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NAFLDsym v2A Release Webinar

April 9, 2019

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DILIsym Services Division
Simulations Plus

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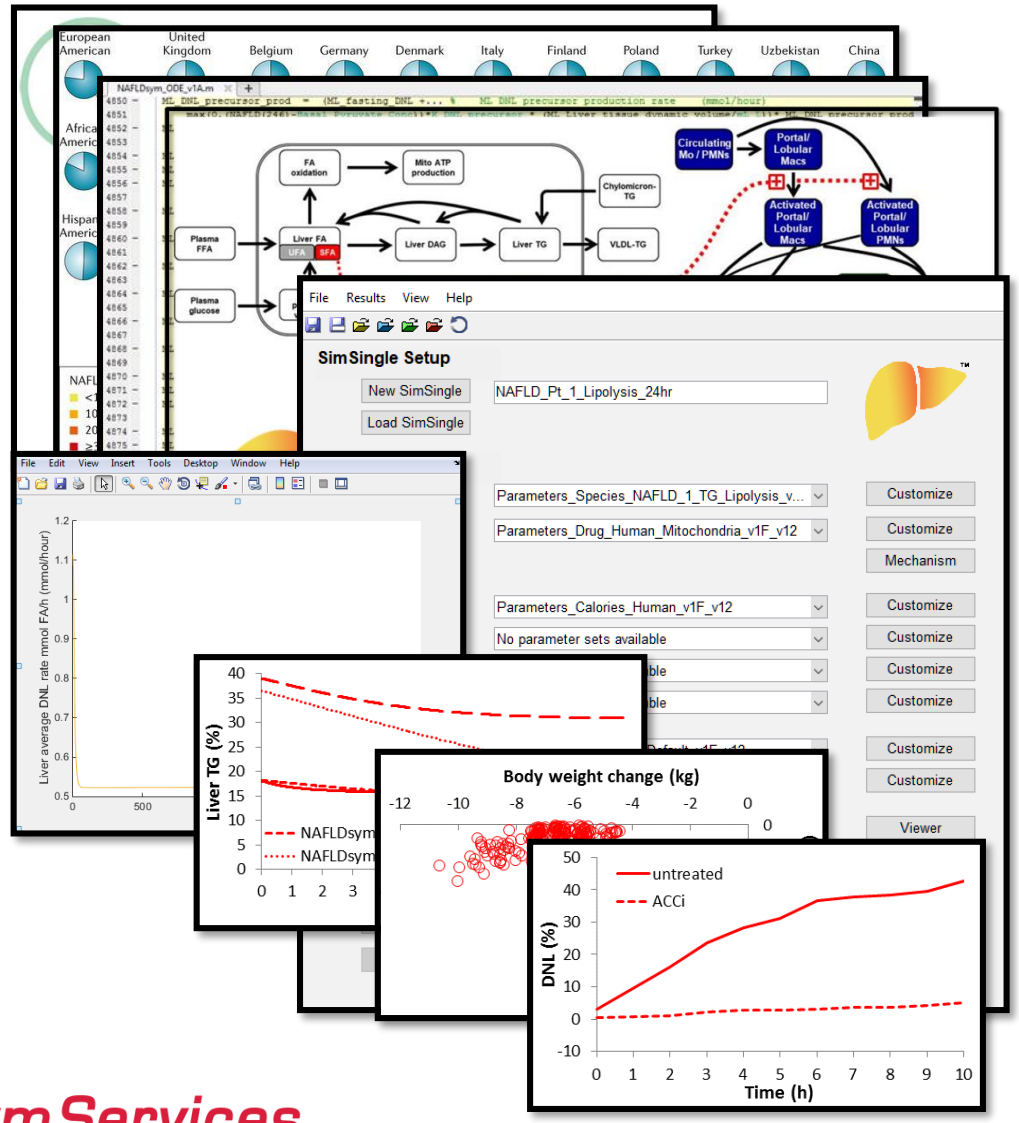
NAFLDsym Is Designed to Support Drug Development with Efficacy Predictions

- NAFLD is a progressive disease of the liver
- 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/NASH
  - NAFLDsym v2A includes steatosis, lipotoxicity, inflammation, and fibrosis sub-models; available Q2 2019
  - Includes pathophysiologically diverse simulated patients in SimPops
- 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
  - Provides ability to evaluate combinations of treatments with different mechanisms of action
- NAFLDsym has been used in collaborative research agreements with Pfizer, Gilead and other companies to inform clinical programs



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DILIsym Services, Inc.

"Our vision is safer, effective, more affordable medicines for patients through modeling and simulation."

DILIsym® RENAsym™ IPFsym™ NAFLDsym®

- DILIsym Services, Inc. offers comprehensive program services:
  - **DILIsym** software licensing, training, development (DILI-sim Initiative)
  - **NAFLDsym** software licensing, training, development
  - **DILIsym** and **NAFLDsym** simulation consulting projects
  - Consulting and data interpretation; *in vitro* assay experimental design and management
  - **RENAsym** and **IPFsym** software in development

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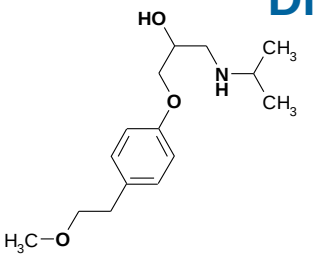
Simulations Plus Inc. (NASDAQ: SLP): Your "End-to-End" Software Provider

Discovery



Preclinical

Clinical

ADMET Predictor® ⟷ **GastroPlus®**

- **MedChem Studio™**
  MedChem Designer™
- **DDDPlus™**
  MembranePlus™
- **PKPlus™**
- **KIWI™**
- **DILIsym®**
- **NAFLDsym®**

Consulting Services and Collaborations

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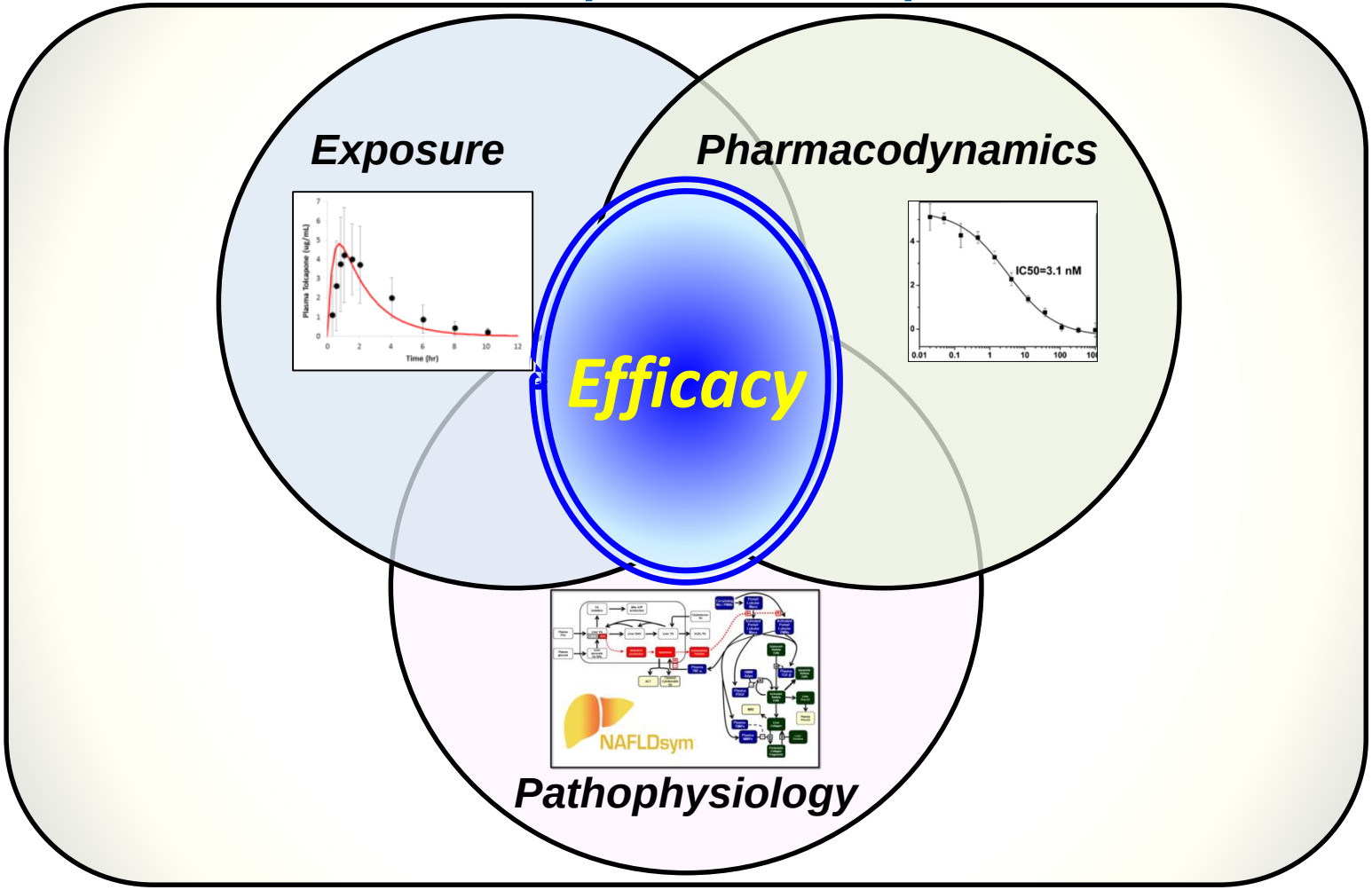
Outline

