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-2Rα 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 5000. 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-STAT3(pSTAT3), 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 Rβγ 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 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 River Laboratories, as approved by the CRL Institution Ethics Board with the following study and approval numbers: CRL-220007; 20222440: PK Cynomolagus 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).

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0566
Abstracts

568 XTX201, A PROTEIN-ENGINEERED IL-2, EXHIBITS TUMOR-SELECTIVE ACTIVITY IN MICE WITHOUT PERIPHERAL TOXICITIES IN NON-HUMAN PRIMATES


Background High-dose recombinant human interleukin-2 (aldesleukin) elicits durable anti-tumor immunity and gained FDA approval two decades prior to checkpoint blockers. However, use of aldesleukin is limited due to treatment-related life-threatening toxicities. Second generation efforts to alleviate toxicities have largely focused on eliminating binding to IL-2Rα, often with half-life extension. We have determined that mice and non-human primates (NHPs) treated with a 2nd generation IL-2 surrogate that does not bind IL-2Rα still experience characteristic dose-limiting toxicities, including vascular leak syndrome (VLS), and exhibit dysregulated peripheral immune function due to reduced Treg activation. To overcome 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 cytolytic 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. This approach may enhance tumor localization of immune memory responses were observed upon tumor rechallenge.

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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0567

569 TARGETING IL-15 DELIVERY TO PD-L1 EXPRESSING TUMORS WITH AN ANTI-PD-L1-IL-15 CYTOKINE FUSION IG TO ENHANCE T CELL AND NK CELL MEDIATED TUMOR CYTOTOXICITY

Angus Sinclair *, Thierry Giffon, Dean Ng, Poornam Yakkundi, Hope Lancero, Marigold Manlusoc, Rodnie Rosete, Ameesh Saini, Madeline Tran, Kevin Carlin, Chitra Saraya, Ramesh Baliga, Bruce Keyt. IGM Biosciences, Mountain View, CA, USA

Background Therapeutic antibodies inhibiting PD-1/PD-L1 have demonstrated clinical efficacy though only a fraction of patients respond. Combinations are being explored to enhance responses including anti-PD-1/PD-L1 IgG antibodies with IL-15-pathway stimulating agents to remove PD-1 immunosuppressive signaling and enhance anti-tumor NK and memory CD8 T cell expansion and survival. We have engineered an anti-PD-L1 pentamer to a ~1000-fold reduction in Treg activation. To target low PD-L1 expressing tumors, with an IL-15 superagonist fused to the joining (J) chain.

Methods An anti-PD-L1 IgM was generated by grafting heavy chain variable regions of a high affinity IgG onto the IgM heavy chain framework and co-expressed with the light chains. The IL-15 superagonist fused to the J chain generated PDL1-ISA. Anti-PD-L1 binding was performed using recombinant antigen ELISAs and on cells by FACS. Reporter assays and PBMCs were used for potency testing. Cytokines were evaluated by CBA assays. In vitro cytotoxicity assays used luciferase tagged MDA-MB-231 cells with PBMCs, NK or CD8 T cells. Pharmacodynamic and efficacy studies were conducted in syngeneic and humanized mouse models.

Results The parental anti-PD-L1 IgM antibody bound recombinant and cellular PD-L1 more potently than an IgG antibody with the same binding domain. In functional PD-L1 and PD-1 blocking studies the anti-PD-L1 IgM was as efficacious as the IgG. PDL1-ISA provided a potent proliferation signal to primary human NK and CD8 T cells in vitro with little/no impact on regulatory or CD4 T cells. Limited cytokines were detected following 3–4 days culture with human PBMCs. PDL1-ISA had similar potencies for both human and cynomolgus CD8 T cells, and a 2–3-fold lower potency for mouse cells. Pharmacodynamic studies in humanized and BALB/c mice showed transient and dose-dependent increases in circulating NK and CD8 T cells. PDL1-ISA enhanced in vitro killing of PD-L1 positive MDA-MB-231-Luc cells by human PBMCs, CD8 T and NK cells compared to the anti-PD-L1 IgM (no IL-15). PDL1-ISA also demonstrated efficacy in a hPD-L1-CT26 HuCELL mouse model, with most treated animals having complete tumor regressions. Durable anti-tumor immune memory responses were observed upon tumor rechallenge.

Conclusions We have engineered an IL-15 immunostimulatory anti-PD-L1 IgM antibody that binds PD-L1 more potently than an IgG, stimulates NK and CD8 expansion in vitro and in vivo and induces complete tumor regressions in mouse models. This approach may enhance tumor localization of