Discovery and Versatile Use of Peptide-Based Agonists of Hetero-Dimeric γ_c Cytokine Receptors

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Chief Executive Officer

Medikine, Inc.

Next Generation Cytokines: Challenges and Modalities

Challenges

- Potency (including attenuation)
- Intrinsic efficacy (partial agonism)
- Receptor specificity (subtypes or decoy receptors)
- Pharmacokinetics

- Targeting to specific cell-types
- Conditional activation in tumor microenvironment
- Additional pharmacology (bispecifics)
- Manufacturing
- Intellectual Property
- Anti-cytokine ADAs

Modalities

Modified Cytokines:

- Mutated cytokine sequence
- Chemically modified cytokine (e.g., PEG)
- Fusion to protein that blocks undesired interaction
- Fc / albumin fusion for PK
- Fusion to targeting antibody

Non-cytokine Modalities

- Bi-specific heavy chain only Ab
- Dual nanobodies

Peptide-based mimetics: PEPTIKINES™

Peptide Mimetics of Homodimerizing Receptors

Small Peptides as Potent Mimetics of the Protein Hormone Erythropoietin

Nicholas C. Wrighton,* Francis X. Farrell, Ray Chang, Arun K. Kashyap, Francis P. Barbone, Linda S. Mulcahy, Dana L. Johnson, Ronald W. Barrett, Linda K. Jolliffe, William J. Dower

SCIENCE • VOL. 273 • 26 JULY 1996

Increased potency of an erythropoietin peptide mimetic through covalent dimerization

Nicholas C. Wrighton*, Palaniappan Balasubramanian, Francis P. Barbone', Arun K. Kashyap², Francis X. Farrell', Linda K. Jolliffe', Ronald W. Barrett, and William J. Dower

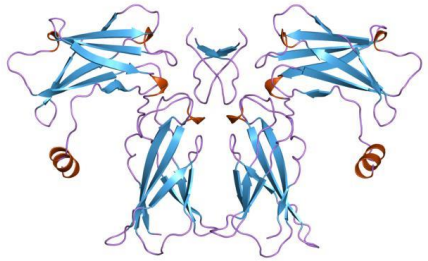
NATURE BIOTECHNOLOGY VOLUME 15 NOVEMBER 1997

Peptide Agonist of the Thrombopoietin Receptor as Potent as the Natural Cytokine

Steven E. Cwirla, Palaniappan Balasubramanian, David J. Duffin, Christopher R. Wagstrom, Christian M. Gates, Sara C. Singer, Ann M. Davis, Robert L. Tansik, Larry C. Mattheakis, Chris M. Boytos, Peter J. Schatz, David P. Baccanari, Nicholas C. Wrighton, Ronald W. Barrett, William J. Dower*

SCIENCE • VOL. 276 • 13 JUNE 1997

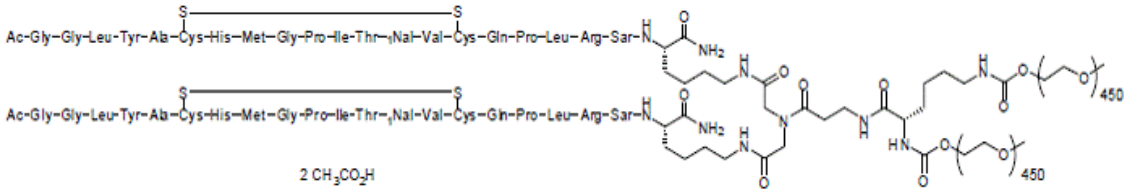
Monomeric Peptide Self-Dimerizes!

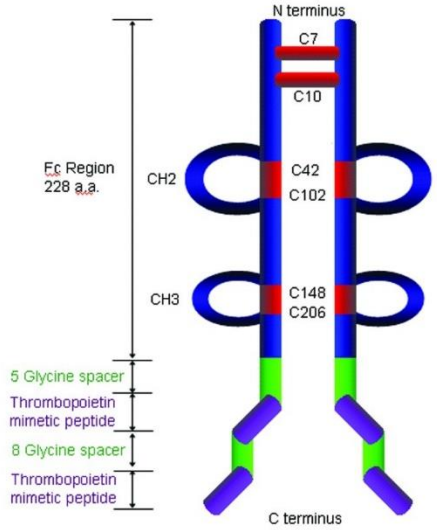


IEGPTLRQWLAARA

IEGPTLRQWLAARA (β-Ala)K

Dimerized PEG-Peptide with Improved Potency and Half-life

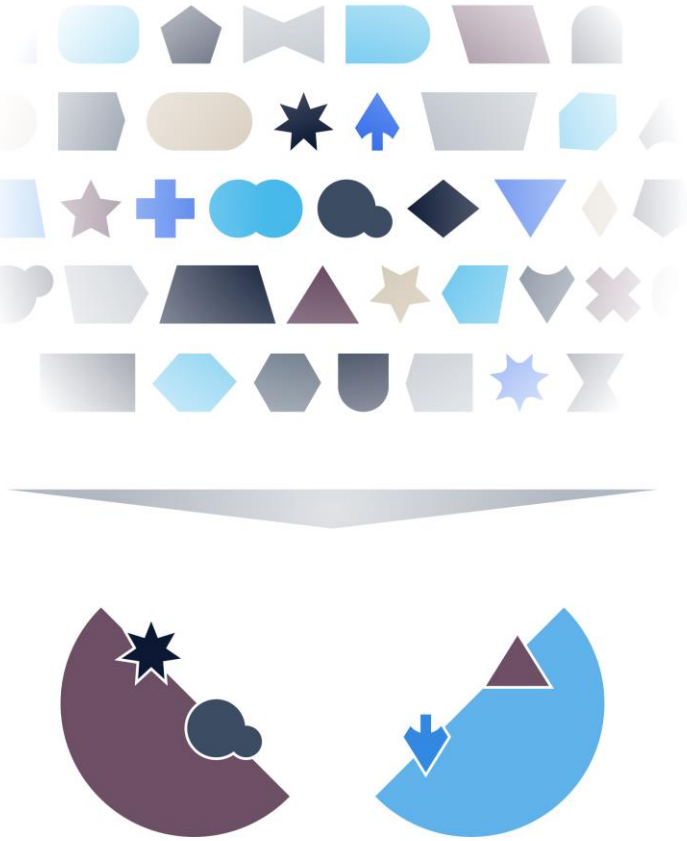




Cytokine Mimetic Discovery Platform – Heterodimeric Receptors

Step 1: Peptide Ligand Discovery

Screen libraries of >10^{10} unique linear and constrained peptides for those that bind to the extracellular domain of each receptor subunit



Keys to Success:

- Design and diversity of primary phage libraries
  - Use of pIII and pVIII to identify many sequences to identify many sequence families, particularly low affinity peptides that serve as starting points for optimization
- Screening for ligands
  - Use of multiple receptor ECD formats
  - Methods to eliminate non-receptor binders
- Secondary library design and affinity maturation methods

Peptides on phage: A vast library of peptides for identifying ligands

(recombinant diversity/N-terminal hexapeptides/fd bacteriophage/avidity panning/antibody specificity)

STEVEN E. CWIRLA, ELIZABETH A. PETERS, RONALD W. BARRETT, AND WILLIAM J. DOWER*

Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 6378–6382, August 1990

Selective Enrichment and Characterization of High Affinity Ligands from Collections of Random Peptides on Filamentous Phage

Ronald W. Barrett,*,[1] Steven E. Cwirla,† Martha S. Ackerman,* Ann M. Olson,* Elizabeth A. Peters,† and William J. Dower†

ANALYTICAL BIOCHEMISTRY **204**, 357–364 (1992)

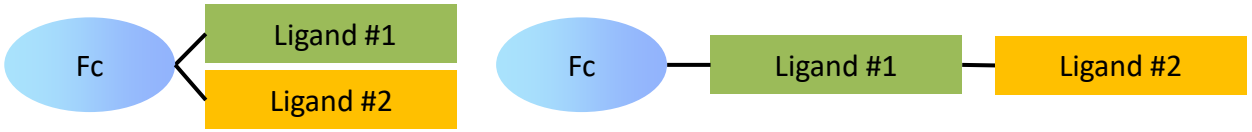
Cytokine Mimetic Discovery Platform – Heterodimeric Receptors

