# Discovery of tumor types highly susceptible to FASN inhibition and biomarker candidates for clinical analysis

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## Introduction

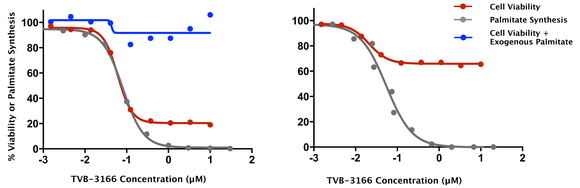
- **3-V Biosciences' lead, oral FASN inhibitor is in Phase I clinical trials for the treatment of solid tumors**
- Fatty acid synthase (FASN) catalyzes the synthesis of palmitate from acetyl-CoA, malonyl-CoA, and NADPH
- Tumor cells have an increased dependence on FASN-synthesized palmitate compared to non-tumor cells
- FASN expression increases with tumor progression in human tumors and associates with chemoresistance, metastasis, and diminished patient survival in many tumor types.
- Palmitate and palmitate-derived lipids comprise diverse cellular components and function in processes required for tumor cell proliferation and survival
- Studies to understand the mechanisms of action and biological consequences of FASN inhibition are guiding the discovery of tumors highly dependent on FASN and biomarkers for assessment of pharmacodynamic activity and patient selection
- Inhibition of the AKT and Wnt/β-catenin pathways, including TCF-promoter-regulated genes such as c-Myc, provide examples of mechanistic responses to FASN inhibition that identify biomarker candidates

## Results

### FASN Inhibition of Tumor Cell Viability is an On-Target Effect

**NSCLC Cell Line**
- Inhibition of palmitate synthesis and cell viability

**Lung Fibroblast Cell Line**
- Inhibition of palmitate synthesis, not cell viability




### Fatty Acid Levels Align with Sensitivity to FASN Inhibition

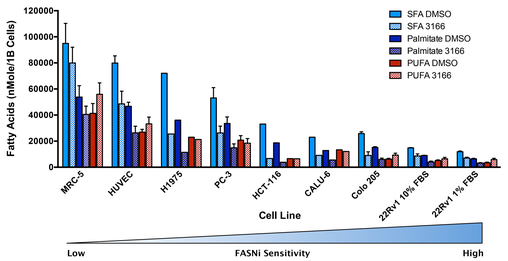



**Figure 1.** Cell-based assays for palmitate synthesis and cell viability show alignment of $IC_{50}$ values in CALU-6 tumor cells. MRC5 lung fibroblasts show inhibition of palmitate synthesis but minimal effect on viability. Palmitate assay measures incorporation of $^{13}C$ into palmitate from $^{13}C$ sodium acetate. Cell viability is measured using the Cell Titer Glo assay following treatment with TVB-3166 for 7 days in Advanced MEM media with 1% charcoal-stripped FBS. Absolute fatty acid levels were measure using Metabolon's FAME analysis.

