Colorectal cancer (CRC) kills over 50,000 people per year in the US. Current drug therapy targets single intracellular pathways. Activation of mTOR plays a key role in many cancers. Over-activation of the pathway mostly occurs from activation or mutation upstream from mTOR, either from PI3K or AKT. Mutations in the mTOR pathway have been identified in roughly 30% of colorectal cancers. Clinical data supports the use of mTOR inhibitors as single agents, but resistance to these agents can develop. Previous studies show that the RAS pathway is up-regulated in the colorectal cancer (CRC) cell lines resistant to TAK228 when compared to the sensitive CRC lines. Our objective was to find a new combination using two current drugs, TAK228, which targets mTOR, and Trametinib which targets MEK in the RAS pathway.

**RESULTS**

### Drug Targets

- MEK
- Trametinib
- mTOR
- LST8
- RICTOR
- raptor
- rictor
- Survivin
- Actin

**Figure 2: Targets of Trametinib and TAK228.**

### IC50 Values of TAK228 in CRC Cell Lines

Figure 3: IC50 values calculated from Cell Titer-Glo assay following 72 hours exposure to TAK228. GSEA analysis was performed on the six most sensitive (green line) and six most resistant (red line) CRC cell lines.

**Cell Proliferation with TAK228 and Trametinib**

- HCT116
- SW620
- RKO

**Figure 4: Proliferation assay using Cell Titer-Glo following 72 hour exposure to various concentrations of TAK228 and Trametinib.**

**IC50 Values of TAK228 in CRC Cell Lines**

Figure 5: Apoptosis/Cell death and proliferation assay using Caspase 3/7 and Incucyte Zoom.

**Figure 6: Cell recovery after drug wash out through a clonogenic assay of CRC cell lines exposed to TAK228 and Trametinib for 72 hours, followed by regrowth for an additional 72 hours. Percent confluence was analyzed by ImageJ and graphed.**

**Protein Concentration in CRC Cell Lines**

**Figure 7: Immunoblot following 24 hour exposure to TAK228 (60nM) and Trametinib (15nM).**

**Human CRC Growth in SCID Mice**

**Figure 8: HCT116 cell line xenograft treated with TAK228 and Trametinib.**

**CONCLUSIONS**

- The combination of TAK228 and Trametinib inhibited proliferation in multiple CRC cell lines
  - As shown using both the Incucyte Zoom (Fig. 5) and clonogenic assays (Fig. 6)
  - The combination of TAK228 and Trametinib maintained tumor volume in SCID mice
  - Immunoblotting shows a decrease in survivin, which may lead to an enhanced anti-proliferative response.
  - Immunoblotting also verified that the compounds were controlling their targets (MEK & mTOR)
  - This control was maintained in the combination treatment (data not shown)

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