AXA1125-Associated Metabolic Profile Changes Are Predictive of Observed Reductions in Liver Fat Content in NAFLD

Poster

Virtual Café

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Introduction

- Nonalcoholic fatty liver disease (NAFLD) is prevalent in >25% of the global population and in 50% to 90% of obese adults. Once nonalcoholic steatohepatitis (NASH) occurs, there may be progression to cirrhosis[1-3]
- Patients with NASH may benefit from approaches that concurrently address multiple metabolic and fibroinflammatory pathways; however, at present, there are no approved therapies[4,5]
- In early NAFLD clinical studies, AXA1125, an investigational oral endogenous metabolic modulator (EMM) composition of 5 selectively combined amino acids (AAs; leucine, isoleucine, valine, arginine, glutamine) and N-acetylcysteine, simultaneously supported multiple metabolic biologies and pathways of NAFLD,[6] improving insulin sensitivity, inflammation, fibrosis, and magnetic resonance imaging-proton density fat fraction (MRI-PDFF) (AXA1125-002,[7] -003[8])
- Changes in proton density fat fraction (PDFF) have been linked to histologic improvement in NASH with fibrosis in other therapeutic trials[9-11]
- In vitro studies in primary human hepatocytes indicated that AXA1125 impacted multiple metabolic pathways such as AA metabolism (involving branched-chain amino acids [BCAAs]), the urea cycle, and peroxisome proliferator-activated receptor pathway which regulates metabolism of fatty acids, among others[12]

Objectives

- To investigate the relationship between AXA1125-associated changes in fasting plasma metabolites and assessment of PDFF by MRI
- To evaluate how observed metabolic profile changes may contribute to the proposed mechanisms of AXA1125

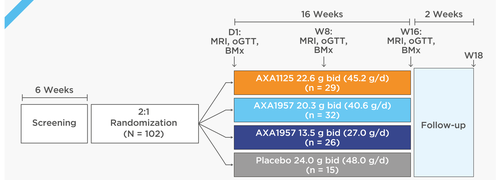
Methods

Sample Sources

- AXA1125-003 (NCT04073368) was a multicenter, 16-week, single-blind study; 102 subjects with NAFLD were randomized to receive twice daily AXA1125 22.6 g (free AAs; 24 g total weight), AXA1957 13.5 or 20.3 g, or calorie-, excipient-, and color-matched placebo 24 g (Figure 1)[8]
  - MRI-PDFF measurements were assessed at baseline and at weeks 8 and 16

FIGURE 1

AXA1125-003 Study Design



bid, twice daily; BMx, biomarkers; D, day; MRI, magnetic resonance imaging; oGTT, oral glucose tolerance test; W, week.

- AXA1125 had more consistent activity across the critical metabolic, inflammatory, and fibrotic pathways relevant for NAFLD pathogenesis over 16 weeks than did AXA1957, so it was chosen to advance for clinical development in NASH
  - Changes from baseline to week 16 with AXA1125 vs placebo: MRI-PDFF –22.9% vs –5.7%, homeostasis model assessment of insulin resistance (HOMA-IR) –4.4 vs +0.7, alanine aminotransferase (ALT) –21.9% vs 7.2%, corrected T1 (cT1) –69.6 vs +18.3 msec, and N-terminal type III collagen propeptide (Pro-C3) –13.6% vs –3.6%[8]
- In the AXA1125-003 study, fasting plasma samples were also collected for AA measurements (baseline, weeks 2, 4, 8, 12, and 16) and polar metabolite measurements (baseline, weeks 8 and 16)
- AXA1125-002 was a multicenter, open-label, 12-week, safety and tolerability study in 32 subjects with NAFLD and type 2 diabetes treated with AXA1125 22.6 g three times a day[7]
  - In subjects with liver fat >10% at baseline (n=23), mean PDFF decreased from 17.8% at baseline to 13.9% at week 12, a mean change of –4.1%

Data Analysis

- Amino acid measurements were baseline corrected to yield percent change from baseline, and polar metabolite measurements were baseline corrected to yield log2-fold change measurements, at each timepoint
  - Two-sided t tests with the null hypothesis that there was no change from baseline were conducted and p values corrected by Benjamini-Hochberg procedure; results were visualized on Volcano plots
- Differences in metabolite profiles between placebo and AXA1125 groups were identified using hierarchical clustering
- Machine learning models (Random Forest [for the AA dataset] and Lasso regression [for both datasets] using leave-one-out cross-validation) were fitted to quantify different metabolites in placebo- and AXA1125-treated samples to correlate to reduction in MRI-PDFF
- Top predictive features shared across models were validated with the AXA1125-002 study data

