

Preclinical studies characterize tumor type sensitivity to FASN inhibition and the mechanism and efficacy of novel drug combinations with TVB-2640

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Introduction

- 3-V Biosciences' lead, oral FASN inhibitor is in Phase I clinical development 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 Inhibition decreases cellular levels of palmitate and saturated fatty acids and induces tumor cell apoptosis
- In vitro and in vivo studies have identified multiple mechanisms of action for FASN inhibition efficacy that include: (1) Membrane and lipid raft architecture remodeling; (2) Tumor cell gene expression reprogramming; (3) Signal transduction pathway inhibition
- 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

Results

Classification of Tumor Lipogenic and Epithelial Gene Expression Aligns with FASN-Inhibitor Sensitivity In Vitro

- Published literature reports gene expression signatures of lipogenic, glycolytic, epithelial, and mesenchymal phenotypes
 - Lipogenic and epithelial phenotypes co-segregate
 - Glycolytic and mesenchymal phenotypes co-segregate
- Application to 3-V In vitro data shows association with FASN inhibitor sensitivity
- Application to TCGA tumor data (8588 samples) to identify candidate tumor types and biomarkers of FASN inhibitor sensitivity
 - Stratification and analysis of molecular genetic features of tumors

References

- Collisson et al. Nat Med. 2011 Apr;17(4):500-3. Epub 2011 Apr 3. PMID: 21460848
- Daemen et al. Proc Natl Acad Sci U S A. 2015 Aug 11;112(32):E4410-7. Epub 2015 Jul 27. PMID: 26216984

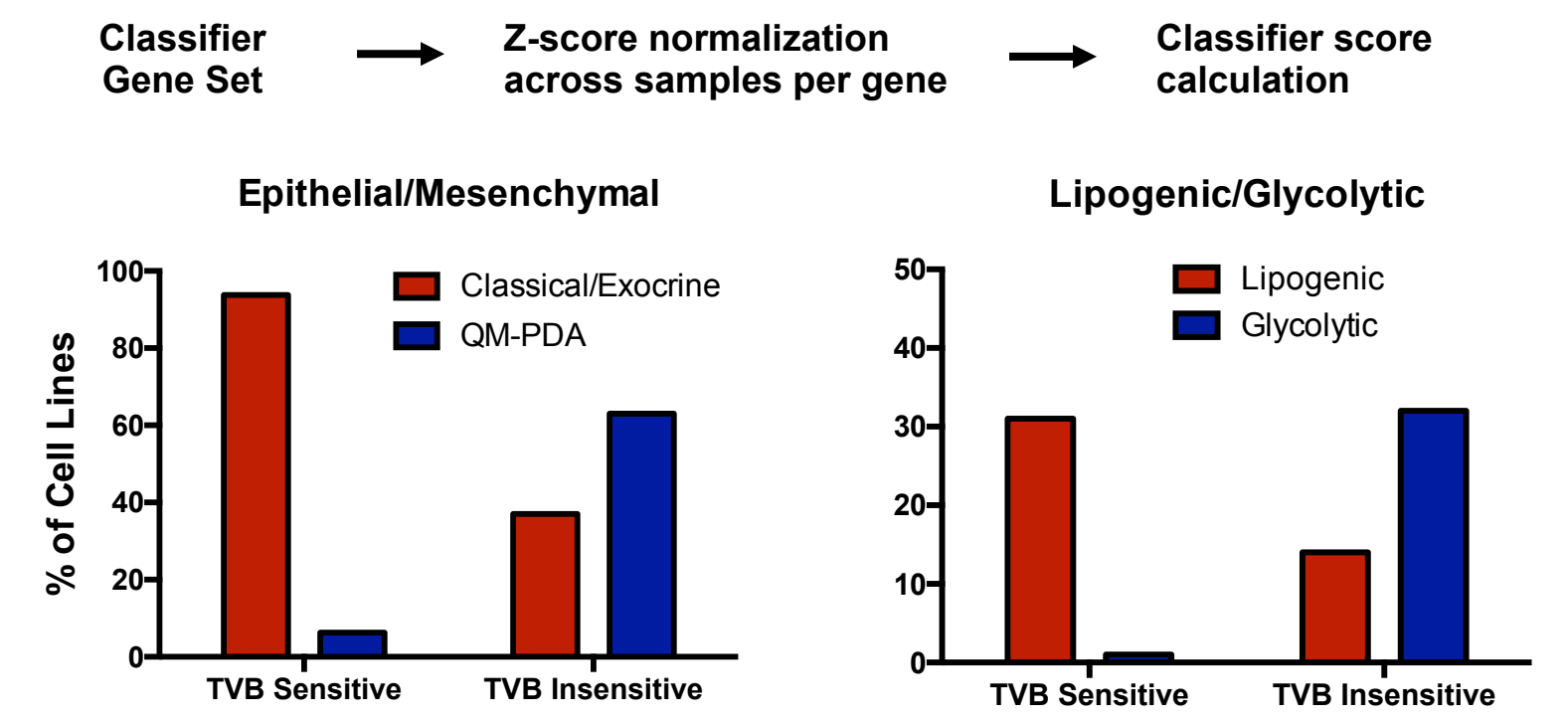


Figure 1. Tumor cell line classification by gene expression analysis. Methods described in the Collisson et al and Daemen et al reports were followed using in vitro viability data (cell titer glo) with TVB-3166. Gene expression data were from the CCLE (Broad Institute, Cambridge, MA).

