Application of a Novel Allergen-Specific Immune Signature Directed Approach to Dietary Elimination Therapy in Patients With Eosinophilic Esophagitis (IDiET)

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Application of a Novel Allergen-Specific Immune Signature Directed Approach to Dietary Elimination Therapy in Patients with Eosinophilic Esophagitis (IDiET)

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Initial version: March 8, 2016
Version 2.0: October 3, 2016
Version 3.0: June 20, 2017

Changes from Previous Version

This version includes the following major changes to the previous version of the protocol, Version 2.0 (03Oct2016).

Section 1.2, Rationale	Added additional possible allergens tested on research samples for data collection.
Section 6.1, Sample Size Determination	The number of consented and enrolled subjects has been increased to 30, with an increased goal of 20 subjects completing the study.
Administrative Changes	Updated protocol version on first page and header of all subsequent pages. Minor typographical errors corrected throughout.

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Protocol Signature Page

I have read and agree to the protocol entitled 'Application of a Novel Allergen-Specific Immune Signature-Directed Approach to Dietary Elimination Therapy in Patients with Eosinophilic Esophagitis (IDiET)' dated 20Jun2017. I am aware of my responsibilities as an Investigator under the guidelines of GCP, local regulations (as applicable) and the trial protocol. I agree to conduct the trial according to these responsibilities and to appropriately direct and assist the staff under my control, who will be involved in the trial.

Principal Investigator:

Print Name	Title
Signature	Date

List of Abbreviations

Item	Definition
CEDAS	Center for Esophageal Diseases and Swallowing
CRF	Electronic Case Report Form
DSQ	Dysphagia symptom questionnaire
EC	Ethics Committee
EGD	Esophagogastroduodenoscopy
EoE	Eosinophilic Esophagitis
EoG	Eosinophilic Gastroenteritis
Eos	Eosinophils
EREFS Score	EoE Endoscopic Reference Score
FDA	Food and Drug Administration
HPF	High Power Field
IDE	Investigational device exemption
IRB	Institutional Review Board
IVD	In-vitro diagnostic
NSR	Non-significant risk
PID	Participant ID

Study Summary

Title	Application of a Novel Allergen-Specific Immune Signature Directed Approach to Dietary Elimination Therapy in Patients with Eosinophilic Esophagitis (IDiET)
Short Title	IDiET
Methodology	Prospective single center clinical trial
Study Duration	2 years
Study Center(s)	University of North Carolina, Chapel Hill, NC
Objectives	To evaluate prospectively the effectiveness of individualized allergen-specific immune signature-based dietary elimination therapy for EoE
Number of Subjects	30
Diagnosis and Main Inclusion Criteria	*Inclusion Criteria*: 1) Age 16-80 years old 2) Meet one of the following: a. Active EoE as per consensus guidelines OR b. Undergoing upper endoscopy for a clinical suspicion of EoE 3) No prior history of dietary elimination therapy *Exclusion Criteria*: 1) Concomitant eosinophilic gastroenteritis 2) Any corticosteroid exposure in the 4 weeks prior to their baseline endoscopic exam 3) Previous esophageal surgery 4) Medical instability that precludes safely performing upper endoscopy 5) Inability to read or understand English 6) Pregnant women
Statistical Methodology	To determine the response rate for the primary outcome, the number of subjects with <15 eos/hpf on follow-up endoscopy will be calculated. For secondary outcomes, median eosinophil counts, endoscopy scores, and symptoms scores will be compared before and after treatment with Wilcoxon signed-rank. These will be contextualized by calculating odds ratios and 95% confidence intervals where appropriate, so as not to over-rely on p values alone. Patients who drop out or who have incomplete data for the outcomes will be excluded from analysis. Because this study's outcomes are determined after the treatment period and follow-up endoscopy, a subject must complete this end of treatment evaluation for us to be able to include their data in the analysis. We do not feel that selection bias would impact the analysis if there are incomplete data due to drop out. We are not performing a comparative analysis between groups, so drop out would not be dependent on different treatments administered in different study arms. In addition, in our experience with previous clinical studies of EoE, dropout rates have been quite low.

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice, applicable government regulations and Institutional research policies and procedures.

1.1 Background

Over the past decade, eosinophilic esophagitis (EoE) has rapidly increased in incidence and prevalence, becoming a major cause of chronic gastrointestinal morbidity. EoE is defined by pathologic infiltration of eosinophils into the esophageal mucosa, leading to dysphagia and progressive esophageal stenosis, and is now the most common cause of food impaction in the U.S. EoE is a chronic, allergic/immune-mediated condition that appears to be driven primarily by IL-5+ and IL-13+ T cell responses to food antigens, based on studies observing disease remission in individuals empirically avoiding all potentially allergenic foods. However, such radical elimination diets are not feasible and there is no currently available test to accurately identify specific food triggers in EoE. Because of this, successful dietary therapy requires empiric elimination of a large number of foods, subsequent reintroduction of single foods, and invasive monitoring (endoscopy with biopsy) to assess response. There is a critical need to develop methods to reliably identify allergen triggers.

In Phase I of the Team Translational Science Award, we began to address these key knowledge gaps. Using prospectively collected biospecimens, we successfully developed a novel T cell directed approach whereby allergen-specific T cells were correlated with known dietary triggers of EoE. We examined esophageal tissue, duodenal tissue, and blood, and found that the blood compartment was the most reliable for extracting, culturing, and stimulating T cells. This demonstration was the first time in humans with EoE that potential food triggers could be detected with a T cell signature in the blood, and it provided proof-of-principle of a clinical application of individualized food elimination regimens. We also performed preliminary studies detecting allergen-specific IgG4 in esophageal biopsies, a recently described pathogenic mechanism of EoE. We feel that the combination of these techniques will allow determination of allergen-specific immune signatures in EoE patients. These hold great promise to impact clinical practice by accurately identifying foods to eliminate in dietary therapy, thus increasing compliance and ultimately response rates. However, additional steps are required before clinical adoption of this approach.

The goal of this study is to conduct a pilot prospective study of allergen-specific immune signature-guided dietary elimination therapy to assess the clinical effectiveness of this technique. The overall hypothesis is that T cells obtained from the blood of a subject with active EoE, when cultured and stimulated with food allergens, and when coupled with allergen-specific IgG4 detected in an esophageal biopsy, will result in a food trigger-specific immunological signature that can be used to design highly effective dietary treatment specific to that individual.

