

Abstract
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Leonid Gorelik¹, George Avgerinos¹, Yune Kunes², Wayne A. Marasco³

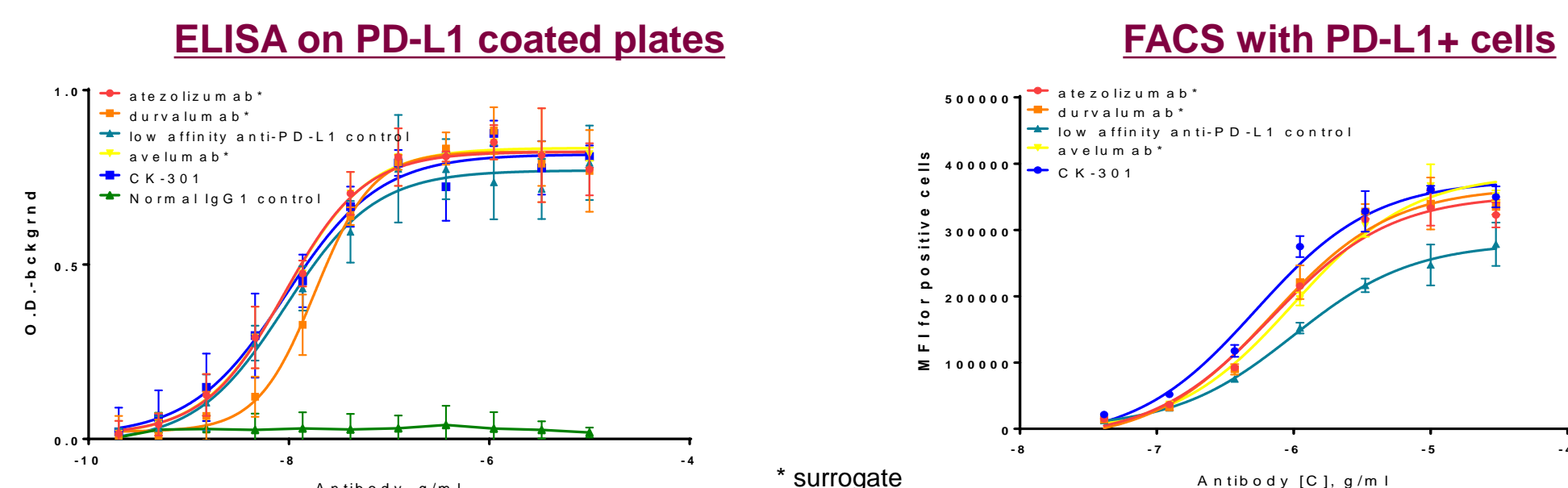
¹Checkpoint Therapeutics, Inc., New York, NY; ²TG Therapeutics, New York, NY; ³Dana-Farber Cancer Institute, Boston, MA.

Abstract

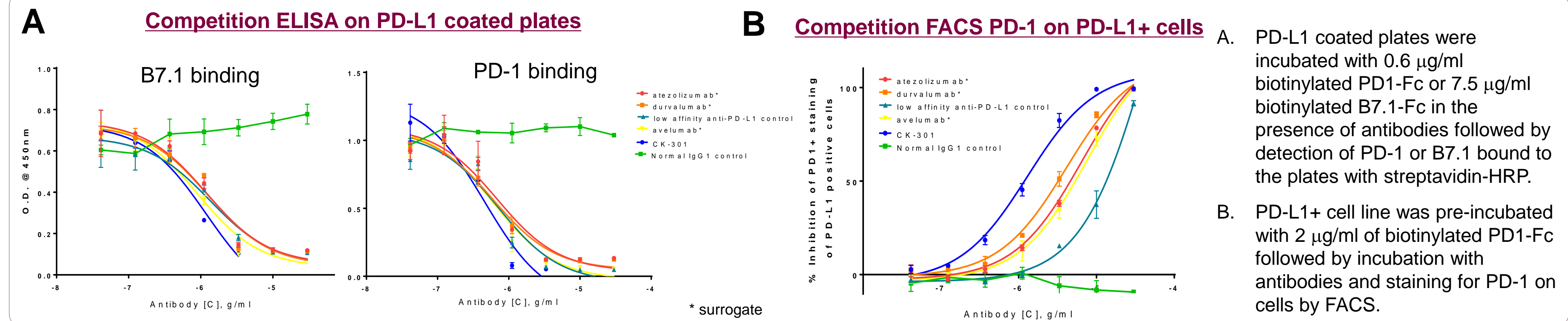
Antibodies targeting Programmed Death-1 (PD-1), or its ligand, PD-L1, have demonstrated remarkable efficacy in subsets of cancer patients, with inhibition of the interaction between PD-1 on T-cells and PD-L1 on tumor cells leading to the recovery of anti-tumor immune response and immune-mediated eradication 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-1 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

