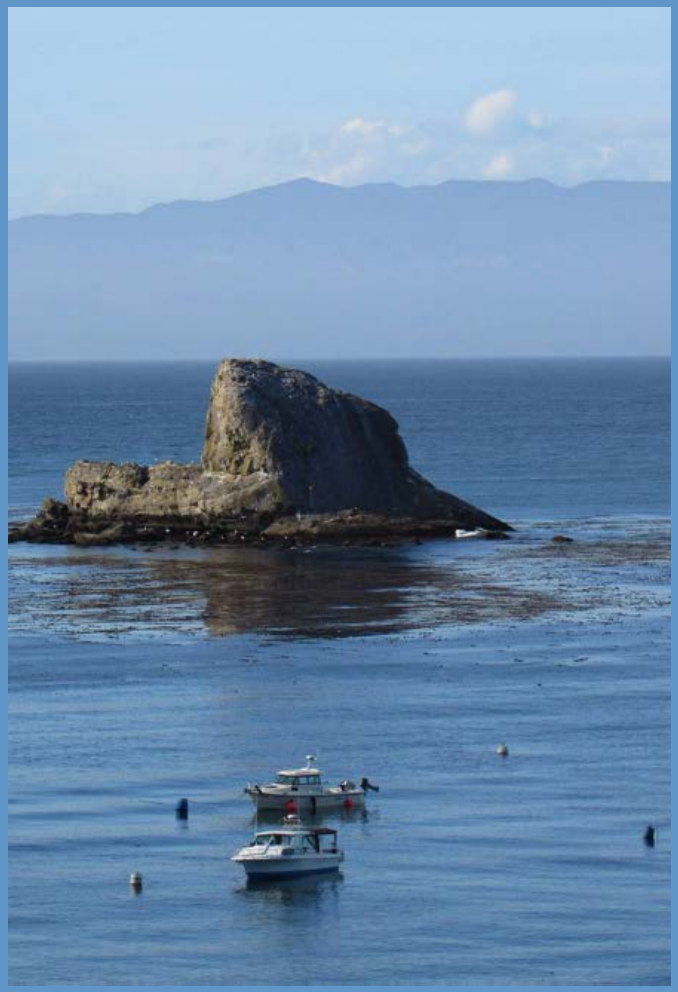
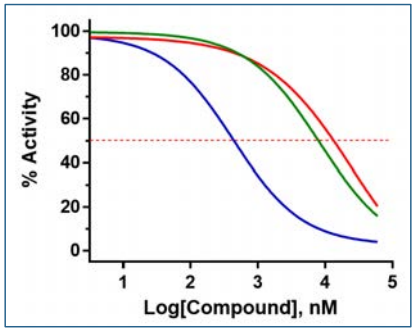


SRT-015: A Second-Generation ASK1 Inhibitor for Liver Diseases Including NASH

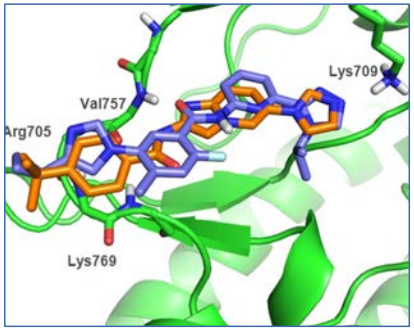
Kathleen Elias, PhD
Seal Rock Therapeutics, Inc.

Seal Rock Therapeutics: Discovery and development of kinase inhibitors

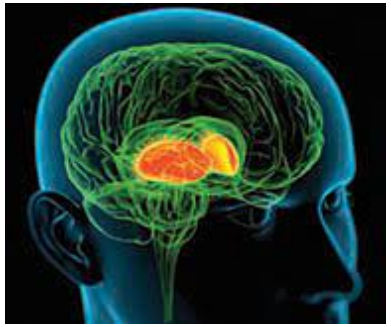
Most advanced program is phase 2-ready | 2nd series compounds are preclinical



Identified off-target liabilities
of all competitors' ASK1 inhibitors



400+ compounds
2 chemical series
7 patents



Discovered first oral CNS-
penetrant LRRK2/ ASK1 inhibitors

SRT-015

Clinical-stage best-in-class ASK1 inhibitor

Preclinically validated in multiple diseases

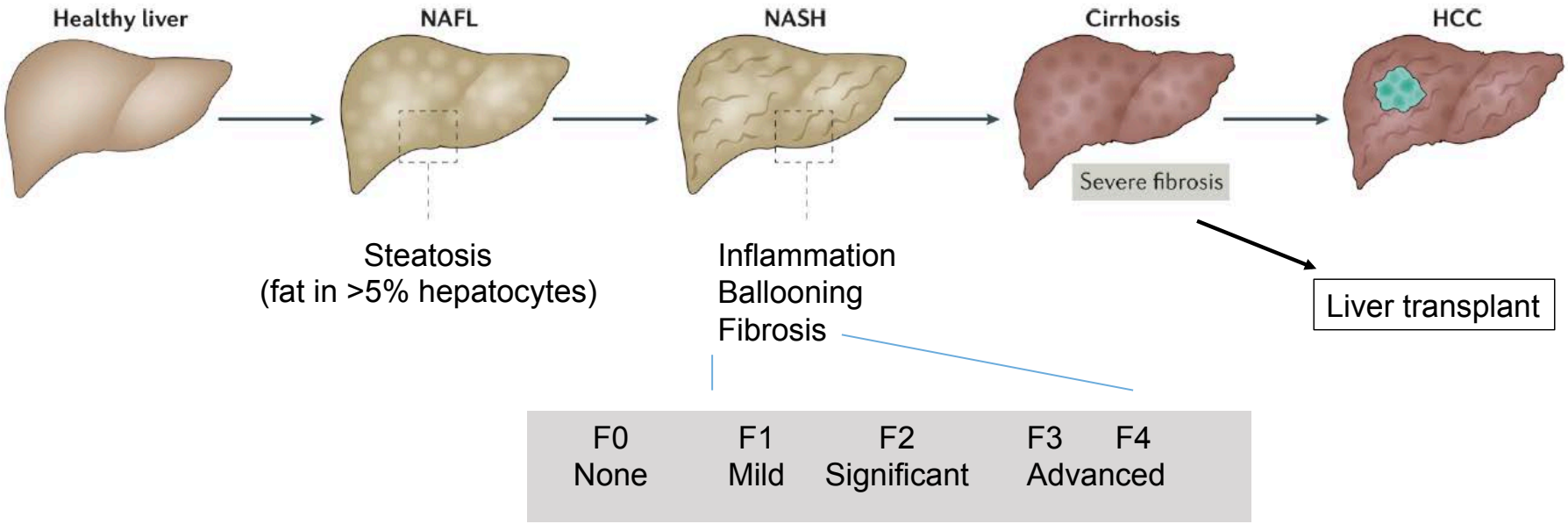
Completed Phase 1 clinical trial

SRT-055 series

1st in class LRRK2/ASK1 dual inhibitors

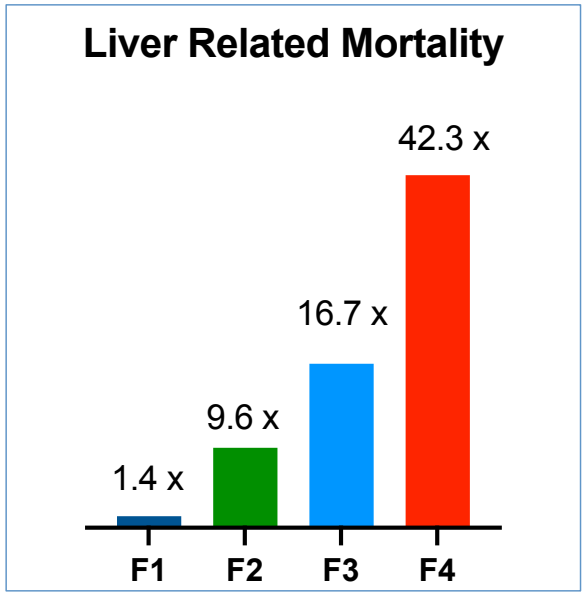
Preclinically validated for CNS diseases