Classification of Lipogenicity in TCGA Tumor mRNA Data

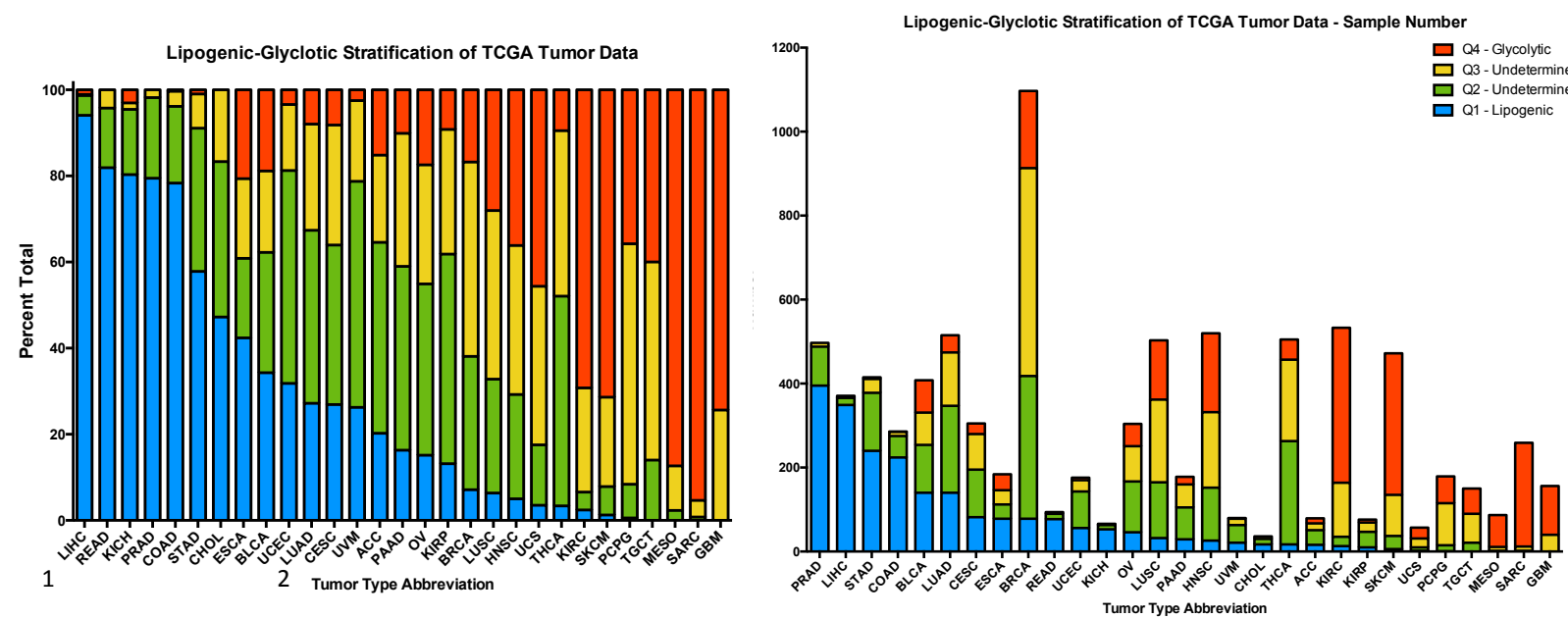


Figure 2. Classification of 8558 TCGA tumor sample data using the Daemen et al 2015 methods. Data were downloaded, processed, and analyzed at 3-V Biosciences. In figure 3 below, tumor cell line xenografts representing (1) liver tumors (LIHC) and (2) lung adenocarcinoma tumors (LUAD) were assessed for tumor growth inhibition efficacy using TVB-3166.

