Protocol Title: Development and Translation of Generator-Produced PET Tracer for Myocardial Perfusion Imaging.

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Exploratory Investigational New Drug (Sponsor-Investigator eIND)

Investigational Drug:	**[^{68}Ga]Galmydar**
FDA IND Number:	157468
IND Date of Activation:	August 11, 2021
IRB/HRPO:	202107042
RDRC:	925
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REVISION HISTORY

Date (yr-mo-day)	Version	Regulatory Committee	Revision History
2021-06-24	1.0	RDRC	Original submission. Approvable letter received.
2021-06-29	2.0	n/a	RDRC revisions recommended to the Protocol, version 2.0 and Rest/Stress Consent, version 2.0: 1. The interval between the rest and stress [^{68}Ga]Galmydar PET acquisitions should be no less than 2 and no greater than 4 hours. 2. Rest/Stress group: ^{13}N-ammonia data acquisition revised to 10-min list-mode (if the anterior-posterior diameter of the chest is >50cm increase to 15-min).
2021-07-09	2.0	IRB	Initial submission.
2021-12-13	2.1	IRB	Revised Protocol from version 2.0 to 2.1 Bottom of pg. 20 Whole-body PET image acquisition from head to mid-thigh. Imaging of subjects will be at three time points spanning from immediately after injection up to 4-hours after [^{68}Ga]Galmydar injection. A low-dose spiral CT scan (120 kVp, 50 mAs) will be obtained for attenuation correction. Begin whole-body PET image acquisition from the head to mid-thigh immediately post [^{68}Ga]Galmydar injection for 60-min and repeat at 2 hours and 4 hours.
2022-01-26	3.0	IRB	Revising Protocol from version 2.1 to 3.0 protocol on pages 18, 19, and 45. This is to add a second IV to the dosimetry group. In this group we will use 1 IV to draw bloods, including the serum metabolites, and the second to inject the [^{68}Ga]Galmydar.
2022-07-26	4.0	IRB	Revising protocol 3.0 to 4.0 Protocol pages 16, 21, 36, 37, and 38. Reducing the dose of the radioactive tracer in the stress portion from 8mCi to 6mCi on Day 1. Revising the reportable events table to page 36 as well.
2022-12-6	4.1	IRB	Increasing the reimbursement for rest/stress Changing galmydar imaging time from “up to 75 mins” to “90 mins” Changing rest/stress from 2 days to possibly 3 days Changing 2 IVs to possibly 1 IV

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1 BACKGROUND

While exploring structure-activity relationships of organic ligands possessing an N^4O^2 donor core [1-3], we identified a gallium-68-radiopharmaceutical (named herein as ^{68}Ga-Galmydar) that demonstrates high extraction into the myocardium of mice, rats, and rabbits while also displaying efficient clearance from the blood pool. Additionally, [^{68}Ga]Galmydar is recognized by MDR1 P-glycoprotein (Pgp) [4] and Breast Cancer Resistance Protein (BCRP, ABCG2) [5] as their efficient transport substrate, and thus it exploits transporter-mediated excretion pathways from the blood and liver, resulting in high heart/blood and heart/liver ratios 60-minute post injection. Live cell-imaging of rat cardiomyoblasts using Galmydar (fluorescent probe) shows its localization within the mitochondria following permeation within cells indicating excellent correlation with radiotracer uptake data [4,5]. [^{68}Ga]Galmydar micro positron emission tomography (PET)/computed tomography (CT) imaging of rat and rabbit hearts show high myocardial uptake and high target/background contrast. Following ligation of the left anterior descending (LAD) coronary artery in rat and rabbits, PET/CT imaging clearly showed the hypoperfused region of the left ventricle wall. These hypoperfused regions of the myocardium correlated well to the same ex vivo regions by histochemical staining post imaging. [^{68}Ga]Galmydar has also shown sensitivity in monitoring ischemia caused by transient ligation of LAD in rabbits on PET imaging, also with positive correlation of the ischemic region with ex vivo histology. Radiation dosimetry studies in mice extrapolated to human dosimetry estimates using Medical Internal Radiation Dosimetry (MIRD) methodology and employing FDA guidelines (21 CFR 312) of maximum allowable dose to any organ of 50 mSV indicate an allowable maximum injection dose of 8 mCi in humans. This is comparable to ^{18}F-Flurpiridaz, another myocardial perfusion imaging (MPI) agent undergoing Phase 3 studies. Importantly, ^{6}[^{68}Ga]Galmydar does not demonstrate any remarkable clinical pathology (toxicology) in critical organs following intravenous administration of a single dose (1000 fold excess of an IV imaging dose) of an unlabeled agent into rats over 14 days. Finally, compared to ^{13}N-ammonia (a positive control), [^{68}Ga]Galmydar also demonstrates similar radiotracer characteristics and, in particular, comparable myocardial first pass extraction of about 70-90% so that regadenosone-induced stress myocardial perfusion and myocardial blood flow (MBF) quantification employing kinetic modeling should be comparable [6,7,8]

Thus, this study is designed to demonstrate feasibility of [^{68}Ga]Galmydar for the noninvasive detection and characterization of subclinical and clinically-manifest coronary artery disease (CAD) in conjunction with PET by its comparative analysis to ^{13}N-ammonia PET rest and stress studies in human subjects (n=20, 10 males and 10 females). Secondary aims are to evaluate dosimetry, biodistribution, and safety, and imaging characteristics following a single injection in normal healthy volunteers at rest (n=8, 4 males; 4 females).

1.1 Significance

Existing state-of-the art MPI tracers have certain limitations, such as low sensitivity, poor spatial resolution, attenuation artifacts, slow liver clearance, and nonlinearity of MBF at high flow rates.

To overcome these shortcomings, new MPI agents are desired. Utility of PET MPI agents, such as ^{13}N-ammonia, and H2^{15}O is limited due to the requirement for proximity to a multimillion dollar cyclotron. In addition, myocardial perfusion examinations using ^{82}RbCl3 suffer from low first pass extraction, low spatial resolution because of the higher positron range, and short radionuclide half-life due to both potential oversaturation of the detectors at the beginning of the acquisition due to injected high dose, and low count statics and suboptimal signal/noise ratios at later time points. Conversely, Gallium-68 based agents offer tremendous advantages as potential PET MPI agents and are likely to widely resolve these shortcomings.

Using Gallium-68, Galmydar incorporates four important components into its pharmacophore design: a) a rationale built upon exploiting transporter-mediated efflux pathways (Pgp and BCRP substrates) to facilitate excretion from neighboring tissues, such as liver and blood, to allow high heart-to-liver and heart-to-blood ratios enabling high target/ background ratios, b) moderately hydrophobic cationic probes possessing a delocalized positive charge on their molecular surface to allow penetration of myocardium in response to favorable negative plasma- and mitochondrial transmembrane potentials, thereby producing high first-pass extraction and minimal washout from myocardium during imaging sessions, c) a PET agent that incorporates a generator-produced radionuclide, ^{68}Ga (t1/2 = 68 min), to allow production of the radiopharmaceutical independent of the availability of a nearby cyclotron, and d) a potential technology conducive for business models of kit distributions comprising a ligand with a long shelf-life, thereby enabling onsite production for imaging in clinics. These combined elements indicate that further development of [^{68}Ga]Galmydar and its translation is based upon the strong scientific premise.

Also, using both rats and rabbits, micro-PET and nano-single photon emission computed tomography (SPECT) imaging directly showed high heart uptake of [^{67}Ga]Galmydar and rapid clearance from the liver within 30 min post injection, indicating its ability to serve as a potential myocardial perfusion agent for both PET and SPECT. In this regard, the faster kinetics and short half-life of [^{68}Ga]Galmydar may lead to improved dosimetry over ^{18}F-Flurpiridaz, another mitochondrial complex I targeted MPI agent. Moreover, Complex I deficiency, which is clinically and genetically heterogeneous, can present with hypertrophic cardiomyopathy that could either be isolated or associated with other comorbidities. . Therefore, the lower uptake of a given mitochondrial Complex I targeted agent, such as ^{18}F-Flurpiridaz could also potentially be influenced by these complications. Compared with the performance of ^{18}F-Flurpiridaz in these areas, the design of [^{68}Ga]Galmydar is based upon an established and successful biochemical model for development of MPI agents. Importantly, the robust biochemical validation of Galmydar in *cellulo* (radiotracer, and optical imaging assays) and in mice, rat, and rabbit models of both permanent and transient LAD ischemia, with response to pharmacological stress, toxicology studies in rats, and dosimetry studies in vivo, provide us with a sound platform for further versatile molecular imaging probes for quantifying myocardial perfusion and MBF in humans.

1.2 Study Rationale

1.2.1 Dosimetry Group

The successful implementation of [68Ga]Galmydar in humans could provide a new mechanism-driven tracer for PET MPI, and successful realization of aims could ultimately take PET MPI to underserved regions nationwide and developing countries worldwide, where an 18F-distribution business model would have limitations. [68Ga]Galmydar PET MPI could enable quantification of myocardial flow reserve (MFR= stress MBF / rest MBF that carries important diagnostic and prognostic information) that may play an additional important role to evaluate or monitor the effectiveness of preventive medical care or the treatment response on the function of the heart vessels in CAD individuals. These benefits combined with the minimal risks weight the ratio in favor of moving the PET radiotracer [68Ga]Galmydar into human subjects. This component of the study will determine the biodistribution and calculate the radiation dosimetry of [68Ga]Galmydar in normal healthy volunteers. Our radiation dosimetry studies in mice [9] extrapolated to human dosimetry estimates using MIRD methodology suggest an allowable maximum injection dose of 8 mCi in humans. The maximum Effective Dose for one 8 mCi intravenous administration of [68Ga]Galmydar and three whole-body CT scans with CT attenuation scans results in a gender average radiation dose of 2.84 rem that is well within 57% of the allowable annual dose for radiation workers.

1.2.2 Rest/stress Group

A rest/stress myocardial 13N-ammonia PET/CT is clinically indicated for the detection of flow-limiting, obstructive coronary artery disease (CAD). Myocardial perfusion PET provides images of a higher quality than SPECT, due to higher spatial and contrast resolution, higher scanner sensitivity, and accurate attenuation correction. In addition, the concurrent quantification of myocardial blood flow (MBF) affords the assessment of hyperemic MBF during pharmacologic vasodilation and at rest and, thus, the computation of the myocardial flow reserve (MFR= MBF stress/MBF rest). The concurrent assessment of hyperemic MBF and/or MFR aids in the identification and characterization of multi-vessel CAD, microvascular dysfunction, and prognostication.

We will compare the results of [68Ga]Galmydar PET/CT MPI to the results of 13N-ammonia PET/CT MPI in a group of 20 symptomatic patients and 10 normal control subjects without symptomatology. Half of the symptomatic patients recruited will have a SPECT MPI study positive for ischemia in two myocardial segments with referral for invasive coronary angiography (ICA) as part of their routine standard-of-care (n=10), and half with normal SPECT MPI and no ICA referral (n=10). Patients referred for ICA will undergo their [68Ga]Galmydar and 13N-ammonia PET/CT MPI studies prior to intervention. In addition, as a completely normal control, another 10 subjects without symptomatology or known cardiovascular disease will also undergo both 13N-ammonia PET/CT MPI and [68Ga]Galmydar PET/CT MPI. In total 30 patients or normal human subjects will be studied.

1.3 Risks and Benefits

[^{68}Ga]Galmydar shows critical traits desirable in PET MPI. Additionally, despite the fact that concentrations of radiotracer needed for PET imaging are extremely low and rarely a concern, preclinical toxicity studies were conducted and thoroughly investigated according to FDA guidelines for new radiopharmaceuticals. The small animal toxicity report is located in the appendices of the initial IND submission.

In summary, a non-GLP 14-day single dose exploratory toxicity study of Galmydar was performed in male and female Sprague Dawley rats (study number, 2682-207370NI, Innotiv Maryland Heights, Missouri). The toxicity of Galmydar, a radiopharmaceutical diagnostic drug, was studied when administered as a single intravenous (IV) dose to male and female Sprague Dawley rats. Ten animals per sex from each group were euthanized for postmortem examination 24 hours after dose administration and 5 animals per sex from each group were euthanized for postmortem examination on Day 14 after administration.

