HRP-503B – BIOMEDICAL RESEARCH PROTOCOL
(2017-1)

Protocol Title: Inhibition of Sterile Inflammation by Digoxin

Principal Investigator: Wajahat Z Mehal

Version Date: 04-19-2023

(*If applicable*) Clinicaltrials.gov Registration #: Click or tap here to enter text.

INSTRUCTIONS
This template is intended to help investigators prepare a protocol that includes all of the necessary information needed by the IRB to determine whether a study meets approval criteria. **Read the following instructions before proceeding:** 1. Use this protocol template for a PI initiated study that includes direct interactions with research subjects. Additional templates for other types of research protocols are available in the system Library. 2. If a section or question does not apply to your research study, type "Not Applicable" underneath. 3. Once completed, upload your protocol in the "Basic Information" screen in IRES IRB system.

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SECTION I: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.

   Alcoholic hepatitis (AH) is a major liver disease. Liver inflammation is a key feature of AH. We are testing if administering digoxin orally to healthy subjects results in a reduction in innate immune inflammatory responses in the peripheral blood, and if digoxin can alter binding of PKM2 to pro-inflammatory loci in primary human monocytes, and identify novel gene targets for PKM2.
   Hypothesis: Pyruvate Kinase M2 (PKM2) regulates liver inflammation by up-regulating HIF-1α and other pro-inflammatory genes, and this can be inhibited by digoxin to provide hepatoprotection in AH.
   Aim 1. Obtain clinical data supporting the therapeutic use of digoxin in alcoholic hepatitis.
   Aim 2. Identify dominant and novel targets that are regulated by PKM2 in alcoholic hepatitis.
   Aim 3. Obtain plasma proteomic and molecular data to allow for early identification of patients with SIRS.

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.
   204 weeks

3. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.
   The development of sterile inflammation after cell death is a ubiquitous response which occurs in all organs. The liver is notable for developing an exceptionally strong sterile inflammatory response. This is seen in experimental and human alcoholic steatohepatitis (AH). It is also notable that for (AH) there is no effective therapy. This is in contrast to suppression of T cell responses, for which potent drugs exist (tacrolimus and cyclosporine). In addition to the lack of effective therapy, we also have a poor ability to identify patients with AH who will develop clinically significant systemic inflammatory response (SIRS), which is associated with higher mortality. The sterile inflammatory response in the liver incorporates many cell types and signaling pathways, and two processes among these are universally present. One is an increased tissue redox state, which plays a key role in the progression of inflammatory disorders. There are many cellular mechanisms of production of reactive oxygen species (ROS), with tissue hypoxia and activation of the HIF-1α pathway being a dominant one. Acute and chronic alcohol exposure result in liver hypoxia leading to HIF-1α pathway activation in AH. The requirement for HIF-1α activation in non-alcoholic steatohepatitis (NASH) was demonstrated by the ability of HIF-1α antisense nucleotide treatment to offer protection. We have demonstrated that HIF-1α activation potentiates and sustains the amplitude of acute inflammatory responses, and is vital for the transition from acute self-limiting to sustained chronic inflammation. There is also a close positive relationship between HIF-1α activation and increased ROS production. These mechanistic insights into the role of the HIF-1α pathway in sterile inflammation may have great clinical relevance due to the ability of cardiac glycosides (CGs) to inhibit HIF-1α activation. CGs are well known in cardiology due to their effects of increasing ionotropy, and decreasing chronotropy, via partial inhibition of the Na+ /K+ ATPase on the plasma membrane of myocytes. Of note the HIF-1α inhibitory effect of CGs is independent of its activity on the Na+ /K+ ATPase, indicating CGs have additional unidentified molecular targets. In this application, we describe a novel and potent role for digoxin in reducing the severity of steatosis, inflammation and hepatocellular damage in livers undergoing AH. Digoxin improves oxidative stress during liver injury through maintaining cellular redox homeostasis, and suppressing HIF-1α pathway activation. We have further identified the key metabolic regulator pyruvate kinase isoform 2 (PKM2) as a digoxin-binding protein. PKM2 isoform exists primarily as an enzymatically inactive dimer which can translocate to the nucleus, where it will interact with HIF-1α and

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regulate expression of numerous genes. Interestingly, digoxin does not alter PKM2 kinase activity but suppresses PKM2 dependent transcriptional activity. This area is significant because sterile inflammation (SI) is the central pathological process in AH. Our results identify PKM2 as a key molecule in liver sterile inflammation which can be therapeutically targeted. The identification of digoxin as a PKM2 binder, which leads to critical anti-inflammatory effects, provides a novel opportunity to reduce liver inflammation in AH. We have further identified a unique molecular profile from a pilot aptamer-proteomic screen which will be expanded to the whole InTeam cohort to provide predictive markers for severe AH.

