Statistical Analysis Plan

EMT-FAP-001

Phase IIa Trial of Encapsulated Rapamycin (eRapa) to Prevent Progression in Familial Adenomatous Polyposis Patients under Active Surveillance

Version & Date: V3.0, 26FEB2025

Prepared by:
LumaBridge, LLC.
1305 East Houston Street
San Antonio, Texas 78205
Phone: +1-726-842-8910

Signature Page

Statistical Analysis Plan V3.0 (dated 26FEB2025) for Protocol EMT-FAP-001.

Author:

John Johnson, Principal Statistician PhD

Upon review of this document, the undersigned approves this version of the Statistical Analysis Plan, authorizing that the content is acceptable for the reporting of this study.

Approved by:

Noreen Bhatti
Associate Vice President of Clinical Operations
Biodexa Pharmaceuticals PLC

Modification History

Version	Date	Author/Updated By	Description
V1.0	16JUL2021	Erika Davis	Initial version
V2.0	1NOV2023	Shikha Gautam	• Updated protocol version and number in Section 1 • Corrected polyp burden derivation to "sum of diameters of all polyps" instead of "total number of polyps multiplied by sum of diameters of all polyps" in Section 15.1.1 • Added explicit number of prescribed doses for each dosing cohort in Section 14.1 • Updated the formula for study treatment overall compliance in Section 14.1 • Replaced written word hypotheses with formulas in Sections 15.1.3 and 15.2.3 • Added exploratory efficacy analyses for total polyp burden to section 15.3 • Added analysis windowing conventions to Section 6.4 • Updated missing data methods in Sections 7.3, 15.1.2, 15.2.2, and 15.3.2.
V3.0	26NOV2024 – 26FEB2025	John Johnson	• Added food effect substudy analyses • Updated analysis of total number of adverse events to adverse events rate for statistical validity • Updated minor wording, formatting, and other administrative items

Final	26FEB2024	John Johnson	• Updated definition of polyp burden to account for excised polyps • Updated missing data handling strategy • Clarified hypotheses to be tested • Clarified t-test and α to be used • Updated food effect modeling strategy to account for sparse data in pharmacokinetic analyses, including adding a sensitivity analysis for excluding subjects without detectable levels

Table of Contents

Signature Page 2
Modification History 4
Table of Contents 6
Abbreviations 10
1. Introduction 11
2. Study Objectives 11
2.1. Primary Objectives 11
2.2. Secondary Objective 12
2.3. Exploratory Objectives 12
2.4. Estimands 13
3. Study Design 17
3.1. General Description 17
3.2. Schedule of Events 18
3.3. Changes to Analysis from the Protocol 18
4. Planned Analyses 19
4.1. Final Analyses 19
5. Analysis Sets 19
5.1. Full Analysis Set 19
5.2. Per-Protocol Set 19
5.3. Safety Set 19
6. General Considerations 20
6.1. Reference Start Date and Study Day 20
6.2. Baseline 20
6.3. Windowing Conventions 20

6.4. Statistical Tests 21
6.5. Common Calculations 21
6.6. Software Version 22
7. Statistical Considerations 22
7.1. Adjustments for Covariates and Factors to be Included in Analysis 22
7.2. Multicenter Studies 22
7.3. Multiple Comparisons/Multiplicity 22
7.4. Examination of Subgroups 22
8. Output Presentations 22
9. Disposition and Protocol Deviations 22
9.1. Disposition 22
9.2. Protocol Deviations 23
10. Demographic and Other Baseline Characteristics 23
10.1. Derivations 24
11. Medical History 24
12. Medications 24
13. Study Treatment Exposure 24
13.1. Derivations 24
14. Study Treatment Compliance 25
14.1. Derivations 25
15. Efficacy Outcomes 25
15.1. Primary Efficacy 25
15.1.1. Primary Efficacy Variable & Derivation 25
15.1.2. Missing Data Methods for Primary Efficacy Variable 26
15.1.3. Primary Analysis of Primary Efficacy Variable 26
15.1.4. Sensitivity Analysis of Primary Efficacy Variable 26
15.2. Secondary Efficacy 27

15.2.1. Secondary Efficacy Variables & Derivations....27
15.2.2. Missing Data Methods for Secondary Efficacy Variables....27
15.2.3. Analysis of Secondary Efficacy Variables....27
15.2.4. Sensitivity Analysis of Secondary Efficacy Variables....28
15.3. Exploratory Efficacy....28
15.3.1. Exploratory Efficacy Variable & Derivation....29
15.3.2. Missing Data Methods for Exploratory Efficacy Variable(s)....29
15.3.3. Analysis of Exploratory Efficacy Variable(s)....29
16. Safety Outcomes....29
16.1. Adverse Events....30
16.1.1. All TEAEs....30
16.1.1.1. Severity....31
16.1.1.2. Relationship to Study Treatment....31
16.1.2. Serious TEAEs....31
16.1.3. TEAEs Leading to Discontinuation of Study Treatment....31
16.1.4. Time to First AE Onset....32
16.2. Deaths....32
16.3. Laboratory Assessments....32
16.3.1. Laboratory Reference Ranges and Abnormal Criteria....32
16.4. Vital Signs....33
16.5. Physical Examination....33
16.6. ECOG Performance Status....33
17. Therapeutic Drug Monitoring....33
18. Pharmacodynamic Analysis....33
19. Pharmacokinetic Analysis....33
20. Data Not Summarized or Presented....35
References....36

Appendix 1: Programming Conventions for Outputs 36
Appendix 2: Algorithm for Imputing Partial or Missing Dates 37

Abbreviations

Abbreviation	Definition of Term
ADaM	analysis data model
AE	adverse event
ATC	Anatomical Therapeutic Class
BLQ	below limit of quantification
C	Celsius
CI	confidence interval
cm	centimeters
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose-limiting Toxicity
ECOG	Eastern Cooperative Oncology Group
eCRF	Electronic case report form
EGD	esophagogastroduodenoscopy
eRapa	Encapsulated Rapamycin
FAP	familial adenomatous polyposis
FAS	full analysis set
FDA	Food and Drug Agency
InSiGHT	International Society for Gastrointestinal Hereditary Tumors
IRB	Institutional Review Board
kg	kilograms
m	meters
MedDRA	Medical Dictionary for Regulatory Activities
min	minutes
mmHg	millimeters of mercury
MTD	Maximum Tolerated Dose
mTOR	mechanistic target of rapamycin
NCI	National Cancer Institute
NSAID	Nonsteroidal Anit-Inflammatory Drug
PD	pharmacodynamics