1.2 Rationale

Eosinophilic esophagitis is a rapidly increasing chronic allergic condition

Over the past decade, eosinophilic esophagitis (EoE) has rapidly increased in incidence and prevalence to become a major cause of chronic gastrointestinal morbidity, with annual health care costs estimated at $1 billion.[1-3] EoE is defined by pathologic infiltration of eosinophils into the esophageal mucosa, leading to dysphagia and progressive esophageal stenosis,[4, 5] and is now the most common cause of food impaction in the U.S.[6, 7] EoE is a chronic, allergic/immune-mediated condition that is driven primarily by IL-5+ and IL-13+ T cell responses to food antigens,[8-11] rather than a typical immediate IgE response.[12] This pathogenesis suggests that dietary elimination would be effective, and disease remission has been demonstrated in individuals empirically avoiding all allergenic foods or consuming only a hypoallergenic elemental formula.[13-18]

Dietary elimination, as currently conceived, is a sub-optimal and cumbersome therapy option
There are multiple problems, however, with the dietary elimination approach to treatment of EoE. Radical elimination diets, although effective, are not feasible given their very restrictive nature. For example, more than 50% of adults are unable to tolerate an elemental formula diet for more than a few days,15 and children often require placement of feeding tubes to obtain adequate nutrition.[14, 19] Further, poor ability to adhere to the so-called "six food elimination diet", which empirically removes dairy, wheat, egg, soy, nuts, and seafood, has led to variable and unpredictable response rates.[20, 21]

A major reason for this is that no currently available allergy test can accurately identify specific food triggers leading to EoE. For example, two studies have shown that an elimination diet based on skin-prick testing correctly identifies food triggers in only 13%, a rate that is far worse than expected by chance alone.[17, 22] Because of this, successful dietary therapy requires empiric elimination of a large number of foods, subsequent reintroduction of single foods, then endoscopy and biopsy to assess for recurrent tissue eosinophilia.5 This leads to dietary changes that are frequently unnecessary, and the serial endoscopies, which can number up to 10 in a single year,[22] are costly and risky.[3] Finally, while alternative treatment with topical corticosteroids is possible in patients who cannot comply with dietary therapy, not all patients respond, and long-term use of this medication class is unsafe.[23, 24] Therefore, there is a critical need to develop methods to reliably identify allergen triggers. This will advance understanding of the biology of EoE, lead to substantially improved therapy, and reduce costs.

This study uses novel and highly innovative techniques to identify dietary triggers and individualize dietary treatment regimens, making dietary elimination practical and more palatable
A novel allergen-specific immune signature-directed approach to food trigger identification has great promise for EoE. The current model of EoE pathogenesis holds that when food antigens are presented to the GI tract, either in the esophagus or in the small intestine, previously sensitized T cells are triggered to produce a cascade of Th2 cytokines, including IL-4, IL-5, and IL-13.[8] In addition, a recent study has identified foodspecific IgG4 as important to the pathogenesis.12 Because this model is neither simply an immediate IgE- nor a classic delayed IgG-mediated response, it is not surprising that skin prick or patch tests are not accurate.

An alternative approach would be to create a food trigger-specific immunological signature using both sensitized T cells and food-specific IgG4 as the basis for elimination diet therapy. The complexity of the immune response in EoE suggests that IgG4-secreting B-cells may receive signals from allergen-responsive T cells after exposure.[25] Our previous work has shown that T cells sensitized to specific foods can be readily expanded and characterized in response to antigen in non-EoE patients with IgE-mediated peanut or egg allergies.[26-28] In our work supported by Phase I of the UNC Team Translational Science Award (TTSA), we showed for the first time in patients with EoE that this could also be done. Specifically, using prospectively collected specimens, we successfully developed a novel T cell directed approach whereby allergen-specific T cells were correlated with known dietary triggers of EoE. We examined esophageal tissue, duodenal tissue, and blood, and found that the blood compartment was the most reliable for extracting, culturing, and stimulating T cells (see below). Moreover, we performed preliminary studies detecting food allergen-specific IgG4 in esophageal biopsies in EoE patients (see below).

Taken together, these findings demonstrate proof-of-principle of a potential clinical application using allergenspecific immune signatures to identify food triggers and create elimination diet regimens in patients with EoE.

Optimization of newly developed techniques of determining allergen-specific immune signatures in patients with well-characterized, food-driven EoE.
Investigators studied two allergen-specific signatures. The first was antigen-specific IgG4 in esophageal tissue. Investigators showed that total and food-specific IgG4 was markedly elevated in esophageal biopsies from EoE cases compared to controls (Table 1). Second, investigators showed that food-specific IgG4 levels of trigger foods decreased in EoE patients who responded to dietary elimination (Figure 1). Third, using the sub-population of EoE patients who successfully responded to dietary elimination and in whom the trigger foods were known, investigators were able to develop optimal thresholds for normalized food-specific IgG4 levels that identified food triggers with a good to excellent agreement (Table 2).

Table 1: Esophageal IgG4 levels are markedly elevated in EoE cases

Median levels (ng/mL)	EoE cases (n = 20)	Controls (n = 10)	p
Total	1847	469	0.008
Peanut	4.05	0.01	0.003
Soy	2.12	0.01	< 0.001
Egg	61.4	2.88	< 0.001
Milk	55.8	0.67	< 0.001
Wheat	19.9	1.10	< 0.001

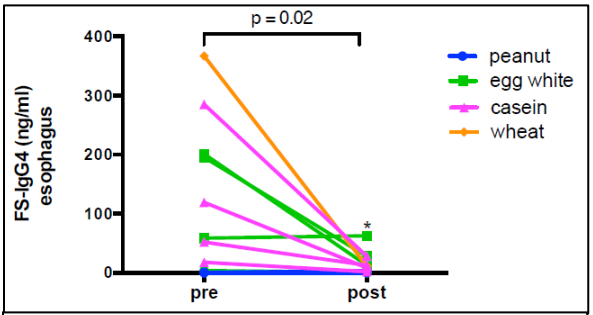


Figure 1: Food specific IgG4 levels decrease after dietary elimination of triggers

Table 2: [redacted] performance of esophageal IgG4 for identifying food triggers in EoE

[redacted]	Nut	Egg	Soy	Wheat	Milk
Testing parameters (%)					
Sensitivity	0	88	0	60	55
Specificity	80	75	100	100	100
Agreement	67	80	67	77	53

The second allergen-specific signature we assessed was the lymphocyte proliferation assay developed by the investigators. Here, investigators again used the sub-population of EoE patients who successfully responded to dietary elimination and in whom the trigger foods were known to develop a threshold for identifying food triggers with good to excellent agreement (Table 3). When both tests were used together, the sensitivity for identifying triggers was improved, with an associated decrease in specificity (Table 4). However, investigators will accept this trade-off for this protocol so that investigators identify as many potential food triggers as possible to optimize the treatment response rate. Moreover, the agreement across all of the tested foods was substantially better than the reported agreement of 13% using traditional skin prick allergy tests. Investigators have confirmed that the lab studies can yield results within 2 weeks, which is a clinically acceptable time frame. In addition, investigators performed cytokine stimulation testing for 14 EoE cases with IL-4, IL-5, and IL-13 but these results did not clearly help to increase the yield for identifying food triggers, so investigators will not pursue this direction further (data not shown).

