‘Agonist Redirected Checkpoint’ Platform (ARC); Engineering Bi-Functional Fusion Proteins (SIRPα-Fc-CD40L), for Cancer Immunotherapy

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Abstract

A majority of clinical responses to PD1/L1 blockade occur in patients with abundant intratumoral PD-L1 expression and lymphocyte infiltration, suggesting that additional efficacy may be found in combination therapies that increase either of these variables. Here we report the generation of a novel, two-sided human fusion protein (***Agonist Redirected Checkpoint, ARC™***), incorporating the ECDs of SIRPα and CD40L, adjoined by a central Fc domain; termed **SIRPα-Fc-CD40L.** This compound was designed to simultaneously enhance antigen uptake and cross-presentation (CD47 axis) and enhance antigen presenting cell maturation and function (CD40 axis), with a single compound.

Human and mouse **SIRPα-Fc-CD40L** were produced and characterized using a range of biochemical assays to determine MW, subunit composition & binding affinity; molecular assays to characterize *in vitro*/*ex vivo* binding, *in vitro* functional activity; and anti-tumor efficacy in multiple syngeneic tumor model systems.

The ARC binds immobilized CD47 and CD40 at 0.628 and 4.74 nM affinities. The CD47 domain binds human tumor cells and the CD40L domain binds primary B cells and dendritic cells (DC), but importantly, did not bind human platelets, RBCs, nor induce hemolysis. **SIRPα-Fc-CD40L** stimulated Fc receptor-independent NFkB-luciferase signaling and induced both TCR- dependent & independent cytokine secretion from human PBMCs. Furthermore, when activated human DCs or macrophages were co-cultured with CD47+ human tumor cells, **SIRPα-Fc-CD40L** enhanced phagocytosis, and induced rapid activation of CD4+ & CD8+ DCs *in vivo*. Finally, the therapeutic activity of SIRPα-Fc-CD40L in established murine tumors was superior to CD47-blocking antibody, CD40-agonist antibody, and combination antibody therapy. Interestingly, anti-tumor response was heightened significantly when **SIRPα-Fc-CD40L** was combined with antibody blockade of CTLA4 or PD1.

ARC Platform / Mechanism of Action

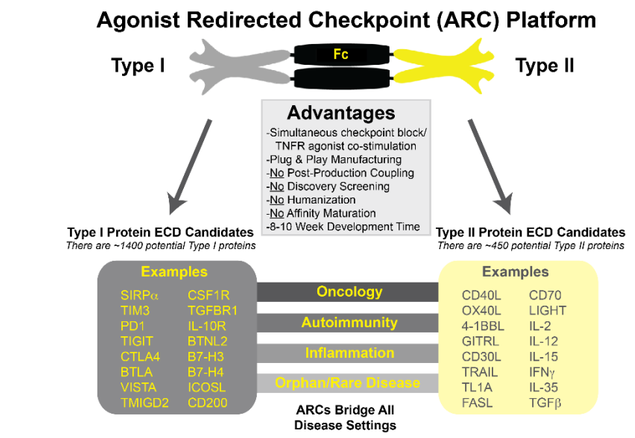
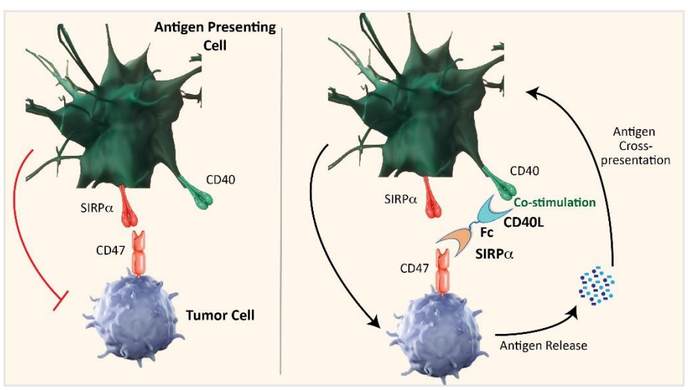


Figure 1. ARC Platform. Advantages, examples of Type I/Type II target combinations, and relevant disease indications.



CD47/SIRPα Ligation
-Prevents tumor recognition and phagocytosis (‘don’t eat me’ signal)

SIRPα-Fc-CD40L
-Binds CD47, blocks APC interaction
-Promotes tumor phagocytosis and antigen release
-Antigens are cross-presented to APCs at the same time as CD40/L co-stimulation

Figure 2. SIRPα-Fc-CD40L Mechanism of Action.

