Characterization of novel humanized FGFR2b antibody-based ADCs site-specifically conjugated with topoisomerase I inhibitor payload in preclinical tumor models

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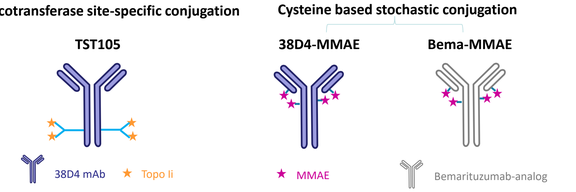
Abstract No: 4769

Abstract

Fibroblast growth factor receptor 2 IIIb (FGFR2b), one of the four FGFR family members that encode transmembrane receptor tyrosine kinases, is overexpressed in a variety of cancers, such as gastric/GEJ (29%)[1], esophageal (41%)[2], squamous NSCLC (31%)[3], TNBC (13%)[3], ovarian (40%)[3], endometrial (86%)[4], cervical (80%)[4], colorectal (62%)[5], and cholangiocarcinoma (22%)[3]. We have developed a FGFR2b-targeting ADC (TST105) with a novel topoisomerase I inhibitor payload by using glycotransferase mediated site-specific conjugation. Significant internalization, specific killing activity and bystander killing effect were observed *in vitro*. TST105 also shows outstanding *in vivo* tumor killing efficacy in gastric and colorectal tumor models, which may be contributed by the site-specific conjugation. These promising preclinical data support further investigations of TST105 in FGFR2b positive solid tumors.

The conjugation structure of ADCs

Fig. 1



TST105 is composed of a FGFR2b-targeting monoclonal antibody 38D4 conjugated to topoisomerase I inhibitor by using glycol based site-specific conjugation technology with DAR4, while 38D4-MMAE and Bema-MMAE were using cysteine based stochastic conjugation with DAR4.

Binding affinity and specificity of FGFR2b antibody 38D4 to hFGFR2b

Tab. 1

Abs	KD (M)	kon(1/Ms)	kdis(1/s)
38D4	2.23E-09	2.10E+05	4.69E-04
Bema-analog	7.90E-09	2.91E+05	2.30E-03

The binding affinity was analyzed by Fortebio Octet.

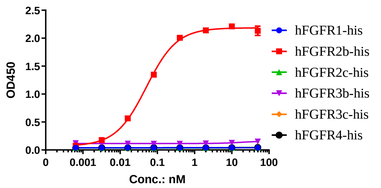
Tab. 2

Species cross-reactivity of 38D4

Species of FGFR2b	human	cyno	rat	mouse
Binding EC50 (nM)	0.05	0.02	0.06	0.02

ELISA assays utilized for analysis

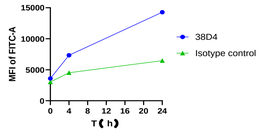
Fig. 2



ELISA assays utilized for analysis

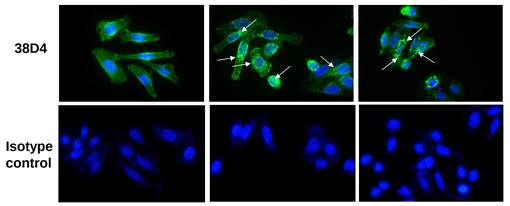
38D4 internalization into FGFR2b expressing cell line

Fig. 3



38D4 or isotype control were labeled with Zenon pHrodo iFL IgG labeling reagents (Invitrogen, Z25611) for 5 minutes before adding to SNU16 cells. After incubation at 37℃ for 0 h, 4 h and 24 h, the fluorescence signal of internalized pHrodoiFL labelled antibody was detected by FACS.

Fig. 4



The internalization images of 38D4 or isotype control by SW480-FGFR2b-high cells. Adherent tumor cells were incubated with 10 μg/ml antibody for 0, 4, 24 hours at 37℃. Then antibodies were detected with goat anti-human IgG Alexa Fluor488 after cells were fixed and permeabilized. Cells were mounted in ProLong Glod Antifade with DAPI. The white arrows refer to the internalized antibodies (Magnification: 400X)

