# Mechanistic Insights Into AXA1125, a Novel Endogenous Metabolic Modulator Composition, Targeting Multiple NASH Drivers

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Poster

Axcella Virtual Café

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## Introduction

- Recent advances in network biology have assisted in unraveling the complex pathophysiological processes underlying nonalcoholic fatty liver disease (NAFLD) and its progression to nonalcoholic steatohepatitis (NASH) and cirrhosis[1]
- The close inter-connectivity of the contributory molecular pathways suggests that effective pharmacotherapy may require a coordinated multitargeted approach
- Per this premise, endogenous metabolic modulators (EMMs) have emerged as a core class of signaling agents and metabolic substrates with the potential to simultaneously impact multiple metabolic and fibroinflammatory pathways in NASH[1]
- EMMs consisting of amino acids are intrinsically fundamental to core NASH pathways[2]

## Aim

- We have designed a novel EMM composition of 5 amino acids (AAs) and N-acetylcysteine (LIVRQNac, also referred to as AXA1125), and here we summarize its effects across a range from *in vitro* systems to human subjects with NAFLD and delineate its underlying mechanisms

## Methods

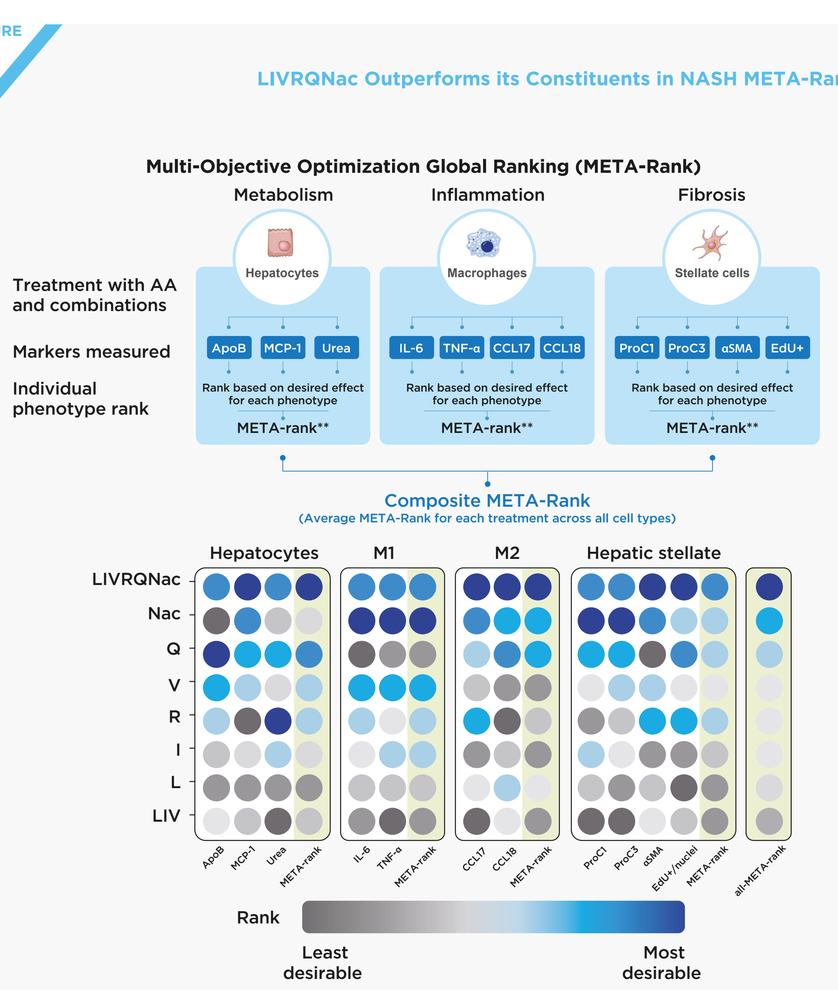
- Primary hepatocytes, macrophages, and stellate cells from multiple human donors were cultured with LIVRQNac as well as its constituents in the presence or absence of key phenotypic inducers, ie, saturated free fatty acids (FFAs; to assess lipotoxicity), lipopolysaccharide and interleukin-4 (to evaluate inflammation), and tumor growth factor-beta (to assess fibrogenesis)
- A comprehensive battery of assessments such as glucose output, triglyceride accumulation, secretion of interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and *N*-terminal type III collagen propeptide (ProC3) were performed, complemented with high-throughput omics: RNASeq and metabolomics
- Composition requirements were assessed using a desirability-based, multi-objective optimization, global ranking approach (META-rank)
- Proof-of-mechanism/concept for AXA1125 were shown in 2 clinical studies in subjects with NAFLD with and without type 2 diabetes (T2D) dosed up to 16 weeks[3,4]

