Mechanism and rationale for inhibition of FASN as a novel therapy for KRAS-mutant non-small-cell lung cancer patients

Tim Heuer

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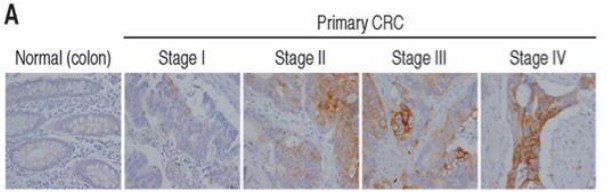
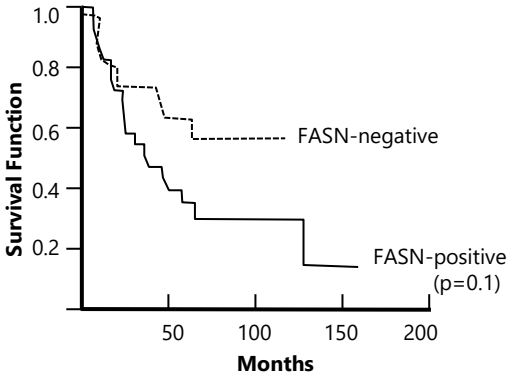
FASN Inhibitor Drug Development

- **FASN has long been a target of interest for drug development**
  - FASN levels are increased in later stage disease
  - High levels of tumor FASN correlates with poor prognosis
  - FASN inhibition is highly selective for tumor cells - tumor cell apoptosis occurs but leaves normal cells unharmed
  - Early attempts at FASN inhibitors did not advance to the clinic due to poor selectivity resulting in significant off-target toxicities



FASN Expression Correlates with Poorer Survival in NSCLC Patients



- **3-V Bio Creates TVB-2640: A first-in-class next generation FASN inhibitor**
  - Oral, potent, highly bioavailable and reversible inhibitor of FASN
  - Selective for FASN - no off target toxicities (such as weight loss) observed at therapeutic doses
  - Other FASN inhibitors are preclinical (GSK, Infinity, Janssen)

Zaytseva et al. CR. 2012
Visca, et al, Anticancer Res, 2004

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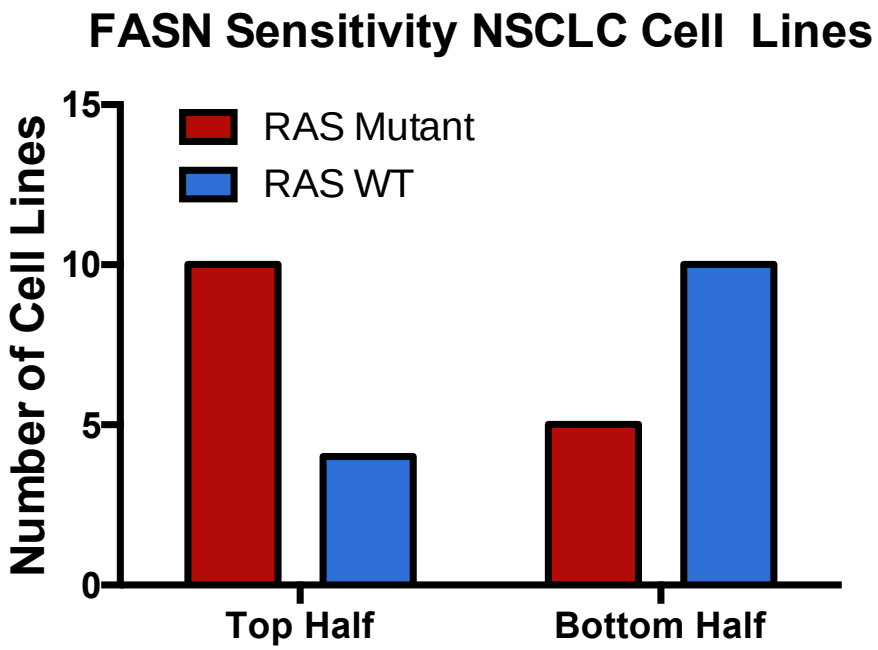
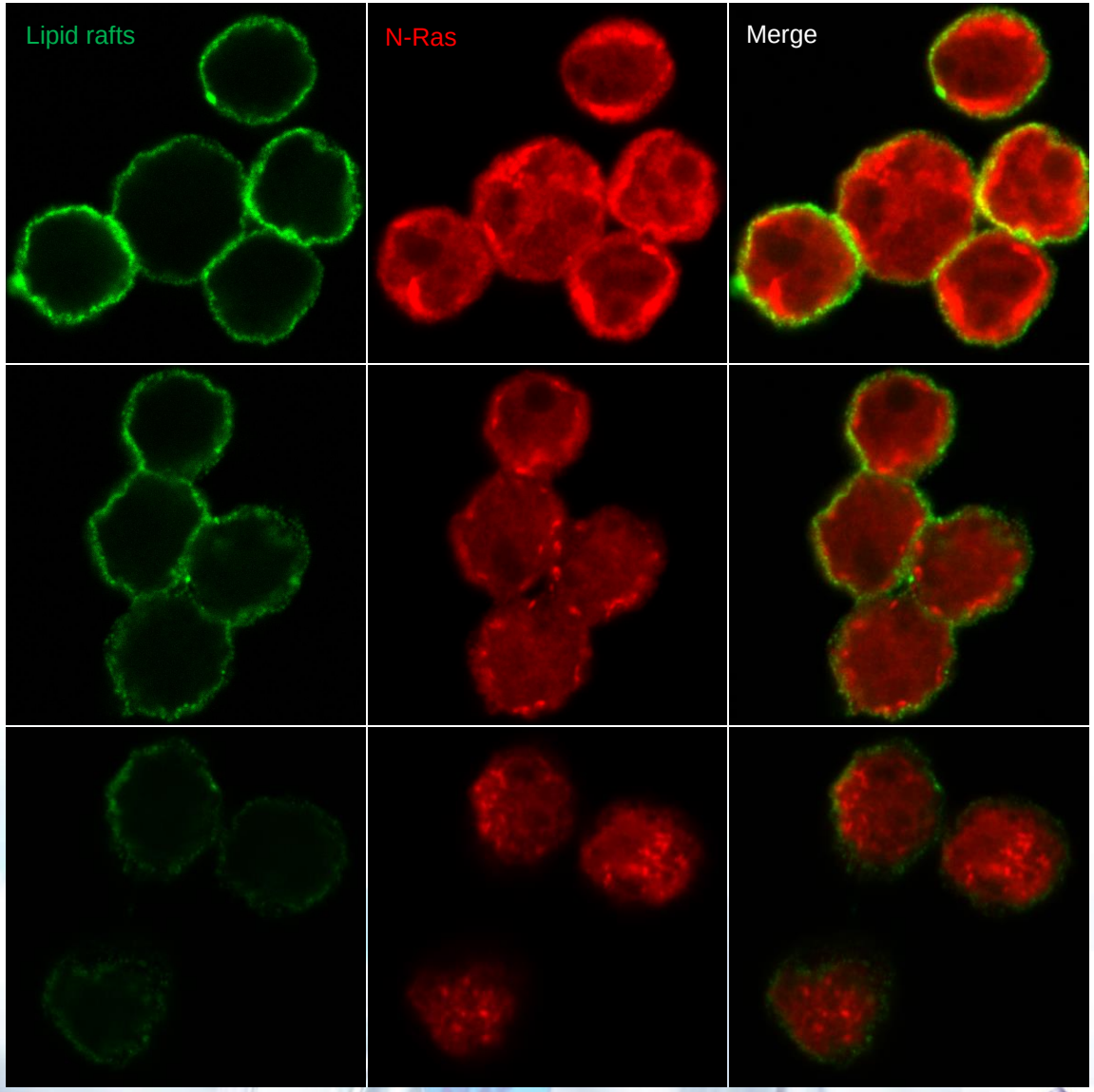
RAS Mutation Associates with FASN Sensitivity in NSCLC Cell Lines



