DILIsym Services

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NAFLDsym Application Showcase: Key Examples of NAFLDsym Use within Drug Development

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May 20, 2021

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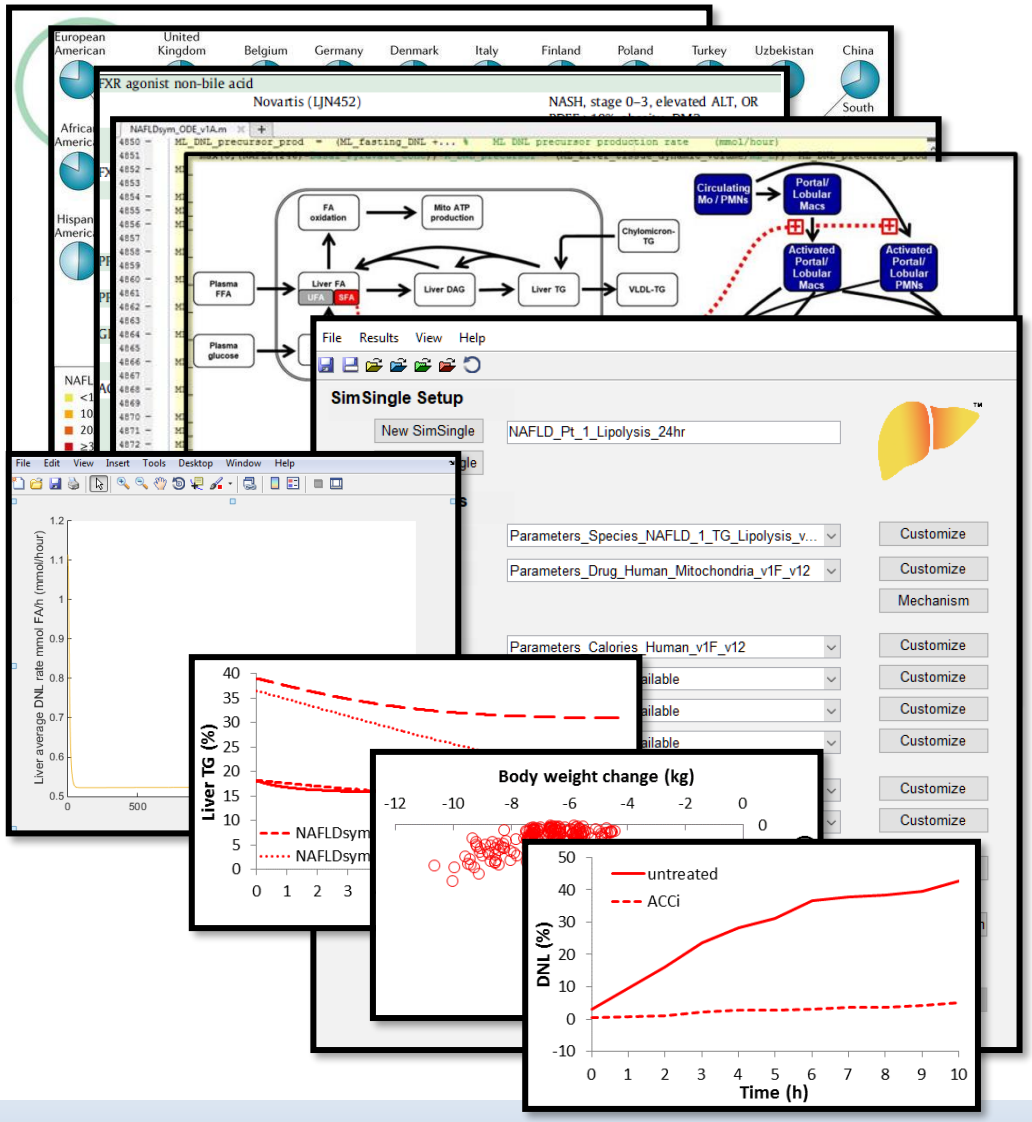
NAFLDsym Is Designed to Support Drug Development with Efficacy Predictions

- NAFLD/NASH 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
- Numerous potential treatments in development
  - Recent setbacks and terminations
- NAFLDsym is a QSP model of NAFLD/NASH
  - NAFLDsym v2A includes steatosis, lipotoxicity, inflammation, and fibrosis sub-models; available now
  - 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 to evaluate >20 NASH compounds and targets
  - Pfizer, Gilead, Genentech, BMS, and other companies to inform clinical programs



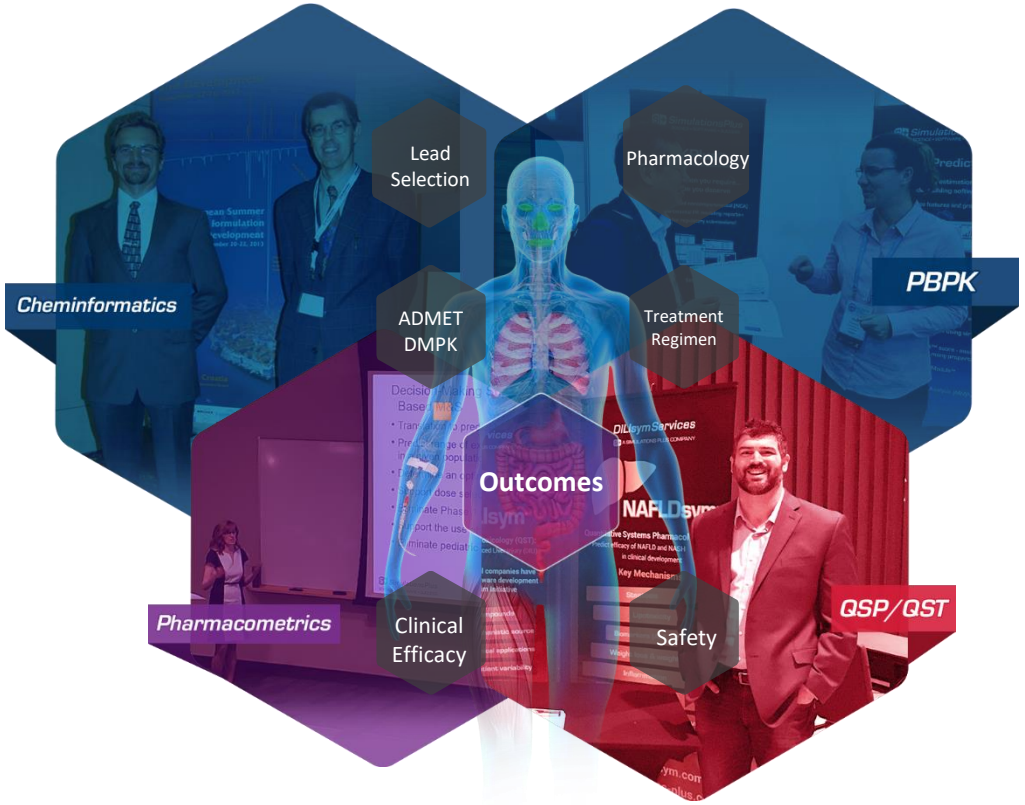
Agenda

- Overview of NAFLDsym v2A
- Simulations of Cenicriviroc Predict Lack of Efficacy
- Simulations of BFKB8488A Provided MoA Understanding and Supported Dosing Paradigm Selection in Subsequent Studies
- Impact of Lifestyle: Impact of Simulated Weight Loss and Weight Gain on Predicted Efficacy
- Q & A

At *SimulationsPlus* We Put It All Together

Science

- Seamless collaboration
- Integrated, innovative solutions to meet <u>your</u> needs



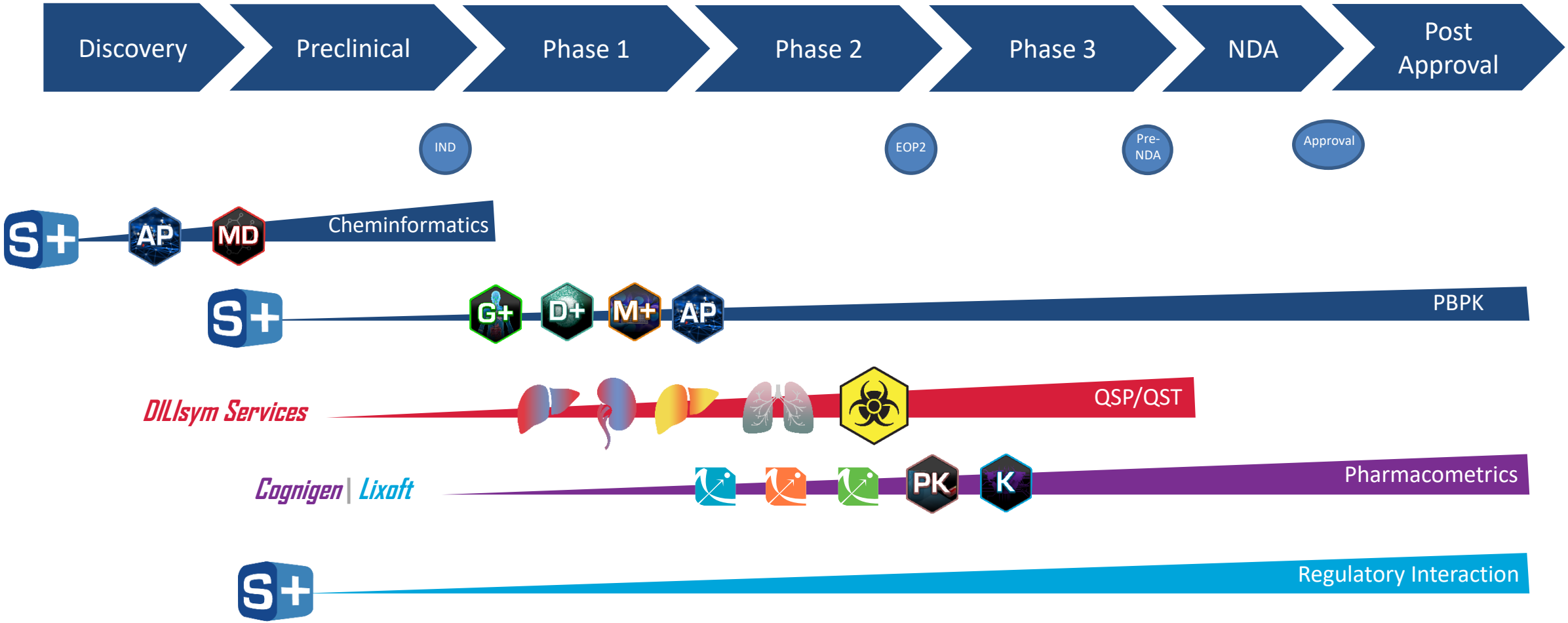
Business

- Resources available to get the job done on time
- One-stop shopping – single vendor for all of your *in silico* drug development needs

We have the *Solutions* and the *People* to Address *<u>Your</u>* Drug Development Questions!

NASDAQ: SLP

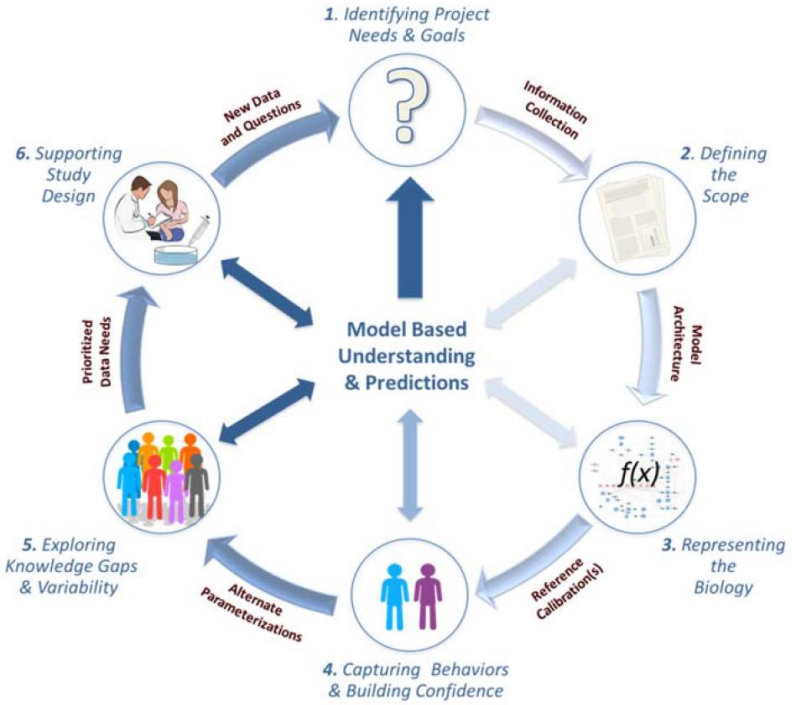
Our Solutions Inform the Entire Drug Development Process



NASDAQ: SLP

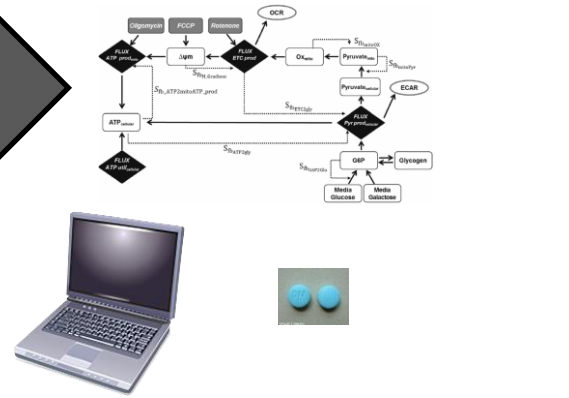
DILIsym Services Division of Simulations Plus: Mechanistic, QSP/QST Modeling

Established QSP and QST modeling methodologies



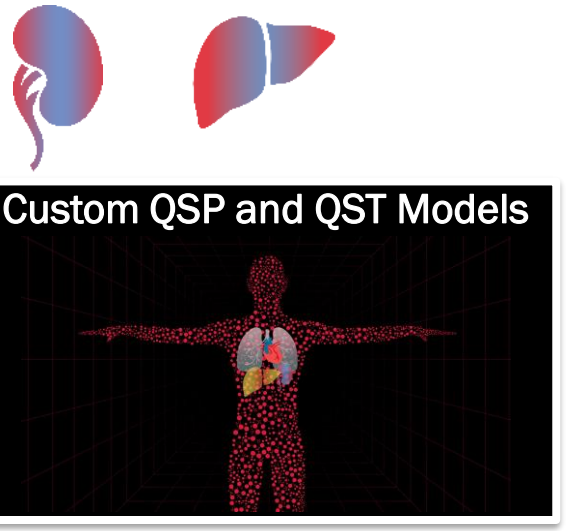
Gadkar 2016

Develop QSP and QST models using established methods



$$\frac{d[ATP]_{cellular}}{dt} = Eff\_Flux^{ATP}_{mito,\ lowgrad} + Eff\_Flux^{ATP}_{gly} - k * [ATP]_{cellular}$$

QSP and QST models for use in supporting compound development



DILIsym Services Division of Simulations Plus: Mechanistic, QSP/QST Modeling

Innovation: **pursuing novel and creative solutions to positively impact the world**

Respect: **promoting a diverse workforce and inclusive culture, while serving our communities**

Integrity: **thoroughly and accurately communicate with uncompromised truth and honesty**

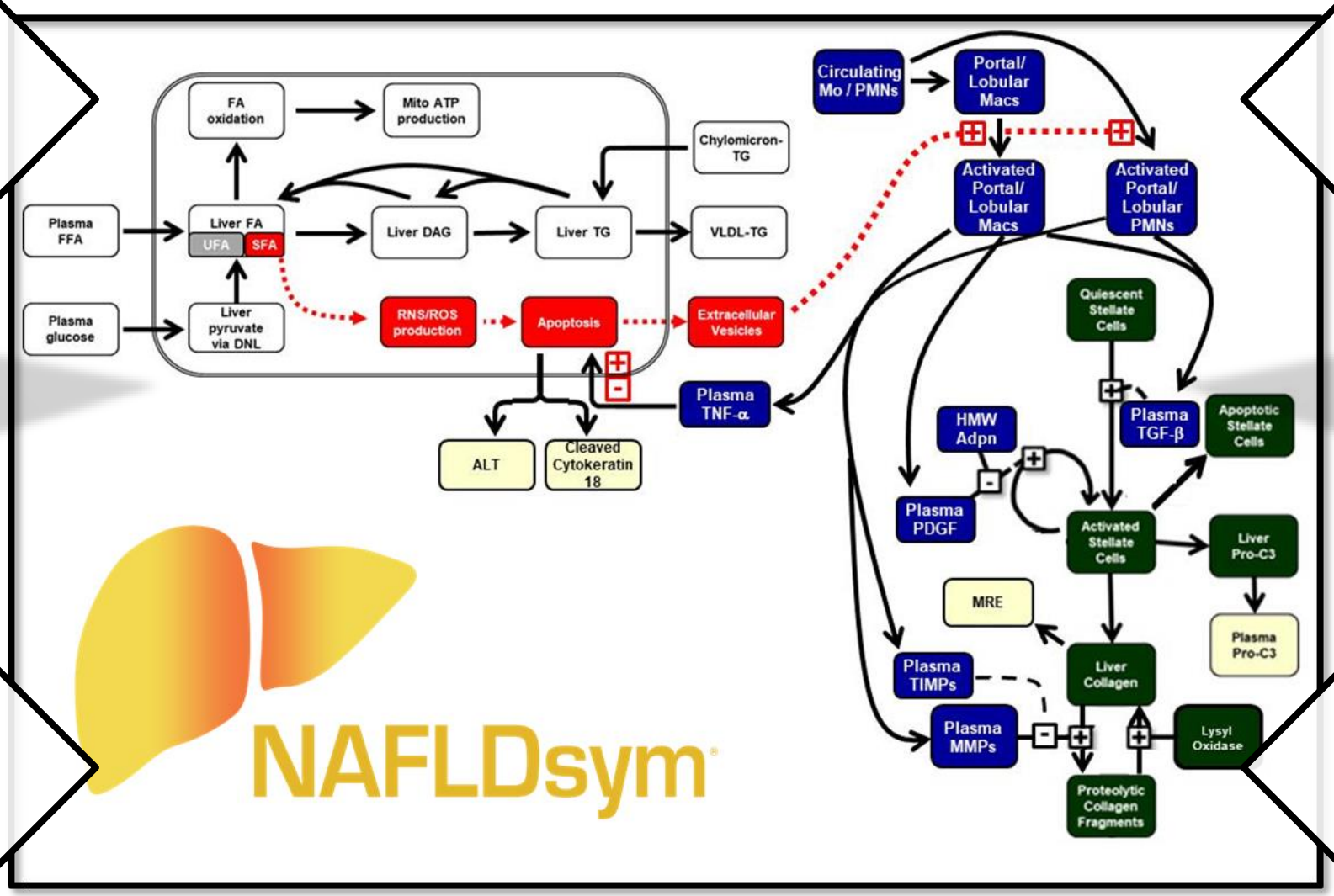
Commitment: **providing quality products and exceptional services that deliver value to our partners and the people we serve**

