‘Agonist Redirected Checkpoint’ Platform (ARC); Engineering Bi-Functional Fusion Proteins (TIM3-Fc-OX40L and TIM3-Fc-CD40L), for Cancer Immunotherapy

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Abstract

Current combination immunotherapy with bispecific modalities have not been able to both block checkpoints and agonize TNF receptors, due to loss in target avidity when engineered to bind multiple targets with monovalent antigen binding arms. Fusion proteins incorporating the extracellular domain (ECD) *of type I membrane proteins* (eg. Enbrel, Orencia) or *type II membrane proteins* (eg. OX40L-Fc, GITRL-Fc), linked to an Fc of antibodies are functional, despite the ECDs being in opposite orientation. We report the generation of two-sided fusion proteins incorporating the ECD of TIM3 and the ECD of either OX40L or CD40L, adjoined by a central Fc domain.

Shattuck synthesizes both murine and human versions of ***ARC proteins***, and assesses them using various biochemical, molecular, *in vitro* functional, and *in vivo* tumor efficacy experiments.

The TIM3 end of the fusion proteins bind GAL9 and phosphtidylserine (PS) on human tumor cells. The OX40L/CD40L ends bind OX40 and CD40, respectively, on the surface of primary PBMCs. Both TIM3-Fc-OX40L & TIM3-Fc-CD40L activate NFkB signaling in cells engineered to overexpress OX40 or CD40 and an NFkB-luciferase reporter, and also in NIK signaling reporter cells. Each TIM3-containing ARC induces a unique cytokine signature in PBMCs incubated with the super-antigen Staphylococcal enterotoxin B (SEB), or when cultured in AIMV media. *In vivo*, the therapeutic activity of TIM3-Fc-OX40L and TIM3-Fc-CD40L in established murine CT26 tumors was superior to TIM3-blocking antibody, OX40-/CD40-agonist antibody monotherapies, or the respective correlating combination antibody therapy. Interestingly, therapeutic activity (anti-tumor in mice or human cytokine secretion in the SEB assay) was enhanced when ARCs were combined with antibody blockade of PD1 or CTLA4.

ARC Platform

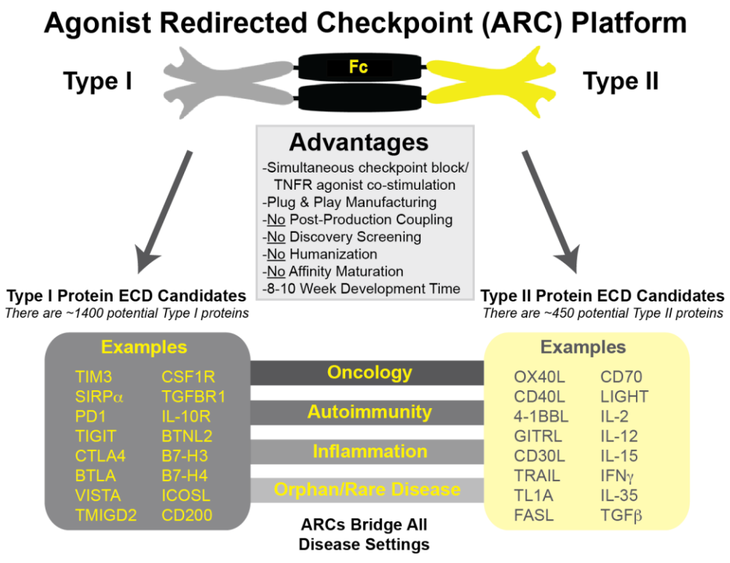


Figure 1. ARC Platform. Agonist Redirected Checkpoints consist of a type I membrane protein extra-cellular domain (ECD), linked to a type II ECD, via a central domain (i.e. Fc). Over 180 ARCs have been synthesized to date, and include combinations of all checkpoint molecules, and the entire family of TNFSF ligands.

Figure 2. TIM3 ARC Structures. Tertiary structure prediction of the TIM3-Fc-OX40L and TIM3-Fc-CD40L ARCs, using the online tool RaptorX.