NAFLD/NASH mechanism progression



Huby 2022
Heyens 2021
Goodman 2007

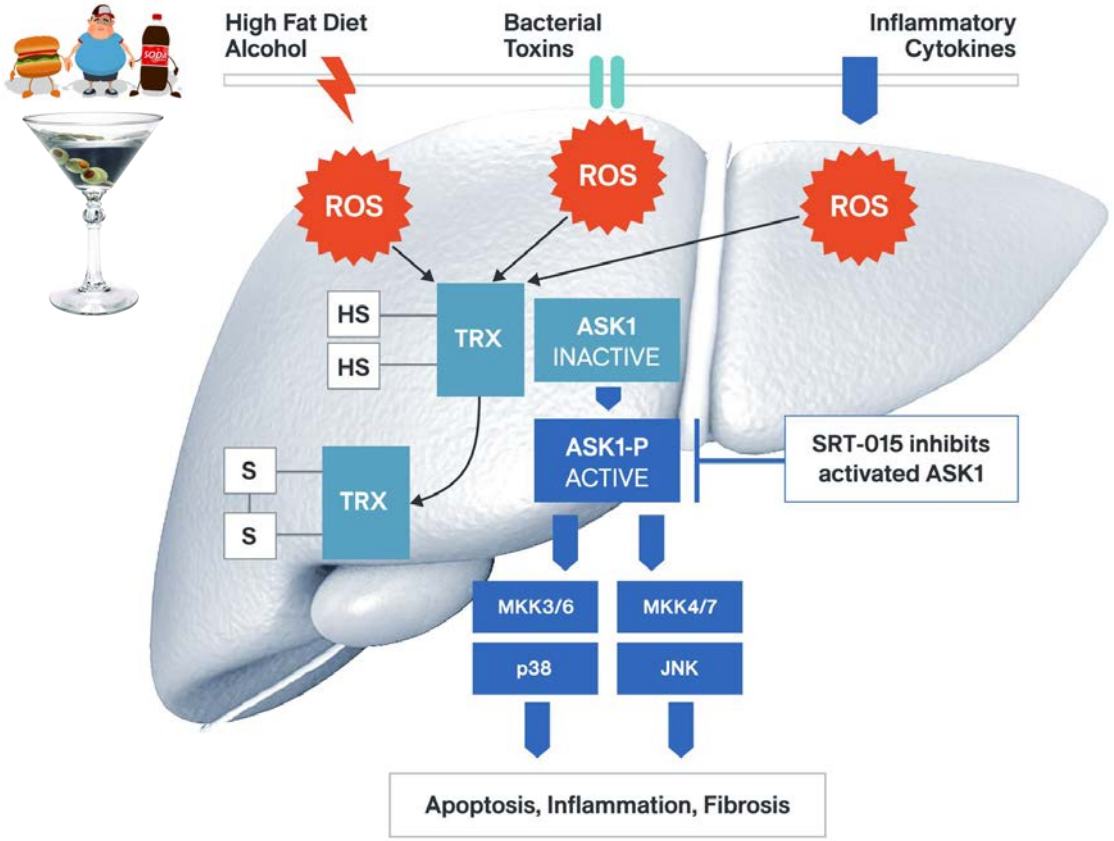
Fibrosis stage is primary determinate of mortality



Compared with FO patients

Noureddin AASLD 2023
Rinella, 2023. Dulai 2017
Hagstrom 201,Taylor 2020

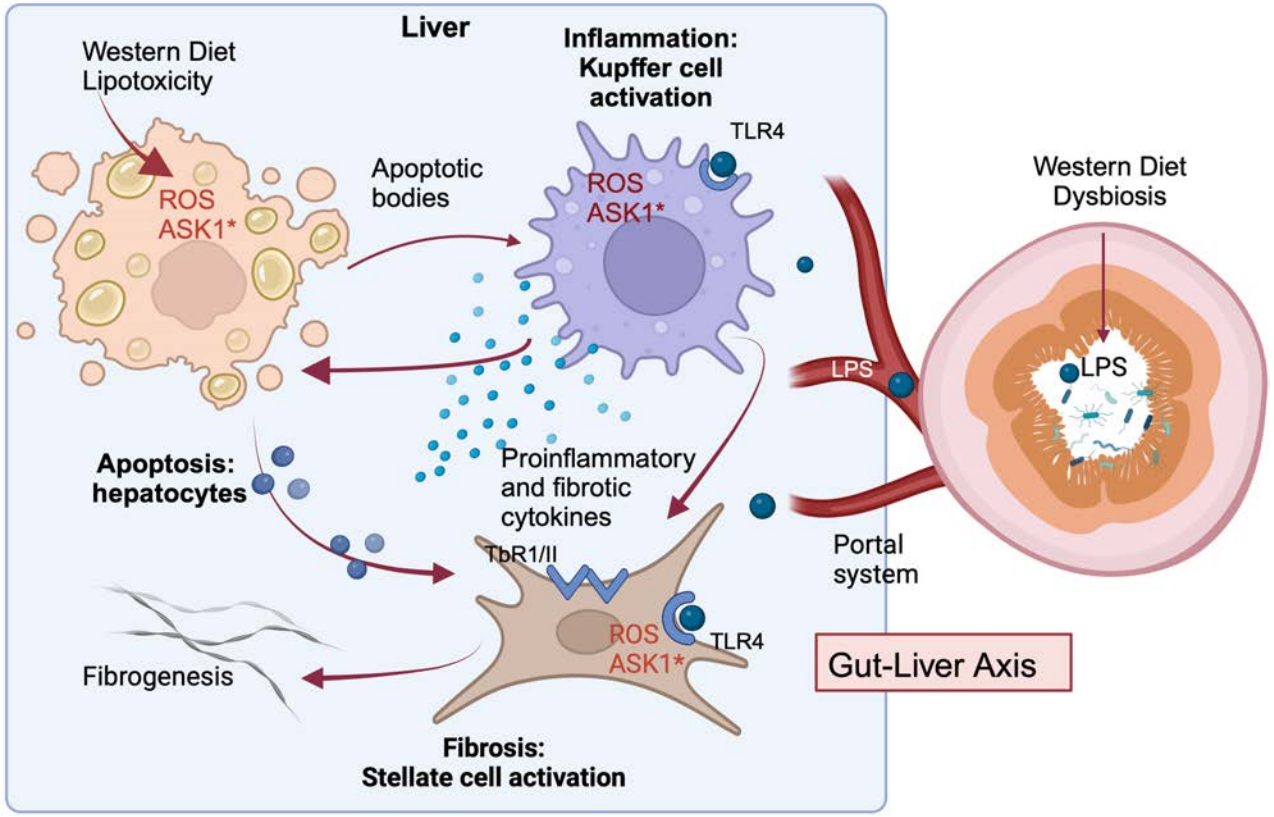
ASK1: Central node in fibrotic, inflammatory and apoptotic cascade



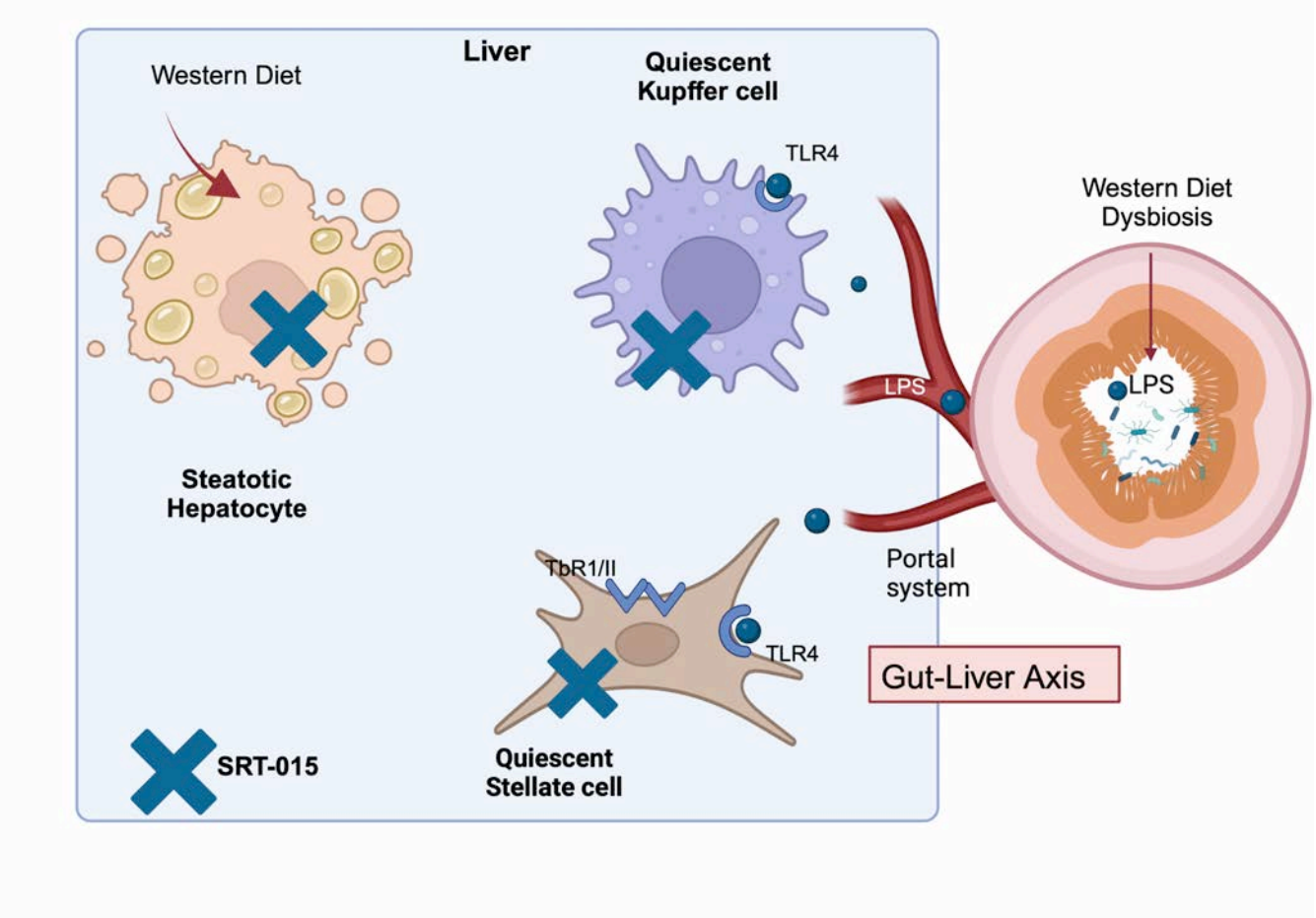
- **Target:** ASK1 is a 1,374 residue polypeptide mitogen-activated kinase kinase kinase 5 (MAP3K5) found in all cells
- **Activation:** Inactive state bound to the redox-regulatory protein thioredoxin (TRX) preventing its activation
- **Function:** When activated by stress factors induces the p38 and JNK pathway apoptosis, inflammation and fibrotic cascade

