Preclinical Characterization of GLS-010 (AB122): A Fully Human Clinical-Stage anti-PD-1 Antibody

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INTRODUCTION

Exhausted T cells express high levels of several immune checkpoint proteins, including the programmed death-1 (PD-1) receptor. Preclinical and clinical data support the role of the PD-1/PD-L1 axis in promoting tumor evasion by curtailing immune responses. We present here the preclinical characterization of GLS-010 (AB122), a novel fully human anti-PD-1 monoclonal antibody currently in Phase 1.

METHODS

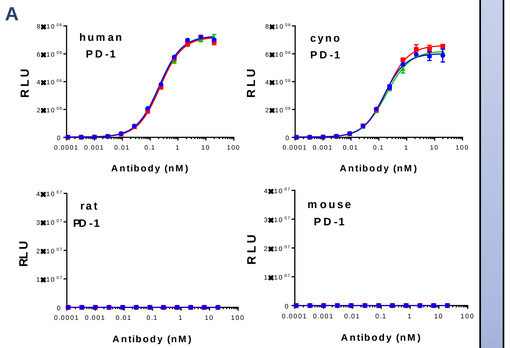
The affinity of GLS-010 (AB122) for human and cynomolgus monkey PD-1, its specificity for PD-1, and its ability to block the PD-1 interaction with PD-L1 and PD-L2 were measured by ELISA, flow cytometry and TCR-activated reporter gene assays. Functional assessment of GLS-010 (AB122) on T cell responses (IFN-γ, IL-2, and proliferation) was performed using mixed lymphocyte reaction (MLR) and re-stimulation with cytomegalovirus (CMV) pp65 peptide. The anti-tumor efficacy of GLS-010 (AB122) was evaluated using a mouse MC-38 tumor model grown in mice transgenic for human PD-1. For PK analysis, GLS-010 (AB122) was given in a single i.v. bolus to male and female cynomolgus monkeys at doses of 2, 6 and 18 mg/kg.

RESULTS AND CONCLUSION

GLS-010 (AB122) is a fully human IgG4 monoclonal antibody that binds to human PD-1 (EC50 ~ 210 pM, ELISA; 770 pM, flow cytometry), cyno PD-1 ((EC50 ~ 150 pM, ELISA), but not rat or mouse PD-1. Lack of GLS-010 (AB122) binding to other related members of the CD28 family, such as ICOS, CD28 and CTLA-4, confirms the specificity of the interaction. Functional studies showed that binding of GLS-010 (AB122) to cell-expressed hPD-1 inhibits the interaction with both hPD-L1 and hPD-L2 with an IC50 of 580 pM and 670 pM, respectively (by flow cytometry) and 2.2 nM and 5.8 nM, respectively (in reporter gene assays). Using allogeneic monocyte-derived dendritic cells, we showed a dose-dependent enhancement of IFN-γ production and proliferation by $CD4^+$ T cells, saturating at concentrations below 100 pM. Similar results were obtained in an antigen-specific T cell recall response assay using CMV-infected donors. GLS-010 (AB122) was very effective at blocking MC-38 tumor growth in hPD-1 transgenic mice. PK profiles following a single i.v. dose of GLS-010 (AB122) administered to male and female cynomolgus monkeys were dose-proportional and the rate of clearance was dose-independent.

GLS-010 (AB122) is a novel and selective antagonistic anti-hPD-1 antibody that potently blocks the interaction of human PD-1 with both PD-L1 and PD-L2. This blockade translates into potent enhancement of T cell activation in a variety of cell culture studies, which combined with its in vivo profile (in mice and monkeys) supports its ongoing clinical development in oncology.

GLS-010 (AB122) IS A HIGH AFFINITY BLOCKING ANTIBODY THAT IS EQUIPOTENT AGAINST HUMAN AND CYNO PD-1



IC_{50} (nM)

	GLS-010 (AB122)	Pembrolizumab	Nivolumab
Human PD-1	0.21	0.23	0.22
Cyno PD-1	0.15	0.19	0.19

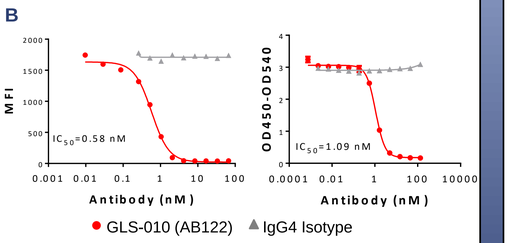


Figure 1: Binding of GLS-010 (AB122), Nivolumab, and Pembrolizumab to plate coated recombinant human, cyno, mouse, and rat PD-1 proteins were assessed by ELISA (A). Functionality of GLS-010 (AB122) in blocking human or cyno PD-1/PD-L1 interactions were assessed by flow cytometry or ELISA respectively.

