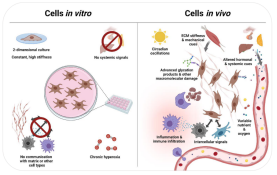
Therapeutic Candidates in a Single Animal Model of NASH

Martin Borch Jensen, Chris Carrico, Linda Chio, Ian Driver, Daniel Fuentes, Akela Kuwahara, Francisco LePort, Chris Towne
Gordian Biotechnology, San Francisco, CA

Background

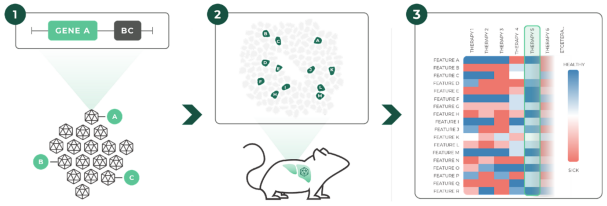
NASH is a disease with a large unmet medical need and complex etiology, involving metabolism, inflammation and fibrosis.
In vivo testing is essential to measure therapeutic efficacy of putative treatments for NASH, since the pathology is dependent on interactions between multiple cell types, and with systemic metabolism. But *in vivo* experiments are difficult to scale, especially in the most realistic animal models.

We have developed technology that enables many therapies to be tested for therapeutic effects inside a single live animal, simultaneously.



Pooled therapeutic screening *in vivo*

We deliver different therapeutics to individual cells of the liver, and extract those cells for phenotypic transcriptome readouts



Generate barcoded gene therapy library

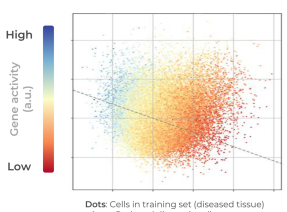
Inject library into living animal, modifying individual cells

Single-cell sequencing measures detailed disease activity for each cell receiving a gene therapy

Biological features curated in transcriptome, representing (individually or in sets) predictions about physiological effects

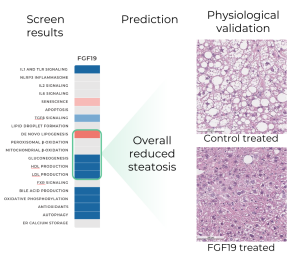
Activity of individual features determined by dimensionality reduction of component genes.

Example: Peroxisomal ß-oxidation



Dots: Cells in training set (diseased tissue)
Axes: Reduced dimensionality gene set
Dotted line: Inferred gene activity gradient

Features validated with orthogonal assays



Screen predicts clinical and preclinical outcomes

Initial screen design

Validated in independent cohorts with a different promoter to reduce gene dose, and using a different NASH model (FAT model, Tsuchida 2018).

AMLN diet-induced NAFLD/NASH mice
High-fat, high-sugar, 2% cholesterol.
7.5 months duration.

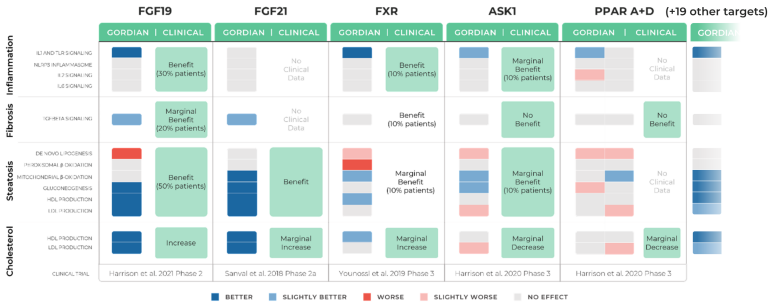
NASH and NAFLD outcomes:
Some animals develop steatosis only, others also inflammation and fibrosis. Screen in each, separately.

AAV gene delivery:
Sacrificed and sequenced 4 weeks after gene therapy library injection

25 gene therapies:
Inert controls
Novel targets
Six clinical targets

Hepatocyte screen:
Cell intrinsic & environment-response effects measured

Predictions about clinical targets included in the screen match 14 of 16 outcomes measured in Ph. II/III trials



Left: Predictions from our *in vivo* pooled screen, based on features grouped by physiological readout.
Right: Significant clinical trial outcomes. Green boxes indicate outcomes consistent with predictions.

In vivo pooled screen predicts majority of physiological outcomes in high dose, single therapy follow-up experiments

Experimental design

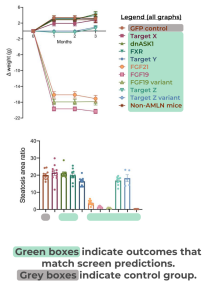
Single therapy
High dose AAV

AMLN diet, 7.5 months (as in screen)

3 months' therapeutic treatment

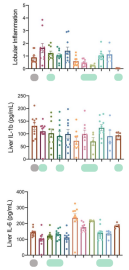
Assays mapping to screen features: Histology (H&E + Sirius red), Luminex cytokine array.

Metabolic phenotypes

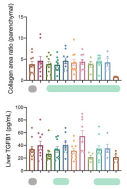


Green boxes indicate outcomes that match screen predictions.
Grey boxes indicate control group.

Inflammation phenotypes



Fibrosis phenotypes



Conclusions & next steps

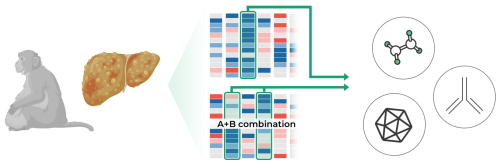
- Controlled delivery of gene therapies to, and phenotypic profiling of, single liver cells feasible.
- Overall, our *in vivo* screen had strong predictive power for both preclinical and clinical outcomes.
- Hepatocyte screen highly predictive for metabolic effects, less so for inflammation. Future stellate & Kupffer screens should capture these features better.
- Thus, *in vivo* pooled screening enables efficacy testing at a dramatically greater scale than has previously been possible.

Upcoming screens will test hundreds of novel candidate therapies per animal

In long-term, diet-induced primate models with greater fibrosis...

...to identify targets *or combinations* with comprehensive therapeutic effects...

...to develop truly effective drugs for NASH.



Join our team!

All authors are employees and stockholders of Gordian Biotechnology. We are grateful to Scott Friedman for advice and insightful discussions.
We thank Mathieu Petijean, Li Chen, Louis Petijean at PharmaNest and Janani Iyer and Robert Brockett at PathAI for their expert analysis of histology data.

We're hiring in San Francisco!

Gordian
www.gordian.bio

Job listing