NGM282 Promotes HDL Biogenesis and Transhepatic Cholesterol Efflux to Prevent Atherosclerosis in Mice

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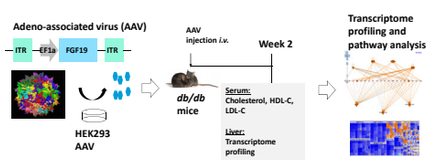
BACKGROUND AND AIMS

- FGF19, an endocrine hormone produced in the gut, acts in the liver to control bile acid synthesis [1-2]
- NGM282 is an engineered, non-tumorigenic analogue of human FGF19 [3-4]
- In phase 2 clinical trials in patients with non-alcoholic steatohepatitis, administration of NGM282 resulted in rapid and profound reductions in liver fat content, liver injury, inflammation and fibrosis [5-6]
- However, NGM282 increased cholesterol levels, and the molecular mechanisms that integrate the FGF19 signaling with cholesterol metabolic pathways are incompletely understood
- Here we investigate these mechanisms using a combination of pharmacological, bioinformatics, metabolomics and biochemical approaches

METHODS

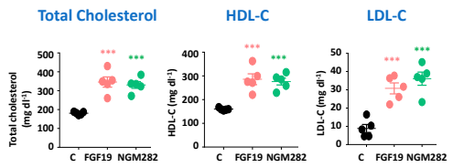
- *db/db* mice (Jax#000642) received an intravenous injection of 1 x 10^{11} vector genome adeno-associated virus (AAV) carrying FGF19, NGM282 or green fluorescent protein (control). 2 weeks later, serum levels of cholesterol, HDL-C and LDL-C were measured. Livers were collected for transcriptome profiling, QPCR, metabolomics and histological analysis
- Hepatocyte-specific ABCA1-deficient mice were obtained by injecting Abca1$^{fl/fl}$Abcg1$^{fl/fl}$ mice with AAV-TBG-Cre, which drives Cre recombinase expression under TBG promoter, allowing hepatocyte-specific expression
- FGFR4-deficient mice or wild-type mice received an intravenous injection of 3 x 10^{11} vector genome AAV carrying FGF19 or green fluorescent protein (control)
- ApoE-deficient mice were placed on a high-fat, high-cholesterol Western diet (Teklad TD88137) immediately following AAV injection, and this diet was continued ad libitum throughout the study. Mice were euthanized 18 weeks after AAV administration for analysis
- En face analyses of the ApoE-deficient mice were conducted at Wake Forest School of Medicine Metabolic Core (Winston-Salem, NC). Aortic lesion area was quantified using Image J software and expressed as percent stained area relative to total aortic area. All quantifications were carried out by an observer blinded to the sample identity
- Concentrations of total cholesterol, HDL-C and LDL-C were measured by enzymatic methods on an automated analyzer (COBAS INTEGRA 400 Plus Clinical Analyzer, Roche Diagnostics)



RESULTS

NGM282 Increases Serum Cholesterol in *db/db* Mice

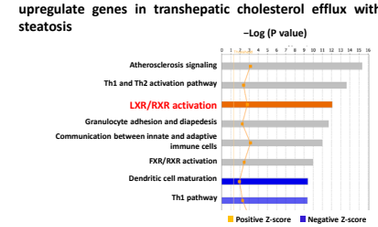
- Administration of FGF19 or NGM282 resulted in elevations in total cholesterol, HDL-C and LDL-C in 2 weeks in *db/db* mice

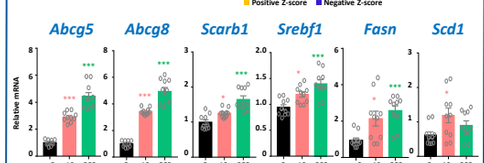


***p<0.001, **p<0.01, *p<0.05 vs the control (C) group

NGM282 Selectively Activates Hepatic LXR Signaling in *db/db* Mice

- Transcriptome profiling and Ingenuity pathway analysis revealed that FGF19 and NGM282 activate the LXR/RXR pathway
- Metabolomics analysis uncovered an increase in intrahepatic hydroxysterols, natural ligands for LXR [7]
- However, FGF19 and NGM282 selectively modulate LXR signaling and upregulate genes in transhepatic cholesterol efflux without causing steatosis





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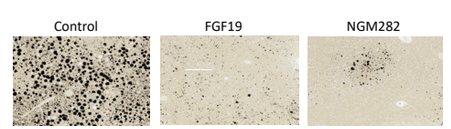
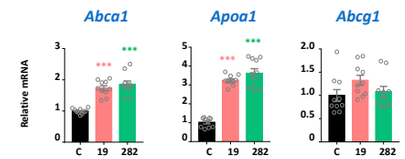


(Oil droplets are shown as black vacuoles in livers stained with osmium tetroxide)

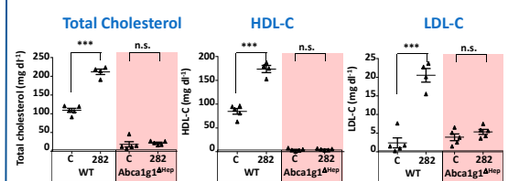
Deficiency in Hepatocellular ABCA1 Abolishes NGM282-Associated Cholesterol Change