Each treatment group (Group 1 and Group 2) was comprised of 15 male and 15 female Sprague Dawley rats. On Day 1, rats were administered either the vehicle control (Group 1) or 0.8 mg/kg Galmydar (Group 2) via IV injection at a dose volume of 1 mL/kg.

Physical examinations were conducted prior to randomization into groups. Clinical observations were recorded hourly at 1, 2, and 3 hours post-dose and once daily on Days 2–14. Body weights were measured prior to initiation of dosing on Day 1 and daily thereafter. Mortality observations were recorded once daily (PM observations). Blood samples for the evaluation of clinical chemistry and hematology endpoints were collected on Day 2 and Day 14 from both groups. Following blood sample collections, necropsy was conducted. Protocol-specified tissues were collected and evaluated grossly, select organs were weighed, and tissues were fixed for microscopic evaluation. A subset of fixed tissues were subsequently processed and evaluated microscopically.

Based on the absence of test article-related effects on body weight, clinical observations, clinical pathology or microscopic findings, a single IV administration of 0.8 mg/kg of Galmydar was well-tolerated in male and female Sprague Dawley Rats at both 24 hours (Day 2) and 14 days after dosing.

The findings above decrease the likelihood that human toxicity will be a hurdle. While information in humans is not yet available, our preliminary animal data shows high agreement in MPI performed with [^{68}Ga]Galmydar and ^{13}N-ammonia. The success of this study of [^{68}Ga]Galmydar in human subjects could provide a mechanism-driven tracer for PET MPI, and ultimately take PET MPI to underserved regions nationwide and developing countries worldwide, wherein ^{18}Fluorine distribution business models have limitations, with requirements for nearby cyclotron facilities. Overall, [^{68}Ga]Galmydar PET MPI could enable quantification of myocardial flow reserve that carries important diagnostic and prognostic information and that may play an important role in the

diagnosis of coronary artery disease and in the evaluation and monitoring of the effectiveness of treatment response. These benefits and minimal risks weight the ratio in favor of this hypothesis-driven diagnostic imaging research project.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the proposed study is to evaluate the potential of [^{68}Ga]Galmydar PET/CT MPI for the noninvasive detection of coronary artery disease. We will evaluate [^{68}Ga]Galmydar PET/CT MPI in comparison to ^{13}N-ammonia PET/CT MPI to quantitatively assess myocardial blood flow (MBF) in patients with suspected or known coronary artery disease (CAD) undergoing clinical SPECT MPI. In addition, perfusion and MBF findings with [^{68}Ga]Galmydar will be compared to the results of coronary morphology or stenosis severity in those patients undergoing invasive coronary angiography (ICA) based on abnormal SPECT MPI. The performance of rest/stress [^{68}Ga]Galmydar PET/CT in comparison to ^{13}N-ammonia PET/CT in normal subjects without cardiovascular disease will be performed as a control.

2.2 Secondary Objectives

To evaluate dosimetry, biodistribution, and safety, and imaging characteristics following a single [^{68}Ga]Galmydar injection in normal healthy volunteers at rest.

3 STATEMENT OF INVESTIGATOR

Vijay Sharma, Ph.D, the Sponsor-Investigator of the IND, Pamela K. Woodard, M.D., Principal Investigator, and Thomas Schindler, M.D., Authorized User will comply with the state and federal regulations related to the conduct of all clinical investigations with the administration of [^{68}Ga]Galmydar. A Form FDA 1572 is included with the IND application.

The study protocol will not be initiated before receiving all required regulatory approvals obtained from the Institutional Review Board (IRB)/Human Research Protection Office (HRPO), the Radioactive Drug Research Committee (RDRC), and receipt of the study activation letter from the Food and Drug Administration (FDA).

4 STUDY DESIGN AND METHODS

A single center, phase 0/1 clinical imaging study designed to assess the role of [^{68}Ga]Galmydar PET/CT imaging in human subjects.

4.1 Study Population

We will recruit up to 38 participants for the dosimetry and rest/stress groups. Healthy adult normal volunteers (n=8, 4 males; 4 females) for whole-body imaging (dosimetry group) and patients who had a prior clinical SPECT MPI examination that is positive or negative for ischemia.

Thirty (30) subjects will be enrolled in the rest/stress group. Symptomatic patients (n=20) will have undergone a SPECT MPI examination. Ten patients in this group will have had a study positive for ischemia in two myocardial segments and referred for invasive coronary angiography (ICA) as part of their routine standard-of-care. [^{68}Ga]Galmydar PET imaging will occur prior to intervention. The other ten patients will have a normal SPECT MPI and no ICA referral. Subjects (n=10) who are asymptomatic and without history of cardiovascular disease will serve as a normal controls.

4.2 Inclusion and Exclusion Criteria

In order for participants to be eligible for study enrollment, an individual must meet the inclusion criteria. An individual who meets any of the exclusion criteria will be excluded from participation in the study.

4.2.1 Dosimetry Group

Healthy adult normal volunteers will be enrolled in the dosimetry group.

4.2.1.1 Inclusion criteria

- Healthy men and women, 18–99 years of age and any race;

4.2.1.2 Exclusion criteria

- Inability to receive and sign informed consent;
- Positive urine screen for drugs of abuse at screening or before dosing or over-the-counter drug use or herbal preparations within the 2-week period prior to enrollment;
- Participation in another research study with a study drug, including a diagnostic or therapeutic radiopharmaceutical, to be administered during this study or which was or will be administered within 10 half-lives of the radiopharmaceutical.
- Severe claustrophobia;
- Pregnant or breastfeeding.
- Body mass index < 18 kg/m^2 or > 40 kg/m^2.

4.2.2 Rest/Stress Group

Twenty symptomatic patients will have undergone a SPECT MPI examination. Ten of these patients will have had a study positive for ischemia in two myocardial segments and referred for invasive coronary angiography (ICA) as part of their routine standard-of-care. [68Ga]Galmydar PET imaging will occur prior to intervention. The other ten patients will have had a negative for ischemia SPECT MPI and no ICA referral. Ten healthy adult normal volunteers, who are asymptomatic, without history of cardiovascular disease or significant cardiovascular risk factors, will serve as control subjects.

Asymptomatic normal controls and symptomatic patients who have undergone a SPECT MPI examination that is normal or positive and prior to ICA intervention, will undergo a rest/stress [68Ga]Galmydar PET MPI and rest/stress ^{13}N-ammonia PET MPI with a minimum of a 3-day separation.

4.2.2.1 Inclusion Criteria

- Male and female, 18-99 years of age and any race;
- Have had a prior clinical SPECT MPI positive for ischemia in two myocardial segments and referred for invasive coronary angiography (ICA) or have had a prior clinical SPECT MPI negative for ischemia;

4.2.2.2 Exclusion Criteria

- Inability to receive and sign informed consent;
- Percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG) within 6-months prior to SPECT or in the intervening days between SPECT and PET examination;
- Participants who have received chemotherapeutic agents within 6 months of enrollment;
- Heart failure (left ventricular ejection fraction ≤ 35%);
- Known non-ischemic cardiomyopathy;
- Inability to undergo pharmacologic stress testing with regadenoson (Lexiscan). Contraindications include:
    - Symptomatic bradycardia or second to third degree atrioventricular (AV) block;
    - Pre-existing obstructive lung disease with active wheezing, i.e., COPD, asthma with active wheezing that precludes the safe administration of the pharmacological stress agent according to the approved label;
    - Uncontrolled and severe hypertension (e.g. systolic blood pressure >200 mmHg, diastolic blood pressure >110 mmHg);
    - Baseline hypotension (e.g. systolic blood pressure < 90 mmHg, diastolic blood pressure <50 mmHg);
- Women who are pregnant or breastfeeding;
- Severe claustrophobia;
- Weight ≥ 500 lbs (weight limit of PET/CT table)

- Administration of any diagnostic or therapeutic radiopharmaceutical, not part of this study, within a period either prior to or after equal to 10 half-lives of the radiopharmaceutical.
- Any condition that in the opinion of the Principal Investigator or designee could increase risk to the participant, limit the participant's ability to tolerate the research procedures or interfere with collection of the data such as:
    - Inability to lie still or unable to tolerate a supine position with arms up over the head for up to a 60-minute PET scan due to chronic back/shoulder pain or arthritis as assessed by physical examination and/or medical history;
    - Current or past history of major medical illness, i.e., severe kidney or liver problems;
    - Patients who suffer an intervening clinical event such as worsening angina pectoris or myocardial infarction or whom undergo a myocardial revascularization procedure or have myocardial ischemia at rest;

4.3 Recruitment of Participants

We will recruit healthy adult normal volunteers for the Dosimetry group and Rest/Stress group from the community through the Research Participant Registry/Volunteer for Health (VFH) Program at Washington University in St. Louis (https://vfh.wustl.edu).

Study patients enrolled in the rest/stress group will be for the assessment of the efficacy of ^{68}Ga-Galmydar. Patients referred for routine SPECT MPI in the Division of Nuclear Medicine or from the cardiology inpatient/outpatient clinics at Barnes-Jewish Hospital/Washington University in St. Louis will be recruited.

Participants' electronic health records (EHR) will be accessed to review general health information for contraindications to planned study procedures and confirm pre-eligibility. A study team member will call potential subjects to discuss the study and answer any questions. Participants who verbally agree to participate will be scheduled for study visits.

4.4 Study Enrollment

Upon arrival to the imaging facility, participants will be provided the informed consent for review. The study coordinator will review the informed consent form with the participant and address any questions or concerns prior to obtaining written consent or performing any research procedures. Participants must be willing and able to undergo all procedures.

Imaging will begin with the healthy adult normal volunteers enrolled in the dosimetry group. The first two, (2) normal volunteers will be separated by 2-3 days to ensure participant safety data is ascertained during the post-injection phone follow-up. Upon completion of the review, the study will continue with imaging of the remaining dosimetry subjects with simultaneous entry of the rest/stress group.

4.4.1 Dosimetry Group

Eight healthy adult normal volunteers (4 males and 4 females) will be enrolled in the dosimetry group and undergo whole-body PET/CT imaging with ^{68}Ga-Galmydar.

4.4.2 Rest/Stress Group

For the rest/stress group, we will recruit 30 subjects. The first set will be symptomatic patients (n=20) who have undergone a SPECT MPI examination. Half of these patients will have had a study positive for ischemia in two myocardial segments and referred for invasive coronary angiography (ICA) as part of their routine standard-of-care (n=10). Subjects will undergo [^{68}Ga]Galmydar PET/CT prior to any intervention. The second half of these patients will have had a normal SPECT MPI and no ICA referral (n=10). A second set of subjects who are normal, asymptomatic, and without a history of cardiovascular disease will serve as controls (n=10).

4.5 Early Discontinuation of Participants

The Principal Investigator or designee may prematurely discontinue a participant at any time if the constraints of the protocol are considered to be not in the best interest of the participant, i.e., increased risks jeopardizes the safety of the participant. Reasons maybe related to an adverse event that in the opinion of the Principal Investigator or designee requires the participant should be withdrawn. Other reasons may include non-compliance with study procedures, lost to follow-up, or a positive pregnancy test.

Participants who develop hypertension, hypotension, or other adverse event secondary to the regadenoson administration during the PET/CT MPI stress component and if in the Principal Investigator or designee's opinion the enrolled participant is deemed ineligible to safely undergo the stress ^{13}N-ammonia PET study, the participant may be allowed to continue participation. The stress portion of the ^{13}N-ammonia PET study will be removed. The participant will only undergo the rest portion of the ^{13}N-ammonia PET study.

4.6 Premature Withdrawal and Replacement of Participants

Any participant who no longer wishes to continue participation may withdraw consent at any time and for any reason without prejudice. Withdrawal will not interfere with routine clinical care. Participants are informed to contact a study team member, e.g., study coordinator or by sending a withdrawal letter to the Principal Investigator or designee as listed in the Informed Consent Form. Attempts will be made to discuss with the participant the reasons for withdrawal and the importance of the study evaluations.

