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MDK1319/MDK-701: A potent peptidyl agonist of IL-7Rαγc, designed with no reference to cytokine or receptor structure and unrelated to IL-7, fused to an Fc-domain for PK enhancement

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Introduction

IL-7 receptor activation is essential for the proper development and homeostasis of T-cell subpopulations and maintenance of TCR clonal repertoire. Emerging evidence indicates potential clinical utility of IL-7 for immunotherapy of lymphopenia, oncology, and other indications. Here we report the discovery of MDK1319, a small novel peptidyl agonist of IL-7R. This peptide is structurally unrelated to IL-7, with MW less than 5000Da. To improve *in vivo* properties, we fused MDK1319 to an IgG Fc-domain to construct MDK-701, which exhibits biological properties similar to those of IL-7 *in vitro*, and when administered to non-human primates.

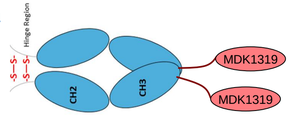
MDK1319 and MDK-701 activate the major IL-7R signaling pathway JAK-STAT (pSTAT5), and induce proliferation (Ki67) in human CD4⁺ and CD8⁺T-cells, exhibiting lymphocyte subpopulation selectivity and efficacy similar to that of IL-7. Agonism is attributable to direct activation of IL-7R, as shown by dependence on the presence of the IL-7Rα subunit for response in test cells, and lack of inhibition by IL-7 neutralizing antibodies. MDK1319 and MDK-701 do not activate nor inhibit any other ("off target") Rγc family receptors at concentrations 100-fold greater than required for maximal IL-7R activation. MDK-701 administered to cynomolgus macaques (single dose IV at 1mg/kg) exhibits a circulating terminal half life of ~32hr; and induces peripheral lymphocyte profiles similar to IL-7 treatment. This includes initial reduction (tissue migration), followed by elevation of peripheral lymphocytes, sustained above baseline for 29 days.

Properties of MDK1319 and MDK-701

MDK1319

- 45 amino acid synthetic peptide
- Novel sequence, unrelated to IL-7 or other cytokines
- Binds to both IL-7Rα and Rγc subunits
- Binds outside of the cytokine binding sites on both subunits
- IL-7R agonist with subnanomolar potency and full efficacy
- Receptor activation requires expression of both IL7Rα and Rγc, and is not inhibited by neutralizing IL-7 antibodies
- Exhibits activation of major IL-7R pathways and proliferation of lymphocytes
- Low predicted immunogenicity (EpiVax[1])

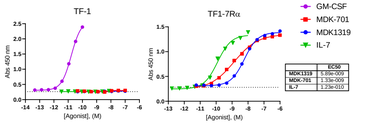
MDK-701



- Single chain C-terminal fusion of MDK1319 to Fc partner
- Retains the potency and full efficacy of MDK1319
- Circulating T1/2 in NHPs of >30hr
- Lymphocyte stimulation in NHP
- Low predicted immunogenicity (EpiVax[1])

Agonist activities of MDK1319 and MDK-701

MDK1319 and MDK-701 directly activate IL-7R



	EC50
MDK1319	5.93e-009
MDK-701	1.21e-009
IL-7	1.23e-010

Fig 1. MDK1319 and MDK-701 activate IL-7R with nanomolar potency and full efficacy. TF-1 cells naturally express Rγc subunit, and are engineered to express IL-7Rα to generate the IL-7 responsive cell line TF1-7Rα. Cells were exposed to compounds for 30min and scored by ELISA for pSTAT5

MDK1319 and MDK-701 stimulate huPBMCs

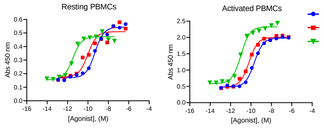
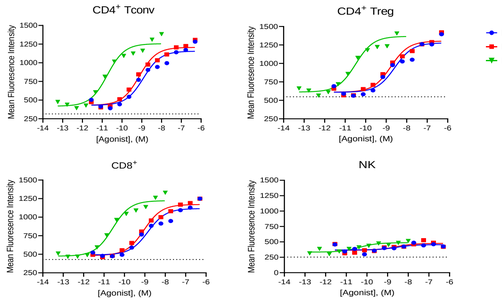


Fig 2. PBMC JAK-STAT signaling is fully induced by MDK1319 and MDK-701. Human PBMCs incubated for 30 min with MDK compounds, and pSTAT5 was determined by ELISA. MDK compounds induce STAT5 phosphorylation with efficacy comparable to IL-7