Table 3: Performance of lymphocyte proliferation testing for identifying food triggers in EoE[redacted]

	Nut	Egg	Soy	Wheat	Milk
Testing parameters (%)					
Sensitivity	100	63	60	20	45
Specificity	82	75	78	50	75
Agreement	75	71	50	42	47

[redacted]

Table 4: Performance of a positive test on either the lymphocyte proliferation or the IgG4 assay for identifying foods

	Nut	Egg	Soy	Wheat	Milk
Testing parameters (%)					
Sensitivity	100	100	75	80	64
Specificity	50	50	45	43	25
Agreement	60	75	53	58	53

These thresholds will be used in this prospective study of allergen-specific immune signature-guided dietary elimination therapy to assess the clinical effectiveness of this technique. Samples may be tested using additional allergens, including but not limited to beef, corn, fish, shellfish, rice, chicken, potato, pork,

legumes, green beans, tomatoes, green peppers, and tree nuts. Results from these additional allergens are for data collection only and will not be used to drive subject diet elimination or communicated to subjects.

2 Study Objectives

Primary Objective: To evaluate prospectively the effectiveness of individualized allergen-specific immune signature-based dietary elimination therapy for EoE.

We hypothesize that at least 75% of patients with active EoE will experience a histologic response following treatment with an individualized dietary regimen based on their own allergen-specific immune signature.

3 Study Design

3.1 General Design

This is a prospective single center clinical trial of allergen-specific immune signature-guided dietary elimination therapy to assess the clinical effectiveness of this technique.

Potential subjects will be approached regarding the study. If eligible and interested, then informed consent will be obtained and they will be enrolled in the study. If the subject has had an esophagogastroduodenoscopy (EGD) at UNC within 3 months since enrollment, clinical biopsies from the EGD show active EoE and the subject continues to meet consensus guidelines for active EoE, and research biopsies were taken during that EGD that can be used for this study, then the subject will complete questionnaires and a blood draw only. The blood draw may be abbreviated if the subject had research blood drawn during the same recent EGD that can be used in this study.

If subjects have not had an EGD with biopsies at UNC within 3 months prior to enrollment, then they will complete questionnaires, a blood draw, and be scheduled to receive a routine care esophagogastroduodenoscopy (EGD) at UNC facilities with clinical and research biopsies. However, in some cases, samples that were obtained prior to this time frame can be used, as long as the samples were obtained when the EoE was active and there were no major changes in clinical status.

During the routine care endoscopy, clinical biopsies will be taken for routine care purposes, and additional research biopsies will be collected for research purposes for diet elimination testing and to be stored for future research studies from the distal, mid, and proximal esophagus. Blood will also be collected during this visit, and questionnaires completed. If research biopsies are unable to be obtained during this EGD the subject will no longer continue in the study and will be considered a screen fail. If pathology from routine care biopsies does not confirm a diagnosis of active EoE, then the subject will no longer continue in the study and will be considered a screen fail. If subjects have had an EGD with clinical and research biopsies within 3 months prior to enrollment, then research biopsies taken during that EGD will be used for this study. As noted above, in some cases, samples that were obtained prior to this time frame can be used, as long as the samples were obtained when the EoE was active and there were no major changes in clinical status.

After completion of the EGD and/or collection of EGD records and previous biopsies if an EGD was previously completed), and confirmation of eligibility, subjects will be scheduled for a routine care nutrition counseling appointment. Two weeks prior to the routine care nutrition counseling appointment, subjects will begin the dysphagia symptom questionnaire (DSQ). During the routine care nutrition counseling appointment, the subject will receive counseling on which foods to eliminate based on the T-cell and IgG4 results from the research biopsies. Subjects will also receive an allergy skin test during this visit. Results from the allergy skin test will not be used to drive food elimination diet.

Subjects will follow their assigned food elimination diet for 6 weeks. At 6 weeks subjects will be scheduled for a routine care esophagogastroduodenoscopy (EGD) with biopsies for clinical purposes. Two weeks prior to the 6 week EGD subjects will restart the DSQ. Data will be collected from the 6 week EGD but no research specific biopsies will be obtained during that visit. Research specific blood will be taken at this visit. Study participation is complete after completion of the 6 week EGD.

3.2 Non-Significant Risk

The algorithm used in this study to diagnose food allergens has been deemed a non-significant risk (NSR) in vitro diagnostic (IVD) device by the UNC IRB as of March 7, 2016. This means the device qualifies for an abbreviated investigational device exemption (IDE). Per the UNC IRB, as used in this study, the device does not pose a serious risk to the health, safety or welfare of a subject and does not otherwise meet the definition of a significant risk device according to 21 CFR 812.3(m). This study has been deemed non-significant risk (NSR); the sponsor and investigator must comply with "abbreviated IDE requirements" described in 21CFR812(b). As the sponsor-investigator, this means Dr. Dellon must ensure he:

(i) Labels the device in accordance with 812.5 (below):

> (a) Contents. An investigational device or its immediate package shall bear a label with the following information: the name and place of business of the manufacturer, packer, or distributor (in accordance with §801.1), the quantity of contents, if appropriate, and the following statement: “CAUTION—Investigational device. Limited by Federal (or United States) law to investigational use.” The label or other labeling shall describe all relevant contraindications, hazards, adverse effects, interfering substances or devices, warnings, and precautions.
>
> (b) Prohibitions. The labeling of an investigational device shall not bear any statement that is false or misleading in any particular and shall not represent that the device is safe or effective for the purposes for which it is being investigated.
>
> (c) Animal research. An investigational device shipped solely for research on or with laboratory animals shall bear on its label the following statement: “CAUTION—Device for investigational use in laboratory animals or other tests that do not involve human subjects.”
>
> (d) The appropriate FDA Center Director, according to the procedures set forth in §801.128 or §809.11 of this chapter, may grant an exception or alternative to the provisions in paragraphs (a) and (c) of this section, to the extent that these provisions are not explicitly required by statute, for specified lots, batches, or other units of a device that are or will be included in the Strategic National Stockpile.

(ii) Obtains IRB approval of the investigation after presenting the reviewing IRB with a brief explanation of why the device is not a significant risk device, and maintains such approval;

(iii) Ensures that each investigator participating in an investigation of the device obtains from each subject under the investigator's care, informed consent under part 50 and documents it, unless documentation is waived by an IRB under 56.109(c).

