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Preclinical characterization of CS1001, an anti-PD-L1 IgG4 monoclonal antibody and its activity beyond T cell regulation

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BACKGROUND

CS1001 is a high-affinity, fully human programmed death ligand-1 (PD-L1) blocking IgG4 monoclonal antibody. So far three therapeutic anti-PD-L1 antibodies (atezolizumab, avelumab, durvalumab) have been approved by US FDA in different cancer indications. CS1001 is developed by OmniRat® transgenic platform, which mirrors natural IgG4 human antibody with expected pharmacokinetic (PK) profiles and may potentially reduce the risk of immunogenicity and toxicity in patients.

CS1001 is currently explored clinically in six registrational trials as monotherapy or in combination with standard of care therapies. CS1001 monotherapy or combo with chemotherapy or target therapy were safe and well tolerated. CS1001 demonstrated promising efficacy in multiple tumor types and support full development of CS1001 as mono/combo therapy for multiple indications in ongoing clinical trials including cHL, ENKTL, GC, EC, and NSCLC.

RESULTS

CS1001 selectively bound to PD-L1 and blocked PD-L1/PD-1 as well as PD-L1/CD80 ligation. The crystal structure of CS1001 complexed with human PD-L1 solved at 2.3Å revealed that CS1001 bound perpendicularly to PD-L1 and the binding epitope region well overlapped with the PD-1 binding region. In the mixed lymphocyte reaction (MLR) assay, CS1001 effectively induced the proliferation of CD4+ T lymphocytes and the production of interferon-γ (IFN-γ) and interleukin-2 (IL-2). CS1001 employs IgG4 isotype and lacks antibody-dependent cellular mediated cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). Furthermore, the in vivo efficacy of CS1001 in inhibiting MC38-hPD-L1 (mouse tumor cells expressing human PD-L1, Biocytogen) tumor growth was demonstrated in both B-hPD-1 and B-hPD-1-hPD-L1 mice (i.e. PD-1 humanized mice and PD-1/PD-L1 dual humanized mice, respectively, Biocytogen). As human PD-L1 is expressed in both tumor and host system, there is an opportunity to evaluate the effect of CS1001 through targeting both tumor and host PD-L1 by using the PD-1/PD-L1 dual humanized mice. It was found that CS1001 led to profound inhibition of Treg population and shifting of myeloid cell profile to benefit anti-tumor immune response.

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CS1001 is a high affinity PD-L1 blocking antibody

Table 1: Measurement of affinity to PD-L1 by surface plasmon resonance (SPR) analysis

Test Antibody	Human PD-L1		
	ka (1/Ms)	kd (1/s)	K_D (M)
CS1001	3.84E+05	5.28E-04	1.38E-09
Benchmark-1[a]	6.80E+06	1.55E-03	2.28E-10
Benchmark-2[b]	1.23E+06	2.17E-03	1.76E-09

[a]Generated based on the sequence of 12A4 in the patent WO2007005874 from BMS;
[b]Generated based on the sequence of 2.7A4 in the patent US8779108 from MedImmune.

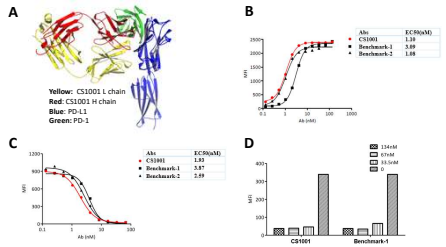

Figure 1. CS1001 binds to PD-L1 and blocks the association of PD-L1 with both PD-1 and CD80. (A) Ribbon representation of the crystal structure of PD-L1 in complex with CS1001 and PD-1. (B) Binding of CS1001 to engineered cells (CHO cells) that express human PD-L1 assessed by FACS analysis. Competitive blocking effect of CS1001 against binding of PD-L1 to PD-1 (C) and PD-L1 to CD80 (D) were assessed by FACS analysis.

CS1001 enhances T cell activation and proliferation

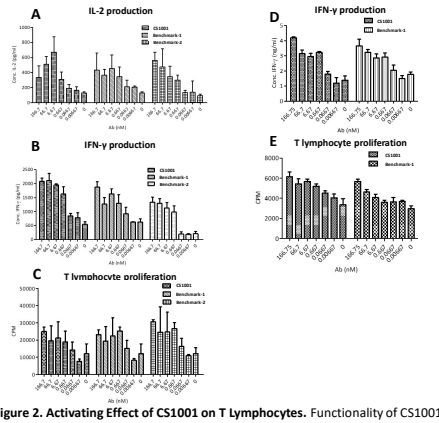

Figure 2. Activating Effect of CS1001 on T Lymphocytes. Functionality of CS1001 on CD4+ T lymphocytes was tested by both (A-C) allogeneic mixed lymphocyte reaction (MLR) and (D-E) CMV pp65-specific T cell recall responses. Cytokine (IL-2 and IFN-γ) secretion and T cell proliferation were quantified by ELISA and ^{3}H-TdR incorporation assay, respectively. Reactions were set up in triplicate for each concentration.

