BIOMEDICAL RESEARCH PROTOCOL
UNIVERSITY OF MISSOURI

Project Title: Role of neuraminidase activity on endothelial dysfunction in type 2 diabetes
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Clinicaltrials.gov Number: *NCT04867707*
Study Drug/Study Device: Zanamivir (Relenza, GlaxoSmithKline)

I. Research Objectives/Background

Neuraminidase inhibition as a novel treatment to improve endothelial function in type 2 diabetes (T2D)

The endothelial glycocalyx is a thin layer of interwoven glycoproteins and proteoglycans that forms a luminal mesh separating the vascular wall from the flow of blood. One of the glycocalyx's primary functions is to serve as a mechanosensor of shear stress in flow-mediated dilation (FMD).[78, 79] In this regard, our preliminary data, and published work by others, show that neuraminidase activity decreases the volume of the glycocalyx on the endothelial surface of arteries, and consequently impairs FMD responses. In this research project, we will determine the role that neuraminidase inhibition has on T2D-associated glycocalyx degradation and FMD impairment.

Currently, the only neuraminidase inhibitors approved for use in humans are those devised as influenza treatments. Therefore, they primarily target viral neuraminidases with IC50s in the nanomolar range. However, of the four FDA approved inhibitors, zanamivir has the highest IC50 for mammalian neuraminidases. In our preliminary experiments, we show that neuraminidase inhibition with zanamivir in cultured endothelial cells protects the glycocalyx from degradation.

Moreover, to test the in vivo effectiveness of neuraminidase inhibition with zanamivir on improving endothelial function in diabetes, we treated T2D mice with zanamivir for five days, which is the treatment regimen approved for influenza. Results indicate that treatment with zanamivir reduced plasma neuraminidase activity and improved endothelial function. On the basis of these exciting results, we are taking a fully translational approach and will determine the capacity of a five-day treatment with zanamivir to improve endothelial function in T2D human subjects in a clinical trial.

The **objective** is to determine if neuraminidase inhibition with zanamivir is efficacious as a therapeutic strategy to restore endothelial function in T2D patients.
Twenty subjects will complete five days of treatment with zanamivir. Baseline measurements will be taken before the initiation of treatment, as well as after the

conclusion of the treatment period (*i.e.*, a total of two assessment visits per subject). Assessment visits will include: vitals (such as blood pressure, heart rate), fasting blood work for plasma neuraminidase activity, plasma sialic acid, plasma glucose and plasma insulin, brachial artery FMD, and glycocalyx integrity assessment via Glycocheck.

II. Drugs/Biologics/Devices

Drug treatment, dose, route and regimen:
Subjects will be treated with 10mg zanamivir twice a day for five days (same dose currently FDA-approved for influenza treatment). Zanamivir will be administered via oral inhalation. Dr. Camila Manrique, MD (co-investigator) and Dr. Guido Lastra, MD (safety officer) will oversee the trial.

Rationale for choosing the drug and dose:
As stated above, the proposed dose and administration route of zanamivir for this study are the same as the dose and administration route currently approved by the FDA for the treatment of influenza. Zanamivir was chosen from the four currently approved neuraminidase inhibitors because of its highest IC50 for mammalian neuraminidases.

Risks and safety information
Per Relenza (zanamivir) packaging insert:

- Medication will not be administered in patients with a history of allergic reaction to any ingredients in the medication, including lactose (which contains milk proteins).
- Most common adverse events are: sinusitis, dizziness (in treatment studies) and fever and/or chills, arthralgia, and articular rheumatism (in prophylaxis studies). Zanamivir will not be administered until two weeks following administration of live attenuated influenza vaccine and the live attenuated influenza vaccine should not be given until 48 hours following cessation of zanamivir.
- Zanamivir also carries warnings/precautions for: bronchospasm, allergic reaction, neuropsychiatric events.

IND:
An Investigational New Drug (IND) is not included as part of this protocol because the study medication, zanamivir is being used at an FDA approved dose in a population that is not anticipated to have increased risk or decreased acceptability of risk. Furthermore, individuals who have COPD or asthma will be excluded from the trial as these conditions may increase risk of adverse events. Lastly, no information will be submitted to the FDA to support a labeling indication, a labeling change, or a change in advertising, as this is not the intent of the current study.

