Reviewer's report

Title: Synthetic high-density lipoprotein nanoparticles for the treatment of Niemann-Pick Diseases

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Reviewer: Daniel Ory

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Schultz et al.

In this manuscript the authors examine the action of synthetic HDL (sHDL) nanoparticles in vitro and in vivo models of Niemann-Pick diseases. They define the optimal peptide and lipid composition for the sHDL, and demonstrate dose-dependent reduction of cholesterol in NPC1 fibroblasts. These findings are extended to an in vivo NPC1 model, in which they show mobilization of cholesterol form tissues, reduced bilirubin and correction of body weight deficits. They show that ICV injection in the brains of these mice can reduce cholesterol storage in Purkinje neurons. They further demonstrate that sHDL is effective in reducing sphingolipid accumulation in NPA fibroblasts.

The study is well executed, data high quality, and conclusions generally justified by the data. This proof of mechanism study represents a solid addition to the NPC field and could prompt further exploration of how to test chronic delivery of sHDL as a therapeutic for NPC (perhaps in the feline NPC1 model?). On the other hand, systemic delivery of the sHDL did not address critical aspects of the NPC liver phenotype, which is curious and should be addressed. Further clarification is needed for some in vitro studies as well.

Specific points:

1. Reduction of SR-B1 and ABCA1 is shown with respect to mRNA abundance but not at the protein level. Western blots are required to effectively rule in or out these proteins in cholesterol reduction. Was ABCG1 knockdown examined?

2. In Fig 3a, the comparison between the actions of cyclodextrin and 5A-SM is not entirely accurate because cyclodextrin and 5A-SM act on different time scales. Cyclodextrin actually decreases SREBP2 target gene expression due to redistribution of lysosomal cholesterol between 6-24 hrs; by 48 hrs homeostasis is restored. What can be said is that the concentration of cyclodextrin necessary for lysosomal cholesterol reduction does not cause net extraction of cellular cholesterol, which is in contradistinction to 5A-SM. This needs clarification.
3. The co-localization of 5A-SM-DiD with LAMP1 and filipin is modest. However, the experiment lacks a positive control, such as LDL, to assess whether this is more or less than one would expect for a lipoprotein endocytic cargo. If not in LE/Ly, then with which compartment is 5A-SM-DiD predominantly associated? Were early endosomal or recycling endosomal markers examined?

4. From the efflux experiment (Fig 4d), all that can be concluded is that the efflux occurs in the same time frame as uptake of sHDL. To determine whether sHDL is required for cholesterol efflux, the experiment would need to include an inhibitor of micropinocytosis such as amiloride.

5. Although the sHDL treatment reduced bilirubin and corrected the weight loss phenotype in the NPC mice, curiously the authors do not address key questions about the in vivo effects. Did treatment reduce sterol or sphingolipid storage or established NPC1 biomarkers in peripheral tissues (e.g., liver or spleen)? In liver sections, was there evidence for reduction of lysosomal cholesterol and/or inflammation (e.g., macrophage staining)? If the answer to these questions is no, is there any significance for the discordant results between the effects of HDL on liver tissue and cerebellar tissue?

6. The conclusion that DiD detection in liver vascular ECs suggests lipid clearance by the liver is not justified. As noted by the authors, the DiD lipid marking the sHDL is not covalently bound to the lipoprotein lipid species. Therefore, detection in the liver is potentially only informative about the clearance of the DiD, not the lipid associated with the sHDL.

Minor:

Pg. 11, line 276: typo for Bregma

Pg. 21, line 468: typo for 2-hydroxypropyl-beta-cyclodextrin

Lines 146, 593, 595, 597: typos for NBD-Sphingomyelin
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
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