In Vitro Characterization, Function & Safety

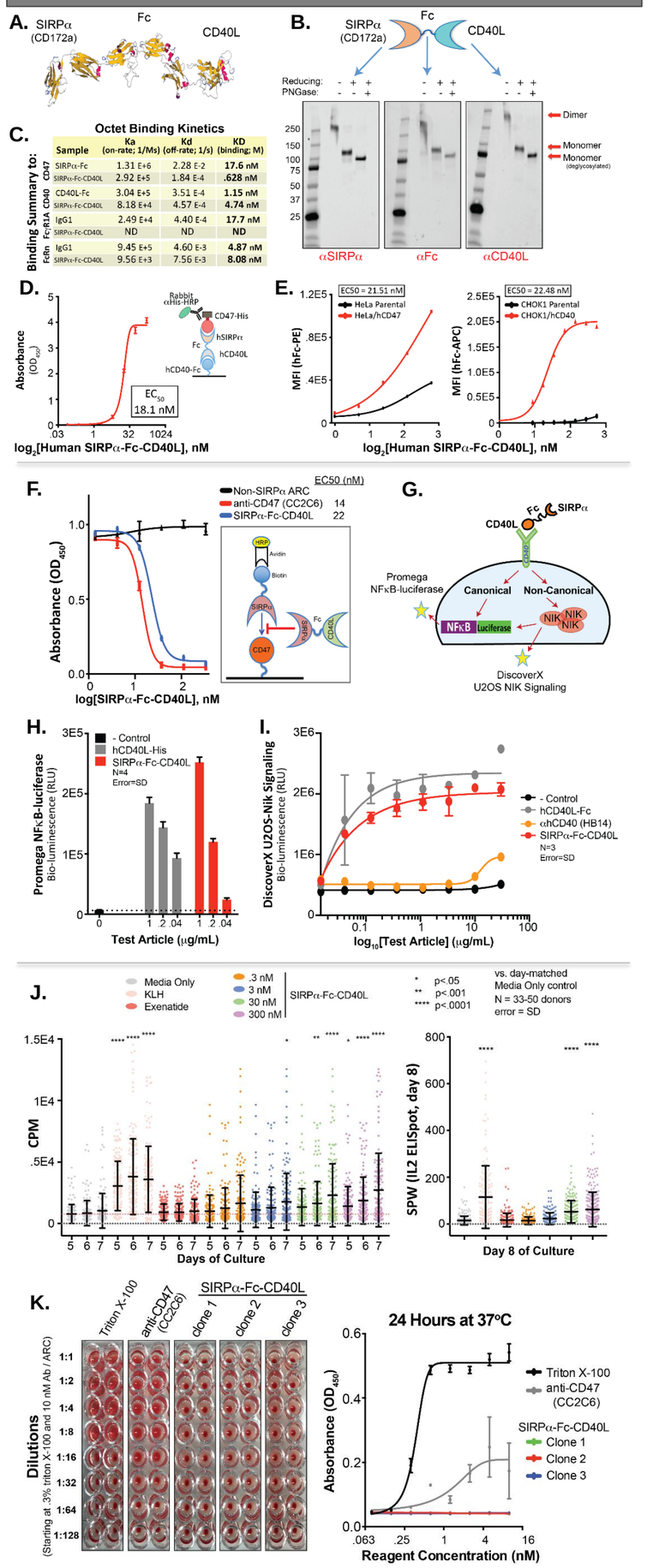


Figure 3. ARC Characterization (A) Tertiary structure prediction (RaptorX). (B) Western blot for all ARC domains. (C) On-/Off- rates and binding affinities (SPR). (D) Dual function ELISA. (E) Cell surface binding to over-expressing cell lines by flow cyt. (F) ELISA competition assay. (G) Schematic of (H) canonical and (I) non-canonical NFκB- signaling assays. (J) TCR-independent proliferation (left) and IL-2 ELISpot in CD8 depleted PBMCs from 33-50 donors, compared to a neoantigen control (KLH) and a clinical stage control molecule (Exenatide). (K) Hemolysis assay visualized in a 96 well plate (left) and quantitated (right).