Step 2: Ligand Dimerization

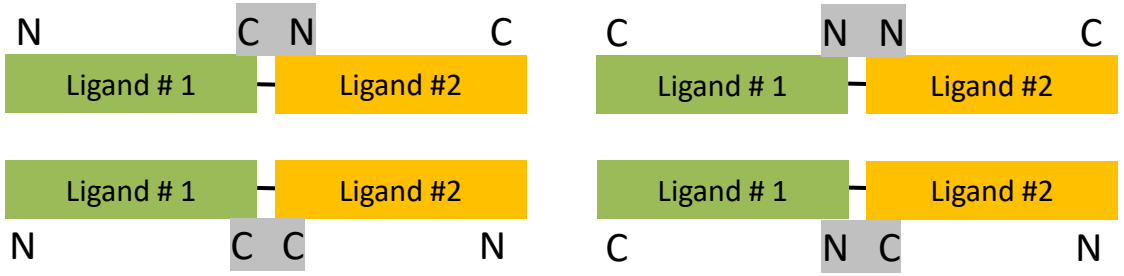
Approaches

- Ligand dimerization with recombinant fusions

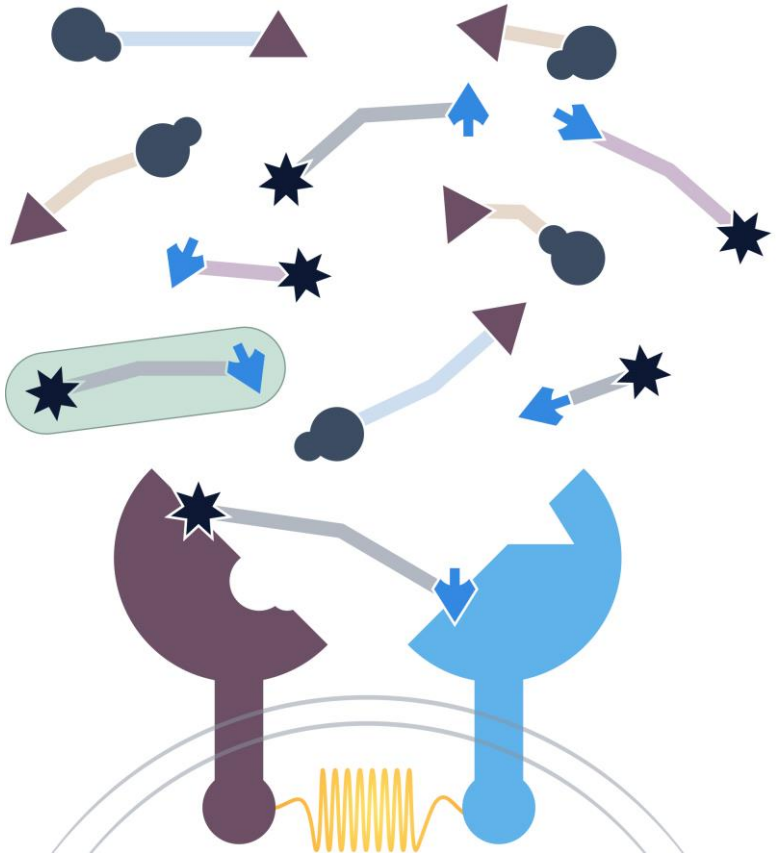
Examples



- Ligand dimerization using click chemistry
  - Useful to survey heterodimer SAR (e.g., ligand affinity, linker)
  - All four orientationmers accessible (C-N, N-C, N-N, C-C)



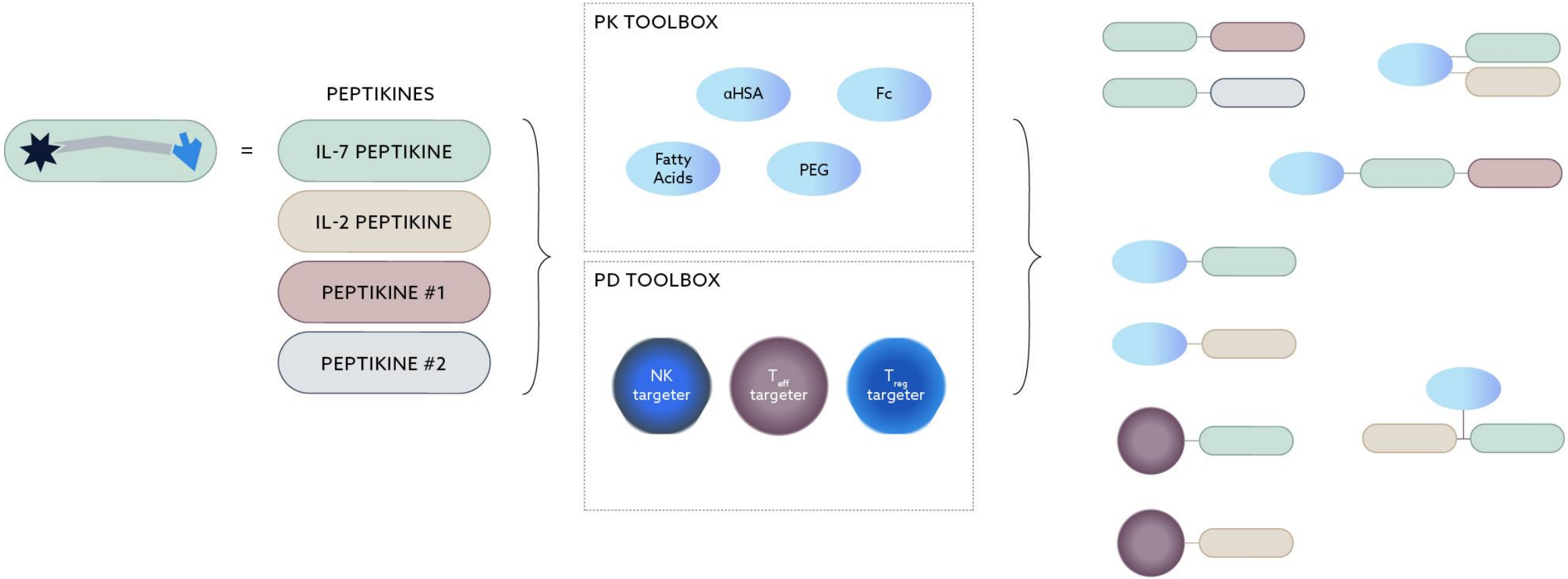
Combinatorially assemble ligated peptides, varying peptide orientation, and linker, to identify PEPTIKINES that activate the cytokine receptor complex in cells



Cytokine Mimetic Discovery Platform – Heterodimeric Receptors

Step 3: Engineering of Cytokine Mimetic Drug

Using synthetic or recombinant fusion technology, modify the PEPTIKINE to impart drug properties such as extended half-life, cell or tissue targeting, and conditional activation



PEPTIKINES:
A Modular and Versatile Modality with Unique Advantages

Ability to Tune Desired Agonist Properties

- Sub-unit binding affinity / selectivity
- Agonist potency and intrinsic activity
- Synthetic PEPTIKINES: not restricted to natural AAs

Pharmacokinetic Modulation Optionality

- Established half-life extension approaches
  - Recombinant fusion (e.g., Fc, HSA binder, etc.)
  - Chemical modification (PEG; fatty acid, etc.)
- Protease sensitivity

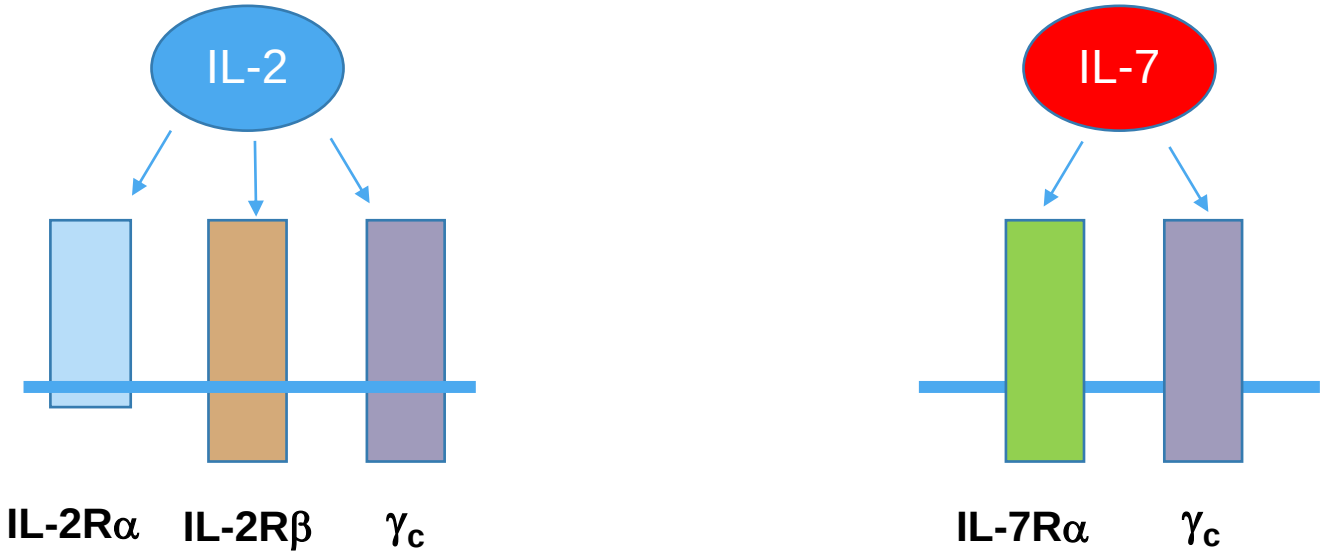
Use in Bispecifics and Cell Therapy

- Dual pharmacology, tissue or cell type targeting, conditional activation
- Synthetic PEPTIKINES for manufacturing cell therapies
- Use of DNA encoding PEPTIKINE in engineered cell therapy and vaccine applications

Lack of Cytokine-Neutralizing ADAs

- PEPTIKINES are selected based on low predicted potential for immunogenicity
- PEPTIKINES are unrelated in sequence or structure to the cytokine and therefore will not generate anti-cytokine Abs

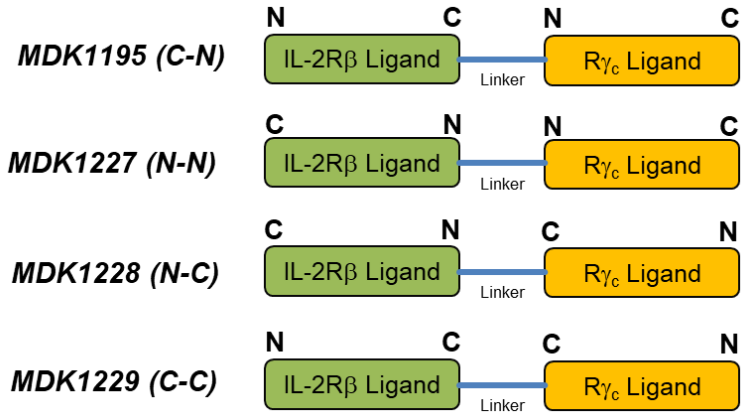
Receptors Discussed Today



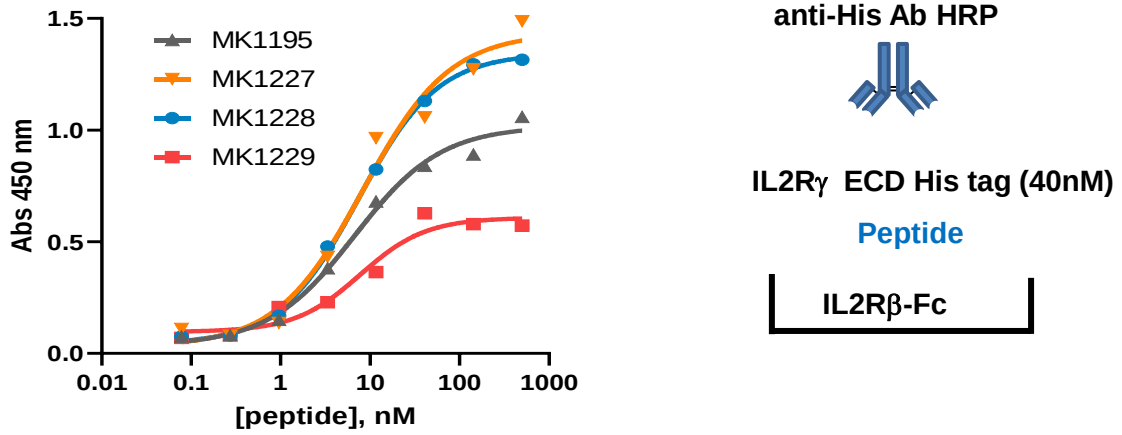
IL-2Rβγ PEPTIKINES – Influence of Orientation

