

**INTRODUCTION**

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 was 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.

**MATERIALS AND METHODS**

IC50 (inhibitory concentration 50) was used to select 7 CRC cell lines (4 presented here). Cell lines were treated with varying concentrations of TAK228 and the MEK inhibitor Trametinib as single agents and in combination. Efficacy of drug combinations were measured using, proliferation and apoptosis assays such as Cell Titer-Glo, imaging with the Incucyte Zoom, and clonogenic assays. The downstream effectors were assessed by immunoblotting to determine the mechanism of the drug combination

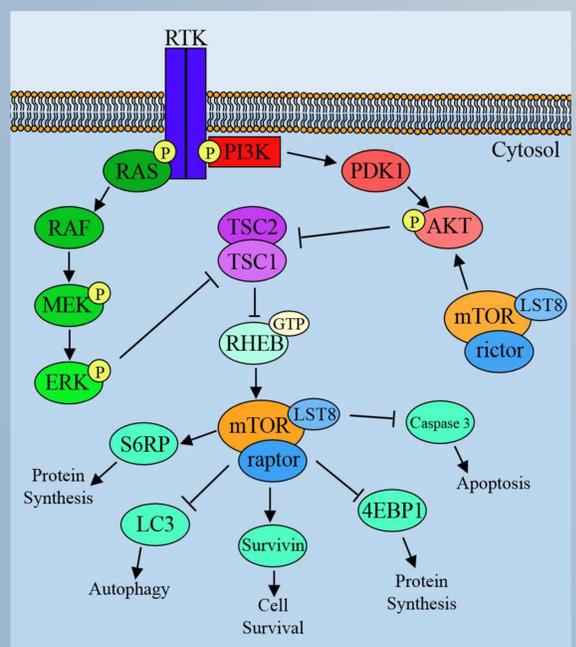


Figure 1: PI3K/AKT/mTOR and MEK pathway

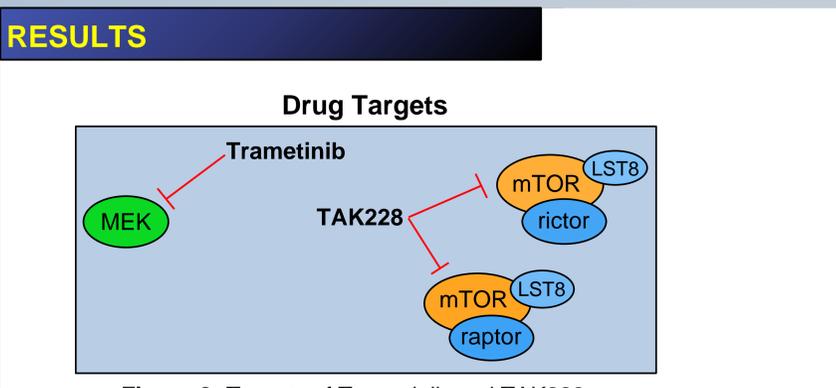


Figure 2: Targets of Trametinib and TAK228.

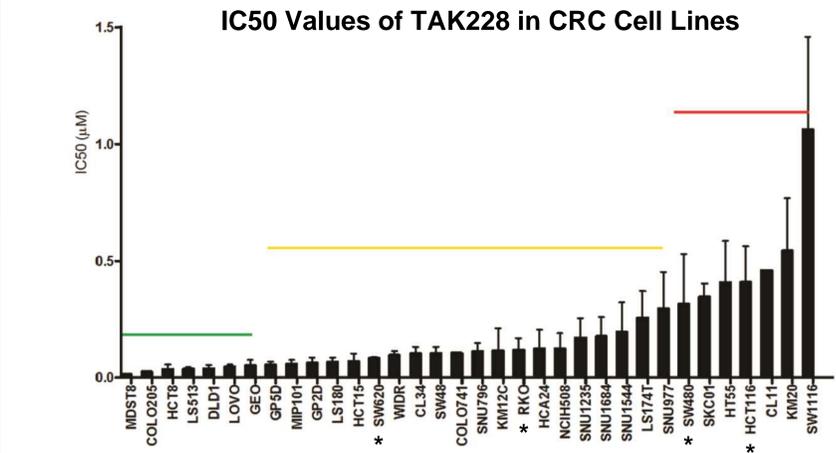


Figure 3: IC50 values calculated from Cell Titer-Glo assay following 72 hours exposure to TAK228. GSEA analysis was performed on the six most sensitive (green line) and six most resistant (red line) CRC cell lines.

