PD-L1 Checkpoint Blockade Using a Single-Chain Variable Fragment Targeting PD-L1 Delivered by Retroviral Replicating Vector Toca 521 Enhances Anti-Tumor Effect in Cancer Models

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Abstract

Immune checkpoint inhibitors are a breakthrough for immunotherapies in treating cancer patients. However, the response rate ranges from 20-30% across tumor types with a number of immune-related adverse events associated with treatment which can lead to a high rate of treatment discontinuation (~ 12-39% patients). An RRV expressing a single-chain variable fragment targeting PD-L1 (RRV-scFv-PDL1) has demonstrated that scFv PD-L1 binds specifically to both mouse and human PD-L1, and the binding specificity of scFv PD-L1 was further confirmed by competitive ELISA showing that RRV-generated scFv PD-L1 was able to compete for target occupancy against a commercially available monoclonal antibody against PD-L1. A bystander effect also has been observed with scFv PD-L1 protein expression from RRV-scFv-PDL1 infected tumor cells in a dose-dependent manner showing saturated receptor binding to the cell surface PD-L1 of bystander cells when co-cultured with as low as 10% scFv-PD-L1 expressing cells. In addition, *in vivo* mouse models showed that tumor cells infected with RRV-scFv-PDL1 conferred robust and durable immune-mediated anti-tumor activity superior to systemically administered anti-PD-1 or anti-PD-L1 monoclonal antibodies. Local scFv PD-L1 production in the tumor and these results support that RRV-scFv-PDL1 checkpoint inhibition is potentially therapeutic and may provide an improved safety and efficacy profile compared to systemic monoclonal antibodies. The anti-tumor activity of RRV-scFv-PDL1 may be a consequence of the delivery approach, which provides a consistent high level of payload and bystander index which is localized within the tumor microenvironment. Furthermore, with selective local production, RRV-scFv-PDL1 may be therapeutically beneficial in combination with other entities as an immuno-oncology agent with less concern of combined autoimmunity adverse events.

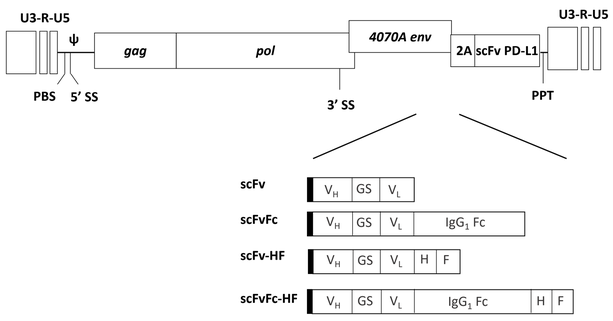
Introduction

Background: RRV-scFv-PDL1 (Toca 521)

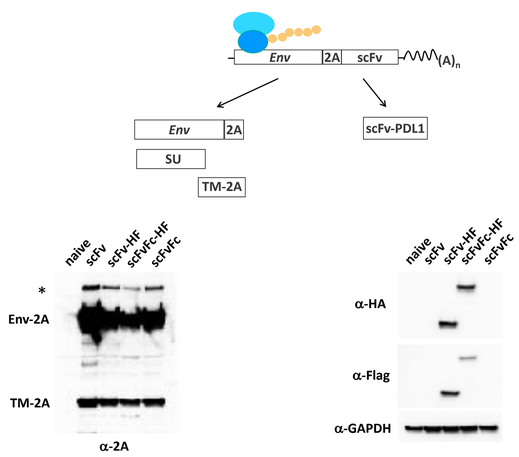
RRV-scFv-PDL1 is a retroviral replicating vector (RRV) expressing a single-chain variable fragment targeting PD-L1 (scFv PD-L1)

- Buds off from tumor cells but does not lyse tumor cells as part of the virus life cycle
- Utilizes self-cleavage 2A peptide which allows proper separation of scFv PD-L1 from the viral envelope protein
- Selectively infects and produces scFv PD-L1 in tumor cells
- scFv PD-L1 produces from the infected tumor cells is secreted into the tumor microenvironment

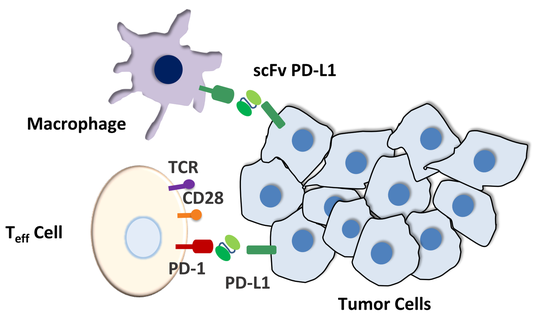
Vector constructs of RRV-scFv-PDL1 (Toca 521):