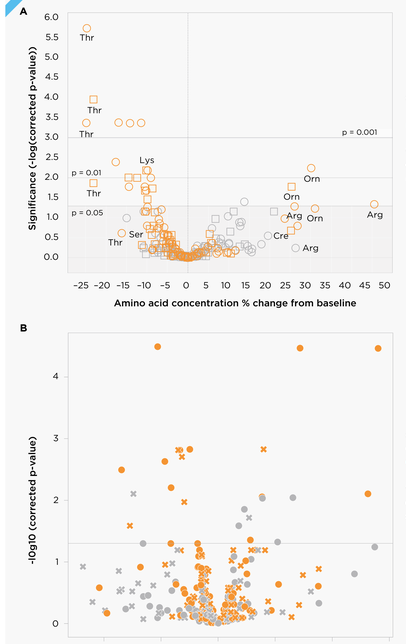
Results

AXA1125 Metabolic Signature

- Among dosed amino acids, significant increases in arginine at weeks 2 and 4 in the AXA1125 group were observed (Figure 2A)
- Multiple non-dosed amino acids significantly decreased from baseline at multiple timepoints (threonine, proline, tyrosine); ornithine, a direct metabolite of arginine, was the only significantly increased non-dosed AA (Figure 2A)
- Polar metabolite profile showed significant changes in AXA1125- and placebo-treated subjects (Figure 2B)
  - At weeks 8 and 16, succinic acid, threonine, γ-amino butyric acid (GABA), and ornithine levels changed significantly from baseline in the AXA1125-treated group; in contrast, asparagine changed significantly in the placebo-treated group
  - At week 8, there were significant changes from baseline in cytosine and 3-phosphoglyceric acid in the AXA1125- and placebo-treated groups, respectively
  - At week 16, significant changes from baseline were observed in:
    - Glutamine and 2-oxoisovaleric acid levels in both the placebo- and AXA1125-treated groups
    - Glutamic acid, cysteine, betaine aldehyde, 2-hydroxybutyric acid, and 2-oxoglutaric acid in the AXA1125-treated group
    - Histidine and N,N-dimethylglycine in the placebo-treated group

FIGURE 2

Amino Acid and Polar Metabolite Changes Specific to AXA1125 Treatment



Arg, arginine; Cre, creatine; Lys, lysine; Orn, ornithine; Ser, serine; Thr, threonine.

- Hierarchical clustering separated the AXA1125 and placebo groups, supporting the notion of a distinct AXA1125 metabolic profile (Figure 3)
  - Clusters within the profile separated most dosed AAs that either increased (arginine and ornithine) or did not change (BCAAs) from non-dosed AAs that decreased from baseline with AXA1125 treatment
  - The observation that BCAAs (valine, isoleucine, leucine) do not change in fasting plasma despite dosing suggests that they are actively metabolized
  - Note that glutamine shows decreases in AXA1125 treatment group despite dosing
- In summary, AXA1125-treated participants had a unique metabolic signature in fasting plasma compared with placebo-treated participants

Metabolic Changes Explaining PDFF Changes

- Feature selection across model classes and metabolomic datasets arrived at a shared set of AAs as most predictive of PDFF changes in study subjects (Figure 4)

Metabolic Changes and PDFF in the AXA1125-002 and -003 Studies

- Linear regression analyses demonstrated significant correlation between increases in amino acids and decreases in PDFF in AXA1125-treated subjects in both early clinical studies
  - In AXA1125-003, the model was trained on all subjects at week 8
  - Subjects receiving AXA1125 and placebo at week 16 show significant linear dependence of MRI-PDFF change and lysine and ornithine change (Figure 5A)
  - In AXA1125-002, ornithine and arginine show significant linear dependence at week 6 (% change in AA – change in PDFF, n=13; Figure 5B)

FIGURE 3

Hierarchical Clustering Groups Treatments Together

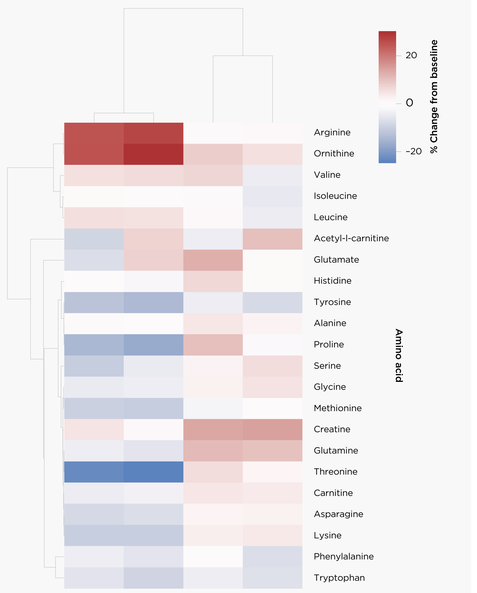
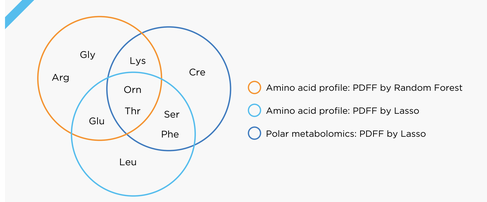
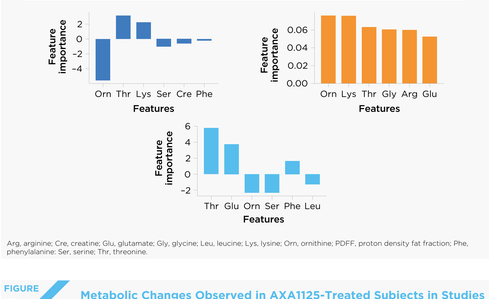


