Abstract # 1519

PD1 TurboCAR™ T cells: PD1-resistant CAR T cells with programmable cytokine signaling outputs

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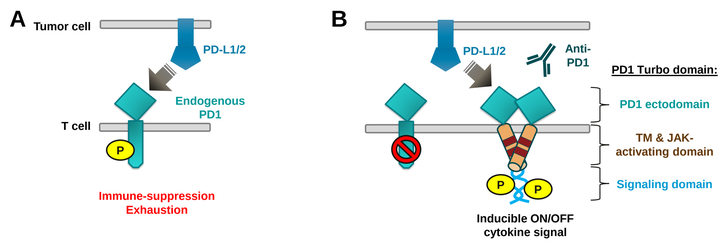
Abstract

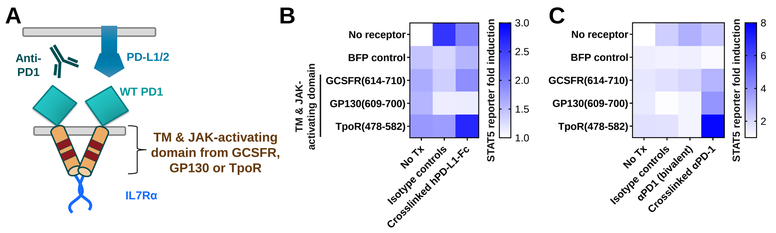
CAR T cell therapy has demonstrated unprecedented efficacy in the treatment of hematological malignancies. However, clinical benefit in solid tumor indications has been limited, potentially in part due to suppressive solid tumor microenvironments (TME) that inhibit T cell effector function and persistence. While the provision of cytokine support can help CAR T cells overcome suppressive TME, combining CAR T therapy with systemically-administered cytokines/cytokine mimetics can result in toxicities and locally secreted cytokines may enhance rejection of allogeneic cell products by host cells. For this reason, we had previously designed and tested a novel cytokine-stimulated CAR T cell designated a TurboCAR. TurboCAR T cells co-express a CAR and a Turbo domain (i.e. a homodimeric cytokine receptor chimera) that transmits CAR T cell-intrinsic cytokine signals. We reported earlier that in preclinical studies, TurboCAR T cells directed towards BCMA demonstrated enhanced potency, expansion and persistence [1,2]. To broaden the TurboCAR platform and tailor it for solid tumors, we employed a two-pronged approach aimed at inhibiting immune-suppressive PD1 signaling while simultaneously transmitting immune-potentiating cytokine signaling. To this end, we engineered PD1 TurboCAR T cells, in which the Turbo domain is fused to a PD1 ectodomain that serves as a dominant-negative receptor. The PD1 ectodomain was further modified for high-affinity binding to PD1 ligands, allowing for preferential ligand sequestration and more effective inhibition of endogenous PD1 signaling. Despite having a larger lentiviral vector cargo, PD1 TurboCAR T cell products remained manufacturable and retained a favorable memory phenotype. PD1 TurboCAR T cells directed towards PDL1-expressing solid tumor target cells showed increased functionality compared to CAR T cells combined with PD1 blockade or to the parental TurboCAR T cells alone. In conclusion, PD1 TurboCARs augmented with a PD1 dominant negative ectodomain conferred CAR T cells with resistance to PD1-mediated inhibition, while simultaneously transmitting cytokine signals in a CAR T cell-intrinsic fashion. As an all-in-one product, PD1 TurboCAR T cells may obviate the need for combination therapy with anti-PD1 antibodies, while circumventing safety risks associated with systemic cytokine administration.