Orientationmers Create Four Potential IL-2Rβγ PEPTIKINES

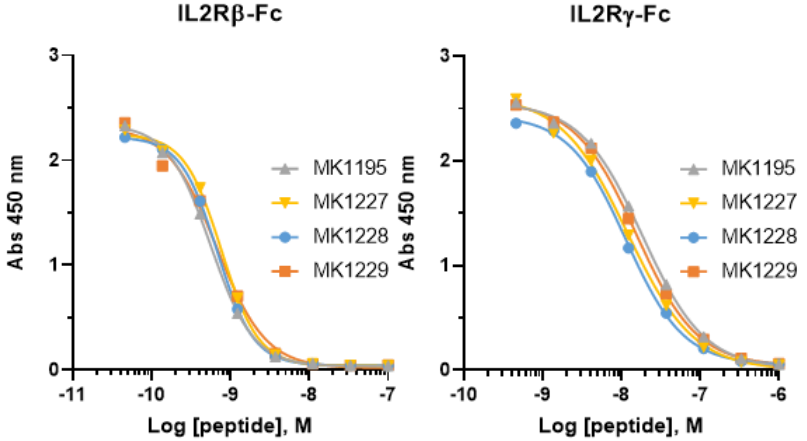
Fixed Length / Flexibility Linker



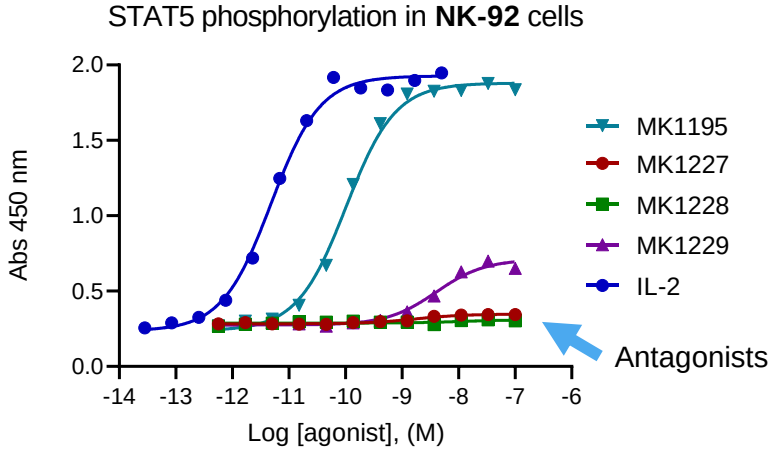
All Four PEPTIKINES Simultaneously Bind to IL2Rβ and IL2Rγ



All Four PEPTIKINES Bind IL-2Rβ and IL-2Rγ

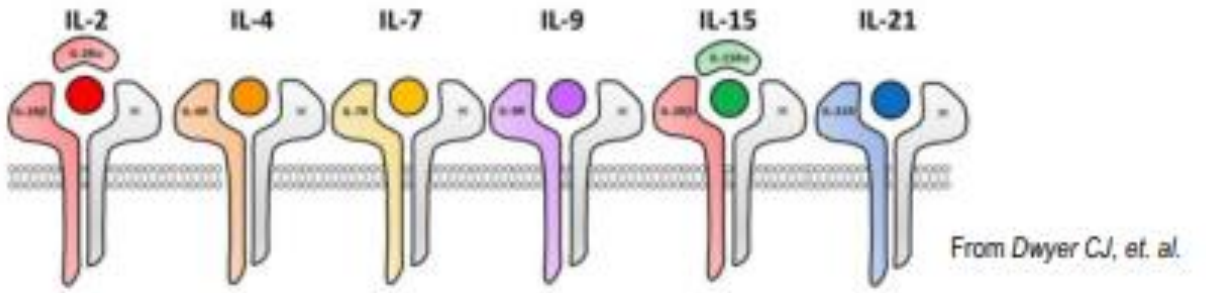


Only One Full Agonist PEPTIKINE (EC50~100 pM)



IL-2Rβγ PEPTIKINES – Unique Binding Sites

MK1169 is a synthetic IL-2Rβγ PEPTIKINE that does not block IL-2 binding to IL-2Rβ



MK1169 does not modify activity of other γ_c cytokines

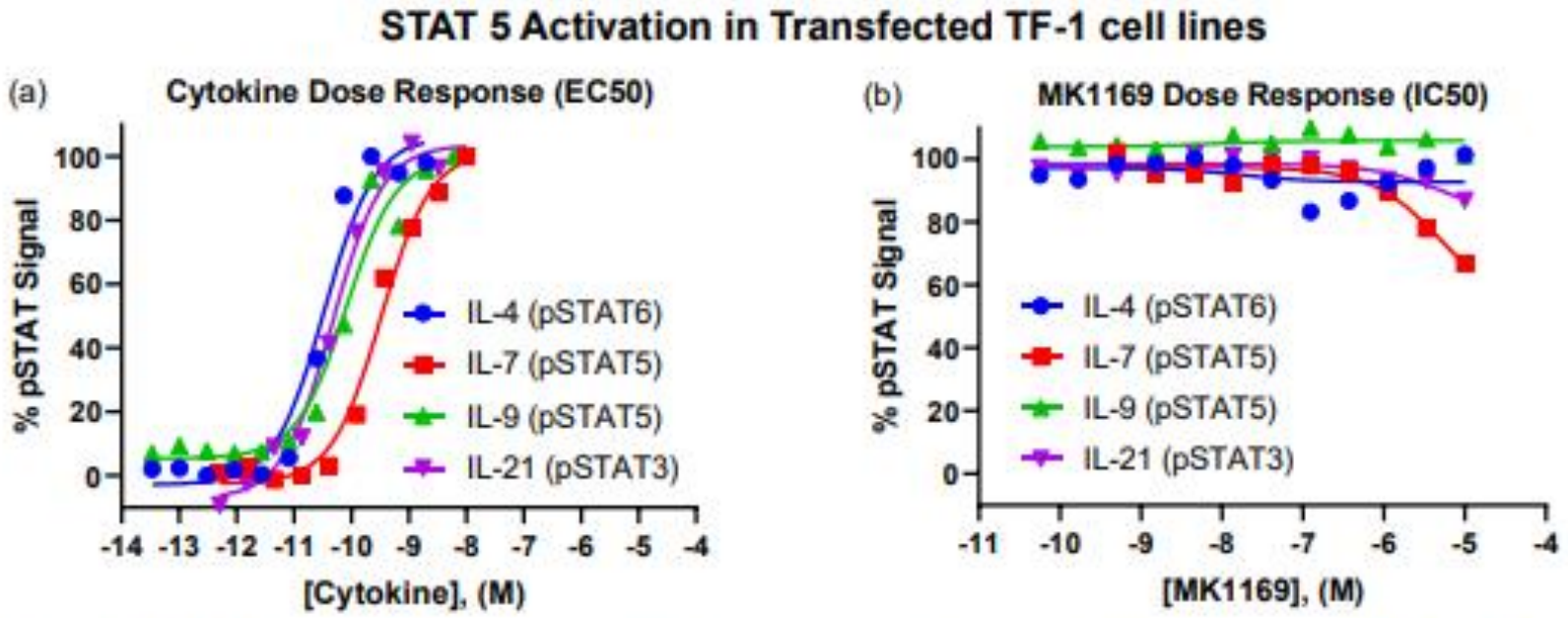
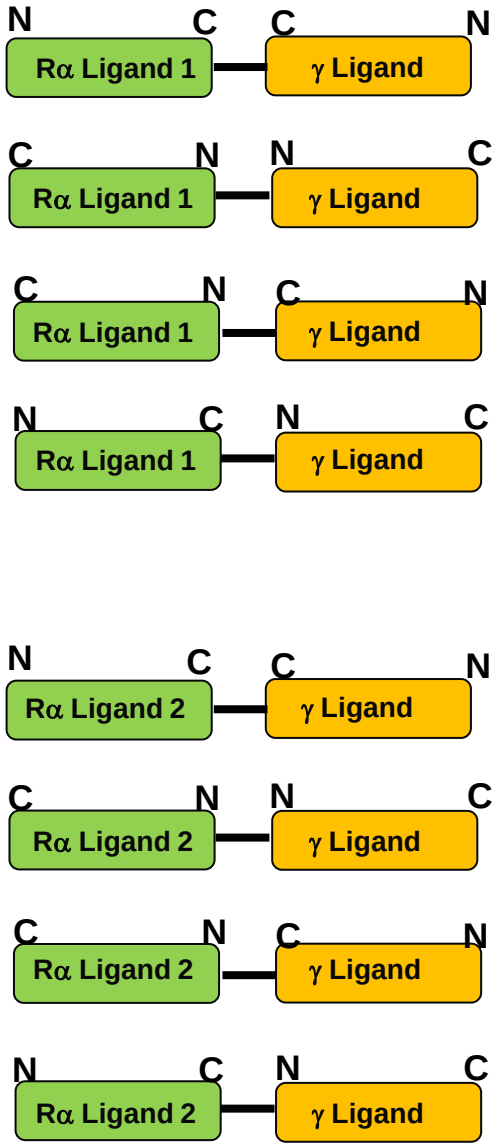
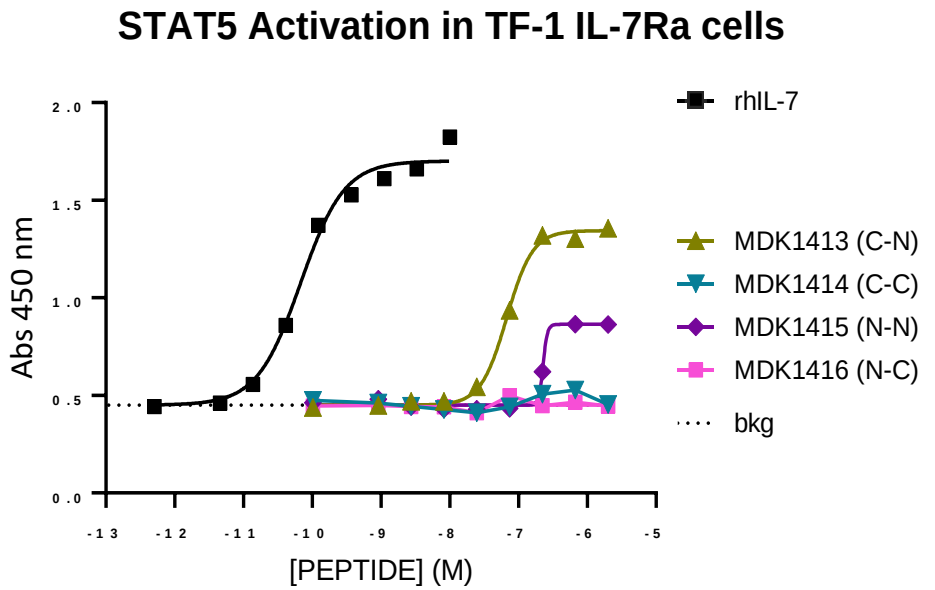