Tumor Cell Line Xenograft Sensitivity Aligns with TCGA Tumor Classification

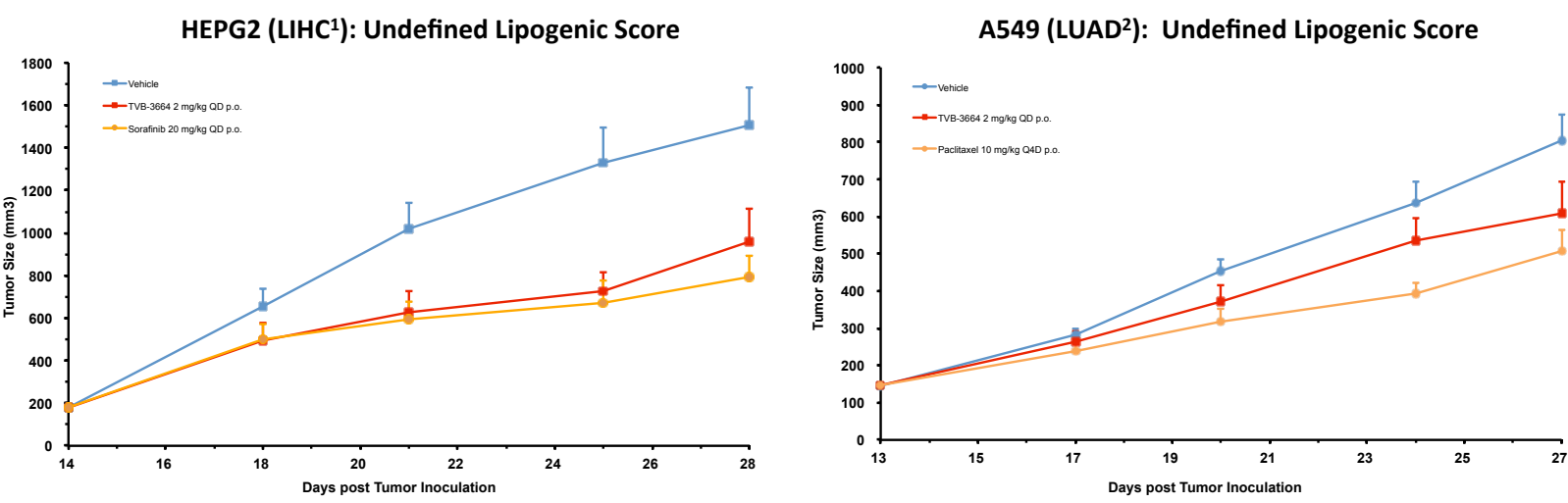
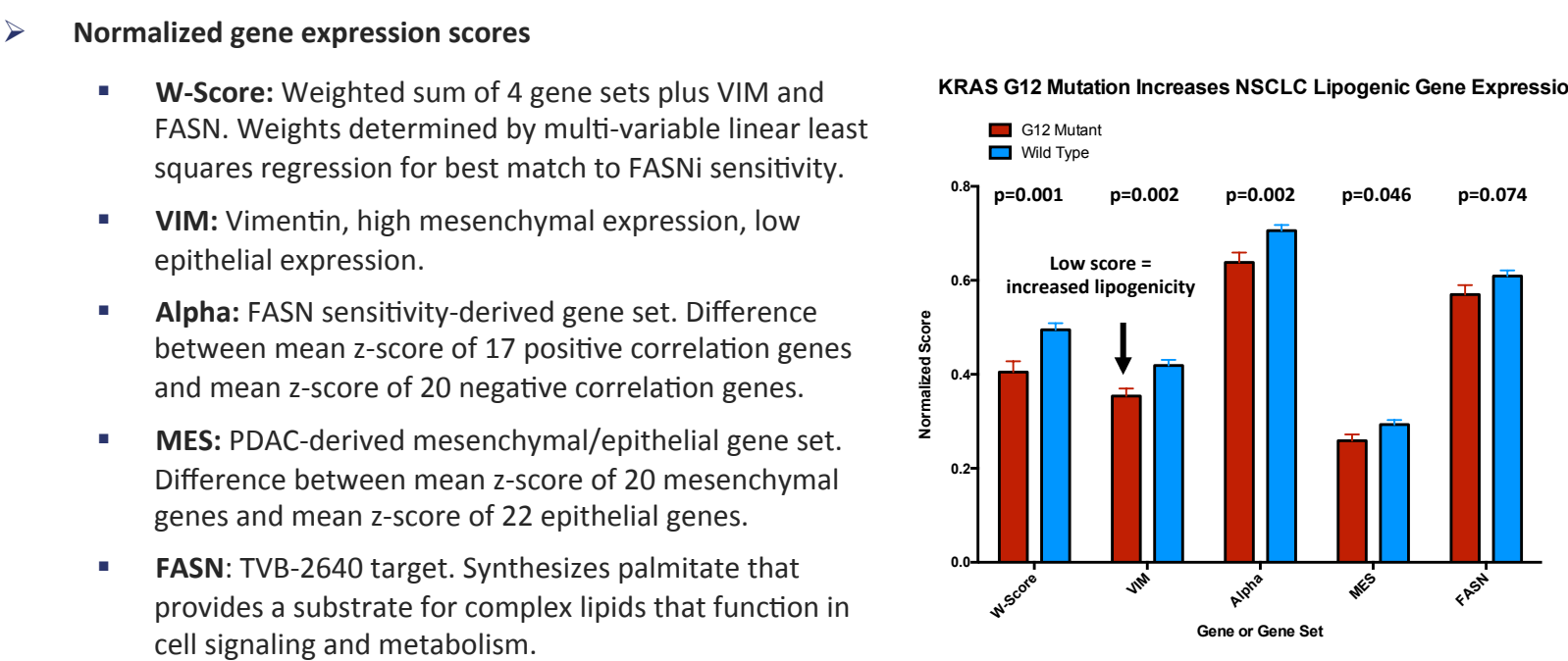


