‘Agonist Redirected Checkpoint’ Platform (ARC); Engineering Bi-Functional Fusion Proteins (PD1-Fc-OX40L), for Cancer Immunotherapy

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Abstract

PD1-Fc-OX40L is a first-in-class biologic derived from the ***Agonist Redirected Checkpoint (ARC™)*** platform developed by Shattuck Labs. The ARC platform was developed to solve a fundamental challenge in cancer immunotherapy, which was to develop combination therapeutics that could block immune checkpoints (such as PD-1), while simultaneously activating Tumor Necrosis Factor (TNF) SuperFamily Receptors (such as OX40, 4-1BB, GITR and CD40).

Pre-clinical development and characterization has been completed, and demonstrated that **PD1-Fc-OX40L** is a potent immune agonist both *in vitro* and *in vivo*. The ARC binds immobilized PD-L1, PD-L2, and OX40 at 2.08, 1.76, and .246 nM affinities, respectively, and binds the respective ligands/receptor on cells *in vitro* and *ex vivo*. High binding affinity on both sides of the construct translated to potent stimulation of OX40 signaling and PD1:PD-L1/L2 blockade, in multiple *in vitro* assays, including improved potency as compared to pembrolizumab, nivolumab, tavolixizumab and combinations of those antibodies. Furthermore, when activated human T cells were co-cultured with PD-L1 positive human tumor cells, **PD1-Fc-OX40L** was observed to concentrate within the immune synapse, which enhanced proliferation of T cells and production of IL-2, IFNγ and TNFα; leading to efficient killing of tumor cells. The therapeutic activity of **PD1-Fc-OX40L** in established murine tumors was superior to PD1 blocking, OX40 agonist, or combination antibody therapy. Importantly, all agonist functions of **PD1-Fc-OX40L** are independent of Fc receptor cross-linking, due to the inherent trimeric and hexameric structure of **PD1-Fc-OX40L**.

The human drug product of PD1-Fc-OX40L, SL-279252, has completed both pre-clinical and non-clinical development, and a phase I trial in human cancer patients will begin by early 2019.

ARC Platform / Mechanism of Action

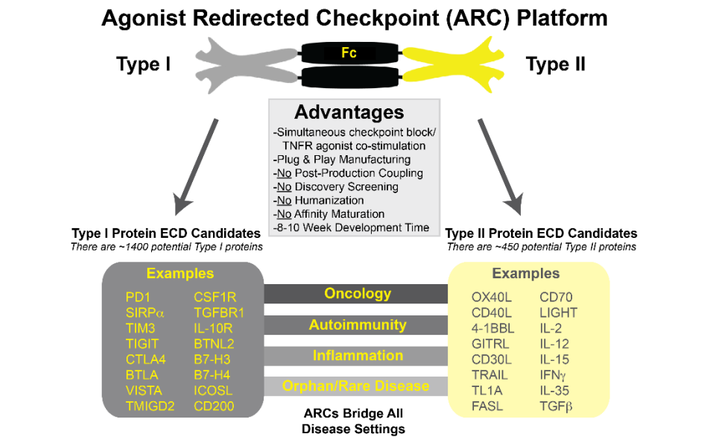


Figure 1. ARC Platform. Advantages, examples of Type I/Type II target combinations, and relevant disease indications.

