Pre-clinical exogenous volatile organic compounds (EVOC®) Probes screening and optimization for chronic liver diseases detection.

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1. Introduction

The sole test available for the diagnosis of non-alcoholic steatohepatitis (NASH) is liver biopsy, an invasive procedure that can lead to complications. Lack of non-invasive methods to assess NASH progression represents a hurdle for clinical trial evaluation of experimental drugs.

Breath analysis coupled with the administration of exogenous volatile organic compounds (EVOC®) Probes represents an attractive means for non-invasive and functional detection of chronic liver diseases, including NASH.

In this study we report novel pre-clinical approaches for the identification and characterization of EVOC Probes that have the potential to be used for a breath test for NASH diagnosis.

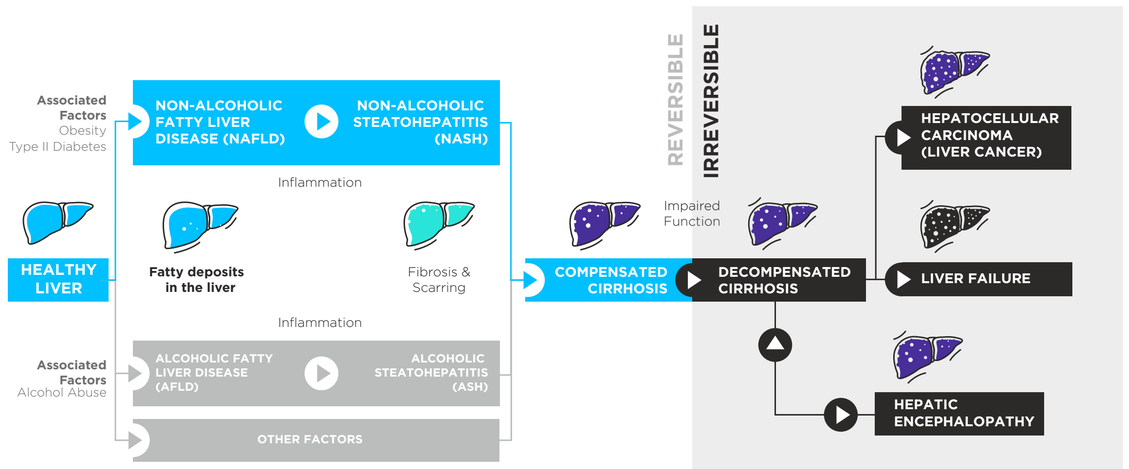

Fig. 1. Non-alcoholic fatty liver disease represents the hepatic component of metabolic syndrome. The initial stage referred to as simple steatosis is often asymptomatic. This condition can progress to non-alcoholic steatohepatitis (NASH), characterized by chronic inflammation and accumulation of fibrotic tissue. Chronic liver injury leads to cirrhosis, the end terminal disease stage, which can induce decompensation and deadly complications. Liver biopsy represents the only approved method for NASH diagnosis but its use is limited due to being invasive and having a higher risk of complications. At Owlstone Medical we have investigated the breath of subjects with cirrhosis to identify biomarkers that can potentially be translated in earlier stages liver disease.

