HRP-592 - Protocol for Human Subject Research with Use of Test Article(s)

Protocol Title:
Pioglitazone Treatment for Hyperglycemic Acute Ischemic Stroke: Effects on the Stress-Immune Response

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1.0 Objectives

1.1 Study Objectives

The long term objective of this study is to determine whether Pioglitazone (PGZ) can improve clinical outcomes in hyperglycemic acute ischemic stroke (IS). We propose to study the efficacy of PGZ treatment and its effects on the peripheral immune-stress response in hyperglycemic stroke patients within a randomized, placebo controlled, double-blinded clinical pilot study with two objectives:

1. **Study the effects of acute PGZ treatment on clinical outcomes at three months after stroke:** Well validated assessment tools of neurological function, including the NIH Stroke Scale (NIHSS), modified Rankin scale (mRS), Barthel index (BI) and Glasgow Outcome Scale (GOS) will be used to assess neurological outcomes. In addition, cognitive function will be assessed by the Montreal Cognitive Assessment (MOCA), and data on in-hospital and after discharge infection rates, re-hospitalization rates, stroke volume and all-cause mortality will be collected.

2. **Explore whether acute treatment with PGZ can improve outcomes in hyperglycemic stroke by modulation of the stress-immune response.** We will measure how PGZ changes blood concentrations of insulin, glucose, cortisol and catecholamines after initiation of treatment. Based on our preliminary preclinical studies, we hypothesize that PGZ will decrease circulating levels of activated neutrophils and monocytes, improve neutrophil function, decrease cytokine levels, normalize the N/L, ratio and increase the anti-inflammatory annexin A-1 pathway, all of which are altered in the hyperglycemic state.

1.2 Primary Study Endpoints

The primary objectives of this pilot study are 1) to determine whether PGZ can improve outcomes at 3 months post-stroke in hyperglycemic acute ischemic stroke, and 2) to study whether there is association between immune-stress response modulation and clinical outcomes after treatment of PGZ following acute ischemic stroke in hyperglycemic patients.

1.3 Secondary Study Endpoints

1) To examine the impact of PGZ on the stress-immune response by measuring cortisol, epinephrine, norepinephrine levels and inflammatory mediators in hyperglycemic ischemic stroke patients.
2) To study the correlation between clinical outcomes and pro and anti-inflammatory mediators after acute treatment of PGZ in hyperglycemic patients.
3) To study the effects of PGZ on stroke volume, cerebral edema and hemorrhagic conversion measured on brain magnetic resonance imaging (MRI) and /or head computed tomography (CT).

2.0 Background

2.1 Scientific Background and Gaps

The global burden of stroke and diabetes is substantial and steadily growing. Stroke-related health care expenditures are projected to increase from $17.5 billion in 2000 to $30.3 in 2030 [1]. Stroke is the second leading cause of death in the diabetic population and the most common cause of chronic disability. A review of our institutional stroke database revealed that about 50% of all acute stroke patients have diabetes or hyperglycemia who have a glucose level of ≥150mg/dl which corroborates observations from previous studies [2]. This high prevalence has far ranging implications for acute stroke care, as patients with diabetes and hyperglycemia have worse clinical outcome, higher mortality rates, more severe disability, larger infarct size, and greater risk for recurrent stroke [3]. The confluence of these effects will result in a dramatic increase in the annual stroke frequency, currently standing at 5.8 million in the U.S., within the next 25 years [4].

In humans and in animals, IS causes an acute stress-immune response through activation of the sympathetic nervous system and the hypothalamic-pituitary axis leading to the release of cortisol and catecholamines [5-7]. Elevated cortisol and catecholamine levels deplete circulating lymphocytes, induce leukocytosis and activate neutrophils which then migrate into the site of brain injury, where they release mediators of inflammation and cause tissue damage. They also undergo apoptosis and become cleared by phagocytosis, which is important for the initiation of tissue repair [8, 9]. Cortisol can have opposing effects on neutrophil apoptosis and subsequent tissue fate. It can induce the release of annexin A1 from neutrophils, which will then bind to their surface receptors, causing neutrophil apoptosis and inhibition of neutrophil extravasation into the infarcted area, downregulating the extent of inflammation [10]. On the other hand, cortisol can increase anti-apoptotic factors such as Mcl-1 and decrease pro-apoptotic molecules, leading to prolonged neutrophil survival and upregulation of the inflammatory response [11, 12].

By reducing circulating lymphocytes, suppressing the T-cell response and inhibiting monocytes, eosinophils and various other components of the innate and adaptive immune response, cortisol and epinephrine also render the host more susceptible to infection, contributing to complications and increased stroke morbidity and mortality [13, 14]. Previous studies have shown that an increase in the peripheral neutrophil to lymphocyte (N/L ratio) after an acute IS is correlated with worse clinical outcome [15, 16].

2.2 Previous Data

Our preliminary data from 24 acute IS patients showed that hyperglycemic (glucose level ≥150mg/dl) stroke patients had significantly higher cortisol levels and more pronounced and earlier peripheral neutrophil activation than their euglycemic counterparts, suggesting an alteration of the post-stroke stress-immune response in the hyperglycemic state. Similarly, in our preclinical studies of diabetic db/db mice, the extent of brain damage after IS was significantly worse in diabetic compared to euglycemic animals [13]. In our preclinical and clinical pilot studies, we observed that the peripheral N/L-ratio was higher in hyperglycemic compared to euglycemic stroke patients and in diabetic compared to non-diabetic mice. In stroke patients, an increased N/L ratio was associated with higher rates of malignant cerebral edema, worse neurological function and greater neurological disability, irrespective of i.v. t-PA use. In addition, hyperglycemic patients showed a more pronounced peripheral lymphocyte suppression for B- and T-lymphocytes, including T-helper, T-suppressor and natural killer cells.

We have previously shown the importance of neutrophils for stroke outcomes where neutrophil depletion prior to induction of IS in db/db mice resulted in significantly smaller stroke volumes. Thus, an intervention aiming at restoration of the altered stroke-induced immune response is a promising approach to improve outcome in hyperglycemic stroke. We have demonstrated that darglitazone, that a a similar drug within the same class and similar mechanism of action, restores the altered pro-inflammatory response and dramatically reduces stroke size in diabetic ob/ob mice when administered before IS was induced [13]. In addition, PGZ given to non-diabetic C57BL6 mice within 2h after acute IS, also reduced the stroke size and the inflammatory response at the site of brain injury. After administration of a single dose of 30/mg/kg (which is equivalent to approximately 150 mg dose in humans) of PGZ immediately after IS, we observed a significant decrease in circulating neutrophils and an increase in lymphocytes at 4h and 24 h compared to baseline, and smaller stroke volumes in PGZ versus placebo (saline) injection-treated mice. Furthermore, we found a decrease in astrocyte and microglia activation following PGZ treatment, suggesting a reduced central inflammatory response. In summary, our preliminary results suggest that the post-stroke stress-immune response is altered in diabetes and that PGZ may improve clinical outcome by modulating this response.

