Statistical Analysis Plan

Study ID: RVLO 121-04

Study Title: A Phase 2a, Double-blind, Placebo-Controlled, Multi-Center Study to Assess the Efficacy, Safety, and Tolerability of IRL201104 in Adult Participants with Active Eosinophilic Esophagitis (EoE)

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STATISTICAL ANALYSIS PLAN

TITLE PAGE

A Phase 2a, Double-blind, Placebo-Controlled, Multi-Center Study to Assess the Efficacy, Safety, and Tolerability of IRL201104 in Adult Participants with Active Eosinophilic Esophagitis (EoE)

Final 1.0: DEC 15, 2022

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DECLARATION

I, the undersigned, declare that I have prepared the statistical analysis plan along with TLF mock shells and that to the best of my knowledge this document is internally consistent with protocol and scientifically rational.

Prepared by:

DocuSigned by: [redacted]
[redacted]
I am the author of this
12/15/2022 | 5:51:43 A
A39056949CED40EB904DBD244165CD2D

Name: [redacted]
Designation \ Role: Project Biostatistician **Sign & Date (MMM DD, YYYY)**

I, the undersigned declare that I have reviewed the statistical analysis plan along with TLF mock shells and that to the best of my knowledge the document is internally consistent with protocol and scientifically rational.

Reviewed by:

DocuSigned by: [redacted]
[redacted]
I have reviewed thi
12/15/2022 | 5:54:0
F28CE448D1C44C74A99D038AECE5BE86

Name: [redacted]
Designation \ Role: Lead Biostatistician **Sign & Date (MMM DD, YYYY)**

AUTHORIZATION: I, the undersigned, declare that I have reviewed the statistical analysis plan along with TLF mock shells and that to the best of my knowledge the document accurately reflects the protocol objectives.

Authorized by:

DocuSigned by: [redacted]
[redacted]
I approve this document
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Sponsor Representative(s) Name: [redacted]
Designation \ Role: Senior Dir. Clinical Development **Sign & Date (MMM DD, YYYY)**

REVISION HISTORY

Version	Date	Author	Reasons

LIST OF ABBREVIATIONS

Abbreviation or Special Term	Explanation
ADA	Antidrug Antibody
AE	Adverse Event
ALT	Alanine Aminotransferase
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CRF	Case Report Form
CSR	Clinical Study Report
CPK	Creatine phosphokinase
DSQ	Dysphagia Symptom Questionnaire
ECG	Electrocardiogram
eCRF	Electronic case report form
EDN	Eosinophil derived neurotoxin
EDP	Eosinophilic esophagitis diagnostic panel
EoE	Eosinophilic esophagitis
EoEHSS	Eosinophilic Esophagitis-Histology Scoring System
eos/hpf	Eosinophilic count per high-power field
EREFS	Eosinophilic Esophagitis-Endoscopic Reference Score
FAS	Full Analysis Set
FSH	Follicle Stimulating Hormone
HBsAg	Hepatitis B surface antigen
HIV	Human Immunodeficiency Virus
ICH	International Council for Harmonisation
ICF	Informed Consent Form
IV	Intravenous
IWRS	Interactive web response system
MedDRA	Medical Dictionary for Regulatory Activities
mTB	mycobacterium tuberculosis
PGI-C	Patient Global Impression of Change
PGI-S	Patient Global Impression of Severity

Abbreviation or Special Term	Explanation
PK	Pharmacokinetic(s)
PRO	Patient-reported outcomes
PT	Preferred Term
RNA	Ribonucleic acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
TEAE	Treatment-Emergent Adverse Event
TLFs	Tables, Listings, and Figures
WHO	World Health Organization
WHODRUG	World Health Organization Drug Dictionary

1 INTRODUCTION

This Statistical Analysis Plan (SAP) describes the statistical methods and data handling procedures to be followed during the final analyses and reporting of data collected for the study Protocol RVLO 121-04. Its purpose is to provide specific guidelines from which the analysis will proceed. It provides in detail the algorithms and conventions to be used in the analysis and presentation of the efficacy and safety data for this study. Any deviations from these guidelines will be documented in the clinical study report (CSR). In the event where discrepancies in statistical analysis may be encountered between the SAP and what is described in the study protocol, the SAP will take precedence.

This SAP should be read in conjunction with the study protocol and case report form (CRF). This version of the plan has been developed using the protocol Version 2.0 dated JUL 01, 2021 and CRF Version 4.0 dated SEP 27, 2022.

This is a Phase 2a, double-blind, placebo-controlled, multi-center study to assess the efficacy and safety of a repeat-dose regimen of IRL201104 in participants with active eosinophilic esophagitis (EoE).

Eosinophilic esophagitis (EoE) is a chronic and progressive disease of the immune system resulting in damage to the esophagus with subsequent loss of function resulting in difficulty swallowing and food impaction. If not adequately treated, symptoms and inflammation can progress, leading to scarring of the esophagus with irreversible damage. In the United States (US), there are approximately 160,000 patients with EoE who are currently treated, of which approximately 50,000 have failed multiple treatments. There are currently no therapies approved by the US Food and Drug Administration (FDA) for EoE.

Eosinophilic esophagitis is caused by the presence of large numbers of eosinophils in the esophagus. The production and accumulation of eosinophils may be caused by many factors, such as immune hypersensitivity responses to particular foods or environmental proteins (allergens) in affected individuals. Eosinophilic esophagitis is characterized by esophageal barrier defects and eosinophilic infiltrates. Therapeutic interventions that target reduction in esophageal eosinophils have been shown to correlate with symptom improvement.

IRL201104 is a novel synthetic peptide derived from the active domain of the mTB-secreted protein chaperonin 60.1. It is being developed for the relief of symptoms in patients with active EoE. The peptide significantly decreases airway eosinophil infiltration in rodent models of allergic and nonallergic inflammation. IRL201104 appears to be effective in resetting the immune system from an inflammatory state and in inducing inflammatory disease remission in nonclinical models.