FIGURE 4

Amino Acid Changes Most Predictive of PDFF Change

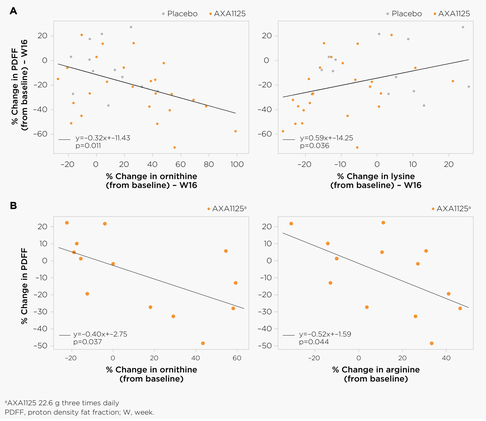




Arg, arginine; Cre, creatine; Glu, glutamate; Gly, glycine; Leu, leucine; Lys, lysine; Orn, ornithine; PDFF, proton density fat fraction; Phe, phenylalanine. Ser, serine; Thr, threonine.

FIGURE 5

Metabolic Changes Observed in AXA1125-Treated Subjects in Studies AXA1125-003 (A) and AXA1125-002 (B) Correlate With PDFF Change as Predicted by Machine Learning Models Across Timepoints and With Cross-Study Validation



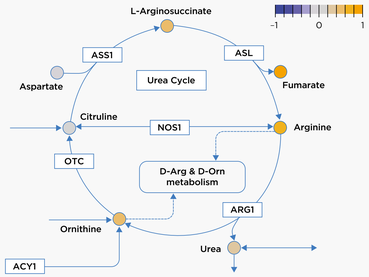
*AXA1125 22.6 g three times daily
PDFF, proton density fat fraction; W, week.

Key Biological Observations

- Key biological observations are consistent with the proposed mechanism of action of AXA1125
  - Increased fasting arginine (dosed) and its direct metabolite ornithine are predictive of MRI-PDFF decrease. This may correspond to AXA1125-associated increases in urea cycle activity and energetics. Increases in several urea cycle metabolites are observed in AXA1125- vs placebo-treated subjects at week 8 (Figure 6)
  - BCAAs are unchanged between AXA1125 and placebo metabolic signature; lack of accumulation despite dosing supports increased metabolism and/or protein synthesis. Similarly, decreased glutamine despite dosing may indicate that AXA1125 treatment promotes oxidation or protein synthesis
  - Non-dosed AA depletions may reflect an AXA1125-driven increased oxidation and/or protein synthesis—hypothesized metabolic remodeling in NAFLD/NASH to greater capacity for hepatic energy expenditure
  - Increase in purine and pyrimidine metabolites observed in AXA1125- vs placebo-treated subjects at week 8 is indicative of improved liver energy metabolism

FIGURE 6

Increase in Urea Cycle Metabolites in AXA1125- vs Placebo-Treated Subjects at Week 8



ACY1, aminocyclase 1; Arg, arginine; ARG1, arginase 1; ASL, argininosuccinate lyase; ASS1, argininosuccinate synthetase 1; NOS1, nitric oxide synthase 1; Orn, ornithine; OTC, ornithine transcarbamoylase.

Conclusions

- AXA1125 is associated with a distinct metabolic signature in fasting plasma from subjects with NAFLD that is predictive of MRI-PDFF reductions, including significant changes in dosed and non-dosed AAs and polar metabolites
- An overlap in top AA predictors of MRI-PDFF reduction identified by different machine learning models between metabolomic datasets, and between the AXA1125-003 and AXA1125-002 studies, strengthens support for this metabolic signature
- These findings add to a growing body of data suggesting an association between the metabolic profile of AXA1125 and MRI-PDFF improvements consistent with the proposed multifactorial metabolic impact of AXA1125

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Disclosures

MR, AD, NT, MJK, KA: employees of Axcella Therapeutics and may own stock options in the company. **SK, MH:** employees of Axcella Therapeutics during the time the studies were conducted.

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