✓ **Inhibition of ASK1:** Reduce apoptosis, inflammation and fibrosis

NASH progression involves all liver cell types and gut-liver axis

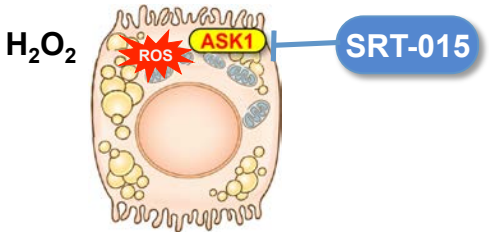


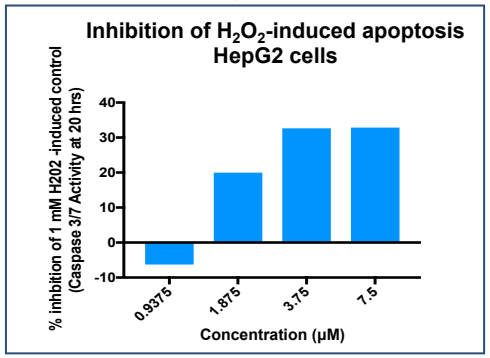
SRT-015 inhibits pathology in all liver cell types



SRT-015: Direct inhibition of apoptosis, inflammation, and fibrosis

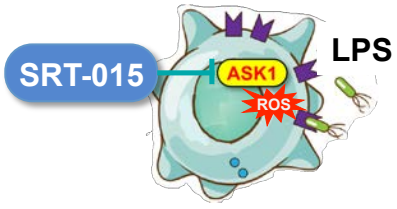
APOPTOSIS

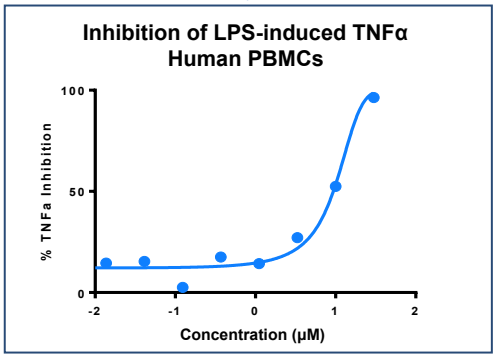




Anti-apoptotic

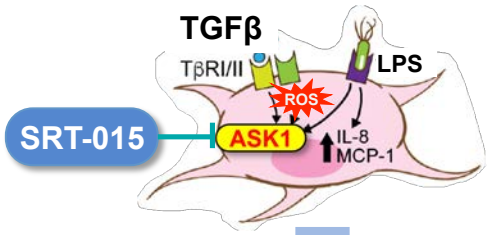
INFLAMMATION

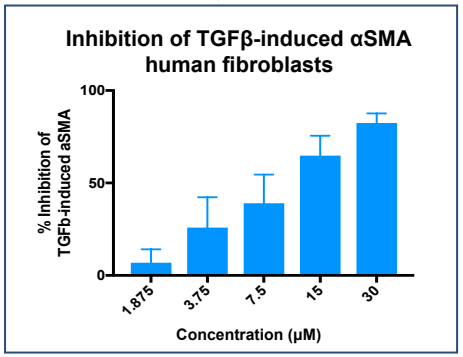




Anti-inflammatory

FIBROSIS





Antifibrotic

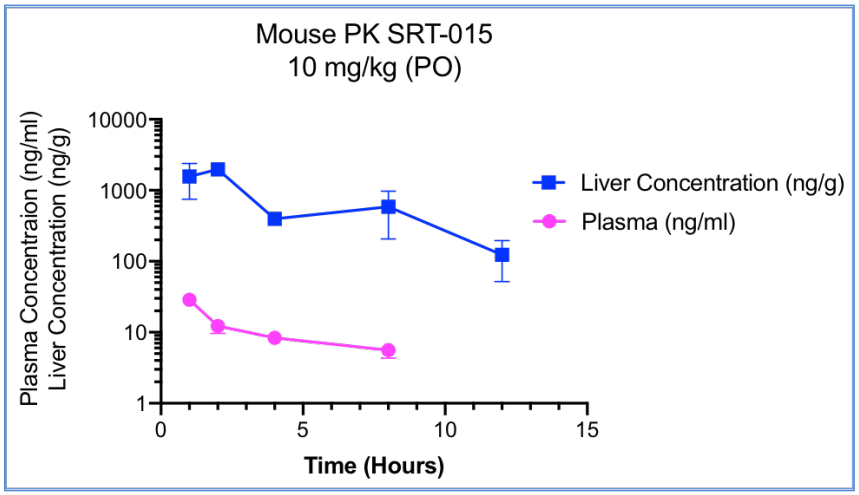
SRT-015 has a unique trimodal mechanism of action

SRT-015: In vivo efficacy in multiple acute and chronic liver models

- **Significant efficacy in liver injury models of varying etiologies**
  - Acetaminophen overdose hepatotoxicity
    - Reduced liver injury and target engagement (Elias EASL 2023)
  - Alcoholic hepatitis (Pyrazole/LPS model)

    Reduced liver injury (Elias EASL 2023)
  - Cholestatic liver disease (bile duct ligation)
    - Reduced fibrosis
  - **Metabolic injury (DIO-NASH therapeutic model)**
    - **Reduced liver injury, inflammation, fibrosis, and apoptosis** (Elias AASD 2020)

SRT-015: Uniquely exhibits a liver-selective distribution PK profile with >10x liver:plasma ratio



Liver-selective distribution of SRT-015 observed in all species evaluated (mice, rats, and non-human primates)

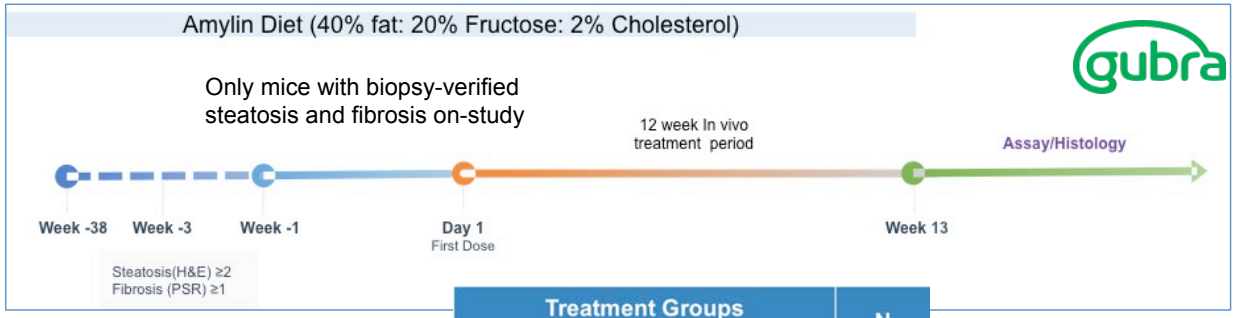
No hepatic accumulation after 12 weeks of chronic administration in DIO-NASH model

No hepatic accumulation in GLP-toxicology studies

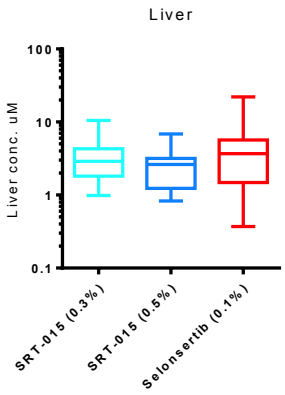
Low plasma exposure of SRT-015 allows exploration of higher doses for greater efficacy while maintaining superior systemic safety profile

