Title: A Phase 1, Four-Part Study to Assess the Safety, Tolerability, Pharmacokinetics, and Gluten Degradation Activity of PvP001, PvP002, and PvP003 in Healthy Adult Volunteers and to Assess the Safety, Tolerability, and Pharmacokinetics of PvP001 and PvP002 in Adults with Celiac Disease

NCT Number:NCT03701555

SAP Approve Date: 24 February 2021

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STATISTICAL ANALYSIS PLAN

Takeda Pharmaceuticals, Inc.

PvP-102-01

Protocol Title:	A Phase 1, Four-Part Study to Assess the Safety, Tolerability, Pharmacokinetics, and Gluten Degradation Activity of PvP001, PvP002, and PvP003 in Healthy Adult Volunteers and to Assess the Safety, Tolerability, and Pharmacokinetics of PvP001 and PvP002 in Adults with Celiac Disease
Protocol Version and Date:	Amendment 5.0; 18 October 2020
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1 STATISTICAL ANALYSIS PLAN APPROVAL

Sponsor:	Takeda Pharmaceuticals, Inc.
Clinical Protocol Number:	PvP-102-01
Protocol Title:	A Phase 1, Four-Part Study to Assess the Safety, Tolerability, Pharmacokinetics, and Gluten Degradation Activity of PvP001, PvP002, and PvP003 in Healthy Adult Volunteers and to Assess the Safety, Tolerability, and Pharmacokinetics of PvP001 and PvP002 in Adults with Celiac Disease
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Approved By:

PPD

2021-02-25

Date

2021-02-25

Date

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3 LIST OF ABBREVIATIONS

Table 1 List of Abbreviations

Abbreviation	Definition
ADA	Anti-drug Antibody
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
AUC	Area Under the Plasma Concentration-Time Curve
BLQ	Below the Limit of Quantification
BMI	Body Mass Index
CeD	Celiac Disease
CI	Confidence interval
CRF	Case Report Form
CSR	Clinical Study Report
CV	Coefficient of Variation
DLT	Dose-limiting Toxicity
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
ET	Early Termination
GSQ	Gastrointestinal Symptoms Questionnaires
ICH	International Council for Harmonisation
ITT	Intent-to-Treat
LC-MS/MS	Liquid Chromatography/Mass Spectrometry
LLOQ	Lower Limit of Quantitation
LOCF	Last Observation Carried Forward
MedDRA	Medical Dictionary for Regulatory Activities
MFD	Maximum Feasible Dose
MTD	Maximum Tolerated Dose
NAb	Neutralizing Antibody
NG	Nasogastric
PEG	Polyethylene Glycol
PK	Pharmacokinetics
PP	Per-Protocol
PPI	Proton Pump Inhibitor
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard deviation

Abbreviation	Definition
SE	Standard error
SI	Système International
TEAE	Treatment-emergent Adverse Event
WHODDE	World Health Organization Drug Dictionary Enhanced

4 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to provide comprehensive and detailed descriptions of the methods and presentation of data analyses proposed for Takeda Pharmaceuticals, Inc., Protocol PvP-102-01 (A Phase 1, Four-Part Study to Assess the Safety, Tolerability, Pharmacokinetics, and Gluten Degradation Activity of PvP001, PvP002, and PvP003 in Healthy Adult Volunteers and to Assess the Safety, Tolerability, and Pharmacokinetics of PvP001 and PvP002 in Adults with Celiac Disease). Descriptions of planned analyses are provided in order to avoid post hoc decisions that may affect the interpretation of the statistical analysis. The statistical methods applied in the design and planned analyses of this study are consistent with the International Council for Harmonisation (ICH) guideline *Statistical Principles for Clinical Trials* (E9) (1998).

This SAP will be finalized prior to data analysis and database lock to provide full details, including templates for tables, listings, and figures, to be presented in the clinical study report (CSR). Any changes between the statistical methods provided in the clinical study protocol and this SAP will be explained herein; any changes or deviations from this SAP relative to the final analysis will be fully documented in the CSR. Minor changes or deviations from the templates for tables, figures, and listings need not be documented in the CSR.

5 STUDY OBJECTIVES

5.1 Primary Study Objectives

Part 1

The primary objective is to determine the safety and tolerability of single doses of PvP001 and PvP002 in healthy volunteers and patients with celiac disease (CeD).

Part 2

The primary objectives are to:

- Evaluate the ability of PvP001 and PvP002 to degrade gluten in healthy volunteers
- Determine the effect of standard dose proton pump inhibitor (PPI) pretreatment on the ability of PvP001 to degrade gluten in healthy volunteers

Part 3

The primary objective is to evaluate ability of PvP003 to degrade gluten in healthy volunteers.

Part 4

The primary objective is to determine the safety and tolerability of multiple doses of PvP003 600 mg in healthy volunteers.

5.2 Secondary Study Objectives

Part 1

The secondary objectives are to:

- Determine the pharmacokinetics (PK) of PvP001 and PvP002 in healthy volunteers and patients with CeD
- Determine the maximum tolerated dose (MTD) (100, 300, or 900 mg) of PvP001 in healthy volunteers for use in Part 2

Part 2

The secondary objectives are to:

- Evaluate the safety, tolerability, and gluten-degradation ability of the MTD of PvP001 compared to the Maximum Feasible Dose (MFD) of PvP002 in healthy volunteers
- Determine the PK of PvP001 and PvP002 in healthy volunteers
- Evaluate the ability of PvP001 300 mg and PvP001 600 mg to degrade 1 g of gluten at 20, 35 and 65 minutes in healthy volunteers
- Evaluate the ability of PvP001 900 mg to degrade 6 g of gluten at 20, 35, and 65 minutes in healthy volunteers

Part 3

The secondary objectives are to:

- Evaluate the ability of single doses of PvP003 600 mg with and without pretreatment buffer solution and PvP003 150 mg without pretreatment buffer solution to degrade 1 g of gluten at 35 and 65 minutes in healthy volunteers when administered before a standardized gluten-containing study meal
- Evaluate the ability of single doses of PvP003 600 mg without pretreatment buffer solution to degrade 1 g of gluten at 35 and 65 minutes in healthy volunteers when administered between two portions of a standardized gluten-containing study meal
- Evaluate the ability of single doses of PvP003 600 mg without pretreatment buffer solution to degrade 1 g of gluten at 65 minutes in healthy volunteers when

administered before a standardized gluten-free study meal followed by a standardized gluten-containing study meal

- Determine the safety and tolerability of single doses of PvP003 150 mg and 600 mg in healthy volunteers
- Determine the PK of single doses of PvP003 150 mg and 600 mg in healthy volunteers
- Measure the development of anti-drug antibodies (ADA) after administration of single doses of PvP003 150 mg and 600 mg in healthy volunteers

Part 4

The secondary objectives are to:

- Determine the PK of multiple doses of PvP003 600 mg in healthy volunteers
- Measure the development of ADA after administration of multiple doses of PvP003 600 mg in healthy volunteers

6 INVESTIGATIONAL PLAN

6.1 Overall Study Design

Subjects who participate in Part 1 or Part 2 of the study, and who are not ADA positive, may participate in Part 3 or Part 4 of the study, and will be analyzed independently by Part. No other subjects may participate in more than one Part/Group of the Study.

Part 1 of this study is a single-blind, placebo-controlled, single ascending dose study of PvP001, followed by administration of a single dose of the MFD of PvP002, in healthy adult subjects and adult patients with well controlled CeD. Approximately 15-30 eligible healthy subjects and 15-30 eligible patients with CeD will participate in Part 1 of the study. The MFD has been determined to be 600 mg. **Table 2** shows the number of subjects planned in each cohort for Part 1.

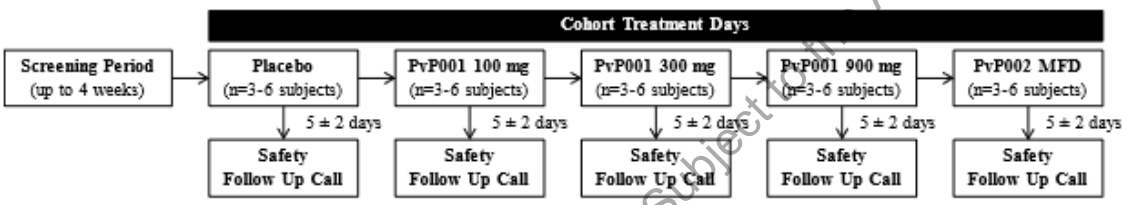
Table 2 Part 1 Number of Subjects per Cohort

PvP001 (mg)	Healthy Subject Cohort	Number of Healthy Subjects per Cohort	Celiac Disease Patient Cohort	Number of Patients with Celiac Disease per Cohort
0	1A-1	3-6	1A-2	3-6
100	1B-1	3-6	1B-2	3-6
300	1C-1	3-6	1C-2	3-6
900	1D-1	3-6	1D-2	3-6
PvP002 (mg)				
600	1E-1	3-6	1E-2	3-6

Enrollment in each of the five dose Cohorts will begin with healthy subjects in Cohort 1A-1 and will proceed sequentially through Cohort 1E-1 according to the dose escalation guidelines described in protocol section 4.1. Once a given PvP001 dose level or 600 mg of PvP002 is deemed safe in healthy subjects based on these guidelines, enrollment of patients with CeD will begin at this dose level and will proceed according to the same dose escalation guidelines.