- Deep knowledge in multiple disease areas, including treatment and toxicity
- **DILIsym and RENAsym** software licensing, training, development (DILI-sim, RENAsym consortia)
- **NAFLDsym** and **IPFsym** software licensing, training, development
- **DILIsym, NAFLDsym,** and **IPFsym** simulation consulting projects
- **Custom QSP model development** and simulation consulting projects
- Drug development consulting and data interpretation; *in vitro* assay experimental design and management

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

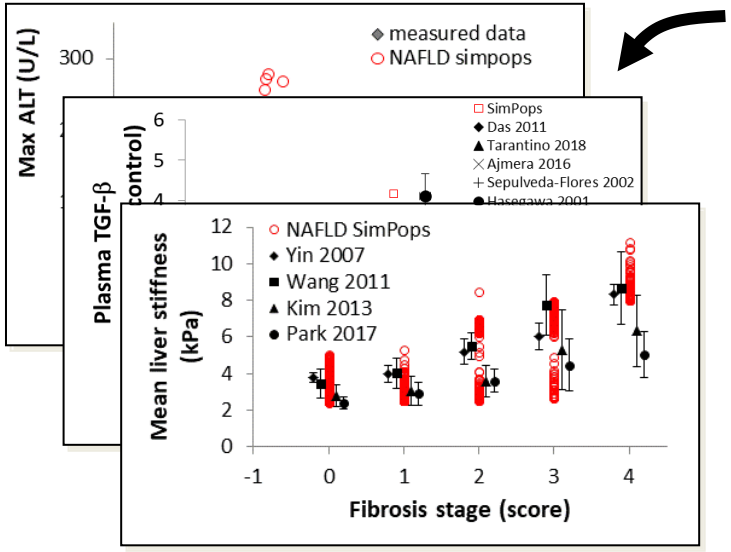
Numerous simulated patients (SimPops) included to account for pathophysiologic and clinical heterogeneity

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 Pro-C3	Activated hepatic stellate cells	
	Hepatic collagen	
	Collagen Percent Area (CPA)	

Pathophysiologic Variability Represented in NAFLDsym with NAFLD/NASH SimPops

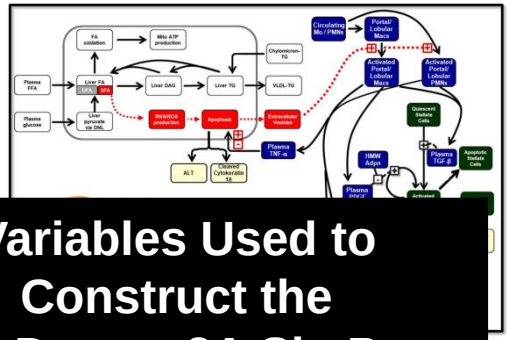
- SimPops are population samples with variability across key areas of NAFLD/NASH pathophysiology
- Multiple parameters are varied to produce diverse possible simulated patients
- Simulated patients are compared with a multitude of clinical data to validate pathophysiology
- Response data (e.g., dietary 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
Body weight
Adipose FA release
De novo lipogenesis
RNS-ROS clearance
Mitochondria function
VLDL-TG secretion rates
Plasma glucose
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

Agenda

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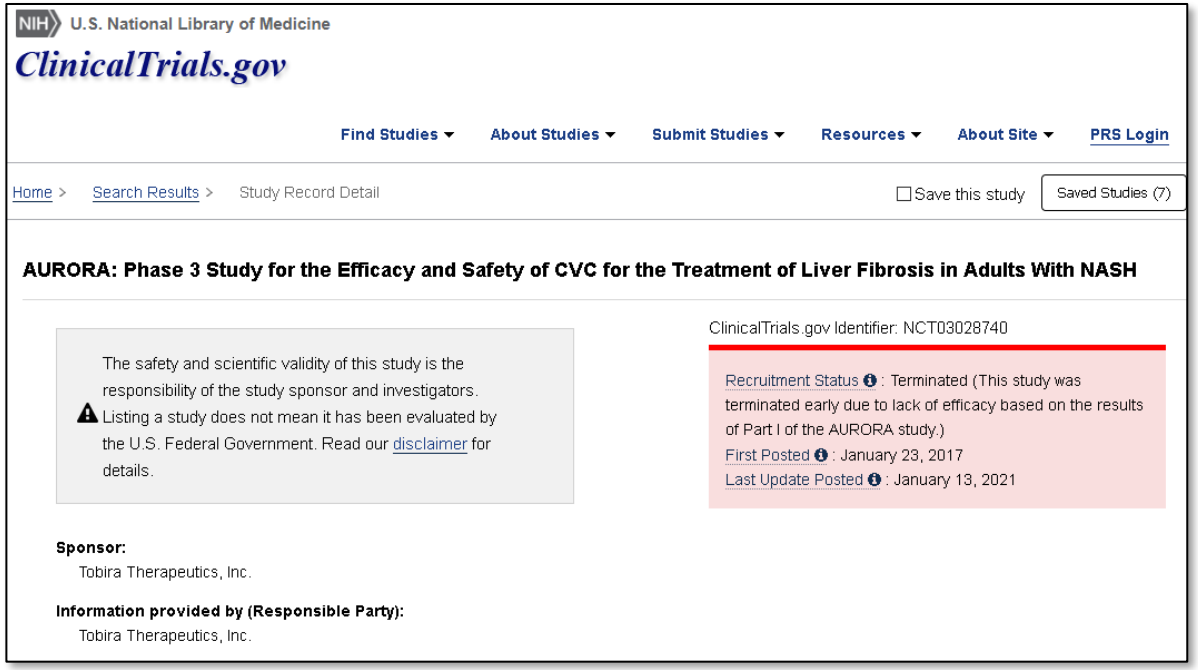
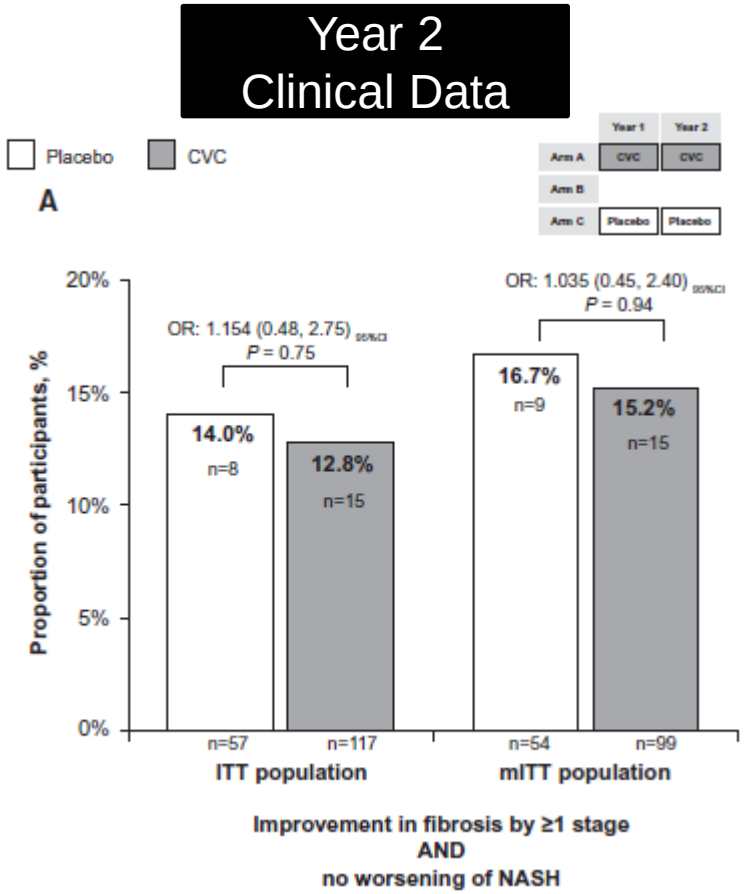
Clinical Potential of CVC Could Have Been Investigated Using NAFLDsym Prior to Clinical Trial

- Cenicriviroc (CVC), a dual CCR2/5 antagonist, was being developed to treat patients with NASH
  - CVC thought to be potent anti-inflammatory and anti-fibrotic therapeutic
  - CVC reduces recruitment of macrophages and activation of hepatic stellate cells
- CVC represented in NAFLDsym in late 2020
  - NAFLDsym predicted minimal efficacy in NASH cohort consistent with clinical patient characteristics from CVC trial
- Phase 3 AURORA study terminated in January 2021 due to lack of efficacy



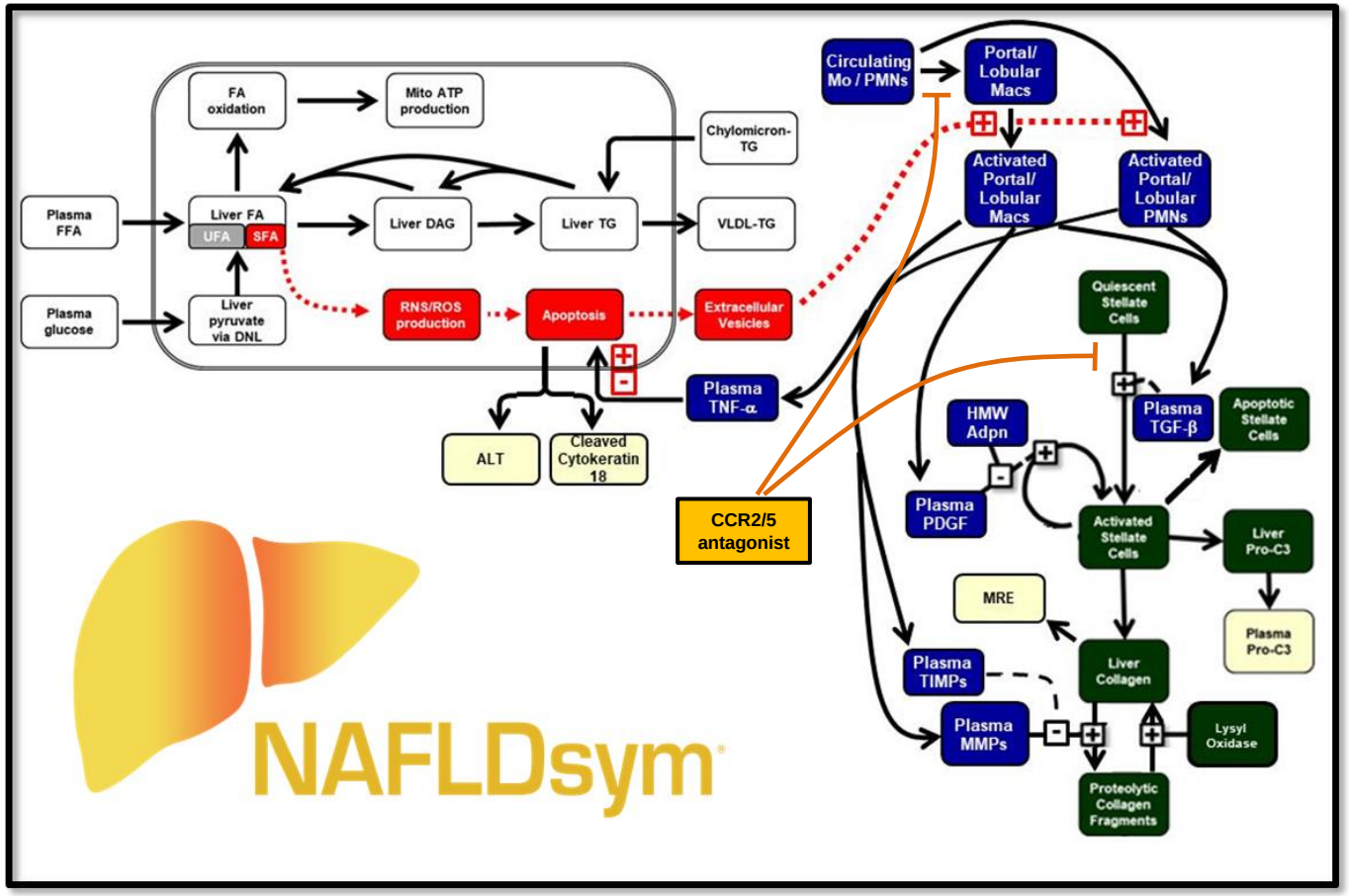
Final CENTAUR Data Indicate Modest Fibrosis Improvement, Similar to Placebo

- Year 1 data indicated CVC primary outcome not met but secondary achieved significance (Friedman 2018)
  - Primary: ≥ 2-point NAS improvement, no fibrosis worsening (*P* = 0.5)
  - Secondary: ≥ 1 stage improvement in fibrosis with no NASH worsening (*P* = 0.02)
- In year 2 final analysis, CVC did not maintain significant difference from placebo (Ratziu 2020)
  - Neither primary nor secondary outcome



Ratziu 2020

CVC Represented as Affecting Monocyte/Macrophages and Stellate Cells in NAFLDsym

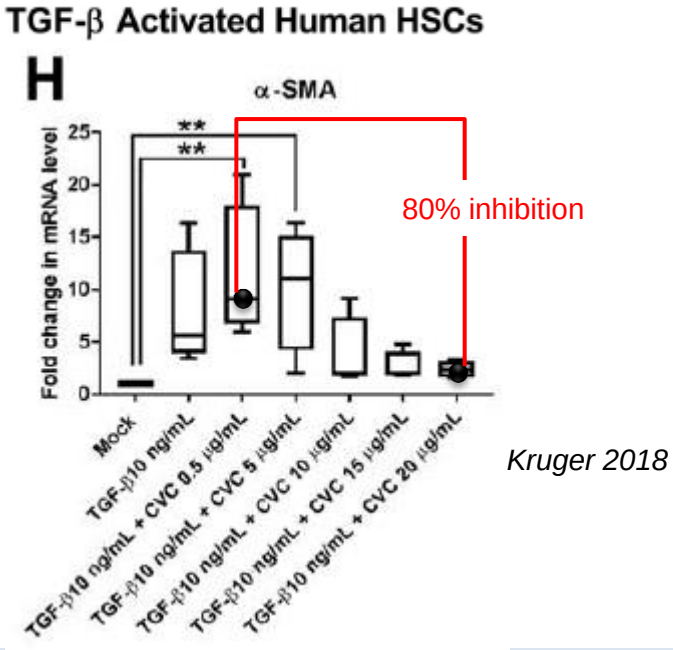


CVC Mechanism of Action Parameter Values Derived from Published Literature

CVC on activation of HSCs

- Data indicate maximum inhibition of 80%
- Inhibitory response engaged only when CVC >5 µg/mL
  - Neglects potentially stimulatory response at low CVC concentrations

in vitro

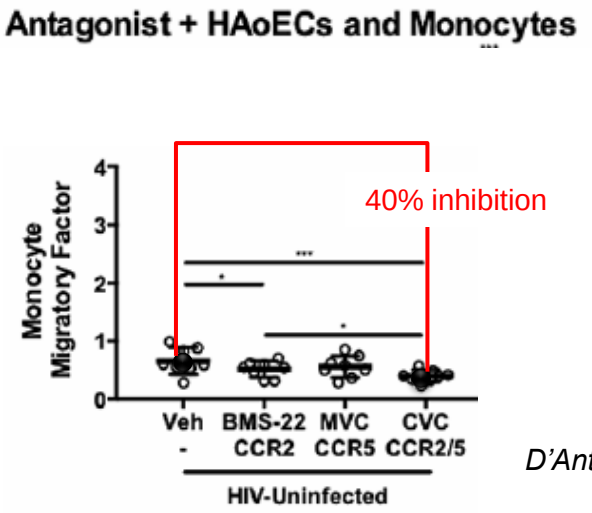


Kruger 2018

CVC on macrophage recruitment

- Data indicate maximum inhibition of 40%
- Max inhibitory response at 0.696 µg/mL

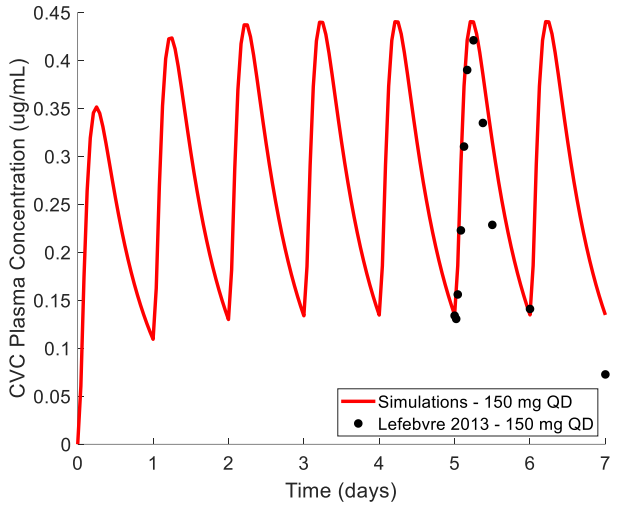
in vitro

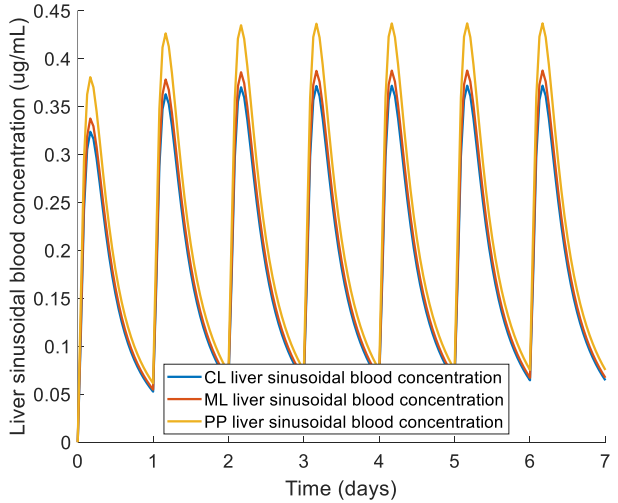


D'Antonio 2018

CVC PBPK Model Captures Clinical Data from Healthy Volunteers

- CVC PBPK model optimized using 150 mg qd clinical data from healthy individuals
  - Predicted liver sinusoidal concentrations to predict compound concentration at site of target
- Where possible, parameter values were taken directly from literature
  - Parameters for which values were not found in literature were estimated using ADMET Predictor (QSAR model) or optimized to plasma profiles following CVC administration
- Liver sinusoidal concentrations exported to NAFLDsym to drive effects of CVC on HSC activation and monocyte recruitment





