Protocol B7981045

AN OPEN LABEL, PHASE 1, TWO-ARM STUDY TO ASSESS TARGET OCCUPANCY AND FUNCTIONAL INHIBITION OF JAK3 AND TEC KINASES BY SINGLE DOSES OF RITLECITINIB IN HEALTHY ADULT PARTICIPANTS

Statistical Analysis Plan
(SAP)

Version: 1

Date: 18 Oct 2021

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1. VERSION HISTORY

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1 18 Oct 2021	Protocol Amendment 1 01 Oct 2021	N/A	N/A

2. INTRODUCTION

Ritlecitinib (previously known as PF-06651600) is a potent, covalent, and irreversible inhibitor of JAK3 with high selectivity over the other three JAK isoforms (JAK1, JAK2, and TYK2. Ritlecitinib also inhibits irreversibly the TEC family kinases BTK, BMX, ITK, RLK, and TEC) with selectivity over the broader human kinome. Ritlecitinib is being developed for the treatment of UC, CD, AA, RA, and vitiligo.

Inhibition of JAK3 by ritlecitinib is expected to modulate γ-common chain cytokine pathways, such as IL-7, IL-9, IL-15, and IL-21, but expected to spare inhibition of other JAK-dependent pathways that do not utilize JAK3, including IL-6 signaling and key immuno-suppressive cytokines, such as IL-10, IL-27 and IL-35. Selective JAK3 targeting may reduce adverse effects from other JAK pathways, including cytopenia's (JAK2 dependent), cardiovascular events, malignancies, and severe infections. Inhibition of TEC kinases by ritlecitinib is expected to modulate T cell and B cell development and function (TEC kinases are activated downstream of the TCR and BCR). These effects by ritlecitinib on TCR signaling have been shown to inhibit the cytotoxic function of CD8+T cells and NK cells. There is limited information on the degree of target occupancy by Ritlecitinib on JAK3 and TEC kinases (ie, irreversible kinase binding) in humans, and how TO and target turnover of both JAK3 and TEC kinases a) is regulated by drug half-life, and b) how TO translates into functional effects on specific cell types and pathways.

TO of JAK3 and the five TEC kinases by ritlecitinib has been demonstrated in vivo in NOD scid IL2rγ-null mice engrafted with human PBMCs which expresses JAK3 and TEC kinases. Despite the large variation in occupancy measurements likely due to differing levels of human PBMC engraftment and the small number of mice (n=3) per time point, approximately 100% occupancy of JAK3 and most of the TEC kinases, except RLK, was observed in the spleen samples from early time points (0.25 to 5 h). A slower recovery of unbound BTK was seen compared to JAK3 and ITK due to relatively longer half-life of BTK (12 24 hours vs. 2-3 hours for JAK3/ITK). Since the PK profile of ritlecitinib is characterized by rapid absorption and rapid elimination half-life, it is important to study the relationship between TO of JAK3 and the TEC kinase family and the functional consequences of their inhibition over time by ritlecitinib in healthy participants to provide insights in assessing the impact in patients with inflammatory and autoimmune diseases. Based on the predicted PK for ritlecitinib, along with the need to assess target occupancy (and functional inhibition) in

a predefined specimen collection window, at least 2 doses would be required to capture expected concentrations in patients at clinically relevant doses and also to better understand the expected hysteresis in exposure and response.

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study B7981045. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

2.1. Study Objectives, Endpoints, and Estimands

Objectives	***Endpoints***
Primary:	***Primary:***
• *To characterize the TO of JAK3 and TEC kinases in peripheral blood mononuclear cells (PBMCs) after single oral dose of ritlecitinib*	• *Percent TO for JAK3 and TEC kinases (BTK, ITK, RLK, TEC and BMX)*
Secondary:	***Secondary:***
• *To evaluate the plasma exposure of ritlecitinib over 48 hours after single oral dose administration*	• *Ritlecitinib plasma PK parameters* • C_{max}, T_{max}, C_{last}, C_{av}, AUC_{last} and, AUC_{24}
• *To evaluate the safety and tolerability of an oral dose of ritlecitinib when administered alone and in healthy adult participants*	• *Assessment of TEAEs, clinical laboratory tests, and vital signs*
Tertiary:	***Tertiary:***
• *To characterize the relationship between TO in PBMCs and plasma ritlecitinib exposure over 48 hours after single oral dose*	• *Maximal inhibition (occupancy) by ritlecitinib (TO_{max})* • *Ritlecitinib plasma concentrations at 50% TO_{max} (IC_{50})*

