Dynamic Positron Emission Tomography Imaging with ^{11}C-ER176 to Delineate Macrophage Activation in Diabetic Gastroparesis

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A. Specific Aims

Diabetic gastroparesis (DG) is a well-established complication of diabetes mellitus and results in significant morbidity for the patients[1, 2]. Mechanistic studies from diabetic animal models of delayed gastric emptying as well as molecular studies on human full-thickness gastric biopsies have significantly advanced our understanding of this disorder[3-6]. An innate immune dysregulation and resulting injury to the interstitial cells of Cajal (ICC) and other components of the enteric nervous system is central to the pathophysiology of DG[7, 8]. Specifically, hyperglycemic animal models have demonstrated that macrophages are critical for the development of delayed gastric emptying[9, 10]. A loss of anti-inflammatory macrophages associates with loss of ICC in both animal models and patients with DG. Our transcriptomic[5] and proteomic[6] studies in gastric full thickness biopsies from DG patients have also demonstrated macrophage-based immune dysregulation as the primary pathway altered. Although they have provided us seminal findings, widespread use of full-thickness biopsies can be challenging for assessment of neuromuscular immunopathology in the stomach. Additionally, these biopsies only provide assessment of a small area in an untargeted manner, which can result in incomplete assessment considering that changes can be patchy[11]. Thus, there is an unmet need for novel noninvasive strategies to assess immune dysregulation in the gastric wall.

Activated state of macrophages is mirrored by upregulation of the 18-kDa translocator protein (TSPO)[12-14]. Positron emission tomography (PET) with TSPO-specific ligands has been developed over the last few years and provides a novel *in vivo* molecular technique to detect macrophage activation[15]. However, results obtained by second-generation radioligands in preclinical models have not been consistently reproduced in humans[15]. Successively more promising PET radiotracers have been developed for this application[16-20]. Recently, one of the newer agents, ER176 {4-(2-chlorophenyl)-N-(methyl-11C)-N-((1R)-1-methylpropyl)- 2-Quinazolincarboxamide}, a third-generation TSPO-specific radioligand (**Figure 1**), has shown high affinity and excellent *in vivo* target-to-background binding[21, 22]. Based on imaging data from several ^{11}C-labeled TSPO PET ligand studies, ER176 has been identified as the most suitable for *in vivo* study of activated macrophages due to: 1) a higher specific binding, 2) a smaller inter-subject variability, and 3) the unique ability to image even a low-affinity binding state[23]. Delineation of activated macrophages with ^{11}C-ER176 PET in the stomach of patients with DG is a novel application. Mayo Clinic has an FDA-approved investigational new drug (IND) application (#149229) for use of ^{11}C-ER176 in a trial of neuroinflammation, which will be amended to include gastric imaging in DG and control participants proposed in this study.

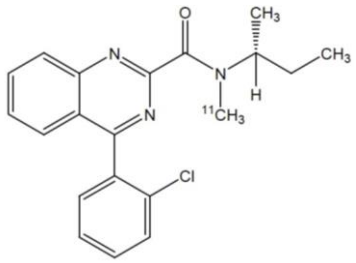

Fig 1: Molecular structure of ^{11}C-ER176.

The **overall goal** of this project is to demonstrate feasibility of dynamic ^{11}C-ER176 PET imaging to identify macrophage-driven immune dysregulation in gastric muscle of patients with DG. Non-invasive quantitative assessment with PET can significantly add to our diagnostic armamentarium for patients with diabetic gastroenteropathy. If a clear immune dysregulation is identified in a subset of patients, it can be targeted using immunosuppressive or immunomodulatory therapies. Furthermore, if validated, this imaging technique can facilitate clinical trials aimed at targeting immune dysregulation in DG, thus offering new therapies in a disorder with significant unmet need. We propose the following specific aim:

Specific Aim: To determine feasibility of dynamic ^{11}C-ER176 PET to identify macrophage-activation in diabetic gastroparesis

Hypothesis 1: Patients with diabetic gastroparesis demonstrate increased uptake for ^{11}C-ER176 in gastric body and antrum compared to matched controls

Hypothesis 2: Increased uptake of ^{11}C-ER176 associates with a shift towards pro-inflammatory macrophage milieu on tissue assessment made by targeted biopsies of gastric muscle

