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In vitro and in vivo properties of MDK-703: An Fc-peptide fusion IL-7Rαγc agonist unrelated in structure to IL-7

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Introduction

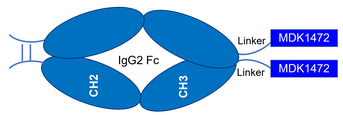
IL-7 receptor (IL-7R) activation is essential for the proper development and homeostasis of T-cell subpopulations and maintenance of the TCR clonal repertoire. Emerging evidence indicates the potential clinical utility of IL-7R agonists for immunotherapy of lymphopenia, oncology, and other indications. However, IL-7-based proteins that have been studied in humans show a propensity to induce anti-drug antibodies (ADAs), including those that neutralize natural IL-7[(1)]. Here we report the discovery of MDK1472, a small novel peptidyl agonist of IL-7R with a MW <5000Da. This peptide is structurally unrelated to IL-7 and has a sequence that makes MDK1472 unlikely to generate IL-7 neutralizing antibodies. MDK1472 is fused to an IgG Fc-domain to improve pharmacokinetics and this fusion, MDK-703, exhibits biological properties similar to those of IL-7 *in vitro* and when administered to animals.

MDK1472 and MDK-703

MDK1472

- Synthetic peptide with sequence unrelated to IL-7 or other human proteins
- Binds to IL-7Rα and Rγc subunits and activates IL-7R with sub-nanomolar potency and full efficacy in pSTAT5 assay
- Rγc-binding portion of the molecule does not block the activity of IL-2, IL-4, IL-9, IL-15, or IL-21[(2)]
- In silico assessment of potential immunogenicity (EPIVAX) indicated a low probability of the presence of Class II (HLA-DR) restricted HLA ligands and putative T cell epitopes[(3)]

Fc fusion MDK-703 (Development lead candidate)



MDK-703 Binding to IL-7Rα and Rγc

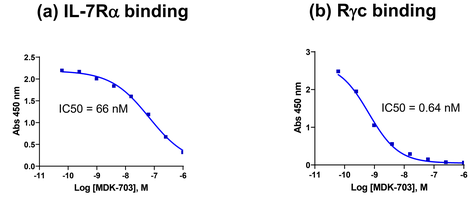
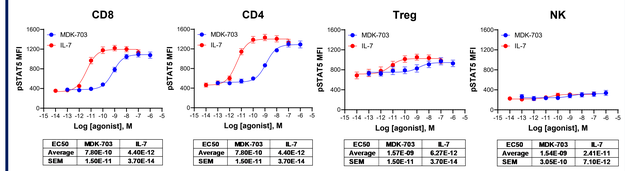


Fig 1. MDK-703 binding to IL-7R subunits

The binding affinity of MDK-703 to IL-7Rα (**a**) and Rγc (**b**) was measured by competition ELISA. Serially diluted MDK-703 was added to plate wells coated with human IL-7Rα-Fc (**a**) or Rγc-Fc (**b**). After 1 hour, NeutrAvidin™-HRP:biotin-IL-7Rα binding peptide complex (**a**) or NeutrAvidin™-HRP:biotin-γc binding peptide complex (**b**) was added and incubated for 45 min. Bound complexes were quantified by measuring HRP activity using TMB substrate. IC50 values were generated using GraphPad Prism software.

MDK-703 Induces pSTAT5 in PBMCs



EC50	MDK-703	IL-7
Average	7.80E-10	4.40E-12
SEM	1.50E-11	3.70E-14

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Average	7.80E-10	4.40E-12
SEM	1.50E-11	3.70E-14

EC50	MDK-703	IL-7
Average	1.57E-09	6.27E-12
SEM	1.50E-11	3.70E-14

EC50	MDK-703	IL-7
Average	1.54E-09	2.41E-11
SEM	3.05E-10	7.10E-12

Fig 2. Induction of pSTAT5 in PBMC treated with MDK-703 or IL-7. Frozen PBMCs from 5 healthy donors were rested overnight and stained for viability, followed by cell surface antibody staining on ice. Cells were washed and incubated with MDK-703 or IL-7 for 30 min at 37°C. After washing, cells were fixed, permeabilized, stained with anti-pSTAT5 antibody, and analyzed by flow cytometry.

