PROTOCOL

Background

1. Provide the scientific background, rationale and relevance of this project.

The Tearney Laboratory at Massachusetts General Hospital invents and translates new noninvasive, high-resolution optical imaging modalities that enable disease diagnosis from living patients without excising tissues from the body. Led by Guillermo (Gary) Tearney, MD, PhD, the lab's large multidisciplinary team invents, validates and translates novel devices that use light to conduct microscopy in living patients. Light is uniquely well suited for noninvasively interrogating the microscopic structure, molecular composition and biomechanical properties of biological tissues. The goal of the laboratory's research is to improve understanding and diagnosis of disease by imaging the human body at the highest possible level of detail in vivo.

The Tearney lab has a long-standing partnership with the Bill and Melinda Gates Foundation for harnessing the ability of Optical Coherence Tomography (OCT) technology to collect detailed information about the gut to obtain more information about diseases like Environmental Enteric Dysfunction EED, which are underserved. This study, funded by the Bill and Melinda Gates foundation seeks to demonstrate the safety and feasibility of using the Trans Nasal Endomicroscopy (TNE) and associated accessory tools to evaluate the intestines of unsedated infants. The goal of this trial is to demonstrate a safe and minimally invasive means for obtaining detailed information on infantile intestinal tissue that is needed for the development of effective environmental enteropathy (EE) dysfunction interventions.

Briefly, this procedure will involve trans-nasal deployment of the device into the intestine of the infant subjects, through a procedure identical to clinical naso-gastric tube placement. Once the device is in place, then intestinal sampling will proceed through the device. Sampling may consist of either microbiome brushings or measurement of the electrical potential differences in the intestine, or both. Once the sampling is complete, the devices will be removed from the subject.

Environmental Enteric Dysfunction (EED)

Environmental Enteric Dysfunction (EED) is a poorly understood condition characterized by villous inflammation and loss and disruption of microscopic intestinal architecture, resulting in decreased intestinal permeability(1). EED is prevalent in regions of the world with inadequate sanitation and hygiene. Bacterial overgrowth and chronic exposure to fecal pathogens is hypothesized to cause inflammation and structural changes in the small bowel, which ultimately result in functional changes(2). As a result, EED is suspected to be the driving factor for carbohydrate malabsorption, malnourishment, poor growth and stunting, poor neurological development, oral vaccine failure and infection affecting 25% of all children globally and causing over a million deaths each year(1–4)

Growth faltering with the resulting poor neurocognitive development and stunting caused by EED in children is largely irreversible after 2 years of life(1). Progress towards understanding EED and developing effective interventions has been hampered by an inability to evaluate the intestinal mucosa of populations in impoverished regions of the world where this condition is endemic. Currently, the only means for directly evaluating the intestine is endoscopy with mucosal biopsy(1). Unfortunately, endoscopy is untenable for the study of EED because of the invasiveness of the method, limited resources and the high cost(4). As a result, there is a clear, unmet need for a less invasive tool that can be used in low- and middle-income countries (LMICs) to evaluate the intestine

[redacted]

Figure 1: [redacted]

Optical Coherence Tomography (OC

Optical Coherence Tomography is an optical diagnostic technology that provides a high- resolution [redacted] cross- sectional images of tissues in a noninvasive way(5,6). [redacted]

Transnasal Endomicroscopy (TNE)

There is a need for effective diagnosis and intervention to be implemented in EED patients before the age of 2 years to prevent the deleterious and permanent sequelae of the disease. The Tearney Lab has developed a new method of visualizing the gastrointestinal tract of unsedated subjects by inserting an endomicroscopy device transnasally, called Transnasal Endomicroscopy. [redacted]

[redacted]

AKUH Infant Imaging:

The TNE device has been used to image 9 infants between the ages of 6 and 18 months at AKUH, Pakistan. [redacted] All infants tolerated the procedure well with no adverse events or complications.

[redacted]

Trans Nasal Endomicroscopy [TNE] Catheter

The Trans Nasal Endomicroscopy (TNE) catheter is comprised of a trans nasal introduction tube and an optical probe. [redacted]

[redacted]

[redacted]

[redacted]

Microbiome Brush

There have been theories proposed that increased microorganism load and/or dysbiosis or an imbalance in the composition of gut microorganisms in the intestine may be important in the establishment and/or maintenance of EED. Development of EED has also been associated closely with the transition of infants from breast feeding to weaning foods. While there is definite association of increased/abnormal microbial load with EED, there have been few papers published which examine this association in detail (10).