- FGF19 and NGM282 promote HDL biogenesis through the induction of *Abca1* and *Apoa1*, both are LXR targets



***p<0.001, **p<0.01, *p<0.05 vs the control (C) group

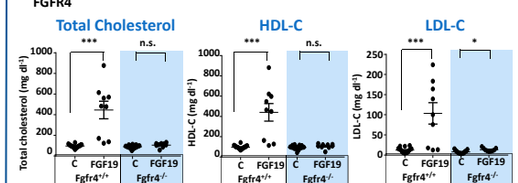
- NGM282-associated cholesterol increases were blunted in mice deficient in ABCA1



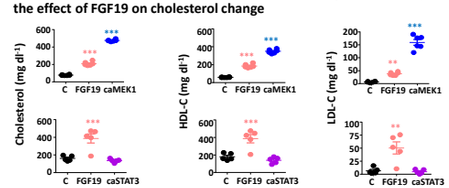
***p<0.001 vs the control (C) group; n.s., not significant

NGM282-Associated Cholesterol Change is Dependent on FGFR4

- FGF19-associated cholesterol increases were abolished in mice deficient in FGFR4



- A constitutively active MEK1, but not a constitutively active STAT3, mimics the effect of FGF19 on cholesterol change



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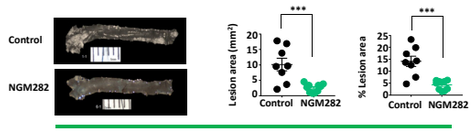
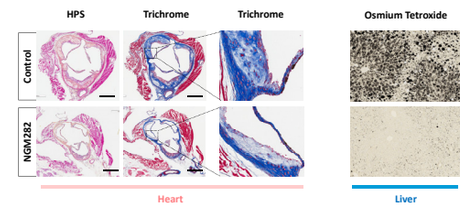
NGM282 Protects Against Atherosclerosis in ApoE-deficient Mice

- In dyslipidemic ApoE-deficient mice fed a Western diet, treatment with NGM282 significantly reduced atherosclerotic lesion area in aortas



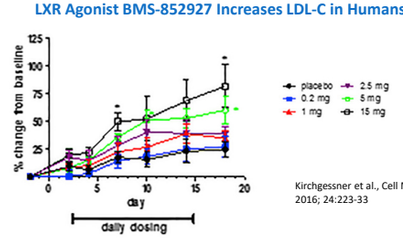
***p<0.001 vs the control group



LXR Agonist Increases Serum LDL-C in Humans

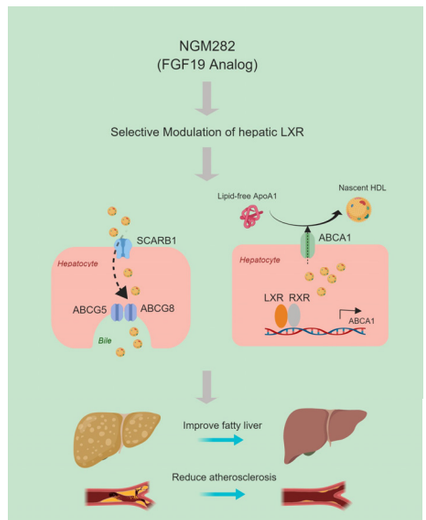
- Activation of LXR signaling is well known to promote reverse transport of cholesterol from peripheral tissues to the liver [8]
- LXR activation induces the expression of genes involved in cholesterol efflux, facilitates cholesterol esterification by promoting fatty acid synthesis, and exhibits anti-inflammatory effects through inhibition of TLR signaling [8]
- The development of LXR agonists as anti-atherogenic agents has been hindered by hepatic steatosis that accompanies activation of LXR in the liver [9]. Furthermore, first-in-human testing of LXR agonists revealed LDL-C elevations [10]
- Similarly, administration of NGM282 in patients with nonalcoholic steatohepatitis resulted in elevations in serum LDL-C levels, but reductions in liver fat content [5]

LXR Agonist BMS-852927 Increases LDL-C in Humans



Kirchgessner et al., Cell Metab. 2016; 24:223-33

Summary



CONCLUSION

- The endocrine hormone FGF19 and its analogue NGM282 have a hitherto unsuspected intrinsic role in promoting transhepatic cholesterol efflux and HDL biogenesis through selectively activating LXR signaling, while ameliorating steatosis and atherosclerosis
- The selective modulation of LXR by NGM282 may provide additional understanding of the observed cholesterol increases in animals and humans, as well as the cardiovascular benefit in animals

References:

1. *Kliewer et al, Dig Dis 2015; 33:327-331*
2. *Degirolamo et al., Nat Rev Drug Discov 2016; 15:51-69*
3. *Zhou et al., Cancer Res 2014; 74:3306-3316*
4. *Luo et al., Sci Transl Med 2014; 6:247ra100*
5. *Harrison et al., Lancet 2018; 391:1174-1185*
6. *Harrison et al., Hepatology 2019; doi: 10.1002/hep.30590*
7. *Janowski et al., Nature 1996; 383:728-731*
8. *Tontonoz et al., Mol Endocrinol 2003; 17:985-93*
9. *Schultz et al., Genes & development 2000; 14:2831-2838*
10. *Kirchgessner et al., Cell Metab 2016; 24:223-33*

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Author disclosures on file at EASL 2019