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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Abstract

CBT-501 (Genolimzumab, or GB-225) is a novel humanized mAb (i.g., against human Programmed Death-1 (PD-1)) CBT-501 demonstrated highly specific binding to PD-1 of human (K\(_{d}\)=950 fm) and cynomolgus (K\(_{d}\)=2.2 mg). It did not completely block the binding of m.o.m. or centrifusorb, suggesting a novel escape. CBT-501 efficiently inhibited the binding of PD-1-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-\(\gamma\). The Fc-fragment bound to FcRn dose-dependently without any effector function. The PKPD 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 weeks after last dose. Single and repeat dose toxicity studies in cynomolgus showed weight gain of the thyroids 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 mice vaccinated with adenovirus-mediated Simian Immunodeficiency Virus, intravenously CBT-501 promoted the antigen-specific T-cell response, as shown by ELISPOT and intracellular staining of IL-2, INF-\(\gamma\) and TNF-\(\alpha\) in PBMC and T-cell subpopulation. CBT-501 injected i.p.3x=3x in the PD-1 HuGeRi isoform Model with Subcutaneous MC38 Mouse Colon Adenocarcinoma demonstrated significant antitumor activity. The tumor growth reduction was dose-dependent with comparable or improved activity over m.o.m., 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 in 1:2 and IFN-\(\gamma\) 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 HuGeRi age: 9 weeks at dosing. Male. The gene encoding humanmurine chimera 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) 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 (50 mg/kg qd) were conducted and receptor occupancy was determined by measuring CD4+ and CD8+ T-cells via FACS along with concomitant pharmacokinetic analysis.

Effect of CBT-501 vs. higmg in mice monkeys vaccinated with adenovirus SIV vaccine (ADDIV / SIV) and HIV vaccine (DJ111). ELISPOT was used to detect the frequency of IFN-\(\gamma\) spot formation in PBMCs to evaluate the antigen-specific cellular immune responses.

Table 1: Study Design

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Dose</th>
<th>Volume</th>
<th>Route</th>
<th>Regimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>2 mg</td>
<td>0.5 mL</td>
<td>i.m.</td>
<td>weekly</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>10 mg</td>
<td>0.5 mL</td>
<td>i.m.</td>
<td>weekly</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>50 mg</td>
<td>0.5 mL</td>
<td>i.m.</td>
<td>weekly</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>2 mg</td>
<td>0.5 mL</td>
<td>i.v.</td>
<td>weekly</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>50 mg</td>
<td>0.5 mL</td>
<td>i.v.</td>
<td>weekly</td>
</tr>
</tbody>
</table>

Legend: i.p. = intraperitoneal injection; WD = weekly

Conclusion

CBT-501 demonstrated significant antitumor activity in the humanized genetically engineered mouse model (hGeRM). The tumor growth reduction was dose-dependent with comparable or improved activity over m.o.m. 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 (NCT0353466).

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Further Information

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