Elias AASLD 2020

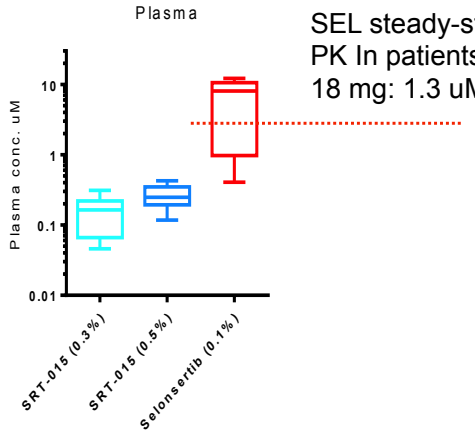
SRT-015 evaluated in therapeutic DIO-NASH model; Compared with selonsertib



Treatment Groups (drug in diet % w/w)	N
CHOW Diet	10
NASH Diet	12
NASH + SRT-015 (0.3%)	11
NASH + SRT-015 (0.5%)	11
NASH + Selonsertib (0.1%)	12



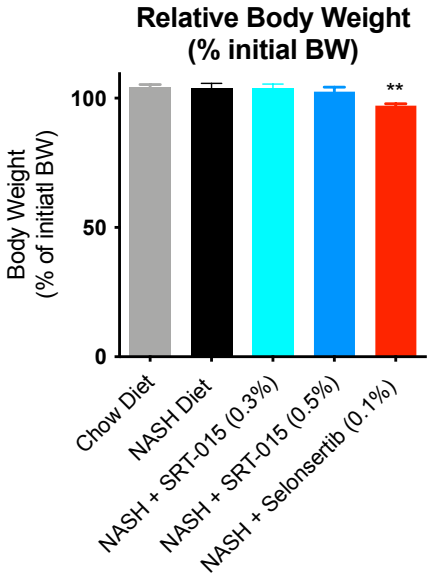
Liver levels of SRT-015 matched selonsertib liver levels

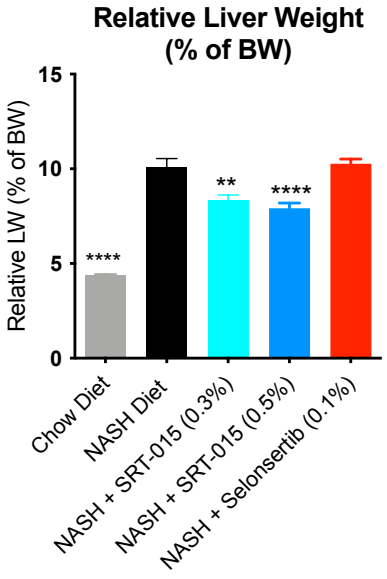


SEL steady-state PK In patients at 18 mg: 1.3 uM

Selonsertib plasma levels matched plasma levels reported in clinical trials

SRT-015 treatment decreased DIO-induced hepatomegaly

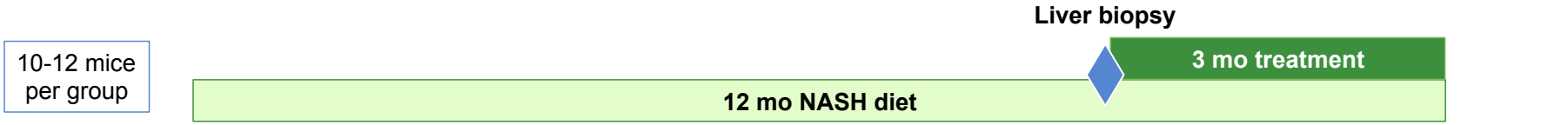




** P< 0.01 compared to NASH Vehicle
*** P< 0.001 compared to NASH Vehicle
****P< 0.0001 compared to NASH Vehicle

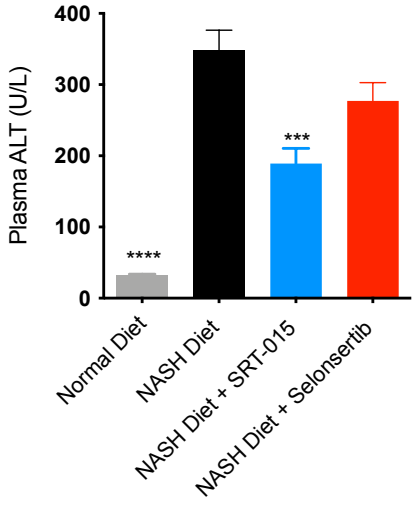
SRT-015 is acting through direct effects, not decreased body weight
At a clinically relevant exposure, selonsertib had no effect on liver weight

SRT-015: In vivo efficacy in gold-standard diet-induced NASH model



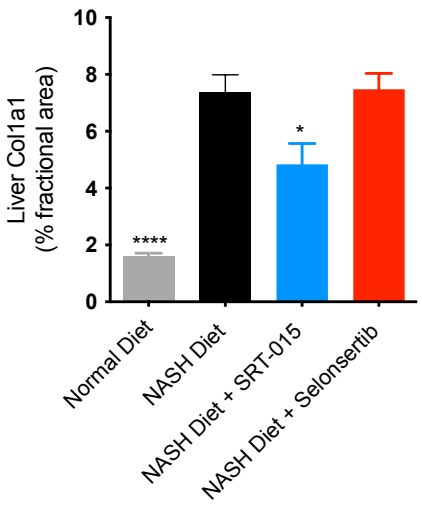
Liver Injury

Reduced Plasma ALT



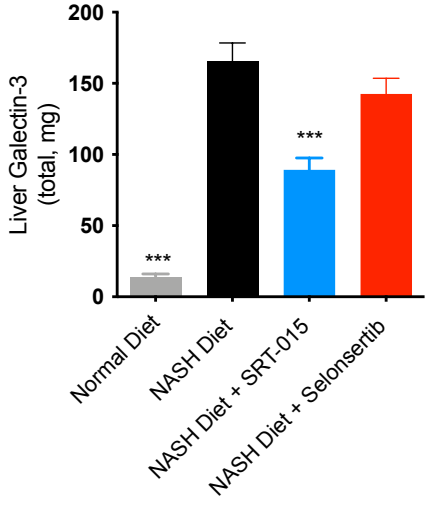
Fibrosis

Reduced Liver Col1a1



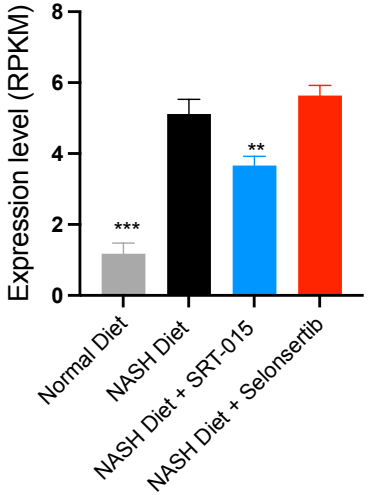
Inflammation

Reduced Liver Galactin-3



Apoptosis

Reduced Liver CASP1



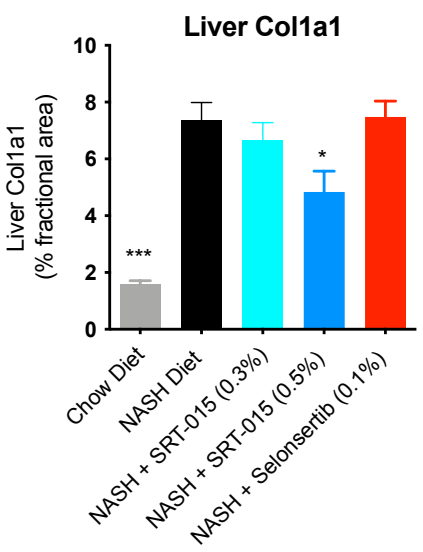
SRT-015 efficacious with no decrease in BW

No significant effects with selonsertib

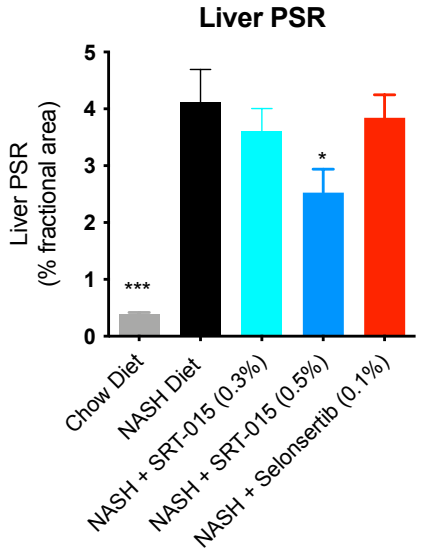
* P < 0.05 vs NASH vehicle
** P < 0.01 vs NASH vehicle
*** P< 0.001 vs NASH vehicle