Include documentation of reason(s) for discontinuation or withdrawal in the enrollment log. Withdrawn participants are not allowed to re-enroll into the study. Participants who do not complete all protocol-required procedures are replaced to ensure adequate evaluable data.

5 INVESTIGATIONAL STUDY DRUG

5.1 Drug Description

The investigational study drug is ^{68}Ga-Galmydar. Gallium-68 is a positron-emitting radioisotope that is produced from a ^{68}Ge/^{68}Ga generator with a physical half-life of 67.7 minutes. An Investigator's Brochure does not exist for this radioactive drug. The pharmacological properties and formulation are described in detail in the Chemistry, Manufacturing, and Control Data section of the IND application.

5.2 Drug Handling, Preparation, and Release

Handling of all radioisotopes will be conducted under the auspices of the Radiation Safety Office at Washington University School of Medicine (WUSM). [^{68}Ga]Galmydar will be produced under the leadership of the Director of the WUSM Cyclotron Facility, and under the supervision of the Associate Director of the Cyclotron Production and the Director of the Radiopharmacy. Trained personnel perform the preparation and quality control tests for the release of [^{68}Ga]Galmydar under the supervision of the radiopharmacist in charge.

5.3 Drug Administration

The Sponsor-Investigator will adhere to the procedures and requirements in accordance with applicable policies of the Radiation Safety Committee (RSC) at Washington University and the U.S. Nuclear Regulatory Commission (NRC).

A PET-certified medical professional will draw, administer, and assay the dosage in a dose calibrator before and after the administration of all radiotracers.

The Dosimetry group will receive one single intravenous administration of 8 mCi ± 20% (6.4–9.6 mCi) of ^{68}Ga-Galmydar. The Rest/Stress Group will receive two intravenous administrations of ^{68}Ga-Galmydar, 4 mCi ± 20% (3.2–4.8 mCi) during rest and 6 mCi ± 20% (4.8-7.2 mCi) during the stress of [^{68}Ga]Galmydar on the PET MPI Imaging Day-1. The acceptable mass for each [^{68}Ga]Galmydar administration is ≤ 10 µg. On Imaging Day-2, subjects will receive two single intravenous administrations each of 10 mCi ± 20% (8–12 mCi) of N-13 Ammonia.

5.3.1 Dosage Variance

If a required administered dosage is outside the allowable range because of unforeseen circumstances, i.e., lack of radiopharmaceutical activity, an authorized user physician before administration of the tracer must approve a dosage in writing or verbally as a variance. The authorized user must provide written approval to the imaging facility within 48-hours after a verbal approval. All members of the research team will comply with the Washington University Radiation Safety Committee Policy on Dispensing of Radiopharmaceutical Dosages for Human Research Subjects.

6 STUDY PROCEDURES

The Schedule of Activities (SoA) tables for the Dosimetry group and Rest/Stress group summarizes the study assessments and procedures, see Attachments. The results of all assessments and evaluations will be recorded on the case report forms. The PI or sub-investigator delegated by the PI will review screening and evaluations to confirm eligibility criteria of all enrolled subjects. The research study team will be maintain study logs, e.g., screening and enrollment logs of participants with details of screen failures, dropouts and adverse event log.

6.1 Screening Assessments

The coordinator will review the informed consent form and address questions or concerns with the participant and family/friends. Relevant medical history includes current and past diseases/illnesses and concurrent medications, as well as surgical and family medical history. A brief physical exam will be conducted by the Principal Investigator or designee. A designee is one who is a qualified, engaged member and protocol trained to assess participants. Exams are performed at baseline prior to the [^{68}Ga]Galmydar injection to determine if the participant is suitable to undergo study procedures and for subject readiness prior to discharge from the imaging center.

Screening will include the following procedures and evaluations to ensure participants meet inclusion and exclusion criteria.

- Written informed consent will be obtained prior to performing any research procedures;
- Demographics, including gender, date of birth, race, and ethnicity;
- Medical history, including family history and surgical history (e.g., pre-existing conditions and shoulder or chest trauma) as obtained from participants and available medical records, as described below;
- PET/CT contraindications;
- Concomitant medications review, including non-prescription medicines, vitamins, herbs, and supplements;
- Body weight and height measurements;
- A brief physical exam;
- Vital sign measurements, including systolic and diastolic blood pressure, heart rate, and respiratory rate, body temperature;
- 12-lead ECG;
- For women of childbearing potential, a urine pregnancy test must be completed, documented, and confirmed as negative within 24-hrs or on the day of the PET scan. Confirm participant is not breastfeeding and agrees not to become pregnant (refrain from sexual activity or is currently using a medically approved method of contraception) to continue for at least 24-hours post-injection of ^{68}Ga-Galmydar;

- Clinical Laboratory Testing: clinical safety labs includes a complete blood count, comprehensive metabolic panel, and urinalysis. All participants will have up to two IV catheters inserted one in each arm. In dosimetry patients, one IV will be used for the collection of blood samples for the lab tests to include serum metabolites, and the second will be used for the administration of ^{68}Ga-Galmydar. For patients enrolled in the rest/stress group, one IV catheter will be used for the [^{68}Ga]Galmydar administration and the second IV will be used for the administration of the pharmacological stress agent, regadenoson.

6.2 PET/CT Imaging

Imaging will be conducted on the preferred scanner, the Siemens Biograph Vision PET/CT. The Biograph TruePoint/TrueView may be used alternatively in the event of a schedule conflict or scanner problem. The scanners are located adjacent to each other in the Center for Clinical Imaging Research (CCIR) at the Washington University School of Medicine (WUSM) and Barnes-Jewish Hospital (BJH) in St. Louis.

The CCIR allows participants to undergo research-related imaging protocols in a safe environment. The CCIR is fully equipped for the treatment of medical emergencies, including cardiac arrest (crash-cart, intubation equipment, and defibrillator), and is served by BJH emergency code team. The CCIR Facility operates in compliance with the Joint Commission on Accreditation of Healthcare Organization (JCAHO), and the American College of Radiology (ACR). All facilities operate in compliance with the safety guidelines for imaging and for privacy as directed by the Health Insurance Portability and Accountability Act of 1996 (HIPAA) and the Nuclear Regulatory Commission (NRC).

PET radiopharmaceuticals will be produced at Washington University School of Medicine (WUMS)-Mallinckrodt Institute of Radiology (MIR) Cyclotron Facility. For investigational PET drugs for human use produced under an Investigational New Drug (IND) application in accordance with 21 CFR Part 312. WUMS MIR Cyclotron Facility follows the requirement for production of research radiopharmaceuticals in 21 CFR Part 212, Current Good Manufacturing Practice (CGMP) For Positron Emission Tomography Drugs, by complying with the regulations in Chapter <823>, *Radiopharmaceuticals for Positron Emission Tomography (PET)—Compounding, Investigational, and Research Use*s.

6.2.1 PET Imaging Assessments and Preparation

Subjects will be instructed to fast overnight for a minimum of 6-hours prior to the scheduled appointment time. The Rest/Stress group subjects who undergo the stress PET MPI will be instructed to avoid theophylline-containing medication or dipyridamole for at least 48 hours prior to the stress imaging scan. Vasoactive medications such as calcium-channel blockers, angiotensin-converting enzyme inhibitors, β-blockers, and nitrates should be discontinued at least 24 hours before the PET perfusion examination.

The following assessments and procedures will be performed for PET imaging visits:

- Confirm eligibility. Review concomitant medications and any changes in health since the screening if assessments were performed on a separate day. Repeat between imaging days;
- Confirm or perform and document a negative urine pregnancy test for women of childbearing potential for each imaging day;
- Subjects will be requested to use the restroom to empty their bladder prior to radiotracer injection and discharge from the imaging facility;
- Place one peripheral intravenous (IV) catheter for the administration of radiotracer injection and draw blood samples for safety labs. Both the dosimetry group and the rest/stress group will have a second contralateral IV catheter placed. In the dosimetry group, one IV will be used for collection of safety labs and radiotracer metabolites and one IV will be used for the administration of [^{68}Ga]Galmydar. In the rest/stress patients, one IV will be used for the administration of [^{68}Ga]Galmydar and one for the administration of the pharmacological stress agent, regadenoson;
- Collect blood and urine samples for clinical safety lab tests for [^{68}Ga]Galmydar participants at screening to be used for a baseline pre-injection measurement and repeated after the imaging prior to discharge post [^{68}Ga]Galmydar injection and;
- Perform a brief physical exam for [^{68}Ga]Galmydar participants. The screening exam may be used for baseline pre-injection measurement and repeated post [^{68}Ga]Galmydar administration;
- Place the participant on the imaging table in supine position with both arms positioned above the head. To ensure participant comfort, place a pillow or folded towels under elbows and pillows or foam wedge under legs of the participant to maintain their position for the duration of the scan;
- Place electrodes on the participant's chest for continuous ECG monitoring and capturing of 12-lead ECG. Place a blood pressure cuff on the opposite arm used for the administration of the radiotracer. Continue to monitor ECG-rhythm and HR until imaging is completed;
- Obtain vital sign (VS) measurements and 12-lead ECG at the time points listed below and repeat as needed for [^{68}Ga]Galmydar participants. VS include heart rate, respiratory rate, and diastolic and systolic blood pressure, body temperature. Obtain vital signs (BP and HR) and 12-lead ECG as required per clinical protocol for the rest/stress Ammonia PET MPI study.
    - Baseline: VS (BP, HR, RR, and body temperature) and a 12-lead ECG within 60-min prior to injection, repeat if >60-min;
    - Pre-injection: BP, HR, and RR within 15-min;
    - Post-injection: BP, HR, and RR within 5-min and 15-min, and hourly during imaging visit;
    - End of imaging scan: BP, HR, and RR;
    - Prior to Discharge: VS (BP, HR, RR, and body temperature) and a 12-lead ECG.

- Instruct participants to not move arms, head, or bend knees throughout the study to reduce motion artifacts;
- To reduce respiratory motion on the CTAC and stabilize heart rate, have participant lie quietly for 2-3 minutes before acquiring scout image;
- Participants will undergo a CT scan for attenuation correction (CT-AC) (50 mAs). Prior to acquiring each CT-AC, provide participant specific instructions to *not* take deep breaths during the CT-AC acquisition. This is to reduce respiratory motion on each CTAC. During the CT-AC scan, the participants will be instructed to perform normal end-expiration breath-holds;
- After completion of the PET/CT study, alignment of the heart as seen on the PET and the CT images should be inspected. In the case of a misalignment, the heart should be re-aligned manually and image reconstruction repeated to ensure there are no artifacts of attenuation correction created by mis-registration of the CT and PET data. Images will be inspected by the technologists;
- Prior to discharge from the CCIR to home or transfer to the CTRU, confirm participants' contact information for the phone follow-up. Provide the participant study team contact information, (i.e., phone number) should an untoward reaction occur;
- *For all regadenoson stress PET MPI studies:*
    - A physician (cardiologist) will be present;
    - Obtain 12-lead ECG, BP and HR pre and post regadenoson injection.
    - Obtain BP and HR every minute for at least 10-min post regadenoson injection and monitor ECG rhythm strip.
    - Aminophylline will be readily available to attenuate severe or persistent adverse reactions. Administer slow intravenous injection (50 mg to 100 mg over 30 to 60 seconds) at least 1-minute after radiotracer injection.

6.3 Dosimetry Group - PET/CT Imaging

Healthy adult normal volunteers enrolled in the dosimetry group will come to the Center for Clinical Imaging Research (CCIR) facility, a Washington University facility, located on the 10th floor of Barnes-Jewish Hospital for screening and PET imaging.

The following assessments and procedures will be performed:

- VS, 12-lead ECG, safety labs, and a brief physical exam pre and post injection and prior to discharge from the CCIR to home or transfer to the CTRU as described above;
- All required safety assessments prior to the administration of [^{68}Ga]Galmydar and monitor ECG rhythm and HR as described above. Monitor ECG-rhythm and HR during imaging;
- Administer an intravenous bolus injection of 8 mCi ± 20% (6.4–9.6 mCi) with a mass of ≤ 10 µg of [^{68}Ga]Galmydar followed by a 10 mL normal saline flush;
- Whole-body PET image acquisition from head to mid-thigh. Imaging of subjects will be at three time points spanning from immediately after injection up to 4-hours after

[68Ga]Galmydar injection. A low-dose spiral CT scan (120 kVp, 50 mAs) will be obtained for attenuation correction. Begin whole-body PET image acquisition from the head to mid-thigh immediately post [68Ga]Galmydar injection for 60-min and repeat at 2 hours and 4 hours.