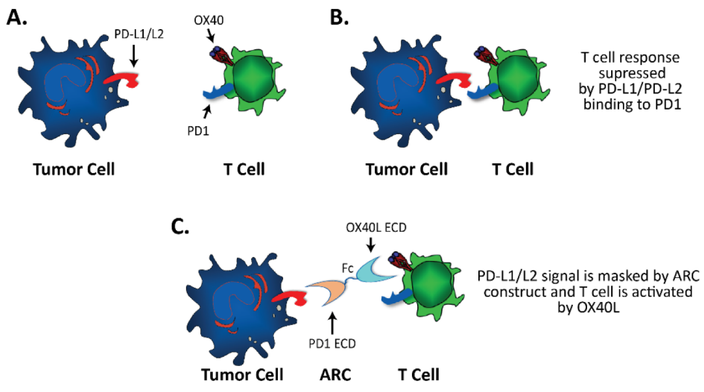
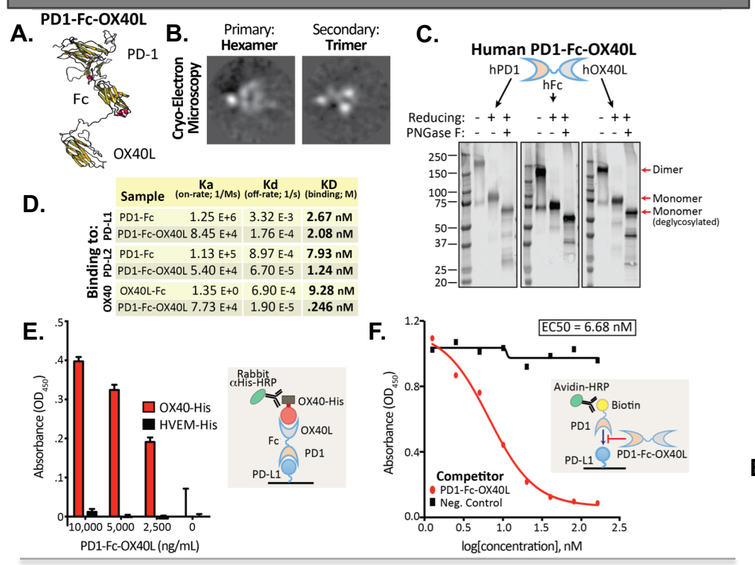
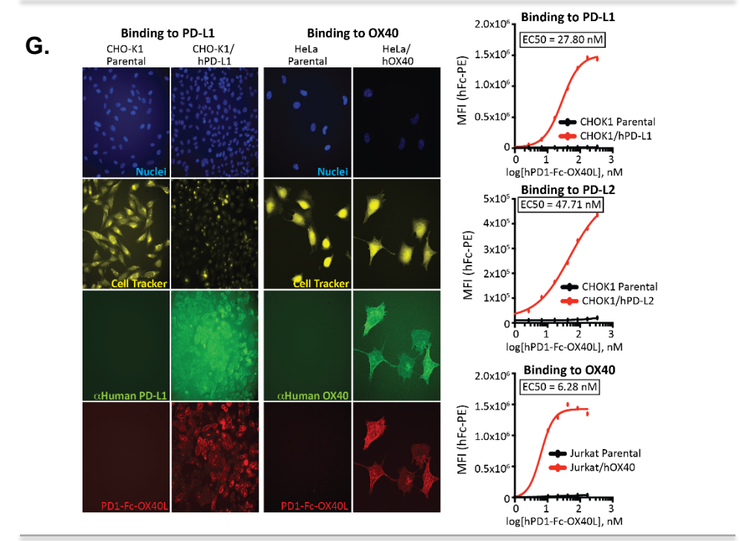


Figure 2. PD1-Fc-OX40L Mechanism of Action. (A) Immune suppressive signaling via PD-L1/PD-L2 can be blocked by the (B) PD1 domain of the ARC, while (C) simultaneously agonizing T cells via OX40L, at the same time T cells encounter tumor antigens – a MOA that the separate administration of two distinct antibodies cannot recapitulate.