Obtaining biopsy samples from the gut remains challenging and requires either an endoscopy or a colonoscopy depending on the region of the gut targeted (11). Duodenal aspiration during esophagogastroduodenoscopy (EGD) is currently used as gold standard for standard of care to collect targeted microbiome. However, the microbiome yield from this procedure is low and the procedure requires an EGD, which is a fairly invasive procedure, and carries all of the risks associated with an EGD. Moreover, EGD is expensive and not easily practicable in LMIC's.(12,13)

The Tearney Lab (MGH) has developed a brush that can collect microbiota samples from the small intestine, in the unsedated subject in a non-invasive manner. [redacted]

Intestinal Potential Difference (IPD) Probe:

Increased gastrointestinal (GI) permeability is associated with several GI conditions that affect millions of people worldwide. Leaky gut is a key feature in celiac disease, Crohn's disease, inflammatory bowel disease (IBD), and environmental enteropathy. It has also been associated with systemic diseases including type 1 diabetes, autoimmune hepatitis, and systemic lupus erythematosus (SLE)(14,15). An alternative approach for measuring mucosal permeability is through measuring the voltage across the intestinal wall (Intestinal potential difference; IPD) that changes with intestinal permeability (16). The Tearney lab has developed an IPD measuring device (IPD probe) that can be deployed trans-nasally through the TNIT to measure the intestinal potential difference in real time at selected locations of the gut. [redacted] The probe has been used to measure IPD in adult subjects at MGH without any adverse events or complications.

OCT Compact Imaging System

The TNE catheter is designed to be compatible with an MGH OCT Compact Imaging System (CIS). [redacted]

M-Mode OCT

[redacted]

[redacted]

Galinstan

Galinstan is a liquid metal alloy of Gallium, Indium and Tin (17). Galinstan has been approved for commercial use by the FDA in various non-mercury thermometers, including pediatric usage(18). Galinstan is chemically non-reactive, biologically inert, and has not been shown to pose safety concerns (17,19)

Objectives/Hypothesis

The purpose of this study is to demonstrate the feasibility of using the TNE platform, using IPD and microbiome brush to evaluate the intestine of unsedated infants.

A total of fifteen (15) subjects, infants 6 months of age to 48 months of age will be enrolled in this study. Study procedures will take place within the Battle Building at either the 5th Floor in the Gastroenterology Clinic (reviewing and/or signing of ICF if during routine care clinic visit), the 3rd Floor CHRC (Child Health Research Center) clinic, and possibly the Battle Outpatient Surgery Center located in the basement.

Study Design: Biomedical

1. Will controls be used? No

►IF YES, explain the kind of controls to be used.

2. What is the study design?
Device feasibility study.

3. Does the study involve a placebo? No
►IF YES, provide a justification for the use of a placebo

Human Participants

Ages: 6 to 48 months of age
Sex: all genders
Race: every race

Subjects- see below
1. Provide target # of subjects (at all sites) needed to complete protocol. 15

2. Describe expected rate of screen failure/ dropouts/withdrawals from all sites.
10% drop-out rate

3. How many subjects will be enrolled at all sites? 15

4. How many subjects will sign a consent form under this UVa protocol? 15

Inclusion/Exclusion Criteria

1. List the criteria for inclusion
- 6 to 48 month old Infants that, that can follow fasting requirements.

2. List the criteria for exclusion
- Any infant under 6kg of weight since this is the minimum weight for 6.5F NG tube
- Any infant whose nasal passage cannot reasonably accommodate a 6.5 French nasoduodenal catheter.
- Any infants with absolute or relative contraindications to transnasal tubes:
  - severe midface trauma and recent nasal, throat, or esophageal surgery.
  - Esophageal varices, esophageal stricture, and alkaline ingestion
  - Congenital anatomical defects affecting the gastrointestinal tract, most specifically cleft lip and/or cleft palate.
- Any infant with absolute or relative contraindication to a duodenal biopsy:
  - coagulation abnormalities

Specific Exclusion Criteria for IPD (applicable only to subjects that will undergo IPD procedure):
- Subjects with uncontrolled Diabetes Mellitus 1 & Diabetes Mellitus 2

- Subjects enrolled in clinical trials involving interventions that affect Intestinal Permeability
- Subjects currently taking H2 Histamine Antagonists (such as Pepcid, Axid, Tagamet, Zantac, etc)
- Subjects currently taking Mast Cell stabilizers

3. List any restrictions on use of other drugs or treatments. Yes, the subject will need to be fasting for the procedure.