4. Research Plan: Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths. Describe the setting in which the research will take place.

Test if administering digoxin orally to healthy subjects results in a reduction in innate immune inflammatory responses in the peripheral blood.
We need to obtain peripheral blood lymphocytes from healthy subjects on different doses of digoxin to test if digoxin has an effect on the immune response of PBLs.

A) There will be three study groups.
Groups:
A: Placebo n=15 B: Digoxin 3 mcg/Kg/day n=15 C: Digoxin 0.15 mcg/Kg/day n=15
Serum digoxin concentrations, BUN, Creatinine, CBC, Electrolytes (Na, K) and ECGs will be obtained weekly. pregnancy testing for female participants of childbearing potential and a baseline screening to determine if you are eligible for this study. Peripheral blood (25ml) in EDTA tubes (purple top) will be obtained at 0, 1, 2, 3 weeks after starting digoxin, with written consent and in accordance with the guidelines of Yale University ethics committee. PMN and monocytes will be isolated using PolymorphPrep (Axis-Shield) according to standard procedure, and purity will be determined by flowcytometric analysis. Spontaneous ROS of PMNs will be measured by culturing neutrophils with CM-H2DCFA for 30 minutes, washing, followed by kinetic analysis of intracellular ROS with fluorescent plate reader. Neutrophils up-regulate ROS spontaneously in culture, and for each sample the un-treated aliquot will be the control for the digoxin treated aliquot. Monocytes will be stimulated LPS (100ng/ml) for 3 hrs after which cDNA will be prepared and qPCR conducted for IL-1B, TNF-α and IL-6. cDNA and supernatant will be stored for further analysis based on initial results.
These initial numbers are based the use of similar assays in the preliminary data. We will obtain additional assistance from Dr. Bruce Barton Professor of Biostatistics at the University of Massachusetts, who will 1) review of study design, including sample size and specimen utilization; 2) develop an appropriate data management system to provide data capture and edit capabilities; 3) develop appropriate statistical analysis, quality assurance, and quality control plans; 4) conduct the statistical analysis and provide material for presentation/publication; and 5) provide resources to assure reproducible research. Please see letter of support.

B) The data management system will be set up in the University of Massachusetts Medical School secure REDCap environment, which is used for all of the pilot studies funded by NIAAA as part of the AlcHepNet Research Network. This HIPAA-compliant, 21 CFR Part 11 compliant environment is accessible only by medical school staff and collaborating clinical sites of the AlcHepNet who are assigned an account in this environment by UMMS IT with approval by the UMMS IRB. The system will be programmed for a single entry with validation rules at the time of entry and additional checking conducted after the data have been submitted to the main data base. These edits will check for validity, consistency, and normal range values. Edit queries will be generated and resolved by clinic staff with corrections posted to the database through the REDCap system, which enforces an audit trail for all changes. The study database will be stored in REDCap within the secure environment to protect any PHI/PII data

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that are collected as part of the study. We will strive to minimize collection of such data and REDCap will be programmed to segregate that data from the study data so that exports for analysis will be deidentified. Data will be exported from REDCap for import into the latest version of SAS (SAS Institute, Cary, NC) or other statistical software for all analysis.

C-E) All reports and analyses will be generated from these files. Data files (and accompanying programs) that are used for reports, presentations, or publication will be archived as required past the end of the study. REDCap will also have the audit trail capability enabled for tracing any data modifications. Data from laboratories can be uploaded directly into REDCap for storage. The REDCap system will be programmed and maintained by study staff at the U Mass Medical School AlcHepNet Data Coordinating Center while data entry will be performed by study staff at the local site.
The sample size for this pilot study was based on results from in vitro experiments, where differences in as few as 5 participants were seen to be significantly different. Given the inherent variability in the absorption of digoxin in humans, a sample size of 15 was determined to be reasonable to provide a reasonable distribution of results from repeated measures of participants at the differing digoxin dose levels.