2 STUDY DETAILS

2.1 Study Objectives

The Primary Objective of the study is:

- To assess the efficacy of repeat IV doses of 4 mg and 8 mg IRL201104 or placebo in adult participants with EoE after 3 doses administered over 14 days as assessed by the change in peak esophageal intraepithelial eosinophilic count per high-power field (eos/hpf) at Week 4.

The Secondary Objectives of the study are:

- To explore the relationship between IRL201104 treatment and change in symptoms of dysphagia in adult participants with EoE, using the patient-reported outcomes (PRO) instrument Dysphagia Symptom Questionnaire (DSQ).

- To assess the efficacy of repeat IV doses of IRL201104 in adult participants with EoE after 3 doses administered over 14 days as assessed by thresholds of histologic response at Week 4.

- To evaluate the safety, tolerability, and immunogenicity of IRL201104 in adult participants with EoE.

The Exploratory Objectives of the study are:

- To explore the relationship between IRL201104 and change in clinical outcomes assessments, to include the PROs PGI-S and PGI-C, and the clinician-reported outcomes EoE-Endoscopic Reference Score (EREFS) and EoE Histology Scoring System (EoEHSS).

- To explore the relationship between IRL201104 and the relative change in RNA gene expression to include EoE diagnostic panel (EDP) transcriptome signature.

- To explore the relationship between IRL201104 treatment on immune cells and markers of inflammation.

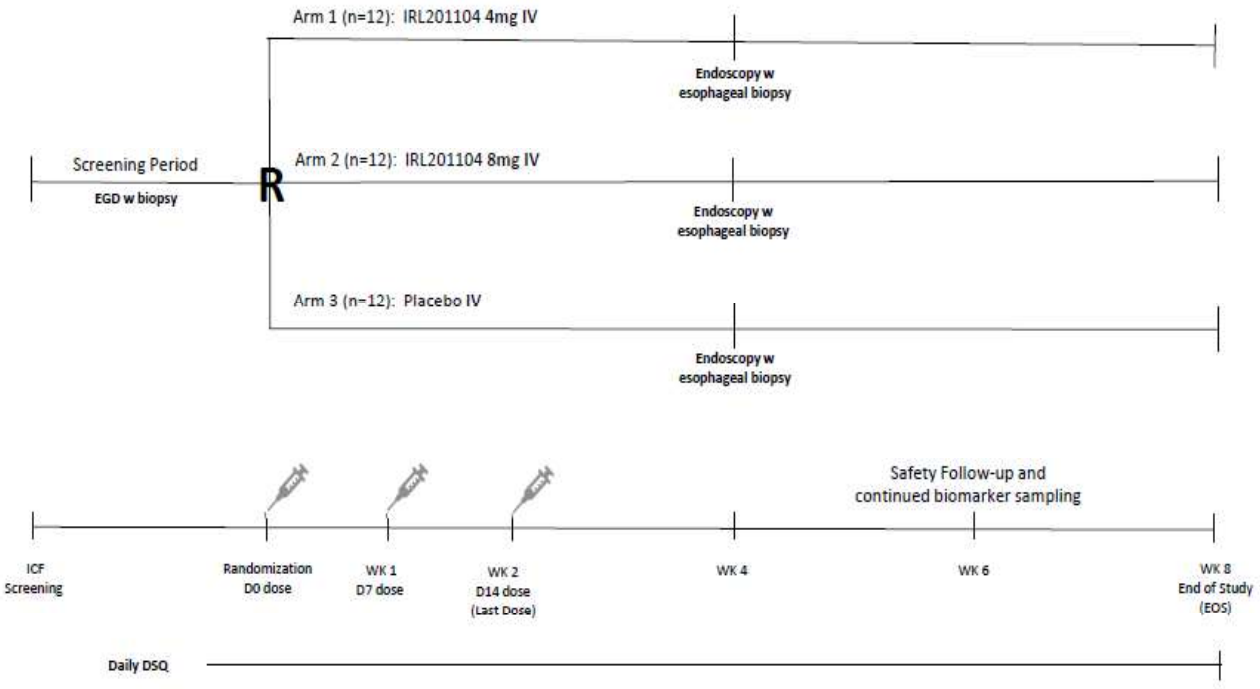
2.2 Study Design

This is a Phase 2a, double-blind, placebo-controlled, multi-center study to assess the efficacy and safety of a repeat-dose regimen of IRL201104 in participants with active EoE. Eligible participants will undergo an up-to-6-week screening period to stabilize current therapy, confirm diagnosis by histologic assessment, and other eligibility criteria. Participant compliance to DSQ reporting will be assessed for at least 2 consecutive weeks prior to randomization. At the end of the screening period, eligible participants will be randomized in a 1:1:1 ratio to one of 3 arms, as displayed in Figure 1

- Arm 1: IRL201104 4 mg IV with endoscopy and esophageal biopsy 2 weeks after last dose (Week 4).
- Arm 2: IRL201104 8 mg IV with endoscopy and esophageal biopsy 2 weeks after last dose (Week 4).
- Arm 3: Placebo IV with endoscopy and esophageal biopsy 2 weeks after last dose (Week 4).

Participants will receive IRL201104 (4 mg or 8 mg IV) or placebo on Days 0, 7, and 14 (3 total doses over 14 days). Participants will be evaluated for clinical and histologic responses at Week 4. All participants will be assessed for safety and continued biomarker sampling through Week 8.

