Discovery of a novel anti-LAG3 antagonist antibody

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ABSTRACT

LAG3 is a negative regulator of T cells. Persistent T-cell activation in a chronic inflammatory environment, as in a tumor or during chronic viral infection, results in sustained LAG3 expression, contributing to a state of exhaustion manifest in impaired proliferation and cytokine production. Clinical studies showed striking synergy between LAG3 and PD1 in multiple settings, highlighting the therapeutic potential of LAG3. Here we reported the discovery of a novel LAG3 antibodies (TJ-A3) with sub-nanomolar binding affinity. Upon binding, it blocked the interaction of LAG3 to its receptor MHCII, leading to increased production of IL-2 in Jurkat cells overexpressing LAG3 and in activated human primary T cells. TJ-A3 can significantly inhibit tumor growth in combination with a PD-L1 blocking antibody in a syngeneic mouse tumor model by using LAG3-humanized mice. Together with favorable cynomolgus monkey PK and cell line development profile, these studies support further clinical development of TJ-A3.

MATERIALS AND METHODS

Antibody generation. A mouse lead monoclonal antibody Mu147H was originally obtained through standard immunization and hybridoma process using the extracellular domain (ECD) of human LAG3 as the antigen. Following sequencing, the molecule was humanized (using CDR-grafting and back mutation strategy) and affinity matured, which resulted in an antibody named TJ-A3, with a human IgG4 Fc domain with a serine to proline mutation at No.228 position to avoid arm exchange.

***In vitro* receptor blocking assay.** LAG3 antibodies were incubated with Tag1-LAG3 and Tag2-MHCII for 15 min at room temperature. Pre-mixed detection antibodies containing anti-Tag1-Tb3+ and anti-Tag2-XL665 antibodies were added and incubated for another 1 hr at room temperature. The plate was analyzed by PerkinElmer Envision plate reader.

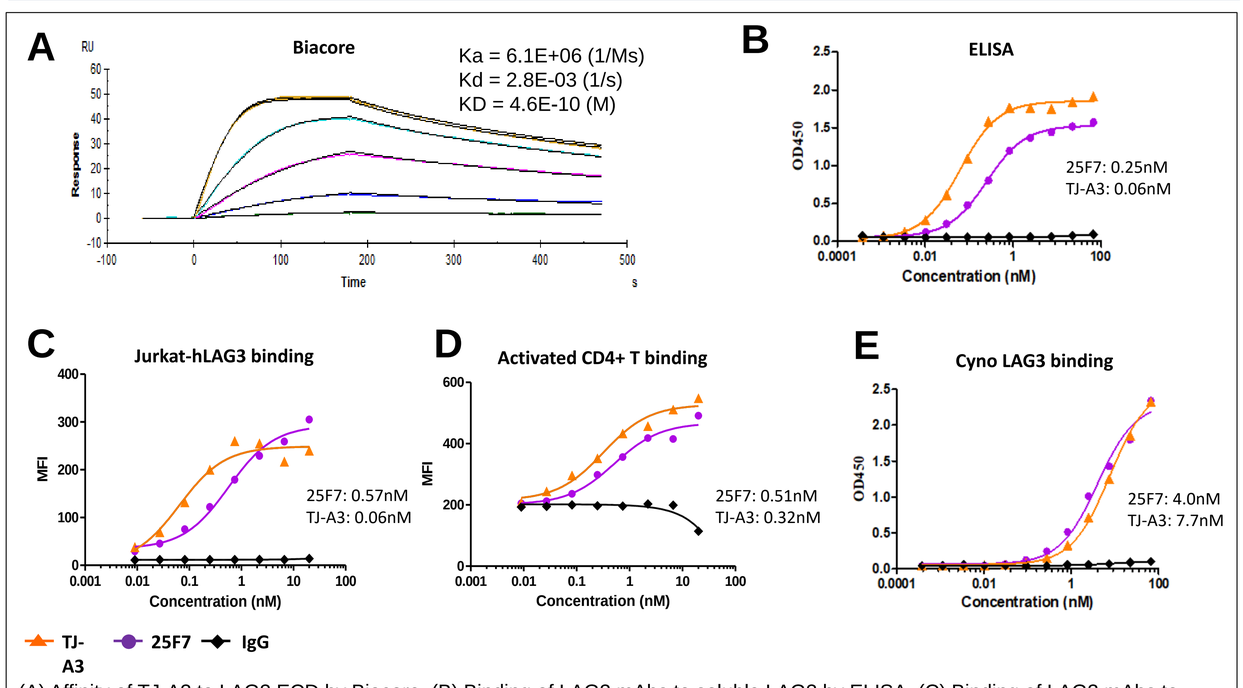
Jurkat-LAG3 cell based IL2 release assay. LAG3 antibodies were incubated with Raji cells and Jurkat-hLAG3 cells for 48 hours in the presence of TCR stimulation. Culture medium was collected and incubated with anti-IL2 antibody pairs. IL-2 concentration was further analyzed by PerkinElmer Envision plate reader.

