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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Introduction

Derivatives of IL-2, IL-15, and IL-7 are in clinical development as immuno-oncology agents. IL-2 and IL-15 primarily stimulate the proliferation and enhance the function of effector T cells and natural killer cells, whereas IL-7 acts primarily on naïve and memory T cells and is crucial for persistent effector T cell generation (stemness). Combining these complementary effects on immune cells may offer benefits over either mechanism alone. Here we report the in vitro pharmacology of a synthetic branched peptide, MDK1654, which functions as a dual agonist for IL-2Rβγ and IL-7R. MDK1654 comprises three peptide ligands that bind separately to IL-7Rα, IL-2/15Rβ, and common γc, and are connected with linkers that provide an appropriate spatial orientation of the ligands. We refer to synthetic peptide mimetics of cytokines as PEPTIKINES. The dual IL7/IL-2 PEPTIKINE MDK1654 was compared to the corresponding non-alpha IL-2/15 and IL-7 PEPTIKINES with regard to STAT5 phosphorylation and immune cell proliferation.

MDK1654

Fig 1. Schematic illustration of MDK1654 structure. The dual PEPTIKINE MDK1654 is a synthetic branched peptide composed of three peptide ligands: one single chain peptide contains the sequences for IL-2Rβ and γc ligands. A second peptide containing an IL-7Rα ligand is attached to a side-chain of in the linker of the first peptide using click-chemistry. The PEPTIKINEs MDK1517 and MDK1319 each contain the same γc ligand as MDK1654, but they only contain the IL-2Rβ ligand or the IL-7Rα ligand, respectively, and are used for comparison. Peptide ligand sequences are unrelated to IL-2 or IL-7 cytokine sequences. Legend: blue rectangle, IL-2Rβ ligand; green rectangle, IL-7Rα ligand; red rectangle, γc ligand; black lines, peptide linkers.

MDK1654 Binds IL-7 and IL-2 Receptor Chains

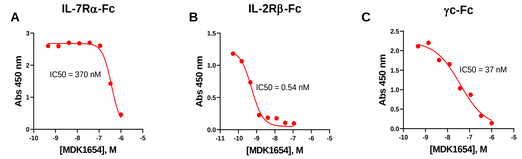


Fig 2. MDK1654 binding to IL-7Rα, IL-2Rβ, and γc chains. The binding affinity of MDK1654 for IL-7Rα, IL-2Rβ, and Rγc was measured by competition ELISA. Serially diluted MDK1654 was added to plate wells coated with human IL-7Rα-Fc (A), IL-2Rβ-Fc (B), or γc-Fc (C). After 1 h, chain-specific competing ligands (15 nM) were added and incubated for 45 min. Bound complexes were quantified by measuring HRP activity using a TMB substrate. Competing ligands are C-terminally biotinylated monomeric forms of IL-7Rα, IL-2Rβ, and Rγc peptide ligands related to those in MDK1654, each pre-complexed with NeutrAvidin™-HRP. IC50 values were generated using GraphPad Prism software.

MDK1654 is an Agonist of Both IL-2 and IL-7 Receptors

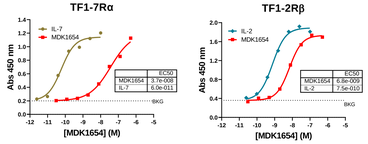


Fig 3. MDK1654 phosphorylation of pSTAT5. IL-7Rα and IL-2Rβ dependent agonist activity induced by MDK1654 in a γc-positive TF-1 cell line engineered to express IL-7Rα (TF1-7Rα) or IL-2Rβ (TF1-2Rβ). Cells were incubated with serially diluted MDK1654 for 30 min, and STAT5 phosphorylation was evaluated by ELISA. EC50 values were calculated using GraphPad Prism software.