- Introduction to NAFLDsym Modeling Software
- Demonstration of NAFLDsym v2A Software
- Example NAFLDsym Application
- NAFLDsym Licensing and Services Projects

NAFLDsym Predicts Efficacy via the Intersection Between Pathophysiology Mechanisms, Compound Exposure, and PD



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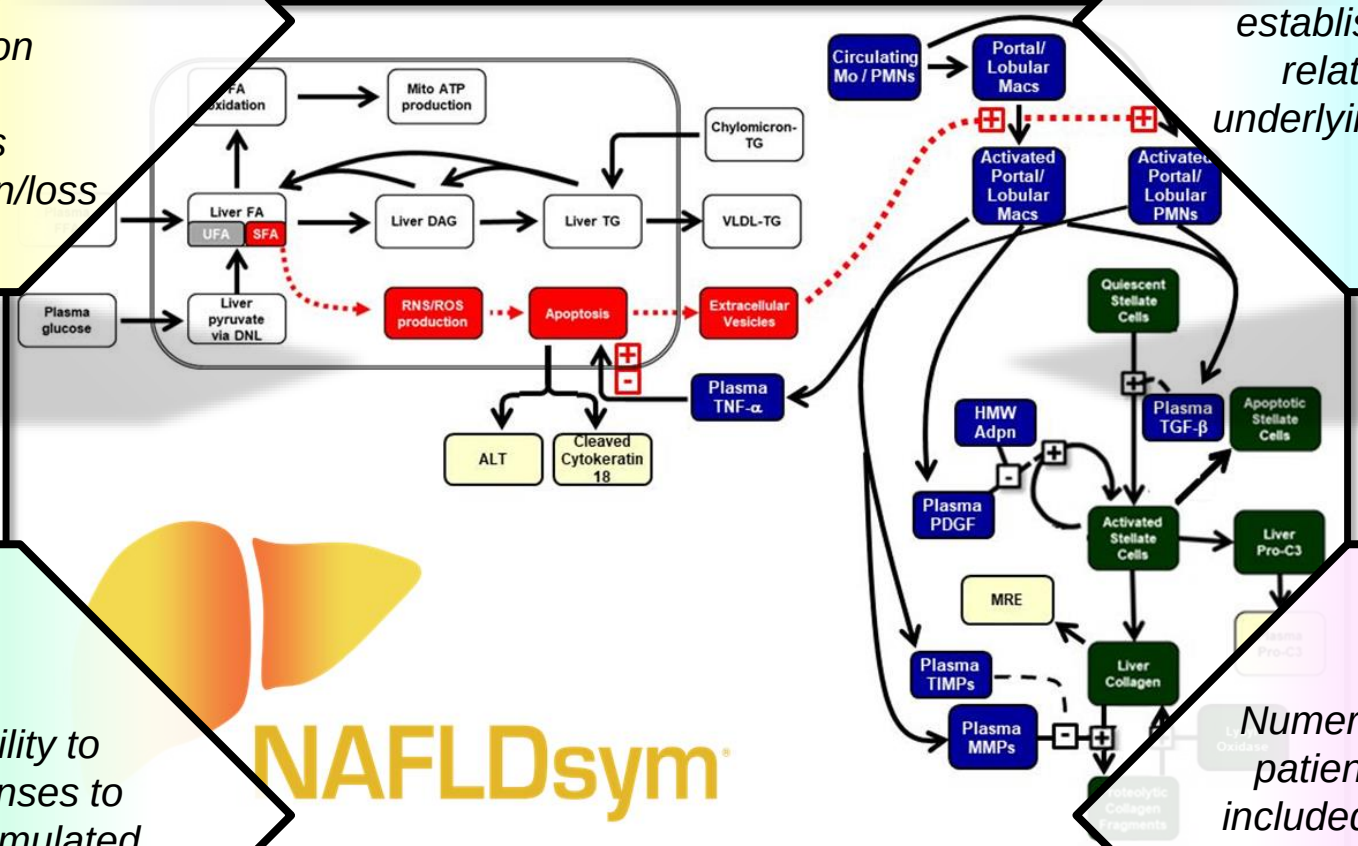
NAFLDsym v2A Overview

Multiple interacting sub-models, including

- *Steatosis*
- *Lipotoxicity*
- *Inflammation*
- *Fibrosis*
- *Biomarkers*
- *Weight gain/loss*

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



Provides ability to predict responses to treatment in simulated clinical trials

Numerous simulated patients (SimPops) included to account for pathophysiologic and clinical heterogeneity

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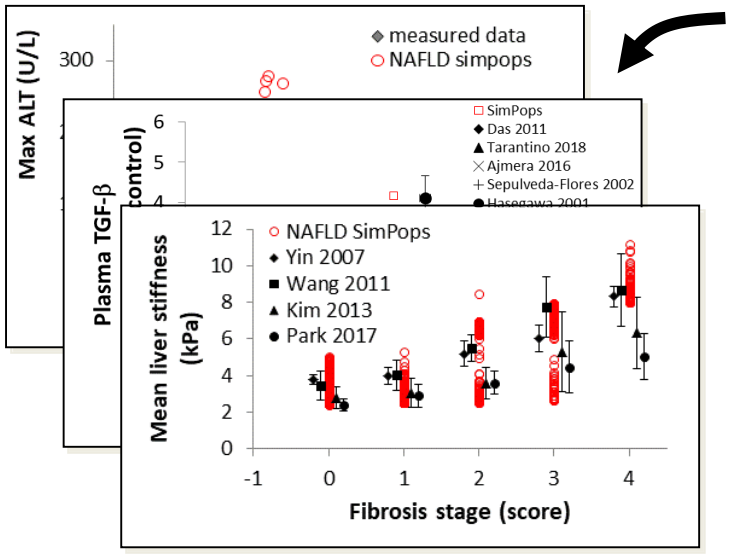
Pathophysiologic Variability Represented in NAFLDsym with NAFLD/NASH SimPops

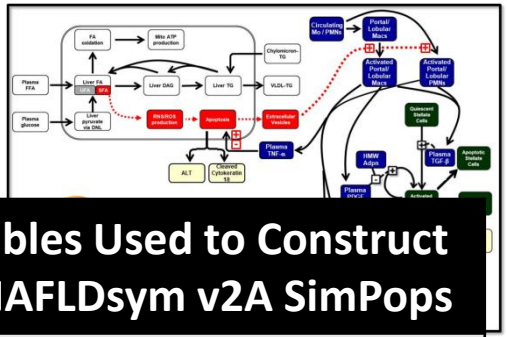
- SimPops are population samples with variability across key areas of NAFLD/NASH pathophysiology
- Multiple parameters are varied to produce diverse possible simulated patients
- Simulated patients are compared with a multitude of clinical data to validate pathophysiology
- Response data (e.g., dietary intervention) have been used to validate the SimPops



Maximos 2015, Das 2011, Tarantino 2018, Ajmera 2016, Sepulveda-Flores 2002, Hasegawa 2001, Yin 2007, Wang 2011, Kim 2013, Park 2017

Measured data

Simulation results



Variables Used to Construct the NAFLDsym v2A SimPops
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De novo lipogenesis
RNS-ROS clearance
Mitochondria function
VLDL-TG secretion rates
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Hepatic glucose uptake
Plasma TG clearance
Apoptotic sensitivity to RNS-ROS
Necrotic sensitivity to ATP reductions
Hepatocyte regeneration
Extracellular vesicle release
Inflammatory mediator production
Stellate cell activation
Collagen synthesis and degradation