Figure 3. In vivo mouse tumor xenograft efficacy analysis of the HEPG2 liver tumor and A549 NSCLC tumor model. Liver tumors classify as the most highly lipogenic by gene expression analysis (GEA); lung adenocarcinomas are moderately lipogenic by GEA. HEPG2 and A549 tumor cell lines classify as undetermined; thus, not as highly lipogenic as other liver or lung adenocarcinoma tumor cell lines. In-life analysis of tumor growth efficacy was conducted by Crown Biosciences (Santa Clara, CA)

KRAS-Mutant NSCLC Tumors are More Lipogenic



RAS Mutation Associated with FASN Sensitivity in NSCLC Cell Lines

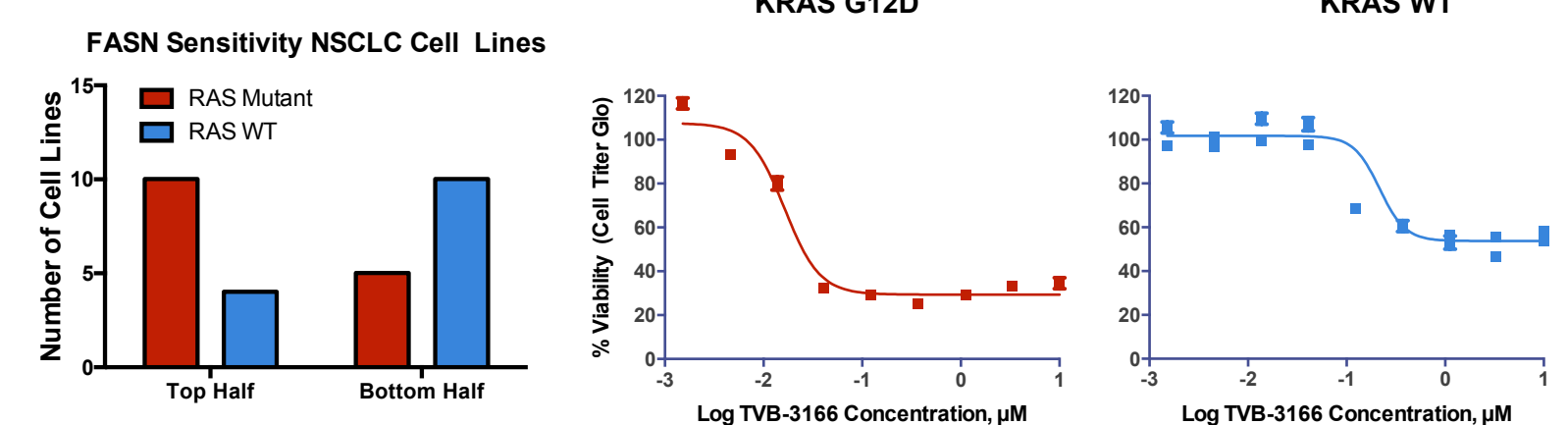
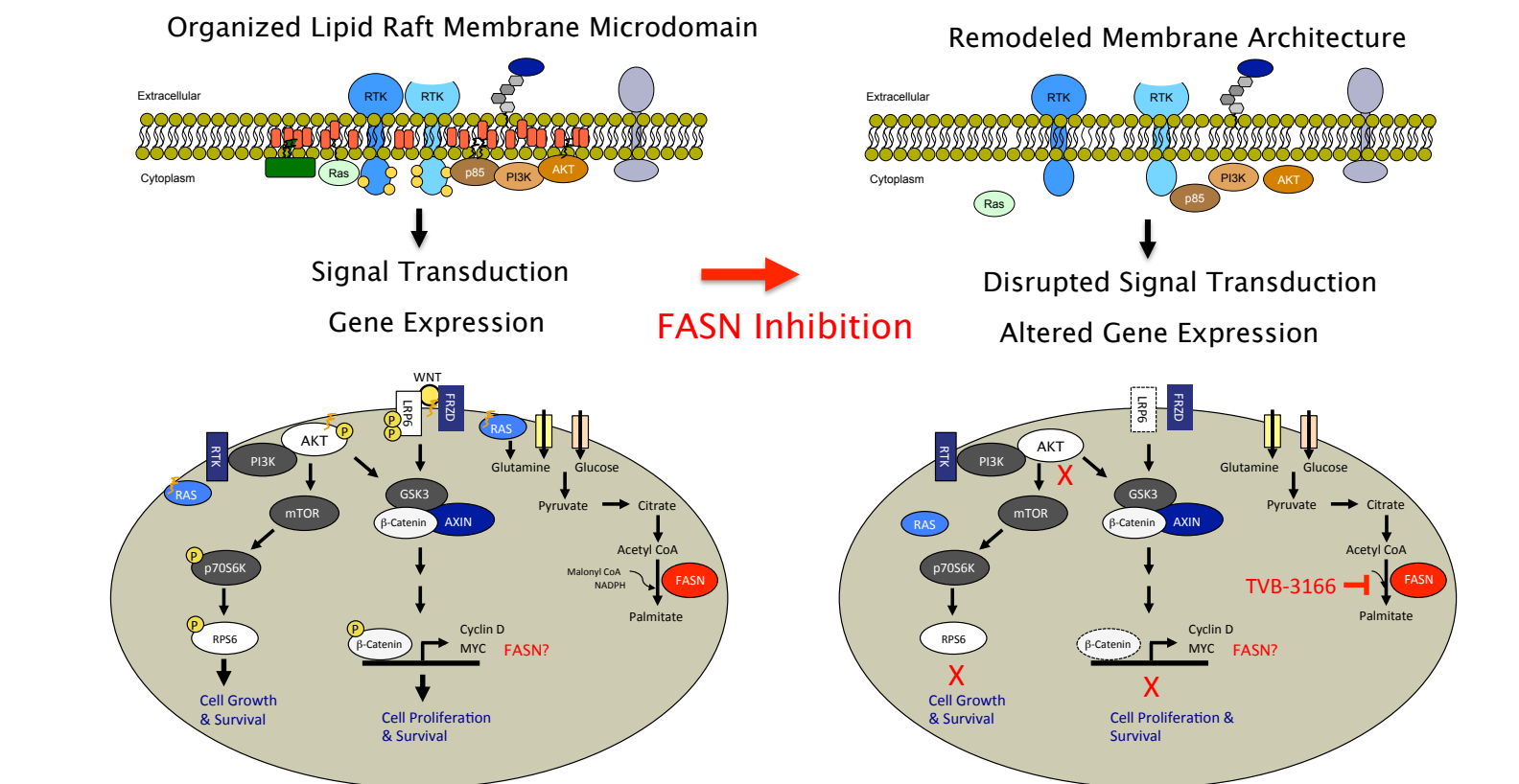


