A novel, tumor-targeted immunocytokine comprising an anti-PD-L1 Affimer® fused to IL-15 exhibits potent anti-tumor activity

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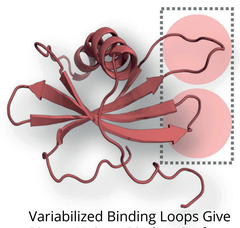
SCAN

Introduction

- Avacta's proprietary Affimer® technology is a novel class of proteins that is designed to have similar molecular recognition characteristics to monoclonal antibodies. Affimer® proteins (15kDa) are based on naturally occurring proteins (Stefin A) that are engineered to stably display two loops forming a binding surface.



Variabilized Binding Loops Give Rise to Unique Binding Surfaces

Affimer® Key Feature 1 - Small & Specific
- 1/10th size compared to antibodies.
- High specificity and ability to generate binders to difficult targets.

Affimer® Key Feature 2 - Mix & Match
- Flexible structure design; Affimer® proteins can be linked to create multi-specific therapeutics.
- Tunable pharmacokinetics using Affimer® XT, a serum albumin-binding Affimer® with half-life extension properties, or using Affimer®-Fc fusions.

Affimer® Key Feature 3 - Excellent Drug Development Properties
- Robust and highly soluble.
- High thermostability.
- No disulfide bonds or other post-translational modifications.
- High expression.
- Low risk of immunogenicity; Affimer® protein scaffold is human.

- Affimer® proteins with high-affinity competitive binding to human PD-L1 which block interaction with PD-1 have been previously identified using phage display.
- Here we show novel immunocytokines that consist of Fc half-life extended Affimer® antagonist to PD-L1 fused to human Interleukin 15 (IL-15) receptor alpha sushi domain and human IL-15 (PDL1-IL15 Affimer® fusion proteins).
- IL-15 signals through the IL-2 receptor beta chain and the common gamma chain (IL-2Rβ/γ) and induces differentiation and proliferation of CD8+ T cells and NK cells; it does not stimulate Tregs. Immunostimulatory cytokines have therapeutic potential but are often associated with toxicity when administered systemically.
- PDL1-IL15 Affimer® fusion proteins may bring the immunomodulatory cytokine directly to the tumor and reverse the immunosuppressive tumor microenvironment by enhancing T and NK cell activation, while also introducing a checkpoint inhibitor to augment the anti-tumor immune response.

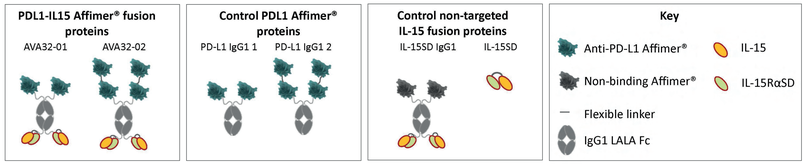


Figure 1: Schematic representation of PDL1-IL15 Affimer® fusion proteins and controls.

PDL1-IL15 Affimer® fusion proteins bind PD-L1 and IL-2Rβ/γ

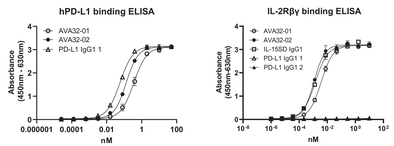


Figure 2 and Table 1. Binding of PDL1-IL15 Affimer® fusion proteins or controls to human PD-L1 and IL-2Rβγ measured by ELISA. NB: no binding; NT: not tested.

Affimer® Fusion Proteins	hPD-L1 Binding EC50 (nM)	IL-2Rβγ Binding EC50 (nM)
AVA32-01	0.355	0.004
AVA32-02	0.131	0.001
IL-15SD IgG1	NT	0.001
PD-L1 IgG1 1	0.059	NB
PD-L1 IgG1 2	NT	NB

PDL1-IL15 Affimer® fusion proteins exhibit PD-1:PD-L1 blocking activity and IL-2Rβ/γ activation

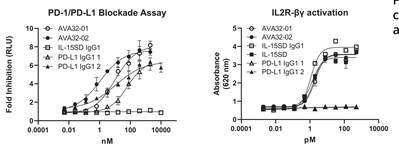


Figure 3 and Table 2. Activity of PDL1-IL15 Affimer® fusion proteins or controls in Promega PD-1/PD-L1 blockade reporter assay and IL-2Rβγ activity JAK3/STAT5 reporter assay. NA: no activity; NT: not tested.

