Combined Drug Efficacy in Preclinical Models of Colorectal Cancer
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**Background:** Colorectal cancer is the third most commonly diagnosed cancer and the second leading cause of cancer death in men and women combined in the U.S. Albeit not widely represented in terms of cancer awareness, about 150,000 Americans are diagnosed with this disease and more than 50,000 die annually. Patients suffer from a change in bowel habits, diarrhea, constipation, discomfort in the abdomen among many other symptoms. Several treatment plans are available including but not limited to surgery, radiation therapy, chemotherapy, as well as targeted therapy. In this study, we sought to optimize targeted therapy more specifically in terms of a combination drug therapy. We hypothesize that combination drug treatment using blockades against cancer proliferation-inducing targets will lead to greater efficacy compared to single-drug therapy. The treatment focuses on inhibiting EGFR, a receptor prominent in cancer cells and promotes their growth, through panitumumab as well as blocking the amino acid transporter, ASCT2, utilized by cancer cells for rapid reproduction, using V-9302 and its variants, the CDP series.

**Methods:** As a preliminary characterization of the colorectal cancer cell lines utilized in the study, a BCA protein assay was conducted to quantitate protein concentration of each sample extracted. Using the results from the protein assays, a Western Blot was conducted to detect proteins related to EGFR and ASCT2 in the samples. The antibodies used were Phospho-EGF Receptor, EGF Receptor, ASCT2, Anti-GAPDH antibody, Anti-rabbit IgG HRP-linked Antibody. From there, the effects of panitumumab and V-9302 on viability of the cancer cells were observed through single-drug therapy at several dose ranges (0.01 - 1000 \(\mu\)g/mL for Panitumumab & 0.001 - 100 \(\mu\)M for V-9302) with a 48 hour incubation. Finally, to determine the efficacy of the combined treatment another cell viability assay was conducted using panitumumab and either V-9302, CDP3, or CDP7.

**Results:** In our Western Blot, of the five cell lines used (DiFi, Caco2, SNUC4, HT29, SW48) it was observed that the relative concentration of EGFR was particularly high in DiFi, and the concentration of ASCT2 was relatively high in HT29 and SW48. From our single-drug therapy using panitumumab, we were able to observe a sizeable decrease in viability in DiFi and showed minimal death in the other cell lines. In treatment using V-9302, DiFi was also much more sensitive compared to the other four cell lines in the study. The combined treatment showed a substantial decrease in viability when compared to the single-drug therapy and ranged between 92% to 98% cancer cell death.

**Conclusion:** These studies provide confirmation that a combined drug treatment lead to not only greater cancer cell death when compared to single-drug treatment, but also to a significant and reliable degree. In the future expanding through further trials as well as a bioinformatics analysis to characterize the cell lines used. Comprehending the relationship between Western Blot results and sensitivity to drug treatment can also be extremely beneficial. The results of this study prompt us to look further into combination therapy and its implication as an important form of anti-cancer treatment for patients who suffer from colorectal cancer.

**Keywords:** Colorectal Cancer; Combined Drug Therapy, Panitumumab, V-9302

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