# Preclinical identification and development of small molecule drug discovery leads with novel MOAs for non-alcoholic steatohepatitis (NASH)

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twoXAR Pharmaceuticals Discovered with AI

## Introduction

- Non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) are metabolic diseases that affect millions of patients globally with a high unmet medical need.
- twoXAR's powerful AI-driven drug discovery approach builds an *in silico* disease model using complex patient-derived biological data combined with clinical health record data and a diverse chemical library of experimental and FDA-approved molecules with associated pharmacology data.
- The AI discovery output is a rank-ordered list of molecules with predicted efficacy for treatment of the disease.
- Discovery hits are reviewed to determine if drugs with known efficacy are re-discovered as a method to quality check the results.
- Highly-ranked hits with novel MOAs are selected for *in vitro* and/or *in vivo* preclinical screening to identify leads for optimization and clinical development.
- twoXAR's platform preserves interpretable data-driven links to disease biology to facilitate efficient validation and optimization studies.

## Methods

### Discovery of Novel MOA Hits

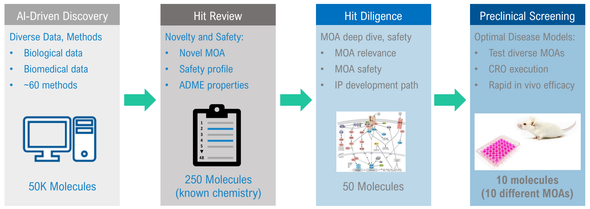



**Figure 1: twoXAR's AI-driven drug discovery approach.** twoXAR's AI-driven drug discovery approach. An *in silico* NASH disease model was constructed using complex data sets from NASH patients. Biological data examples include gene expression, micro-RNA, and GWAS data. Biomedical data includes claims-like data. The computational process integrated disease features with chemical features from a large library of drug and drug-like molecules to produce a rank-ordered list of molecules with predicted efficacy for treatment of NASH. Predictions were reviewed to select 10 hits with novel MOAs for evaluation in a preclinical *in vitro* and *in vivo* efficacy screening study.

- NASH computational disease model was built using twoXAR's AI platform, and efficacy predictions were made from a diversified library of 50,000+ molecules.
- 10 molecules with unique novel mechanisms of action (untested in NASH clinical trials) were selected as drug discovery hits.
- *In vitro* and *in vivo* methods were used to evaluate the hits. The expression of fibrosis-associated genes was evaluated using primary human hepatic stellate cells (HHSCs) isolated from a NASH donor. *In vitro* validated discovery hits were tested *in vivo* using a STAM™ model. Dose levels for each molecule were selected using published data.

### *In Vivo* Efficacy Study Design Using STAM Mouse Model of NASH

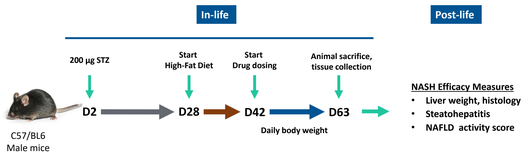
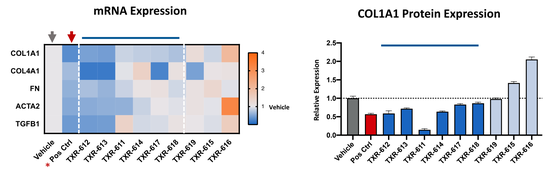



**Figure 2: Hit evaluation in STAM mouse model of NASH.** Five discovery hits that showed efficacy in the *in vitro* study were evaluated in the STAM NASH mouse model. Each treatment group include 10 mice. All 5 discovery hits were dosed for 21 days at 2 dose levels once-daily (QD) starting at Day 42 by oral gavage or subcutaneously. The positive control group was treated with telmisartan. Body weight was measured daily to monitor drug tolerability. Liver tissue was collected at study termination for histopathology analysis (H&E and PSR)