FASN inhibitor-sensitive NSCLC cell lines enriched for mutant KRAS

FASN Inhibition Disrupts Tumor Cell Lipid Rafts, Ras Localization



COLO-205

DMSO

Rafts: High density
NRas: Membrane-associated

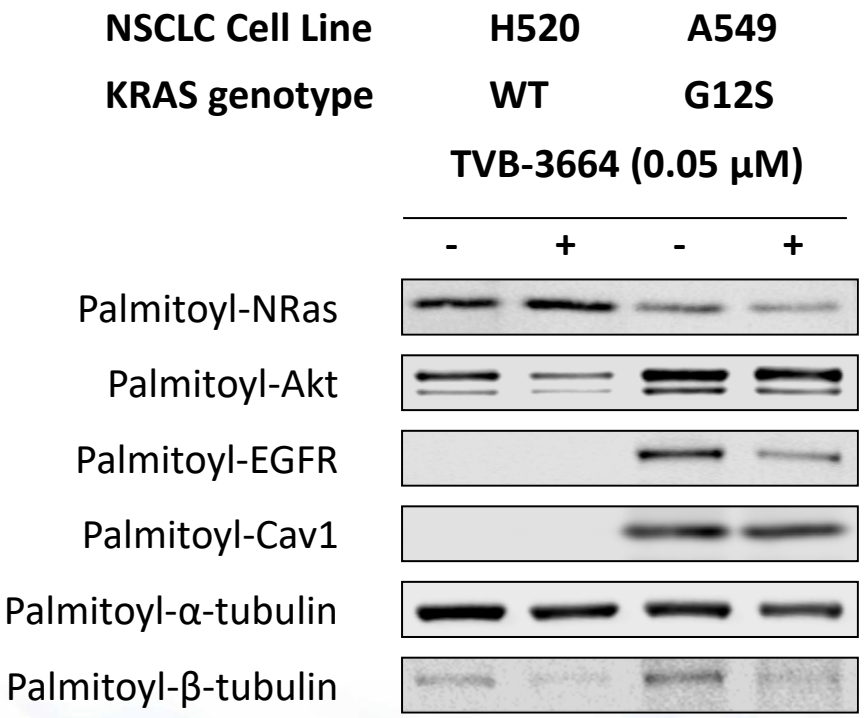
TVB-3166
0.1 µM

Rafts: Decreased density
NRas: Punctate, intermittent

TVB-3166
1 µM

Rafts: Very low intensity
NRas: Cytoplasmic

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

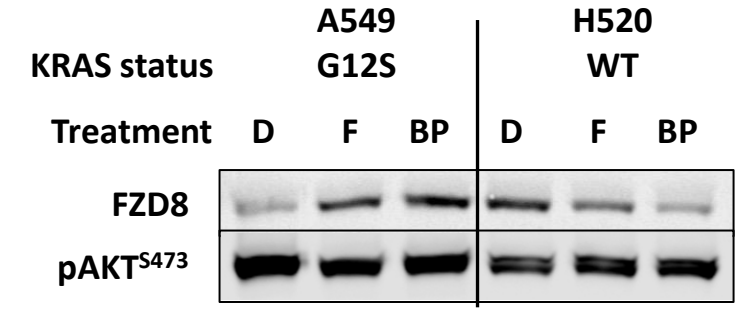


- KRAS activating mutation increases protein palmitoylation
- FASN inhibition diminishes palmitoylation of oncogenic proteins

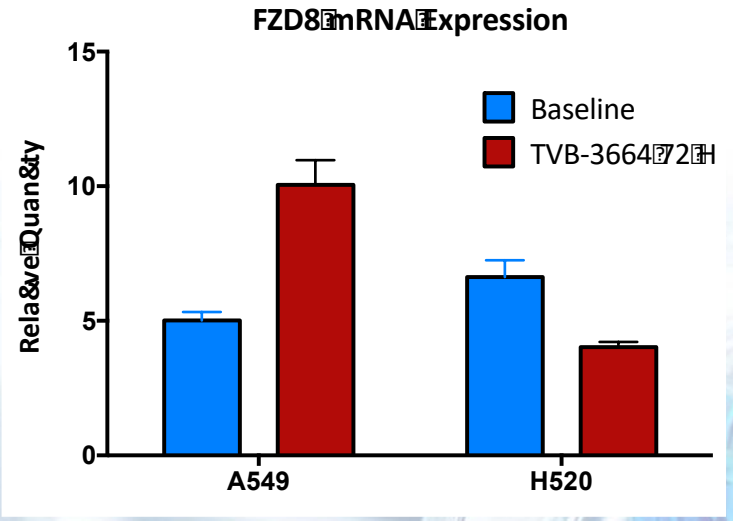
R. Ventura

FASN Inhibition Inhibits Wnt Signaling in KRAS Mutant NSCLC Cell Lines

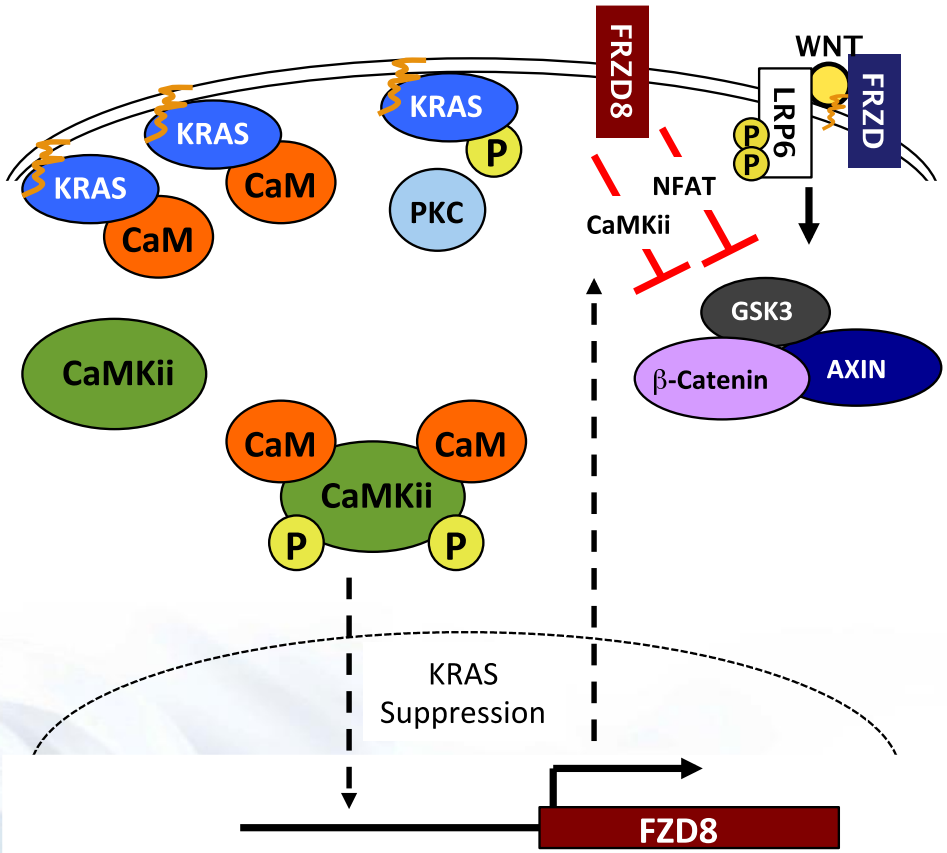
D: DMSO
F: TVB-3664 (0.05 µM)
BP: 2-Bromopalmitate (20 µM)



