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GSK-3 inhibitor elraglusib (9-ING-41) to enhance tumor-infiltrating immune cell activation in tumor biopsies and synergize with anti-PD-L1 in a murine model of colorectal cancer.

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Background: Glycogen synthase kinase 3 (GSK-3) is a serine/threonine kinase with key roles in myriad biological processes such as tumor progression, and inhibition of GSK-3 using a novel small-molecule elraglusib has shown promising preclinical antitumor activity in multiple tumor types. **Methods:** We characterize the effects of elraglusib *in vitro* on tumor and immune cells including cell killing and cytokine profiling, *in vivo* in combination with checkpoint inhibitors in a syngeneic murine colon carcinoma BALB/c model using MSS cell line CT-26, and in human tumor biopsies and plasma samples from patients with refractory solid tumors of multiple tissue origins enrolled in a Phase 1 clinical trial investigating elraglusib (NCT03678883).We used human plasma samples from patients treated with elraglusib, paired pre- and post-treatment tumor biopsies and performed digital spatial proteomics. **Results:** Elraglusib promoted immune cell-mediated tumor cell killing, enhanced tumor cell pyroptosis, decreased tumor cell NF-κB-regulated survival protein expression, and increased immune cell effector molecule secretion. Synergy was observed between elraglusib and anti-PD-L1 in an immunocompetent murine model of colorectal cancer. Murine responders had more tumor-infiltrating T-cells, fewer tumor-infiltrating Tregs, lower tumorigenic circulating cytokine concentrations, and higher immunostimulatory circulating cytokine concentrations. Murine responders had lower serum concentrations of BAFF, CCL7, CCL12, VEGF, VEGFR2, and CCL21, and higher serum concentrations of CCL4, TWEAK, GM-CSF, CCL22, and IL-12p70 as compared to non-responders. We utilized human plasma samples from patients treated with elraglusib and correlated cytokine profiles with survival. Elevated baseline plasma levels of proteins such as IL-1 β and reduced levels of proteins such as VEGF correlated with improved PFS and OS. PFS was also found to be positively correlated with elevated plasma levels of immunostimulatory analytes such as Granzyme B, IFN-γ, and IL-2 at 24 hours post-treatment with elraglusib. Several of these secreted proteins correlated with results from the *in vivo* study where expression of proteins such as IL-1 β, CCL22, CCL4, and TWEAK was positively correlated with improved response to therapy while expression of proteins such as BAFF and VEGF negatively correlated with response to therapy. Using paired tumor biopsies, we found that CD45+ tumor-infiltrating immune cells had lower expression of inhibitory immune checkpoints and higher expression of T-cell activation markers in post-elraglusib patient biopsies. **Conclusions:** These results introduce several immunomodulatory mechanisms of GSK-3 inhibition using elraglusib, providing a rationale for the clinical evaluation of elraglusib in combination with immunotherapy. Research Sponsor: Some funding from Actuate Therapeutics; U.S. National Institutes of Health.