Affimer® Fusion Proteins	PD1/PDL1 blockade EC50 (nM)	IL-2Rβ/γ activation EC50 (pM)
AVA32-01	10.67	1.986
AVA32-02	1.103	1.737
IL-15SD IgG1	NA	1.085
IL-15SD	NT	1.289
PD-L1 IgG1 1	65.11	NA
PD-L1 IgG1 2	10.74	NA

PDL1-IL15 Affimer® fusion proteins reverse T cell exhaustion in primary human MLR assay

- One-way mixed lymphocyte reaction (MLR) assay with immature monocyte-derived dendritic cells (moDC) and exhausted T cells (Tex) was used to assess reversal of Tex hypo-responsiveness in the presence of test molecules and controls.
- IFN-γ, a major cytokine released by cytotoxic T cells important for anti-tumor response, was measured.
- PDL1-IL15 Affimer® fusion proteins induce higher levels of IFN-γ release than PD-L1 only or non-targeted IL-15 control proteins, and higher levels than anti-PD1 (Nivolumab) or anti-PDL1 (Atezolizumab).

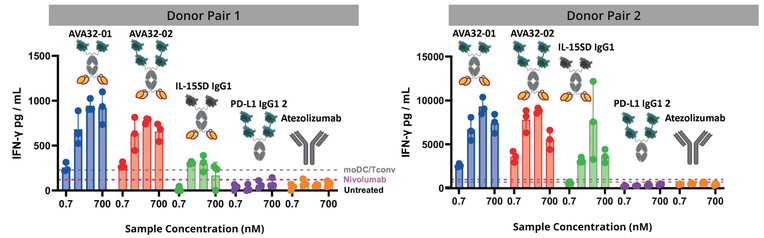


Figure 4. IFN-γ release after exposure with indicated molecules for 5 days in human MLR assay. Tex were generated by isolating pan-T cells (CD3+) from PBMC donors and repeatedly stimulating using CD3/CD28 Dynabeads®. ***In vitro*** generated moDCs were combined with Tex cells to create MLR pairs (N=2 donor pairs), which were then cultured with indicated molecules, nivolumab or left untreated for 5 days. As a control, MLR cultures with moDCs and non-exhausted T cells (Tconv) were included. IFN-γ was measured by ELISA.

PDL1-IL15 Affimer® fusion proteins induce proliferation and activation of human CD8+ αβ T cells, γδ T cells and NK cells

- In human PBMC cultures, PDL1-IL15 Affimer® fusion protein AVA32-02 induces proliferation and activation of CD8+ αβ T cells, γδ T cells and NK cells with highest sensitivity in NK cells and γδ T cells.
- The response is primarily driven by IL-15 as IL-15SD and IL-15SD IgG1 response is comparable to AVA32-02 in some donors; in other donors AVA32-02 is more potent such as in the example shown below.
- CD8+ αβ T cells, γδ T cells and NK cells play an important role in anti-tumor response by releasing pro-inflammatory cytokines and cytotoxic molecules (e.g. granzyme B, perforin).

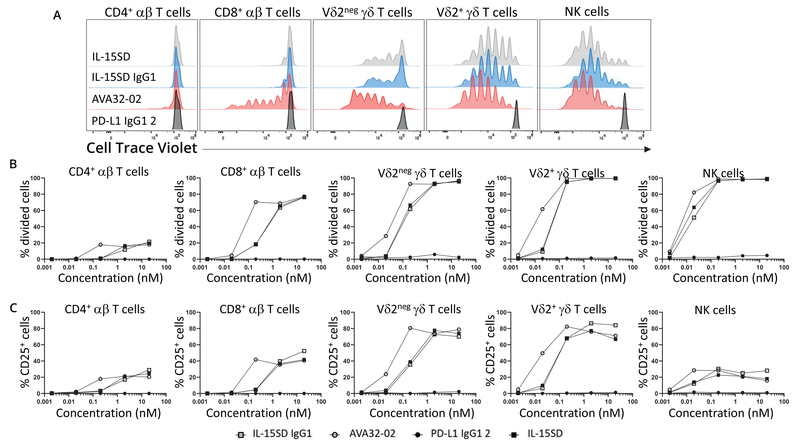


Figure 5. Human PBMCs were stained with Cell Trace Violet and cultured with the indicated molecules for 7 days. Cells were analyzed on day 7 via flow cytometry. Cells were defined as CD4 αβ T (CD3+γδTCR-CD8-); CD8 αβ T (CD3+γδTCR-CD8+); Vδ2neg γδ T (CD3+ γδTCR+Vδ2-); Vδ2pos γδ T (CD3+ γδTCR+Vδ2+) and NK (CD3-γδTCR-CD56+). Representative data from 1 donor is shown (N=2 biological replicates). (A) Example cell proliferation flow plots of cell subsets cultured with target molecules at 0.2nM. (B) Proportion of divided cells in the indicated subset. (C) Proportion of CD25+ cells in the indicated subset cultured at different compound concentrations.

