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Characterization of CS1003, A Novel Clinical-Stage PD-1 Monoclonal Antibody

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BACKGROUND

The programmed cell death protein 1 (PD-1) is an immune-checkpoint that negatively regulates the immune system to avoid collateral damage to self-tissues. Tumors hijack this mechanism as a way to avoid immune detection and destruction. The FDA has approved three anti-PD-1 antibodies for the treatment in more than ten cancer types. CS1003 is a novel anti-PD-1 mAb developed to disrupt the PD-1 interaction with PD-L1/PD-L2 to restore or improve T-cell function as stand-alone therapy or in combination with other anticancer reagents.

METHODS

A panel of rat PD-1 mAbs were generated through conventional hybridoma technology. Several mAbs with favorable characteristics were further humanized. CS1003, a humanized, hinge-stabilized IgG4 mAb, was selected as our lead candidate for further characterization including antigen binding, biophysical properties as well as functional characterization.

RESULTS

CS1003 binds mouse, cynomolgus monkey and human PD-1 with similar affinity. CS1003 bound PD-1-expressing cell lines and chronically-activated T cells, blocked PD-1 interactions with PD-L1/PD-L2, resulting in inhibition of PD-1 signaling, and enhanced T cell cytokine secretion and proliferation to levels comparable to those observed with reference mAb 1 and mAb 2 molecules. CS1003 showed significantly anti-tumor activity in conventional MC38 as well as in hPD-1 knock-in mice. CS1003 showed no unexpected cross-reactivity in human tissues, with specific staining observed primarily in lymphocytes of lymphoid organs. In a pharmacokinetic (PK) study in cynomolgus monkeys following single intravenous administration at multiple dose levels, PK properties were linear with the proportionally increasing exposures from 2-18 mg/kg. In a repeated-dose toxicity study, CS1003 was well tolerated in cynomolgus monkeys and the major finding was mononuclear infiltration in multiple organs, which was consistent with its pharmacologic activity. CS1003 demonstrated a favorable safety profile with the highest non-severely toxic dose (HNSTD) of 100 mg/kg.

SUMMARY

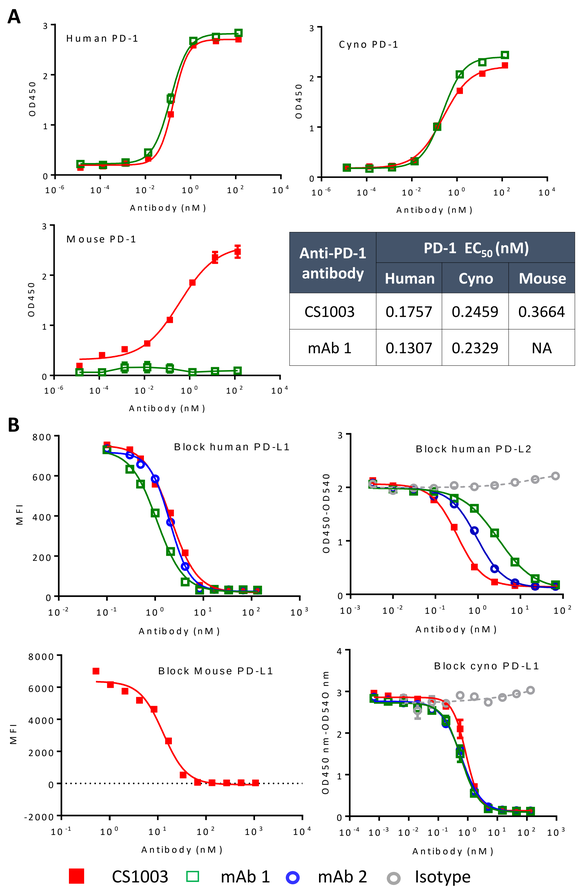
- CS1003 is a humanized PD-1 targeted IgG4 (S223P) monoclonal antibody (mAb) developed by the rat platform.
- CS1003 is equipotent against human, cyno and mouse PD-1 and this should greatly facilitate further evaluation its potential for combination therapy in various syngeneic mouse tumor models.
- CS1003 blocks PD-1/PD-L1 and PD-1/PD-L2 interactions, interrupts PD-1 signaling and enhances antigen-induced IFN-γ release with potency comparable to reference mAb 1 and mAb 2.
- A Phase 1 study [NCT03475251] evaluating the safety, tolerability and PK of CS1003 in patients with advanced solid tumors is on-going.

RESULTS

CS1003 IS A HIGH AFFINITY BLOCKING ANTIBODY THAT RECOGNIZES HUMAN, CYNO AND MOUSE PD-1

SPR ANALYSIS

Ligand	Human PD-1		
	ka (1/Ms)	kd (1/s)	K_D (M)
CS1003	5.97E+05	3.66E-03	6.13E-09
mAb 1	8.79E+05	2.28E-03	2.59E-09
mAb 2	4.02E+05	1.35E-03	3.37E-09
Ligand	**Mouse PD-1**		
	ka (1/Ms)	kd (1/s)	K_D (M)
CS1003	3.23E+05	1.29E-03	3.99E-09



Anti-PD-1 antibody	PD-1 EC_{50} (nM)		
	Human	Cyno	Mouse
CS1003	0.1757	0.2459	0.3664
mAb 1	0.1307	0.2329	NA

Figure 1: Binding of CS1003, reference mAb 1 and mAb 2 to plate coated recombinant human, cyno and mouse PD-1 proteins were assessed by ELISA (A). Functionality of CS1003 in blocking human PD-1/PD-L1(L2), cyno or mouse PD-1/PD-L1 interactions were assessed by flowcytometry or ELISA respectively.