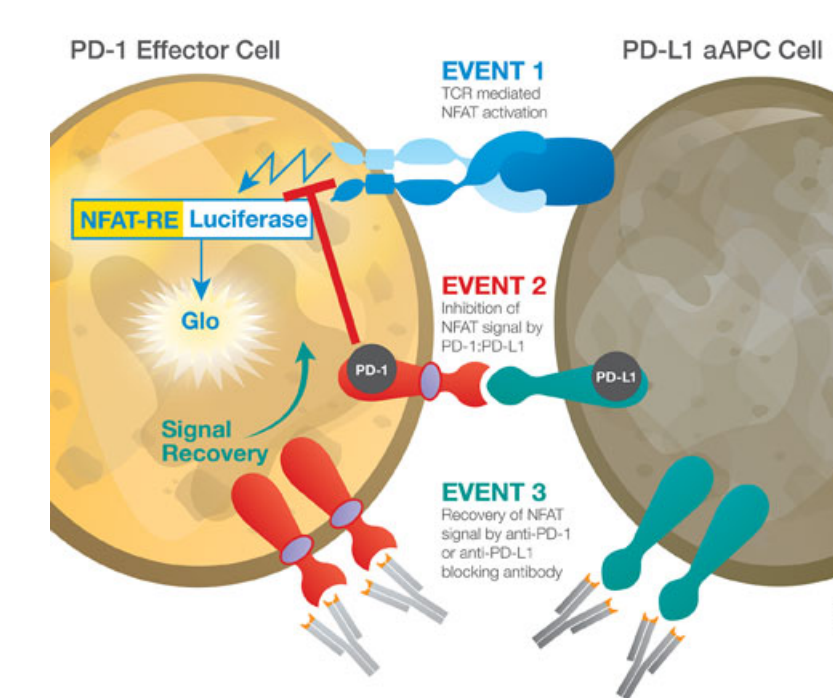
Target Protein	Antibody	KD (M)	kon(1/Ms)	kdis(1/s)
huPDL1	CK-301	8.47E-10	7.20E+05	6.10E-04
cynoPDL1	CK-301	5.55E-10	1.14E+06	6.35E-04
huPDL1	atezolizumab*	2.02E-09	4.52E+05	9.11E-04
cynoPDL1	atezolizumab*	8.95E-09	6.10E+05	5.46E-03