III. Recruitment Process

Patients with T2D (n=20) will be recruited from the MU Cosmopolitan Diabetes Center, MU Department of Medicine clinics, and the Columbia community by our research team. MU Health Care's electronic medical record has informatics tools and databases used for developing research studies, including i2b2 (Informatics for Integrating Biology and the Bedside) and RedCap (Research Electronic Data Capture). As in prior studies, with IRB approval, we contact patients and inform them of the potential to participate in this research.

Recruitment:

Subjects will be recruited from the University of Missouri-Columbia as well as the local community by Dr. Manrique and our research team.

1. A chart review of clinic patients from the Division of Endocrinology, the Department of Medicine, and the Department of Medicine, and the Department of Family Medicine who are between the ages of 45 and 64 and have been diagnosed with type 2 diabetes will be completed in Powerchart by study coordinator/staff based on the study inclusion/exclusion criteria. A letter, chart message, or phone call will be utilized to notifying them of their eligibility to participate in a research study. Study coordinator/staff will work in conjunction with healthcare providers that have a direct patient care relationship to recruit subjects from clinics. If patients indicate an interest in the study after they are initially contacted by the study team, they will be sent a follow up email with a Qualtrics screening link and more details about the study.
2. Subjects may respond to the recruitment flyers, word of mouth, or advertisements posted in MU Hospital clinics, MU Hospital television screens, MU Info, or any place generally accessible to the public (i.e., grocery stores, community centers, newspapers, Facebook, etc.). Only recruitment materials approved by IRB will be posted. For all recruitment, subjects will be given an email address and/or a phone number to contact. After email or phone contact is established, subjects will be given a link to complete a screening questionnaire via Qualtrics: (https://missouri.qualtrics.com/jfe/form/SV_2truBrLOYWemj3g).
3. Subjects may also be identified and contacted via Studykik services. (Studykik will be provided with copies of IRB-approved recruitment materials to post)

IV. Consent Process

Preliminary screening

As part of preliminary screening, all individuals who are interested in participating in the research study will be required to complete a screening questionnaire (via Qulatrics or phone) to determine preliminary eligibility before being scheduled for a study screening visit. These questions are for preliminary screening only and are not used as study data. Questions include queries about health history/habits, age, sex, height, weight, tobacco

use, medications/ supplements, over the counter medications use, illness, and chronic conditions. The study design is described in general terms to subjects, with mention of factors most likely to impact subject interest in participating. If the subject is considered to fulfill the inclusion/exclusion criteria, an appointment will be scheduled for a review of medical history and written consent.

Screening and Consenting Visit

For all subjects, informed written consent will be obtained using HIPAA Research Description and Consent Forms approved by the MU IRB. The study physician will be available to answer any potential questions. During that process, the subject will be asked to describe, in their own words, what the study procedures will be like. They will be provided with the study details. Review of the consent form will occur with study personnel in a quiet, unhurried setting. Comprehension will be assessed by asking the subject to explain the study in their own words. Participants will have adequate opportunity to review the informed consent and to ask any questions they may have about the research protocol, compensation, risks, and benefits of taking part in the study. This study visit will also include collection of medical history and payment information and Glycocheck instruction and practice with the participant.

V. Inclusion/Exclusion Criteria

Inclusion:

1. Men and women with a BMI of 25-39 kg/m^2
2. Ages 45-64 years at randomization
3. Diagnosis of T2D based on physician diagnosis
4. No vulnerable populations (*e.g.*, prisoners, pregnant, children) will be enrolled

Exclusion criteria:

1. Cardiovascular disease including myocardial infraction, heart failure, coronary artery disease, stroke
2. History of chronic renal or hepatic disease
3. Active cancer
4. Autoimmune diseases
5. Immunosuppressant therapy
6. Hormone replacement therapy
7. Excessive alcohol consumption (>14 drinks/week for men, >7 drinks/week for women)
8. Current tobacco use
9. Pregnancy (premenopausal women will be required to complete a urine pregnancy test before participation)
10. History of asthma or chronic obstructive pulmonary disease
11. History of allergic reaction to lactose or milk proteins
12. Intranasal live attenuated influenza vaccine (LAIV) given within 2 weeks before zanamivir administration or a planned dose within 48 hours after zanamivir administration. Product insert states to avoid zanamivir administration with intranasal live attenuated influenza vaccine (LAIV).