Proper protein translation and separation from the 2A peptide



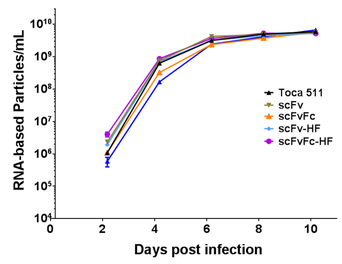
Proposed mechanism of action: Alleviate tumor suppression mediated via PD-1/PD-L1 and reinvigorate T_{eff} function in the tumor microenvironment



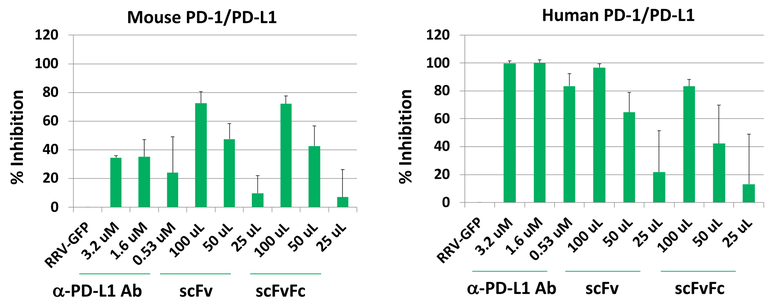
Methods and Results

RRV-scFv-PDL1 Vectors Replicate Efficiency and Produce Viral Titers

	TU/mL	Std Dev
RRV-scFv	2.09E+06	4.80E+05
RRV-scFv-HF	2.08E+06	6.73E+05
RRV-scFvFc	1.98E+06	4.38E+05
RRV-scFv Fc-HF	1.29E+06	1.87E+05

