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Randomized Control Trial Investigating the Use of anti-MiR33 in Adults to Increase High Density Lipoprotein (HDL) Cholesterol

A. Study Purpose and Rationale

Introduction: More than 18 million people in North America have cardiovascular disease in spite of novel lipid-lowering therapy. Cardiovascular disease continues to be one of the leading causes of mortality in the United States. Specifically, elevated low-density lipoprotein (LDL) cholesterol has been established as a predictor of cardiovascular health and is associated with increased risk of cardiovascular events. As such, statin therapy has revolutionized the treatment of high cholesterol by reducing the risk of cardiovascular events by up to 30%[1].

However, studies show that in conjunction with high LDL, low levels of high-density lipoprotein (HDL) cholesterol can also increase the risk of cardiovascular disease. The VA-HIT trial suggested that increasing HDL levels with gemfibrozil was associated with few cardiovascular events in men[2]. The HDL-Atherosclerosis Treatment Study showed that simvastatin plus niacin therapy was able to cause the regression of coronary atherosclerosis as measured by angiography and decrease the rate of cardiovascular events[1]. The Treating to New Targets Trial showed that when an LDL of 70mg/dl was achieved, rates of cardiovascular events was decreased by approximately 25% in those subjects who were in the highest quintile of HDL levels[3]. However, the degree to which raising HDL vs lowering LDL contributed to the atheroprotective effects of the therapy is unclear.

New studies suggest that HDL functionality rather than mass may be a more accurate indicator of its ability to decrease cardiovascular risk. Free cholesterol is toxic to cells; as such, intracellular cholesterol is modulated by efflux through ATP-binding cassette receptors ABCA1 and ABCG1. The measurement of increased HDL-mediated cholesterol efflux from macrophages may actually be a better predictor of cardiovascular disease. Novel therapies that can increase HDL levels, such as CETP inhibitors, were developed but many have failed to show clinical benefit[4]. Indeed, although the AIM-HIGH trial showed an increase in HDL levels, no benefit was seen in terms of cardiovascular outcome. The Heart Protection Study-2 Treatment of HDL to Reduce the Incidence of Vascular Events (HPS2-THRIVE) trial showed that the use of niacin/laropiprant with statin therapy did not reduce the risk of cardiovascular events more than statin therapy alone[4]. In addition to this, niacin, one of the oldest HDL-raising therapies, has many side effects, including hepatotoxicity, hyperglycemia, and flushing.

The recent development of using microRNAs as potential post-transcriptional regulators of cholesterol metabolism has emerged as a possible target for future therapies.

MicroRNAs are often encoded in the intronic regions of the genome and bind to the 3' untranslated regions of mRNAs, causing repression of translation or destabilization of the mRNA itself. Importantly, a single microRNA can have multiple targets, which can provide a mechanism for the simultaneous repression of multiple genes involved in a specific pathway, such as cholesterol regulation[5].

MiR33: MiR33 became a potential therapeutic target after a genome-wide screen of miRNAs showed that it was modulated by cholesterol content and was encoded within the SREBP1 and SREBP2 genes which are involved in triglyceride regulation and cholesterol synthesis, respectively[5].Target prediction algorithms showed that predicted targets of miR33 were ABCA1 and ABCG1, which play important roles in the reverse cholesterol transport and HDL biogenesis in the liver. Effluxed cholesterol is transported via HDL to the liver for excretion into bile.

Preliminary studies showed that in mice, anti-miR33 was associated with a 50% increase in hepatic ABCA1 and an increase in HDL levels by approximately 25%. Of potential clinical significance, the inhibition of miR33 led to extensive decreases in the macrophage and lipid content of plaques as well as an increase in the collagen content of the plaques. In mice, atherosclerotic lesion areas decreased by approximately 35% after treatment with anti-miR33 as compared to controls. These plaques were also significantly more stable, suggesting that this novel therapy may have atheroprotective properties[6].