2.3 Study Rationale

The rationale for the proposed research is to develop an acute intervention that can improve neurological recovery and decrease mortality and morbidity in hyperglycemic stroke patients who are a high risk group for poor outcome. Understanding how the immune-stress response is altered in hyperglycemic stroke and how PGZ may modulate this response is an entirely novel undertaking. If PGZ shows a trend towards efficacy and the study design proves feasible, we aim to conduct a multi-center trial of PGZ in acute hyperglycemic stroke patients.

3.0 Inclusion and Exclusion Criteria

3.1 Inclusion Criteria

To be eligible to participate in this study, candidates must meet the following eligibility criteria at screening:

1. Aged 21 or older
2. Diagnosis of acute IS
3. Must present within 18 hours of stroke symptom onset or within 18 hours of their last known time being well.
4. Blood glucose level greater than 150 mg/dl at the time of presentation.
5. Brain MRI or head CT proven ischemic stroke or clinical presentation suggestive of IS.
6. Initial NIH SS of ≥ 2.

3.2 Exclusion Criteria

Patients will be excluded from study entry if any of the following exclusion criteria exist at screening:

1. Known hypersensitivity to PGZ.
2. Infection at the time of presentation as defined by body temperature > 38^{o}C , pneumonia evident on chest X-ray, urinary tract infection (positive tests for nitrites, leukocyte esterase, and bacteria on urine analysis), other acute infection per history or current use of antibiotic or antiviral treatment.
3. Active malignancy and / or autoimmune disease requiring treatment.
4. Use of immunomodulatory drugs or chemotherapy.
5. History of stroke or brain injury within the last 90 days prior to presentation.
6. Acute illness within the last 30 days which could have affected the white blood cell count.
7. Known history of clinically significant hypoglycemia.
8. Patients already taking PGZ.
9. Active liver disease (ALT and /or AST 2.5 times the upper limit of normal, total bilirubin > 1.2 mg/dl).
10. Acute decompensated heart failure, and/or admission for an acute coronary syndrome, myocardial infarction (MI), cardiac arrest, coronary artery surgery within the past 3 months and patients with New York Heart association Class III and IV heart failure.
11. History of bladder cancer
12. Pregnant and nursing women.
13. Currently incarcerated patients.

3.3 Early Withdrawal of Subjects

3.3. 1 Criteria for removal from study

Patients with a need for treatment with immunomodulatory drugs or those who develop any new conditions described in the exclusion criteria during treatment phase of clinical trial will be removed from the study. Additionally, cognitively impaired subjects will be withdrawn if they are unduly distressed as required by IRB policies.

3.3. 2 Follow-up for withdrawn subjects

Subjects will be withdrawn from the study if the study subject or Legally Authorized Representative (LAR) withdraws consent for any reason. The primary reason for the subjects' withdrawal from the study will be recorded.

If a subject is withdrawn due to pregnancy, experiences an adverse event (AE), a medical emergency, or is unwilling or unable to comply with the protocol, he/she must undergo a 90 day assessment unless the withdrawal is due to death or the withdrawal of consent.
Subjects who withdraw from the study may be replaced.

4.0 Recruitment Methods

4.1 Identification of subjects

Patients who present to the Emergency Department (ED) or who are already hospitalized at Hershey Medical Center (HMC) will be identified via the "brain attack" alert system. This system is updated in real-time when patients are having an acute IS in the ED or on the inpatient services of hospital. All stroke attending team physicians, residents, stroke fellows, the clinical stroke team and the stroke research coordinators are paged. All study personnel for this study have access to the brain attack alert system and will be notified. Upon presentation, all stroke patients have a neurological examination and are assessed using the NIHSS and the mRS as part of their routine care.

4.2 Recruitment process

4.2. 1 How potential subjects will be recruited.

Research team members will review the electronic medical record (EMR) for each patient arriving in the ED or presenting on the inpatient services at HMC with acute stroke-like symptoms for their eligibility to participate in this study.

4.2. 2 Where potential subjects will be recruited.

Potential subjects will be recruited in the Emergency Department (ED), inpatient or by telephone if the subject is found to be not capable of consenting for him or herself, and LAR is not present..

4.2. 3 When potential subjects will be recruited.

Potential subjects will be recruited prior to any study procedures taking place.

4.2. 4 Describe the eligibility screening process and indicate whether the screening process will occur before or after obtaining informed consent. Screening begins when the investigator obtains information about or from a prospective participant in order to determine their eligibility. In some studies, these procedures may not take place unless HIPAA Authorization is obtained OR a waiver of HIPAA Authorization when applicable for the screening procedures is approved by the IRB.

The EMR will be reviewed for inclusion/exclusion criteria prior to approaching a potential subject and/or subject's LAR.

5.0 Consent Process and Documentation

5.1 Consent Process:

Check all applicable boxes below:

☒ **Informed consent will be sought and documented with a written consent form *[Complete Sections 5.2 and 5.6]***

☐ **Implied or verbal consent will be obtained – subjects will not sign a consent form (waiver of written documentation of consent) *[Complete Sections 5.2, 5.3 and 5.6]***

☐ **Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception). *[Complete section 5.2, 5.4 and 5.6]***

☐ **Informed consent will not be obtained – request to completely waive the informed consent requirement. *[Complete Section 5.5]***

5.2 Obtaining Informed Consent

5.2. 1 Timing and Location of Consent

An approved study team member will meet with the subject and/or their LAR in a quiet private area. After careful and complete explanation of the study details, risks, and other options is provided to the subject and/or their LAR, a copy of the consent form will be provided to them for review. If they are interested in consenting to this study, they will be given additional time to ask questions. When all questions have be answered to their satisfaction by a study team member, the subject or their LAR will be asked to sign the informed consent document. A note will also be documented in the patient's medical record describing the process and discussion.

Telephone Consent Process

The physician/resident/ study coordinator will go to talk to potential subject and/ or family to determine if they are able to consent. If it is found that the potential subject cannot consent and their LAR is not present, an attempt to reach the LAR will be made to discuss the potential study enrollment. Whenever possible the potential subject will be present in the room while discussing the study over the phone with his/her LAR.

