Preclinical Characterization of a Novel Fully Human IgG1 Anti-PD-L1 mAb CK-301

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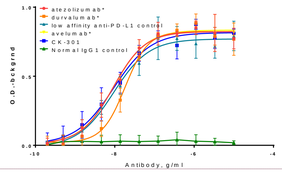
Abstract

Antibodies targeting Programmed Death-1 (PD-1), or its ligand, PD-L1, have demonstrated remarkable efficacy in subsets of cancer patients, with inhibition of the interaction between PD-1 on T-cells and PD-L1 on tumor cells leading to the recovery of anti-tumor immune response and immune-mediated eradication of tumors. However, not all patients respond to existing PD-1 and PD-L1 targeting agents and relapses to therapy still occur. Therefore, there exists a need to identify additional therapeutics and approaches to engage the immune system to enhance the efficacy of current anti-cancer therapies. Using phage and yeast display approaches, we have discovered and optimized a novel, fully human PD-L1 specific IgG1 antibody, CK-301, which exhibits sub-nanomolar binding affinity for PD-L1. CK-301 blocks binding of PD-L1 to both PD-1 and B7-1 in enzyme-linked immunosorbent assays (ELISA) and cell-based competition assays. Using an assay measuring inhibition of a nuclear factor of activated T-cells (NFAT) reporter caused by PD-L1 binding to PD-1, we demonstrate that CK-301 completely reverses reporter inhibition at concentration of less than 1 µg/ml, IC50 of the dose response curve is 80ng/ml. CK-301 enhances IFN-gamma secretion in allogeneic mixed lymphocyte reaction (MLR) using primary human T-cells and immature dendritic cells. CK-301 can also trigger antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) mediated killing of PD-L1+ cell lines, including lymphoma cells. CK-301 has similar sub-nanomolar affinity for cynomolgus monkey PD-L1 as for human PD-L1, hence we chose Macaca fascicularis for pre-clinical toxicology and safety pharmacology studies. Single dose administration of CK-301 to monkeys up to the highest tested dose of 100 mg/kg was shown to be safe and demonstrated linear dose-dependent pharmacokinetic (PK) properties over the dose range from 1 to 100 mg/kg with a half-life of 15 days at 100 mg/kg. A first-in-human Phase 1 study of CK-301 is planned to commence in mid-2017.

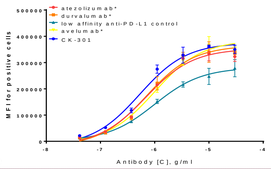
High affinity binding of CK-301 to huPD-L1

Target Protein	Antibody	KD (M)	kon(1/Ms)	kdis(1/s)
huPDL1	CK-301	8.47E-10	7.20E+05	6.10E-04
cynoPDL1	CK-301	5.55E-10	1.14E+06	6.35E-04
huPDL1	atezolizumab*	2.02E-09	4.52E+05	9.11E-04
cynoPDL1	atezolizumab*	8.95E-09	6.10E+05	5.46E-03

ELISA on PD-L1 coated plates



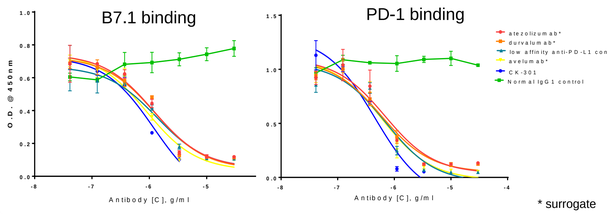
FACS with PD-L1+ cells



* surrogate

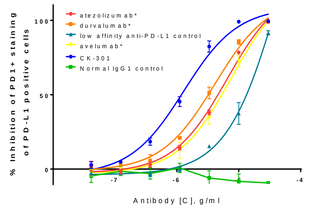
CK-301 shows strong competition with PD-1 and B7.1 for binding to PD-L1

A **Competition ELISA on PD-L1 coated plates**



* surrogate

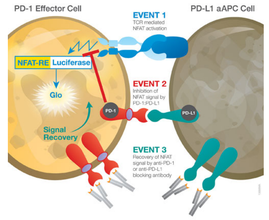
B **Competition FACS PD-1 on PD-L1+ cells**



A. PD-L1 coated plates were incubated with 0.6 µg/ml biotinylated PD1-Fc or 7.5 µg/ml biotinylated B7.1-Fc in the presence of antibodies followed by detection of PD-1 or B7.1 bound to the plates with streptavidin-HRP.