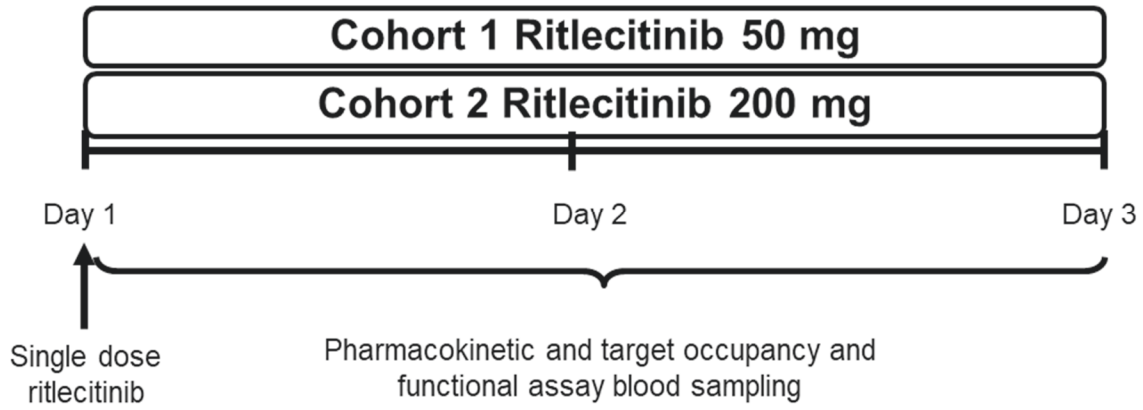
CCI [redacted]

2.2. Study Design

This is a Phase 1, single dose, open label, two-arm study to assess TO of JAK3 and TEC kinases by ritlecitinib in healthy adult participants. A total of approximately 16 healthy male and/or female participants, 8 in each cohort, will be enrolled and dosed to achieve at least 6 participants completing each dose level. Participants who discontinue from the study for non safety reasons may be replaced at the sponsor's discretion.

Participants will be enrolled to receive either 50 mg or 200 mg single dose of ritlecitinib on Day 1 (See Figure 1)*. Blood samples to assess ritlecitinib PK, target occupancy and functional assays will be collected over 48 hours. Participants will remain in the CRU until Day 3 when they will be discharged after completion of study related activities as indicated in the SoA.*

Figure 1. Study Schema



3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

The primary endpoint in human PBMCs is TO of JAK3 BTK, ITK, RLK, TEC and BMX after a single dose of ritlecitinib. Target occupancy for each kinase at time *t* will be evaluated as follows:

$$\%\,Target\ Occupancy_t = 100\,\% - \%\,Free\ Target_t$$

with TO_0 as the baseline variable of target occupancy, which is the mean of the two pre-dose measurements at Hour -1 and Hour 0 on Day 1. If only one of the two pre-dose measurements is available, TO_0 is the available pre-dose measurement. (see Section 3.4).

3.2. Secondary Endpoint(s)

3.2.1. PK Parameters

The secondary endpoints are pharmacokinetic parameters of ritlecitinib after single oral dose. PK parameters will be derived, given available data, following single dose administration from the concentration-time profiles for ritlecitinib as follows:

Table 2. Plasma PK Parameters

Parameter	***Definition***	***Method of Determination***
AUC_{24}	*Area under the plasma concentration-time curve from time 0 to 24 hours*	*Linear/Log trapezoidal method*
AUC_{last}	*Area under the plasma concentration-time curve from time 0 to the time of the last quantifiable concentration (C_{last})*	*Linear/Log trapezoidal method*
C_{av}	*Average plasma concentration from time 0 to the time of the last quantifiable concentration*	*$AUC_{24}/24$*
C_{max}	*Maximum plasma observed concentration*	*Observed directly from data*
C_{last}	*Plasma concentration at 48 hours post-dose*	*Observed directly from data*
T_{max}	*Time to reach C_{max}*	*Observed directly from data as time of first occurrence*

3.3. Other Endpoint(s)

3.3.1. Tertiary Endpoints

CCI [redacted]

Maximal target occupancy by ritlecitinib (TO_{max}) and Ritlecitinib plasma concentrations at 50% TO_{max} (IC_{50}) will be derived, if data permit.