PK	pharmacokinetics
PT	preferred term
RP2D	Recommended Phase 2 Dose
SAE	serious adverse event
SAP	statistical analysis plan
SAS	Statistical Analysis System Institute software
SDTM	study data tabulation model
SOC	system organ class
TEAE	treatment-emergent adverse event
TLF	tables, listings, and figures
ULQ	upper limit of quantification
WHODD	World Health Organization Drug Dictionary

1. Introduction

This statistical analysis plan (SAP) describes the rules and conventions to be used in the presentation and analysis of efficacy and safety data for Protocol EMT-FAP-001. It describes the data to be summarized and analyzed, including specifics of the statistical analyses to be performed. This SAP is based on protocol version 3.0, dated 04DEC2023.

2. Study Objectives

2.1. Primary Objectives

The primary objectives of the study are:

1. To study the frequency and severity of adverse events associated with low dose encapsulated rapamycin (eRapa) in familial adenomatous polyposis (FAP).
2. To determine the recommended Phase 2 dose (RP2D) based on assessment of safety, long-term tolerability, optimal blood levels, and clinical benefit.
3. To determine the efficacy of eRapa in delaying polyp progression in patients with FAP compared to historic controls.

2.2. Secondary Objectives

The secondary objectives of the study are as follows:

- To further examine the clinical effect of eRapa on other markers of cancer prevention.
- To determine the effect of a high fat, high calorie meal on the pharmacokinetics of eRapa when administered at the anticipated RP2D.

2.3. Exploratory Objectives

The exploratory objective of the study is to explore the correlation between immune markers influenced by mechanistic target of rapamycin (mTOR) inhibition and clinical outcomes. The analyses for these objectives are not discussed in this document.

2.4. Estimands

The primary and secondary estimands (quantities to be estimated in a statistical analysis) to support regulatory decisions are described in the following table:

Table 1: List of Estimands

Estimand	Definition	Attributes			
		Population	**Variable/Endpoint**	**Intercurrent event handling strategy**	**Population-level summary measure**
Primary Estimand #1: Frequency and severity of AEs associated with eRapa in subjects with FAP	Number of subjects who report AEs related to eRapa	Subjects enrolled in the study who received at least one dose of eRapa	AE graded toxicity assessed throughout the trial per CTCAE v5.0	Missing data handled using multiple imputation	Proportion of subjects with reported AE graded toxicities compared between each dose cohort

Primary Estimand #2: RP2D of eRapa in subjects with FAP	RP2D based on safety, long-term tolerability, optimal blood levels, and clinical benefit	Subjects enrolled in the study who received at least one dose of eRapa	AEs, dose modifications including delays, reductions, withdrawal of treatment secondary to low-grade toxicities, and serum PK monitoring	Missing data handled using multiple imputation	Proportion of subjects with reported AE graded toxicities compared between each dose cohort
Primary Estimand #3: Efficacy of eRapa in delaying polyp progression in patients with FAP at 6 months	Percent change from baseline in colorectal polyp burden at 6 months	Subjects enrolled in the study	Percent change from baseline in colorectal polyp burden at 6 months	Missing data handled using multiple imputation	Proportion of subjects with reported AE graded toxicities compared between each dose cohort

Estimand	**Definition**	**Attributes**			
		Population	**Variable/Endpoint**	**Intercurrent event handling strategy**	**Population-level summary measure**

Secondary Estimand #1: Clinical effect of eRapa on colorectal polyp burden in subjects with FAP at 12 months	Percent change from baseline in colorectal polyp burden at 12 months	Subjects enrolled in the study	Percent change from baseline in colorectal polyp burden at 12 months	Missing data handled using multiple imputation	Proportion of subjects with reported AE graded toxicities compared between each dose cohort
Secondary Estimand #2: Clinical effect of eRapa on InSiGHT Stage in subjects with FAP at 6 and 12 months	Change from baseline in InSiGHT Stage at 6 and 12 months	Subjects enrolled in the study	Change from baseline in InSiGHT Stage at 6 and 12 months	Missing data handled using multiple imputation	Proportion of subjects with reported AE graded toxicities compared between each dose cohort
Secondary Estimand #3: Clinical effect of eRapa on Spigelman Stage Score in subjects with FAP at 6 and 12 months	Change from baseline in Spigelman Stage Score at 6 and 12 months	Subjects enrolled in the study and have polyps at baseline on EGD	Change from baseline in Spigelman Stage Score at 6 and 12 months	Missing data handled using multiple imputation	Proportion of subjects with reported AE graded toxicities compared between each dose cohort
Secondary Estimand #4: Clinical effect of eRapa on duodenal polyp number and size in subjects with FAP at 12 months	Percent change from baseline in duodenal polyp number and size at 6 and 12 months	Subjects enrolled in the study and have polyps at baseline on EGD	Percent change from baseline in duodenal polyp number and size at 6 and 12 months	Missing data handled using multiple imputation	Proportion of subjects with reported AE graded toxicities compared between each dose cohort
Secondary Estimand #5: Effect of a high fat, high calorie meal on the pharmacokinetics of eRapa when administered at the anticipated R2PD	$AUC_{0-\infty}$ and C_{max} after two weeks of treatment of eRapa taken with food vs. eRapa taken fasted	Subjects enrolled in food effect substudy	$AUC_{0-\infty}$ and C_{max} after two weeks of treatment of eRapa taken with food vs. eRapa taken fasted	None	Log ratio of $AUC_{0-\infty}$ and C_{max} after two weeks of treatment of eRapa taken with food vs. eRapa taken fasted (two [2] weeks and six [6] weeks).

					compared to tolerance limits of (-0.2231, 0.2231)

3. Study Design

3.1. General Description

This study is an open-label phase IIa pilot trial of encapsulated rapamycin (eRapa) to reduce polyp burden in subjects with FAP who are under active surveillance. The study will consist of 3 cohorts where subjects are administered eRapa orally at doses of either 0.5 mg every other day, 0.5 mg daily every other week, or 0.5 mg daily. Subjects will serve as their own controls and polyp burden will be compared via endoscopy at 6 months (180 days) and 12 months (360 days) to the baseline pre-intervention endoscopy results.