Figure 4. Cell Titer Glo in vitro assay was used to assess cell viability in response to treatment with the FASN inhibitor TVB-3166. Cell were treated for 7 days with TVB-3166.

FASN Inhibition Blocks RAS-Associated Signaling Pathways



Ventura et al. EBioMedicine. 2015 Jul 2;2(8):806-22 PMID: 26425687; 3-V Biosciences.

TVB-3664 Inhibits Increased Palmitoylation of RAS-Associated Signaling Proteins in KRAS-Mutant NSCLC Lines

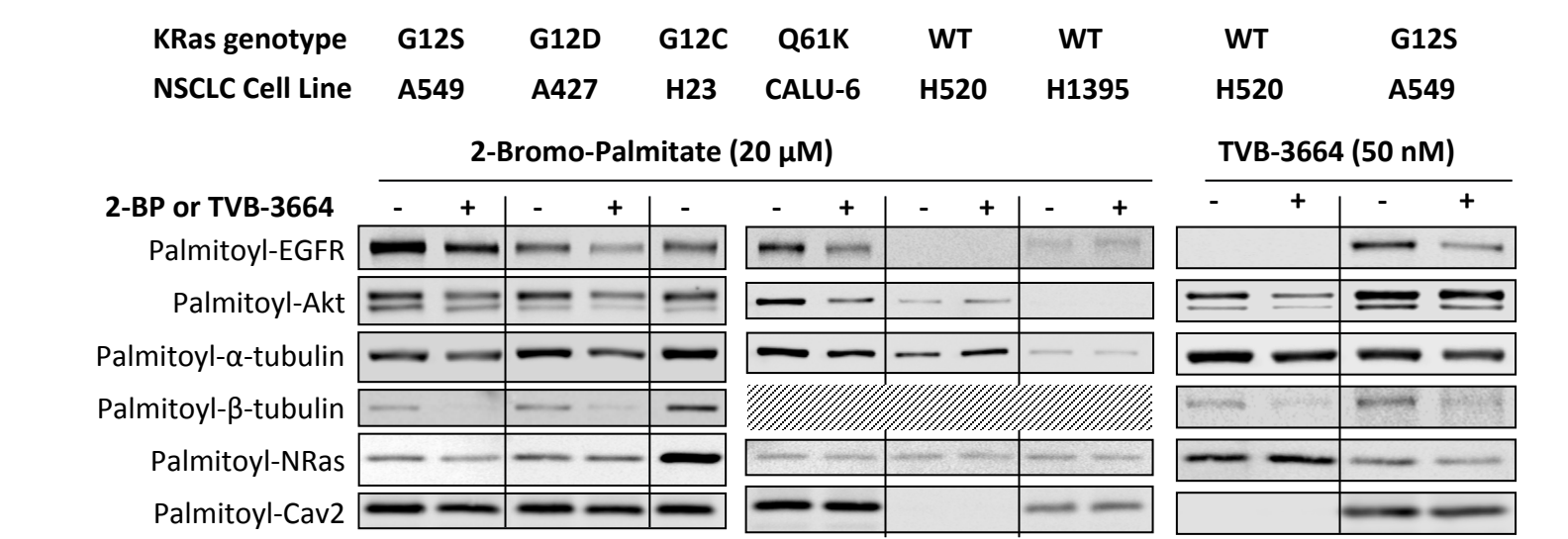


Figure 5. In vitro acyl-biotinyl exchange and Western blot analysis of protein palmitoylation and inhibition by treatment with 2-bromopalmitate or the FASN inhibitor TVB-3664. TVB-3664 treatment for 72 hours. 2-BP treatment for 16 hours.