SPR ANALYSIS

Ligand	k_a (1/Ms)	K_d (1/s)	K_D (M)	Rmax (RU)
GLS-010 (AB122)	1.12E+06	1.96E-04	1.75E-10	54.14
Nivolumab	7.76E+05	8.97E-04	1.16E-09	68.9

Figure 2: GLS-010 (AB122) and Nivolumab were immobilized on protein A/G chip. Soluble hPD-1.ECD.His was used as analyte

SELECTIVITY OF GLS-010/AB122 AGAINST CD28, ICOS, AND CTLA-4

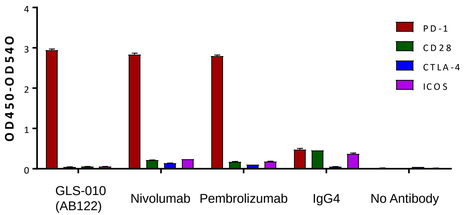


Figure 3: Binding of 66.7 nM of GLS-010 (AB122), Nivolumab, Pembrolizumab, and IgG4 to plate coated PD-1.mFc, CD28.mFc, CTLA-4.His, or ICOS.mFc was assessed by ELISA. Assay background was determined using no antibody wells.

GLS-010 (AB122) EFFECTIVELY BLOCKS SUPPRESSION OF TCR ACTIVATION MEDIATED BY PD-1/PD-L1 OR PD-1/PD-L2 INTERACTION

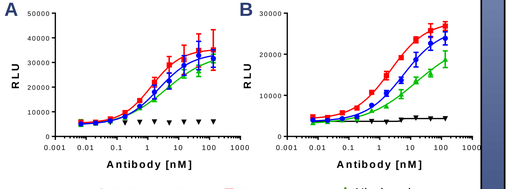


Figure 4: Functionality of GLS-010 (AB122) in blocking PD-1/PD-L1 (A) and PD-1/PD-L2 (B) interactions were measured using NFAT-response element (NFAT-RE) luciferase reporter cells (Promega Bioassay kit).

GLS-010 (AB122) ENHANCES T CELL ACTIVATION

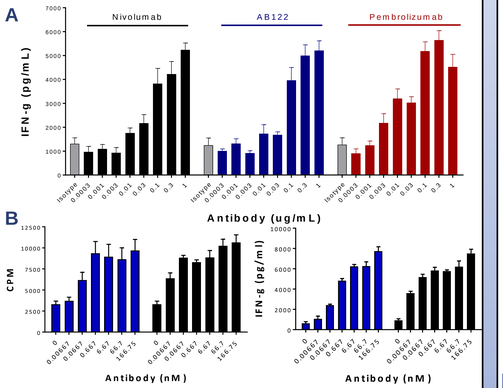
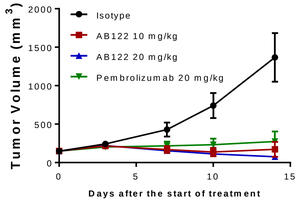


Figure 5: Functionality of GLS-010 (AB122) in enhancing T cell responses were assessed using (A) allogeneic Mixed Lymphocyte Reaction (MLR) and (B) CMVpp65-specific T cell recall responses. T cell proliferation (^{3}H-TDR incorporation) and effector function (IFN-γ production) were quantified. Ab denotes antibody

GLS-010 (AB122) IS EFFECTIVE IN REGULATING TUMOR GROWTH



	Tumor-free Mice
Isotype	0/8
GLS-010 (AB122) 10 mg/kg	5/8
GLS-010 (AB122) 20 mg/kg	5/8
Pembrolizumab 20 mg/kg	4/8

Figure 6: The efficacy of AB122 was evaluated in hPD-1 knock-in model with subcutaneous MC38 mouse colon adenocarcinoma. All antibodies were administered IP twice a week for 3 weeks starting at 150 mm^3 tumor volume.

PHARMACOKINETIC ANALYSIS OF GLS-010 (AB122) IN CYNOMOLGUS MONKEYS

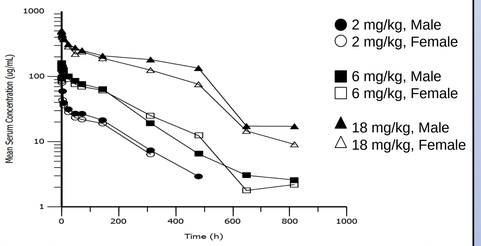


Figure 7: Naïve male and female cynomolgus monkeys (3 per gender for a total of 6 per dose group) were administered with the indicated concentration of GLS-010 (AB122).

PRE-CLINICAL SUMMARY OF GLS-010 (AB122)

Assay	GLS-010/AB122	Nivoumab	Pembrolizumab
Human Binding FACS (EC50, nM)	2.1	3.1	N/A
Cynomolgus cross reactivity	Yes	Yes	Yes
Mouse cross reactivity	Negative	Negative	Negative
Homolog activity	None	None	None
Human PD-1/PD-L1 blocking (IC50, nM)	0.13	0.10	0.11
Human PD-1/PD-L2 blocking	Yes	Yes	Yes
Human PD-1 binding affinity by SPR (M)	1.75E-10	1.16E-09	N/A
Epitope binning	Same or Overlapping		
Increase T cell activation in human allogeneic MLR	Comparable for all 3		
Increase T cell activation in human antigen specific response	Comparable for both molecules		N/A
Reversal of T_{reg} suppression	Comparable for both molecules		N/A
ADCC test	Negative	Negative	N/A
CDC test	Negative	Negative	N/A

REFERENCES:

Pembrolizumab: CAS 1374853-91-4

Nivolumab: CAS 946414-94-4