VI. Number of Subjects

The design for this clinical trial is a 5-day pharmaceutical treatment with pre- and post-assessment.

The clinical trial is powered to detect a 25% effect of zanamivir on FMD. Based on pilot data, this represents approximately one-third of the distance in mean response between healthy and T2D subjects. Our pilot data were also used to estimate the within-subject variance, assuming a correlation of r=0.6 between serial measurements on the same subject (Harris, Padilla *et al.*, 2006). Sample size was estimated using the software package PASS and confirmed using code from Jones & Kenward (2015). A total size of 18 subjects will provide 80% power to detect a 25% difference when testing a two-sided alternative with alpha of 0.05. The final sample size will be increased to 20 to accommodate as much as a 10% drop-out rate. A $p<0.05$ will be considered significant throughout all analyses.

VII. Study Procedures/ Design/Treatment Plan

Assessment Visits

Subjects will complete two separate assessment visits over the course of five days. Pre-menopausal women will be required to complete a urine pregnancy test at both assessment visits, prior to the completion of other study activities. Subjects will undergo brachial artery FMD, Glycocheck, and blood chemistries taken at baseline testing. They will then complete a five-day treatment course with zanamivir. At the end of the treatment course, subjects will have all measurements repeated. Study procedures take approximately 3 hours to complete at each assessment.

Special consideration in case of unforeseeable hardships such as public health emergencies or weather-related events

If subjects are not able to complete study visits, the following remedial steps will be taken:

1. Medication may be mailed to the subject through the investigational pharmacy.
2. In the case that subjects are unable to complete their follow up assessments at the originally scheduled dates, their medication course maybe delayed to better accommodate scheduling availability. Treatment duration will remain the same.
3. In the event of the Clinical Research Center (CRC) not being available or if it is considered by the study physician that performing studies there can be an increased risk of exposure to infectious agents or related hazards, the following alternative sites will be made available for screening, safety and study visits:
    a. MUPAW, Diabetes Center.

These locations are not carpeted, and we do not anticipate an increased risk for the subjects given that medical supervision and nursing staff assistance will be unchanged.

Study procedures:

Brachial artery FMD: Arterial measurements will be performed by imaging the brachial artery longitudinally using high-resolution duplex ultrasonography. Arterial vasodilatory responses to hyperemia (*i.e.*, FMD) will be examined by inflating a forearm cuff up to 250 mmHg for five minutes. Before, during and after rapid release of the cuff, brachial artery blood flow velocity and diameter will be continuously measured. In addition, during the FMD measurements, near-infrared spectroscopy (NIRS) probes will be placed on the forearm; the probe will be secured via an elastic tensor bandage which will be loosely wrapped around the site. These probes will stay attached to the participant for the duration of testing. The FMD procedure will be performed in duplicate.

Glycocheck: The Glycocheck video microscope instrument will be placed under the subject's tongue to measure capillaries, blood vessel density, red blood cell concentration, flow rate, and red blood cell penetration of the glycocalyx lining. Glycocheck is an FDA Class 1 Medical Device. This procedure takes approximately 1 hour to complete.

Study medications

Zanamivir: Used as the study medication. Subjects will inhale 10mg of zanamivir twice daily for five days.

Sources of research material

Sources will include the subject's medical history, physical exam, blood tests, brachial artery FMD, and Glycocheck. Demographic data (plus other related data: emergency contact person, pregnancy status), blood pressure, and anthropometrics (height and weight) will be measured. Each subject will donate up to 40 mL of blood.

Changes to existing therapies

For scientific purposes, it would be best to have patients who are not on medications; however, it would neither be safe nor ethical to completely discontinue medications. Therefore, we will study patients on their existing drug therapy but will limit recruitment to patients on the fewest medications. Common medications in T2D (*e.g.*, insulin, metformin, gliptins, GLP-1 analogs, SGTL2i, antihypertensives, antidepressants, statins) will be considered during recruitment, analyses (*e.g.*, using covariate analysis) and interpretation.

Subject safety

Should subjects exhibit changes in their health status across any system related or unrelated to the study treatment, Dr. Guido Lastra, the safety officer, will be consulted for advice.