	A549			H520		
FZD8	100	173	231	100	62	40
pAKT(S473)	100	56	69	100	116	125



KRAS → FZD8 regulate Wnt/β-catenin signaling



Wang et al 2015, *Cell*

R. Ventura, C. Rubio

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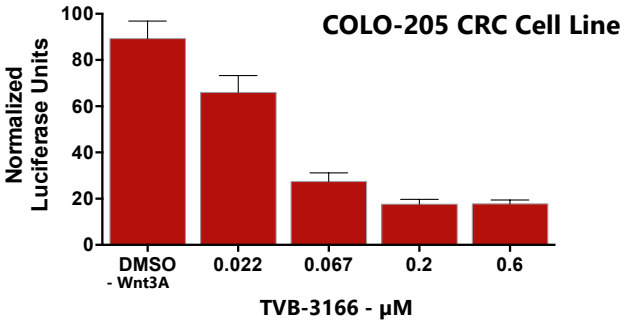
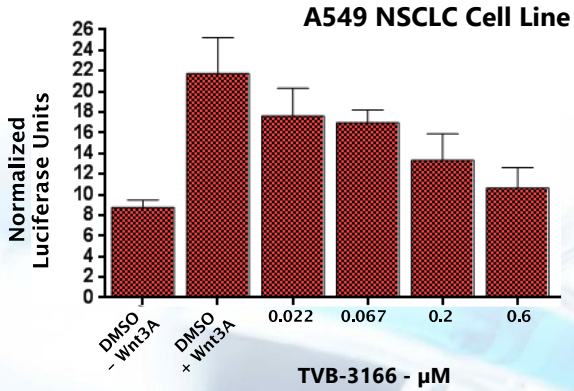
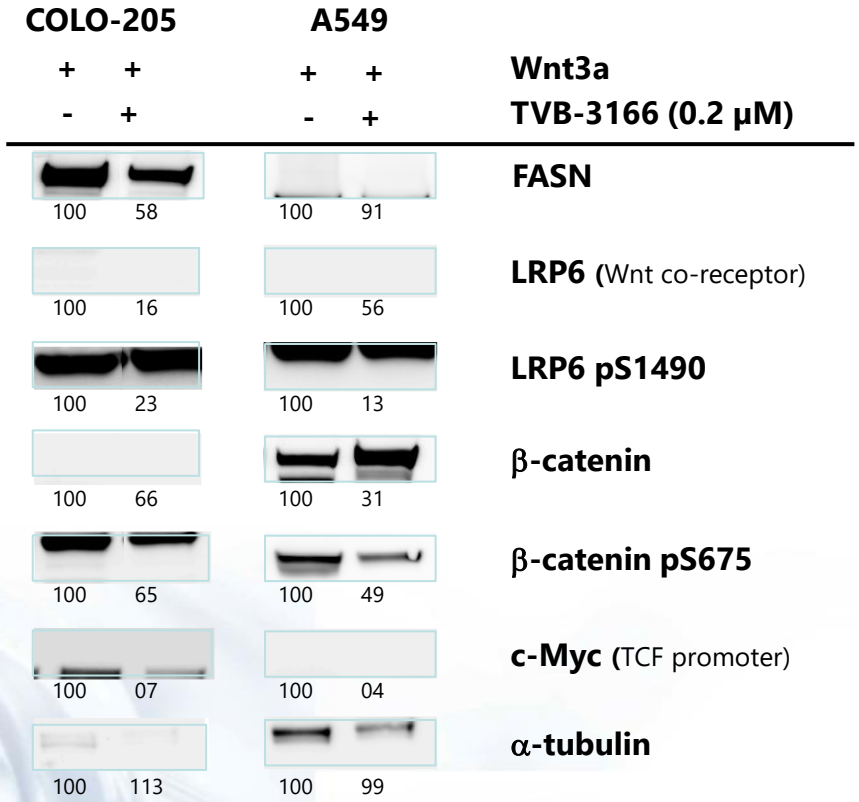
FASN Inhibition Blocks β-catenin Pathway Activity and Myc Expression

TCF Promoter Luciferase Expression





Pathway Signal Transduction and Wnt-Responsive Protein Expression



K. Mordec

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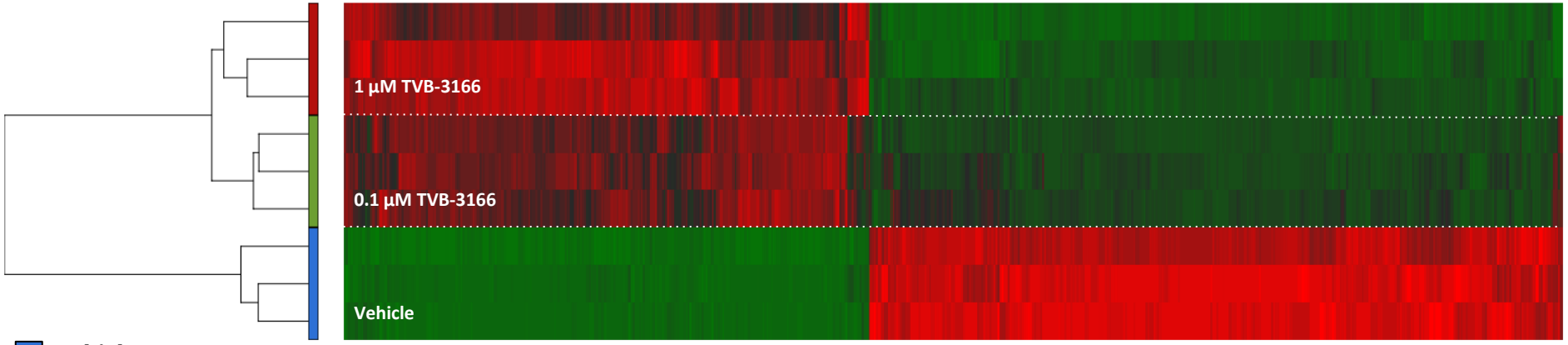
FASN Inhibition Modulates Gene Expression in Metabolism, Proliferation and Survival Pathways

22Rv1 Cells



Vehicle

0.1 µM TVB-3166

1 µM TVB-3166

Up-Regulated Pathways (p<0.01)