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

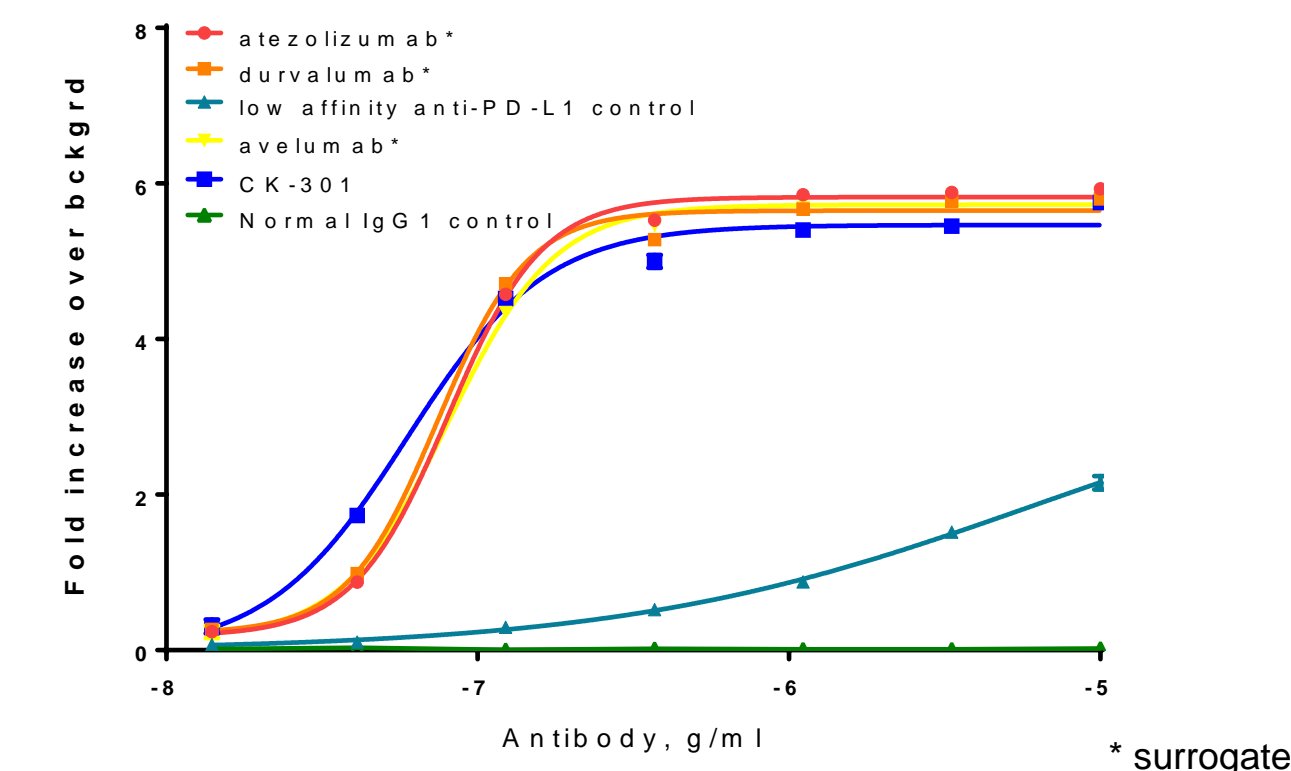


CK-301 reverses NFAT inhibition caused by PD-L1/PD1 interaction

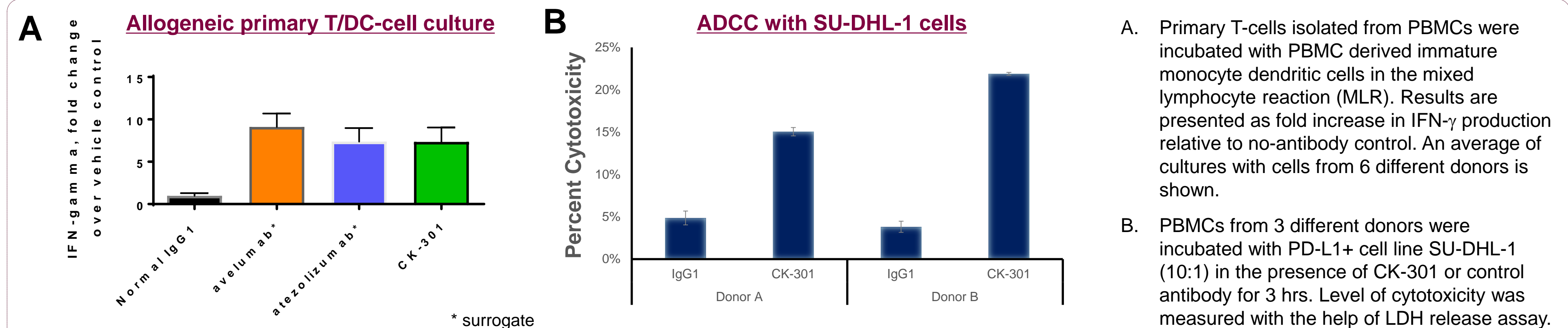


Promega homogeneous PD-1/PD-L1 blockade bioassay

- ❖ 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.



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



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.

Acknowledgements & Disclosures

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