B. Background and Significance

Gastroparesis as a complication of diabetes mellitus: Gastroparesis is a chronic disorder of the upper GI tract characterized by delayed gastric emptying of solids and/or liquids in the absence of mechanical obstruction of the gastric outlet[1]. Cardinal symptoms include nausea, vomiting, bloating, postprandial fullness, early satiety and abdominal pain. In one study, DG accounted for almost a third of the overall gastroparesis cases[24]. In the diabetes control and complications trial, delayed gastric emptying was associated with greater HgbA1c, longer duration of diabetes, lower R-R variability on electrocardiogram, nephropathy, retinopathy, and greater GI symptoms[25]. In a study from the type 1 diabetes exchange clinic registry, females are more likely to have gastroparesis than males[26]. Another population-based study showed a 4-fold higher prevalence of gastroparesis in women when compared with men[27]. Patients with DG have a significant impairment of their quality of life; especially symptoms of nausea, bloating and chronic pain[28, 29]. In spite of the frequent occurrence of this highly morbid complication of diabetes mellitus, there is a significant dearth of treatment options.

Immune dysregulation in pathophysiology of diabetic gastroparesis: ICC are the pacemaker cells, which create the bioelectrical slow wave potential in the GI tract[30]. Syncytial network of ICC generate electrical slow waves in a spatiotemporal manner driving rhythmic contraction of smooth muscle cells[31, 32]. ICC are also involved in integrating and mediating sequential excitatory and inhibitory neuro-transmissions and in mechanotransduction, the critical components of normal gastrointestinal motility[33]. Variants in Ano-1 transcripts, a protein specifically expressed in ICC has been associated with symptoms in DG[34]. Macrophage activity is a reflection of both pro-inflammatory and anti-inflammatory subtypes. In the Non-obese diabetic (NOD) mice, the development of delayed gastric emptying was associated with an increase in iNOS expression, a marker for macrophages with a “M1” phenotype (classically activated or pro-inflammatory).[4] Macrophage-deficient CSF1$^{op/op}$ mice did not develop delayed gastric emptying despite the presence of severe hyperglycemia.[9] Administration of CSF1 resulted in replenishment of macrophages and development of delayed gastric emptying and ICC damage in >75% of the mice. Conditioned media from macrophages exposed to oxidative stress resulted in damage to cultured ICC, which could be prevented by neutralizing antibodies against IL6R and TNF.[35] We showed that decreased CD206+ (alternatively activated, anti-inflammatory M2 macrophages) in the gastric antrum of patients with DG correlated with the loss of ICC.[36] Additionally, patients with DG had long GT alleles in the HMOX1 gene, and these were associated with worse nausea symptoms.[7] In our deep transcriptomic analysis of full thickness (excluding mucosa) gastric body biopsies, 111 genes were found to be differentially abundant in DG (log_2fold difference of |≥2| with a false detection rate (FDR) < 5%)[5]. Top canonical pathways in DG included genes involved with macrophages, fibroblasts and endothelial cells. In a more recent study, we used the SOMAscan proteomics platform that quantitates expression of 1300 human proteins. We found that 73 proteins were differentially expressed in DG. “Role of Macrophages, Fibroblasts and Endothelial Cells” was the most statistically significant altered pathway. Additionally, in DG, properdin expression strongly correlated with bloating scores on the Gastroparesis Cardinal symptom Index and expressions of prostaglandin G/H synthase 2, protein kinase C zeta type and complement C2 correlated with 4 hr gastric retention. Overall, these complimentary mice and human studies suggest an immune dysregulation driven by activated macrophages as central to driving injury to the ICC that are central to the physiology of gastric emptying.

Lack of noninvasive tools for assessment of immune dysregulation in DG: There are no imaging techniques that can reliably assess activated macrophages *in vivo*. Metabolic imaging with the ubiquitous PET radiotracer, F18 fluorodeoxyglucose (FDG), is insufficient for this purpose because of its non-specificity. There can be both diffuse or focal uptake of FDG in the stomach either physiologically or due to inflammatory and neoplastic conditions. A representative image showing diffuse uptake of FDG in the stomach is shown (**Figure 2**). On the other hand, ^{11}C-ER176

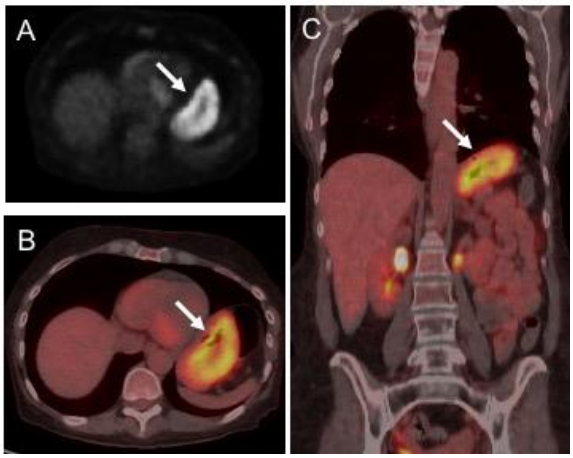

Fig 2: Non-specificity of FDG uptake in stomach: Axial corrected PET **(A)**, and fused axial **(B)** and coronal PET/CT images **(C)** demonstrate physiologic pattern of diffuse uptake of FDG in the stomach (white arrows).

