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Clinical Evidence of Safety, Prolonged Half-life, and Low Immunogenicity of Monoclonal Antibodies TAVO101 and TAVO103 Engineered with Multiple Fc Mutations

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PURPOSE

Engineering functional mutations in the Fc region of antibody-based therapeutics can enhance safety, extend circulation half-life, and improve *in vitro* and *in vivo* functionalities. While clinical data support half-life extension and modulation of the Fc effector functions, there is limited information regarding molecules incorporating multiple Fc functional mutations. Additionally, no clinical safety and immunogenicity data have been reported for a protease resistance mutation in the human IgG1 antibody hinge area to improve stability in protease-rich disease conditions found in inflammatory tissues. Moreover, protein engineers and clinicians seek more clinical data on fit-for-purpose antibodies with multiple functional mutations in the Fc region. We present the clinical profiles of TAVO101 and TAVO103, human IgG1-based anti-TSLP and anti-IL-1β antibodies (mAbs), respectively. These two mAbs were engineered to extend their half-life and reduce Fc effector functions. In addition, TAVO103 has the protease-resistance modifications. Both molecules were evaluated *in vitro* and in clinical trials for safety, pharmacokinetics (PK), and immunogenicity.

OBJECTIVES

- To characterize TAVO101, anti-TSLP, and TAVO103, anti-IL-1β, antibodies *in vitro* for engineered functional mutations
- To test TAVO101 and TAVO103 in clinical settings for safety, tolerability, PK, and immunogenicity
- To analyze and summarize clinical results for guiding future clinical studies

METHOD(S)

Protein construct and generation:

TAVO101 and TAVO103 were engineered with M428L/N434S (LS) mutations to extend their half-life, and complementary L234A/L235A (LALA) mutations to silence Fc effector functions. TAVO103 was also engineered with an E233P mutation and a G236 deletion to improve hinge region protease resistance. Both molecules were produced for clinical trials using CHO cell expression systems and typical human mAb downstream processes.

***In vitro* testing of the antibodies**:

Binding affinities to FcRn at pH 6.0 were assessed using ELISA-based assays.

Binding to FcγRI (CD64), FcγRIIIA (CD16a), and C1q were evaluated on plates coated with recombinant human FcγRI, FcγRIIIA, or C1q proteins.

The stability against IgG protease IdeZ was tested at 37°C and analyzed using SDS-PAGE.

Phase 1 clinical study:

First-in-human, double-blinded, single-dose escalation Phase 1 clinical studies were conducted in healthy volunteers in Australia (for TAVO101) and the US (for TAVO103) to assess safety, tolerability, PK profiles, and immunogenicity.

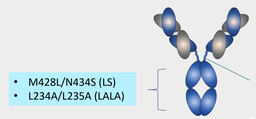
Subjects received a single intravenous administration of the study drug or placebo, followed by a 195-day safety and PK assessment.

Drug concentrations in serum samples were measured using validated and specific ELISA-based assays. Anti-drug antibodies (ADA) were analyzed using screening and specificity assays, and the ADA titer in the confirmed serum sample was measured.

Treatment-emergent adverse events (TEAE) were coded using the System Organ Class (SOC) and Preferred Term (PT). Individual PK profiles were fitted into a two-compartment population model.

RESULT(S)

Functional Fc mutations

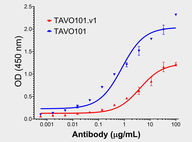


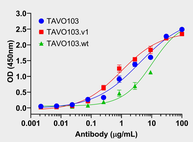
TAVO101 – anti TSLP
TAVO101: IgG1, LALA, LS
TAVO101.v1: LALA only

TAVO103 – anti IL-1β
TAVO103: IgG1, LALA, LS, EP/Gdel
TAVO103.v1: without EP/Gdel
TAVO103.wt: IgG1 wild type

In vitro results

Figure 1. TAVO101's FcRn binding at pH6.0 was enhanced





TAVO101 (left) was compared to an isotype mAb with LALA mutation; TAVO103 (right) was compared with an isotype mAb without EP/Gdel mutation and a wild-type mAb.

