KROS 101: A next-generation GITR agonist boosting anti-tumor T cell responses and reprogramming the tumor microenvironment

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Abstract

Glucocorticoid-induced Tumor Necrosis Factor Receptor (GITR) and its ligand GITRL are members of the tumor necrosis factor receptor (TNFR) superfamily, pivotal in T cell activation and modulation. GITR is expressed on activated T cells and regulatory T cells (Tregs), while GITRL is predominantly found on antigen-presenting cells (APCs), such as dendritic cells, macrophages, and B cells. The GITR-GITRL interaction induces receptor trimerization, essential for downstream signaling and effective T cell activation. In mouse models, this trimerization leads to robust receptor clustering, enhancing T cell proliferation and Treg suppression. However, clinical trials using GITR agonist antibodies in humans have shown limited efficacy, likely due to suboptimal trimerization and receptor clustering of human GITRL. To address this, we conducted a chromatography analysis of soluble human GITRL, revealing an equilibrium between dimers and trimers. We then developed a novel small molecule GITR agonist, KROS 101, aimed at stabilizing human GITRL trimerization and enhancing GITR signaling. Using surface plasmon resonance binding assay, we demonstrated that KROS 101 binds to hGITRL with high affinity. In in vitro functional assay, KROS 101 treatment significantly increased the proliferation of CD3+ T cells, with marked expansion of both CD4+ and CD8+ subsets, while concurrently decreasing Treg cell proliferation. KROS 101-treated T cells also exhibited significantly enhanced cytotoxicity against glioblastoma (GBM) cell lines and patient-derived glioma cancer stem cells (CSCs), indicating potent anti-tumor activity compared to untreated control T cells. Given the reduced trimerization efficiency of human GITRL compared to mouse counterparts, we utilized a humanized GITR/GITRL double knock-in mouse model to determine the in vivo effects of KROS 101. In a melanoma model using B16-F10-Luc2 cells, KROS 101 treatment significantly inhibited tumor growth, as measured by reduced luminescence intensity. Tumor sizes in KROS 101-treated mice were markedly smaller than those in mice treated with the GITR antibody TRX518, which is currently in clinical trials for various cancers. Comprehensive immune profiling of tumor-infiltrating lymphocytes (TILs) revealed that KROS 101 significantly increased infiltration of CD3+ and CD8+ T cells while reducing Treg cell population within the tumor microenvironment. Notably, these immune effects were superior to those observed with TRX518. Additionally, KROS 101 increased the number and percentage of effector CD4+ and CD8+ T cells in the TME, suggesting a shift towards a more effective anti-tumor immune response. In summary, our novel small molecule, KROS 101, is a potent GITR agonist that enhances T cell infiltration, proliferation, and cytotoxicity, while effectively reducing Treg-mediated suppression. The in vivo data support KROS 101 as a promising candidate for clinical trials in cancer immunotherapy, warranting further investigation as a therapeutic agent for melanoma and glioblastoma.