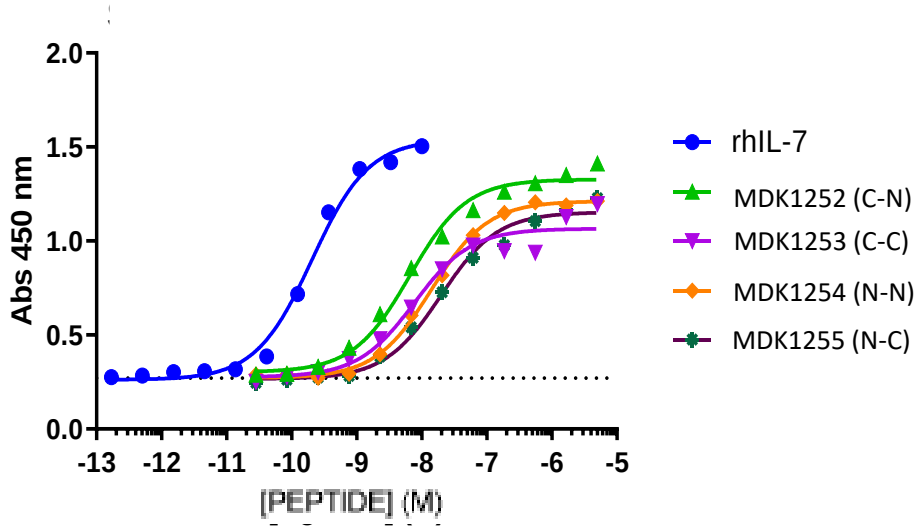
Fig. 7 (a) TF-1 cells (γ_c only) stably transfected with cytokine-specific receptors become responsive to respective cytokine (b) MK1169 was tested for potential to antagonize the activity of an approximate EC50 concentration of cytokine

IL-7R PEPTIKINE: Influence of Ligand & Orientation

- Identified two IL-7Rα ligands unrelated in sequence that cross compete (bind to same site)
- For each IL-7Rα ligand, constructed four possible orientations with the same γc ligand
- Similar linker used in all constructs



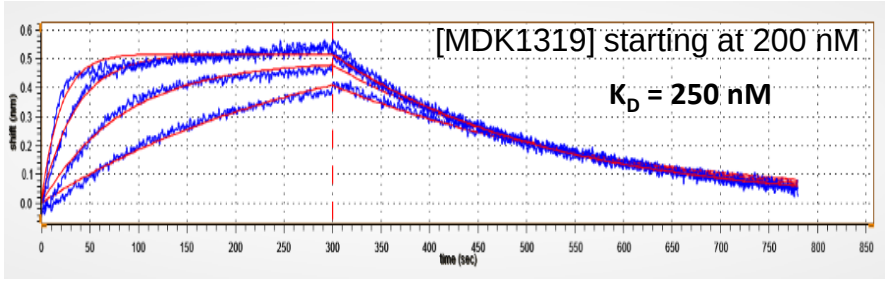




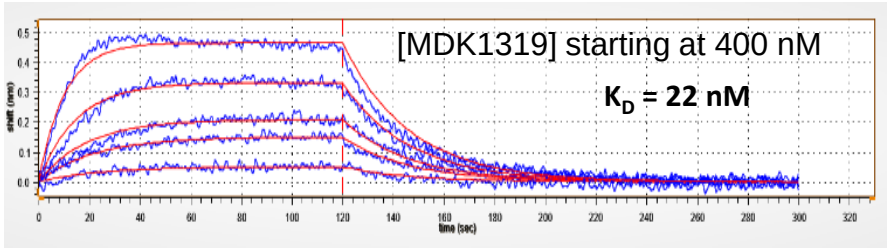
IL-7R PEPTIKINE: Binding Sites Differ from IL-7

Synthetic PEPTIKINE Binding to Receptor ECD

IL-7Rα

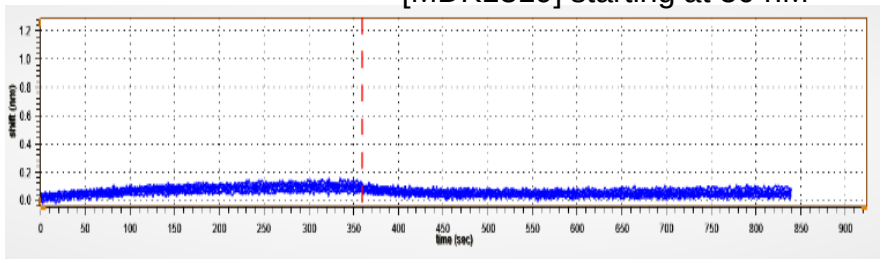


IL-2Rγ



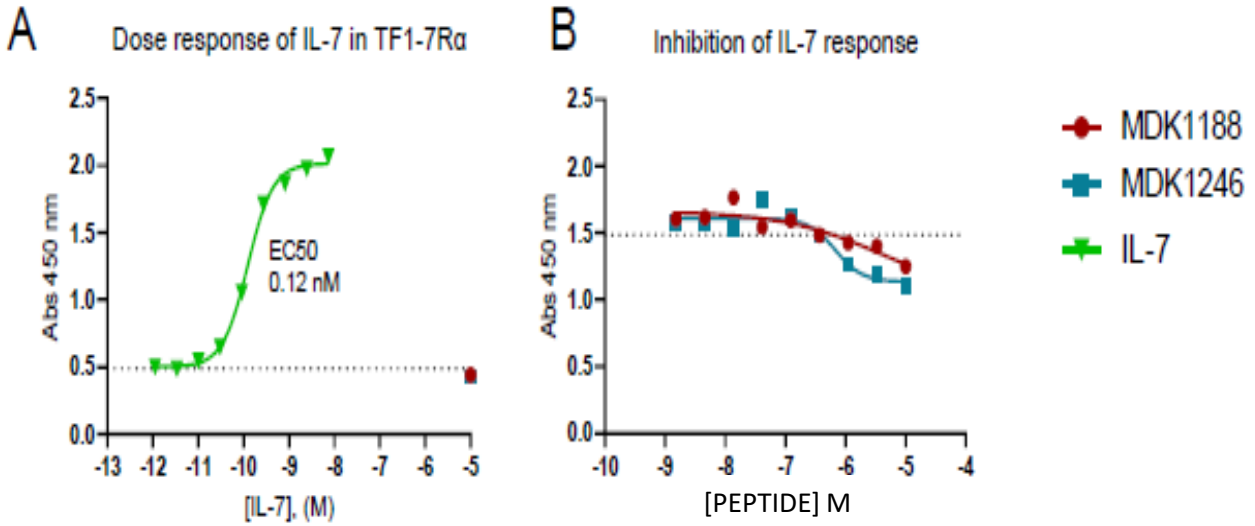
IL-2Rβ

[MDK1319] starting at 50 nM



Receptor	MDK1319		
	kon (M-1 sec-1)	koff (sec-1)	KD (M)
IL-2Rb	no measurable binding		
IL-2Rg	1.9E+05	4.1E-03	**2.2E-08**
IL-7Ra	1.5E+05	3.8E-02	**2.5E-07**

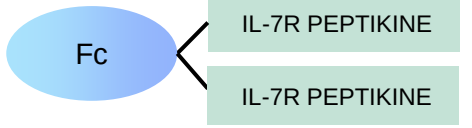
IL-7Rα and IL-2Rγ Monomer Peptides Do Not Inhibit IL-7