- Lipid, Sterol, Pyruvate, Amino Acid Metabolism
- P53 Signaling

Down-Regulated Pathways (p<0.01)

- DNA Replication
- DNA Repair (base excision and mismatch)
- Cell Cycle
- PI3K-AKT Signaling

TVB-3166 vs Vehicle (DMSO) Analysis

- p<0.01
- ≥1.6 Fold Change
- 1304 Genes
- Dose Dependent Gene Expression Changes

Expression Scale

Hierarchical Clustering

-2 **+2**

Gene expression changes following FASN inhibition are glutamine dependent in KRAS-mutant tumor cells

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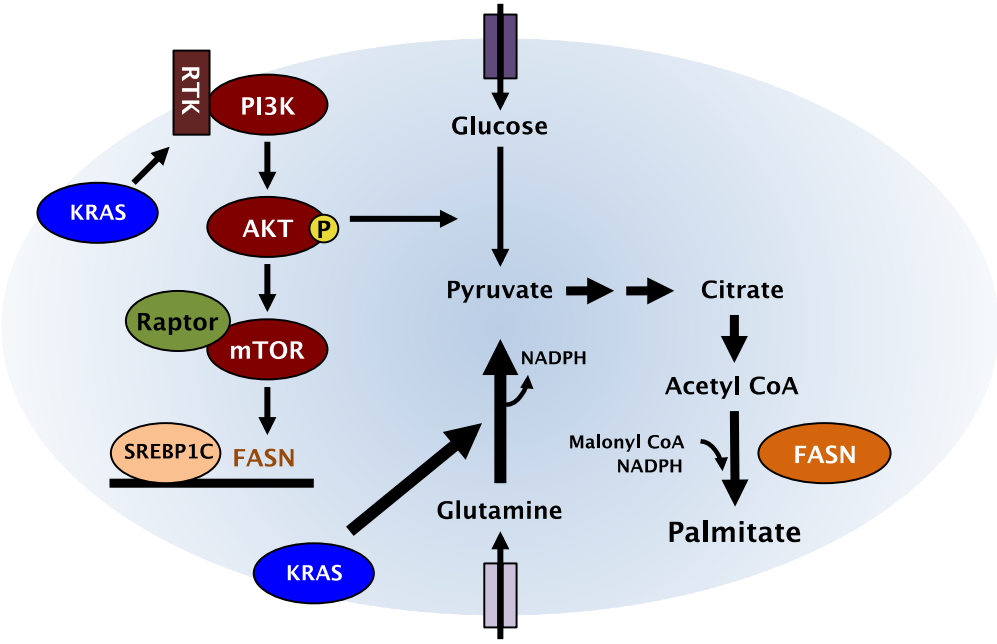
Oncogenic KRAS Reprograms Tumor Cell Metabolism

- **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



- Glutamine enhances FASN sensitivity in KRAS-mutant tumor cell lines
- Model: glutamine and glucose metabolism products enable increased FASN activity in KRAS-mutant tumors

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.

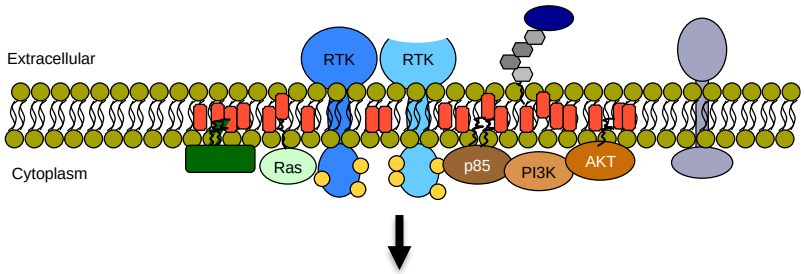
FASNi Mechanism of Action in KRAS-Mutant NSCLC

FASN inhibition disrupts multiple Ras-driven effects on tumor cell biology and phenotype including:

- **Increased palmitoylation of Ras-associated signaling proteins**
  - Examples include NRas, EGFR, Akt, Cav1
- **Ras-associated pathways involved in tumor cell growth, proliferation, and survival**
  - Lipid raft architecture and membrane-associated protein localization is disrupted, e.g. NRas
  - Akt/mTor and b-catenin pathway signal transduction is inhibited
  - Gene expression of metabolic and non-metabolic biological pathways
- **Canonical Wnt/β-catenin signaling – via KRAS-mediated suppression of non-canonical Wnt signaling**
  - FZD8 expression decreased in KRAS mutant cells, induced by FASN inhibition
  - RNA and protein expression of beta-catenin-regulated genes is diminished, e.g. Myc
- **Glutamine and glucose metabolism reprogramming, increased FASN activity**
  - Glutamine enhances FASN sensitivity of KRAS mutant tumor cell lines
  - FASN inhibition blocks lipid synthesis and interferes with tumor cell metabolic requirements

FASN Inhibition Mechanism of Action Model

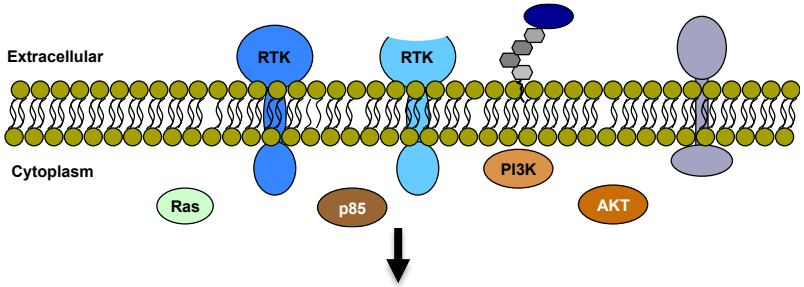
Organized Lipid Raft Membrane Microdomain