All fibrosis biomarkers significantly decreased with SRT-015 treatment

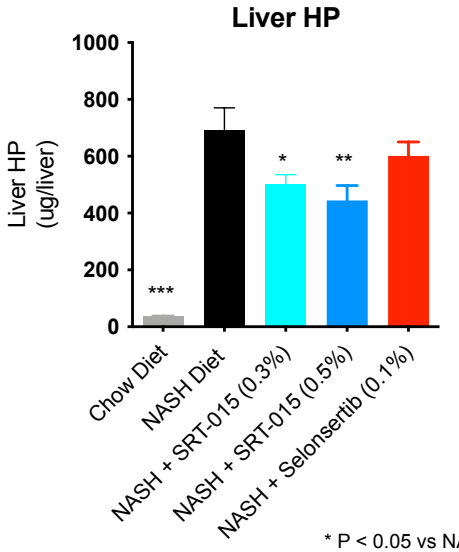
Col1a1 quantified by morphometry



Pico Sirius Red quantified by morphometry



Hydroxyproline quantified by biochemistry

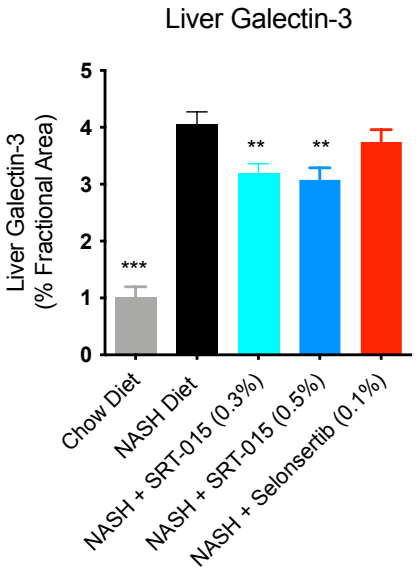


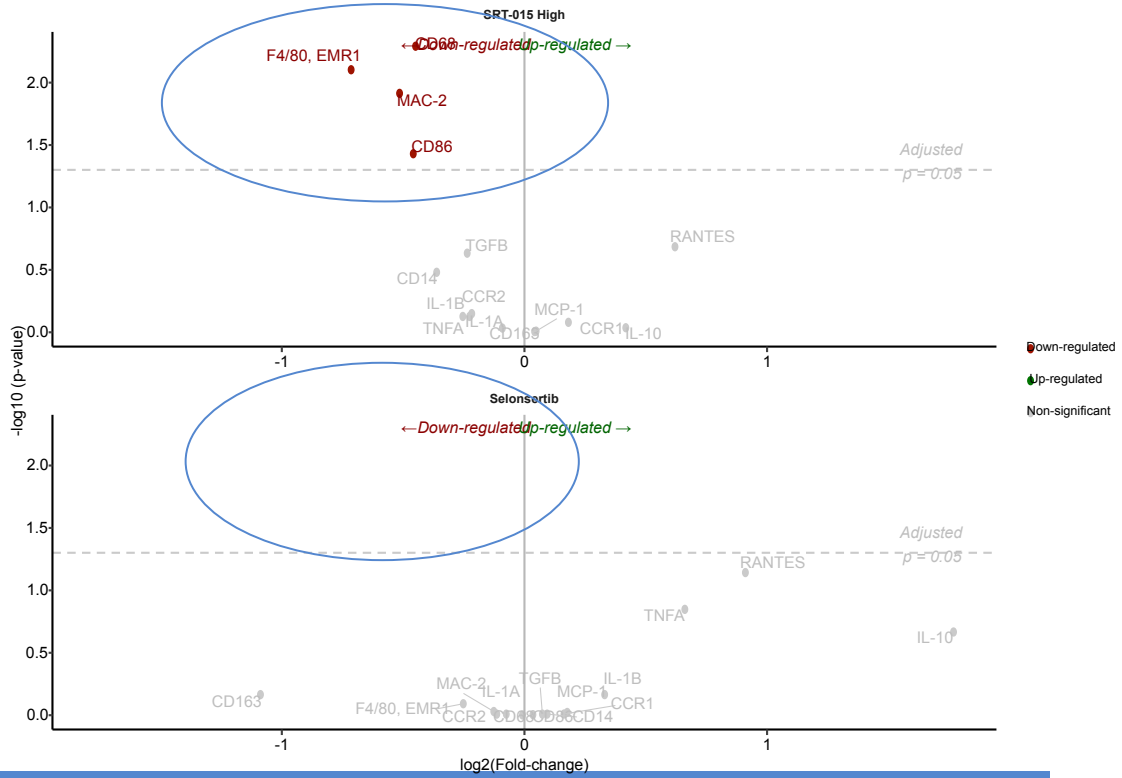
* P < 0.05 vs NASH diet
** P < 0.01 vs NASH diet
*** P< 0.001 vs NASH diet

SRT-015 decreasing fibrosis directly, not by decreasing body weight
At a clinically relevant exposure, selonsertib had no antifibrotic activity

SRT-015: Decreased inflammation, monocyte recruitment factors

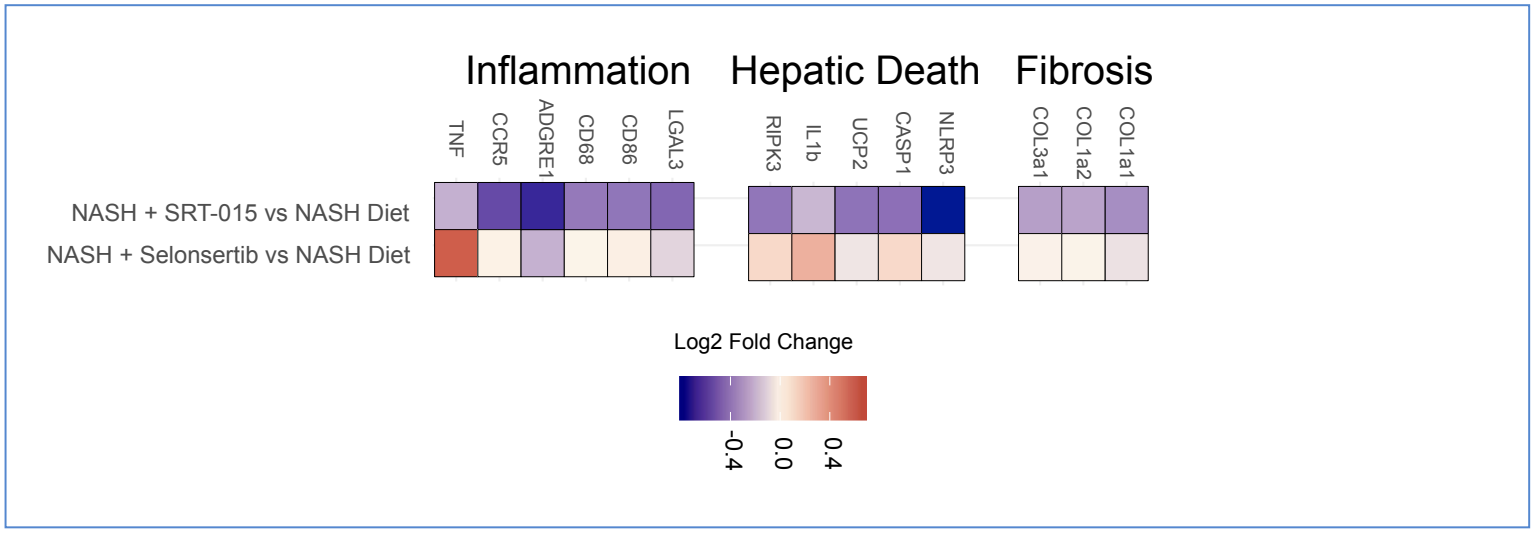
Galectin-3 quantified by morphometry





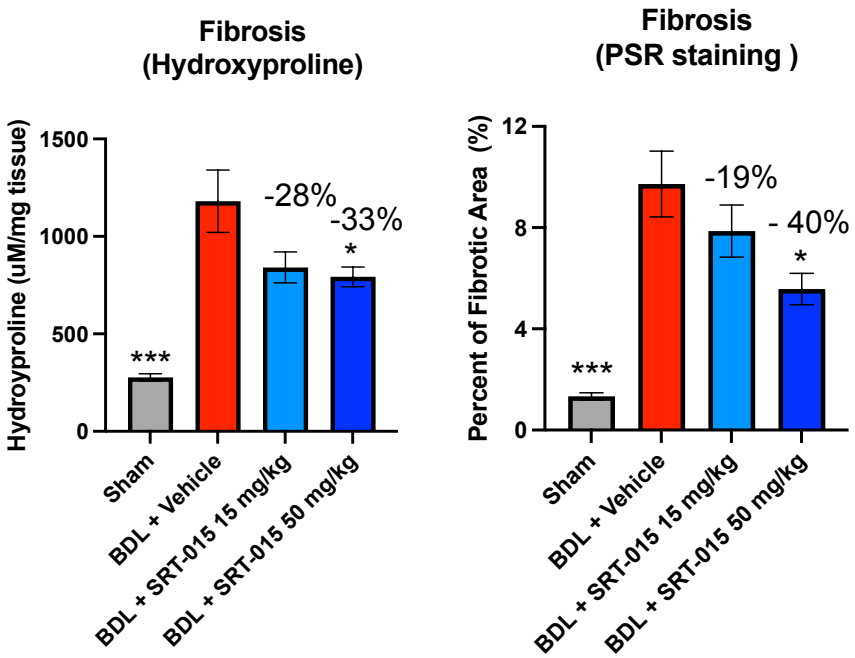
CD86, CD68, F4/80, EMR1, Galectin-3 (MAC-2) all down regulated only by SRT-015