A total of 30 subjects with genetic or clinical diagnosis of FAP will be enrolled in this study (10 subjects in each arm). Each subject is expected to participate in the study for at least 12 months (360 days) for treatment and follow-up. Subjects who do not meet the criteria for participation in the trial (i.e., screen failure) because of polyp burden may be rescreened. Rescreened subjects will be assigned the same participant number as for the initial screening.

The trial is anticipated to last approximately 24 months (12 months for enrollment and 12 months for treatment and follow-up).

Dose-Limiting Toxicities and Cohort Safety Review Procedures

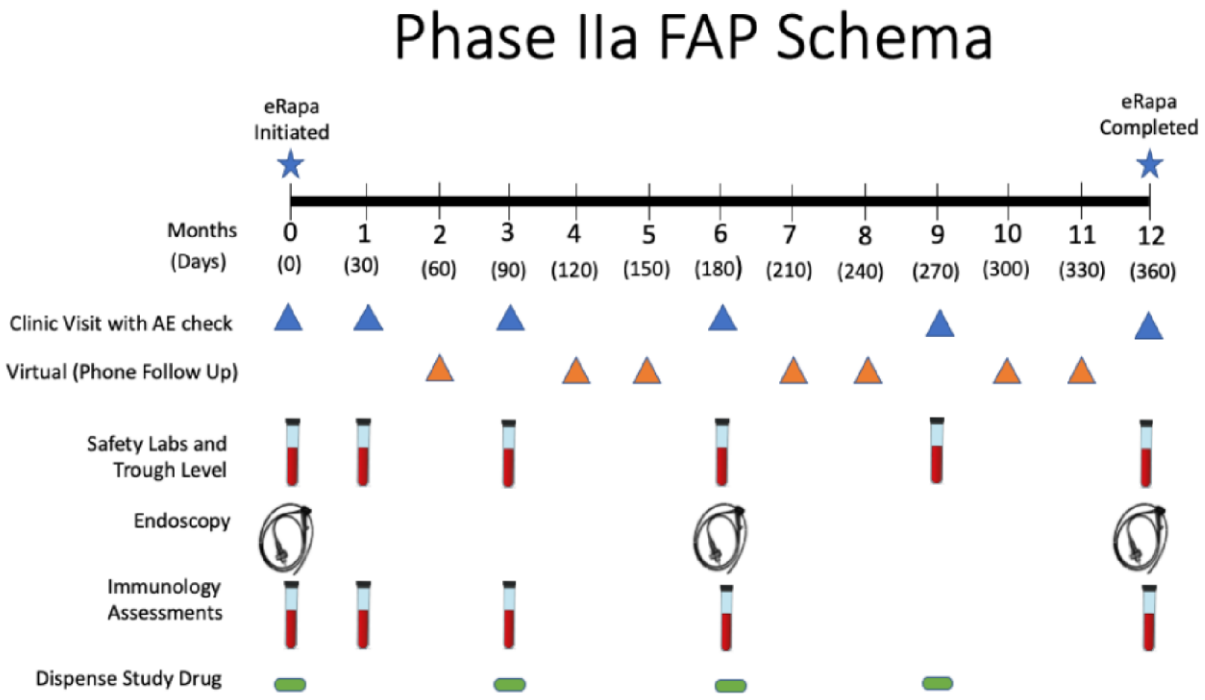
A formal safety review will occur after the first 3 subjects enrolled in the first cohort have been on treatment for 1 month (30 days). The review will include all labs, exams, and interviews performed during that period. Until the safety review occurs, no additional subjects in the cohort will be enrolled. A dose-limiting toxicity (DLT) is defined as any ≥ Grade 3 event occurring during the DLT evaluation period that is at least possibly related to eRapa administration. In order to be included in the safety review, subjects must have received > 75% of the eRapa dose unless treatment was discontinued secondary to a DLT or a lower-grade toxicity. If there are no DLTs in the first 3 subjects of the cohort, the remainder of the cohort will be enrolled. If there is a single DLT in the first 3 subjects, then an additional 3 subjects will be enrolled, and a formal safety review will be repeated at that time. If an additional DLT occurred (i.e., 2 DLTs in the first 6 subjects), then escalation will cease, and the dose immediately prior to the dose of that cohort will be determined to be the maximum tolerated dose (MTD), and all future subjects as well as those currently receiving the DLT dose will be transitioned to the MTD.

Once an entire cohort is enrolled, a second safety review will occur. Cohort enrollment is defined as the time from the first subject dosing through the completion of the 10th subject's Month 1 (Day 30) visit. If there are ≤3 DLTs in the entire cohort of 10 subjects, then the next cohort will be initiated. If there are >3 DLTs in the cohort, then escalation will cease, and the dose immediately prior to the dose of that cohort will be determined to be the MTD, and all future subjects as well as those currently receiving the DLT dose will be transitioned to the MTD.

Once safety has been shown at two assessments in the first cohort (i.e., after 3rd and 10th subject have been dosed in the cohort), the formal safety assessment after the 3rd subject in cohorts 2 and 3 will be discontinued. The safety assessment initiated after the 10th subject is dosed in each cohort will be reviewed and the DLT guidelines established will continue to be applied to this review.

In addition to the DLT criteria, if a significant reaction or toxicity occurs (as defined in Section 4.1 in the protocol), dose escalation will cease. If a fatal adverse event related to eRapa occurs, the study will be suspended until further review by the sponsor, FDA, and the IRB.

Figure 1: Study Design Diagram



3.2. Schedule of Events

Schedule of events can be found in Section 1.3 of the protocol.

3.3. Changes to Analysis from the Protocol

The follow changes to analysis from the protocol were made:

- Section 8.4.2 and 8.4.3 of the protocol specifies that the change in polyp burden, InSiGHT score, or Spigelman score will be compared using a one-sided paired samples t-test. However, the comparison will be done using a one-sided t-test based by planned treatment group to compare if the change from baseline is <0. This is corrected in Section 15.
- Section 8.4.8 of the protocol incorrectly specifies that immunologic response expression percentages will be analyzed using a Chi-square test. Expression percentages should be analyzed as a continuous variable. This is corrected in Section 15.3.3.
- Added exploratory efficacy analysis of upper tract polyp burden (only Apex Duodenal Bulb to Tattoo location) and total polyp burden (all reported location).