Statistical Considerations

1. Is stratification/randomization involved? No

►IF YES, describe the stratification/ randomization scheme.

►IF YES, who will generate the randomization scheme?

_____ Sponsor
_____ UVa Statistician. Insert name Answer/Response:
_____ UVa Investigational Drug Service (IDS)
_____ Other: Specify Answer/Response:

2. What are the statistical considerations for the protocol?
The objective of this study is to perform a feasibility study in 15 infant subjects in order to demonstrate that we can successfully perform the TNE technique and associated procedures.

3. Provide a justification for the sample size used in this protocol.
This is a feasibility study to generate preliminary data for a larger study, so a power calculation was not performed. Based on the conditions needed for the TNE feasibility study, for each of the accessory devices, we determined that an enrollment of 15 subjects would be sufficient.

4. What is your plan for primary variable analysis?
This is not applicable in this feasibility study, as our primary objective is to demonstrate our ability to using the TNE platform to evaluate the intestine of unsedated infants.

5. What is your plan for secondary variable analysis?
This is not applicable in this feasibility study, as our primary objective is to demonstrate our ability to using the TNE platform to evaluate the intestine of unsedated infants.

6. Have you been working with a statistician in designing this protocol?
Not for this feasibility study

7. Will data from multiple sites be combined during analysis? No, data from each site will be analyzed independently and may later be compared if appropriate. Data acquired from UVA will be the control, representing the non-EED mucosa. It will not be combined with the data from the Low to Middle Income Counties (LMIC) sites.

7(a). Does the study involve randomization?

IF YES, will randomization be done at each site or among sites?

7(b). Has the sample size calculation considered the variation among sites?

7(c). When combining the data from multiple sites to assess the study results, is the effect of the treatment to be tested (or the association to be tested) assumed to be the same across sites or vary among sites? What is the modelling strategy?

7(d). Is there a common protocol used in all sites?

IF NO, how will differences among sites, such as those related to the implementation, inclusion criteria, patient characteristics, or other sites characteristics, be considered to assess the study results?

Study Procedures-Biomedical Research

1. What will be done in this protocol?
Overview

Briefly, the procedure will be conducted as follows:

1. The TNIT (with optical probe inside) is inserted, confirmation of placement within the esophagus is confirmed via OCT image and the TNIT is advanced down the esophagus
2. Once in the gut, the balloon(s) may be inflated.
3. Once at a location of interest, the optical probe is removed while the TNIT stays in position.
4. The appropriate accessory device can then be inserted for microbiome brushing/aspiration, and/or IPD measurement.

The infant may receive any or all of the evaluation methods that are detailed in this protocol - intestinal potential difference measurement (IPD) and microbiome sample collection. When both evaluation methods are performed, IPD will be completed first.

The research procedure can be stopped at any time. If at any point the family, clinical staff, study staff, or physician feels that the subject will not be able to tolerate the TNIT procedure, the procedure will be terminated and the device removed from the subject. The procedure is described in detail below.

Preprocedure

The PI and other pediatric GI physicians will identify potential subjects aged 6-48 months and contact the CRC with the potential patient's contact information and/or ask the parents of the potential subjects if they'd like a CRC to come speak to them in the clinic. The CRC will verify possible enrollment of this infant with the Pediatric Gastroenterologist and contact the family of the patient to gauge their interest. The CRC will offer trial information to the infant's family (ie: informed consent). The CRC will then inform the UVA and MGH teams about the interested family. The study team will coordinate the timing and logistics of the research and clinical procedures. Subjects will need to follow this study's guidelines for fasting. Children over 12 months will be asked to fast after midnight the day of the procedure. Formula fed infants under 12 months may be given formula up to 6 hours prior to the procedure. Breastfed infants under 12 months may feed up to 4 hours before the procedure.

The procedure will take place in the Battle building on the 3rd Floor CHRC Clinic, 5th Floor Gastroenterology Clinic (when consent is reviewed and/or signed during a routine clinic visit) as well as possibly the Battle Outpatient Surgery Center. On the day of the procedure, to be certain that infant's family understands the study procedure, they will be able to discuss the study again with the research nurse/research coordinator on the day of their appointment. Consent will be obtained prior to the commencement of the procedure. The option to discuss their concerns with a physician will be offered to all subjects.

