2.6.2 PHARMACOLOGY WRITTEN SUMMARY

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Abbreviation	Definition
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
ANOVA	Analysis of variance
AUC	Area under the curve
C1q	Complement component 1q
CI	Confidence interval
EC_{50}	Half-maximal effective concentration
ELISA	Enzyme-linked immunosorbent assay
Fc	Fragment crystallizable
FcγRI	Fc-gamma receptor I
GLP	Good laboratory practice
HEK	Human embryonic kidney
HuIgG1	Human immunoglobulin G1
IC_{50}	Half-maximal inhibitory concentration
IgG1	Immunoglobulin G1
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-6	Interleukin-6
IL-18	Interleukin-18
mAb	Monoclonal antibody
MRC-5	Medical Research Council-cell strain 5
NFκB	Nuclear factor κB
NIH3T3	Immortalized mouse embryonic fibroblast cell line
SEAP	Secreted embryonic alkaline phosphatase

2.6.2.1. SUMMARY

TAVO103A is a humanized IgG1 monoclonal antibody (mAb) that binds specifically to interleukin-1 beta (IL-1β). IL-1β is a cytokine produced by many cell types and mediates immune responses during infection and inflammation. By binding to IL-1β, TAVO103A prevents IL-1β interaction with its receptor and inhibits multiple downstream inflammatory pathways. TAVO103A is being developed by Tavotek for treatment of IL-1β mediated inflammatory diseases.

TAVO103A is composed of 2 heavy chains and 2 light chains (kappa) which are stabilized by multiple disulfide bonds. The constant region of each heavy chain has a single N-linked glycan site. TAVO103A uses a humanized anti-IgG1 framework with mutations (L234A, L235A, M428L, N434S, E233P and G236 deletion) in the heavy chains to increase safety, half-life and stability of the antibody. It is produced by fermentation in mammalian Chinese hamster ovary M cell suspension culture. The theoretical deglycosylated molecular weight of TAVO103A is 146558.6 Daltons (Da).

Multiple, nonclinical studies were conducted to elucidate the binding specificity, efficacy and safety of TAVO103A. "Canakinumab analogue" (manufactured at Tavotek from published sequence amino acid sequences of Novartis' canakinumab) is a human IgG1 mAb that was used as a comparator anti- IL-1β antibody molecule.

A human IL-1β binding study showed concentration-dependent binding of TAVO103A and canakinumab analogue to human IL-1β by enzyme-linked immunosorbent assay (ELISA). The half-maximal effective concentration (EC_{50}) value for human IL-1β binding with TAVO103A was determined to be comparable with canakinumab analogue. In another study to assess the inhibition of IL-1β binding to its receptor, the IC_{50} in transfected human embryonic kidney (HEK)293T cells showed comparable results for TAVO103A and canakinumab analogue. In a later study that assessed inhibition of IL-1β induced IL-6 release from MRC-5 cells, the TAVO103A IC_{50} was again comparable to that of canakinumab analogue.

Binding specificity and species cross-reactivity studies of TAVO103A and canakinumab analogue were compared by ELISA. TAVO103A and canakinumab analogue did not bind to other members of the interleukin-1 family, including interleukin-1 alpha (IL-1α), and to IL-18 which are structurally similar to IL-1β.

TAVO103A showed strong binding to monkey IL-1β (rhesus and cynomolgus monkeys have identical sequences), but undetectable binding to mouse and rat IL-1β. The EC_{50} values for binding of TAVO103A to IL-1β of monkey and human were similar. The IC_{50} values for the inhibition of monkey and human IL-1β binding to its receptor in transfected HEK293T cells also were similar. Based on these data, the cynomolgus monkey was chosen as a relevant species for in vivo toxicity testing and no toxicity studies were performed in rodents.