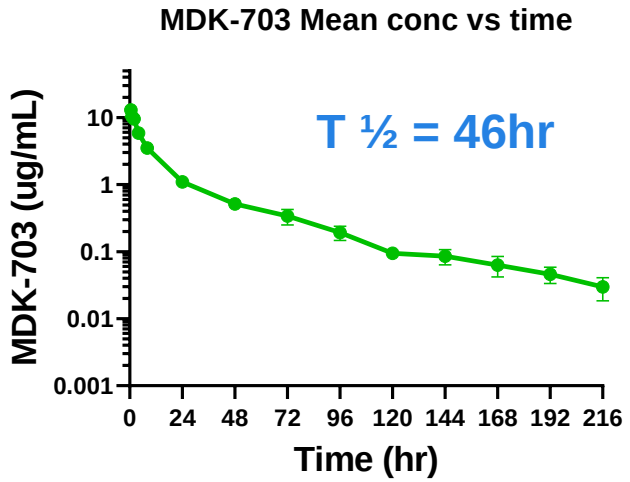


Half-life Extension: Fc Fusions

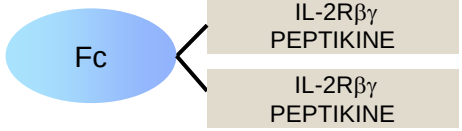
Single IV dose (1 mg/kg) in NHP (n=3)

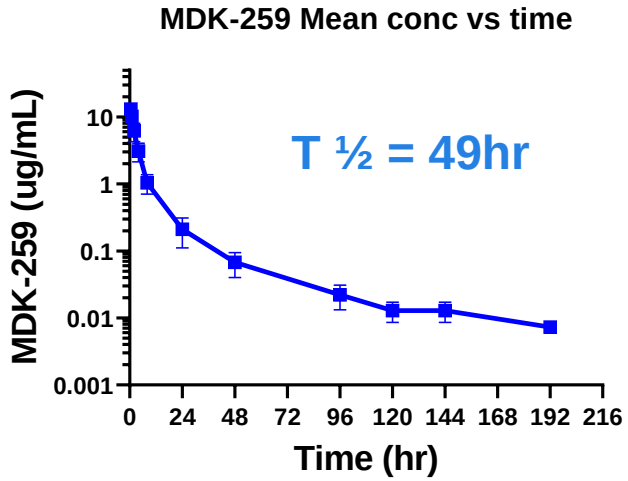
MDK-703





MDK-259





Half-life Extension: Lipidation for HSA Binding

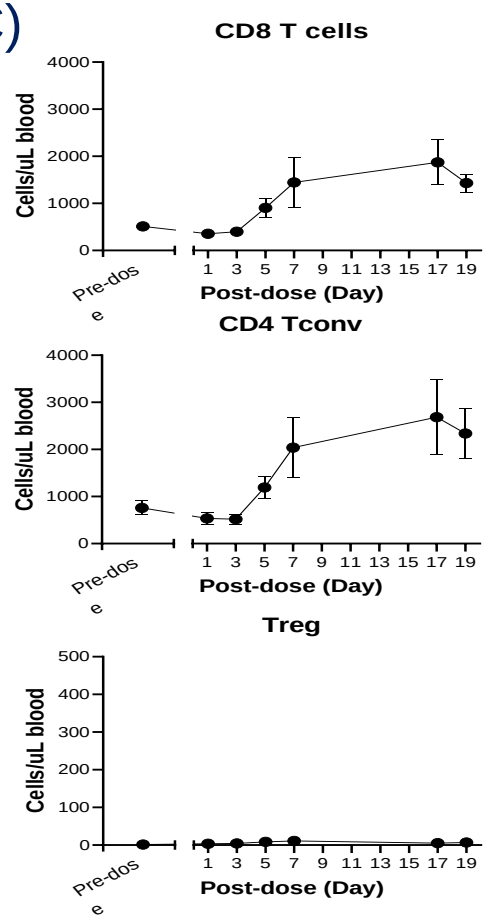
PEPTIKINE in MDK-703

MDK1664

HSA Binding Fatty Acid — MK1472

Synthetic

(0.3mg/kg via SC)

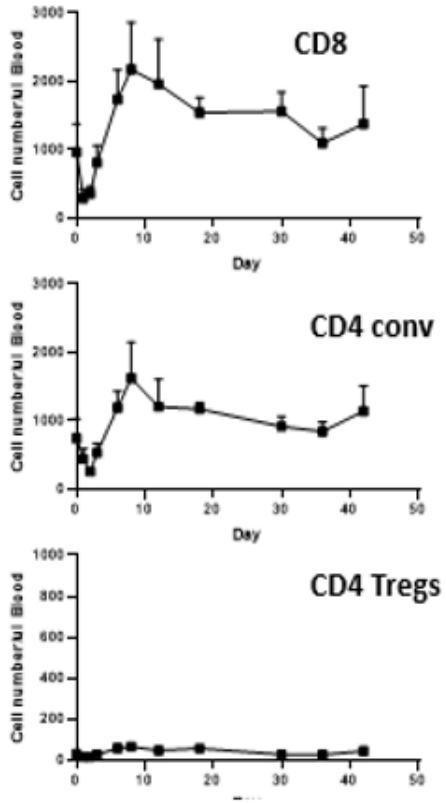


MDK-703

Fc — IL-7R PEPTIKINE, IL-7R PEPTIKINE

Recombinant Protein

(0.3mg/kg via SC)



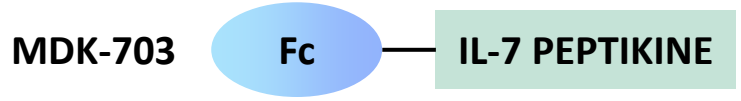
Medikine's IL-7 PEPTIKINE and MDK-703

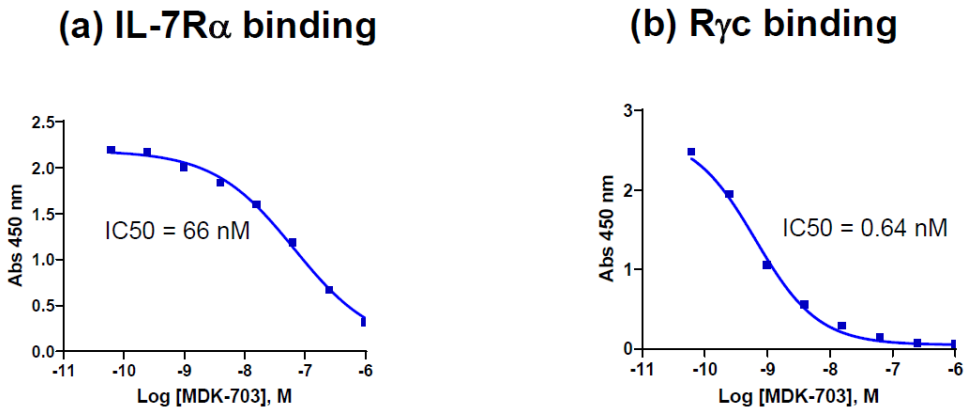
IL-7 PEPTIKINE

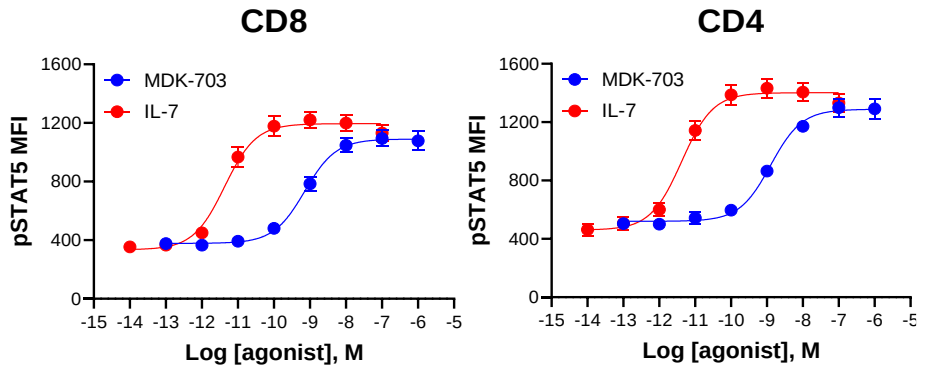
- Synthetic peptide with sequence unrelated to IL-7 or other human proteins
  - MW less than 5000Da
- Binds to IL-7Rα and Rγc subunits and activates IL-7R with sub-nanomolar potency and full efficacy in pSTAT5 assay
- Rγc-binding portion of IL-7 PEPTIKINE does not block the activity of IL-2, IL-4, IL-9, IL-15, or IL-21
- *In silico* assessment of potential immunogenicity indicated low probability of the presence of Class II (HLA-DR) restricted HLA ligands and putative T cell epitopes
  - Low predicted immunogenicity
  - Avoids generation of neutralizing ADAs to endogenous IL-7
- Novel Composition-of-Matter IP

MDK-703 – BEST-IN-CLASS IL-7 MIMETIC

- To improve *in vivo* properties, IL-7 PEPTIKINE is fused to an IgG Fc-domain to construct MDK-703





