564 POTENCY-REDUCED AND EXTENDED HALF-LIFE IL12 HETERODIMERIC FC-FUSIONS EXHIBIT STRONG ANTI-TUMOR ACTIVITY WITH POTENTIALLY IMPROVED THERAPEUTIC INDEX COMPARED TO NATIVE IL12 AGENTS

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Background Interleukin-12 (IL12) is a proinflammatory cytokine produced by activated antigen-presenting cells that induces differentiation of Th1 cells and increased proliferation and cytotoxicity of T and NK cells. Stimulation of these cells by IL12 leads to production of high levels of IFNγ. These immune-stimulating aspects of IL12 may help to establish an inflammatory tumor microenvironment critical for anti-tumor responses. Preclinical studies in mice revealed that native IL12 can dramatically shrink syngeneic tumors, however clinical studies in humans resulted in severe toxicity and a small therapeutic window, limiting response rates. Prior work at Xencor demonstrated that reduced-potency IL15/IL15Rα-Fc fusion proteins exhibited superior pharmacokinetics, pharmacodynamics, and safety in non-human primates through reduction of receptor-mediated clearance. Applying similar principles to IL12, we created IL12 heterodimeric Fc-fusions (IL12-Fc) with reduced potency to improve tolerability, slow receptor-mediated clearance, and extend half-life.

Methods IL12 is a heterodimeric protein consisting of two subunits, so we engineered IL12-Fc fusions by fusing the IL12p35 subunit to one side of a heterodimeric (and inactive) Fc domain, and the IL12p40 subunit to the other side. These Fc-fusions were tuned for optimal activity by introducing amino acid substitutions at putative receptor-interface positions and screening for reductions of in vitro potency. In vitro activity was assessed on human PBMCs by measuring signaling in a STAT4 phosphorylation assay and IFNγ production in a mixed-lymphocyte reaction (MLR). In vivo anti-tumor activity was assessed by engrafting MCF-7 cells into PBMC engrafted NSG MHC class I and II double-knockout mice and by measuring tumor volume, lymphocyte activation/proliferation, and IFNγ production over time.

Results IL12-Fc were produced with good yield and purity. An IL12-Fc potency series was created, and variants had up to a 10,000-fold reduction in STAT4 signaling potency and IFNγ production in an MLR assay compared to native IL12-Fc. Anti-tumor activity in the huPBMC-MCF7 model was achieved with potency-reduced IL12-Fc as a single-agent and in combination with anti-PD1, with weaker variants maintaining anti-tumor activity at higher dose levels. Analysis of peripheral lymphocytes indicated increased numbers of T and NK cells as well as activation of CD8+ T cells, as evidenced by upregulation of CD25. Increased expression of immune checkpoints including PD1 was also observed. Analysis of serum indicated up to 200-fold increases in IFNγ levels.

Conclusions Combined, these data indicate that potency-reduced IL12-Fc retain strong anti-tumor activity, while potentially overcoming safety and tolerability issues related to small therapeutic index associated with recombinant native IL12 or IL12-Fc agents.

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565 A NOVEL LONG-ACTING INTERLEUKIN-7 AGONIST, NT-I7, INCREASES CYTOTOXIC CD8+ T CELLS AND ENHANCES SURVIVAL IN MOUSE GLIOMA MODELS

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Background Radiation (RT) and temozolomide (TMZ), which are standard of care for patients with glioblastoma (GBM), can cause prolonged severe lymphopenia. Lymphopenia, in turn, is an independent risk factor for shorter survival. Interleukin-7 (IL-7) is a cytokine that is required for T cell homeostasis and proliferation. IL-7 levels are inappropriately low in GBM patients with lymphopenia. NT-I7 (efineptakin alfa) is a long-acting recombinant human IL-7 that supports the proliferation and survival CD4+ and CD8+ cells in both human and mice. We tested whether NT-I7 rescues treatment-induced lymphopenia and improves survival.

Methods Immunocompetent C57BL/6 mice bearing two intracranial glioma models (GL261 and CT2A) were treated with RT (1.8 Gy/day x 5 days), TMZ (33 mg/kg/day x 5 days) and/or NT-I7 (10 mg/kg on the final day of RT completion). We profiled the CD3, CD8, CD4, FOXP3 cells in peripheral blood over time. We also immunoprofiled cervical lymph nodes, bone marrow, thymus, spleen, and the tumor 6 days after NT-I7 treatment. Survival was monitored daily.