F1, F2, F3 SimCohorts Characteristics for CVC Simulations

		SimCohorts			Clinical Cohort
		F1	**F2**	**F3**	**CENTAUR§**
Baseline characteristics	Units	Mean ± SD	Mean ± SD	Mean ± SD	
N		41	35	73	289
Body weight	kg	116.4 ± 20.5	95.8 ± 20.6	85.8 ± 17.5	96.1 ± 21.1
Liver fat	%	23.9 ± 6.2	16.2 ± 8.4	15.0 ± 7.6	-
NAS score	score	5.9 ± 0.8	4.0 ± 1.6	5.3 ± 1.4	-
Fibrosis stage	score	1.0 ± 0	2.0 ± 0.0	3.0 ± 0.0	-
ProC3	ng/mL	15.4 ± 5.4	18.0 ± 6.3	23.2 ± 8.5	-
Alanine aminotransferase (ALT)	U/L	40.2 ± 11.0	49.7 ± 16.4	62.2 ± 25.1	63.4 ± 37.5
Serum triglycerides	mM	1.7 ± 0.4	1.9 ± 0.5	2.0 ± 0.6	*
Collage percent area (CPA)	%	0.4 ± 0.8	4.0 ± 0.6	6.5 ± 1.0	-
F1 (% of total)	%	27.5	-	-	32.9
F2 (% of total)	%	-	23.5	-	28.4
F3 (% of total)	%	-	-	49.0	38.4

§ Baseline patient characteristics reported by Friedman 2018
* Discrepancy between TG levels reported in Friedman 2018 main text vs. supplement

CVC Simulation Protocol for Comparison Against CENTAUR Data

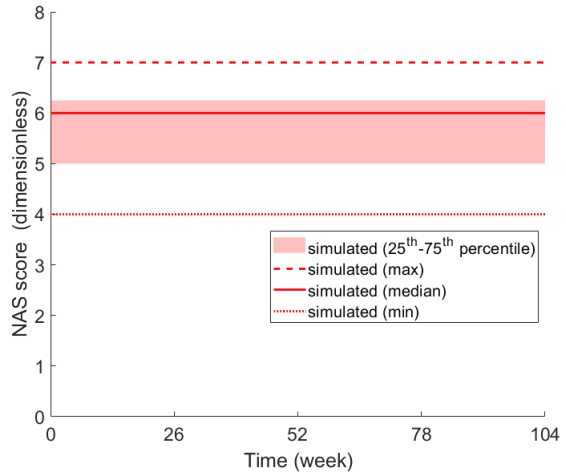
- Simulated CVC administration of 150 mg qd exposure for 2 years (104 weeks) to align with CENTAUR study
- Utilized CVC-informed monocyte/macrophage and HSC parameter values
  - Liver sinusoidal blood concentrations drive effects on HSCs and monocytes/macrophages
- Simulated in F1, F2, F3 SimCohorts
  - F1, F2, F3 baseline characteristics align with CENTAUR patients

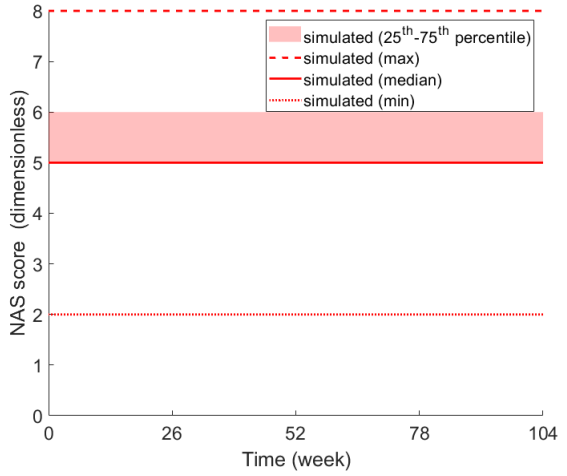
Treatment	Patient Types	Timing	Dose	SimCohorts
CVC	F1, F2, F3	104 weeks	150 mg qd	

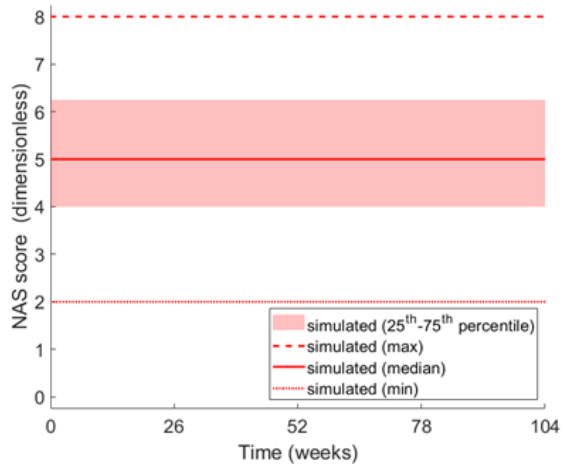
CVC Has No/Minimal Improvement on NAS and Fibrosis Stage after 104 Weeks of Treatment

F1 F2 F3

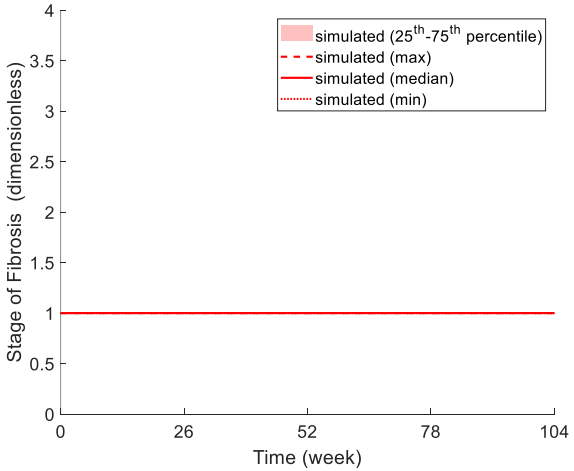
NAS

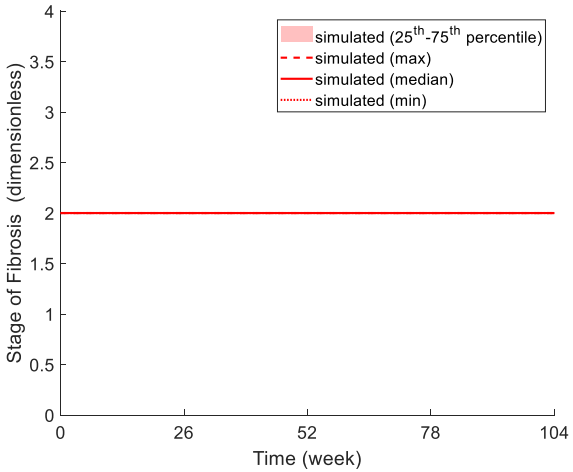


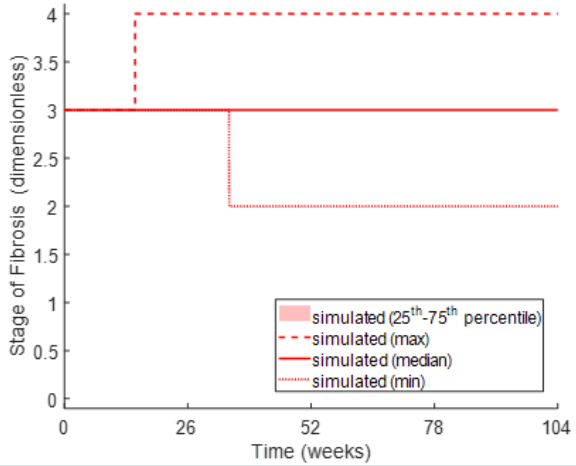




Fibrosis Stage



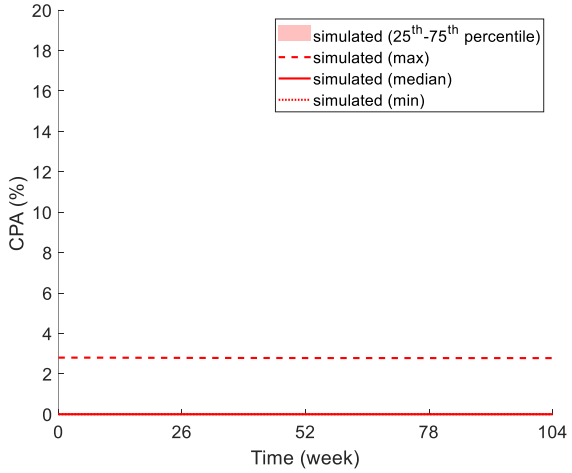


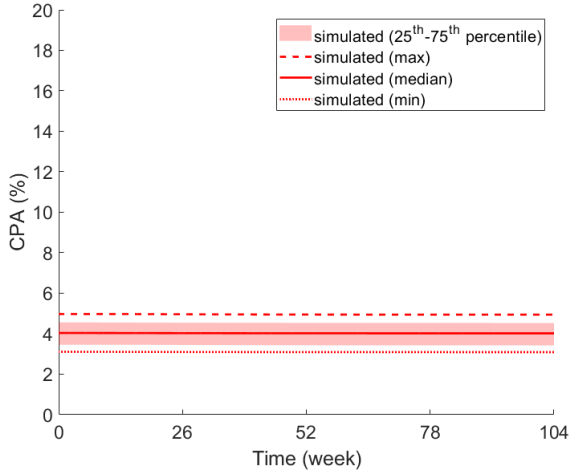


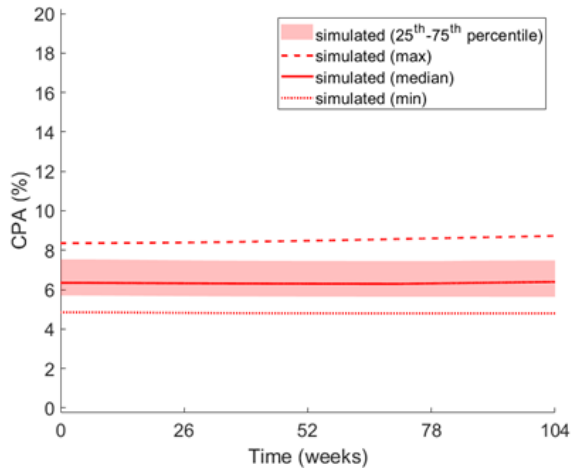
CVC Has No/Minimal Improvement on Collagen Outcomes after 104 Weeks of Treatment

F1 F2 F3

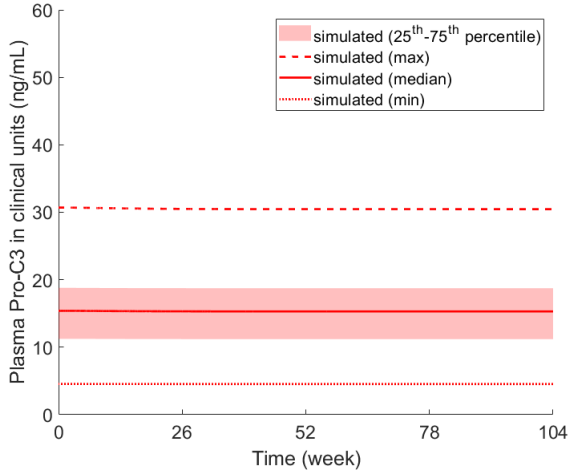
Collagen Percent Area

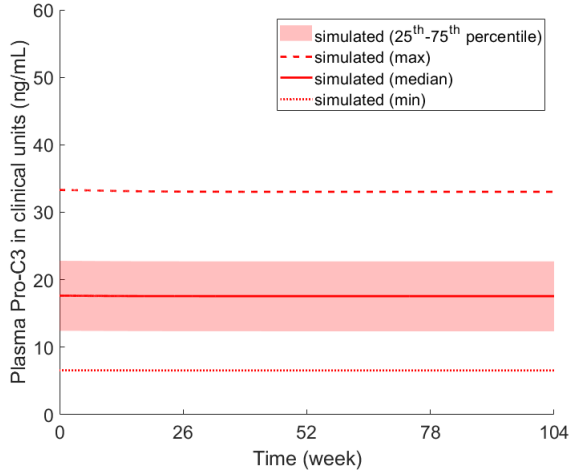


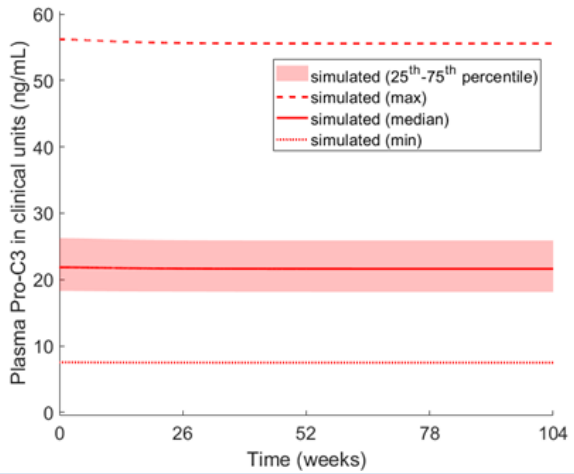




Pro-C3



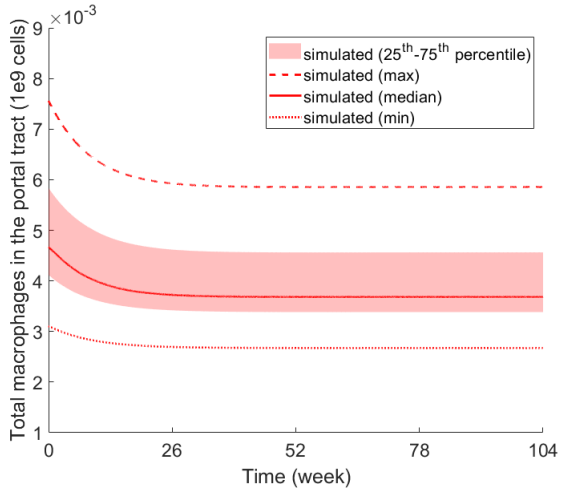


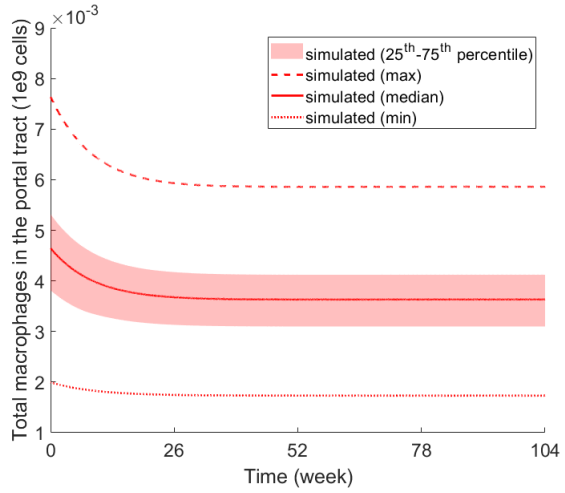


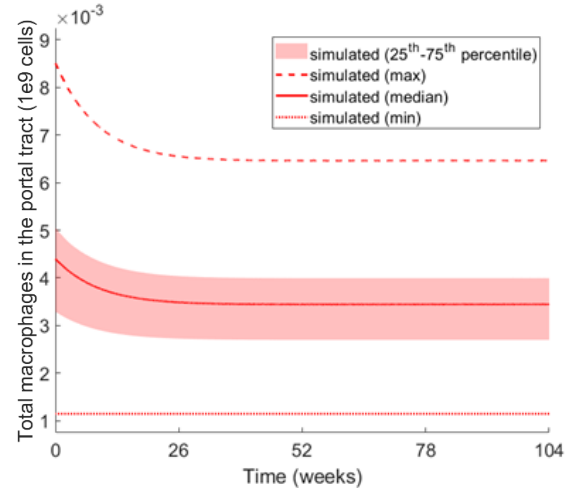
CVC Exhibits More Potent Effect on Monocytes/Macrophages, Less Potent Effect on Hepatic Stellate Cells

F1 F2 F3

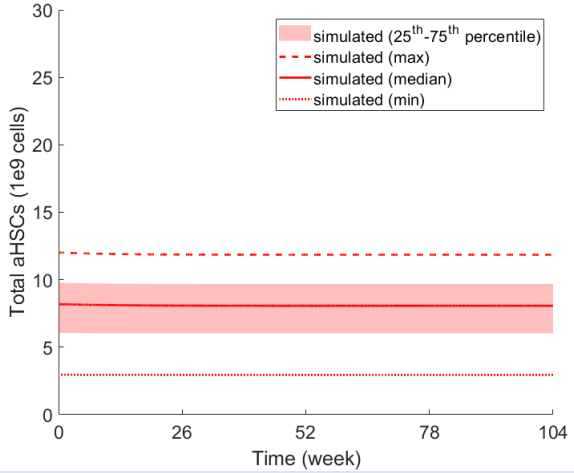
Portal macrophages

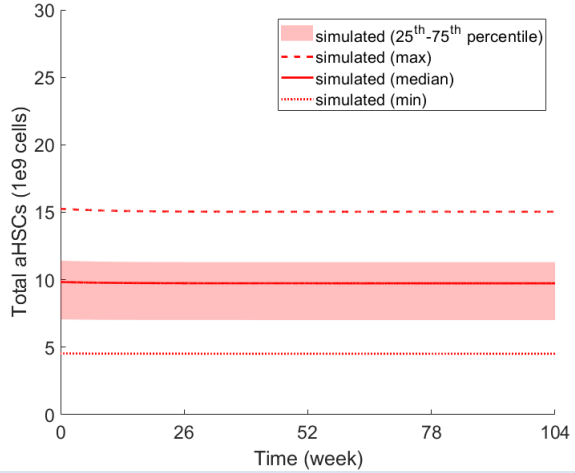


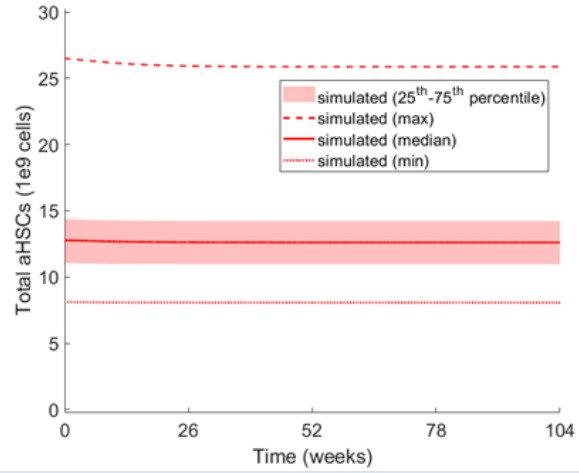




Total activated hepatic stellate cells







CVC Simulation Results Align with CENTAUR Study Results

		Fibrosis improvement (≥1) with no worsening of NASH	NASH improvement (≥2) with no worsening of fibrosis
SimCohorts	F1 SimCohorts	0%	0%
	F2 SimCohorts	0%	0%
	F3 SimCohorts	4%	0%
Clinical Cohort	CENTAUR Arm A (CVC + CVC), ITT population	13%	Not reported
	CENTAUR Arm C (Placebo + Placebo), ITT population	14%	Not reported
	CENTAUR Arm A (CVC + CVC), mITT population	15%	Not reported
	CENTAUR Arm C (Placebo + Placebo), mITT population	17%	Not reported

