MDK-271: A dual function molecule consisting of empirically-designed peptidyl agonists of IL-2/15Rβγc and IL-7Rαγc, unrelated to IL-2, IL-15, or IL-7, incorporated into a bispecific Fc fusion protein

William J. Dower, Steven E. Cwirla; Alice V. Bakker, Praechompoo Pongtornpipat, Blake M. Williams, Prarthana Joshi, Sandra M. Wang, Michael C. Needels, Ronald W. Barrett,
Medikine, Inc. Menlo Park, CA

Introduction

Modified versions of IL-2 or IL-7, compounds activating either IL-2/15Rβγc or IL-7R, are under investigation as monotherapy, or in combination with checkpoint inhibitors, engineered T or NK cells, or neo-antigen vaccines for immuno-oncology. We have previously described small synthetic peptides, unrelated to IL-2, IL-15, or IL-7, that selectively activate either IL-2/15Rβγc or IL-7R[(1-3)]. IL-2/15Rβγc and IL-7R agonists exhibit some complementary effects on immune cells, which combined may offer benefits over each independent mechanism. We now report the creation of an IgG-Fc-fusion protein that incorporates both IL-2/15Rβγc and IL-7R agonist peptides, and describe its properties in cell lines and human (PBMC) lymphocyte populations.

Peptide agonists of IL-2/15Rβγc (MDK1169), and IL-7R (MDK1319) were separately fused to each chain of an asymmetric heterodimeric Fc molecule. The Fc-fusion was purified by protein-A and size exclusion chromatography, and characterized by LC-MS. Biological properties were initially characterized by examining activation of the shared Jak-STAT5 pathway of IL-2R and IL-7R in cell lines and human lymphocyte populations

Cell-based assay of MDK-271 demonstrates potent, fully efficacious phosphorylation of STAT5 in TF-1 cells naturally expressing Rγc, and engineered to express either IL-2/15Rβ or IL-7Rα. PBMCs exposed to MDK-271 exhibit additive and complementary effects among various lymphocyte subpopulations. The mono-specific agonists MDK-202 and MDK-701 produce proliferative effects and signaling patterns in responsive cell lines and lymphocyte subsets similar to those induced by IL-2v (an IL-2/15Rβγc-biased mutant of IL-2)[(4)] and IL-7[(3)], respectively. Combining both activities in MDK-271 induces response profiles that differ in some T-cell subsets from those of mono-specific agonists of the two receptors.

Dual Agonist MDK-271

- Knob-in-hole forced Fc heterodimer (asymmetric) construction
- IgG-Fc molecule, one chain fused at the C-terminus to IL-2/15Rβγc agonist MDK1169, and the other chain to IL-7Rαγc agonist MDK1319
- Retains the full efficacy of MDK1169 and MDK1319
- Respective agonist activities depend on IL-2Rβ and IL-7Rα chain expression

IL-2/15Rβγc and IL-7αγc Dual Agonist MDK-271

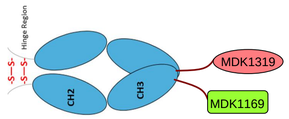


Fig 1. Schematic of dual agonist MDK-271. By knob-in-hole forced Fc heterodimer construction, an IgG-Fc molecule containing one chain fused at the C-terminus to IL-2/15Rβγc agonist MDK1169, and the other chain to IL-7Ragc agonist MDK1319.

MDK1169 [(1, 2)]

- 42 amino acid synthetic peptide
- Novel sequence, unrelated to IL-2 or IL-15, or other cytokines
- 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 Rβγc-restricted agonist to avoid preferential stimulation of Treg cells relative to Teff/NK cells, for more effective anti-tumor activity
- Low predicted immunogenicity (EpiVax [(4)])

MDK1319 [(3)]

- 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 cytokine binding site 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 [(4)])

Purification and structural characterization of MDK-271

SDS-PAGE gel
Non-reduced

LCMS analysis of MDK-271
(reduced & deglycosylated)

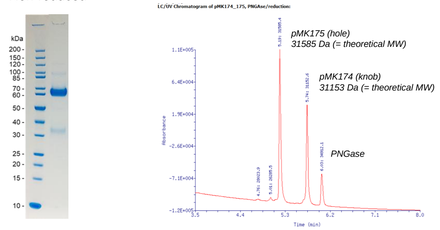


Fig 2. Structural confirmation of dual agonist MDK-271. Supernatant from HEK-293 cell culture expressing MDK-271 was passed over protein A column. PrA-captured fraction was further purified by SEC. Non-reduced SDS-PAGE indicated the expected MW for the assembled Fc-fusion. Deglycosylation and LCMS analysis of SEC purified MDK-271 confirmed that the protein contained two chains, each of the size expected for the individual KiH-Fc chains fused to either MDK1169 or MDK1319 agonist peptides.