MDK-703 Induces Proliferation in PBMCs

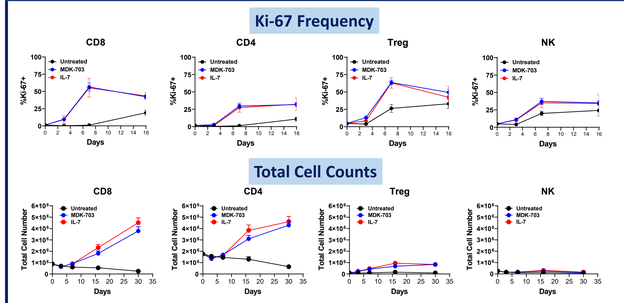


Fig 3. Proliferation of PBMC subpopulations following treatment with MDK-703 or IL-7. Frozen PBMCs from 5 healthy donors were rested overnight and left untreated or treated with 100nM MDK-703 or 1nM IL-7 in culture. On days 3, 7, 16, and 30, cell aliquots were taken to determine Ki-67 expression in immune cells and total cell counts in the culture.

MDK-703 Expands Tscm in PBMCs

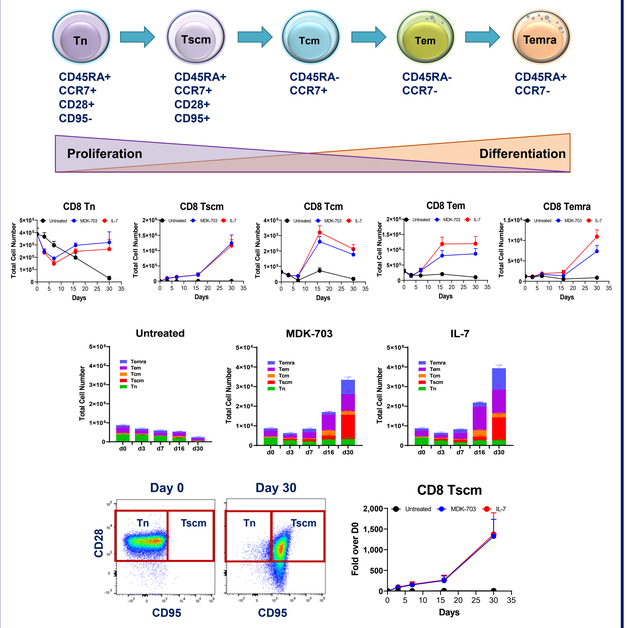


Fig 4. Changes in memory T-cell populations mediated by MDK-703 or IL-7 in PBMCs. Frozen PBMCs from 5 healthy donors were rested overnight and treated with vehicle, 100nM MDK-703 or 1nM IL-7 in a serum-free culture medium. On days 3, 7, 16, and 30, cell aliquots were analyzed by flow cytometry for memory T cell populations and cell counts.

MDK-703 PK/PD in NHP

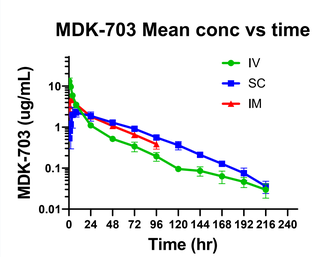


Fig 6. Pharmacokinetics (PK) of MDK-703 in cynomolgus macaques. Animals (n=3) were administered a single dose of MDK-703 via IV, SC, or IM at 1mg/kg. The serum concentration of MDK-703 at the indicated time points was determined by sandwich ELISA.

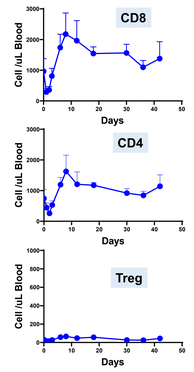


Fig 7. Pharmacodynamic (PD) effects of MDK-703 in cynomolgus macaques. Three animals were dosed once subcutaneously at 0.3 mg/kg, and blood samples were collected at the indicated time points for immune profiling by flow cytometry.