Figure 1: Overview on the Study Procedures



D = day; EGD = esophagogastroduodenoscopy; EOS = End of Study; ICF = informed consent form; IV = intravenous; R = randomization; WK = week

Table 1: Study Schema

The Schedule of Events for the study is

Procedures	Screening Period	Prior to Rand	Rand/ Baseline	Treatment Period				End of Study/ Final Safety	Notes
Visit	**V -1**	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	
Week	**-6**	-	**0**	**1**	**2**	**4**	**6**	**8**	
Day	-	-	**0**	**7**	**14**	**28**	**42**	**56**	
Window	**≤ 6 weeks**	-	-	**± 3 days**	**± 3 days**	**± 3 days**	**± 3 days**	**± 3 days**	
Screening/ Baseline									
Informed consent	X	-	-	-	-	-	-	-	
Demographics/ Medical history review	X	-	-	-	-	-	-	-	
Inclusion/exclusion criteria review	X	X	X	-	-	-	-	-	
Treatment									
Randomization IWRS	-	-	X	-	-	-	-	-	
Administer study treatment	-	-	DBL	DBL	DBL	-	-	-	Pharmacy site staff will be unblinded to prepare the treatment. Investigators, study staff members, and study participants will be blinded to the treatment and dose level (4 mg or 8 mg or placebo) and will remain blinded throughout the study.
Efficacy									
DSQ training and issue handset	X	X	-	-	-	-	-	-	Daily completion of the DSQ starts on the first day it is issued to the participant.

Procedures	Screening Period	Prior to Rand	Rand/ Baseline	Treatment Period				End of Study/ Final Safety	Notes
Visit	V -1	V0	V1	V2	V3	V4	V5	V6	
Week	-6	-	0	1	2	4	6	8	
Day	-	-	0	7	14	28	42	56	
Window	≤ 6 weeks	-	-	± 3 days	± 3 days	± 3 days	± 3 days	± 3 days	
DSQ compliance assessment	-	-	X	X	X	X	X	X	
Retrieval of DSQ handset	-	-	-	-	-	-	-	X	
Esophageal endoscopy and biopsy with EoE-EREFS	EGD	-	-	-	-	X		-	
EoEHSS - histology scoring	X	-	-	-	-	X		-	
PGI-S	-	-	X	-	X	X	X	X	
PGI-C	-	-	-	-	X	X	X	X	
Safety									
Physical examination	X	-	X	X	X	X	X	X	Predose on Day 0.
ECG	X	-	X	X	X	X	X	X	
Height	X	-	-	-	-	-	-	-	
Weight	X	-	X	X	X	X	X	X	
Vitals	X	-	X	X	X	X	X	X	Should be measured within 15 minutes prior to dosing, and +15 minutes (+/- 5 minutes) postdose on Days 0, 7, and 14.
Diet history	X	X	X	X	X	X	X	X	
Concomitant medications and procedures	X	X	X	X	X	X	X	X	
Review of adverse events	-	X	X	X	X	X	X	X	

Procedures	Screening Period	Prior to Rand	Rand/ Baseline	Treatment Period				End of Study/ Final Safety	Notes
Visit	V -1	V0	V1	V2	V3	V4	V5	V6	
Week	-6	-	0	1	2	4	6	8	
Day	-	-	0	7	14	28	42	56	
Window	≤ 6 weeks	-	-	± 3 days	± 3 days	± 3 days	± 3 days	± 3 days	
Local laboratory									
Hematology and serum chemistry	X	-	X	X	X	X	X	X	
Coagulation	X	-	X	X	X	X	X	X	Should be performed before the procedure of endoscopy with esophageal biopsies on days the procedure is performed.
Urinalysis	X	-	X	X	X	X	X	X	
Serology	X	-	-	-	-	-	-	-	
Pregnancy testing	X	-	X	-	-	-	-	X	Serum pregnancy test at screening; urine pregnancy test at all subseqeuent time points.
FSH testing	X	-	-	-	-	-	-	-	FSH of 2.25 U/mL must be documented to confirm post-menopausal status in female participants who have had at least 12 months without menses.
Research samples/ Central laboratory									
Blood PK sampling	-	-	X	X	X	X	X	X	Blood sample for PK concentrations collected predose on Day 0 only; 4min (+/- 1 min) postdose on Days 0, 7, and 14.
Antidrug antibodies	-	-	X	-	-	-	-	X	Predose on Day 0.

Procedures	Screening Period	Prior to Rand	Rand/ Baseline	Treatment Period				End of Study/ Final Safety	Notes
Visit	V -1	V0	V1	V2	V3	V4	V5	V6	
Week	-6	-	0	1	2	4	6	8	
Day	-	-	0	7	14	28	42	56	
Window	≤ 6 weeks	-	-	± 3 days	± 3 days	± 3 days	± 3 days	± 3 days	
Biopsies for central lab	X	-	-	-	-	X		-	Screening endoscopy with esophageal biopsies and photographs are to be performed during screening period to allow results to be available prior to Visit 1.
Central histology result received prior to visit	-	X	-	-	-	-	-	-	
Whole blood for PBMC: Immune cell phenotyping (T, B, & Mo-subsets) and A20	X	-	X	X	X	X	X	X	Predose and 2 hours (+/- 10 minutes) postdose on Days 0, 7, and 14.
Serum cytokines	X	-	X	X	X	X	X	X	Predose and 2 hours (+/- 10 minutes) postdose on Days 0, 7, and 14.
Serum IgE/IgG4 (total)	X	-	X	X	X	X	X	X	Predose and 2 hours (+/- 10 minutes) postdose on Days 0, 7, and 14.
Serum EDN	X	-	X	X	X	X	X	X	Predose and 2 hours (+/- 10 minutes) postdose on Days 0, 7, and 14.

