Mechanistic analysis of reversible FASN inhibition in preclinical tumor models identifies highly susceptible tumor types and enriches biomarker discovery for clinical applications

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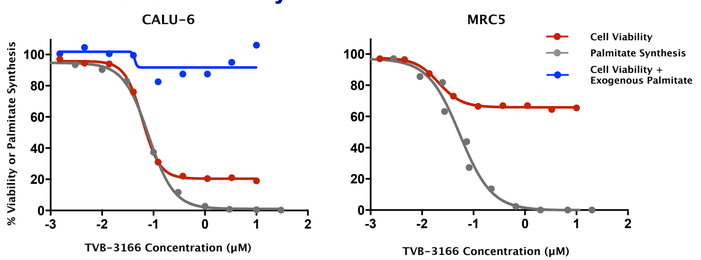
Introduction

- **3-V Biosciences' first-in-class, 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
- Palmitate and palmitate-derived lipids function in vital cellular processes such as energy metabolism and cellular membrane biosynthesis
- Palmitate is conjugated directly to specific proteins as a mechanism to affect protein localization and activation
- FASN tumor expression has been found to be increased in a stage-dependent manner with high expression associated with diminished patient survival
- FASN activity promotes the tumorigenic capacity of cells by multiple mechanisms including enhanced macromolecular biosynthesis and glucose metabolism, cell growth and survival signal transduction, cellular stress response, and resistance to chemotherapeutics
- In vitro and in vivo studies in preclinical tumor models demonstrate that FASN inhibition reduces tumor cell proliferation and induces apoptosis
- Preclinical studies have discovered biomarker candidates and provide insight into FASN inhibition anti-tumor mechanisms of action

Results

FASN Inhibition Lowers Palmitate and Saturated Fatty Acid Levels, Inhibits Tumor Cell Viability



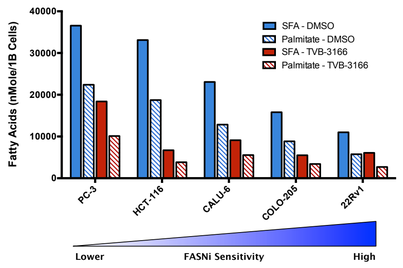


Figure 1. Cell-based assays for palmitate synthesis and cell viability show alignment of IC_{50} values in CALU-6 tumor cells. MRC5 lung fibroblasts show inhibition of palmitate synthesis but minimal effect on viability. Palmitate assay measures incorporation of ^{13}C into palmitate from ^{13}C sodium acetate. Cell viability is measured using the Cell Titer Glo assay (ATP levels). Cells were treated 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.

FASN Inhibition Blocks β-catenin Pathway Activity and c-Myc Expression

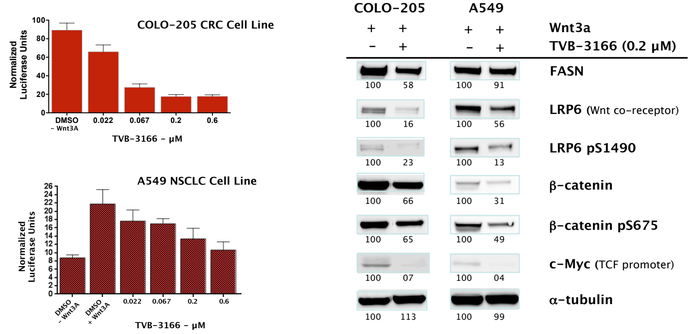


Figure 2. In vitro analysis inhibition of β-catenin pathway activity by FASN inhibition. COLO-205 and A549 cells were treated with 0.2 μM TVB-3166 for 48 hours for TCF promoter-driven luciferase expression analysis (left) or 96 hours for Western blot analysis (right). COLO-205 cells have constitutive pathway activity. Where indicated, cells were stimulated with 200 ng/ml Wnt3A for 18 hours before treating with TVB-3166.