## Results

### Discovery Hits Modulate *In Vitro* mRNA and Protein Expression in HHSCs Similar to Galunisertib




* Reference treatment with galunisertib provides a positive control (TGF-β receptor type 1 kinase inhibitor)

**Figure 3: Discovery hits modulated fibrosis associated gene and protein expression in HHSCs.** Primary human hepatic stellate cells (HHSCs) isolated from NASH donors were treated with respective discovery hits for 24 hours and cells were harvested for qRT-PCR or immunofluorescence analysis. Left panel: mRNA expression analysis of Collagen Type I Alpha 1 (COL1A1), Collagen Type IV Alpha 1(COL4A1), Fibronectin (FN), Actin Alpha 2 (ACTA2), Transforming Growth Factor Beta 1 (TGFB1) levels in HHSCs. Right panel: Quantification of relative signal intensities for COL1A1 following immunofluorescence staining of treated HHSCs. Vehicle: DMSO; Pos Ctrl: Galunisertib (TGF-β receptor type 1 kinase inhibitor).

### Discovery Hits Well-Tolerated in STAM Mouse Model

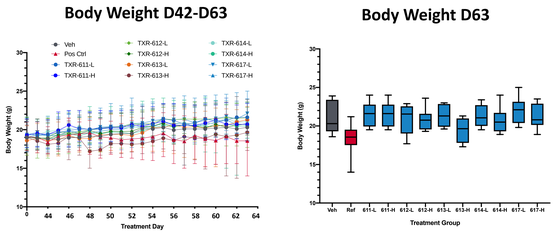



**Figure 4: Discovery hits demonstrate good tolerability *in vivo***. Daily body weight measurements of STAM mice during the in-life phase provides a method to assess drug tolerability. Left panel: Mean body weight during the entire 21-day period (D42-D63) of the in-life study phase. Right panel: Body weight distributions on Day 63 at study termination. N=10 per Group. Abbreviations: L: Low-dose, H: High-dose. Pos Ctrl/Ref: telmisartan.

### TXR-611-H and TXR-612-H Decrease Liver Weight

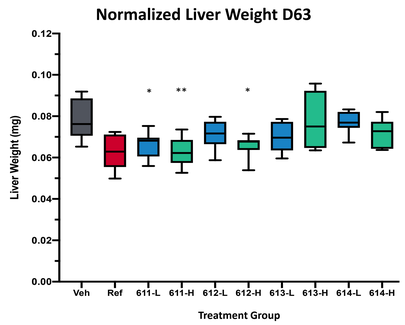



**Figure 5: Discovery hits demonstrated efficacy as measured by liver weight.** TXR-611-H and TXR-612-H demonstrated the strongest efficacy by these measures. Decrease in liver weight by TXR-611-H achieved significance with a p-value < 0.01. TXR-612-H achieved statistical significance with a p-value < 0.05. Tissues were collected and weighed at study termination (Day 63) and normalized to total mouse body weight. N=10 per Group. Ref: telmisartan. Significance: * p<0.05 ** p<0.01. *** p<0.001

### NASH Hits Reduce Steatosis and Hepatocyte Ballooning

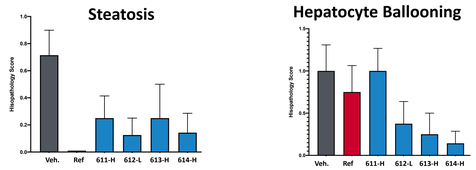



**Figure 6: Discovery hits reduced steatosis and hepatocyte ballooning in STAM mice.** Histological analysis demonstrated that TXR-611-H, TXR-612-L, TXR-613-H, and TXR-614-H reduced steatosis. TXR-612-L, TXR-613-H, and TXR- 614-H also reduced hepatocyte ballooning. Left panel: Steatosis. Right panel: Hepatocyte ballooning. N=10 per Group. Ref:telmisartan.