(iv) Complies with the requirements of 812.46 with respect to monitoring investigations (below);

> (a) Securing compliance. A sponsor who discovers that an investigator is not complying with the signed agreement, the investigational plan, the requirements of this part or other applicable FDA regulations, or any conditions of approval imposed by the reviewing IRB or FDA shall promptly either secure compliance, or discontinue shipments of the device to the investigator and terminate the investigator's participation in the investigation. A sponsor shall also require such an investigator to dispose of or return the device, unless this action would jeopardize the rights, safety, or welfare of a subject.
>
> (b) Unanticipated adverse device effects. (1) A sponsor shall immediately conduct an evaluation of any unanticipated adverse device effect.
>
> (2) A sponsor who determines that an unanticipated adverse device effect presents an unreasonable risk to subjects shall terminate all investigations or parts of investigations

presenting that risk as soon as possible. Termination shall occur not later than 5 working days after the sponsor makes this determination and not later than 15 working days after the sponsor first received notice of the effect.

(c) Resumption of terminated studies. If the device is a significant risk device, a sponsor may not resume a terminated investigation without IRB and FDA approval. If the device is not a significant risk device, a sponsor may not resume a terminated investigation without IRB approval and, if the investigation was terminated under paragraph (b)(2) of this section, FDA approval.

(v) Maintains the records required under 812.140(b) (4) and (5) and makes the reports required under 812.150(b) (1) through (3) and (5) through (10);

812.140(b) 4 and 5:

(4) For each investigation subject to §812.2(b)(1) of a device other than a significant risk device, the records described in paragraph (b)(5) of this section and the following records, consolidated in one location and available for FDA inspection and copying:

(i) The name and intended use of the device and the objectives of the investigation;

(ii) A brief explanation of why the device is not a significant risk device:

(iii) The name and address of each investigator:

(iv) The name and address of each IRB that has reviewed the investigation:

(v) A statement of the extent to which the good manufacturing practice regulation in part 820 will be followed in manufacturing the device; and

(vi) Any other information required by FDA.

(5) Records concerning adverse device effects (whether anticipated or unanticipated) and complaints

812.150(b) (1) through (3) and (5) through (10);

(b) Sponsor reports. A sponsor shall prepare and submit the following complete, accurate, and timely reports:

(1) Unanticipated adverse device effects. A sponsor who conducts an evaluation of an unanticipated adverse device effect under §812.46(b) shall report the results of such evaluation to FDA and to all reviewing IRB's and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests.

(2) Withdrawal of IRB approval. A sponsor shall notify FDA and all reviewing IRB's and participating investigators of any withdrawal of approval of an investigation or a part of an investigation by a reviewing IRB within 5 working days after receipt of the withdrawal of approval.

(3) Withdrawal of FDA approval. A sponsor shall notify all reviewing IRB's and participating investigators of any withdrawal of FDA approval of the investigation, and shall do so within 5 working days after receipt of notice of the withdrawal of approval.

(5) Progress reports. At regular intervals, and at least yearly, a sponsor shall submit progress reports to all reviewing IRB's. In the case of a significant risk device, a sponsor shall also submit progress reports to FDA. A sponsor of a treatment IDE shall submit semi-

annual progress reports to all reviewing IRB's and FDA in accordance with §812.36(f) and annual reports in accordance with this section.

(6) Recall and device disposition. A sponsor shall notify FDA and all reviewing IRB's of any request that an investigator return, repair, or otherwise dispose of any units of a device. Such notice shall occur within 30 working days after the request is made and shall state why the request was made.

(7) Final report. In the case of a significant risk device, the sponsor shall notify FDA within 30 working days of the completion or termination of the investigation and shall submit a final report to FDA and all reviewing the IRB's and participating investigators within 6 months after completion or termination. In the case of a device that is not a significant risk device, the sponsor shall submit a final report to all reviewing IRB's within 6 months after termination or completion.

(8) Informed consent. A sponsor shall submit to FDA a copy of any report by an investigator under paragraph (a)(5) of this section of use of a device without obtaining informed consent, within 5 working days of receipt of notice of such use.

(9) Significant risk device determinations. If an IRB determines that a device is a significant risk device, and the sponsor had proposed that the IRB consider the device not to be a significant risk device, the sponsor shall submit to FDA a report of the IRB's determination within 5 working days after the sponsor first learns of the IRB's determination.

(10) Other. A sponsor shall, upon request by a reviewing IRB or FDA, provide accurate, complete, and current information about any aspect of the investigation.

(vi) Ensures that participating investigators maintain the records required by 812.140(a)(3)(i) and make the reports required under 812.150(a) (1), (2), (5), and (7) (below); and

812.140(a)(3)(i)
(a) *Investigator records.* A participating investigator shall maintain the following accurate, complete, and current records relating to the investigator's participation in an investigation:

(3) Records of each subject's case history and exposure to the device. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. Such records shall include:

(i) Documents evidencing informed consent and, for any use of a device by the investigator without informed consent, any written concurrence of a licensed physician and a brief description of the circumstances justifying the failure to obtain informed consent. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

812.150(a) (1), (2), (5), and (7);
(a) Investigator reports. An investigator shall prepare and submit the following complete, accurate, and timely reports:

(1) Unanticipated adverse device effects. An investigator shall submit to the sponsor and to the reviewing IRB a report of any unanticipated adverse device effect occurring during an investigation as soon as possible, but in no event later than 10 working days after the investigator first learns of the effect.

(2) Withdrawal of IRB approval. An investigator shall report to the sponsor, within 5 working days, a withdrawal of approval by the reviewing IRB of the investigator's part of an investigation.

(5) *Informed consent.* If an investigator uses a device without obtaining informed consent, the investigator shall report such use to the sponsor and the reviewing IRB within 5 working days after the use occurs.

(7) *Other.* An investigator shall, upon request by a reviewing IRB or FDA, provide accurate, complete, and current information about any aspect of the investigation.

(vii) Complies with the prohibitions in 812.7 against promotion and other practices (below):

A sponsor, investigator, or any person acting for or on behalf of a sponsor or investigator shall not:

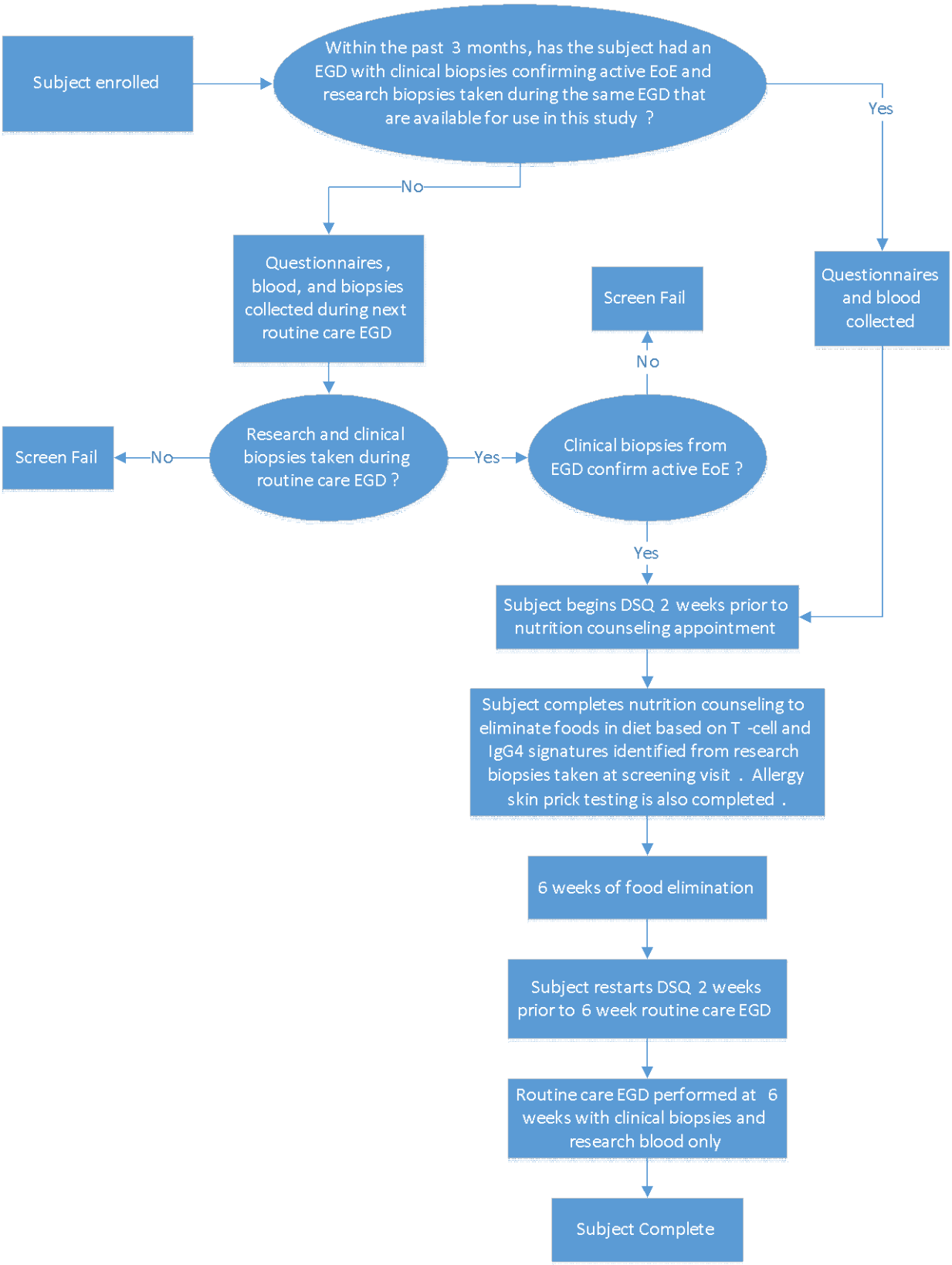
(a) Promote or test market an investigational device, until after FDA has approved the device for commercial distribution.

(b) Commercialize an investigational device by charging the subjects or investigators for a device a price larger than that necessary to recover costs of manufacture, research, development, and handling.

(c) Unduly prolong an investigation. If data developed by the investigation indicate in the case of a class III device that premarket approval cannot be justified or in the case of a class II device that it will not comply with an applicable performance standard or an amendment to that standard, the sponsor shall promptly terminate the investigation.

(d) Represent that an investigational device is safe or effective for the purposes for which it is being investigated.

3.3 Protocol Map



3.4 Study Outcomes

The primary outcome will be histologic response, defined as a post-treatment esophageal eosinophil count of <15 eos/hpf.41 The esophageal eosinophil counts will be quantified using our previously validated protocol.[42]

The secondary outcomes will be: 1) change in the absolute esophageal eosinophil count; 2) improvement in the endoscopic appearance, as measured by a validated endoscopy score;[43] and 3) improvement in symptoms, as measured by a validated dysphagia symptom score.[44]

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1) Age 16-80 years old
2) Meet one of the following:
    a. Active EoE as per consensus guidelines OR
    b. Undergoing upper endoscopy for a clinical suspicion of EoE
3) No prior history of dietary elimination therapy

4.2 Exclusion Criteria

1) Concomitant eosinophilic gastroenteritis
2) Any corticosteroid exposure in the 4 weeks prior to their baseline endoscopic exam
3) Previous esophageal surgery
4) Medical instability that precludes safely performing upper endoscopy
5) Inability to read or understand English
6) Pregnant women

4.3 Subject Recruitment and Screening

Patients with active or suspected EoE or who are undergoing or recently underwent routine care upper endoscopy will be screened by research personnel. These patients will be identified by screening the endoscopy and CEDAS clinic schedules or via referrals from other physicians. Once a potential subject is identified, study personnel will contact the potential subject to describe the study and gauge interest in participating. Initial contact may be in person or on the phone. If the patient is interested in participating, then the study coordinator or other study staff will obtain written informed consent from the subject.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects have the right to withdraw from the study at any time, for any reason, and without repercussion. The investigator, Dr. Evan Dellon, has the right to withdraw a patient from the study in the event of an intercurrent illness, adverse event (AE), treatment failure, protocol violation, and for administrative or other reasons. An excessive rate of withdrawals would reduce the amount of data available for analysis and limit the ability to interpret the study results; therefore, unnecessary withdrawal of patients should be avoided.

If subjects become pregnant during the study, then their participation will be stopped.

4.4.2 Lost to Follow-Up

A subject will be considered lost to follow-up after documentation has been made of at least three documented attempts to contact (via phone or email). At that point a certified letter should be mailed to the subject's home address. If there is still no response after the certified letter is delivered, then the subject will be withdrawn from the study as lost to follow-up.

4.4.3 Data Collection and Follow-up for Withdrawn Subjects

Patients who withdraw prematurely from the study may be asked to complete an end of study visit per the study procedures. In addition, any ongoing adverse drug reactions or study-related adverse events will be followed until resolution or documentation of why there will be no resolution if the event will be ongoing.

5 Study Procedures

Procedure	Screening Visit	Day 1 Visit	6 Week Follow-Up Visit (+/- 7 days)
Eligibility Review	X		
Informed Consent	X		
Demographics	X		
Medical History	X		
Current Medications	X	X	
Vital Signs	X		X
Questionnaires	X		X
Research blood collection	X[1]		X
EGD with EREF and biopsies	X[1,2]		X[2]
Adverse Events	X	X	X
Dysphagia Symptom Questionnaire (DSQ)		X[3]	X[3]
Nutrition Counseling		X[2]	
Allergy Skin Testing		X[4]	
Diet Elimination		X[5]	

[1] Subjects must be scheduled for a routine care EGD with biopsies (clinical and research) at UNC or have had a routine care EGD with clinical and research biopsies at UNC within 3 months prior to enrollment. If subjects have had a recent EGD as defined above, then results from clinical biopsies taken during that EGD will be used to assess eligibility for this study, and research biopsies and EREFs from that EGD will also be used for this study. In these cases, an additional EGD does not need to be performed. In addition, if research blood was collected during the recent EGD and can be used in this study, then subjects will have an abbreviated blood collection during the screening visit. If subjects have not had an EGD with research and clinical biopsies at UNC within 3 months of enrollment, then they must be scheduled to receive a routine care EGD with biopsies at UNC during which clinical and research biopsies will be collected for this study.