### TVB-2640 Inhibits COLO-205 Rat Xenograft Tumor Growth in Association with β-Catenin, c-Myc and pAKT Inhibition

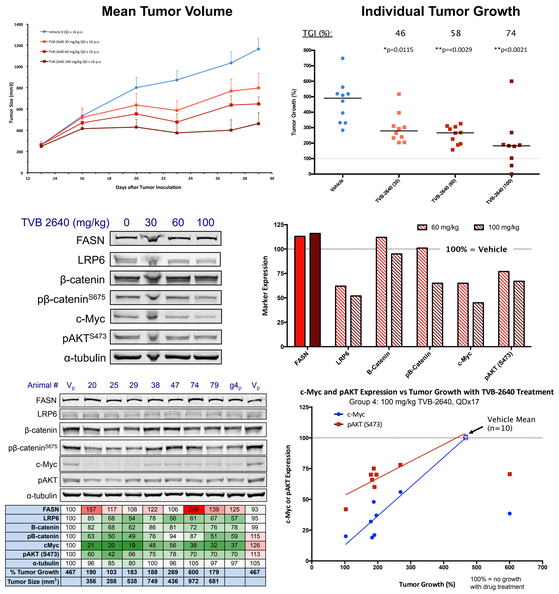



**Figure 2.** Tumor growth and pharmacodynamic analysis of of COLO-205 tumors. Female Rowett nude rats (NIH-Foxn1$^{rnu}$ ), 10-12 weeks of age, were inoculated subcutaneously at the right flank with COLO-205 tumor cells (2 x $10^7$) in 0.2 mL of PBS with matrigel (1:1). Tumor growth inhibition (TGI) was calculated as the percentage of tumor growth, relative to tumor size at the start of treatment in drug-treated compared to vehicle-treated groups. The Mann-Whitney U test was used to assess statistical significance. In-life phase of the efficacy studies were performed by Crown Biosciences (Santa Clara, CA; Beijing, China). Western blot analysis of tumor lysates was performed at 3V Biosciences.

### TVB-2640 Induces Gene Expression Changes in COLO-205 Rat Xenograft Tumors In Association with Treatment Efficacy

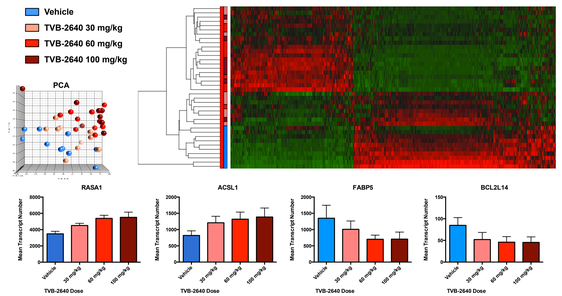
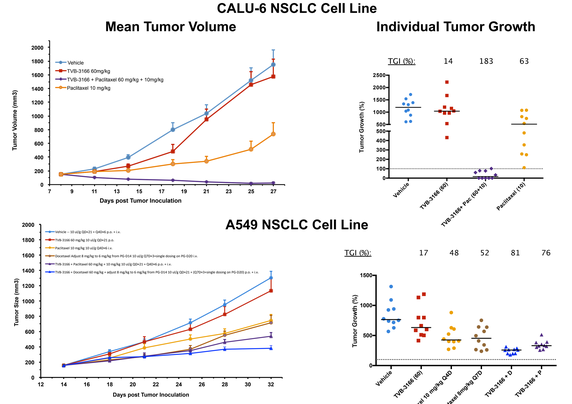
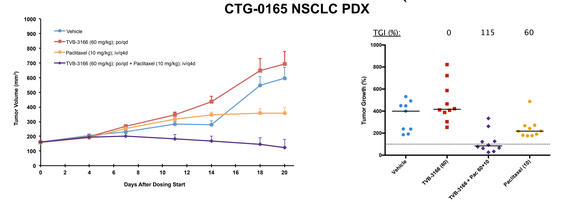
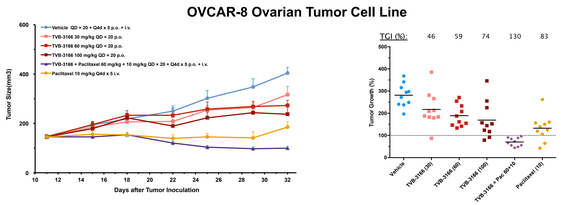



**Figure 3**. COLO-205 rat xenograft tumors were harvested after 17 days of once-daily, oral treatment with TVB-2640 or vehicle (2 hours post final dose). RNA isolation and data analysis was performed at 3-V Biosciences. RNA sequencing (RNASeq-25, Illumina, Inc.) was performed by Expression Analysis (Durham, NC). Differential gene expression data analysis was performed at 3-V Biosciences using Partek Genomics Suite software (St. Louis, MO).

