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Applying the QSP Model NAFLDsym® to Predict and Understand NASH Treatments

April 24, 2018

Scott Q Siler, CSO

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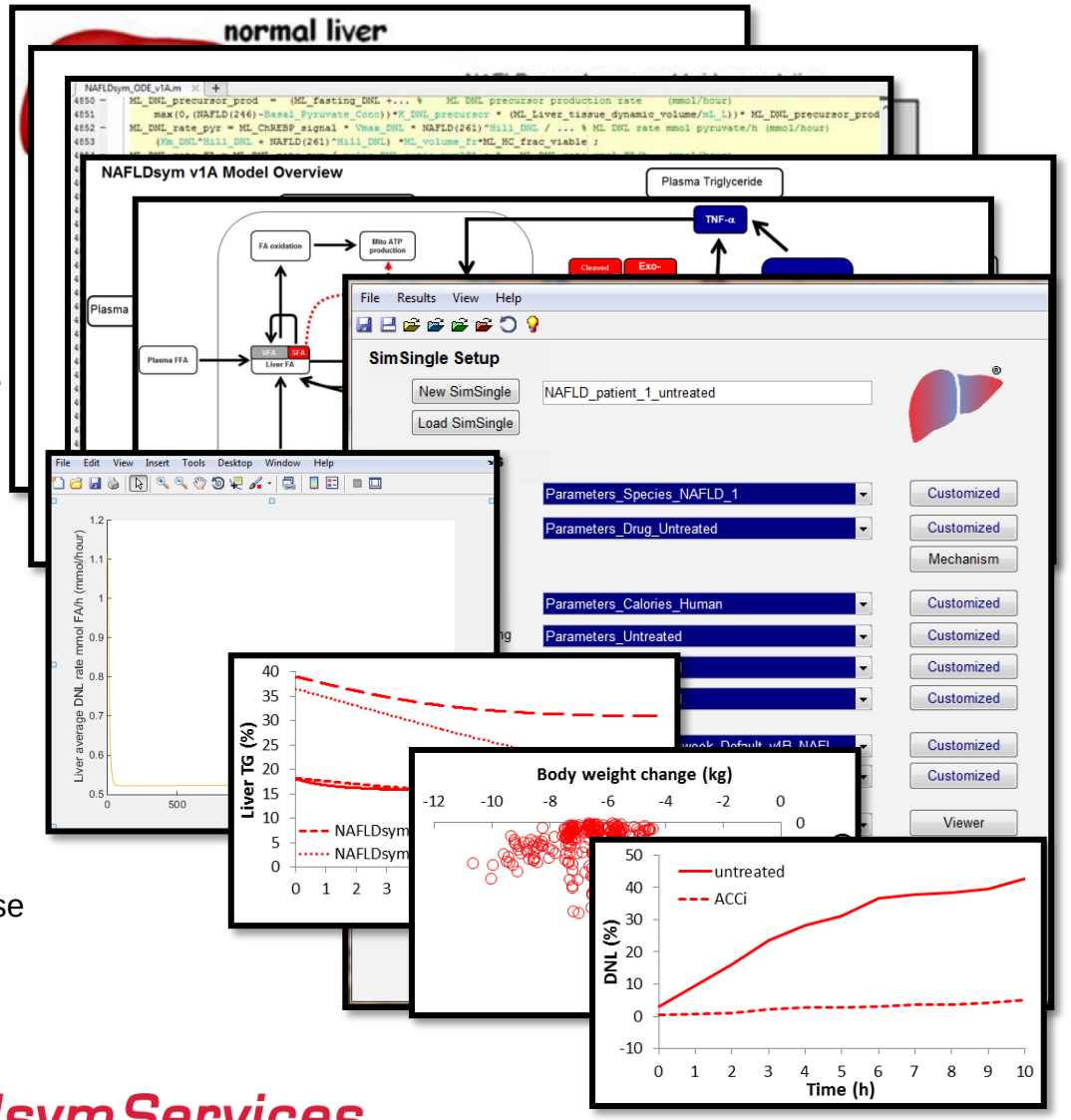
NAFLDsym Is Designed to Support Drug Development with Efficacy Predictions

- NAFLD incidence is growing worldwide with few treatment options
  - Substantial opportunity to improve health for many patients by developing treatments
- NAFLDsym is a QSP model of NAFLD and NASH
  - v1A focuses on key pathways that contribute to steatosis and lipotoxicity; currently in use
  - Currently developing v2A, which will include inflammation and fibrosis sub-models; available Q4 2018
  - Includes >300 diverse simulated patients in SimPops™
  - NAFLDsym utilizes many key aspects of DILIsym®
- NAFLDsym can be used to support NAFLD drug development
  - Combines PK, PD, pathophysiology to predict efficacy of novel treatments
  - Flexible framework facilitates addition of new targets as needed
  - Can be used to optimize clinical trial protocols and identify key hypotheses related to mechanistic underpinnings of predicted response to treatment
- NAFLDsym has been used in collaborative research agreement with Pfizer, Gilead and other companies to inform clinical programs



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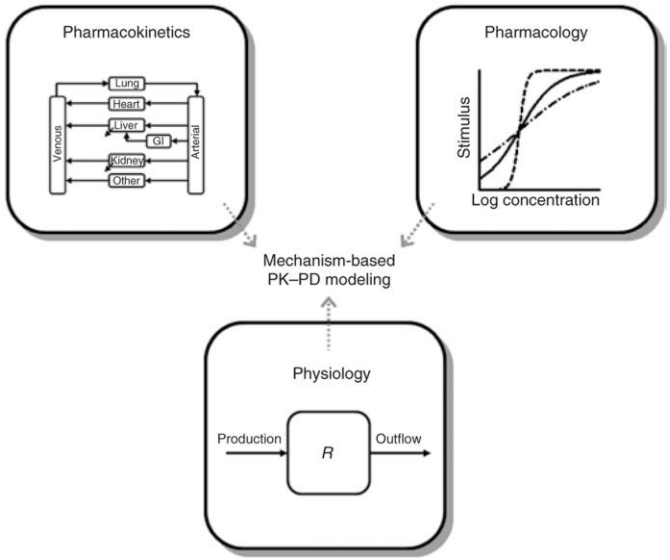
Slide courtesy of CJ Musante, Pfizer

The Intersection of PK, PD, and QSP

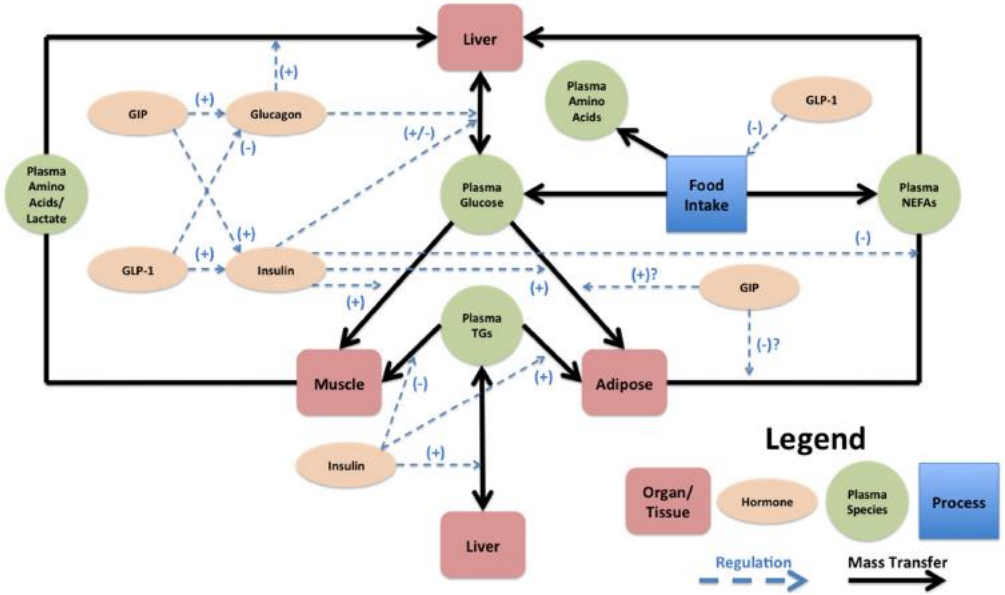
Pharmacokinetics (PK): What the body does to a drug
Pharmacodynamics (PD): What a drug does to the body
QSP: PK and PD extended to effects at the systems level (e.g., disease modification)

PK and PD



Mager and Jusko 2008

QSP



Rieger and Musante 2016

http://www.merckmanuals.com

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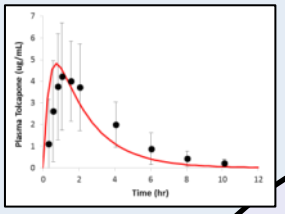
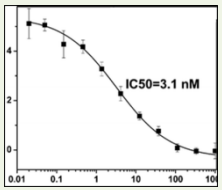
NAFLDsym Predicts Efficacy via the Intersection Between Exposure, PD, and Inter-Patient Pathophysiologic Variability

PBPK modeling capabilities provide ability to predict intracellular compound concentrations at site of target

Exposure



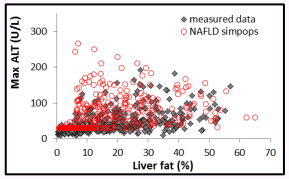
Pharmacodynamics



Efficacy

Direct PD and downstream (e.g. gene expression) effects can be represented, relying on existing data

Mechanisms of NAFLD pathophysiology represented in equations, including characteristics and interpatient variability that can influence predicted efficacy