In non-human primates (African green monkeys), the systemic introduction of an anti-sense miR33 via subcutaneous injection was shown to increase HDL levels by 50% within 8 weeks. These cholesterol particles were also significantly larger than their control counterparts, suggesting increased cholesterol efflux from macrophages. This study also showed that treating monkeys with anti-miR33 decreased several proteins involved in fatty acid synthesis and increased protein levels of those involved in fatty acid oxidation potentially leading to better overall cardiovascular health. There was a significant decrease in triglyceride levels in the monkeys as well. Of importance, although the anti-miR33 was introduced as a subcutaneous injection in these monkeys, there were no associated toxicities. Blood counts and chemistries, coagulation markers, body weights and serum cytokines remained normal throughout the duration of the study[7].

The purpose of this study is to conduct a randomized, double masked, control trial to investigate whether anti-miR33 may be a potential atheroprotective therapy by increasing HDL levels in humans who have established cardiovascular disease.

Study Aims: To study the effect of anti-miR33 on HDL levels in patients who have stable coronary heart disease with HDL levels lower than 35mg/dl and to determine if this therapy will have atheroprotective effects on patients with established cardiovascular disease.

Study Hypothesis:

1. Anti-miR33 therapy will increase HDL levels in subjects by at least 5mg/dl at the end of a twelve week trial.

B. Study Design and Statistical Analysis:
The proposed study will be a randomized, placebo-controlled, double blind, single-center clinical trial. The study arms will include patients who are randomized to either the anti-miR33 treatment group or the placebo group. The patients will be randomized using random number generation.

Primary and Secondary Outcomes: The primary outcome measure will be HDL levels at 4, 8, and 12 weeks, based on previous data in the non-human primate model. The core laboratory at Columbia University Medical Center will measure lipid panels at each visit. Secondary outcomes may include increased cardiovascular health as measured by decreased incidence of cardiovascular events such as unstable angina or myocardial infarction. Potential atheroprotective effects of therapy may also follow the regression of coronary atherosclerotic plaques via catheterization.

Statistical analysis: In order to achieve 80% power with a p value of 0.05, a sample size of at least 17 patients in each arm was calculated using an unpaired t-test analysis, assuming that a 5mg/dl difference between the treatment and placebo groups would be clinically significant. This model also assumes a standard deviation of approximately 5mg/dl for each group. In order to account for attrition, 25 patients will be recruited for each arm.

C. Study Procedure: Patients will be recruited through referral from their primary care physicians in the AIM clinic at Columbia University Medical Center. Subjects will be enrolled in the study based on their HDL level (less than or equal to 35mg/dl) in the setting of a normal LDL (with or without statins). They will be screened for previous cardiovascular events and other risk factors such as hypertension and diabetes. After randomization into the treatment and placebo arms, patients will be required to return to the AIM clinic twice a week for the first two weeks of the trial, and once a week thereafter for twelve weeks. At these appointments, they will be given a subcutaneous injection of either anti-miR33 or placebo. Every fourth week, the subjects will have blood drawn in order to trend their HDL and lipid panel, as well as their blood counts and blood chemistries in order to monitor for potential side effects. Patients will be informed of any potential adverse effect of the medications. They will be advised to report any signs of bleeding, chest pain, urinary symptoms, or other adverse events to their physicians.

D. Study Drugs: Anti-miR33 has not yet been approved for use in humans. Based on non-human primate studies, the therapy does not have any adverse effects as measured by blood counts, blood chemistries, hepatic function tests, or coagulation markers. The drug will be administered subcutaneously. Previous studies did not suggest local reactions to the injected medication.

E. Medical Device: Not applicable

F. Study Questionnaires: Not applicable

G. Study Subjects:
Inclusion criteria:

1. Age: 45 years or older
2. HDL levels of 35mg/dl or less
3. LDL less than 120mg/dl (with or without statin therapy)
4. Established cardiovascular disease, defined as stable coronary heart disease, cerebrovascular or carotid disease, or peripheral arterial disease
5. Ability to discontinue any lipid-modifying drugs prior to enrollment in the trial with the exception of statins

Exclusion criteria:

1. Age: Less than 45 years of age.
2. HDL levels 40mg/dl or greater
3. LDL levels of greater than 120mg/dl
4. Hospitalization for acute coronary syndrome or revascularization procedure within 4 weeks prior to enrollment
5. Stroke within 8 weeks prior to enrollment

Note: Criteria is influenced by the AIM-HIGH trial[1].