The Part 1 visit scheme will consist of a screening period, a Cohort Treatment Day with a post-dose 24-Hour Safety Assessment, and Follow Up Anti-Drug Antibody (ADA) Blood Sampling Visits, with each subject participating for up to approximately 8 weeks. **Figure 1** shows the study visit schematic for Part 1.

Figure 1 Part 1 Study Visit Schematic



MFD = maximum feasible dose (600 mg)
Note: Blood sample for anti-drug antibody testing will be obtained 14 ± 2 and 28 ± 2 days after the Cohort Treatment Day.

Part 2 of this study is a single-blind, placebo-controlled, single dose study of PvP001 and PvP002 using gastric sample aspiration to evaluate gluten degradation in healthy adult subjects. Approximately 46 eligible subjects will participate in Part 2 of the study. Twelve subjects will participate in Group 1 and will receive PvP001 placebo, the MTD of PvP001, and the MTD of PvP001 following 7 days of treatment with a standard dose of a PPI; each of these 12 subjects will receive all three treatments but will be randomized to the treatment order. Ten unique subjects will participate in Group 2 and will receive the PvP002 comparator (sterile water) and 600 mg of PvP002; each of these 10 subjects will receive both treatments but will be randomized to the treatment order. Twenty-four unique subjects will participate in Group 3. Each subject will receive two treatments but will be randomized to the treatment order. Eight subjects will be randomized to receive a 1 g gluten-containing study meal; the two treatments will be PvP001 placebo and PvP001 300 mg. Eight subjects will be randomized to receive a 1 g gluten-containing study meal; the two treatments will be PvP001 placebo and PvP001 600 mg. Eight subjects will be randomized to receive a 6 g gluten-containing study meal; the two treatments will be PvP001 placebo and PvP001 900 mg. The MTD of PvP001 has been determined from Part 1 of the study in healthy adult subjects to be 900 mg.

Table 3 shows the number of subjects planned in each cohort for Part 2.

Table 3 Part 2 Number of Subjects per Cohort

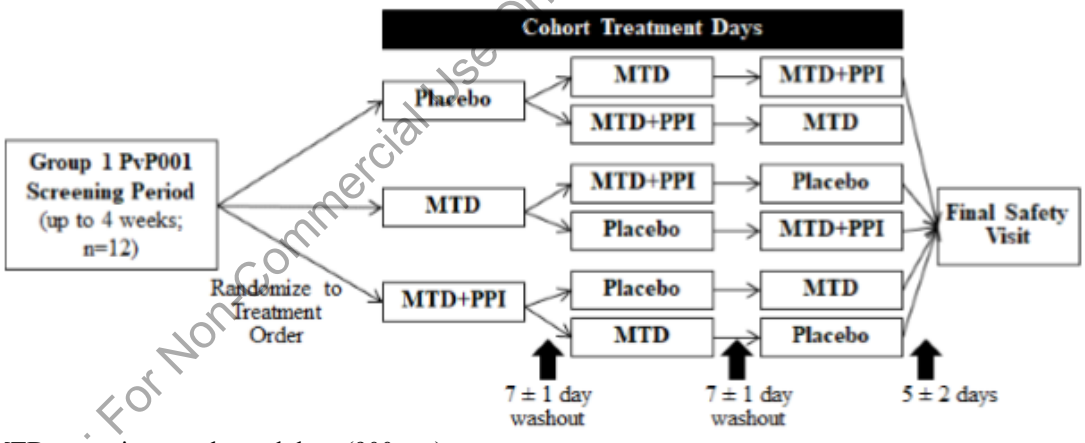
Group	Cohort	PvP001 (mg)	Healthy Subjects per Group
1	2A	0	12

Group	Cohort	PvP001 (mg)	Healthy Subjects per Group
	2B	900	
	2C	900 with PPI	
		PvP002 (mg)	
2	2D	0	10
	2E	600	
3	2F	0	24
	2G	300	
	2H	600	
	2I	0	
	2J	900	

PPI = proton pump inhibitor

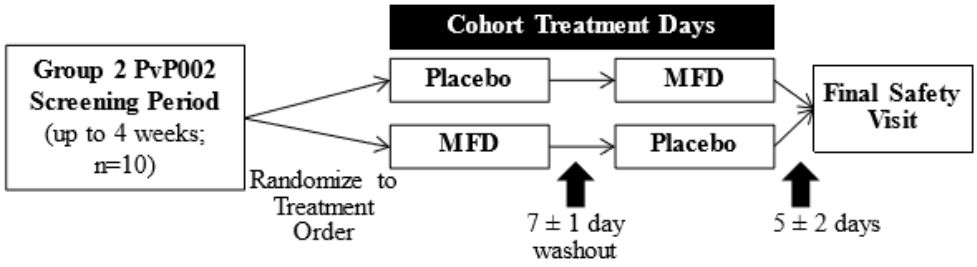
The Part 2 visit scheme will consist of a screening period, three Cohort Treatment Days with a washout period between each of the Cohort Treatment Days, a Safety Visit after the final Cohort Treatment Day, and two Follow Up Anti-Drug Antibody Blood Sampling Visits, with each Group 1 subject participating for up to approximately 10 weeks and each Group 2 subject participating for up to approximately 9 weeks. **Figure 2** shows the study visit schematic for Part 2 Group 1. **Figure 3** shows the study visit schematic for Part 2 Group 2. **Figure 4** shows the study visit schematic for Part 2 Group 3.

Figure 2 Part 2 Group 1 Study Visit Schematic



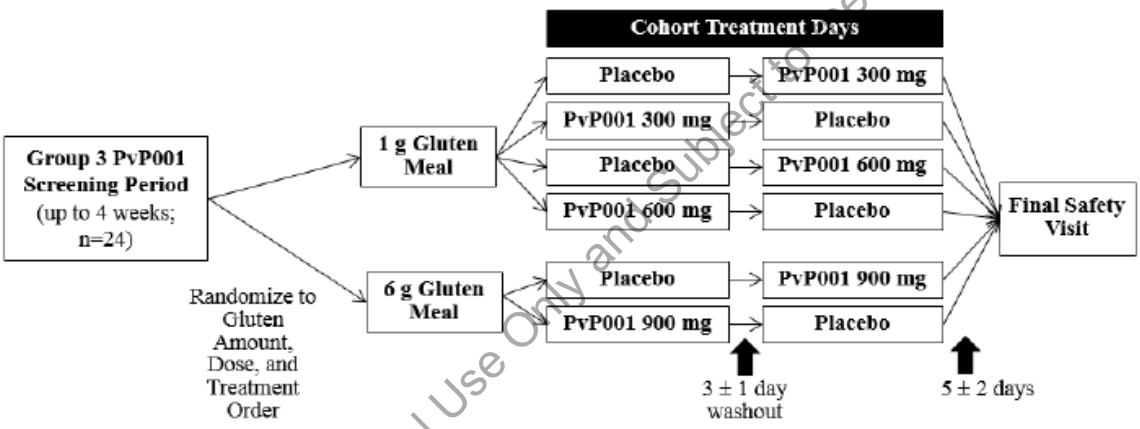
MTD = maximum tolerated dose (900 mg)
Note: Blood sample for anti-drug antibody testing will be obtained 14 ± 2 and 28 ± 2 days after the final Cohort Treatment Day.

Figure 3 Part 2 Group 2 Study Visit Schematic



MFD = maximum feasible dose (600 mg)
Note: Blood sample for anti-drug antibody testing will be obtained 14 ± 2 and 28 ± 2 days after the final Cohort Treatment Day.

Figure 4 Part 2 Group 3 Study Visit Schematic



Note: Blood sample for anti-drug antibody testing will be obtained 14 ± 2 and 28 ± 2 days after the final Cohort Treatment Day.

Part 3 of this study is a single-blind, placebo-controlled, single dose study of PvP003, a tablet formulation, using gastric sample aspiration to evaluate gluten degradation in healthy adult subjects under the following conditions: (a) In Group 1, administration of PvP003 placebo and PvP003 600 mg with pretreatment buffer solution before a standardized 1 g gluten-containing study meal, (b) In Group 2, administration of PvP003 placebo and PvP003 600 mg without pretreatment buffer solution before a standardized 1 g gluten-containing study meal, (c) In Group 3, administration of PvP003 placebo and PvP003 600 mg without pretreatment buffer solution after an approximately 50 mL portion of a standardized 1 g gluten-containing study meal, (d) In Group 4, administration of PvP003 placebo and PvP003 600 mg without pretreatment buffer solution before a standardized gluten-free study meal followed approximately 30 minutes later by a standardized 1 g gluten-containing study meal and (e) In Group 5, administrations of PvP003 placebo and PvP003 150 mg without pretreatment buffer solution before a standardized 1 g gluten-containing study meal. Thirty-six unique subjects (6 subjects in Group 1, Group 2, Group 3, and Group 4 and 12 subjects in Group 5) will participate in Part 3 and will receive PvP003 placebo and PvP003 150 mg or 600 mg as noted in **Table 4**; each of these 36 subjects will receive both treatments, but will be randomized to the

treatment order. Part 3 subjects will be blinded to which of the two treatments is active study drug. A subject may not participate in more than one Group. Enrollment of subjects in Group 1, Group 2, Group 3, Group 4 and Group 5 will occur sequentially. **Table 4** shows the number of subjects planned in each cohort for Part 3.