Pathophysiologic Variability

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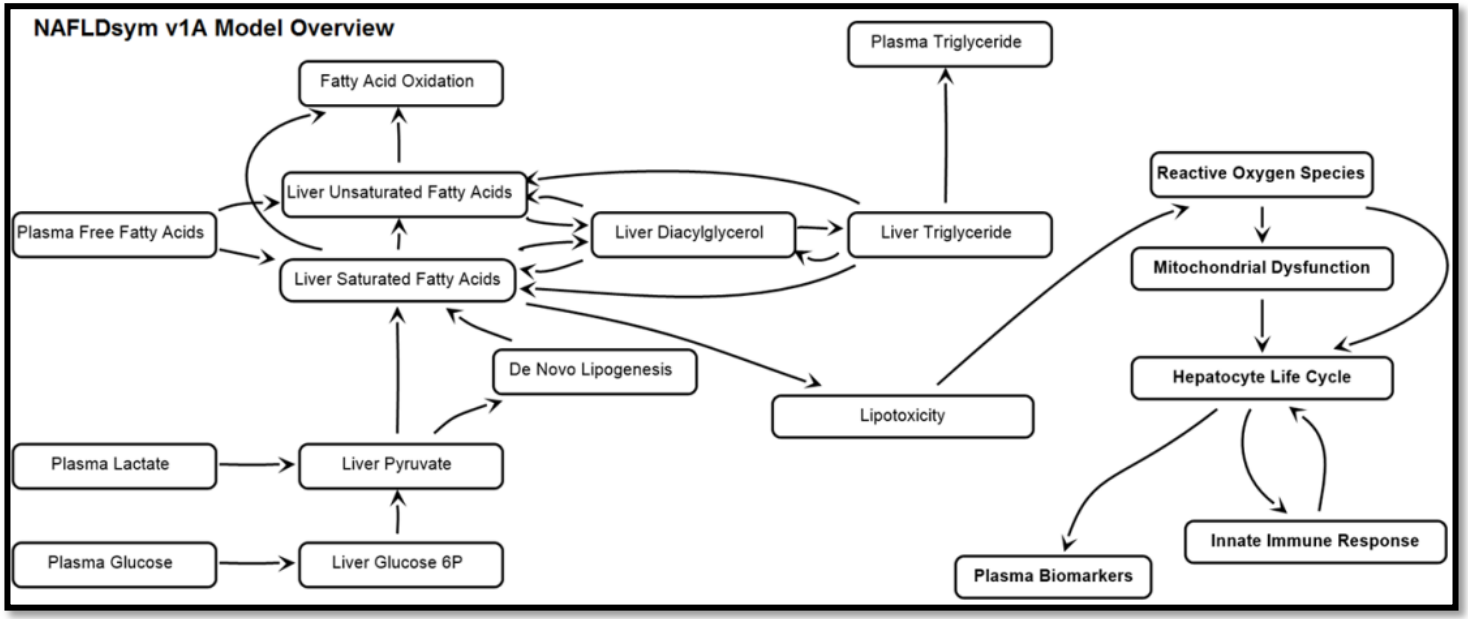
NAFLDsym v1A Overview



Essential cellular processes represented to multiple scales in interacting sub-models

- Steatosis and lipotoxicity
- PBPK Pharmacokinetics
- Injury progression
- Mitochondrial dysfunction
- Cellular energy balance
- Biomarkers
- Hepatocyte apoptosis and necrosis
- Hepatocyte proliferation
- Macrophage, LSEC life cycles
- Immune mediators
- Caloric intake

Clinical data from literature used to establish quantitative relationships for underlying biochemistry

Clinical responses to several drugs utilized to determine quantitative effects of lipotoxicity: *juxtapid, kynamro, etomoxir*

NAFLDsym Includes numerous simulated patients with pathophysiologic and clinical inter-patient variability (SimPops)

NAFLDsym v1A excludes fibrosis and inflammatory aspects of NAFLD and NASH. These sub-models are currently under development and will be included in v2A

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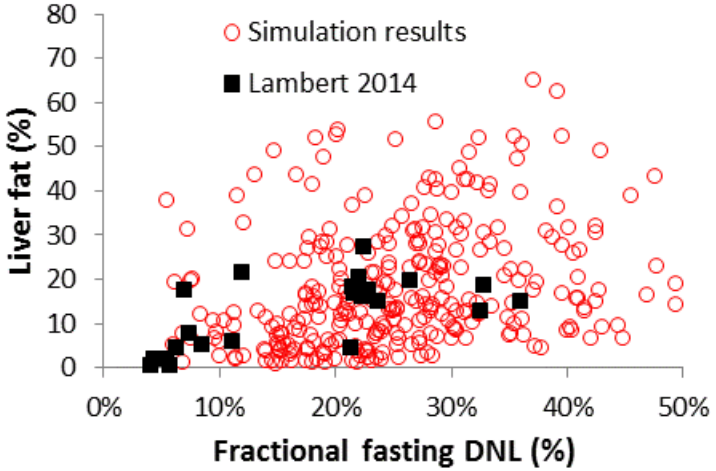
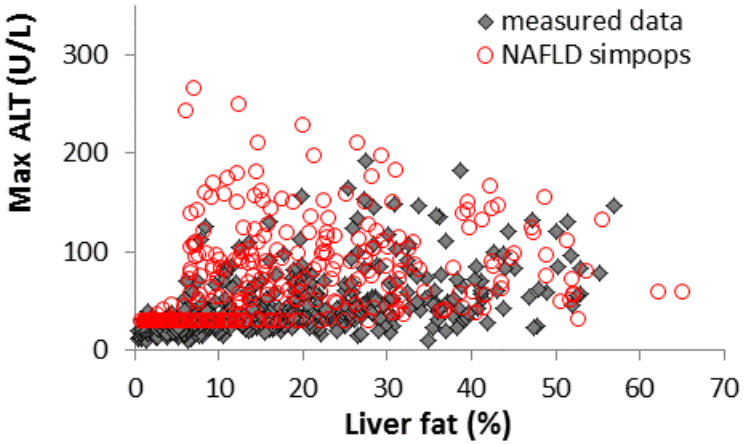
NAFLD SimPops Include Steatosis, DNL, and Liver Injury Consistent with Clinical Data

- Simulated patients have wide range of contributions to steatosis from DNL
  - Consistent with Lambert 2014 observation that frequency of elevated DNL higher in patients with extensive steatosis
- Majority of simulated patients within range of liver fat-ALT clinical data (Maximos 2015)
  - Indicates that relationship between steatosis and lipotoxicity is captured within SimPops
- NAFLD SimPops also consistent with data describing (*not shown*)
  - Plasma fatty acid levels (Zhang 2014)
  - Proportion of patients with type 2 diabetes (Browning 2004, Copaci 2015, others)
  - Liver DAG levels (Kotronen 2009)
  - Liver oxidative stress (Hardwick 2010)
  - BMI distributions (Dudekala 2014)



Lambert 2014



Maximos 2015

Clinical Data and Simulation Results

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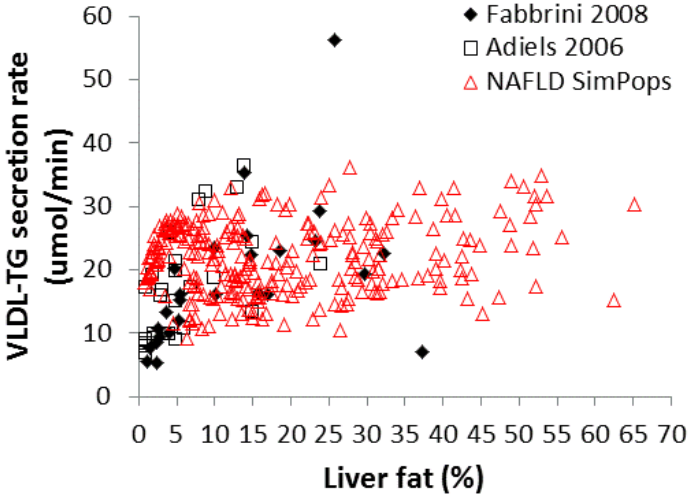
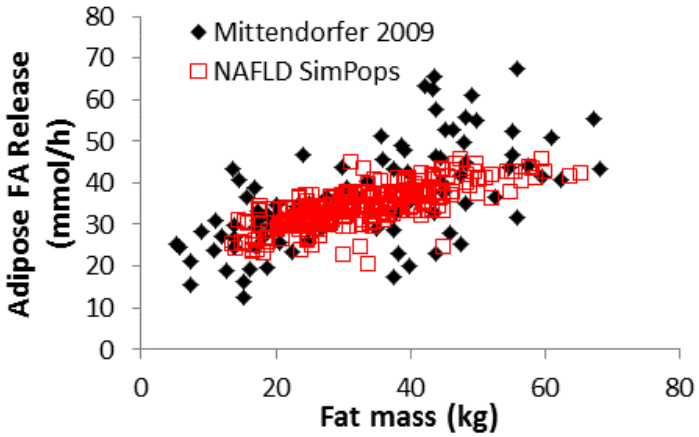
NAFLD SimPops Has Range of VLDL-TG and Adipose FA Release Rates Consistent with Clinical Data

- Simulated patients have wide range of VLDL-TG release that is consistent with clinical data
  - Generally greater VLDL-TG release rates with higher degrees of steatosis
- Adipose fatty acid release rates dependent upon fat mass in clinical and simulated patients
  - Consistent with Mittendorfer 2009 clinical data



