Protocol Title Dyslipidemia and Diabetic Retinopathy

Study Protocol & Statistical Analysis Plan

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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:	Dyslipidemia and Diabetic Retinopathy
Study Description:	Measurement of acid sphingomyelinase (ASM) activity in myeloid angiogenic cells
Objectives:	Determine if myeloid angiogenic cells isolated from diabetics with diabetic retinopathy have higher levels of ASM than age matched controls
Endpoints:	N/A
Study Population:	Diabetics and Age matched controls
Phase:	N/A
Description of Study Intervention:	N/A
Study Duration:	N/A
Subject Duration:	N/A

2 INTRODUCTION

Study Rationale The purpose of this study is to determine if hematopoietic reparative cells are defective in individuals with diabetes. Diabetic retinopathy (DR) is an eye disease related to diabetes. It can cause blurred vision and possible bleeding from the blood vessels in the back of the eye (retina). Damage to the cells of the blood vessels from DR can initiate pathology that then may cause vision loss or blindness. Even with current treatments, the quality of life for people with DR is much reduced.

Overall, this proposal will address the novel concept that the diabetes-induced decreases in DHA, and associated endothelial- specific increase in the central enzyme in sphingolipid metabolism ASM, affects both retinal endothelial cells and bone marrow-derived EPCs. Understanding both mechanisms of damage and repair have the potential to lead to therapeutic strategies that will address the dyslipidemia observed in diabetic retinopathy and may ultimately lead to the prevention of diabetes-induced retinal damage.

3 STUDY DESIGN

3.1 OVERALL DESIGN

Peripheral blood (200 cc) is removed from diabetics with and without diabetic retinopathy. Blood sampling is done in the AM between 8-10 due to circadian variation in bone marrow cell release.

3.2 END OF STUDY DEFINITION

N/A

4 STUDY POPULATION

4.1 INCLUSION CRITERIA

1. **Able to understand and sign an informed consent and HIPAA privacy document.**
2. **18 years of age or older at the time of informed consent**
3. **Able and willing to follow protocol instructions.**
4. **Presence of diabetic retinopathy by history, eye examination or by chart review.**

4.2 EXCLUSION CRITERIA

1. **Current use of tobacco products.**
2. **Use of medication other than prescribed antidiabetic agents.**

3. **Other ocular disease**
4. **Medical history of psychiatric illness.**
5. **Illicit drug use and drug addictions.**

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4.3 SCREEN FAILURES

N/A

4.4 STRATEGIES FOR RECRUITMENT AND RETENTION

Clinic subjects will be recruited.

4.5 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

There is minimal risk with a single blood draw and only infrequently is pain, bruising or infection at the site of the blood draw experienced. Occasionally a patient may feel faint during the blood draw and if this is the case, the patient will remain sitting for approximately 10 minutes until faint feeling dissipates.

5 STATISTICAL CONSIDERATIONS

5.1 STATISTICAL HYPOTHESES AND ANALYSIS

Diabetic individuals with diabetic retinopathy and age and gender matched controls were enrolled and CD34+ cells were isolated, and levels of ASM determined. All analyses were performed in GraphPad Prism v9.1 software. Prior to hypothesis testing, data were assessed for outliers, as indicated by the ROUT method, and outliers excluded from further analyses. Data underwent assessment for adherence to a normal (Gaussian) distribution using the Shapiro-Wilks Normality test. Data which were found to be normally distributed were analyzed by an unpaired Student's t-test (categorical predictor with 2 levels). For all statistical analyses, each input measure was indicative of a single, independent sample from one human subject. All data was analyzed at $\alpha=0.05$.

There have been 4 publications from this study.

Cholesterol crystal formation is a unifying pathogenic mechanism in the development of diabetic retinopathy. Hammer SS, Dorweiler TF, McFarland D, Adu-Agyeiwaah Y, Mast N, El-Darzi N, Fortmann SD, Nooti S, Agrawal DK, Pikuleva IA, Abela GS, Grant MB, Busik JV.Diabetologia. 2023 Sep;66(9):1705-1718. doi: 10.1007/s00125-023-05949-w. Epub 2023 Jun 14. PMID: 37311879

Intravitreal Administration of AAV2-SIRT1 Reverses Diabetic Retinopathy in a Mouse Model of Type 2 Diabetes. Adu-Agyeiwaah Y, Vieira CP, Asare-Bediako B, Li Calzi S, DuPont M, Floyd J, Boye S, Chiodo V, Busik JV, Grant MB. Transl Vis Sci Technol. 2023 Apr 3;12(4):20. doi: 10.1167/tvst.12.4.20.PMID: 37070938.

Lipids, hyperreflective crystalline deposits and diabetic retinopathy: potential systemic and retinal-specific effect of lipid lowering therapies. Jenkins AJ, Grant MB, Busik JV.Diabetologia. 2022 Apr;65(4):587-603. doi: 10.1007/s00125-022-05655-z. Epub 2022 Feb 11. PMID: 35149880