4. Planned Analyses

4.1. Final Analyses

All final, planned analyses identified in this SAP will be performed by LumaBridge Biostatistics following Emtora authorization of this SAP, database lock, and sponsor authorization of analysis sets.

5. Analysis Sets

5.1. Full Analysis Set

The Full Analysis Set (FAS) will contain all enrolled subjects who provided informed consent in the study and subjects will be classified according to assigned treatment.

5.2. Per-Protocol Set

The Per-Protocol Set will contain all subjects in the FAS who complied with eRapa administration at least 80% of the time within the treatment period. Prior to database lock, a review of each subject's Per Protocol set assignment will be performed.

5.3. Safety Set

The Safety Set will contain all subjects who enrolled in the study and received at least one dose of study treatment. Subjects will be classified according to actual treatment received.

5.4. Food Effect Set

The food effect set consists of all subjects enrolled in the food effect substudy who successfully complete at least one of the two food effect treatment periods, comply with the dosing schedule, and complete at least one pharmacokinetic blood draw at the appropriate time point (two [2] weeks or six [6] weeks). Subjects will serve as their own controls in this population. This population will serve as the basis for all food effect analyses.

Several subjects had issues with assessment of the concentration. To assess the impact of these issues, a sensitivity analysis will be performed with a modified food effect set. The issues will be handled as follows:

- Subjects with elimination half-lives that could not be assessed: summaries of elimination constant and terminal half-life will still exclude these assessments. They will be included in other summaries as appropriate.

- Subjects with non-detectable levels of eRapa throughout the sampling period: these subjects will be excluded from the food effect set, but included in the modified food effect set. In summaries, values will be set to those as if they had shown one-half of the lower level of quantification (LLOQ) throughout the sampling period, *i.e.* 0.5 ng/mL. Hence, the C_{max} will be set to 0.5 ng/mL, and the AUC will be set to 720 ng×min/mL. T_{max} will be set to 360 min.

6. General Considerations

6.1. Reference Start Date and Study Day

Study day will be calculated from the reference start date and will be used to show start/stop day of assessments and events.

Reference start date is defined as the day of the first dose of study treatment and will appear in every listing where an assessment date or event date appears.

- If the date of the event is on or after the reference date then:
  Study Day = (date of event – reference date) + 1

- If the date of the event if prior to the reference date then: Study Day = (date of event – reference date)

In the situation where the event date is partial or missing, Study Day, and any corresponding durations will appear partial or missing in the listings. Study day, and any corresponding durations, will be presented based on the imputations specified in Appendix 2.

6.2. Baseline

Unless otherwise specified, baseline is defined as the last non-missing measurement taken prior to the first dose of study treatment, including unscheduled assessments. In the event when the last non-missing measurement and the reference start date are on the same day, time will be used to determine if a record can be considered baseline. If time is not available and the protocol schedule of events specifies that the assessment is to be performed prior to first dose of study treatment, then the measurement will be considered baseline.

For efficacy assessments, subjects will serve as their own control. Results will be compared to the baseline pre-intervention endoscopy.

6.3. Windowing Conventions

Table 2 and Table 3 present the analysis visit windows assigned for assessments in this study. Table 2 will be used for general data analysis, including safety, exploratory efficacy, and immunological tests, while Table 3 will be used for primary and secondary efficacy endpoints data analysis.

If multiple assessments (scheduled or unscheduled visits) are taken within an analysis window, the assessment obtained on the day closest to the target day will be used. If two assessments are equidistant to the target day, the assessment obtained on the later day will be used in the analysis. If two assessments are on the target day, the worst value visit will be used.

Unscheduled measurements will not be included in by-visit summaries but will contribute to the Last Observation Carried Forward (LOCF) value, or best/worst case value where required (e.g., shift table).

Table 2: General Data Analysis Visit Windows

Visit Name	Target Study Visit Day	Analysis Visit Window Study Days
Baseline	0	0 – <15
1 month	30	15 – <45
3 months	90	45 – <135
6 months	180	135 – <270
12 months	360	270 – 450

Table 3: Primary and Secondary Efficacy Analysis Visit Windows

Visit Name	Target Study Visit Day	Analysis Visit Window Study Days
Baseline	0	0 – <90
6 months	180	90 – <270
12 months	360	270 – 450

Listings will include all performed assessments.

6.4. Statistical Tests

A nominal one-sided alpha of 0.05 will be used for all significance tests and confidence intervals (CIs) will be constructed at the two-sided 90% level unless otherwise specified.

6.5. Common Calculations

For quantitative measurements, common calculations will be calculated as:

- Change from baseline: Test Value at Visit X – Baseline Value
- Percent change from baseline: 100*([Test Value at Visit X – Baseline Value]/Baseline Value)

Descriptive statistics for continuous variables will be summarized with the number of non-missing observations, mean, standard deviation, median, minimum, and maximum. Categorical data will be summarized as counts and percentages.

Differences between treatment groups will be determined using a Chi-square test for categorical variables and a t-test for continuous variables.

6.6. Software Version

All analyses will be conducted using SAS version 9.4 or higher.

7. Statistical Considerations

7.1. Adjustments for Covariates and Factors to be Included in Analysis

No adjustments for covariates and factors will be included in the analysis unless otherwise indicated.

7.2. Multicenter Studies

Data from multiple sites will be pooled for use in analyses for this study.

7.3. Multiple Comparisons/Multiplicity

No adjustments for multiplicity will be performed for this study.

7.4. Examination of Subgroups

No formal subgroup analyses will be performed for this study. Additional exploratory subgroup analyses may be performed outside the scope of this analysis plan.

8. Output Presentations

Appendix 1 shows conventions for presentation of data in outputs. The TLF shells provided with this SAP describe the format and content of outputs provided by LumaBridge.

9. Disposition and Protocol Deviations

9.1. Disposition

All subjects who provide informed consent will be accounted for in the study. A subject is considered to have completed the study if he or she has completed all phases of the study including the last visit or the

last scheduled procedure shown in the Schedule of Events found in Section 1.3 of the protocol. Number of subjects enrolled, number of subjects who completed the study, number of subjects who discontinued treatment, the reasons for early discontinuation of treatment, number of subjects who discontinued from the study, and the reasons for early discontinuation from the study will be summarized overall and by cohort for the FAS.

Reasons for exclusion from each analysis set, inclusion criteria not met, and exclusion criteria met will be listed.

