TST004, a Humanized IgG4 Anti-MASP2 Antibody, Demonstrates Potent In Vitro / In Vivo Inhibitory Activities on MASP2 Complement Pathway and Excellent Safety Profiles in Non-Human Primate (NHP)

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ABSTRACT

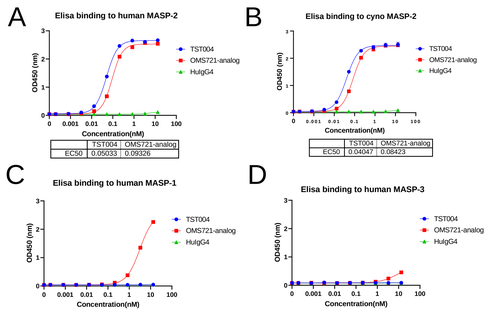
Introduction: MASP2, mannose-binding protein-associated serine protease 2, is a key enzyme in the lectin pathway initiation of complement activation. Studies have shown that lectin pathway activation contributes to multiple human diseases such as immunoglobulin A nephropathy (IgAN), hematopoietic stem-cell transplantation–associated thrombotic microangiopathy (HSCT-TMA). Therefore, inhibition of MASP2 might be a potential treatment approach for diseases related to lectin pathway activation. TST004 is a humanized IgG4 anti-MASP2 antibody. Here we report the in vitro characterization of TST004, as well as in vivo pharmacokinetic (PK) and pharmacodynamic (PD) and safety profiles in cynomolgus monkeys.

Methods: Affinity, and binding specificity of TST004 were evaluated using Fortebio octet and ELISA method respectively. PD assays including C3/C4/MAC deposition were measured with HRP linked anti-C3c, anti-C4c, anti-C5b-9 antibodies. The complement pathway selectivity was determined by ELISA methods using HRP linked anti-C5b-9 antibody, in which mannan, IgM and LPS were used as initiators for the MBL pathway, classical pathway and alternative pathway respectively. The PK/PD and safety profiles of TST004 were assessed in a single dose cynomolgus monkey (cyno) PK/PD study, and single and repeated dose toxicity studies. The concentrations of TST004 in monkey serum were determined with an ELISA-based method. The PK parameters were estimated by noncompartmental analysis with Phoenix WinNonlin.

Results: Fortebio and ELISA analysis demonstrated that TST004 specifically bound to human and cyno MASP2, but not to rodent MASP2, with higher affinity compared to the reference molecule (Narsoplimab analog, a human Mab against MASP2). In vitro pharmacology assay showed TST004 had better inhibition of lectin pathway using human and cyno serum compared to the reference molecule. TST004 only blocked complement activation initialized from the lectin pathway, but not the other two complement pathways (classical and alternative). In the 10mg/kg single dose cyno PK/PD study TST004 showed inhibition of C4 activity up to 14 days, twice as long compared to the reference molecule. In the tox studies TST004 displayed a linear PK profile in cyno. TST004 was well tolerated in cyno following a single subcutaneous administration up to 500 mg/kg. In the repeated dose toxicity study, no test article-related adverse effects were observed following once weekly dosing up to 300 mg/kg for 4 weeks via subcutaneous injection, and no remarkable findings were noted in routine observations, clinical pathology, histopathology examination, injection site evaluation, as well as safety pharmacology assessment. Furthermore, a dose and time dependent reduction on serum C4c by TST004 was also demonstrated in the repeated dose toxicity studies.

Conclusions: TST004 demonstrated potent and selective in vitro and in vivo activities for the MASP2 dependent lectin complement pathway. TST004 displayed excellent tolerability and safety profiles in non-human primate (NHP). The sustained and dose-dependent reduction of serum C4c level upon TST004 dosing provides a PD signal for MASP2 dependent complement pathway inhibition. These data warrant further evaluation of the potential utility of TST004 in blocking MASP2 dependent diseases.

Specific Binding of TST004



	TST004	OMS721-analog
EC50	0.05033	0.09326

	TST004	OMS721-analog
EC50	0.04047	0.08423

Figure 1. The 96-well plates were coated with human (A), or cyno (B) MASP-2 protein, or with MASP-2 family members: human MASP-1 (C), human MASP-3 (D). Binding specificity of TST004 and OMS721 analog were evaluated by ELISA. TST004 had a higher binding to human and cyno MASP-2 compared to OMS721 analog, no binding to human MASP-1 and MASP-3. No binding to rodent MASP-2 (data not shown).

Binding Affinity of TST004

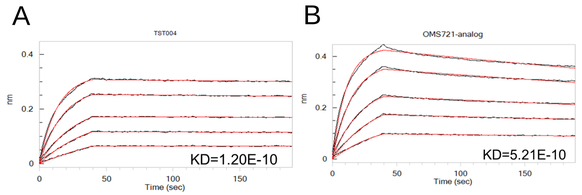
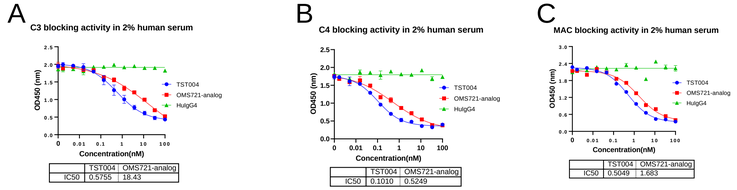


Figure 2. Affinities of TST004 (A) and OMS721 analog (B) were evaluated using Fortebio octet with a human MASP-2 protein loading method. All the kinetics data were analyzed by Octet data analysis software following global fit method. TST004 had a higher binding affinity to human MASP-2 compared with OMS721 analog. HulgG4 showed no binding (data not shown).