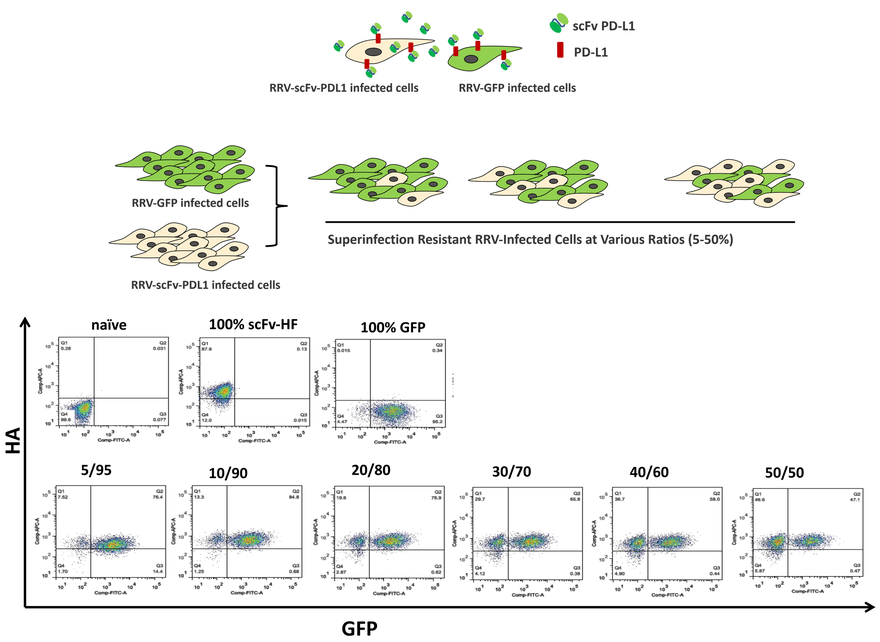


scFv PD-L1 Competes with PD-1 for PD-L1 Binding



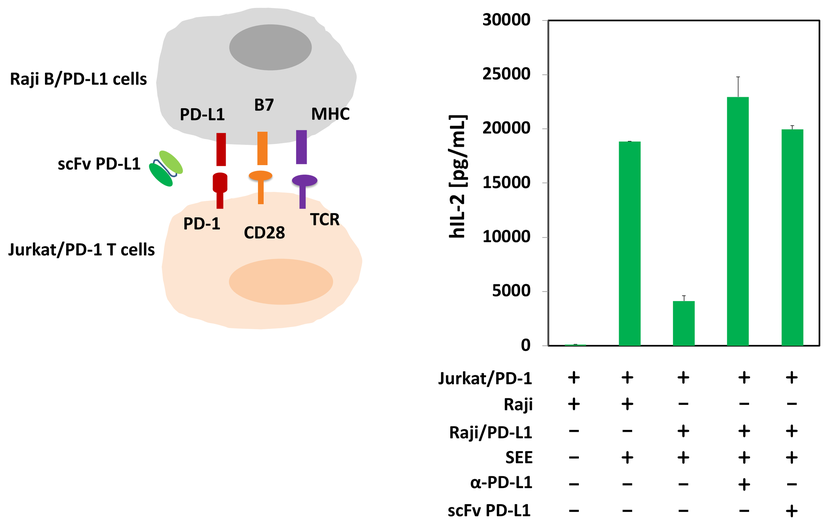
Wells in a 96-well plate were coated with 100 µL of recombinant mouse or human PD-L1 at 8 µg/mL. Various volume of supernatant from CT26 cells expressing scFv PD-L1 or scFvFc PD-L1 were co-incubated with His-tagged recombinant mouse or human PD-1 at 8 µg/mL followed by incubation with anti-His antibody for detection of PD-1 binding. Anti-PD-L1 antibodies were included as a positive control. Percentage of inhibition was calculated relative to supernatant from RRV-GFP infected cells that do not express scFv PD-L1 nor scFvFc PD-L1. Data are presented as means ± SD from one of three independent experiments.

scFv PD-L1 Bystander *Trans*-Binding Activity to PD-L1 on the Cell Surface



Mixtures of HA-tagged scFv PD-L1 (scFv-HF) expressing and GFP-tagged non-scFv PD-L1 expressing EMT6 cells at various ratios were stained with anti-HA antibody for detection of PD-L1 binding on the cell surface. The numbers on the second row indicate the ratios of scFv PD-L1 to non-scFv PD-L1 cells. Data presented are from one of three independent experiments.

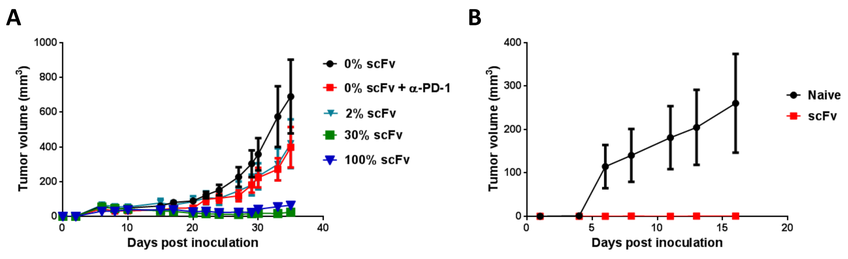
scFv PD-L1 Reverses PD-1/PD-L1 Mediated Immune Suppression



Co-incubation of Jurkat T cells with Raji B cells presenting the superantigen staphylococcal enterotoxin E (SEE), which binds to the TCR and to MHC class II molecules, leads to stimulation of Jurkat T cells and IL-2 production. Co-incubation of Jurkat T cells overexpressing PD-1 and Raji B cells overexpressing PD-L1 blocks the stimulation and IL-2 production. Anti-human PD-L1 antibody was included as a control. Data are presented as means ± SD from one of three independent experiments.

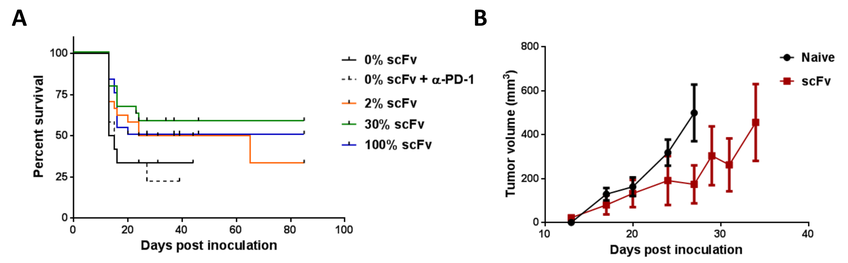
Results

scFv PD-L1 Inhibits Tumor Growth and Elicits Immune Memory Function in Tu-2449SC Mouse Model



