Efficacy of FASN-selective small molecule inhibitors in preclinical tumor models

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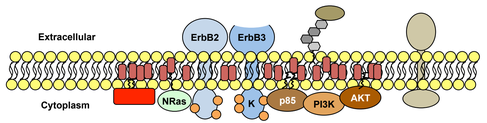
Introduction

- **3-V Biosciences lead, oral FASN inhibitor has initiated Phase I clinical trails 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 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
- Preclinical studies have discovered biomarker candidates and provide insight into FASN inhibition anti-tumor mechanisms of action

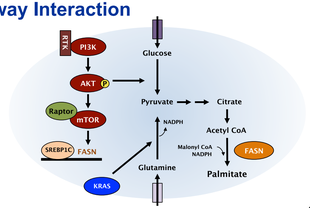
Lipid Regulation of Protein Function

- 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



Metabolic and Signaling Pathway Interaction

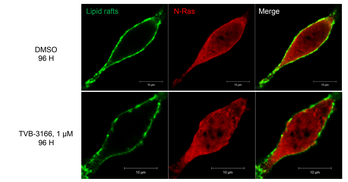
- Metabolic and signal transduction pathway cross-talk reprograms tumor cell metabolism
- Tumor cells require increased energy and macromolecular biosynthesis
- FASN inhibition blocks cellular lipid synthesis and signal transduction



Results

FASN Inhibition Disrupts Lipid Rafts and Palmitoylated Protein Localization

- Discontinuous, lower intensity lipid raft staining following FASN inhibition
- N-Ras localization altered following FASN inhibition



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 in vitro immunoflouresent staining of lipid rafts (cholera toxin) and N-Ras. CALU-6 cells were treated with TVB-3166 for 96 hours in Advanced MEM media with 1% charcoal-filtered FBS.

FASN Inhibition Blocks Signal Transduction and Induces Apoptosis

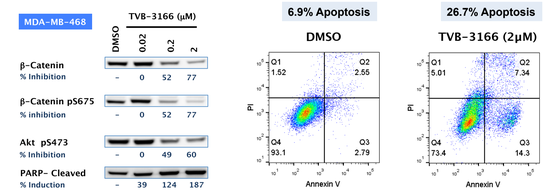


Figure 2.FASN inhibition in MDA-MB-468 cells blocks β-catenin and AKT signaling. Annexin V flow cytometry shows induction of apoptosis. Cells were treated with TVB-3166 for 96 hours (Western) or 120 hours (Annexin V) in Advanced MEM media with 1% CF FBS.

FASN Inhibition Blocks Anchorage-Independent Tumor Cell Growth

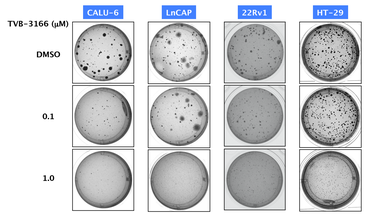
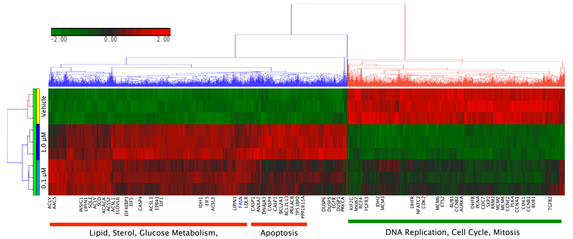


Figure 3. Soft agar colony formation assays show inhibition of anchorage-independent growth by FASN inhibition in lung, prostate, and colon tumor cell lines. Cells were treated with TVB-3166 for 21-28 days in IDEM plus 10% FBS.

FASN Inhibition Induces mRNA Expression Changes in Tumor Cell Growth, Survival, and Metabolism Pathways

- Lipid, sterol, glucose, metabolism signatures
- Apoptosis, cell cycle, DNA replication signatures
- Concerted modulation of pathway-associated gene expression



