Hepatocyte Targeted Therapy to Enhance Efficacy and Safety of Drugs for Liver Diseases

LP21

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Background

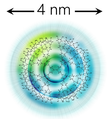
Problem: Hepatocytes are involved in many liver diseases, but drugs are not selective for hepatocytes resulting lack of efficacy or off target toxicity.

Approach: Selectively target drug to hepatocytes to improve therapeutic window and safety profile of an farnesoid X receptor agonist (FXRa).

Goal: Demonstrate the potential for novel hepatocyte targeted hydroxyl dendrimers (htHD) to selectively target and treat liver disease (NASH model)

Hydroxyl Dendrimer (HD) Technology

- Only taken up by reactive inflammatory cells in diseased tissues (broad range of diseases)
- Targeted systemic therapy (Oral or injectable)
  - Crosses tissue barriers (BBB, retina, tumor)
  - Safe at high doses in animals & humans
  - Sustained duration of effect
- Low cost manufacturing, rapid discovery process (Over 65 HDTs to date)
- Broad license to technology from Johns Hopkins University (JHU) (>15 yrs, >$30 M NIH, >30 JHU collaborators, >70 papers, 22 issued & 50 pending patents)



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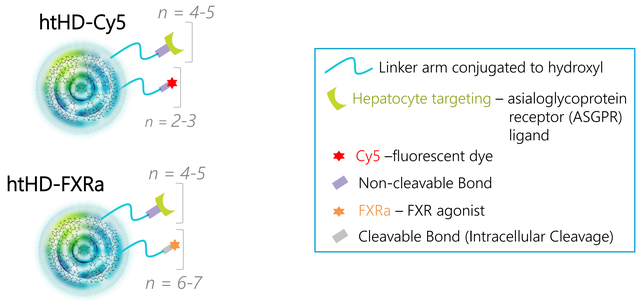
- Water-like Surface (hydroxyls only) (novel finding)
- Targets Key Cells
- No Ligand Needed
- 64 hydroxyls

STAM™ Model of NASH

- C57BL/6J mice 2 days after birth treated with streptozotocin (diabetes)
- After 4 weeks, initiate high fat diet
- 5-6 weeks: steatosis phase
- 7-8 weeks: steatohepatitis phase
- 9-12 weeks: fibrosis phase (transition to chronic fibrosis)

(Fujii et al 2013)

HD Construct Design



- Minimal Release of FXRa from htHD-FXRa under plasma conditions with complete release under intracellular conditions (pH 5.5 esterase) in 1 month
- After intracellular uptake of htHD-FXRa, slow sustained release of FXRa inside hepatocytes
- Plasma clearance within 2 days (human Phase 1 data of HDT, OP-101)

STAM Mice Study of htHDs

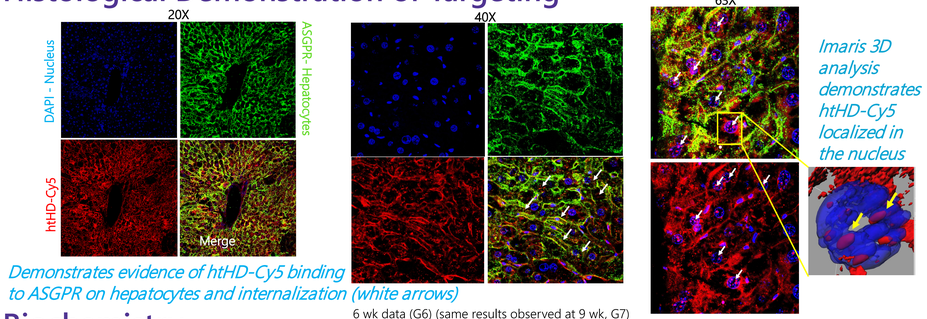
Groups		n	Treatment	Dose (mg/kg)	Regimen	Sacrifice (wks)
1	Normal	8	None	--	--	9
2	STAM	8	Vehicle	--	IP QOD 6 wks- 9 wks	9
3	STAM	8	Free FXRa	30	PO QD 6 wks- 9 wks	9
4	STAM	8	htHD-FXRa	6 (FXRa)*	IP QOD 6 wks- 9 wks	9
5	STAM	8	htHD-FXRa	30 (FXRa)*	IP QOD 6 wks- 9 wks	9
6	STAM	4	htHD-Cy5	50 (total)	IP Single Dose 6 wks	6 (24 hr post dose)
7	STAM	4	htHD-Cy5	50 (total)	IP Single Dose 9 wks	9 (24 hr post dose)

