CN-BIO

SUMMER WEBINAR SERIES

The scars of fat

The transability of the 3D NASH microtissues to model human/murine NASH and TGFβ signalling (in a Jar)

Q&A

A full run down of questions & answers from our June 2nd webinar

Abbreviations

NASH – Nonalcoholic steatohepatitis

NAFLD - Nonalcoholic fatty liver disease

PH - Primary hepatocytes

KC - Kupffer cells

HSC - Hepatic stellate cells

OOC - Organ-on-chip

MPS - Microphysiological system

BMP8B - Bone Morphogenic protein 8B

NGS - Next generation sequencing

WD - Western diet

BCD - Butyryl CoA dehydrogenase

Q&A participants

Michele Vacca

University of Cambridge

Gareth Guenigault

Senior Scientist

Another question?

Drop an email to one of our experts - **sales@cn-bi.com**

Missed the webinar?

Watch an on demand - **recording of the webinar here**

Questions

Q1

Q: Now that you have first-hand experience using CN Bio's NASH model, in your opinion, how important is it to incorporate organ on chip models into the mix when investigating its mechanism, or developing drugs to target NASH?

A: Michele Vacca MD PhD, University of Cambridge

This is the first time I have been able to fully translate data generated *in vitro* in hepatocytes (using a co-culture of primary hepatocytes (PH), Kupffer cells (KC) and hepatic stellate cells (HSC), with *in vivo* data. Since the data generated using the Microphysiological System (MPS) and *in vivo* NASH were reproducible, with regards to TGFβ signalling, there is no discussion - I would start from the MPS skipping the 2D cultures next time. Are we at the point a point where we can skip animal research? Probably it is too early, but this is certainly the way to go.

Q2

Q: Did you look at any other models apart from CN Bio's Organ-On-Chip (OOC) NASH assay in such detail and if so, could you comment on the genomic overlap?

A: Michele Vacca MD PhD, University of Cambridge

From direct experience or through handling publicly available GEO datasets, I can comment on the following: PHH, AML12, HepG2, HEPA16 and stem-cell derived hepatocytes either cultured in 2D or organoids. These cell types are not well differentiated, have high rates of cell proliferation, and *in vitro* data need to be carefully interpreted when it comes to metabolism, as it might not directly translate to *in vivo*.

Q3

Q: The hepatic stellate cells (HSC) activated by Bone Morphogenic Protein 8B (BMP8B) is it due to BMP8B secreted by hepatocytes or by HSC? have you considered deleting BMP8B specifically in hepatocytes and see whether this reduces HSC activation and fibrosis?

With regards to HSC activation by BMP8B, is it due to BMP8B secreted by hepatocytes or by HSC? Have you considered deleting BMP8B specifically in hepatocytes to see whether this reduces HSC activation and fibrosis?

A: Michele Vacca MD PhD, University of Cambridge

That is a good point. Because of time constraints, I only showed the data associated with the possible autocrine effect of BMP8B using activated wild type and BMP8B knock out primary HSC. However, within the associated publication (**Vacca et al., 2020**) you will find the results of an additional challenge where a recombinant protein was added to both wild type and knock out cells. Here, knock out cells recover, whilst a potentiation of the activation process is observed using wild type cells. So, although we did not use the strategy suggested in the question, we have shown that if you top up with recombinant BMP8B, HSC activation is accelerated even in wild-type cells.

Q4

Q: Could BMP8B gene expression analysis be used to investigate the effects of different steatosis treatments in HepG2 cells?

A: Michele Vacca MD PhD, University of Cambridge

I would not suggest using HepG2 cells for any metabolic study focussing on NAFLD as they are cancer cells. Lipid metabolism in hepatocellular carcinoma (HCC) is substantially different from that of normal liver (see this paper we recently published – **Hall et al., 2020**). Also, HepG2 cells differ significantly in terms of TGFβ receptor expression compared to normal hepatocytes. In both MPS cells and *in vivo* experiments, we did not find any direct evidence (From Next generation sequencing (NGS) or lipidomics) that BMP8B could have a direct intrahepatic metabolic effect on hepatocytes. In my opinion, exploring these paradigms using HCC cells would generate misleading results.