Fabbrini 2008, Adiels 2006



Mittendorfer 2009

Clinical Data and Simulation Results

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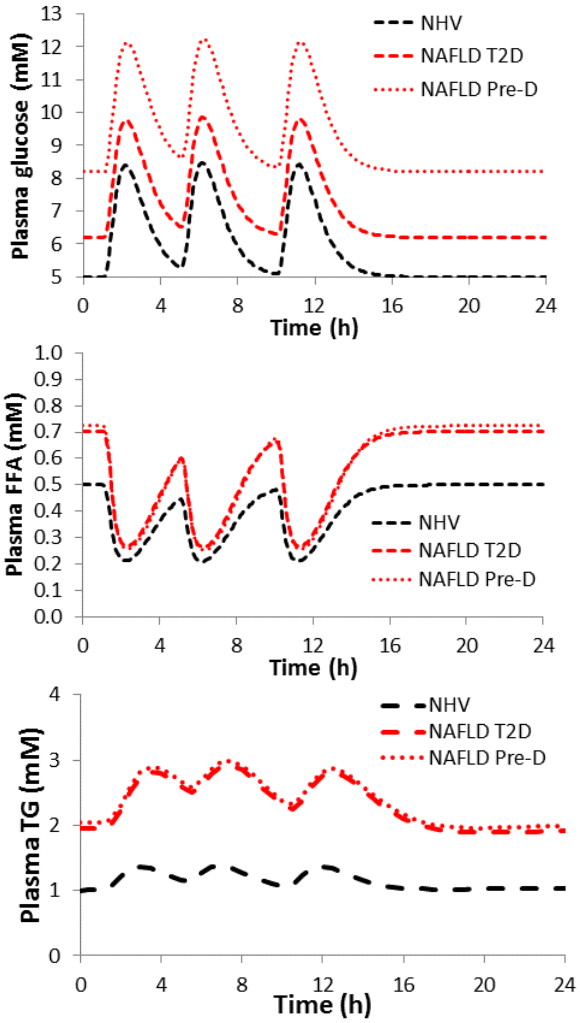
Appropriate Simulated Responses to Meals for Circulating Nutrients

- Dynamic, meal-related changes to circulating nutrients included in NAFLDsym
- Plasma glucose
  - Pre-diabetes and type 2 diabetes levels of glycemia in NAFLD patients
  - Post-prandial increases simulated
- Plasma FFA
  - Increased fasting FFA due to increased adiposity in NAFLD patients
  - Post-prandial decreases simulated
- Plasma triglycerides (TG)
  - Increased fasting TG in NAFLD patients
  - Post-prandial chylomicron-TG increases simulated

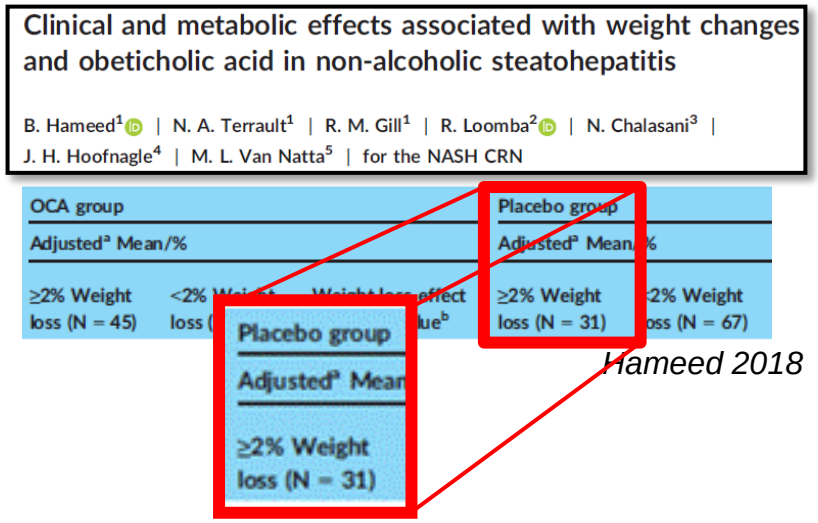


NAFLDsym v1A SimPops Patients Include Common Measurements of Treatment Efficacy

Biomarker/ Measurement	NAFLDsym output	Pathophysiology represented by biomarker
Liver TG (biopsy)	Liver TG concentration	Steatosis
Liver fat percentage (imaging)	Liver fat percentage	Steatosis
Plasma TG	Plasma TG	Steatosis, Cardiovascular risk
Plasma ALT	Plasma ALT	Hepatocellular health
Plasma cytokeratin cleaved 18 (cK18)	Plasma cK18	Hepatocellular apoptosis
Steatosis score (histology)	Steatosis score	Steatosis
Ballooning score (histology)	Ballooning score	Hepatocellular apoptosis
Inflammation score (histology)	*In development*	Inflammation
NAFLD Activity Score (NAS)	*In development*	Histological assessment of steatosis, cell health, and inflammation

Histologic Steatosis and Ballooning Scores Show Appropriate Responses to Weight Loss

- Weight loss shown to be an effective treatment for NAFLD
  - Hameed 2018 reported changes in liver histology with different degrees of weight loss
  - Comparator with OCA
- Performed simulations of 1 year of weight loss in SimCohort (n=36)
  - Compare histologic outputs with Hameed 2018
  - Simulated ~10% weight loss
- Magnitude of change in histologic steatosis consistent with clinical data
  - Reduced food intake impacts liver fat by decreasing adipose FA release and hepatic DNL
- Magnitude of change in histologic ballooning consistent with clinical data
  - Reduced food intake impacts ballooning by reducing lipid levels and associated lipotoxicity
- Provides confidence in representation of histologic NAS components


Clinical and metabolic effects associated with weight changes and obeticholic acid in non-alcoholic steatohepatitis

B. Hameed[1] | N. A. Terrault[1] | R. M. Gill[1] | R. Loomba[2] | N. Chalasani[3] | J. H. Hoofnagle[4] | M. L. Van Natta[5] | for the NASH CRN

OCA group

Adjusted[a] Mean/%

≥2% Weight loss (N = 45)

Placebo group

Adjusted[a] Mean/%

≥2% Weight loss (N = 31)

Placebo group

Adjusted[a] Mean

≥2% Weight loss (N = 31)

Hameed 2018

Histologic improvement with weight loss		Clinical Data	Simulation Results
Histologic steatosis	Baseline	2.1+/-0.8	2.2+/-1.0
	Change from baseline	-0.8	-0.5+/-0.7
Histologic ballooning	Baseline	1.5+/-0.7	0.8+/-0.9
	Change from baseline	-0.2	-0.2+/-0.5

Clinical Data and Simulation Results

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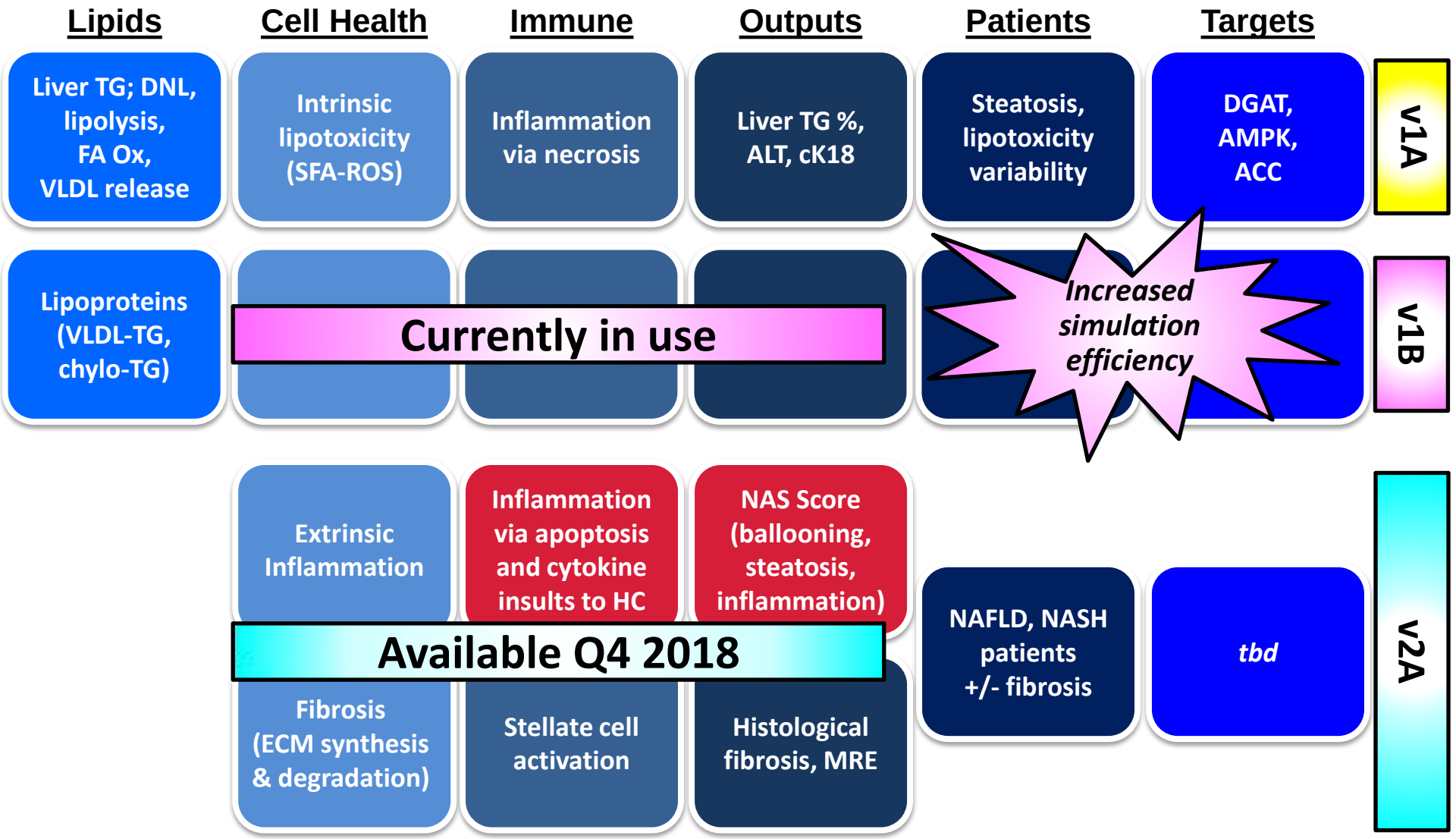
NAFLDsym Development Plan