6.4 Rest/Stress Group - PET/CT Imaging

On Imaging Day-1, all participants enrolled in the rest/stress group will come to the CCIR facility for < 24-hours for screening, pre and post rest/stress [68Ga]Galmydar PET MPI safety assessments.

On Imaging Day-2, all participants will report directly to the CCIR facility for the rest/stress 13N-Ammonia PET MPI. [68Ga]Galmydar and 13N-ammonia imaging visits will be separated by a minimum of 3-days and not before the follow-up phone call is complete for capturing participant adverse events following the administration of 68Ga-Galmydar.

In some instances, participants may be split the Day-1 imaging into two visits and complete a Day-3 study. Therefore, on imaging Day-1, participants enrolled in the rest/stress group will come to the CCIR for screening, pre [68Ga]Galmydar PET MPI safety assessments and the rest imaging. On Day-2 participants will return to the CCIR for their stress imaging and post [68Ga]Galmydar PET MPI safety assessments. On Imaging Day-3, participants will report to the CCIR facility for the rest/stress 13N-Ammonia PET MPI.

6.4.1 Imaging Day-1, Rest/Stress [68Ga]Galmydar PET MPI

6.4.1.1 Rest [68Ga]Galmydar PET

- Perform all required safety assessments pre-injection of [68Ga]Galmydar and monitor ECG rhythm and HR as described above. Obtain baseline VS and ECG (BP, HR, and RR) within 15-min [68Ga]Galmydar pre-injection;
- Administer an intravenous bolus injection of 4 mCi ± 20% (3.2–4.8 mCi) with a mass of ≤ 10 μg of [68Ga]Galmydar followed by a 10 mL normal saline flush.
- Begin up to a 30-min list-mode data acquisition for a single bed position at the level of the heart immediately post-injection;
- Remove subject from the scanner. Transfer participant to CTRU for a break.
- The interval between the rest and stress [68Ga]Galmydar PET acquisitions *should be no less than 2 hours and no greater than 4 hours.*

6.4.1.2 Stress [68Ga]Galmydar PET

- Perform all required safety assessments pre-injection of regadenoson and [68Ga]Galmydar as described above. Monitor ECG rhythm and HR continuously during the PET scan;
- Administer a rapid (10-sec) regadenoson intravenous injection (0.4 mg/5 mL), followed by 5 mL saline flush;

- 30-sec post regadenoson injection, administer a maximum intravenous bolus injection of 6 mCi ± 20% (4.8-7.2 mCi) with a mass of ≤ 10 μg of [^{68}Ga]Galmydar followed by a 10 mL normal saline flush. If the same IV line is used for both the regadenoson and radiotracer injection, wait 10 to 20 seconds after the normal saline flush post regadenoson injection before injecting the radiotracer;
- Begin up to a 30-min list-mode data acquisition for a single bed position at the level of the heart immediately post-injection;
- Perform all required safety assessments post-injection of ^{68}Ga-Galmydar, end of imaging and prior to discharge from the imaging facility as described above;
- Transfer participant to CTRU for post imaging assessments and procedures prior to discharge as described above.

6.4.2 Imaging Day-2, Rest/Stress ^{13}N-Ammonia PET MPI

[^{68}Ga]Galmydar and ^{13}N-ammonia imaging visits will be separated by a minimum of 3-days but not before the participant follow-up phone call is complete for capturing adverse events following the administration of ^{68}Ga-Galmydar. Participants will report to the CCIR. The PI, study cardiologist or trained designee will evaluate symptomatic patients to confirm there has been no change in clinical status since the [^{68}Ga]Galmydar PET MPI. If in the judgment of the investigator/designee the patient's clinical condition has changed since the [^{68}Ga]Galmydar imaging day, the patient may undergo the rest only ^{13}N-ammonia PET imaging scan or be rescheduled for Imaging Day-2, ^{13}N-ammonia PET MPI.

6.4.2.1 Rest ^{13}N-Ammonia PET

- Review of concomitant medications and any changes in health between the rest/stress PET MPI imaging day-1, ^{68}Ga-Galmydar, and imaging day-2, ^{13}N-ammonia;
- Confirm a negative urine pregnancy test for women of childbearing potential;
- Obtain baseline vital signs (BP and HR) pre and post ^{13}N-ammonia injection as described above. Continue to monitor ECG-rhythm and HR until imaging is completed;
- Administer an intravenous bolus injection of 10 mCi ± 20% (8–12 mCi) of ^{13}N-ammonia followed by a 10 mL normal saline flush.
- Begin a 10-min list-mode data acquisition (if the anterior-posterior diameter of the chest is >50cm increase to 15-min) for a single bed position at the level of the heart immediately post-injection;
- Obtain vital signs (BP and HR) and 12-lead ECG as required per clinical protocol;
- Remove patient from scanner for a break. The interval between the rest and stress ^{13}N-ammonia acquisitions should be per the clinical protocol (*no less than 1-hour*).

6.4.2.2 Stress ^{13}N-Ammonia PET

- Obtain baseline vital sign measurements and 12-lead ECG pre-injection of regadenoson or ^{13}N-ammonia administrations. Continue to monitor ECG-rhythm, and HR, until stress PET MPI is completed;
- Administer regadenoson intravenous injection (0.4 mg/5 mL) over 10 sec, followed by 5-10 mL saline flush;
- Obtain vital signs (BP and HR) and 12-lead ECG as required per clinical protocol;
- 30 sec post regadenoson injection, administer an intravenous bolus injection of 10 mCi ± 20% (8–12 mCi) of ^{13}N-ammonia followed by a 10 mL normal saline flush. If the same IV line is used for both the regadenoson and radiotracer injection, wait 10 to 20 seconds after the normal saline flush post regadenoson injection before injecting the radiotracer;
- Begin a 10-min list-mode data acquisition (if the anterior-posterior diameter of the chest is >50cm increase to 15-min) for a single bed position at the level of the heart immediately post-injection;
- Obtain vital signs (BP and HR) as described above prior to discharge.

7 SAFETY ASSESSMENTS

Safety will be evaluated by monitoring changes from screening or baseline assessments in vital signs, physical findings, electrocardiograms, clinical laboratory tests values, and the incidence of adverse events (AE). AEs will be reviewed for potential significance and clinical importance.

7.1 Clinical Laboratory Tests

The Department of Laboratories, Barnes-Jewish Hospital, is an accredited and Clinical Laboratory Improvement Act (CLIA) certified lab that will perform all laboratory blood tests for this study. A list of the current established reference ranges are available on the General Information page of the Barnes-Jewish Hospital (BJH) Lab website, https://bjhlab.testcatalog.org/.

All participants will have baseline blood and urine samples obtained for screening and for comparison with post-imaging complete blood count, comprehensive metabolic panel, and urinalysis. For abnormal changes from baseline, the Investigator or designee will determine if additional assessments for clinical significance or change in participant management if needed. Any adverse events/serious adverse events must also be documented in the case report forms (source documents) and appropriately reported.

Participants may request to receive a copy of their lab results or their rest/stress ^{13}N-Ammonia PET MPI results to become part of their medical record at Barnes-Jewish Hospital.

7.2 Vital Sign Measurements

Vital sign (VS) measurements will be obtained at screening to determine eligibility, at baseline within 60-min, within 15-min pre-injection and within 5 min, 15-min, and hourly post-injection of

68Ga-Galmydar, and end of imaging prior to discharge. VS measurements may be repeated as needed. Follow standard-of-care stress regadenoson and rest imaging clinical protocol for the N-ammonia imaging. VS measurements include heart rate (HR), systolic and diastolic blood pressure (BP), respiratory rate (RR), and body temperature. Temperature will be taken at baseline and prior to discharge on imaging days and as needed. Time points listed may only include BP and HR; see Imaging PET Assessments section for details. BP and HR must be obtained at least once prior to injection and once post-injection before discharge with the participant in the supine position. Care will be taken to obtain subsequent recordings with the participant in the same position (supine or upright).

The following variables are considered clinically significant if changes occur from baseline. A systolic BP of < 90 or > 160 mmHg or a diastolic BP of < 50 or > 100 mmHg or a 20 mmHg change from baseline in the SBP or DBP. A heart rate of < 50 BPM or > 100 or a 20 BPM change from baseline. A respiratory rate of < 12 or > 20 breaths/min and oral temperature of >100ºF.

Participants who develop bradycardia (heart rate < 40 BPM, Mobitz 2:1 AV Block (Type 1 or Type 2) or QTc ≥ 500ms) will be monitored until heart rate and conduction returns to baseline. Any abnormalities that meet the definition of an adverse event/ serious adverse event must be documented and appropriately reported.

7.3 12-lead electrocardiogram

A standard 12-lead electrocardiogram (ECG) will be obtained at screening for all participants. Baseline is within 60-min pre-injection, within 5-min post-injection, and prior to discharge, and repeated as needed. For the Rest/Stress group, ECGs will be obtained per standard-of-care rest/stress regadenoson imaging clinical protocol. A study cardiologist delegated by the PI will review and interpret the ECG, sign, and date.

The following variables are considered abnormal if changes occur from baseline. A PR interval of <120 or >200 msec, QRS interval <50 or >100 msec, RR interval <600 or >1000 msec, or QT interval ≥500 msec. Participants who develop second or third degree AV block and/or QTc ≥500 post-injection, will be monitored until the ECG returns to normal and will be excluded from additional study procedures. Any abnormalities that meet the definition of an adverse event/ serious adverse event must be documented and appropriately reported.

7.4 Follow-up

Study staff will contact dosimetry and rest/stress participants by phone following the administration of [68Ga]Galmydar to confirm the subject's well-being and to ascertain if an adverse event (AE) occurred post-imaging procedures. If any of the specified days are not business days, the phone call may occur the following business day. In the event of a serious AE or unanticipated problem, we will ask participants to return to the imaging facility for evaluation/safety visit. Follow-up may

consist of an in-person visit(s) or additional telephone contact. Study staff may access medical records during the study to follow AE/problems that developed because of study participation. We will follow all participant AEs until resolution or stabilization, as evaluated by the PI or designee. Study participation is complete at the end of the follow-up or the final monitoring visit of serious AEs.

7.4.1 Dosimetry group

Dosimetry subjects will receive a phone call within 2-3 days and repeated at 5-7 days or as late as 14 days post [68Ga]Galmydar injection.

7.4.2 Rest/Stress group

Rest/stress participants will receive a phone call within 2-3 days post [68Ga]Galmydar injection. The follow-up phone call to collect safety data must be complete before scheduling a participant for the rest/stress 13N-ammonia PET imaging visit.

8 IMAGE PROCESSING AND ANALYSIS

8.1 Rest Stress Group

8.1.1 PET Images

All images will be reconstructed at 5-min (a delay to allow for blood-pool clearance) using reconstruction matrix at 168 × 168 × 109, with a pixel size (2 × 2 × 2 mm). Scatter, decay, and random corrections will also be applied to the reconstructed images. Static myocardium perfusion and gated myocardial function images will be generated by skipping the first 5 minutes of the list mode data acquisition. Dynamic image sequence will be reconstructed for quantitative blood flow evaluation. Comparative quantitative blood flow will be evaluated with Carimas and also using QPET or 4DM software[65] available through our departmental image processing servers (MIM/Vista and Hermes). Myocardial image processing techniques will then be applied to the gated datasets. The myocardial wall segment analysis will be performed using the 17-segment American Heart Association model[66].