[2] These procedures are considered routine care and are billed to the subject and/or subject's insurance.

[3] The DSQ is started 2 weeks prior to the Day 1 and 6 week visits.

[4] Results from the allergy skin testing will not be used to drive the food elimination diet.

[5] Subjects will begin diet elimination on day 1 and continue for 6 weeks.

5.1 Screening Visit

During the screening visit, eligibility is assessed (based on inclusion/exclusion criteria) and those eligible and interested in participating will provide informed consent. Potential cases will be identified by screening endoscopy and clinic schedules. This will require approval of a limited HIPAA waiver to access the personal health information (PHI) of potential research subjects prior to their formal enrollment.

The following procedures will be completed during the screening visit:

- Informed consent
- Inclusion/exclusion criteria review to determine eligibility
- Demographics
- Medical history
- Current medications
- Vital signs

- Questionnaires
- Blood collection
- EGD with EREF score and biopsies for pathology, per routine care, and biopsies for research purposes***
- Adverse events
- CRF completion

***If subjects have had an EGD at UNC within 3 months of enrollment including clinical and research biopsies that can be used in this study, then the EGD will not be performed and biopsies and EREF scores from the recent EGD will be used in this study.

5.1.1 Consenting Procedure

If a subject is screened eligible and interested in the study, the subject will be consented on the study prior to any study procedure. Written informed consent will be obtained by qualified study personnel. Documentation of the consent process will be maintained in the subject's research record.

Subjects will be given ample time to review the consent document and ask any questions they may have. A copy of the written consent form will be provided to the subject and the original maintained in the subject's research record.

If subjects meet all inclusion and none of the exclusion criteria and consent to the study, they will be enrolled in the study. Subjects will be assigned a unique subject code.

5.1.2 Biopsy Collection and Processing

The research biopsy protocol below is frequently used in CEDAS research projects. If subjects have had an EGD with biopsies at UNC within 3 months prior to enrollment and the biopsies are available for use in this study, and the subject consented to their use in future research studies, then the subject does not need to return for an EGD with biopsies, unless necessary for routine care.

If subjects have not had an EGD within 3 months of enrollment in which biopsies below are available for this study, then they can be scheduled for a routine care EGD with biopsies at UNC to be eligible for this study. However, in some cases, samples that were obtained prior to this time frame can be used, as long as the samples were obtained when the EoE was active and there were no major changes in clinical status. During the routine care EGD, clinical biopsies will be obtained for pathology assessment. In addition, 4 research-specific biopsies will be obtained from each of the following locations: Distal esophagus (3cm from TGF), middle esophagus (8cm from TGF), and proximal esophagus (13cm from TGF). Additionally, 2 research-specific biopsies will be obtained from the duodenum, and 2 research-specific biopsies will be obtained from the stomach (1 from antrum, 1 from body). The 4 distal biopsies will be separated into 4 cryovials: 2 filled with formalin for histology, 1 filled with RNALater, and 1 empty to be frozen immediately in liquid nitrogen.

Research biopsies will be used in this study, and will be stored indefinitely for future research. Subjects will sign a separate stored specimens consent form for this purpose. If a complete set of clinical and research biopsies is not collected during the routine care EGD, then he subject will be considered a screen fail.

Research Related Biopsy Collection Protocol:

Biopsy Location	Description	Processing/Labeling Instructions
4 distal esophageal (d) 	4 single biopsies 3cm from GEJ	2 formalin (h): • **Label:** PID_eh_d1 and PID_eh_d2 + collection date 1 RNALater (r): Refrigerate, store long term in -80deg.F • **Label:** PID_er_d + collection date 1 frozen (f): • **Label:** PID_ef_d + collection date
4 mid esophageal (m) 	4 single biopsies 8cm from GEJ	1 formalin (h): • **Label:** PID_ eh_m + date 1 RNALater (r): • **Label:** PID_er_m + date 2 frozen(f): • **Label:** PID_ef_m + date and PID_ef_m2 + date
4 proximal esophageal (p) 	4 single biopsies 13cm from GEJ	2 formalin (h): • **Label:** PID_eh_p+ date and PID_eh_p2 + date 1 RNALater (r): • **Label:** PID_er_p + date 1 frozen (f): • **Label:** PID_ef_p + date

Participant IDs (PIDs) will be assigned in numerical order and follow the convention of ID-###. For example, the first person enrolled will be assigned a PID of ID-001.

Missed biopsies will not be considered protocol deviations or violations.

5.1.3 Blood Sample Collection and Processing

Blood samples will be collected and used in this study, and will be stored indefinitely for future research studies. Subjects will sign a separate stored specimens consent form for this purpose.

Items	Processing
6 - 5 mL green top tubes	Hand to PI or representative upon collection **Label**: PID and collection date **Example**:ID-001 08Feb16

The following blood samples should be collected only if the subject has no research blood samples previously collected during a recent EGD (within 3 months of enrollment) that can be used in this study.

Items	Processing
The following blood samples should be collected only if the subject does not have these samples previously collected from a recent EGD (within 3 months of enrollment) and available for use in this study.	
2 red tops	Spin, aliquot serum into 9 cryovials **Label**: PID_s# and collection date **Example**:ID-001_s1 08Feb16
1 yellow top	Spin, aliquot plasma into 9 cryovials **Label**: PID_p# and collection date buffy into 1 cryovial **Label**:PID_b#
1 purple top	Aliquot whole blood into 1mL aliquots – minimum of 3 cryovials **Label**: PID_w# and collection date
1 PAXgene RNA blood	Room temp at least 2 hours, then freeze **Label**: PID and collection date

Participant IDs (PIDs) will be assigned in numerical order and follow the convention of ID-###. For example, the first person enrolled will be assigned a PID of ID-001.

Missed blood samples will not be considered protocol deviations or violations.

5.2 Day 1 Visit

Two weeks prior to the day 1 visit, and while the lab performs T-cell and IgG4 signatures testing, subjects will be asked to fill out the Dysphagia Symptom Questionnaire (DSQ). The DSQ is a daily 3-question symptom diary to be completed nightly over a 2 week time period. This can be done on paper or by email.

During the day 1 visit, subjects will be scheduled for a routine care nutrition counseling appointment. During this appointment subjects will be advised how to effectively follow an elimination diet based on the specific triggers identified between Screening and Day 1 using samples obtained for the research study and will under allergy skin prick testing as a research procedure. Results from the allergy skin prick testing will not be used to drive the food elimination diet.