Figure 1: The homodimeric PD1 Turbo domain activates cytokine signaling in response to PD1 ligands or anti-PD1



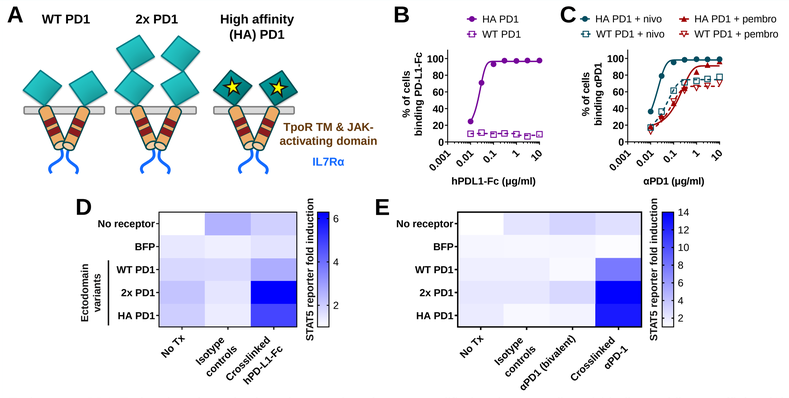
(A) Ligation of the PD1 receptor by its ligands transmits inhibitory signals into the T cell, resulting in functional suppression and exhaustion. (B) PD1 Turbo domains inhibit immune-suppressive PD1 signaling while simultaneously transmitting immune-potentiating cytokine signaling. PD1 Turbo domains are homodimeric chimeras comprised of: (1) an ectodomain derived from the PD1 receptor, (2) transmembrane (TM) and JAK-activating domain, and (3) an intracellular signaling domain containing phosphorylable tyrosine residues derived from a cytokine receptor of interest. The PD1 ectodomain competes with endogenous PD1 for binding to PD1 ligands. Engagement of the PD1 ectodomain by PD1 ligands or anti-PD1 induces Turbo domain clustering and activation, leading to downstream cytokine signaling.

Figure 2: The homodimeric TpoR transmembrane and JAK-activating domain was selected for its ability to induce Turbo domain signaling



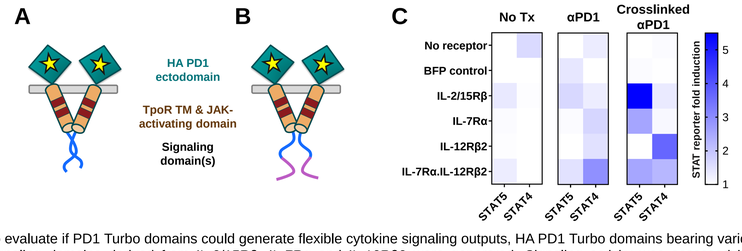
To identify homodimeric TM and JAK-activating domain(s) capable of eliciting downstream signaling in the chimeric receptor, Turbo domains bearing various homodimeric TM and JAK-activating domains were screened for responsiveness to PD-L1 and the anti-PD1 antibody nivolumab. (A) Schematic of Turbo domain comprising the WT PD1 ectodomain, an IL7Rα signaling domain, as well as TM and JAK-activating domains derived from GCSFR, GP130 or TpoR. In a HEK293T cell reporter assay, responsiveness of TM and JAK-activating domain variants following ligation with (B) PD-L1 and (C) anti-PD1 was assessed. Crosslinking of primary antibodies (10 μg/ml) was achieved using an anti-human IgG Fc secondary antibody (25 μg/ml). Untransfected (No receptor) and mock-transfected (BFP control) cells were included as negative controls. The TpoR TM and JAK-activating domain induced Turbo domain signaling most efficiently, as seen by STAT5 reporter activation.

Figure 3: Ectodomain modifications improved Turbo domain signaling



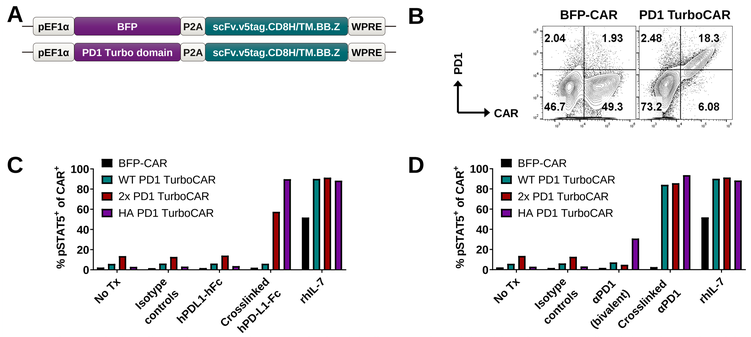
To improve PD1 Turbo domain activation, the ectodomain was modified to increase ligand binding avidity or affinity. (A) Schematic of ectodomain variants. Two WT PD1 ectodomains were fused in tandem (2x PD1) to increase ligand binding avidity. Alternatively, point mutations were introduced to generate a high affinity (HA) PD1 ectodomain with improved binding to (B) PD-L1 and (C) the anti-PD1's nivolumab and pembrolizumab . In a HEK293T cell reporter assay, responsiveness of JAK-activating domain variants following ligation with (D) PD-L1 and (E) the anti-PD1 nivolumab was assessed. The 2x PD1 and HA PD1 ectodomain variants improved Turbo domain signal strength, based on STAT5 reporter activation.