In Vivo Immune Profiling and Anti-Tumor Efficacy

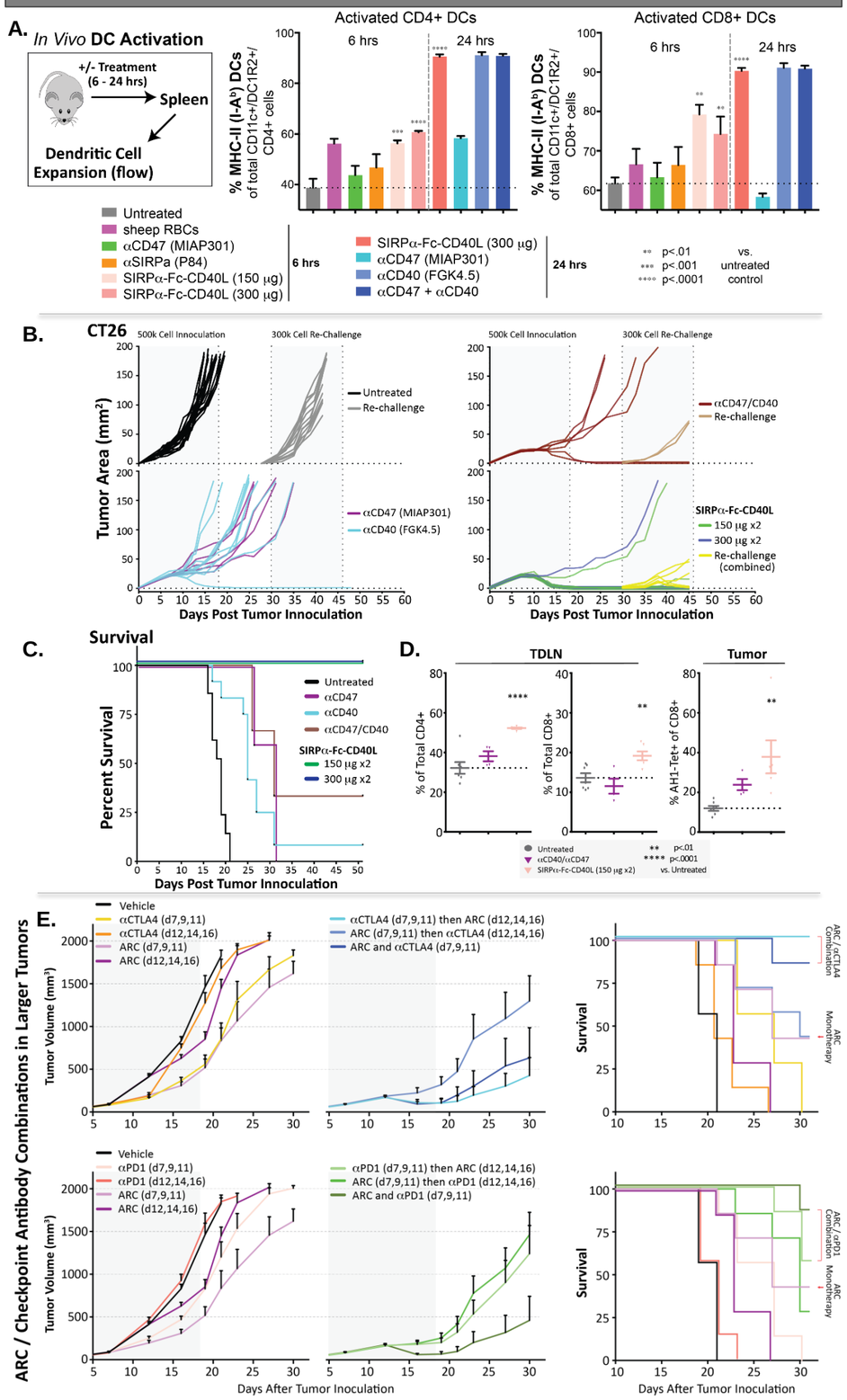


Figure 4. *In vivo* Activity (A) *In vivo* DC expansion/activation in spleens from treated mice. (B) CT26 tumor efficacy with 2 treatments starting at tumor volumes of **30-60 mm³**. On day 30, surviving mice were re-challenged with a 2nd tumor, with no additional treatment. (C) 50-day survival and (D) Immune phenotyping on day 13 after tumor inoculation, showing $CD4^+/CD8^+$ populations within tumor draining lymph nodes (TDLN) and antigen-specific $CD8^+$ cells ($AH1^+$) within the tumor. (E) CT26 tumor efficacy with 3 treatments of ARC and/or antibodies on days shown; at larger starting tumor volumes of **85-130 mm³**. Shown are various sequencing combinations with anti-CTLA4 (9D9; top) or anti-PD1 (RMPI-14; bottom).

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.

-**SIRPα-Fc-CD40L** can stimulate both the innate and adaptive immune systems, promoting dendritic cell activation/co-stimulation, phagocytosis, tumor antigen presentation/processing, and increased antigen-specific anti-tumor response.