Abstract No. 108

CBT-501, a novel humanized anti-PD-1 antibody shows differentiated activity in a humanized genetically engineered mouse model of colon carcinoma

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Introduction

CBT-501 (Genolimzumab, or GB-226) is a novel humanized mAb (IgG4κ) against human Programmed Death-1 (PD-1). CBT-501 demonstrated highly specific binding to PD-1 of human (K_D=505 pM) and cynomolgus (K_D=7.2 nM). It did not completely block the binding of nivolumab or pembrolizumab, suggesting a novel epitope. CBT-501 efficiently inhibited the binding of PD-L1/L2 to PD-1 through a competitive action for both human and monkey. It enhanced human T-cell activation in the Mixed Lymphocyte Reaction (MLR), as shown by increased release of IL-2 and INF-γ. The Fc-fragment bound to FcRn dose-dependently without any effector function. The PK/PD studies in cynomolgus showed a linear dose exposure, with a $T_{1/2}$ of 115-142 h for single and repeat dosing. The PD-1 receptor occupancy after a single dose was dose-dependent reaching 100% at peak, maintained after multiple dosing and continued for 6 week after last dose. Single and repeat dose toxicology studies in cynomolgus showed weight gain of the thyroid and mild expansion of the thyroid follicles at the highest dose of 100 mg/kg which were reversible. No abnormal drug-related toxicity was found. In rhesus vaccinated with adenovirus-mediated Simian Immunodeficiency Virus, biweekly IV CBT-501 promoted the antigen-specific T-cell response, as shown by ELISPOT and intracellular staining of IL-2, INF-γ and TNF-α in PBMC and T-cell subpopulation. CBT-501 injected i.p.q2w×3wk in the PD-1 HuGEMM Isograft Model with Subcutaneous MC38 Mouse Colon Adenocarcinoma demonstrated significant anti-tumor activity. The tumor growth reduction was dose dependent with comparable or improved activity over nivolumab, and with the highest inhibition rate at 83%.

Materials and Methods

DCs and CD4+ T-cells were isolated from human PBMCs from different donor sources in a mixed lymphocyte reaction (MLR), and the DCs were induced to differentiate and mature in vitro. DCs and CD4+ T-cells were mixed and IL-2 and IFN-γ was measured.

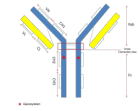
A humanized genetically engineered mouse model expressing human PD-1 extracellular domain with a fully functional murine immune system was developed. Mouse strain: PD-1 HuGEMM; age: 9 weeks at dosing; male. The gene encoding human/murine chimeric PD-1 was obtained by replacing exon 2 encoding the extracellular domain of C57BL/6 mouse PD-1 gene with exon 2 of human PD-1 gene. Subcutaneous colon adenocarcinoma MC38 syngeneic tumor graft was established in mice.

Pharmacokinetic and receptor occupancy studies were carried out in cynomolgus monkeys. Single dose (2, 10, and 50 mg/kg) and multi-dose studies (10 mg/kg qwx4) were conducted and receptor occupancy was determined by measuring CD4+ and CD8+ T-cells via FACS along with concomitant pharmacokinetic analyses.

Effect of CBT-501 vs hIgG4 in rhesus monkeys vaccinated with adenovirus SIV vaccine (AD5 / SIV) and HPV vaccine (ZR111). ELISPOT was used to detect the frequency of IFN-γ spot formation in PBMCs to evaluate the antigen-specific cellular immune responses.

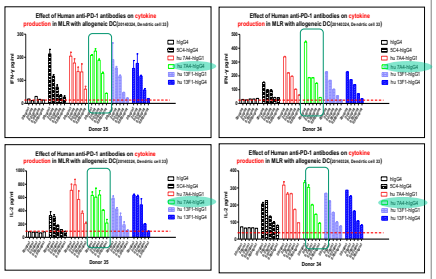
Results

Figure 1. CBT-501 (Genolimzumab)



- Binding to human PD-1 is concentration-dependent EC50 is 2.24 ng/mL (ELISA) or 63 ng/mL (FACS, on 293T/PD-1) or 13 ng/mL (FACS, on Jurkat/PD-1)
- Binding constant (Biacore) Kd ~ 505 pM
- Disrupts binding to PD-L1 and PD-L2 ligand IC50 (PD-L1) ~ 0.32 nM (ELISA); 0.44 nM (FACS) IC50 (PD-L2) ~ 0.54 nM (ELISA); 0.60 nM (FACS)
- No binding to CD28, CTLA-4, ICOS & BTLA (ELISA)
- Binds to Cyno PD-1; not to mouse PD-1
- CBT-501 binding to PD-1 is competitive with pembrolizumab and nivolumab, suggesting their binding sites are in close proximity to each other or overlapping, but distinct

