"Switch" Transmembrane Immunomodulatory Proteins (TIPs) Consisting of High-Affinity PD-1 Extracellular Domains (PD-1 vIgDs) and Costimulatory Intracellular Domains Potently Enhance the Activity of TCR-Engineered T Cells

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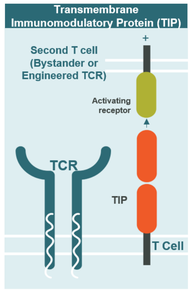
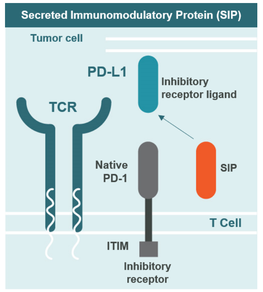
Abstract

Background: T cell receptor (TCR)-engineered T cell therapies hold great promise as personalized, adoptive anticancer treatments, but clinical experience to date has demonstrated generally only modest efficacy, attributed in large part to unfavorable factors in the tumor microenvironment. This includes the presence of receptors, such as PD-L1, that can inhibit T cell responses, and/or insufficient engineered T cell longevity. Common strategies to address such limitations have involved attempts to provide additional costimulatory signals to T cells. However, addition of costimulatory signals alone may not be sufficient to overcome PD-L1-mediated inhibition. We have utilized our variant immunoglobulin domain (vIgD™) platform, based on the directed evolution of immunoglobulin superfamily (IgSF) members, to develop PD-1 domains with higher affinity for PD-L1 and then substituted costimulatory intracellular signaling domains for the native PD-1 inhibitory intracellular region with the hypothesis that PD-L1 engagement of these Transmembrane Immune-modulatory Proteins (TIPs™) would result in costimulation rather than inhibitory signaling. Such "Switch" TIPs consisting of a checkpoint-inhibitory extracellular PD-1 vIgD and intracellular costimulatory domains may therefore improve the activity of TCR-engineered T cells by providing costimulation while preventing inhibitory signaling through native PD-1.
Methods: Variant PD-1 extracellular domains (PD-1 vIgDs) were generated using random mutagenesis and FACS-based selection of yeast displayed proteins selecting for increased binding to PD-L1. These variants were then fused to intracellular signaling domains from CD28, ICOS and CD137 either singly or together in combinations. These constructs were expressed via lentivirus in primary human T cells along with a TCR recognizing an HPV16 E6 peptide (E6 TCR). Surface expression was confirmed by flow cytometry, and activity was assessed against HPV+ tumor cell lines via proliferation, cytokine production (IFNγ, TNFα and IL-2) and cytotoxicity.
Results: TIPs including PD-1 vIgDs selected for increased PD-L1 binding and various combinations of intracellular signaling domains potently enhanced the activity of E6 TCR-engineered T cells, including killing of HPV+ target cells (Figure 1a) as well as target-driven proliferation and cytokine production (Figure 1b). Activity was consistently superior to TIPs consisting of only a PD-1 extracellular vIgD, or TIPs which included a wild-type extracellular PD-1 domain. Importantly, TIP activity was abrogated in the presence of an anti-PD-L1 antibody, demonstrating PD-L1 dependence of the costimulatory activity.
Conclusions: "Switch" TIPs consisting of high-affinity PD-1 vIgD extracellular domains and costimulatory intracellular signaling domains potently augment the antitumor activity of TCR-engineered T cells as judged by proliferation, cytokine production and cytotoxicity. Ongoing studies continue to explore this and analogous strategies with other IgSF-based vIgDs and/or costimulatory domains and will hopefully significantly enhance the clinical efficacy of engineered T cells in both solid and hematological malignancies.