Signal Transduction

Gene Expression

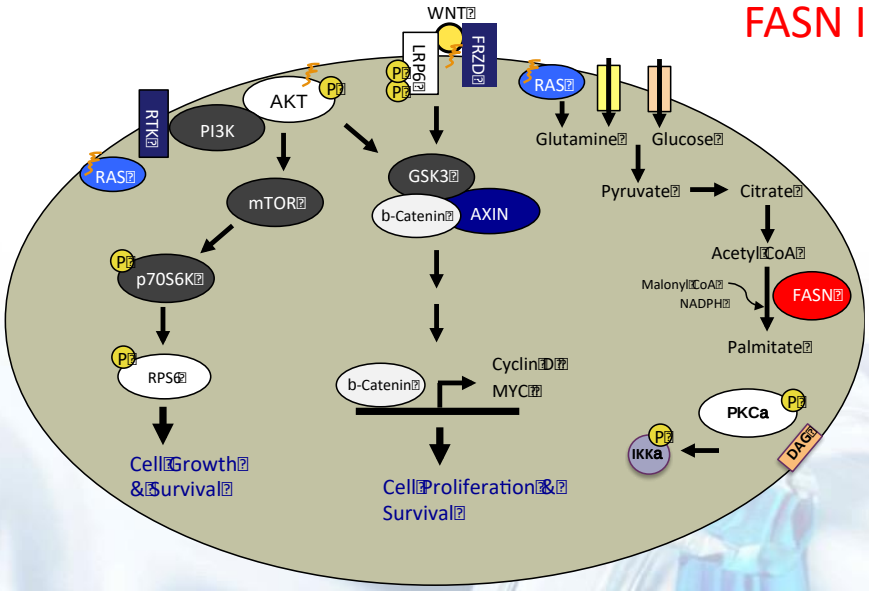
Remodeled Membrane Architecture

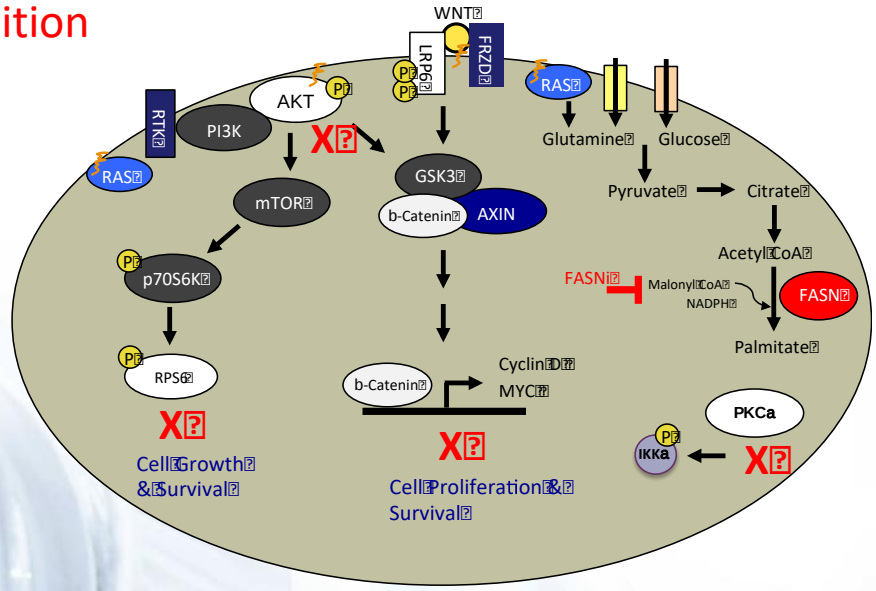


Disrupted Signal Transduction

Altered Gene Expression

FASN Inhibition





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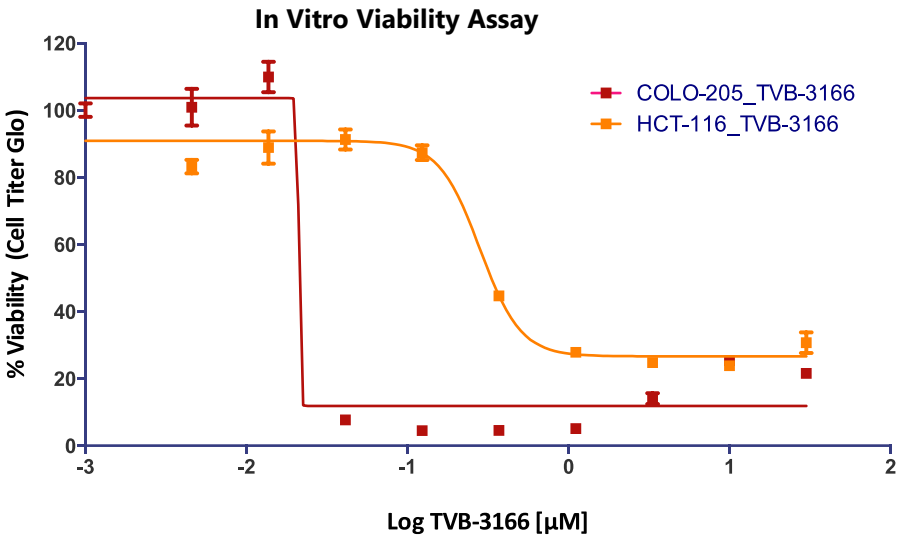
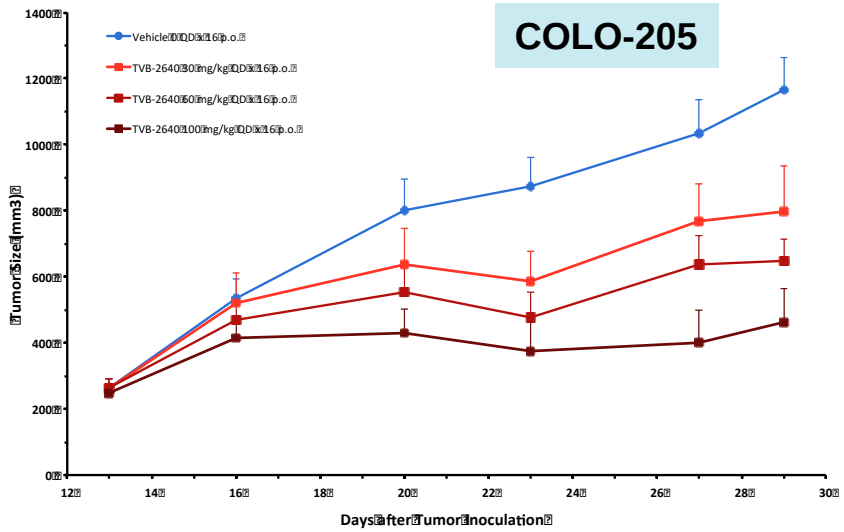
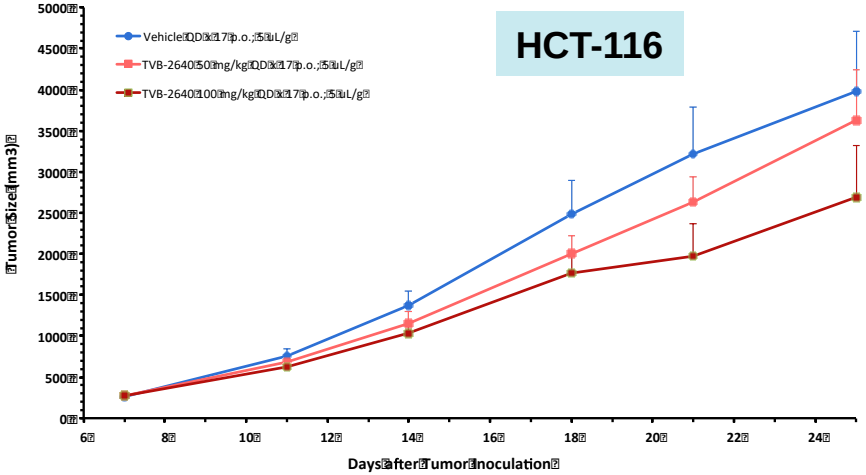
FASN Biomarker Discovery

- Diverse molecular and cell biology approach
- Utilize literature and current knowledge to investigate association between marker candidates and FASNi sensitivity
  - e.g. FASN expression in tumor or plasma/serum
- Develop preclinical in vitro and in vivo assays that enable the discovery of pathways, genes, and markers that:
  - Change in response to FASN inhibition (pharmacodynamic marker)
  - Predict sensitivity to FASN inhibition (prognostic marker)
- IHC, ELISA, DNA and RNA Sequencing, Western, mass spectrometry
- Example: Integrated analysis of gene expression and lipid/metabolite changes in response to FASN inhibition