Adapted from Ratziu 2020

- Simulation results demonstrate minimal improvement in inflammatory or fibrotic endpoints in F1, F2, or F3 SimCohorts
  - As expected, CVC does not affect liver fat, plasma triglycerides, or body weight (upstream of CVC mechanisms of action)
- Results consistent with 2-year CENTAUR data and with early termination of the phase 3 AURORA trial due to lack of efficacy

NAFLDsym CVC Results Summary

- Simulated 2-year treatment with CVC predicted to result in no/minimal improvement in NAS and fibrosis score endpoints consistent with 2-year CENTAUR results and with early termination of the phase 3 AURORA trial due to lack of efficacy
  - PBPK modeling applied to reproduce CVC exposure
  - *In vitro* PD and MoA data informed parameter values
  - SimCohorts representing inter-individual variability provide heterogeneity in response
- Clinical potential of CVC could have been investigated using NAFLDsym *prior* to clinical trial

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NAFLDsym Used to Increase Understanding of Mechanism of Action and to Inform Selection of Dosing Paradigms

- BFKB8488A, agonist anti-FGFR1/KLB bispecific antibody, is being developed to treat patients with NASH
- BFKB8488A represented in NAFLDsym and simulations indicated that
  - BFKB8488A administration was predicted to increase serum adiponectin 40-80% over 12 weeks of dosing in an exposure-related manner, within clinical data range
  - Adiponectin appeared to play a mechanistic role in potential for efficacy in NASH patients
  - Simulation results used to evaluate and guide selection of dosing paradigms for subsequent clinical studies

Mathematical Modeling with NAFLDsym Supports the Role of Adiponectin in the Reduction of Steatosis by the Anti-FGFR1/KLB Bispecific Antibody

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ABSTRACT

The agonist anti-FGFR1/KLB bispecific antibody, BFKB8488A, has been shown to be effective at reducing liver fat in NAFLD patients in a Ph1b study [1]. However, FGFR1/KLB receptors are primarily expressed in adipose rather than liver, suggesting a role for adipokine mediators such as adiponectin (Adpn). Adpn levels have been shown to increase with BFKB8488A treatment. NAFLDsym, a QSP mechanistic, mathematical model of NAFLD and NASH, was employed to evaluate the plausibility of Adpn increases mediating the reduction in liver fat observed with BFKB8488A treatment.

Exposure of BFKB8488A was predicted from PopPK modeling and combined with a mechanistic representation of the effects of BFKB8488A interaction with the FGFR1/KLB complex in adipose. The mechanistic model incorporated the effects of increased Adpn to elicit changes in several hepatic pathways that can act in concert to reduce the hepatic lipid burden. This included decreases in hepatic de novo lipogenesis and mono-acyl glycerol transferase activity along with an increase in hepatic fatty acid oxidation. Subcutaneous administration of 50 mg Q2W, 75 mg Q2W, 100 Q2W or 250 Q4W BFKB8488A was simulated for 12 weeks in a virtual cohort of NAFLD patients with steatosis (n=42).

Generally, simulations of BFKB8488A-mediated increases in Adpn were able to predict comparable reduction in liver fat as those observed in the Ph1b study. Simulated BFKB8488A administration was predicted to increase serum Adpn 40-80% over 12 weeks of dosing in an exposure-related manner (Figure 1), which was within range of the clinical data (except for 100 mg Q2W). Liver fat reductions were predicted to increase in magnitude with increasing dose within the simulated patient population, ranging from 0% to >90% relative to baseline. The inter-patient variability in the liver fat reduction was reasonably predicted. Alternative simulations without Adpn increase did not predict any effects on liver fat.

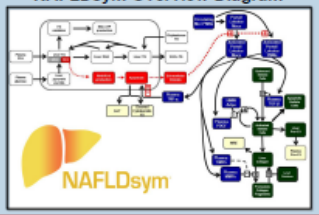
The hypothesis that BFKB8488A-induced increases in Adpn mediate the observed effects on liver fat in NAFLD patients is consistent with NAFLDsym simulations. The similarity between the clinical observations and model predictions utilizing the simulated mechanistic effects of Adpn on hepatic lipid pathways suggests that Adpn participates in mediating the potentially beneficial response to BFKB8488A.

INTRODUCTION

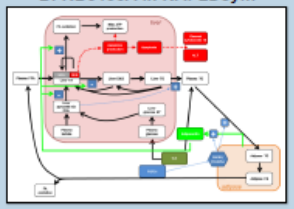
- BFKB8488A, an agonist anti-FGFR1/KLB bispecific antibody, has been shown to be effective at reducing liver fat in NAFLD patients in a Ph1b study (Kunder et al., AASLD 2019)
- FGFR1/KLB receptors are primarily expressed in adipose rather than liver, suggesting a role for adipokine mediators such as adiponectin (Adpn). Adpn levels have been shown to increase with BFKB8488A treatment
- NAFLDsym, a QSP model of NAFLD pathophysiology, was employed to evaluate the plausibility of Adpn increases mediating the reduction in liver fat observed with BFKB8488A treatment

RESULTS

NAFLDsym Overview Diagram



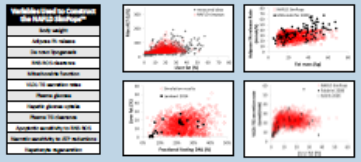
Representation and Optimization of BFKB8488A in NAFLDsym



Description

- BFKB8488A agonist anti-FGFR1/KLB acts on adipose tissue to increase adiponectin secretion from the adipose and increase uptake of triglycerides from the plasma to the adipose. These PD effects were included in the simulations
- The simulations also downstream effects in the liver mediated by changes in the adiponectin receptor which stimulates AMPK [2-4]; these changes decrease de novo lipogenesis, decrease processing of saturated fatty acids into mono-, dia-, and triglycerides, increase liver secretion of triglycerides, and increase fatty acid oxidation [5-7].

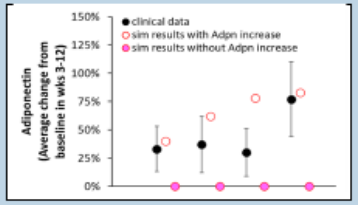
NAFLD SimPops Validation



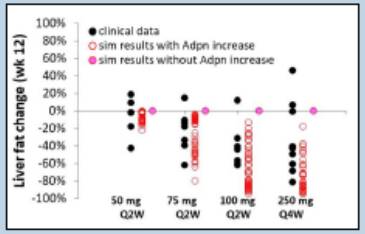
Construction and validation of NAFLD SimPops

- Simulated NAFLD patients (n=1707) include combinations of parameter ranges based on reported responses from literature [8-12].
- Simulated patients within SimPops have pathophysiologic and clinical characteristics consistent with what has been reported in literature [8-12]

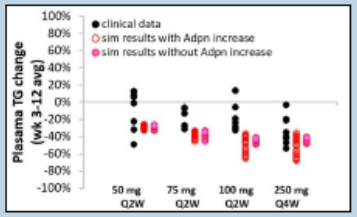
Accurate Prediction of Phase I Clinical Response to BFKB8488A with NAFLDsym



- NAFLDsym accurately predicted (red) clinical responses (black) for adiponectin (Adpn) in representative SimCohorts
- NAFLDsym simulations parameterized without Adpn increase (pink) did not represent clinical Adpn response



- NAFLDsym accurately predicted (red) clinical responses (black) for liver fat in representative SimCohorts, based on dose-dependent Adpn increases mediating liver effects
- NAFLDsym simulations parameterized without Adpn increase (pink) did not represent clinical liver fat response



- NAFLDsym reasonably predicted (red) the clinical responses (black) for plasma TG changes in representative SimCohorts, accounting for wide clinical variability in plasma TG responses

METHODS

Overview NAFLDsym is a mechanistic, mathematical, QSP model that was utilized for all simulations. NAFLDsym includes a representation of the primary pathways controlling liver fatty acid and triglyceride fluxes in addition to the effects of lipotoxicity on hepatocellular health. NAFLDsym v2A also contains submodels describing the pathophysiology of inflammation and fibrosis; these submodels were not the focus for the simulations described herein. The primary simulated NAFLDsym outputs utilized were adiponectin, ALT, liver fat, and plasma TG.

Simulated patients A simulated population of patients with the pathophysiological aspects of NAFLD are included in NAFLDsym. This SimPops (n=1707) includes a number of characteristics that are consistent with the observed heterogeneity of pathophysiologic and clinical features of NAFLD. For this study, a subset of all simulated patients (SimCohorts, n=42) with similar characteristics as the clinical cohort was utilized.

Simulated effects of BFKB8488A High molecular weight (HMW) adiponectin has been shown to increase the activity of hepatocellular AMPK following its interaction with the ADIPO R1 and R2 receptors [2-4]. In separate studies employing pharmacologic activators of AMPK in hepatocytes or HepG2 cells, AMPK activity has been demonstrated to reduce the expression and/or activity of ACC and FAS [5]. These are rate controlling enzymes of the de novo lipogenesis (DNL) pathway; reductions in expression/activity of these enzymes reduce flux through the DNL pathway. ACC also regulates the entry of fatty acids into the mitochondria; reduced ACC activity allows for greater fatty acid entry into the mitochondria to support fatty acid oxidation [6]. Additional studies have shown that AMPK activation reduces the hepatocellular expression/activity of MGAT, one of the enzymes that participates in the esterification of fatty acids to triglycerides [7]. Exposure-response relationships between HMW adiponectin and DNL inhibition, enhanced fatty acid oxidation, enhanced VLDL-TG secretion, and inhibition of fatty acid esterification, respectively were included within NAFLDsym v2A.

A subset of Genentech's ANTI-FGFR1/KLB MAB Phase Ib clinical data (50 mg Q2W and 250 mg Q4W) were used to optimize the quantitative relationships of each effect; the quantitative relationships based on the in vitro studies [5], [7] were not employed due to uncertainty of translating the quantitative aspects to humans. Validation of the optimized quantitative effects on DNL inhibition, fatty acid oxidation, and MGAT inhibition was performed by comparing simulation results with additional Phase Ib clinical data (75 mg Q2W and 100 mg Q2W).

Simulations were also conducted without parameterizing an adiponectin increase, to test the key method of action hypothesis for BFKB8488A.

Simulated Protocols Subcutaneous administration of 50 mg Q2W, 75 mg Q2W, 100 Q2W or 250 Q4W BFKB8488A was simulated for 12 weeks in a virtual cohort of NAFLD patients with steatosis

CONCLUSIONS

NAFLDsym simulated predictions of 12 weeks of treatment with the agonist anti-FGFR1/KLB bispecific antibody BFKB8488A indicate that:

- BFKB8488A administration was predicted to increase serum Adpn 40-80% over 12 weeks of dosing in an exposure-related manner, within the clinical data range
- Liver fat reductions in the simulated patients were predicted to increase in magnitude with increasing dose, and simulated magnitudes were consistent with the observed liver fat reduction
- Simulations parameterized without an adiponectin increase did not represent the clinical response

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[2]. T. Yamauchi et al. Nat. Med. 2002 Oct; 8(11): 1288-95
[3]. T. Yamauchi et al. Nat. Med. 2007 Mar; 13(3): 332-9.
[4]. H. Wakil et al. J. Biol. Chem. 2003 Oct; 278(41): 40352-63.
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[6]. L. Tong and H.J. Harwood. J. Cell. Biochem. 2006 Dec; 99(6): 1476-88
[7]. R.W. Hunter et al. Cell Metab. 2017 Aug; 26(2): 394-406.
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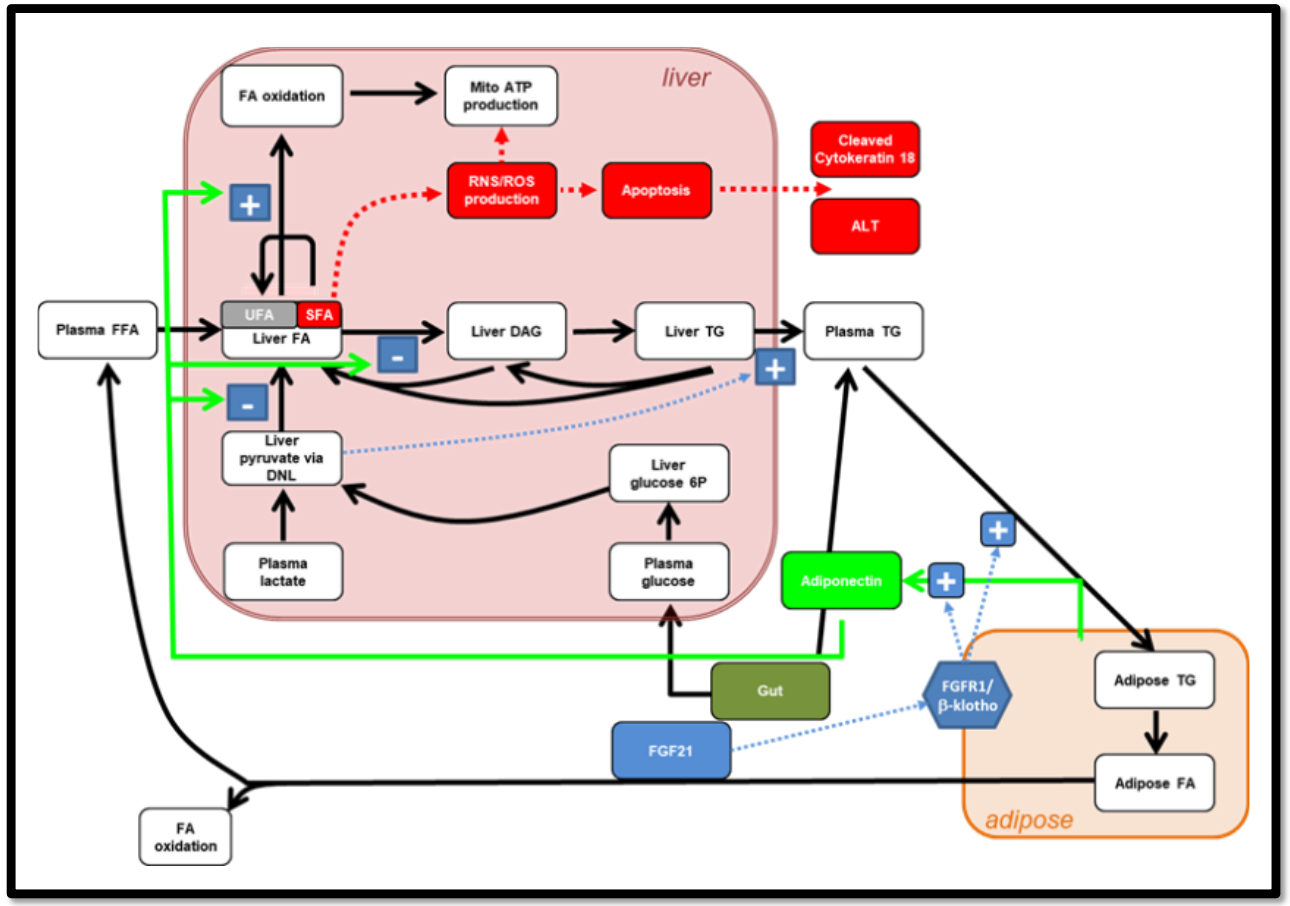
DILIsymServices — A SIMULATIONS PLUS COMPANY

BFKB8488A Represented as Targeting Adipose Tissue with Subsequent Adiponectin-Mediated Effects on Liver Fat in NAFLDsym

- BFKB8488A agonist anti-FGFR1/KLB acts on adipose tissue
  - Increase adiponectin section
  - Enhance plasma TG lipolysis and uptake

- Adiponectin interacts with hepatic receptors to enhance hepatocellular AMPK [2-4] and
  - Decrease de novo lipogenesis
    - Downstream effect to increase hepatic secretion of VLDL-TG
  - Decrease esterification of fatty acids
  - Increase fatty acid oxidation [5-7]



[2]. T. Yamauchi et al. Nat. Med. 2002 Oct; 8(11): 1288-95
[3]. T. Yamauchi et al. Nat. Med. 2007 Mar; 13(3): 332-9.
[4]. H. Waki et al. J. Biol. Chem. 2003 Oct; 278(41): 40352-63.
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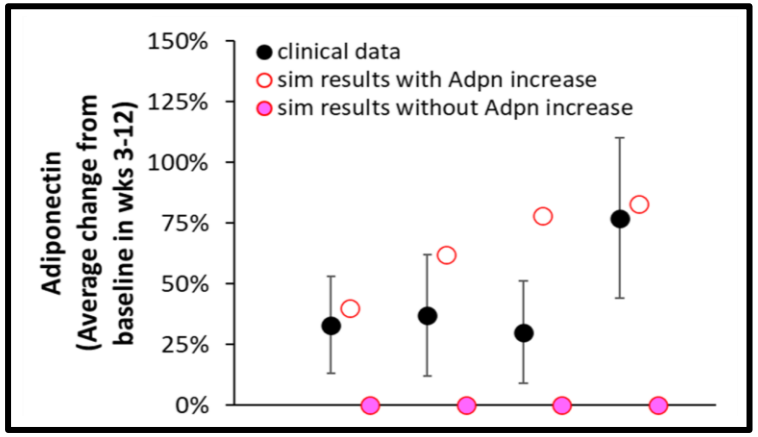
[6]. L. Tong and H.J. Harwood. J. Cell. Biochem. 2006 Dec; 99(6): 1476-88
[7].R.W. Hunter et al. Cell Metab. 2017 Aug; 26(2): 394-406..

