Peptidyl IL-2/15Rβγc-restricted agonists, highly-attenuated and linked to anti-PD-1 antibodies to achieve selectivity and amplify potency in stimulating PD-1^{high} lymphocytes

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INTRODUCTION

Recent reports demonstrate that targeting "non-alpha" IL-2/15Rβγc agonists to a PD-1^{high} CD8^{+} stem-like population induces proliferation and differentiation into a highly functional cytotoxic effector phenotype (1-3). We have previously described small synthetic peptides, unrelated to IL-2 or IL-15, that are potent, full agonists of IL-2/15Rβγc, faithfully emulating the signaling and transcriptional profiles induced by IL-2 and IL-15. These peptides do not bind IL-2Rα or IL-15Rα, and are therefore IL-2/15Rβγc-restricted agonists (4). We now describe potency-attenuated variants of these peptidyl agonists, fused to an anti-PD-1 antibody to achieve selective targeting to PD-1^{high} cells, generating cis-mediated enhancement in potency of IL-2R stimulation of the targeted cells.

PROPERTIES OF IL-2R AGONIST COMPOUNDS

MDK1169

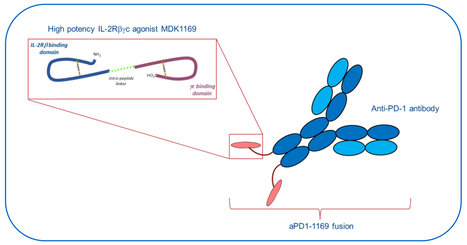
- *42 amino acid synthetic peptide*
- *Novel sequence, unrelated to IL-2 or IL-15*
- *Binds to both IL-2Rβ and Rγc subunits*
- *Binds outside of cytokine binding site on both subunits*
- *IL-2/15Rβγc agonist with sub nM potency and full efficacy*
- *Receptor activation requires expression of both Rβ and Rγc, and is not inhibited by neutralizing IL-2 or IL-15 antibodies*
- *Induces activation of major IL-2/15R pathways, proliferation of lymphocytes, and promotion of CD8^{+} cytotoxicity*
- *Does not bind to IL-2Rα or IL-15Rα*
- *Designed as Rβγc-restricted agonist to avoid preferential stimulation of Treg cells relative to Teff/NK cells, for more effective anti-tumor activity*
- *Low predicted immunogenicity*
- *High stability in serum*

MDK-202

- *MDK1169 fused to C-termini of IgG2 Fc*
- *Fusion retains the potency and full efficacy of MDK1169*
- *Highly stable in human plasma (T1/2 > 72 hr)*
- *Circulating T1/2 in mice and cynomolgus macaques of ~30hr*
- *Exhibits increase in lymphocyte counts in hPBMC-engrafted NCG-mice*
- *Low predicted immunogenicity*

αPD1-1169, -1406, -1442, -1445

- *MDK1169, and attenuated analogs, fused to C-termini of αPD-1 IgG HC*
- *Fusions retain binding affinity of αPD-1 for PD-1*
- *αPD1-1169 retains IL-2Rβγc potency of MDK-202*



RESULTS

Schematic of cis activation mechanism

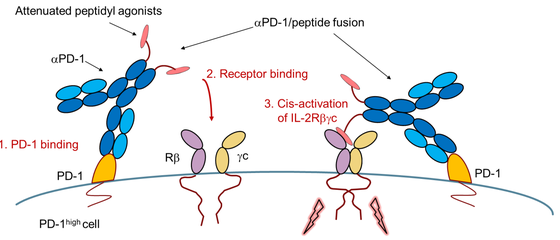
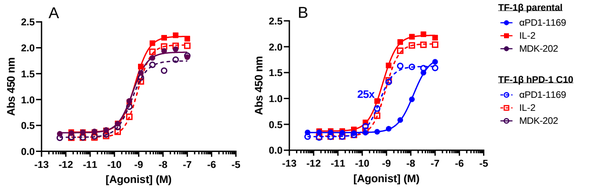


Fig 1: Proposed cooperative cis mechanism of PD-1 binding and IL-2/15R activation. (1) Compounds engage target cell via PD-1 binding, (2) cooperative binding of agonist peptides to proximal IL-2/15Rβγc receptor subunits, (3) receptor activation, initiating signaling.