In Vitro Characterization and Activity





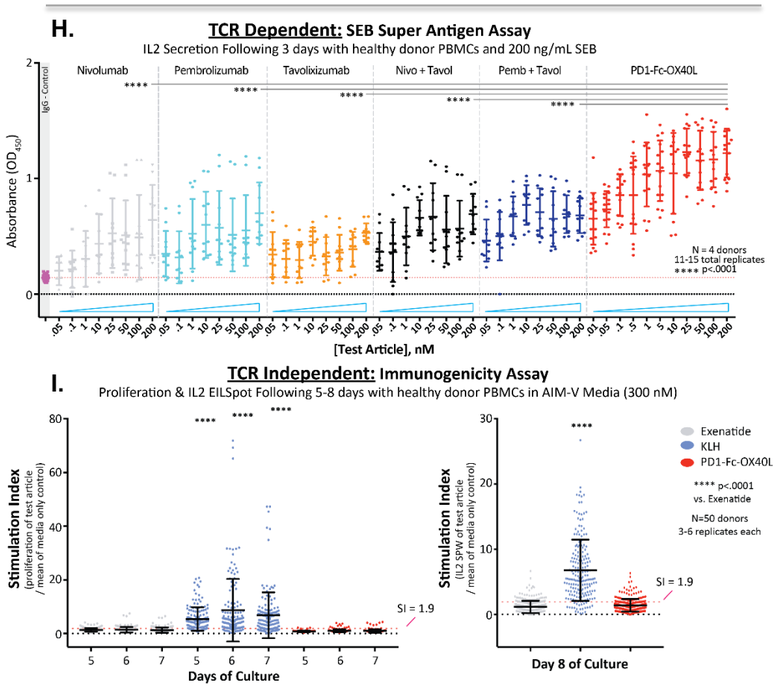
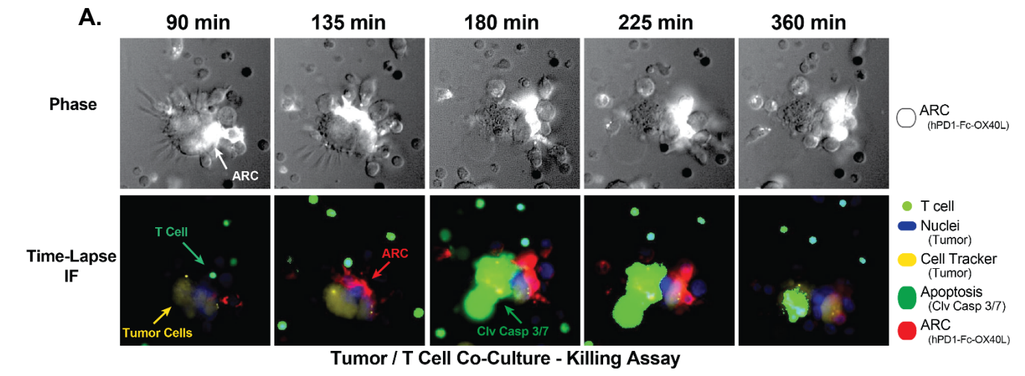


Figure 3. ARC Characterization (A) Structure analysis by RaptorX and (B) cryo-EM. (C) Western blot for all ARC domains. (D) On-/Off- rates and binding affinities (KD by SPR). (E) Dual ELISA detecting simultaneous PD-L1 and OX40 binding (F) ELISA-based PD1 competition assay. (G) Cell membrane binding of PD1-Fc-OX40L to over-expressing cell lines, detected by IF (left) and flow cytometry (right). (H) SEB super-antigen IL-2 stimulation assay in human PBMCs treated with a titration of PD1-Fc-OX40L or clinical antibody equivalents. (I) TCR-independent proliferation (left) and IL-2 ELISpot (right) in CD8-depleted PBMCs from 50 donors, compared to a neoantigen positive control (KLH) and an example of a clinical-stage, non-immunogenic molecule (Exenatide).