Base = baseline; DSQ = Dysphagia Symptom Questionnaire; ECG = electrocardiogram; EDN = eosinophil derived neurotoxin; EDP = EoE diagnostic panel; EGD = esophagogastroduodenoscopy; EoE = eosinophilic esophagitis; EoEHSS = EoE Histology Scoring System; EREFS = Endoscopic Reference Score; d = days; FSH = follicle stimulating hormone; FU = follow-up; Ig = immunoglobulin; IWRS = Interactive Web Response System; IV = intravenous; Mo = macrophage; q2wk = once every 2 weeks; PBMC = peripheral blood mononuclear cell; PGI-C = Patient Global Impression of Change; PGI-S = Patient Global Impression of Severity; PK = pharmacokinetic; QoL = quality of life; Rand = randomization; DBL = double-blind; Screen = screening; TBD = to be determined; V = visit

2.3 Determination of Sample Size

The chosen sample size for this study reflects reasonable clinical expectations of the number of participants who could be evaluated in this observational investigation for the purpose of obtaining preliminary estimates of histological response to treatment. It also provides for some level of accounting of the variability of estimates and the repeatability of results among a small group of participants treated in each proposed arm.

Approximately up to 50 participants are planned to be enrolled to yield 36 participants who are randomized and complete the final endoscopy at Week 4.

2.4 Randomization

Approximately 36 participants will be randomized in a 1:1:1 ratio to receive IRL201104 4 mg or 8 mg or placebo according to a central randomization scheme provided by an interactive web response system (IWRS).

Participants who discontinue from the study will be replaced if they did not receive 3 doses of study treatment and the final endoscopy as randomized.

2.5 Blinding

This is a double-blind study using open-label investigational product. Pharmacy staff will be unblinded for investigational product preparation. Investigators, study team members and study participants will be blinded to the dose level given (4 mg, 8 mg, or placebo) and will remain blinded throughout the study.

3 DATA ANALYSIS CONSIDERATION

Output processing will be undertaken by [redacted], using SAS Version 9.3 (or higher). All Tables, Listings and Figures (TLFs) will be produced in landscape format. In general, all data will be listed by the participant and visit. The number of variables presented in each listing can vary. Please refer to mock tables, listings and figures.

No formal statistical analysis will be conducted for this study. Summary statistics appropriate for categorical and continuous variables will be presented by treatment group and all inferences will be based on clinical evaluation of those results.

Data will be summarized by treatment group where appropriate. The total number of participants (N) in each treatment group under the stated population will be displayed in the header of summary tables.

Continuous data will be summarized using descriptive statistics for continuous variables. Unless otherwise stated, descriptive statistics will include the number of participants (n), mean, standard deviation (SD), minimum, median and maximum. When n=0, summary statistics (other than n) will be displayed as blank fields. When n=1, only the minimum and maximum will be reported; all other summary statistics will be displayed as blank entries. The number of missing values associated with an n that is less than the expected n for the visit or time point of interest will also be presented.

For summary tables of categorical variables, counts and percentages will be presented. The count [n] indicates the actual number of participants with a particular value of a variable or event, which should always be less than or equal to the total number of participants in the respective treatment group [N]. The number and percentage of participants with missing values for a variable/category/event will also be presented for the resulting visits/time points under the “Missing” category.

Percentage will be obtained by: % = (n/N) *100.

All dates will be displayed in DDMMMYYYY format.

Decimal Precision Convention:

The minimum and maximum statistics will be presented to the same number of decimal places as the original data. The mean and median will be presented to one more decimal place than the original data, whereas the standard deviation will be presented to two more decimal places than the original data. Unless otherwise stated, all percentages will be expressed to one decimal place.

Handling of Missing Data: Imputation of missing data will not be performed and will be analyzed as missing. To handle missing or partial AEs and concomitant medication dates, the following rules will be applied.

For partial Start Dates:

1. If the year is unknown, then do not impute the date but assign a missing value.

2. If the month is unknown, then:
   i. If the year matches the year of the first dose date, then impute the month and day of the dose date.
   ii. Otherwise, assign "January."

3. If the day is unknown, then:
   i. If the month and year match the month and year of the first dose date, then impute the day of the dose date.
   ii. Otherwise, assign "01."

For partial end dates:

1. If the year is unknown, then do not impute the date but assign a missing value.
2. If the month is unknown, then assign "December."
3. If the day is unknown, then assign the last day of the month.

After implementing the rules above, to determine whether AEs (or medications) with missing start or stop dates are pre-treatment or on/after treatment, the following strategy will be used:

1. If the start date and stop date are both missing, then the most conservative approach is taken and the AE (or medication) is considered to be treatment emergent (or concomitant medication).

2. If the start date is missing but the stop date is not missing and is on or after the date of signed informed consent, then the most conservative approach is taken, and the AE (or medication) is considered to be treatment emergent (or concomitant medication).

3. If the start date is missing but the stop date is not missing and is before the date of signed informed consent, then the AE (or medication) is considered to be before treatment (or prior medication).

4. If the start date is not missing but the stop date is missing, then the most conservative approach is taken, and medication is considered to be concomitant while the AE is defined by start date.

5. If the Adverse Event Relationship flag is missing, the relationship for adverse event will be imputed and will be considered as Definitely related. If the Adverse Event Severity flag is missing, the severity will be imputed and will be considered as severe & undesirable (Grade 3).

4 DEFINITIONS AND DERIVATIONS

Baseline: Baseline is defined as the last non-missing value (whether from scheduled or unscheduled visit) prior to the first study drug administration.

Change from Baseline: The change from baseline values at all post-baseline visits will be calculated by subtracting the baseline value from the corresponding post-baseline value.

Change from Baseline = Post baseline value - Baseline value.

Percent reduction: The percent reduction is the ratio of the reduction in the post-baseline value to its baseline value multiplied by 100.

$$\textbf{Percent reduction} = \frac{\text{Post Baseline value} - \text{Baseline value}}{\text{Baseline value}} \times 100$$

End of the Study: The end of the study is defined as the final follow-up visit by the last participant. If the study is terminated prematurely, the study ends when the sponsor notifies the investigator in writing that the study has finished, or when the last participant attends the final follow-up visit, whichever is later.