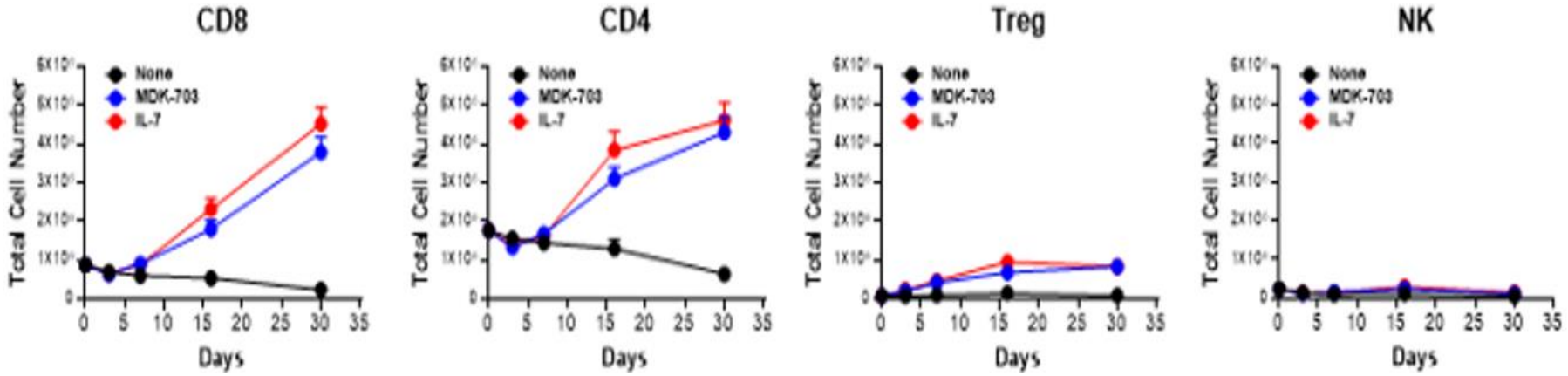


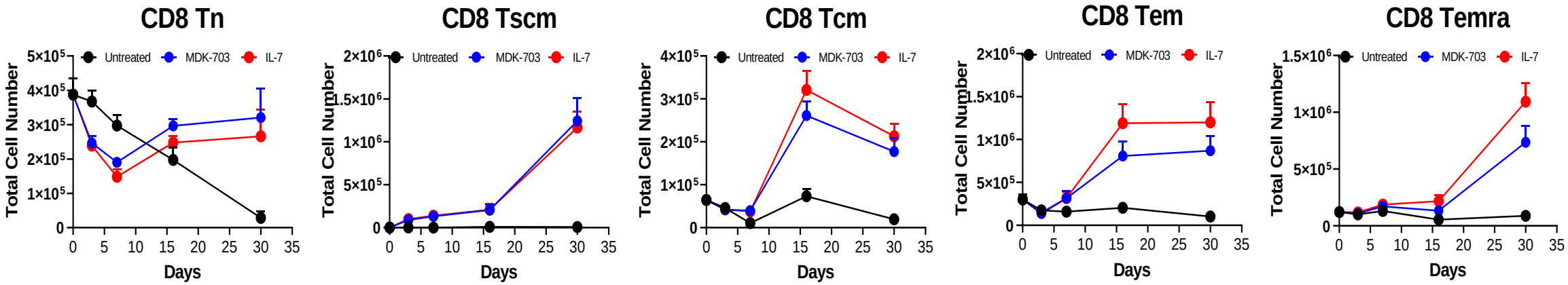
MDK-703

In Vitro Effect on Human T and NK Cells

MDK-703 Expands CD8/CD4 T Cells including Tscm in PBMCs

- Frozen PBMCs from five healthy donors were treated with vehicle, 100nM MDK-703 or 1nM IL-7
- On days 3, 7, 16, and 30, cell aliquots were analyzed by flow cytometry for naïve and memory T cell counts

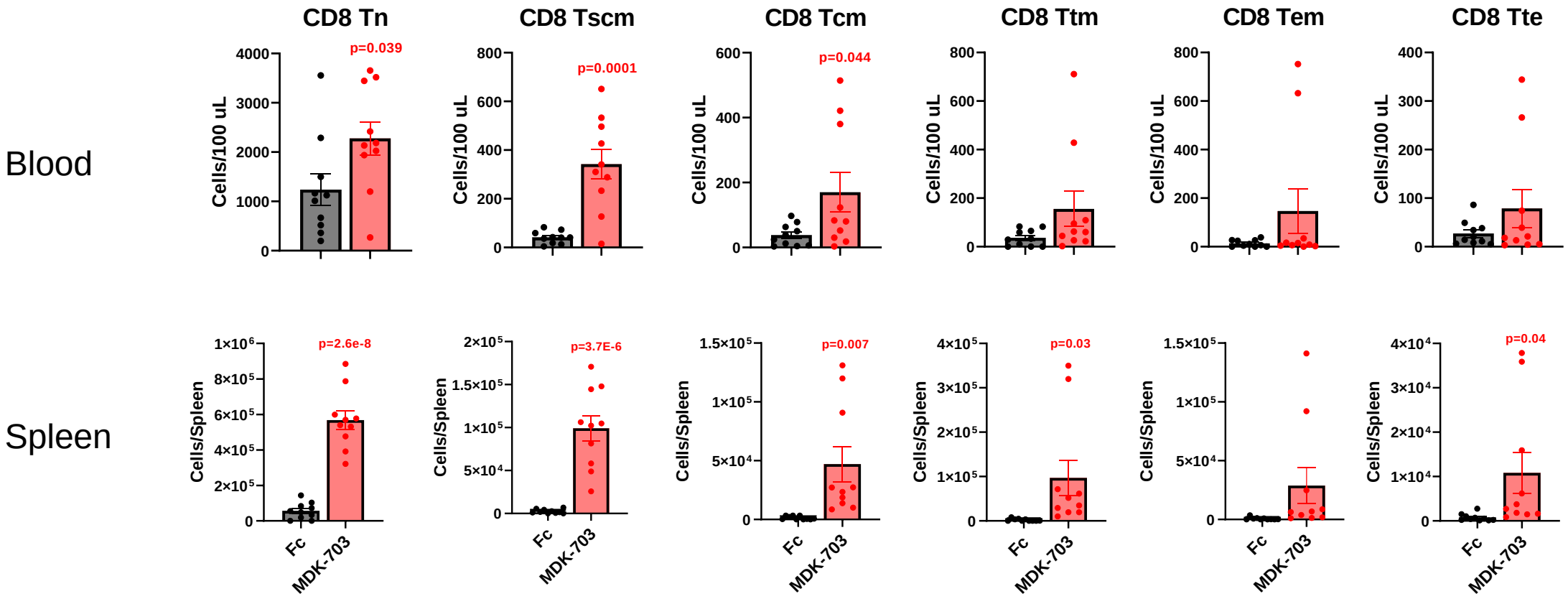




MDK-703
Activity in Humanized Mouse

MDK-703 Increases CD8, CD4, and Memory T cells

- NSG mice separately engrafted with human CD34+ cells from two donors were dosed once IV with 1 mg/kg Fc or MDK-703
- On day 12, terminal blood and spleen samples were collected for immune profiling



MDK-703 drives the expansion of memory cell populations in blood and spleen on day 12

IL-2/15Rβγc PEPTIKINE

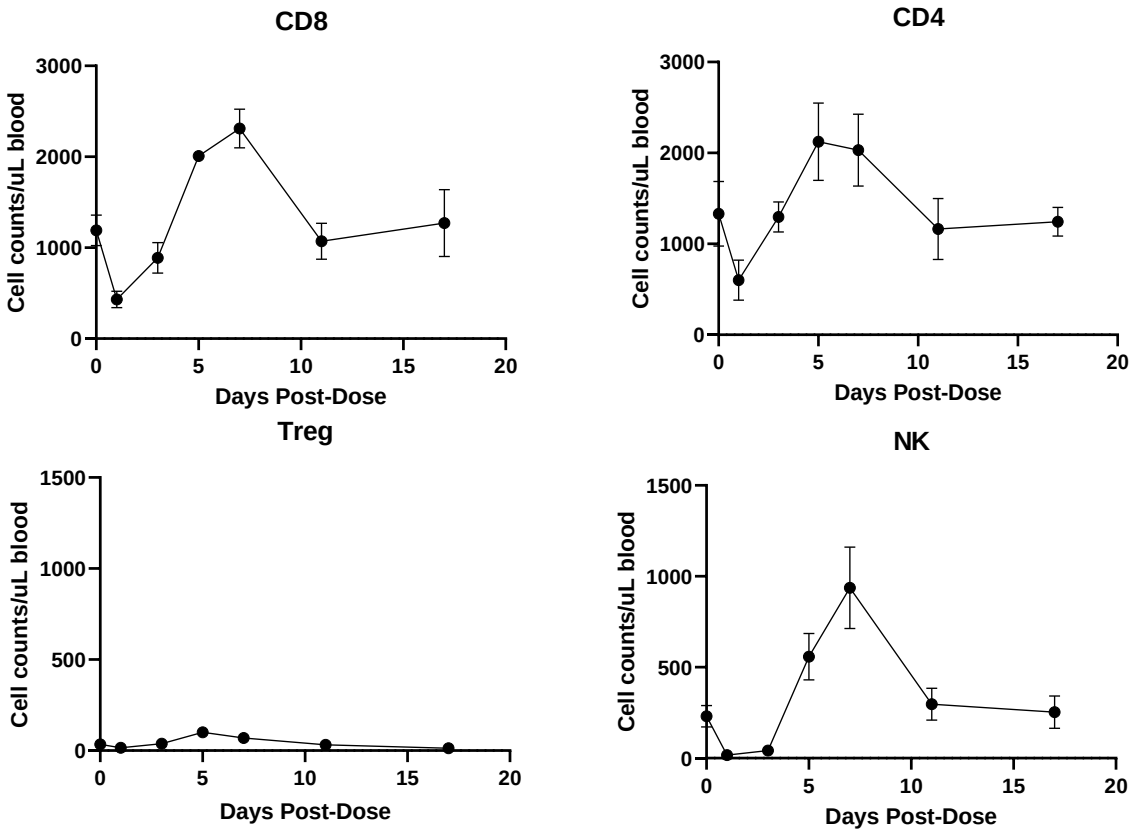
PROPERTIES OF IL-2 PEPTIKINE

- Potent, full agonist of IL-2/15Rβγc
  - No evidence for biased agonism
  - Binds to different sites on IL-2/15Rβ and γc chain than IL-2
  - Does not interfere with agonist activity of γc cytokines
  - Does not bind to IL-2Rα or IL-15Rα
- Constructed without reference or similarity to cytokine structure or receptor contacts
  - Eliminates chance of IL-2 or IL-15 nAbs
- Readily amenable to optimization for use in bispecific constructs

Current Focus: Novel uses of IL-2Rβγ PEPTIKINE

EFFECTS in NHP

- Single IV dose (1mg/kg) of IL-2 PEPTIKINE fused to an Fc-domain in NHP (n=3)