Agonist Properties on Cell Lines

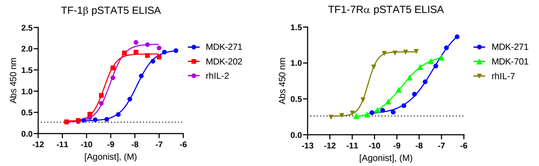
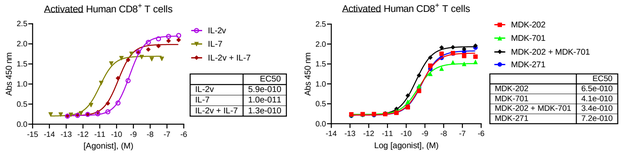


Fig 3. Potency and efficacy of MDK-271 on TF-1β (engineered to express IL-2/15 Rβγc) and TF1-7Rα (expressing IL-7Rαγc) cell lines

Agonist Properties on Major T-cell Populations

pSTAT5 CD8⁺ cells (ELISA)

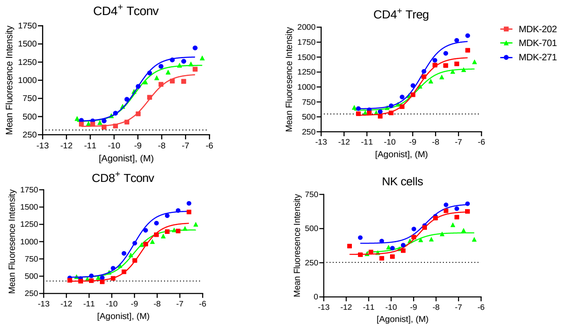


	EC50
IL-2v	5.9e-010
IL-7	1.0e-011
IL-2v + IL-7	1.3e-010

	EC50
MDK-202	6.5e-010
MDK-701	4.1e-010
MDK-202 + MDK-701	3.4e-010
MDK-271	7.2e-010

Fig 4. Potency and efficacy of STAT5 activation by dual agonist MDK-271 compared with Medikine IL-2Rβγc and IL-7R agonists, and cytokines IL-2v[(4)] and IL-7

MDK-271 stimulation of major lymphocyte populations (flow)

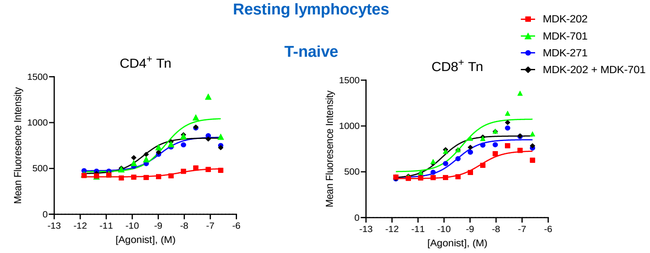


	CD4⁺ Tconv	CD4⁺ Treg	CD8⁺ Tconv	NK
	EC50 (M)			
MDK-202	2.8E-09	1.8E-09	2.0E-09	1.9E-09
MDK-701	7.5E-10	1.7E-09	9.1E-10	8.1E-10
MDK-271	8.7E-10	2.6E-09	9.5E-10	3.4E-09

Fig 5. Effect of MDK-271, MDK-202 and MDK-701 on STAT5 phosphorylation in activated CD4⁺, CD4⁺Tregs, CD8⁺, and NK cells

Effects of MDK-271 on Naive and Memory T-cells

Resting lymphocytes



T-memory

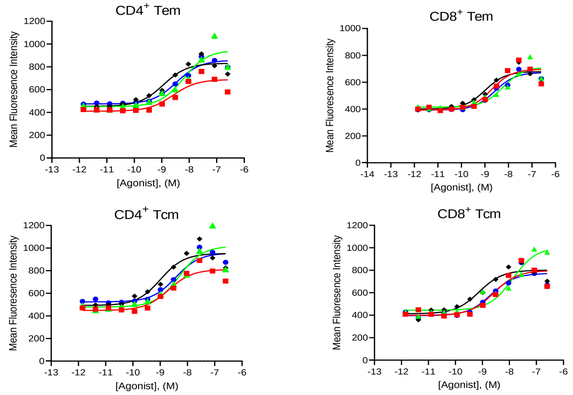


Fig 6. MDK-271 stimulates resting T-cell naive and memory populations with efficacy comparable to IL-7 full agonists. Fresh human PBMCs were isolated from a buffy coat by density gradient centrifugation, then rested overnight in T cell medium. The following day, the PBMCs were plated in a 96-well plate 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

Conclusions

IL-2/15Rβγc and IL-7R are both currently undergoing extensive scrutiny as potential immuno-oncology therapeutic targets. The biology of these cytokines is both overlapping and complementary in stimulating and supporting T-cell populations; and some recent evidence suggests possible superiority of the combination. Based on in vitro properties, the Fc-peptide fusion reported here, exhibiting both IL-2/15Rβγc-biased agonist and IL-7Rαγc agonist activities, may be valuable in anti-tumor therapeutic applications.

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. Dower,W. et al. (2020) 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 SITC 2020 Poster #566
3. Dower,W. et al. (2020) 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. SITC 2020 Poster #567
4. Klein,C. et al. (2017) Oncoimmunology 6(3) e1277306
5. Moise, L. et al. (2015) Human vaccines and immuno-therapeutics 11:2312-23.