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MDK-202: an empirically-designed peptidyl agonist of IL-2/15βγc receptor, devoid of Rα interaction, unrelated to IL-2 or IL-15, and fused to an Fc-domain for PK enhancement

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Introduction

Efforts to adapt IL-2 for immuno-oncology applications focus on modifying the receptor selectivity of IL-2 by reducing Rα interaction to bias effects against Rα+ immune cells (e.g.Tregs). IL-2/15Rβγc-biased agonists also typically incorporate PK enhancement to extend duration of action and reduce side effects associated with peak drug levels. We previously described a small synthetic peptide (MDK1169), unrelated to IL-2 or IL-15, that binds IL-2/15Rβγc to induce receptor signaling[1]. Here we demonstrate that peptide, when fused to an IgG-Fc partner for PK enhancement (MDK-202), induces biological responses in human lymphocytes emulating those induced by natural cytokine agonists of IL-2/15Rβγc.

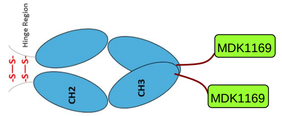
MDK-202 exhibits *in vitro* potency similar to the synthetic peptide (MDK1169), activating the major IL-2/15R signaling pathways and inducing proliferation (Ki-67) in human PBMCs, with kinetics and efficacy similar to IL-2. DGE profiling of CD8⁺ transcription patterns induced by IL-2v, IL-15, and MDK1169 show very high correlation of gene expression induction. Neither MDK1169 nor the bulkier MDK-202 interferes with the activities of other Rγc family receptors. In studies of CD-1 mice and non-human primates, MDK-202 exhibited half-life of ~30hr; and in hPBMC-engrafted NCG mice, affected lymphocyte population dynamics similarly to those reported for "non-Rα" variants of IL-2.

Properties of MDK1169 and MDK-202

MDK1169[1]

- 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 subnanomolar 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
- Exhibits activation of major IL-2/15R pathways, proliferation of lymphocytes, and induction of CD8⁺ cytotoxicity
- Does not bind to IL-2Rα or IL-15Rα
- Designed as IL-2/15Rβγc-restricted agonist to avoid preferential stimulation of Treg cells relative to Teff/NK cells, for more effective anti-tumor activity
- Low predicted immunogenicity (EpiVax[2])

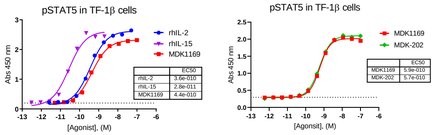
MDK-202



- Single chain C-terminal fusion of MDK1169 to Fc partner
- Retains the potency and full efficacy of MDK1169
- Low proteolysis in human plasma (T1/2 > 72 hr)
- Circulating T1/2 in mice and NHPs of ~30hr
- Lymphocyte stimulation in hPBMC-engrafted NCG-mice
- Low predicted immunogenicity (EpiVax[2])

Agonist Activities of MDK1169 and MDK-202

MDK1169 and MDK-202 activate IL-2/15Rβγc

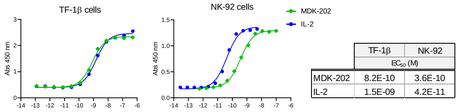


	EC50
rhIL-2	3.6e-010
rhIL-15	2.8e-011
MDK1169	4.4e-010

	EC50
MDK1169	5.9e-010
MDK-202	5.7e-010

Fig 1. MDK1169 and MDK-202 activate IL-2/15Rβγc with subnanomolar potency and full efficacy. TF-1 cells naturally express Rγc subunit, and are engineered to express Rβ to generate the IL-2/15 responsive cell line TF-1β. Cells were exposed to compounds for 30min and scored by ELISA for STAT5 phosphorylation.

Effect of IL-2Rα expression on potency of compounds



	TF-1β	NK-92
	EC50 (M)	
MDK-202	8.2E-10	3.6E-10
IL-2	1.5E-09	4.2E-11

Fig 2. Potency of MDK compounds is unaffected by presence of Rα subunit, in contrast to affinity boost exhibited for IL-2. Comparison of MDK-202 with IL-2 in responsive TF-1β cells (Rα^{lo}) and NK-92 cells (Rα^{hi}) demonstrates that the expression of IL-2Rα boosts the potency of IL-2 by ~100-fold, but has no effect on potency of the IL-2Rβγc-restricted MDK-202.