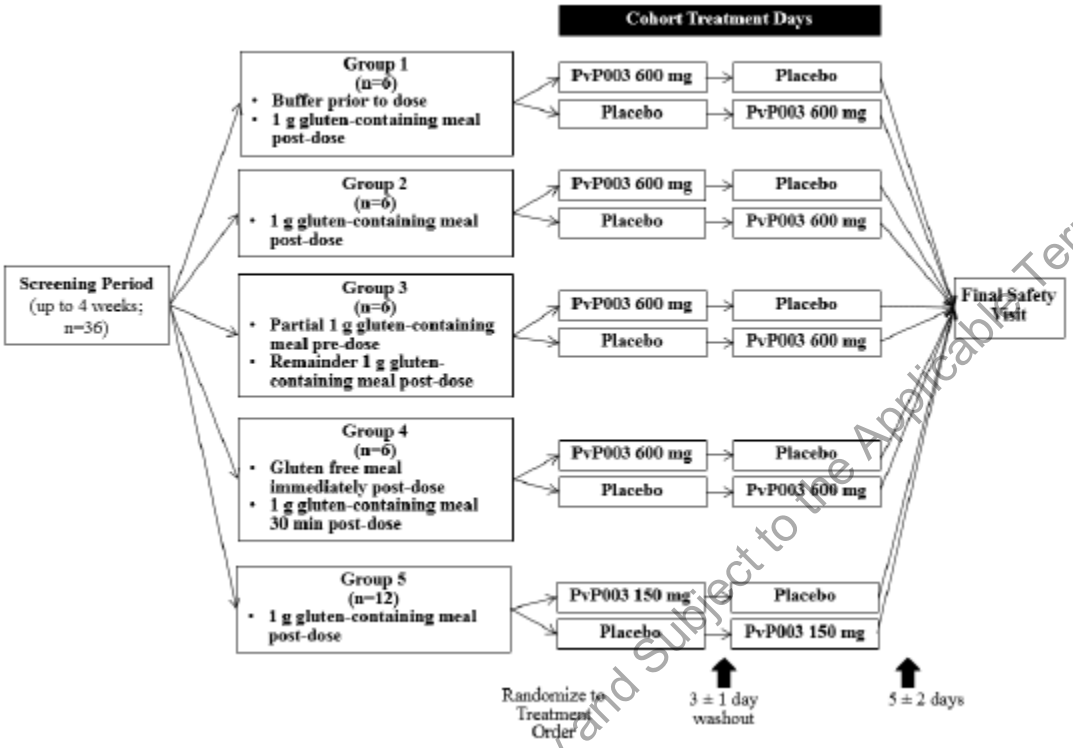
Table 4 Part 3 Number of Subjects per Cohort

Group	Cohort	PvP003 (mg)	Pretreatment Buffer Administered	Healthy Subjects per Group
1[a]	3A	0	Yes	6
	3B	600	Yes	
2[a]	3C	0	No	6
	3D	600	No	
3[a]	3E	0	No	6
	3F	600	No	
4[b]	3G	0	No	6
	3H	600	No	
5[a]	3I	0	No	12
	3J	150	No	

[a] Group 1, Group 2, Group 3 and Group 5 subjects will ingest a standardized 1 g gluten-containing study meal.
[b] Group 4 subjects will ingest a standardized gluten-free study meal followed by a standardized 1 g gluten-containing study meal.

The Part 3 visit scheme will consist of a screening period, two Cohort Treatment Days with a washout period between each of the Cohort Treatment Days, a Safety Visit after the final Cohort Treatment Day, and two Follow Up Anti-Drug Antibody Blood Sampling Visits, participating for up to approximately 9 weeks. **Figure 5** shows the study visit schematic for Part 3.

Figure 5 Part 3 Study Visit Schematic



Note: Blood sample for anti-drug antibody testing will be obtained 14 ± 2 and 28 ± 2 days after the final Cohort Treatment Day

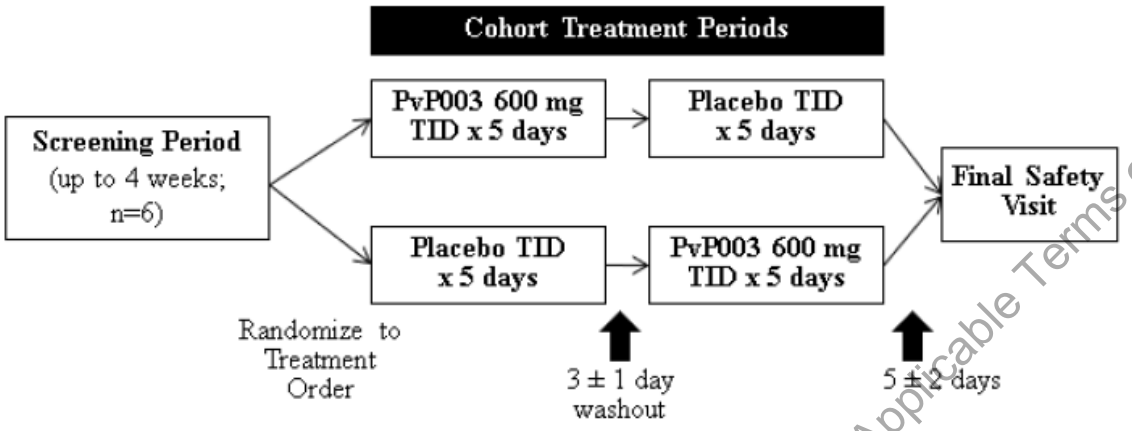
Part 4 of this study is a single-blind, placebo-controlled, multiple dose study of PvP003 in healthy adult subjects to evaluate safety following repeated administrations.
Six unique subjects will participate in Part 4 and will receive PvP003 placebo and PvP003 600 mg; each of these 6 subjects will receive both treatments three times a day (TID) for 5 days, but will be randomized to the treatment order. Part 4 subjects will be blinded to which of the two treatments is active study drug. No pretreatment buffer solution will be administered. **Table 5** shows the number of subjects planned in each cohort for Part 4.

Table 5 Part 4 Number of Subjects per Cohort

Cohort	PvP003 (mg) TID X 5 Days	Number of Healthy Subjects
4A	0	6
4B	600	

The Part 4 visit scheme will consist of a screening period, two 5-day Cohort Treatment Periods with a washout period between each of the Cohort Treatment Periods, a Safety Visit after the final Cohort Treatment Period, and two Follow Up Anti-Drug Antibody Blood Sampling Visits, participating for up to approximately 9 weeks. **Figure 6** shows the study visit schematic for Part 4.

Figure 6 Part 4 Study Visit Schematic



TID = three times a day
Note: Blood sample for anti-drug antibody testing will be obtained 14 ± 2 and 28 ± 2 days after the final Cohort Treatment Day.

6.2 Schedule of Assessments

Part 1

For the complete schedule of assessments, refer to Table 5 of the clinical study protocol.

Part 2

For the complete schedule of assessments, refer to Table 6 of the clinical study protocol.

Part 3

For the complete schedule of assessments, refer to Table 7 of the clinical study protocol.

Part 4

For the complete schedule of assessments, refer to Table 8 of the clinical study protocol.

6.3 Treatments

6.3.1 Treatments Administered

Part 1

A single dose of PvP001 placebo, PvP001 100 mg, PvP001 300 mg, PvP001 900 mg, or PvP002 will be administered on the Cohort 1A, 1B, 1C, 1D, or 1E Treatment Day, respectively.

Part 2

A Group 1 subject will be treated with a single dose of PvP001 placebo, 900 mg of PvP001, and 900 mg of PvP001 following 7 days of treatment with a standard dose of a

PPI on Cohort 2A, 2B, and 2C Treatment Days, respectively. Each Group 1 subject will take Nexium 20 mg capsules once daily at bedtime beginning 7 days prior to the subject's scheduled Cohort 2C Treatment Day. A Group 2 subject will be treated with a single dose of the PvP002 comparator (sterile water) and 600 mg of PvP002 on the Cohort 2D and 2E Treatment Days, respectively. A Group 3 subject will be treated with a single dose of PvP001 placebo on the Cohort 2F or 2I Treatment Day and a single dose of PvP001 300 mg, 600 mg, or 900 mg on the Cohort 2G, 2H, or 2J Treatment Day, respectively.

Part 3

Subjects will be treated with a single dose of PvP003 placebo on Cohort 3A, 3C, 3E, 3G and 3I Treatment days and a single dose of 600 mg of PvP003 on Cohort 3B, 3D, 3F and 3H Treatment days. Subjects will be treated with a single dose of 150 mg of PvP003 on the Cohort 3J Treatment day.

Part 4

Subjects will take three doses of PvP003 placebo daily for 5 days in the Cohort 4A Treatment Period, and take three doses of PvP003 600 mg daily for 5 days in the Cohort 4B Treatment Period.

In Parts 1 and 2 of the study, each subject will ingest a standardized study meal within 10 minutes of study drug administration. The standardized study meal in Part 1 will be gluten-free. The standardized study meal for Groups 1 and 2 in Part 2 will contain 3 g of gluten. The standardized study meal for Group 3 in Part 2 will contain either 1 g or 6 g of gluten.