Clinical Data and Simulation Results

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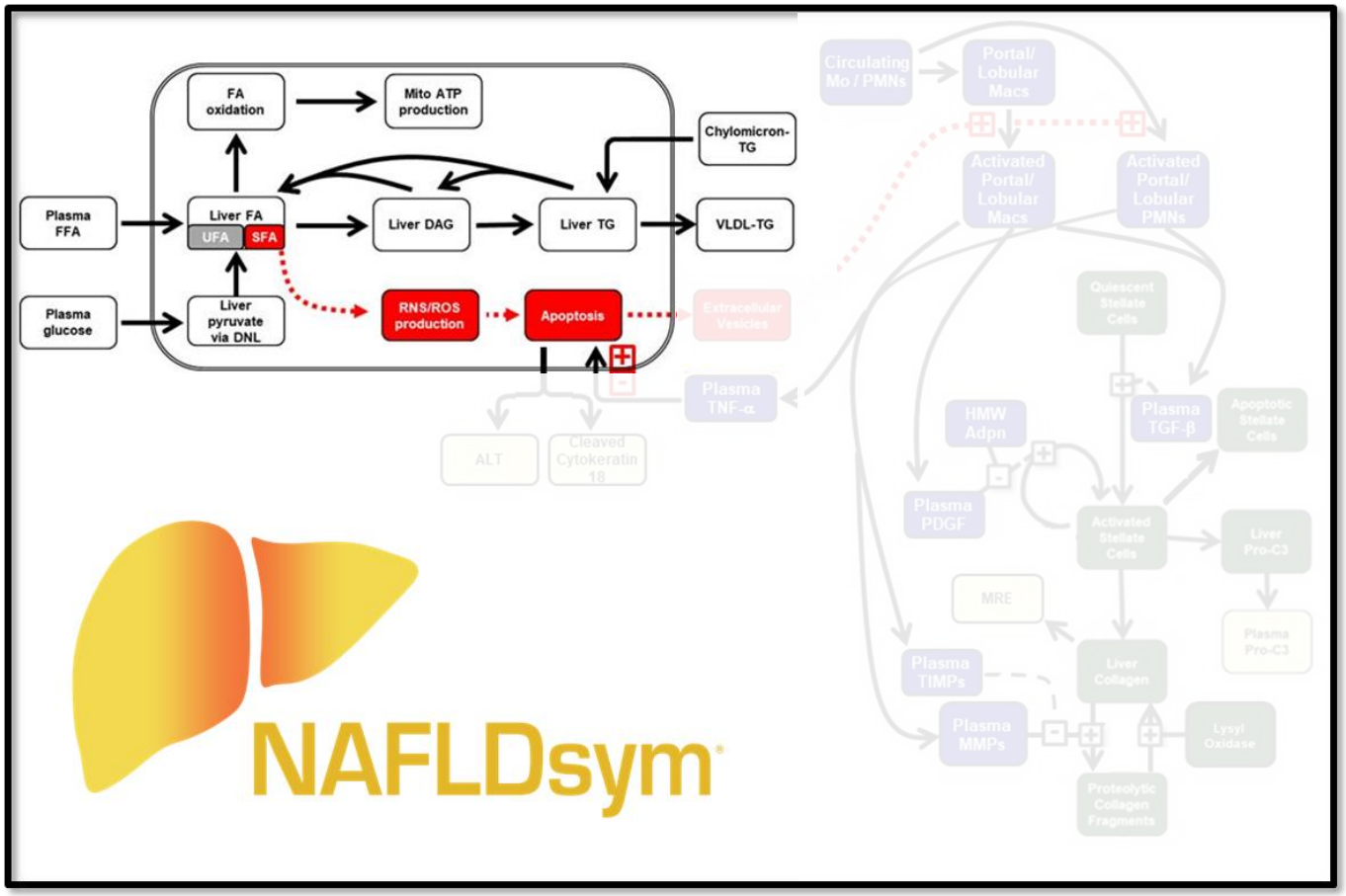
NAFLDsym v2A Overview: Steatosis-Lipotoxicity



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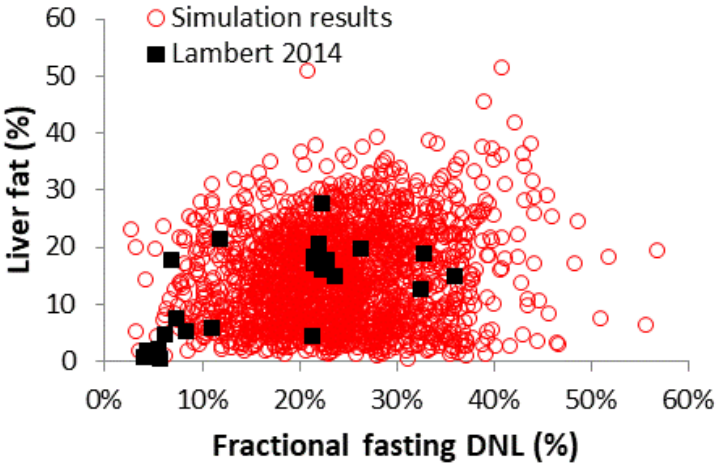
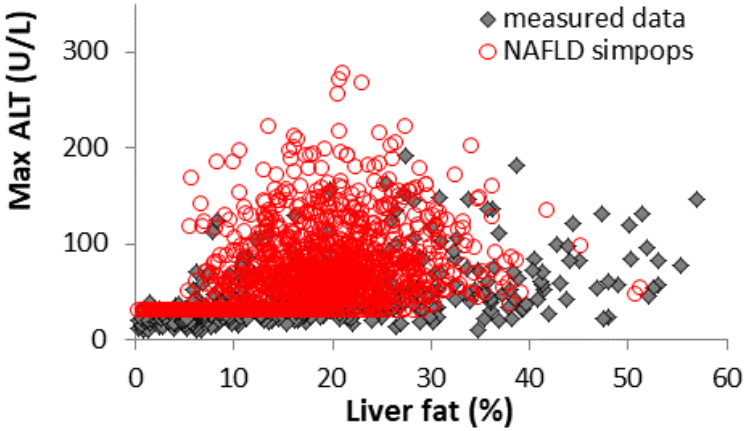
NAFLDsym v2A Includes Simulated Patients with Steatosis, DNL, and Liver Injury Consistent with Clinical Data

- 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



Lambert 2014



Maximos 2015

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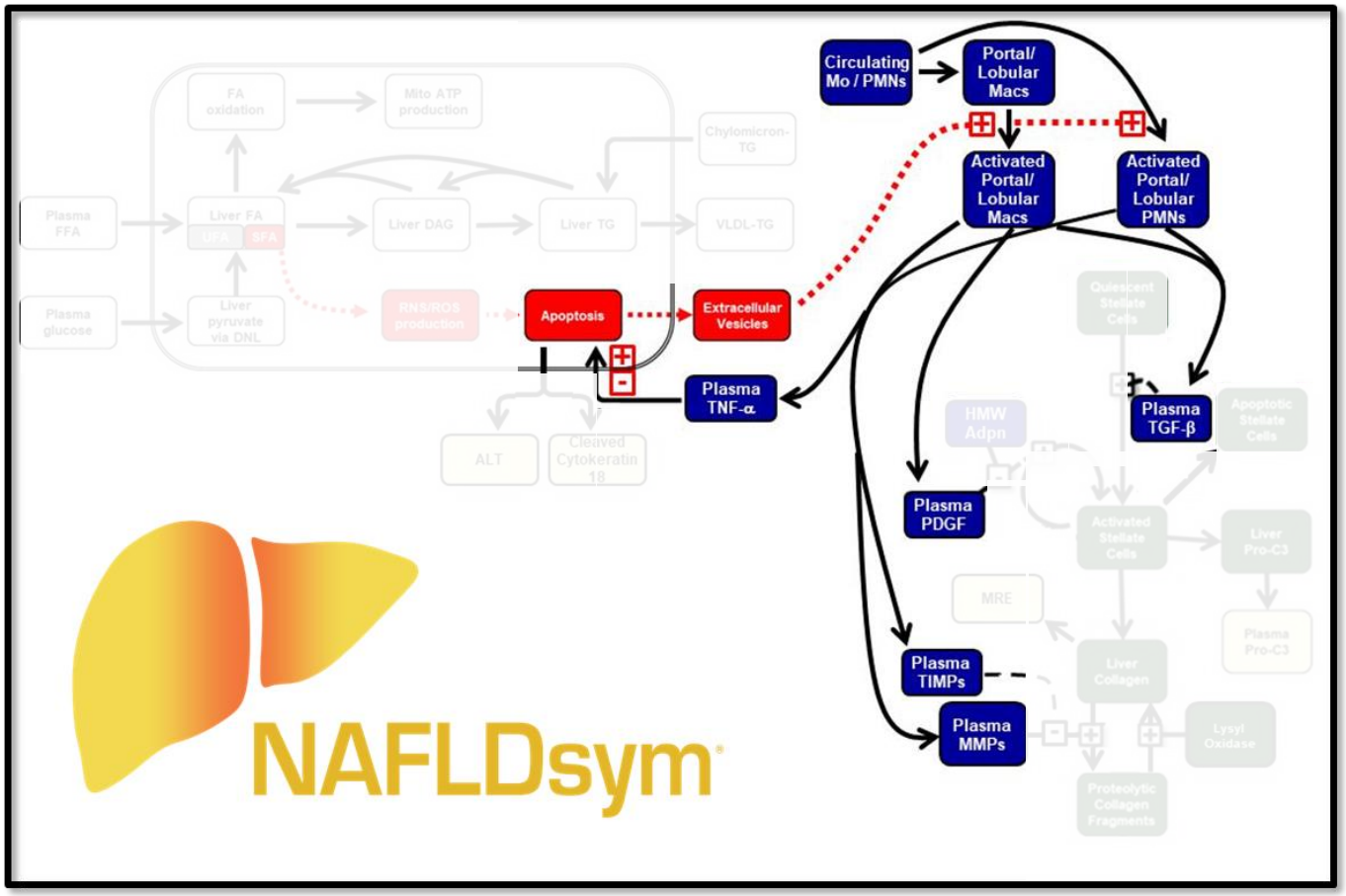
NAFLDsym v2A Overview: Inflammation