Lectin Pathway Inhibition of TST004



	TST004	OMS721-analog
IC50	0.5755	18.43

	TST004	OMS721-analog
IC50	0.1010	0.5249

	TST004	OMS721-analog
IC50	0.5049	1.683

Figure 3. The 96-well plates were coated with mannan which was used as an initiator for the Lectin pathway. TST004 and OMS721 analog were serially diluted with assay buffer containing 2% human serum. PD assays including C3 (A),C4 (B) and MAC (C) deposition were conducted with HRP linked anti-C3c, anti-C4c, anti-C5b-9 antibodies. TST004 showed better inhibition of lectin pathway mediated by MASP-2 in the presence of human serum.

Pathway Selectivity of TST004

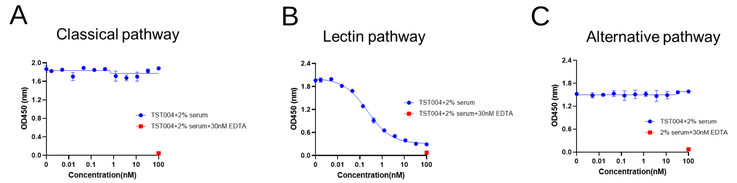


Figure 4. The complement pathway selectivity was determined by ELISA methods using HRP linked anti-C5b-9 antibody, in which mannan, IgM and LPS were used as initiators for the lectin pathway (B), classical pathway (A) and alternative pathway (C) respectively. TST004 only blocked complement activation initialized from the lectin pathway, but not the other two pathways, demonstrating that TST004 selectively blocks the Lectin pathway complement activation.

Cyno PK/PD Study of TST004 with Single Dose

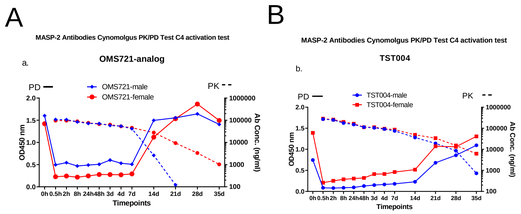


Figure 5. Two cynomolgus monkeys each group were administrated intravenously with 10 mg/kg TST004 or OMS721 analog. Serum samples were collected at 0, 0.5h, 2h, 8h, 24h, 48h, D3, D4, D7, D14, D21, D28, D35 post infusion. Antibody concentrations in serum (A) and the potency of lectin pathway activation (B) were tested. For the PK result, TST004 systemic exposure (AUC0-t) in cynomolgus was 40347.458 μg·h/mL, higher than that for OMS721 analog (17633.199 μg·h/mL). For the PD result, TST004 showed inhibition of C4 activity up to 14 days, twice as long compared to OMS721 analog.

Cyno PK/PD Study of TST004 with Repeated Dose

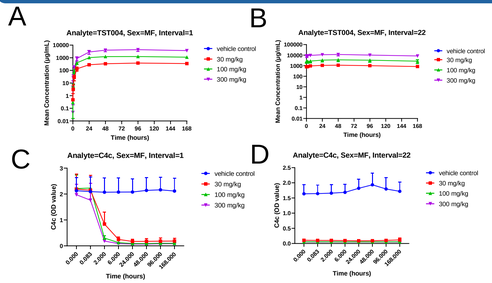


Figure 6. TST004 were administered once weekly via subcutaneous injection. Mean (+SD) concentrations (μg/mL) of TST004 from all monkeys on Days 1 and 22 pre-dose and at approximately 0.083 (5 min), 0.5, 2, 6, 24, 48, 96, and 168 (prior to next dose) hours post-dose were shown (A, B). TST004 caused decrease of C4c .

Safety Profiles of TST004 in NHP

Study Species	Administration Route	Dosing Duration	Dose Levels	GLP Compliance	Key Results
Monkey/Cynomolgus	Subcutaneous injection	Single-dose	250, 500 mg/kg	Yes	MTD is 500 mg/kg. Toxicokinetic: exposures were comparable between sexes and exhibited dose-proportional.
Monkey/Cynomolgus	Subcutaneous injection.	4 weeks (Once weekly for five doses)	30, 100, 300 mg/kg	Yes	Toxicity: no noteworthy test related safety issues. NOAEL is 300 mg/kg.

Table 1. Brief summary of in vivo toxicity studies in NHP for TST004.

CONCLUSIONS

- TST004, a humanized IgG4 anti-MASP2 antibody, binds to MASP2 selectively in high affinity and showed potent in vitro and in vivo blocking activities for the MASP2 dependent lectin complement pathway.
- TST004 displayed excellent tolerability and safety profiles in NHP in single and repeated dose toxicity studies following subcutaneous administration.
- These data warrants further evaluation of the clinical activity of TST004 for complement dependent disease in patients.

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