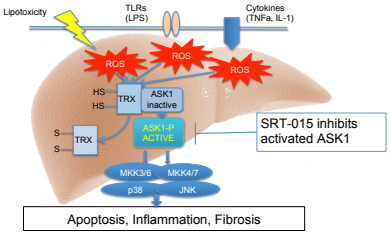
P-P38 CORRELATION WITH IN VIVO EFFICACY BY BEST-IN-CLASS ASK1 INHIBITOR SRT-015 BUT NOT SELONSERTIB

Keystone 2023 Abstract 1526
Metabolic and Molecular Mechanisms of NAFLD/NASH

Kathleen Elias, S. David Brown, Neil D. McDonnell, Artur Plonowski
Seal Rock Therapeutics Inc., Seattle WA, USA

Introduction

- Apoptosis signal-regulating kinase 1 (ASK1) is a ubiquitous redox-sensitive kinase that remains inactive under normal conditions and is activated by specific stimuli including oxidative stress, lipotoxicity, or TLR4-mediated pro-inflammatory signaling (1).
- Activation of ASK1 causes activation of the JNK and p38 MAPK downstream kinase cascades and the NLPR3 inflammasome pathway (2,3) to result in inflammation, apoptosis and fibrosis, all key components of liver diseases including NASH.
- Inhibition of the ASK1 target is well validated in in preclinical models, yet the first-generation ASK1 inhibitor, selonsertib, failed to show clinical efficacy in NASH (4) and other trials and has limited in vitro activity (5).
- SRT-015, a second generation ASK1 inhibitor demonstrates all the key features of an ASK1 inhibition (5) and recently completed Phase 1 clinical trial in healthy subjects (6).



Objectives

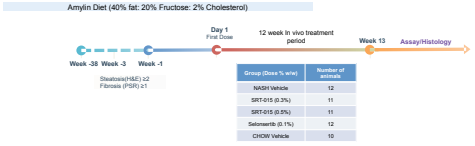
ASK1 is a highly validated preclinical target for NASH and other indications yet selonsertib has multiple clinical efficacy failures with only p-p38 decreases observed.

Here we investigated the correlation between p-p38 inhibition and preclinical efficacy by ASK1 inhibitors selonsertib and SRT-015 in specific liver populations, using a biopsy confirmed, therapeutic DIO-NASH model.

Methods

In vivo DIO-NASH model

Gubra therapeutic DIO-NASH mouse study design



- After 38 weeks on AMLN diet, in mice with biopsy confirmed fibrosis and steatosis, SRT-015 or selonsertib dosing in AMLN chow (ad libitum) was initiated for an additional 12 weeks.
- Compound dosing concentrations were chosen to match compound liver exposures with SRT-015 and selonsertib.
- Histological, serum chemistry and biochemical analysis was performed at study termination.
- RNAseq analysis of liver tissue was performed on NASH vehicle, 0.5% SRT-015 and selonsertib groups; historical lean control group was used for comparison.
- Statistical analysis by ANOVA followed by Tukey Multiple Comparisons Test; * P<0.05, ** P< 0.01, ***P < 0.001, ****P < 0.0001 comparing to NASH vehicle.

p-p38 IHC

p-p38 (Cell Signaling/4511S) activity in hepatocytes and non-hepatocytes (presumed immune and stellate cells) in DIO-NASH liver sections was assessed using an AI-powered digital assay pathology platform for cellular segmentation and analysis (Reveal Biosciences)

Acknowledgements

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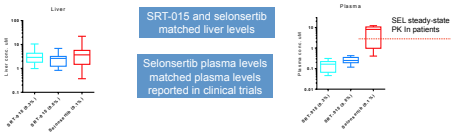
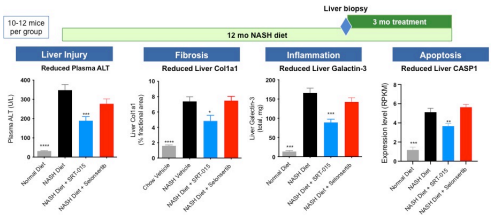
Results: SRT-015 efficacious in DIO-NASH therapeutic model