is a radiotracer with specific binding to TSPO in activated macrophages and has many favorable imaging properties. For instance, preliminary studies have noted physiologic organ uptake of ^{11}C-ER176 in brain, thyroid, lung, heart, liver, spleen and kidneys but not in the stomach[22]. Finally, there have been no adverse or clinically detectable pharmacologic effects of ^{11}C-ER176 in healthy subjects[22]. Therefore, ^{11}C-ER176 represents a promising PET radiotracer for delineation of activated macrophages in patients with DG.

Innovation: This proposal is innovative from both biological and technical standpoints. Strong animal and human data generated by our group and others demonstrate macrophage activation in gastroparesis. This has been mechanistically shown to play a role in gastric motor dysfunction via injury to ICC. There is currently no noninvasive way to determine immune dysregulation in the gastric muscle. More broadly, macrophage dysfunction also plays a role in other inflammatory disorders of the gut such as inflammatory bowel disease. Technological advancements in ligands for PET imaging have already led to emergence of its role in studying neuroinflammation as well as joint inflammation. We plan to use the latest generation TSPO radioligand, ^{11}C-ER176, for delineating macrophage activation in DG. We will validate imaging findings with state-of-art sampling of gastric muscle followed by isolation of immune cells and using single cell-based techniques to determine transcriptional changes in immune cells. If successful, these studies will provide pilot data for larger studies aimed at determining immune dysregulation in diabetic gastroenteropathy as well as other inflammatory GI disorders.

C. Preliminary Studies

Macrophage cell population in gastric muscle expresses TSPO: In preliminary studies, human gastric muscle tissues (idiopathic gastroparesis) were dissociated followed by magnetic separation and isolation of CD45$^+$ immune cells. Libraries were prepared using 10x Genomics Library Kits and sequenced on HiSeq4000. The t-distributed stochastic neighbor embedding (t-SNE) plot revealed 12 clusters of CD45$^+$ cells (**Figure 3a**). CD68, a type I transmembrane glycoprotein, heavily expressed in cells of monocyte/macrophage lineage forming a discrete cluster (**Figure 3b**). TSPO showed predominant expression in the CD68$^+$ subset of cells (**Figure 3c**). This suggests that gastric muscle contains immune cells that are TSPO$^+$, hence, providing proof-of-concept data that ^{11}C-ER176 PET imaging targeting TSPO should demonstrate uptake *in vivo* in humans. TSPO has been strongly associated with an activated or pro-inflammatory macrophage subtype. In a recent study, during M2 (anti-inflammatory) polarization, TSPO expression was decreased[14]. A TSPO antagonist enhanced anti-inflammatory polarization. Additional studies have also demonstrated TSPO being an imaging target for identifying pro-inflammatory macrophage milieu[12, 19].

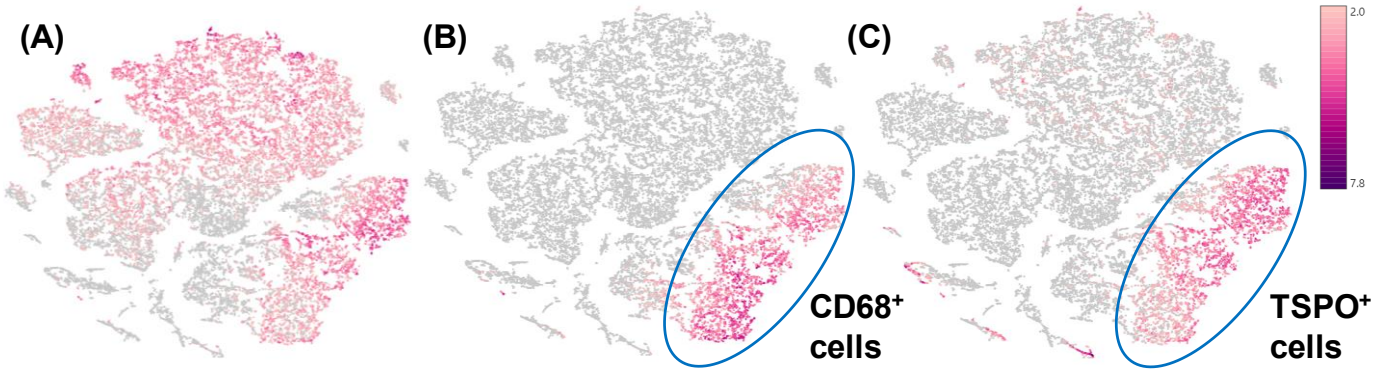

Fig 3: Gastric muscular propria single cells on tSNE plots. **(A)** Overall immune cells (CD45$^+$). **(B)** CD68$^+$ cell subset, a marker for macrophage and phagocytic cell population. **(C)** TSPO$^+$ cells closely overlapping with the CD68$^+$ population suggesting macrophage subset of immune cells in gastric muscle express TSPO.