### Additive and Synergistic Inhibition of Xenograft Tumor Growth by FASN Inhibition in Combination with Taxanes



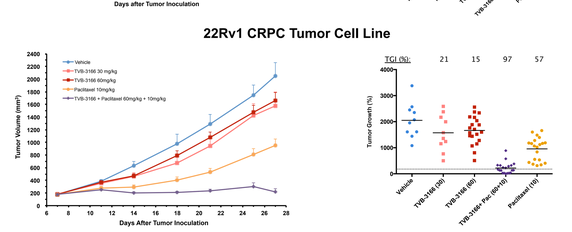




**Figure 4.** Combination treatment of patient-derived and cell-line-derived xenograft tumors with TVB-3166 and paclitaxel (or docetaxel) shows synergistic tumor growth inhibition and tumor regression. TGI was calculated as the percentage of tumor growth, relative to tumor size at the start of treatment in drug-treated groups compared to vehicle-treated groups. The efficacy studies were performed by Champions Oncology and Crown Biosciences.

### Mechanism of Taxane Synergy Insights: FASN Inhibition Affects β-tubulin Expression and Microtubule Organization

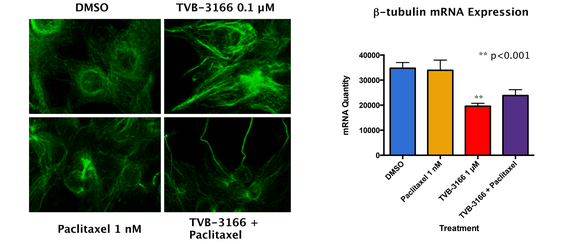



**Figure 5.** Immunofluorescence of β-tubulin in 22Rv1 cells shows decreased expression and disrupted cellular organization following treatment with TVB-3166 for 72 hours. Imaging was performed using a Zeiss LSM510 confocal microscope (100X objective). β-tubulin mRNA expression was measured by RNA sequencing (RNASeq-25, Illumina, Inc.) following 48 hours of TVB-3166 treatment. RNA sequencing was performed by Expression Analysis (Durham, NC).

### Combined FASN-Paclitaxel Inhibition Induces PDX mRNA Changes in Apoptosis, Metabolism, and Tubulin-Associated Genes

**7 days Treatment**
**TVB-3166 + Paclitaxel vs Vehicle**
- p<0.01
- 1.5 fold change
- 1143 genes
- Counter selection for paclitaxel-induced genes (n=151)

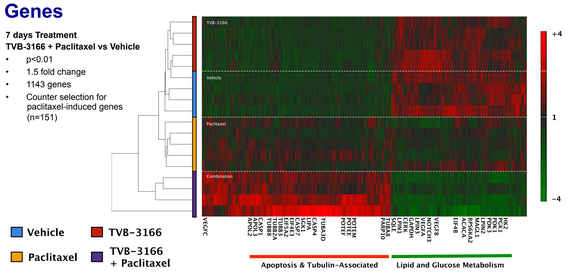



**Figure 6**. CTG-0165 NSCLC PDX xenograft tumors (Champions Oncology) were harvested after 20 days of once-daily, oral treatment as indicated (6 hours post final dose). RNA isolation and data analysis was performed at 3-V Biosciences. RNA sequencing (RNASeq-25, Illumina, Inc.) was performed by Expression Analysis (Durham, NC). Differential gene expression data analysis was performed at 3-V Biosciences using Partek Genomics Suite software (St. Louis, MO).

## Conclusions and Status

- TVB-2640 a first-in-class oral FASN inhibitor, is in Phase I clinical development for the treatment of solid tumors.
- TVB-2640 demonstrates dose-dependent single agent tumor growth inhibition in the COLO-205 rat xenograft tumor model.
- Tumor growth inhibition by TVB-2640 is associated with inhibition of β-catenin, c-Myc and AKT and modulation of tumor gene expression.
- FASN inhibition combined with paclitaxel or docetaxel shows synergistic tumor growth inhibition, including tumor regression in many different xenograft tumor models that include NSCLC, ovarian, and prostate tumors types.
- The mechanism of FASN-taxane synergy likely includes: (1) decreased expression of β-tubulin and disrupted microtubule organization as a result of FASN inhibition; (2) taxane-mediated sensitization of tumor cells to modulation of gene expression by FASN inhibition.
- In-vitro and in-vivo evaluation of additional, potent drug combinations is ongoing

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