In vivo efficacy of TAVO103A compared to canakinumab analogue was evaluated in a mouse model of knee joint inflammation. Since human IL-1β can activate receptors in mice to induce inflammation, joint inflammation was induced upon continuous secretion of human IL-1β from transfected murine fibroblast NIH3T3 cells injected into the mouse knee joint. Evaluation of

treatment efficacy was based on increased right knee diameter (injected with human IL-1β secreting NIH3T3 cells) normalized by left knee diameter (injected with NIH3T3 null cells, as well as area under the curve (AUC) for increase in (normalized) right knee diameter over time. Treatment with 10 mg/kg TAVO103A significantly suppressed knee joint inflammation relative to the control over the four days following injection of 3T3- IL-1β cells. Mean AUC for the 10 mg/kg TAVO103A group was 57% lower than the control group, and the difference was significant.

In a similar mouse knee joint inflammation study, TAVO103A and canakinumab analogue were administered at a wide range of doses (0.3, 1, 3 and 10 mg/kg). Treatment of TAVO103A (3 or 10 mg/kg) or canakinumab analogue (1, 3 or 10 mg/kg) resulted in significant reductions in overall increase in right knee diameter compared with the control treatment. Treatment with TAVO103A (0.3 or 1 mg/kg) and canakinumab analogue (0.3 mg/kg) did not show statistically significant reductions, however both antibody treatments showed statistically similar AUC reductions at doses of 3 and 10 mg/kg. These studies suggest that TAVO103A is comparable to canakinumab analogue and that TAVO103A may offer promising treatment of IL-1β related inflammatory conditions.

Safety of TAVO103A compared to canakinumab analogue was assessed by measuring ELISA based binding activity to recombinant human proteins: CD16A, CD64, and C1q. Canakinumab analogue and human IgG1 (HuIgG1) were included as comparator molecules in these assays.

Canakinumab analogue and HuIgG1 showed strong binding to recombinant human CD16A, whereas TAVO103A showed moderate and weak binding, respectively. Canakinumab analogue, and huIgG1 showed strong binding to human CD64, whereas TAVO103A showed weak binding. Canakinumab analogue and HuIgG1 showed moderate binding to recombinant human C1q protein, whereas TAVO103A showed minimal binding. In general, TAVO103A shows reduced binding to recombinant human CD16A, CD64, and C1q proteins compared to HuIgG1 control and canakinumab analogue; suggesting potentially lower levels of Fc-mediated effector function for TAVO103A.

Overall, these in vitro binding, specificity and in vivo efficacy studies demonstrate that TAVO103A has comparable and potentially more favorable preclinical profiles than canakinumab analogue.

Currently, three anti-IL-1 related biologic drugs have been approved in the US: Rilonacept (ARCALYST®, a dimeric fusion protein consisting of extracellular portion of IL-R1 and IL-1 receptor accessory protein), Anakinra (KINERET®, a modified form of human interleukin 1 receptor antagonist), and canakinumab (ILARIS®, an anti-IL-1β mAb). Among them, only canakinumab is IL-1β specific. TAVO103A is comparable to canakinumab analogue in binding and functional activity.

2.6.2.2. PRIMARY PHARMACODYNAMICS

2.6.2.2.1. Binding and Specificity of TAVO103A to Human IL-1β

Binding of TAVO103A to human IL-1β was compared with canakinumab analogue using enzyme-linked immunosorbent assay (ELISA) (Study TAVO103A-0002.A). Each antibody demonstrated concentration-dependent binding to human IL-1β. However, TAVO103A showed comparable binding to human IL-1β than canakinumab analogue with relative EC_{50} values of 19.85 ng/mL [95% confidence interval (CI): 18.01-21.88 ng/mL] for TAVO103A and 85.55 ng/mL [95% CI: 77.27-94.93 ng/mL] for canakinumab analogue.

The specificity of TAVO103A compared to canakinumab analogue was investigated for binding to human IL-1α and human IL18, since these molecules share amino acid sequence identity and similarity with IL-1β. Neither TAVO103A nor canakinumab analogue demonstrated binding to either human IL-1α or IL-18 by ELISA.