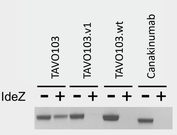


Figure 2. The EP/Gdel mutation improved the stability of TAVO103 in the presence of IdeZ protease.

Figure 3. The LALA mutation decreased FcγRI (CD64), FcγRIIIA (CD16), and C1q binding potencies of TAVO101

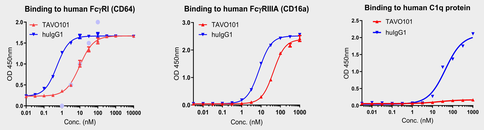
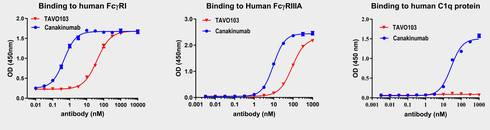


Figure 4. The LALA mutation decreased FcγRI (CD64), FcγRIIIA (CD16), and C1q binding of TAVO103



* "Canakinumab" is a canakinumab analogue made for comparison

Clinical Safety Profile

TAVO101 Ph1 trial had 7 dose escalation cohorts (n=6 per cohort, 4:2 active to placebo) from 0.01 to 10 mg/kg. TAVO101 was safe and well tolerated with all TEAEs being mild to moderate. The Ph1 trial of 27 TAVO101-treated healthy subjects showed possible drug-related AEs such as laryngitis (1), throat irritation (1), headache (1), thrombocytopenia (1), fatigue (1), dysgeusia (1), and neutropenia (1).

TAVO103 Ph 1 trial had 5 dose escalation cohorts (n=6, 4:2 active to placebo) from 0.1 to 10 mg/kg. TAVO103 was safe and well tolerated, with all TEAEs being mild to moderate. The Ph1 trial of 20 TAVO103-treated healthy subjects showed possible drug-related AEs that included lightheadedness (1), headache (2), and nausea (1).

The severity and number of events in the treated groups of both study drugs were comparable to those in the pooled placebos, and no dose-correlation trends.

Immunogenicity

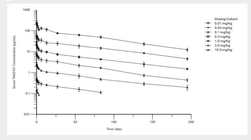
All 27 treated subjects tested negative (-) for anti-TAVO101 antibodies

One of 20 (1/20) treated subjects tested positive (+) for anti-TAVO103 antibodies with a low titer. All other treated subjects tested negative (-).

Clinical Pharmacokinetics

TAVO101 – anti-TSLP antibody

Figure 5. Mean serum concentrations of TAVO101



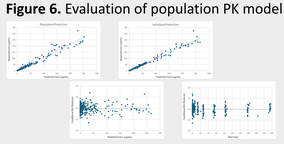
PK parameters of TAVO101

Parameter (Unit)	Statistic	TAVO101						
		0.01 mg/kg (N=1)	0.03 mg/kg (N=4)	0.1 mg/kg (N=4)	0.3 mg/kg (N=4)	1 mg/kg (N=4)	3 mg/kg (N=4)	10 mg/kg (N=4)	Overall (N=25)
C_{max} (μg/mL)	GM	0.152	0.496	2.56	7.97	29.3	58.6	251	NC
	CV%	NA	15	21.8	13.7	37.7	17.1	21.6	NC
AUC_{inf} (d·μg/mL)	GM	NA	26.9	126	359	1057	3271	10683	NC
	CV%	NA	14.4	15.1	14.4	5.5	11.6	11.8	NC
CL (L/d)	GM	NA	0.0898	0.0691	0.0682	0.0677	0.0624	0.0698	0.0706
	CV%	NA	3.3	14.4	17.5	16	10.4	20	17.4
V_d (L)	GM	NA	8.27	7.56	5.73	7.21	5.91	6.02	6.72
	CV%	NA	8.6	10.6	18.5	22.6	18	13.7	20.2
$t_{1/2}$ (day)	Median	NA	65.3	74.5	58.6	73.9	67.1	58.1	67.4
	Min	NA	55.5	67.9	54.6	66.8	58.9	55.6	54.6
	Max	NA	70.5	88.5	61.6	81.8	70	68.1	88.5

