(P618)

MK1169, a peptide unrelated to IL-2, is a potent IL-2Rβγc agonist

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ABSTRACT

Background

Efforts to modify IL-2 for immuno-oncology applications have focused on alterations that reduce interaction with the Rα subunit of the receptor complex via mutation, chemical modification, or complexation with antibodies or the Rα-ectodomain. IL-2Rβγc agonists may also incorporate features to reduce side effects associated with peak drug levels, and to extend duration of action.

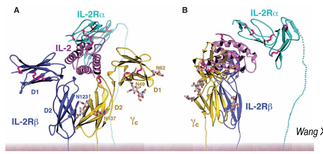
Methods

Peptides were selected from recombinant peptide libraries to identify molecules binding simultaneously to the β and γc subunits of the IL-2 receptor. Identified peptides were synthesized by both chemical and recombinant means, and evaluated for properties of IL-2Rβγc activation in IL-2-responsive cell lines and human lymphocytes.

Conclusions

PK-enhanced versions of these IL-2Rβγc agonist peptides are an attractive alternative to engineered IL-2 variants. Dual pharmacology therapeutics incorporating these molecules also offer promise for immuno-oncology. To our knowledge, this work is the first demonstration of small peptide agonists of a heterodimeric cytokine receptor.

IL-2R BIOLOGY



Structure of the quaternary IL-2/IL-2Rαβγ signaling complex

- IL-2R consists of 3 receptor subunits, Rα, β, γc
- Rβ and γc are signaling subunits, activated upon IL-2 binding
- Rα is non-signaling subunit on some IL-2 responsive cells
- Rα, by boosting the affinity of IL-2 for the complex, enhances the potency of IL-2 on cells expressing Rα, such as Treg cells

MK1169 PROPERTIES

- 42 amino acid synthetic peptide (includes linker AAs)
- Novel sequence, unrelated to IL-2
- Binds to both IL-2Rβ and Rγc subunits
- Does not bind to IL-2Rα or IL-15Rα
- MK1169 interaction restricted to Rβγc to avoid preferential stimulation of Treg cells relative to Teff/NK cells, and provide more effective anti-tumor effects
- Low proteolysis in human plasma ($t_{1/2}$ > 72 hr)
- Low predicted immunogenicity (EpiVax, *Moise, et. al.*)

AGONIST ACTIVITY

MK1169 induces major signaling pathways of IL-2R with full efficacy

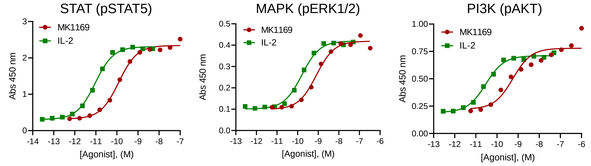


Fig. 1 Compounds were added to starved NK-92 cells for 30 min at 37C. Cells were lysed and pSTAT5, pERK1/2, and pAKT were measured by ELISA

MK1169 stimulates proliferation of human IL-2-responsive cells

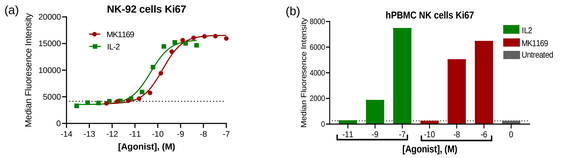
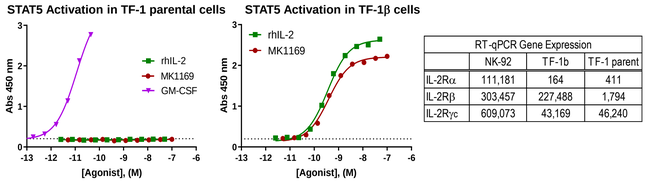


Fig. 2 (a) NK-92 cells exposed to compounds for 48 hr, stained for Ki67 levels, and scored by flow cytometry (b) Human PBMCs exposed to compounds for 72 hr, stained for cell markers and Ki67 levels, and scored for NK proliferation by flow cytometry

MK1169 acts by directly activating IL-2Rβγc



RT-qPCR Gene Expression			
	NK-92	TF-1b	TF-1 parent
IL-2Rα	111,181	164	411
IL-2Rβ	303,457	227,488	1,794
IL-2Rγc	609,073	43,169	46,240

Fig. 3 Comparison of pSTAT5 activation in TF-1 cells (which express Rγc only) and TF-1β (engineered to also express Rβ) indicates dependence of MK1169 activity on presence of Rβ subunit

MK1169 is equipotent to IL-2 in cells lacking IL-2Rα

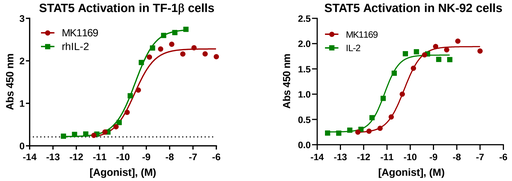


Fig. 4 pSTAT5 activation in TF-1β and NK-92 cells is consistent with lack of MK1169 interaction with IL-2Rα, in contrast to IL-2

