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

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Introduction

Modified versions of IL-2 or IL-7 compounds activating either IL-2/15Rβγc or IL-7Rαγc are under investigation as immunotherapy, or in combination with checkpoint inhibitors, engineered T or NK cells, or neo-antigen vaccines for immune-oncology. We have previously described empirical synthetic peptides, unrelated to IL-2, IL-15, or IL-7, that selectively activate either IL-2/15Rβγc or IL-7Rαγc. The IL-2/15Rβγc and IL-7Rαγc 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-7Rαγc agonists, and describe its properties in cell lines and human (PBMC) lymphocyte populations.

Peptide agonists of IL-2/15Rβγc (MDK1169), and IL-7Rαγc (MDK1319) were separately fused to each chain of an asymmetric heteromeric 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-STAT pathway of IL-2R and IL-7R in cell lines and human lymphocyte populations.

Cell-based assay of MDK-271 demonstrates potent, totally efficacious phosphorylation of STATS in TF-1 cells naturally expressing Rγc, and engineered to express either IL-2/15Rβγc or IL-7Rαγc. 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-2 (an IL-2/15Rβγc-biased mutant of IL-22) and IL-7, respectively. Combining both activities in MDK-271 induces responses that differ in some T-cell subsets from those of mono-specific agonists of the two receptors.

Dual Agonist MDK-271

- Knock-in forced Fc heterodimer (asymmetric) construction
- IgG Fc molecule, one chain fused at the C-terminus to IL-2/15Rβγc agonist MDK1179, and the other chain to IL-7Rαγc agonist MDK1319
- Retains the full efficacy of MDK1169 and MDK1319
- Respective agonist activities depend on IL-2/15Rβγc and IL-7Rαγc chain expression

Fig. 1. Schematic of dual agonist MDK-271. By co-fusing a forced Fc heterodimer, combinations of IL-2/15Rβγc agonist MDK-202 and IL-7Rαγc agonist MDK-701 were generated and characterized. The IL-2/15Rβγc chain included a cysteine at position 271 to minimize aggregation, and in some cases a cysteine at position 331 to enhance PK stability.

Purification and structural characterization of MDK-271

Fig. 2. Structural confirmation of dual agonist MDK-271. Comparison of SEC gradient elution for MDK-271, compared with the monospecific agonists MDK-202 and MDK-701, from which MDK-271 was purified. LCMS analysis of SEC-purified MDK-271 confirmed the protein contained two chains, each of the size expected for the individual mono-Fc chains fused to either MDK1179 or MDK1319 agonist peptides.

Conclusions

IL-2/15Rβγc and IL-7Rαγc are both currently undergoing extensive scrutiny as potential immune-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.

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

Fig. 4. Potency and efficacy of STATS activation by dual agonist MDK-271 compared with Medikine IL-2/15Rβγc agonist, and cytokines IL-2v and IL-7

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

Fig. 6. MDK-271 stimulates resting T-naive and memory T-cells with efficacy comparable to IL-2/15Rβγc agonist. Fresh human PBMCs were isolated from buffy coat material and placed into T cell culture. The following day, the PBMCs were plated into 96-well plates at 10^6 cells and treated with test compounds for 48 hours. After the incubation, the cells were fixed, permeabilized, and stained with fluorescein-conjugated antibodies for FACS analysis.

Fig. 7. MDK-271 stimulation of major lymphocyte populations in flow cytometry.