Subjects will be instructed to eliminate between 1 and 5 foods (dairy, wheat, egg, soy, and/or nuts) based on results from the T-cell and IgG4 signatures. Subjects will adhere to the elimination diet for 6 weeks

The following procedures will be completed during the day 1 visit

- DSQ (started 2 weeks prior to day 1 visit)
- Current medications
- Adverse events
- Nutrition counseling as per routine care
- Begin 6 week diet elimination therapy
- CRF completion

5.3 6 Week Follow-Up Visit (+/- 7 days)

Two weeks prior to the 6 week follow-up visit, subjects will be asked to fill out the Dysphagia Symptom Questionnaire (DSQ). The DSQ is a daily 3-question symptom diary to be completed nightly over a 2 week time period. This can be done on paper or by email.

After completion of the 6 week food elimination diet, subjects will return to one of the UNC endoscopy centers and undergo repeat EGD for routine care. During the EGD, clinical biopsies will be obtained. No research biopsies are collected for this study during the 6 week follow-up EGD. Follow up blood samples are collected. Subjects will complete the same questionnaires as the screening visit.

The following procedures will be completed during the 6 week follow-up visit

- DSQ (started 2 weeks prior to 6 week follow-up visit)
- Vital Signs
- Questionnaires
- EGD with EREF score and biopsies for pathology, per routine care (no research biopsies are obtained at the 6 week follow-up visit for this study)
- Blood collection
- Adverse events

Study participation is complete after completion of the 6 week follow-up visit.

6 Statistical Plan

6.1 Sample Size Determination

Because this is a proof-of-principle effectiveness study without a comparison group, there is not a formal sample size calculation. However, we plan to consent and enroll up to 30 subjects with a goal of 20 subjects completing the study to show that 75% (15) have a histologic response to the allergen-specific immune-signature dietary elimination. We feel that this is a robust number of patients to demonstrate a preliminary effect, while also being a realistic number to enroll during this pilot study. In addition, while the 95% CIs around this goal are somewhat wide (47-92%), current allergy testing only has the ability to accurately predict triggers 13% of the time, so even if we are at the lower end of the range, it's still a major clinical step forward in practice.

In the proposed study, we will be performing the first prospective effectiveness study of an allergen-specific immune signature-based individualized dietary elimination regimen in patients with EoE. Because we are conducting what will essentially be a pilot or proof-of-principle study, the data we collect, including response rates and magnitude of response, will directly inform a planned large scale (ie R01) grant application for a randomized study of immune-signature-based elimination versus empiric food elimination.

6.2 Statistical Methods

To determine the response rate for the primary outcome, the number of subjects with <15 eos/hpf on follow-up endoscopy will be calculated. For secondary outcomes, median eosinophil counts, endoscopy scores, and symptoms scores will be compared before and after treatment with Wilcoxon signed-rank. These will be contextualized by calculating odds ratios and 95% confidence intervals where appropriate, so as not to

over-rely on p values alone. Patients who drop out or who have incomplete data for the outcomes will be excluded from analysis. Because this study's outcomes are determined after the treatment period and follow-up endoscopy, a subject must complete this end of treatment evaluation for us to be able to include their data in the analysis. We do not feel that selection bias would impact the analysis if there are incomplete data due to drop out. We are not performing a comparative analysis between groups, so drop out would not be dependent on different treatments administered in different study arms. In addition, in our experience with previous clinical studies of EoE, dropout rates have been quite low.

6.3 Subject Population(s) for Analysis

Patients who drop out or who have incomplete data for the outcomes will be excluded from analysis

7 Safety and Adverse Events

7.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRSOs)
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Adverse Event
An ***adverse event*** (AE) is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, experience, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. Adverse events encompass both physical and psychological harms. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization, or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

Serious vs. Severe
The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on patient/event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Unanticipated Adverse Device Effect
Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (231CFR812.3(s)).

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if <u>any one of the following</u> conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

7.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

7.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 7.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

7.3.1 Notifying the UNC IRB

Dr. Evan Dellon is responsible for reporting adverse events to the UNC IRB per the UNC IRB SOPs for reporting adverse events. Federal regulations require investigators to report unanticipated problems involving risks to subjects or others to the IRB. Historically, there has been confusion about what needs to be reported. OHRP and FDA have issued guidance that clarifies that investigators need only report “unanticipated problems involving risks to subjects or others” (or UPIRSOs). The UNC-Chapel Hill policy is based on this guidance. “Adverse events” that are not UPIRSOs are not required to be reported to the IRB. Site should refer to the most recent version of UNC IRB SOPs for up to date reporting requirements.

7.3.1.1 Differentiating between an UPIRSO and an Adverse Event

By definition, an UPIRSO is unexpected, whereas an "adverse event" may be anticipated or unanticipated. Additionally, an UPIRSO may involve the increased risk of harm—whether or not any actual harm occurred. In order to decide which events or circumstances constitute an UPIRSO, it is important to bear in mind the following:

- Not all Adverse Events are UPIRSOs. Only a small subset of "adverse events" occurring in FDA-regulated clinical trials and other types of studies constitute UPIRSOs. Many events that are required to be reported to the sponsor or federal agency are not UPIRSOs.
- An UPIRSO may not be an adverse event. It is possible for an event that does not involve actual physical, psychological, social, or economic harm to a research subject or another person nevertheless to constitute an UPIRSO that must be reported to the IRB. This is the case if the event places subjects or others at increased or different risk of harm, regardless of whether actual harm has occurred.

There are other types of incidents, experiences and outcomes that occur during the conduct of human subjects research that represent UPIRSOs but are not considered adverse events. Some UPIRSOs involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, UPIRSOs place subjects or others at risk of harm, but no harm occurs. For example, an investigator conducting behavioral research collects individually identifiable sensitive information about illicit drug use and other illegal behaviors by surveying college students. The data are stored on a laptop computer without encryption, and the laptop computer is stolen from the investigator's car. This is an UPIRSO and must be reported because the incident was (a) unexpected (i.e., the investigators did not anticipate the theft); (b) related to participation in the research; and (c) placed the subjects at a greater risk of psychological and social harm from the breach in confidentiality of the study data than was previously known or recognized.

Other examples of UPIRSOs that should be reported to the IRB, even though they are not adverse events, include:

- Publication in the literature, safety monitoring report (e.g., DSMB report), interim result, or other finding that indicates an unexpected change to the risk/benefit ratio of the research;
- Breach in confidentiality resulting from a disclosure of confidential information or from lost or stolen confidential information;
- Unresolved complaint of a participant, family member or other individual;
- Laboratory or medication errors that may involve potential risk to that individual or others;
- Change in FDA labeling because of adverse consequences or withdrawal from marketing of a drug, device, or biologic used in a research protocol;
- Disqualification or suspension of investigators;
- Accidental or unintentional change to the IRB-approved protocol that involves risks or has the potential to recur;
- Deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant
- Any deviation from the IRB-approved protocol that increases the risk or affects the participant's rights, safety, or welfare.