D. Research Design and Methods

We plan to recruit 4 patients with DG, 4 patients with diabetes without gastroparesis, and 4 age-matched healthy volunteers. Considering the clear higher prevalence of DG in females compared to males, in this pilot and feasibility study, we will recruit only females. DG patients and diabetic patients will be recruited using the inclusion and exclusion criteria listed in **Table 1**. Healthy volunteers will be screened to have no clinical history of diabetes or any GI symptoms. These will be screened using a simple GI questionnaire. Additionally, they will

be screened to have no inflammatory disorders of the GI tract or use of anti-inflammatory or immunosuppressive therapies. Participants will undergo a screening visit which will involve informed consent, history and physical

examination by the PI to ensure eligibility for participation in the study. Participants will also have a blood draw that will occur prior to the PET/CT scan. Participants will have 25 mL of blood drawn for PBMC isolation, proteomic, metabolomics, and genetic analysis. PBMCs will be isolated and their transcriptional and epigenomic profile will be studied. DG patients will complete a self-reported Patient Assessment of Upper Gastrointestinal Disorders Symptoms (PAGI SYM) questionnaire. This is composed of 20 items and 6 subscales to assess for symptoms of gastroparesis, dyspepsia, and gastroesophageal reflux disease. The severity of each symptom item over a 2-week recall period is scored from 0 (none or absent) to 5 (very severe). The Gastroparesis Cardinal Symptom Index (GCSI)[37], a 9 symptom survey relevant to gastroparesis stratified in 3 subscales (nausea/vomiting, fullness/early satiety, and bloating) is contained within the PAGI-SYM. We will recruit DG patients with a score ≥3 suggesting moderate-severe symptoms. In the second visit, participants will present NPO after midnight and undergo the ^{11}C-ER176 PET/ CT scanning followed by upper endoscopy and biopsies. The imaging and endoscopy details are as below:

Table 1: Enrollment criteria for DG and Diabetic patients

Inclusion criteria	Exclusion criteria
-Female - Ability to provide informed consent - Age 18-70 years - Type I or II diabetes mellitus - Clinical gastric emptying testing within 6 months of study enrollment (for DG patients only) -Gastroparesis defined by gastric retention of Tc-99m >20% at 4 hrs on scintigraphy (for DG patients only) - Average Gastroparesis Cardinal Symptom Index (GCSI) ≥3 suggesting moderate-severe symptoms (for DG patients only)	- Women who are pregnant or cannot stop breast feeding for 24 hrs - Using anti-coagulants, anti-inflammatory medications (NSAIDs, corticosteroids, etc.) or immunosuppressive therapies within 4 weeks prior (for DG patients only) - Opioid use within last 4 weeks of gastric emptying scintigraphy (for DG patients only) - Prior gastric surgery - History of IBD, celiac disease, eosinophilic gastroenteritis, microscopic colitis

Imaging: ^{11}C-ER176 will be produced in full accordance with GMP requirements in the Mayo Clinic Cyclotron Facility. All PET/CT imaging will take place in the PET/CT Molecular Imaging Research Center on [redacted] of Mayo Clinic. PET/CT imaging will be performed on a state-of-the art digital PET/CT system (Siemens Biograph Vision 600, Siemens Healthineers). This system utilizes silicon photomultiplier PET detectors to attain a spatial resolution of 3.7 mm, a timing resolution of 210 picoseconds and a sensitivity of 16 cps/kBq over a 26-cm axial field-of-view.

Prior to imaging, a pregnancy test will be administered to women of child-bearing potential. The subject will void their bladder and an intravenous (IV) line will be placed in an arm for administration of ^{11}C-ER176. Participants will be given small amount of water (≤ 250 ml) immediately before the PET scan to allow gastric distension. The subject will be positioned supine on the imaging table with their head in a head-holder. Participants will be monitored visually and intermittently for adverse events during injection.