BFKB8488A Simulation Protocol for Comparison with Clinical Data

- Subcutaneous administration of 50 mg Q2W, 75 mg Q2W, 100 Q2W or 250 Q4W BFKB8488A was simulated for 12 weeks
- A subset of Genentech's ANTI-FGFR1/KLB MAB Phase Ib clinical data (50 mg Q2W and 250 mg Q4W) were used to optimize the quantitative relationships of each effect;
  - Validation of the optimized quantitative effects was performed by comparing simulation results with additional clinical data (75 mg Q2W and 100 mg Q2W).
- Simulations were also conducted without parameterizing an adiponectin increase, to test the key method of action hypothesis for BFKB8488A.
- For this study, SimCohorts (n=42) with similar characteristics as the clinical cohort was utilized.

Accurate Prediction of Phase I Clinical Response to BFKB8488A with NAFLDsym



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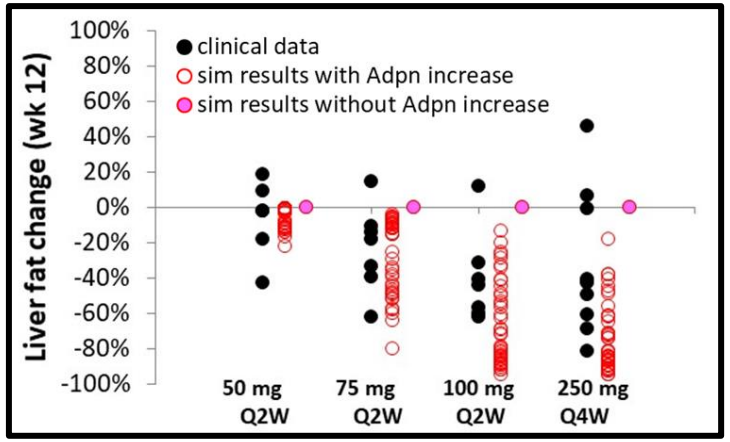

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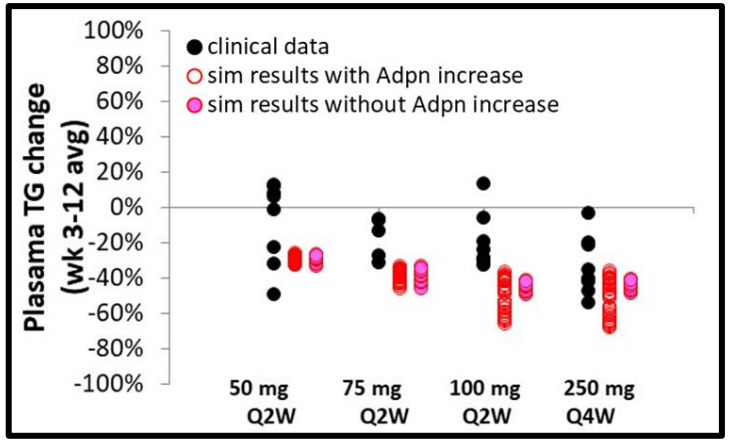


- NAFLDsym reasonably predicted (red) the clinical responses (black) for plasma TG changes in representative SimCohorts, accounting for wide clinical variability in plasma TG responses

NAFLDsym BFKB8488A Results Summary

- Accurately predicted liver fat reductions with BFKB8488A treatment
  - Simulated mechanisms of action within NAFLDsym
- Simulations highlighted mechanistic role for adiponectin in BFKB8488A mechanism of action
- Simulations informed dosing paradigm selection for subsequent clinical studies

Agenda

- Overview of NAFLDsym v2A
- Simulations of Cenicriviroc Predict Lack of Efficacy
- Simulations of BFKB8488A Provided MoA Understanding and Supported Dosing Paradigm Selection in Subsequent Studies
- Impact of Lifestyle: Impact of Simulated Weight Loss and Weight Gain on Predicted Efficacy
- Q & A

Lifestyle Can Influence Outcomes of NASH Clinical Trials

- Patient behavior can influence clinical trial outcomes
  - Dosing compliance
  - Weight gain or weight loss
- Unexpectedly substantial responses in placebo cohort can make it challenging to establish pharmacologic efficacy with compound
- Recent example in NASH clinical development: Elafibranor
  - Lack of statistical significance between treatment and placebo cohorts for primary and secondary endpoints
  - 72 week interim efficacy results
  - Improvements within placebo cohort were substantial
- QSP modeling can help delineate pharmacologic efficacy from lifestyle contributions
  - Simulate each effect separately
  - Simulate all effects simultaneously

Interim efficacy results at week 72

ITT (missing biopsy = non-responder)		Elafibranor 120mg		*Placebo*		*P-Value*
		N	%	N	%	
Primary Endpoint	NASH Resolution without worsening of fibrosis	138/717	19.2	52/353	14.7	0.0659
Key secondary Endpoint	Fibrosis improvement of at least one stage	176/717	24.5	79/353	22.4	0.4457

Genfit May 11, 2020 Press Release

Changes in Body Weight Associated with NASH Disease Progression and Reversal

- Change in body weight appeared to influence NASH disease progression
  - Weight gain imposes greater lipid burden upon liver
  - Weight loss relieves lipid burden upon liver
- Weight loss shown to be effective treatment for NASH patients
- Body weight increases appeared to worsen NASH status
  - Wong 2010
  - Based on histologic scoring
  - Patients with increased NAS had increased BMI
- NAFLDsym includes mechanisms linking changes in body weight to lipid burden of liver
  - Alterations in lipid burden can lead to downstream changes in lipotoxicity, inflammation, and fibrosis

Gastroenterology 2015;149:367–378

CLINICAL—LIVER

Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis

Eduardo Vilar-Gomez,[1,2] Yadina Martinez-Perez,[1] Luis Calzadilla-Bertot,[1] Ana Torres-Gonzalez,[1] Bienvenido Gra-Oramas,[3] Licet Gonzalez-Fabian,[3] Scott L. Friedman,[4] Moises Diago,[5] and Manuel Romero-Gomez[2]

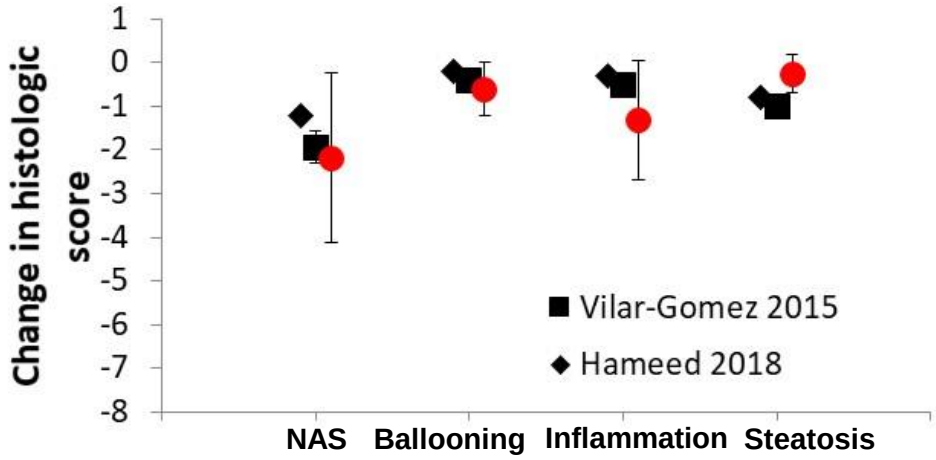
Table 4 Factors associated with increased non-alcoholic fatty liver disease (NAFLD) activity score from baseline to month 36

Factors	Increased NAFLD activity score	Static or decreased NAFLD activity score	p
N	26	26	
Age (years)	45±9	44±9	0.65
Male gender, n (%)	16 (62)	18 (69)	0.56
Diabetes mellitus, n (%)	15 (58)	11 (42)	0.27
Hypertension, n (%)	12 (46)	14 (54)	0.58
Metabolic syndrome, n (%)	18 (69)	17 (65)	0.77
Body mass index (kg/m^2)	27.4±4.1	27.4±3.3	0.99
Change in body mass index (kg/m^2)*	0.6±1.6	−0.8±1.7	0.003
Waist circumference (cm)	92.8±11.1	92.5±6.7	0.91

Wong 2010

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

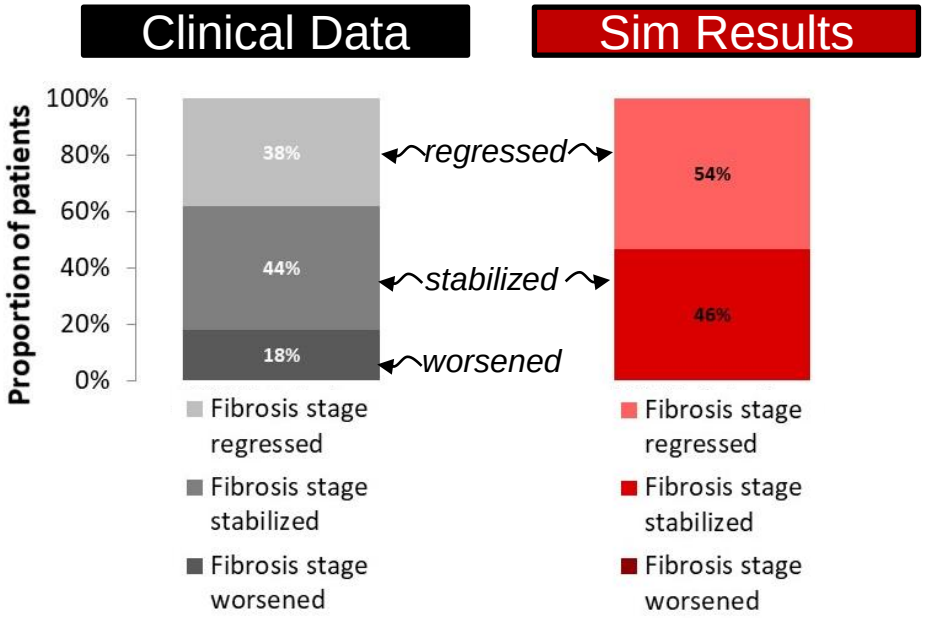
- Weight loss has been shown to improve NASH and fibrosis
  - Current standard of care
  - Greater efficacy with greater weight loss
- Simulated ≈5% weight loss over 1 year in SimCohorts
  - Comparable to protocols from clinical studies by Vilar-Gomez 2015 and Hameed 2018
  - Compared predicted changes in NASH biomarkers with clinical data
- Good agreement between predicted changes in NAS score and components with clinical data



Vilar-Gomez 2015, Hameed 2018

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

- 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 in SimCohorts
  - Simulating study undertaken by Vilar-Gomez et al.
- Proportion of patients with reductions in fibrosis scores comparable with clinical data
  - No simulated patients predicted to have fibrosis stage worsened with weight loss



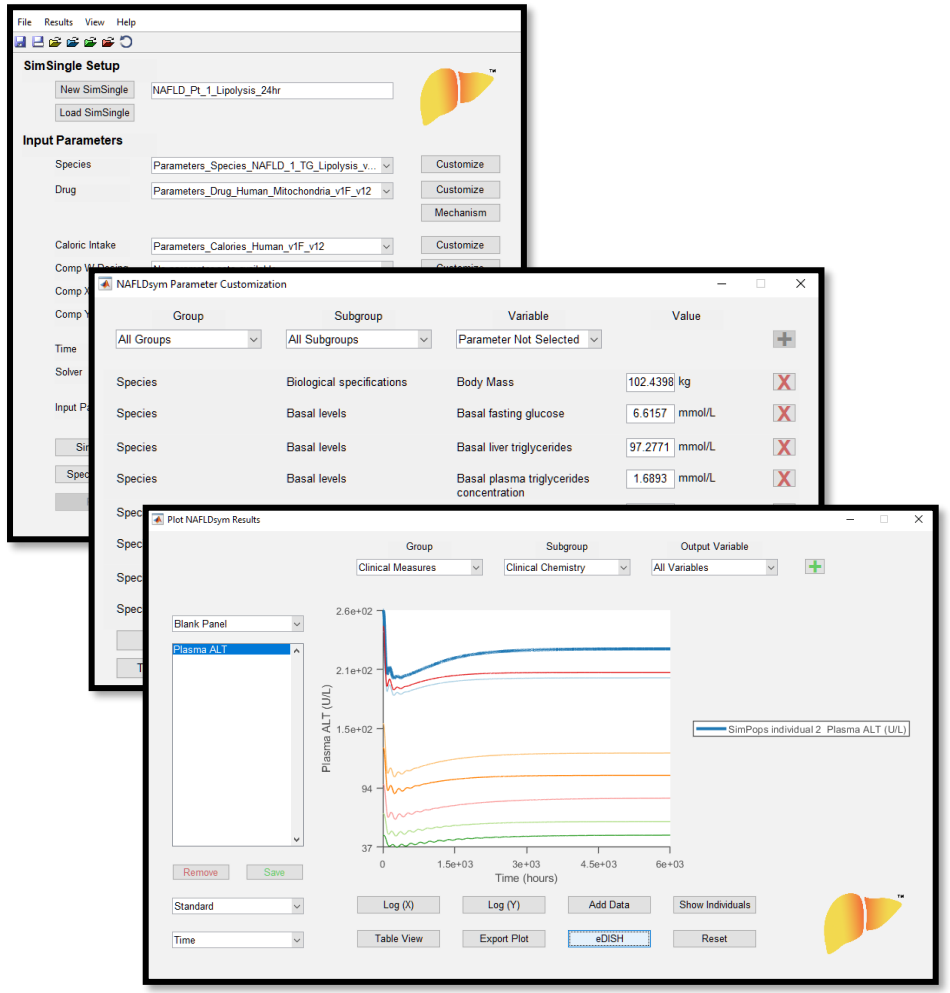
Vilar-Gomez 2015

NAFLDsym Weight Loss Results Summary

- Modest weight loss can yield improvements in NASH and fibrosis
  - NAS and fibrosis score reductions reported with ≥5% weight loss
- NAFLDsym predicted reductions of NAS and fibrosis score consistent with clinical reports
- NASH patients undergoing weight loss in clinical trials may obfuscate efficacy due to pharmacologic effects of drug
  - Particularly challenging when this occurs in placebo cohort
- NAFLDsym simulations be used to delineate weight loss effects from pharmacologic benefits

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



NAFLDsym Services Projects Include Multiple Stages to Ensure Alignment of Simulations with Compound Development Goals

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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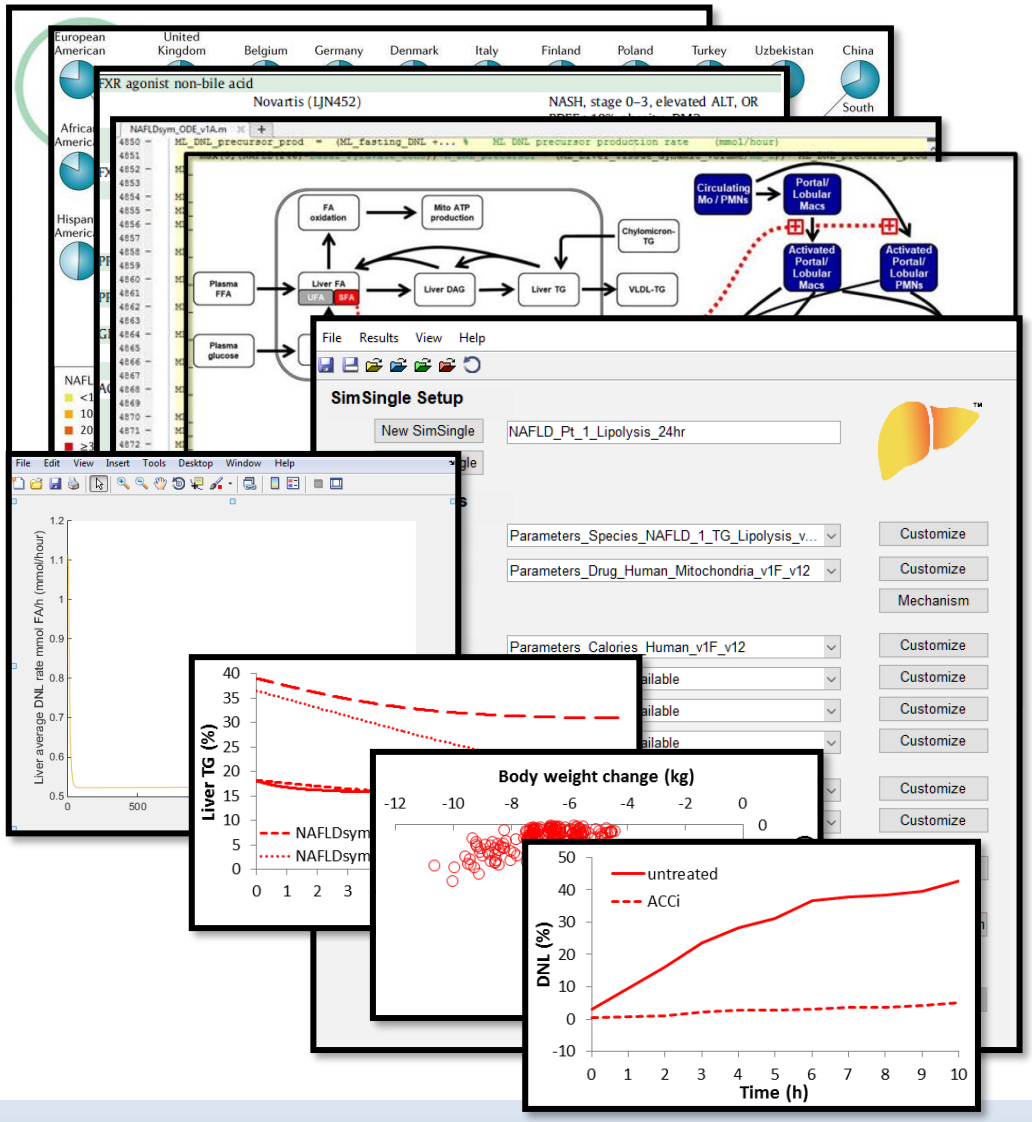
Agenda

