858 TILKINE-2: A NOVEL BEST-IN-CLASS TUMOR INFILTRATING LYMPHOCYTE (TIL) TARGETING ENGINEERED IL-2 WITH SUPERIOR PRE-CLINICAL EFFICACY AND SAFETY FOR IMMUNOTHERAPY OF CANCER

Sreerupa Challa, Jonathan Carnino, Andrea Umana, Yuesheng Li, Jing Xu, Lingyun Rui, Yao-Te Hsieh*. *Cugene, Waltham, MA, USA*

Background High-dose Interleukin-2 is the earliest FDA-approved immunotherapy for metastatic melanoma and renal cell carcinoma. Unfortunately, its application is limited due to its short half-life and severe toxicity at the therapeutic dose. To limit systemic toxicity, tumor-targeting antibody-based delivery of IL-2 has been developed, however with poor outcomes. We here deploy a novel strategy to deliver IL-2 to the tumor microenvironment by binding to Tumor-Infiltrating Lymphocytes (TILs). TILKine-2 is a recombinant bifunctional protein comprised of an antibody directed against TILs (TILAb) fused to an engineered IL-2, which simultaneously revives and expands antigen-primed exhausted T cells. The IL-2 portion of TILKine-2 was engineered to have improved tolerability, slower receptor-mediated clearance, and prolonged half-life.

Methods Target binding of TILKine-2 was evaluated by cell-free and cell-based methods. In vitro functional characterization was performed using human peripheral blood mononuclear cells (PBMCs). Pharmacokinetics (PK), pharmacodynamics (PD), and anti-tumor activity of murine TILKine-2 surrogate (TILKine-2s) were evaluated in various syngeneic models. The safety and immune cell activation of TILKine-2 were assessed in non-human primates (NHPs).

Results Structure-based design and activity-guided fine-tuning resulted in an optimized IL-2 variant that was fused to TILAb to generate TILKine-2. TILKine-2 demonstrated TIL-target antigen binding and blocking activity with sub-nM potency. TILKine-2 has a binding activity abolished to IL-2Rα and fine-tuned to IL-2Rβγ. In PBMCs, TILKine-2 potently induced intracellular signaling and cell proliferation in IL-2Rβγ dominant effector CD8+T and NK cells along with IFN-γ secretion. In vivo, TILKine-2 displayed significantly prolonged half-life with sustained proliferation, expansion, and Granzyme B expression on CD8+T and NK cells. Notably, the effects were more pronounced in the tumor than periphery, leading to massive immune hot tumors. Consequently, TILKine-2s exhibited robust anti-tumor primary and memory response in both cold and hot tumor models (MC38, CT26, B16F10, PAN02). Furthermore, TILKine-2s demonstrated superior and synergistic anti-tumor efficacy compared to TILAb alone, engineered IL-2 alone, or their combination, with 100% tumor regression resulting in ~80% tumor free mice in MC38 and Pan02 models. In NHPs, TILKine-2 preferentially induced memory CD8+T, total CD8+T, and NK cell expansion. TILKine-2 was safe and well-tolerated in NHPs with no notable changes in body weight, temperature, clinical pathology, or signs of vascular leakage after repeated dosing.

Conclusions By targeting TILs, TILKine-2 demonstrated robust anti-tumor efficacy by preferentially inducing proliferation, expansion, and activation of intra-tumoral lymphocytes while reducing systemic toxicity and improving therapeutic window. In conclusion, TILKine-2 is a promising therapeutic agent for clinical development.

Ethics Approval For mouse studies, the practices and procedures used were reviewed and approved by Brandeis University IACUC committee (Protocol #22001). For monkey studies, the practices and procedures used were in accordance with the safety and Quality Assurance guidelines set out in the Guideline for Experiments document of Kunming Biomed International (KBI–01-GEv2.0).

http://dx.doi.org/10.1136/jitc-2021-SITC2021.858