The participants will have a low-dose, non-gated, non-contrast-enhanced, free-breathing CT from the orbits to upper thigh for attenuation correction (CTAC) and anatomic co-localization. Immediately following the start of the PET scan, 518 MBq (14 mCi) (range 370-666 MBq; 10-18 mCi) of ^{11}C-ER 176 will be administered intravenously followed by a saline flush. The first 6 min of PET scanning will be a dynamic acquisition over the heart for measurement of the bolus phase of an input function (IF) which represents the amount of ^{11}C-ER176 in blood supplying tissue as a function of time. Next, 8-10 static images will be acquired sequentially by automatically shuttling the imaging table and scanning the patient from upper chest through abdomen, which will include the heart for late phase of the IF in multiple passes. A whole-body PET scan from the orbits to upper thigh will then be acquired at the end (60-min post injection). All PET data will be reconstructed with fully-3D iterative reconstruction algorithms that include corrections for attenuation (using the CTAC scan), scatter, randoms, dead time, decay and normalization.

Image analyses: PET and CT images will be transferred to a dedicated workstation (MIM Software Inc., OH). Image analyses will be done by a board-certified nuclear radiologist. Diagnostic image quality of the late PET images will be rated on a 5-point Likert scale. Ratings of ≥3 will be considered acceptable for diagnostic use whereas ratings of 1-2 will be considered unacceptable. On each PET image, volumes of interest (VOIs) will be

drawn around the stomach and other organs that may show radiotracer accumulation. The uptake in each VOI in each PET image will be measured and decay-corrected to the time of injection and normalized to the administered activity. The quantified VOI activity for all PET images will be converted to time-activity curves, and the retention of activity in each region will be used along with the IF to model the biodistribution of ^{11}C-ER 176. The radiopharmaceutical biodistribution will also be quantified by standard metrics [maximum & mean standardized uptake value (SUVmax and SUVmean respectively)]. The image quality, time-activity curves, and semi-quantitative metrics will be compared between diabetic gastroparesis patients and controls.

Upper endoscopy with endoscopic ultrasound and targeted biopsies of gastric muscle: A pregnancy test will be administered to women of child-bearing potential. Upper endoscopy will be performed only on DG patients only. The endoscopy will be performed within 12 weeks of the participant's PET/CT scan, this will allow more scheduling flexibility for the participant and study team. During the endoscopy procedure an additional procedure known as endotracheal intubation may be performed to keep the airway open and assist with breathing. This will vary from case to case and is determined by the anesthesiologist assisting with or the physician performing the endoscopy. The echoendoscope (Aloka Arietta 850; Olympus, Center Valley, PA) will be advanced into the gastric lumen and a site targeted for EUS-guided core biopsies based on findings of the PET scan. Fine needle biopsy of the gastric wall will be performed using a 19 G needle (SharkCore; Medtronic, Minneapolis, MN) passed through the echoendoscope and across the entire gastric wall ensuring a full thickness (mucosa to serosa) core tissue sample. Sampling of the uninvolved region will be done to serve as control. In patients who do not have clear signal on PET/CT, sampling will be done from gastric antrum and body to ask as negative control. All endoscopic procedures will be done by advanced endoscopist [redacted]). EUS-guided core biopsy is a safe procedure associated with a low risk of adverse events.

Assessment of gastric macrophages: After tissue dissociation, using technique similar to that shown in the preliminary data, cells will be sorted and profiled using the Fluidigm (San Francisco, CA) Helios cytometry by time of flight (CyTOF) mass spectrometry system. After cleanup in FloJo to obtain singlets, data will be analyzed using the R-based CytofKit tool[38, 39]. In an approach that was not biased towards a limited subset of cell types, cellular subtypes will be identified and counted after labeling with a panel of 32 antibodies to distinguish monocytes and tissue myeloid cells from the other immune cells (CCR2, CD24, CD64, Ly6C, CD115/CSF1R, CD38, CD8a, Ly6G, CD11b (Mac-1), CD3e, CX3CR1,MERTK, CD11c, CD4, ESAM, PDL-1, CD14, CD44, F4/80, PDL-2, CD161 (NK1.1), CD45, FcER1,SiglecH, CD19, CD45R (B220), I-A/I-E, SIRPa (CD172a), CD206 (Mrc1), CD62L, IL2RA, CD25, TER-119). Data will be displayed as tSNE maps and heatmaps.

Endpoints and statistical analysis: The biodistribution of ^{11}C-ER 176 in the stomach using time activity curves and other described imaging metrics will be the primary endpoint. Correlative analysis will be done between biodistribution on ^{11}C-ER176 and pro-inflammatory markers on cellular expression using CyTOF. The proposed study will provide means and standard deviations for larger investigation in this area. Considering the robust expression of TSPO in gastric macrophages in our preliminary data, we expect to see uptake of TSPO ligand ^{11}C-ER 176 in the gastric muscle.