TVB-2640 Inhibition of Rat Xenograft Tumor Growth Aligns with In Vitro Cell Line Sensitivity







COLO-205 CRC

- BRAF V600E mutant
- APC LOF

Treatment	TGI D17	p vs Veh
• Vehicle		
• TVB-2640 30 mg/kg	46%	0.0039
• TVB-2640 60 mg/kg	58%	0.0001
• TVB-2640 100 mg/kg	74%	0.0021

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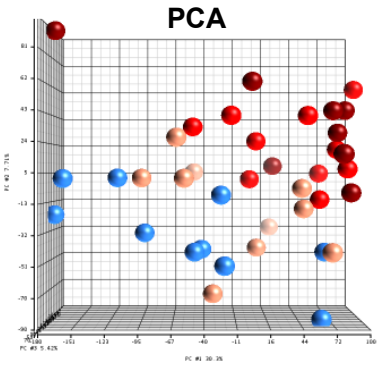
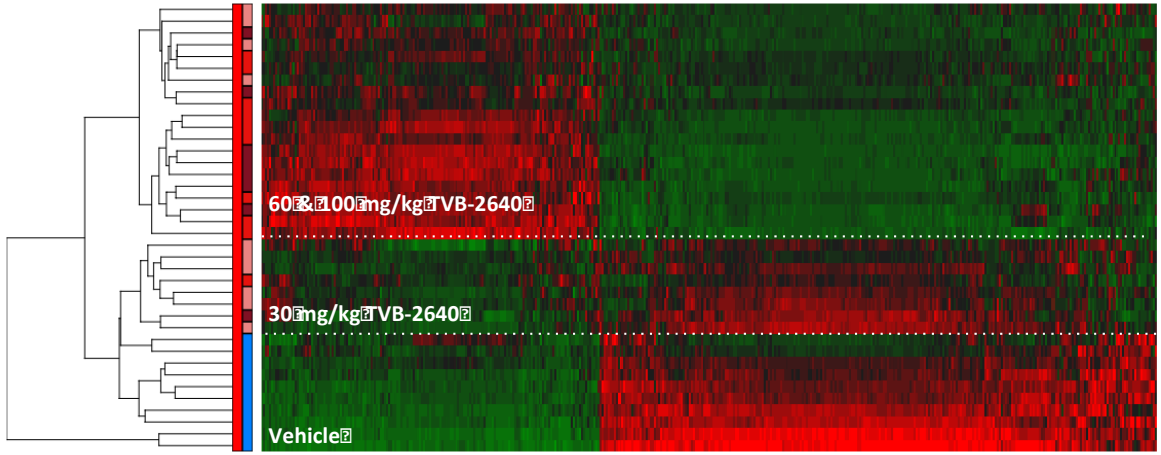
TVB-2640 Induces Gene Expression Changes in Metabolic, Growth, Proliferation, and Survival Pathways

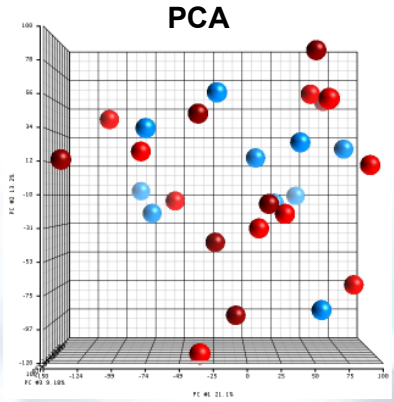


COLO-205

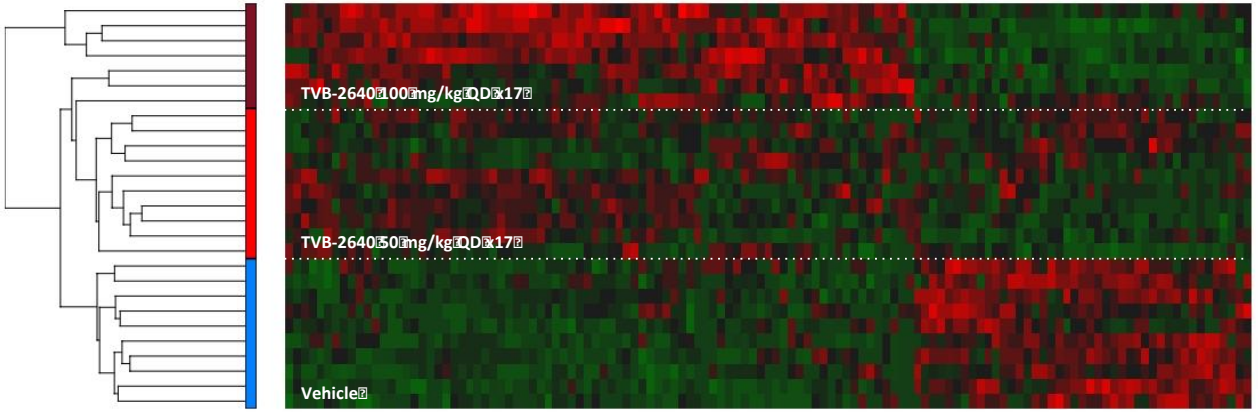


477 genes

- **p<0.001**
- **≥1.5 mean fold change in 60 and 100 mg/kg TVB-2640 dose groups**