αPD1-1169 exhibits modest potency enhancement on PD-1^{high} cells



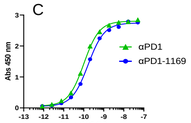


Fig 2: pSTAT5 response of TF-1β cells +/- PD-1 expression, treated with peptide agonist MDK1169 fused to αPD-1 antibody. TF-1β cells (TF-1 cells naturally express γc, engineered to express IL-2Rβ), and TF-1β/PD-1 cells (TF-1β engineered to express PD-1) were starved overnight and exposed to test compounds for 30 min at 37ºC. Cells were lysed and analyzed for pSTAT5 by Pathscan Phospho-Stat5 (Tyr694) ELISA Kit. (A) MDK-202, a fusion of MDK1169 to IgG Fc (no-targeting control); (B) αPD1-1169 tested on TF-1β (filled symbols/solid lines) and TF-1β/PD-1 (open symbols/dotted lines). (C) ELISA assay of αPD-1 and αPD1-1169 binding to immobilized PD-1.

Targeting attenuated IL-2/15Rβγc agonists to PD-1^{high} cells

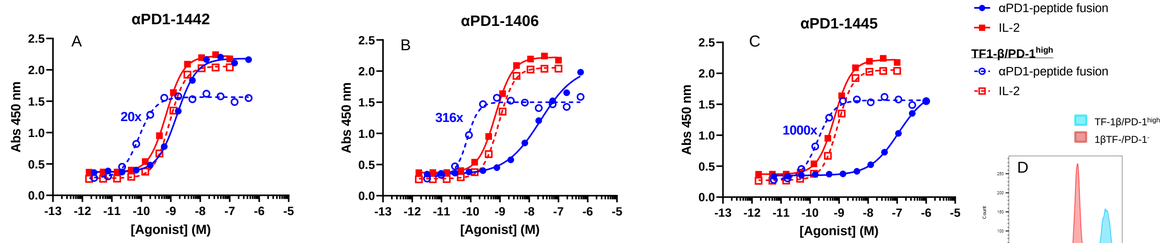


Fig 3: pSTAT5 response of TF-1β cells +/- PD-1 expression, treated with αPD1-directed IL-2Rβγc agonists. Test compounds were anti-PD-1 fusions of potency-attenuated derivatives of MDK1169: (**A**) αPD1-1442, (**B**) αPD1-1406, (**C**) αPD1-1445. (**D**) Histogram (MFI) of relative PD-1 expression on TF-1β (red) and TF-1β/PD-1 (blue) cells. Assay conditions as in Fig 2.

Targeting attenuated IL-2/15Rβγc agonists to activated CD8+ cells high in PD-1 expression

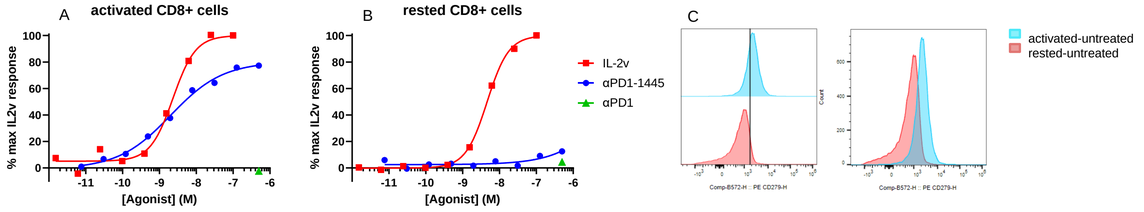


Fig 4: pSTAT5 response of CD8^{+} cells (+/- activation) to treatment with αPD1-1445. PBMCs isolated by density gradient centrifugation from buffy coat, and either rested overnight in T-cell medium, or activated three rounds, each for 3 days in anti-CD3 coated wells with anti-CD28 in solution, and rested overnight before treatment with compounds. Test compounds added to cells and incubated at 37°C for 30 min. Samples were fixed and permeabilized, then stained and analyzed for pSTAT5 accumulation and PD-1 expression by flow cytometry. (**A**) activated CD8^{+} cells (**B**) rested CD8^{+} cells. (**C**) flow analysis of CD279 (PD-1) expression in untreated cells.