F) Pregnancy: ***The chance of a female subject to become pregnant on a 4-week study is very less, if in case this does happen they will be asked to stop the drug and withdraw from the study immediately. We do not intend to follow the pregnancy and birth outcome.***

Drug preparation: Digoxin is commercially available as a 0.05mg/ml elixir (Boehringer Ingelheim). The appropriate dosing of digoxin and control will be prepared with the help of the Yale Center for Clinical Investigation (YCCI) as detailed in the facilities and resources. Stopping criteria: Subjects will be removed from the study if any serum digoxin level is above 0.8ng/ml, or if there are any abnormalities in blood tests or ECGs. Data from these subjects will not be used in the analysis. Analysis, expected outcome and alternative approaches: It is expected that there will not be a significant difference in the neutrophil ROS and monocyte qPCR results in control group A between the four time points. It is expected that for group B and C, both neutrophil ROS and monocyte qPCR levels will be lower at time points 1, 2, 3 weeks as compared to time 0, and as compared to all time points of group A. It is not clear if in group D time points of week 1, 2, and 3 will be any different from time point 0. For the time points where significant differences are found we will perform additional luminex assay with the supernatant using the luminex five plex panel (GM-CSF, IL-1B, IL-6, IL-8, TNF-α). There will also be an analysis of the production of ROS and qPCR levels in relation to serum digoxin levels at the time that the analysis samples were obtained. The combinations of these analysis will establish that digoxin taken orally is effective in reducing inflammatory responses, and will also provide information on dose response for oral dosing as well as serum concentrations.

5. Genetic Testing N/A ☒
   A. Describe
      i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned *Write here*
      ii. the plan for the collection of material or the conditions under which material will be received *Write here*
      iii. the types of information about the donor/individual contributors that will be entered into a database *Write here*
      iv. the methods to uphold confidentiality *Write here*

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B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects? *Write here*
C. Is widespread sharing of materials planned? *Write here*
D. When and under what conditions will materials be stripped of all identifiers? *Write here*
E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials? *Write here*
    i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)? *Write here*
F. Describe the provisions for protection of participant privacy *Write here*
G. Describe the methods for the security of storage and sharing of materials *Write here*

6. Subject Population: Provide a detailed description of the types of human subjects who will be recruited into this study.
In total 60 healthy subjects ≥18 years and ≤70 years with no known clinical history *will be recruited for this study.*

7. Subject classification: Check off all classifications of subjects that will be specifically recruited for enrollment in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☐Children	☒ Healthy	☐Fetal material, placenta, or dead fetus
☐Non-English Speaking	☐ Prisoners	☐Economically disadvantaged persons
☐Decisionally Impaired	☐ Employees	☐Pregnant women and/or fetuses
☐Yale Students	☐ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects?
Yes ☐ No ☒

8. Inclusion/Exclusion Criteria: What are the criteria used to determine subject inclusion or exclusion?

Inclusion Criteria

1. Age >18 y ≤ 70 years

2. subjects with normal serum creatinine, normal EKG and currently not taking any medication.

Exclusion criteria

1. Autoimmune liver disease (ANA > 1/320)
2. Chronic viral hepatitis
3. Hepatocellular carcinoma
4. Complete portal vein thrombosis
5. Extrahepatic terminal disease
6. Pregnancy
7. Treatment with prednisolone or pentoxifyllin for more than 3 days prior to inclusion/start date

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8. Active alcohol abuse (>50 g/day for men and >40 g/day for women) in the last 3 months
9. AST > ALT and total bilirubin > 3 mg/dl in the past 3 months
10. Liver biopsy and/or clinical picture consistent with alcoholic hepatitis
11. Lack of signed informed consent.
12. Known hypersensitivity to digoxin or other forms of digitalis, ventricular fibrillation.
13. Any significant medical conditions, any electrolyte abnormalities, over the counter medications, natural products and prescription drugs.
14. Breastfeeding mothers.

9. How will **eligibility** be determined, and by whom? Write here

The eligibility will be determined by PI and his team according to their IRB protocol.

10. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

The following risks are associated with participating in the study:

- *Adverse effects from digoxin and its toxicity. Symptoms of digoxin toxicity include: confusion, irregular pulse, loss of appetite, nausea, vomiting, diarrhea, fast heartbeat, vision changes (which could be unusual, but includes blind spots, blurred vision, changes in how colors look, and seeing spots), decreased consciousness, decreased urine output, difficulty breathing when lying down, excessive nighttime urination, and overall swelling*
- *Allergic reaction to digoxin (nausea, vomiting, diarrhea, loss of appetite, weakness, dizziness, headache, anxiety, depression, slow heart rate, enlarged or tender breasts in men, or skin rash.*
- *Risks and discomforts of phlebotomy: To obtain detailed biochemical liver function tests and blood samples for research, blood sampling will be required. Patients will have 4 venipunctures during the period of evaluation (28 days). Each venipuncture will remove ~18 ml of blood. However, no more than 6 ml/kg will be drawn from any one person during this 4-week period. Blood collection by venipuncture is associated with mild discomfort, and possible risk of localized bruising, phlebitis, or extravasation. The risk of infection or fainting is extremely small.*
- *Risks and Inconveniences of an electro-cardiogram (EKG): EKG is very safe procedure which is being performed very commonly for evaluation of cardiac status. There is no risk of performing EKG to the subjects, however, there may be slight inconvenience of cold sensations to the skin related to the application of gel at the site of EKG leads placement.*

11. **Minimizing Risks:** Describe the manner in which the above-mentioned risks will be minimized.

1. Each participant will be enrolled only after signing an informed consent in the presence of a study coordinator. 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.
2. Blood draws and other biological sample collections will be performed by trained personnel. pregnancy testing for female participants of childbearing potential and a baseline screening to determine if you are eligible for this study.
3. A written detailed collection procedure is provided to minimize spilling during collection.
4. Serum digoxin concentrations, BUN, Creatinine, CBC and ECGs will be obtained weekly to monitor the adverse effect of digoxin. In case, subject is having adverse effect related to digoxin, they will be removed from study and medical care will be provided at Yale new haven hospital if needed.
5. In terms of potential drug allergies, all subjects will be asked standardized questions about previous allergic reactions to digoxin. If a participant reports a digoxin allergy, they will be excluded. We will contact (telephone, email, or text message) each participant within three days of starting the oral digoxin in order to assess tolerability.

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6. There may be low risk of breach of confidentiality, however we will follow appropriate data safety plan to minimize the breach of confidentiality. In addition, unauthorized personnel will not be allowed to have access to subject's confidential information.
7. If study subjects express a desire to discontinue the protocol, for any reason, the intervention will be immediately terminated and consent to participate may be rescinded at the subject's discretion.

12. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.)
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study? *Risks are minimal.*
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? *Write here*
    c. Include an appropriate Data and Safety Monitoring Plan. Examples of DSMPs are available here http://your.yale.edu/policies-procedures/forms/420-fr-01-data-and-safety-monitoring-plans-templates for
        i. Minimal risk
        ii. Greater than minimal

Greater Than Minimal Risk DSMP

1. Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency, which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator, the IRB have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed greater than minimal for the following reasons: (choose those that apply)

1. We do not view the risks associated with the _____ as minimal risks.
2. We do not view the risks associated with the combined use of _____and_____ as minimal risks.
3. Given the now established safety and validity of the current _drug_____ in our prior work, we do not view the proposed studies as high risk.
4. Given our experience with the combined co-administration_____, we do not view the proposed studies as high risk.

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand

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experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:

Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator (*Dr Wajahat Mehal*) according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event
2. Moderate adverse event
3. Severe

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events:

In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

1. Death;
2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
3. A persistent or significant disability or incapacity;
4. A congenital anomaly or birth defect; OR
5. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

6. Plan for reporting UPIRSOs (including Adverse Events) to the IRB

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The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – *serious, unexpected, and related adverse events* and *unanticipated adverse device effects*. ***Please note*** that adverse events are reportable to the IRB as UPIRSOs only if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have occurred at the expected frequency and level of severity as previously documented. In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

7. Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g., industrial sponsor, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

□ All Co-Investigators listed on the protocol.
□ Yale Cancer Center Data and Safety Monitoring Committee (DSMC)