- Overview of NAFLDsym v2A
- Simulations of Cenicriviroc Predict Lack of Efficacy
- Simulations of BFKB8488A Provided MoA Understanding and Supported Dosing Paradigm Selection in Subsequent Studies
- Impact of Lifestyle: Impact of Simulated Weight Loss and Weight Gain on Predicted Efficacy
- Q & A

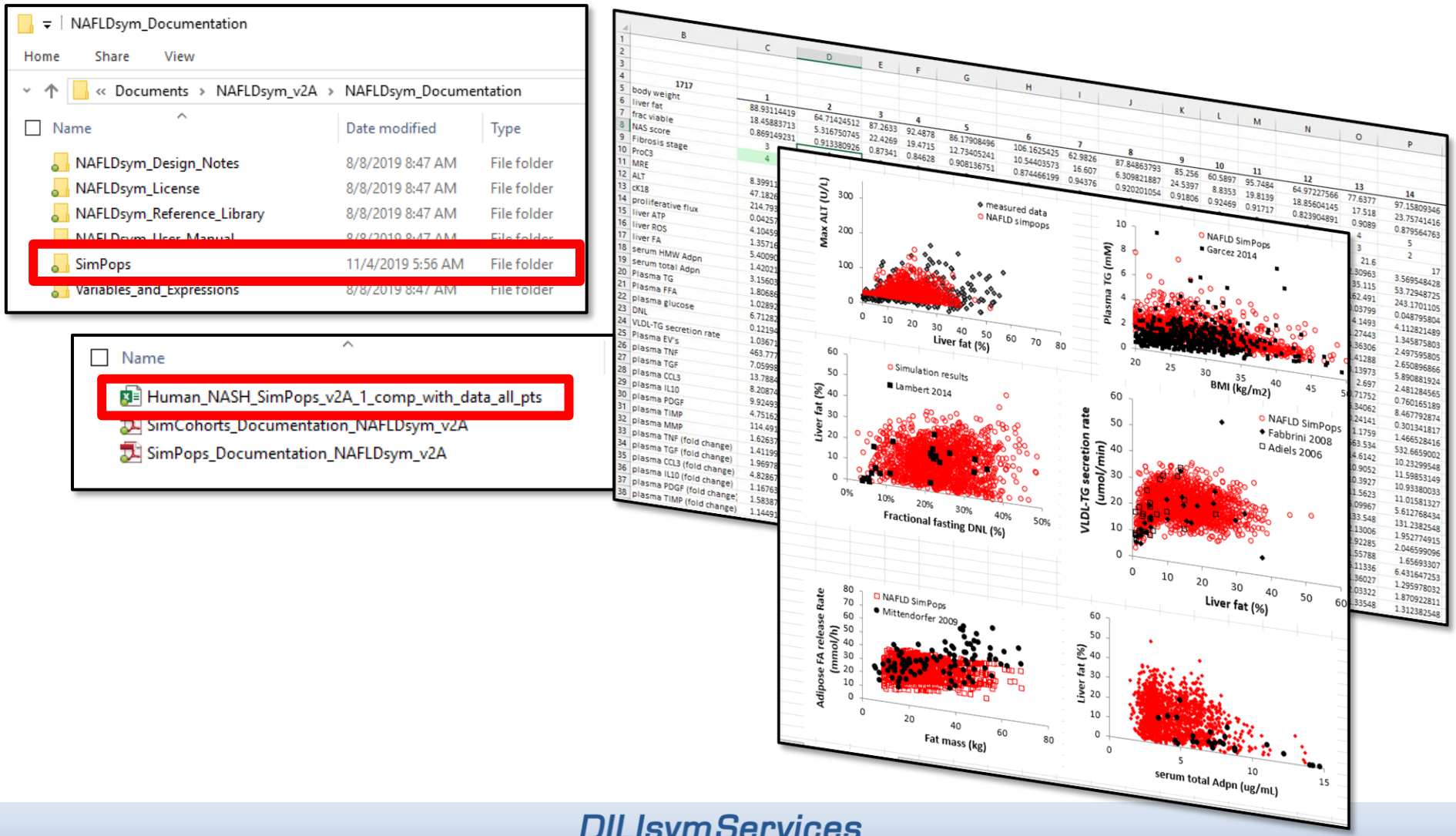
NAFLDsym Is Designed to Support Drug Development with Efficacy Predictions

- NAFLD/NASH 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
- Numerous potential treatments in development
  - Recent setbacks and terminations
- NAFLDsym is a QSP model of NAFLD/NASH
  - NAFLDsym v2A includes steatosis, lipotoxicity, inflammation, and fibrosis sub-models; available now
  - 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 to evaluate >20 NASH compounds and targets
  - Pfizer, Gilead, Genentech, BMS, and other companies to inform clinical programs

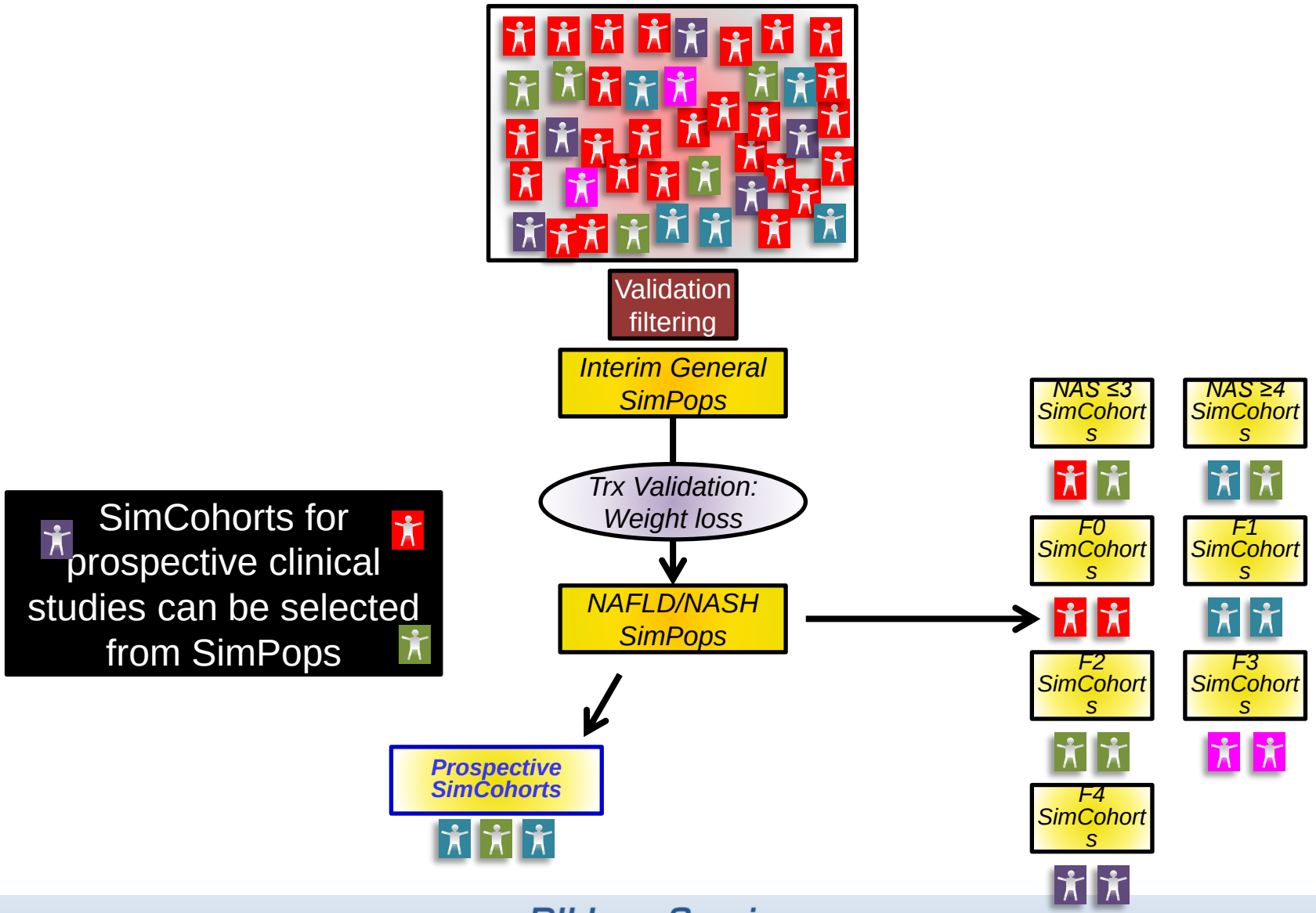


Backup Slides

NAFLD SimPops Patient Characteristics Summarized in Excel Spreadsheet



NAFLDsym v2A SimPops and SimCohorts

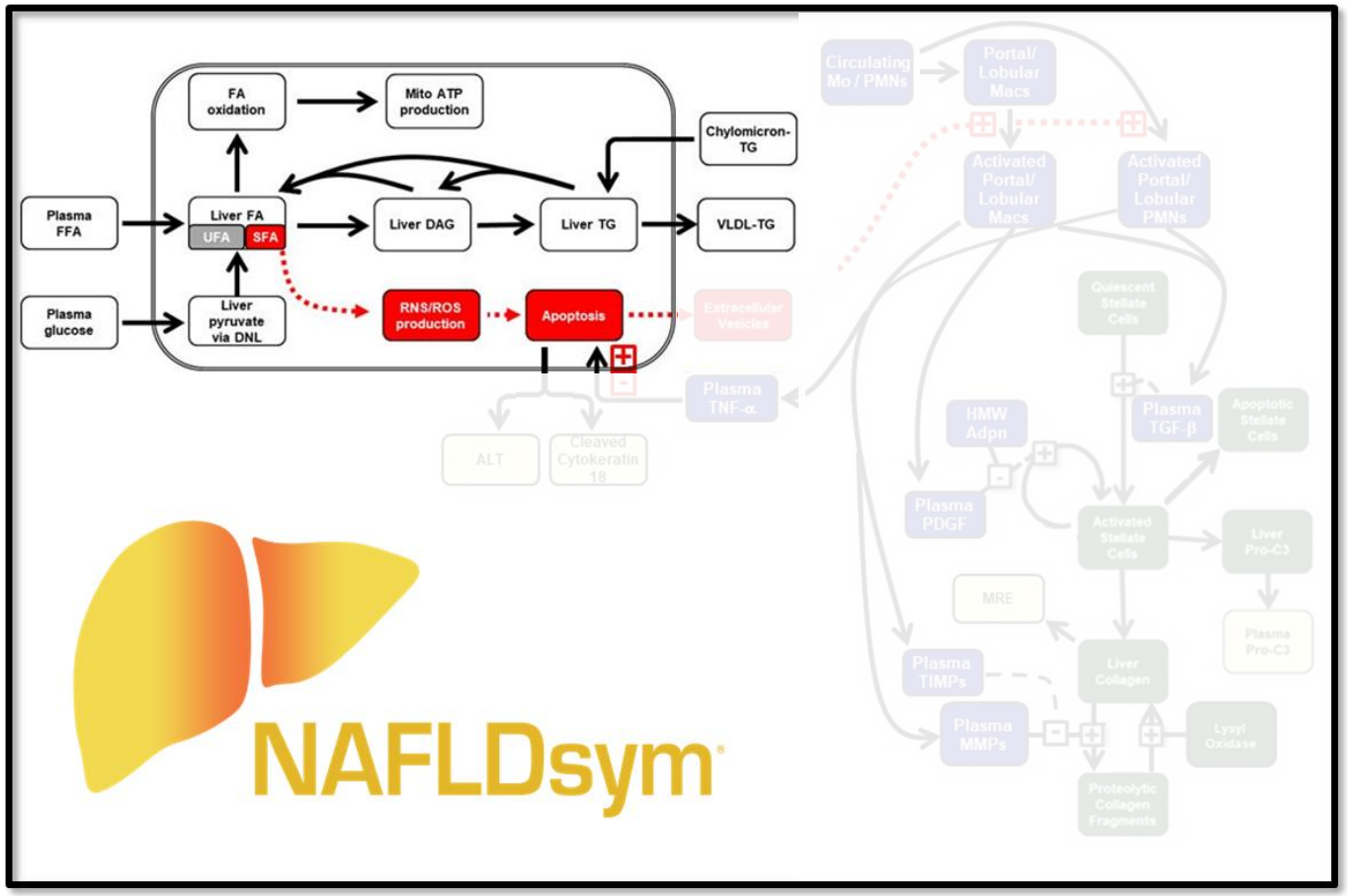


NAFLDsym v2A Comparisons with Measured Data for Different Sub-Models to Validate SimPops

- NAFLDsym parameters optimized in accordance with standard QSP modeling practices
- Generated SimPops (n=1717) with diverse pathophysiology
  - Adjusted parameters to create variability in steatosis and lipotoxicity
  - Adjusted parameters to create variability in inflammation and fibrosis sub-models
  - Followed general approach to calibration/validation for QSP models
    - Calibrate sub-models as separate units as 1st step
    - Confirm calibration is retained when sub-units interact (i.e., full model)
    - Evaluate influence of inter-patient variability in pathophysiology
  - Compared simulation results with numerous data sets to ensure model is properly calibrated;

- Steatosis/lipotoxicity comparisons with data:
  - Liver fat vs. ALT
  - Fat mass vs. adipose FA release
  - Liver fat vs. Fractional DNL
  - Liver fat vs. VLDL release
  - Liver fat vs. adiponectin
- Inflammation comparisons with data:
  - Liver macrophages and neutrophils
  - TNF-α
  - IL-10
  - TGF-β
  - PDGF-BB
  - TIMP-1
  - MMP
- Fibrosis comparisons with data:
  - aHSC
  - Collagen levels
  - MRE
  - ProC3
  - Fibrosis scores

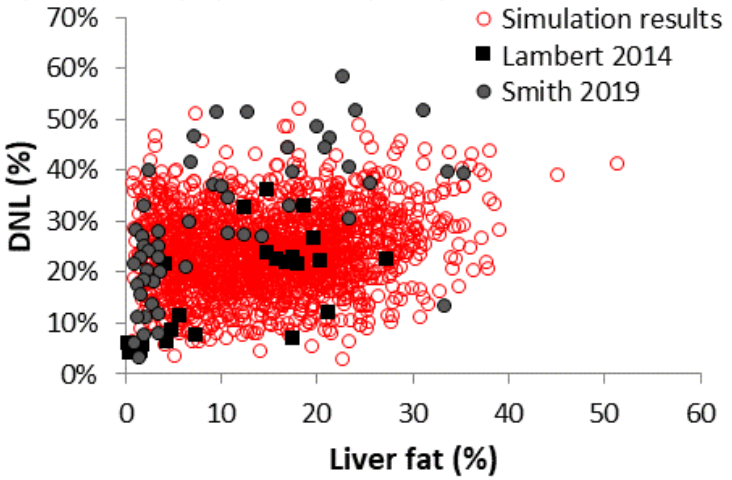
NAFLDsym v2A Overview: Steatosis-Lipotoxicity



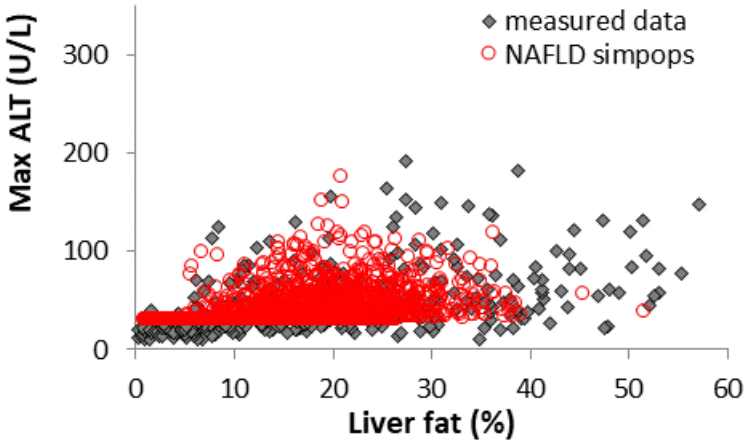
NAFLDsym v2A Includes Simulated Patients with Steatosis, DNL, and Liver Injury Consistent with Clinical Data

- Simulated patients have wide range of contributions to steatosis from DNL
  - Consistent with Lambert 2014 and Smith 2019 observations 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