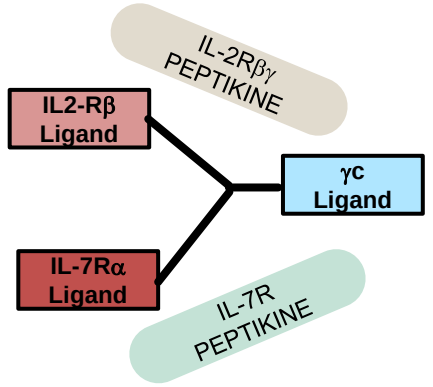


Results similar to other non-alpha IL-2 derivatives

IL-7R x IL-2Rβγ Dual Agonist

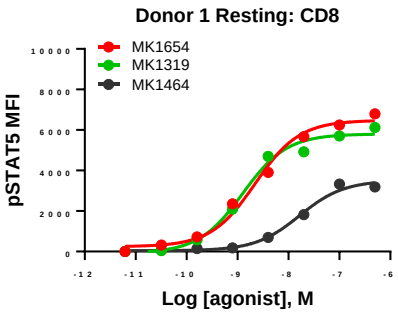
MDK1654 – Branched Synthetic Peptide

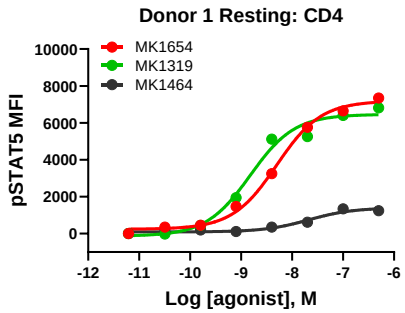
Activates IL-2Rβγ and IL-7R in engineered cell lines

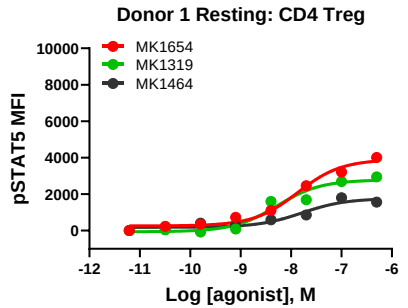


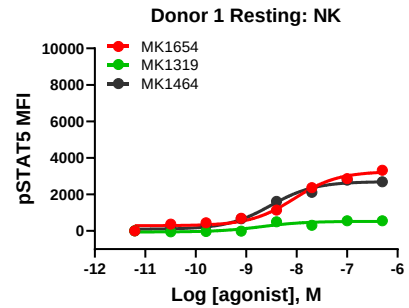
Resting PBMCs

- IL7R STAT5 activation > IL2R activation
- MK1654 behaves like an IL7R agonist (except for NK)



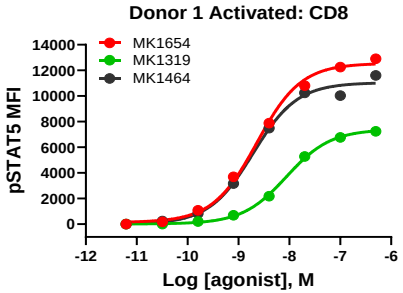


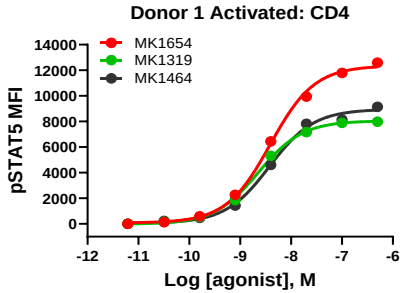


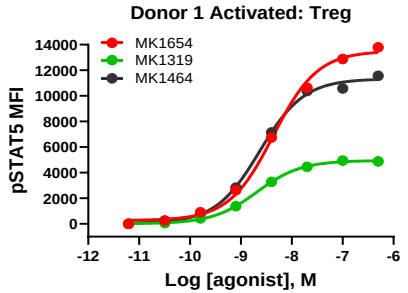


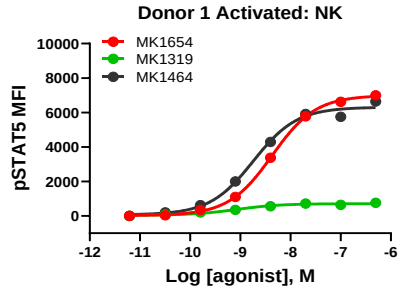
Activated PBMCs

- IL2R STAT5 activation > IL7R STAT5 activation
- MK1654 behaves like an IL2R agonist





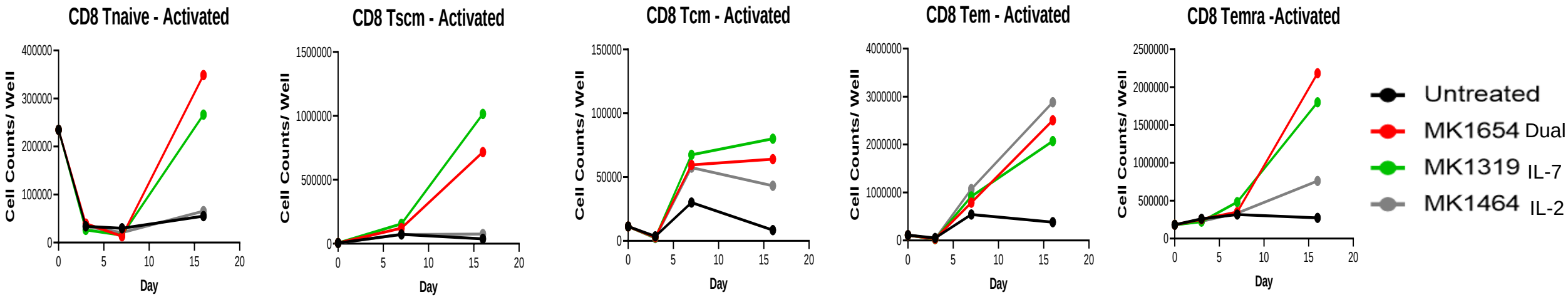




IL-7R x IL-2/15Rβγ Dual Agonist: MK1654 Effects on Naïve and Memory CD8 Cells

- 2 healthy donor PBMCs were activated with 10ng/mL aCD3 mAb in the absence or presence of **MK1654** (5nM), **MK1319** (2.5nM), **MK1464** (2.5nM) for the duration of the experiment
- Concentrations were chosen based on previously determined pSTAT5 EC50 using PBMCs

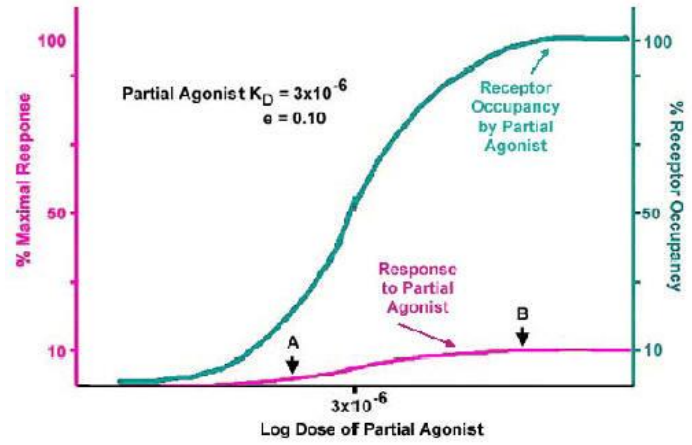
Results from Donor 1



IL-2/15Rβγ PEPTIKINE has a Different Profile Than IL-7R PEPTIKINE
Dual Agonist Behaves like IL-7 PEPTIKINE

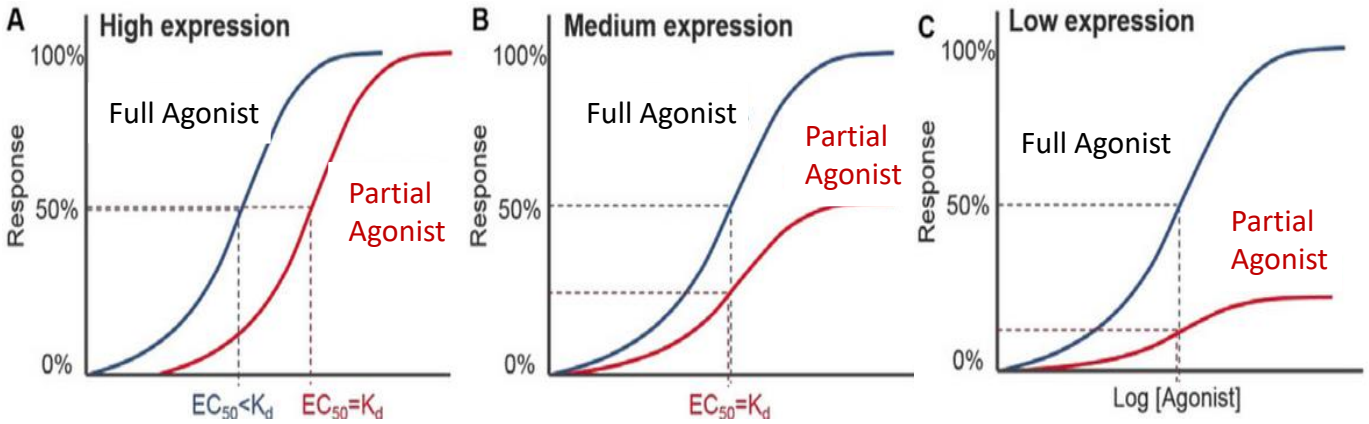
PEPTIKINES – Partial Agonism / Cell Selectivity

Partial Agonism



Partial Agonists Can Be Cell Selective

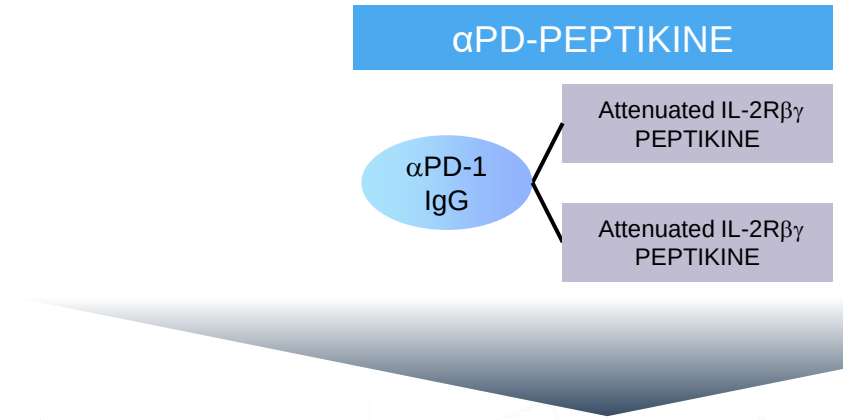
Partial agonists can show cellular selectivity based on receptor expression