2.6.2.2.2. Cross-Reactivity with IL-1β in Other Species

The binding specificity of TAVO103A for mouse, rat, and rhesus/cynomolgus monkey IL-1β proteins were evaluated using ELISA (Study TAVO103A-0002.B). The amino acid sequence of rhesus monkey IL-1β is 100% identical to cynomolgus monkey IL-1β. Hereafter, we reference rhesus monkey IL-1β data as monkey IL-1β data.

TAVO103A at a single concentration of 10 µg/mL showed strong binding to monkey IL-1β compared to canakinumab analogue at the same concentration. Binding of TAVO103A or canakinumab analogue to mouse and rat IL-1β was not detected at the concentrations tested.

2.6.2.2.3. Neutralizing Activity in IL-1β-Mediated Reporter Assays

The neutralizing ability of TAVO103A and related antibodies on human, monkey, and mouse IL-1β was tested in the HEK Blue™ IL-1β cell reporter assay (Study TAVO103A-0003). Binding of IL-1 to its receptor on the surface of HEK Blue™ IL-1β cells trigger a signaling cascade leading to the activation of NFκB /AP-1 and subsequent production of Secreted Embryonic Alkaline Phosphatase protein (SEAP). SEAP levels are monitored using QUANTI-Blue™ Solution.

Various concentrations of TAVO103A or canakinumab analogue were applied to HEK Blue™ IL-1β cells along with 1 ng/mL human IL-1β. After overnight incubation, IL-1β induced reporter gene expression was quantitated. Results showed that TAVO103A was able to neutralize human IL-1β with an IC_{50} =11.29 ng/mL [95% CI: 9.91-12.86 ng/mL], which is comparable with that of canakinumab analogue with an IC_{50} =32.00 ng/mL [95% CI: 27.12-37.74 ng/mL].

In a similar experiment, various concentrations of TAVO103A or canakinumab analogue were applied to HEK Blue™ IL-1β cells along with 1 ng/mL monkey IL-1β. Quantitation of overnight IL-1β driven reporter gene expression showed that TAVO103A and canakinumab analogue were able to neutralize monkey IL-1β. TAVO103A showed an IC_{50}=4.74 ng/mL [95% CI: 3.90-5.69

ng/mL] whereas canakinumab analogue showed a neutralizing effect mainly at high concentrations (IC_{50} >2800 ng/mL).

Various concentrations of TAVO103A or canakinumab analogue were applied to HEK Blue™ IL-1β cell along with 100 ng/mL mouse IL-1β. After overnight incubation, IL-1β driven reporter gene expression was quantitated. Results showed neither canakinumab analogue nor TAVO103A neutralized mouse IL-1β.

2.6.2.2.4. Neutralizing Activity in IL-1β-Mediated Cytokine Release Assays

The abilities of TAVO103A and canakinumab analogue to block the functional effects of human, monkey, and mouse IL-1β were evaluated in the Medical Research Council-cell strain 5 (MRC-5) cell-based cytokine release assay (Study TAVO103A-0003). The objective of this assay was to measure IL-6 production upon IL-1RI stimulation by IL-1β. The level of IL-6 production was quantified using an IL-6 ELISA.

Various concentrations of TAVO103A or canakinumab analogue were applied to MRC-5 cells along with 1 ng/mL human IL-1β. After overnight incubation, TAVO103A neutralized human IL-1β driven IL-6 release from the MRC-5 cells with an IC_{50} = 69.8 ng/mL [95% CI: 55.91-87.53 ng/mL] which was comparable with canakinumab analogue with an IC_{50}=234.7 ng/mL [95% CI: 148.5-401.9 ng/mL].

Various concentrations of TAVO103A or canakinumab analogue were applied to MRC-5 cells along with 1 ng/mL monkey IL-1β. After overnight incubation, TAVO103A neutralized monkey IL-1β driven IL-6 release from MRC-5 cells with an IC_{50} =32.2 ng/mL [95% CI: 27.7-37.5 ng/mL], whereas canakinumab analogue did not neutralize monkey IL-1β activity in this assay.