## Results

- META-rank analysis demonstrated that, compared with its individual constituents, the LIVRQNac combination was effective in addressing the full range of dysfunction across NASH-relevant metabolic and fibroinflammatory phenotypes (**Figure 1**)

FIGURE 1

**LIVRQNac Outperforms its Constituents in NASH META-Rank**




αSMA, α-smooth muscle actin; AA, amino acid; ApoB, apolipoprotein B; CCL, C-C motif chemokine ligand; EdU, 5-ethynyl-2′-deoxyuridine; I, isoleucine; IL-4, interleukin 4; IL-6, interleukin 6; L, leucine; LIV, leucine/isoleucine/valine; LIVRQNac, 5 amino acids and N-acetylcysteine; LPS, lipopolysaccharide; M, macrophage; MCP-1, monocyte chemoattractant protein 1; Nac; N-acetylcysteine; Q, glutamine; ProC1, *N*-terminal type I collagen propeptide; ProC3, *N*-terminal type III collagen propeptide; R, arginine; sFFA, saturated free fatty acid; TGF-β, tumor growth factor-beta; TNF-α, tumor necrosis factor-alpha; V, valine.

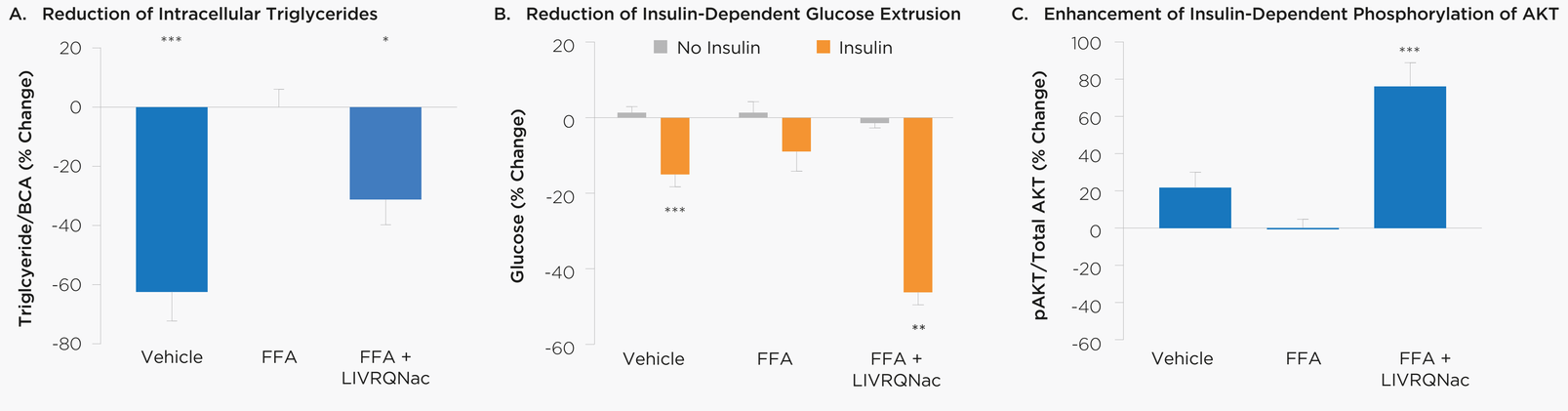
**Mean of the ranks for phenotypes measured in each cell type.

Ranking system: 1 is the most desirable and 9 is the least desirable.

- Treatment of primary human hepatocytes (PHHs) with FFAs + LIVRQNac significantly reduced intracellular triglyceride levels (↓31%; p<0.05), compared with untreated cells (**Figure 2A**)
- LIVRQNac reduced insulin-dependent glucose output (↓50%, p<0.01) (**Figure 2B**) and enhanced insulin-dependent protein kinase B (AKT) phosphorylation (↑80%, p<0.001) (**Figure 2C**)

FIGURE 2

**LIVRQNac Reduces Lipotoxicity and Improves Glucose Homeostasis**




*p<0.05; **p<0.01; ***p<0.001.