Anticipated results, potential pitfalls and alternative strategies: We anticipate finding that patients with DG will have significantly higher PET uptake of ^{11}C-ER176 compared to healthy volunteers. Additionally, we anticipate that the uptake will strongly associate with upregulation of TSPO and other pro-inflammatory markers like Ly6C and downregulation of anti-inflammatory markers like CCR2, CX3CR1 and CD206. Most second-generation TSPO radioligands have varying degrees of sensitivity to the single nucleotide polymorphism rs6971 in the TSPO gene[40], and this genotype sensitivity may vary depending upon the organ where it is expressed[22]. Therefore, some subjects who are low-affinity binders are excluded from analyses and the remaining subjects are corrected for being either high- or mixed-affinity binders. On the other hand, ^{11}C-ER176, a third-generation ligand, has shown little *in vitro* sensitivity to rs6971. However, *in vivo*, ^{11}C-ER176 was found to be sensitive to rs6971. Nevertheless, it allowed quantitation even in low affinity binders and had high non-displaceable binding potential, which suggests that its relative *in vivo* sensitivity to rs6971 may not impact clinical imaging[22]. Due to the small sample size of this feasibility study, we will be unable to select subjects with varying TSPO affinity genotype. However, if we see insufficient binding, we will determine the genotype.

Timeline: We plan to finish recruitment of 4 DG patients and 4 healthy volunteers including ^{11}C-ER176 PET/CT as well as endoscopic ultrasound with core biopsies within 7 months. Images will be processed in parallel. The next 3 months will be used for finalizing image analysis as well as CyTOF for the immune markers followed by manuscript submission in the last 2 months. Considering the large volume of DG patients seen at Mayo Clinic and strengths of the PI's team in recruitment, we are confident we will be able to recruit. The PI's laboratory is housed within the Enteric Neuroscience Program (Director, [redacted]) and the group has considerable experience in cellular and molecular assessment of human gastric tissue[1, 5, 6, 10, 41-43]. The nuclear medicine group at Mayo Clinic has a long-standing track record and expertise in studies of this nature as well as an established infrastructure to perform the dynamic PET scanning. The preliminary data, availability of participants, and the expertise of the investigating groups (GI, radiology) will ensure that the proposed studies are completed within the 1-year time frame allowing time for dissemination of results and generating data for a larger grant proposal.

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F. Protection of Human Subjects

1. Risks to the Subjects

a. Human subject involvement and characteristics

Patients with diabetic gastroparesis (n=4) , diabetic patiens, and age-and BMI-matched healthy volunteers (n=4) will be recruited for the study (all female) using following criteria.

Participants:

Inclusion criteria:

1) Age: 18 to 70 years of age
2) Ability to provide informed consent
3) Type I or II diabetes mellitus
4) Gastroparesis defined by gastric retention of Tc-99m >60 % at 2 hrs and/or >10% at 4 hrs on scintigraphy (for DG patients only)
5) Average Gastroparesis Cardinal Symptom Index (GCSI) ≥3 indicating moderate-severe symptoms (for DG patients only)

Exclusion criteria:

1) Women who are pregnant or cannot stop breast feeding for 24 hrs
2) Using anti-coagulants, anti-inflammatory medications (NSAIDs, corticosteroids, etc.) or immunosuppressive therapies within the 4 weeks prior (for DG patients only)
3) Opioid use within the last 4 weeks of gastric emptying scintigraphy
4) 4. Prior gastric surgery
5) 5. History of IBD, celiac disease, eosinophilic gastroenteritis, microscopic colitis

Dietary, fluid and other restrictions:

During recruitment, informed consent review, and visits, the subjects were informed and reminded of the following restrictions:

1. No alcohol for 72 hours before 1st visit and until after end of study period
2. Use of tobacco products (last 3 months)

Sites where research will be performed

Mayo Clinic in Rochester, Minnesota

b. Sources of research materials

Research material will be the ^{11}C ER 176 PET/CT images, upper endoscopy with gastric muscle biopsies and cells derived from the biopsies. Additionally, questionnaire responses and medical records will be research materials. Results will be maintained in the research laboratories that are ultimately the sole responsibility of the PI, and will be accessed by a secure password available only to authorized study personnel. Data will be merged for studies of associations by the biostatistician. All of the information collected in this study will be for research purposes only.

c. Potential risks

The potential risks associated with the study are those associated with completing questionnaires, ^{11}C ER 176 PET/CT scan and EUS with biopsies. EUS-guided core biopsy is a safe procedure associated with a low risk of adverse events. Mild self-limiting serosal bruising and/or localized hematoma formation at the sites of EUS biopsies were reported in a small cohort of patients with gastroparesis who underwent EUS-guided FNA biopsy of the gastric muscle. Questionnaires come with a risk of privacy erosion and emotional distress. However, the

questionnaires proposed in the study are extensively validated and well received. Participants will have the option of leaving any question unanswered that makes them uncomfortable.