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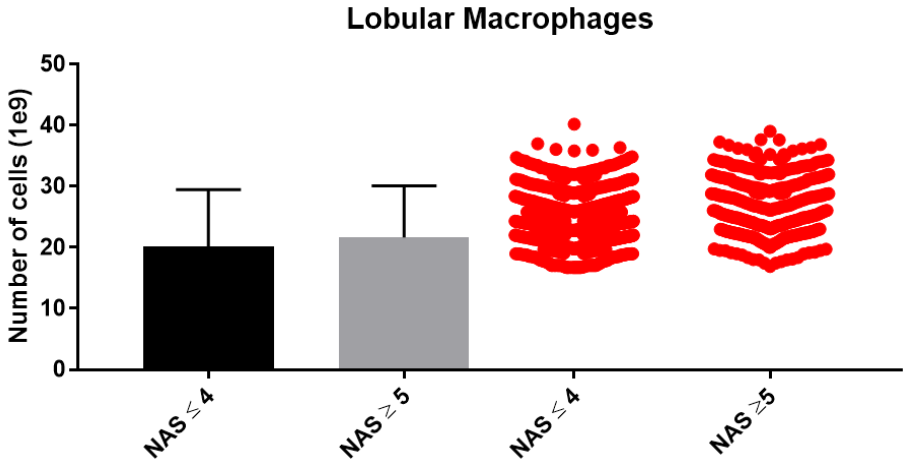
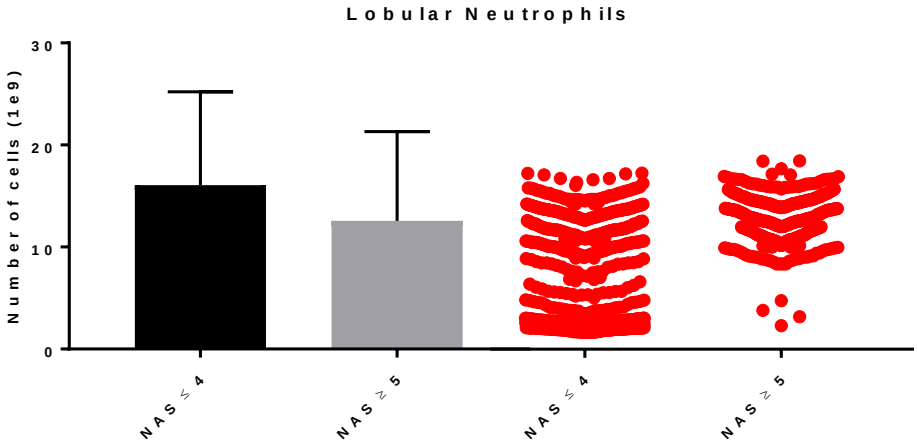
NAFLDsym v2A Includes Lobular Macrophage and Neutrophil Numbers Consistent with Clinical Data

- Multiple papers indicate that lobular inflammatory cell numbers do not appear to change with NAFLD disease severity (Krenkel 2017, Tajiri 2009, Rensen 2009, Leicester 2006)
- Lobular macrophages and neutrophils from simulated patients compare favorably with measured data





Tajiri 2009

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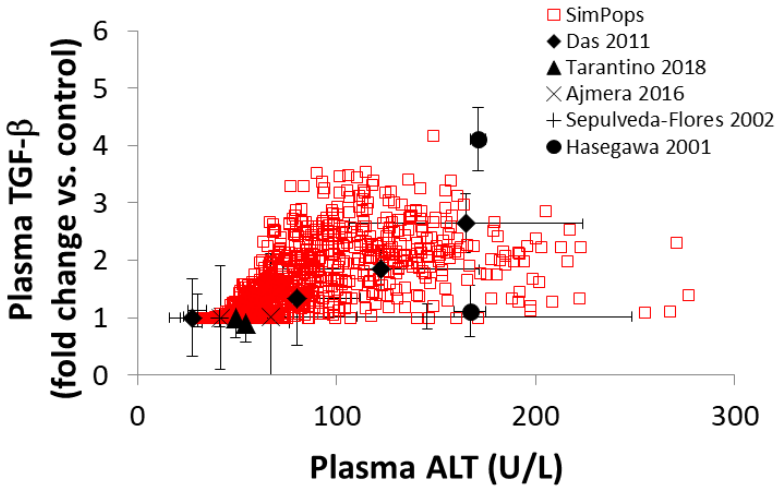
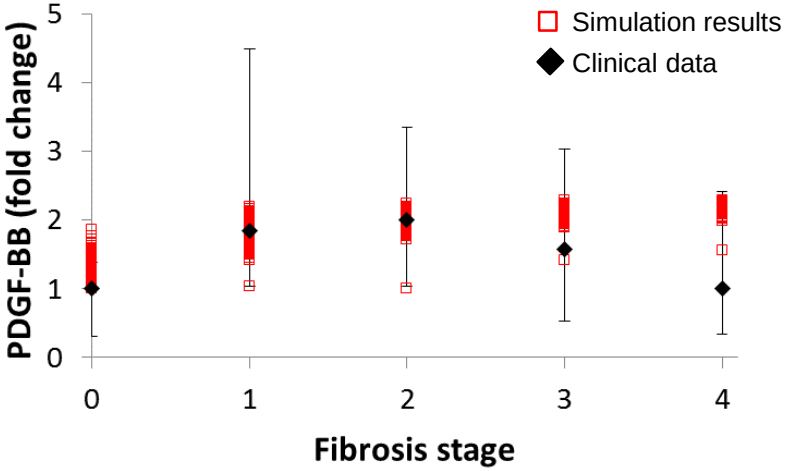
NAFLDsym v2A Include Mediators Consistent with the Majority of Data: TGF-β, PDGF

- Simulation results demonstrate modest increases in TGF-β consistent with the reported range (Das 2011)
  - Other data show no change or modest increases with disease severity
- Simulation results demonstrate modest increases in PDGF consistent with reported increases at lower Metavir fibrosis scores
  - Limited NAFLD data available; Yoshida et al. (2014) report serum levels from a cohort that includes 24% NAFLD patients