Various concentrations of TAVO103A or canakinumab analogue were applied to MRC-5 cells along with 10 ng/mL mouse IL-1β. After overnight incubation, neither TAVO103A nor canakinumab analogue was able to neutralize mouse IL-1β driven IL-6 release from MRC-5 cells.

2.6.2.2.5. In Vivo Potency of TAVO103A in Neutralizing IL-1β Activities

A mouse model of knee joint inflammation was used to evaluate the *in vivo* efficacy of TAVO103A (Study TAVO103A-0004.A, TAVO103A-0004.B). Since human IL-1β can activate receptors in mice to induce inflammation, joint inflammation in this model was induced due to continuous secretion of human IL-1β from transfected murine fibroblast NIH3T3 cells injected into the mouse knee joint (**Alten 2008**).

DBA-1 mice were dosed by intraperitoneal route with TAVO103A at 1 and 10 mg/kg or with control IgG at 10 mg/kg. Two hours post dose, mice received an intra-articular injection of inflammatory cells (10 x 10^4 3T3-IL-1β) into the right knee and an equivalent number of control cells (10 x 10^4 3T3-null cells) into the left knee. Calliper measurements of right and left knee diameters were made pre-injection and at 24-, 48-, 72- and 96-hours post-injection. The difference between right and left knee diameters was calculated for each animal to determine the

absolute increase in right knee diameter. Evaluation of efficacy was based on increased right knee diameter as well as area under the curve (AUC) for increase in right knee diameter over time.

Treatment with 10 mg/kg TAVO103A significantly suppressed knee joint inflammation relative to the control over the four days following injection of 3T3-IL-1β cells. On Days 1 and 2 post injection, the right-to-left knee differences were significantly lower in the TAVO103A treated group with p values below 0.05 and 0.01, respectively. On Day 3, the difference for 1 mg/kg dose reached statistical significance with $p < 0.05$. Mean AUC for the 10 mg/kg TAVO103A group (0.628 mm*day) was 57% lower than the control group (1.06 mm*day) and this difference was significant (p=0.027) (**Figure 1**). AUC difference for the 1 mg/kg cohort did not result in statistically-significant reductions.

Figure 1. Effect of TAVO103A Treatment on the Right (Affected) - Left (Control) Knee Diameter Difference