For Part 3 of the study, subjects in groups 1, 2 and 5 will ingest a standardized 1 g gluten containing study meal within 10 minutes of the study drug administration. In Group 1, pretreatment buffer solution will be administered, while in Groups 2 and 5, pretreatment will not be administered. Subjects in Group 3 will ingest approximately 50 mL portion of a standardized 1 g gluten-containing study meal within 5 minutes. Study drug will be administered orally immediately after completing the ingestion of the approximately 50 mL portion of the study meal. The remaining portion of the standardized 1 g gluten-containing study meal will begin to be ingested immediately after study drug administration; this remaining portion of the study meal will be ingested within 10 minutes of study drug administration. No pretreatment buffer solution will be administered. In Group 4, study drug will be administered orally immediately prior to beginning the ingestion of a standardized gluten-free study meal. The entire gluten-free study meal will be ingested within 10 minutes of study drug administration. A standardized 1 g gluten-containing study meal will begin to be ingested 30 minutes after study drug administration. The entire gluten-containing study meal will be ingested within 10 minutes. No pretreatment buffer solution will be administered.

6.3.2 Method of Assigning Subjects to Treatment Groups

Part 1

Part 1 of this study is non-randomized. Subjects will receive a single dose of PvP001 placebo, PvP001 100 mg, PvP001 300 mg, PvP001 900 mg, or PvP002.

Part 2

Each Group 1 subject will receive three treatments but will be randomized to one of six possible treatment orders. Subjects will be randomized to treatment order in a 1:1:1:1:1:1 ratio.

- Cohort 2A, Cohort 2B, Cohort 2C
- Cohort 2A, Cohort 2C, Cohort 2B
- Cohort 2B, Cohort 2C, Cohort 2A
- Cohort 2B, Cohort 2A, Cohort 2C
- Cohort 2C, Cohort 2A, Cohort 2B
- Cohort 2C, Cohort 2B, Cohort 2A

Each Group 2 subject will receive two treatments but will be randomized to one of two possible treatment orders. Subjects will be randomized to treatment order in a 1:1 ratio:

- Cohort 2D, Cohort 2E
- Cohort 2E, Cohort 2D

Each Group 3 subject will be randomized to a 1 g gluten-containing study meal or a 6 g gluten-containing study meal in a 2:1 ratio, respectively, and to PvP001 dose and treatment order in a 1:1 ratio. A subject randomized to receive the 1 g gluten-containing study meal will receive both PvP001 placebo and PvP001 300 mg in one of two possible treatment orders or will receive both PvP001 placebo and PvP001 600 mg in one of two possible treatment orders. Each subject randomized to receive the 6 g gluten-containing study meal will receive both PvP001 placebo and PvP001 900 mg in one of two possible treatment orders.

- Cohort 2F, Cohort 2G
- Cohort 2G, Cohort 2F
- Cohort 2F, Cohort 2H
- Cohort 2H, Cohort 2F
- Cohort 2I, Cohort 2J
- Cohort 2J, Cohort 2I

Table 6 Treatment Description for Each Cohort

Group	Cohort	Treatment
1	2A	PvP001 0 mg (Placebo)
1	2B	PvP001 900 mg
1	2C	PvP001 900 mg with PPI
2	2D	PvP002 0 mg (Comparator)
2	2E	PvP002 600 mg
3	2F	PvP001 0 mg (Placebo)
3	2G	PvP001 300 mg
3	2H	PvP001 600 mg
3	2I	PvP001 0 mg (Placebo)
3	2J	PvP001 900 mg

PPI = proton pump inhibitor

Part 3

Each Group 1 subject will receive two treatments, but will be randomized to one of two possible treatment orders. Subjects will be randomized to treatment order in a 1:1 ratio.

- Cohort 3A, Cohort 3B
- Cohort 3B, Cohort 3A

Each Group 2 subject will receive two treatments, but will be randomized to one of two possible treatment orders. Subjects will be randomized to treatment order in a 1:1 ratio.

- Cohort 3C, Cohort 3D
- Cohort 3D, Cohort 3C

Each Group 3 subject will receive two treatments, but will be randomized to one of two possible treatment orders. Subjects will be randomized to treatment order in a 1:1 ratio.

- Cohort 3E, Cohort 3F
- Cohort 3F, Cohort 3E

Each Group 4 subject will receive two treatments, but will be randomized to one of two possible treatment orders. Subjects will be randomized to treatment order in a 1:1 ratio.

- Cohort 3G, Cohort 3H
- Cohort 3H, Cohort 3G

Each Group 5 subject will receive two treatments, but will be randomized to one of two possible treatment orders. Subjects will be randomized to treatment order in a 1:1 ratio.

- Cohort 3I, Cohort 3J
- Cohort 3J, Cohort 3I

Part 4

Each subject will receive two treatments, but will be randomized to one of two possible treatment orders. Subjects will be randomized to treatment order in a 1:1 ratio.

- Cohort 4A, Cohort 4B
- Cohort 4B, Cohort 4A

Randomization will occur only after the subject has been determined to be eligible for study participation based on the inclusion and exclusion criteria. The randomization schedule will be generated using a computer program and verified for accuracy using strict quality control procedures. Prior to each eligible subject's participation in the first Cohort Treatment Day, the next unique, sequentially available randomization number will be assigned to the subject using an interactive web response system. The randomization number will be recorded into the electronic Case Report Form (eCRF).

6.4 Efficacy and Safety Variables

6.4.1 Efficacy Variables

Efficacy analyses only apply to Part 2 and Part 3 of the study.

Efficacy endpoints include the following:

- Amount of total gluten (corrected for PEG) in the subject's stomach 20, 35, and 65 minutes after study drug administration as measured through gastric sampling and quantified using enzyme-linked immunosorbent assays (ELISA)

- Percent of gluten degraded 20, 35, and 65 minutes after study drug administration

The 35 minute time point for percent of gluten degraded will be collected and assessed for Part 2, Groups 1, 2, and 3. The 20 and 65 minute time points for percent of gluten degraded will be collected and assessed for Part 2, Group 3. The 35 and 65 minute time points for percent reduction in recovered gastric gluten will be collected and assessed for Part 3, Groups 1, 2, 3 and 5. Part 3, Groups 2 and 3 will be assessed both separately and in a combined analysis. The 65 minute time point for percent reduction in recovered gastric gluten will be collected and assessed for Part 3, Group 4. Concentration of PEG in the gastric sample will not be collected for Part 3 subjects and percent of gastric gluten degraded will not be analyzed.

6.4.2 Description of Safety Variables

Safety assessments will include adverse events (AEs), Gastrointestinal Symptoms Questionnaires (GSQs), physical examinations, vital signs, weight assessments, clinical laboratory tests (chemistry, hematology, and urinalysis), ADA testing, serum and urine pregnancy tests (in females), and electrocardiograms (ECGs).

6.4.2.1 Adverse Events

An AE is defined as any untoward medical occurrence in a clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with the product. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a pharmaceutical product, whether or not it is considered related to the pharmaceutical product.

6.4.2.2 Laboratory Parameters

Table 7 Clinical Laboratory and Other Tests

Chemistry	Hematology	Urinalysis	Other Tests
Alkaline phosphatase Aspartate aminotransferase Alanine aminotransferase Total bilirubin Total protein Albumin Glucose Carbon dioxide Blood urea nitrogen Creatinine Sodium Potassium Chloride	Hemoglobin Hematocrit Mean corpuscular hemoglobin Mean corpuscular hemoglobin concentration Mean corpuscular volume Erythrocyte count Leukocyte count Neutrophil count and percentage Lymphocyte count and percentage Monocyte count and percentage Eosinophil count and percentage Basophil count and percentage Platelet count	Glucose Protein Specific gravity pH Nitrite Bilirubin Urobilinogen Ketone Leukocyte esterase	Serum and urine pregnancy tests (beta-human chorionic gonadotropin) in females Urine drug (amphetamines, benzodiazepines, tetrahydrocannabinol, Cocaine, Opiates) screen Helicobacter pylori breath test Serum tissue transglutaminase immunoglobulin A antibodies in patients with celiac disease Total immunoglobulin A in patients with celiac disease

6.4.2.3 Gastrointestinal Symptoms Questionnaires (GSQ)

The GSQ will include nine items (abdominal discomfort, abdominal pain, abdominal bloating, constipation, diarrhea, passing gas, belching/burping, nausea, and heartburn), each rated on a five-point Likert scale on which the lowest score, 0, denotes no symptoms and the highest score, 4, denotes the most pronounced symptoms. In Part 1 of the study, each subject will complete the GSQ at screening and on the Cohort Treatment Day pre-dose (i.e., before pretreatment buffer solution administration) to ensure that the subject is free of clinically relevant gastrointestinal symptoms; each subject will complete the GSQ on the Cohort Treatment Day post-dose (i.e., after completion of study meal ingestion) and at the 24-Hour Safety Assessment to ensure that all gastrointestinal complaints are reported by the subject. In Part 2 and Part 3 of the study, each subject will complete the GSQ at screening and on each Cohort Treatment Day pre-dose (i.e., before nasogastric [NG] tube placement) to ensure that the subject is free of clinically relevant gastrointestinal symptoms; on each Cohort Treatment Day pre-dose (i.e., after NG tube

placement) to ensure that all gastrointestinal complaints that occur in association with the placement or presence of the NG tube are reported by the subject; and post-dose (i.e., after completion of study meal ingestion) to ensure that all gastrointestinal complaints are reported by the subject. In Part 4 of the study, each subject will complete the GSQ on Day 1 and Day 5 of each Cohort Treatment Period pre-dose (i.e., before administration of the first daily dose of study drug) and on Day 1 of each Cohort Treatment Period post-dose (i.e., after completion of breakfast ingestion), as well as every day at bedtime from Day 1 of the first Cohort Treatment Period through the day prior to the Safety Visit, to ensure that all gastrointestinal complaints are reported by the subject.