Yoshida 2014

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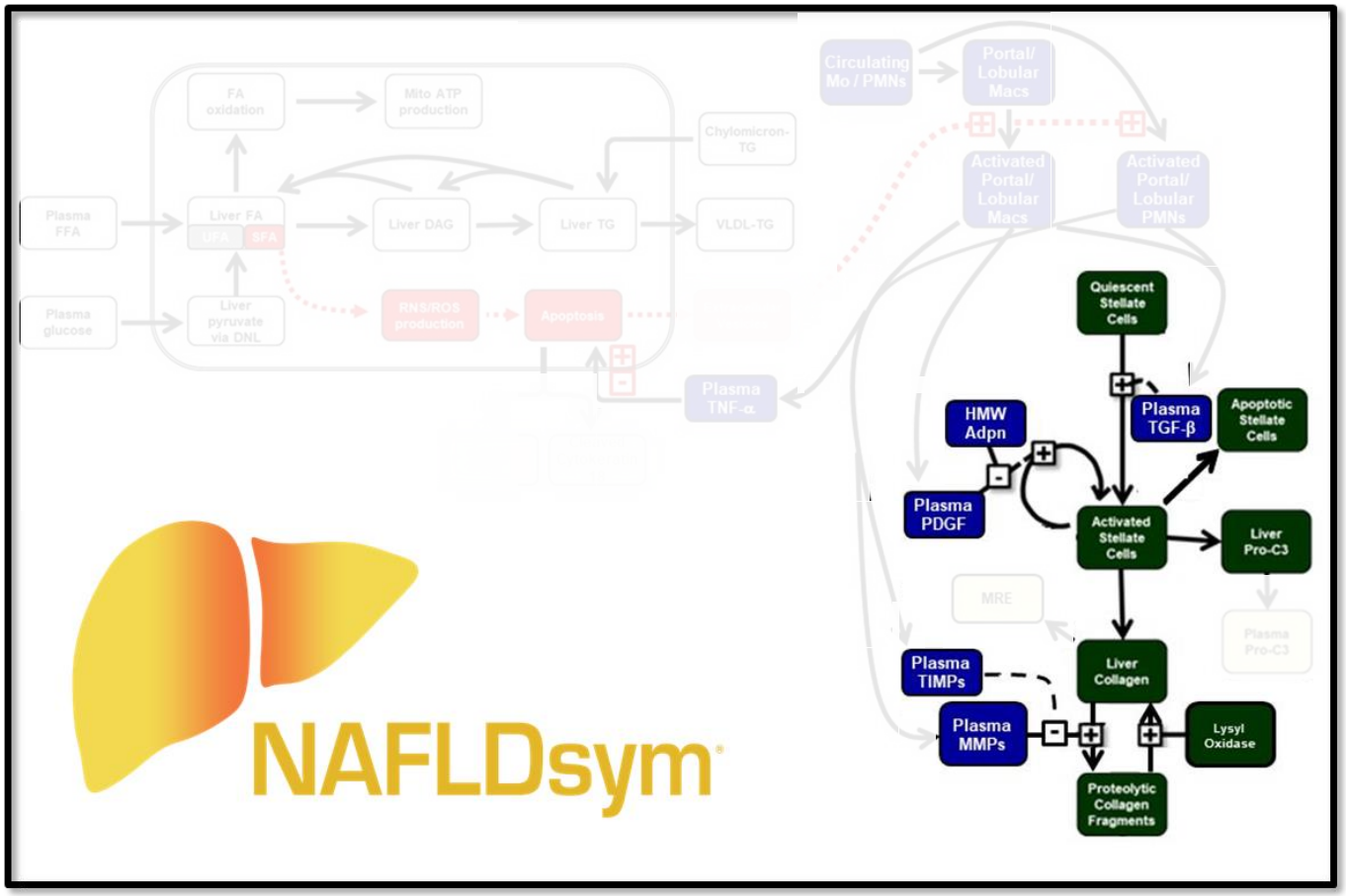
NAFLDsym v2A Overview: Fibrosis



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NAFLDsym v2A Includes Activated HSC Consistent with Clinical Data

- Simulated patients have increasing number of activated hepatic stellate cells (aHSC) with increasing fibrosis stage
  - Consistent with clinical data showing increased HSC activation with more extensive fibrosis (Abdeen 2009, El Gendi 2012, Washington 2000)

- Simulated number of aHSC varies across zones, with centrilobular (CL) predominance
  - Consistent with clinical data showing the degree of stellate cell activation in NASH patients was highest in CL (Washington 2000)
  - Driven by different Km values for TGF-β-induced HSC activation across zones
  - Contributes to zonal differences in histologic fibrosis stages (F1, F2, F3)

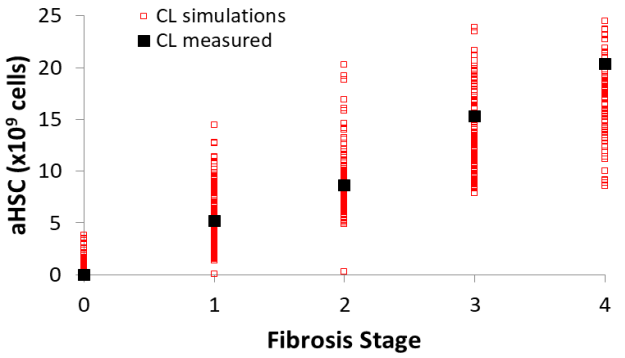
Increasing Km of HSC activation

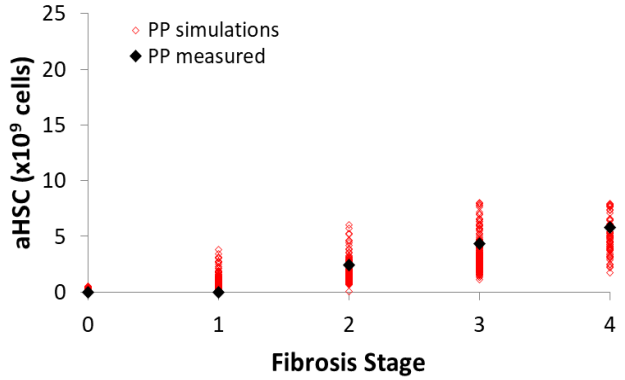
CL

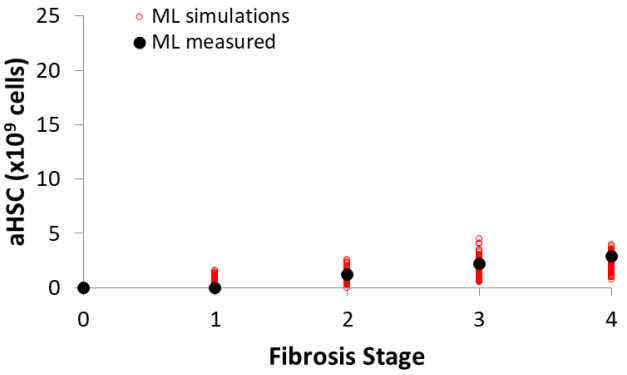
PP

ML

Abdeen 2009, El Gendi 2012, Washington 2000







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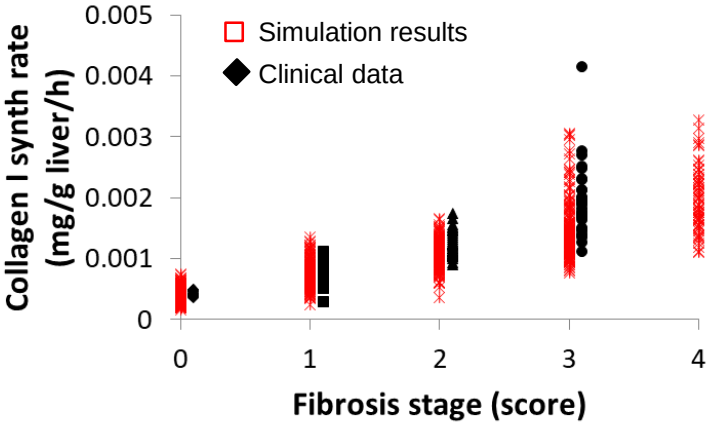
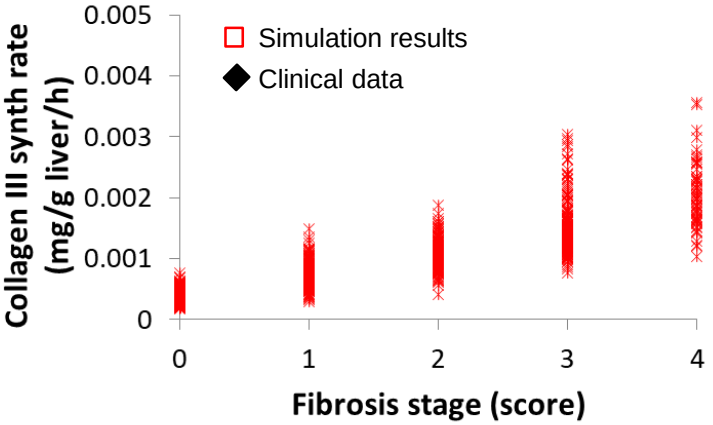
NAFLDsym v2A Has a Range of Collagen Synthesis Rates Consistent with Clinical Data

- Rates of collagen I synthesis are greater in higher fibrosis stages
  - Consistent with clinical data showing increased collagen synthesis rates in NASH patients (Decaris 2017)
  - Rates from Decaris et al. combined with collagen quantities from Masugi et al.
- Rates of collagen III synthesis are predicted to be greater in higher fibrosis stages
  - No clinical data in NASH patients for collagen III synthesis rates
  - Comparable to collagen I synthesis rates