Oncogenic KRAS Reprograms Tumor Cell Metabolism

- Increased Glutamine metabolism
 - Reductive glutamine metabolism can promote lipid synthesis via pyruvate and NADPH production
- Increased Glucose metabolism
 - Glycolytic pyruvate anabolism can promote lipid synthesis
 - Increased non-oxidative PPP biosynthesis and NADPH production

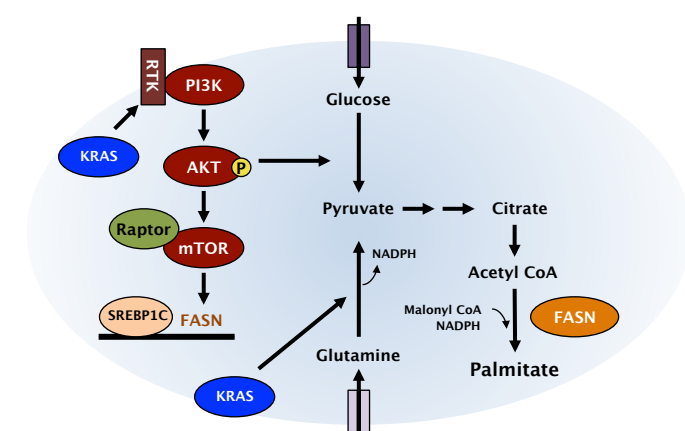


Figure 6. Activated KRAS reprograms tumor cell metabolism to increase utilization of glutamine and glucose. One of the consequences is high levels of pyruvate production. Son et al. 2013 Nature; Ying et al. 2012 Cell. A possible fate of pyruvate is metabolism to citrate and acetyl CoA, the substrate for palmitate synthesis by FASN.

Glutamine Sensitizes KRAS-Mutant Tumor Cells to FASN Inhibition

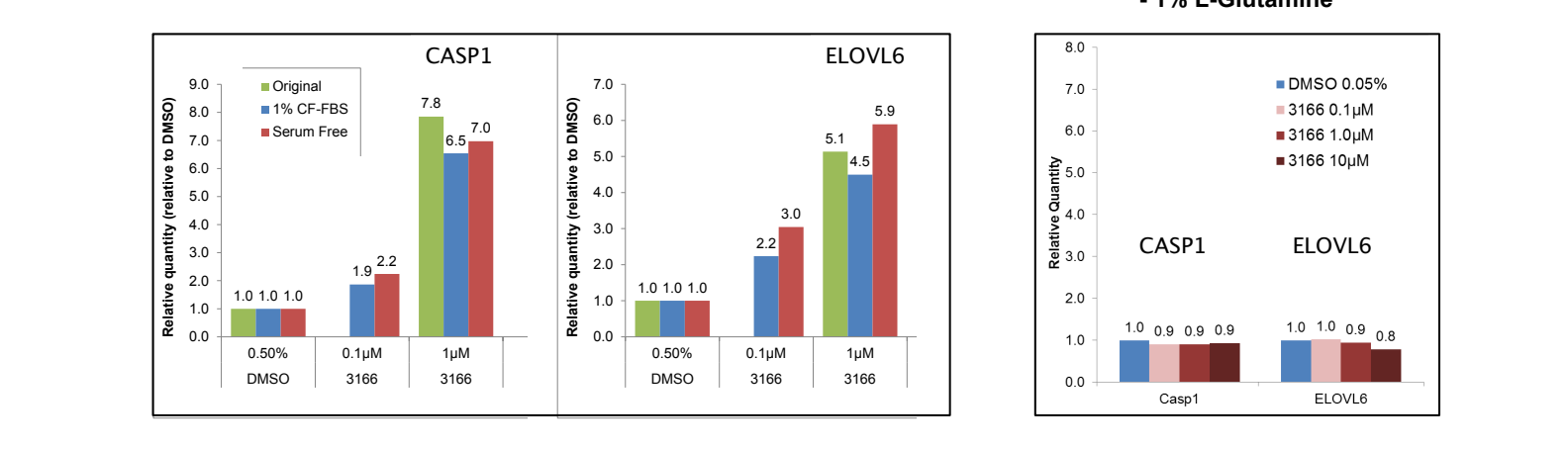


Figure 7. CALU-6 NSCLC cells respond to FASN inhibition in the presence of 1% glutamine but not glutamine-free media. Similar results observed in PANC-1 tumor cells. CASP1 and ELOVL6 gene expression by qPCR are representative of more global gene expression effects.