FASN Inhibition Modulates Gene Expression In Vitro and In Vivo

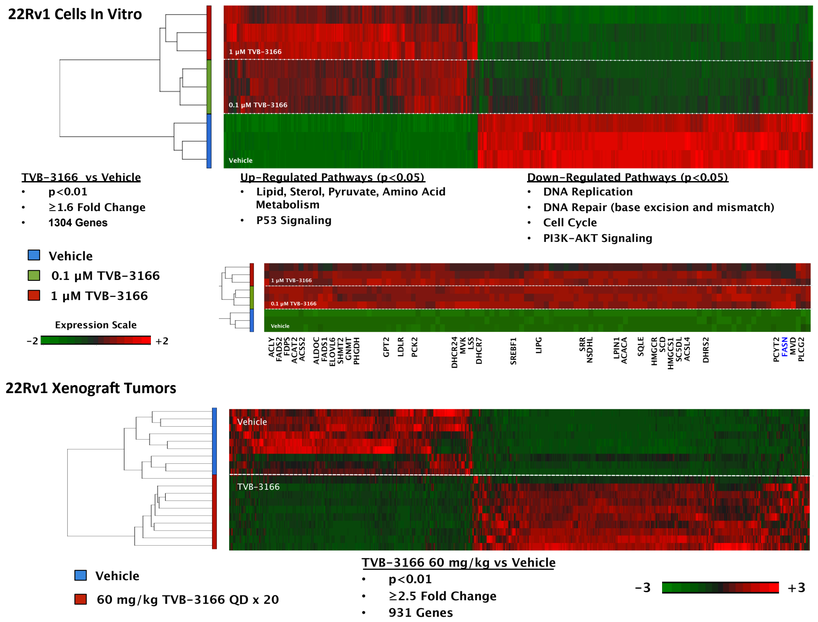


Figure 3. RNA sequencing analysis of gene expression changes induced by FASN inhibition includes unsupervised hierarchical clustering and pathway enrichment analysis of genes with significant TVB-3166 treatment-dependent variance. 22Rv1 tumor cells were treated with TVB-3166 for 48 hours in Advanced MEM plus 1% CS FBS and L-glutamine. 22Rv1 xenograft tumors were harvested after 20 days of once-daily, oral treatment with TVB-3166. RNA isolation and data analysis was performed at 3-V Biosciences. RNA sequencing (RNASeq-25, Illumina, Inc.) was performed by Expression Analysis (Durham, NC). The in-life phase of the xenograft study was conducted by Crown Biosciences (Santa Clara, CA; Beijing, China).

Gene Expression Classifies In Vitro FASN Inhibitor Sensitivity

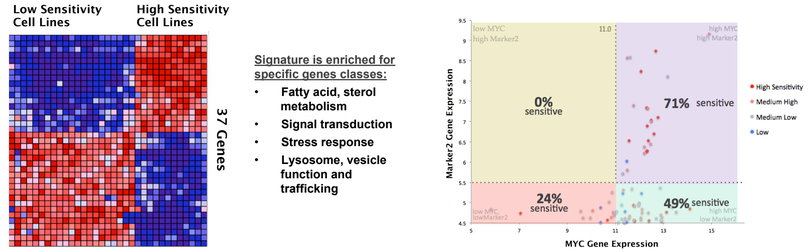
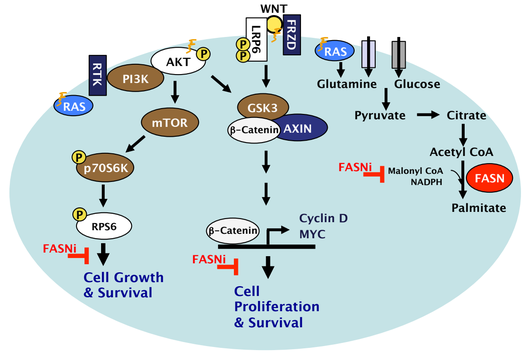


Figure 4. Discovery of genes expression profiles that correlate with in vitro sensitivity to FASN inhibition. (A) 37-gene signature classifies FASN inhibitor sensitivity. Linear regression analysis identified genes with positive or negative correlation to cell viability after TVB-3166 treatment ($r > 0.5$ or $r < -0.4$). (B) MYC gene expression combined with a second marker stratifies tumor cell lines into classes with varied sensitivity to FASN inhibition. The CCLE gene expression data set (Broad Institute, Cambridge, MA) was used for all analyses.

FASN Inhibition Blocks Tumor Cell Growth Through Multiple Pathways and Mechanisms of Action



Mechanistic findings guide biomarker discovery

Conclusions and Status

- FASN inhibition lowers palmitate and unsaturated fatty acid levels in tumor cells and lower pre-inhibition levels correlate with increased inhibitor sensitivity.
- FASN inhibition of β-catenin pathway activity and c-Myc expression provides biomarker candidates for selecting tumors susceptible to FASN inhibition.
- FASN inhibition modulates expression of metabolism, proliferation, and survival pathway genes in vitro and in xenograft tumors, identifies biomarker candidates.
- Bioinformatics analysis identifies a gene expression signature that classifies in vitro sensitivity to FASN inhibition. In vivo utility is being investigated.
- FASN inhibition provides multiple mechanisms of action to inhibit tumor cell growth, proliferation, and viability. Discovery and analysis of biomarker candidates for selecting highly susceptible tumors is proceeding.
- **Biomarker candidates from mechanistic studies are under development to support active clinical trials with 3-V Biosciences' oral FASN inhibitor**