8.1.2 MPI Interpretation

All images will be interpreted and blindly scored by a board certified radiologists (Drs. Schindler, Woodard, and Gropler) in nuclear medicine. Both overall qualitative diagnosis and semi-quantitative 17 segment with 5 point (0=normal, 4=absent tracer uptake) scoring will be employed in the independent blind read out by 3 expert radiologists in PET MPI interpretation. To maintain consistency, the same three readers will interpret all PET studies in independent reading sessions. Importantly, these readers will be blinded to type of radiotracer, the presence or absence of stress agent, and all clinically relevant data. Image quality will also be classified as excellent, good, fair, poor, or uninterpretable. For purpose of assessing diagnostic efficacy, each blinded reader will rate the rest/stress perfusion and gated images as normal, ischemic, ischemic and scar, or scar. A

patient will be defined as MPI negative if the rating will be normal. Summed stress scores (SSS), summed rest scores (SRS), and summed difference scores (SDS) will also be calculated [64]. Absolute regional myocardial blood flow will also be reported.

8.1.3 Coronary Angiography Interpretation

All coronary angiograms will be interpreted using quantitative coronary analysis (QCA). Separate research analysis of the anonymized data will be performed. A coronary stenosis will be considered present when there is = 50% diameter stenosis in any epicardial coronary artery. The presence of one or more coronary stenosis will be used to define the presence of significant CAD. Presence or absence of wall motion abnormality will not be considered in interpreting the presence or absence of CAD. The determination of the presence or absence of CAD will be strictly related to coronary stenosis in the native coronary arteries; arteries with patent stents will be classified as no significant CAD, regardless of evidence of prior myocardial infarction. Arteries with bypass grafts but native coronary stenosis will be classified as significant CAD.

8.1.4 Kinetic Modeling for Imaging Quantification to Calculate Myocardial Uptake, MBF, and Retention

Carimas software incorporates the needed compartmental models suitable for this study. [^{68}Ga]Galmydar MBF (at rest and stress) will be quantitatively analyzed using a 1-compartment tracer kinetic model as used previously for other mitochondrial targeted agents.[52] The standard 1-compartment model is governed by two rate constants: K1 represents activity from blood to myocardium, and is related to blood flow and K2 characterizes the activity from myocardium to the blood. In general, blood flow is related to K1 by a flow-dependent extraction fraction with a relationship of the form: K1 = EF (Extraction Fraction).MBF with EF = (1-exp(-(αMBF + β)/MBF) with α and β being tracer dependent parameters. Importantly, [^{13}N]-NH_3 is a validated quantitative blood flow tracer which will be modeled using the 2-compartment model described earlier. Further, [^{13}N]-NH_3 shows first pass extraction of 90% and will serve as a reliable reference control. In both of these models, the effects of partial recovery of the counts in the myocardium and spill-over from the blood pool have been fitted along the model parameters.

To investigate the relationship between the radiotracer extraction and flow, the tracer-derived MBF data will be plotted against both the absolute microsphere-derived MBF values and ^{13}N-ammonia MBF values. This relationship will allow us to characterize the flow dependent extraction fraction for the radiotracer. We will first determine K1 from compartmental modeling and then determine the extraction fraction parameters (α and β) by relating [^{68}Ga]Galmydar K1 to radioactive microsphere MBF and [^{13}N]-NH_3 derived MBF. We will then compare and analyze this extraction fraction to flow dependent extraction of other known MBF tracers, such as ^{99m}Tc-sestamibi, ^{99m}Tc-Tetrafosmin, ^{201}Tl or ^{82}Rb. Furthermore, [^{68}Ga]Galmydar excretes from blood pool much faster than ^{99m}Tc-sestamibi due to low logP value, and heart/blood (5min/60 min) ratios in mice are also superior to that of ^{18}F-Flurpiridaz ([^{68}Ga]Galmydar: %ID/g; 12.0/0.1= 120 REF; ^{18}F-BMS-747158: 9.5/0.5 = 1914; 120/19 = 6.3 folds), these data point to superior flow dependent

extraction fraction of [^{68}Ga]Galmydar than ^{99m}Tc-sestamibi. Furthermore, the MFR (myocardial flow reserve), which is defined as the ratio of maximal to basal MBF, will be compared between the myocardial retention ($MFR_{retention}$) and SUV-derived data (MFRSUV) versus both either [^{68}Ga]Galmydar MFR ($MFR_{Galmydar}$) or ^{13}N-Ammonia MFR ($MFR_{Ammonia}$). Because myocardial perfusion will be homogeneous, only mean values in the left ventricle will be used in the analysis. These proposed experiments will allow us to accurately measure this factor in an animal model closer to an anatomical reference for application in humans thus providing modeling paradigms to enable quantification of the myocardial blood flow in humans. MBF will also be evaluated with clinically approved software such as QPET or 4DM software[65] available through our departmental image processing servers (MIM/Vista and Hermes).

9 STATISTICAL PLAN

Continuous variables will be expressed as mean (or median) and range or standard deviations. Categorical variables will be expressed as frequencies. The diagnostic efficacy of [^{68}Ga]Galmydar and ^{13}N-ammonia PET MPI will be assessed following blinded and independent reads of PET MPI data using ICA as the truth standard. Analysis of diagnostic efficacy will be performed by the reader. The analysis of diagnostic certainty will be a modified majority rule in which the median rating will be used when three readers have 3 different ratings. Similarly, summaries and comparisons of Summed Stress Score (SSS), the Summed Rest Score (SRS), and the Summed Difference Score (SDS) measures will use the median of the results of 3 blinded readers. The sensitivity and specificity and corresponding 95% confidence interval of each radiotracer will be reported. Normalcy rate will be determined in the patients with a low likelihood of CAD and no ICA and be calculated as % of patients with normal/probably normal studies. Agreement for the diagnostic efficacy (presence/absence of CAD) between [^{68}Ga]Galmydar and ^{13}N-ammonia PET MPI will be compared using a kappa statistic. The agreement between the quality of scan will be assessed using a weighted kappa and the SUV uptake will be assessed using an intra-class correlation coefficient. The ROC analysis will be performed using 17 segment model (according to the American Heart Association Guidelines) to assess the diagnostic efficacy in detection of obstructive coronary disease. The area under ROC and confidence intervals will use the empirical approach developed by DeLong, et al.[68] The inter-reader agreement between 3 blinded readers will be estimated by comparison of dichotomized ratings (normal/abnormal) and presented using kappa and percent agreement. Intra-reader agreement in each of the reader will be performed by randomizing 10% of the repeat images in the blinded read and will be estimated using kappa and percent agreement. All tests will be two-sided and a significance level of 5%.

9.1 Sample size justification

While information in humans is not available, our preliminary animal data shows high agreement between [^{68}Ga]Galmydar and ^{13}N-ammonia. Therefore, we expect to see strong agreement between the diagnostic efficacies of these two radiotracers in this sample of patients. A sample size of 20 patients results in a two-sided 95% confidence interval with a width of 0.26 (lower

limit=0.669, upper limit=0.931) assuming strong agreement between the radiotracers (a kappa of 0.8) and a standard deviation of 0.3. Sample size calculations were conducted in PASS v14.

10 REGULATORY AND SAFETY REPORTING

10.1 Regulatory Compliance

The Sponsor-Investigator and Principal Investigator will conduct the protocol in adherence to the guidelines set forth by the Institutional Review Board (IRB) /Human Research Protection Office (HRPO) and the Radioactive Drug Research Committee (RDRC)/Radiation Safety Office at Washington University and the U.S. Food and Drug Administration (FDA). The Sponsor-Investigator will ensure members of the research team will comply with the adverse event reporting as described below.

The Principal Investigator, Sponsor-Investigator, Authorized User, and members of the research team will promptly review all available safety information relevant to the safety of the drug, including any findings from another source not related to this study that suggest a significant risk for human subjects. Subjects will be instructed to inform a member of the research team as soon as possible should they experience symptoms of an adverse event to begin an adverse event evaluation. AE follow-up will occur as ordered by the Principal Investigator or designee. Additional follow-up will occur until the abnormal measurements return to baseline or acceptable limits, or until monitoring is no longer warranted or stabilization as evaluated by a physician. A study team member will review the subject's medical record for clinical history and, if applicable, monitor for test results as made available.

10.2 Safety Event Definitions

For this study, the following standard definitions for reporting will be used:

10.2.1 Investigational Drug

The investigational drug named [^{68}Ga]Galmydar is a radioactive drug held under an Investigational New Drug (IND) application.

10.2.2 Adverse Event

Adverse Event (AE) is any untoward medical occurrence in human subjects who receive the investigational radioactive drug ^{68}Ga-Galmydar, whether or not considered related to the drug. An AE (also referred to as an adverse experience) may include any unfavorable and unintended sign (i.e., abnormal lab test finding), symptom, or disease temporally associated with the use of the drug, and does not imply any judgement about causality.

10.2.3 Suspected Adverse Reaction

A suspected adverse reaction is any adverse event that is determined that there is a reasonable possibility that the investigational drug caused the adverse event. For IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

10.2.4 Adverse Reaction

Adverse Reaction means any adverse event caused by the investigational drug. Adverse reactions are a subset of all suspected adverse reactions where there is a reason to conclude that the drug caused the event.

10.2.5 Serious Adverse Event

An adverse event or suspected adverse reaction is considered serious if the event or reaction, in the view of the Principal Investigator, results in any of the following serious outcomes:

- Death,
- A life-threatening adverse event as defined as placing the participant at immediate risk of death from the AE as it occurred,
- Initial or prolonged inpatient hospitalization (hospital admission defined as ≥ 24 hours or prolongation of a hospital stay due to adverse event),
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- Congenital anomaly or birth defect.

10.2.6 Life-threatening

An adverse event or suspected adverse reaction is “life-threatening” if, in the view of the Principal Investigator, its occurrence places the participant 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.

Important Medical Events (IMEs) are not immediately life-threatening, nor do they place a participant at immediate risk of death or require hospitalization. Examples of IMEs include allergic bronchospasm requiring intensive treatment in the emergency room or at home, or convulsions that do not result in inpatient hospitalization. IMEs may be serious if in the judgement of the Principal Investigator or designee they require medical or surgical intervention to prevent one of the above serious outcomes.

10.2.7 Unexpected

An adverse event or suspected adverse reaction is unexpected if is not listed at the specificity or severity that is currently

observed, or the event is not consistent with the risk information described in the protocol, informed consent form, general investigational plan or elsewhere in the current IND application. An Investigator's Brochure does not exist nor is it required for this IND. The Principal Investigator or designee will determine if an adverse event is unexpected.

Expected adverse reactions are adverse events that are known to occur for the investigational drug and should be collected in a standard, systematic format using a severity grading scale. Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study drug. For risk related to this IND protocol, see Expected Risks section below.

10.3 Causality and Severity Assessment

The Principal Investigator or designee will make a clinical determination as to the likelihood that an AE is related to the administration of the radioactive drug or research visit procedure. Other etiologies such as pre-existing conditions, concomitant therapy, study-related procedures, accidents that occur while on study, and other external factors will be considered during the evaluation of relatedness.

Pre-existing Conditions that are present at the time of informed consent will be considered participant baseline. All baseline conditions will be recorded on the medical history in the source document forms. Worsening of the severity or frequency of a pre-existing condition in a research participant which does not necessarily have a causal relationship with the investigational drug is a reportable adverse event. Any changes, i.e., deterioration or worsening in these condition(s) shall be documented and evaluated by the Principal Investigator or designee. AEs with signs and symptoms that are believed to be due to the pre-existing condition(s) will not be reported as an AE unless there is an increase in frequency and severity.

10.3.1 Severity/Intensity

An adverse event will be classified as one of the following to describe the severity (intensity) and any necessary action taken in an attempt to resolve the event:

- **Mild**: A mild AE is usually transient in nature and generally not interfering with normal activities. Participants are asymptomatic or have mild symptoms; intervention is not indicated.
- **Moderate**: A moderate AE is sufficiently discomforting to interfere with normal daily activities. Participants may need minimal, local, or noninvasive intervention;

- **Severe**: Also referred to as medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization is indicated; disabling; limiting self-care activities of daily living (ADL). Note that a severe event is not necessarily a serious event.