2. Adequacy of Protection against Risks

a. Recruitment and informed consent

All participants will provide written informed consent using IRB-approved consent forms. The consent form becomes a permanent part of the medical record at Mayo Clinic. All subjects will be given a verbal explanation of the study, provided time to read and understand the written consent form, given opportunities to ask questions and provided a copy of the consent form. Participants will be informed of their right to withdraw from the study at any time without prejudice to their clinical management at Mayo Clinic then or in the future. Consent will be sought by the PI or the assigned study coordinator, and consent will be documented by the participant's signature on the consent form. Specific information is provided in the Mayo consent form regarding storage and future use of the samples. The participants will be informed that any genetic information collected is not yet pertinent to clinical practice, the information will not be included in the medical record, but will be maintained in a coded fashion accessible only to study personnel. The consent form includes specific details about sharing sequencing data on public databases in a coded fashion at the time of publication. All recruitment material will be approved by the IRB at Mayo Clinic.

b. Protection against risk

Responsible conduct of human research by study personnel

The personnel involved in the study have completed the required education on the protection of human research participants. This is done through IRB's website "Protecting Human Research Participants" online training program. At the conclusion of the instruction, individuals are required to complete a quiz for competency assessment. In addition, Dr. Grover has completed CCaTS Core 6001 (Refresher in Responsible Conduct of Research) and Collaborative Institutional Training Initiative (CITI) training on Good Clinical Practice Course for Clinical Trials Involving Drugs. He is also up-to-date on the CITI instruction in human subjects protection. The course is designed to help users understand human subjects protection issues through a web-based program.

Specific risks and precautions

1) Survey questionnaires: Questionnaires pose no more than minimal risk to the participants. There is a potential for perception of privacy intrusion. However, the subjects have the right to refuse to answer any of the questions or stop at any time, and there is no penalty for refusing to participate. No complications have been recorded. The proposed questionnaires are validated, widely used, and were received well by the participants.
2) ^{11}C ER 176 PET/CT scan: PET scans and the tracers used are generally safe. In a recent study, no adverse or clinically detectable pharmacologic effects were observed with 11C-ER176 for any of the 17 subjects
3) EUS-guided core biopsy: Mild self-limiting serosal bruising and/or localized hematoma formation at the sites of EUS biopsies. Other standard risks of an upper endoscopy include pain (abdominal and throat), aspiration and extremely rarely, death. Serious risk of bleeding and frank perforation is rare (<1 in 1000).

3. Potential Benefits of the Proposed Research to the Subjects and Others

The proposed research has the potential to remarkably enhance our understanding of visualizing immune dysregulation in the gastric muscle of patients with diabetic gastroparesis. This will have a downstream effect of identifying immune dysfunction in other GI conditions. Broadly, this can revolutionize assessment of inflammatory milieu in areas that are not easily or safely amenable for a biopsy. As of now, there likely will not be a direct benefit to the gastroparesis patients as we still do not have clear therapies to target immune dysfunction in gastroparesis. Given the anticipated mechanistic insights to the participants in this research proposal, and to others with diabetic gut dysfunction, the benefits outweigh the risks.

4. Importance of the Knowledge to be Gained

The proposed research has the potential to develop a new approach to diagnose immune dysregulation in diabetic gastroparesis, a disorder with unmet clinical need. Specifically, this project is aiming to determine if a special radio ligand ^{11}C ER 176 based PET/CT imaging can detect activated macrophages in the gastric muscle of patients with diabetic gastroparesis. Additionally, these results will be validated using actual assessment of gastric muscle tissue to determine cellular markers that associate with pro- or anti-inflammatory macrophages. This study will open a novel paradigm for assessment of immune dysregulation in the GI tract which will have utility for a broad range of GI conditions. This pilot and feasibility proposal will generate novel preliminary evidence for future investigations in this area and treatment trials. The risks to the participants are fairly low with questionnaires, PET/CT scan and EUS with biopsies which are safe procedures. Given the repertoire of the PI and study team in conducting mechanistic, endoscopic and imaging studies of this nature as well as the precautions taken to minimize discomfort or adverse effects, the overall risk of this study is fairly low. There is a high likelihood (e.g., investigator team's record and expertise, validated methods) of obtaining meaningful, useful information for the proposed aim.