Q5

Q: Have you scored fibrosis using the CN-Bio Physiomimix™ NASH model?

A: Michele Vacca MD PhD, University of Cambridge

The short answer is no because the experiment was designed to study BMP8B mechanism of action and we looked at quite early time points. I moved to western diet (WD) murine models to show the long-term evidence of its "inhibition" (knock out) *in vivo*. However, in the Hepatology Communications study (**Kostrzewski et al., 2019**), CN Bio has shown that longer-term challenge with TGFβ leads to collagen accumulation, so the model is indeed suitable for studying fibrosis as an endpoint.

Q6

Q: Could you comment on the reproducibility and reliability between culture batches using the CN Bio Physiomimix™ system and NASH model?

A: Michele Vacca MD PhD

We repeated the experiments twice and both times we ended up with the same kind of results.

A: Gareth Guenigault, Senior Scientist, CN Bio

As a rule, reliability is good between different batches. One of the advantages of CN Bio's system is the ability to run a lot of experiments at the same time. We have 12 wells available on a single plate and we can run 6 plates at the same time. Generally, we get great reproducibility using the same donor within an experiment. When you start using different donors you do start to see some variability, but outcomes tend to trend the same. You would, however, expect to see donor to donor variations in the same way as variations are observed patient to patient.

All cell lines used within the model are subjected to significant quality control checks prior to experiments to ensure they give the best quality data.

Q7

Q: Does BMP8 knock out in mice alleviate injury from NASH and restore the normal phenotype?

A: Michele Vacca MD PhD, University of Cambridge

We know that the absence of BMP8B prevents NASH progression but does not impact the development or the degree of steatosis.

Due to time constraints, I didn't have time to include these results, however, in the supplementary data of the associated research paper (**Vacca et al., 2020**) we demonstrated that the absence of BMP8B in the context of a chronic WD model (Leading to NASH F1) interferes with inflammation and delays fibrosis without interfering with the amount of steatosis either quantified histologically by the pathologist, or by looking at hepatic fat content.

Q8

Q: Can the CN Bio NASH model be used to understand key mechanistic data?

A: Michele Vacca MD PhD, University of Cambridge

Certainly, for TGFβ and BMP signalling and for modelling NASH, the answer is yes. It is clear from our investigations that SMAD phosphorylation occurs and at 5 hours the target genes of SMAD are upregulated as you would expect. We were able to inhibit both the branches of the family using chemical inhibitors thus affecting the transduction of the signal so, for TGFβ signalling and for modelling NASH, the MPS system represents a nice model for preclinical science.

Q9

Q: Would you say the CN Bio NASH model represents a genuine alternative to animal models?

A: Michele Vacca MD PhD, University of Cambridge

In the future, once enough data has been generated to further support our findings, this is a trajectory that I think the field will take, however, currently we all know that if you want to publish a paper you need animal evidence.

Mid-term, advanced 3D co-culture systems certainly represent the future for pre-clinical liver disease research. Longer term, the 3 R principles request that we to try to reduce the number of animals used in studies and to find viable alternatives to replace animal research. At least based on our insights, where recombinant protein data generated using CN Bio's NASH model beautifully mirrored data generated using knock out mice, this is a step in the right direction.

Q10

Q: Is the CN Bio system uniquely enabled for NASH disease modelling?

A: Michele Vacca MD PhD, University of Cambridge

With respect to the models I have tested so far, such as cell lines or primary cells co-cultured in 2D or in 3D, I have not found a system capable of keeping hepatocytes so highly differentiated. Additionally, since you can clearly see signals from stellate cells and inflammatory cells, in my experience this is an excellent model to begin any kind of pre-clinical research focussing on the disease mechanism of NASH.

Q11

Q: Would inhibiting BMP8B impair wound healing elsewhere?