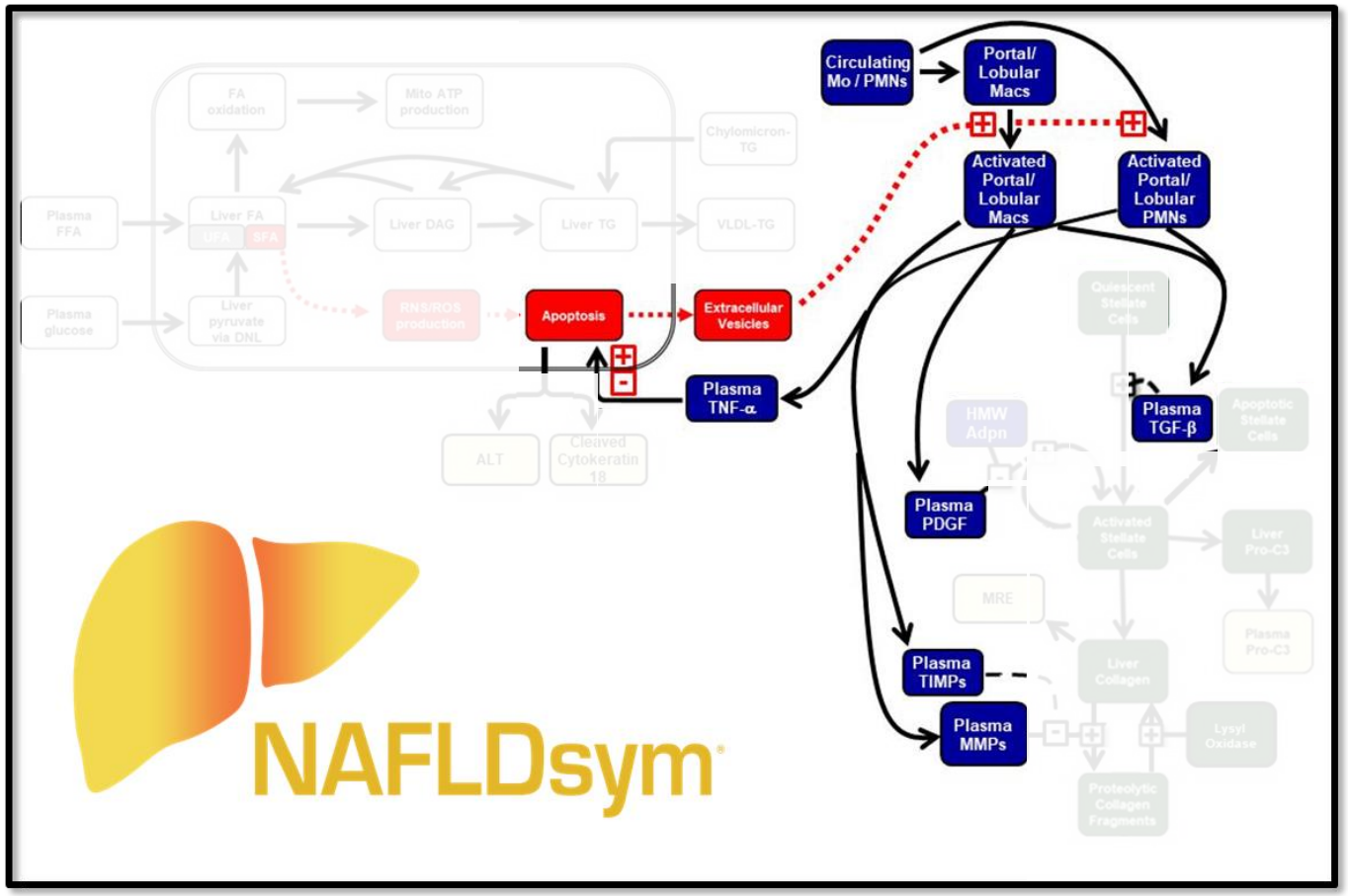


Lambert 2014



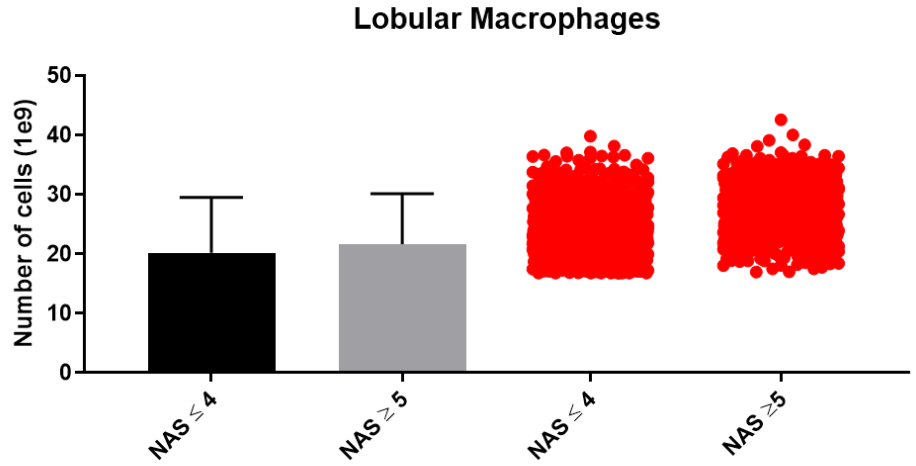
Maximos 2015

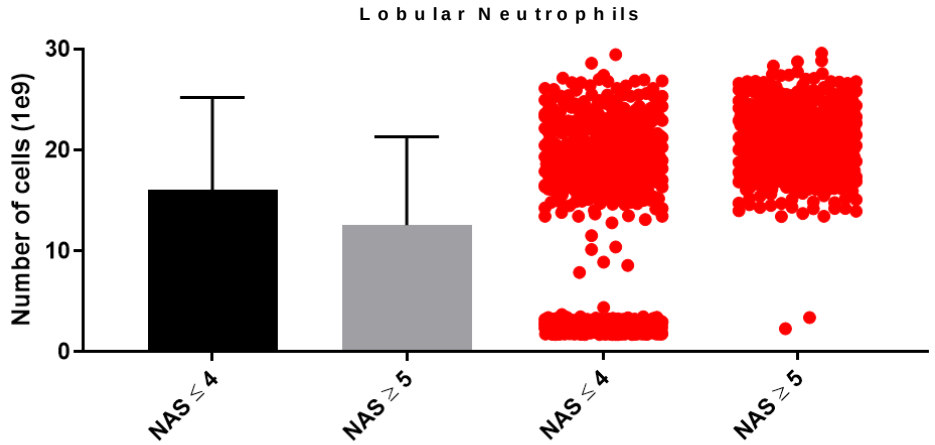
NAFLDsym v2A Overview: Inflammation



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

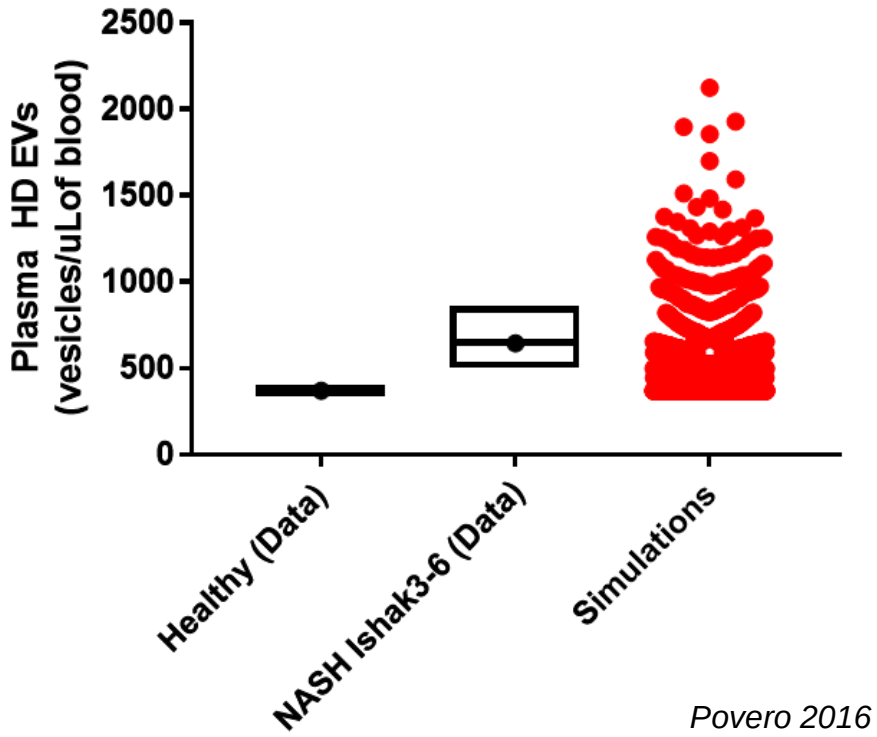
- Multiple papers indicate that lobular inflammatory cell numbers do not appear to change with NAFLD disease severity (Krenkel 2017, Tajiri 2009, Rensen 2009, Leicester 2006)
- Lobular macrophages and neutrophils from simulated patients compare favorably with measured data





NAFLDsym v2A Includes Extracellular Vesicle (EV) Levels Consistent with Available Clinical Data

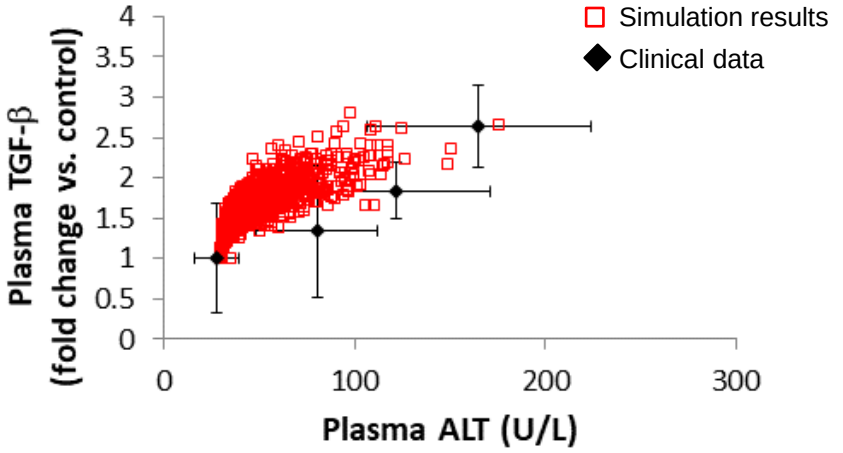
- EVs released from lipid-laden hepatocytes can activate innate immune cells (Ban 2016, Ban 2017, Povero 2014)
  - EV release from apoptotic HCs drives activates inflammation in NAFLDsym
- Human plasma EV levels from NAFLD patients reported by Povero 2016
- SimPops demonstrate range of EV level consistent with reported data
  - Simulated range extends beyond data based on assumption that larger n could reveal greater variability
  - SimPops (n>1500), Povero 2016 (n = 50 NASH patients)

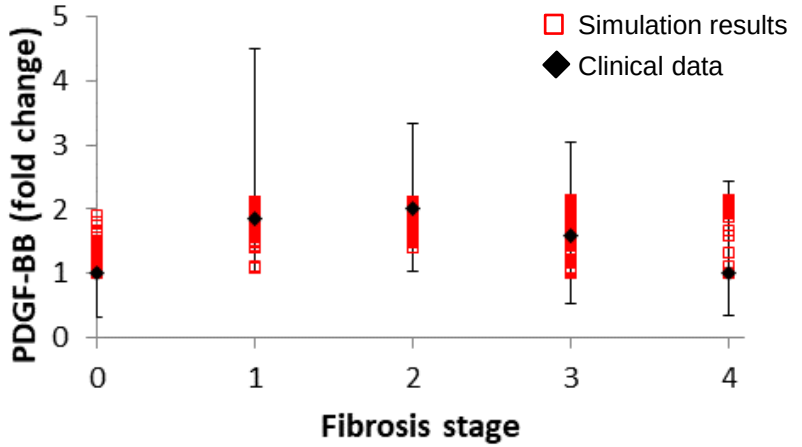


Povero 2016

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

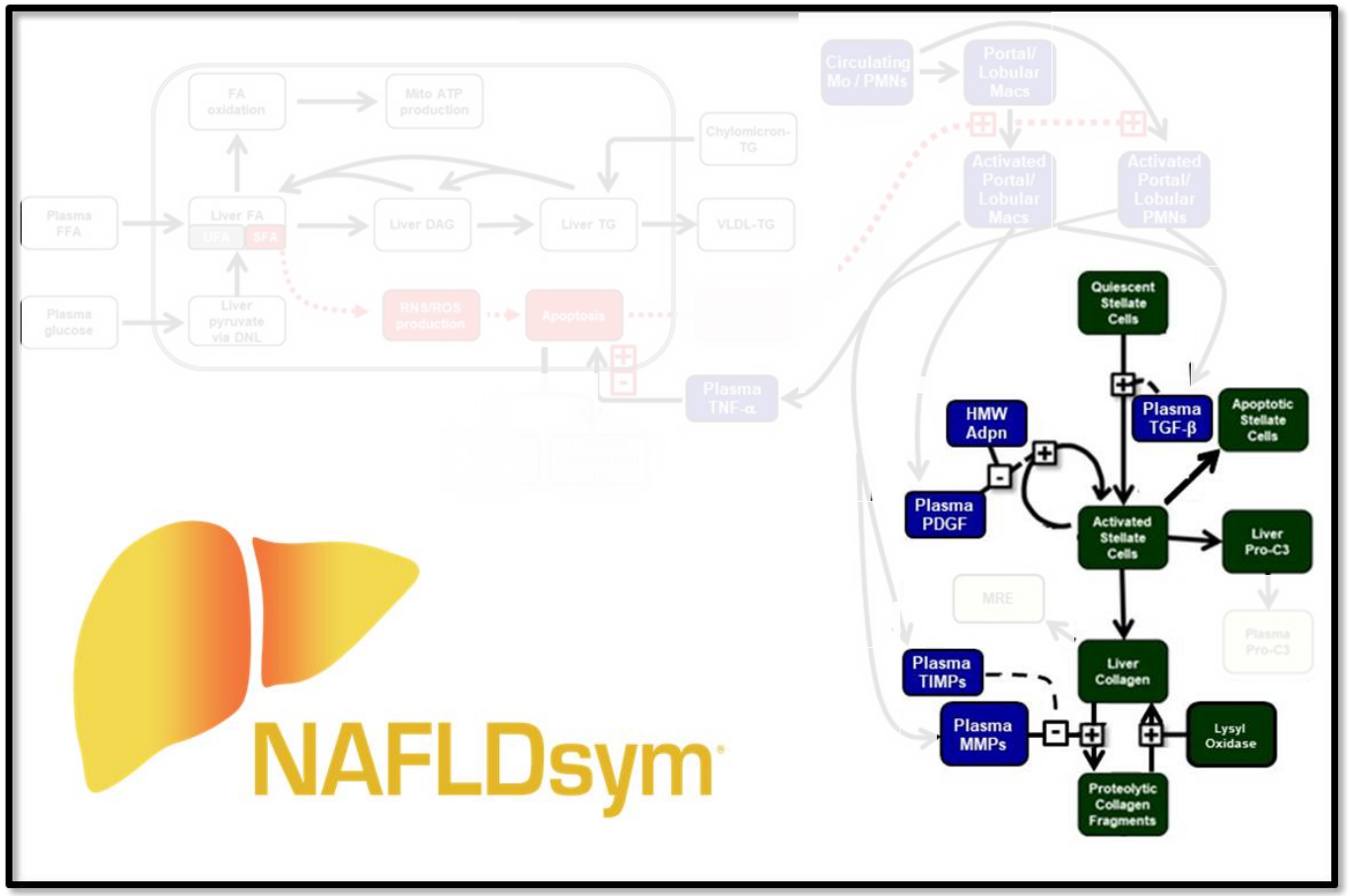
- Simulation results demonstrate modest increases in TGF-β consistent with the reported range (Das 2011)
  - Other data show no change or modest increases with disease severity
- Simulation results demonstrate modest increases in PDGF consistent with reported increases at lower Metavir fibrosis scores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





Yoshida 2014

NAFLDsym v2A Overview: Fibrosis



NAFLDsym Representation of Fibrosis in Acinar Zone Enables Prediction of Histological Fibrosis Scoring

- Long-standing guidance for NASH fibrosis scoring based on location of fibrosis (Brunt 1999)
  - Histologic visualization of collagen deposition in specific acinar zones of liver
- NAFLDsym includes Zones 1, 2, and 3 in liver, enabling mechanistic predictions of Fibrosis scores

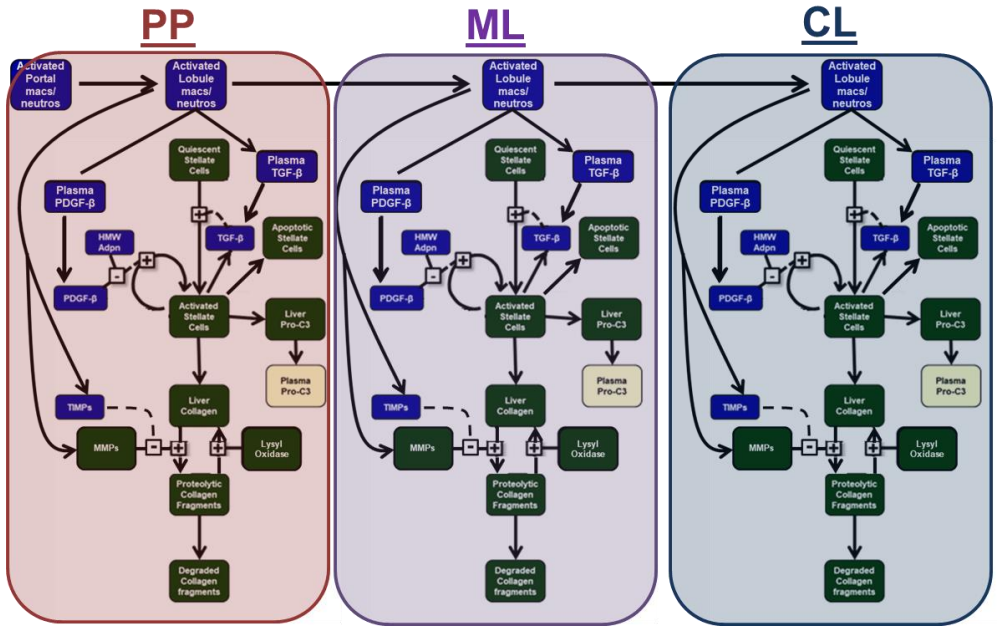
Stage 1. Zone 3 perisinusoidal/pericellular fibrosis; focally or extensively present.

Stage 2. Zone 3 perisinusoidal/pericellular fibrosis with focal or extensive periportal fibrosis.

Stage 3. Zone 3 perisinusoidal/pericellular fibrosis and portal fibrosis with focal or extensive bridging fibrosis.

Stage 4. Cirrhosis.