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NAFLDsym Was Used to Support the Clinical Development of the ACCi GS-0976

- Early clinical results indicated GS-0976 MoA may be more complex than initially believed

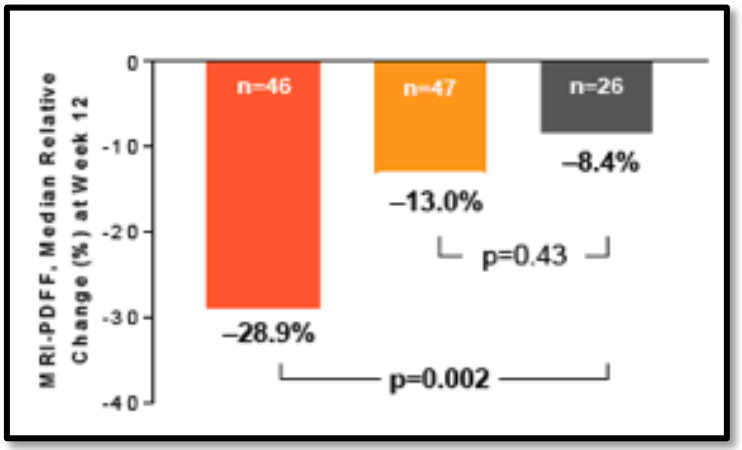
- NAFLDsym employed to evaluate MoA hypotheses
  - Developed PBPK model of GS-0976 (exposure)
  - Utilized existing preclinical and clinical data to determine PD parameters
  - Utilized existing simulated patients to generate appropriate SimCohorts

- Simulation study conducted in parallel with Phase 2 clinical trial; comparison between clinical data and NAFLDsym predictions provided validation
  - Further validated in comparisons with MK-4074

- Simulation study identified key pathways that were activated via downstream gene expression effects and contributed to clinical response
  - Enhanced understanding of MoA helped provide guidance to clinical development program

ACC Inhibitor GS-0976 Has Demonstrated Ability to Reduce Liver Fat

- GS-0976: Liver-targeted ACC inhibition
  - Direct pharmacological effects on de novo lipogenesis and fatty acid oxidation
- Single dose clinical study revealed rapid, potent ability to inhibit hepatic DNL
  - Oral fructose challenge; Steide 2017
- 12 week POC study revealed efficacy potential in obese, non-diabetic patients
  - Reduced liver fat, ALT, DNL
  - Increases in plasma TG in some patients
- 12 week Phase 2 study demonstrated efficacy in NAFLD patients
  - Improved steatosis and ALT
  - Increases in plasma TG
- Combination treatments may prevent plasma triglyceride increases
  - Fibrates appear to offset downstream gene expression changes caused by ACCi



Loomba et al. The Liver Meeting (AASLD) 2017

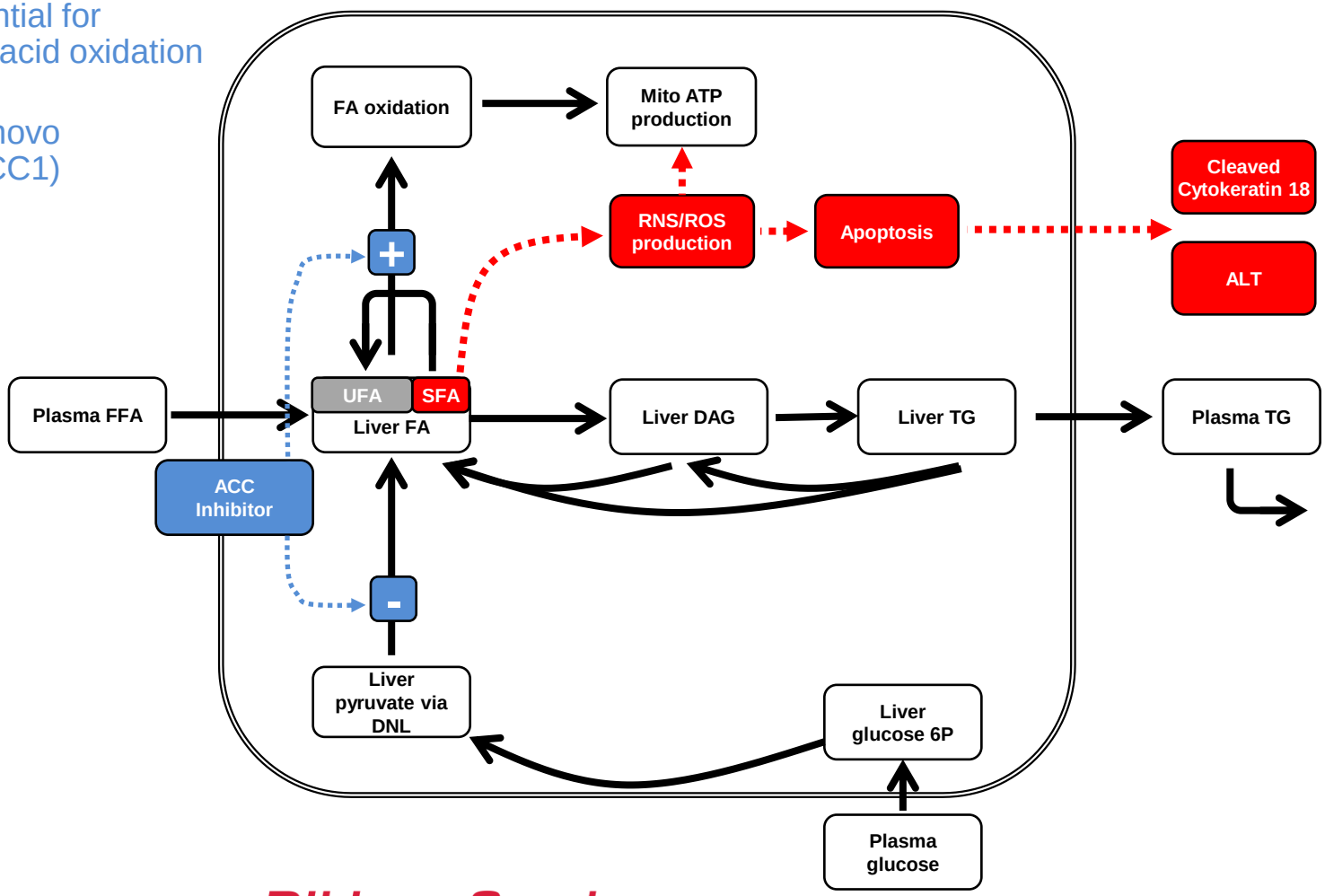
Table: Lipid Parameters in Subjects Treated with GS-0976 20 mg Daily for 12 Weeks

Lipid Parameter	BL	W1	W4	W12
TG (mg/dL)	160 (125,201)	191 (136,290)*†	188 (142,270)*	177 (116,277)*
VLDL-TG (nmol/L)	93 (66,133)	120 (73,236)*†	97 (69,200)*	96 (68,175)
VLDL-P (nmol/L)	53 (36,73)	72 (39, 121)*	52 (43,90)	52 (31,93)

Mantry et al. International Liver Congress (EASL) 2018

GS-0976 Direct PD Effects Included in NAFLDsym

- ACC inhibitor directly reduces Malonyl CoA levels
  - Increases potential for increased fatty acid oxidation (ACC2)
  - Decreases de novo lipogenesis (ACC1)



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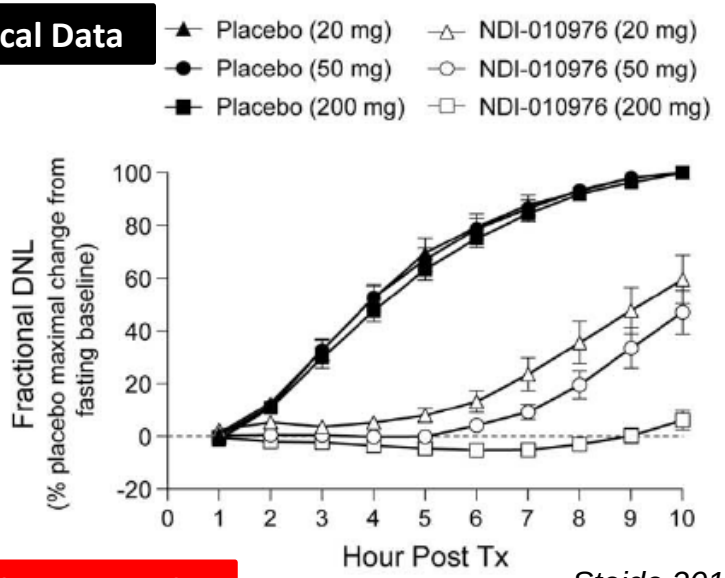
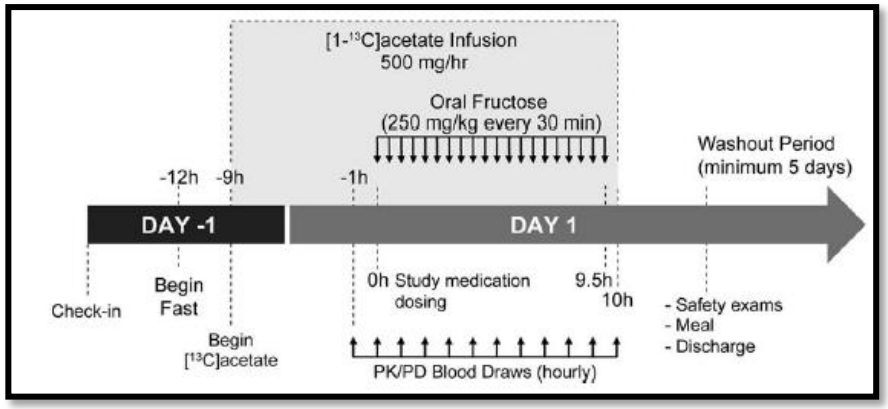
Accurate Recapitulation of GS-0976 DNL Response to Fructose Challenge Protocol

