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Identification of a Novel Raf-1 Activating Drug That Inhibits Gastrointestinal Carcinoid Growth

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Background: Surgery is the only curative option currently available for carcinoid cancer. Recent work has identified the Raf-1 pathway as a potential therapeutic target. In order to identify possible Raf-1 activating drugs, we examined Leflunomide (LFN), currently FDA approved for rheumatoid arthritis and its metabolite, Teriflunomide (TFN). We hypothesized these compounds would inhibit carcinoid growth through activation of the Raf-1 pathway.

Methods: Gastrointestinal carcinoid cells treated with LFN or TFN were analyzed using the MTT colorimetric growth assay, propidium iodide exclusion flow cytometry, serotonin ELISA, quantitative PCR, and western blotting for cyclin B1, Achaete-Scute Complex-Like 1 (ASCL1), Chromogranin A (CgA) and markers of Raf-1 pathway activation. *In vivo* studies were performed using a subcutaneous xenograft in nude mice.

Results: Treatment of carcinoid cells with LFN and TFN resulted in dose dependent inhibition of growth and induction of cell cycle arrest. These changes were associated with Raf-1 pathway activation. In our *in vivo* model, oral administration of LFN resulted in significant inhibition of tumor growth. Cellular levels of serotonin and CgA protein were reduced. Importantly the protein and mRNA levels of the transcription factor, ASCL1, were dose dependently suppressed. The effects on cyclin B1 as well as ASCL1 were reversed by pretreatment with U0126, a Raf-1 pathway inhibitor, illustrating that the anti-cancer effects of these drugs are mediated via the Raf-1 pathway.

Conclusions: LFN and TFN induce *in vitro* cell cycle arrest while suppressing *in vivo* carcinoid growth. Importantly, levels of key carcinoid markers are suppressed. These effects are mediated through the Raf-1 pathway. LFN thus represents one of the first clinically applicable Raf-1 pathway activators in carcinoid cancer, and given its clinical safety record in humans, is worthy of further investigation.