3.4. Baseline Variables

Baseline TO, CCI [redacted] will be defined as the mean of the two pre-dose measurements at Hour -1 and Hour 0 on Day 1. If only one of the two pre-dose measurements is available, the baseline will be defined as the available pre-dose measurement.

In general, for all other analyses, baseline will be defined based on observations collected prior to first dose. Baseline values for demographics, medical and other history will be based on measures collected at Visit 1/Screening visit. Study Day 1 is defined as the day the subject receives first dose of study drug. For purposes of all other analyses including analyses for change from baseline, the baseline value will be defined as measured on Day 1 pre-dose. If a value is missing on Day 1, then the last available observation before Day 1 will be used.

3.5. Safety Endpoints

The following data are considered in standard safety summaries (see protocol for collection days, baseline assessment, and list of parameters):

- AEs;
- clinical laboratory tests;
- vital signs;
- ECG.

3.5.1. Adverse Events

An adverse event (AE) is considered a Treatment-Emergent Adverse Event (TEAE) if the event started during the effective duration of treatment. All events that start on or after the first dosing day and time/start time, if collected, but before the last dose plus the lag time, will be flagged as TEAEs. The lag time is defined by the Pfizer Standard of 365 days post last dose of Ritlecitinib. The algorithm will not consider any events that started prior to the first dose date. If an AE starts on the same day as the first dose date, it will be considered treatment emergent unless the CRF data indicates otherwise via explicitly recording time for AE onset which was occurred before the first treatment dosing.

3.5.2. Laboratory Data

Safety laboratory tests will be performed as described in the protocol.

To determine if there are any clinically significant laboratory abnormalities, the haematological, clinical chemistry (serum) and urinalysis safety tests will be assessed against the criteria specified in the sponsor reporting standards. The assessment will not take into account whether each subject's baseline test result is within or outside the laboratory reference range for the particular laboratory parameter.

Baseline of safety laboratory tests is defined as the pre-dose measurement on Day -1.

3.5.3. Vital Signs

Supine blood pressure and pulse measurements will be taken at all time points listed in the Schedule of Activities given in the protocol.

The baseline measurement is the pre-dose measurement at Hour 0 taken on Day 1. The maximum decrease and increase from baseline over all measurements taken postdose for supine systolic and diastolic blood pressures will be determined. The maximum increase from baseline will be calculated by first subtracting the baseline value from each postdose measurement to give the change from baseline. The maximum of these values over the entire study will then be selected, except in the case where a subject does not show an increase. In such an instance, the minimum decrease should be taken.

Similarly, the maximum decrease from baseline will be determined by selecting the minimum value of the changes from baseline. In cases where a subject does not show a decrease, the minimum increase should be taken.

3.5.4. ECG

Single supine 12-lead ECG will be taken at all time points listed in the Schedule of Activities given in the protocol. The baseline measurement is the pre-dose measurement at Hour 0 taken on Day 1.

The QT, QTc, PR, QRS and heart rate will be recorded at each assessment time.

If not supplied, QTcF will be derived using Fridericia's heart rate correction formula:

$$QTcF = QT/(RR)^{1/3} \qquad \text{where } RR = 60/HR \text{ (if not provided)}$$

The maximum absolute value (post-dose) and the maximum increase from baseline for QTcF, QT, heart rate, PR and QRS, will be determined for all measurements taken post-dose for QTcF, heart rate, PR and QRS.