- Simulated fructose challenge protocol in NAFLDsym
  - Utilized glucose meals and removed Randle cycle-type regulations to provide appropriate substrate flux
  - Utilized 12 simulated, overweight non-steatotic volunteers (SimCohorts)
- Accurate depiction of kinetics of fractional DNL due to fructose challenge with NAFLDsym
  - Magnitude and kinetics of DNL suppression appropriately captured

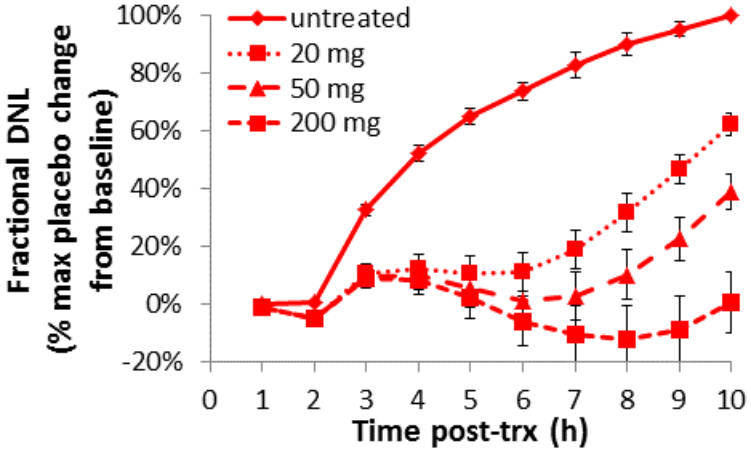
Clinical Data



Steide 2017



Simulation Results



Steide 2017

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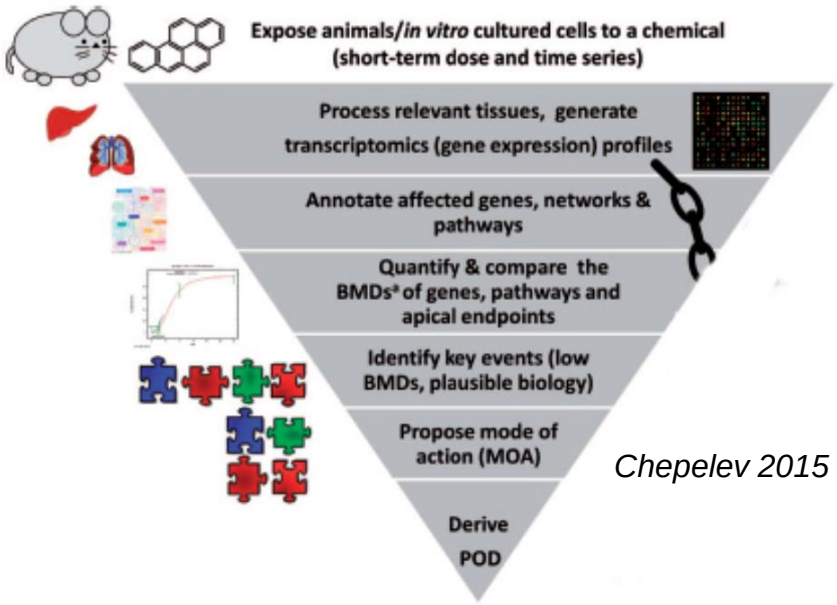
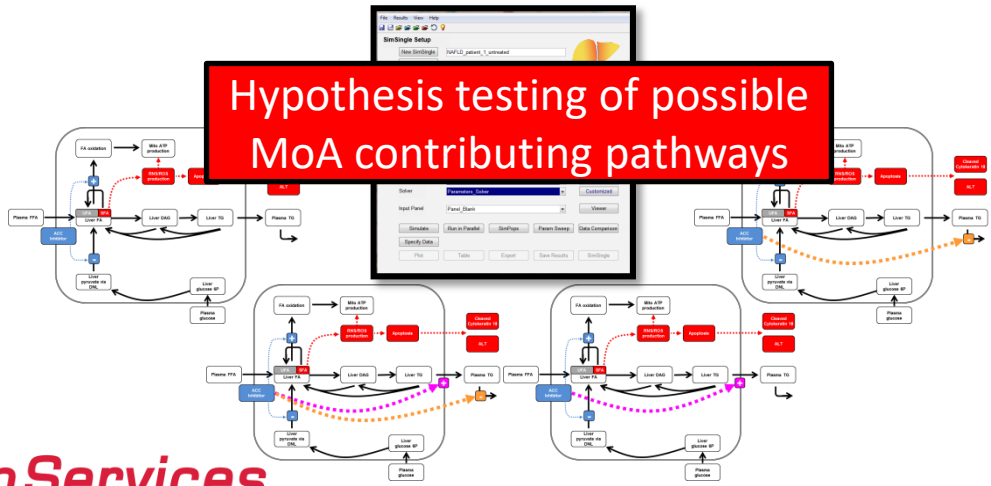
QSP Modeling and Gene Expression Analyses Have Complementary Approaches

- Gene expression analyses provide large amounts of data in evaluation of drug MoA
  - Identifies specific enzyme(s) that may participate in drug action
  - Challenging to identify causative roles
  - Challenging to determine biochemical context
- QSP modeling provides insight into plausibility of contributions of specific pathways
  - Provides insight into dynamics of pathways affected by drug actions
  - Challenging to identify specific enzyme(s) that may participate in drug action
- Combination of NAFLDsym simulation analyses and preclinical data provided insight into GS-0976 MoA
  - Changes to liver fat and plasma TG



Chepelev 2015



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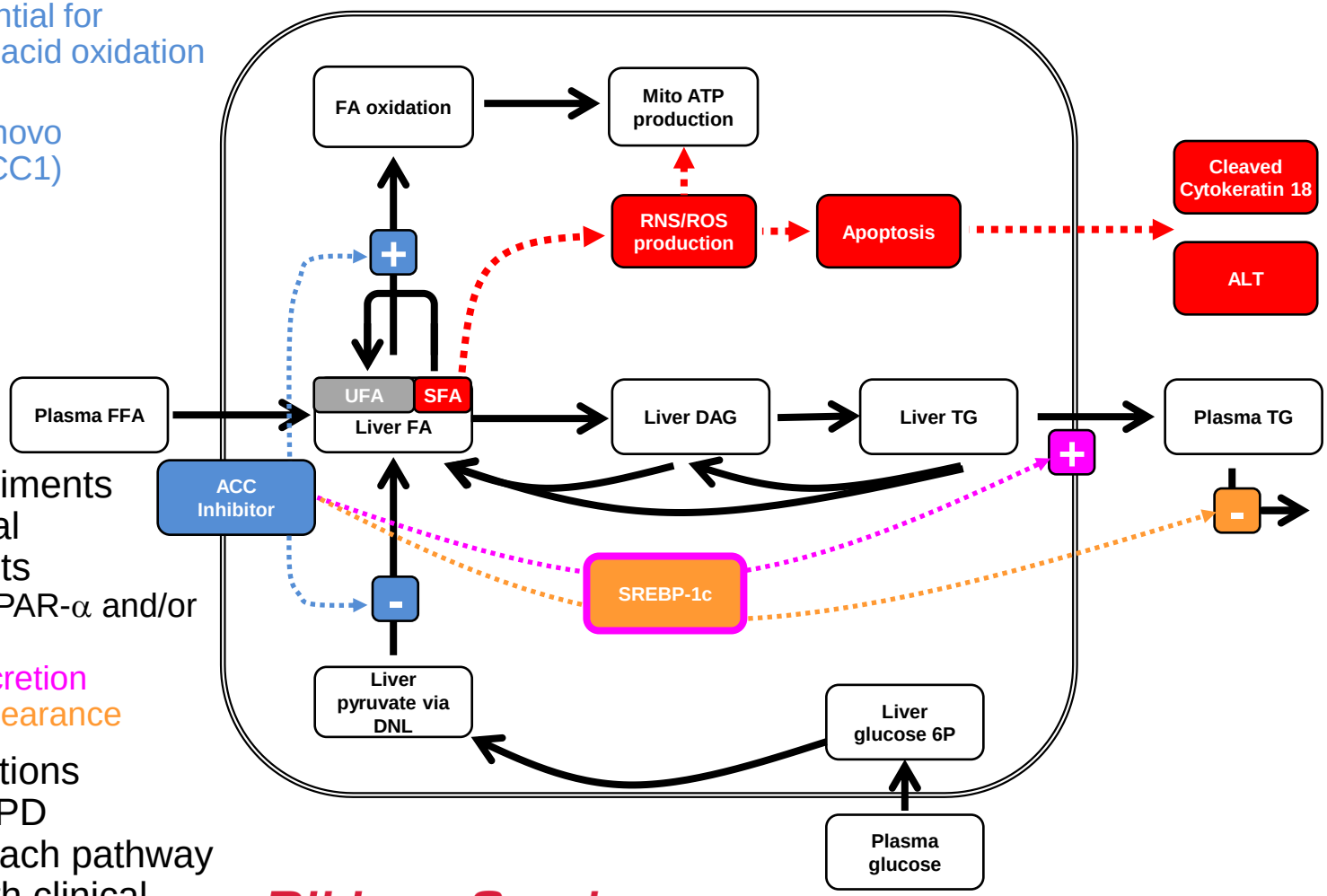
GS-0976 Direct and Downstream PD Effects Included in NAFLDsym