If there is interest by the potential subject's LAR we will continue to obtain information (email address, telephone or fax number, if available) so a copy of the consent and any other information can be sent to them.

The potential subject's representative (LAR) will be provided/sent a copy of the consent form and any other required information about the study, via email telephone, or fax, as requested by the LAR.

The physician and the study coordinator will conduct the telephone consent discussion with the subject's representative. This study involves greater than minimal risk, so we will have a witness present with the study team member during the telephone consent discussion. A review of the consent will be completed. All questions and concerns will be answered prior to the end of the discussion. Time will be given for the subject and/or subject's representative to review and discuss with other family members, if needed, prior to completion of the consent process.

The subject's representative will be asked if he/she would like to participate. If yes, the subject's representative will be asked to sign, date, time, and print their name on the consent form.

The subject's representative will be asked to return the signed consent form to the research site via email, phone text message with an attached picture of the signed consent page, or fax. If in route to the hospital, we will ask for the original signed document, as well. In order to return the signed document a telephone number, or email address, or fax number will be given to the subject's representative. A reminder of possible loss of

confidentiality may occur with email, telephone or fax, but we will be careful to give and review the telephone or fax number, and/or email address given to the subject and/or their representative.

We will begin study procedures once the signed copy of the consent form is received from the subject's representative via email, test message with photo attachment of signed consent, or fax.

The team member obtaining informed consent will complete and sign a Signature Page for Telephone Consent Process, which was approved by the IRB.

A complete copy of the signed consent form and the signed Signature Page for Telephone Consent Process will be given to the subject's representative. The signed copies may be given to the subject's representative in-person or sent to the subject's representative by email, mail or fax, depending on how the subject's representative requests the signed documents to be sent to them

The phone consent procedure should be included in the note to file used to document the informed consent process in the research record or medical record. A copy of the signed consent form will be placed in the medical record and given to the subject.

All subjects will be required to assent when able to sign an informed consent form. The same consenting process will be completed with the subject – the discussion will be maintained in a private setting, he/she will be given time to review and discuss the research study and consent with his/her family. Questions will be answered and LAR will be present during the discussion.

5.2. 2 Coercion or Undue Influence during Consent

The study will first be introduced to subjects by one of the clinical investigators. The study coordinator or other member of the study team who is not directly involved in the clinical care of the patient will meet with the subject for a detailed discussion of the study and to complete the consent process.

Study team members will stress that participation is completely voluntary. Additionally, it will be carefully and clearly explained that if they should decide not to participate in this study, that decision will have no impact on the care the patient will receive.

5.3 Waiver of Written Documentation of Consent

Not applicable

5.3. 1 Indicate which of the following conditions applies to this research:

☐ The research presents no more that minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

OR

☐ The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern.

OR

☐ If the subjects or legally authorized representatives are members of a distinct cultural group or community in which signing forms is not the norm, that the research presents no more than minimal risk of harm to subjects and provided there is an appropriate alternative mechanism for documenting that informed consent was obtained.

Describe the alternative mechanism for documenting that informed consent was obtained:

5.3. 2 Indicate what materials, if any, will be used to inform potential subjects about the research (e.g., a letter accompanying a questionnaire, verbal script, implied consent form, or summary explanation of the research)

5.4 Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception).

Not applicable

5.4. 1 Indicate the elements of informed consent to be omitted or altered
5.4. 2 Indicate why the research could not practicably be carried out without the omission or alteration of consent elements
5.4. 3 Describe why the research involves no more than minimal risk to subjects.
5.4. 4 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects.
5.4. 5 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.
5.4. 6 Debriefing

5.5 Informed consent will not be obtained – request to completely waive the informed consent requirement

Not applicable

5.5. 1 Indicate why the research could not practicably be carried out without the waiver of consent
5.5. 2 Describe why the research involves no more than minimal risk to subjects.
5.5. 3 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects.
5.5. 4 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.
5.5. 5 Additional pertinent information after participation

5.6 Consent – Other Considerations

5.6. 1 Non-English-Speaking Subjects

At this time, we do not anticipate enrolling any non-English speaking subject. However, if we do, an approved short form or oral translation process will be used to obtain informed consent.

5.6. 2 Cognitively Impaired Adults

5.6.2.1 Capability of Providing Consent

All stroke patients undergo a detailed neurological examination by the treating physician team which includes an assessment of their capacity to give informed consent.

In addition potential study subjects will be evaluated by a study team member for any cognitive or language impairment or other physical limitations that does prevent him/her from making a reasoned, informed decision. All members of the research team have prior experiences in participating in acute stroke research.

5.6.2.2 Adults Unable to Consent

This study involves individuals who have an ischemic stroke. Due to the type of the insult, these individuals may be compromised and unable to communicate. If the subject is unable to provide consent, the research team will obtain informed consent from an (LAR). The LAR will be defined as a court-appointed legal guardian, a health care power of attorney or a health care representative.

5.6.2.3 Assent of Adults Unable to Consent

All subjects who are physically able to provide assent will be asked if unable to sign an informed consent form. The same consenting process will be completed with the subject – the discussion will be maintained in a private setting, he/she will be given time to review and discuss the research study and consent with his/her family. Questions will be answered and an impartial individual or LAR will be present during the discussion.

5.6.3.1 Parental Permission

5.6.3.2 Assent of subjects who are not yet adults

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

☐ **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.** *[Mark all parts of sections 6.2 and 6.3 as not applicable]*

☒ **Authorization will be obtained and documented as part of the consent process.** *[If this is the only box checked, mark sections 6.2 and 6.3 as not applicable]*

☒ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).** *[Complete all parts of sections 6.2 and 6.3]*

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

6.2. 1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2 .1.1 Plan to protect PHI from improper use or disclosure

Information is included in the "Confidentiality, Privacy and Data Management" section of this protocol.

6.2 .1.2 Plan to destroy identifiers or a justification for retaining identifiers

Minimal screening activities prior to consent may include accessing patient charts to confirm eligibility. No identifiers will be retained for any patient who does not meet eligibility requirements or whose LAR does not provide consent to participate in the study.

6.2. 2 Explanation for why the research could not practicably be conducted without access to and use of PHI

The study requires the screening of patients identified through the brain attacks call system to evaluate them for potential participation in this study. This prescreen takes place prior to study enrollment. Depending on their diagnosis, they may be able to be enrolled in the study once a quick review of their chart is completed, in order to permit a review of inclusion/exclusion criteria. A partial waiver is needed to recruit the patients that meet criteria for the study.