Figure 1: TIP/SIP Formats for Engineered Cell Therapy (ECT)

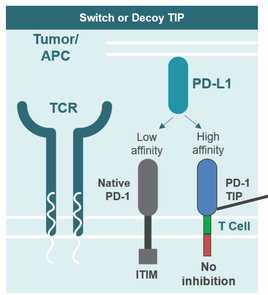


- Costimulatory signaling to other engineered T cells and/or endogenous T cells can be provided by a costimulatory TIP
- T cells have endogenous capability to express IgSF costimulatory domains, so TIPs can be expressed at high levels
- Option to add intrinsic signaling capability to TIPs through signaling domain fusion



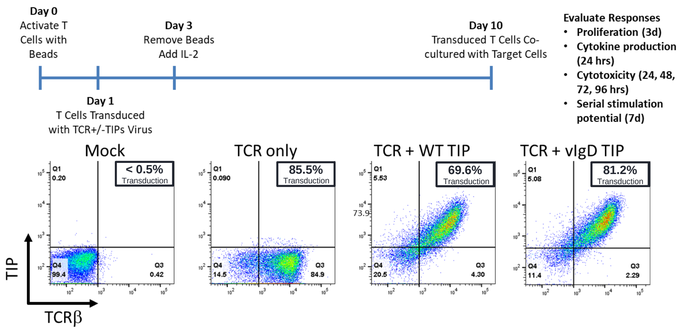
- A vIgD can be engineered to bind inhibitory receptors/ligands with higher affinity than native inhibitory ligands
- Multiple antagonistic domains could be "stacked" such as PD-1, TIGIT, CTLA-4, LAG-3, and TIM-3
- No antibody heavy and light chain pairing required of the T cell protein machinery
- Small SIP molecular weight provides potential tumor penetration advantages while avoiding systemic toxicity

Figure 2: Strategy for Switch Transmembrane Immunomodulatory Proteins (Switch TIPs)



Generate vIgD PD-1 domains with higher affinity for PD-L1 and replace the PD-1 inhibitory intracellular signaling domain (ICD) with costimulatory ICD, "switching" normal PD-1 inhibitory response to a positive, T cell activating signal.

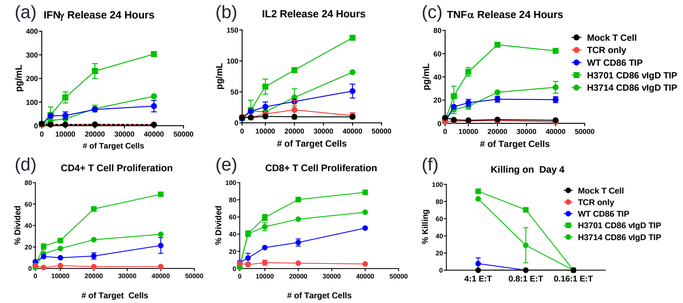
Figure 3: Alpine's Proprietary Lentiviral Vector Produces High Transduction Efficiencies with TCR and TIP in a Single Vector



Primary human T cells were activated and transduced with HPV-specific T cell receptor (TCR) virus with or without TIPs. Expression of each TIP and the TCR were assessed on day 10.

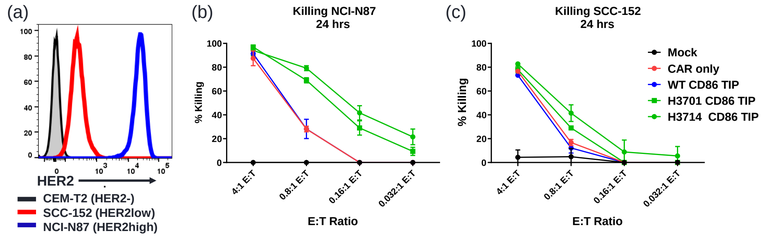
Figure 4: Expression of CD86 Costimulatory vIgD TIPs Enhances Antigen Specific T Cell Responses in TCR and CAR Transduced T Cells