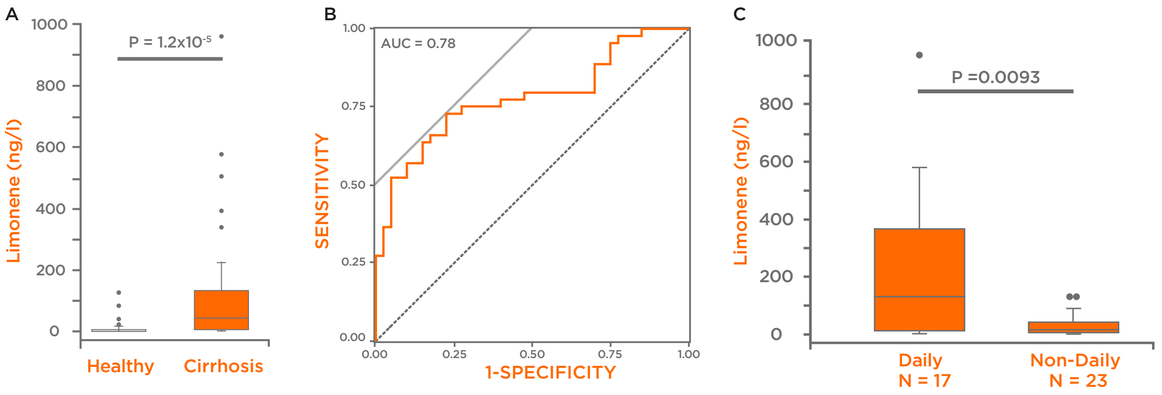

Fig. 2. (A) We confirmed the results of previous studies[1] showing that levels of limonene on breath are elevated in subjects with cirrhosis compared to controls. (B) Limonene shows a discriminatory performance with an AUC of 0.78. (C) Besides cirrhosis severity, levels of limonene on breath depends on how often the subjects have been exposed. (D) In principle, a standardized EVOC Probe exposure before breath collection could improve diagnostic performance making it more sensitive to detecting early-stage liver disease, such as NASH.

Top-down approach: Identify additional VOCs using Breath Biopsy OMNI

Bottom-up approach: identify disease associated pathways using transcriptomics analysis

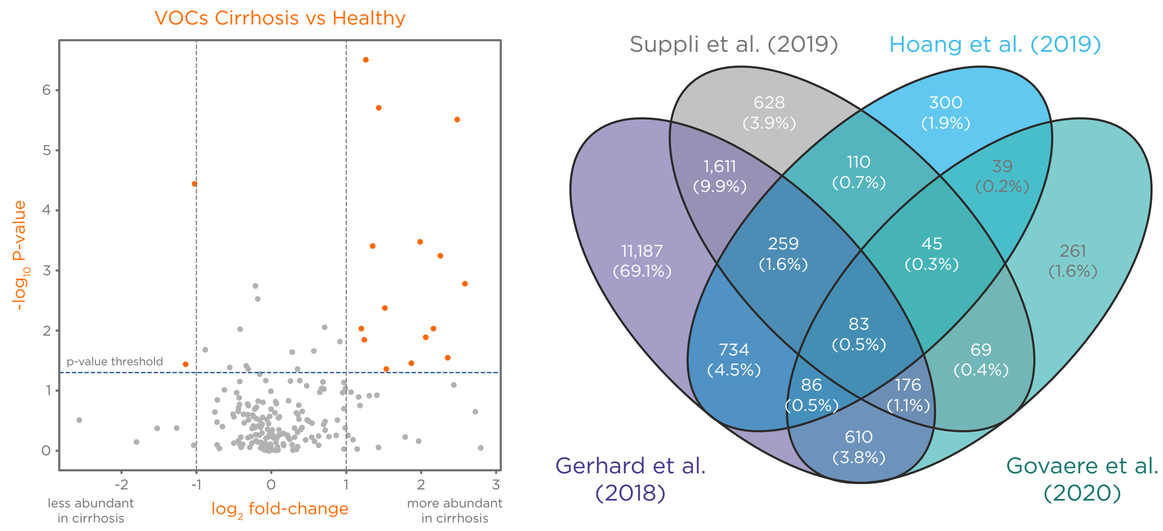

Fig. 3. To identify additional EVOC Probes, besides limonene, we have used a top-down and a bottom-up approach. For the top-down approach, we performed untargeted analysis of breath samples, using Breath Biopsy® OMNI, and compared the abundance of molecular features between subjects with cirrhosis and controls. We found several features elevated in the breath of subjects with cirrhosis. Some of these have the potential to be used as EVOC Probes. For the bottom-up approach, we have compared hepatic transcription datasets between NASH and controls and identified differentially expressed genes that metabolize potential EVOC Probes.

References

1. Fernandez Del Rio R, O'Hara ME, Holt A, Pemberton P, Shah T, Whitehouse T, et al. Volatile Biomarkers in Breath Associated With Liver Cirrhosis - Comparisons of Pre- and Post-liver Transplant Breath Samples. EBioMedicine 2015;2:1243-1250.
2. Pijls KE, Smolinska A, Jonkers DM, Dallinga JW, Masclee AA, Koek GH, et al. A profile of volatile organic compounds in exhaled air as a potential non-invasive biomarker for liver cirrhosis. Sci Rep 2016;6:19903.
3. Sinha R, Lockman KA, Homer NZM, Bower E, Brinkman P, Knobel HH, et al. Volatomic analysis identifies compounds that can stratify non-alcoholic fatty liver disease. JHEP Reports 2020:100137.