MDK1654 Induces pSTAT5 in T and NK cells

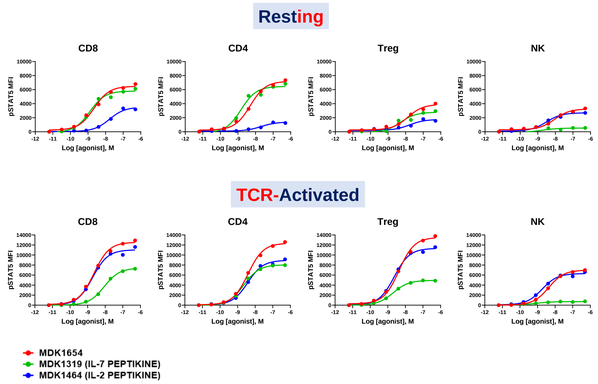


Fig 4. Induction of pSTAT5 in PBMC treated with PEPTIKINES. Frozen PBMCs from a healthy donor were rested overnight or CD3/CD28-activated and stained for viability, followed by cell surface antibody staining on ice. Cells were washed and incubated with the PEPTIKINES for 30 min at 37 ºC. After washing, cells were fixed, permeabilized, stained with anti-pSTAT5 antibody, and analyzed by flow cytometry. MDK1464 is a close analog of MDK1517. Similar results were observed in another donor.

MDK1654 Expands CD8 and CD4 T cells

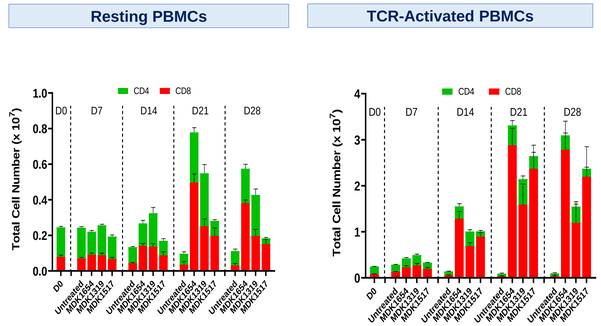


Fig 5. Proliferation of CD8 and CD4 T cells in response to PEPTIKINES. Frozen PBMCs from 5 healthy donors were rested overnight. The following day, cells either remained rested or were activated with anti-CD3 antibody, and all cells (except untreated control) were treated with 250 nM mono-specific PEPTIKINES (MDK1319 or MDK1517) or 500 nM of MDK1654. PEPTIKINEs were replenished every 2-3 days. Cell aliquots were taken for cell counting and immunophenotyping on days 7, 14, 21, and 28.

MDK1654 Expands Memory T cells

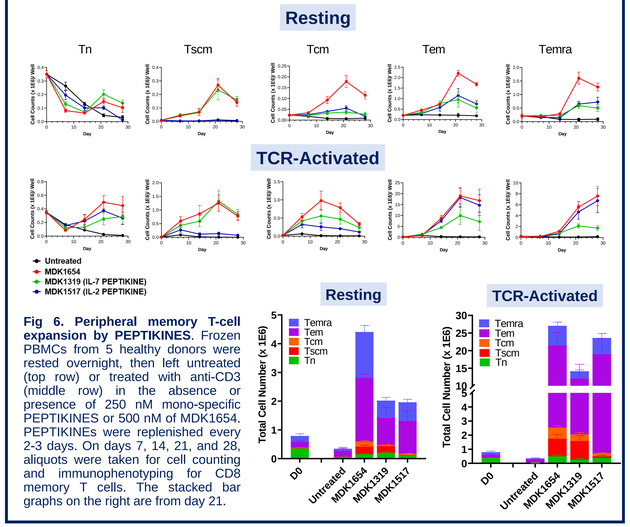


Fig 6. Peripheral memory T-cell expansion by PEPTIKINES. Frozen PBMCs from 5 healthy donors were rested overnight, then left untreated (top row) or treated with anti-CD3 (middle row) in the absence or presence of 250 nM mono-specific PEPTIKINES or 500 nM of MDK1654. PEPTIKINEs were replenished every 2-3 days. On days 7, 14, 21, and 28, aliquots were taken for cell counting and immunophenotyping for CD8 memory T cells. The stacked bar graphs on the right are from day 21.