Study Day: This is the number of days from the date of the first study drug administration to a target date:

Study Day = (Target Date - Date of the first study drug administration) + 1
(If target date is greater than or equal to the date of the first study drug administration)

or

Study Day = (Target Date – Date of the first study drug administration)
(If target date is less than date of the first study drug administration.)

5 PRIMARY, SECONDARY AND EXPLORATORY ENDPOINTS

5.1 Primary Endpoint

The Primary evaluation parameter is:

- Change from baseline in the peak esophageal intraepithelial eosinophil count at Week 4.

5.2 Secondary Endpoints

- Absolute change in DSQ score from baseline (see DSQ score definition in Section 7.7.2)
- Proportion of participants achieving peak esophageal intraepithelial eosinophil count of < 15 eos/hpf (Week 4)
- Percent reduction in peak esophageal intraepithelial eosinophil count (eos/hpf) (Week 4)
- The safety endpoints are Monitoring of AEs, physical examinations, vital signs, electrocardiograms (ECGs), clinical laboratory tests, and clinical evaluations. Participants will be asked to monitor all AEs experienced from the time of informed consent until their last study visit.

5.3 Exploratory Endpoints

- Proportion of participants achieving peak esophageal intraepithelial eosinophil count of ≤ 6 eos/hpf (400×) at Week 4
- Proportion of participants achieving peak esophageal intraepithelial eosinophil count of ≤ 1 eos/hpf (400x) at Week 4
- Relative change in the EDP transcriptome signature (Week 4)
- Relative change in immune cells and markers of inflammation pre- and postdose administration at screening, baseline, and Weeks 1, 2, 4, 6, and 8. Immune cells and markers of inflammation include: immune cell phenotyping (T-subsets, B-subsets, and macrophage subsets); A20; serum cytokines; serum IgE/IgG4; and serum eosinophil derived neurotoxin (EDN) for eosinophil activation.
- Absolute change in EoE-EREFS (Week 4)

- Absolute change in EoE grade score from the EoE Histology Scoring System (EoEHSS) (Week 4)
- Absolute change in EoE stage score from the EoEHSS (Week 4)
- Proportion of participants with use of rescue medications or procedures (up to Week 8)
- Concentration of functional IRL201104 (up to Week 8)
- Incidence of treatment-emergent antidrug antibody (ADA) responses (Week 8)
- Absolute days without dysphagia.
- Change in PGI-S and PGI-C.

Note that an additional exploratory endpoint: absolute change in severity and/or frequency of EoE symptoms other than dysphagia (up to Week 8) was included in the protocol but will not be evaluated because the relevant information is being captured only as part of the screening visit evaluation. It was inadvertently left in the most current version of the protocol.

6 ANALYSIS POPULATION AND TREATMENT GROUPS

6.1 Analysis Population

Full Analysis Set: The full analysis set (FAS) will consist of all participants randomized at baseline who receive at least one dose of treatment and who had a baseline and at least one post-baseline measure of the primary or any one of the secondary endpoints. Efficacy evaluations will consider participants according to the treatment group to which they were assigned.

Safety Analysis Set: The safety analysis set will include all participants who were documented to have taken at least 1 dose of study treatment. Safety evaluations will consider participants according to the actual treatment they received.

6.2 Treatment Groups

Participants will be randomized 1:1:1 to 4 mg or 8 mg IRL201104 or placebo respectively. Participants will receive a dose of IRL201104 4 mg IV with endoscopy, IRL201104 8 mg IV with endoscopy or Placebo IV with endoscopy.

The 3 Arms will be labelled as **"IRL201104 4 mg IV", "IRL201104 8 mg IV**" and **"Placebo"** in respective header of TLFs.

The below table includes the treatment labeling for all Tables, Listings and Figures (wherever as appropriate):

Treatment	Treatment Label for TLF
IRL201104 4 mg IV with endoscopy and esophageal biopsy 2 weeks after last dose (Week 4).	IRL201104 4 mg IV
IRL201104 8 mg IV with endoscopy and esophageal biopsy 2 weeks after last dose (Week 4).	IRL201104 8 mg IV
Placebo IV with endoscopy and esophageal biopsy 2 weeks after last dose (Week 4)	Placebo
All treatments	Overall

7 ANALYSIS METHODS AND REPORTING DESCRIPTIONS

7.1 Disposition

Participant disposition will summarize the number and percentage of participants included in the FAS and Safety Analysis Sets, along with a summary of those who completed the study and those who discontinued from the study along with the reasons for discontinuation from study by treatment group using FAS. Study discontinuation will also be summarized by study week interval (through Week 1, >Week 1 through Week 2, >Week 2 through Week 4, and >Week 4 through Week 8).

A listing of participant disposition will be also presented describing all details of the reason for discontinuation, investigator comments, date and study day from randomization of the discontinuation, and any other relevant information, e.g., adverse events or other information that determined the grounds for discontinuation.

7.2 Demographic and Baseline Characteristics

Demographic and baseline characteristics will include age (years), sex, ethnicity, race, data for childbearing potential, height (cm), weight (kg) and BMI (kg/m^2) of the participants and will be summarized by treatment group for the FAS and repeated for the Safety Analysis Set if the two sets are different.

Descriptive statistics (n, mean, SD, minimum, median, and maximum) will be generated for continuous variables and the number and percentage of participants in each class of the categorical variables will be tabulated.

All individual participant demographic and baseline characteristics data will be listed by treatment group.

7.3 Medical History

Medical history will be coded using Medical Dictionary for Regulatory Activities (MedDRA) version 24.0.

Medical/Surgical history will be summarized by treatment group with the number and percentage of participants in each system organ class (SOC), preferred term (PT), and overall, on the Safety Analysis Set. Participants will be counted only once at the PT, only once at the SOC, and only once at participant level for the counting of total number of participants with a medical history term.

Listings of medical history events for participants in the Safety Analysis Set will be provided.

EOE-specific medical history of participants will be listed and summarized separately.