MK1169 activates STAT5 phosphorylation in human lymphocytes

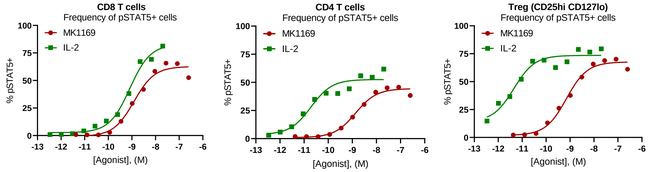


Fig. 5 pSTAT5 was measured by flow cytometry after incubation of rested hPBMCs with compound for 30 min

MK1169-treated hPBMCs induce tumor cell PD-L1 expression and lysis

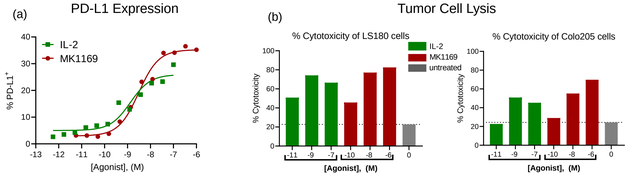
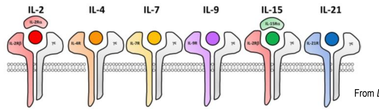


Fig. 6 (a) Human PBMCs plated on A549 cells (10:1 E:T) and exposed to compounds for 48 hr. PBMCs were removed, and A549 cells assayed for PD-L1 expression by flow cytometry. (b) LS180 or Colo205 cells plated with hPBMCs (20:1 E:T). Treated with compound for 48 hr. Lysis determined by LDH release

EFFECT ON IL-2Rγc CYTOKINE RECEPTORS



MK1169 does not modify activity of other γc cytokines

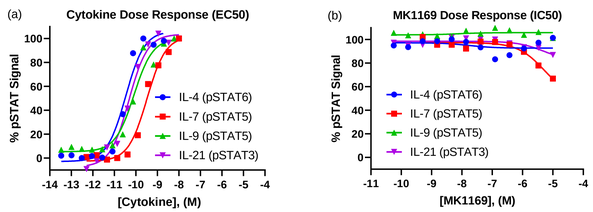
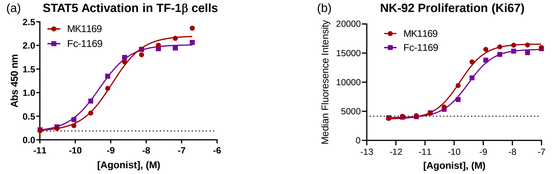


Fig. 7 (a) TF-1 cells (γc only) stably transfected with cytokine-specific receptors become responsive to respective cytokine (b) MK1169 was tested for potential to antagonize the activity of an approximate EC50 concentration of cytokine

MACROMOLECULAR FORMATS

MK1169 fused to IgG-Fc



MK1169 fused to Pembrolizumab (Dual Pharmacology)

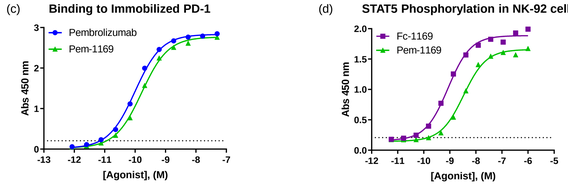


Fig. 8 (a) TF-1β cells exposed to compounds for 30 min, and scored for pSTAT5 by ELISA (b) NK-92 cells exposed to compounds for 48 hr, stained for Ki67 levels, and scored by flow cytometry (c) Compounds tested for direct binding to PD-1 (d) NK-92 cells exposed to compounds for 30 min, and scored for pSTAT5 by ELISA

PEGylated MK1169

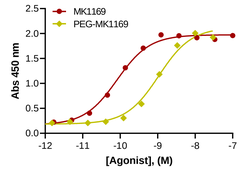


Fig. 9 MK1169 linked to 40kDa PEG. NK-92 cells exposed to compounds for 30 min, and scored for pSTAT5 by ELISA

CONCLUSIONS

- MK1169 is a potent IL-2Rβγc agonist as measured by pSTAT5 (in cell lines and primary blood cells) and proliferation (NK cells)
- MK1169 does not bind to IL-2Rα, is structurally unrelated to IL-2, appears to be stable to proteolysis and is predicted not to be immunogenic
- MK1169 can be fused to IgG and Fc domains with no loss of IL-2Rβγc agonist activity
- Fusion of MK1169 to PD-1 antibodies to create a single recombinant protein with dual pharmacology has been demonstrated
- Potential Utilities: Fc-1169 / PEG-1169 used in combo therapy, dual pharmacology fusions, tissue/cell targeted IL-2Rβγc agonist, vaccine adjuvant, cell therapy (NK/T cell exhaustion)

Citations: Wang X, et. al. (2005) Science 310:1159
Dwyer CJ, et. al. (2019). Front. Immunol. 10:263
Moise L, et al. (2015) Human Vaccines & Immunotherapeutics 11:2312