Figure 4: Signaling domains can be tailored to mimic diverse, combinatorial cytokine outputs



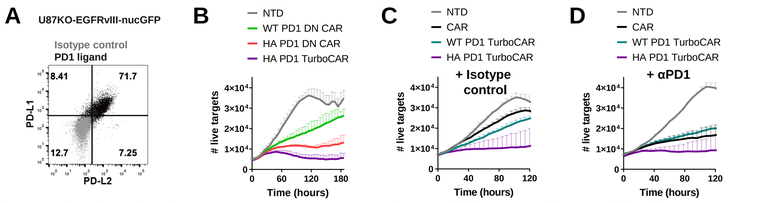
To evaluate if PD1 Turbo domains could generate flexible cytokine signaling outputs, HA PD1 Turbo domains bearing various signaling domains derived from IL-2/15Rβ, IL-7Rα and IL-12Rβ2 were generated. Signaling activity was assessed in a HEK293T cell reporter assay. Schematic of HA PD1 Turbo domain bearing (A) one or (B) two signaling domains fused in tandem. (C) Treatment with the anti-PD1 nivolumab activated STAT signaling expected from the parental receptor. Moreover, signaling domains derived from distinct cytokine receptors (i.e., IL-7Rα and IL-12Rβ2) fused in tandem could simultaneously activate multiple STAT pathways from a single homodimeric PD1 Turbo domain.

Figure 5: PD1 Turbo domains signal in human CAR T cells



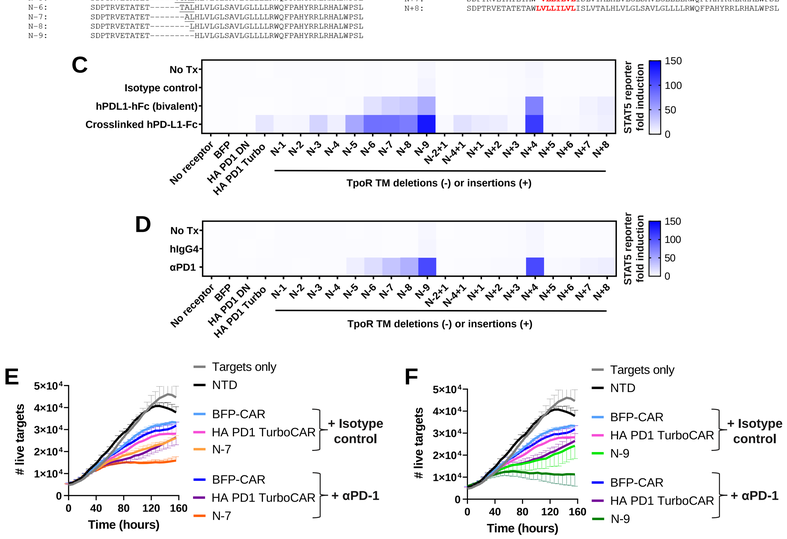
Expression and signaling of PD1 TurboCARs in lentivirally transduced healthy donor T cells. (A) Schematic of lentiviral vectors used. A v5 epitope tag was inserted between the scFv and the CD8 hinge to facilitate CAR detection. (B) PD1 Turbo CAR T cells co-express both transgenes. (C & D) Responsiveness of PD1 Turbo CAR T cells bearing an IL7Rα signaling domain following ligation with (C) PD-L1 and (D) the anti-PD1 nivolumab was assessed by phosphoflow. 2x PD1 TurboCAR T cells showed a higher background signal and activated STAT5 in response to crosslinked hPD-L1, but not bivalent anti-PD1. By contrast, HA PD1 TurboCAR T cells had little to no background signaling and activated STAT5 more efficiently in response to crosslinked hPD-L1 and bivalent anti-PD1, and was therefore selected for further studies.