7.4 Prior and Concomitant Medications

Prior Medications

Prior medications are those medications that are taken before the ICF signed. i.e., prior medications have a stop date/time before ICF signed. Medications stopped on the same day as the ICF signed will be considered as prior medication only.

Concomitant Medications

Any treatment (including nutritional supplements) or procedure administered from the time of informed consent to the end of the final study visit is considered concomitant medication/procedures. This includes medications that were started before the study and are ongoing during the study.

Prior and concomitant medication will be categorized by preferred name and ATC level 4 class per World Health Organization Drug Dictionary (WHODRUG; Version 01MARCH GLOBAL 2021), will be summarized overall for the Safety Analysis Set. If ATC level 4 class is missing, we will use highest non missing available ATC level data. The number and percentage of participants using each medication will be displayed together with the number and percentage of participants using at least one medication.

All prior and concomitant medications will be presented in separate listings for the Safety Analysis Set.

7.5 Protocol Deviation

A summary table of protocol deviations will be presented by treatment group using the Safety Analysis Set. A listing of participants with protocol deviations will be presented along with the specific deviation(s) and recorded time/visit of the deviation(s) for each participant.

7.6 Study Drug Exposure

A listing will be provided for study drug administration information, including date and time of IRL201104 4 mg and 8 mg and placebo administration using the Safety Analysis Set. That listing will also contain, if applicable, relevant information recorded by the investigational site that describes or otherwise explains deviations from the protocol not already captured in the protocol deviation listing. Such information may be included as part of a post-hoc appendix to the clinical study report (CSR).

7.7 Efficacy Analysis

7.7.1 Analysis of Primary Endpoint

The primary efficacy endpoint is change from baseline in peak esophageal intraepithelial eosinophil count at Week 4.

The change from baseline will be summarized as the n, mean, standard deviation, median, minimum, and maximum by the treatment group of interest using the FAS. Analysis will also be done using the SAS.

7.7.2 Analysis of Secondary Endpoint(s)

For the secondary endpoints, continuous variables will be summarized using the descriptive statistics (n, mean, standard deviation, median, minimum, and maximum) for each observed measurement and change from baseline at each time point by treatment group while categorical variables will be summarized using the counts and percentages as applicable. The FAS will be used for this evaluation. Analysis will also be done using the SAS.

Dysphagia Symptom Questionnaire (DSQ)

The DSQ is used to measure the frequency and intensity of dysphagia. The DSQ scores can range from 0 to 84, with a lower score indicating less frequent or less severe dysphagia.

The questionnaire will be completed by participants daily. Each evening before bedtime, participants will be asked to indicate if they experienced dysphagia symptoms (eg, food passing slowly or food sticking) during that day. Participants must have experienced dysphagia (response of "yes" to Question 2 on DSQ) on a minimum of 2 days total and completed the DSQ on ≥ 70% of days in at least 2 consecutive weeks (qualifying period) prior to the baseline visit (Visit 1). Participants must fill out the DSQ at least 5 or more days during a given week in order to be compliant. Calculations will be performed on daily ePRO entries from the qualifying period prior to baseline, and prior to each study visit during the treatment and follow-up periods. The DSQ score for the secondary endpoints will be calculated by summing the scores of responses to Questions 2 and 3 only. Questions 1 and 4 will be excluded from the DSQ score.

$$\text{DSQ score for 14-day period} = \frac{(\text{Sum of points from questions 2+3 in the daily DSQ with non-missing data over the interval being reported}) \times 14}{\text{Number of diaries reported with non-missing data over the interval being reported}}$$

For each DSQ calculation, the sum of the specified question(s) from each reported daily diary with non-missing data over the interval being reported will be obtained, dividing this by the number of reported daily diaries with non-missing data in that same interval. This result will then be multiplied by the interval. This normalizes each DSQ sum over the interval. For the DSQ score, if a response of "No" is recorded for Question 2, the DSQ score will be set to zero for that day. The DSQ calculations during the interval being reported prior to the baseline visit will be defined as the baseline DSQ evaluation. For each 14-day period, at least 8 reported diaries are needed to effectuate a DSQ calculation for that period. If at least 8 diaries are not available, the DSQ result for that 14-day period will be set to missing.

The absolute change in DSQ score from baseline will be summarized as the n, mean, standard deviation, median, minimum, and maximum by treatment group.

Additionally, exploratory endpoint analysis of absolute days without dysphagia will be summarized as the n, mean, standard deviation, median, minimum, and maximum by treatment group. Absolute days without dysphagia will be obtained through a counting process of the number of DSQ records between baseline and Week 8 that the participant reports DSQ question #2 as "No."

Esophageal intraepithelial Eosinophil

The proportion of participants achieving peak esophageal intraepithelial eosinophil count of < 15 eos/hpf (Week 4) will be summarized using frequency and percentage.

Also, percent reduction in peak esophageal intraepithelial eosinophil count (eos/hpf) (Week 4) will be summarized as the n, mean, standard deviation, median, minimum, and maximum by treatment group.

Also, exploratory endpoint analysis will be performed for esophageal intraepithelial eosinophil. The proportion of participants achieving peak esophageal intraepithelial eosinophil count of ≤ 1 and ≤ 6 eos/hpf (400×) at Week 4 will be summarized separately using frequency and percentage.

7.7.3 Analysis of Exploratory Endpoint(s)

The Exploratory endpoints will be summarized using the FAS.

Patient Global Impression of Severity (PGI-S)

The PGI-S is a PRO measure that uses a verbal rating scale of severity. The PGI-S uses a recall period that aligns with the assessment period used for calculating DSQ endpoint scores (ie, DSQ endpoints are based on 14-day scores, and the PGI-S is administered on the last day of the 14- day assessment period. The PGI-S instructs participants to recall over the past 14 days, such that the anchor covers the same 14-day period as the 14-day DSQ endpoint score).