The maximum increase from baseline will be calculated by first subtracting the baseline value from each postdose measurement to give the change from baseline. The maximum of these values over the entire study will then be selected, except in the case where a subject does not show an increase. In such an instance, the minimum decrease should be taken.

3.5.5. Other Safety Data

Additional safety data will be collected as described in the protocol and will be listed if collected in the sponsor's database.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

Data for all participants will be assessed to determine if participants meet the criteria for inclusion in each analysis population prior to unblinding and releasing the database and classifications will be documented per standard operating procedures.

Population	Description
Enrolled	All participants who sign the ICD.
Randomly assigned to investigational product	All participants who sign the ICD and meet all eligibility criteria.
Evaluable	The PK concentration population is defined as all participants enrolled and treated who have at least 1 concentration measurement of ritlecitinib. The PD population is defined as all enrolled and treated who have at least 1 TO measurement.
Safety Analysis Set	All participants who take at least 1 dose of investigational product.

5. GENERAL METHODOLOGY AND CONVENTIONS

5.1. Hypotheses and Decision Rules

No formal hypothesis testing will be performed in this study.

5.2. General Methods

Descriptive analyses will be performed. Some measures will be summarized using graphical representations by dose, where appropriate.

5.2.1. Analyses for Continuous Endpoints

For continuous variables, the data will be summarized using the number of subjects, mean, median, standard deviation, minimum, and maximum in accordance with current Pfizer's data and reporting standards. For appropriate PK parameters, geometric mean and geometric coefficient of variation (geocv%) will also be summarized. For the summaries of TO and baseline corrected TO, the 90% CI for the mean will also be presented.

Summaries will be presented by dose and nomial time post-dose, if appropriate.

5.2.2. Analyses for Categorical Endpoints

For categorical or ordinal variables, number of subjects, numbers and percentages of subjects meeting the categorical criteria will be supplied in accordance with current Pfizer's data and reporting standards.

5.3. Methods to Manage Missing Data

For the analysis of safety endpoints, the sponsor data standard rules for imputation will be applied.

Methods to handle missing PK data are described below.

Concentrations Below the Limit of Quantification:

In all data presentations (except listings), concentrations below the limit of quantification (BLQ) will be set to zero. (In listings BLQ values will be reported as "<LLQ" where LLQ will be replaced with the value for the lower limit of quantification).

Deviations, Missing Concentrations and Anomalous Values:

In summary tables and plots of median profiles, statistics will be calculated having set concentrations to missing if 1 of the following cases is true:

1. A concentration has been collected as ND (ie, not done) or NS (ie, no sample),

2. A deviation in sampling time is of sufficient concern or a concentration has been flagged anomalous by the pharmacokineticist.

Note that summary statistics will not be presented at a particular time point if more than 50% of the data are missing.

Pharmacokinetic Parameters:

Actual PK sampling times will be used in the derivation of PK parameters. If a PK parameter cannot be derived from a subject's concentration data, the parameter will be coded as NC (ie, not calculated). (Note that NC values will not be generated beyond the day that a subject discontinues). In summary tables, statistics will be calculated by setting NC values to missing; and statistics will be presented for a particular dose with ≥3 evaluable measurements.

If an individual subject has a known biased estimate of a PK parameter (due for example to an unexpected event such as vomiting before all the compound is adequately absorbed from the gastrointestinal tract), this will be footnoted in summary tables and will not be included in the calculation of summary statistics or statistical analyses.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoint(s)

The primary analysis will be performed on the PD population (see Section 4).

The primary endpoint is TO of JAK3, BTK, ITK, RLK, TEC and BMX after a single dose of ritlecitinib. TO profiles, CFB and percent CFB in TO profiles of each kinase will be assessed graphically and summarized by dose and nominal timepoints using appropriate descriptive statistics as described in Section 5.2.1.

6.2. Secondary Endpoint(s)

6.2.1. PK Parameters

The PK parameters detailed in Table 2 will be listed and summarized for participants in the PK concentration population (see Section 4). Missing values will be handled as detailed in Section 5.3. The PK parameters will be calculated using standard non-compartmental methods. Each PK parameter will be summarized by dose and will include the set of summary statistics as specified in the table below.