Results Median survival in mice treated with NT-I7 combined with RT was significantly longer than RT alone (GL261: 40d vs 34d, $p<0.0021$; CT2A: 90d vs 40d, $p<0.0499$) or NT-I7 alone (GL261: 40d vs 24d, $p<0.008$; CT2A: 90d vs 32d, $p<0.0154$). NT-I7 with RT was just as effective as NT-I7 combined with RT and TMZ in both GL261(40d vs 47d) and CT2A (90d vs 90d). Cytotoxic CD8+ T cells were increased in both peripheral blood (0.66 x 105 to 3.34 x 105; $P\leq0.0001$) and tumor (0.53 x 103 to 1.83 x 103; $P\leq0.0001$) in mice treated with NT-I7 when compared to control. Similarly, NT-I7 in combination with RT increased the CD8+ T cells in peripheral blood (0.658 x 105 to 1.839 x 105 $P\leq0.0001$) when compared to RT alone. There were decreases in tumor infiltrating FOXP3+ T-reg cells in mice treated with NT-I7 (1.9 x 104 to 0.75 x 104 $P\leq0.0001$) and NT-I7 + RT (1.9 x 104 to 0.59 x 104 $P\leq0.0001$) when compared to the control group without NT-I7. In addition, NT- I7 treatment increased CD8+ T cells in thymus, spleen, and lymph nodes.

Conclusions NT-I7 enhances cytotoxic CD8+ T lymphocytes systemically and in the tumor microenvironment, and improves survival. A phase I/II trial to evaluate NT-I7 in patients with high-grade gliomas is ongoing (NCT03687957).

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566 MDK-202: AN EMPIRICALLY-DESIGNED PEPTIDYL AGONIST OF THE IL-2/15βγC RECEPTOR, DEVOID OF Rα INTERACTION, UNRELATED TO IL-2 OR IL-15, AND FUSED TO AN FC-DOMAIN FOR PK ENHANCEMENT

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Background Efforts to modify IL-2 for immuno-oncology applications focus on modifying the receptor selectivity of IL-2 to bias effects on immune cells; in particular, to reduce Rα interaction via mutation, chemical modification, complexation with antibodies, or fusion to the Rα-ectodomain. IL-2/15Rβγc-biased agonists also incorporate PK enhancement to extend duration of action, and reduce side effects associated with peak drug levels. We previously reported discovery of small synthetic peptides, unrelated to IL-2 or IL-15, that simultaneously bind IL-2Rβ and γc subunits to induce IL-2/15R signaling. These peptides do not bind IL-2Rα, and are therefore IL-2/15Rβγc-selective agonists with MW less than 5000D. We now describe properties of an IL-2/15Rβγc agonist peptide fused to an Fc-domain (MDK-202).

Methods Peptides were selected from recombinant peptide libraries to identify molecules binding simultaneously to the β and γc subunits of IL-2/15R. Active peptides were fused to Fc-domains to evaluate efficacy, potency, and quality of signaling upon activating IL-2/15Rβγc in cell lines and human lymphocytes. PK and PD properties in mice and NHP were also determined.

Results MDK-202 exhibits in vitro potency similar to the synthetic peptide (MDK1169). MDK-202 does not bind IL-2Rα, activates the major IL-2/15R signaling pathways: JAK-STAT(pSTAT5), MAPK (pERK1/2), PI3K (pAKT), and induces proliferation (Ki-67) in human PBMCs, with kinetics and efficacy similar to IL-2. Agonism is attributable to direct activation of IL-2/15Rβγc as shown by dependence on Rβ expression in test cells, and insensitivity to blockade by neutralizing antibodies against IL-2 and IL-15. At concentrations greatly exceeding that required for maximum IL-2/15R activation in vitro, MDK-202 does not interfere with the activities of other Rγc family receptors. The predicted immunogenicity potential for MDK-202 is very low, and in the unlikely event of MDK-202-induced ADA, neutralization of endogenous IL-2 or IL-15 would not be expected. MDK-202 is highly stable in human serum, showing no significant degradation after 4 days at 37C. In ex vivo human PBMC and in vivo studies in normal mice, hPBMC-engrafted NCG mice, and non-human primates, MDK-202 exhibited extended half-life, and activation, proliferation, and population dynamics of lymphocytes similar to those induced by 'non-Rα' variants of IL-2.

