The Preclinical Characterization of TST001, A Novel Humanized Anti-Claudin18.2 mAb with Enhanced Binding Affinity and Anti-Tumor Activity

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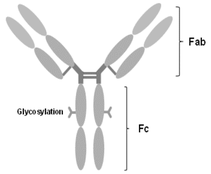
ABSTRACT

Claudin-18 isoform 2 (CLDN18.2) is a member of the human claudin family of tetraspan membrane proteins that are crucial structural and functional components of tight junctions[1]. Unlike other family members, CLDN18.2 expression is strictly limited to differentiated epithelial cells of gastric mucosa[1][2]. Interestingly CLDN18.2 was ectopically expressed at a significant level in multiple tumor types including gastric, esophageal, pancreatic and lung cancers, which makes it an attractive anti-cancer target[2]. TST001 is a novel humanized IgG1 monoclonal antibody, which specifically binds to cells expressing human CLDN18.2 with high affinity but not to the closely related Claudin 18.1. TST001 binds to CLDN18.2 via a distinct epitope. By reducing fucosylation during cell culture process, TST001 has further enhanced binding affinity to FcγRIIIa, and has more potent ADCC activity. Indeed, TST001 showed sub-nanomolar ADCC activity against gastric cancer cells expressing medium to low CLDN18.2 in the presence of human PBMC and NK cells, which is significantly more potent than IMAB362 analog. TST001 also showed more potent CDC and ADCP activities against CLDN18.2 expressing cells than IMAB362 analog. In both Sprague Dawley Rat and Cynomolgus Monkey, the systemic exposure of TST001 increased proportionally in a dose-dependent manner. In gastric cancer cell line and patient derived xenograft tumor models, TST001 showed more potent anti-tumor activity as compared with IMAB362 analog. Furthermore, the combination with chemotherapeutic agents resulted in enhanced anti-tumor activity of TST001 in these tumor models. In addition, we have also generated and characterized an antibody that is selective to CLDN18.2 over CLDN18.1 and is suitable for IHC based detection. Altogether, these preclinical findings warrant further clinical evaluation of TST001 in patients with CLDN18.2 expressing tumors.

About TST001



TST001 is a humanized antibody and produced from a stable CHO cell line with low levels of fucose in the Fc region. TST001 was humanized from 18B10, a mouse derived hybridoma antibody. The amino acid N298 of each heavy chain is the conserved N-glycosylation site and process optimization resulted in reduced fucosylation at N298 which enhanced the affinity of Fc binding to FcγRIIIa and ADCC activity [3].

TST001 specifically binds to CLDN18.2

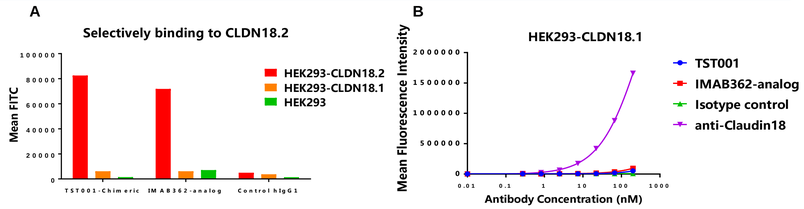


Figure 1. FACS analysis revealed TST001 specificity to CLDN18.2 over CLDN18.1. (A) FACS analysis using HEK293-CLDN18.1 or HEK293-CLDN18.2 or parental cell lines. The concentrations of CLDN18.2 antibodies were 10μg/ml. (B) FACS analysis using HEK293-CLDN18.1 cell line head to head with rabbit anti-human Claudin18.1/2 (Abcam, Cat#ab222513) as positive control.

TST001 has a high affinity to both CLDN18.2 and Fc receptors

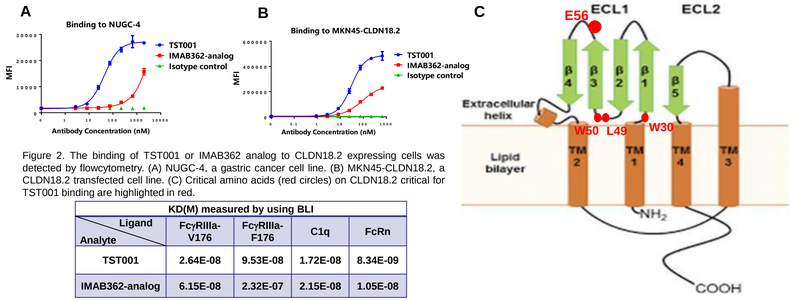


Figure 2. The binding of TST001 or IMAB362 analog to CLDN18.2 expressing cells was detected by flowcytometry. (A) NUGC-4, a gastric cancer cell line. (B) MKN45-CLDN18.2, a CLDN18.2 transfected cell line. (C) Critical amino acids (red circles) on CLDN18.2 critical for TST001 binding are highlighted in red.

KD(M) measured by using BLI				
Ligand / Analyte	FcγRIIIa-V176	FcγRIIIa-F176	C1q	FcRn
TST001	2.64E-08	9.53E-08	1.72E-08	8.34E-09
IMAB362-analog	6.15E-08	2.32E-07	2.15E-08	1.05E-08

Table 1. The binding of TST001 or IMAB362-analog to Fc receptors was detected by ForteBio Octet.