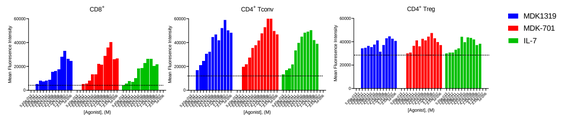
MDK1319 and MDK-701 emulate IL-7-induced pSTAT5 response in lymphocytes



	CD4⁺ Tconv	CD4⁺ Treg	CD8⁺	NK
	EC50 (M)			
MK1319	1.2E-09	2.6E-09	1.4E-09	
MDK-701	7.5E-10	1.7E-09	9.1E-10	
IL-7	1.8E-11	3.2E-11	2.6E-11	4.3E-11

Fig 3 Major subpopulations of PBMC lymphocytes respond to MDK1319 and MDK-701 with full efficacy of receptor activation (equivalent to IL-7). PBMCs were pre-activated and exposed to compounds for 30min, stained for pSTAT5, and analyzed by flow cytometry.

Proliferation (Ki67) of major lymphocyte populations

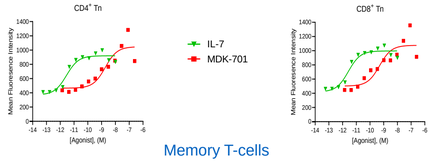


	CD8⁺	CD4⁺ Tconv	CD4⁺ Treg
	EC50 (M)		
MDK1319	3.7E-09	4.4E-10	2.0E-08
MDK-701	5.2E-10	4.3E-10	3.8E-11
IL-7	8.9E-11	3.3E-11	2.8E-11

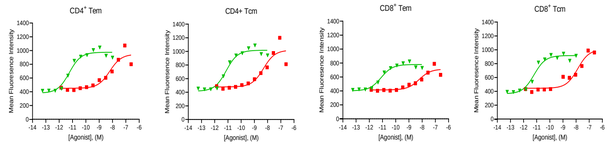
Fig 4. Proliferation (Ki67) of PBMCs exposed to compounds for 4 days. Freshly-isolated human PBMCs were cultured with test compounds (MDK1319, MDK-701, IL-7) for 4 days at 37°C. Cells were stimulated with soluble αCD3 mAb (1ng/mL; OKT3 clone) throughout the duration of the assay. After incubation, cells 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 line indicates Ki67 background.

MDK compound effects on CD4⁺ and CD8⁺ naive and memory

Naive T-cells



Memory T-cells



	Naïve T-cells		Memory T-cells			
	CD4⁺ Tn	CD8⁺ Tn	CD4⁺ Tem	CD4⁺ Tcm	CD8⁺ Tem	CD8⁺ Tcm
	EC50 (M)					
IL-7	2.9E-12	3.3E-12	5.8E-12	7.4E-12	7.9E-12	6.4E-12
MDK-701	1.8E-09	9.7E-10	6.2E-09	4.9E-09	6.0E-09	1.3E-08

Fig 5. MDK-701 stimulates STAT5 phosphorylation in resting naive and memory T-cell populations with efficacy comparable to IL-7. Fresh human PBMCs were isolated from a buffy coat by density gradient centrifugation, then rested overnight in T cell medium. The following day, the PBMCs were plated in a 96-well plate at 10^6 cells/well and treated with test compounds for 30 minutes. After the incubation, the cells were fixed, permeabilized, and stained with fluorophore-conjugated antibodies for FACS analysis

Differential gene expression in naive and memory T-cells

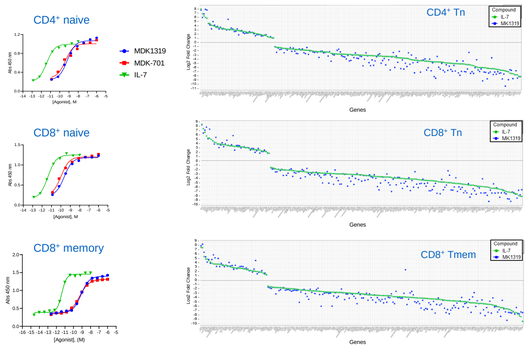


Fig 6. MDK1319 emulates the gene expression profile induced by IL-7 in CD4⁺Tn, CD8⁺ Tn, and CD8⁺ Tmem cells. CD4⁺Tn, CD8⁺Tn, and CD8⁺Tmem cells were isolated in bulk from fresh PBMCs by negative selection. Cells were incubated with the test compounds or buffer alone for 24hr at concentrations inducing 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 buffer control were selected. Differential expression data is sorted by IL-7 signal in descending order of Log2-Fold change. These genes exhibited similar differential expression upon stimulation of the respective T-cell populations with both compounds, indicating, at this resolution of analysis, that MDK1319 stimulation emulates the transcription patterns of IL-7 in these samples of IL-7-responsive T-cell populations