Intraprocedure

The subjects will not be sedated during the procedure. The TNIT catheter will be introduced transnasally in a manner nearly identical to the standard of care procedure in place for NG tube insertion,(20) described below. The procedure will be performed by a licensed health care provider under the supervision of an MD or by an MD.

The tip of the TNIT catheter (2-3 inches) will be lubricated with clinically used lubricant gel prior to insertion into the nostril. Once the catheter passes the pharynx the imaging will be turned on and confirmation of placement will be obtained via OCT images (Figure 3a). The balloon(s) [redacted] may be inflated slightly to provide better tissue contact. The catheter will be further advanced. If there is some resistance or no further advancement, a pacifier dipped in sugar water or small drops of sugar water may be given and the advancement will be coordinated with swallowing. Water administered during the TNE placement will be limited in volume and the time of administration will be monitored. If unable to advance tube after 2 trials in each nostril the insertion attempt will be discontinued and the procedure terminated.

Once the TNE is inside the GI tract, as confirmed by the OCT images, the balloon(s) may be inflated with air or a biocompatible liquid (such as Galinstan, sugar water, saline) to add weight (Fig:3b) to facilitate its motion towards the pylorus and into the duodenum (Fig: 3c). Once the balloons are inflated, the team may wait for the device to pass into the duodenum/region of interest by peristalsis. The operator may assist in the placement of the TNE device by gently maneuvering the device. The study team may have the subjects change their body position or sip water during the procedure to facilitate placement or movement of the device and improve imaging, as required. During the device transit from insertion to duodenum, images will be periodically acquired to determine and confirm the TNE's position in the GI tract.

It is estimated that the catheter will enter the duodenum in anywhere from a few minutes to up to 1 hour from the time it is introduced, based on the range of previous gastric emptying times demonstrated in TNE studies in infants and children at AKUH. The entire research procedure (insertion, IPD, brush, removal) will not exceed 90 minutes. If the device does not pass into the desired region at the end of the IRB allotted procedure time, the device will be removed from the subject.

Once the catheter has passed through the pyloric sphincter, images of the duodenum and potentially jejunum will be continuously acquired as it is propelled down the small intestine via peristalsis. The distance from the nose will be measured via marks on the outer sheath of the tether. OCT images of the small intestine will be obtained as the catheter descends the small intestine via peristalsis. During the procedure, air, water, and/or saline may be used to flush the lumen of the TNIT to allow for easier insertion of the probes and improve image quality. This will be done at the discretion of the pediatric gastroenterologist performing the procedure. The optical probe may be removed and reintroduced or replaced while the TNIT is in situ inside the subject. Once the TNIT has been placed into the region of interest, as confirmed by OCT images, any or all of the following procedures may be performed while the TNIT is in situ. When both evaluation methods are performed, IPD will be completed first.

a. Microbiome brushings and aspiration:

Once the target site has been identified, the optical probe will be removed while the TNIT remains in place. The operator will then introduce the microbiome brush through the accessory port of the TNIT until the tip of the microbiome brush is in contact with the mucosa, [redacted] The brush will then be manipulated back and forth over the mucosa, [redacted], a couple of times and then will be removed completely out of the working channel of the TNIT tube. Additionally, a small amount of suction throughout the duration of brushing may also be applied via the outer sheath of the brush using a syringe in order to collect a small amount of aspirate for processing.

A small tube may also be threaded down the TNIT before or after the brush to collect aspirations. Similar to the brush, suction will be applied for no more than 30 seconds. [redacted]

In order to improve tissue contact, either while imaging or collecting samples, we may apply a small amount of suction [redacted]

This specimen collection procedure may be repeated up to a total of 5 times, with a new brush, tube, and syringe each time to ensure adequate specimen collection. We may collect up to a total of 5 brush samples and/or 5 aspirate samples. The tip of the TNIT may be moved slightly (by approximately a couple of centimeters) to avoid brushing/aspirating the same region multiple times. This will improve the quality of the specimen and avoid excessive mucosal abrasion. Each brushing will not last longer than 30 seconds.