X National Institutes of Health
□ Food and Drug Administration (Physician-Sponsored IND #_____)
□ Medical Research Foundation (Grant_____)

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□ Study Sponsor
□ Other Data Safety Monitoring Board (DSMB) or Committee (DSMC)

The principal investigator (*Dr Wajahat Mehal)* will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

Please note: For any study that may be considered high risk, the IRB will be more focused on the safety requirements for the study and a DSMB will likely be required.

d. For multi-site studies for which the Yale PI serves as the lead investigator:N/A
   i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed? *Write here*
   ii. What provisions are in place for management of interim results? *Write here*
   iii. What will the multi-site process be for protocol modifications? *Write here*

13. Statistical Considerations: Describe the statistical analyses that support the study design.

Primary analyses of the biochemical measures will be conducted using mixed effects models to account for the correlation among the biochemical measures obtained from repeated blood draws on the same participant. The models will include digoxin dose (3 mcg/kg/day, 0.15 mcg/kg/day, none/placebo), time on study (weeks 0, 1, 2, and 3), and relevant patient characteristics (i.e., age, gender). A random intercept will be included so that each participant will have their own starting point and time will be a categorical predictor to better model a potential curvilinear relationship between time on study and biochemical levels without assuming a linear slope. Because treatment group is a three-level variable, we will first conduct a Type III multi-degree of freedom test to determine if any difference exists among the three groups and, if that is significant, conduct all pairwise comparisons to determine where the difference is. Because these tests are not independent, we will apply a Bonferroni correction to the critical p-value to reduce the possibility of a Type I error by chance alone. In this case, the critical p-value will be set at 0.05/3 = 0.017 so that any p-value less than 0.017 will be declared statistically significant.
Adherence analysis will be ascertained using pill counts for each participant at the weekly visits. For safety analyses, we will record any adverse events, both serious (SAE) and not, described by the participant at each weekly visit. Each AE will include a description of the AE/SAE, relatedness to digoxin, and resolution. These will be summarized by treatment group and, if there are three or more participants in any one group with a specific AE/SAE, we will conduct a statistical test (Likelihood Ratio Chi-Square) to determine any statistically significant difference among the treatment groups using the analogous approach as above to first test for any difference in the incidence of AE/SAE among the three treatment groups followed by conducting the three pairwise comparisons, again using the Likelihood Ratio Chi-Square. Because this is a safety analysis, the critical p-value is left at 0.05 for all comparisons to avoid increasing the probability of a Type II error.

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SECTION II: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

If this section (or one of its parts, A or B) is not applicable, check off N/A and delete the rest of the section.

A. RADIOTRACERS ☒N/A

1. Name of the radiotracer: *Write here*

2. Is the radiotracer FDA approved? ☐YES ☐NO

If NO, an FDA issued IND is required for the investigational use unless RDRC assumes oversight.

3. Check one: ☐IND# *Write here* or ☐RDRC oversight (RDRC approval will be required prior to use)

4. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this radiotracer is being administered to humans, include relevant data on animal models.
Write here

4. **Source:** Identify the source of the radiotracer to be used. *Write here*

5. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, method of sterilization and method of testing sterility and pyrogenicity.
Write here

B. DRUGS/BIOLOGICS ☒N/A

1. If an **exemption from IND filing requirements is** sought for a clinical investigation of a drug product that is lawfully marketed in the United States, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Exempt Category 1: The clinical investigation of a drug product that is lawfully marketed in the United States can be exempt from IND regulations if all of the following are yes:	
1. The intention of the investigation is NOT to report to the FDA as a well-controlled study in support of a new indication for use or to be used to support any other significant change in the labeling for the drug.	☒
2. The drug that is undergoing investigation is lawfully marketed as a prescription drug product, and the intention of the investigation is NOT to support a significant change in the advertising for the product.	☒
3. The investigation does NOT involve a route of administration or dosage level or use in populations or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product	☒

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4. The investigation will be conducted in compliance with the requirements for institutional (HIC) review and with the requirements for informed consent of the FDA regulations (21 CFR Part 50 and 21 CFR Part 56).	☒
5. The investigation will be conducted in compliance with the requirements regarding promotion and charging for investigational drugs.	☒

Exempt Category 2 (all items i, ii, and iii must be checked to grant a category 2 exemption)

☐i. The clinical investigation is for an *in vitro* diagnostic biological product that involves one or more of the following (check all that apply):

☐ Blood grouping serum
☐ Reagent red blood cells
☐ Anti-human globulin

☐ii. The diagnostic test is intended to be used in a diagnostic procedure that confirms the diagnosis made by another, medically established, diagnostic product or procedure; and

☐iii. The diagnostic test is shipped in compliance with 21 CFR §312.160.