RESULTS

Potency and affinity of targeted IL-2/15Rβγc agonists

	EC50 (M)		Ratio	Subunit binding	
Compound	TF-1β/PD-1^{high}	TF-β (PD-1lo)	TF-1β(PD-1lo) :TF-β/PD-1^{high}	IL-2Rβ monomer	IC50(M)
IL-2	9.2E-10	7.1E-10	1		
MDK-202	5.3E-10	5.7E-10	1		
αPD1-1169	5.2E-10	1.3E-08	25	1134	3.0E-09
αPD1-1442	8.2E-11	1.7E-09	20	1442	8.0E-09
αPD1-1406	7.6E-11	2.4E-08	316	1406	2.0E-07
αPD1-1445	1.10E-10	1.1E-07	1000	1445	1.0E-07

DISCUSSION AND CONCLUSIONS

We previously constructed a fusion of an anti-PD-1 antibody to MDK1169, a potent peptidyl IL-2/15Rβγc agonist, to create αPD1-1169 (3). That construct exhibits a 15-fold increase in potency of pSTAT5 accumulation on TF-1β/PD-1^{high} cells. This initial experiment served to demonstrate PD-1 targeting of the peptide agonist; but the potency of MDK1169 in this construct (~500pM, EC50 pSTAT5 induction) is too high, relative to the affinity of the antibody for PD-1, to achieve substantial αPD-1-directed-selectivity for PD-1^{high} cells over PD-1^{lo} cells.

To improve selectivity, a collection of peptide agonists, related to MDK1169, but reduced in potency for IL-2R activation in the absence of PD-1 targeting, were identified for fusion with αPD-1. We show here examples of these fusions exhibiting increases of up to 1000-fold in potency on TF-1β/PD-1^{high} compared to the parental TF-1β(PD-1^{lo}) cells. The most highly-selective of these PD-1-targeted compounds, αPD1-1445, was also evaluated on CD8^{+} lymphocytes, either resting, or activated to express elevated PD-1 levels, and found to exhibit substantially higher IL-2R potency in the activated lymphocytes. The activated CD8^{+} cells exhibit >3 log greater sensitivity to αPD1-1445 compared to the rested CD8^{+} cells low in PD-1.

Peptide agonists utilized in these experiments constitute a large sequence-related family, with members varying in potency, maximal efficacy, and the potential of signal pathway bias. The malleability of these novel agonists for facile analog generation provided compounds greatly reduced in potency, useful for construction of cell-selective cis-acting agonists that exhibit very low potency on IL-2-responsive cells low in PD-1 expression, and potent effects on PD-1^{+} cells. The collection of peptidyl IL-2R agonists may also be paired with a variety of antibody-based agents targeting useful cell surface markers to produce cis-mediated cell or tissue selectivity.

Targeting IL-2R agonists to a stem-like CD8+ population, characterized in part by high PD-1 expression, is reported to redirect differentiation from a transient effector pathway leading to an exhausted phenotype, into highly proliferative polyfunctional effectors with high cytotoxic potential (1-3). Those reports demonstrate IL-2/15 derivatives fused to blocking αPD-1 antibodies in a cis-acting molecule exhibit anti-tumor activity in animal models that is enhanced over IL-2/15R agonists and PD-1 blockade administered individually or in combination.

One aspect of the PD-1 targeting of MDK peptidyl agonists observed in our experiment, is a modest drop in maximum efficacy of IL-2R activation of PD-1 targeted agonists in PD-1^{high} cells compared to IL-2; yet exhibiting full efficacy in PD-1^{lo} cells. This effect may be attributable to an "anchor effect" of binding cell surface PD-1. Codarri-Deak et al (2) observed a cell-surface anchoring effect of PD-1, finding that internalization of IL-2v was slowed when fused to an anti-PD-1 antibody, suggesting that PD-1 binding impedes internalization of IL-2/15R agonists.

References

1) Wullshleger et al. 2021 AACR Abs 71
2) Codarri-Deak, et al. 2021 Pre-print in review at https://www.researchsquare.com/article/rs329812/v1
3) Xu, et al. Cancer Immunol Res 2021 (9)1141-1157
4) Dower et al 2019 SITC P618; Dower et al 2020 SITC P566