TST005, a bifunctional fusion protein of PD-L1/TGF-βRII, demonstrates potent anti-tumor activities with good safety profiles

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ABSTRACT

Not all patients with PD-L1 expression respond to the PD1/PD-L1 immunotherapy. A growing body of data demonstrated the pivotal roles of regulators from the tumor microenvironment (TME), such as transforming growth factor β (TGF-β), in the development of resistance to Immune checkpoint inhibitors. Therefore, co-inhibition of TGF-β signaling is expected to enhance the antitumor responses of PD1/PD-L1-based immunotherapies. TST005 is a bifunctional fusion protein composed of the truncated extracellular domain of the TGF-βRII receptor (a TGF-β trap) fused to a humanized anti-PD-L1 IgG1 antibody with ablated Fc immune effector function. Here we report TST005 anti-tumor activities in MC38 colorectal cancer and EMT-6 breast cancer models as compared to M7824 (Merck's PD-L1/TGF-βRII) analog, and its safety profiles following single or repeated doses in rats and non-human primates (NHP). TGF-β1 depletion from the plasma of the MC38 tumor–bearing mice was observed at the lowest dose (1mg/kg). At the higher dose (10mg/kg), TGF-β1 depletion persisted longer. The same trend was also found in the MC38 tumor tissue. In EMT-6 tumor model upon treatment (30mg/kg), TST005 showed a better tumor inhibition compared to M7824 analog, which correlates well with the extent of TGF-β1 depletion. For TST005, TGF-β1 depletion was persistent throughout all time points tested (up to Day 14) in plasma and tumor tissue compared to M7824 analog. TST005 treatment (3mg/kg, 10mg/kg) significantly lowered phosphorylated SMAD2 (pSMAD2) in the MC38 tumors. TST005 was well tolerated in both rats and monkeys, and no significant safety issues were observed following single or repeated doses in general toxicity studies. The exposure of TST005 in both species was comparable between two genders and increased in an approximately dose-proportional manner. No cytokine release was observed in monkeys. Based on the exposure of TST005 in monkeys and predicted exposure at First in Human (FIH) dose, the safety margin of TST005 could be higher than 200 and 5000 folds calculated on Cmax and AUC, respectively. In conclusion, we have demonstrated the antitumor activity of TST005 in PD1/PD-L1 sensitive and resistant tumor models as well as the safety profile in rat and NHP, a phase 1 clinical trial of TST005 in patients with advanced malignancies and have failed prior standard therapies is ongoing in USA (NCT04958434).

Efficacy of TST005 vs. M7824 Analog in Tumor Models

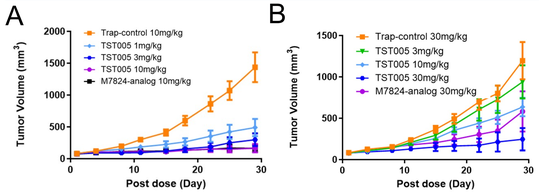


Figure 1. Tumor growth curve of TST005 and M7824 analog (mean± S.E.M, n=10). A. In the MC38/hPD-L1 colorectal tumor model, At 10mg/kg, TST005 showed similar tumor inhibition compared to M7824 analog. B. In the EMT6/hPD-L1 breast tumor model, a model with higher TGF-β expression, TST005 treatment with 30 mg/kg resulted better tumor inhibition compared to M7824 analog with the same dose.

TST005 Depletes TGF-β1 in MC38/hPD-L1 Tumor Model

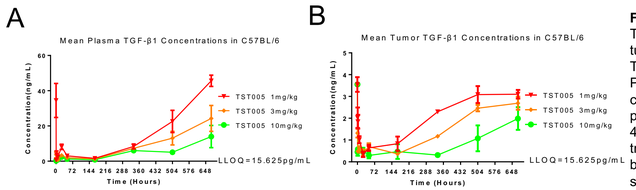
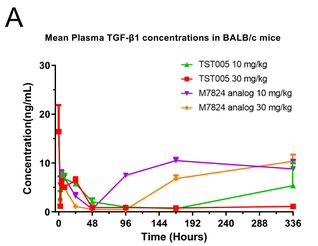


Figure 2. The concentration of TGF-β1 in the MC38/hPD-L1 tumor–bearing mice treated with TST005 via single IV injection. Plasma (A) and tumor (B) TGF-β1 concentration were analyzed at pre-dose and 30min, 2h, 8h, 24h, 48h, D7 and D14, D21, D28 after treatment. TGF-β1 depletion in both plasma and tumor tissue showed in a dose dependent manner with maximum depletion at 10mg/kg.