Exempt Category 3

☐ The drug is intended solely for tests in vitro or in laboratory research animals if shipped in accordance with 21 CFR 312.60

Exempt Category 4

☐ A clinical investigation involving use of a placebo if the investigation does not otherwise require submission of an IND.

2. Background Information: Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

Cardiac glycosides have been used since ancient Egyptians, and the clinical effects of digoxin were first characterized in 1785, and was approved by the FDA in the mid-1990's. Since then many hundreds of thousands of patients have taken digoxin and there are no side-effects when serum levels are maintained in or below the therapeutic range. We have selected a dose that will maintain serum levels in or below the therapeutic range and in addition we serum levels will be monitored weekly.

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3. **Source:** Identify the source of the drug or biologic to be used. *Prashant Patel, PharmD Manager, Investigational Drug Service Department of Pharmacy Services Yale-New Haven Hospital O: 203-688-8515*

a) Is the drug provided free of charge to subjects? ☒YES ☐NO
If yes, by whom? *Write here*

4. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.

This is under the management of the investigational Drug Service, Department of Pharmacy Services Yale-New Haven Hospital. We can provide the location of initial delivery, elixir preparation, and other storage places including details of refrigerators at YCCI if required.

Check applicable Investigational Drug Service utilized:

☒ YNHH IDS ☐ CMHC Pharmacy ☐ West Haven VA
☐ PET Center ☐ None
☐ Other:

Note: *If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.*

5. Use of Placebo: ☐Not applicable to this research project

YNHH IDS will be utilized.

If use of a placebo is planned, provide a justification which addresses the following:

a) Describe the safety and efficacy of other available therapies. If there are no other available therapies, state this. *Write here*

b) State the maximum total length of time a participant may receive placebo while on the study. *Write here*
c) Address the greatest potential harm that may come to a participant as a result of receiving placebo. *Write here*
d) Describe the procedures that are in place to safeguard participants receiving placebo. *Write here*

6. Continuation of Drug Therapy After Study Closure ☒Not applicable to this project

Are subjects provided the opportunity to continue to receive the study drug(s) after the study has ended?

☐ Yes If yes, describe the conditions under which continued access to study drug(s) may apply as well as conditions for termination of such access. *Write here*

☐ NO If no, explain why this is acceptable. *Write here*

B. DEVICES ☒N/A

1. Are there any investigational devices used or investigational procedures performed at Yale-New Haven Hospital (YNHH) (e.g., in the YNHH Operating Room or YNHH Heart and Vascular Center)? ☐Yes ☐No

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If Yes, please be aware of the following requirements:

A YNHH New Product/Trial Request Form must be completed via EPIC: **Pull down the Tools tab in the EPIC Banner, Click on Lawson, Click on "Add new" under the New Technology Request Summary and fill out the forms requested including the "Initial Request Form," "Clinical Evidence Summary", and attach any other pertinent documents. Then select "save and submit" to submit your request**; AND

Your request must be reviewed and approved **in writing** by the appropriate YNHH committee before patients/subjects may be scheduled to receive the investigational device or investigational procedure.

2. **Background Information:** Provide a description of previous human use, known risks, and any other factors that might influence risks. If this is the first time this device is being used in humans, include relevant data on animal models.
   Write here
3. **Source:**
   a) Identify the source of the device to be used. *Write here*
   b) Is the device provided free of charge to subjects? ☐Yes ☐No

4. **Investigational device accountability**: State how the PI, or named designee, ensures that an investigational device is used only in accordance with the research protocol approved by the HIC, and maintains control of the investigational device as follows:

   a) Maintains appropriate records, including receipt of shipment, inventory at the site, dispensation or use by each participant, and final disposition and/or the return of the investigational device (or other disposal if applicable): *Write here*

   b) Documents pertinent information assigned to the investigational device (e.g., date, quantity, batch or serial number, expiration date if applicable, and unique code number): *Write here*

   c) Stores the investigational device according to the manufacturer's recommendations with respect to temperature, humidity, lighting, and other environmental considerations: *Write here*

   d) Ensures that the device is stored in a secure area with limited access in accordance with applicable regulatory requirements: *Write here*

   e) Distributes the investigational device to subjects enrolled in the IRB-approved protocol: *Write here*