If Microbiome and aspirate samples are collected, then, the tip of the brush and any aspirate collected then, the tip of the brush and any aspirate collected, will be stored in appropriately labelled and sealed container with **DNA/RNA agent.**

The samples will be anonymized and sent to a Mass General Brigham (MGB) approved vendor for processing, as per institutional policies for transport of biological/other research materials for microbiological processing including culture and genomic sequencing.

b. IPD Measurement

Once a preferable location for IPD measurements is confirmed via OCT imaging, the optical probe will be removed from the TNIT. [redacted]

To create a reference potential for IPD measurements, the study team will apply a small skin patch taped to abraded skin. The site chosen for the skin patch will be wiped with an alcohol swab. Next, the most superficial epidermal layer will be removed using a dermabrasion device (similar to a nail file), or by rubbing dry, unwoven gauze on the site. Once the dermal layer has been removed, the skin patch or sponge will be applied (23).

[redacted]

When IPD and skin probe have been calibrated, te[redacted] The operator will then introduce the IPD probe [redacted]

[redacted]

[redacted]

During the IPD measuring period, the IPD probe may be moved within the TNIT or the whole TNIT itself may be moved to find different tissue contact sites within the GI tract for more data points. Once the IPD measurements are complete, the IPD probe will be removed.

The same or a different imaging probe may then be reintroduced to reimage the gut. The imaging probe(s) may also be introduced at any point between the 2 gut evaluation procedures to reimage the gut as well. Once the transnasal procedure is complete, the balloon (s) will be deflated, and the introduction tube will be removed. The entire procedure,

including the introduction of the TNIT, transit into the duodenum, imaging and acquisition of samples/IPD measurement will not take more than 90 minutes.

Post Procedure

Approximately 1 day following the research procedure the CRC will call the subject's family.

Data Collection & Review

For each of the consented subjects, characteristics such as age, sex, body mass index (BMI), pertinent medical diagnostic information will be recorded.

TNE imaging will be used for research purposes only and will not be used for diagnostic purposes. An investigator will also assess the quality of the recorded images after the imaging is completed. Coded imaging data and other relevant data will be processed and analyzed.

Risks

Overall, the risks associated with this study procedure are low. The most common risk is discomfort associated with the initial placement of the transnasal device. These devices have been used in studies conducted at MGH and AKUH with no serious complications or injuries. OCT images and/or length markings on the outside of the TNIT can be used to determine the location of the device. At any moment the device can be removed.

Expected Risks related to study participation.	**Frequency** Frequently, Rarely,
Temporary sensation of gagging and or coughing. Bleeding at brush site. Swelling, redness, pain, and or bruising at IPD reference probe skin patch site.	__X_Occurs Frequently

Temporary sensation of choking. Injury to nasal passages.* Nose bleed. There is a very small risk of infection. This risk is the same as when having a regular endoscopy.* Bleeding at IPD site.* Feeling dizzy or fainting. Balloon(s) burst.* Device breaking while inside the research participant.* Duodenal hematoma.* Placement of the device into the lungs. This is prevented this by visually confirming the placement of the tube using the camera at the tip of the device. If the device is in the wrong place, it will be removed immediately.* Infection at IPD reference probe skin patch site.* Confidentiality breach; mitigated by use of unique subject ID.* Injury or perforation of GI tract. Mitigated by only having trained medical professionals perform the research procedure.*	_X__Occurs Rarely
* In studies performed with these devices at MGH and the Aga Khan University Hospital (AKUH) in Karachi, Pakistan, this has never occurred.	

Remuneration

In order to adequately compensate the subject and their family for their time, they will receive a check for up to $600 for completing this study.

Payment	Research Procedure Completion
$600	TNE Device Inserted IPD Measurements Attempted **AND**

	Microbiome Brushings Attempted
$400	TNE Device Inserted OCT Imaging Performed **AND** IPD Measurements Attempted **OR** Microbiome Brushings Attempted
$200	Subject consents for study but the team is unable to successfully insert the TNE, for any reason

2. **If this protocol involves study treatment, explain how a subject will be transitioned from study treatment when they have completed their participation in the study.** N/A

Subject Compliance with Study Procedures

1. **Explain how the study team will monitor the subject for compliance with the study procedures.** (e.g. study team will administer study drug/ study interventions, study drug inventory of dispensed and returned drug, diary etc.)

   The procedure is performed on the consented subject. Subject compliance is not a factor.

   If, at any point, the physician/endoscopist or study staff feels that the subject will not be able to tolerate the procedure, the study procedure will be terminated.

2. **Describe criteria for when a subject is considered to be non-compliant with study procedures.** (e.g. subject returns more than 20% of the study drug, subject misses 20% of study visits)

   N/A

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