10.3.2 Attribution

The degree of certainty about causality will be graded using the following relationship categories:

- **Unrelated**: An adverse event is clearly not related to the drug or procedure.
- **Unlikely Related**: An adverse event is doubtfully related to the drug procedure.
- **Possibly Related**: An adverse event is may be related to the drug or procedure.
- **Probably Related**: An adverse event is likely related to the drug or procedure.
- **Definitely Related**: An adverse event is clearly related to the drug or procedure.

For the purposes of regulatory reporting, a causality assessment of unlikely related will be managed as "unrelated." An adverse event that is definitely, probably, or possibly related will be managed as "related" and considered a "reasonable possibility" that the drug caused the adverse event and, therefore, meets the criteria for reporting to the FDA.

10.4 Documenting Adverse Events

All serious and non-serious adverse events as well as abnormal test results/findings, regardless of suspected causal relationship will be documented in the subjects' research record. Complete appropriate reporting forms according to the guidelines set forth by the Institutional Review Board (IRB) at Washington University Human Research Protection Office (HRPO) and the U.S. Food and Drug Administration (FDA).

Adverse events that are classified as reportable will be followed until resolution or stabilization as evaluated by a physician. Any new relevant clinically significant findings/abnormalities or changes from the subjects' baseline that meet the definition of an adverse event must also be recorded and documented as an adverse event. All clinically significant changes, relevant medical history and concomitant medications and action taken will be documented in the subjects' case report forms (source document forms). Events that which do not meet the requirements of SAE reporting will be reviewed and confirmed by the Investigator and reported with the IRB annual continuing review, Data Safety and Monitoring report and the FDA annual report.

The investigator's clinical determination of any adverse event will be recorded and maintained in the subjects' research record. All AEs shall be documented on the adverse event log maintained in the regulatory binder.

10.5 Food and Drug Administration Mandatory Reporting

The Principal Investigator and Sponsor Investigator will promptly review all study information relevant to the safety of the drug obtained or otherwise received from any source, foreign or domestic, including information derived from clinical investigations, animal investigations,

commercial marketing experience, reports in the scientific literature, and unpublished scientific papers. The Principal Investigator and Sponsor Investigator will identify previously reported similar adverse events in IND safety reports and analyze the significance of the current event in light of the previous reports.

The Sponsor-Investigator must notify the Food and Drug Administration (FDA) of any adverse events that are observed or voluntarily reported according to the reporting criteria and timeframe as described below.

10.5.1 "7-day" Reportable Events

Report any study event that is serious (fatal or life-threatening), unexpected, and associated with the use of the investigational drug. Report as soon as possible but no later than 7-calendar days following the Sponsor-Investigator/ Principal Investigator's initial receipt of the information. Identify the submission as "7-day IND Safety Report."

10.5.2 "15-day" Reportable Events

Report any study event that is serious, but not fatal or life-threatening, unexpected, and associated with the use of the investigational drug. Other findings that suggest significant risk to human subjects, any clinically important increased rate of events or any previous unreported adverse event that was not initially reportable because the event did not fit the criteria for reporting. Report within 15-calendar days from when Sponsor-Investigator/Principal Investigator's initial receipt of the information or determination of the previous event is currently reportable. Identify the submission as "IND Safety Report."

10.5.3 Follow-up Reporting

The Sponsor-Investigator/ Principal Investigator will report any additional information that pertains to an IND safety report previously submitted. Any relevant follow-up information that pertains to an IND safety report previously submitted will be submitted within 15-calendar days from day zero, date of the 7-day report. Identify the submission as "Follow-up IND Safety Report."

10.5.4 Annual Report

A summary of all IND safety reports, including the most frequent and most serious AEs, will be submitted each year within 60 days of the anniversary of the date that the IND became active, the date clinical studies were permitted to begin.

10.5.5 Reporting Events to the FDA

IND safety reports may be submitted electronically on an FDA Form 3500A (MedWatch) or in a narrative format via secure e-mail, facsimile transmission or telephone to the FDA Regulatory Project Manager in accordance with 21 CFR 312.32 to the address below.

Division Director

U.S. FDA CDER - Division of Medical Imaging Products
Attention: FDA Central Document Room
5901-B Ammendale Road
Beltsville, MD 20705-1266
FAX: 301-796-9899

10.6 Institutional Review Board Mandatory Reporting

The Principal Investigator or designee will report to the Institutional Review Board (IRB) at Washington University (WU) and the Human Research Protection Office (HRPO) in accordance with WU IRB policies and procedures. The Investigator or designee will report immediately to the IRB any observed or volunteered self-reported adverse event that meets the unanticipated problem criteria or if the Investigator believes, the information meets the exception criteria as described below.

10.6.1 IRB/HRPO Definitions

10.6.1.1 Unanticipated Problem (UP) involving risks to participants or others:

- Are unexpected in terms of nature, severity, and/or frequency given the research procedures that are described in the protocol and consent form and the characteristics of the subject population being studied; and
- Are related or possibly related to participation in the research; and
- Suggest that the research places participants or others at a greater risk of harm including physical, psychological, economic, or social harm, than previously known or recognized.

10.6.1.2 Unexpected adverse drug event:

Any adverse drug experience, associated with the use of the drug, of which the frequency, specificity, or severity is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information provided to the participants and the IRB.

10.6.1.3 Protocol Exceptions and Deviations

- **Protocol exceptions** apply only to a single participant or a singular situation. The IRB is required to review and approve a planned protocol change before initiation or implementation of a change, except when necessary to eliminate immediate hazards to a participant. In the event a situation occurs which requires deviation from the protocol, the Principal Investigator or designee will make the final judgement on whether a participant has completed the study. The PI should report the change after the occurrence as a reportable event within 10 working days.
- **Protocol deviations** from the investigational plan or protocol that affect the life or physical well-being of a participant in any emergency, the Principal Investigator or designee will promptly notify the FDA and IRB as required. Deviations because of unforeseen

circumstances, .e.g., low yield tracer production, technical problems with a scanner, or participant unable to complete the full imaging protocol will not result in cancellation of the scan and is not reportable as a protocol deviation. Data analysis will account for a shorter imaging protocol. The Authorized User verbally approves a dose variance before the administration of a low dosage that is outside the approved allowable range. A shorter imaging protocol or dose variance will be documented in the participant research record, but will not be reported as a protocol deviation.

10.6.1.4 Non-Compliance and Serious Non-Compliance

- **Noncompliance** may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB. Noncompliance is the failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB.
- **Serious noncompliance** is noncompliance that materially increases risks that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

10.6.2 Reporting Events to IRB/HRPO

Table 1 is a summary of reportable events that do not meet the unanticipated problem definition will be reported with the IRB annual continuing review, i.e., IND external safety reports. Federal regulations do not require immediate reporting to the IRB of IND/outside safety reports for events that do not meet the criteria outlined in 21 CFR 312.32(c). Thus, IND Safety reports that do not meet the regulatory requirement for immediate reporting will be reported in an aggregate summary format at the time of IRB continuing review.

10.6.2.1 "1-day" Reportable Events

The Principal Investigator or designee will promptly notify the IRB of the events below within one working day of the occurrence or notification of the event to the PI or a member of the research team.

- Any unanticipated problem involving risk to participants or others and results in the death of a participant at WU.
- An unexpected adverse drug event that results in the death of a participant at WU.
- A major deviation that results in the death of a participant at WU.

10.6.2.2 "10-day" Reportable Events

The Principal Investigator or designee will promptly notify the IRB of the events below within 10 working days of the occurrence or notification of the event to the PI or a member of the research team.

- An unanticipated problem involving risk to participants or others, or a major deviation that does NOT result in the death of a participant at WU.
- A series of minor deviations that represent a systemic issue with the conduct of the study.
- New information that may influence the willingness of subjects to participate in the research study, e.g., an interim analysis or safety monitoring report that indicates the frequency or magnitude of harms or benefits, or a paper published from another study that shows a difference in risks or potential benefits.
- Participant complaints that result from an unanticipated problem or noncompliance.
- Breach of confidentiality
- Incarceration of a participant enrolled in the protocol
- Withdrawal of the participant poses a safety issue

10.7 Reporting to Radioactive Research Drug Committee

According to applicable policies of the Radioactive Research Drug Committee (RDRC) at Washington University (WU), the Sponsor-Investigator/ Principal Investigator or designee will notify the RDRC of any excessive radiation exposure or serious adverse reaction to the radioactive drug within 24 hours to the Chairman of the RDRC.

10.8 Timeframe for Reporting Required Events

Table 1 below summarizes the timeframe requirements for reportable events after initial receipt of the information by the Sponsor-Investigator (Vijay Sharma, Ph.D.), Principal Investigator (Pamela K. Woodard, M.D.), Authorized User (Thomas Schindler, M.D.), and Sub-Investigators, or a member of the research team as described above.

Table 1 - Reportable Events

IRB and RDRC REPORTING		FDA REPORTING	
• Any unanticipated problem involving risk to participants or others and results in the death of a participant. • An unexpected adverse drug event that results in the death of a participant. • A major deviation that results in the death of a participant.	The **PI** will report to the IRB and the **AU** will report to RDRC within "**1-working day**".	• Any serious (fatal or life-threatening), unexpected suspected adverse reactions.	The IND **Sponsor-Investigator** will report to the FDA as soon as possible but no later than "**7-calendar days**" following initial receipt of the information.
• An unanticipated problem involving risk to participants or others, or a major deviation that does not result in the death of a participant. • New information that may influence the willingness of participants to take part in the study, e.g., an interim analysis or safety monitoring report that shows the frequency or magnitude of harms or benefits, or a published paper from another study that shows a difference in risks or potential benefits, revised Investigator's Brochure (IB), FDA labeling change, a marketing drug withdrawal, or DSMB reported safety issue. • A series of minor deviations that represent a systemic issue with the conduct of the study. • Participant complaints that result from an unanticipated problem or noncompliance. • Breach of confidentiality. • Participant withdrawal poses a safety issue. • Incarceration of an enrolled participant.	The **PI** will report to the IRB and the **AU** will report to RDRC if deemed reportable within "**10-working days**".	• Any serious, unexpected, suspected adverse reactions. • Findings from other clinical, animal, or in-vitro studies that suggest significant human risks. • Any clinically important increased rate of serious suspected adverse reaction.	The IND **Sponsor-Investigator** will report to the FDA and all investigators within "**15-calendar days**" after determining the information qualifies for reporting.
• Adverse events that do not meet the definition of unanticipated problem.	The **PI** will include an adverse event summary in the IRB annual continuing review.	• Adverse events that do not meet the definition of serious, unexpected, and suspected adverse reaction.	The IND **Sponsor-Investigator** will include a summary of adverse events in the FDA annual report.

• External IND Safety reports (non-WU) for a cross-referenced IND drug that does not meet the above reporting requirements.	The **PI** will include a summary of external IND Safety reports in the IRB annual continuing review.	• Adverse events that do not meet the definition of serious, unexpected, and suspected adverse reaction.	The IND **Sponsor-Investigator** will include a summary of external IND Safety reports in the FDA annual report.

11 EXPECTED RISKS

11.1 [^{68}Ga]Galmydar Risks

We consider the risk associated with an investigational new PET radioactive drug extremely low based on the short half-life of [^{68}Ga]Galmydar of 68 minutes, the prescribed dosage is at the required microdose level, and observations made from the preclinical toxicity studies that no histopathologic changes were attributable to drug toxicity nor were there abnormalities attributable to the single drug dose. Despite this, there is nevertheless the possibility of a rare allergic reaction. See IND application appendices for the toxicity report.

Based on common responses to other tracers, we anticipate that some participants may experience dysgeusia (bad taste in mouth), flushing, headache, dizziness or lightheadedness, mild gastroenteritis, pruritus, urticaria.

11.2 ^{13}N-Ammonia Risks

^{13}N-Ammonia is an FDA approved drug. There are no known side/adverse effects associated with the use of ^{13}N-Ammonia other than radiation exposure.

11.3 Radiation Exposure

Participants will be exposed to radiation from ^{68}Ga-Galmydar, ^{13}N-ammonia, and low-dose computerized tomography (CT) imaging conducted on the Vision Edge PET/CT or Biograph-40 TruePoint PET/CT scanner. The effective dose (ED) calculations are for worst-case scenarios depending on the number of CT-attenuation correction (CT-AC) scans with topogram. An additional CT-AC scan with topogram will be performed for participants who may need to be rescheduled due to technical problems, e.g., scanner issues, radiotracer failure, or for participants who require a break during a scan and resume imaging after the break. The risk associated with the amount of radiation exposure participants receive during this research study is low and comparable to other everyday risk.