TST005 Depletes TGF-β1 in EMT6/hPD-L1 Tumor Model



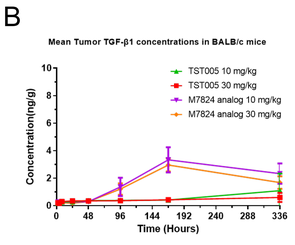


Figure 3. TGF-β1 concentration in the EMT6/hPD-L1 tumor–bearing mice treated with TST005 and M7824 analog via single IV injection. Plasma (A) and tumor (B) TGF-β1 concentration were analyzed at 2h, 4h, 8h, 24h, 48h, D4, D7 and D14 after treatment. TST005 showed more complete TGF-β1 depletion compared to M7824 analog.

TST005 Lowers pSMAD2 in MC38/hPD-L1 Tumor Model

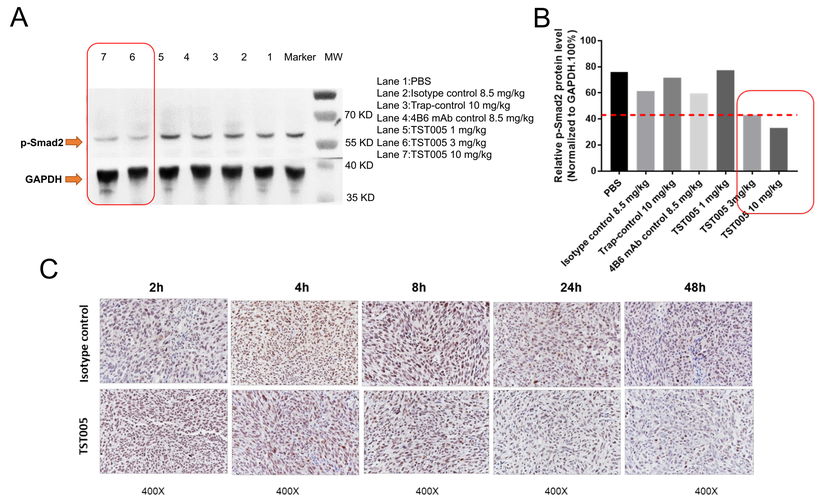


Figure 4. A. Western blot (WB) analysis of phosphorylated SMAD2 (pSMAD2) in MC38/hPD-L1 tumor–bearing mice treated with TST005 via IV injection twice weekly for two weeks, tumors were analyzed at 24h after 4th dosing. B. Relative pSMAD2 protein level normalized to GAPDH based on the WB result. C. Representative images of anti-pSMAD2 immunohistochemistry (IHC) of tumors at 2h, 4h, 8h, 24h and 48h after TST005 treatment with 10mg/kg via single IV injection. TST005 treatment significantly lowered pSMAD2 in MC38 tumors relative to isotype control.

Safety Profiles of TST005 in Rat and NHP

Study Species	Administration Route	Dosing Duration	Dose Levels	GLP	Key Results
Rat/Sprague-Dawley	Intravenous infusion	Single dose	100, 300, 600 mg/kg	Yes	Well tolerated up to 600 mg/kg
Rat/Sprague-Dawley	Intravenous infusion	Repeated dose (QW × 4 weeks, 5 doses in total)	20, 60, 200 mg/kg	Yes	Toxicokinetic: exposure was comparable between sexes and roughly dose-proportional
					Toxicity: no significant test article related safety issue
Monkey/Cyno molgus	Intravenous infusion	Single dose	150, 300 mg/kg	Yes	Well tolerated up to 300 mg/kg
Monkey/Cyno molgus	Intravenous infusion	Repeated dose (QW × 4 weeks, 5 doses in total)	20, 60, 200 mg/kg	Yes	Toxicokinetic: exposure was comparable between sexes and exhibited approximately dose-proportional.
					Toxicity: no significant test article related safety issue
					Cytokine release: no
					Safety margin: 231 folds based on C_{max}; 5135 folds based on $AUC_{T,ss}$

Table 1. A summary of in vivo toxicity studies for TST005. Note: safety margin was calculated by dividing Cmax and AUC0-t in monkeys at 200 mg/kg following the 4th dose with predicted Cmax and AUCT,ss in human at FIH, respectively.

CONCLUSIONS

- TST005 is a bi-functional fusion protein designed to simultaneously block PD-L1 and reduce TGF-β signaling, and it displayed better anti-tumor activities in pre-clinical studies as compared to M7824 analog with tolerable safety profiles.
- TST005 showed comparable depletion of TGF-β1 in MC38/hPD-L1 and EMT6/hPD-L1 tumor models in both plasma and tumor tissues.
- TST005 depleted TGF-β1 more efficiently in EMT6/hPD-L1 tumor model compared to M7824 analog.
- TST005 inhibited downstream pSMAD2 signal dose-dependently in MC38/hPD-L1 tumor model.
- TST005 showed tolerable safety profiles in rat and NHP species in single and repeated dose GLP toxicity studies.

REFERENCES

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