- ACC inhibitor directly reduces Malonyl CoA levels
  - Increases potential for increased fatty acid oxidation (ACC2)
  - Decreases de novo lipogenesis (ACC1)



- Preclinical experiments revealed additional downstream effects
  - Likely due to PPAR-α and/or SREBP-1c
  - ↑ VLDL-TG secretion
  - ↓ Plasma TG clearance
- Performed simulations including varying PD combinations of each pathway and compared with clinical data to determine feasibility

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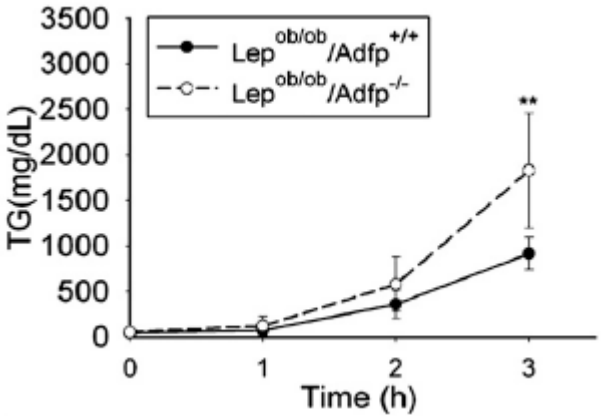
Hepatic Enzymes Identified in Preclinical Literature that Could Participate in Increased VLDL-TG Secretion

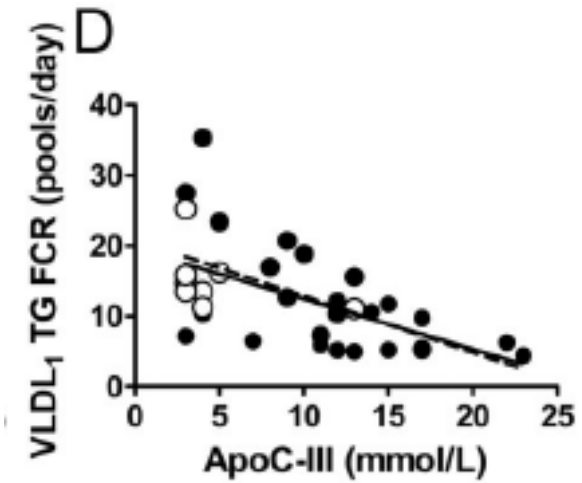
- Decreased perilipin expression can lead to increased VLDL secretion
  - Chang 2010, Najt 2016
- Increased ATGL expression can lead to increased lipolysis of liver TG
  - Reid 2008, Ong 2011
  - Shown to be increased in ACC knockout mice (Kim 2017)
- Increased DGAT expression can lead to increased VLDL-TG secretion rates
  - Yamazaki 2005



Chang 2010

Hepatic Enzymes Identified in Preclinical Literature that Could Participate in Reducing Plasma TG Clearance

- Increased hepatic angiopoietin-like-3 expression can lead to inhibition of LPL and decreased TG clearance
  - Koishi 2002
- Decreased ApoE (Apoe) expression can lead to decreased TG clearance
  - Knouff 1999, Stanford 2009
- Increased ApoCIII correlated with reduced VLDL-TG clearance in obese patients
  - Taskinen 2011



Taskinen 2011

Preclinical and Clinical Data

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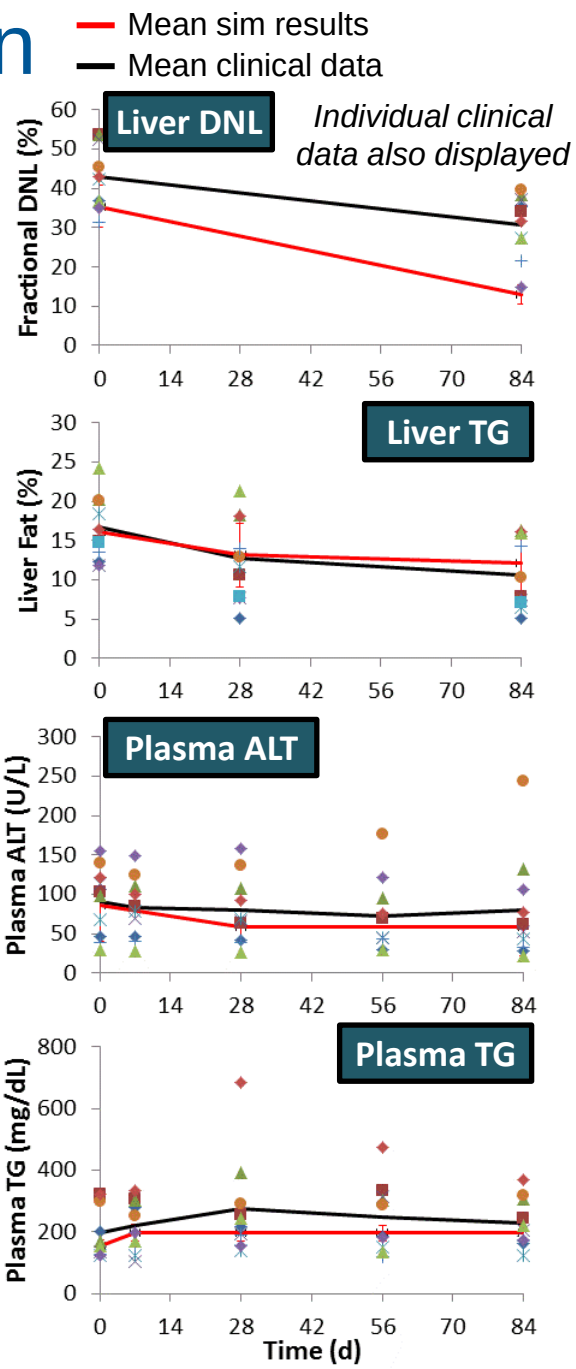
ACCi with ↑VLDL-TG Secretion and ↓TG Clearance Effects Agrees with 12 wk POC Data

- Simulated 12 wk POC study
  - SimCohorts with comparable clinical characteristics (NAFLD)
  - 20 mg q.d. AM GS-0976
  - 12 weeks
  - Includes direct pharmacologic effects of ACCi DNL inhibition and FA oxidation enhancement
- Simulations included downstream effects that increase hepatic VLDL-TG secretion and reduced TG clearance
  - Simulated 20-30% reduction in plasma TG clearance
  - Simulated 20-30% increase in VLDL-TG secretion
- Good agreement between clinical data and simulation results
- NAFLDsym helped identify relevant genes/pathways based on expression data
  - Simulation results excluding downstream effects not consistent with clinical data



Clinical Data and Simulation Results

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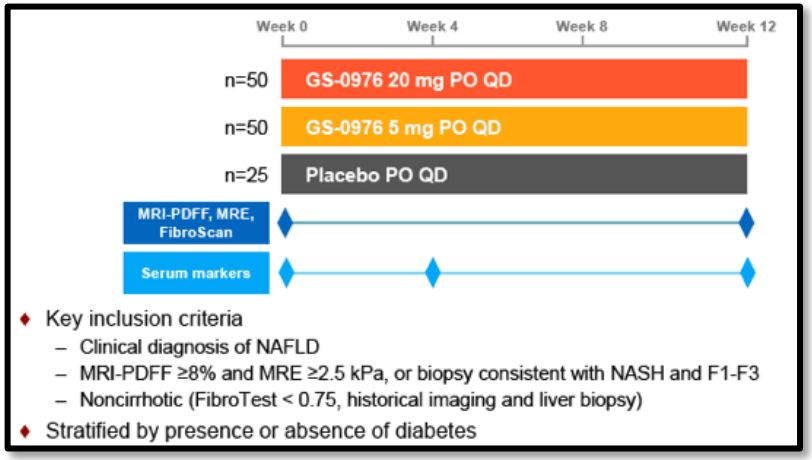
Good Agreement between GS-0976 Phase 2 Clinical Data and NAFLDsym Simulation Results Validates MoA Hypotheses



Loomba et al.
The Liver Meeting (AASLD) 2017

Measure	Dose	Sampling time	Clinical data	Simulation results
Liver fat (%)	20 mg	Baseline	16.3	16.5
Liver fat (%)	20 mg	Week 12	11.6	11.0
Liver fat (%)	5 mg	Baseline	16.4	16.5
Liver fat (%)	5 mg	Week 12	14.9	12.7
Plasma TG (mg/dL)	20 mg	Baseline	181.3	187.3
Plasma TG (mg/dL)	20 mg	Week 12	251.9	231.9
Plasma TG (mg/dL)	5 mg	Baseline	173.3	187.3
Plasma TG (mg/dL)	5 mg	Week 12	209.8	223.1
Plasma ALT (U/L)	20 mg	Baseline	64.7	69.9
Plasma ALT (U/L)	20 mg	Week 12	49.8	48.4
Plasma ALT (U/L)	5 mg	Baseline	70.2	69.9
Plasma ALT (U/L)	5 mg	Week 12	65.9	56.9