11.3.1 Low-dose CT

The effective dose (ED) for one whole-body CT protocol conducted at a single time point is 0.49 rem (50 mAs). A one-bed position low dose CT (50 mAs) over the heart for attenuation correction (CT-AC) is 0.18 rem.

11.3.2 [^{68}Ga]Galmydar and ^{13}N-Ammonia PET

The effective dose for [^{68}Ga]Galmydar is 0.103 rem/mCi (gender averaged). The effective dose for ^{13}N-ammonia is 0.007 rem/mCi.

11.3.2.1 Dosimetry Group

A single dosage of 8 mCi ± 20% (6.4–9.6 mCi) with a mass of ≤ 10 µg is planned for [^{68}Ga]Galmydar administration for the Dosimetry group. The effective dose for a whole-body PET/CT imaging protocol includes a single intravenous administration of 8 mCi of [^{68}Ga]Galmydar, three whole-body CT scans and four CT-AC scans is 2.81 rem and 2.87 rem for male and females.

11.3.2.2 Rest/Stress Group

Participants will receive two [^{68}Ga]Galmydar intravenous administrations, 4 mCi during rest and 6 mCi during stress for the PET MPI performed on Imaging Day-1. The effective dose for a single intravenous administration of 6 mCi of [^{68}Ga]Galmydar is 0.62 rem and 0.41 rem for a 4 mCi administration performed. The acceptable mass of [^{68}Ga]Galmydar is ≤ 10 µg per administration. On Imaging Day-2, participants will receive two single administrations each of 10 mCi of ^{13}N-Ammonia during the rest and stress PET MPI.

- **Rest/Stress [^{68}Ga]Galmydar PET MPI**

  The effective dose for a rest and stress PET MPI study includes an intravenous administration of 4 mCi during the rest PET and an 6 mCi administration during the stress PET MPI, and two CT-AC scans with topogram is 1.40 rem.

- **Rest/Stress ^{13}N-Ammonia PET MPI**

  The effective dose for a single intravenous administration of 10 mCi of ^{13}N-Ammonia is 0.07 rem. The effective dose for a rest/stress ^{13}N-Ammonia PET MPI study includes two administrations each of 10 mCi of ^{13}N-Ammonia and two CT-AC scans is 0.51 rem.

- **Total Rest/Stress PET MPI**

  The total effective dose for Imaging Day-1, rest/stress [^{68}Ga]Galmydar PET MPI and Imaging Day-2, rest/stress ^{13}N-Ammonia PET MPI study is 1.91 rem. This includes one administration each of ^{68}Ga-Galmydar, 4.0 mCi during rest and 6 mCi during stress, and two CT-AC scans and two administrations each of 10 mCi of ^{13}N-Ammonia and two CT-AC scans.

11.4 Regadenoson

Regadenoson, the pharmacological stress agent may cause a flushed feeling during the intravenous injection. After injection of regadenoson, mild headache, nausea, or dizziness, an irregular and/or fast heartbeat, shortness of breath, mild chest pressure or chest pain may ensue for a short while. If needed as prescribed by the study doctor (cardiologist), intravenous aminophylline may be given to resolve these side effects.

11.5 PET/CT Imaging Scanner

Other risks may include feeling uncomfortable while lying down during the scan. Participants may experience claustrophobia (anxiety due to being restrained in a confined area) while some experience dizziness or feel faint. If the participant experiences these symptoms and does not wish to continue, the study will be stopped immediately.

There is a rare risk of malfunction of worn or implanted electronic medical devices during the low-dose CT attenuation scan conducted prior to the PET scan. Participant screening of such devices will be performed by the investigator or designee and trained research staff. The CT scan may cause a malfunction of worn or implanted electronic medical devices, i.e., pacemaker or a drug pump.

11.6 Intravenous Catheter Placement

Participants may experience pain, bruising, and/or bleeding at the site of needle insertion for the intravenous (IV) catheter. There is a rare risk of infection from the needle insertion.

11.7 Questionnaires

Participants may experience emotional discomfort when answering some questions in the questionnaires (i.e., PET/CT safety screening assessments). If any particular question makes the participant uncomfortable, the participant may discuss its importance and the need to answer it with a trained interviewer. Participants have the right to refuse to answer any question for any reason. If safety screening cannot be completed, the imaging exams will be canceled.

11.8 Breach of Confidentiality

Disclosure of confidential information may occur accidentally during participation in this study. We will use our best efforts to keep the information about participants secure. All private information is stored in secured areas inside locked cabinets or on encrypted and password protected Washington University computers.

A radiologist will review all CT scans. If there is a clinically meaningful abnormality identified or other medical need for sharing, workflows have been established for sharing the images and reports. The Principal Investigator or a designee communicates any potentially clinically meaningful abnormal findings to the participant and, if the participant permits, to their primary

physician. If requested for clinical use, following hospital HIPAA policies, a copy of the CT scan and the report will be uploaded to the participant's electronic health record (EHR). Once the CT is part of the EHR, then participants may request additional copies to be sent to additional physicians using a standard medical record request.

11.9 Other risks

There may also be unknown risks. Although allergic or other immediate adverse reactions are not anticipated, participants will be monitored in an area where emergency equipment is available. There are no alternative methods to gather comparable data.

12 DATA SAFETY MONITORING PLAN

In compliance with the Washington University School of Medicine Institutional Data Safety Monitoring (DSM) Plan, the PI will provide a DSM report to the IRB/HRPO annually. All members of the research team will review safety data as it becomes available. The DSM report will include, but not be limited to the protocol title, IRB protocol number, IND number, the activation date of the study, the number of subjects enrolled to date, a summary of all adverse events, number of subjects who dropped out/withdrew for any reason whether thought to drug related with a corresponding reason for dropout, and any new information related to the investigational drug or that may affect the safety or ethics of the study.

Drs. Woodard (PI) and Wahl and the Sponsor-Investigator, and at least one additional sub-investigator, will review and assess drug biodistribution and safety data through the 2-3 day post-injection phone follow-up from the first two (2) normal volunteers prior to imaging of the remaining normal volunteers. Upon completion of the review, the study will continue with imaging of the remaining dosimetry subjects with simultaneous entry of the rest/stress group. Asymptomatic normal controls and symptomatic patients who have undergone a SPECT MPI examination that is normal or positive and prior to ICA intervention, will undergo a rest/stress [^{68}Ga]Galmydar PET MPI and rest/stress ^{13}N-ammonia PET MPI with a minimum of a 3-day separation.

13 DATA COLLECTION, RETENTION, AND DATA MANAGEMENT

13.1 Data Collection Forms

Each participant will have a research chart with case report forms (source document forms) designed for documenting all observations and other pertinent study related activity. The research coordinator or designee will document eligibility criteria and other pertinent information such as demographic information, imaging visit sessions with pregnancy test results, if applicable and administration of radiopharmaceutical, adverse events and any in-person or correspondence with participants.

13.2 Records Management

The Sponsor-Investigator/ Principal Investigator will maintain records in accordance with Good Clinical Practice Guidelines. The Investigator will delegate such tasks to research team members to ensure all study files are accurate and organized at all times. The study coordinator will review participant research records, including hard copy and electronic, for completion and accuracy. The study team will follow best practice guidance with implementation of electronic storage of research study documents. Electronic records may be created, maintained, and stored where possible. A certified copy of the signed informed consent form may be stored in a secure web-based data storage application that is HIPAA compliant. All electronic records are password protected in an electronic data capture system that is on a (the?) Washington University Secure Network and in compliance with university policies. Paper records that are required for the study will be stored under a 2-tiered locked system.

Imaging Safety questionnaires and other imaging data required for radiation safety will be collected in accordance with the Code of Federal Regulations (21 CFR 312) procedures and requirements governing the use of investigational new drugs and the monitoring of serious adverse events per for the protocol to allow for oversight of annual radiation exposure of participants. We may share the data with other investigators if a participant takes part in future studies requiring imaging procedures.

13.3 Retention and Availability of Records

The Sponsor-Investigator/ Principal Investigator will maintain records in accordance with standards of Good Clinical Practice, and applicable local institutional policies and procedures. IND records and reports will be retained for a minimum of 2 years after study discontinuation, or the FDA has been formally notified (21 CFR 312.57). Participants provide consent to authorization for the use of private health information. Therefore, all research records, including signed consent forms and data/case report forms (source documents) will be retained for at least seven, (7) years beyond the close of the study.

The Sponsor-Investigator/ Principal Investigator will make study data and research records available for audit purposes and inspections if so requested by authorized representatives of Washington University Institutional Review Board, the Human Research Protection Office or Regulatory Agencies, i.e., Food and Drug Administration or National Institutes of Health, the source of grant funding.

13.3.1 Archival of Imaging Data

Only code numbers will appear on any data and documents to be used for evaluation or statistical analysis. Publications emanating from this research will not identify individual patients. HIPAA compliance will be enforced as per Washington University policy and HRPO approval.

Processed and raw PET/CT data will be transferred to the Central Neuroimaging Data Archive (CNDA). Request for access to CNDA must be reviewed and approved by the Principal Investigator or an engaged sub-investigator. Data access is limited to approved user roles as well as assigned access privileges. CNDA complies with Section 164.310 of the Health Information Portability and Accountability Act (HIPAA) that specifies physical safeguards that are required for protection of Electronic Protected Health Information (EPHI) to avoid unauthorized disclosure, modification, or destruction. The CNDA system has the functionality to create audit tables used to monitor traffic through the system and to identify unauthorized attempts to upload or change information or otherwise cause damage.

14 PROTECTIONS, RIGHTS, AND CONFIDENTIALITY

14.1 Informed Consent

The informed consent process will occur in accordance with the Declaration of Helsinki, ICH GCP, U.S. Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA), and applicable institutional research policies and procedures.

Written informed consent will be obtained from each research participant prior to the initiation of any research procedure. All participants will be given ample opportunity to review the informed consent form to consider participation in the study, under circumstances that would eliminate undue influence, and time to discuss with family and the research team. Participants will be informed of the right to refuse to participate that will not involve a penalty or loss of benefits. A participant may withdraw from study participation at any time without interfering with routine clinical care. The informed consent process will take place in a private area. The Investigator or designee will review the consent form with the participant, confirm eligibility criteria and assess the participants' level of understanding. The informed consent process shall be documented, and a copy of the consent will be provided to the participant.

14.2 Confidentiality

See the Data Archiving section for details. All data are safeguarded in accordance with the Health Insurance Portability and Accountability Act (HIPAA) and by the principles and practices of strict confidentiality. Protected Health Information (PHI) on study participants will be managed according to Washington University Policies and Security Requirements. Studies are done for research purposes only. Reports from patients' records concerning research observations will not be available to outside medical facilities without written consent of the participant. Participants will be given the contact information for the University's Privacy Officer for questions or concerns about privacy and the use of PHI.

Electronic data obtained as part of the research project will be stored on a secure network with password access. Hardcopy data will be stored in a 2-tiered locked system. The research team will

follow state and federal laws. The research team will obtain consent to use and share the subject's information. When possible, the research team will de-identify information that could be linked to the subject.

Participant study related electronic data may be saved to Box, a HIPAA compliant cloud-based file storage and collaboration tool that allows for file sharing. Any electronic data saved to local computers are encrypted, password-protected with limited access. Participant hardcopy data (e.g., participant signed consent forms) will be stored under a 2-tiered locked system with limited access to only research team members.

14.2.1 Certificate of Confidentiality

To further protect the privacy of study participants, a Certificate of Confidentiality (CoC) will be issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure of research participants. It allows the investigator and study team members or others who have access to research records to refuse to disclose identifying information on research participants in any civil, criminal, administrative, legislative, or other proceedings, regardless of the type of whether at the federal, state, or local level.

