**Introduction**

Derivatives of IL-2, IL-15, and IL-7 are in clinical development as immune-oncology agents. IL-2 and IL-15 primarily stimulate the proliferation and enhance the function of effecting T cells and natural killer cells, whereas IL-7 acts primarily on naive and memory T cells and is crucial for persistent effector T cell generation (clonal expansion). In contrast with these cytokines, MDK1319, which functions as a dual agonist of TCR and IL-7R, inhibits T cell proliferation in TCR and IL-7R double-negative T cells, which are connected with the licensing of TCR and IL-7R expression. We report the in vitro pharmacology of a synthetic branched peptide, MDK1517, which functions as a dual agonist of TCR and IL-7R.

**MDK1654 induces pSTAT5 in T and NK cells**

- **Resting PBMCs**
  - **TCR-Activated PBMCs**

**MDK1654 expands CD8+ T cells**

- **Resting**
  - **TCR-Activated**

**MDK1654 binds IL-7R and IL-2 Receptor Chains**

- **MDK1654 binding to IL-7R**
  - **CD16**

**MDK1654 is an agonist of both IL-7 and IL-2 Receptors**

- **Resistant**
  - **TCR-Activated**

**MDK1654 Expands Memory T cells**

- **Resting**
  - **TCR-Activated**

**Summary & Conclusion**

MDK1654 acted as an agonist in engineered TF-1 cells expressing 4EGRF (2.2 ng/mL) or 4EGRF (10 ng/mL). In resting PBMCs, MDK1654 induced STAT5 and expanded T cells similarly to the IL-7 PEPTIKINE. MDK1654’s expansion of CD8+ T cells displayed a greater phenotype than that of MDK1517, and MDK1654 induced CD4+ T cell expansion on cells activated by IL-2 but not PEPTIKINE.

**MDK1654 Expands CD56+ NK cells**

- **Resting**
  - **TCR-Activated**

**MDK1654 Expands γδ T Cells**

- **Resting**
  - **TCR-Activated**

**MDK1654: A Branched Synthetic Peptide that Activates Both the IL-7 Receptor and the βγc Form of the IL-2/15 Receptor**

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D. Data from Hodge et al. Scandinavian J of Immunol (2000) 51, 67

**Fig. 5. Proliferation of CD8+ and CD4+ T cells in response to PEPTIKINES.** Proliferation assays were performed overnight. The following day, cells were harvested and stained with live/dead dye and CD8 and CD4 antibodies. Cells were then evaluated by flow cytometry and plotted for CD8, CD4, and CD69. Data are expressed as mean + standard error of the mean (SEM).

**Fig. 7. The proliferation of peripheral NK cells following treatment with PEPTIKINES.** Proliferation assays were performed overnight. The following day, cells were harvested and stained with live/dead dye and CD3 antibodies. Cells were then evaluated by flow cytometry and plotted for CD3, CD16, and CD56. Data are expressed as mean + standard error of the mean (SEM).

**Fig. 1. Schematic illustration of MDK1654 structure.** The dual PEPTIKINE MDK1654 is a synthetic branched peptide composed of three peptide ligands: one single IL-2 peptide chain for TCR activation and two single IL-7 peptide chains for IL-7R activation. A second chain composed of 20 Cys residues is added to MDK1654 as a protective chain equipped with cysteine residues. The unprotected and the protected MDK1654 peptides were incubated in a labeled cell line and observed for IL-7R binding capacity. After washing, cells were fixed, permeabilized, stained with anti-STAT5 antibody, and analyzed by flow cytometry. MDK1644 is a close analog of MDK1654. Similar results were observed in another donor.

**Fig. 4. Induction of pSTAT5 in PBMC treated with PEPTIKINES.** Proliferation from PBMCs from a healthy donor were rested overnight or CDDO-COOH activated and stained for viability. Followed by cell surface antibody staining as shown in figure. Cells were harvested and incubated with the PEPTIKINES for 20 and 40 minutes. After washing, cells were fixed, permeabilized, stained with anti-pSTAT5 antibody, and analyzed by flow cytometry. MDK1644 is a close analog of MDK1654. Similar results were observed in another donor.

**Fig. 2. Western blot analysis of MDK1654 expression.** Western blot analysis of MDK1654 expression in cell lines. Resting PBMCs were harvested, and the expression of MDK1654 was confirmed by Western blotting. Data are expressed as mean ± standard error of the mean (SEM).

**Fig. 3. MDK1654 phosphopeptide of pSTAT5, pSTAT6, and pSTAT7 is detected by MDK1654 binding to IL-7R.** The expression of MDK1654 in cell lines was confirmed by Western blotting. Data are expressed as mean ± standard error of the mean (SEM).

**Fig. 6. MDK1654 binds IL-7R and IL-2 Receptor Chains.** The binding affinity of MDK1654 for IL-7R (IC50, 10.2 nmol/L) and IL-2 (IC50, 13.2 nmol/L) and for the 7R chain was determined by flow cytometry. Data are expressed as mean ± standard error of the mean (SEM).

**Fig. 7. The proliferation of peripheral NK cells following treatment with PEPTIKINES.** Proliferation assays were performed overnight. The following day, cells were harvested and stained with live/dead dye and CD3 antibodies. Cells were then evaluated by flow cytometry and plotted for CD3 and CD56. Data are expressed as mean ± standard error of the mean (SEM).

**Fig. 8. The proliferation of γδ T cells following treatment with PEPTIKINES.** Proliferation assays were performed overnight. The following day, cells were harvested and stained with live/dead dye and CD3 antibodies. Cells were then evaluated by flow cytometry and plotted for CD3 and CD4. Data are expressed as mean ± standard error of the mean (SEM).