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. **Targeted Enrollment: Give the number of subjects:**
   a. Targeted for enrollment at Yale for this protocol: 60
   b. If this is a multi-site study, give the total number of subjects targeted across all sites: *Write here*

2. **Indicate recruitment methods below .** Attach copies of any recruitment materials that will be used.

☒ Flyers	☒ Internet/web postings	☐ Radio
☐ Posters	☒ Mass email solicitation	☐ Telephone
☐ Letter	☐ Departmental/Center website	☐ Television
☐ Medical record review*	☐ Departmental/Center research boards	☐ Newspaper

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☐ Departmental/Center newsletters ☐ Web-based clinical trial registries ☐ Clinicaltrails.gov
☒ YCCI Recruitment database ☐ Social Media (Twitter/Facebook):
☐ Other:

* Requests for medical records should be made through JDAT as described at
http://medicine.yale.edu/ycci/oncore/availableservices/datarequests/datarequests.aspx

3. Recruitment Procedures:

a. Describe how potential subjects will be identified. We will use the YCCI subject database.
b. Describe how potential subjects are contacted. We will use the YCCI subject database.
Who is recruiting potential subjects? *The investigators have employed the services of the Yale Center for Clinical Investigation/CTSA for recruitment for this study, including dedicated recruitment staff, community outreach personnel, as well as the use of the websites www.yalestudies.org and www.researchmatch.org with targeted recruitment information for both patients and healthcare professionals.*
The project manager will primarily oversee screening and recruitment.

4. Assessment of Current Health Provider Relationship for HIPAA Consideration:

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?
☐Yes, all subjects
☐Yes, some of the subjects
☒No

If yes, describe the nature of this relationship. *Write here*

5. Request for waiver of HIPAA authorization: (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

Choose one:
☐ For entire study
☒ For recruitment/screening purposes only
☐ For inclusion of non-English speaking subject if short form is being used and there is no translated HIPAA research authorization form available on the University's HIPAA website at hipaa.yale.edu.

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data: *We are requesting a waiver of signed HIPAA authorization in order to undertake telephone and online survey screening to determine potential eligibility of interested subjects. Without a request of signed HIPAA authorization for telephone and online survey recruitment only, we would not be able to telephone screen or provide an online screening survey to participants.*

ii. If requesting a waiver of signed authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data: *By signing this protocol application, the investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.*

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The investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

6. Process of Consent/Assent: Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.
   Prior to signing the consent document, study staff will provide subjects with additional information about the study and review the consent forms and other study-related documentation. All subjects participating in the study will provide written consent. All subjects will be informed of the option of not participating, or of stopping at any time during the interview.
7. Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent: Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.
   The Principal Investigator and project manage/coordinator will determine the ability and capacity of individuals to give consent by questioning during the process of consent. Potential subjects will be asked, "Could you explain to me what we are going to ask you to do in this study? This will help me to be sure that you understand the research," as well as, "What more would you like to know about this study?" All subjects will be informed of the option of not participating, or of stopping at any time during the study
8. Non-English Speaking Subjects: Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.
   As needed, we will utilize bilingual translators for the informed consent process.

As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment? YES ☐ NO ☒

Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are available on the HRPP website (yale.edu/hrpp) and translated HIPAA Research Authorization Forms are available on the HIPAA website (hipaa.yale.edu). If the translation of the short form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via modification prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

9. Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study. If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

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☐Not Requesting any consent waivers

☐Requesting a waiver of signed consent:

☐ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study (Note that an information sheet may be required.)