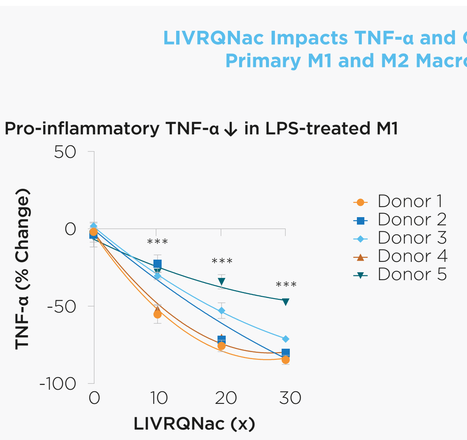
Error bars represent ± standard error of the mean.

AA, amino acid; AKT, protein kinase B; BCA, bicinchoninic acid; FFA, free fatty acid; LIVRQNac, 5 amino acids and N-acetylcysteine; pAKT, phosphorylated protein kinase B.

- LIVRQNac suppressed secreted levels of proinflammatory IL-6 and TNF-α (↓ up to 78%–85%; p<0.001) and induced anti-inflammatory C-C motif chemokine ligand (CCL18) (up to 411.23%; p<0.001) in primary M1 and M2 macrophages, respectively (**Figure 3**)

FIGURE 3

**LIVRQNac Impacts TNF-α and CCL18 in Primary M1 and M2 Macrophages**




***p<0.001.

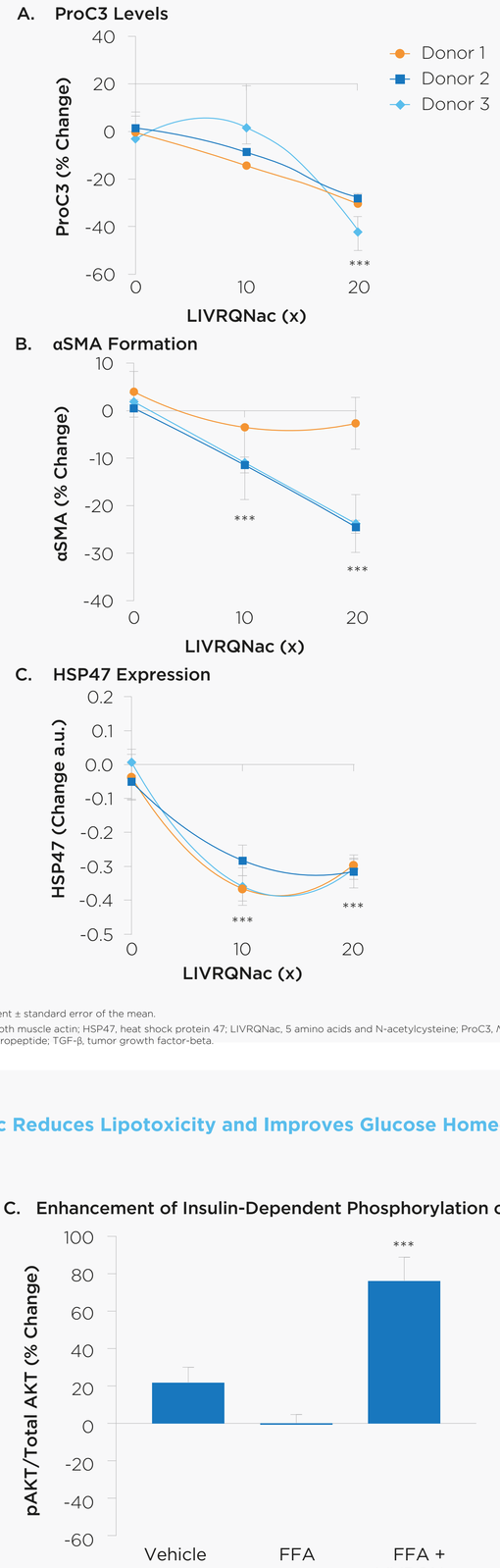
Error bars represent ± standard error of the mean.

CCL18, C-C motif chemokine ligand 18; IL-4, interleukin 4; LIVRQNac, 5 amino acids and N-acetylcysteine; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-alpha.

