Characterization of small-molecule FASN inhibitors in preclinical tumor models

Timothy S. Heuer, Minchao Chen, Richard Ventura, Joanna Waszczuk, Satya Yendluri, Julie Lai, Samnang Tep, Shirley Feng, Russell Johnson, Cristiana Zaharia, Douglas Buckley, and George Kemble

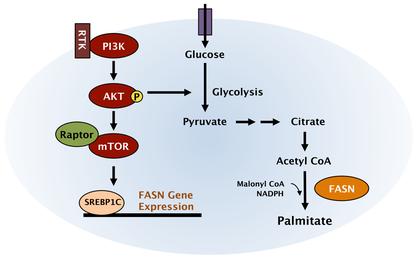
3-V Biosciences Inc., 1050 Hamilton Court, Menlo Park, CA

Introduction

- 3-V Biosciences has discovered and developed a series of potent, selective, orally available, and reversible FASN inhibitors with excellent pharmaceutical properties
- 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, cellular membrane biosynthesis, and protein localization and function
- Palmitate is conjugated directly to specific proteins as a mechanism to affect protein localization and activation
- FASN tumor over-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 chemotherapeutic agents
- FASN inhibition can restore sensitivity to chemotherapeutics
- In vitro and in vivo studies in preclinical tumor models demonstrate that FASN inhibition reduces tumor cell proliferation and induces apoptosis

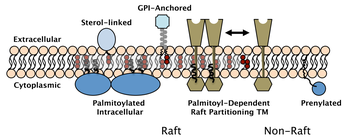
Metabolic and Signaling Pathway Interaction

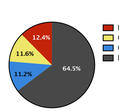
- Metabolic and signal transduction pathway cross-talk reprograms tumor cell metabolism



Lipid Regulation of Protein Function

- Membrane-associated lipid rafts localize proteins for cell signaling
  - Protein palmitoylation required for architecture and signal transduction
- Lipid-associated protein activation fuels cancer cell growth and survival
  - Ras, Raf, RTKs (e.g. EGFR, ErbB2), Akt, etc.
- FASN inhibition is a mechanism to inhibit vital tumor-cell-activated pathways regulated by lipid modification or lipid association

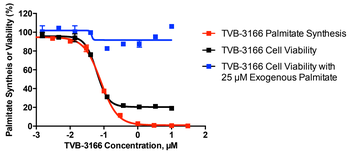




Results

Inhibition of Palmitate Synthesis Causes Tumor Cell Death

- Cell death induction by FASN inhibition is an on-target effect reversed by exogenous palmitate



Assay	TVB-3166 IC_{50} (µM)
Hu FASN Biochem	0.042
CALU-6 Cell Palmitate	0.081
CALU-6 Cell Viability	0.061

Figure 1. CALU-6 cell-based assays for palmitate synthesis and cell viability show alignment of IC_{50} values in each assay. Palmitate assay measures incorporation of ^{13}C into palmitate from ^{13}C sodium acetate. Cell viability is measured using the Cell Titer Glo assay, which measures cellular ATP levels. CALU-6 cells were treated with TVB-3166 for 7 days in Advanced MEM media with 1% charcoal-stripped FBS.

Inhibition of Tumor Cell Viability in Diverse Tumor Types

- Range of FASNi sensitivities among tumor cell lines from diverse tumor types
- FASN inhibition does not induce non-specific cell toxicity
- Tumor cell lines identified with different sensitivity for MOA/biomarker ID studies

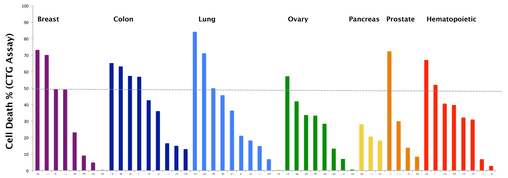
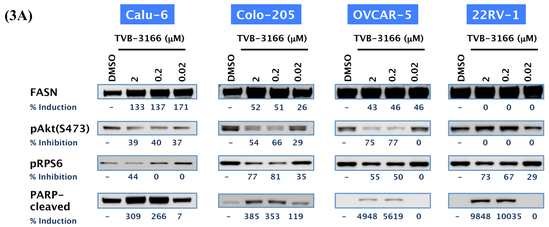


Figure 2. 50 tumor cell lines representing 6 different tumor types were profiled in vitro with TVB-3166 for cell viability affects using the Cell Titer Glo assay. Cells were treated with TVB-3166 for 7 days in Advanced MEM media with 1% charcoal-stripped FBS.