Disposition for the food effect study will be presented by sequence and overall and based on the food effect population. Subjects who entered and successfully completed each period (first and second) and also who started and successfully completed each treatment (fed vs. fasted) will be presented. Reasons for not completing any period (e.g. adverse event, withdrawal of consent) will be presented.

9.2. Protocol Deviations

Protocol deviations will be reported by CTMS. Type and severity (Critical, Major, and Minor) of protocol deviation will be summarized overall and by cohort for the FAS.

10. Demographic and Other Baseline Characteristics

Demographic data and other baseline characteristics will be presented for the FAS.

The following demographic will be reported for this study:

- Age (years)
- Sex
- Race
- Ethnicity
- Weight (kg)
- Height (cm)
- BMI (kg/m^2)
- Baseline ECOG
- Smoking History (Yes/No)
- Alcohol Use History (Yes/No)

The following FAP baseline characteristics will be reported for this study:

- Age at diagnosis (years) – date of diagnosis as reported on the FAP History at Screening eCRF
- Documented APC Mutation (Yes/No)
- Intact Colon (Yes/No)
- Total Abdominal Colectomy with Ileorectal Anastomosis (Yes/No)
- FAP Phenotype for Polyposis Post Colectomy (Yes/No)
- History of Desmoid Tumors (Yes/No) ○ Tumor Size (cm)
- History of Extraintestinal Malignancies (Yes/No)

- Prior Colorectal Cancer (Yes/No)
- Prior Esophagogastroduodenoscopy (EGD) (Yes/No)
- Worst Stage of Duodenal Polyposis (Stage 0, Stage I, Stage II, Stage III, Stage IV)
- NSAID Use (Yes/No)
- PPI Use (Yes/No)
- Family History of FAP (Yes/No)
- Family History of Colorectal Cancer (Yes/No)
- Family History of Duodenal Cancer (Yes/No)

A separate demography and baseline characteristics table will be presented for the food effect substudy by sequence and overall and will be based on the food effect population.

10.1. Derivations

- Age at diagnosis (years) = [(date of diagnosis – date of birth) + 1]/365.25

11. Medical History

Medical history will be presented in a listing.

12. Medications

Medications will be coded using the World Health Organization Drug dictionary (WHODD), version March 2020, and presented for the Safety Set overall and by cohort. Summaries will be presented by Anatomical Therapeutic Class (ATC) Level 2 and Drug Name.

Prior medications are medications which started and stopped prior to the first dose of study treatment. Concomitant medications are medications which 1) started prior to, on or after the first dose of study treatment, and 2) ended on or after the date of first dose of study treatment or were ongoing at the end of the study.

13. Study Treatment Exposure

Exposure to study medication in months will be presented for the Safety Set. The date of first and last study treatment administration will be taken from the eRapa Drug Diary eCRF. Summaries will include duration of exposure, number of subjects with dose adjustments, the reason for the dose adjustment, number of subjects with missed doses, and the reason for dose(s) missed.

Interruptions, compliance, and dose changes are not taken into account for duration of exposure.

13.1. Derivations

- Duration of exposure (months) = (date of last study treatment administration – date of first study treatment administration +1)/30.4375

14. Study Treatment Compliance

Overall compliance to study treatment will be presented for the Safety Set, overall and by cohort, and based on the drug accountability data. Number of capsules dispensed, number of capsules returned, and overall compliance will be summarized.

14.1. Derivations

Compliance will be calculated as the number of tablets taken (total dispensed – total returned) divided by the prescribed number of tablets expressed as a percentage, as described in the calculations below.

- Overall Compliance to study treatment will be calculated as follows:

$$\frac{No.\ of\ doses\ taken}{(Date\ of\ Last\ Dose\ of\ Treatment - Date\ of First\ Dose\ of\ Treatment) \times N_{daily}} \times 100$$

  Where $No.\ of\ doses\ taken$ is the total number of doses administered as reported in the Drug Diary and N_{daily} is the number of capsules required to be taken per day. The denominator is the duration of treatment from the first dose of treatment to the last dose of treatment.

N_{daily} is calculated as the following for each cohort:

- Cohort 1, dosed every other day: 16 doses per 31 days
- Cohort 2, dosed 7 days on, 7 days off: 17 doses per 31 days
- Cohort 3, dosed every day: 31 doses per 31 days

15. Efficacy Outcomes

15.1. Primary Efficacy

15.1.1. Primary Efficacy Variable & Derivation

The primary efficacy variable is percentage change from baseline in colorectal polyp burden as measured by endoscopy at 6 months (180 days). Colorectal polyp burden is calculated as the sum of the diameter of all polyps reported on the Polyp Burden section of the Endoscopy eCRF. Colorectal polyps are those with a reported location of anastomosis to anal verge, cecum, ascending colon & hepatic flexure, transverse colon, splenic flexure and descending colon, sigmoid colon, and rectum. Excised polyps will be excluded

from this calculation at baseline. In the event all polyps are excised, the baseline polyp burden will be imputed to be 1 for purposes of change and percent change from baseline calculations.

15.1.2. Missing Data Methods for Primary Efficacy Variable

Multiple imputation will be used to address missing polyp burden data. For variables where this method is applied, each missing value will be replaced multiple times based on estimated relationships among subject variables, resulting in multiple complete datasets that are conventionally analyzed. The results from these conventional analyses are combined into estimates of treatment effect and standard error that are unbiased under the assumption of missing at random. Furthermore, this approach lends itself to a tipping point analysis that assesses the robustness of this result under the missing at random assumption.

For colorectal and duodenal polyp burden data, 6-month and 12-month missing results will be multiply imputed. Baseline covariates to be included are site, age, sex, and race. In addition, 12-month burden will include 6-month burden data where it was actually collected (*i.e.* not imputed). Imputed total polyp burden scores will be calculated from colorectal and duodenal polyp burdens.

15.1.3. Primary Analysis of Primary Efficacy Variable

The primary objective of this study is to test the hypothesis that eRapa delays polyp progression in subjects with FAP compared to baseline controls. The primary efficacy analysis will be performed for the FAS by cohort.

$$H_0 : \mu_{\%CFB} \geq 0$$

$$H_A : \mu_{\%CFB} < 0$$

Where %CFB is the percent change in colorectal polyp burden from baseline to 6 months (180 days). The percent change from baseline less than zero signifies a decrease in the total polyp burden at month 6.