6.4.2.4 Anti-Drug Antibody Blood Sampling

A blood sample will be obtained at screening and the Follow Up Anti-Drug Antibody Blood Sampling Visits 14 ± 2 days and 28 ± 2 days after the Cohort Treatment Day (Part 1), after the final Cohort Treatment Day (Part 2 and Part 3), or after Day 5 of the second Cohort Treatment Period (Part 4) to test for ADA to PvP001, PvP002, and PvP003. Testing of samples for ADA will be performed using validated assays. Confirmed positive samples yielding a measurable titer will be further tested for neutralizing antibody (NAb) activity. In a Part 1 and Part 2 subject who develops ADA, the ADA level will be monitored until it returns to the pre-dose baseline. In a Part 3 and Part 4 subject who develops ADA, the ADA level will be monitored monthly until it returns to the pre-dose baseline or for 6 months, whichever occurs first. As appropriate, the potential impact of ADA on safety, efficacy, and PK will be assessed.

6.4.3 Description of Pharmacokinetic Variables

Pharmacokinetic testing will be done to evaluate systemic exposure to PvP001, PvP002, and PvP003. A blood sample for PK testing will be obtained before pretreatment buffer solution administration and approximately 15, 30, 45, 60, 120, 180, 240, 360, and 480 minutes after study drug administration on the Cohort Treatment Day in Part 1 and Part 2 of the study. A blood sample for PK testing will also be obtained at the 24-Hour Safety Assessment, approximately 24 hours after study drug administration in Part 1 of the study. A blood sample for PK testing will be obtained before pretreatment buffer solution administration (Group 1), before study drug administration (Group 2 and Group 4), or before ingestion of a 50 mL portion of a standardized 1 g gluten-containing study meal (Group 3), and approximately 15, 30, 45, 60, 120, 180, 240, 360, and 480 minutes after study drug administration on the Cohort Treatment Day in Part 3 of the study. A blood sample for PK testing will be obtained before and approximately 15, 30, 45, 60, 120, 180, and 240 minutes after administration of the first daily dose of study drug on Day 1 and Day 5 of each Cohort Treatment Period in Part 4 of the study. Testing of samples will first be performed for PvP001, PvP002 and PvP003 using a validated ELISA. The ELISA will be considered negative if the result is below the limit of quantification (BLQ). If the ELISA is positive, testing of samples for total enzyme, as well as intact (full-length) enzyme and processed (mature) enzyme, will be performed using a validated liquid chromatography/mass spectrometry (LC-MS/MS). The LC-MS/MS assay results will be considered negative if the result is BLQ.

The following PK parameters will be estimated by non-compartmental methods using actual elapsed time from dosing:

- $AUC_{0\text{-last}}$: Area under the concentration-time curve from time zero (pre-dose) to the last measureable concentration, calculated by the linear trapezoidal rule
- $AUC_{0\text{-}\infty}$: Area under the concentration curve from time zero (pre-dose) to infinity, calculated by the linear trapezoidal rule and extrapolated to infinity by addition of the last quantifiable concentration divided by the elimination rate constant ($AUC_{0\text{-last}} + C_{last}/\lambda_z$). If the extrapolated area (C_{last}/λ_z) is greater than 30% of $AUC_{0\text{-}\infty}$, then $AUC_{0\text{-}\infty}$ will be set to missing.
- C_{max}: Maximum concentration (ng/mL), obtained directly from the observed concentration versus time data
- T_{max}: Time to maximum concentration (hours), obtained directly from the observed concentration versus time data
- $T_{1/2}$: Apparent terminal half-life (hours), determined as $\ln(2)/\lambda_z$

Calculations will use the linear trapezoidal rule. The following formula will be used:

$$AUC = \frac{1}{2}(C_1 + C_2)(t_2 - t_1)$$

6.5 Data Quality Assurance

Report summaries will be generated using validated Base SAS® software, version 9.4 or higher, on a personal computer (PC) or server-based platform. Additional validated software may be used to generate analyses, as needed.

All SAS programs that create outputs or supporting analysis datasets will be validated by a second statistical programmer or biostatistician. At a minimum, validation of programs will consist of a review of the program log, review of output or dataset format and structure, and independent confirmatory programming to verify output results or dataset content. Additionally, all outputs will undergo a review by a senior level statistical team member before finalization.

The content of the source data will be reviewed on an ongoing basis by project statistical programmers and statisticians. Data will be checked for missing values, invalid records, and extreme outliers through defensive programming applications, analysis-based edit checks, and other programmatic testing procedures. All findings will be forwarded to the project data manager for appropriate action and resolution.

7 STATISTICAL METHODS

7.1 General Methodology

Data will be analyzed by Precision for Medicine biostatistics personnel. Statistical analyses will be reported with tables, figures, and listings, presented in rich text format, and using recommended ICH numbering. Output specifications for all tables, figures, and listings will be in conformance with guidelines specified by the ICH in Appendix 7 of the *Electronic Common Technical Document Specification* (Apr 2003).

7.1.1 Reporting Conventions

Part 1, Part 2, Part 3 and Part 4 analyses will be presented separately. Part 1 analyses will be presented by dosage level of PvP001 and the MFD of PvP002. Healthy subjects and patients with CeD will be presented separately and combined. Part 2 efficacy analyses will present Group 3 separately from Groups 1 and 2. Part 2 safety and PK analyses will present Groups 1, 2, and 3 together. Part 2, Part 3 and Part 4 analyses will be presented by group and cohort. Part 2 Groups 1 and 2 safety and PK analyses will present placebo and comparator separately. Part 2 Group 3, and Part 3 safety and PK analyses will combine placebo cohorts. Select analyses will also be presented by cohort order in each group. Part 3, Groups 2 and 3 efficacy analyses will be presented both separately and combined.

Tables and figures will be summarized by treatment group. Tables summarizing demographics and other baseline characteristics will also include a column for all subjects combined. In general, all data collected and any derived data will be presented in subject data listings, for all enrolled subjects. Listings will be ordered by site, subject number, treatment group, and assessment or event date. The treatment group presented in listings will be based on the planned assignment, unless otherwise noted.

Overall trial summaries will be produced for disposition, demographics and other baseline characteristics, as well as safety data summaries. Subject disposition, demographics and baseline characteristics will be summarized by study part, and overall. Safety data summaries will be summarized by single dose level, combining Parts 1, 2 and 3. Part 4 will not be included in the overall safety summaries due to multiple dosing of study drug.

The total column in Overall trial summaries will represent the number of unique subjects. The following list clarifies how subjects who are enrolled in multiple study parts are handled:

- Subject disposition – The total column will show subjects experiencing the condition in any study part.
- Demographics, Baseline Characteristics, Medical History, and Prior Medications – The total column will use data from the first study part in which they are enrolled.

- Adverse Events and Concomitant Medications – The total column will represent incidence in any study part.

- Shift tables – The baseline in the total column will represent the baseline from the first study part in which they are enrolled. The worst post-baseline value will be derived as worst post-baseline from any study part.

- Univariate summary tables by Visit – The total column will use the values from their first study part in which they are enrolled.

- Anti-Drug Antibody – The total column will report positive ADA from any study part.

In general, continuous variables will be summarized to indicate the population sample size (N), number of subjects with available data (n), mean, standard deviation (SD), median, minimum, and maximum values. Categorical variables will be summarized by the population sample size (N), number of subjects with available data (n), number of subjects in each category, and the percentage of subjects in each category. Unless otherwise noted, the denominator to determine the percentage of subjects in each category will be based on the number of subjects with available data. Select ordinal data may be summarized using both descriptive statistics and counts and percentages of subjects in each category, as appropriate.

Non-zero percentages will be rounded to one decimal place. Rounding conventions for presentation of summary statistics will be based on the precision of the variable of summarization, as it is collected in its rawest form (i.e., on the eCRF or as provided within an external file) and are outlined as follows:

- The mean and median will be rounded to one more decimal place than the precision of the variable of summarization;

- Measures of variability (e.g., SD, standard error [SE]) will be rounded to two more decimal places than the precision of the variable of summarization; and

- Minimum and maximum values will be presented using the same precision as the variable of summarization.

Other statistics (e.g., confidence intervals [CIs]) will be presented using the same general rules outlined above, or assessed for the most appropriate presentation based on the underlying data.

No formal statistical analysis will be performed to compare treatment groups. Descriptive statistics will be tabulated by treatment group and reviewed to evaluate all study endpoints.

7.1.2 Summarization by Visit

Data summarized by study visit will be based on the nominal, scheduled visit label as reported on the eCRF.

7.1.3 Data Handling Rules

Unless otherwise noted, values reported as greater than or less than some quantifiable limit (e.g., "< 1.0") will be summarized with the sign suppressed in summary tables and figures, using the numeric value reported. Data will display on subject listings to include the sign.

7.1.4 Standard Calculations

Where appropriate, the calculated study day of each assessment or event will be presented with the assessment or event date on subject data listings, where study day will be determined as:

- The assessment/event date minus the date of first dose, if the assessment/event date is prior to the date of first dose;
- The assessment/event date minus the date of first dose, plus one, if the assessment/event date is on or after the date of first dose.