MDK1654 Expands CD56^{hi} NK cells

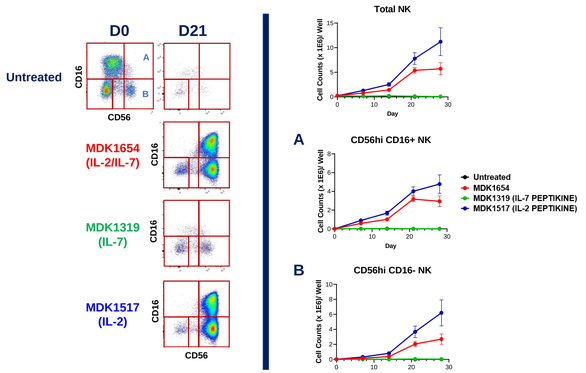
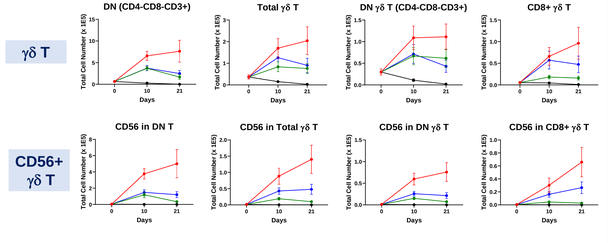


Fig 7. The proliferation of peripheral NK cells following treatment with PEPTIKINES. Frozen PBMCs from 5 healthy donors were rested overnight, then incubated in the absence or presence of 250 nM mono-specific PEPTIKINES or 500n M of MDK1654. PEPTIKINEs were replenished every 2-3 days. On days 7, 14, 21, and 28, aliquots were taken for cell counting and immunophenotyping for NK cell markers. Representative cytometry plots on days 0 and 21 are shown on the left, and the cell counts over time are shown on the right.

MDK1654 Expands γδ T cells



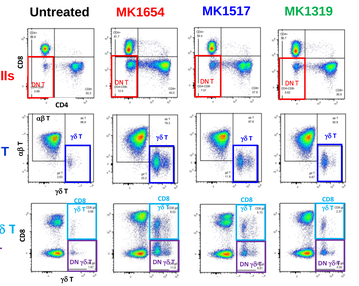
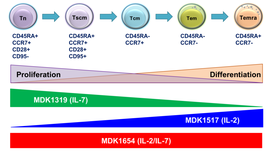


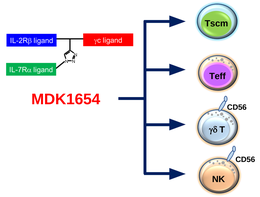
Fig 8. The proliferation of γδ T cells by PEPTIKINES. Frozen PBMCs from 5 healthy donors were rested overnight and left untreated or treated with 250 nM mono-specific PEPTIKINES or 500 nM of MDK1654. γδ T cell subsets were analyzed, and cell numbers are determined on days 10 and 21. Representative flow cytometry plots are shown on the right using one donor at day 10.

Summary & Conclusion



MDK1654 acted as an agonist in engineered TF-1 cells expressing IL-2/15Rβγ or IL-7R. In rested PBMCs, MDK1654 induced pSTAT5 and expanded T cells similarly to the IL-7 PEPTIKINE. MDK1654's expansion of Tcm, Tem, and Temra cells was greater than that observed with either IL-7 or non-alpha IL-2 PEPTIKINEs.

In CD3-activated PBMCs, which are known to express higher levels of IL-2R[2], MDK1654 effects on T cells were similar to the non-alpha IL-2 PEPTIKINE.



MDK1654 expanded conventional NK cells, as did the non-alpha IL-2 PEPTIKINE. MDK1654 produced more γδ T cells (including CD56^{hi}) than the non-alpha IL-2 or IL-7 PEPTIKINEs. CD56+ γδ T cells have shown a high cytotoxic capacity[3].

As MDK1654 maintains and expands memory T cells via IL-7R signaling and effector T cells, NK, and γδ T cells via IL-2R signaling, the combined action of MDK1654 on both the adaptive and innate immune cells warrants development for the treatment of solid tumors.

1. Dower et al. JITC 2020 8, Issue Suppl 3 #691
2. Hodge et al. Scandinavian J of Immunol (2000) 51, 67-72
3. Clin Cancer Res (2008) 14 (13): 4232–4240