MOA, Immune Profiling and Anti-Tumor Efficacy



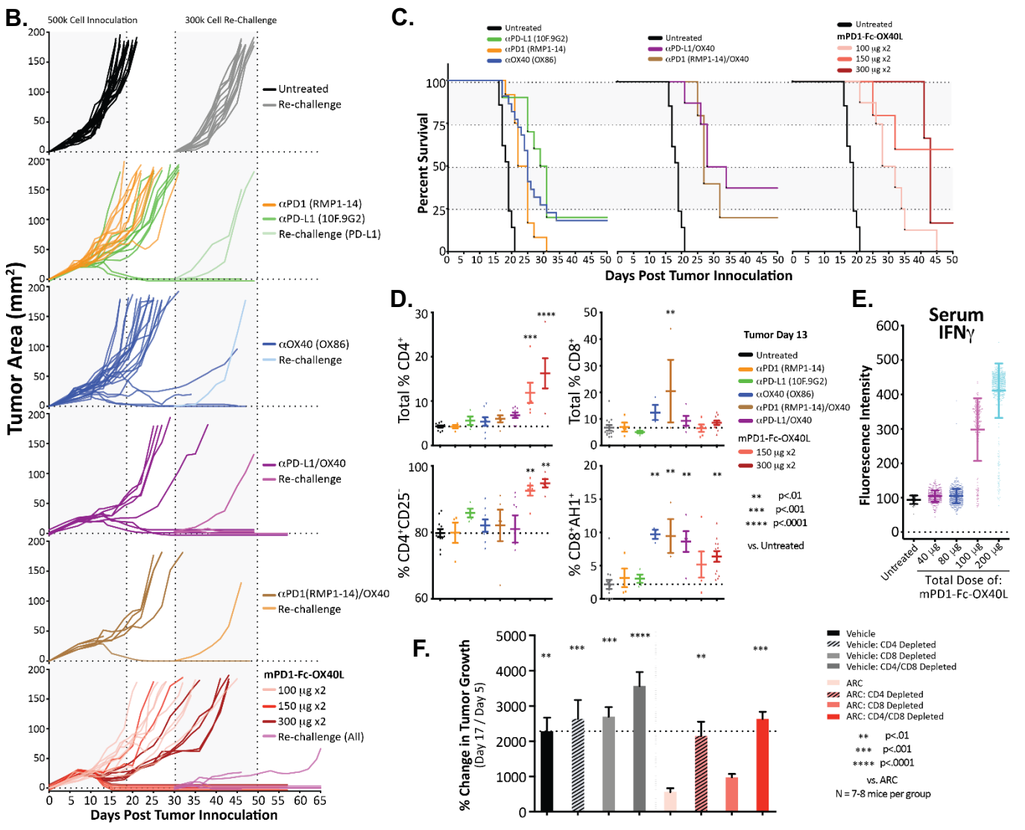


Figure 4. *In vitro* tumor killing activity, *in vivo* immune-profiling and anti-tumor efficacy (CT26). (A) Human NCI-H2023 cells were co-cultured with stimulated T cells. Tumor cells (yellow; nuclei in blue), T cells (green), PD1-Fc-OX40L ARC (red), and a cleaved caspase 3/7 reporter (green) were imaged over 6 hrs using time-lapse live-cell IF imaging. (B) 500k CT26 cells were inoculated on the rear flank, indicating day 0. Mice were treated with antibodies or ARC on days 4 and 6 (IP), when tumors were ~4-6 mm in diameter. Curves show growth with individual mice. On day 30, mice that rejected primary tumors, were inoculated with a secondary tumor on the opposing rear flank, without further treatment. (C) Overall survival through day 50 of the time-course. (D) A cohort of mice were sacrificed 9 days after treatment to assess CD4+/CD25- effector cells and CD8+AH1+ antigen-specific cells in the tumor, and (E) serum cytokine levels of IFNγ. (F) In a similar experiment, mice were pre-treated with CD4, CD8, or both CD4/CD8 depleting antibodies on days -1, 1, and 10. Tumors were inoculated on day 0 and mice were treated with 2 doses of 300 µg ARC on days 4 & 6.

Summary

-***ARCs*** are a novel class of bi-functional biologics capable of targeting type-I and type-II membrane proteins, and can target all checkpoint molecules and the entire family of TNFR superfamily receptors.

-**>180 ARCs** have been synthesized/characterized by Shattuck to date.

-**PD1-Fc-OX40L** can link checkpoint-blocking and T cell co-stimulation signals in the same microenvironment, at the time in which T cells are engaging cognate tumor antigens.

-**PD1-Fc-OX40L** has completed NHP GLP tox studies, is on track for a Nov 2018 IND submission, and is anticipated for first-in-man treatment Q1 2019.