(A) Enhanced responses in TCR transduced T cells



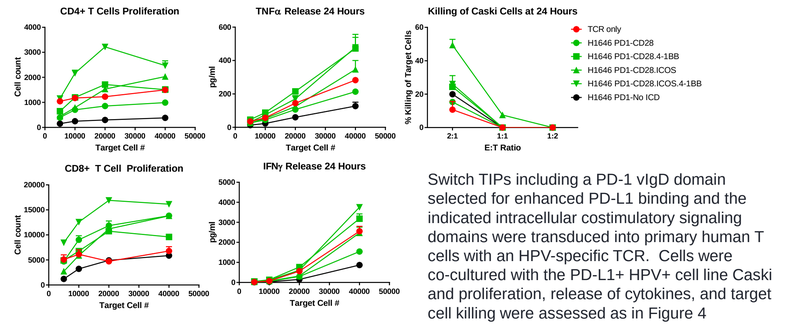
Expression of CD86 vIgD TIPs enhances HPV-specific tumor responses in vitro. Primary human T cells were transduced with an HPV-specific TCR and expanded for 10 days. Transduced cells were then incubated with varying numbers of HPV+ tumor cell line SCC-152 and the release of (a) interferon-gamma (IFNγ), (b) IL-2, or (c) TNFα were assessed after 24 hours. Transduced cells were also CFSE labeled and incubated with target cells and proliferation was evaluated after 3 days on (d) CD4+ and (e) CD8+ T cells. Finally, (f) target cell killing was tested using a luciferase expressing target cell line and percent killing after 4 days of co-culture is plotted versus the effector to target (E:T) ratio

(B) Enhanced responses in CAR-T cells



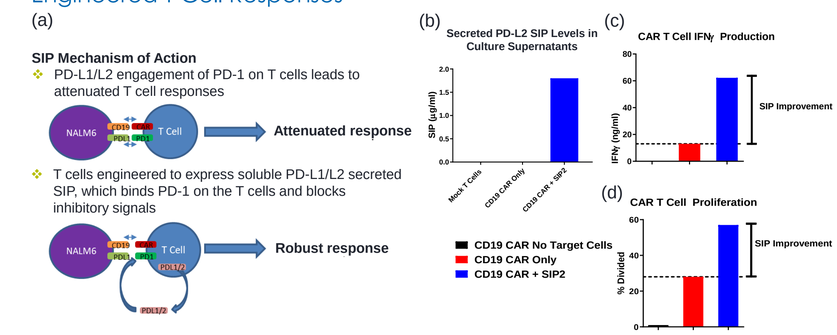
Expression of CD86 TIPs enhances anti-HER2 CAR-T cell killing of target cells expressing either high or low levels of antigen. (a) HER2 is expressed at different levels on NCI-N87 and SCC-152 cell lines. (b) Primary human T cells transduced with an anti-HER2 CAR more effectively kill (b) NCI-N87 and (c) SCC-152 cell lines if they also express CD86 vIgD TIPs compared to WT CD86 TIP or no TIP

Figure 5: Switch TIPs Improve Key Measures of T Cell Activity and Survival



Switch TIPs including a PD-1 vIgD domain selected for enhanced PD-L1 binding and the indicated intracellular costimulatory signaling domains were transduced into primary human T cells with an HPV-specific TCR. Cells were co-cultured with the PD-L1+ HPV+ cell line Caski and proliferation, release of cytokines, and target cell killing were assessed as in Figure 4

Figure 6: Secreted Immunomodulatory Proteins (SIPs) Also Enhance Engineered T Cell Responses



(a) Schematic of SIP mechanism of action. Primary human T cells were transduced with a second generation CD19 CAR with or without a PD-L2 vIgD SIPs. CD19+ NALM6 cells were engineered to express PD-L1. Transduced CFSE labeled T cells were co-cultured for three days with CD19+ NALM6 cells, resulting in attenuated CAR-T cell responsiveness compared to NALM6 cells not engineered for PD-L1 expression (not shown). Culture supernatants were analyzed for (b) secreted SIP and (c) IFNγ production and (d) T cell proliferation was evaluated by CFSE dilution.

Summary and Conclusions

- vIgD TIPs, Switch TIPs, and SIPs can be highly expressed in engineered T cells, potently enhancing *in vitro* responses such as proliferation, cytokine production, and/or cytotoxicity
- Proprietary lentiviral vector system can achieve TCR transduction efficiencies of over 80% with TCR and TIP in a single vector
- vIgD TIPs, Switch TIPs, and SIPs may embody a next generation strategy for engineered T cells, particularly TCR-based therapies where limitations in persistence and potency of current TCR-based platforms may restrict clinical utility
- Additional potential applications include non T cell-based cellular technologies, such as oncolytic viruses or dendritic cell therapeutics

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