6.2. 3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization

There is a short (18 hour) window in which patients are eligible for enrollment and the waiver will allow the study staff and/or coordinator to review the chart as soon as possible, in order to assist in the recruitment process.

6.3 Waiver or alteration of authorization statements of agreement

Protected health information obtained as part of this research will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other permitted uses and disclosures according to federal regulations.

The research team will collect only information essential to the study and in accord with the 'Minimum Necessary' standard (information reasonably necessary to accomplish the objectives of the research) per federal regulations.

Access to the information will be limited, to the greatest extent possible, within the research team. All disclosures or releases of identifiable information granted under this waiver will be accounted for and documented.

7.0 Study Design and Procedures

7.1 Study Design

This double-blinded, randomized, placebo-controlled pilot study will evaluate the effects of acute PGZ treatment on clinical outcomes and markers of the stress-immune response in hyperglycemic patients (blood glucose ≥ 150 mg/dl at the time of presentation) who present with acute IS symptoms within 18 hours of symptom onset or their last known time being well. In addition feasibility and safety of acute PGZ treatment in stroke patients with hyperglycemia will be established.

Study enrollment will not delay administration of standard of care stroke therapies, such as thrombolysis and/or thrombectomy. If patients undergo a thrombectomy prior to study enrollment, the blood sugar level will be rechecked after the procedure to ensure that patients still meet the criteria to participate in this study (blood glucose ≥ 150 mg/dl).

To study the effects of PGZ on the immune system, we will collect blood from all study participants before initiation of study drug and 24 hours after each dose of study drugs. The following will be performed:

- Measurement of immune cells: Absolute counts of neutrophil, monocytes and lymphocytes will be measured by performing differential counts on blood smears of blood samples from study participants and by flow cytometry.
- Measurement of activated neutrophils and monocytes: We will measure the neutrophil and monocyte activation activated cell surface markers by flow cytometer and data will be analyzed using FlowJo software Tree Star, Inc.

- Neutrophil function: Neutrophil function will be measured by phagocytosis assay and oxidative burst using flow cytometry.
- Annexix A1, apoptotic and anti-apototic markers: Apoptotic marker caspase 3-PE, anti-apoptotic factor MCl-1 FITC and Annexin A1-APC will be measured by flow cytometry.
- Cytokine assay: Pro-inflammatory circulating cytokines (TNFα, IL-1α, IL-1β, IL-2, IL-6, IL-10 which are known to be up-regulated after ischemic stroke will be measured by Bio Plex multiplex immunoassays.
- Serial measurements of Insulin and Glucose levels, cortisol, catecholamines ESR and hsCRP will be performed.
- An additional blood sample will be stored for future biomarker analysis.

7.2 Study Procedures

7.2. 1 Baseline

Baseline activity will occur within 18 hours of symptom onset (or last known well time) and will include screening, enrollment, randomization, medical history, physical and neurological examinations, clinical assessments (including the NIHSS, mRS, BI, GCS and MOCA), collection of blood biomarkers and liver function tests.

1. **Screening and Enrollment:** The study will enroll participants over one year. Each patient in the ED or on the inpatient service who presents with acute stroke-like symptoms and/or suspected cerebral infarction within 18 hours of symptom onset or their last known time being well, will be identified and screened for the study. The time of symptom onset will be determined based on discussion between the clinical care team and the patient, the patient's care givers and the treating physicians. If the stroke onset time is not known, such as when a patient awakens with stroke symptoms, the last known time when the patient was well will be defined as onset time. Acute ischemic stroke (AIS) will be diagnosed based on non-contrast head CT, perfusion head CT and/or brain MRI or based on neurological assessment, all of which is part of the routine assessments by the clinical physician team. The severity of stroke will be determined by the NIHSS and mRS, which are completed by the treating physicians at time of presentation.

   Prior to performing any study-related activities, written informed consent will be obtained from the subject or the subject's LAR. The background of the proposed study, all study procedures, the benefits and risks of the study, and that study participation is voluntary for the subject will be explained to the subject or the subject's LAR, who will be given sufficient time to consider whether to participate in the study.

   After obtaining written consent, the subject's data will be collected from the medical record for research purposes.

2. **Randomization:** After obtaining written consent, subjects will be randomized to either the PGZ or placebo treatment arm of the study using an equal allocation ratio (1:1). See section 7.4.3.

3. **Pretreatment assessments:** Prior to the administration of the investigational product, the following will be done.

   The following standard of care data will be **collected from the EMR for research purposes:**
   - Demographic information (age, gender, race, contact information)

- Medical history
- Admission diagnoses
- Time of symptom onset or last known well time
- Current medication usage
- Results from the standard care laboratory workup including complete blood count, coagulation studies, basic metabolic panel (including serum glucose, renal function, and electrolytes), and if applicable results of pregnancy test and urinary drug screen)
- Health care services rendered (diagnostic workup procedures, use of t-PA, and thrombectomy, use of antibiotics, treatment of cerebral edema and hemorrhagic conversion)
- Images and results of the interpretation of the neuroimaging studies (brain MRI and head CT
- NIHSS and mRS scale

The following assessments will be **performed only for research**:

- BI, GOS will assess neurological outcomes and will be performed by certified study personnel.
- MOCA will assess cognitive function and will be performed by certified study personnel.
- Blood will be collected (approximately 15 mls.) for the following tests:
  - ESR, hsCRP, CBC with differential, blood glucose, insulin, cortisol and catecholamines
  - T Cells, B cells, activated myelocytes
  - Inflammatory markers, cytokines, apoptotic markers
  - Other biomarkers.
  - AST, ALT and total bilirubin

4. **Administration of the Investigational Product:** Study treatment administration will be initiated within 18 hours after the onset of stroke symptoms or last known well time. Liver function tests (serum ALT, AST, and total bilirubin) will be assessed prior to initiating study drug treatment. The treatment group will receive a 45 mg Pioglitazone capsule orally every 24 hours for 3 days for a total of 3 doses. The control group will receive placebo at the same time points. Both groups will be treated identically and receive best standard of care.

   If subject will be unable to swallow the study drug, will be administered via feeding tube (See study drug detailed in sections 7.4.2 and 7.4.5).

   In addition, for research purposes finger stick glucose measurements will be performed 2h after initial study drug administration (time of peak of PGZ concentration) and subsequently at regular time points as determined by the standard of care protocol for stroke patients (see attached standard of care protocols for hypoglycemia and hyperglycemia for acute stroke patients, Appendix 1).