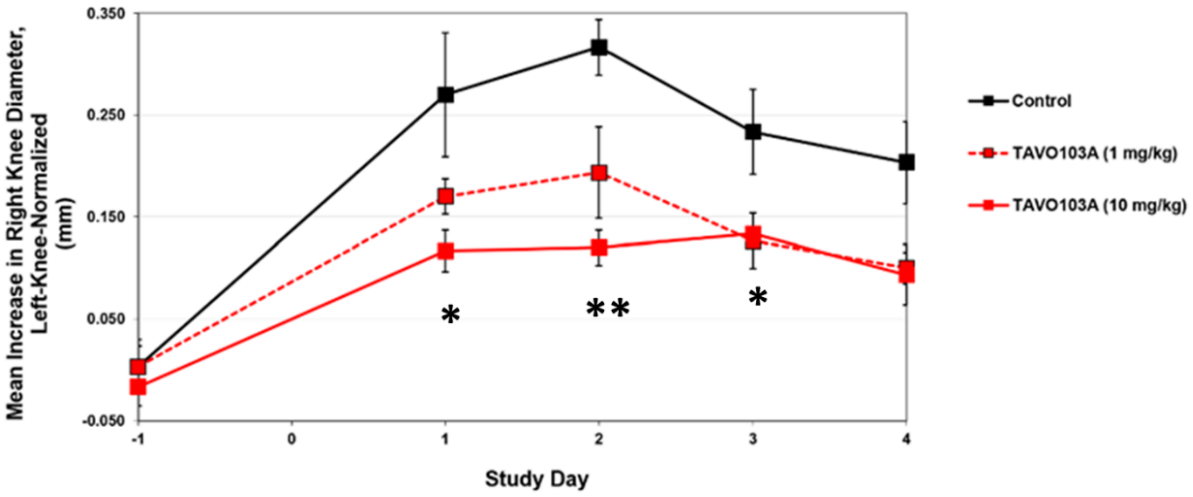


Figure 1 shows the relative right-left-knee diameter difference increase on every study day (x axis) in male DBA-1 mice administered with TAVO103A (1 or 10 mg/kg) or control substance. The values shown are means (n=3) with standard error. The y axis shows the mean difference between the diameter of the right and the left knee diameters. On Day -1, baseline measurements were taken. On Day 0, animals were given a single dose of TAVO103A (1 mg/kg), TAVO103A (10 mg/kg), or Control (10 mg/kg) followed by an intra-articular injection (20 µL) of inflammatory cells (10×10^4 3T3-IL-1β cells) into the right knee and an intra-articular injection of control cells (10×10^4 3T3-null cells) into the left knee. *p<0.05, **p<0.01; one-way ANOVA with a Dunnett's post-hoc analysis.

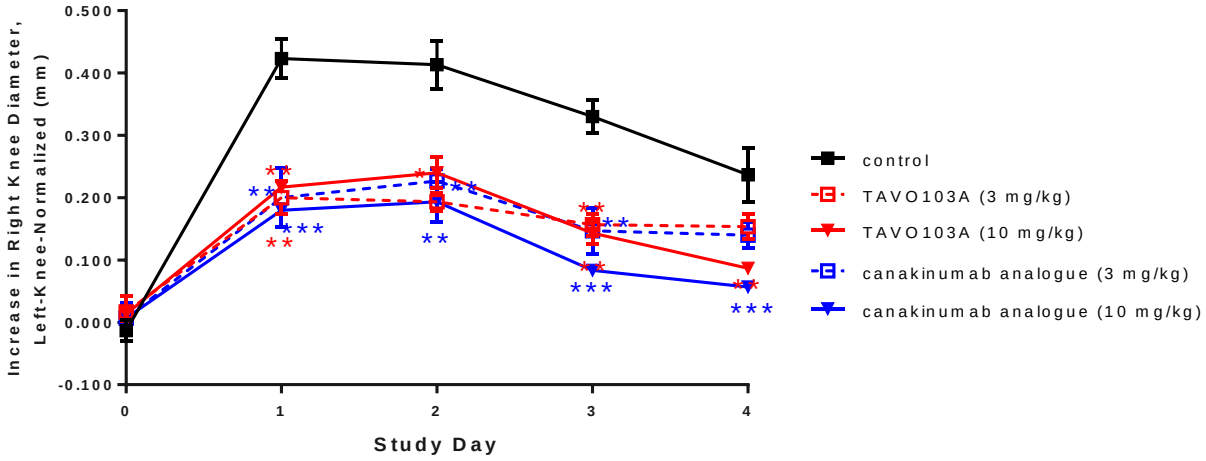
Source: Nonclinical Study Report TAVO103A-0004.A

In a similar study, TAVO103A and the canakinumab analogue were tested for their abilities to reduce knee joint inflammation over a wide range of doses (0.3, 1, 3 and 10 mg/kg).

Dose-dependent responses to TAVO103A and canakinumab analogue were observed in the DBA/1 mouse model of IL-1β-induced knee inflammation. Right-left knee differences were

significantly lower in the TAVO103A group at 3 and 10 mg/kg compared to control on 3 of 4 days of the study. The canakinumab analogue displayed a similar profile (**Figure 2**). Treatment of TAVO103A (3 or 10 mg/kg) or canakinumab analogue (1, 3 or 10 mg/kg) resulted in significant reductions in overall increase in right knee diameter compared with control over the four days following injection of 3T3-IL-1β cells. Treatment with TAVO103A (0.3 or 1 mg/kg) and canakinumab analogue (0.3 mg/kg) did not result in statistically significant reductions. TAVO103A and canakinumab analogue resulted in statistically similar AUC reductions at doses of 3 and 10 mg/kg (**Figure 3**).