For each dose cohort, the 6-month percent change from baseline colorectal polyp burden will be compared to 0 using a one-sided single-group t-test with an alpha level of 0.05. Descriptive statistics for continuous variables will be shown in the summaries for colorectal polyp burden values including change from baseline, and percent change from baseline. The corresponding two-sided 90% CI and one-sided p-value will also be displayed.

15.1.4. Sensitivity Analysis of Primary Efficacy Variable

In the event of a significant result (at α=0.10) for the t-test described in Section 15.1.3, a tipping point analysis will be used to assess the robustness of the analysis to missing data.

15.2. Secondary Efficacy

The secondary efficacy analyses will be performed for the FAS by cohort.

15.2.1. Secondary Efficacy Variables & Derivations

The secondary efficacy variables are:

- Percent change from baseline in colorectal polyp burden as measured by endoscopy at 12 months
- Change from baseline in InSiGHT Stage at 6 months and 12 months
- Change from baseline in Spigelman Stage Score at 6 months and 12 months
- Percent change from baseline in duodenal polyp burden at 6 months and 12 months

Percent change from baseline in colorectal polyp burden as measured by endoscopy at 12 months is derived in the same method described in Section 15.1.1, with the exception that to account only for drug effect and not surgical effect, the total burden excised at 6 months will be added to the 12 month burden for each location.

InSiGHT Stage and Spigelman Stage Score are captured on the Endoscopy eCRF. InSiGHT Stage is a categorical outcome but, for the purposes of this analysis, will be treated as a continuous variable (i.e., Stage 0 corresponds to a numeric value of 0 while Stage IV corresponds to a numeric value of 4).

Percent change from baseline in duodenal polyp number and size at 6 months and 12 months are captured in the Polyp Burden section of the Endoscopy eCRF. Duodenal polyps are those with a reported location of apex duodenal bulb to tattoo.

15.2.2. Missing Data Methods for Secondary Efficacy Variables

Missing secondary efficacy data will be handled using multiple imputation methods as described for the primary efficacy analysis in Section 15.1.2 (including covariates used for the multiple imputation model).

15.2.3. Analysis of Secondary Efficacy Variables

The secondary objectives of this study are to test the hypotheses that eRapa delays polyp progression in subjects with FAP compared to baseline controls for each secondary efficacy endpoint. The analyses of secondary efficacy will be performed for the FAS by cohort and follow the same methods as described in Section 15.1.3. The hypotheses for each secondary efficacy endpoint are specified below:

- Percent change from baseline in colorectal polyp burden as measured by endoscopy at 12 months

$H_0 : \mu_{\%CFB} \geq 0$

$H_A : \mu_{\%CFB} < 0$

Where %CFB is the percent change in colorectal polyp burden from baseline to 12 months.

- Change from baseline in InSiGHT Stage at 6 months and 12 months

$H_0 : \mu_{CFB} \geq 0$

$H_A : \mu_{CFB} < 0$

Where CFB is the change in InSIGHT Stage from baseline to 6 months and 12 months.

- Change from baseline in Spigelman Stage Score at 6 months and 12 months

$H_0 : \mu_{CFB} \geq 0$

$H_A : \mu_{CFB} < 0$

Where CFB is the change in Spigelman Stage Score from baseline to 6 months and 12 months.

- Percent change from baseline in duodenal polyp burden at 6 months and 12 months

$H_0 : \mu_{\%CFB} \geq 0$

$H_A : \mu_{\%CFB} < 0$

Where %CFB is the percent change in duodenal polyp burden from baseline to 6 months and 12 months.

Descriptive statistics for continuous variables will be shown in the summaries for secondary efficacy variables. The corresponding two-sided 90% CI and p-values will also be displayed.

15.2.4. Sensitivity Analysis of Secondary Efficacy Variables

The same sensitivity analysis described in Section 15.1.4 will be performed for each secondary efficacy variable.

15.3. Exploratory Efficacy

The exploratory efficacy analyses will be performed for the FAS by dose cohort. Additional exploratory analysis may be performed that are not included in this SAP.

15.3.1. Exploratory Efficacy Variable & Derivation

The exploratory efficacy variables are:

- Immunological response
- Percent change from baseline in total polyp burden as measured by endoscopy at 6 months
- Percent change from baseline in total polyp burden as measured by endoscopy at 12 months

The exploratory efficacy variable of immunologic response is analyzed and measured by a central laboratory and includes overall assessment of T cell phenotype and function, specifically analyzing T cell naïve/memory/effector phenotype, exhaustion markers (PD-1, LAG3, TIM3), and homing markers (CD62L, CCR7) prior to, during, and after treatment with eRapa. Immunologic response is assessed prior to eRapa treatment and 1 month (30 days), 3 months (90 days), 6 months (180 days), and 12 months (360 days). The analysis of these data are not described in this document.

The exploratory efficacy variable of change in total polyp burden polyp burden is calculated as the sum of the diameter of all polyps reported on the Polyp Burden section of the Endoscopy eCRF. Polyps with all reported locations will be included for total polyp burden analysis.

15.3.2. Missing Data Methods for Exploratory Efficacy Variable(s)

For exploratory analyses, missing data will be handled as with secondary endpoints as necessary.

15.3.3. Analysis of Exploratory Efficacy Variable(s)

Descriptive statistics for continuous variables will be shown in the summaries including change from baseline. A one-sided t-test will be used to compare values within cohorts and a two-sided two-sample t-test will be used to compare between cohorts at each timepoint. The corresponding 95% CI and p-values will also be displayed.

A line plot for each immunologic response assessment by visit and cohort will be generated displaying mean and standard error. An additional line plot for each immunologic response assessment by visit showing individual subject data will be generated and output for each cohort.

For the analysis of change in upper tract polyp burden and change in total polyp burden, the 6-month (or 12-month) endoscopy result will be compared to the baseline pre-intervention endoscopy result using a one-sided t-test with an alpha level of 0.05. Descriptive statistics for continuous variables will be shown in the summaries for polyp burden values, change from baseline, and percent change from baseline. The corresponding 90% CI and p-value will also be displayed.

16. Safety Outcomes

All outputs for safety outcomes will be based on the Safety Set and summarized overall and by cohort. There will be no statistical comparisons between the treatment groups, unless otherwise specified in the relevant section.