1) In therapeutic DIO-NASH model: Well-matched SRT-015 and selonsertib liver concentrations were observed in the study

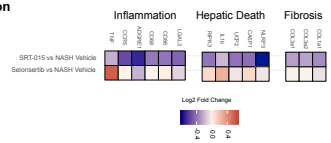
- As expected, SRT-015 plasma levels are lower than selonsertib due to liver selective distribution
- Selonsertib plasma levels in this study were within range of clinical steady-state level at 18 mg: 1.3 uM



2) SRT-015 treatment significantly lowered plasma ALT, and demonstrated direct effects on all the mechanisms of ASK1 inhibition – decreased fibrosis, inflammation and apoptosis.



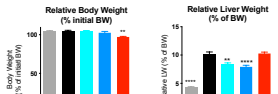
3) Liver RNAseq analysis confirmed and extended SRT-015 mechanisms of action



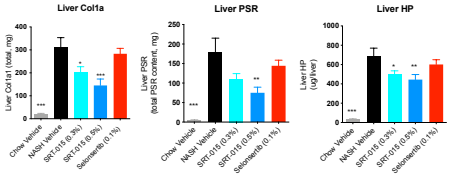
In contrast to SRT-015 treatment, at comparable liver drug levels, no efficacy was observed with selonsertib after 3 months treatment in a therapeutic DIO-NASH model replicating clinical plasma exposure.

4) Direct effects of SRT-015 treatment as no body weight loss observed.

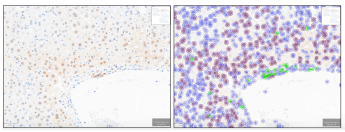
DIO-induced hepatomegaly decreased with SRT-015 treatment



5) Multiple methods were used to demonstrate decreased fibrosis with SRT-015 treatment including IHC morphometry using col1a1 and Pico Sirius Red (PSR) and biochemical analysis of hydroxyproline (HP) levels

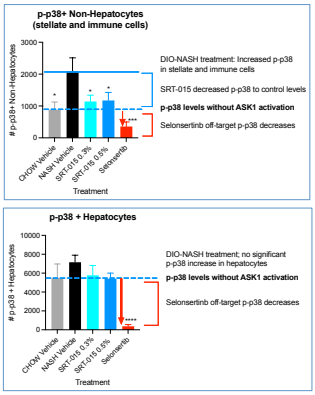


Results: SRT-015 decreased DIO-induced p-p38 in stellate and immune cells



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

6) 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



- DIO NASH treatment significantly increased p-p38 in non-hepatocyte cells (immune/stellate cells) but not in hepatocytes
- SRT-015 treatment significantly decreased p-p38 in non-hepatocyte population demonstrating target engagement and correlating with anti-inflammatory and anti-fibrotic efficacy
- Selonsertib significantly reduced p-p38 significantly below lean controls in all cell types (red arrows) where ASK1 is expected to remain in an inactive state, indicating an ASK1-independent mechanism

Summary and Conclusions

- SRT-015 demonstrates preclinical efficacy with anti-fibrotic and anti-inflammatory mechanisms of action in a therapeutic DIO-NASH model and corresponding p-p38 target engagement in stellate and immune cells.
- Selonsertib demonstrated no preclinical efficacy yet uniformly and profoundly decreased p-p38 in all cell types below levels observed in lean controls where ASK1 remains in an inactive state indicating an ASK1-independent mechanism.
- These data suggest caution is warranted when interpreting ASK1 target engagement by selonsertib in clinical trials based solely on p-p38 in liver biopsies.
- These data also indicate that p-p38 is a useful target engagement biomarker for SRT-015 and supports the advancement of SRT-015 as a therapeutic for liver diseases including NASH.

References

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4 Harrison et al, J Heptol 2020
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