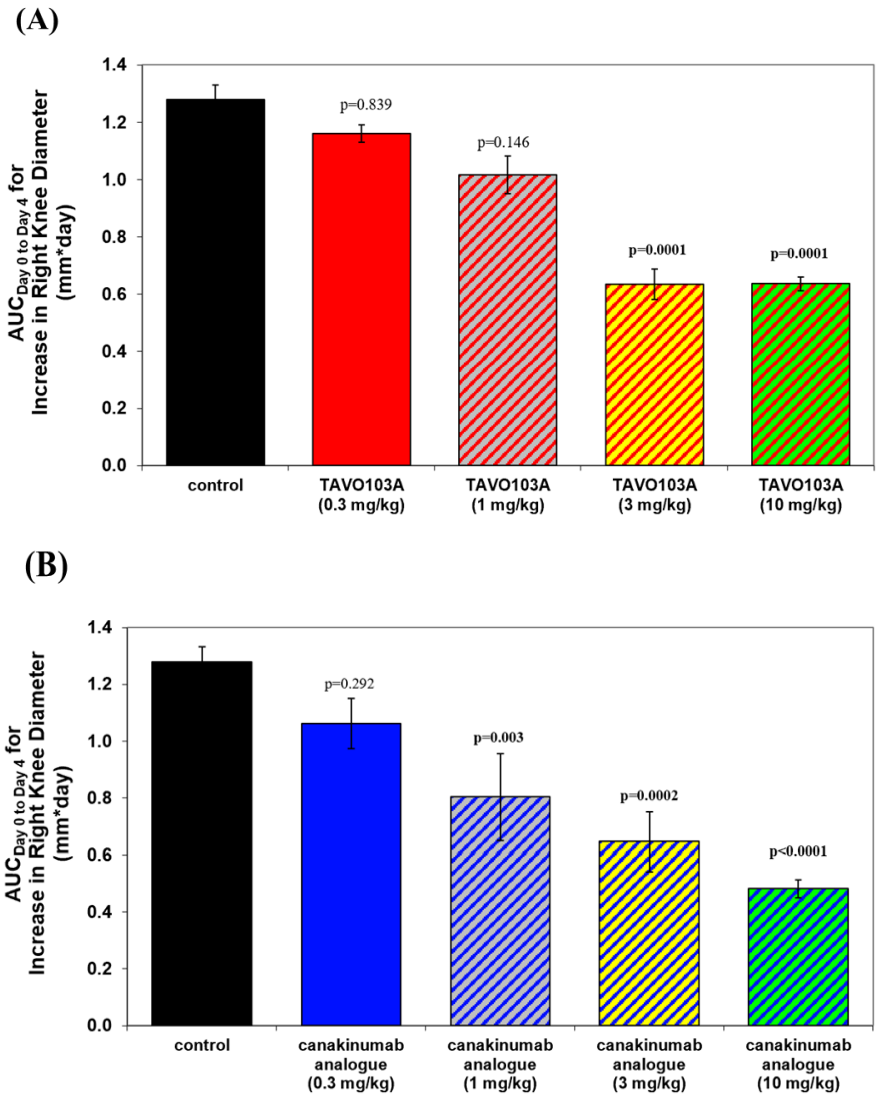
Figure 2. Normalized Increase in Right Knee Diameter Over Time in Mice Administered TAVO103A or Canakinumab analogue



Each value represents a mean of differences between the diameter of the right knee (treated, n=3) and the left knee (control, n=3) calculated for each animal at every time point. Error bars represent means with the standard error. On Day 0, baseline measurements were taken. On Day 1, animals were given a single dose of TAVO103A (3 or 10 mg/kg), canakinumab analogue (3 or 10 mg/kg), or control (10 mg/kg) followed by an intra-articular injection (20 µL) of inflammatory cells (10×10^4 3T3 IL-1β cells) into the right knee and an intra-articular injection of null control cells (10×10^4 3T3 IL-1β null cells) into the left knee.