In Vitro Characterization and Function

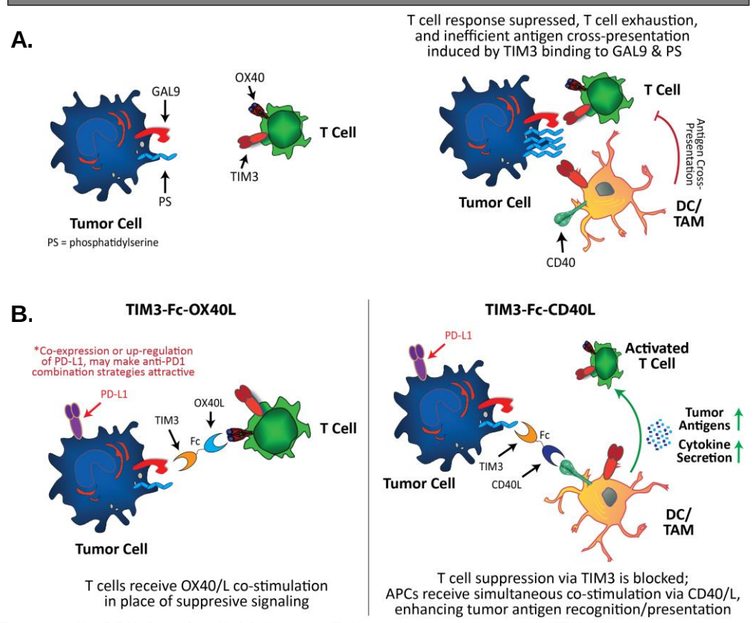


Figure 3. MOA. (A) GAL9 and PS can interact with TIM3 expressed on T cells or dendritic cells (DC) and tumor associated myeloid cells (TAM) to suppress T cell activation. (B) TIM3-Fc-OX40L (left) and TIM3-Fc-CD40L (right) can block the immune suppressive signals and simultaneously provide T cell / DC/TAM co-stimulation.

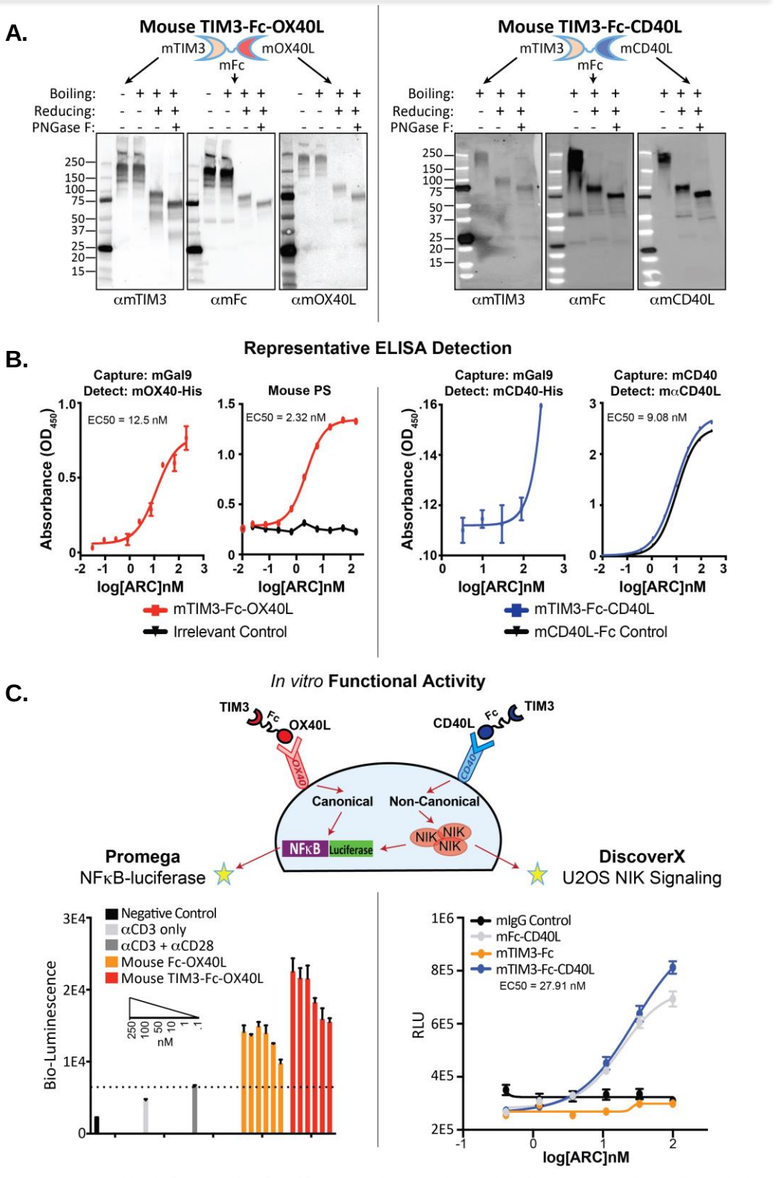
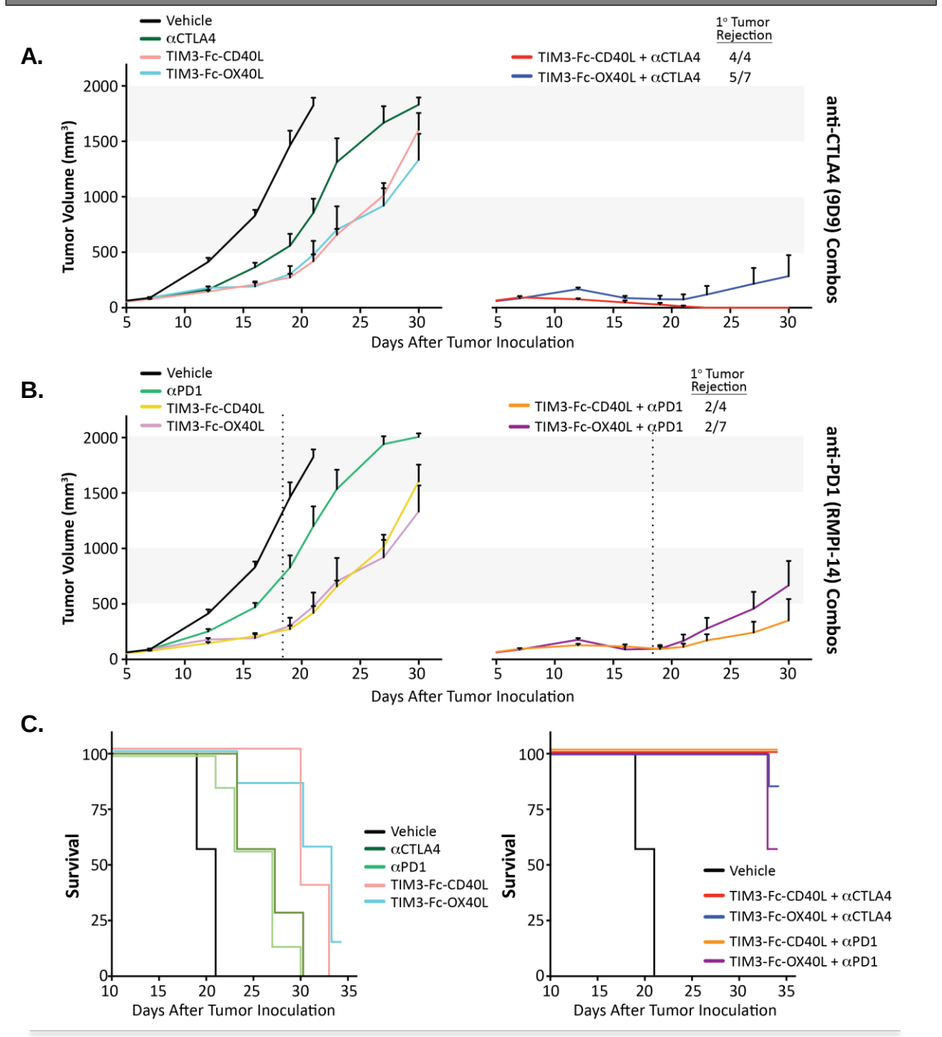