CS1001 doesn't induce ADCC or CDC effect

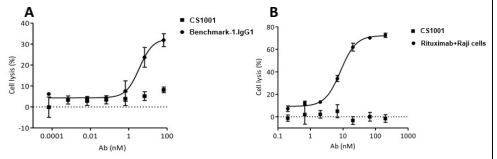

Figure 3. ADCC and CDC activities of CS1001. (A) ADCC activity towards PD-L1 positive CD4 T cells was observed in the presence of an PD-L1 control Ab utilizing IgG1 isotype , but not in the presence of CS1001 that employs IgG4 isotype as expected. (B) Similarly, CS1001 didn't induce CDC activity towards PD-L1 positive CD4 T cells. Rituximab and target cells Raji served as the positive control of the CDC activity. Reactions were set up in triplicate for each concentration of the samples.

CS1001 is effective in inhibiting tumor growth

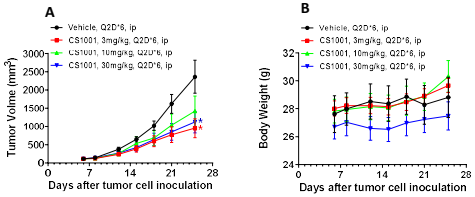

Figure 4. Effect of CS1001 on tumor growth of MC38-hPD-L1 murine syngeneic colon carcinoma model. Murine colon cancer cell line MC38-hPD-L1 (expressing human PD-L1) were subcutaneously inoculated in B-hPD-1 C57BL/6 mice (expressing humanized PD-1). The treatment started on Day 6 after inoculation when the TV reached ~110 mm^3. The mice were treated with CS1001 intraperitoneally (i.p.) at 3, 10 or 30 mg/kg, every two days for a total of 6 doses. Tumor volume and body weights were measured 2~3 times a week. Data were represented as mean ± SEM. Statistical analysis was performed by independent t-test. N=7/arm. *$p<0.05$ vs. vehicle group.

CS1001 modulates both tumor infiltrating T lymphocytes and myeloid cells

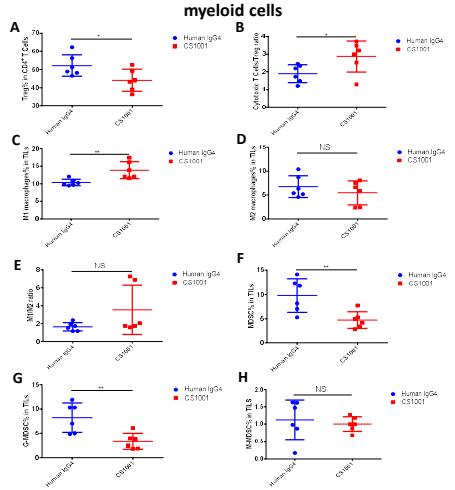

Figure 5. CS1001 altered the phenotypes of tumor-infiltrating leukocytes (TILs). Murine colon cancer cell line MC38-hPD-L1 (expressing human PD-L1) was subcutaneously inoculated in B-hPD-1/hPD-L1 mice (expressing humanized PD-1 and PD-L1). Mice were treated with CS1001 or human IgG4 at 3mg/kg, twice a week for a total of 6 doses. CS1001 produced significant efficacy (TGI=49%, p=0.031 vs. isotype control, data not shown). Tumor samples were collected for TIL analysis 24h post the last dose. (A) Percentage of Treg cells (FoxP3$^+$CD4$^+$ T cells) over CD4$^+$ T cells, (B) Cytotoxic T cells/ Treg ratio, (C) percentage of M1 macrophages (IA-IE$^+$CD206$^-$ F4/80$^+$CD11b$^+$CD45$^+$) in TILs (CD45+), (D) percentage of M2 macrophages (IA-IE$^-$CD206$^+$F4/80$^+$CD11b$^+$CD45$^+$) in TILs (CD45+), (E) M1/M2 ratio, (F) percentage of MDSC (Gr-1$^+$CD11b$^+$CD45$^+$), (G) G-MDSC (Ly6c$^-$Ly6G$^+$ MDSC)and (H) M-MDSC (Ly6c$^+$Ly6G$^-$ MDSC) in TILs were analyzed. N=6/arm. *$p<0.05$ vs control; ** $p<0.01$ vs control.

Discussion

This work suggested beyond the well-known mechanism of blocking the interaction between PD-L1 and PD-1 to reinvigorate the dysfunctional tumor-infiltrating effector T cells, the PD-L1 blocking antibody may also modulate PD-L1 expressing myeloid cells. Further more, CS1001 potentially play the therapeutic role through at least three other mechanisms: 1) inducing antibody-dependent cellular phagocytosis (ADCP)-mediated tumor cell killing through crosslinking PD-L1 positive tumor cells with macrophages; 2) blocking PD-L1 on DCs to relieve B7.1 (CD80) sequestration in cis by PD-L1, which allows the B7.1/CD28 interaction to enhance T cell priming; 3) blocking tumor-intrinsic PD-L1 signaling to inhibit tumor cell proliferation. CStone is actively investigating the aforementioned MOAs that may shed more light on our understanding on the therapeutic functions of CS1001 in the future.