5. Data and Safety Plan

Subject Safety: Adverse Events

Adverse Event Grading

Attribution scale: An adverse event is defined as either an expected side effect that is of a serious nature, or an unexpected side effect regardless of severity. All events will be graded as to their attribution (unrelated to protocol: possibly, probably, or definitely related to protocol). Any event that is reported to either the PI or his designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented as such.

Expected risks: These risks will be addressed in the protocol and consent form. The expected risks include:

- Loss of confidentiality through unauthorized release of private medical information. This is unlikely since all data are collected in electronic form and access is protected by user identification ID.
- Questionnaires pose some risk of causing emotional distress but these are validated and widely used questionnaires which are typically well received.
- ^{11}C ER 176 based PET/CT imaging is generally safe and no adverse effects were noted in published studies. However, there is radiation exposure which can be harmful. Additionally, there could be intolerance to the IV injection of radio ligand.
- EUS with core gastric biopsies are generally low risk, especially with an advanced endoscopist like Dr. Rajan who will perform this procedure on all proposed participants. Adverse effects include abdominal pain, bleeding, and perforation. Rarely, these adverse events can lead to sepsis and death. However, the probability of a serious adverse event is 1 in 2000 or less, making the procedure quite safe.

Plan for Reporting both Anticipated and Unanticipated Adverse Events.

Any adverse event that is reported generates a report, which will be submitted to the Mayo Clinic IRB. The report will include a description of the event, when and how it was reported, as well as any official chart records or documentation to corroborate the event or the reporting of the event. All adverse events will be graded as mild, moderate, or severe. Any severe and/or unanticipated adverse event will be reported within 24 hours to the IRB. All other adverse events will be reported in a timely fashion to the IRB, preferably within 2 weeks. All adverse events will be summarized annually and submitted to the IRB.

Subject Safety: Safety review plan and monitoring

Safety Reviews: The PI will review the safety and progress of this study on a quarterly basis.

Annual Review: The PI will review this protocol on a continuing basis for participant safety and include results of the review in the annual progress reports submitted to the IRB. The annual report will address: (1) whether adverse event rates are consistent with pre-study assumptions; (2) reason for dropouts from the study; (3) whether all participants met entry criteria; (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study; and (5) conditions whereby the study might be terminated prematurely.

Data Integrity
Adherence Statement: The DSMP outlined will adhere to the protocol approved by the Mayo Clinic IRB (IRB-C which handles research conducted in the Mayo Clinic CCaTS).

Subject Privacy: Data are kept in strict confidence. The template consent form required by Mayo Clinic's IRB includes appropriate language addressing the risks and confidentiality protection. Confidentiality is assured by the use of study number identifiers assigned to each participant. All data will be identified with the use of the study number identifier to the participant. Participants are assured protection of confidentiality as specified by the HIPAA language contained within the standardized template.

Data Confidentiality.
Database protection: The Mayo Clinic software programs are secured with password protection. All information kept within Excel spreadsheets use a study number identifier. Any electronic communication with collaborators outside the Mayo Clinic would include data identified by study number only. The only people with access to the spreadsheet are the study coordinator and the study statistician. The remaining investigators only review group data that have been summarized by the biostatistician.

Secure storage of biospecimens: Tissue samples collected are submitted to a designated laboratory technologist after anonymizing with a study number that is unrelated to the patient's Mayo Clinic registration number. Thus, the technologist does not have access to clinical information. The database that includes sample numbers and Mayo Clinic numbers is maintained in an Excel spreadsheet by the study coordinator.

G. Sharing Plan

We will share materials and manage intellectual property as described in the NIH Sharing Policies and Related Guidance on NIH-Funded Research Resources including the NIH Public Access Plan for Increasing Access to Scientific Publications and Digital Scientific Data from NIH Funded Scientific Research (February, 2015) and the NIH Genomic Data Sharing Policy (August, 2014).

Following publication, we will share our results, methodology, and reagents with the research community at large in a manner consistent with data/resource sharing policies of the National Institute of Health, the Mayo Clinic and the publishers of the journals where our reports will appear. Should any intellectual property arise which requires a patent, we will ensure that the technology (materials and data) remain widely available to the research community according to the NIH Principles and Guidelines document.

As a means of sharing knowledge, publication of NIH-supported original research in primary scientific journals will be our priority. The PI will concentrate on publishing these findings in a timely manner and acknowledge that the research is supported by the NIH. Upon acceptance, publications will be submitted to the NIH Manuscript Submission (NIHMS) system for open access via PubMed Central.