A: Michele Vacca MD PhD, University of Cambridge

We have not found any evidence so far in other organs, however fibrosis occurring in the kidneys could be worth considering. We know that BMP8B does not result in significant fibrosis into the adipose tissue but where expression levels are found within the kidneys, its potential role is worth investigating to establish if it associates with butyryl CoA dehydrogenase (BCD), or chronic kidney disease occurring secondary to diabetes. That is a good point and potentially an intriguing avenue for future development.

Q12

Q: Have you investigated patient hepatocytes for BMP8 upregulation?

A: Michele Vacca MD PhD, University of Cambridge

Yes, we investigated this in two independent cohorts. As I showed you in two the initial slides, upon NASH progression BMP8B was observed to be upregulated in the whole liver and colocalised with cytokeratin 18 in human hepatocytes and albumin in data generated from murine models. The more damage, the more BMP8B upregulation was observed.

Q13

Q: How long can CN Bio's NASH model be cultured for?

A: Gareth Guenigault, Senior Scientist, CN Bio

Cultures can be run for up to one month. It utilizes CN Bio's proprietary liver-on-chip technology which enables longer term *in vitro* culture (>1 month) of primary liver cells in 3D microtissue structures, providing plenty of time to include fat loading, genetic manipulation and compound dosing.

Q14

Q: What end point measurements, or read-outs, are offered in the *in vitro* 3D NASH model?

A: Gareth Guenigault, Senior Scientist, CN Bio

A range of microscopy, transcriptomics, lipidomics, biomarkers end points are available. Examples of end point measurements are listed below:

- Steatosis: Oil Red O Quantification
- Inflammation: Secretion of key cytokines/chemokines (IL-6, IL-8, IL1RA, IP-10, MCP-1)
- Fibrosis: Secretion of biomarkers (TIMP-1 and Fibronectin) and imaging of ECM proteins and quantification
- Cell health: Albumin, LDH release, microtissue size

Q15

Q: How flexible is the *in vitro* 3D NASH model, can it be customised?

A: Gareth Guenigault, Senior Scientist, CN Bio

Our model has been designed to be highly adaptable allowing culture of a range of cells. Our standard model uses hepatocytes, Kupffer cells and stellate cells, however, we can run simpler versions without parenchymal cells, or more complex models (such as immune cells).

One of the advantages of the model is the large cell number and volume of media, enabling higher content data from a single replicate. This means that a large range of different assays can

be performed from a single sample, whether they be secreted or cellular biomarkers.

Q16

Q: What throughput can be achieved, or how many conditions can be evaluated in one experiment using CN Bio's *in vitro* model?

A: Gareth Guenigault, Senior Scientist, CN Bio

One PhysioMimix™ OOC unit can operate up to 6 plates at once with 12 wells in each plate. This results in 72 individual samples. As standard we run experiments in triplicate providing the ability to investigate 24 independent conditions. More complex studies can be scaled up using more than one PhysioMimix™ unit.

Q17

Q: Tell us a little more about CN Bio's NASH Fee-For-Service program?

A: Gareth Guenigault, Senior Scientist, CN Bio

CN Bio's **NASH Fee-For-Service** provides access to the Company's expertise in the field and one of the most advanced *in vitro* models currently available.

The NASH model captures key aspects of the human disease at different stages of NAFLD/NASH in a range of culture conditions: intracellular fat accumulation, inflammation and fibrosis.

This program enables researchers to screen new NAFLD and NASH therapeutic candidates, investigate mechanisms of action, integrate effects on different target cell types of the liver and generate actionable data in weeks.

By providing translatable human data, CN Bio supports researchers in planning and optimising clinical trial design and to prioritise pre-clinical candidates.

Our summer webinar series continues

2nd JULY

Go with the flow

Register here

Application of microfluidic 3D liver chip models to genotoxicity testing

Dr Renato Cardoso
Dr Thalita Zanoni
Charles River Laboratories

15th JULY

A regulators viewpoint

Register here

Establishing strategies to evaluate microphysiological systems for drug development

Dr Alexandre Ribeiro
FDA

CN-BIO

332 Cambridge Science Park
Milton Road, Cambridge, CB4 0WN

+44 (0) 1223 737941 | **sales@cn-bio.com**

visit **cn-bio.com**