7.2. 2 Day 1

This will occur 24 (+/- 2) hours after the initial dose of the investigational product is administered. At this time, the following **research-only activities** will occur:

- AEs and concomitant medications will be documented.
- Prior to administration of the investigational product, blood will be collected (approximately 10 mls) for the following tests: ESR, hsCRP, CBC with differential, blood

glucose, insulin, cortisol and catecholamines, T Cells, B cells, activated myelocytes, inflammatory markers, cytokines, apoptotic markers, other biomarkers.
- The second dose of investigational product will be administered.

7.2. 3 Day 2

This will occur 24 (+/- 2) hours after the second dose of investigational product is administered. At this time, the following **research-only activities** will occur:

- AEs and concomitant medications will be documented.
- Prior to administration of the investigational product, blood will be collected (approximately 10 mls) for the following tests: ESR, hsCRP, CBC with differential, blood glucose, insulin, cortisol and catecholamines, T Cells, B cells, activated myelocytes, inflammatory markers, cytokines, apoptotic markers, other biomarkers.
- Prior to administration of the investigational product, blood will be collected (approximately 5 mls) and sent for liver function testing (AST, ALT and total bilirubin). Liver function test results will be confirmed prior to the administration of the investigational product.
- The third dose of investigational product will be administered.

7.2. 4 Day 3

This will occur 24 (+/- 2) hours after the third dose of investigational product is administered. This may occur at the discharge visit, if that occurs prior to the scheduled day 3 visit. At this time, the following **research-only activities** will occur:

- AEs and concomitant medications will be documented.
- Blood collection (approximately 10 mls) for the following tests: ESR, hsCRP, CBC with differential, blood glucose, insulin, cortisol and catecholamines, T Cells, B cells, activated myelocytes, inflammatory markers, cytokines, apoptotic markers, other biomarkers.

7.2. 5 Discharge

This will occur on the day of discharge from the hospital. A standard of care routine physical and neurological examination will be conducted. The following assessments will be performed;

- NIHSS and mRS scale
- BI, GOS (for research only)
- MOCA (for research only)
- AEs and concomitant medications will be documented for **research-only**.

7.2. 6 Day 90 Visit

This visit will occur 90 days (+/- 30 days) from the day of discharge. A standard of care routine physical and neurological examination will be conducted. If the patient is unable to travel to the clinic, the following assessments may be done via phone call:

- NIHSS and mRS scale
- BI, GOS (for research only)
- MOCA (for research only)
- AEs and concomitant medications will be documented for **research-only**.

Schedule of Study Activities

	Baseline (0-18h from symptom onset)	**Day 1:** 24+/-2h after first dose of study drug/ placebo 24 hrs	**Day 2:** 24+/-2h after second dose of study drug/ placebo	**Day 3:** 24+/-2h after third dose of study drug/ placebo	**Discharge**	**Day 90:** ± 30 days follow-up visit

Screening/Enrollment	X					
Informed consent	X					
Administration of study drug or placebo	X	X	X			
Clinical scales: NIHSS, mRS, BI, GOS, MOCA	X				X	X
Blood for laboratory tests and biomarkers	X (pre-treatment)	X (pre-treatment)	X (pre-treatment)	X		
Liver function test	X (pre-treatment)		X (pre-treatment)			
Medical history	X (pre-treatment)					
Physical and neurological examination	X (pre-treatment)				X	X
AEs and concomitant Medications		X	X	X	X	X
Blood glucose assessment via finger stick	X*					

BI = Barthel Index; GOS = Glasgow Outcome Scale; MOCA = Montreal Cognitive Assessment: mRS = modified Rankin Scale; NIHSS = NIH Stroke Scale

*finger stick glucose measurements will be performed 2h after initial study drug administration (time of peak of PGZ concentration) and subsequently at regular time points as determined by the standard of care protocol for stroke patients (see attached standard of care protocols for hypoglycemia and hyperglycemia for acute stroke patients, Appendix 1).

7.3 Duration of Participation

The total duration of study participation for each subject will be approximately three months, which consists of:

- Screening and enrollment period (within 18 hours of stroke symptom onset)
- Treatment period of 72 hours (3 days)
- A 90 day follow-up visit occurring during the standard of care outpatient visit to the neurology clinic.

7.4 Test Article(s) (Study Drug(s) and/or Study Device(s))

7.4. 1 Description

Pioglitazone hydrochloride 45mg tablets will be overencapsulated to match placebo capsules for this study.

Pioglitazone, a peroxisome proliferator-activated (PPARγ) receptor agonist, is FDA-approved as an anti-glycemic agent for the treatment of type-2 diabetes that has shown promise for secondary stroke prevention in clinical stroke trials [3-5], but has thus far not been studied as an acute intervention for acute diabetic stroke. PGZ acts primarily by decreasing insulin resistance.

7.4. 2 Treatment Regimen

Following oral administration, PGZ is first measurable in serum within 30 minutes, with peak concentrations observed within 2 hours. It is relatively insoluble but can be crushed and given through a feeding tube. Serum concentrations of total PGZ (PGZ plus active metabolites) remain elevated 24 hours after a once daily dosing.

The treatment group will receive 45 mg of PGZ orally within 18 hours of stroke symptom onset or last known well and subsequently at 24h and 48h after the initial dose. This represents the highest recommend dose in humans and is selected to achieve the highest blood concentration of PGZ as quickly as possible following the stroke event. The control group will receive placebo orally at the same time points. Both groups will be treated identically and receive best standard of care.

Some acute ischemic stroke patients experience trouble swallowing (dysphagia), and all stroke patients are therefore routinely assessed for dysphagia as part of their routine clinical care. If patients are not able swallow, PGZ/placebo will be crushed and administered via a feeding tube.

To avoid unblinding of the study team if the capsules have to be opened/crushed for administration, a warning label will be place on study drug package telling the bedside nurse not to reveal the contents of the capsule to study team members. Study team members will not administer drug to any patients who are unable to swallow.

There is no provision to provide study treatment after the study.

7.4. 3 Method for Assigning Subject to Treatment Groups

Participants will be randomized to either PGZ or placebo using an equal allocation ratio (1:1). The randomization will be performed using a random number generator via the PLAN procedure within SAS software, version 9.4 (SAS Institute Inc., Cary, NC). Personnel in the Department of Public Health Sciences (PHS) will use the PLAN procedure within SAS software to generate a randomization list using variable-size, random permuted blocks to ensure that the number of participants in each arm is balanced after each set of B randomized participants, where B is the block size. Personnel in PHS will choose the block sizes without revealing it to any of the investigators or study personnel who will be collecting and reviewing outcomes data. The PHS generated randomization list will be provided to Investigational Drug Services (IDS) who will be implementing the randomization for this trial.