VIII. Potential Risks/Adverse Events

The following are potential risks:

Venous blood draw: The potential risks of a venous blood draw include infection, swelling and discomfort at the puncture site. Some bleeding may occur as well after the needle is removed. There is also the possibility of fainting, dizziness, and possible pain and bruising as a result of the puncture. These risks will be greatly minimized by using sterile procedures and having an experienced registered nurse do the blood draw. Risk of bleeding is reduced by applying pressure at the site of puncture. Bruising is treated with ice. Fainting is prevented by drawing blood in the semi-recumbent position.

Brachial artery FMD with NIRS: This is a measurement of endothelial function. There are no risks associated with this procedure. When assessing FMD, the blood pressure cuff will squeeze the arm tightly; however, any discomfort will be alleviated as soon as the pressure in the cuff is released.

Glycocheck: This procedure poses no risks.

Zanamivir: Potential side effects are outlined in package insert. Medication should not be administered to individuals with COPD or asthma.

Protection against risks

Risks to loss of confidentiality are reduced by assigning all subjects a data identifier code. Hard copies of data are stored in a locked office, and only the study personnel have access to the locked files. Individual names or initials are not used in any discussions or publications of the data. We have assembled a research team which includes scientists, physicians, and clinician-scientists with significant experience in human research and cardiometabolic diseases to help anticipate and reduce the risks to subjects.

Plan for reporting study deviations

Any minor problem/deviation will be summarized and reported to the IRB within five working days of awareness, including any event that does not: 1) increase the risk to the subject, 2) decrease the benefit to the subject, or 3) significantly affect integrity of the research data. Any major problem/deviation will be summarized and reported to the IRB within five working days of awareness, including any event that: 1) increases the risk to the subject, or 2) significantly affects integrity of the research data.

Stopping rules

We will stop an individual study if a serious adverse event occurs. If four or more subjects experience a serious adverse event requiring termination of the study, the study will be stopped and the events will be discussed with the IRB to determine whether it is appropriate to continue and/or determine appropriate modifications to the protocol to avoid further adverse events. All unanticipated serious adverse events will be submitted for review according to current protocols. The medication will be discontinued at the discretion of the study safety officer.

Definition of serious adverse event (SAE)

A SAE is classified as any untoward medical occurrence that, at any dose, meets any of the following criteria (a – f):

a. Results in death

b. Is life-threatening

The term 'life-threatening' in the definition refers to an event in which the subject was at risk of death at the time of the event, it does not refer to an event which hypothetically might have caused death if it were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

A hospitalization or prolongation of hospitalization will not be regarded as a serious adverse event if

at least one of the following exceptions is met:

- The admission results in a hospital stay of less than 12 hours
- The admission is pre-planned
- The admission is not associated with an adverse event related to the study medication or study procedures as determined by the study safety officer and data safety monitoring board.

d. Results in persistent or significant disability / incapacity

Disability means a substantial disruption of a person's ability to conduct normal life's functions.

e. Is a congenital anomaly / birth defect

f. Is another serious or important medical event as judged by the investigator.

Breach of confidentiality

Subject confidentiality will be rigorously maintained. The data collected as part of this study will be for research only. It will be de-identified after collection. Confidentiality of data will be assured by coding of unique subject identities and that coding will be known only to the research team, including the use of secure files, locked in an office, and a unique subject coding system. The original study data will be kept in locked in an office (hard copy) or entered into a secure computer database password protected under a secure server space allocated for use by only the study team (electronic). Furthermore, data analysis will be appropriately blinded and any individual data presented in manuscripts will also be presented in an anonymous nature. No identifying information will be disclosed. Confirming with University of Missouri policy, all research records will be retained for a period of seven years following completion of the study.

All protocols and techniques to be used will be approved by the Institutional Review Board (IRB) prior to initiation of any studies. Each subject will give written informed consent after all questions have been answered by a study team member. The consent form will also include a statement guaranteeing confidentiality. Adverse event reports and annual

summaries will not include subject-identifiable material. No information will be given to anyone without permission from the subject. Electronic communication with study team members will involve only coded, unidentifiable information. Any unanticipated breach of confidentiality will be summarized and reported to the IRB within five working days of awareness.