16.1. Adverse Events

AEs will be coded using MedDRA coding dictionary, Version 23.0.

Treatment-emergent adverse events (TEAEs) are defined as AEs that started or worsened in severity on or after the first dose of study treatment.

See Appendix 2 for handling of partial dates for AEs to determine treatment-emergence. In the event when it is not possible to define an AE as treatment-emergent or not, the AE will be classified by the worst case, i.e, treatment-emergent.

Number of subjects and percentages will be presented for all AE summaries overall and by cohort. An overall summary for each of the categories described below will be provided in addition to individual summary tables presented by System Organ Class (SOC) and Preferred Term (PT), unless otherwise specified. Listings will include all AEs and a column for duration of treatment and duration of AE.

16.1.1. All TEAEs

Incidence and number of events of TEAEs will be summarized overall and by maximum severity and relationship to study medication. If a subject reports a TEAE more than once within a SOC/PT (or within a PT if sponsor chooses), then they will only be counted once, at highest grade.

The summary tables will include:

- Overall Summary of AEs
- All TEAEs by SOC/PT/CTCAE Grade
- Dose-Limiting Toxicity (DLT) events by SOC/PT
- Related TEAEs by SOC/PT
- Serious TEAEs by SOC/PT
- TEAEs Leading to Discontinuation of Study Treatment by SOC/PT

For the Overall Summary of AEs table, the following analyses will be performed:

- A Fisher's exact test will be performed to compare the differences between cohorts in each summary category (i.e., Related TEAEs, Serious TEAEs, TEAEs Leading to Discontinuation). The p-value and corresponding 95% CI will be presented.
- Summary of duration of all TEAEs in days
- Summary of duration of any AEs with >= 10% subject incidence – a two-sample t-test will be performed to compare the differences between cohorts*

- Summary of total numbers of TEAEs, serious TEAEs, and related TEAEs divided by total exposure on study – a two-sample t-test will be performed to compare the differences between cohorts*

*Depending on the distribution of the data, a Wilcoxon rank sum test may be used. For the purposes here, total exposure is defined as the (last treatment date + 7 days – first treatment date + 1) / 365.25 and will be expressed in patient-years.

In addition, the overall summary of AEs will be presented for the food effect substudy by treatment (fed vs. fasted) and overall.

16.1.1.1. Severity

AEs will be graded using the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 5.0. The guidance can be found here: https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

AEs with missing severity will be classified as severe (Grade 3). Summaries by maximum severity will be provided. If a subject reports a TEAE more than once within a SOC/PT, the AE with the worst-case severity will be used in the summaries.

16.1.1.2. Relationship to Study Treatment

Relationship is determined by the investigator and is classified as “Not Related”, “Unlikely to be Related”, “Possibly Related”, “Probably Related”, and “Definitely Related”. A related TEAE is defined as a TEAE with a relationship to study treatment reported as “Possibly Related”, “Probably Related”, and “Definitely Related”. TEAEs with a missing relationship to study treatment will be considered related to study treatment. Summaries by maximum relationship to study treatment will be provided. If a subject reports the same AE more than once within a SOC/PT, the AE with the worst-case relationship to study treatment will be used in the summaries.

16.1.2. Serious TEAEs

Serious TEAEs are those events reported as “Serious” on the Adverse Events eCRF. A summary of serious TEAEs will be provided. TEAEs with missing seriousness will be considered serious.

16.1.3. TEAEs Leading to Discontinuation of Study Treatment

TEAEs leading to discontinuation of study treatment will be determined by AEs that have “Drug Withdrawn” reported as the action taken with study treatment on the Adverse Event eCRF or “Adverse Event” reported as the reason for treatment discontinuation on the Visit Date eCRF.

16.1.4. Time to First AE Onset

Time to first TEAE onset in days will be presented and analyzed using Kaplan-Meier methods. Time to first AE onset will be calculated as the date of first AE onset minus the date of first dose of study treatment to +1. Subjects who do not experience an AE will be censored at the date of last dose of study treatment or study discontinuation, whichever is later.

Time to first AE onset will be summarized in a table including number of subjects with an AE, number of subjects censored, median number of days, and the corresponding 95% CI. A Kaplan-Meier plot will also be presented. A log rank test will be used to compare differences between cohorts. The p-value and corresponding 95% will also be presented.

16.2. Deaths

All deaths as reported as "Fatal" on the Adverse Events eCRF or reported as a reason for treatment discontinuation on the Visit Date eCRF will be presented in a summary table and a data listing.

16.3. Laboratory Assessments

Hematology, Serum Chemistry (including Complete Metabolic Panel, Lipid Panel, and LDH), Coagulation, and Urinalysis results from the sites' local laboratories will be summarized.

Presentations will use SI units. Local laboratory results reported in conventional units will be converted to SI units using the conversion factors outlined in: https://www.labcorp.com/resource/si-unit-conversionhttps://www.labcorp.com/resource/si-unit-conversion-tabletable.

Quantitative laboratory measurements reported as "<X", i.e., below the lower limit of quantification (BLQ), or ">X", i.e., above the upper limit of quantification (ULQ), will be converted to X for the purpose of quantitative summaries, but will be represented as reported, i.e., "<X" or ">X" in the listings.

The following summaries will be provided:

- Values and change from baseline by visit (for quantitative measurements)
- Shift from baseline to last visit on-treatment according to normal range criteria (for quantitative and categorical measurements)
- Incidence of abnormal values according to normal range criteria
- Listing of female subjects who report a positive pregnancy test

16.3.1. Laboratory Reference Ranges and Abnormal Criteria

Quantitative laboratory measurements will be compared with the laboratory reference ranges in SI units and categorized as Low, Normal, and High.

16.4. Vital Signs

The following vital signs measurements will be summarized:

- Height (cm)
- Weight (kg)
- BMI (kg/m^2)
- Temperature (°C)
- Heart Rate (beats/min)
- Respiratory Rate (breaths/min)
- Systolic Blood Pressure (mmHg)
- Diastolic Blood Pressure (mmHg)

A tabular summary of vital signs with values and change from baseline by visit will be presented overall and by cohort. A listing with all subject data will also be provided.

16.5. Physical Examination

A listing with subjects who any clinically significant findings during the physical exam will be provided.

16.6. ECOG Performance Status

A tabular summary of Eastern Cooperative Oncology Group (ECOG) performance status with values and change from baseline by visit will be presented overall and by cohort. A listing with all subject data will also be provided.