A numeric scoring for the PGI-S would be 0, 1, 2, and 3. Therefore, while the PGI-S will be summarized categorically, this measure will be also summarized as change from baseline to post-baseline visits.

Patient Global Impression of Change (PGI-C)

The PGI-C is a PRO measure that assesses change compared with prior to starting treatment and reflects the participant's belief about the efficacy of treatment. The PGI-C is a scale depicting a participant's rating of overall improvement.

A numeric scoring for the PGI-C it would be -2, -1, 0, 1, 2. Therefore, the PGI-C will be summarized using categorical and continuous variable summary statistics.

Eosinophilic Esophagitis-Endoscopic Reference Score (EoE-EREFS)

The EoE-EREFS will be used to measure the endoscopically identified EoE esophageal mucosal inflammatory and remodeling features. This instrument includes a total of 17 items related to the presence and severity of esophageal features. The specific esophageal features include: rings (absent, mild, moderate, severe, not applicable); stricture (yes, no, not applicable); diameter of the stricture (if applicable); exudates (absent, mild, severe); furrows (absent, mild, severe); edema (absent, present); crepe paper esophagus (absent, present); overall general appearance incorporating all endoscopically identified EoE findings (ie, fixed rings, strictures, whitish exudates, furrowing, edema, and crepe paper mucosa).
In addition, mucosal changes associated with gastroesophageal reflux disease (GERD) will also be recorded using the Los Angeles classification system for erosions (No Erosions or LA Classification A, B, C, D). The EoE esophageal characteristics will be analyzed based on the EoE-EREFS, a validated scoring system for inflammatory and remodeling features of disease using both overall scores and scores for each individual characteristic (Hirano 2013). The EoE-EREFS should be performed by the physician who performs the endoscopy procedure at the time of the endoscopy. In addition to the local read, images will be submitted to the central imaging laboratory for central reading.

The EoE-EREFS will be summarized descriptively by absolute change from baseline to week 4 by treatment group. Centrally and individual site locally read data will be summarized both across sites and by site. Maximum features (highest score for each feature including both proximal and distal areas) EoE-EREFS will also be summarized across sites.

Eosinophilic Esophagitis-Histology Scoring System

The EoEHSS is a validated histology scoring system for esophageal biopsies that evaluates 8 features: eosinophil density, basal zone hyperplasia, eosinophil abscesses, eosinophil surface layering, dilated intercellular spaces, surface epithelial alteration, dyskeratotic epithelial cells, and lamina propria fibrosis (absent/present) (Collins 2017). Severity (grade) and extent (stage) of abnormalities will be scored using a 4-point scale (0 normal; 3 maximum change).

Absolute change in EoE grade score and EoE stage score from the EoE Histology Scoring System (EoEHSS) (Week 4) will be summarized separately using descriptive statistics.

For the exploratory EoE-EREFS and EoEHSS endpoints, a categorical summary of the scores will be obtained using frequency count and percentage along with the mean, standard deviation, median, minimum, and maximum change from baseline.

Rescue Medications

Use of rescue medication will be summarized separately. The proportion of participants with use of rescue medications or procedures (up to Week 8) will be summarized using frequency and counts. Separate listing will be provided.

ADA (Antidrug antibody)

Antidrug antibody status (negative or positive based on whether results are lower or higher, respectively, than the optical density OD cut-off value) will be listed and summarized by treatment group and time point at scheduled visit based on the Safety Analysis Set.

Incidence of treatment-emergent antidrug antibody (ADA) responses (Week 8). This data will be summarized using frequency, count and percentage.

7.8 Safety Analysis

The analysis of safety endpoint variables will include assessments of AEs, vital signs, 12-lead ECGs, physical examinations, and clinical laboratory tests. All Safety assessments will be summarized and tabulated as appropriate and will be listed by participant as applicable. All safety analyses will be performed on Safety Analysis Set. No formal statistical analysis will be performed on safety outcomes.

Example: The following variables will be evaluated to assess the safety:

- Adverse Events
- Clinical Laboratory Data
- 12 Lead Electrocardiogram (12-Lead ECG)
- Vital Sign measurements
- Physical Examination

Adverse Events:

Adverse Events will be coded using (MedDRA version 24.0 or latest) AE coding system for purpose of summary tables. Adverse events will be summarized using frequency count and percentage by MedDRA SOC and PT.

"Treatment-emergent" will be defined as starting or worsening after the first dose of investigational product. If the start date is missing, the event is assumed to be treatment emergent.

All AEs and TEAEs during the study will be summarized for the respective treatment group. A participant experiencing the same AEs and TEAEs multiple times will be counted only once for that PT. Similarly, if a participant experience multiple AEs and TEAEs (preferred terms) within the same SOC then that participant will be counted only once for that SOC. When summarizing by severity or relationship, only event with highest severity / relationship will be counted. Adverse events will be dichotomized into related (definitely, probably, and possibly) and unrelated (unlikely and not related) All AEs will be listed in chronological order of the events occurred.

An overview of AE summary will be presented. It will include the following:

- AEs
- Serious AEs
- TEAEs
- Serious TEAEs
- Treatment related TEAEs
- Treatment related Serious TEAEs
- TEAEs leading to study discontinuation
- TEAEs leading to death

The incidence and frequency of AEs will be summarized by treatment according to SOC and PT.

Treatment-Emergent Adverse Events (TEAEs)

A summary of the frequency (number and percentage of participants) of TEAEs will be presented by SOC, PT.

TEAEs will be summarized by treatment, system organ class, preferred term, and severity (mild, moderate, severe and undesirable, life-threatening or disabling and death).

TEAEs will be summarized by treatment, system organ class, preferred term and relationship to the study treatment is recorded as related (definitely, probably, and possibly) and unrelated (unlikely and not related). The number (%) of participants having relationship for each event and the number of events will be presented.
All AEs (including non-TEAEs) recorded on the CRF will be listed.

