Comprehensive characterization of an off-the-shelf AMLN mouse model and considerations for experimental design in NASH pre-clinical research

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INTRODUCTION

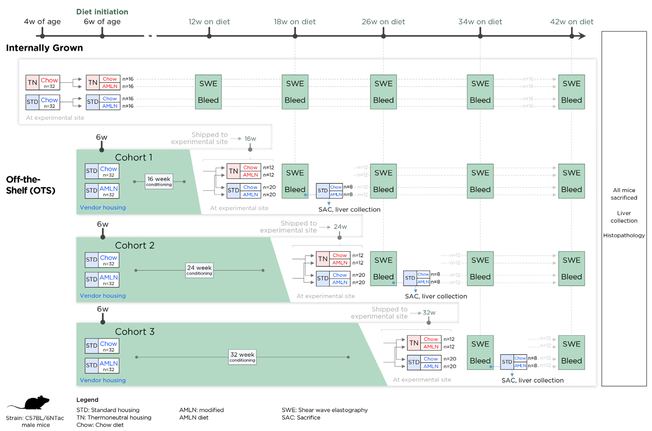
There is still an unmet need for a reproducible, efficient metabolically driven preclinical NASH model with clinical translatability. The AMLN NASH mouse model recapitulates the multifactorial disease mechanisms of human NASH, however, this model requires lengthy time on diet and substantial space requirements, limiting its utility. The availability of an off-the-shelf model could provide space, cost and time savings for NASH pre-clinical researchers.

AIM

To compare the disease progression of an off-the-shelf (OTS) Taconic AMLN model, relative to internally grown mice (IG), to assess the feasibility of using an OTS AMLN mouse for preclinical drug development.

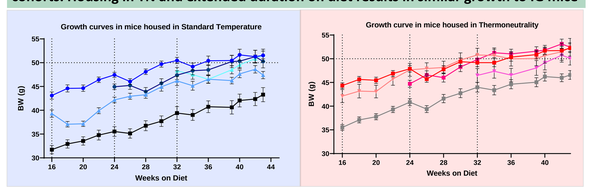
MATERIAL & METHODS

- "internally grown cohort" = C57BL/6N Mice arrived at 4 weeks of age to Pfizer and started on Chow or modified AMLN diet at 6 weeks
- "OTS cohorts" = C57BL/6N Mice arrived from Taconic at 16, 24 and 32 weeks on modified AMLN diet or chow
- All cohorts divided into housing at standard lab temperature (STD; 72°F) or thermoneutral (TN; 80°F)
- Diet used = Research Diets # D09100310 (modified AMLN diet)
  - 40% kcal fat (primarily palm oil)
  - 20% kcal fructose
  - 2% w/w cholesterol
- Disease Progression evaluated by
  - Body Weight
  - Shear Wave Elastography (SWE)
  - Plasma biomarkers (Lipid panel, Liver Function Tests (LFTs))
  - Histopathology
    - Picrosirius Red (PSR)
    - Ionized calcium-binding adaptor molecule 1 (Iba1)
    - Alpha Smooth Muscle Actin (αSMA)

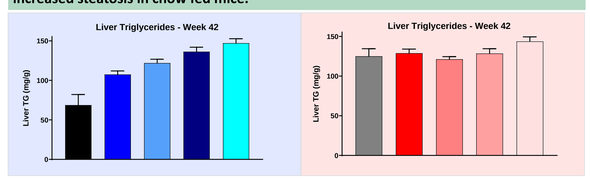


RESULTS

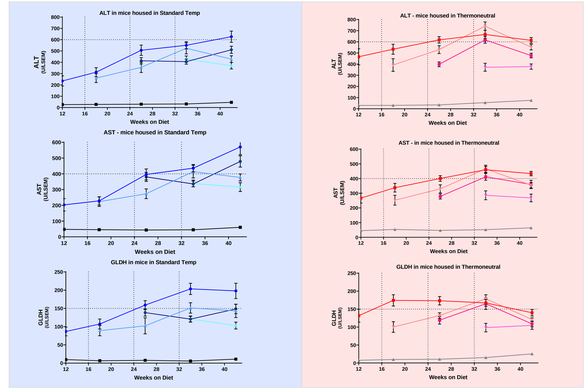
OTS mice shipped at 16 weeks in STD housing were significantly smaller than the other cohorts. Housing in TN and extended duration on diet results in similar growth to IG mice



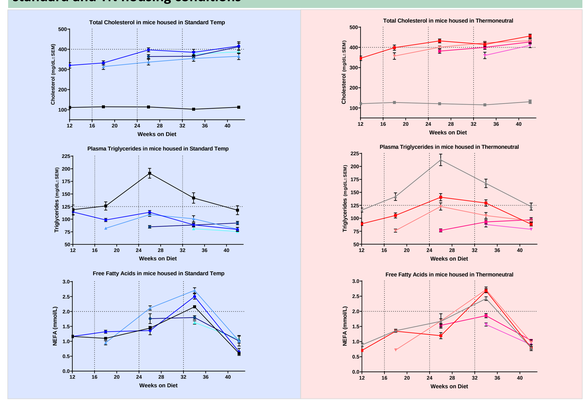
Steatosis was similar in OTS mice relative to internally grown mice. TN housing drives increased steatosis in chow fed mice.