Decaris 2017, Masugi 2018



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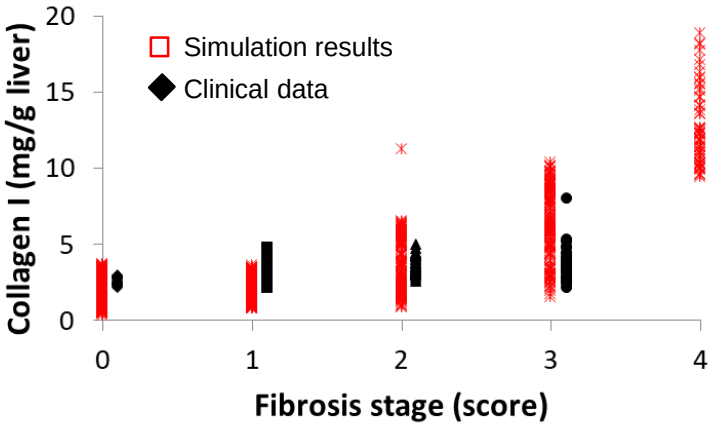
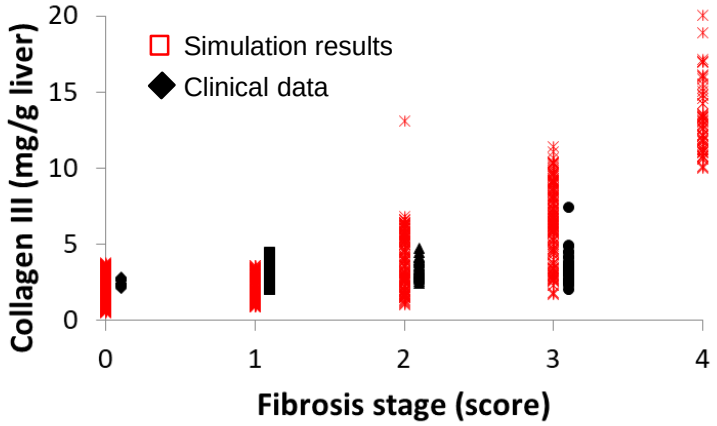
NAFLDsym v2A Has a Range of Hepatic Collagen Levels Consistent with Clinical Data

- Hepatic collagen I and III levels are comparable in fibrosis stages 0, 1, 2
  - Consistent with clinical data showing collagen levels in NASH patients (Masugi 2018)
  - Histologic assessment of collagen levels by Masugi et al. converted to collagen quantities by incorporating data from Aycock and Seyer and Nakabayashi et al.
- F3 and F4 collagen levels can dramatically exceed levels in F0-F2
  - Wide variability in clinical data and simulation results





Masugi 2018, Aycock 1989, Nakabayashi 1993

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NAFLDsym v2A SimPops Patients Include Common Measurements of Treatment Efficacy

Plasma Biomarkers	Histology Measurements	Imaging measurements
Plasma TG	Steatosis score	Liver fat percentage (MRI)
Plasma ALT	Ballooning score	Liver stiffness (MRE)
Plasma cytokeratin cleaved 18 (cK18)	Inflammation score	
Plasma free fatty acids	NAFLD Activity Score (NAS)	
Plasma adiponectin	Fibrosis stage	
Plasma TNF-α	Activated hepatic stellate cells	
Plasma TGF-β	Hepatic collagen	
Plasma Pro-C3		

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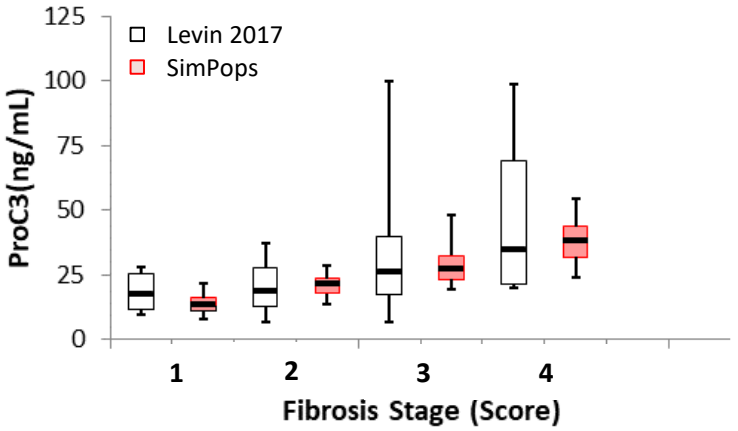
NAFLDsym SimPops Simulated ProC3 is Consistent with Reported Clinical Data

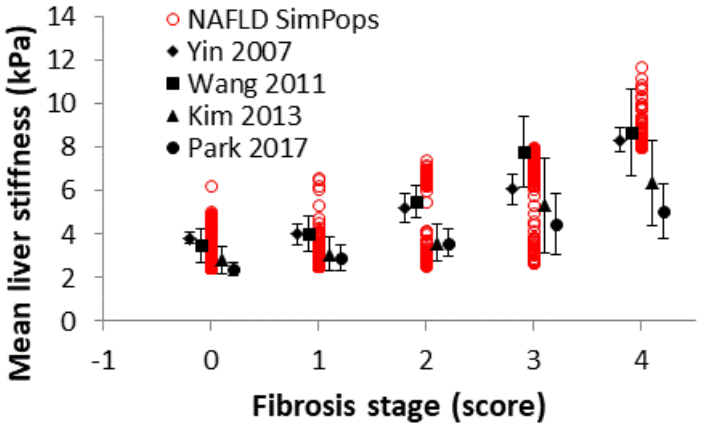
- ProC3 is a biomarker of collagen synthesis
  - Proteolytic fragment of procollagen 3 that enters the circulation
  - Production rate of ProC3 is based on the synthesis rate of collagen 3
- Simulated ProC3 aligns well with clinical data
  - Levin 2017 AASLD abstract/presentation



Levin 2017

NAFLDsym SimPops Simulated Liver Stiffness (MRE) is Consistent with Reported Clinical Data

- Liver stiffness is an imaging biomarker of hepatic collagen levels
  - Magnetic resonance elastography (MRE)
- NAFLDsym includes liver stiffness as an output
  - Liver stiffness levels correspond with amount of hepatic collagen
- Simulated liver stiffness aligns well with clinical data
  - Yin 2007, Wang 2011, Kim 2013, Park 2017



Yin 2007, Wang 2011, Kim 2013, Park 2017

Pathophysiologic Variability Represented in NAFLDsym with NAFLD/NASH SimPops

- SimPops are population samples with variability across key areas of NAFLD/NASH pathophysiology
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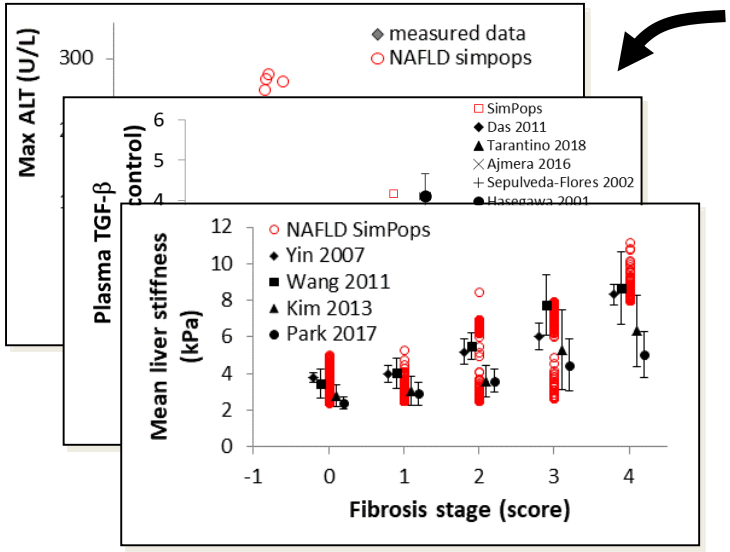
- **Response data (e.g., dietary intervention) have been used to validate the SimPops**



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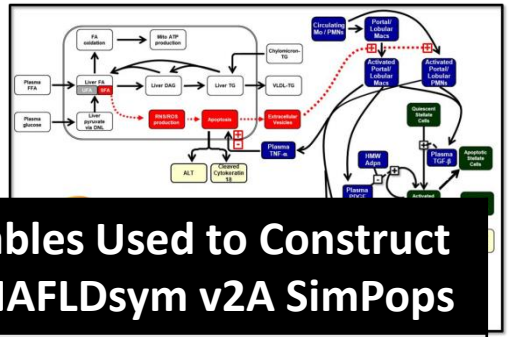
◆ Measured data