* Dose based on mass of FXRa in htHD-FXRa

Body Weight and Liver Weight at 9 weeks

Parameter (Mean ± SD)	Normal (G1)	Vehicle (G2)	Free FXRa (G3)	htHD-FXRa low (G4)	htHD-FXRa high (G5)
Body Weight (g)	28.7 ± 0.5	22.1 ± 1.8	21.6 ± 3.1	21.5 ± 3.1	20.9 ± 3.8
Liver Weight (mg)	1400 ± 64	1752 ± 235	1520 ± 259	1554 ± 276	1566 ± 277
Liver-body weight ratio (%)	4.9 ± 0.2	8.0 ± 1.4	7.2 ± 1.3	7.3 ± 1.1	7.6 ± 1.2

Histological Demonstration of Targeting



Imaris 3D analysis demonstrates htHD-Cy5 localized in the nucleus

Demonstrates evidence of htHD-Cy5 binding to ASGPR on hepatocytes and internalization (white arrows)

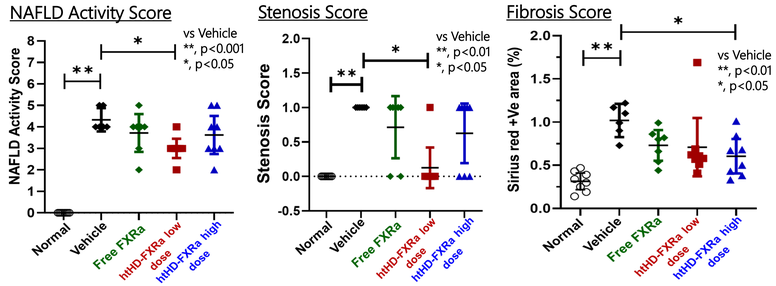
6 wk data (G6) (same results observed at 9 wk, G7)

Biochemistry

Parameter (Mean ± SD)	Normal (G1)	Vehicle (G2)	Free FXRa (G3)	htHD-FXRa low (G4)	htHD-FXRa high (G5)
Serum ALT (U/L)	22 ± 7	48 ± 7	57 ± 22	28 ± 7	41 ± 11
Liver Triglyceride (mg/g liver)	5.4 ± 1.2	59.1 ± 26.1	54.7 ± 25.9	37.5 ± 10.0	56.9 ± 20.0

Improvements in Biomarkers

Immunohistochemistry



- *htHD-FXRa significant reductions NAFLD, stenosis and fibrosis scores.*
- *Free FXRa has no significant effect*

Conclusions & Next Steps

- Demonstrated selective targeting to hepatocytes including internalization in nucleus
- Targeting FXRa (htHD-FXRa) to hepatocytes provides significantly better therapeutic benefit compared to free FXRa
- Potential to reduce toxicity and increase efficacy of FXRa compounds
- Sustained intracellular effects (up to one month) and potential for oral administration may yield a once per month NASH therapeutic
- Approach is also being applied to other liver diseases such as hepatocellular carcinoma

References

Fujii, M, Shibazaki, Y, Wakamatsu, K, Honda, Y, Kawauchi, Y, Suzuki, K, Arumugam, S, Watanabe, K, Ichida, T, Asakura, H, Yoneyama, H 2013. A murine model for non-alcoholic steatohepatitis showing evidence of association between diabetes and hepatocellular carcinoma. Medical Molecular Morphology **46**, 141–152.

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