TST105 *in vitro* cytotoxicity in gastric and colorectal tumor cell lines

Fig. 5A

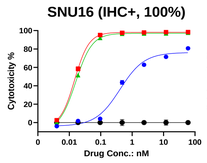


Fig. 5B

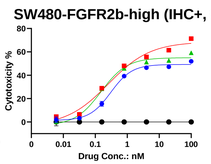
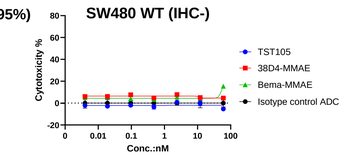
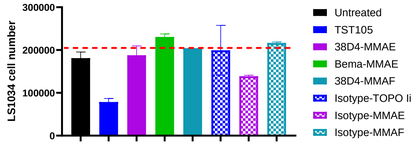


Fig. 5C



TST105 has strong bystander killing effect *in vitro*

Fig. 6



SW480-FGFR2b-high cells were co-cultured with LS1034 cells (FGFR2b negative) for 120 hrs. Ratio of SW480-FGFR2b-high: LS1034=1:1. 38D4-MMAF was using the same stochastic conjugation and MMAF, which has poor membrane permeability and no bystander effect[6].

TST105 exhibited more potent anti-tumor activity in gastric tumor model *in vivo*

Fig. 7A

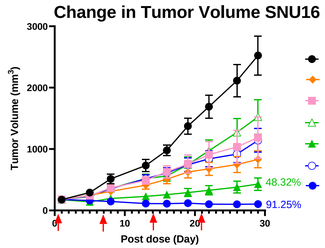


Fig. 7B

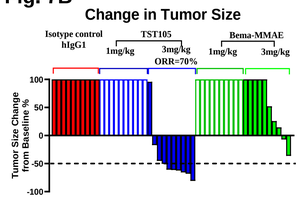
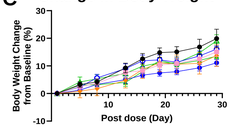


Fig. 7C



Efficacy of FGFR2b ADCs in SNU16 tumor model on BALB/c nude mice. Mice were inoculated with piece of approximately 2-3 mm^3 in diameter of SNU16 tumor blocks, when tumor size reached 150-200 mm^3, ADCs were i.v injected once a week. ORR: overall response rate (50% reduction of tumor volume from baseline).

TST105 induced a better anti-tumor efficacy than 38D4-MMAE in colorectal tumor model *in vivo*

Fig. 8A

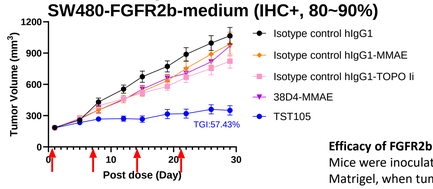
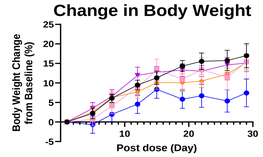


Fig. 8B



Efficacy of FGFR2b ADCs in SW480-FGFR2b-medium tumor model at 5 mg/kg. Mice were inoculated with 5X10^6 SW480-FGFR2b-medium tumor cells with 50% Matrigel, when tumor size around 180 mm^3, ADCs were i.v injected once a week.

Summary and Conclusions

- **TST105** is a novel and potent FGFR2b-targeted monoclonal antibody 38D4 conjugated to a novel topoisomerase I inhibitor by using glycol based site-specific conjugation technology.
- As 38D4 specifically binds to FGFR2b and can be internalized into FGFR2b expressing tumor cells, **TST105** could induce specific cytotoxicity to these tumor cells with a potency between 0.3 nM and 0.4 nM *in vitro*.
- **TST105** expresses higher potent bystander effect than MMAE based ADCs.
- **TST105** produced a greater *in vivo* anti-tumor efficacy (SNU16) than Bema-MMAE (TGI: 91.25% vs 48.32%, ORR: 70% vs 0%).
- **TST105** produced a greater *in vivo* tumor inhibition effect (SW480-FGFR2b-medium) than 38D4-MMAE (TGI: 57.43% vs 1.92%).
- **TST105** is one of several potent, next-generation therapeutic agents under development targeting FGFR2b expression in solid tumors.

References
[1] Based on the 910 patients screened for potential participation in the FIGHT Phase 2 clinical trial of bemarituzumab. [2] Yoshino et al Int J Oncol 2007. [3] Based on IHC staining conducted by Five Prime and Ventana of commercially sourced tissue samples. [4] Kurban et al Oncol Rep 2004. [5] Yoshino et al Oncol Rep 2005. [6] Hingorani et al. Molecular cancer therapeutics 2020. [7] Mangeat et al. EJNMMI Research 2023.