○ Simulation results



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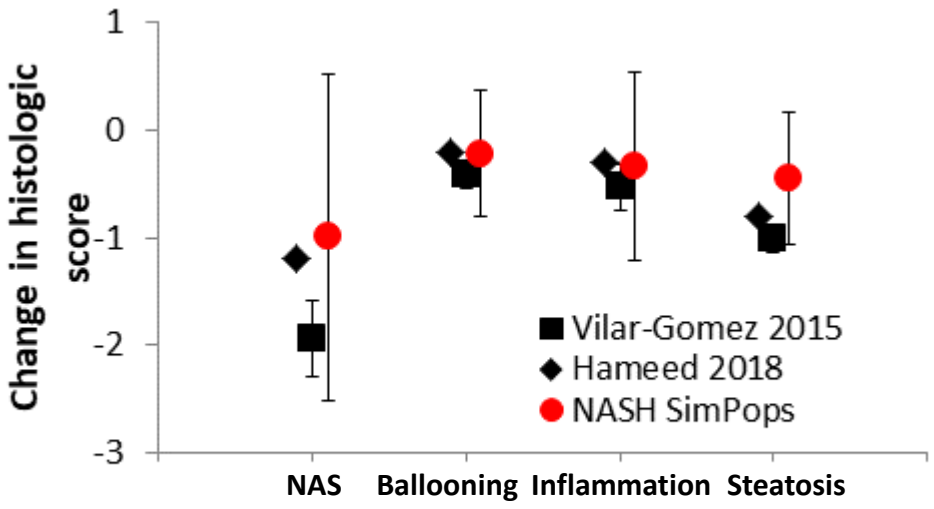
NAFLDsym v2A SimPops Predicted Response to Weight Loss is Consistent with Clinical Data: NAS

- Weight loss has been shown to improve NASH and fibrosis
  - Current standard of care
  - Greater efficacy with greater weight loss

- Simulated ≈5% weight loss over 1 year
  - Comparable to data from clinical studies by Vilar-Gomez 2015 and Hameed 2018
  - Compared predicted changes in NASH biomarkers with clinical data
  - Good agreement between predicted changes in NAS score and components and clinical data

- Provides validation for NAFLDsym v2A SimPops

Emergent behavior



Vilar-Gomez 2015, Hameed 2018

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NAFLDsym v2A SimPops and SimCohorts

Validation filtering

Interim General SimPops

Trx Validation: Weight loss

NAFLD/NASH SimPops

SimCohorts for prospective clinical studies can be selected from SimPops

Prospective SimCohorts

NAS ≤3 SimCohorts

NAS ≥4 SimCohorts

F0 SimCohorts

F3 SimCohorts

F1 SimCohorts

F4 SimCohorts

F2 SimCohorts

Progression SimCohorts: Weight gain/loss

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- Introduction to NAFLDsym Modeling Software
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- NAFLDsym Licensing and Services Projects

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- Introduction to NAFLDsym Modeling Software
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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

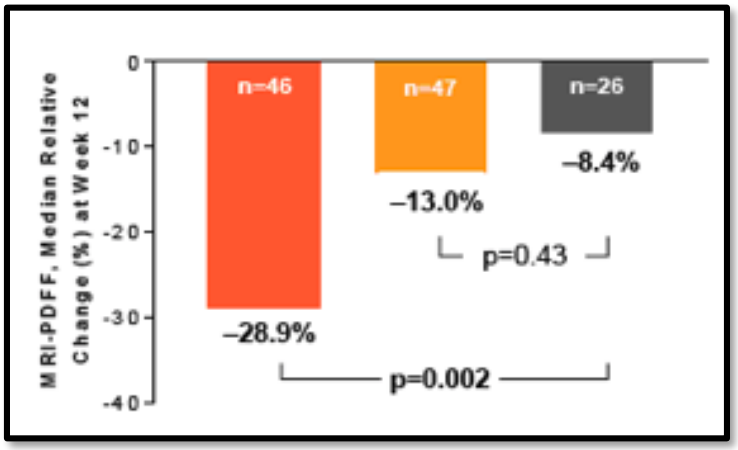
- NAFLDsym employed to evaluate MoA hypotheses
  - Developed PBPK model of GS-0976 (exposure)
  - Utilized existing preclinical and clinical data to determine PD parameters
  - Utilized existing simulated patients to generate appropriate SimCohorts

- 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

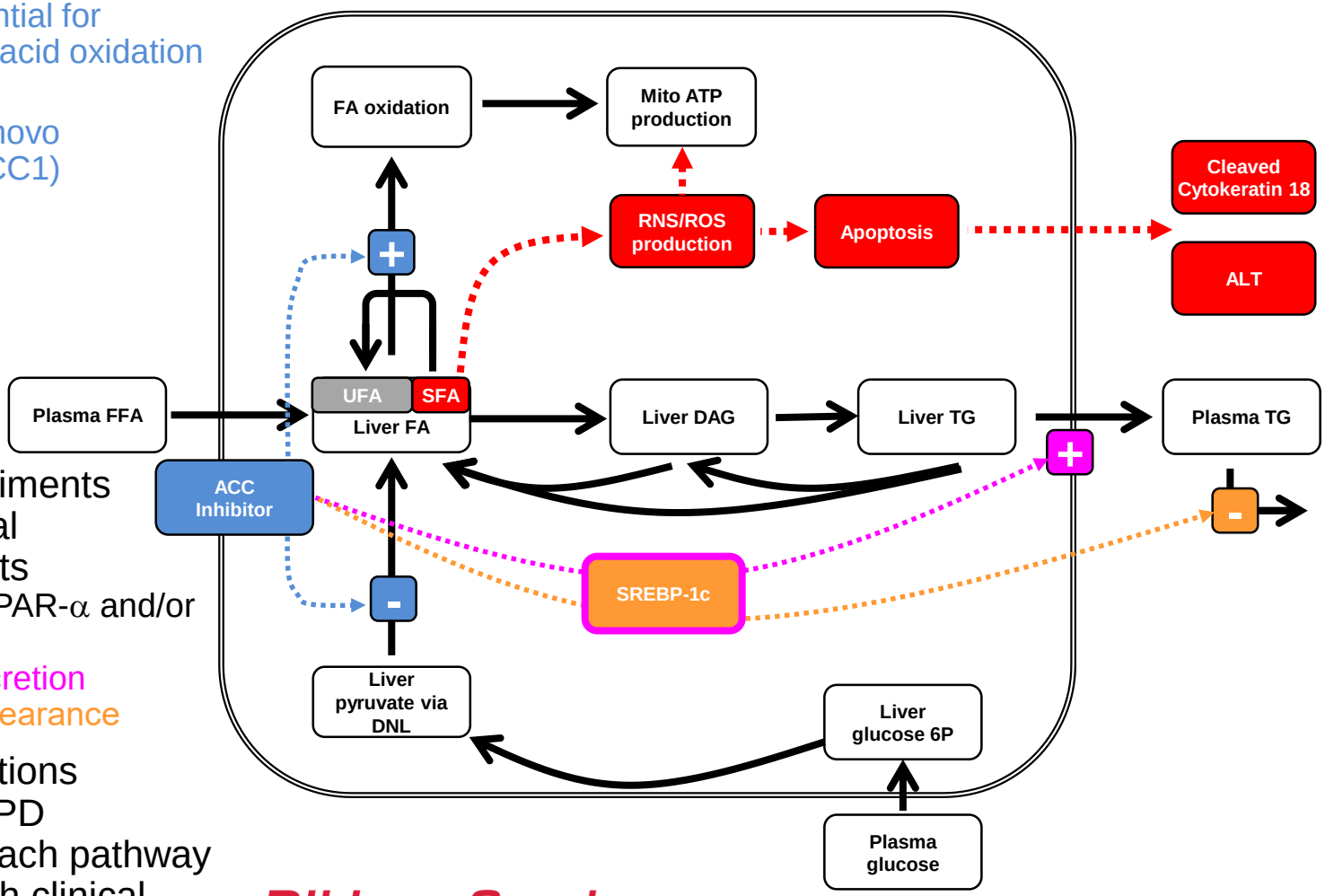
Table: Lipid Parameters in Subjects Treated with GS-0976 20 mg Daily for 12 Weeks

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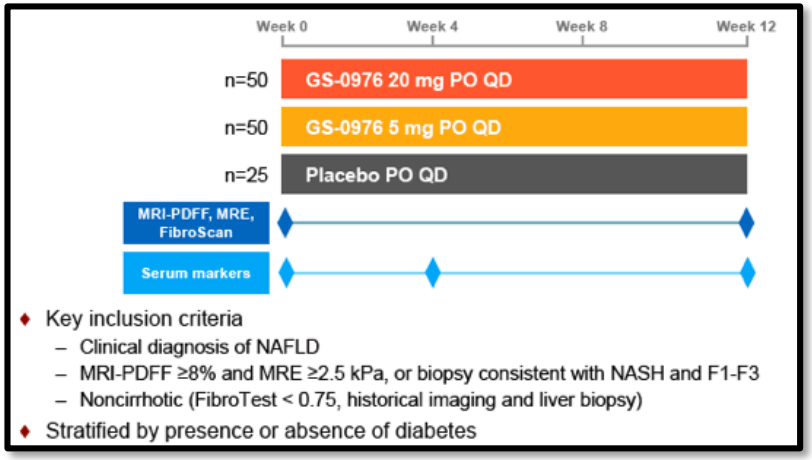
Good Agreement between GS-0976 Phase 2 Clinical Data and NAFLDsym Simulation Results Validates MoA Hypotheses