HCT-116



123 genes

- **p<0.005**
- **≥1.5 mean fold change in 100 mg/kg TVB-2640 dose group**

- **QD x 17 dosing**
- **2 hours post last dose**

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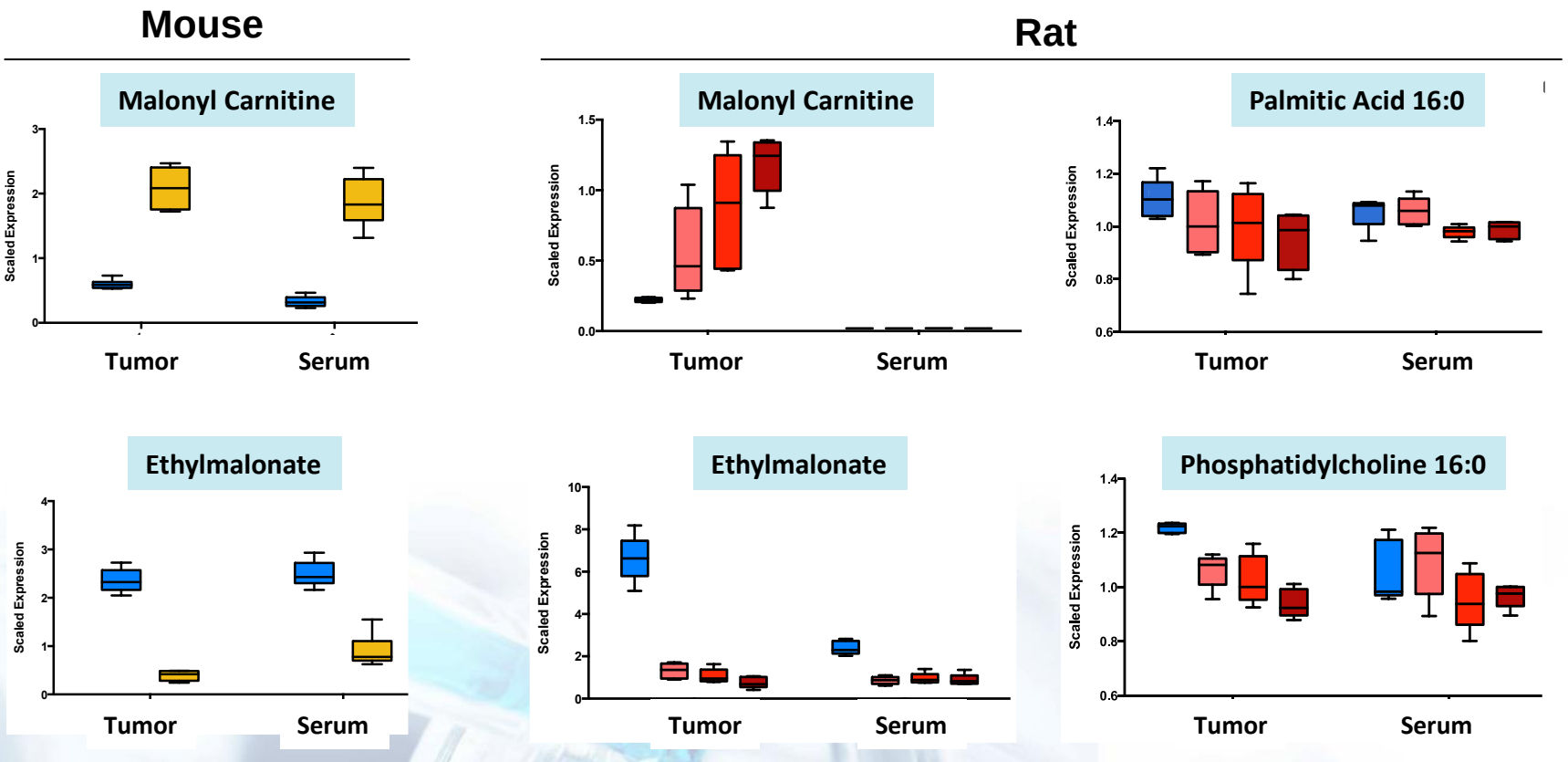
TVB-2640 Induces a Metabolic Signature of FASN Inhibition in COLO-205 Xenograft Tumors

- Elevated malonyl carnitine, decreased palmitic acid, and altered beta-oxidation among significant changes observed by metabolomic profiling
  - Concerted changes in gene expression



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TVB-2640 Phase 1 Study

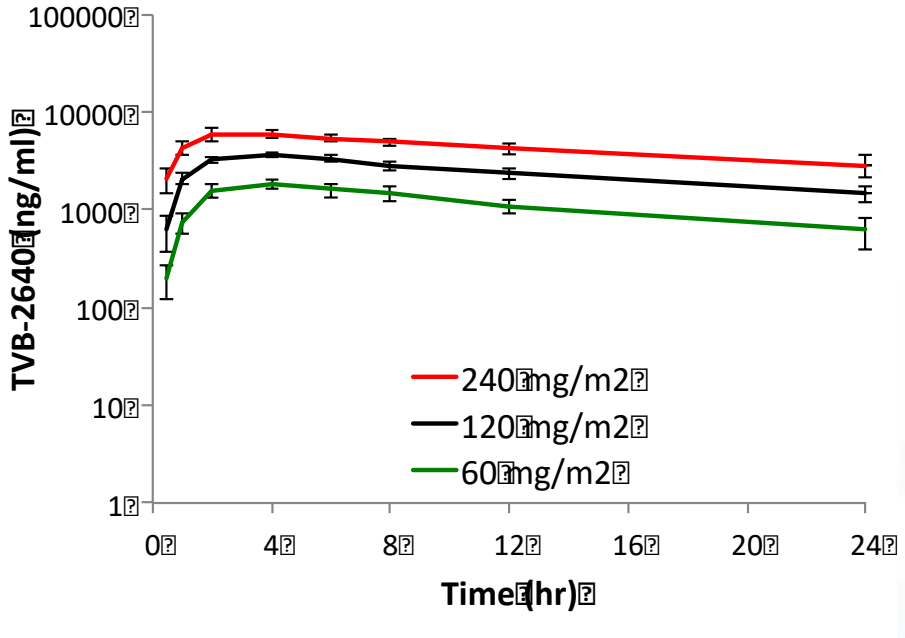
- **Standard Phase I Design**
  - Oral, once daily dosing
  - Solid tumors
- **Primary Objectives:**
  - Safety, MTD, recommended Phase-2 dose
- **MTD identified, currently in expansion cohorts**

TVB-2640: Oral, Potent, Once Daily FASN Inhibitor with Excellent Human Exposure

- **TVB-2640 FASN inhibitor**
  - First in class
  - Potent
  - Highly selective
  - Reversible
- **Pharmacokinetics in human**
  - Plasma levels increase with dose
  - Mean half-life ~15 hours
  - Steady state by day 8
  - Exceeds preclinical efficacy threshold at all doses

Dose Escalation (n=30)
TVB-2640 Monotherapy

- **Evaluated doses from 60-240mg/m^2 including flat dosing**
- **6 DLTs*:**
  - **3 Skin @ 120mg/m^2 & 240mg/m^2**
  - **3 Eye @ 240mg/m^2 and 250mg**



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TVB-2640 Safety Summary

- Reversible DLTs (Hand/Foot Syndrome, Eye Toxicity)
- Manageable RP2D skin and eye toxicities: typically ≤ Grade 2
- Onset of target-related AE's typically >14-21 days of dosing

Most Common (Incidence >10%) **TEAEs (across all doses)**	**TVB-2640 Monotherapy (n = 53)**	
	Any Incidence n (%)	Grade 3/4 n (%)
Alopecia	30 (57)	1 (2)
Eye disorders	26 (49)	3 (6)
Palmar-plantar erythrodysesthesia syndrome	20 (38)	4 (8)
Decreased appetite	7 (13)	0
Diarrhoea	9 (17)	0
Nausea	9 (17)	0
Asthenia	4 (8)	0
Dry skin	11 (21)	0
Vomiting	4 (8)	0
Abdominal pain	13 (25)	0
Dehydration	1(2)	0
Skin exfoliation	8 (15)	0
Constipation	1 (2)	0
Pyrexia	2 (4)	1 (2)
Urinary tract infection	5 (11)	1 (2)

TVB-2640 Pharmacodynamic Activity in Patient Tumor and Serum

Tumor RNA Sequencing

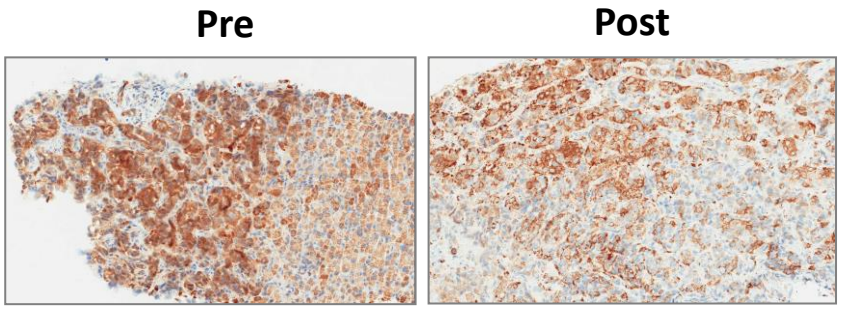
Tumor tissue changes much greater than normal tissue changes