Figure 4. ARC Characterization. (A) Western blot probing for all ARC domains. (B) Representative ELISA binding of TIM3 ARCs to GAL9, OX40, PS, and CD40. (C) TIM3 ARCs signal through both the canonical and non-canonical NFkB-signaling pathways; shown here are example assays performed using the Promega and DiscoverX platforms.

Anti-Tumor Efficacy and Synergy with Checkpoint Blockade



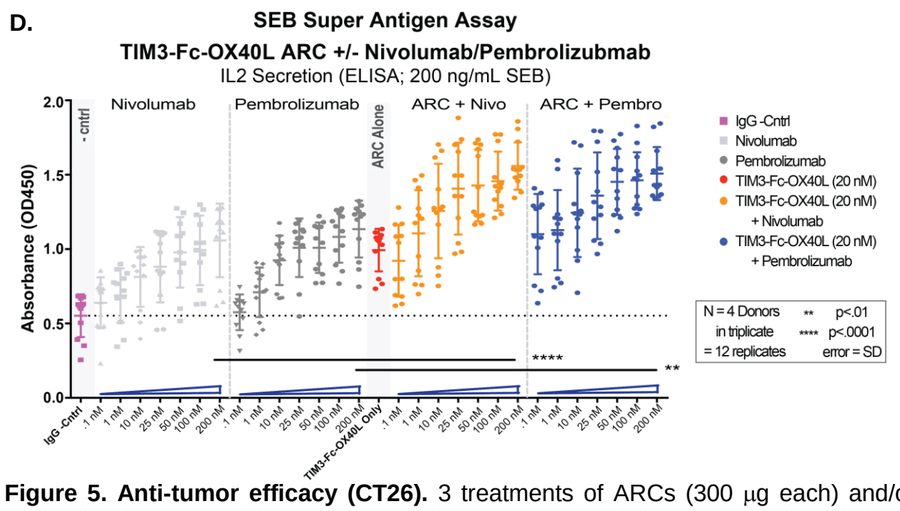


Figure 5. Anti-tumor efficacy (CT26). 3 treatments of ARCs (300 μg each) and/or antibodies (100 μg each) were given on days 7, 9, and 11 following tumor inoculations, when starting tumor volumes were **85-130 mm³**. Monotherapies and combinations with (A) anti-CTLA4 (9D9) and (B) anti-PD1 (RMP1-14) are shown, along with (C) 33-day survival. (D) Combination activity of the human TIM3-Fc-OX40L ARC with nivolumab and pembrolizumab were assessed in a *Staphylococcal* enterotoxin B, super-antigen assay; using human donor PBMCs and IL-2 ELISA as a read-out.

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.

-**TIM3-Fc-OX40L** and **TIM3-Fc-CD40L** can block Gal9/PS interactions while simultaneously co-stimulating T cells via OX40L and DC/TAMs via CD40L, resulting in enhanced antigen-cross presentation and T cell activation; promoting greater antitumor activity.