2. Methods

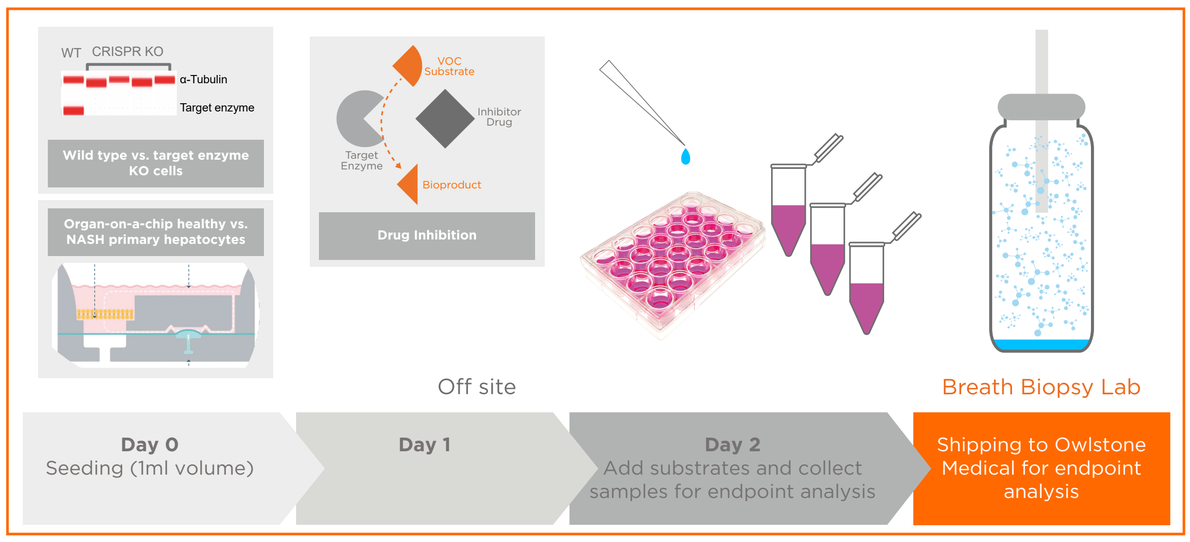

Fig. 4. Different cell culture models were tested, as well as primary hepatocytes representing healthy or NASH liver. After culturing, investigated EVOCs were added to the culture media. Aliquots of medium were collected at different timepoints, and the headspace was analysed to quantify substrates and bioproducts.

3. Results

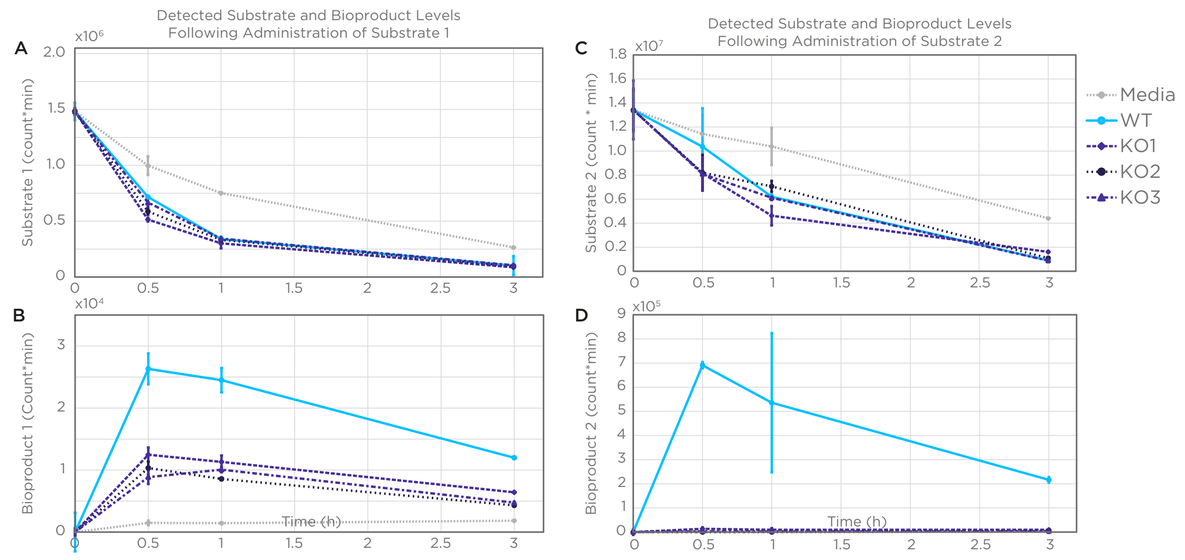

Fig. 5. Quantification of substrates and bioproducts after treatment of WT or target enzyme KO cells. (A) Substrate 1 reduction over time in the presence of WT or KO cells, or in the absence of cells (Medium). (B) Bioproduct formation from substrate 1. (C) Substrate 2 reduction over time. (D) Formation of bioproduct from substrate 2.