IX. Anticipated Benefits

There may be no benefit to the subjects in this study. These data will aid in the understanding of the efficacy of neuraminidase inhibition as therapeutic strategy for restoring endothelial function in T2D patients as well as determining the efficacy of treating glycocalyx degradation as a means of improving endothelial function in T2D.

X. Compensation

We have completed multiple clinical studies involving human subjects with adherence rates of greater than 95% and subject retention rates of greater than 85%. We utilize a validated retention strategy published by Jeffrey et al (Am J Clin Nutr. 2003;78(4):684-689) that has been successful. With respect to subject honoraria, subjects will be paid $50 after completion of the screening visit, $250 after the baseline visit, and $300 after the final visit. Thus, a total compensation of $600 will be provided as study events are completed.

Visit	Compensation
Screening	$50
Pre-assessment	$ 250
Post-assessment	$ 300

XI. Costs

The subjects will not be charged for any procedures that are part of this research study. The costs of the study will be covered by a federal grant from the NIH.

XII. Data Safety Monitoring Plan

The data safety monitoring plan (DSMP) for this study is focused on the supervision performed by the PIs, the Co-Investigator, Dr. Manrique, and the safety officer, Dr. Guido Lastra. Monitoring will include enrollment, attrition, efficacy end-points, and adverse events.

Plan for data management

A password-protected database will be used to manage all study data. To ensure confidentiality only subject ID numbers will be entered into the database. Signed informed

consent forms are kept in a locked office. Participants will not be individually identified in any publication. Participants' right to privacy will be protected.

Frequency of monitoring, including any plans for interim analysis and stopping rules

Monitoring will include enrollment, attrition, efficacy endpoints, and adverse events. The safety officer will routinely review the safety information related to this project at three-month intervals. In addition to monitoring by the PIs, study physician, study coordinator, and safety officer, the DSMC conducts its reviews on a semiannual basis. The frequency of the structured data review for this study can be summarized in the following table:

Data type	Frequency of review
Subject accrual (adherence to protocol regarding demographics, inclusion/exclusion)	At the end of each recruitment
Adverse event rates (injuries)	semi-annually
Compliance to supplementation	semi-annually
Stopping rules report regarding statistical power implications of dropouts and missing data	semi-annually
IRB review	annually
DSMC review	semi-annually

Plan for adverse event reporting:

Serious adverse events will be reported to the NIH. Unanticipated events will be reported to the IRB, and both serious and non-serious adverse events will be reported to the MU School of Medicine DSMC. For reporting to the DSMC, adverse events will be categorized and classified according to the Common Terminology Criteria for Adverse Events Scale (CTCAE v3.0). Safety reports will be sent to the safety officer. The PIs will be responsible for assembling the data and producing these reports, as well as assuring that all parties obtain copies of these reports.

Qualifications and responsibilities of the safety officer:

Guido Lastra, MD, Associate Professor of Clinical Medicine, Division of Endocrinology, and board-certified endocrinologist will serve as the independent safety officer for this project. Dr. Lastra is an experienced patient-oriented investigator, who is not part of the key personnel involved in this project but is familiar with all the proposed methodologies and their potential risks to human subjects. His expertise is in cardiovascular function and diabetes. As safety officer, Dr. Lastra will review eligibility criteria with the PIs and Dr. Manrique. He will review all reports sent by the PIs to determine whether there is any corrective action, trigger of an ad hoc review, or stopping rule violation that should be communicated to the University of Missouri IRB and the Data and Safety Monitoring Committee. In addition, the safety officer may comment on whether the study investigators need to report any specific out-of-range laboratory data.

MU data and safety monitoring committee (DSMC)

The MU School of Medicine DSMC was formed by the MU Institute for Clinical and Translational Science and activated as an advisory committee within the MU School of

Medicine. Its structure and processes are consistent with NIH guidelines. The DSMC helps investigators, and MU research compliance oversight mechanisms protect human-research participants and ensure the integrity and scientific validity of research data. The DSMC provides education for investigators, research teams, and faculty regarding data and safety monitoring; reviews proposed Data and Safety Monitoring Plans; establishes Data and Safety Monitoring Boards; conducts independent, interim reviews of study safety and progress; and makes recommendations concerning the continuation of studies, including recommendations regarding the modification, suspension, or termination of a study.

XIV. References/Appendices

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