Primary T functional assay. LAG3 antibodies were incubated with MHC-mismatched DC and CD4+ T cells for 5 days in the presence of TCR stimulation. Culture medium was collected and incubated with anti-IL2 antibody pairs. IL-2 concentration was further analyzed by PerkinElmer Envision plate reader.

***In vivo* antibody treatment in MC38 huLAG3 syngeneic model.** Humanized-Lag3 mice were subcutaneously implanted with 1×10^6 MC38 cells on day 0. On day 6, mice with an average tumor volume of 52mm^3 were randomized into four treatment groups (N=7/group). Mouse were intraperitoneally administered saline, anti-PDL1 (Tecentriq) at 1 mg/kg, and anti-PDL1 (Tecentriq) at 1 mg/kg plus anti-LAG3 antibodies (10 mg/kg) every 3 days for 4 doses. Tumor volumes were monitored by caliper measurement twice per week for the duration of the experiment.

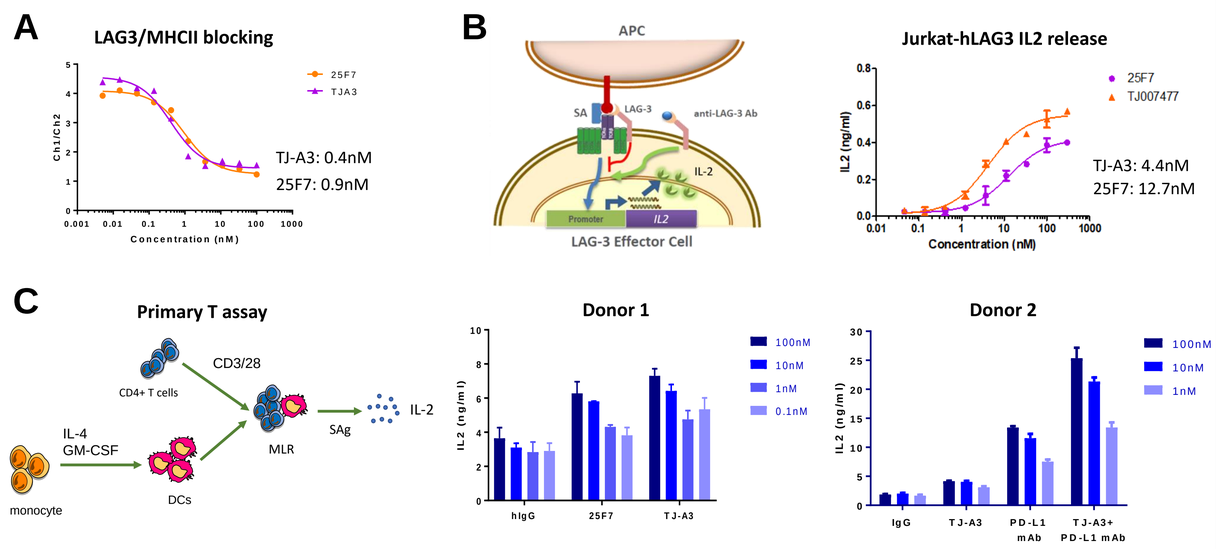
RESULTS

Binding properties of TJ-A3



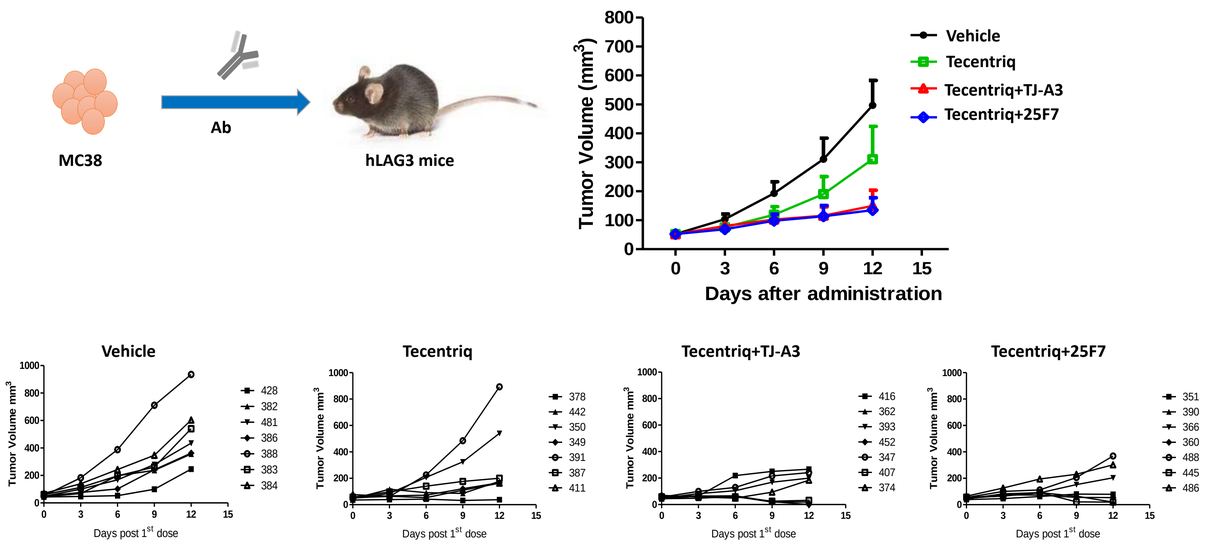
(A) Affinity of TJ-A3 to LAG3 ECD by Biacore. (B) Binding of LAG3 mAbs to soluble LAG3 by ELISA. (C) Binding of LAG3 mAbs to Jurkat-hLAG3 cells by FACS. (D) Binding of LAG3 mAbs to activated human CD4+ T cells by FACS. (E) Binding of LAG3 mAbs to cyno LAG3 by ELISA.

In vitro functional assays of TJ-A3



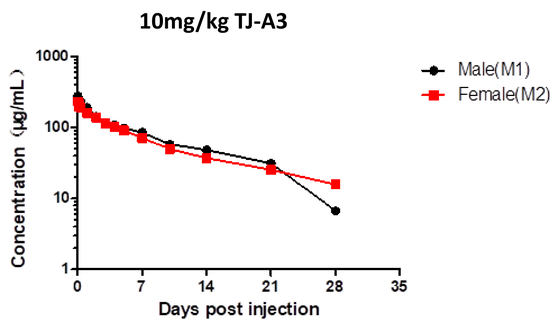
(A) Receptor blocking assay. (B) Left, assay principle of Jurkat IL2 assay. Right, Stimulation of IL2 release in Jurkat-hLAG3 by LAG3 mAbs. (C) Left, assay principle of primary assay. Middle, stimulation of IL2 release in activated CD4+ T cells by LAG3 mAbs or PD-L1 mAb or combination treatment.

In vivo efficacy of TJ-A3