Clinical Data and Simulation Results

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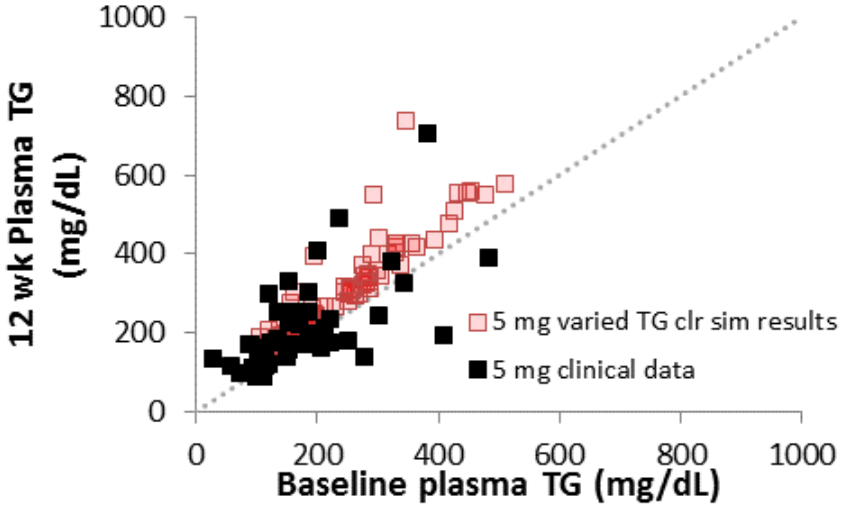
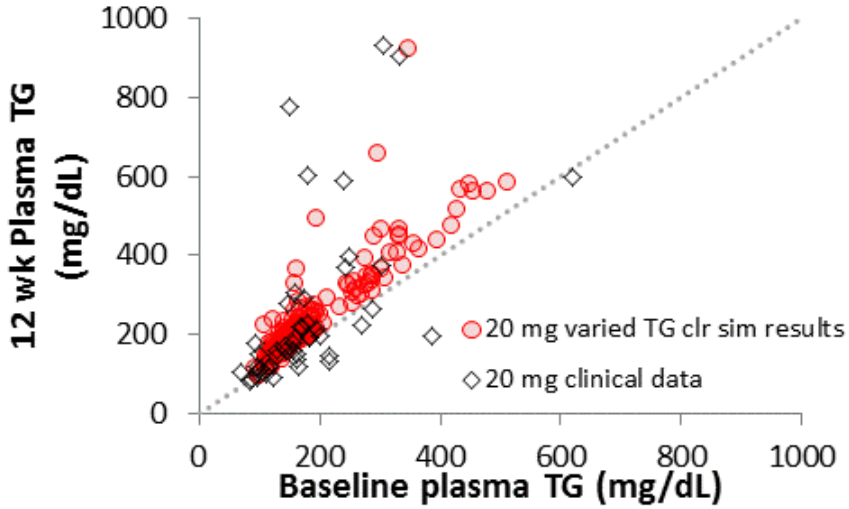
Predicted Plasma TG Change Relative to Initial Levels Comparable to Clinical Response

- Patients in each cohort had mildly increased plasma TG levels
  - Simulation results align well with this range of response at each dose
- Some patients had greater increases in plasma TG
  - Mostly patients with hypertriglyceridemia
  - Simulations with SimCohorts reproduce this observation
  - Likely due to greater changes in ApoE, ApoCIII, or syndecan-1 expression
- Inclusion of downstream changes to hepatic VLDL-TG and plasma TG clearance to overall ACCi effects provided ability to align with clinical data





Clinical Data and Simulation Results

DILIsymServices

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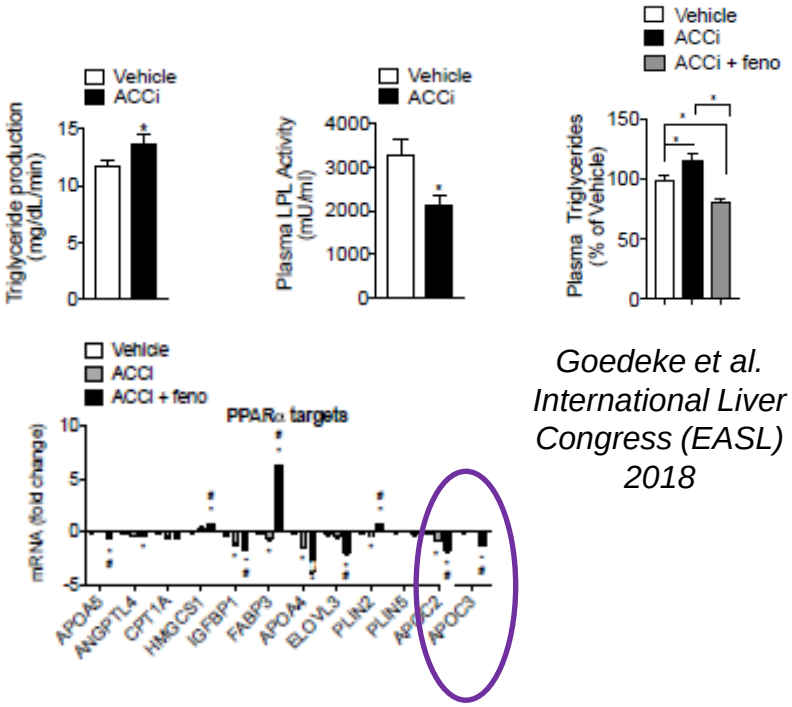
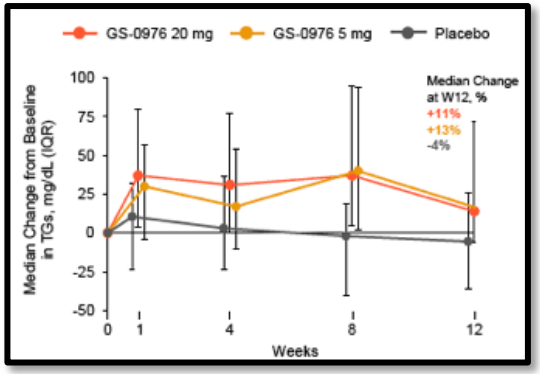
Co-administration of Fibrates with GS-0976 Can Ameliorate Plasma TG Increases

- Preclinical and clinical data suggest that co-administration of ACCi with a PPAR-α agonist can ameliorate hypertriglyceridemia induced by ACCi
- Preclinical studies have shown ACCi can lead to increased plasma TG
  - Due to increases in VLDL-TG production and decreased plasma TG clearance (LPL activity)
  - HFSD-fed rats
- Co-administration of fenofibrate prevents plasma TG increase due to ACCi
  - PPAR-a agonist elicits reduced expression of ApoC3
  - Reduced ApoC3 relieves LPL inhibition
  - Goedeke et al. EASL 2018
- Fenofibrate restricted plasma TG increases when administered to patients with asymptomatic plasma TG > 500 mg/dL in patients treated with GS-0976
  - Loomba et al. AASLD 2017



Goedeke et al. International Liver Congress (EASL) 2018



Loomba et al. The Liver Meeting (AASLD) 2017

Preclinical and Clinical Data

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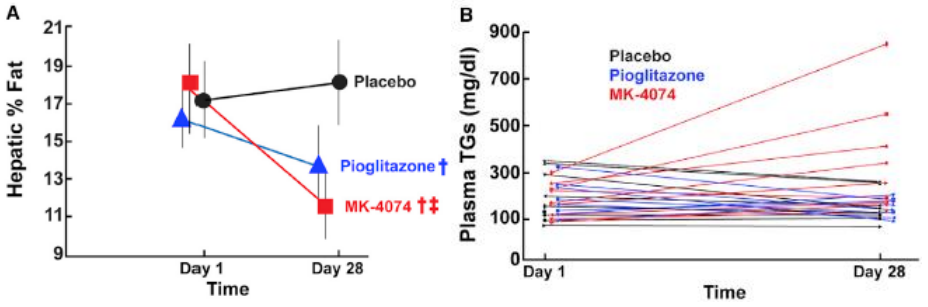
ACC Inhibitor MK-4074 Decreases Liver Fat, Increases Plasma TG and VLDL-TG Secretion

- Recent publication by Kim et al., from Merck, reported clinical observations for the ACC inhibitor, MK-4074
  - Reductions in liver fat
  - Increases in plasma TG
- Preclinical mechanistic studies showed lipid changes were dependent upon downstream changes to specific pathways
  - Hepatic VLDL-TG secretion rates elevated in ACC knockout mice
  - Restricting hepatic VLDL-TG secretion (via GPAT siRNA) mitigated liver and plasma TG changes (*not shown*)
- Simulated enduring, nearly complete ACC inhibition and compared predicted results to those reported in Kim et al. 2017
  - Utilized NAFLD SimCohorts (n=196)
  - Simulations included direct effects on DNL, VLDL-TG secretion, and plasma TG clearance

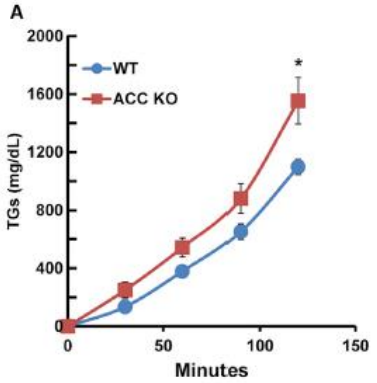
Acetyl CoA Carboxylase Inhibition Reduces Hepatic Steatosis but Elevates Plasma Triglycerides in Mice and Humans: A Bedside to Bench Investigation

Chai-Wan Kim,[1] Carol Addy,[3] Jun Kusunoki,[3] Norma N. Anderson,[1] Stanislaw Deja,[2] Xiaorong Fu,[2] Shawn C. Burgess,[2] Cai Li,[3] Manu Chakravarthy,[3] Steve Previs,[3] Stuart Milstein,[4] Kevin Fitzgerald,[4] David E. Kelley,[3] and Jay D. Horton[1,5,*]