7.4. 4 Subject Compliance Monitoring

Nursing staff will chart administration of study drug into the patient's EMR and study personnel will monitor and document the completion of all study procedures.

7.4. 5 Blinding of the Test Article

The investigators, study staff, subjects, and caregivers will be blinded to the subject's treatment assignments for the duration of the trial. Investigational pharmacy will be placed the investigational product into a plain capsule to insure that the investigative team remains blinded (see Section 7.4.6.3). To avoid unblinding of the study team members, if the capsules have to be

opened/crushed for administration by nursing staff, a warning label will be placed on study drug package telling the bedside nurse not to reveal the contents of the capsule to any study team member. Study team members will not administer any study drug.

All blood samples will be coded to maintain masking prior to analysis. The laboratory staff will be unaware of the condition and the treatment of the participants for whom they will conduct the assays as the samples will be identified only with a study number.

The results of the laboratory measurements will not be shared with the treating physicians, and will not be impact patient care.

7.4. 6 Receiving, Storage, Dispensing and Return

7.4 .6.1 Receipt of Test Article

The IDS pharmacy will obtain commercially available Pioglitazone 45mg tablets from a pharmacy wholesaler, and pharmaceutical grade empty gelatin capsules and USP grade methylcellulose from an appropriate pharmacy supplier. IDS pharmacy will then compound the active pioglitazone 45mg and matching placebo capsules.

7.4 .6.2 Storage

The study drugs will be stored at controlled room temperature in the pharmacy department with the inpatient IDS study medications.

7.4 .6.3 Preparation and Dispensing

IDS pharmacy will compound the active capsules as follows: the 45mg pioglitazone tablets will be split and placed in an empty gelatin capsule with filler (USP grade methylcellulose). The placebo capsules will be compounded by placing USP grade methylcellulose in an empty gelatin capsule. IDS pharmacy will maintain compounding records for each batch of active and placebo capsules compounded and drug accountability logs for all study medications prepared and dispensed. The compounded capsules will be marked with an appropriate expiration date and expired capsules will be removed from study inventory and destroyed as per the IDS Destruction Policy.

Study treatment capsules (PGZ or placebo) will look identical.

Once a subject has consented and enrolled in the study, confirmation of consent and a study order will be sent to the pharmacy. Pharmacy will then randomize the subject and dispense the 3-day supply of the study medication to the study team for delivery to the subject's nurse. The study medication will be labeled in a blinded manner.

Once the study drug is prepared for the subject, it can only be administered to that subject by the patient's bedside nurse.

7.4 .6.4 Return or Destruction of the Test Article

Once the study drug is prepared for the subject, it can only be administered to that subject. If a subject is withdrawn from the study (See section 3.3), the study drug will be returned to IDS and it will be destroyed according to the IDS drug destruction policy.

7.4 .6.5 Prior and Concomitant Therapy

A concomitant therapy is any medication which is administered between study enrollment and the end of the study drug treatment phase. All concomitant therapies will be monitored daily and recorded by study team members during the duration of the treatment phase with the study drug.

Precautions: PGZ is metabolized in the liver. Therefore, any drug which is a strong CYP2C8 inhibitor or inducer may affect the efficacy of PGZ. Subjects who require these medications during study drug treatment are permitted into the study with additional monitoring/precautions in place:

1. Strong CYP2C8 inhibitors (such as gemfibrozil) increase PGZ concentrations. Therefore, gemfibrozil will be held for the 3 day duration of the study treatment and started after 24 hours of the last dose of study drug.
2. CYP2C8 inducers (e.g., rifampin) may decrease PGZ concentrations. Therefore, these medications will be spaced 4 hours after administration of the study drug.

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

This study will enroll 50 hyperglycemic acute ischemic stroke patients.

8.2 Sample size determination

This is an exploratory study to accumulate pilot data. The sample size of this proposed pilot study is a total of 50 hyperglycemic adult patients with acute IS, (n=25 per treatment arm, study drug and placebo, respectively). The data from this pilot study will help the power analysis for a future clinical trial pf PGZ in hyperglycemic acute ischemic stroke.

Based on preliminary studies, we predict that about 30% (n=15/50) of patients with acute stroke symptoms will be diagnosed as “stroke mimics” after enrollment, having conditions such as a complicated migraine, seizure, metabolic disturbances that are not strokes. These individuals will continue study participation and will serve as controls. We also predict an attrition rate of 18% (n=5) at 3 months. Therefore we aim to include a total of 44 enrolled patients (n=22/group) for the intention-to-treat analysis.

8.3 Statistical methods

Biomarkers, such as cortisol and neutrophils are anticipated to have skewed distributions and thus will be logarithmically transformed in order to meet parametric modeling assumptions, such as normality. Descriptive statistics will be provided in the form of geometric means and coefficients of variations for these biomarkers at each of the time point. For repeated measurements over time, linear mixed-effects models will be used and applied to the logarithmically-transformed data to derive the biomarker-by-time profile for the PGZ treated and placebo group. In addition, correlation coefficients among all of the biomarkers and stroke volumes will be derived along with their 95% confidence intervals.

Stroke outcomes will be in the form of volume variables, which are anticipated to be ordinal in nature. Therefore, nonlinear mixed-effects models, with a cumulative logitlink function, will be applied to compare the PGZ and placebo groups with respect to clinical outcomes based on differences in various neurological scales at three months compared to admission. A third set of outcomes will be in the form

of neuroimaging variables, which are anticipated to be ordinal. Therefore, a nonlinear mixed-effects model, with an ordinal logistic link function, will be applied to compare the PGZ treated vs placebo in diabetic IS.

9.0 Data and Safety Monitoring Plan

9.1 Periodic evaluation of data

To monitor safety throughout the course of the study, every effort will be made to remain alert to possible AEs/SAEs. If an AE/SAE occurs, the first concern should be for the safety of the subject. If necessary, appropriate medical intervention will be provided. At the signing of the written consent form, each subject or his/her legally authorized representative and/or main caregiver must be given the names and telephone numbers of study site staff for reporting AEs/SAEs and medical emergencies.

As recommended by NCATS, our study team has identified an independent safety monitor for our study. Dr. Raja Khan (endocrinologist) has kindly agreed to serve in this role. She will review every adverse event during the study (as described in the next paragraph) based on severity and relationship to the study treatment. She will also review patient safety regularly after every 10th patient enrollment.