RNAseq Analysis: Confirmation of SRT-015 direct in vivo mechanisms



Confirmed SRT-015: Direct decrease of inflammation, hepatocyte cell death and fibrosis
Hepatic cell death inhibition partially via the inflammasome pathway

SRT-015: Fibrotic efficacy in 14-day rat BDL cholestatic model



* P< 0.05 ANOVA, Dunnett's

SRT-015 decreased fibrosis even in model so severe there were no changes in liver enzymes

Why did selonsertib fail in NASH/ALD clinical trials?

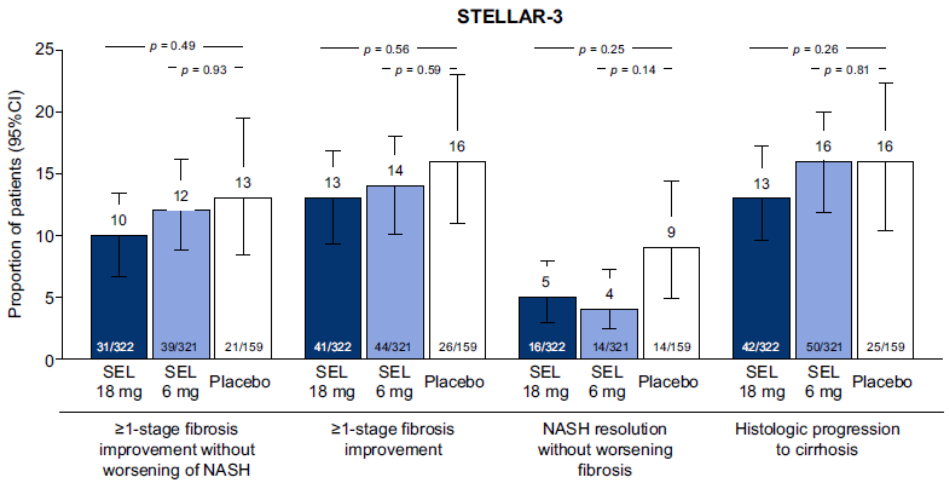
- Preclinical
  - No efficacy in gold-standard therapeutic DIO-NASH model (Elias 2020; Choi 2019)
  - Direct in vitro cytotoxicity (Elias 2020), CDK6 inhibition (Biag 2022), microtubule stabilizer (Ramirez-Rios 2020)
  - Selonsertib is potent inhibitor of BSEP, P-gp, and BCRP, key efflux pumps (Nelson 2017)
- Clinical: Why was selonsertib dose limited to 18 mg in the clinic?

  Off-target liabilities of selonsertib that may have necessitated limiting its clinical dose include:
  - QT prolongation at high doses in human subjects (Nelson 2015)
  - Selonsertib is extensively metabolized (Nelson 2017) and may have been dosed lower to limit the significant accumulation of a CYP3A4 generated metabolite
  - One metabolite accumulates at 6x parent after 14 days in human subjects (Nelson 2020)
  - Over-reliance on p-p38 as marker of target engagement (Phase 3, Harrison 2020)

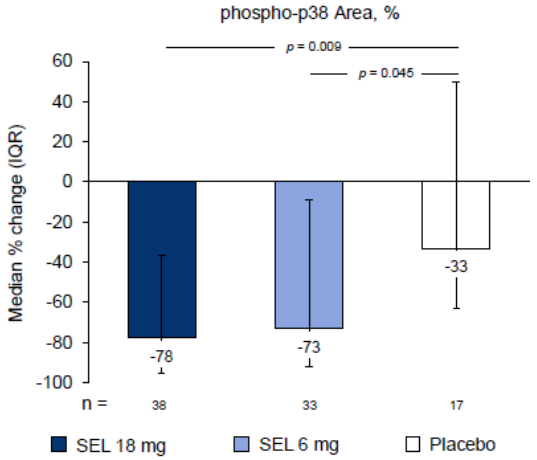
Off-target effects specific to selonsertib, not p-ASK1 target nor SRT-015

No clinical response with selonsertib in Phase 3 STELLAR clinical trial

No clinical response with selonsertib



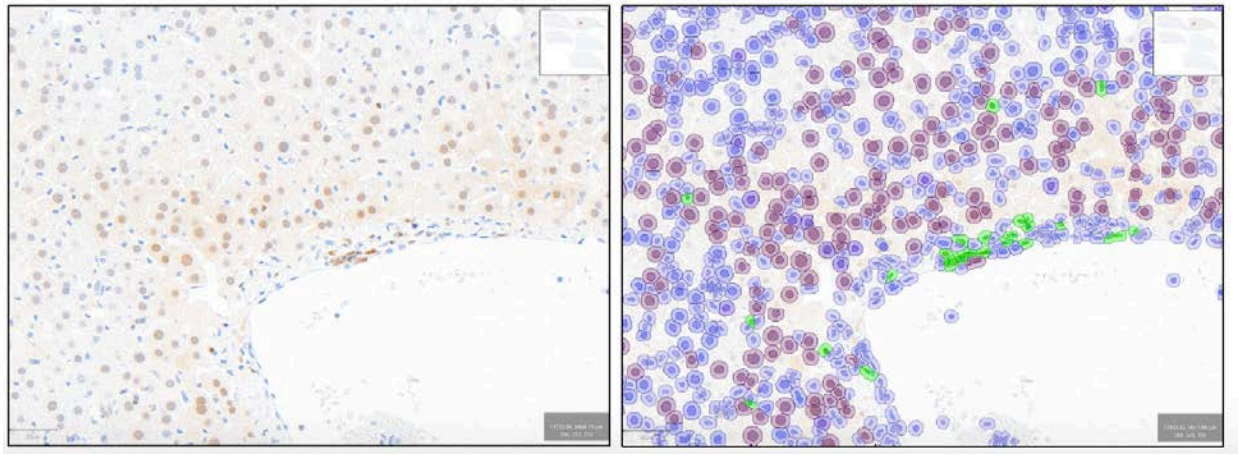
Yet positive p-p38 biomarker response



Harrison et al. Results from randomized phase III STELLAR trials. J Hepatol. 2020

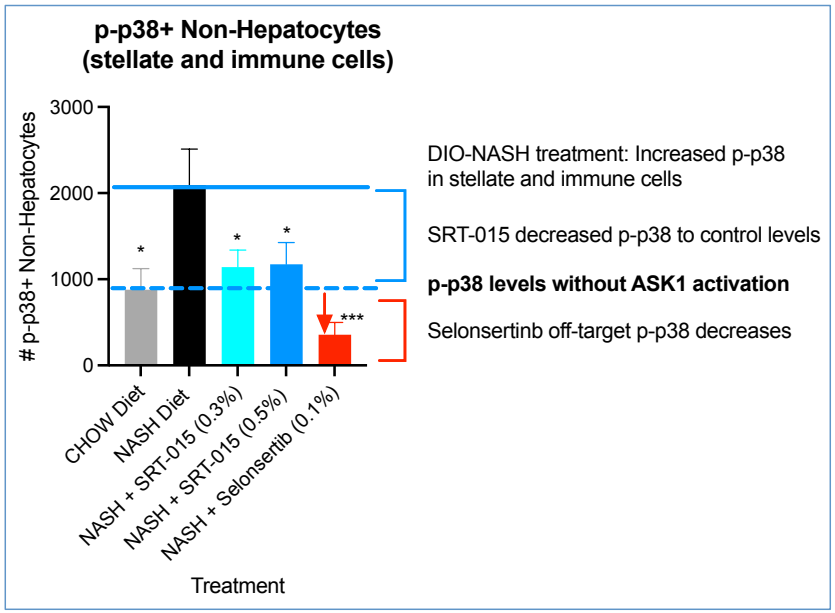
AI analysis of p-p38 positive immune/stellate cells and hepatocytes