Clinical Data



Preclinical Data



Kim 2017

Clinical Data and Preclinical Data

DILIsym Services

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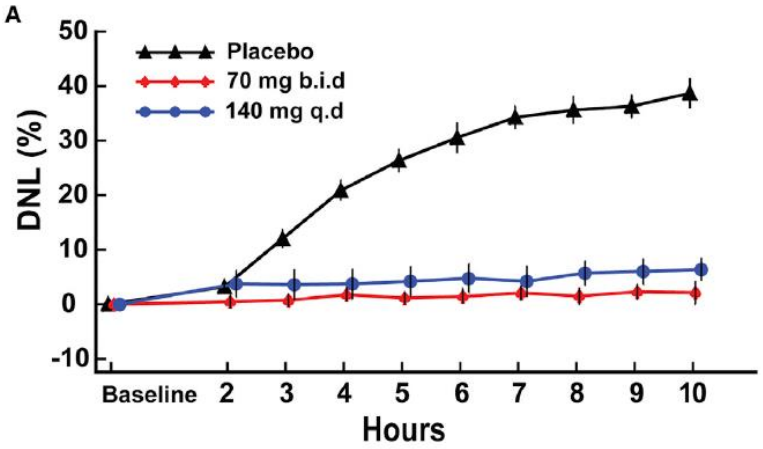
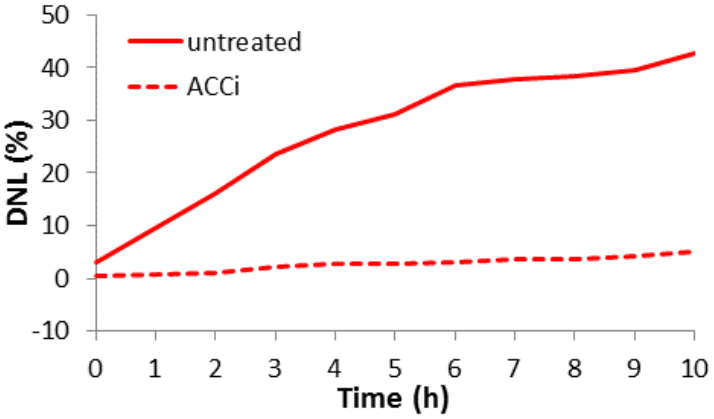
Good Agreement between Simulation Results and MK-4074 Fructose Challenge Data

Clinical Data

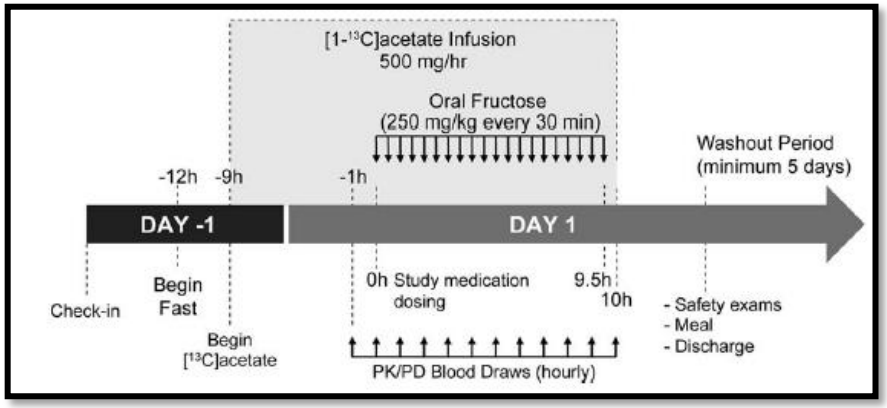


Kim 2017

Simulation Results



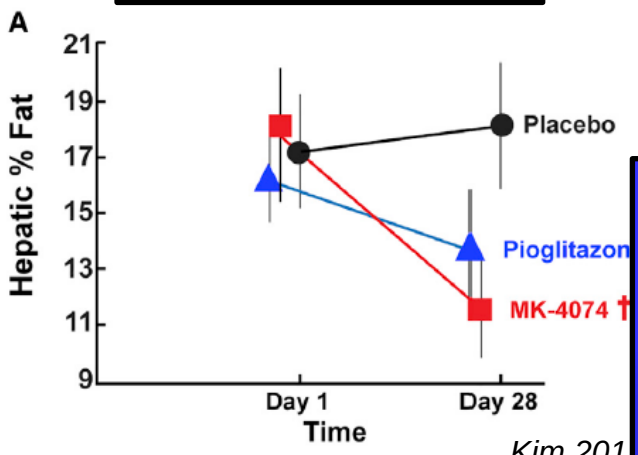
- Simulated fructose challenge protocol
- Simulated ACCi with potent, enduring DNL inhibition, comparable to MK-4074
  - Simulated NHV (N=1); clinical volunteers (n=11)
- Full suppression of DNL, despite substantial stimulus from fructose challenge



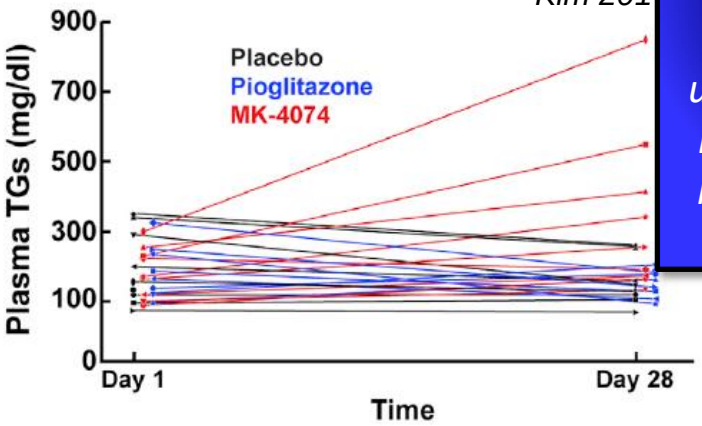
Steide 2017

Good Agreement between Simulation Results and MK-4074 Clinical Data

Clinical Data

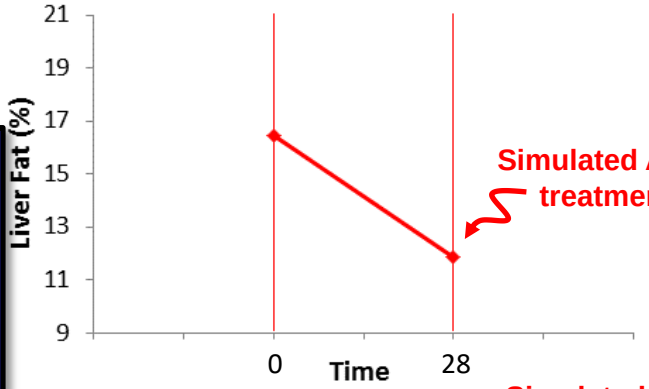


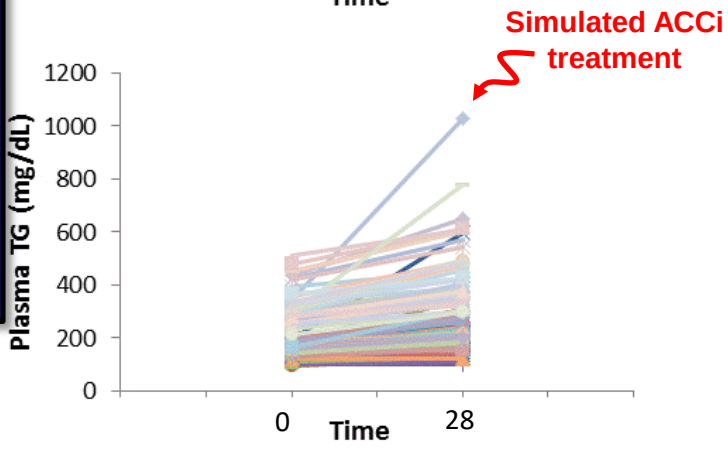
Kim 201



Good agreement between simulation results and clinical data provide support for hypotheses describing mechanistic underpinnings driving reduced liver fat and increased plasma TG

Simulation Results





- Simulated 28d trx ACCi (comparable to MK-4074) with DNL inhibition, ↑ VLDL-TG secretion, and ↓ plasma TG clearance
  - SimCohorts (n=196); clinical cohort (n=10)

Acknowledgements

DILIsym Services

Gilead

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Justin Lutz
Bryan McColgan
Robert P Myers
G. Mani Subramanian
John G McHutchison

Overall Summary

- NAFLDsym is a mechanistic, mathematical, QSP model
  - Can predict efficacy for compounds that modulate lipids and/or lipotoxicity with v1A
  - NAFLDsym v2A will also include inflammation and fibrosis submodels (available Q4 2018)
  - Flexible platform facilitates incorporation of additional targets of interest, if needed
  - Inter-patient variability represented with SimPops, providing pathophysiologic and clinical variability
  - Derived from DILIsym®, the gold standard QST mathematical model for predicting DILI risk

- NAFLDsym has been used to help Gilead gain a better understanding of the mechanism of action of the ACC inhibitor, GS-0976
  - Helped guide the clinical development program
  - Accurate prediction of clinically-observed liver fat and ALT reductions

- NAFLDsym has been constructed to support clinical development of NAFLD treatments
  - Can be used to optimize clinical trial protocols
  - Can identify key hypotheses related to mechanistic underpinnings of predicted response to treatment that can be verified with key measurements and/or studies
  - Can be used to determine efficacy potential for targets across clinical development pipeline