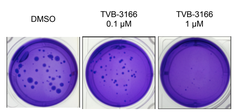
Inhibition of Cell Signaling and Induction of Apoptosis

- FASN inhibition causes AKT/mTOR inhibition and induction of cleaved PARP in diverse tumor cell lines

(3A)



(3B)



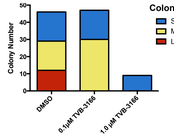
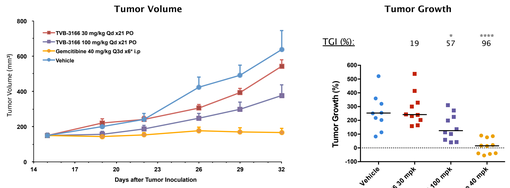


Figure 3. (A) CALU-6, COLO-205, OVCAR-5, and 22RV-1 tumor cells were treated with TVB-3166 at 0.02, 0.2, 0r 2.0 µM for 4 days and analyzed by Western Blot for FASN expression, AKT S473 phosphorylation, RPS6 phosphorylation and cleavage of PARP. (B) Soft agar colony formation assay with CALU-6 cells shows inhibition of colony growth by TVB-3166.

Oral Dosing of TVB-3166 Inhibits Xenograft Tumor Growth

(4A)

- TVB-3166: 57% PANC-1 tumor growth inhibition p=0.0431



Tumor Growth Inhibition p-values

• TVB-3166	p=0.0431
• Gemcitabine	p<0.0001

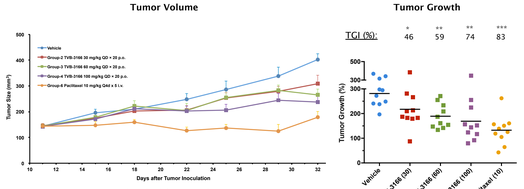
(4B)

TVB-3166		VEH	
30	100		mg/kg
			FASN
406	537	100	% Induction
			Total AKT
			S473 pAKT
0	52	0	% Inhibition (total AKT-normalized)
19	57	0	Tumor Growth inhibition
3.9	7.0	0	Plasma Drug concentration (µM)
1.1	2.9	0	Tumor Drug concentration (µM)

Figure 4. (A) TVB-3166 inhibits PANC-1 xenograft tumor growth. (B) Western blot PD analysis of PANC-1 xenograft tumors shows FASN induction and AKT-S473 phosphorylation inhibition. (C) TVB-3166 inhibits OVCAR-8 xenograft tumor growth. Female BALB/c-nude mice, 6-7 weeks of age, were inoculated subcutaneously at the right flank with COLO-205 tumor cells (5 x 10^6) in 0.1 mL of PBS with matrigel (1:1). The day of tumor cell inoculation is denoted as day 0. Tumor growth inhibition (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 Mann-Whitney U test was used to assess statistical significance. In-life phase of efficacy studies was performed by Crown Biosciences.

(4C)

- TVB-3166: 74% OVCAR-8 tumor growth inhibition p=0.0052



Tumor Growth Inhibition p-values

• TVB-3166	p=0.0052
• Paclitaxel	p=0.0001

TVB-3166 Inhibits Patient-Derived Xenograft Tumor Growth and Causes Tumor Regression

- TVB-3166: 87% mean TGI in NSCLC PDX tumor model
- 2 of 3 tumors show 49 and 55% regression

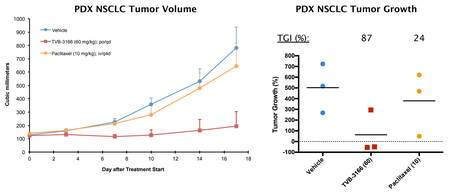


Figure 5. TVB-3166 inhibits growth of non-small-cell lung patient-derived tumor xenograft tumors. Tumor growth inhibition (TGI) was calculated as the percentage of tumor growth, relative to tumor size at the start of treatment, in drug-treated groups compared to vehicle-treated groups. The efficacy study was performed by Champions Oncology.

In Vivo FASN Inhibition is Well Tolerated

- TVB-3166 causes minimal or no weight loss at doses that produce tumor growth inhibition

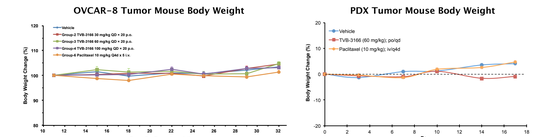


Figure 6. TVB-3166 is well tolerated with once daily oral dosing. Doses up to 100 mg/kg that produce significant tumor growth inhibition cause minimal to no body weight loss in murine xenograft tumor studies.

Conclusions

- **3-V Biosciences is initiating clinical development of a first-in-class, orally active, potent, and reversible FASN inhibitor as a novel cancer therapeutic.**
- **In vitro FASN inhibition suppresses tumor cell palmitate synthesis, tumor cell growth, AKT/mTOR signal transduction, and induces tumor cell apoptosis with an aligned concentration dependence for all of these effects.**
- **In vivo FASN inhibition demonstrates significant tumor growth inhibition or tumor regression in a broad range of murine xenograft models of human cancer including pancreatic, ovarian and lung cancer.**
- **In vivo FASN inhibition is well tolerated in murine xenograft tumor studies at doses that result in significant anti-tumor efficacy.**
- **Additional studies are ongoing to further investigate single and combination agent efficacy, biomarker discovery, and further elucidate mechanism of action effectors downstream of palmitate synthesis inhibition.**

3-V BIOSCIENCES

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