Discovery of a novel TIGIT therapeutic antibody with strong efficacy in tumor xenografts as monotherapy

Feifei Cui, Lei Fang, Zhengyi Wang, Taylor B. Guo

Suite 500, 2275 Research BLVD, Rockville Maryland 20850, I-Mab Biopharma US
I-Mab Biopharma, Inc., Shanghai, China

ABSTRACT

The immune checkpoint co-inhibitory receptor TIGIT (T cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif) is expressed on activated CD4+ T, CD8+ T and NK cells and on regulatory T cells (Tregs). Blocking TIGIT interaction with its ligand CD155 can re-energize tumor antigen-specific CD8+ T cells, unleash NK cells and inhibit Treg-mediated immunosuppression in the tumor microenvironment. Previous reports showed strong efficacy of anti-TIGIT antibodies in combination with anti-PDL1 agents in multiple tumor models, indicating a potential for TIGIT blocking antibodies as a combination partner for cancer treatment. Here we report discovery of a novel, humanized, Fc-enabled, sub-nanomolar anti-TIGIT antibody with significantly enhanced tumor inhibition as monotherapy and combo-therapy with anti-PDL1 antibody, thus differentiating it from current anti-TIGIT molecules.

MATERIALS AND METHODS

Antibody generation. A mouse monoclonal antibody TJ-T6-M was originally obtained through standard immunization and hybridoma process using the extracellular domain (ECD) of human TIGIT as the antigen. Following sequencing, TJ-T6-M was humanized using CDR-grafting and back mutation strategy and resulted in a humanized antibody named TJ-T6, with a human IgG1 Fc domain. In vitro cell-based receptor blocking assay. Serially diluted antibodies were incubated with K562-TIGIT cells for 30 min at 4 °C. hCD155-Fc protein was then incubated with antibody-cell complex for 30 min at 4 °C. PE-anti-human CD155 antibody was used to detect hCD155 when it bound to TIGIT expressed on cell surface by Flow cytometry.

Jurkat cell-based IL2 release assay. Jurkat-TIGIT-CD226 cells were incubated with Raji-CD155 cells and for 48 hours in the presence of TCR stimulation and TIGIT antibodies. Concentration of IL2 in the supernatant was determined by standard ELISA kit. For functional assay, Jurkat-TIGIT-CD226-PD1 and Raji-CD155-PDL1 cells were used in the coculture systems.

Primary T cell functional assay. Human primary CD8+ T cells isolated from PBMCs from healthy donors were incubated with CHO-TCR activator-CD155 cells for 3 days in the presence of anti-TIGIT antibodies. Concentration of IFNγ in the supernatant was determined by standard ELISA kit. For functional assay, CHO-TCR activator-CD155-PDL1 cells were used to coculture with CD8+ T cells.

In vivo MC38 syngeneic model. Humanized-TIGIT mice or double-humanized TIGIT/PDL1 mice were subcutaneously implanted with 1 x 10^6 MC38 cells or MC38 with humanized PDL1 cells on day 0. On day 7, mice with an average tumor volume of 110 mm^3 were randomized into indicated treatment groups (N=7/group). Mice were intraperitoneally administered indicated antibodies every 3 days for 6 doses. Tumor volumes were monitored by caliper measurement twice per week for the duration of the experiment.

RESULTS

Binding properties of TJ-T6

CONCLUSIONS

• TJ-T6, an Fc-enabled human IgG1 antibody, binds human and cynomolgus TIGIT with similar sub-nanomolar binding.
• TJ-T6 blocked the interaction of TIGIT to its ligand CD155, leading to increased production of IL-2 in a Jurkat cell line which overexpressed TIGIT and CD226.
• Mono-treatment of TJ-T6 significantly suppressed tumor growth in a dose-dependent manner in a syngeneic MC38 tumor model compared to vehicle, achieving maximal 60% tumor growth inhibition at 10 mg/kg. Percentages of tumor infiltrating CD4+ T and CD8+ T cells were significantly enhanced after TJ-T6 treatment.
• Combination of anti-PDL1 antibody with TJ-T6 show synergistic effect in vivo.
• Combination of anti-PDL1 antibody with TJ-T6 significantly inhibit tumor growth in a syngeneic MC38 tumor model compared to control or monotherapy groups.