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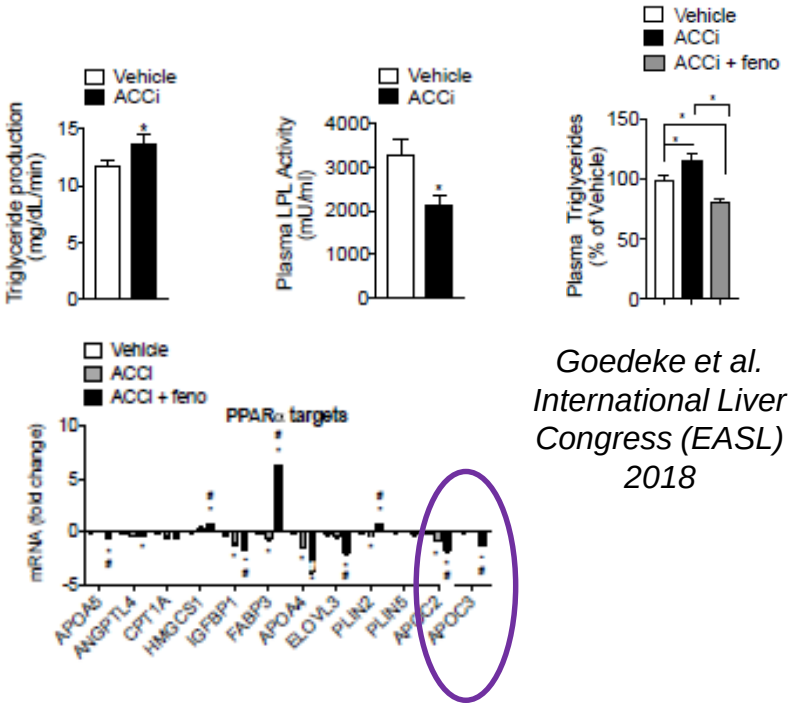
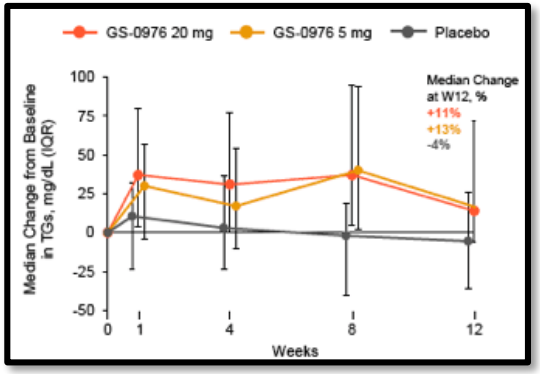
Co-administration of Fibrates with GS-0976 Can Ameliorate Plasma TG Increases

- 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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Outline

- Introduction to NAFLDsym Modeling Software
- Demonstration of NAFLDsym v2A Software
- Example NAFLDsym Application
- NAFLDsym Licensing and Services Projects

General Project Timeline and Deliverables

Stage I Project Activities
- Updates to NAFLDsym to represent NAFLD therapeutic strategies
- Represent Compound A PK and PD

Stage II Project Activities
- Simulations of Compound A prior studies in representative NAFLD patients
- Simulations of Compound A prior studies in NAFLD human SimPops

Stage III Project Activities
- Simulations of Compound A future clinical trial protocols in representative NAFLD patients
- Simulations of Compound A future clinical trial protocols in NAFLD human SimPops

Stage 1 | Stage 2 | Stage 3

Kickoff meeting → **Milestone 1 meeting** → **Milestone 2 meeting** → **Milestone 3 meeting**

Final Deliverables
- Final report summarizing findings shortly after project completion

Project costs are dependent upon required resources

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NAFLDsym v2A License Provides Opportunity to Actively Utilize QSP Model

- A license to NAFLDsym v2A is $67,500 per year for 1 instance
  - Includes capabilities of predicting effects of treatments on steatosis, lipotoxicity, inflammation, and fibrosis in NAFLD/NASH patients
  - Includes 10 hours of training
  - Local desktop installations only
  - No network shareable licenses
  - Must be renewed annually
  - Additional licenses can be made available at reduced, volume pricing
- Equations can be viewed by users
  - Can be modified to represent novel targets within NAFLDsym v2A
  - No original NAFLDsym v2A code can be ported out to other MATLAB files or languages without the permission of DILIsym Services

DILIsym Services

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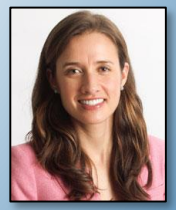

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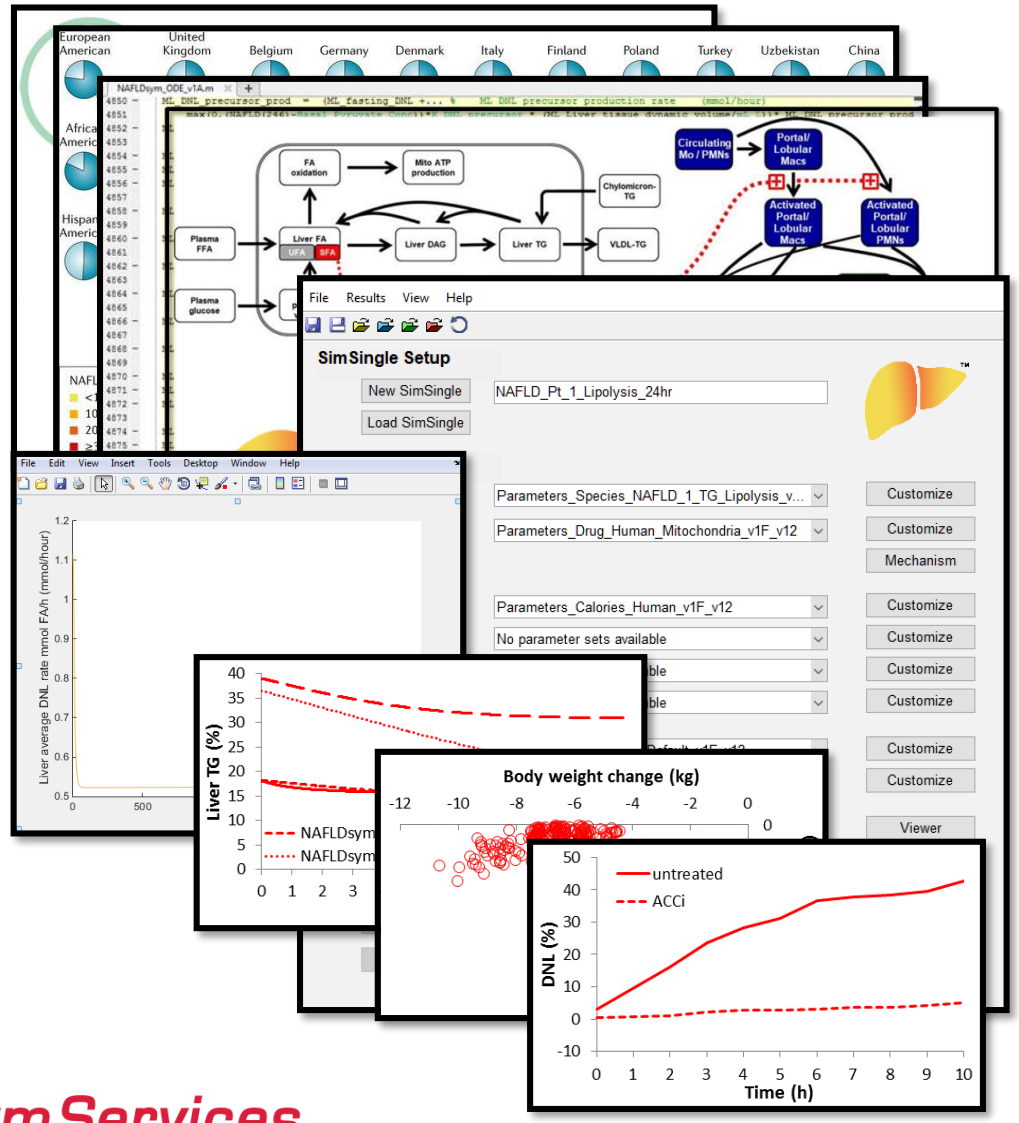
NAFLDsym Is Designed to Support Drug Development with Efficacy Predictions

- NAFLD is a progressive disease of the liver
- 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/NASH
  - NAFLDsym v2A includes steatosis, lipotoxicity, inflammation, and fibrosis sub-models; available Q2 2019
  - Includes pathophysiologically diverse simulated patients in SimPops
- 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
  - Provides ability to evaluate combinations of treatments with different mechanisms of action
- NAFLDsym has been used in collaborative research agreements with Pfizer, Gilead and other companies to inform clinical programs



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