In this study, any AE/SAE experienced by the subject between the time of first dose of study treatment and 24 hours after the last dose will be recorded on the electronic case report form, regardless of the severity of the event or its relationship to study treatment. During study treatment and visit after 90 days, the research team will assess the subject for AEs and will record any new AEs/SAEs or updates to previously reported AEs on the electronic case report form.

The data will be reviewed by the study statistician in the Public Health Science department. If our trial allows us to reach our predetermined alpha with fewer patients than our predicted sample size, than periodic evaluation will allow us to avoid randomizing patients to a treatment that is potentially inferior. The data will be reviewed once, when 50% of patients have been enrolled in each arm.

9.2 Data that are reviewed

Data collected as primary outcomes and safety profile for the study will be reviewed. Neurological function determined by NIHSS, mRS, MOCA, BI and GOS, discharge disposition and rate of infection will be reviewed. Liver function tests will be reviewed.

9.3 Method of collection of safety information

Safety information will be collected in the research database throughout the study. The safety of the study drug will be evaluated by clinical assessments and laboratory tests (see section 7.2).

9.4 Frequency of data collection

The data of neurological function (NIHSS, BI, mRS, GOS and MOCA) will be collected before initiation of study drug, at discharge from hospital, and at 90 days during the follow-up visit. Inflammatory markers and all biomarkers will be collected at baseline and 24 hours after each dose of study drug for three consecutive days (see also table of study events, section 7.2).

9.5 Individuals reviewing the data

Dr. Raja Khan (endocrinologist) will review the efficacy and safety of the PGZ intervention.

9.6 Frequency of review of cumulative data

Data will reviewed periodically, i.e after collecting data of 5 patients, and will be discussed with team members approximately every three months.

9.7 Statistical tests

For details, see the statistical section under 8.3.

9.8 Suspension of research

This is pilot study, but after periodic evaluation of study result and safety profile, the PI can terminate the research anytime based on outcomes of periodic safety monitoring and benefit/risk outcomes and will inform the IRB if such a decision is being made.

10.0 Risks

The following risks are associated with PGZ during study treatment

Hypoglycemia: PGZ along with sulfonyl urea, other oral diabetic agents or insulin can cause mild to moderate hypoglycemia when used in conjunction with insulin or an insulin secretagogue. Under these conditions a lower dose of the insulin or insulin secretagogue may be needed to reduce the risk of hypoglycemia. Therefore, finger sticks glucose measurements will be performed 2h after initial study drug administration (time of peak of PGZ concentration) and subsequently at regular time points as determined by the standard of care protocol for stroke patients (see attached standard of care protocols for hypoglycemia and hyperglycemia for acute stroke patients, **Appendix 1**).

Cold-like symptoms such as headache, sinus infection, muscle pain and sore throat.
Edema: Dose-related edema may occur following long-term treatment with PGZ. However in this acute study of 3 doses edema is unlikely to occur, would be temporary and not cause a risk to study subjects.

Liver dysfunction: Long-term PGZ treatment may cause liver dysfunction, nausea, vomiting, stomach pain, unusual or unexplained tiredness, loss of appetite, dark urine, or jaundice which is unlikely to occur during the short treatment duration in this study. However, patients with type 2 diabetes may have fatty liver disease or cardiac disease with episodic congestive heart failure, both of which may cause liver test abnormalities, and they may also have other forms of liver disease, many of which can be treated or managed. Therefore, liver tests (serum ALT, AST, and total bilirubin) will be assessed prior to initiating study drug treatment and 24 hours after the second dose of study drug has been administrated. If after the second dose of study administration the ALT and/or AST should raise to 2.5 times the upper limit of normal and/or total bilirubin > 1.2 mg/dl study treatment will be stopped and patients will continue to be monitored and treated as deemed appropriate by the treating physician.

Congestive heart failure: Fluid retention may occur and can exacerbate or lead to congestive heart failure. Combination use of PGZ with insulin and use in congestive heart failure NYHA Class I and II may increase the risk. Although this is unlikely to occur during the brief treatment duration in this study, patients will be closely monitored for signs and symptoms of an acute exacerbation of congestive heart failure. If this should occur, treatment with the study drug will be stopped and patients will continue to be monitored and treated as deemed appropriate by the treating physician.

Bladder cancer: Long term use of PGZ may increase the risk of bladder cancer. Patients with active bladder cancer or history of bladder cancer will therefore not be included in this study.

Risk of randomization: The active research treatment may prove to be more/less effective or have more side effects than the placebo research treatment.

During blood draws, discomfort associated with the insertion of a needle into a vein is a slight pinch or pinprick when the sterile needle enters the skin. The risks of a blood draw include mild discomfort and/or a black and

blue mark at the site of puncture. Less common risks include a small blood clot, infection, or bleeding at the puncture site, and on rare occasions fainting during the procedure.

Loss of confidentiality is always a risk, but every attempt will be made to prevent any loss of data and to protect our patients' information, maintaining privacy, and keeping all data in a de-identified and password protected storage area.

11.0 Potential Benefits to Subjects and Others

11.1 Potential Benefits to Subjects

Subjects may or may not benefit from participation in this study. It is unclear whether PGZ treatment will lead to any benefits in study subjects as no data are currently available. However, possible benefits the subject may experience from this research study include the possible reduction of infection, which may reduce the brain injury following stroke, and a feeling of improvement of short and long term symptoms and day-to-day function.

11.2 Potential Benefits to Others

The potential benefit to society and others includes increasing knowledge regarding the effects of PGZ on clinical outcomes and its effect on the stress-immune response in acute hyperglycemic patients. New knowledge gained from this study could give way to novel treatments to help stroke recovery for a high-risk population.

12.0 Sharing Results with Subjects

Study results of individual subjects result will not be shared with subjects, the LAR or any treating physician.

13.0 Subject Payment and/or Travel Reimbursements

Not applicable

14.0 Economic Burden to Subjects

14.1 Costs

There will be no added costs to the subject enrolled in this study. Most data collected will be obtained from standard of care treatment. Any additional research-only laboratory tests and neurological tests completed will be paid for by the research study (detailed in section 7.2.3).

14.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

15.0 Resources Available

15.1 Facilities and locations

All study procedures will be performed at the Penn State Milton S. Hershey Medical Center. Study specific analyses of blood samples will be conducted at the PI's laboratory, the proteomics and mass spectrometry laboratory and the flow cytometry core. Statistical data analysis will be performed by Public Health Sciences.

15.2 Feasibility of recruiting the required number of subjects

The stroke service of the Department of Neurology at PSHMC admits over 800 patients annually and completes well over 1200 consultations/year for "brain attacks" in the ED and on the inpatient services at HMC, providing adequate access to potential study participants. A review of the institutional stroke data base has shown that more than 40% of patients with acute IS are hyperglycemic at the time of initial evaluation.

