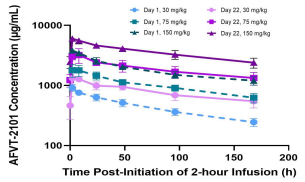
AFVT-2101 PK Profile in Nonhuman Primates

AFVT-2101 NHP Serum Concentration Following Single IV Administration



- **Single IV Dose non-GLP NHP PK and non-GLP NHP IV DRF mean serum concentration-time data**
- Single dose study: n=3M/group at 3 and 30 mg/kg
- DRF study: n=3F/group at 30, 75, and 150 mg/kg
- Non-linearity of pharmacokinetics apparent at 3 mg/kg relative to higher doses – consistent with TMDD
- Good agreement between studies at 30 mg/kg
- Elimination slopes generally parallel between dose levels ≥30 mg/kg
- Qualified sandwich ELISA LBA quantitation method

AFVT-2101 NHP Serum Concentration Following Single and Repeat IV Dosing



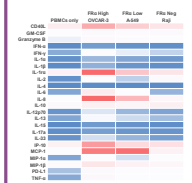
- **28-day NHP GLP IV Tox/TK repeat dose mean serum concentration-time data**
- n=3/sex/group at 30 mg/kg/dose, n=5/sex/group at 75 and 150 mg/kg/dose
- Combined male and female data (<2-fold difference between sexes)
- Modest accumulation (~2-fold) apparent between first and fourth doses of AFVT-2101 at each dose level
- Elimination slopes generally parallel between dose levels and with multiplicity of administration
- No impact of treatment-emergent ADA on PK
- Validated hybrid immunoaffinity-LC-MS/MS quantitation method

AFVT-2101 Preclinical Toxicology

- Nonhuman primate (specifically, cynomolgus monkey) was determined to be the only pharmacologically relevant toxicology species on the basis of in vitro binding to FRα & CD16A homologs; as such, in vivo single dose PK (non-GLP) along with dose range finding (DRF, non-GLP) and 28-day repeat dose (GLP) toxicity/TK studies were performed in cynomolgus monkey
- In agreement with >91% sequence homology for both targets in human and cynomolgus monkey, a GLP tissue cross-reactivity assessment with fluoresceinated AFVT-2101 against a panel of human and cynomolgus monkey cryosections demonstrated similar binding against various cell types as expected based on expression of FRα & CD16A
- The NOAEL for AFVT-2101 was determined to be 150 mg/kg following weekly administration of AFVT-2101 at 30, 75, & 150 mg/kg/dose via 2-hour IV infusion, in a GLP 28-day toxicity and toxicokinetic study in cynomolgus monkey including 28-day recovery and safety pharmacology assessment
  - No impact of AFVT-2101 on bodyweight, clinical observations, ophthalmology, ECG measurements, respiratory parameters, clinical pathology, organ weights, or macroscopic observations were reported
  - No adverse AFVT-2101 related histopathological findings were reported
  - No significant impact on cytokines or chemokines and no AFVT-2101 related changes in absolute counts or relative percentages of immune cell populations in peripheral circulation were observed
  - No impact of treatment-emergent ADA on the safety, tolerability, or toxicokinetic profile of AFVT-2101 was observed

AFVT-2101 *in vitro* and *in vivo* Cytokine Release Profile

***in vitro* Human PBMC cultures with or without target cells + 100 µg/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 100 µg/mL AFVT-2101
- OVCAR-3 & A-549 are FRα positive cell lines (high & low target expression 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**
- **Good concordance between *in vitro* cytokine release profile and that seen in NHPs administered AFVT-2101 weekly via IV infusion**
- **No red flags to indicate a risk for cytokine release syndrome in patients**

Cytokine Release in NHPs Following Repeated Weekly IV Infusion Administration of AFVT-2101

Mean NHP serum cytokine levels in 28-Day GLP Tox/TK study
- Pre-screening
- Prior to first dose on Day 1
  - 2 & 24h after first dose
- Prior to fifth dose on Day 29
  - 2 & 24h after fifth dose
- Transient increases in MCP-1 at ≥30 mg/kg
- No impact on safety or tolerability of AFVT-2101

* Below the limit of quantitation (<LLOQ)

Summary

- AFVT-2101 was well-tolerated in cynomolgus monkeys, the only pharmacologically relevant preclinical species, and possesses a pharmacokinetic and safety profile congruent with clinical development for the treatment of solid tumors
- 150 mg/kg/dose was determined to be the NOAEL in a 28-Day GLP repeat IV dose Tox/TK study in NHPs with 28-Day recovery period
- Following IV infusion administration, AFVT-2101 exhibited non-linear pharmacokinetics with supra-proportional increases in AUC relative to dose between 3 and 30 mg/kg and approximately dose-proportional increases in exposure at higher doses, suggesting target-mediated drug disposition (TMDD)
- This profile is consistent with the PK of AFVT-2101 being driven, at least in part, by the interaction with a high-affinity, high-specificity, relatively low-capacity pharmacological target; i.e., innate immune cells through CD16A engagement
- There was no observed impact of treatment-emergent ADA on the safety or pharmacokinetics of AFVT-2101
- AFVT-2101 is engineered to exert potent and selective immune-mediated antitumor activity with minimal off-tumor toxicity and an exceptional preclinical safety profile
- AFVT-2101 is expected to enter Phase I clinical trials in 2023

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Abbreviations
FRα: folate receptor alpha; **CD16A**: FcγRIIIA receptor; **ICE**: innate cell engager; **NHP**: nonhuman primate; **PK**: pharmacokinetic; **TK**: toxicokinetic; **DRF**: dose range finding; **TMDD**: target-mediated drug disposition

References
1. 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
2. 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