Other variables requiring calculations will be derived using the following formulas:

- **Days:** A duration between two dates expressed in days will be calculated using the following conventions:
    - Later date – earlier date + 1, if the earlier date is on or after the date of first dose of study drug; or
    - Later date – earlier date, if the earlier date is prior to the date of first dose of study drug.
- **Months:** A duration expressed in months will be calculated by dividing the duration in days by (365.25 / 12)
- **Baseline:** Last value reported prior to study drug administration in a given cohort
- **Change from Baseline:** Change from baseline will be calculated as the post-baseline value minus the baseline value.

7.2 Analysis Populations

The analysis populations are defined as follows:

- Safety Population: Includes all subjects who receive at least one dose of study drug. Subjects will be analyzed based on treatment received.

- Intent-to-Treat (ITT) Population: In Part 2 and Part 3 of the study, includes all subjects who receive at least one dose of study drug. Subjects will be analyzed based on the treatment to which they were randomized.

- Per-Protocol (PP) Population: In Part 2 and Part 3 of the study, includes all treated subjects who have no major protocol violations. Subjects will be analyzed based on the treatment received.

- PK Population: Includes all subjects who received at least one dose of study drug and have any PK data.

Data summaries in Part 2 and Part 3 to be presented on both the Safety Population and the ITT Population will only be produced on both analysis sets if there is a difference in the population groups (e.g., at least one subject receives a different treatment than the subject was originally assigned).

7.3 Study Subjects

7.3.1 Disposition of Subjects

Subject disposition will be summarized for all enrolled subjects in Part 1 by dosage level of PvP001 and 600 mg of PvP002. Healthy subjects and patients with CeD will be presented separately and combined. Subject disposition will be summarized for all randomized subjects in Part 2, Part 3 and Part 4 by cohort order in each group, overall by group, and over all subjects combined. Summaries will include the number and percentage of subjects in each analysis population, completing the study, and discontinuing the study early by the primary reason for discontinuation.

7.3.2 Protocol Deviations

Major protocol violations will be summarized in Part 1 by dosage level of PvP001 and 600 mg of PvP002 and over all subjects combined for the Safety Population. Healthy subjects and patients with CeD will be presented separately and combined. Major protocol violations will be summarized in Part 2, Part 3 and Part 4 by cohort order in each group, overall by group, and over all subjects combined for the Safety Population. Major protocol violations may include, but will not be limited to the following:

- Violation of eligibility criteria;

- Randomization error;

- Non-compliance with study drug dosing; and

- On-study administration of a prohibited medication.

All major protocol violations will be determined and appropriately categorized prior to database lock. The number and percentage of subjects with any major protocol violations as well as the number and percentage of subjects with major protocol violations within each category will presented.

7.4 Efficacy Evaluation

7.4.1 Datasets Analyzed

Part 1 does not include any efficacy summaries. Efficacy summaries will be based on both ITT and PP Populations for Part 2 and Part 3 of the study only. A listing of subjects excluded from the ITT or PP Population, to include the reason for exclusion, will be presented. All efficacy data will be provided as summary tables as well as individual subject listings. Efficacy summaries will be presented by group and cohort. For efficacy analysis presented by study visit, the baseline value will be defined as the last value reported prior to study drug administration in a given cohort. All PK summaries will be based on the PK Population.

7.4.2 Demographic and Other Baseline Characteristics

Demographic variables including age, sex, ethnicity, and race in Part 1 will be summarized for the Safety and PK Populations by dosage level of PvP001 and 600 mg of PvP002. Healthy subjects and patients with CeD will be presented separately and combined. Demographic variables in Part 2 and Part 3 will be summarized for the Safety, PK, ITT, and PP Populations by cohort order in each group, and over all subjects combined. Demographic variables in Part 4 will be summarized for the Safety and PK Populations by cohort order in each group, and over all subjects combined.

Age will be summarized using descriptive statistics. Sex, ethnicity, and race will be summarized with the number and percentage of subjects in each parameter category.Baseline characteristics include medical history, CeD method of diagnosis and time since diagnosis for patients with CeD, height, weight, and body mass index (BMI). Body mass index will be auto-calculated in the database. Time since CeD diagnosis will be calculated in years relative to informed consent date. Baseline characteristics in Part 1 will be summarized for the Safety Population by dosage level of PvP001 and the MFD of PvP002. Healthy subjects and patients with CeD will be presented separately and combined. Baseline characteristics in Part 2, Part 3 and Part 4 will be summarized for the Safety Population by cohort order in each group, overall by group, and over all subjects combined. Height, weight, BMI, and time since CeD diagnosis at baseline will be summarized using descriptive statistics. Frequency counts and percentages to summarize subjects reporting positive medical history by body system and CeD method of diagnosis will be presented. Medical history is coded based on the Medical Dictionary for Regulatory Activities (MedDRA), version 23.0.

An overall trial summary will be produced for demographics and baseline characteristics, summarized by study part and overall. Overall baseline characteristics will summarize height, weight and BMI only.

7.4.3 Measurements of Treatment Compliance

In Part 2 of the study, compliance with the PPI treatment, defined in this study as Nexium 20 mg once daily for 7 days prior to the Cohort 2C Treatment Day, will be assessed using the subject's Daily Nexium Dosing diary and an inventory of the subject's returned Nexium capsules. Compliance with Nexium dosing will be determined as the number of doses received divided by the expected 7 doses, multiplied by 100. Compliance with Nexium dosing will be considered acceptable if the subject has dosed 4 to 7 days and no doses were missed during the 4-day period before Cohort 2C Treatment Day -1. Dosing compliance will be summarized using descriptive statistics, by cohort order, based on the Safety Population. The number and percentages of subjects who have acceptable compliance and non-acceptable compliance within each cohort order and overall will be summarized.

In Part 4 of the study, compliance with the study drug that was dispensed on Day 1 of the Cohort Treatment Period will be assessed on Day 4 of each Cohort Treatment Period using the subject's Daily PvP003 Dosing Diary and an inventory of the subject's returned study drug. Compliance with PvP003 and PvP003 placebo dosing will be determined as the number of doses received divided by the expected doses, multiplied by 100. Expected doses are calculated as the number of Cohort Treatment Days x 3. Compliance with study drug during the current Cohort Treatment Period will be considered acceptable if the subject receives at least two (approximately 67%) of the three expected daily doses each day on Day 1, Day 2, Day 3, and Day 4 (and Day 5, if applicable), including the third daily dose that site personnel will administer before dinner on Day 4 (or Day 5, if applicable). The subject will be questioned regarding any discrepancies from expected dosing. The subject will be withdrawn from the study if the subject's compliance during the current Cohort Treatment Period is less than 67%.Compliance is calculated separately for each cohort 4A and 4B.

7.4.4 Efficacy Endpoint Analysis Methods

The following section applies to Part 2 and Part 3 of the study only. The primary analysis set is the ITT Population with no imputation of missing data. The following data will be reported for each individual and summarized for each cohort using descriptive statistics:

- Measured gluten concentration in gastric sample at post-dose time points: Gluten Concentration (G_{CN}) (µg/mL) using ELISA based on the monoclonal R5 and G12 antibodies
- Measured gastric volume recovered at post-dose time point: Recovered Volume (V_R) (mL)

The following calculations will be performed and reported for each individual, and summarized for each cohort using descriptive statistics:

- Percent reduction in gluten concentration in gastric sample relative to placebo at corresponding post-dose time point: % Reduction in Gluten Concentration Relative to Placebo = $(1-(G_{CN\text{-}ACTIVE} / G_{CN\text{-}PLACEBO})) * 100$

  Where:

  $G_{CN\text{-}ACTIVE}$ = Gluten Concentration after Active Study Drug

  $G_{CN\text{-}PLACEBO}$ = Gluten Concentration after Placebo

- Gastric gluten recovered at post-dose time point: Gluten Recovered (G_R) (mg) = $G_{CN} * V_R / 1000$ (µg/mg)
- Percent reduction in recovered gastric gluten relative to placebo at post-dose time point: % Reduction in Recovered Gluten Relative to Placebo = $(1-(G_{R\text{-}ACTIVE} / G_{R\text{-}PLACEBO})) * 100$

  Where:

  $G_{R\text{-}ACTIVE}$ = Gluten Recovered after Active Study Drug

  $G_{R\text{-}PLACEBO}$ = Gluten Recovered after Placebo

The following calculations pertain to Part 2 subjects only:

- Polyethylene glycol (PEG)-corrected gastric volume at post-dose time point: Corrected Volume (V_C) (mL) = $PEG_{INPUT} / PEG_{RECOVERED}$

  Where:

  PEG_{INPUT} = 1,000 mg

  $PEG_{RECOVERED}$ = Concentration of PEG_{3350} in gastric sample (mg/mL)

- PEG-corrected total gastric gluten at post-dose time point: Corrected Total Gluten (G_C) (mg) = $(G_{CN} * V_C) / 1000$ (µg/mg)

- Percent reduction in PEG-corrected total gastric gluten relative to gluten input at post-dose time point: % Reduction in Corrected Total Gluten Relative to Input = $(1-(G_C / G_{INPUT})) * 100$

  Where:

  G_{INPUT} = 1,000 mg, 3,000 mg, or 6,000 mg depending on which standardized meal received

- Percent PEG-corrected gastric gluten degraded relative to placebo at post-dose time point: % Gluten Degraded Relative to Placebo (Corrected for Volume) = $(1-(G_{C\text{-}ACTIVE} / G_{C\text{-}PLACEBO})) * 100$

  Where:

  $G_{C\text{-}ACTIVE}$ = Corrected Gluten after Active Study Drug

  $G_{C\text{-}PLACEBO}$ = Corrected Gluten after Placebo

Part 3, Groups 2 and 3 will be analyzed both separated and in a combined manner.