15.3 PI Time devoted to conducting the research

The PI will work closely with the study team members and has allocated sufficient time to successfully complete research related procedures, including study enrollment, data collection and data analysis. There will be regular meetings with study investigators to allow ongoing discussions, interim analyses and to address any operational issues.

15.4 Availability of medical or psychological resources

Penn State Milton S. Hershey Medical Center's ED is available 24/7.

15.5 Process for informing Study Team

The PI and study coordinator will regularly review the protocol as well as any study updates. Patient recruitment and follow-up will be reviewed during this time. The PI and/or study coordinator will communicate updates to all team members as they become aware of any changes or updates.

16.0 Other Approvals

16.1 Other Approvals from External Entities

Penn State CTSI

16.2 Internal PSU Committee Approvals

Check all that apply:

☐ Anatomic Pathology – **Penn State** Health **only** – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of "HRP-902 - Human Tissue For Research Form" in CATS IRB.

☐ Animal Care and Use – **All campuses** – Human research involves animals and humans or the use of human tissues in animals

☒ Biosafety – **All campuses** – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

☒ Clinical Laboratories – **Penn State Health only** – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids

that had been collected for clinical purposes but are no longer needed for clinical use. Upload a copy of "HRP-901 - Human Body Fluids for Research Form" in CATS IRB.

☐ Clinical Research Center (CRC) Advisory Committee – **All campuses** – Research involves the use of CRC services in any way.

☐ Conflict of Interest Review – **All campuses** – Research has one or more of study team members indicated as having a financial interest.

☐ Radiation Safety – **Penn State Health only** – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of "HRP-903 - Radiation Review Form" in CATS IRB.

☐ IND/IDE Audit – **All campuses** – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

☒ Scientific Review – **Penn State Health only** – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Health Cancer Institute (PSCI) Protocol Review Committee or the PSCI Disease Team is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website.

17.0 Multi-Site Study

17.1 Other sites

Not applicable

17.2 Communication Plans

17.3 Data Submission and Security Plan

17.4 Subject Enrollment

17.5 Reporting of Adverse Events and New Information

17.6 Audit and Monitoring Plans

18.0 Adverse Event Reporting

18.1 Adverse Event Definitions

For drug studies, incorporate the following definitions into the below responses, as written:	
Adverse event	Any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related
Adverse reaction	Any adverse event caused by a drug
Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction". • *Reasonable possibility*. For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
Serious adverse	Serious adverse event or Serious suspected adverse reaction: An adverse event

event or Serious suspected adverse reaction	or suspected adverse reaction that in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Life-threatening adverse event or life-threatening suspected adverse reaction	An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that had it occurred in a more severe form, might have caused death.
Unexpected adverse event or Unexpected suspected adverse reaction.	An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure, general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

18.2 Recording of Adverse Events

All AEs (serious or non-serious) and abnormal test findings observed or reported to study team which are believed to be associated with the study drug will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.

18.3 Causality and Severity Assessments

The investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s) or device(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s) or device(s)", the adverse event will be classified as associated with the use of the study drug(s) or device(s) for reporting purposes. If the investigator's final determination of causality is "unknown but not related to the study drug(s) or device(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

18.4 Reporting of Adverse Reactions and Unanticipated Problems to the FDA

If any drug adverse reactions will occur, they will be reported to the FDA.

18.4. 1 Written IND/IDE Safety Reports

Not applicable

18.4. 2 Telephoned IND Safety Reports – Fatal or Life-threatening Suspected Adverse Reactions

Not applicable

18.5 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

18.6 Unblinding Procedures

In case of a medical emergency, when the knowledge of the subject's treatment assignment may influence the subject's clinical care, the Investigator may access the subject's treatment assignment by PHS. The research team members must document the reasons for unblinding in the subject's source documentation. Study team members will not share the information to personnel involved with the analysis and conduct of the study.

18.7 Stopping Rules

In the following situations the treatment of subjects with the study drug will discontinue:

- The subject experiences a severe hypersensitivity reaction due to study treatment.
- The subject or legal representative wishes to no longer continue with the study at any time point.
- The subject is unwilling or unable to comply with the protocol.
- Hypoglycemia cannot be rectified by the treating team despite treatment, following the standard patient care protocol for hypoglycemia.
- The subject experiences an AE that necessitates permanent discontinuation of study treatment.
- Patient exhibits incidental abnormal finding of clinical evidence of active liver disease (AST or ALT exceed 2.5 times the upper limit of normal or total bilirubin >1.2 mg/dl) during study treatment.
- Patient develops an acute decompensated heart failure (NYHA Class III or IV), acute myocardial infarct or coronary syndrome, cardiac arrest, or requires coronary intervention (percutaneous coronary intervention or coronary artery surgery).

19.0 Study Monitoring, Auditing and Inspecting

19.1 Study Monitoring Plan

19.1. 1 Quality Assurance and Quality Control

Quality control procedures will be implemented at each stage of data handling to ensure that all data are reliable and have been processed correctly. Data anomalies will be communicated and discussed with Dr. Raja Khan (endocrinologist) and/or study team members as appropriate.

The investigator will permit study-related monitoring, audits, and inspections by the Penn State quality assurance program office(s), the IRB, the sponsor, and government regulatory bodies, of all study related documents (e.g., source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g., pharmacy, diagnostic laboratory, etc.).

19.1. 2 Safety Monitoring

Dr. Raja Khan will complete safety monitoring review for the study. She will review every adverse event during the study (as described in the protocol) based on severity and relationship to the study treatment. She will also review patient safety regularly after every 10th patient enrollment. This plan will be amended if an increase in monitoring is needed.

20.0 Future Undetermined Research: Data and Specimen Banking

20.1 Data and/or specimens being stored

Stored biological specimens will consist of blood and plasma. Data will be collected from subjects' medical records and completed tests and collected images will be stored electronically on password protected computers. Data will be entered into a password protected Redcap data base.

20.2 Location of storage

Biological specimens will be stored in the PI's laboratory (room C3771 in the Neural and Behavioral Sciences Department of the College of Medicine). All electronic and paper based data will be stored in the PI's research office.

20.3 Duration of storage

Specimens will be kept indefinitely until used up or not needed. The images will be stored for an undetermined time.

20.4 Access to data and/or specimens

Only research team members will have access to any data or blood samples.

20.5 Procedures to release data or specimens

Not applicable

20.6 Process for returning results

Not applicable

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22.0 Confidentiality, Privacy and Data Management

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