TST001 has potent killing activities on CLDN18.2-expressing cells

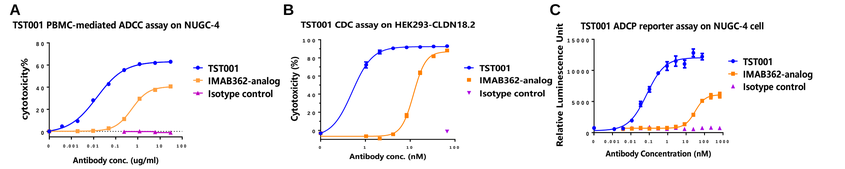
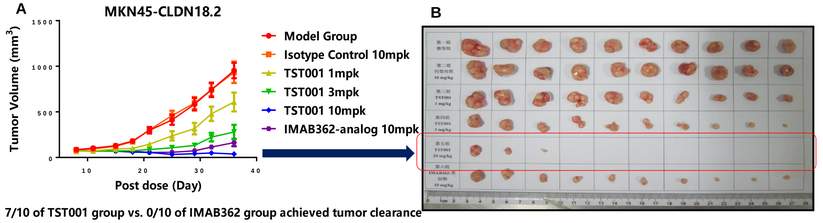


Figure 3. The cell killing activities of TST001 and IMAB362-analog were compared by using ADCC/CDC/ADCP assays. (A) PBMC-mediated ADCC assay. (B) CDC assay using human serum as complement and HEK293-CLDN18.2 as target cell. (C) ADCP reporter assay using NUGC-4 cell as target cell and Jurkat-NFAT-Luc-FcγRIIa cell as effector cell. The luminescence signal of effector cell indicates ADCP activity.

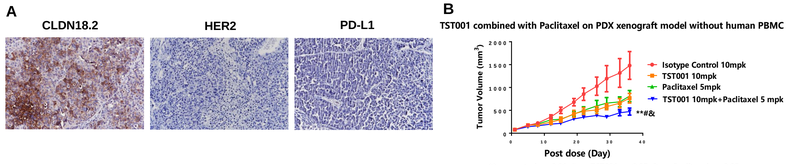
TST001 regresses tumor growth in MKN45-CLDN18.2 xenograft model



7/10 of TST001 group vs. 0/10 of IMAB362 group achieved tumor clearance

Figure 4. TST001 and IMAB362-analog were compared head to head in *in vivo* MKN45-CLDN18.2 model with human PBMC co-inoculated. (A) Average tumor volume. (B) Tumor size on Day 36.

Chemotherapeutic agents enhances tumor inhibition of TST001



****: vs control, p<0.01; #, vs TST001, p<0.05; &, vs Paclitaxel, p<0.05**

Figure 5. The chemotherapeutic agent Paclitaxel enhanced anti-tumor activity of TST001 on a Patient-Derived tumor Xenograft (PDX) model without human PBMC *in vivo*. (A) Immunohistochemistry was used to characterize the expression of CLDN18.2, HER2 and PD-L1 expression on tumor tissue derived from adenocarcinoma/gastric cancer patient used for PDX model. The photos are taken as 200×. (B) The tumor tissue was transplanted subcutaneously into nude mice without human PBMC to set up a PDX tumor model. In PDX tumor bearing mice, 5mg/kg of Paclitaxel was i.v. injected once a week, 10mg/kg of TST001 and isotype control were i.p. injected twice a week.

14G11 is selective to CLDN18.2 and suitable for IHC detection

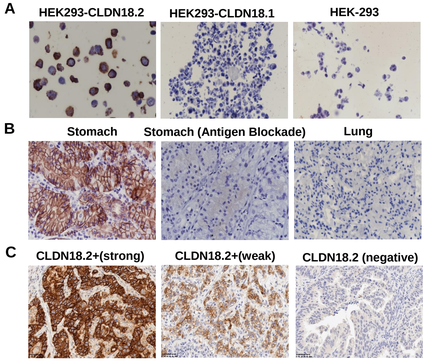


Figure 6. Evaluation of 14G11, an antibody specific for CLDN18.2, for IHC detection. (A) Cell lines were made into cell blocks for IHC analysis. 14G11 selectively binds to HEK293-CLDN18.2 but not to HEK293-CLDN18.1 cells. (B) 14G11 showed specific staining on cell membrane of human stomach but not on other tissues, such as lung. And this staining could be blocked by the CLDN18.2 peptide. (C) 14G11 based staining identified tumors with strong , medium or low expression of CLDN18.2 using human Gastric-Esophageal Adenocarcinoma samples. All the photos are taken as 400x.

Summary and Conclusions

- TST001 displayed specific, high affinity binding to CLDN18.2.
- With reduced fucosylation, TST001 has an enhanced affinity to FcγRIIIa and ADCC activity.
- In gastric cancer cell xenograft model, TST001 resulted in tumor regression in a dose-dependent manner.
- TST001 combined with chemotherapeutic agent, such as Paclitaxel, resulted in greater tumor inhibition than single agent in a CLDN18.2 positive PDX model.
- TST001 has a linear PK profile and is well tolerated in Cynomolgus monkeys.
- Antibody 14G11 displayed high specificity and sensitivity to CLDN18.2 in fixed tumor sample and is suitable for IHC detection.
- **Conclusions:** These studies demonstrated that TST001 is capable of potently inducing tumor killing both *in vitro* and *in vivo* and warrant further clinical evaluation of TST001 in patients with CLDN18.2 expressing tumors.

References

[1] Ö. Türeci et al. Gene 481 (2011) 83–92. [2] Sahin U et al. Clinical Cancer Research (2008), 14(23): 7624-7634. [3] Hristodorov, D.et al. Mol Biotechnol (2013) 54, 1056–1068

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