### NASH Hits Reduce Inflammation and Reduce NAFLD Activity Score

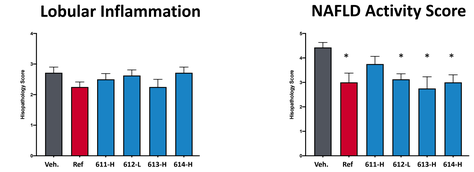



**Figure 7: Discovery hits decreased NAFLD activity score in STAM mice. Histological analyses revealed** TXR-612-L, TXR-613-H, and TXR- 614-H significantly reduced NAFLD activity score. The NAFLD activity score is a composite score that includes steatosis, hepatocyte ballooning, and lobular inflammation. Left panel: Lobular Inflammation. Right panel: NAFLD activity score. N=10 per Group. Ref:telmisartan. Significance: * p<0.05

### NASH Hits Decrease Fibrillar Collagen Deposition

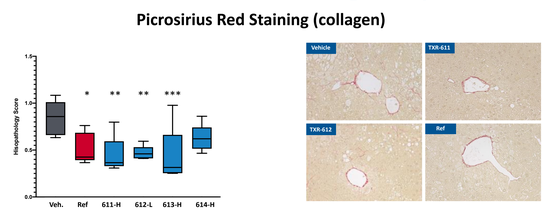
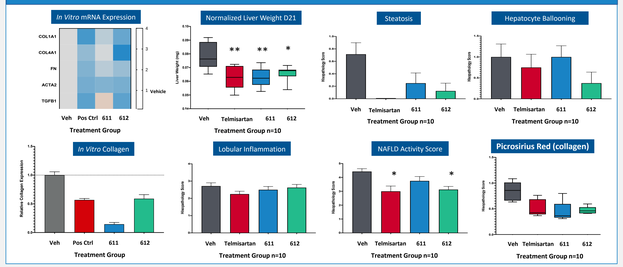


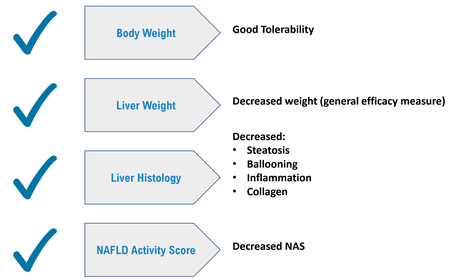

**Figure 8: Discovery hits decreased fibrillar collagen deposition in STAM mice.** TXR-611-H, TXR-612-L, TXR-613-H, and TXR-614-H significantly significantly decreased collagen expression and deposition. Picrosirius red was used to stain collagen in liver sections (right panel) and quantified using a histopathological scoring system (left panel). N=10 per Group. Significance: * p<0.05 ** p<0.01. *** p<0.001

### TXR-611 and TXR-612 NASH Efficacy Summary




## Conclusions

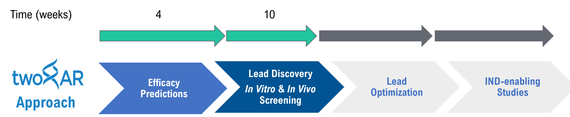
### TXR-611 and TXR-612 Have Attributes of a Drug Discovery Lead




- Significant efficacy in STAM NASH model
- Efficacy compares favorably with telmisartan.
- Known compound chemistries to facilitate lead optimization.

### TXR-611 and TXR-612 Continued Development

- TXR-611 and TXR-612 MOAs are unique and novel for NASH
- Immediate studies:
  - Characterize drug pharmacokinetics and pharmacodynamics
  - Show efficacy reproducibility in the $CCl_4$ mouse model
  - Establish PK/PD/efficacy relationships




- Rapid progression through discovery hit validation to lead optimization
  - 10 discovery hits with novel MOAs selected and evaluated using *in vitro* and *in vivo* NASH preclinical models
  - 15 weeks from program initiation to completion of *in vivo* efficacy screen
  - 2 leads with novel MOAs discovered from *in vivo* screening data
  - PK/PD characterization to inform selection of lead for optimization
  - Known chemistry facilitates lead optimization

## Acknowledgements and References

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- Correspondence: isaac@twoxar.com.