Combined FASN Inhibition with PD1 Immunotherapy Enhances Xenograft Efficacy

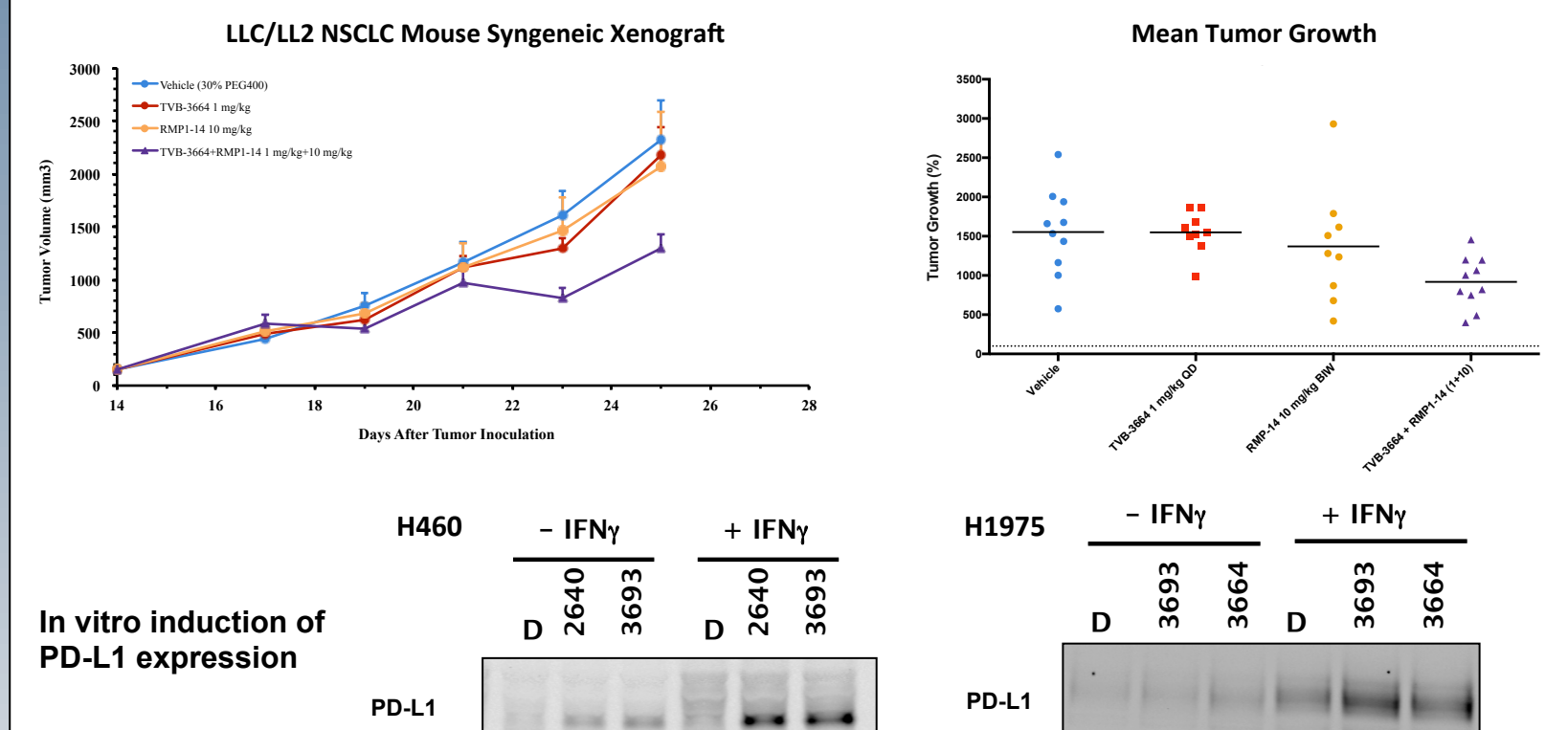


Figure 8. FASN inhibition sensitizes the LLC/LL2 NSCLC mouse tumor model to anti-PD immunotherapy. In vitro, FASN inhibition induces PD-L1 expression in NSCLC tumor cell lines