Brunt 1999



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)

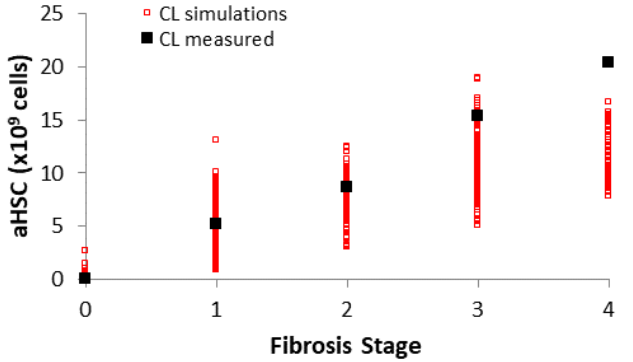
Increasing Km of HSC activation

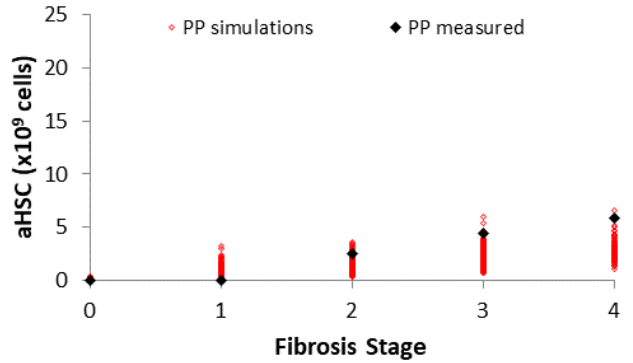
CL

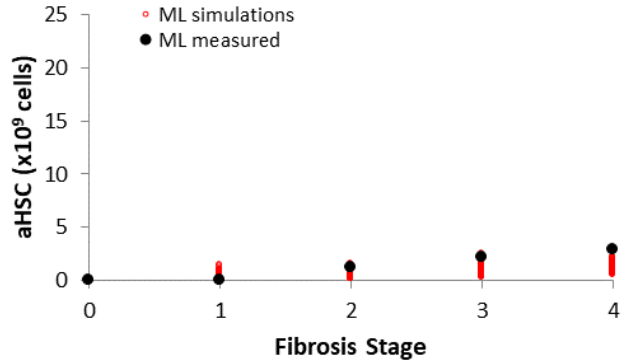
PP

ML

Abdeen 2009, El Gendi 2012, Washington 2000



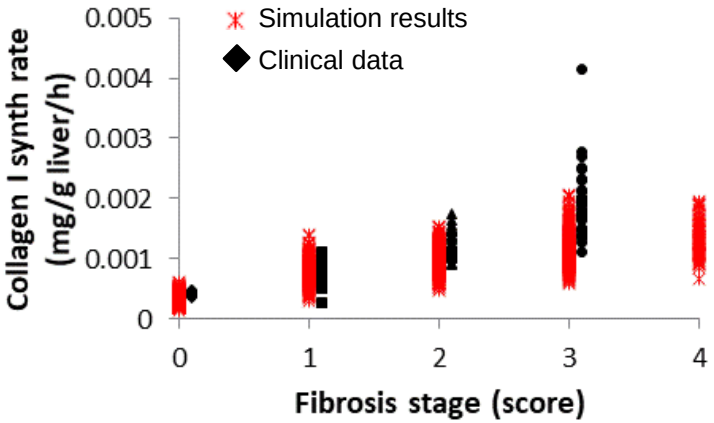




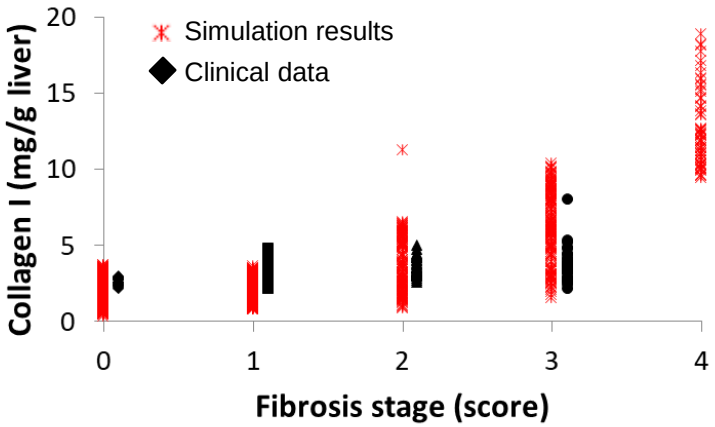
NAFLDsym v2A Includes Ranges of Collagen Synthesis Rates and Levels Consistent with Clinical Data

- 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.

- Hepatic collagen I levels are comparable in fibrosis stages 0, 1, 2, 3
  - 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.



Decaris 2017, Masugi 2018



Masugi 2018, Aycock 1989, Nakabayashi 1993

Weight Gain Correlated with NASH Disease Progression

- NASH patients studied longitudinally, including liver biopsies and histology
  - Wong 2010
  - n=52 patients
  - 3 year time interval between biopsies
- Change in body weight appeared to influence NASH disease progression
  - Based on histologic scoring
  - Patients with increased NAS had increased BMI
- Other studies have shown equivocal results for weight loss effect on progression
  - Variability in body weight over time likely factor
- Disease progression in NAFLDsym driven primarily by changes in body weight
  - Variable alterations in lipids, inflammation and fibrosis

Table 4 Factors associated with increased non-alcoholic fatty liver disease (NAFLD) activity score from baseline to month 36

Factors	Increased NAFLD activity score	Static or decreased NAFLD activity score	p
N	26	26	
Age (years)	45±9	44±9	0.65
Male gender, n (%)	16 (62)	18 (69)	0.56
Diabetes mellitus, n (%)	15 (58)	11 (42)	0.27
Hypertension, n (%)	12 (46)	14 (54)	0.58
Metabolic syndrome, n (%)	18 (69)	17 (65)	0.77
Body mass index (kg/m^2)	27.4±4.1	27.4±3.3	0.99
Change in body mass index (kg/m^2)*	0.6±1.6	−0.8±1.7	0.003
Waist circumference (cm)	92.8±11.1	92.5±6.7	0.91

Table 3 Distribution of fibrosis stage at baseline and month 36

Month 36	F0	F1	F2	F3	F4	Total
Baseline						
F0	17	7	0	1	1	26
F1	7	7	1	2	0	17
F2	4	1	0	1	1	7
F3	0	0	1	0	0	1
F4	0	0	0	0	1	1
Total	28	15	2	4	3	52

Table 2 Distribution of disease activity at baseline and month 36

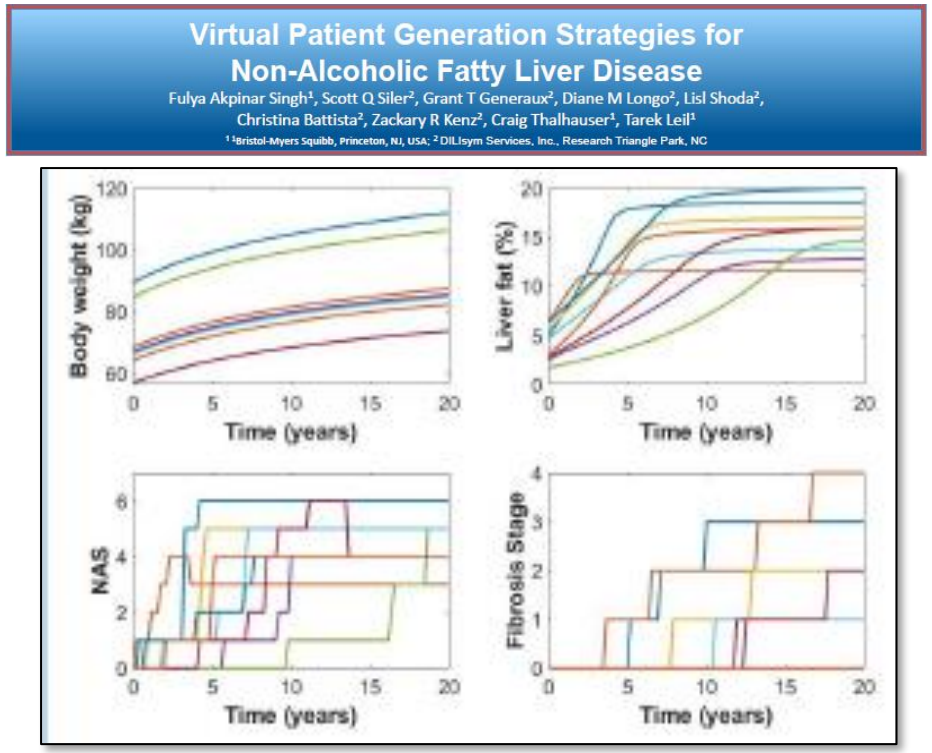
NAFLD activity score at month 36	<3	3–4	≥5	Total
NAFLD activity score at baseline				
<3	12	16	1	29
3–4	5	10	3	18
≥5	0	5	0	5
Total	17	31	4	52

NAFLD, non-alcoholic fatty liver disease.

Wong 2010

NASH Disease Progression via Weight Gain Predicted in NAFLDsym

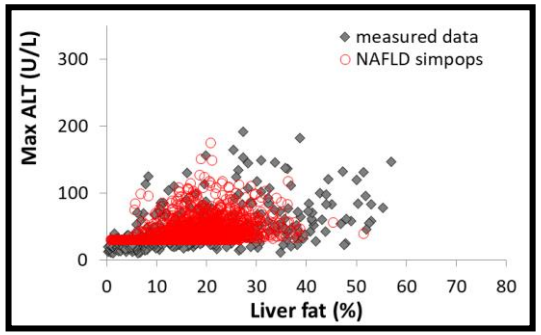
- Simulated weight gain over 20 years in SimCohorts
  - 20-30% increase in body weight
  - McTigue 2002
- Increase in food intake and weight gain elicit increases in steatosis
  - Driven by increases in de novo lipogenesis and adipose fatty acid release
- Increased NAS score over time due to lipotoxicity and increased hepatocellular apoptosis and hepatic inflammation
  - Release of pro-fibrotic mediators also drives increased fibrosis

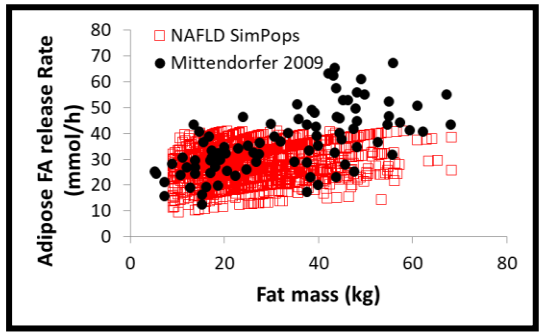


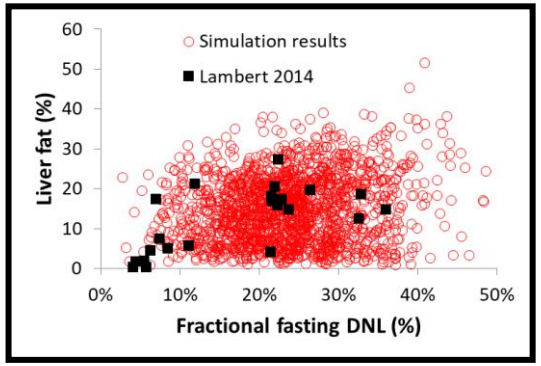
Akpinar Singh 2019

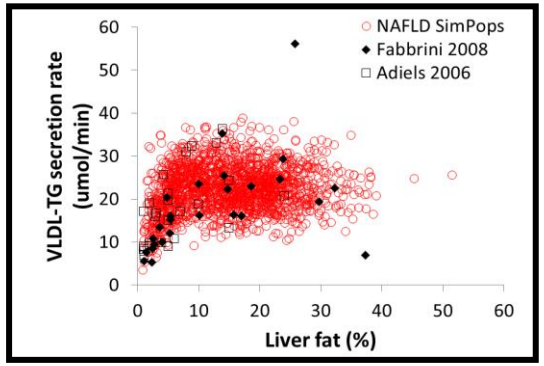
NAFLD SimPops Validation

- Simulated NAFLD patients (n=1707) include combinations of parameter ranges based on reported responses from literature [8-12].
- Simulated patients within SimPops have pathophysiologic and clinical characteristics consistent with what has been reported in literature [8-12]









Variables Used to Construct the NAFLD SimPops™
Body weight
Adipose FA release
De novo lipogenesis
RNS-ROS clearance
Mitochondria function
VLDL-TG secretion rates
Plasma glucose
Hepatic glucose uptake
Plasma TG clearance
Apoptotic sensitivity to RNS-ROS
Necrotic sensitivity to ATP reductions
Hepatocyte regeneration

[8]. Maximos et al. Hepatology. 2015 Jan;61(1):153-60.
[9]. Lambert et al. Gastroenterology. 2014 Mar;146(3):726-35
[10]. Fabbrini et al. Gastroenterology. 2008 Feb;134(2):424-31
[11]. Adiels et al. Diabetologia. 2006 Apr;49(4):755-65
[12]. Mittendorfer et al. Obesity. 2009 Oct;17(10):1872-7

NAFLDsym v2A SimPops Predicted Worsening of NAS Score with Weight Gain is Consistent with Clinical Reports

- Simulated4-10% weight gain in SimCohorts
  - Simulating study undertaken by Wong et al.
  - SimCohorts comprised of F3 and F4 simulated patients
- Simulated patients with increased NAS score had association with weight gain
  - Consistent with correlations reported by Wong et al.
  - Heterogeneity of response in clinical cohort and SimCohorts
- Simulated weight gain can contribute to disease progression in NAFLDsym

Clinical Data

Table 4 Factors associated with increased non-alcoholic fatty liver disease (NAFLD) activity score from baseline to month 36

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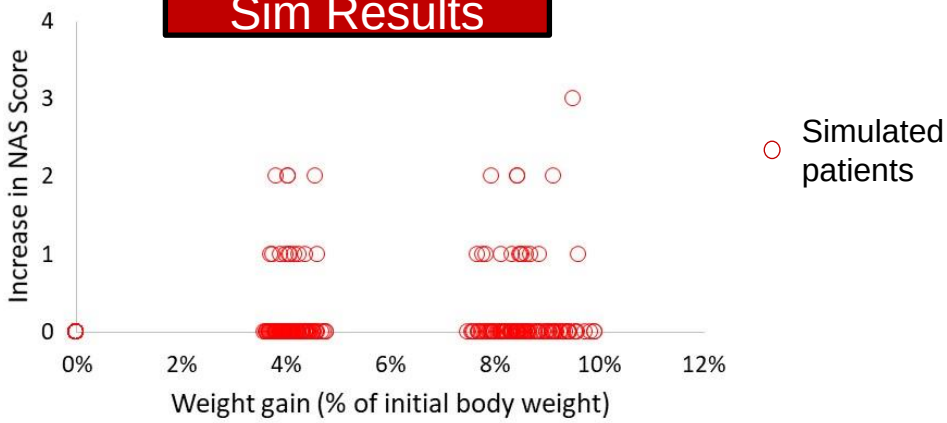
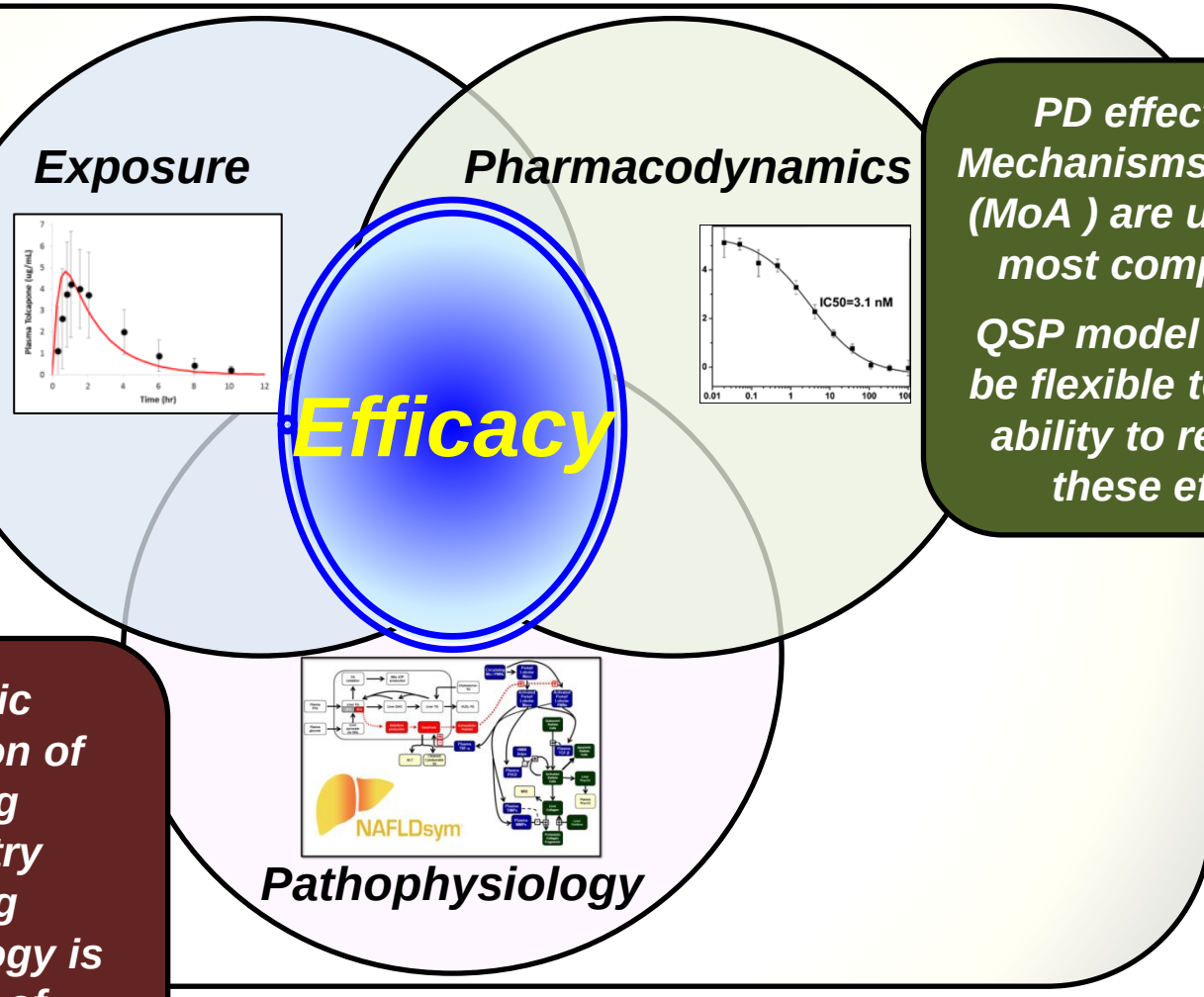
Wong 2010

Sim Results



DILIsym Services Is Using QSP Modeling to Predict Efficacy and Safety of Drugs in Development

Predicted compound concentrations at site of target often require PBPK models



PD effects and Mechanisms of Action (MoA) are unique for most compounds;

QSP model needs to be flexible to provide ability to represent these effects

Mechanistic representation of underlying biochemistry describing pathophysiology is foundation of QSP models