In vivo efficacy of combo treatment of TJ-A3 and anti-PDL1 in an MC38 syngeneic mouse model. MC38 cells were subcutaneously implanted into huLAG3 mice. When the tumor grew to around 50 mm^3, mice were intraperitoneally treated with control, anti-PDL1 only or anti-PDL1 plus the indicated LAG3 mAb. Tumor growth was monitored by volumetric measurement. Shown are means±SEM (top) and individual mouse data (bottom).

Pilot pharmacokinetics of TJ-A3



PK parameters	
t 1/2 (hr)	156
Tmax (hr)	0.08
Vss (mL/kg)	55.53
CL (mL/hr/kg)	0.24
Cmax (ug/mL)	255.50
AUC (h*mg/mL)	40.59

Naïve cynomolgus monkeys were treated with a single i.v. injection of TJ-A3 (10 mg/kg, n=2). Serum samples from each individual monkey were collected at various time points and measured for the levels of anti-LAG3 mAbs by a generic ELISA method using recombinant LAG3 protein ECD as the capture antigen.

CONCLUSIONS

- TJ-A3 is an antagonist antibody against LAG3 with sub-nanomolar affinity.
- TJ-A3 binds to human and cynomolgus LAG3, blocking the binding of LAG3 to its receptor MHCII molecule.
- Upon binding, TJ-A3 leads to the increased production of IL-2 in Jurkat cells overexpressing LAG3 and in activated human primary T cells.
- TJ-A3 showed strong synergistic effects on the T cell activation in combo with anti-PDL1 antibody. Consistently, when combined with PD-L1 antibody, TJ-A3 can significantly inhibit tumor growth in a syngeneic MC38 tumor model using LAG3-humanized mice.
- Time-concentration profiles indicated that the PK of TJ-A3 was consistent with normal human IgG.
- Developability assessment of the TJ-A3 sequence indicates that it is a reasonably developable molecule to be taken forward. CMC-wise, a cell line with decent titer have been developed.