Q: How does Tafazzin mutation cause plasmalogen deficiency?
A: We currently do not know. However, one possibility would be the following sequence of events. Tafazzin affects cardiolipin remodeling. Immature CL does not seal the MIM well and allows the production of ROS that oxidizes plasmalogens. It also goes the other way. Loss of plasmalogens results in more ROS that oxidizes CL and causes less efficient ox-phos.

Q: Can you highlight some of the complexities of a dietary approach eg. intestinal absorption of HG, bioavailability, and possible hydrolysis but gut lipases?
A: In order to test a drug effect on a specific metabolic pathway one could use living cells or whole animals. In addition to eliminating many processes found in whole animals, using cells greatly limits the variation between individual cells and makes the effect of the drug easier to test and lowers the error. Best is a single cell line in which the genetics of the cell are identical for all cells. We have used two Barth and two control cell lines – allowing for some genetic variation. Even using mice, one would try to limit genetic factors by using a particular mouse strain and possibly even sex. In addition to genetic difference, animals have greater variations in their metabolic state dependent in part on their physical activity and diet. This will vary much more between one animal and another than between one cell and another. There are additional issues of drug delivery in whole animals. With oral administration there is the issue of intestinal absorption and of enzymatic degradation in the GI-tract. With HG it has been shown that the lipid is distributed throughout the body, except perhaps for the brain which is not a major target organ for Barth Syndrome. If delivery becomes a problem, a simple solution would be to simply use more drug than was used with cells.
**Q: What is your next step? Try alkylglycerol in the mouse model?**

A: The “bottom line” for testing this approach would be to try it on humans. However, given the importance of not exposing humans to treatments that do not work ideally, the limited number of individuals with Barth Syndrome and the expected variability between individuals because of genetic and environmental factors, we would like to extend our test with the lymphoblast cell lines before going into whole animals. In addition to optimizing and further testing the hexadecylglycerol administration, we would also like to compare this strategy with administering linoleic acid that has been shown to result in the recovery of cardiolipin. Testing the effect of linoleic acid administration on plasmalogen levels will help us to understand the relationship between plasmalogens and cardiolipin to aid in the design of future therapeutic strategies.

**Q: How long plasmalogen treatment takes to take effect for BTHS in real-life (estimation)?**

A: Given that the HG distributes quickly but CL turnover is slow, it should take days to start to improve symptoms in a Barth patient.

**Q: Does AG restore the cardiomyopathy in TAZ Barth Syndrome mice model?**

A: That is a very interesting question. Our initial studies with a cell model are encouraging, but at the same time other questions need to be addressed. Our plan is to optimize the protocol to fully restore the lipid and mitochondrial phenotype in the cell model; specifically, we need to find the best AG molecular species and administration schedule. Once we find those conditions, we intend to use a BTHS mice model and evaluate the effectiveness of the therapy on other manifestations of BTHS, including cardiomyopathy.

**Q: You show membrane potential alterations, did you measure mtROS at all?**

A: Unfortunately we have not measured the effects of the therapy on mtROS yet. However, the ability of our treatment to restore the mitochondrial membrane potential, suggests a more robust electron transport chain activity. This would lead to a decrease in the production of mtROS. Also, the increased plasmalogen levels might act to scavenger mtROS, again leading to a decrease in mtROS. We do propose looking directly at mtROS in the next grant cycle. This is one of the reasons we think it prudent to spend one more year in optimizing the procedure using cells before going on to animals or humans.

**Q: Did you measure mitochondrial function after treatment?**

A: We showed increased ATP production per mitochondria. In the coming grant cycle we will look further at mitochondrial function and morphology.
Q: We have done alkylglycerol supplementation in a mouse model in which plasmalogens are deficient and you can have COMPLETE restoration of plasmalogens except in the brain!
A: Thank you for reminding us of this result. It provides a good basis for eventually extending our study into whole animals.

Q: The levels of alkylglycerols are also quite high in human breast milk. I know some BTHS patients getting troubles when weaned from breast milk and started on formula. Could there be a link?
A: This is an interesting observation. It might be a good place to start a clinical trial. Hexadecylglycerol is not expensive and is approved for human administration.

Q: Does AG restore the cardiomyopathy in TAZ Barth Syndrome mice model?
A: That is a very interesting question. Our initial studies with a cell model are encouraging, but at the same time other questions need to be addressed. Our plan is to optimize the protocol to fully restore the lipid and mitochondrial phenotype in the cell model; specifically, we need to find the best AG molecular species and administration schedule. Once we find those conditions, we intend to use a BTHS mice model and evaluate the effectiveness of the therapy on other manifestations of BTHS, including cardiomyopathy.