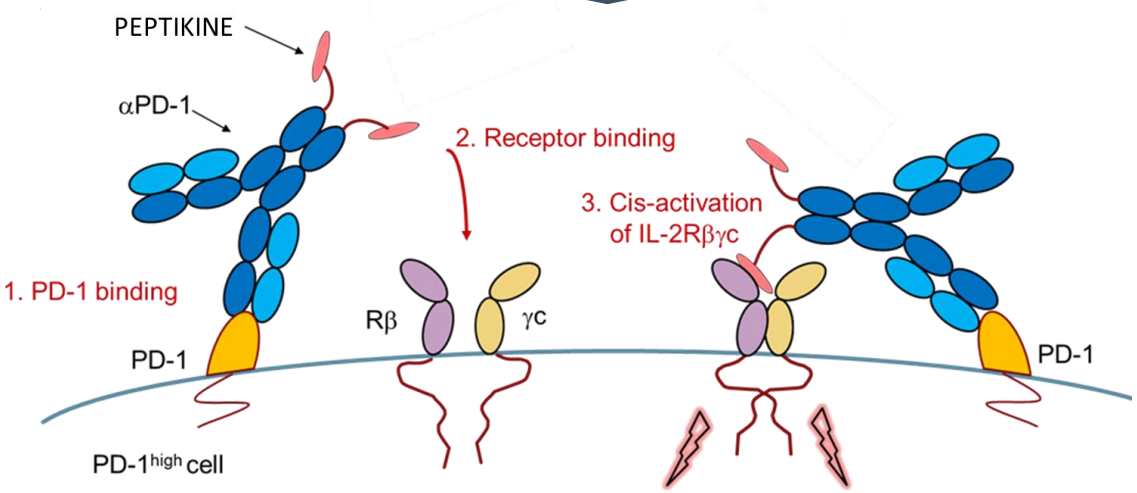


Natural cytokines generally have higher affinity for private receptor unit and low affinity for shared receptor subunit.
By definition, they are full agonists.

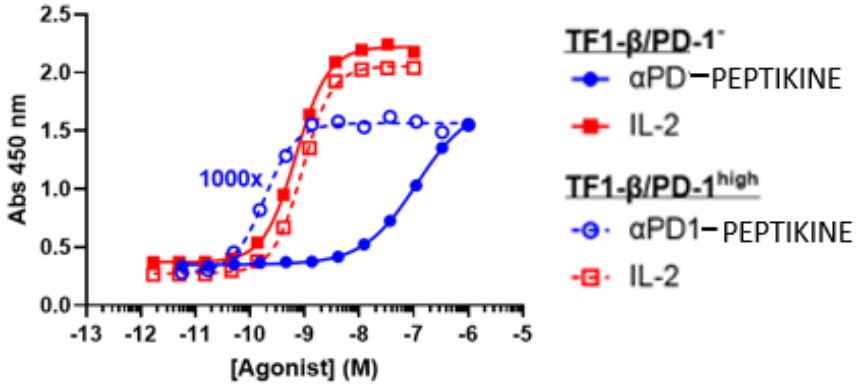
PEPTIKINES can be readily engineered for desired affinity of each subunit and for level of intrinsic efficacy.
Depending on affinities and receptor expression, PEPTIKINES can behave as partial agonists and achieve cell selectivity.

Cell Targeting via Cis-Activation

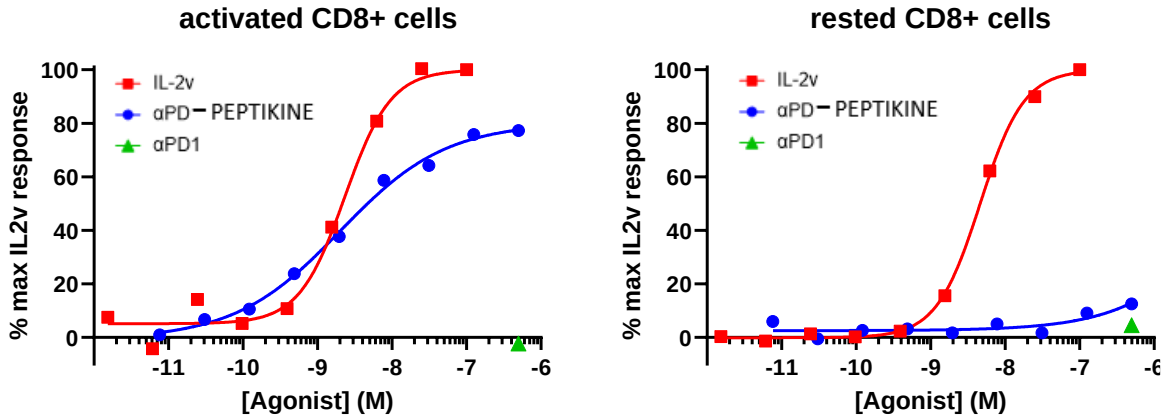




Engineered TF-1 cells expressing hIL-2Rβγ
(pSTAT5 ELISA)



hCD8+ cells in rested or activated PBMCs
(pSTAT5 Flow Cytometry)

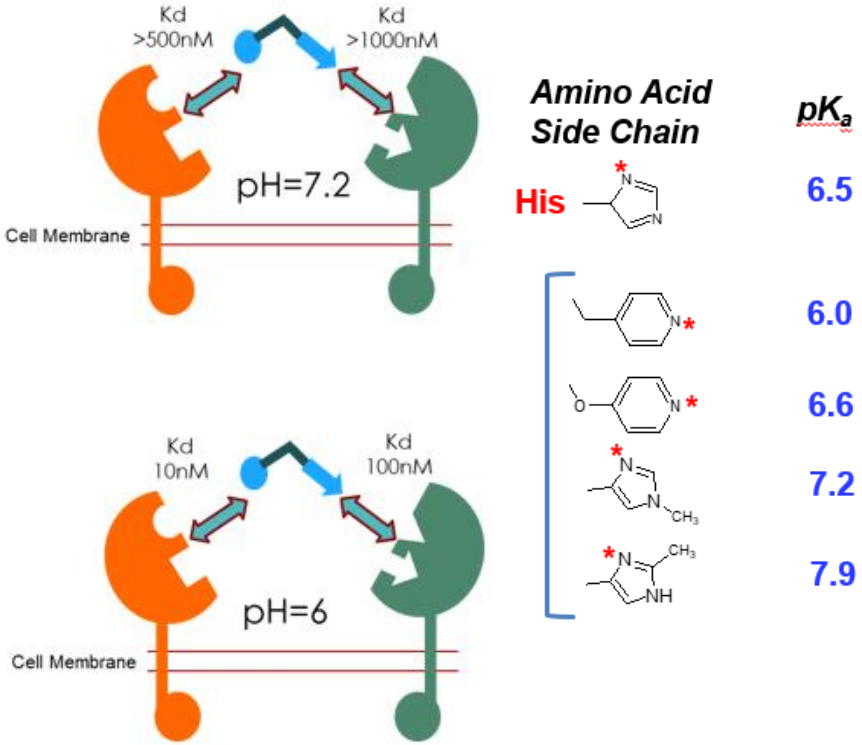


αPD1-PEPTIKINE has nM potency on PD-1^{high} cells and an estimated >3 log loss of potency on PD-1^{low} cells

PEPTIKINES: Tumor-Specific Activation

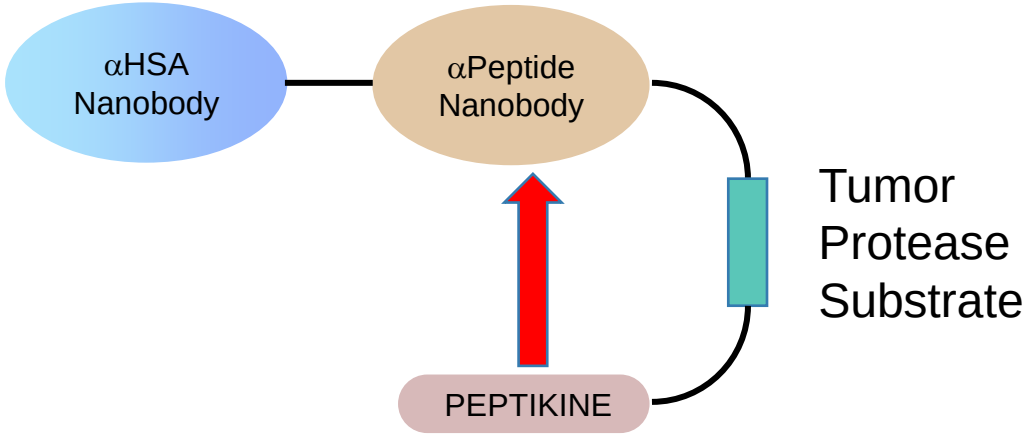
pH Dependent Potency

- Peptides with pH dependent binding can be selected from phage libraries
- Further optimization can be achieved with incorporating unnatural AAs into synthetic PEPTIKINE



Tumor Protease Activation

- PEPTIKINE activity is “masked” outside tumor
- Masked PEPTIKINE has extended half-life
- Tumor protease “releases” PEPTIKINE within TME
- Any peripheral “free’ PEPTIKINE has short-half life



PEPTIKINES:
A modular and versatile modality for cytokine mimetics with optimal properties

Synthetic OR Recombinant
Potency
Intrinsic Efficacy
Half-life
Lack of Immunogenicity
Dual Pharmacology
Cell Targeting
Conditional Activation

Versatile Modality for Best in Class Cytokine Receptor Agonists

PEPTIKINES:
A modular and versatile modality for cytokine mimetics with optimal properties

<u>Medikine Colleagues</u>

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Prae Pongtornpipat

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