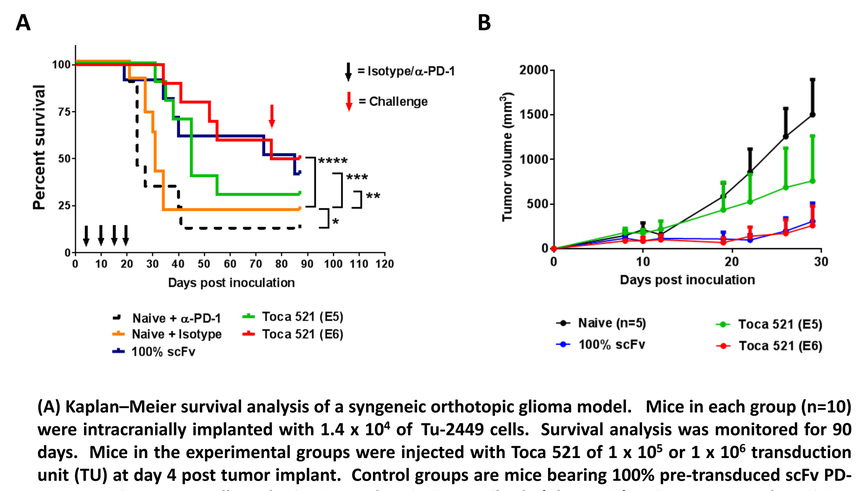
(A) Mixture of 2 x 10^6 Tu-2449SC tumor cells (Mitchell et al., 2017) pre-transduced with RRV-scFv-PDL1 and RRV-GFP at indicated ratios were implanted subcutaneously in B6C3F1 mice (n = 10 per group). Tumor growth was monitored over time. Anti-PD-1 antibody (Clone J43) was included as a control and was intraperitoneally administered on day 0 (300µg/mouse), day 3, 6 and 9 (200µg/mouse). (B) All Mice that cleared their initial tumor implant from scFv treated groups (n = 15), were challenged with Tu-2449SC on the other side of the flank as were naïve control animals and tumor growth was monitored overtime. Naïve group (n=5) was included as a control. Error bars indicate the standard error of the dataset.

scFv PD-L1 Inhibits Tumor Growth and Elicits Immune Memory Function in EMT6 Orthotopic Breast Cancer Model



(A) Kaplan–Meier survival analysis of orthotopic breast cancer model. Mixture of 5 x 10^4 EMT6 tumor cells pre-transduced with RRV-scFv-PDL1 or RRV-GFP at indicated ratios were implanted in the mammary fat pad in BALB/C mice (n = 10 per group). Survival was monitored for 90 days. Anti-PD-1 antibody (Clone J43) was included as a control and was intraperitoneally administered on day 10 (300µg/mouse), day 13, 16, 19 (200µg/mouse). Animals with necrotic tumors were censored from analysis (indicated by tick marks). (B) Mice that survived initial tumor implant from scFv treated groups (n = 5) were challenged with EMT6 tumor cells on the flank and tumor growth was monitored overtime. Naïve animals were included as controls. Error bars indicate the standard error of the dataset.

Intracranial Injection of RRV-scFv-PDL1 (Toca 521) Improves Survival in Syngeneic Orthotopic Glioma Model



(A) Kaplan–Meier survival analysis of a syngeneic orthotopic glioma model. Mice in each group (n=10) were intracranially implanted with 1.4 x 10^4 of Tu-2449 cells. Survival analysis was monitored for 90 days. Mice in the experimental groups were injected with Toca 521 of 1 x 10^5 or 1 x 10^6 transduction unit (TU) at day 4 post tumor implant. Control groups are mice bearing 100% pre-transduced scFv PD-L1 expressing tumor cells and mice treated anti-PD-1 antibody (Clone J43) or isotype control. Anti-PD-L1 antibody was intraperitoneally administered at indicated time points (300µg/mouse induction dose followed by 200µg/mouse maintenance dose). Statistical significance of survival between mice treated with isotype and scFv PD-L1 or Toca 521 treated groups was determined by the Log-rank (Mantel-Cox) test and is indicated by the brackets; **** p = 0.0252, *** p = 0.0649, ** p = 0.0832, * p = 0.3960. (B) Mice which had survived from initial tumor implant from scFv treated or Toca 521 treated groups were challenged with Tu-2449SC tumor cells on the flank and tumor growth was monitored overtime.

Conclusions

- scFv PD-L1 can be expressed in the RRV-2A configuration and shows efficient separation from the viral envelope protein.
- scFv PD-L1 expressed and secreted from Toca 521 infected cells competes with PD-1 binding to PD-L1 in a dose-dependent manner.
- scFv PD-L1 can rescue PD-1/PD-L1 mediated immune suppression *in vitro*.
- scFv PD-L1 expressed from Toca 521 infected tumor cells shows anti-tumor response in several tumor models.
- With selective local production, Toca 521 may have the potential to improve safety and/or efficacy over current check-point inhibitors as a monotherapy or in combination.