Adverse Events of Special Interest

A summary of the frequency (number and percentage of participants) of AESIs will be presented by SOC, PT and Listed separately.

Serious Adverse Events

SAEs and treatment related SAEs will be summarized separately by SOC and PT. All SAEs recorded on the CRF will be listed for all participants.

Adverse Events Leading to Study Drug Discontinuation

All AEs leading to discontinuation recorded on the CRF will be listed.

Deaths

Deaths occurring in the study will be listed for all participants.

Clinical Laboratory Data

The Clinical Laboratory test category will include the following assessments:

Hematology: Hemoglobin, Hematocrit, Red blood cells count, white blood cell count, red cell indices, Platelet count and differential (neutrophil, lymphocyte, monocyte, eosinophil, and basophil). Visits include screening, Day 0, Day 7, ……. Day 56.

Biochemistry: Sodium, Total protein, Total and indirect bilirubin, Potassium, Creatinine, Total cholesterol, Chloride, Blood urea nitrogen, Low-density lipoprotein, Carbon dioxide, AST, High-density lipoprotein, Calcium, ALT, Triglycerides, Glucose, Alkaline phosphatase, Uric acid, Albumin, Lactate dehydrogenase, CPK. Visits include screening, Day 0, Day 7, ……. Day 56.

Coagulation: Prothrombin time and partial thromboplastin time. Visits include screening, Day 0, Day 7, ……. Day 56.

Urinalysis: Color, Glucose, Red blood cells, Clarity, Blood, Hyaline and other casts, pH, Bilirubin, Bacteria, Specific gravity, Leukocyte esterase, Epithelial cells, Ketones, Nitrite, Crystals, Protein, White blood cells, Yeast. Visits include screening, Day 0, Day 7, ……. Day 56.

Serology: HIV-1/2 antibodies, HBsAg Hepatitis, C virus antibodies. Collecting at screening visit only.

Pregnancy: Serum pregnancy tests will be performed for females of reproductive potential at screening, after which urine pregnancy tests will be performed. Visits include screening, Day 0 and Day 56.

FSH: Follicle stimulating hormone testing will be performed at screening to confirm post-menopausal status in female participants who have had at least 12 months without menses. Collecting at screening visit only.

All observed and change from baseline results of hematology, biochemistry, and coagulation parameters data will be summarized by treatment group, at each scheduled visit using descriptive statistics (n, mean, SD, median, minimum, and maximum) for each scheduled study assessment for the Safety Analysis Set.

For urinalysis data, the continuous variables will be summarized by treatment group at each scheduled visit using descriptive statistics (n, mean, SD, median, minimum, and maximum).

Individual participant data listings of laboratory results will be presented. Values outside of the laboratory's reference range (i.e., those with low or high values) will be flagged in the listings.

The other laboratory tests: FSH, serology and serum pregnancy test will be listed.

12-Lead ECG

The 12-lead ECG will include the following measurements: Ventricular Rate, PR Interval, RR Interval, QRS Duration, QT Interval, QTcF Interval. And it will be summarized for the Safety Analysis Set.

Overall evaluation of safety ECGs will be summarized by treatment group, using frequency counts and percentage of participants as normal or abnormal, and the relevance of the abnormality will be summarized by clinically significant (CS) or not clinically significant (NCS).

Observed and change from baseline in ECG parameters will be summarized by treatment over each scheduled time-point/visit in terms of absolute values using descriptive statistics (n, mean, SD, median, minimum, and maximum).

Individual data listings of ECG results along with the Investigator-identified ECG abnormalities will be presented for each participant by treatment group.

Vital Signs

Vital signs (systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature) will be summarized using the descriptive statistics for each vital sign measurement and change from baseline by treatment group, visit and timepoint using the Safety Analysis Set.

Vital Sign data will be listed by individual time course for each parameter.

Physical Examination

Physical examinations will include examination of the following body systems: head, eyes, ears, nose and throat (HEENT); cardiovascular, respiratory, gastrointestinal, dermatological, musculoskeletal, nervous systems, lymph nodes and general appearance.

The physical examination findings (normal, abnormal NCS, abnormal CS) will be summarized using counts and percentages by treatment group for each body system using the Safety Analysis Set.

All physical examination findings will also be listed individually for each participant by treatment.

7.9 PK/PD Analysis

Concentrations of IRL201104 will be listed by participant and summarized by treatment group and time point from predose to Week 8 based on the Safety Analysis Set.

PK analysis will be detailed in the PK plan.

8 POOLED ANALYSES

No pooled analyses will be conducted in this trial.

9 SUBGROUP ANALYSES

No subgroup analyses will be conducted in this trial.

10 INTERIM ANALYSIS

No interim analyses will be conducted in this trial.

11 CHANGES TO ANALYSES SPECIFIED IN PROTOCOL

The below mentioned endpoints will not be analysed for this study.

Eosinophilic Esophagitis Diagnostic Panel (EDP) Transcriptome

RNA sequencing and EDP transcriptome will be evaluated. A molecular EDP has been identified that is composed of 94 EoE genes and distinguishes EoE from control individuals without esophagitis or with GERD (Wen 2013).

The EDP signature will be evaluated in biopsy samples. Using a method reported by (Wen 2015), the EoE transcriptome will be determined using the EDP from extracted RNA.

The Relative change will be provided for EDP transcriptome signature by treatment group.

Relative change = {(Post baseline value – Baseline value)/ Baseline value} *100.

Immune Cells and Markers of Inflammation

Immune cells and markers of inflammation include: immune cell phenotyping (T-subsets, B-subsets, and macrophage subsets); A20; serum cytokines; serum IgE/IgG4; and serum eosinophil derived neurotoxin (EDN) for eosinophil activation.
Relative change in immune cells and markers of inflammation pre- and post dose administration at baseline, and Weeks 1, 2, 4, 6, and 8 will be provided. Separate listing will be provided.

12 REFERENCE

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