17. Therapeutic Drug Monitoring

eRapa trough levels will be assessed at quarterly visits for all subjects by the central laboratory at University of Texas Health – Long School of Medicine. A tabular summary of the values and changes from baseline by visit will be presented by cohort. A listing of eRapa trough levels for all subjects will also be provided.

18. Pharmacodynamic Analysis

Pharmacodynamic analysis is not discussed in this document.

19. Pharmacokinetic Analysis

19.1. Estimation of Pharmacokinetic Parameters

Pharmacokinetic (PK) analyses will be performed on the PK set and provided by the central laboratory at University of Texas Health – Long School of Medicine. PK concentrations and parameters will be summarized by cohort. The following PK parameters will be summarized:

- C_{max}
- T_{max}
- C_{last}
- T_{last}
- K_e
- Half-life
- $AUC_{0-\infty}$

PK concentration tables and figures will display number of non-missing values, median, minimum, maximum, geometric mean and coefficient of variation (CV%) at each protocol-specified time point. The PK parameters summaries will display similar summary statistics as the PK concentration summaries except T_{max} and T_{last} which will display mean and standard deviation instead of geometric mean.

19.2. Evaluation of Food Effect

In the food effect substudy, subjects are randomized to a sequence of fed vs. fasted administration of eRapa at the anticipated R2PD. Blood draws are obtained after two (2) and six (6) weeks,

As a two-period crossover design, the designated endpoints ($AUC_{0-\infty}$ and C_{max}) will be analyzed as follows:

A mixed model

$$log(Y_{ijk}) = \mu + \pi_i + \tau_j + \lambda_k + \epsilon_{ijk}$$

Where $log(Y_{ijk})$ is the AUC or concentration difference on log scale (end of period – beginning of period), μ is the overall mean, π_i is the ith subject effect as a random effect, τ_j is the jth period effect (1 or 2, regardless of treatment during that time), and λ_k is the kth treatment effect (1 or 2, either fed or fasted). ε_{ijk} is normal independent and identically distributed error with zero mean. To determine whether the fed vs. fasted states have an effect, the least squares mean of the treatment difference and its standard error will be used to construct a 90% confidence interval, to be compared to the tolerance limits of (-0.2231, 0.2231) (which corresponds to 80%-125% on the ratio scale). If both C_{max} and $AUC_{0-\infty}$ confidence intervals fall completely within the tolerance limits, the food effect will be considered to be zero.

In the event the model does not converge, it will be simplified as follows. Effects will be removed in the order of subject and then period effect until one it converges. If the model still does not converge, no further attempt will be made to interpret food effect.

As a sensitivity analysis, the model will be repeated considering all subjects who did not have detectable levels at a visit. In such cases, the parameters will be imputed as if the subject had one-half of the lowest

level of quantification (i.e. 0.5) at each timepoint. E.g. Cmax will be imputed with 0.5, and AUC will be calculated with a 0.5 at each sampled timepoint. This imputation will be done by visit.

20. Data Not Summarized or Presented

The other variables and/or domains not summarized or presented here are:

- Comments

These domains and/or variables will not be summarized or presented, but will be available in the clinical study database, Study Data Tabulation Model (SDTM) and/or Analysis Data Model (ADaM) datasets.

References

International Conference on Harmonisation. (1995). *E3: Structure and Content of Clinical Study Reports*. Retrieved from https://database.ich.org/sites/default/files/E3_Guideline.pdf

International Conference on Harmonisation. (1998). *E9: Statistical Principles for Clinical Trials*. Retrieved from https://database.ich.org/sites/default/files/E9_Guideline.pdf

International Conference on Harmonisation. (2019). *E9 (R1): Addendum on Estimands and Sensitivity Analysis in Clinical Trials to the Guideline on Statistical Principles for Clinical Trials*. Retrieved from https://database.ich.org/sites/default/files/E9-R1_Step4_Guideline_2019_1203.pdf

Food and Drug Administration. (2017). *Multiple Endpoints in Clinical Trials Guidance for Industry*. Retrieved from https://www.fda.gov/files/drugs/published/Multiple-Endpoints-in-Clinical-Trials-Guidance-forhttps://www.fda.gov/files/drugs/published/Multiple-Endpoints-in-Clinical-Trials-Guidance-for-Industry.pdfIndustry.pdf

National Cancer Institute. (2017). *Common Terminology Criteria for Adverse Events, version 5.0*. Retrieved from https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

Borgan, Ø. (1997). Three Contributions to the Encyclopedia of Biostatistics: The Nelson-Aalen, KaplanMeier, and Aaalen-Johansen Estimators. Norway: Department of Mathematics, University of Oslo.

Appendix 1: Programming Conventions for Outputs

- TLF shells are presented in a separate document.
- Depending on the data available, dates and time will take the form DDMMMYYY:hh:mm:ss
- The cohorts will be presented in the outputs as shown below and in the given order (main study only):

Cohort	**For Tables and Figures**
Cohort 1: 0.5 mg every other day	Cohort 1
Cohort 2: 0.5 mg daily every other week	Cohort 2
Cohort 3: 0.5 mg daily	Cohort 3

- Food effect table columns are presented in the shells. These tables are presented by sequence (Fed/Fasted, Fasted/Fed) or fed or fasted state as appropriate.
- All listings will be ordered by the following (unless otherwise indicated in the TLF shells):
    - Cohort, in ascending order ○ Site ○ Subject ID ○ Assessment (where applicable), ○ Date (where applicable),
- Footnotes will be ordered by the following:
    - Abbreviations ○ General output notes
    - Footnotes with superscripts or symbols

Appendix 2: Algorithm for Imputing Partial or Missing Dates

Imputed dates will not be presented in the listings. Imputed dates are only meant for determining treatment-emergence of an AE, if a medication is prior or concomitant, or calculating a duration of time.

Date	**Action**
Start Date	
Partial, Missing day	Impute missing day to the first day of the month
Partial, Missing day and month	Impute missing day and month to the earliest possible date (i.e., 1st of January)
Missing	Impute to date of first dose of treatment
End Date	
Partial, Missing day	Impute missing day to the last day of the month
Partial, Missing day and month	Impute missing day and month to the latest possible date (i.e., 31st of December)
Missing	Impute to date of first dose of treatment