Three gluten quantification methods will be used in this study: ELISAs based on monoclonal R5 and G12 antibodies, and for Part 2, an LC-MS/MS-based method that quantifies 33mer peptide. All parameters above are summarized for both ELISA methods. Gluten concentration in gastric samples at the pre-dose time point using the ELISA methods as, well as measurements for the 33mer peptide concentration (ng/mL) at pre-dose and post-dose time points, will appear in listings only. The calculated amount of gluten recovered in the subject's stomach after active treatment relative to the amount of gluten recovered in the same subject's stomach after placebo treatment is based on the formula utilized by Siegel 2012. Placebo includes PvP001 placebo, PvP002 comparator (i.e., PvP002 placebo) and PvP003 placebo. For Part 2, Group 1, calculations involving comparisons to placebo will be performed for both active study drug using 900 mg (Cohort 2B) and active study drug using 900 mg with PPI pretreatment (Cohort 2C). The percentage reduction in gluten concentration relative to placebo, percentage reduction in recovered gluten relative to placebo, percentage reduction in total gluten relative to input, and percentage total gluten degraded relative to placebo will be summarized for both ELISA gluten quantification methods using counts and percentages for the following categories: $\geq 80\%$, $\geq 90\%$ and $\geq 95\%$. Only percent reduction in gluten concentration relative to placebo and percent reduction in recovered gluten relative to placebo will be summarized for Part 3 due to not collecting PEG concentrations. For the purpose of summarizing percentage reduction in total gluten relative to placebo in Part 2, Group 3 and Part 3 for each time point, the recovered volume, which is only collected at the final time point, will be used for derivation for each previous time point, with the assumption that recovered volume would be static at each time point. The absolute percentage of gluten degraded relative to placebo/comparator, percentage of recovered gluten relative to placebo, corrected and uncorrected total gluten, and gluten concentration will be presented in box plots separately by ELISA. Percentage of recovered gluten relative to placebo/comparator, gluten concentration and gastric gluten recovered will be plotted for Part 3. For both Part 3 and subjects in Part 2, Group 3 that receive a standard 1 g gluten

meal (Cohorts 2F, 2G, 2H), a summary table using counts and percentages along with a bar chart of the proportion of subjects with < 50 mg of gastric gluten recovered will be summarized each time point. For the Part 2, Group 3 subjects that receive a standard 1 g gluten meal, a summary table using counts and percentages along with a bar chart of the proportion of subjects with < 50 mg of PEG-corrected total gastric gluten will be summarized each time point. For the purposes of performing the calculations listed above and plotting the data, gluten concentrations BLQ will be set to the lower limit of quantitation (LLOQ) for each analyte. The LLOQ will be 10 µg/mL for the R5 ELISA and the LLOQ will be 6.4 µg/mL for the G12 ELISA. The number and percentage of subjects with BLQ values will be summarized by time point and treatment group.

7.4.5 Statistical/Analytical Issues

7.4.5.1 Adjustments for Covariates

There are no planned applications of covariate adjustments for any of the statistical models on this trial. Data may be analyzed post-hoc to explore the effects of various factors. Such analyses will be labeled as post-hoc. Subjects enrolled in multiple parts of the study will be analyzed independently by part, and no adjustment for within-subject correlation will be made.

7.4.5.2 Handling of Dropouts or Missing Data

No imputations will be performed on missing data; all analyses will be based on observed data only.

7.4.5.3 Interim Analyses and Data Monitoring

An interim analysis will be conducted on a subset of subjects in Part 1 and Part 2 of the study. All analyses are descriptive in nature; therefore, no alpha spending is required.

There is no plan to establish a data monitoring committee for this study.

7.4.5.4 Multicenter Studies

This study is to be conducted at a single center.

7.4.5.5 Multiple Comparisons/Multiplicity

There will be no adjustments for multiple comparisons in the efficacy analysis for this study. Efficacy analyses are exploratory in nature.

7.4.5.6 Use of an "Efficacy Subset" of Subjects

The primary efficacy analysis will be performed on the ITT Population; the PP Population will be utilized as a sensitivity analysis. The PP Population will exclude subjects with major protocol violations.

7.4.5.7 Active-Control Studies Intended to Show Equivalence

This study does not include an active-control product and is not intended to demonstrate equivalence between any two drug products.

7.4.5.8 Examination of Subgroups

There are no planned analyses to assess efficacy results by subgroups; the study population is too small to warrant any meaningful interpretations.

7.4.6 Plasma Concentrations

Raw plasma concentration values of PvP001, PvP002 and PvP003 for the total enzyme, as well as the intact (full-length) enzyme and processed (mature) enzyme, will be summarized for the PK Population by treatment group and sampling time point using descriptive statistics, to include the geometric mean and coefficient of variation (CV) (%) on the HPLC/MS data. The geometric CV is calculated as $100*\text{sqrt}[\exp(\sigma^2)-1]$, where σ^2 is the variance of the log-transformed data. Each subject's plasma concentrations will be plotted over time for the total enzyme, intact (full-length) enzyme, and processed (mature) enzyme. Mean study drug concentrations will be plotted over time for total enzyme only. For summaries that are produced, figures and estimation of individual PK parameters, all concentrations assayed as BLQ should be set to zero irrespective of where they occur within a profile. The number and percentage of subjects with BLQ values will be summarized by time point and treatment group.

7.4.7 Pharmacokinetic Analysis

Pharmacokinetic parameters for total enzyme, as well as intact (full-length) enzyme and processed (mature) enzyme, will be analyzed based on the PK Population. For each subject, the PK parameters described in Section 6.4.3 will be determined by a non-compartmental approach. For the purpose of the non-compartmental PK analysis, all plasma concentration BLQ values will be set to zero as detailed in Section 7.4.6.

PK parameters will be summarized by treatment group using descriptive statistics that includes the CV. Summaries of C_{max}, AUC, and $T_{1/2}$ will also include point estimates for the geometric mean and geometric CV. The geometric mean is calculated by computing the mean of the log-transformed concentration values and then presented in the original scale by calculating the anti-log of the mean result. The geometric CV is calculated as $100*\text{sqrt}[\exp(\sigma^2)-1]$, where σ^2 is the variance of the log-transformed data. Listings of calculated PK parameters will be provided. T_{max} summaries will include point estimates for median and range.

7.5 Safety Evaluation

Safety analysis will be carried out for the Safety Population, to include all subjects who receive at least one dose of study drug. Subjects who do not complete the study, for whatever reason, will have all available data up until the time of termination included in the analysis. For safety analyses presented by study visit, the baseline value will be

defined as the last value reported prior to study drug administration in a given cohort. Screening values will be summarized on by-visit summaries.

Unless otherwise specified, overall summaries will be produced as described in section 7.1.1.

7.5.1 Extent of Exposure

Extent of exposure to Nexium will be summarized for Part 2 Cohort 2C using the Safety Population. The duration of exposure will be presented in days and calculated as the date of last dose of Nexium minus the date of first dose of Nexium, plus one. Duration of exposure and total dose received (mg) will be summarized using descriptive statistics.

Extent of exposure to PvP003/Placebo will be summarized for Part 4 using the Safety Population. The duration of exposure will be presented in days and calculated as the date of last dose of PvP003/Placebo minus the date of first dose of PvP003/Placebo, plus one. Duration of exposure and total dose received (mg) will be summarized using descriptive statistics.

No overall summaries will be produced for exposure.

7.5.2 Adverse Events

Treatment-emergent AEs (TEAEs) are defined as those AEs with onset after the first dose of study drug or existing events that worsened after the first dose of study drug. Treatment-emergent AEs will be summarized by treatment group. Events reported with a partial onset date (e.g., month and year are reported but the day is missing) will be considered to be treatment-emergent if it cannot be confirmed that the event onset was prior to the first dose of study drug based on the available date entries. Adverse events occurring after NG tube placement, but before the first dose of study drug, will not be considered treatment-emergent and will be displayed in the AE listing only.

Verbatim terms on CRFs will be mapped to preferred terms and system organ classes using MedDRA, version 23.0.

Summaries that are displayed by system organ class and preferred terms will be ordered by descending incidence of system organ class and preferred term within each system organ class. Summaries displayed by preferred term only will be ordered by descending incidence of preferred term. Summaries of the following types will be presented:

- Overall summary of number of unique TEAEs and treatment-emergent serious AEs (TESAEs) and subject incidence of TEAEs meeting various criteria;
- Subject incidence of TEAEs by MedDRA system organ class and preferred term;
- Subject incidence of TEAEs by Common Terminology Criteria for Adverse Events (CTCAE) grade, MedDRA system organ class, and preferred term;

- Subject incidence of TEAEs by relationship to study drug, MedDRA system organ class, and preferred term;
- Subject incidence of TESAEs by MedDRA system organ class and preferred term; and
- Subject incidence of non-serious TEAEs by MedDRA system organ class and preferred term.