	CD8⁺	Treg
	EC50 (M)	
MDK1169	1.2E-09	7.0E-10
IL-2	8.1E-10	4.5E-12

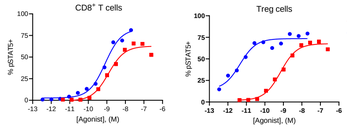
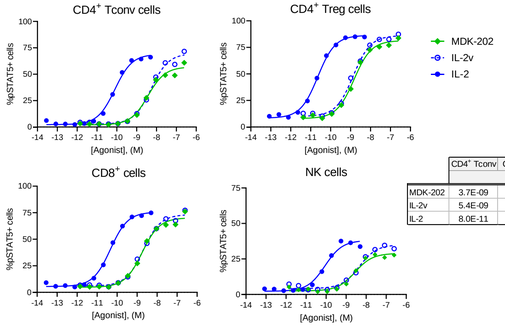


Fig 3. IL-2 stimulates CD4⁺Treg cells much more potently than rested CD8⁺ cells. Tregs constitutively express high levels of IL-2Rα, and are ~100-fold more responsive than rested CD8+ cells (Rα^{lo}) to IL-2. MDK-202 does not bind to Rα, exhibits equivalent potency on Tregs and CD8+, therefore avoiding preferential stimulation of Tregs.

MDK-202 emulates IL-2 in efficacy of stimulation of activated lymphocytes



	CD4⁺ Tconv	CD4⁺ Treg	CD8⁺	NK
	EC50 (M)			
MDK-202	3.7E-09	1.5E-09	1.8E-09	2.8E-09
IL-2v	5.4E-09	1.4E-09	2.0E-09	4.5E-09
IL-2	8.0E-11	2.3E-11	4.6E-11	8.8E-11

Fig 4. Major subpopulations of PBMC lymphocytes respond to MDK-202 with full efficacy of receptor activation (equivalent to IL-2). PBMCs were pre-activated and exposed to compounds 30min, stained for pSTAT5, and analyzed by flow cytometry.

Proliferation of CD4⁺, CD8⁺, and NK Cells

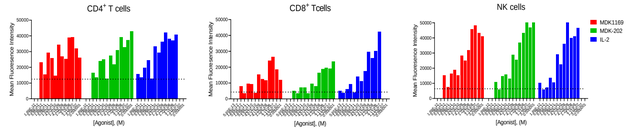


Fig 5. Proliferation (Ki67) of PBMCs exposed to compounds for 4 days. Freshly-isolated human PBMCs were cultured with test compounds (MDK1169, MDK-201, IL-2) for 4 days at 37°C. Cells were stimulated with soluble αCD3 mAb (1ng/mL; OKT3 clone) throughout the duration of the assay. After the incubation, cells were fixed, permeabilized, and stained with fluorophore-conjugated antibodies for FACS analysis. Lymphocyte proliferation was assessed by detection of Ki-67 expression in viable cells. Dotted lines indicate background Ki67 level for each population.

IL-2/15R Signaling Pathways in Activated CD8⁺ Cells

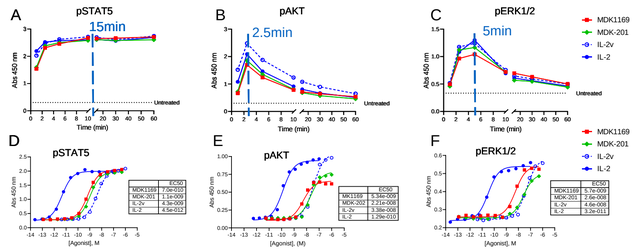


Fig 6. MDK compounds activate the three major signaling pathways of the IL-2Rβγc receptor, exhibiting kinetics and efficacy similar to natural agonists of the receptor. Panels **A-C** exhibit the time course of induction of Jak-STAT, PI3K, and MapK pathways by saturating (Emax) concentrations of each test compound. For each pathway, the kinetics of induction was identical for all compounds. Dose response curves (**D-F**) were performed at the Tmax, showing efficacy of MDK compounds to be fully efficacious (comparable to IL-2, and IL2v, an Rβγc-restricted variant of IL-2[3]) in inducing Jak-STAT (pSTAT5) and MapK (pERK) pathways, and partial agonists (~75%) of PI3K (pAKT). The relative potencies among the compounds are similar for induction of each pathway.

Differential Gene Expression in Activated CD8⁺ Cells

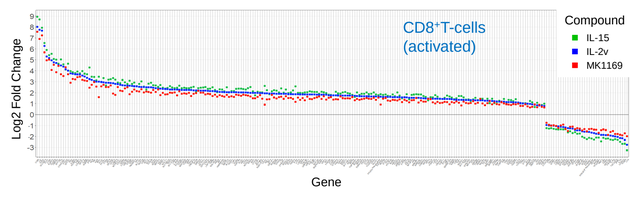


Fig 7. MDK1169 emulates the gene expression profiles induced by IL-2v and IL-15 in CD8⁺ T-cells. Activated CD8⁺ T-cells were incubated with the test compounds or buffer alone for 4hr at concentrations inducing maximum STAT5 phosphorylation (Emax). Samples were prepared in triplicate for RNA-seq analysis, and the 200 genes exhibiting highest statistical significance of differential expression for each compound compared to control were selected. Consolidating the top 200 genes for each compound generated a universe of 250 genes. These are displayed, sorted by IL-2v signal in descending order of Log2-Fold Change. All 250 genes exhibited similar differential expression upon stimulation of CD8⁺ cells with the three compounds, indicating, at this resolution of analysis, that MDK1169 stimulation faithfully emulates the transcription patterns of known IL-2/15Rβγc agonists in activated human peripheral CD8⁺ Tcells.