Summary of Results

- MDK-703 binds to and activates human and NHP IL-7 receptors and emulates IL-7 in pSTAT5 assays in vitro
- In human PBMCs, MDK-703 induces T-cell differentiation and proliferation, resulting in substantial increases in memory T cell populations, including T memory stem cell (Tscm) population
- In human CD34⁺ engrafted NSG (humanized) mice, MDK-703 increases Ki-67 expression and cell number in CD8 and CD4 cells. MDK-703 drove the expansion of memory cell populations and TCF1+ memory cells in both blood and spleen on day 12
- In cynomolgus macaques, MDK-703 exhibited a $T_{1/2}$ of 46 hours after IV administration and showed high bioavailability after IM and SC dosing. A single subcutaneous dose at 300 ug/kg induced a sustained increase in CD8⁺ and CD4⁺ T-cells for up to 40 days

MDK-703 Increases CD8, CD4, and memory T cells in Humanized Mice

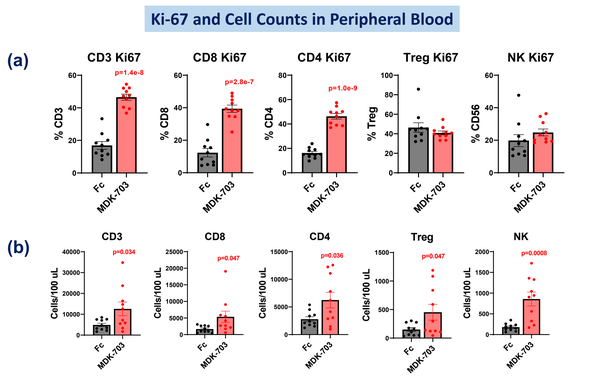
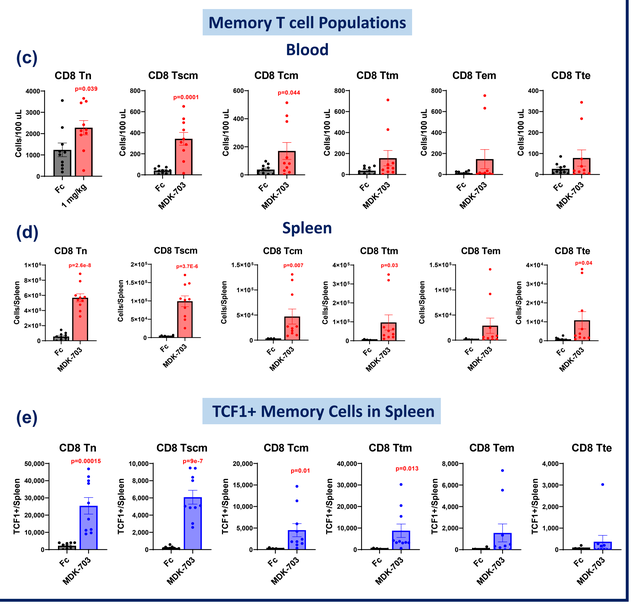


Fig 5. T-cell subpopulations in humanized mice treated with MDK-703. NSG mice separately engrafted with human CD34+ cells from two donors were dosed once intravenously with 1 mg/kg Fc or MDK-703. On day 7, peripheral blood was collected to determine Ki-67+ cells in immune populations (**a**). On day 12, terminal blood samples and spleens were collected for immune profiling (**b**). Markers used for memory T cell populations (**c** and **d**) are shown in the inset box. TCF1 expression (**e**) in memory cells was also determined. Population gates were drawn based on FMO controls. Statistical analysis was done using Student's T-Test.

Tn:	CD45RA+ CCR7+ CD28+ CD95-
Tscm:	CD45RA+ CCR7+ CD28+ CD95+
Tcm:	CD45RA- CCR7+ CD28+ CD95+
Ttm:	CD45RA- CCR7- CD28+ CD95+
Tem:	CD45RA- CCR7- CD28- CD95+
Tte:	CD45RA+ CCR7- CD28- CD95+



Conclusions

MDK-703 is a full agonist of the IL-7 receptor and expanded CD8, CD4, and memory T cell populations in PBMC cultures and in vivo in a humanized mouse model. Especially noteworthy was the expansion of TCF1+ stem-like memory cells which have been shown to possess self-renewal potential.

MDK-703 offers an attractive alternative to IL-7-based drug candidates currently in clinical development. The structural novelty of MDK-703 eliminates the risk of ADAs that neutralize endogenous IL-7. A first human trial of MDK-703 is anticipated in 2022.

References

1) Clin Transl Sci (2020) 13, 1161–1169
2) JITC-2020-SITC2020.0567
3) Human Vaccines & Immunotherapeutics (2015) 11, 2312-2321