H. Recruitment of Subjects: Subjects will be recruited through their primary care physicians at the AIM clinic based on their HDL levels and their history of stable cardiovascular disease.

I. Confidentiality of Study Data: All study data will be stored in a confidential matter via coding with unique identifiers. No personal identifiers will be included.

J. Potential Conflict of Interest: There are no conflicts of interest.

K. Location of the Study: The study will be conducted at the AIM clinic at Columbia University Medical Center.

L. Potential Risks: Potential risks include the risk of physical discomfort and/or infection at the time of injection of anti-miR33 or placebo, as well as at the time of blood draws for lipid studies. There may also be local inflammatory reactions at the site of the injection. Although previous studies in non-human primates did not elucidate any adverse reactions, liver function, blood counts, and blood chemistries will be monitored throughout the trial.

M. Potential Benefits: The main benefit of this study is increased HDL levels, which is expected in all patients in the treatment arm. This increase in HDL may have atheroprotective properties. Some patients may not benefit from this study.

N. Alternative Therapies: Not applicable

O. Compensation of Subjects: Subjects will receive all study medications free of cost. All medical visits and laboratory test will also be provided with no cost to the patients enrolled in the trial. There will be no other compensation offered to the patients.

P. Cost to Subjects: There will be no cost to the patients enrolled in the trial.

Q. Minors as Research Subjects: Patients under the age of 18 will not be included in this trial.

R. Radiation or Radioactive Substances: Not applicable

S. References:

1. AIM-HIGH investigators, Boden WE, Probstfield JL, Anderson T, Chaitman BR, Desvignes-Nickens P, Koprowicz K, McBride R, Teo K, Weintraub W. Niacin in patients with low HDL cholesterol levels receiving intensive statin therapy. New England Journal of Medicine 2011; 365(24): 2255-67.

2. Robins SJ, Collins D, McNamara JR, Bloomfield HE. Body weight, plasma insulin, and coronary events with gemfibrozil in the Veterans Affairs High-Density Lipoprotein Intervention Trial (VA-HIT). Atherosclerosis 2008; 196(2):849-55.

3. Preiss D, Seshasai S, Welsh P, Murphy S, Ho J, Waters D, DeMicco D, Barter P, Cannon C, Sabatine M, Braunwald E, Kastelein J, Lemos J, Blazing M, Pedersen T, Tikkanen M, Sattar Naveed, Ray K. Risk of incident diabetes with intensive-dose compared with moderate-dose statin therapy. JAMA 2011; 305(24):2556-64.

4. Hafiane A, Genest J. HDL, Atherosclerosis, and Emerging Therapies. Cholesterol 2013; 2013: 1-18.

5. Rayner KJ, Saurez Y, Davalos A, Parathath S, Fitzgerald M, Tamehiro N, Fisher E, Moore KJ, Fernandez-Hernando C. MiR-33 contributes to the regulation of cholesterol homeostasis. Science 2010; 328(5985):1570-1573.

6. Rayner KJ, Sheedy F, Esau C, Hussain FN, Temel R, Parathath S, van Gils J, Rayner AJ, Chang AN, Suarez Y, Fernandez-Hernando C, Fisher E, Moore KJ. Antagonism of miR-33 in mice promotes reverse cholesterol transport and regression of atherosclerosis. Journal of Clinical Investigation 2011; 121(7): 2921-2931.

7. Rayner KJ, Esau C, Hussain FN, McDaniel A, Marshal S, van Gils J, Ray T, Sheedy F, Goedeke L, Liiu X, Khatsenko O, Kaimal V, Lees C, Fernandez-Hernando C, Fisher E, Temel R, Moore KJ. Inhibition of miR-33a/b in non-human primates raises plasma HDL and lowers VLDL triglycerides. Nature 2011; 478: 405-409.