PDL1-IL15 Affimer® fusion proteins enhance immune cell killing of tumor cells in *in vitro* co-culture assays

- In MDA-MB-231 tumor cell co-cultures with human PBMCs, PDL1-IL15 Affimer® fusion proteins induce more tumor cell death than PD-L1 only control protein.

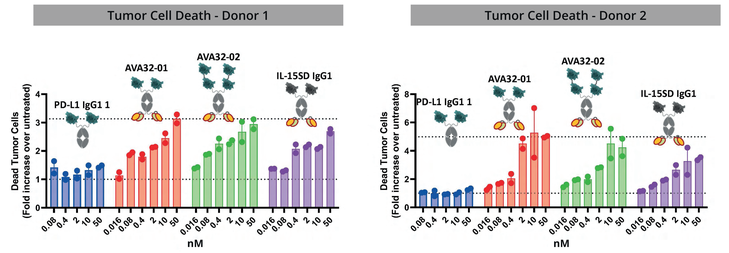


Figure 6. MDA-MB-231 tumor cells were cocultured with human PBMCs from 2 donors in the presence of indicated molecules with no further stimulation for 3 days. Tumor cell death was measured by flow cytometry analysis of harvested tumor cells stained with a viability dye.

PDL1-IL15 Affimer® fusion proteins show *in vivo* efficacy in hPD-L1 MC38 syngeneic tumor model

- AVA32-02 inhibits growth of hPD-L1-MC38 tumors with transient body weight loss which recovers.
- AVA32-02 increases tumor-infiltrating CD8+ T cells and NK cells without increasing NK cell numbers in peripheral blood.

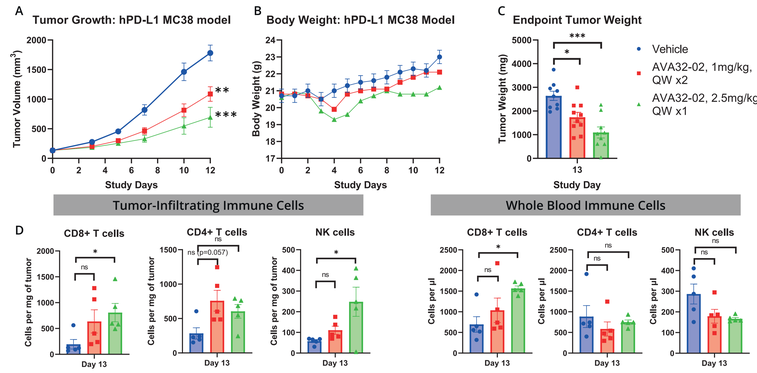


Figure 7. AVA32-02 induces CD8+ T cell and NK cell infiltration into tumor and inhibits growth of MC38 tumors. Female hPD-L1 HuGEMM C57BL/6 mice were subcutaneously injected with hPDL1-MC38 tumor cells. Following tumor formation, mice were administered intravenously with vehicle control or AVA32-02 at 1, or 2.5 mg/kg at day 0 post randomization. A second dose was administered at day 7 to vehicle and AVA32-02, 1 mg/kg group. Tumor volume (A) and body weight (B) are shown as mean -/+ SEM (n= 9-10 mice/group). At day 13, mice were culled, tumors were weighed (C) and tumors and whole blood from 5 mice per group were processed for flow cytometry. (D) Cell count of CD4+, CD8+ T cells and NK cells was determined by flow cytometry. Mean -/+ SEM, n=5, is shown. . *p<0.05; **p<0.01; ***p<0.001; NS, no significance.

Summary

- PDL1-IL15 Affimer® fusion proteins show the following characteristics:
  - ✓ Bind both PD-L1 and IL-2Rβ/γ and exhibit PD-1:PD-L1 blocking activity and IL-2Rβ/γ activation.
  - ✓ Reverse T cell exhaustion in a human MLR assay: PDL1-IL15 Affimer® fusion proteins show greater activity than either the PD-L1 or the IL-15 component alone.
  - ✓ Induce tumor cell death in human PBMC co-culture assays with greater activity than controls.
  - ✓ Activate CD8+ T cells, γδ T cells and NK cells.
  - ✓ Have favourable therapeutic index and exhibit tumor growth inhibition in MC38 tumor *in vivo* model.
- Further preclinical development of PDL1-IL15 Affimer® fusion proteins is ongoing.