Potential interference with Rγc-family cytokines

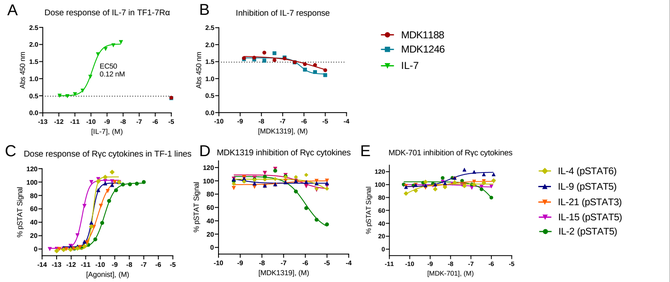
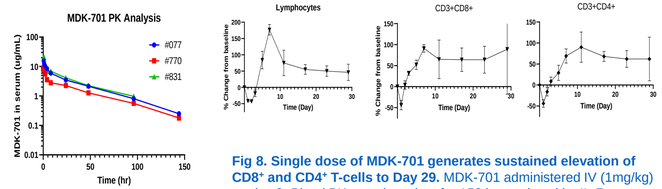


Fig 7. MDK compounds do not significantly interfere with activities of "off-target" members of the Rγc cytokine family. (A) pSTAT5 dose response of IL-7 in TF1-7Rα cells, naturally expressing Rγc, and engineered to express IL-7Rα subunit. **(B)** Inhibition of IL-7 (~EC_{50}) responses by Rα-binding (MDK1246) and Rγc-binding (MDK1188) peptide fragments of MDK1319. IL-7 activity was not significantly inhibited by either MDK1246 or MDK1188, indicating that MDK1319 interacts with both receptor subunits at sites outside of the cytokine binding sites. **(C)** Dose response of IL-2, IL-4, IL-9, IL-15, and IL-21 STAT activation in TF-1 cells expressing the respective Rα or β subunits. **(D)** Evaluation of MDK1319 and **(E)** MDK-701 inhibition of Rγc cytokine responses indicate detectable interference with only the IL-2 response, with an IC_{50} of >1uM. The potency of this effect is ~100-fold lower than the affinity of MDK1319 for Rγc binding, indicating weak non-competitive inhibition; and >1000-fold lower than the EC_{50} of the MDK IL-7R agonists, thus likely to have no practical impact in therapeutic applications. Slight IL-2R inhibition is also seen with MDK-701 at the highest concentration tested (1uM). Receptor activation induced by the other "off-target" Rγc cytokines is not inhibited by either MDK agonist.

Cynomolgus macaque studies

Single Dose Study of MDK-701 in cynomolgus macaques



Mean Terminal $T_{1/2}$ ~ 32 hrs

Fig 8. Single dose of MDK-701 generates sustained elevation of CD8⁺ and CD4⁺ T-cells to Day 29. MDK-701 administered IV (1mg/kg) on day 0. Blood PK samples taken for 150 hr; analyzed by IL-7 responsive reporter cell assay. For PD analysis ALC was determined, and CD4⁺ and CD8⁺ lymphocyte counts determined by flow cytometry to day 29.

Conclusions

MDK-701 offers a potential alternative to recombinant forms of IL-7 as a monotherapy for treatment of chemotherapy- or radiation-induced lymphopenia, and as a combination with checkpoint inhibitors, cell therapy, or neo-antigen vaccines. The small peptidyl nature of the active peptide MDK1319 makes it readily fusable to recombinant protein partners and offers opportunities for incorporation into bispecific molecules, linking IL-7 activity to a variety of useful functions. The active peptide component of MDK-701 has been selected for low immunogenicity; and its novel primary structure, unrelated to IL-7, avoids the possibility of generating neutralizing IL-7 antibodies that have been reported in human studies with glycosylated or Fc-fused IL-7.

Citations

1. Moise, L. et al (2015) Human vaccines and immunotherapeutics 11:2312-23.