Source: Nonclinical Study Report TAVO103A-0004.B

Figure 3 $AUC_{Day0\ to\ Day4}$ for Increase in Right Knee Diameter in Male DBA/1 Mice Administered TAVO103A (A) or Canakinumab analogue (B)



ANOVA=analysis of variance; AUC=area under the curve.

Notes: **Figure 3** presents $AUC_{Day\ 0\ to\ Day\ 4}$ values calculated for TAVO103A (A) and canakinumab analogue (B) treatments. AUC values (mm•day) were calculated using the dose-response curves for each treatment for the total treatment period (Day 0 through Day 4). AUC values of the dose-response curve were differences of mean values of the right and left knee diameters at each time point. Values on this graph represent means with the standard error in each group (n=3). Control group of animals received injection of inactivated anti- IL-1β antibody instead of TAVO103A (0.3, 1, 3, or 10 mg/kg) or canakinumab analogue (0.3, 1, 3, or 10 mg/kg). Statistical differences presented on the graph were calculated using one-way ANOVA with a Dunnett's post-hoc analysis.

Source: Nonclinical Study Report TAVO103A-0004.B

2.6.2.3. SECONDARY PHARMACODYNAMICS

2.6.2.3.1. Fc Mediated Effector Function

The binding activities of TAVO103A and comparators/controls to recombinant human CD16A, CD64, and C1q proteins were evaluated by an ELISA method (Study TAVO103A-TX-0001). Both CD16A and CD64 are Fcγ receptors responsible for the immunoglobulin Fc-mediated effector functions in antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP). The complement component 1q (C1q) protein is the subunit of the C1 enzyme complex that activates the serum complement system. The C1q protein complex is responsible for the binding of the Fc domain of immunoglobulins that results in the activation of the classical complement pathway.

The recombinant human CD16A ELISA assay demonstrated that the canakinumab analogue, which is an IgG1 molecule, and the control HuIgG1 displayed strong binding to recombinant human CD16A protein, with EC_{50} values of 9.770×10^{-9} M [95% CI: 9.161-10.42×10^{-9} M] and 7.987×10^{-9} M [95% CI: 7.623-8.368×10^{-9} M], respectively. TAVO103A had moderate binding to recombinant human CD16A, with an EC_{50} value of 8.794×10^{-8} M [95% CI: 8.271-9.349×10^{-8} M].

The recombinant human CD64 ELISA assay demonstrated that canakinumab analogue and HuIgG1 displayed strong binding to recombinant human CD64 protein, with EC_{50} values of 4.575×10^{-10} M [95% CI: 3.920-5.340 × 10^{-10} M] and 5.234×10^{-10} M [95% CI: 4.636-5.909 × 10^{-10} M], respectively. TAVO103A had moderate binding to recombinant human CD64 with an EC_{50} value of 4.049×10^{-8} M [95% CI: 3.669-4.467 × 10^{-8} M].

TAVO103A and canakinumab analogue showed low levels of affinity to recombinant human C1q protein, while HuIgG1 displayed strong binding to recombinant human C1q protein, with higher maximum activity compared to other antibodies $EC_{50} = 4.299\times10^{-8}$ M [95% CI: 3.244-5.696 × 10^{-8} M]. Canakinumab analogue had moderate binding to recombinant human C1q with the EC_{50} value of 2.704×10^{-8} M [95% CI: 2.294-3.187 × 10^{-8} M]. .

Overall, TAVO103A exhibited lower affinities for recombinant human CD16A, CD64, and C1q proteins, than the control HuIgG1 and canakinumab analogue, suggesting potentially lower levels of Fc-mediated effector function. Details of this study are further elaborated in Toxicology Review in **Section 2.6.6**.