CS1003 ENHANCES T CELL ACTIVATION AND PROLIFERATION

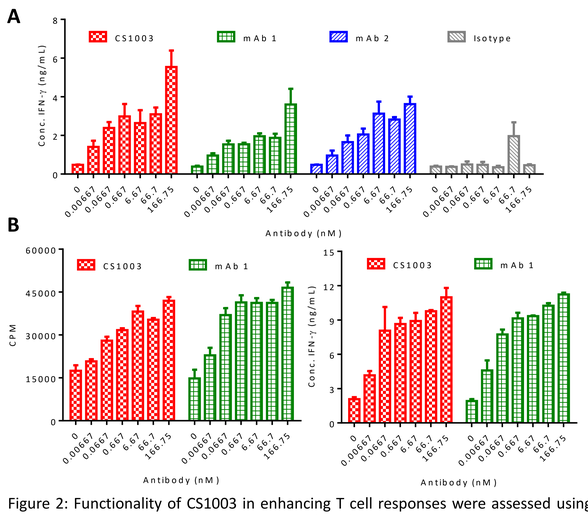


Figure 2: Functionality of CS1003 in enhancing T cell responses were assessed using (A) allogeneic Mixed Lymphocyte Reaction (MLR) and (B) CMV pp65-specific T cell recall responses. T cell proliferation (3H-TDR incorporation) and effector function (IFN-γ production) were quantified.

SELECTIVITY OF CS1003 AGAINST CD28, CTLA-4, ICOS AND BTLA

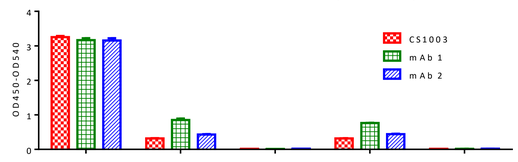


Figure 3: Binding of 66.7 nM of CS1003, reference mAb 1 and mAb 2 to plate coated hPD-1.mFc, hCD28.ECD.mFc, hCTLA-4.ECD.His, hICOS.ECD.mFc or hBTLA.ECD.His were assessed by ELISA. Assay background was determined using no antibody wells.

ADCC AND CDC Activity of CS1003 on Activated T Cells

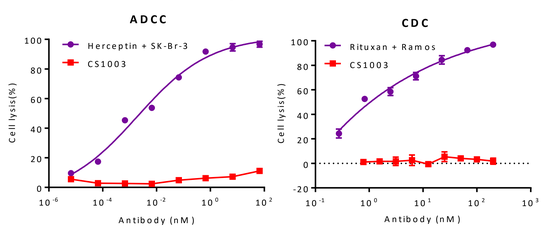
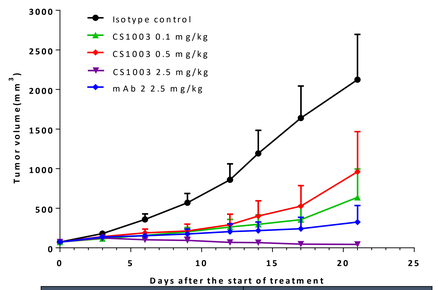


Figure 4. ADCC and CDC activities of CS1003 were evaluated. Herceptin induced SK-Br-3 cell lysis and Rituximab-induced Ramos cell lysis were used as positive control of ADCC and CDC respectively.

CS1003 IS EFFECTIVE IN INHIBITION TUMOR GROWTH



Treatment	Tumor-free Mice
Isotype control	0/8
CS1003 (0.1mg/kg)	2/8
CS1003 (0.5mg/kg)	3/8
CS1003 (2.5mg/kg)	5/8
mAb 2 (2.5mg/kg)	4/8

Figure 5: The efficacy of CS1003 was evaluated in established MC38-huPD-L1 colon cancer model in hPD-1 knock-in mice. All antibodies were administered IP once every three days (Q3D) for a total of 5 doses starting at 74 mm^3 tumor volume.

PHARMACOKINETIC ANALYSIS AND RECEPTOR OCCUPANCY OF CS1003 IN CYNOMOLGUS MONKEYS

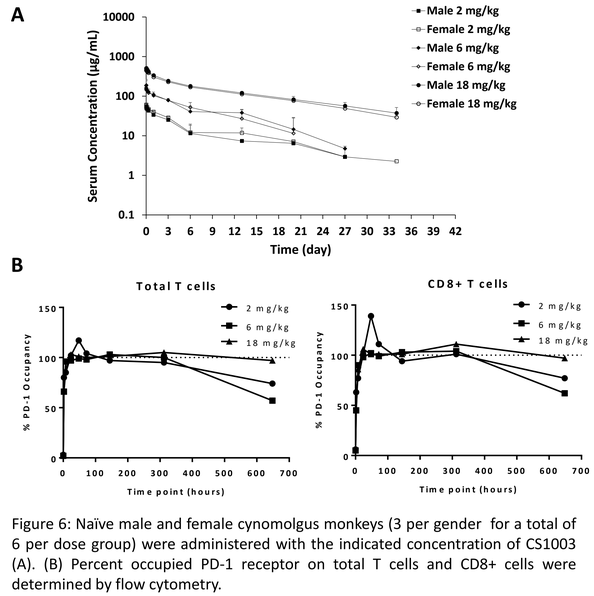


Figure 6: Naïve male and female cynomolgus monkeys (3 per gender for a total of 6 per dose group) were administered with the indicated concentration of CS1003 (A). (B) Percent occupied PD-1 receptor on total T cells and CD8+ cells were determined by flow cytometry.

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