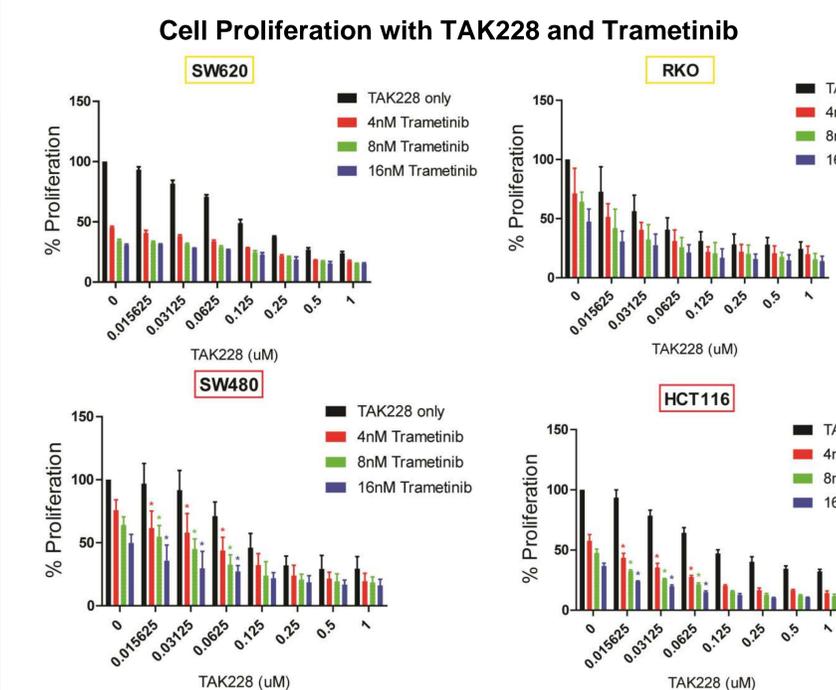


Figure 4: Proliferation assay using Cell Titer-Glo following 72 hour exposure to various concentrations of TAK228 and Trametinib.

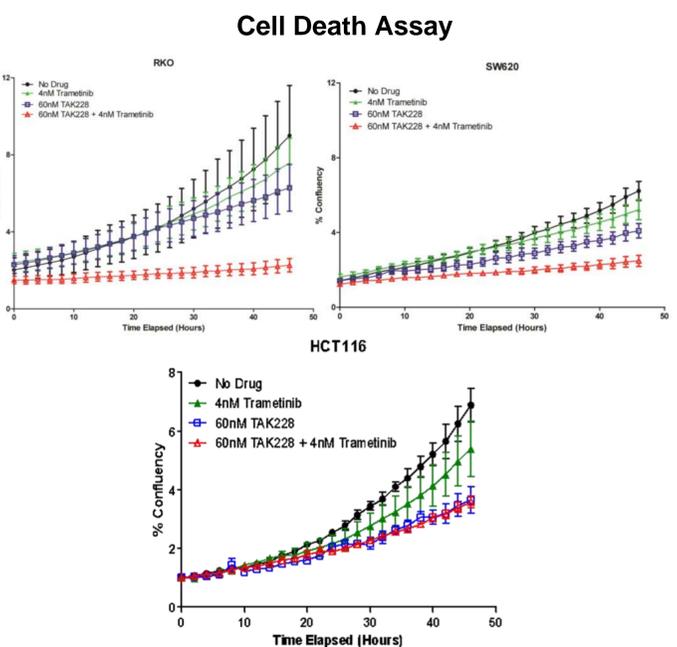


Figure 5: Apoptosis/Cell death and proliferation assay using Caspase 3/7 and Incucyte Zoom.