Population PK parameters of TAVO101

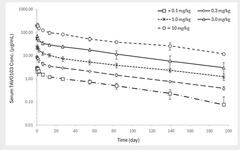
Parameter	Mean	%RSE	STD
Clt (mL/d)	69.7	6.18	--
Vc (mL)	3610	13.5	--
Cld (mL/d)	945	22.1	--
Vp (mL)	2940	8.03	--
$t_{1/2}$ β (day)	66.2	--	15.8

Figure 6. Evaluation of population PK model



TAVO103 – anti-IL-1β antibody

Figure 7. Mean serum concentrations of TAVO103



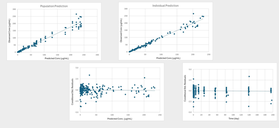
PK parameters of TAVO103

Parameter (Unit)	Statistic	TAVO103					
		0.1 mg/kg (N = 4)	0.3 mg/kg (N = 4)	1.0 mg/kg (N = 4)	3.0 mg/kg (N = 4)	10 mg/kg (N = 4)	Overall (N = 20)
C_{max} (μg/mL)	GM	2.99	9.05	24.3	69.3	228	NA
	CV%	14.9	8.29	20.2	12.5	14.6	NA
AUC_{inf} (d*μg/mL)	GM	112.9	347.6	966.7	2722	9933	NA
	CV%	25.1	5.9	22.4	28.1	8.63	NA
CL (L/d)	GM	0.071	0.066	0.076	0.073	0.068	0.071
	CV%	27.1	17.2	14.4	35.1	15.3	21.8
V_d (L)	GM	5.27	5.51	7.29	5.11	6.26	5.48
	CV%	9.27	15.8	15.1	31.7	20.8	22.0
$t_{1/2}$ (day)	Median	56.6	59.8	65.7	63.4	63.5	63.0
	Min	35.0	49.2	64.2	20.4	57.9	20.4
	Max	63.2	62.8	71.3	65.4	69.4	71.3

Population PK parameters of TAVO103

Parameter	Mean	%RSE	STD
Clt (mL/d)	70.6	15.6	--
Vc (mL)	3030	10.1	--
Cld (mL/d)	433	28.9	--
Vp (mL)	2480	28.7	--
$t_{1/2}$ β (day)	56.8	--	15.2

Figure 8. Evaluation of population PK model



CONCLUSION(S)

1. The results from the studies provide clinical evidence of the safety of TAVO101 and TAVO103, antibodies that incorporate multiple functional mutations for: silencing Fc effector functions, hinge region protease resistance, and half-life extension.
2. The LS mutations of TAVO101 and TAVO103, in combination with other functional mutations, were effective in extending half-life in humans.
3. The LALA + LS mutations for TAVO101 and LALA + LS + E233P/G236 del mutations for TAVO103 did not increase the risk of immunogenicity in humans.

Ongoing Study

- TAVO101 is being studied in a Phase 2a clinical study in Atopic Dermatitis patients in Australia
- Four dose cohorts were designed based on modeling and simulation results using a population pharmacokinetic model developed from healthy subject data.

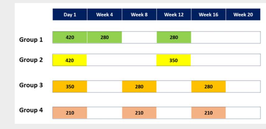
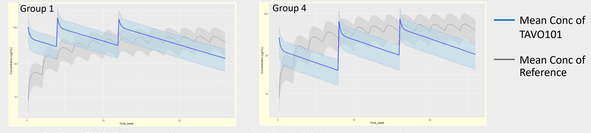


Figure 9. Simulated drug exposures



Predicted TAVO101 concentrations were compared with the clinical exposures of a reference drug

- Preliminary results showed that TAVO101 PK in atopic dermatitis patients were consistent with the model-predicted values.
- TAVO101 exhibited preliminary efficacy signals in all four dose groups.

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