At each level of summarization (e.g., any AE, system organ class, and preferred term), subjects experiencing more than one TEAE will be counted only once. In the summary of TEAEs by CTCAE grade, subjects will be counted once at the highest CTCAE grade reported at each level of summarization. In the summary of TEAEs by relationship, subjects will be counted once at the closest relationship to study drug. Related events include those assessed as "Possibly Related," "Probably Related," or "Definitely Related" to study drug; events considered not related are those assessed as "Unlikely Related" or "Not Related" to study drug. Adverse event data will be presented in data listings by subject, treatment group, and event. Serious AEs and AEs leading to discontinuation of the study drug will be presented in separate data listings.

7.5.3 Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

All deaths during the study, including the post treatment follow-up period, will be listed by subject, to include the primary cause of death. Serious AEs and other significant AEs, e.g., those that led to withdrawal from the study, withdrawal of drug, or change of study drug dose, will be provided in separate subject listings.

7.5.4 Clinical Laboratory Evaluation

All descriptive summaries of laboratory results will be based on data analyzed by the central laboratory and presented in Système International (SI) units, as suggested by the Center for Biologics Evaluation and Research and the Center for Drug Evaluation and Research *Position on Use of SI Units for Lab Tests* (Oct 2013). All data will be included in by-subject data listings. Laboratory measurements identified as abnormal (i.e., outside of the central laboratory reference range) will also be listed separately by subject, laboratory test, and unit. In addition, reference ranges provided by the central laboratory will be presented in a separate listing.

Clinical laboratory measurements, (i.e., serum chemistry and hematology), will be summarized by treatment group. Descriptive statistics will be presented for observed values and change from baseline at each visit where parameters were scheduled to be collected per the clinical study protocol. In Part 2, Part 3 and Part 4 of the study, Safety Visit values will be summarized for each cohort and change from baseline will be calculated relative to each cohort's baseline value. Observed values at the Safety Visit will be the same for all cohorts within each Part 2, Part 3 and Part 4 groups.

Change from baseline to post-dose for glucose values will not be summarized since there is a difference in the fasting status of subjects at the pre-dose (fasting) and post-dose (non-fasting) time points; change from baseline to 24-Hour Safety Assessment/Safety Visit for glucose values will be summarized since subjects are fasting at both time points.

Where applicable, laboratory results will be classified as "low," "normal," or "high" with respect to the parameter-specific reference ranges (i.e., below the lower limit of the reference range, within the reference range, or above the upper limit of the reference range).

Three-by-three contingency tables will be presented separately for each laboratory parameter and cohort to summarize the shift from the baseline category to the worst post-baseline category across all post-baseline assessments through the end of a cohort or the 24-Hour Safety Assessment/Safety Visit. If a laboratory value is low or high on more than one post-baseline assessment, the worst post-baseline category will be the category that applies to the value numerically farthest outside of the normal range. Shift from the baseline category to the worst-post baseline category will not be summarized for glucose values.

Abnormal clinical laboratory measurements will be categorized as results that are classified as "low" or "high". Abnormal clinical laboratory will be summarized in counts and percentages by treatment group, visit, and laboratory test, and categorized as "low", "high", and abnormal (either low or high).

7.5.5 Vital Signs, Physical Findings, and Other Observations Related to Safety

7.5.5.1 Vital Signs

Vital sign parameter measurements will be summarized by treatment group. Descriptive statistics will be presented for results and change from baseline at each visit where parameters were scheduled to be collected per the clinical study protocol. In Part 2, Part 3 and Part 4 of the study, Safety Visit values will be summarized for each cohort and change from baseline will be calculated relative to each cohort's baseline value. Observed values at the Safety Visit will be the same for all cohorts within each Part 2, Part 3 and Part 4 groups.

7.5.5.2 12-Lead Electrocardiogram

Each 12-lead ECG will be classified by the investigator as "normal," "abnormal, not clinically significant," or "abnormal, clinically significant." Three-by-three contingency tables will be presented separately for each cohort to summarize the shift from the baseline category to the worst post-baseline category across all post-baseline assessments through the end of a cohort or the 24-Hour Safety Assessment/Safety Visit. Summary results will include the count and percentage of subjects within each shift category and treatment group.

7.5.5.3 Physical Examination

Results of the physical examination will be presented in subject listings by subject and study visit.

7.5.5.4 Prior and Concomitant Medications

Medications will be coded using the World Health Organization Drug Dictionary Enhanced (WHODDE), version B2 March 1, 2018. Medications entered on the eCRF will be mapped to Anatomic Therapeutic Chemical (ATC) drug class (level 4) and drug name.

Prior and concomitant medications will be summarized separately and the study phase of each medication will be determined programmatically based on medication start and end dates. A prior medication is defined as any medication administered prior to the date of the dose of study drug (Part 1) or the dose of study drug in the cohort (Part 2, 3 and 4). A concomitant medication is defined as any medication administered on or after the date of the dose of study drug (Part 1) or the dose of study drug in the cohort (Part 2, 3 and 4). A medication may be defined as both prior and concomitant. If it cannot be determined whether a medication was received prior to the start of study drug dosing due to partial or missing medication start and/or end dates, it will be considered a prior medication. Likewise, if it cannot be determined whether a medication was received after the start of study drug dosing, it will be considered concomitant.

For both prior and concomitant medications summaries, the number and percentage of subjects receiving any medication will be summarized by treatment group, as will the number and percentage receiving any medication by ATC drug class and generic drug name. Prior medications will also be summarized over all subjects combined. Subjects reporting use of more than one medication at each level of summarization (any medication received, ATC class, and generic drug name) will be counted only once. Anatomic Therapeutic Chemical class terms will be displayed by descending order of incidence, as will generic drug names within each ATC class. The study phase during which each medication was received (e.g., prior, concomitant, or both) will be presented on the listing of prior and concomitant medications.

7.5.5.5 Gastrointestinal Symptoms Questionnaire

GSQ will be rated on a five-point Likert Scale as “none,” “mild,” “moderate,” “severe,” or “very severe.” Five-by-five contingency tables will be presented separately for each symptom and cohort to summarize the shift from the baseline category to the worst post-baseline category across all post-baseline assessments through the end of a cohort or the 24-Hour Safety Assessment/Safety Visit. Summary results will include the count and percentage of subjects within each shift category and treatment group.

7.5.5.6 Anti-Drug Antibody Blood Sampling

Results from ADA blood sampling tests will be presented in subject listings by subject and study visit. Percentage of positive ADA blood sampling results will be summarized

by study visit and presented in tables by treatment group. Each sample will be tested first by a screening (initial) test for ADA, followed by a confirmatory test for ADA if the screening (initial) test is positive. An ADA test will be considered positive only when both the screening (initial) and confirmatory tests show positive values. Testing for NAb will be performed only if the ADA testing is considered positive with a reportable Titer result. Results of the NAb assay will appear in listings only.

7.6 Determination of Sample Size

No formal sample size calculations were conducted for Part 1, Part 2, or Part 4 of this study. The sample sizes in Part 1, Part 2, and Part 4 of the study were selected to meet the objectives of the clinical trial (i.e., to assess the safety and PK of PvP001, PvP002, and PvP003 in Part 1, Part 2, and Part 4, as well as the gluten degradation ability of PvP001 and PvP002 in Part 2). In addition, in Part 2 of the study, the number of subjects in Group 1 was selected so that at least two subjects would be randomized to each of the six possible treatment orders and the number of subjects in Group 3 was selected so that at least four subjects would be randomized to each of the six possible gluten amount, PvP001 dose, and treatment order combinations; in Part 4 of the study, the number of subjects was selected so that at least three subjects would be randomized to each of the two possible treatment orders.

Sample size determination and justification were conducted only for Part 3 of this study. As residual gluten <50 mg is regarded as meaningful in reducing gluten-induced symptoms, the sample size calculation for Part 3 of the study is based on the proportion of subjects with <50 mg residual gluten. For pooled Part 3 Group 2 and Part 3 Group 3, and for Part 3 Group 5,with a sample size of 12 (i.e., six subjects in Group 2 and six subjects in Group 3), assuming the true (population) proportion is 85%, the 80% exact confidence interval (CI) will be (62%, 95%) when the observed rate is 83% (10 out of 12 subjects). Based on the width of this CI, the chosen sample size was considered to provide an acceptable level or precision in the estimation of the primary endpoint.

7.7 Changes in the Conduct of the Study or Planned Analyses

Changes to the conduct of the study and planned analysis from version 1.0 to 2.0 of the SAP included the addition of Part 2, Group 3 subjects and analyses, as well as an addition to the interim analysis as described in Section **7.4.5.3** of the current SAP. Changes to the conduct of the study and planned analysis from version 2.0 to 3.0 of the SAP included the addition of Part 3 and Part 4 subjects and analyses, along with overall study analyses.

There were no changes to the study conduct or planned analyses identified within the development of this SAP, relative to the descriptions provided within the clinical study protocol Amendment 5.

8 REFERENCE LIST

1. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Statistical principles for clinical trials (E9). International Conference on Harmonization; 1998.

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3. Data Standards: Position on Use of SI Units for Lab Tests. U.S Food and Drug Administration; 25 October 2013. Available from: http://www.fda.gov/ForIndustry/DataStandards/StudyDataStandards/ucm372553.htm

4. U.S. Department of Health and Human Services. Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03. 14 June 2010. Available from: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf.

5. Siegel M, Garber ME, Spencer AG, Botwick W, Kumar P, Williams RN, Kozuka K, Shreeniwas R, Pratha V, Adelman DC. Safety, tolerability, and activity of ALV003: results from two phase 1 single, escalating-dose clinical trials. Dig Dis Sci. 2012;57(2):440-50.