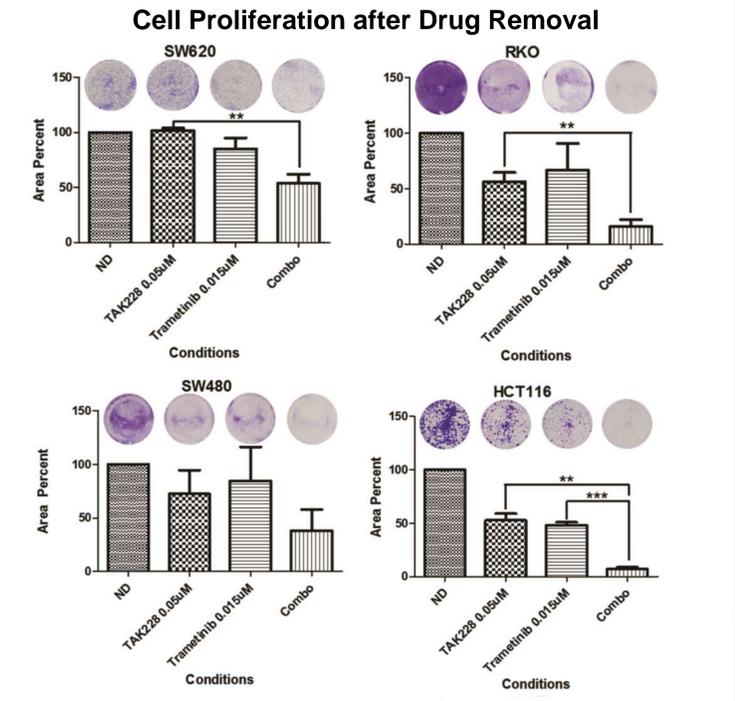


Figure 6: Cell recovery after drug wash out through a clonogenic assay of CRC cell lines exposed to TAK228 and Trametinib for 72 hours, followed by regrowth for an additional 72 hours. Percent confluency was analyzed by ImageJ and graphed.

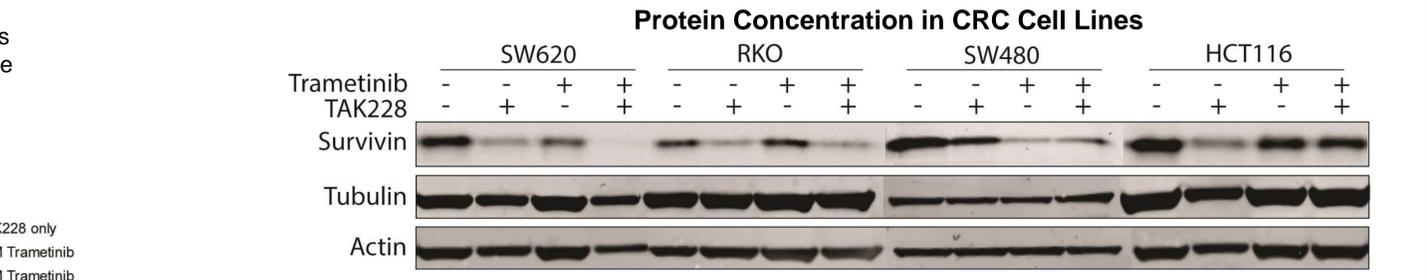


Figure 7: Immunoblot following 24 hour exposure to TAK228 (60nM) and Trametinib (15nM).

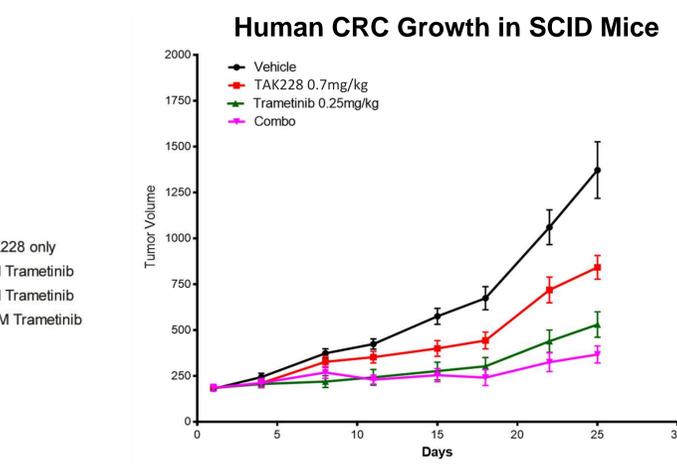


Figure 8: HCT116 cell line xenograft treated with TAK228 and Trametinib.

**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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