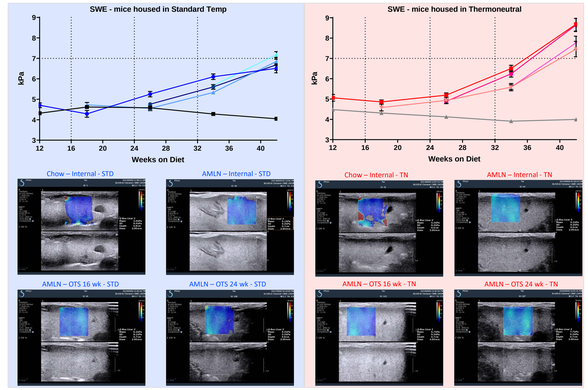
OTS mice demonstrated lower LFTs upon arrival however TN housing and extended duration of diet mitigates this effect. TN accelerates increases in LFTs.



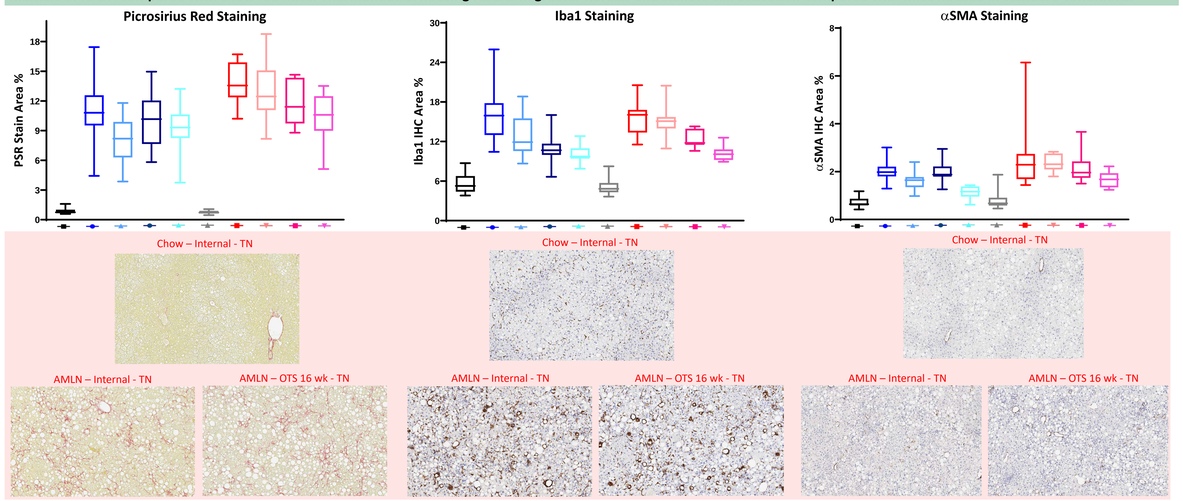
Circulating analytes in OTS mice trended similar to Internally grown mice in both standard and TN housing conditions



OTS mice reach the same degree of liver stiffness in STD housing but not all OTS mice in TN housing. TN housing significantly drives more severe liver stiffness in all AMLN mice.



At 42 week on diet, OTS mice have significantly less fibrosis (lower PSR staining), inflammation (less Iba1 staining) and stellate cell activation (lower αSMA staining) compared with internally grown mice, statistically determined using fit linear models (P < 0.001 for weeks on diet as a predictor of the endpoints). OTS mice have significant increases in all these markers of NASH compared with chow fed mice. Thermoneutral housing results in greater fibrosis and stellate cell activation compared with mice housed in standard conditions.



SUMMARY

- OTS mice develop all the hallmarks of NASH as indicated by SWE and histopathology though not to the severity of internally grown mice
- OTS mice demonstrate reduced body weight and LFTs upon arrival, relative to internally grown mice, resulting from shipping stress
- Longer acclimation times and housing in thermoneutrality mitigates the shipping induced stress insult observed in the OTS mice and results in a similar phenotype to internally grown mice
- Thermoneutral housing accelerates early disease progression and results in increased hallmarks of NASH, including liver stiffness and fibrosis
- Shear Wave Elastography, which measures liver stiffness as a result of elevated fibrosis and inflammation, correlates well with histopathology

STUDY CONSIDERATIONS

- Receive mice based on the NASH disease stage of interest (< lipotoxicity < inflammation < fibrosis). Study goals and endpoints of interest should be determined ahead of ordering mice to achieve adequate window of therapeutic significance
- Utilize appropriate acclimation strategies based on desired study outcomes. Recommend minimum acclimation of 4 weeks. Housing in TN will broaden the window for key fibrosis endpoints
- Power group size based on degree of changes in specific endpoints desired. Based on this study, an n=10-15/group would provide sufficient power to detect biologically relevant therapeutic effects.
- Utilize non-invasive methods (SWE, biomarkers) to monitor disease progression and to identify target stages of disease for intervention strategies

CONCLUSION

Using specific acclimation strategies and study designs which focus on disease stage, the OTS Taconic AMLN mouse serves as a reliable metabolically-driven NASH model which permits efficacy assessment for drugs targeting steatosis, inflammation and fibrotic endpoints in a single model and reduces the time and cost burden to researchers, providing greater flexibility in study planning.

REFERENCES

Boland et al. World J Gastroenterology (2019) 25:33
Giles et al. Nature Medicine (2017) 23:7
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DISCLOSURES

BB, RB, DD, CT, MB and TR are employees at Pfizer Inc.
MM and JR are employees at Taconic Biosciences

Figure Legend:

- Chow - Internal grown, STD
- AMLN - Internal grown, STD
- AMLN - OTS 16 wk - STD
- AMLN - OTS 24 wk - STD
- AMLN - OTS 32 wk - STD
- Chow - Internal grown, TN
- AMLN - Internal grown, TN
- AMLN - OTS 16 wk - TN
- AMLN - OTS 24 wk - TN
- AMLN - OTS 32 wk - TN

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