Serum Metabolomics

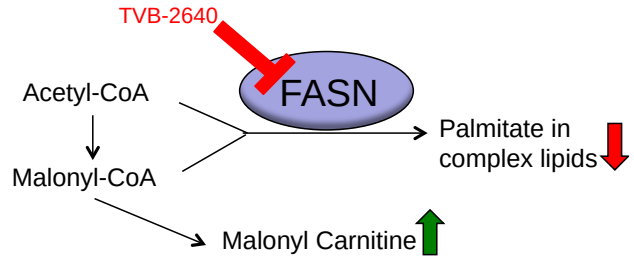
Increased malonyl carnitine

Decreased palmitate-containing lipids

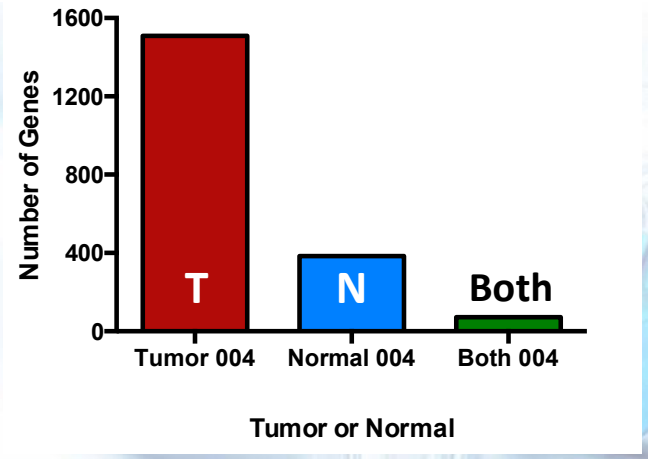
PIK3CA mutant BCa, pAKT S473 IHC

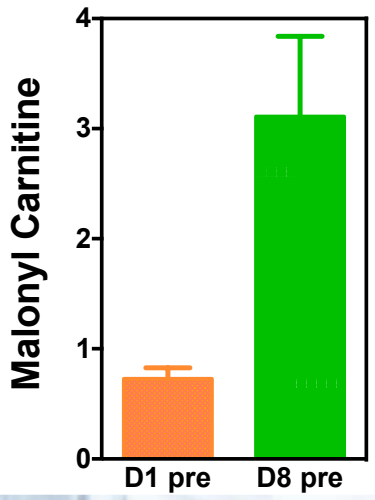


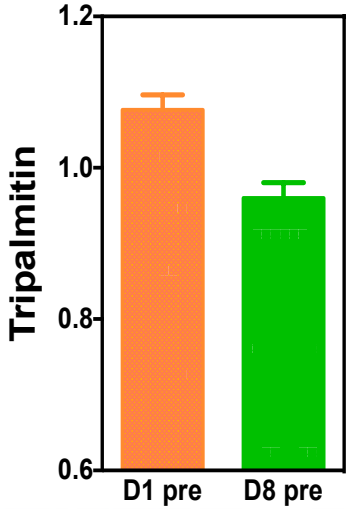
50% inhibition



mRNA: 5-fold change post TVB-2640







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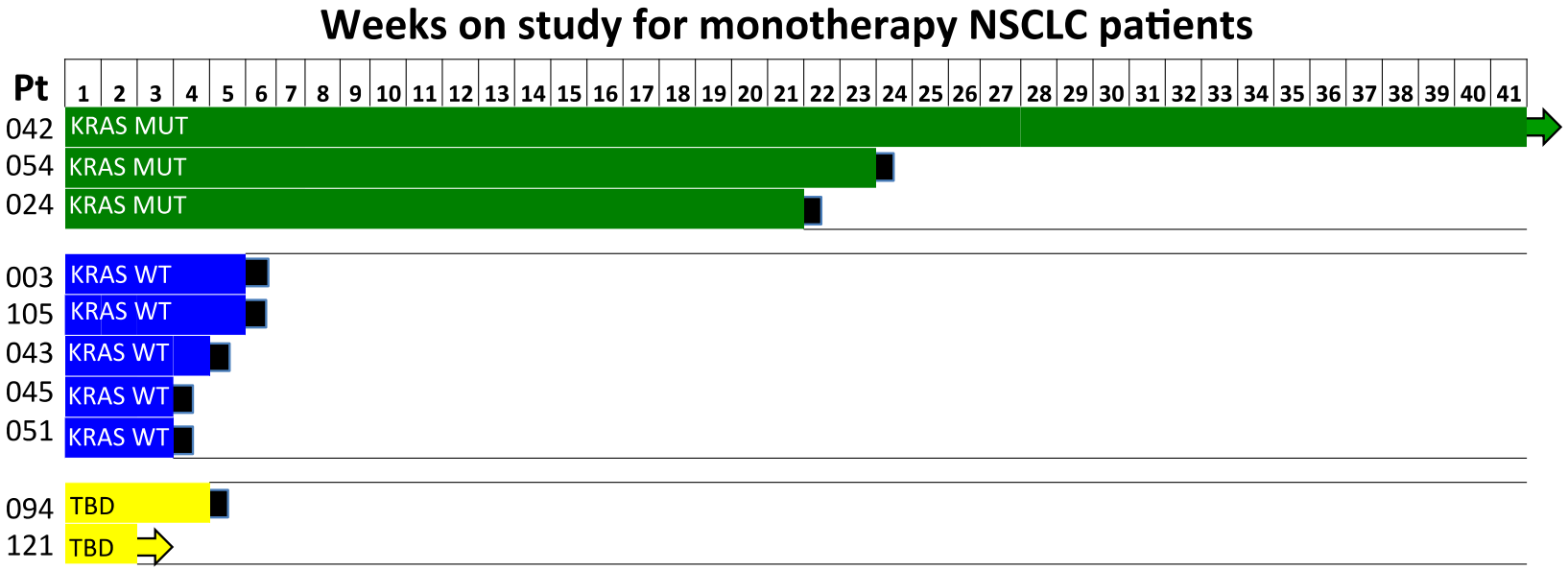
NSCLC Patients with Mutant KRAS Remain On Study Longer



ASCO 2016

- 10 NSCLC patients enrolled on TVB-2640 monotherapy
  - 3 Kras-mutant
  - 5 Kras-wild-type
  - 2 unknown Kras status
- 100% Kras-mutant patients on study > 12 weeks
  - 0% KRAS-wild-type
- Similar plasma TVB-2640 exposure across mutant and wild-type patients

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Summary and Conclusions

- KRAS activating mutations in NSCLC associate with sensitivity to FASN inhibition

- Preclinical studies identified multiple mechanisms of action for FASN inhibition efficacy that include:
  - Membrane and lipid raft architecture remodeling
  - Tumor cell gene expression reprogramming
  - Inhibition of c-Myc expression and AKT phosphorylation associates with preclinical in vitro and in vivo FASN sensitivity

- Preclinical and translational studies identified clinical PD markers and putative prognostic biomarkers
  - Malonyl carnitine, tripalmitin, saturated triglycerides
  - KRAS activating mutations, β-catenin expression/signaling

Acknowledgements

- 3-V Biosciences Scientific and Management Teams

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