Potential Interference with Rγc Family Cytokines

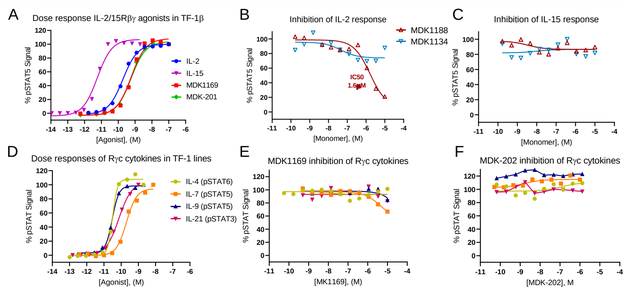
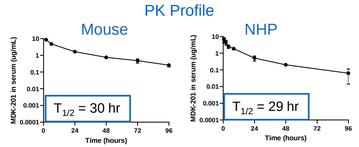


Fig 8. MDK compounds do not interfere with activities of "off-target" members of the Rγc cytokine family. (A) pSTAT5 dose response of IL-2R agonists in TF-1β cells, naturally expressing Rγc, and engineered to express Rβ subunit. **(B)** Inhibition of IL-2 and **(C)** IL-15 (~EC$_{50}$) responses by Rβ-binding (MDK1134) and Rγc-binding (MDK1188) peptide fragments of MDK1169. IL-2 and IL-15 activity was not inhibited by MDK1134, and IL-2 activity was only weakly inhibited by MDK1188. This suggests that MDK compounds interact with both receptor subunits at sites outside of the cytokine binding sites. **(D)** Dose response of IL-4, IL-7, IL-9, and IL-21 STAT activation in TF-1 cells expressing the respective Rα subunits. **(E)** Evaluation of MDK1169 and **(F)** MDK-202 inhibition of Rγc cytokine responses, indicating no significant interference with the biology of "off-target" Rγc cytokine receptor activation.

Animal Studies

Fig 9. MDK-202 exhibits extended half life in CD-1 mice and African green monkey. Single dose of MDK-202 was administered IV to CD-1 mice (1mg/kg) and African green monkeys (0.5mg/kg). Blood samples were collected into serum separator vials over 96h. The amount of MDK-202 in each sample was determined using a TF-1β pSTAT5 luciferase reporter cell line and calculated from standard curve. (T1/2 of MDK1169 in mice was <2 hr).



NCG mice (hPBMC-engrafted)

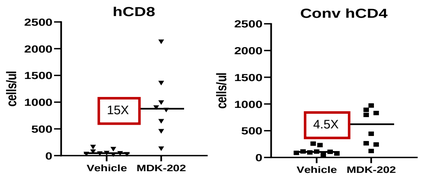


Fig 10. MDK-202 stimulates expansion of human CD8⁺ and CD4⁺T-cells in NCG mice engrafted with human PBMCs. NCG Mice (Charles River) engrafted with 2x10^7 hPBMCs from a single donor. At 14 days post-engraftment, mice exhibiting 10% hCD45⁺ lymphocytes were dosed (sc) with vehicle or 1mg/kg MDK-202. Cell counts were determined by flow cytometry 4 days after dosing.

Conclusions

MDK-202 is an attractive alternative to IL-2/15 variants for use in immuno-oncology applications. Constructed without reference or similarity to cytokine or receptor structures or contacts, the peptidyl agonist component (MDK1169) is completely unique and therefore unlikely to generate ADA's that neutralize IL-2 or IL-15. By construction of an Fc-peptide fusion, half-life was extended beyond that seen with PEGylated IL-2 variants, and avoids potential complications associated with anti-PEG antibodies know to occur in some individuals. The peptide has been shown to be active when fused to other proteins such as anti-PD-1 antibodies and other cytokine receptor agonists[1,4].

Citations

1. Dower,W. et al. (2019) MK1169, a peptide unrelated to IL-2, is a potent IL-2Rβγc agonist. SITC 2019 Poster #618
2. Moise, L. et al (2015) Human vaccines and immunotherapeutics 11:2312-23.
3. Klein,C. et al (2017) Oncoimmunology 6(3) e1277306
4. Dower, et al. (2020) MDK-271: A dual function molecule consisting of empirically-designed peptidyl agonists of IL-2/15Rbgc and IL-7Rαgc, unrelated to IL-2, IL-15, or IL-7, incorporated into a bispecific Fc fusion protein. SITC 2020 Poster #691