Figure 2. Mixed Lymphocyte Assay from Healthy Donor CD4+ Cells



- A concentration dependent effect shown. Elevated levels of IL-2 and IFN-γ observed in presence of the anti PD-1 antibody isoforms. Robust elevation for CBT-501 (7A4-hIgG4 isoform) compared to nivolumab (5C4-hIgG4).

Table 1. Study Design

	Group	N	Dose	Volume	Route	Regimen
1	Isotype Control (IgG4)	8	10 mg/kg	10 μL/g	i.p.	BIW×3
2	nivolumab	8	10 mg/kg	10 μL/g	i.p.	BIW×3
3	CBT-501	8	5 mg/kg	10 μL/g	i.p.	BIW×3
4	CBT-501	8	10 mg/kg	10 μL/g	i.p.	BIW×3
5	CBT-501	8	20 mg/kg	10 μL/g	i.p.	BIW×3

i.p. = intraperitoneal injection; BIW = twice weekly

Figure 3. Tumor Volume Time Course over 21 days

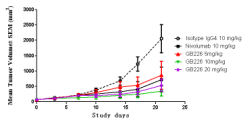


Figure 4. Body Weight Increase over Treatment Period

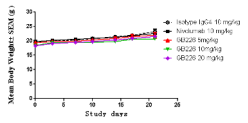
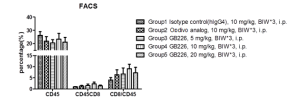
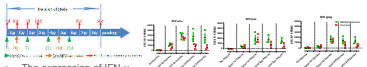


Figure 5. CD45+ and CD8+ cells measured in PBMCs



- Colon cancer MC38 isografts grow rapidly in PD-I HuGEMM mice.
- Tumor growth was significantly inhibited by intraperitoneal injection of CBT-501.
- Tumor growth of tumor-bearing mice in the 5, 10 and 20 mg/kg CBT-501 groups was significantly inhibited, with tumor inhibition rates (TIC) of 58, 83, and 74%.
- Nivolumab group at 10 mg/kg showed a tumor inhibition rate of 65%.
- Good correlation between tumor volumes and percent positive CD8/CD45 cells.

Figure 6. Effect on T-cell Response by INF-γ Spots using ELISPOT



- The expression of IFN-γ, TNF-α and IL-2 in CD4+ and CD8+ T-cells was significantly increased at 2 and 4 weeks after the immunization.

Receptor Occupancy and Pharmacokinetics

Figure 7. Single Dose Receptor Occupancy and Pharmacokinetics in Cynomolgus Monkey

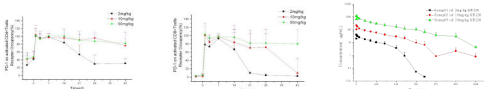
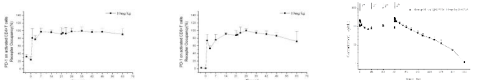


Figure 8. Multi-Dose (QW x 4) Receptor Occupancy and Pharmacokinetics in Cynomolgus Monkey



- CBT-501 occupancy in activated CD4+ and CD8+ T cells increases rapidly after a single dose. Upon 2nd dosing, the occupancy rises to nearly 100%. Six weeks after the last dose, occupancy on CD4+ and CD8+ cells does not decline significantly.
- Drug exposure was significantly dose-dependent. No statistical difference between the half-life and systemic clearance among the three dose levels with the effective half-life $t_{1/2}$ (115–142 hours).

Conclusions

CBT-501 demonstrated significant anti-tumor activity in the humanized genetically engineered mouse model (huGEMM). The tumor growth reduction was dose dependent with comparable or improved activity over nivolumab. The drug was well tolerated at all dose levels tested. Based on the strong nonclinical data and differentiation, and an acceptable safety profile, a first-in-human Phase 1 trial is planned in Australia (NCT03053466).

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Further Information

Please email Sanjeev.Redkar@cbtpharma.com or visit website at www.cbtpharma.com for a PDF version of the poster presentation.