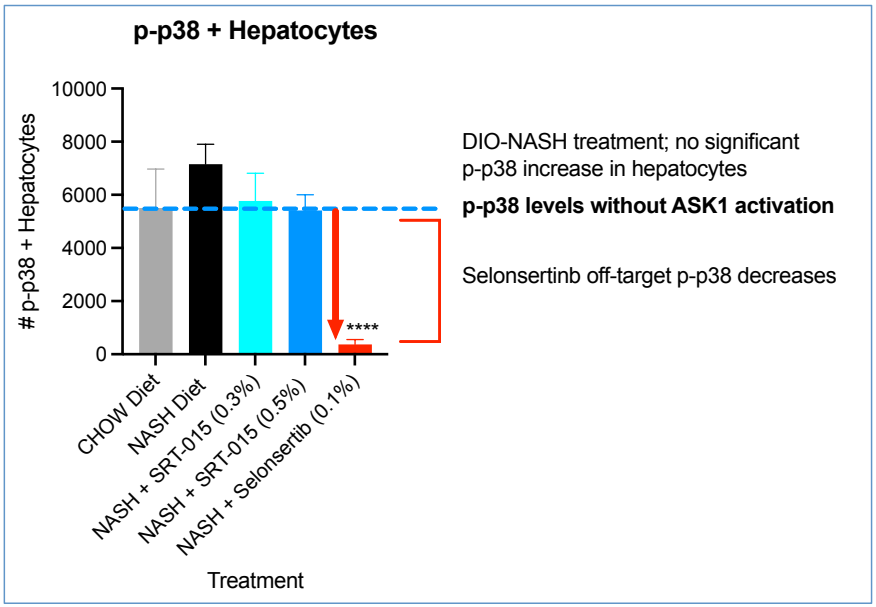
Evaluate p-p38 in therapeutic DIO-NASH study with SRT-015 efficacy and no effect of selonsertib



p-p38 IHC (p-p38 antiboty Thr180/Tyr182; left, brown) and corresponding segmentation over the same section of tissue identifying p-p38 positive hepatocytes (right, large cells, red), positive immune/stellate cells (right, small cells, green) and negative cells (right, blue) used for quantification.

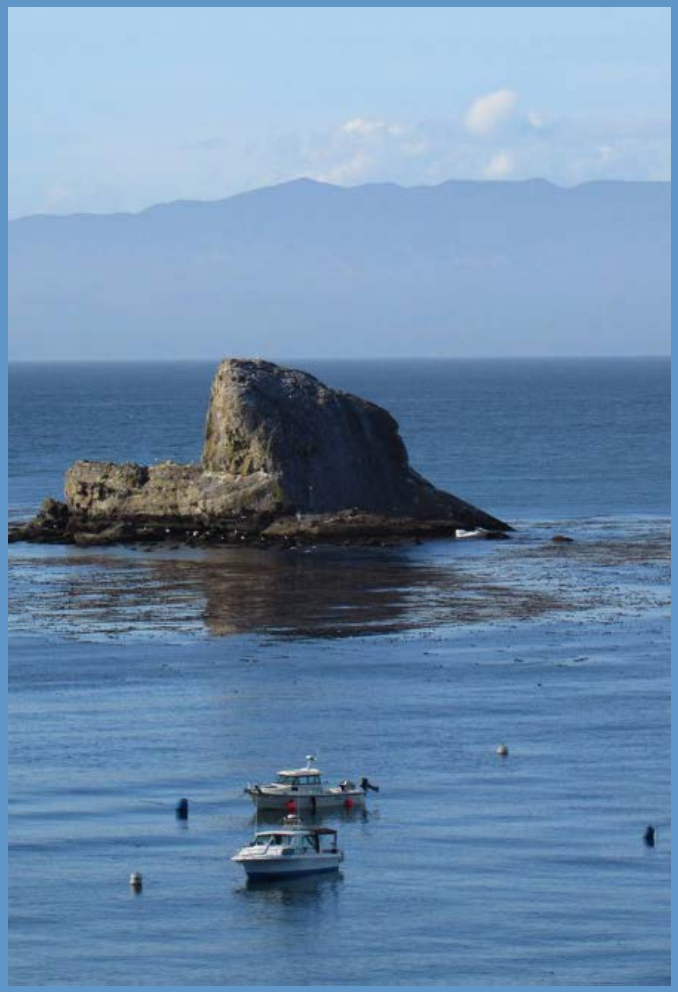
SRT-015 p-p38 target engagement corresponds with preclinical efficacy
Selonsertib p-p38 preclinical responses ASK1-independent effect





DIO-induced p-p38 in immune/stellate cells decreased by SRT-015 treatment
Selonsertib decreased p-p38 in all cell types by ASK1-independent mechanism

SRT-015 treatment demonstrates p-p38 correlation with efficacy in contrast to selonsertib



SRT-015 Phase 1 SAD/MAD trial results

Phase 1, randomized, double-blind, placebo controlled single-ascending (SAD) and multiple-ascending dose (MAD)

- SRT-015 administered as oral suspension in fasted state
- Subjected randomized 3:1 (active:placebo)
- SAD phase
  - 5 cohorts of 7-8 subjects
  - Single dose
  - Cohort dose levels 40 mg, 80 mg, 160 mg, 320 mg and 640 mg
  - Evaluated for safety and PK for 7 days after single dose
- MAD phase
  - 4 cohorts of 8 subjects
  - Twice daily dosing for 13 doses (BID over 7 days)
  - Cohort dose levels BID: 40 mg, 80 mg, 160 mg, and 320 mg
  - Evaluated for safety and PK for 14 days (through 7 days after last dose)

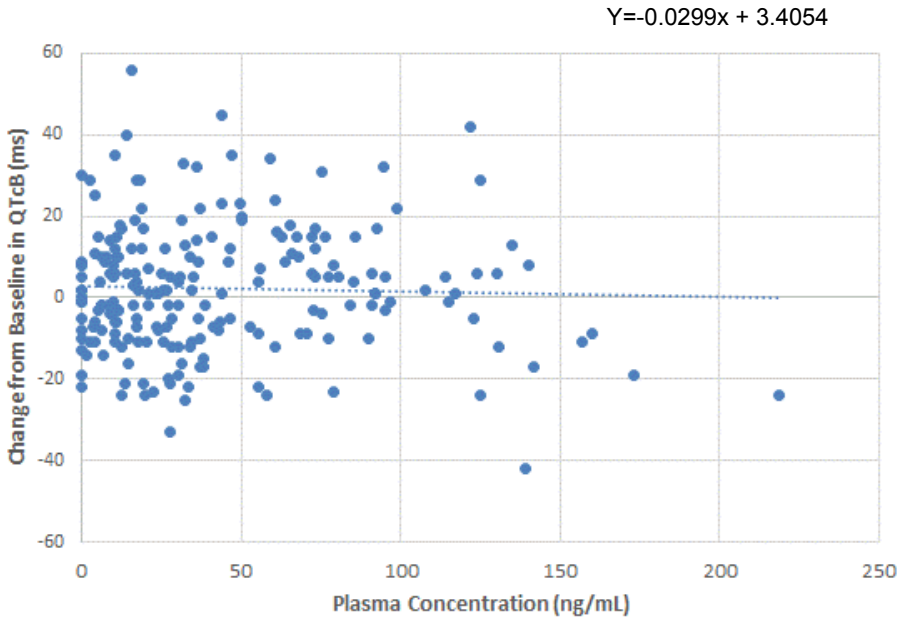
SRT-015: Safe and well-tolerated in healthy volunteers

Single Ascending Dose: 5 cohorts (40, 80, 160, 320 and 640 mg) 27 active:10 placebo

Multiple Ascending Dose: 4 cohorts (40, 80, 160, and 320 mg BID for 7 days) 24 active:8 placebo

	SAD	MAD
Serious adverse events	none	none
Adverse events	11 events in 8 subjects	20 events in 11 subjects
Dose relationship	none	none
Events reported by >1 subject	• headache (3; 2 in 40 mg, 1 in 640 mg) • extremity pain (2; 1 each in 40 and 160 mg)	• headache (2; both 80 mg BID) • contact dermatitis (2; both 40 mg BID)
Intensity	All events mild	All events were mild or moderate

SRT-015 in phase 1 trial demonstrated no drug effect on QT Interval



- No association between QTc and plasma concentration

QTc calculated using Bazett's correction formula

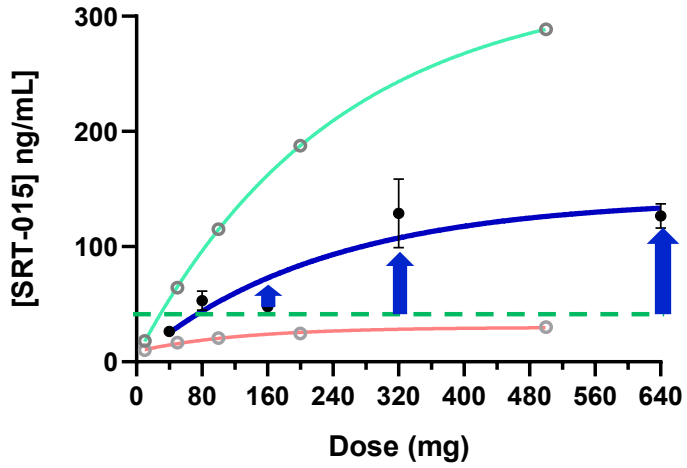
Optimal pharmacokinetic profile in phase 1 trial