- TGF-β–driven induction of profibrogenic factors (ProC3, α-smooth muscle actin [SMA], and heat shock protein [HSP]47) in stellate cells were suppressed by 25%–40% (p<0.001) by LIVRQNac (**Figure 4**)

FIGURE 4

**LIVRQNac Suppressed TGF-β-mediated Induction of Profibrotic Factors**




***p<0.001.

Error bars represent ± standard error of the mean.

αSMA, alpha-smooth muscle actin; HSP47, heat shock protein 47; LIVRQNac, 5 amino acids and N-acetylcysteine; ProC3, *N*-terminal type III collagen propeptide; TGF-β, tumor growth factor-beta.

- Global transcriptomic changes in core biochemical pathways (glycolysis, branched-chain AA degradation, and peroxisome proliferator-activated receptor [PPAR]- and hypoxia-inducible factor 1 alpha [HIF-1α] signaling pathways) mediating these cellular effects were observed (**Figure 5**)

FIGURE 5

**Hepatocyte Gene Expression Changes Following LIVRQNac Treatment**




BCAA, branched chain amino acid; FFA, free fatty acid; HIF-1, hpoxia-inducible factor 1; LIVRQNac, 5 amino acids and N-acetylcysteine; MTOR, mechanistic target of rapamycin; PPAR, peroxisome proliferator-activated receptors.

- Consistent with the underlying mechanism of AXA1125, we observed clinically relevant changes across various metabolic and fibroinflammatory markers in NAFLD subjects with and without T2D treated with AXA1125 for up to 16 weeks (**Table 1**)

**Table 1: Change From Baseline at Week 16 in Metabolic and Fibroinflammatory Biomarkers in NAFLD Subjects (Overall Safety Population and Subgroup With T2D)**

| | Overall Safety Population | | T2D Subgroup | |
|---|---|---|---|---|
| | Placebo (n=15) | AXA1125 (n=29) | Placebo (n=6) | AXA1125 (n=12) |
| **Metabolism** | | | | |
| MRI-PDFF, % | -5.7 | -22.9 | -8.3 | -31.2 |
| Subjects (%) with ≥30% reduction in MRI-PDFF | 8.3 | 38.5 | 0 | 54.5 |
| HOMA-IR | 0.7 | -4.4 | -0.8 | -9.2 |
| Fasting glucose, mg/dL | -3.6 | -12.6 | -9.01 | -23.4 |
| Fasting insulin, mIU/L | 3.0 | -5.0 | -4.0 | -14.0 |
| HbA1c, % | -0.17 | -0.30 | -0.27 | -0.7 |
| **Inflammation** | | | | |
| ALT, U/L | -8.9 | -14.4 | -15.3 | -23.7 |
| Subjects (%) with ≥17 U/L reduction in ALT | 25.0 | 38.5 | 33.0 | 63.6 |
| cT1, msec | 18.3 | -69.6 | -42.7 | -105.1 |
| Subjects (%) with ≥80 msec reduction in cT1 (%) | 16.7 | 34.6 | 33.3 | 45.5 |
| **Fibrosis** | | | | |
| ProC3, ng/mL | -0.68 | -3.4 | -3.5 | -5.3 |
| FIB-4 | -0.28 | -0.18 | -3.5 | -3.6 |

ALT, alanine aminotransferase; cT1, corrected T1; FIB-4, fibrosis-4 index; HbA1c, glycosylated hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; MRI-PDFF, magnetic resonance imaging-protein density fat fraction; NAFLD, nonalcoholic fatty liver disease; ProC3, *N*-terminal type III collagen propeptide; T2D, type 2 diabetes.

## Conclusion

- The potential of AXA1125 to simultaneously address the multifactorial pathogenesis of NASH and its key comorbidities (eg, T2D) represents a novel modality with a unique mechanism of action. Future development of AXA1125 for the treatment of adult and pediatric subjects with NASH is planned

## References

**1.** Hamill M, et al. *iScience.* 2020;23:101628.

**2.** Chakravarthy M, et al. *Gastroenterol Clin North Am.* 2020;49:63–94.

**3.** Marukian S, et al. *Hepatology.* 2018;68(Suppl 1):67A. (Abstract 106).

**4.** Harrison SA, et al. NASH TAG 2021, Park City, Utah, March 11–13, Poster 33.

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## Disclosures

All authors are employees of Axcella Health Inc.

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