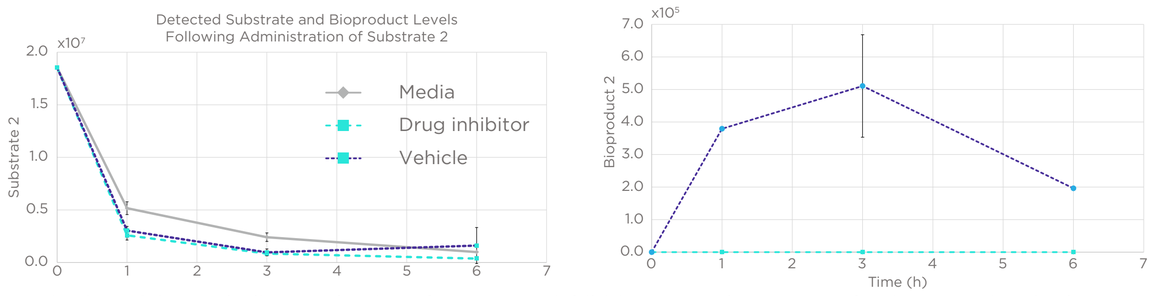

Fig. 6. EVOC metabolism evaluation after enzyme inhibition. (A) Substrate 2 reduction over time in the presence of drug-treated cells, vehicle, or in the absence of cells (Medium). (B) Substrate 2 respective bioproduct formation.

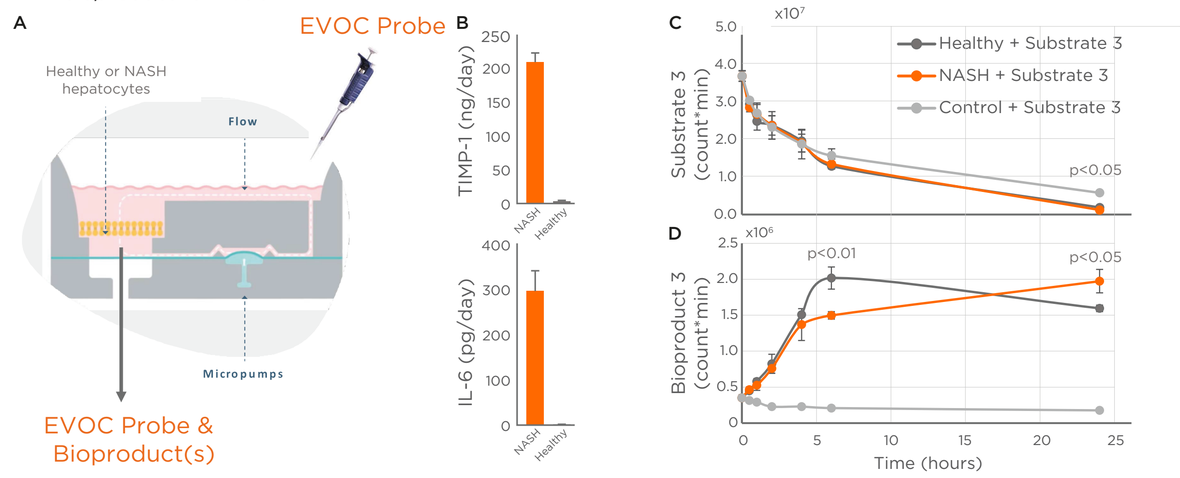

Fig. 7. Comparison of EVOCs metabolism between healthy and NASH hepatocytes. (A) EVOC Probe candidates were added to culture medium of healthy or NASH hepatocytes. Aliquots of medium were collected for headspace measurement of added substrates and corresponding bioproducts. (B) Measurement of NASH biomarkers in the culture medium to assess establishment of the NASH phenotype. (C) Substrate measurement over time in the presence or the absence of hepatocytes. (D) corresponding bioproduct formation over time.

4. Conclusions

EVOC headspace analysis represents a valuable tool for pre-clinical screening and characterization of candidate EVOC Probes.

Potential applications for EVOC Probes:

- Pre-clinical assessment of target engagement
- Functional phenotyping following genetic perturbations
- Functional metabolic characterization after drug treatment
- Supporting translational potential for clinical implementation

Use of pre-clinical models to evaluate the relationship between EVOC Probes and metabolism alterations represents a step-forward for the identification and assessment of biomarkers for breath diagnosis of NASH and other diseases.