Gene Expression Pathway ANOVA Analysis

Pathway Name	Database	Enrichment p-value
Steroid biosynthesis	kegg	9.58E-09
Cell cycle	kegg	5.47E-07
Metabolic pathways	kegg	0.0001
DNA replication	kegg	0.0002
p53 signaling pathway	kegg	0.0003
One carbon pool by folate	kegg	0.0004
Terpenoid backbone biosynthesis	kegg	0.0005
Insulin signaling pathway	kegg	0.0009
Viral carcinogenesis	kegg	0.0016
Aminoacyl-tRNA biosynthesis	kegg	0.0023
HIF-1 signaling pathway	kegg	0.0037
NF-kappa B signaling pathway	kegg	0.0053
Pyruvate metabolism	kegg	0.0065
Glycine, serine and threonine metabolism	kegg	0.0138
Biosynthesis of unsaturated fatty acids	kegg	0.0155
Folate biosynthesis	kegg	0.0156
Glycolysis / Gluconeogenesis	kegg	0.0166
Glutathione metabolism	kegg	0.0189
2-Oxocarboxylic acid metabolism	kegg	0.0200
Mismatch repair	kegg	0.0229
Propanoate metabolism	kegg	0.0261
Dorso-ventral axis formation	kegg	0.0271
Selenocompound metabolism	kegg	0.0311
Pyrimidine metabolism	kegg	0.0336
Gap junction	kegg	0.0506
Mineral absorption	kegg	0.0632
Vitamin B6 metabolism	kegg	0.0655
Fatty acid biosynthesis	kegg	0.0655

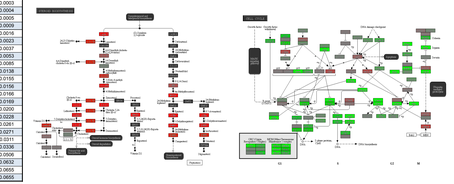


Figure 4 Affymetrix HU133 Plus 2.0 microarray analysis of gene expression changes induced by FASN inhibition. PANC-1 tumor cells were treated with TVB-3166 for 72 hours in Advanced MEM plus 1% CF FBS and L-glutamine. Unsupervised hierarchical clustering and pathway enrichment analysis of 911 genes with significant TVB-3166 treatment-dependent variance (FDR <0.0001).

TVB-3166 Oral PK and Inhibition of De Novo Lipogenesis

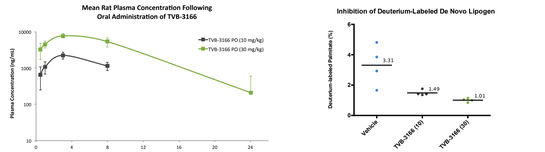


Figure 5 TVB-3166 plasma exposure in Sprague Dawley rats following oral dosing at 30 mg/kg. Inhibition of de novo lipogenesis in Sprague Dawley rats, measured 8 hours following oral dosing at 10 or 30 mg/kg. Lipogenesis is measured as incorporation of deuterium from D_2O into newly synthesized palmitate (Kinemed Inc., Emeryville, CA).

TVB-3166 Inhibits In Vivo Tumor Growth

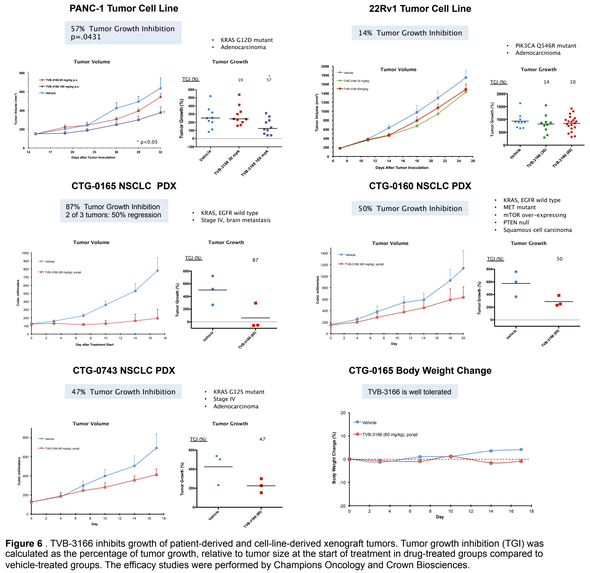


Figure 6 . TVB-3166 inhibits growth of patient-derived and cell-line-derived xenograft tumors. Tumor growth inhibition (TGI) was calculated as the percentage of tumor growth, relative to tumor size at the start of treatment in drug-treated groups compared to vehicle-treated groups. The efficacy studies were performed by Champions Oncology and Crown Biosciences.

Conclusions and Status

- Clinical development of TVB-2640, a first-in-class oral FASN inhibitor, is initiated
- TVB-3166 demonstrates single agent activity in tumor cell line and patient-derived xenograft tumor models
- FASN inhibition effects on tumor cell biology include: (1) membrane and protein localization disruption, (2) signal transduction inhibition, (3) concerted gene expression pathway modulation, (4) apoptosis induction, and (5) anchorage-independent tumor cell growth inhibition
- FASN inhibition-mediated pathway modulation informs selection of drug combinations and discovery of mechanisms of action and biomarker candidates
- Biomarker discovery for patient and expansion cohort selection is proceeding
- In vitro and in vivo evaluation of multiple, potent drug combinations is ongoing

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