Efficacious exposure in all but lowest dose level

SimulationsPlus

Plasma PK

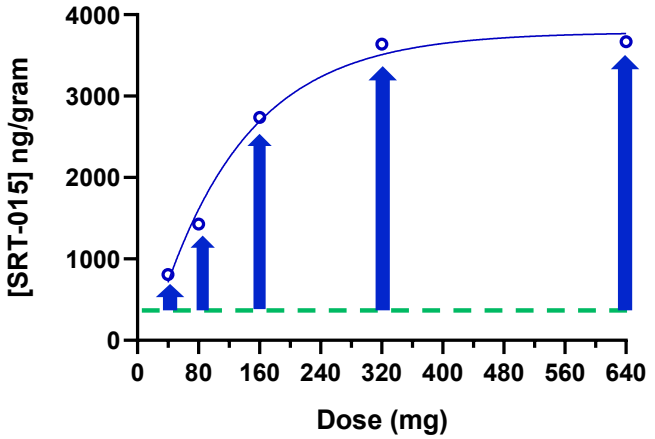
Predicted and actual plasma exposure (C_{max})



- Predicted optimistic
- Actual
- Predicted conservative
- Minimum efficacious exposure

Predicted Liver PK

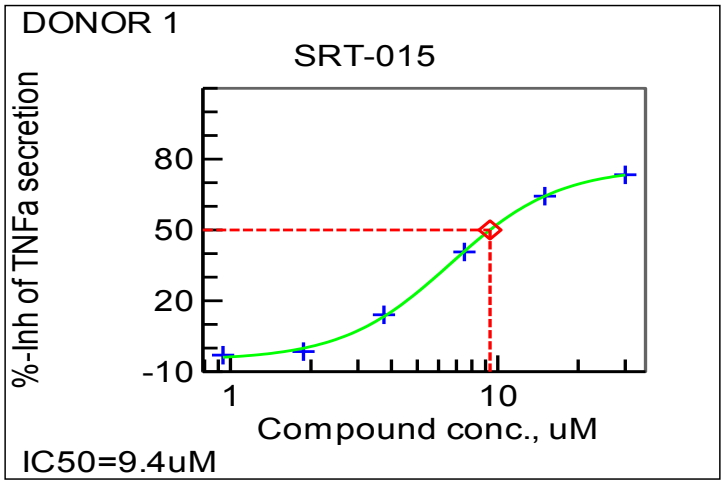
PBPK model of human liver exposure (C_{max})



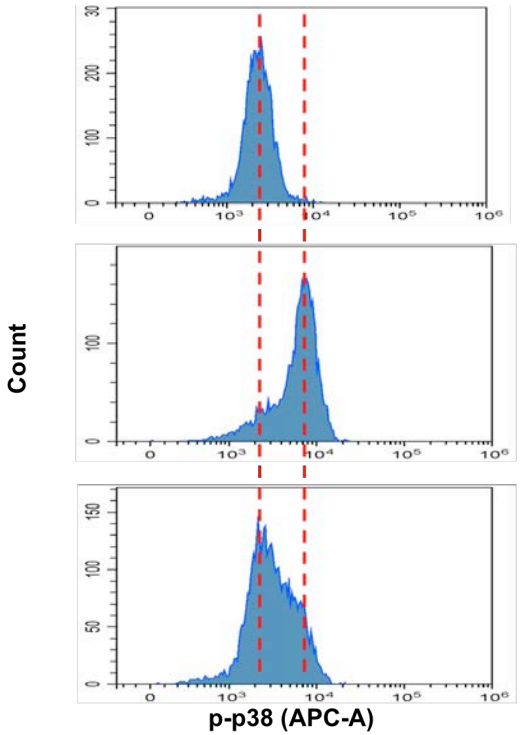
$t_{1/2}$ of 17 hours supports once daily dosing

SRT-015: TNF inhibition and p-p38 in whole blood from human subjects Stimulated with LPS to activate ASK1 and inhibit with SRT-015

TNF Secretion



Flow Cytometry



Unstimulated control

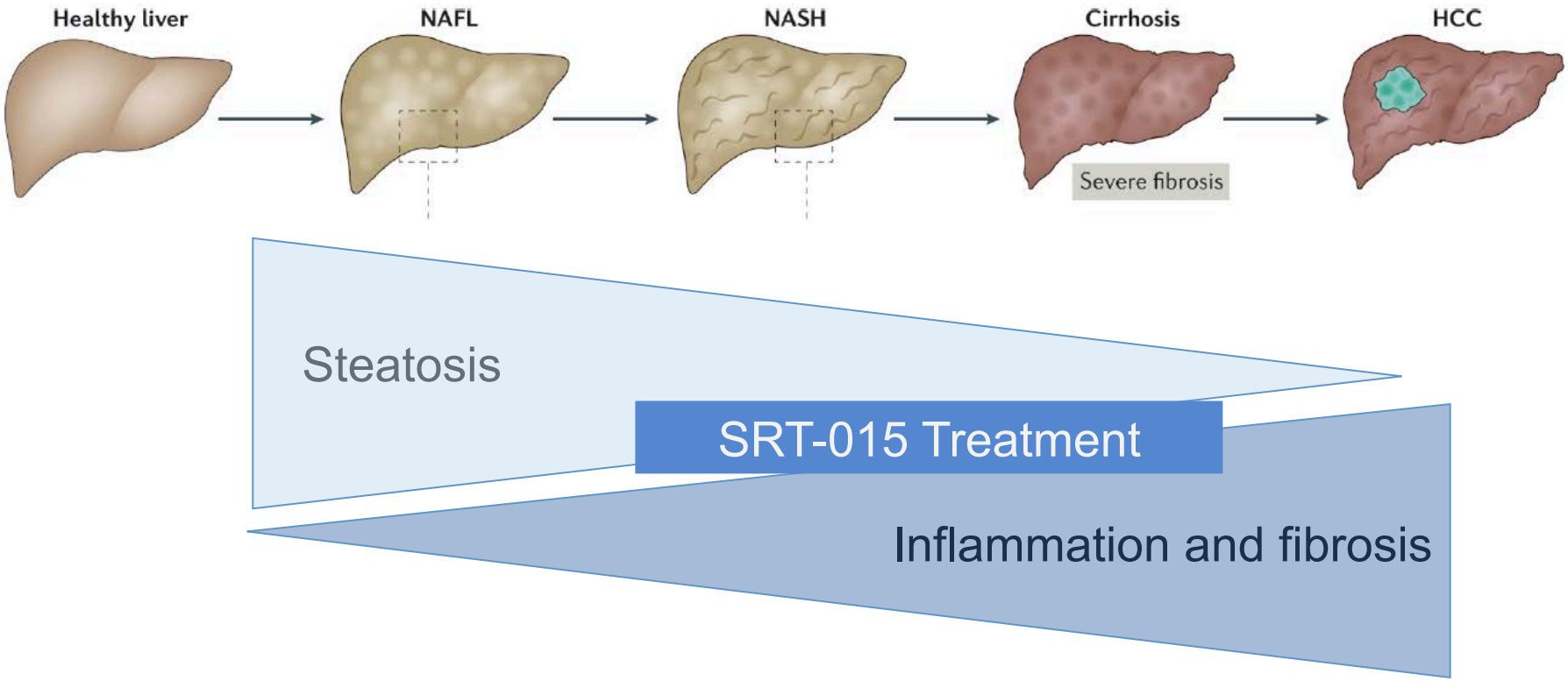
Stimulated control (15min LPS, 0.1ug/mL)

Stimulated sample plus SRT-015

Elias AASLD 2022

SRT-015 demonstrates anti-inflammatory activity and target engagement

SRT-015: Ideal drug for heterogeneous diseases like NASH, ALD



SRT-015 Summary

- Safe, oral, small molecule inhibitor of ASK1
- Direct inhibition fibrosis, inflammation and apoptosis, key NASH pathologies
- Demonstrated efficacy in multiple acute models (APAP, AH, BDL)
- Efficacious in gold-standard DIO-NASH therapeutic model
- Mechanistically, can be used in mono or combination therapy

➢ Phase 1: Demonstrated safe, achieving PK levels that predicted hepatic C_{max} values well above the efficacious exposure levels observed in preclinical efficacy models.

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Thank you!

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