Figure 6: Efficient PD1 blockade coupled to cytokine signaling underlie the superior activity of HA PD1 TurboCAR™ T cells



(A) Expression of PD1 ligands on U87KO-EGFRvIII-nucGFP, a human glioblastoma cell line in which wildtype EGFR was knocked out by TALEN®-mediated gene editing, and EGFRvIII and nuclear GFP was stably overexpressed by lentiviral transduction. (B-D) In vitro cytotoxicity against PD-L-expressing U87KO-EGFRvIII-nucGFP was assessed using the Incucyte Live Cell Imaging System. (B) Functional comparison to PD1 dominant negative (DN) CAR T cells which co-express the PD1 ectodomain and transmembrane domain. HA PD1 TurboCAR T cells were most active, demonstrating the added benefit of cytokine signaling from the Turbo domain. Functional comparison to CAR T cells in the presence of (C) hIgG4 isotype control or (D) the anti-PD1 nivolumab (10 μg/ml). Consistent with (B), HA PD1 TurboCAR T cells were most active in the absence of anti-PD1. Although combination with anti-PD1 improved the cytotoxicity of control CAR T cells and WT PD1 TurboCAR T cells, they remained less active than HA PD1 TurboCAR T cells. Taken together, HA PD1 TurboCAR T cells outperform PD1-inhibited CAR T cells due to the simultaneous activation of cytokine signaling via the Turbo domain.

Figure 7: Responsiveness of the HA PD1 Turbo domain was further enhanced by modifications to the transmembrane domain



(A-B) TpoR protein sequence, with the wildtype transmembrane (TM) domain highlighted in yellow. Amino acid (A) deletions as indicated by "-" or (B) insertions as indicated in bold red were made to the transmembrane domain. In a HEK293T cell reporter assay, responsiveness of TpoR TM domain variants following ligation with (B) PD-L1 and (C) the anti-PD1 nivolumab was assessed. Compared to the HA PD1 Turbo domain bearing the wildtype TpoR TM sequence, the N-5, N-6, N-7, N-8, N-9 and N+4 TM variants showed increased signal strength in response to PD-L2 and anti-PD1. These TpoR TM variants were therefore selected for further testing in the context of human CAR T cells. (E-F) In vitro cytotoxicity of the exemplary TpoR TM variants (E) N-7 and (F) N-9 against PD-L-expressing U87KO-EGFRvIII-nucGFP in the absence or presence of the anti-PD1 nivolumab. Both TpoR TM variants were slightly more active than the HA PD1 TurboCAR in the absence of anti-PD1, and were further enhanced by anti-PD1-induced Turbo domain activation.

Conclusions

- PD1 Turbo domains are designed to overcome the inherent challenges in solid tumors associated with the immuno-suppressive TME by blocking suppressive PD1 signaling and turning it into positive signals
- PD1 Turbo domains were optimized for efficient PD-L sequestration and enhanced cytokine signaling
- PD1 Turbo domains can be tailored for diverse, programmable and combinatorial signaling outputs
- PD1 TurboCAR T cells outperform PD1-inhibited CAR T cells

References: 1. Lin R, et al. TurboCAR™ T Cells: CAR T Cells with Constitutive, Programmable Cytokine Signaling Outputs. ASGCT 2020. 2. Sommer C, et al. Preclinical Evaluation of ALLO-605, an Allogeneic BCMA TurboCAR™ T Cell Therapy for the Treatment of Multiple Myeloma. ASH 2020.

Acknowledgements: Allogene's AlloCAR T™ programs utilize Cellectis technology. The EGFRvIII AlloCAR T™ program is licensed exclusively from Cellectis by Allogene. Allogene holds exclusive U.S. rights and has granted to Servier rights to EGFRvIII product candidates for all other countries.