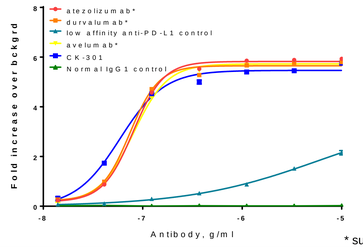
B. PD-L1+ cell line was pre-incubated with 2 µg/ml of biotinylated PD1-Fc followed by incubation with antibodies and staining for PD-1 on cells by FACS.

CK-301 reverses NFAT inhibition caused by PD-L1/PD1 interaction



Promega homogeneous PD-1/PD-L1 blockade bioassay

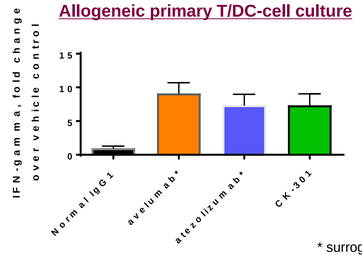
- Event 1: TCR-mediated NFAT activation occurs when engineered Jurkat PD-1 Effector cells and aAPC (artificial antigen presenting cell) PD-L1 cells are engaged through TCR/TCR activator interaction.
- Event 2: Inhibition of NFAT signal by PD-1:PD-L1 ligation when no blocking antibodies are present.
- Event 3: Recovery of NFAT signal by addition of anti-PD-1 or anti-PD-L1 blocking antibody.



* surrogate

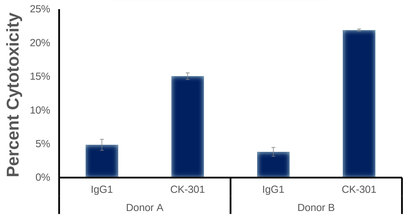
CK-301 increases IFN-γ production by primary T-cells in MLR culture

A **Allogeneic primary T/DC-cell culture**



* surrogate

B **ADCC with SU-DHL-1 cells**



A. Primary T-cells isolated from PBMCs were incubated with PBMC derived immature monocyte dendritic cells in the mixed lymphocyte reaction (MLR). Results are presented as fold increase in IFN-γ production relative to no-antibody control. An average of cultures with cells from 6 different donors is shown.

B. PBMCs from 3 different donors were incubated with PD-L1+ cell line SU-DHL-1 (10:1) in the presence of CK-301 or control antibody for 3 hrs. Level of cytotoxicity was measured with the help of LDH release assay.

CK-301 PK in cynomolgus monkeys

Dose Level (mg/kg)	C_{max} (µg/mL)	DN [(µg/mL)/(mg/kg)]	AUC_{0-168} (µg·hr/mL)	DN [(µg·hr/mL)/(mg/kg)]	$t_{1/2}$ (hr)
1	21.2	21.2	1540	1540	128
10	221.0	22.1	17400	1740	100
100	2370.0	23.7	188000	1880	361

Summary

- CK-301 is a high affinity PD-L1 specific fully human IgG1 antibody. CK-301 blocks binding of PD-L1 to PD-1 and B7.1 and is capable of reversing PD-L1 mediated inhibition of T-cell function(s).
- Activity of CK-301 in all assays performed was similar to that of the surrogate antibodies produced in 293HEK cells from the sequences of avelumab, atezolizumab or durvalumab.
- CK-301 has functional Fc domain and is capable of inducing ADCC mediated killing of PD-L1+ tumor cell lines similar to avelumab (but not atezolizumab or durvalumab).
- IND-enabling GLP toxicology studies and GMP manufacturing are substantially complete to support a first-in-human Phase 1 study of CK-301, planned to commence in mid-2017.

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