Combined FASN and Angiogenesis Inhibition Enhances Efficacy

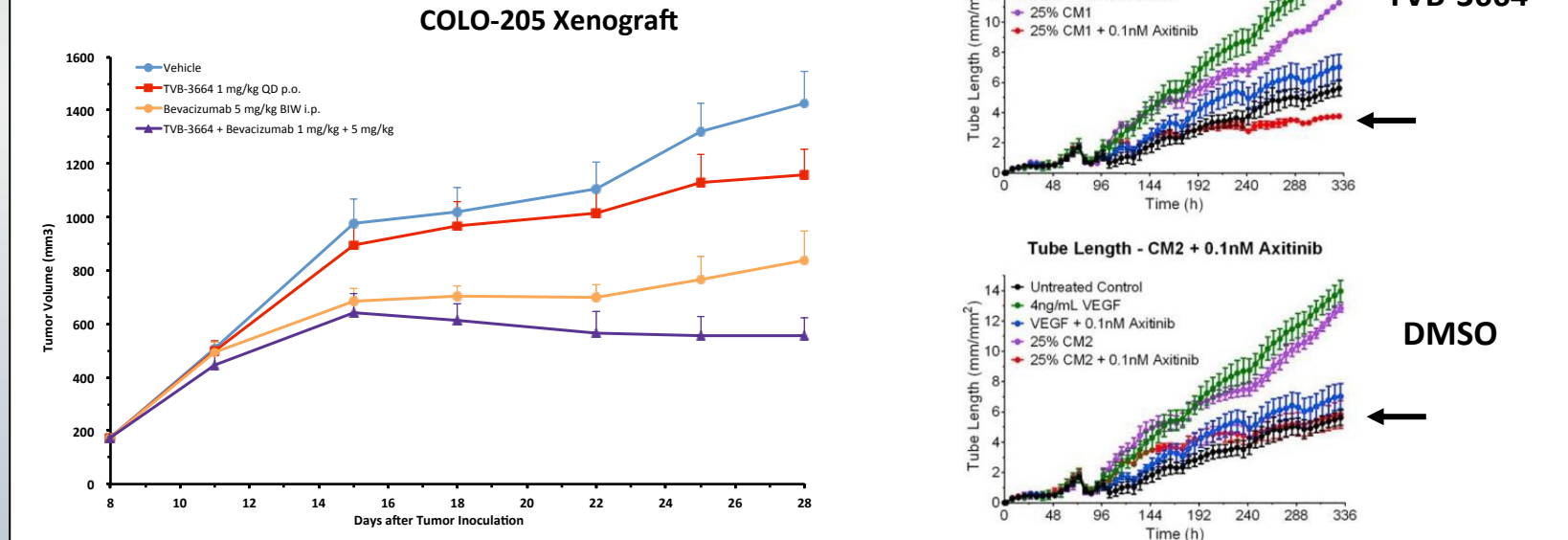
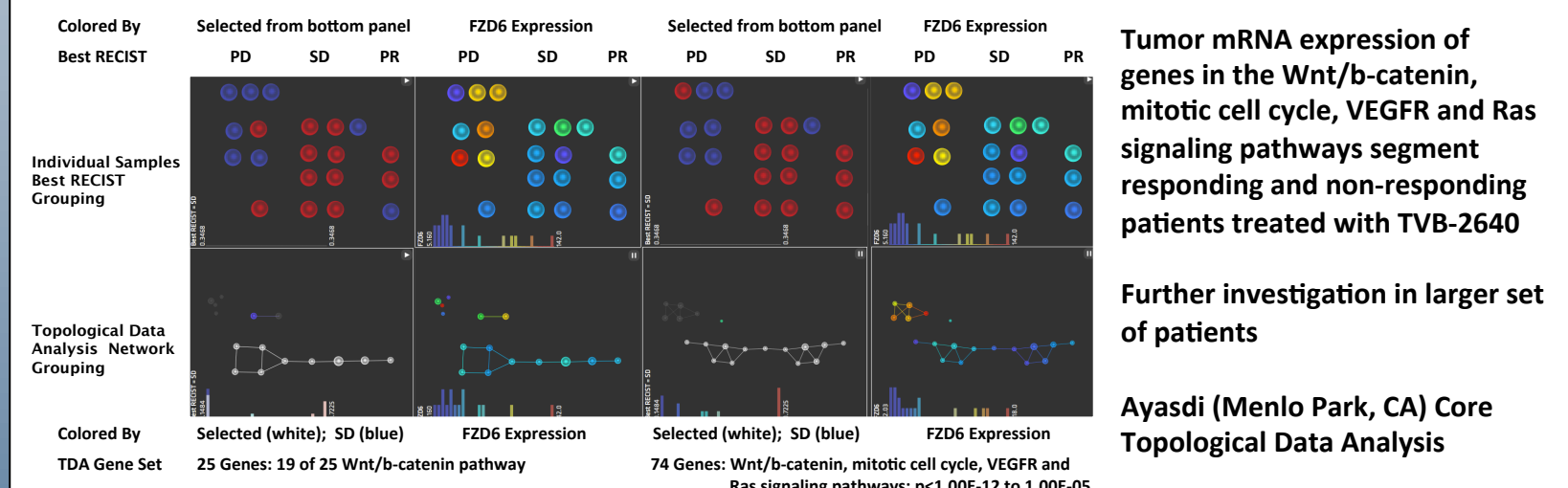


Figure 9. TVB-3664 combined with bevacizumab shows increased tumor growth inhibition compared to single agent treatment. In vitro assays measuring endothelial cell tube formation (Incucyte analysis, Essen biosciences) shows that media conditioned with COLO-205 cells treated with TVB-3664 inhibits tube length in combination with axitinib better than axitinib alone or COLO-205 cells treated with vehicle (DMSO).

TVB-2640 Biomarker Analysis in CLIN-002 Tumor RNA



Conclusions

- Lipogenic gene expression signatures classify FASNi sensitivity
- Lipogenic gene expression is increased in Kras-mutant NSCL tumors; Kras-mutant NSCLC cell lines have increased FASNi sensitivity
- Palmitoylation of Ras-associated signaling proteins is increased in Kras-mutant NSCLC cell lines and is inhibited by FASN inhibition with TVB-3664
- FASN inhibition combines with PD1 immunotherapy or VEGF inhibition (bevacizumab) to increase significantly in vivo tumor xenograft efficacy
- TVB-2640 CLIN-002 tumor mRNA shows b-catenin, mitotic cell cycle, VEGFR and Ras pathway dysregulation in responsive, not unresponsive, patients