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-2R 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 37°C. 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 peptideyl 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 Instution 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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Background High-dose recombinant human interleukin-2 (aldesleukin) elicits durable anti-tumor immunity and gained FDA approval two decades prior to checkpoint blockers. However, aldesleukin is limited due to treatment-related life-threatening toxicities. Second generation efforts to alleviate these toxicities and improve the therapeutic index of IL-2 as an anti-tumor immunotherapy, we employed protein engineering to generate XTX201, a highly potent 3rd generation IL-2 that is designed to be selectively active in tumors, stimulating cytolysis responses against tumor cells while sparing systemic immune activation.

Methods XTX201 binding interactions were measured with SPR, and bioactivity was measured using STAT-5 phosphorylation in human PBMCs and reporter cell lines. Anti-tumor efficacy and immune activation was evaluated in tumors compared to peripheral organs in syngeneic tumor mouse models. Safety and pharmacokinetics were evaluated in rodents and NHPs.

Results Non-activated XTX201 showed no detectable binding to IL-2Rα or IL-2Rβ, and limited IL-2R-dependent STAT-5 signaling in vitro. Activation of XTX201 resulted in high-affinity binding to IL-2Rβ and no binding to IL-2Rα, leading to a ~100-fold reduction in Treg activation as compared to WT IL-2, while retaining CDS+ T and NK cell activation. Mice and NHPs treated with a 2nd generation IL-2 surrogate experienced toxicities that are commonly observed in patients treated with aldesleukin, including pulmonary edema, VLS, fever and lethality. However, XTX201 did not induce toxicities at exposures 100-fold higher than the MTD of the activated version, and achieved similar anti-tumor efficacy in tumors. Pharmacodynamic and efficacy studies were conducted in syngeneic and humanized mouse models.

Conclusions Our data demonstrate that 2nd generation IL-2s that are systemically active and lack binding to IL-2Rα exhibit dose-limiting toxicities unless further engineered for selective activity in tumors. XTX201, a 3rd generation, tumor-selective IL-2, exhibits a long half-life and is innocuous outside of tumors. XTX201 is activated within tumors to release an IL-2Rβ/γ biased cytokine that inhibits tumor growth in syngeneic models, and exhibits tumor-specific pharmacodynamic effects without peripheral toxicities. XTX201 has the potential to be a best-in-class IL-2 immunotherapy by expanding the curative anti-tumor activity of aldesleukin while minimizing dose-limiting toxicities.

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