2.6.2.4. SAFETY PHARMACOLOGY

The effects of TAVO103A on cardiovascular, respiratory, and central nervous system function were evaluated in the pivotal repeat dose GLP study in cynomolgus monkeys (Study P21-S154-RD). Cynomolgus monkeys (2.5 to 5 years old) were randomly assigned to 4 groups, with 5/sex in each group, and administered TAVO103A at 0 (placebo control), 10, 30, and 100 mg/kg/week for 4 consecutive weeks (5 doses in total). The first dosing day was defined as Day 1; main study animals were euthanized and necropsied on Day 30; recovery animals were euthanized and necropsied on Day 57.

The first 4 animals/group/sex were jacketed for telemetric measurement of cardiovascular and respiratory function. Recordings were evaluated prior to the first dosing, and at 2, 6, 48, 96 and 144 hours after the end of dosing on Day 1 and Day 22. Parameters measured were heart rate, P R, R-R and Q-T intervals, QRS duration, ST voltage, respirations per minute and tidal volume. Corrected Q-T interval (QTc) was calculated using Bazett's formula. Blood pressure was obtained for all animals once prior to first dosing, 2 to 4 hours after the end of dosing on Days 1 and 22. In addition, Limb lead II ECG recording was obtained for all animals once prior to first dosing, 2 to 4 hours after the end of dosing on Days 1, 15 and 29, and prior to the end of recovery (Day 56); parameters measured and recorded were heart rate, electrocardiographic waves, P-R and Q-T interval, QRS duration, and QTc. Central nervous system function was evaluated by daily observation and weekly detailed clinical observations.

There were no TAVO103A-related effects on cardiovascular, respiratory, or central nervous system function. Details of this repeated dose GLP Study are further elaborated in the Toxicology Summary in **Section 2.6.6**.

2.6.2.5. PHARMACOKINETIC DRUG INTERACTIONS

Pharmacokinetic-based drug interactions are not relevant for monoclonal antibody therapeutics; therefore, no studies were conducted.

2.6.2.6. DISCUSSION AND CONCLUSIONS

IL-1β, a proinflammatory cytokine with diverse physiological functions and pathological significances, plays an important role in health and disease. TAVO103A is being developed by Tavotek for treatment of IL-1β mediated inflammatory diseases. Currently, three anti-IL-1 related biologic drugs have been approved in the US: Rilonacept (ARCALYST®, a dimeric fusion protein consisting of extracellular portion of IL-R1 and IL-1 receptor accessory protein), Anakinra (KINERET®, a modified form of human interleukin 1 receptor antagonist), and canakinumab (ILARIS®, an anti-IL-1β mAb). Among them, only canakinumab is IL-1β specific. TAVO103A is comparable to canakinumab analogue from the various non-clinical studies presented in this document.

TAVO103A is a human monoclonal IgG1 antibody which binds specifically to human IL-1β with high affinity to neutralize IL-1β mediated signaling and reduce inflammation in IL-1β mediated inflammatory diseases. TAVO103A has mutations: L234A, L235A, M428L, N434S, E233P and G236 deletion in the heavy chains to increase safety, half-life and stability for patient compliance and convenience.

Evidence from pharmacological studies determines that TAVO103A:

- Binds to human IL-1β with high affinity and high selectivity
- Neutralizes the biologic effects of human and monkey IL-1β in cell-based assays in vitro
- Reduces knee joint inflammation in a dose-dependent manner in an artificial mouse model of joint inflammation following injection of inflammatory 3T3-IL-1β cells
- Demonstrates relatively low binding to CD16A, CD64, and C1q, suggesting reduced Fc-mediated effector function
- Has no effects on cardiovascular, respiratory, or central nervous system function

These data confirm the potential of TAVO103A as a therapeutic agent in IL-1β mediated inflammatory diseases.

2.6.2.7. REFERENCES

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Alten R**,** Gram H, Joosten LA, van den Berg WB, Sieper J, Wassenberg S, Burmester G, van Riel P, et al. The human anti-IL-1 beta monoclonal antibody ACZ885 is effective in joint inflammation models in mice and in a proof-of-concept study in patients with rheumatoid arthritis. Arthritis Res Ther. 2008; 10(3): R67.