MDK1319/MDK-701: A potent peptidyl agonist of IL-7Rαc, 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 subsets and maintenance of TCR clonal repertoires. Emerging evidence indicates potential clinical utility of IL-7 for immunotherapy of lymphoproliferative, oncologic, and other indications. Here we report the discovery of MDK1319, a small novel peptidyl agonist of IL-7Rαc. This agonist is structurally unrelated to IL-7, with MW less than 5500Da. To improve in vivo properties, we fused MDK1319 to an Fc Fc-domain to construct MDK-701, which exhibits biological properties similar to those of IL-7 in vitro and in vivo, and when administered to non-human primates.

MDK1319 and MDK-701 activate the major IL-7Rα signaling pathway JAK-STAT (pSTAT), and induce proliferation (K67) in human CD4+ and CD8-T cells, exhibiting targeted potency, selectivity and efficacy similar to that of IL-7. Agonism is attributable to direct activation of IL-7Rα, as shown by dependence on the presence of the IL-7Rα subunit for response in T 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-7Rα activation. MDK1319 administered to cynomolgus macaques (single dose 1µg 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 (dose migration), followed by elevation of peripheral lymphocytes, sustained above baseline for 29 days.

Properties of MDK1319 and MDK-701

MDK1319

- ECαααα-cannonic acid C-terminus fusion of MDK1319 to Fc partner
- Contains the potent and efficacious IL-7Rα agonist described in MDK-701
- Contains 112 µM Fc Fc-domain
- Lyophilized formulation stored at 4°C
- Lyophilized formulation is ≥80% intact
- Has predicted immunogenicity (Epitope)

MDK-701

- Single chain C-terminus fusion of MDK1319 to Fc partner
- Contains the potent and efficacious IL-7Rα agonist described in MDK-701
- Contains 112 µM Fc Fc-domain
- Lyophilized formulation stored at 4°C
- Lyophilized formulation is ≥80% intact
- Has predicted immunogenicity (Epitope)

Agonist activities of MDK1319 and MDK-701

Fig 1. MDK1319 and MDK-701 activate IL-7Rα with nanomolar potency and full efficacy. TF-1 cells stably expressing Rγc and IL-7Rα were seeded at 75% confluence, with IL-7Rα activation induced by 70nM, to determine the 4-7 response at low TF-1 cell numbers. Cells were fixed and stained with fluorophore conjugated anti-Fc antibodies for FACS analysis. pSTAT5 was assessed by detection of 4-7 expression in viable cells. Cells exhibiting the background (B) were subtracted. The panel on the left shows the secretory profile of MDK1319 and MDK-701 directly activated IL-7Rα. The panel on the right shows the degranulation profiles of MDK1319 and MDK-701 stimulated hPBMCs.

MDK compound effects on CD4+ and CD8+ naive and memory T-cells

Fig 4. Proliferation (K67) of PBMCs exposed to compounds for 4 days. freshly isolated human PBMCs were cultured with test compounds (MDK1319, MDK-701, IL-7) at 4°C at 37°C. Cells were stimulated with IL-7 (7.6 nM) and Fc receptor stimuli (IL-7Rα agonist). An ELISA assay is used to assess the amount of secreted antibodies. Cells exhibiting the background (B) were subtracted. The panel on the left shows the IL-7Rα agonist results for MDK1319 and MDK-701, respectively.

Differential gene expression in naive and memory T-cells

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 bulk cost to be used for this experiment. The PBMCs were fixed, permeabilized, and stained for pSTAT5, and analyzed by flow cytometry. The panel on the left shows the immediate (8 min) responses of MDK1319 and MDK-701, respectively. The panel in the middle shows the IL-7Rα agonist responses of MDK1319 and MDK-701, respectively. The panel on the right shows the IL-7Rα agonist responses of MDK1319 and MDK-701, respectively.

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) STAT5 dose response of IL-7, 7α,7β-T cells, naturally expressing Rγc and IL-7Rα, to MDK-701 is shown in the left panel. (B) IL-21 dose response of MDK-701, IL-21Rα+βγc, to MDK-701 is shown in the middle panel. (C) IL-21 dose response of MDK-701, IL-21Rα+βγc, to MDK-701 is shown in the right panel. The data in (A) and (C) are presented to show the potential of MDK-701 to induce STAT5 phosphorylation with efficacy comparable to IL-7.

Cynomolgus macaque studies

Single Dose Study of MDK-701 in cynomolgus macaques

Fig 6. Single dose of MDK-701 generates sustained elevation of CD4+ and CD8+ T-cells to Day 26. MDK-701 administered IV (1mg/kg) daily for 14 days to cynomolgus macaques exposed to IL-7Rα agonist on Day 0. The samples are free from any potential inhibitors or interference in the biological or pharmacological effects of the test compound.

Conclusions

MDK1319 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 on-anogen vaccines. The small peptide length of the active peptide MDK1319 makes it readily fusible to recombinant protein partners and offers opportunities for incorporation into bioparticulate molecules, linking IL-7 activity to a variety of useful functions. The active peptide component of MDK1319 has been selected for low immunoreactivity, and its novel primary structure, unrelated to IL-7, avoids the possibility of generating neutralizing IL-7- or other cytokine antibodies that have been reported in human studies with glycoylated or Fc-fused IL-7.

Citations