14.3 Data Protection

Participants will be assigned a unique research ID code for the research study. Imaging scans and the data and study documents produced as a result of the research and used for evaluation or statistical analysis are labeled with the ID code and the date of the scan.

Publications emanating from this research will not identify individual participants. Reports from patients' records concerning research observations will not be available to outside medical facilities without the written consent of the participant.

14.4 Data Security

Informed consent will be obtained to use and share information. When possible, the research team will de-identify information that could be linked to the participant. Signed hard copy consent forms will be stored in a 2-tiered locked system with limited access to research team members.

14.5 Future Use of Data/ Data Sharing

Important elements for data sharing are included in the consent form and are discussed during the informed consent process. All data is coded and linked with the subject's unique identifier. Information generated by this study, including data and stored blood samples, if applicable will be to be used now and in the future to address research questions in similar fields or other unrelated areas. Research investigators may be at Washington University or outside the university at other research centers, institutions, or industry sponsor of the research.

Coded study data will be maintained at Washington University. This coded data may also be shared with other investigators doing research in similar fields. These investigators may be at Washington University or other research institutions. We may also share coded data with large repositories for broad sharing with the research community. If the participant's research data is placed in one of these repositories, only qualified researchers who have received prior approval from individuals that monitor the use of the data will be able to look at this information.

15 ETHICAL AND FINANCIAL CONSIDERATIONS

15.1 Washington University Institutional Review Board

This imaging study will be conducted in compliance with the protocol approved by Washington University Institutional Review Board, the Human Research Protection Office (HRPO) and in accordance with state and federal government regulations standards of Good Clinical Practice, and applicable institutional research policies and procedures.

The Sponsor-Investigator/ Principal Investigator agrees the study will not be initiated prior FDA correspondence to proceed and not before receipt of IRB or other required regulatory committee approvals. The protocol and informed consent form will be reviewed and approved by IRB/HRPO and the Radioactive Research Drug Committee (RDRC) at Washington University. All subsequent amendments will be submitted to IRB/HRPO and if applicable, the RDRC for approval and other applicable committees as needed.

Washington University complies with the regulations outlined in 45 CFR 46, 21 CFR 50, and 21 CFR 56. The Association for the Accreditation of Human Research Protection Programs has accredited the Institutional Human Research Protection Program since 2004. WU HRPO has two duly appointed IRBs established and is registered in accordance with 45 CFR 46.107, 21 CFR 56.106, and 21 CFR 56.107.

15.2 Funding Source

The U.S. National Institutes of Health is funding this imaging study.

15.3 Conflict of Interest

The Sponsor-Investigator, Principal Investigator, and Sub-Investigators of the research team will follow applicable University Conflict of Interest policies statements as required under Part 54 of Title 21 of the CFR. All members of the research team will verify their financial interests related to the study. The Sponsor-Investigator will promptly report any changes to financial disclosure information according to institutional policies and FDA guidelines for financial interest under 21 CFR 312.57b and 312.64.

15.4 Participant Compensation

Research participants are compensated for their time, research-related inconveniences and discomforts experienced during the study. Any expenses incurred in reimbursing participants are for reasonable and customary medical costs incurred related to the treatment of an adverse event experienced that is determined, in consultation with the Sponsor-Investigator/Principal Investigator, was reasonably related to the administration of the investigational radiotracer, ^{68}Ga-Galmydar.

16 ATTACHMENTS

The Schedule of Activities (SoA) tables for each group begins on the next page, see Table 2 for the Dosimetry group and the Table 3 for the Rest/Stress group.

Table 2. Schedule of Activities–Dosimetry Group

			Whole-Body PET Imaging			
Procedure	**Pre-Screen[a]**	**Screening Visit[b,c]**	**[68Ga]Galmydar PET/CT[i]**	**Delayed Imaging[i]**	**Discharge[i]**	**Phone Follow-up[j]**
Written Informed Consent[b]		X				
Inclusion/Exclusion Criteria[b]	X	X				
Adverse Events Monitoring[b]		X	X	X	X	X
Medical Records (EHR)[c]	X	X	X	X	X	X
Demographics[b]	X	X				
Medical History[b]	X	X				
Concomitant Medication[b]	X	X				X
Physical Exam/Evaluation[b]		X			X	
Body Weight and Height[b]		X				
IV line placement[d]		X				
Clinical safety labs[e]		X	X		X	
Vital Signs[f,h,i]		X	X	X	X	
12-lead Electrocardiogram[h,j]		X	X		X	
Safety Monitoring[j]			X	X		
Whole-Body PET/CT imaging[i,k]			X	X		

a) A phone call will be conducted to determine participant interest, eligibility, and schedule study visits.
b) Screening will occur in the CCIR facility.
c) Safety monitoring for adverse events (AE) will occur from the time of injection to the end of participation. Participants' medical records (EHR) are followed in the event of an adverse event.
A physician or designee must evaluate participants at baseline or [68Ga]Galmydar pre-injection and post-imaging before discharge.
d) Two peripheral IV catheters will be placed for the [68Ga]Galmydar injection and collection of blood samples.
e) Blood and urine samples will be obtained for clinical safety labs (complete blood count, comprehensive metabolic panel, and urinalysis) at screening and post imaging prior to discharge. For women of child-bearing potential, a urine pregnancy test must be obtained and confirmed as negative. See Safety Assessments section.
f) Vital signs will be obtained at screening, baseline within 5 to 15-min pre-injection and within 5 to 15-min -min post injection, end of first imaging session, beginning of each delayed imaging session, and prior to discharge from the imaging facility. Repeat as needed.
g) A 12-lead ECG will be performed at screening, baseline within 60-min prior to injection, within 5 to 15-min post-injection, end of first imaging session, and prior to discharge from imaging facility. Repeat as needed.
h) Monitoring during the PET study: Place a blood pressure cuff on the opposite arm used for the radiotracer administration to measure blood pressure and heart rate. Place electrodes on the participant's chest for continuous ECG monitoring and electrodes to obtain 12-lead ECG.
i) Obtain a low-dose CT-AC (50 mAs) prior to the 8 mCi ±20% (6.4–9.6 mCi) IV injection of [68Ga]Galmydar followed by a 10 mL saline flush. Begin whole-body PET image acquisition from the head to mid-thigh immediately post [68Ga]Galmydar injection for 30-min and repeat at 2 hours and 4 hours.
j) Phone follow-up will occur within 2-3 days and repeated at 5-7 days or as late as 14-days post [68Ga]Galmydar injection. If any of the days are not business days, the follow-up phone call may occur the following business day.

Table 3. Schedule of Activities–Rest/Stress Group

Procedure	Pre-Screen[a]	Screening Visit[b]	IMAGING DAY-1 [68Ga]Galmydar PET/CT[k]			IMAGING DAY-2 13N-Ammonia PET/CT[l]			Phone Follow-up[o]
			Rest	Stress	Discharge[m]	Rest	Stress	Discharge[m]	
Written Informed Consent		X							
Inclusion/Exclusion Criteria	X	X							
Adverse Events[b]		X	X	X	X	X	X	X	X
Medical Records (EHR)[c]	X	X	X	X	X	X	X	X	X
Demographics	X	X			X				
Medical History[a,d]	X	X							
Concomitant Medication[b]	X	X	X	X		X	X		X
Physical Exam/Evaluation[d]		X						X	
Body Weight and Height[b]		X			X				
IV placement[e]			X			X			
Clinical safety labs[f]		X	X	X				X	
Vital Signs[g]		X	X	X	X	X	X	X	
12-lead Electrocardiogram[h,i]		X	X		X	X		X	
Safety Monitoring[g,h,i]			X	X	X	X	X	X	
PET/CT imaging[j,k,l,m]			X	X		X	X		

a) A phone call will be conducted to determine participant interest, eligibility, and schedule study visits.
b) Participants will be admitted to the Clinical and Translational Research Unit (CTRU) facility for screening, during the break between rest and stress [68Ga]Galmydar PET, and post-imaging safety assessments before discharge.
c) Safety monitoring for adverse events (AE) will occur from the time of injection to the end of participation. Participants' medical records are followed in the case of an adverse event.
d) A physician or designee must evaluate participants at baseline or[68Ga]Galmydar pre-injection and post-imaging before discharge.
e) Two peripheral IV lines will be placed for the radiotracer injections, collection of blood samples and for the regadenoson administration during the stress PET imaging.
f) Confirm fasting (≥ 6 hrs). Collect blood and urine samples for [68Ga]Galmydar safety labs (complete blood count, comprehensive metabolic panel, and urinalysis) at screening, baseline, and post imaging prior to discharge from the imaging facility. For all women of childbearing potential, a urine pregnancy test must be obtained and confirmed as negative. See Safety Assessments section.
g) Obtain vital signs at screening, baseline rest [68Ga]Galmydar scan within 5 to15-min pre-injection and within 5 to 15-min post injection, end of rest imaging, and prior to participant transfer to the CTRU or discharge from the imaging facility. Repeat as needed. Follow standard-of-care clinical protocol for regadenoson stress imaging scans for both radiotracers, [68Ga]Galmydar and 13N-ammonia.
h) A 12-lead ECG will be performed at screening/baseline, within 60-min prior to injection, within 5 to 15-min pre-injection, and within 5 to 15-min post injection and end of imaging prior to discharge from imaging facility. For both radiotracer stress regadenoson imaging, follow the standard-of-care clinical protocol.
i) Monitoring: Place a blood pressure cuff on the opposite arm used for the radiotracer administration to measure blood pressure and heart rate. Place electrodes on the participant's chest for continuous ECG monitoring and electrodes to obtain 12-lead ECG.
j) PET/CT imaging: Obtain a low-dose CT-AC (50 mAs) prior to each rest and stress radiotracer injection. If the same IV line is used for both the regadenoson and radiotracer injections, wait 10-20 sec after the normal saline flush post regadenoson injection before injecting the radiotracer.

k) [^{68}Ga]Galmydar Imaging Day-1:
   - *Rest PET*: Administer 4 mCi ±20% (3.2–4.8 mCi) of [^{68}Ga]Galmydar IV injection followed by a 10 mL saline flush and immediately begin up to a 30-min list-mode data acquisition for a single bed position at the level of the heart. The interval between the rest and stress [^{68}Ga]Galmydar PET acquisitions *should be no less than 2 hours and no greater than 4 hours.*
   - *Stress PET MPI*: Administer a rapid (10-sec) regadenoson IV injection (0.4 mg/5 mL), followed by 5 mL saline flush; 30-sec post regadenoson injection, administer 8 mCi ±20% (6.4–9.6 mCi) of [^{68}Ga]Galmydar IV injection followed by a 10 mL saline flush. Begin up to a 30-min list-mode data acquisition for a single bed position at the level of the heart.

l) ^{13}N-Ammonia Imaging Day-2:
   - *Rest PET*: Administer an IV bolus injection of 10 mCi ±20% (8–12 mCi) of ^{13}N-Ammonia followed by a 10 mL saline flush and immediately begin a 10-min list-mode data acquisition (if the anterior-posterior diameter of the chest is >50cm increase to 15-min) for a single bed position at the level of the heart. The interval between the rest and stress ^{13}N-ammonia acquisitions should be *no less than 1-hour*.
   - *Stress PET MPI*: Administer a rapid (10-sec) regadenoson IV injection (0.4 mg/5 mL), followed by 5 mL saline flush; 30-sec post regadenoson injection, administer 10 mCi ±20% (8–12 mCi) of ^{13}N-Ammonia IV bolus injection followed by a 10 mL saline flush. Begin a 10-min list-mode data acquisition (if the anterior-posterior diameter of the chest is >50cm increase to 15-min) for a single bed position at the level of the heart.

m) Transfer [^{68}Ga]Galmydar participants to CTRU for post imaging safety assessments and discharge. Discharge ^{13}N-Ammonia participants from the CCIR.

n) Contact [^{68}Ga]Galmydar participants by phone within 2-3 days post [^{68}Ga]Galmydar injection. [^{68}Ga]Galmydar and ^{13}N-ammonia imaging visits will be separated by a minimum of 3-days and not before the follow-up phone call is complete for capturing participant adverse events following the administration of [^{68}Ga]Galmydar. If any of the days are not business days, the phone call may occur the following business day.

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