Conclusions MDK-202 is an attractive alternative to IL-2/15 variants for use in immuno-oncology therapy. Constructed without reference or similarity to cytokine or receptor structures or contacts, the peptidyl agonist component (MDK1169) is completely unique, and shown to be active when fused to other proteins such as anti-PD-1 antibodies and other cytokine receptor agonists.

Ethics Approval Animal studies were performed by Charles Rivers Laboratories, as approved by the CRL Instuition Ethics Board with the following study and approval numbers: CRL-220007; 20222440 : PK Cynomolgus Monkeys: BA-e451;BA-e451: PD NCG mice BA-e411; BA-e411:PD NCG miceKey 2152; US19001: PK mice: The use of human PBMC in this study was authorized under Minimal Risk Research Related Activities at Stanford Blood Center (SQL 79075).

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567 MDK1319/MDK-701: A POTENT FULLY EFFICACIOUS PEPTIDYL AGONIST OF IL-7RαγC, DESIGNED WITH NO REFERENCE TO CYTOKINE OR RECEPTOR STRUCTURE AND UNRELATED TO IL-7, FUSED TO AN FC-DOMAIN FOR PK ENHANCEMENT

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Background IL-7 receptor activation is essential for the proper development and homeostasis of T-cell subpopulations, and maintenance of the TCR clonal repertoire. Emerging evidence indicates potential clinical utility of IL-7 for immunotherapy of lymphopenia, oncology, and other indications. Here we report the discovery of MDK1319, a small novel peptidyl agonist of IL-7R. This peptide is structurally unrelated to IL-7, with a MW less than 5000D. To improve in vivo properties, we fused MDK1319 to an IgG-Fc-domain to construct MDK-701, which exhibits biological properties similar to those of IL-7 in vitro, and when administered to non-human primates.

Methods Peptides were selected from peptide libraries by screens designed to identify molecules binding simultaneously to the Rα and γc subunits of the human IL-7 receptor. Synthetic peptides, and peptides fused to IgG Fc-domains were evaluated for efficacy, potency, and quality of signaling in IL-7-responsive cell lines and human lymphocytes. PK/PD properties in non-human primates were also determined.

Results MDK1319 and MDK-701 activate the major IL-7R signaling pathways, JAK-STAT (pSTAT5), and PI3K (pAKT), and induce proliferation in human PBMCs, exhibiting lymphocyte subpopulation selectivity, kinetics, efficacy, and potency similar to those of IL-7. Agonism is attributable to direct activation of IL-7R, as shown by dependence on the presence of the IL-7Rα subunit for response in test cells, and insensitivity to IL-7 neutralizing antibodies. MDK1319 and MDK-701 do not activate nor inhibit any other (off target) Rγc family receptors at concentrations 100-fold greater than required for maximal IL-7R activation. MDK-701 administered to cynomologous macaques (single dose, IV at 1 mg/kg) exhibits a circulating terminal half life of ~32 hr; and induces peripheral lymphocyte profiles similar to IL-7 treatment, including initial reduction (tissue migration), followed by sustained elevation of peripheral lymphocytes remaining above baseline for 29 days, with no observed adverse effects.

Conclusions In addition to the utility of Fc-fusion MDK-701 for monotherapy, the small peptidyl nature of the active peptide MDK1319, fusable to recombinant protein partners, offers opportunities for incorporation into bispecific molecules, linking IL-7 activity to a variety of useful functions. These include synergistic cytokine activities, checkpoint blockade, and tissue targeting. Cells engineered to secrete MDK1319 display autocrine stimulation potentially useful in T-cell therapeutics. The structural novelty of MDK1319 substantially decreases risk of cross reactivity of any anti-drug immune response with endogenous IL-7, and may provide a safer alternative to modified forms of IL-7 reported to produce significant anti-IL-7 immunogenicity.

Ethics Approval Animal studies were performed by Envol Biomedical or Charles Rivers Laboratories, as approved by the Institution Ethics Boards with the following study and