For a waiver of signed consent, address the following:

- Would the signed consent form be the only record linking the subject and the research? YES ☐ NO ☐
- Does a breach of confidentiality constitute the principal risk to subjects? YES ☐ NO ☐

OR

- Does the research pose greater than minimal risk? YES ☐ NO☐
- Does the research include any activities that would require signed consent in a non-research context? YES ☐ NO ☐

☐ Requesting a waiver of consent:

☐ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study

For a full waiver of consent, please address all of the following:

- Does the research pose greater than minimal risk to subjects?
  ☐ Yes *If you answered yes, stop. A waiver cannot be granted.*
  ☐ No
- Will the waiver adversely affect subjects' rights and welfare? YES ☐ NO☒
- Why would the research be impracticable to conduct without the waiver? *Write here*
- Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date? *Write here*

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

1. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research? *Name, date of birth, telephone number(s), mailing address, email address, gender, weight and height (to calculate BMI), medications, other diagnoses. Only the investigators will have access to PHI.*

2. How will the research data be collected, recorded and stored? *Paper forms will be kept in each study participant's record and will be stored in a locked file cabinet in PI office at 1 Gilbert Street TAC building, room*

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S223A. Only the PI and study staff will have access to the participant's records. Forms will be coded with subject number; no personally identifiable information will be associated with these forms.

3. How will the digital data be stored? ☐CD ☐DVD ☐Flash Drive ☐Portable Hard Drive ☒Secured Server ☐Laptop Computer ☐Desktop Computer ☐Other

4. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study? *All documents and subject information will be strictly maintained according to HIC and HIPAA regulations to ensure confidentiality at all times. Access to subject information will be limited to a "need to know" basis and all data will be coded to maintain confidentiality. Only those investigators with appropriate Human Subjects training will have access to subject data. All electronic files are encrypted and password protected; paper files are kept in locked file cabinets. All data will be managed to assure strict confidentiality of subjects at all times. In addition, all portable devices will contain encryption software per University policy 5100.*

All portable devices must contain encryption software, per University Policy 5100. If there is a technical reason a device cannot be encrypted please submit an exception request to the Information Security, Policy and Compliance Office by clicking on url http://its.yale.edu/egrc or email it.compliance@yale.edu

5. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured. *Once the research is complete, the data will be analyzed by all members of the study staff and a statistician. Data will be kept for five years after the study ends. Data will then be de-identified using a "Safe Harbor" (45CFR164.514(b)(2)) approach consistent with the HIPAA Privacy rule. De-identified data will be certified by a statistician that there is a very small risk that use of the protected health information could lead to a subject being identified. The principal investigator (Dr. Wajahat Mehal) is responsible for the implementation of data de-identification.*

6. If appropriate, has a Certificate of Confidentiality been obtained?

This is NIH funded and is covered a COC..

SECTION V: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)
There will be no direct benefits of the proposed research to the participating human subjects.

SECTION VI: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?
   Alternatives to participating in the study is not to participate. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.
   Subjects will be remunerated $75 at each visit through ePay system set up by the CSRU.

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2. Costs for Participation (Economic Considerations): Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.

   Subjects will not incur any costs associated with participation in the research besides transportation costs. Subjects will be provided parking passes at each visit to cover parking if they drive to their visit and park in the ProPark lot surrounding the building.

   You will be compensated in the amount of $75 at each visit for 4 weeks. The total amount you will be given in four weeks will be $300 ($75x4) if you complete all the visits required by the study. You will be provided parking passes at each visit to cover parking if you park in the ProPark lot surrounding the building.

In Case of Injury: This section is required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).
This research does not involve more than minimal risk.

a. Will medical treatment be available if research-related injury occurs? *YNHH emergency room.*

b. Where and from whom may treatment be obtained? *YNHH emergency room.*
c. Are there any limits to the treatment being provided? *Treatment will be provided at the current standard of care.*
d. Who will pay for this treatment? *This will vary from subject to subject. The clinical trial will not pay for the treatment.*
e. How will the medical treatment be accessed by subjects? *Subject will walk into the YNHH emergency room. If this is not possible they will be transported there by ambulance.*

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IMPORTANT REMINDERS

Will this study have a billable service? Yes ☒ No☐

A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects.

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? Yes ☐ No ☒

If Yes, please answer questions a through c and note instructions below.

a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform? Yes ☐ No ☐

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? Yes ☐ No ☐

c. Will a novel approach using existing equipment be applied? Yes ☐ No ☐

If you answered "no" to question 4a, or "yes" to question 4b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

IMPORTANT REMINDER ABOUT RESEARCH AT YNHH

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. **By submitting this protocol as a PI, you attest that you and any co-investigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH.**

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