7.3.1.2 UNC IRB Reporting Timelines

Reporting is required of all UPIRSOs, including those which may occur after the participant has completed or is withdrawn from the study, or following study closure. Reporting is completed via IRBIS, UNC's online IRB information system.

Events that meet the criteria for an UPIRSO and are also serious adverse events should be reported to the IRB within **one (1) week** of the investigator becoming aware of the event.

Any other events that meet the criteria for a UPIRSO should be reported to the IRB within **two (2) weeks** of the investigator becoming aware of the problem.

If the report cannot be completed in its entirety within the required time period, a preliminary report should be submitted. The report should be amended once the event is resolved and/or more information becomes available.

Site should refer to the most recent version of UNC IRB SOPs for up to date reporting requirements.

7.3.2 Notifying the FDA – Unanticipated Adverse Device Effects

Unanticipated adverse device effect means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects (231CFR812.3(s)).

Per 21CFR812.46(b) the sponsor (Dr. Dellon) shall immediately conduct an evaluation of any unanticipated adverse device effect.

A sponsor who determines that an unanticipated adverse device effect presents an unreasonable risk to subjects shall terminate all investigations or parts of investigations presenting that risk as soon as possible. Termination shall occur not later than 5 working days after the sponsor makes this determination and not later than 15 working days after the sponsor first received notice of the effect

Per 21CFR812.150(b), a sponsor (Dr. Dellon) who conducts an evaluation of an unanticipated adverse device effect under §812.46(b) shall report the results of such evaluation to FDA and to all reviewing IRB's and participating investigators within 10 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests.

7.4 Stopping Rules

This study does not have stopping rules, and there are no plans for an interim analysis.

7.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9). Medical monitoring will include a regular assessment of the number and type of adverse events.

Safety data will be aggregated and reviewed by the principal investigator, Dr. Evan Dellon periodically. Individual adverse events will be reviewed weekly, or as necessary, by study investigators. Adverse events will be reported to the IRB per local IRB SOPs.

While an overall study stop is not anticipated due to the low risk profile of the study, Dr. Evan Dellon will closely monitor adverse events. Any unusual or unanticipated events will be carefully considered and reported to the IRB as per IRB SOPs. The site PI, Dr. Evan Dellon, will review all safety data and determine severity, and causality on an ongoing basis. Research personnel will communicate safety information to the PI in a timely manner.

8 Data Handling and Record Keeping

8.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study

- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

8.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

8.3 Case Report Forms

The study will utilize paper case report forms (eCRFs). All data requested on the CRF must be recorded. All missing data must be explained in the comments section of the CRF. Data will then be entered into a secure Excel workbook.

8.4 Records Retention

Per 21CFR812.140(d), an investigator or sponsor shall maintain the records required by this subpart during the investigation and for a period of 2 years after the latter of the following two dates: The date on which the investigation is terminated or completed, or the date that the records are no longer required for purposes of supporting a premarket approval application or a notice of completion of a product development protocol.

9 Study Monitoring, Auditing, and Inspecting

9.1 Study Monitoring Plan

This study will be monitored regularly by a study monitor who is not directly involved in the project to ensure accurate data entry and reporting, as well as compliance with all applicable federal regulations. Refer to the most recent study monitoring plan for additional details.

The monitor is responsible for ensuring:

(a) the rights and well-being of trial participants are protected,
(b) reported trial data are accurate, complete, and verifiable from source documents,
(c) the conduct of the trial is in compliance with the currently approved protocol/amendments, with GCP, and with the applicable regulatory requirements, and data reported are verifiable to support meeting the study objectives.

Procedures and sources of information:

A) Protection of the rights and well-being of study subjects by verifying:
1. Investigator(s) have adequate qualifications, education and training, and resources necessary to conduct the study.
2. Verify the investigator and his staff follow the approved protocol and all approved amendments, if any
3. The investigator personally conducts or adequately supervises his study staff
4. Study staff are adequately trained and study functions are delegated to authorized individuals
5. Written informed consent was obtained before each subject's participation in the study
6. Enrollment of only eligible subjects

7. Subjects are instructed in the proper use, storage and return of the investigational product
8. Adequate and accurate case histories that record all observations are maintained
9. Unanticipated adverse events are reported in accordance with the protocol

B) Trial data are accurate, complete and verifiable from source documents as verified from review of subject file, case report forms, and medical procedures reports
   1. Verify that the investigative staff are performing the specific trial functions in accordance with the protocol
   2. Checking the accuracy and completeness of case report form entries, source documents and other trial related records against each other
   3. Adequate and accurate case histories that record all observations are maintained
   4. All withdrawals and dropouts are properly reported and explained
   5. Investigator is informed of protocol deviations, data entry errors and omissions and illegibility

C) Study is conducted in compliance with the currently approved protocol/amendments, with GCP, and applicable regulatory requirements
   1. Verify the investigator follows the approved protocol and all approved amendments, if any
   2. Ensuring that an IRB approval is obtained prior to study initiation and that the IRB is kept informed of changes in research activity and unanticipated problems involving risk to subjects
   3. Determine if investigator is maintaining essential documents in a regulatory binder
   4. Investigational product is properly procured, stored and destroyed accordingly
   5. Documenting deviations from the protocol, SOPs, GCP and applicable requirements to the investigator and taking appropriate action designed to prevent recurrence of the detected deviations.
   6. Monitor will provide a written report after each study visit or study visit communication

D) Data reported are verifiable to support meeting the study objectives of the study

If other agencies choose to monitor the site such as the FDA, or institution (UNC), then the Investigator will ensure the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

9.2 Auditing and Inspecting

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

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10 Ethical Considerations

This trial will be conducted in compliance with institutional review board (IRB) and ICH GCP Guidelines including Title 21 Part 56 of the United States of America (USA) Code of Federal Regulations (CFR) relating to IRBs and GCP as described in the United States Food and Drug Administration (FDA) CFR (21 CFR § 50, 56, 812) in accordance with applicable ICH regulations regarding clinical safety data management (E2A, E2B(R3)), with ICH regulations regarding scientific integrity (E4, E8, E9 and E10) and with FDA regulations regarding financial disclosure (21 CFR § 54). In addition this trial will adhere to all local regulatory requirements, and requirements for data protection.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before

commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided an IRB-approved consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

11 Study Finances

11.1 Funding Source

This study is funded by a Translational Team Science Award (TTSA) through the North Carolina Translational & Clinical Sciences Institute.

11.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of North Carolina investigators will follow the University conflict of interest policy.

11.3 Subject Stipends or Payments

Subjects will receive up to $100 for participation in this study. The subject consent form provides details on how payment will be remunerated.

12 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

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