Table 3. PK Parameters to be Summarized Descriptively

Parameter	Summary Statistics
AUC_{24}, AUC_{last}, C_{av}, C_{max}, C_{last}	N, arithmetic mean, median, cv%, standard deviation, minimum, maximum, geometric mean and geometric cv%.
T_{max}	N, median, minimum, maximum.

For the derivation of PK parameters, actual PK sampling times will be used.

To assess the relationship between the PK parameters and dose, AUC_{24}, AUC_{last} and C_{max} will be plotted against dose (using a logarithmic scale), and will include individual subject values and the geometric means for each dose. Geometric means will have a different symbol than the individual values. These plots will be presented for both the dose-normalized (to a 1mg dose, by dividing the individual values and raw geometric means by dose) and non dose-normalized versions of these parameters.

Presentations for Ritlecitinib concentrations will include:

- A listing of all concentrations sorted by subject ID, dose and nominal time postdose. The concentration listing will also include the actual times. Deviations from the nominal time will be given in a separate listing.

- A summary of concentrations by dose and nominal time postdose, where the set of statistics will include n, mean, median, standard deviation, coefficient of variation (cv), minimum, maximum and the number of concentrations above the lower limit of quantification.

- Median concentrations time plots (on both linear and semi-log scales) against nominal time postdose by dose (all treatments on the same plot per scale, based on the summary of concentrations by dose and time postdose).

- Mean concentrations time plots (on both linear and semi-log scales) against nominal time postdose by dose (all treatments on the same plot per scale, based on the summary of concentrations by dose and time postdose).

- Individual concentration time plots by dose (on both linear and semi-log scales) against actual time postdose (there will be separate spaghetti plots for each dose per scale).

- Individual concentration time plots by subject (on both linear and semi-log scales) against actual time postdose [there will be separate plots for each subject (containing all doses) per scale].

For summary statistics, median and mean plots by sampling time, the nominal PK sampling time will be used, for individual subject plots by time, the actual PK sampling time will be used.

Additional PK analyses may be performed if deemed appropriate.

6.2.2. Safety Endpoints

All safety analyses will be performed on the safety population (see Section 4).

The safety endpoints detailed in Section 3.5 will be listed and summarized descriptively in accordance with the sponsor reporting standards based on the safety, with more details provided below.

A set of summary tables split by dose will be produced to evaluate any potential risk associated with the safety and toleration of administering Ritlecitinib.

6.2.2.1. Adverse Events

Adverse events will be reported in accordance with the sponsor reporting standards.

Subject discontinuations due to adverse events will be detailed by dose. Data will be reported in accordance with sponsor reporting standards.

6.2.2.2. Laboratory Data

Laboratory data will be listed and summarized by dose in accordance with the sponsor reporting standards. The baseline measurement is the pre-dose measurement on Day -1.

6.2.2.3. Vital Signs

The baseline measurement is the pre-dose measurement at Hour 0 taken on Day 1.

Baseline values and change from baseline values within each dose will be summarized with descriptive statistics (using sponsor default standards). The thresholds used in the categorical summarization of vital signs data are presented in the Appendix 1.

Maximum and minimum absolute values and maximum changes from baseline for supine vital signs will also be summarised descriptively by dose using categories as defined in Appendix 1. Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned post-dose time points will be counted in these categorical summaries.

6.2.2.4. Electrocardiograms

The baseline measurement is the pre-dose measurement at Hour 0 taken on Day 1 of each period.

Baseline values and change from baseline values within each dose will be summarized with descriptive statistics (using sponsor default standards). The thresholds used in the categorical summarization of ECG data are presented in the Appendix 1.

Maximum absolute values and increase from baseline for QTcF, PR and QRS will also be summarised descriptively by dose using categories as defined in Appendix 1. Numbers and percentages of participants meeting the categorical criteria will be provided. All planned and unplanned post-dose time points will be counted in these categorical summaries. For QT and heart rate, there was no related criteria regarding the maximum absolute value and maximum increase.

6.3. Other Endpoint(s)

6.3.1. Tertiary Endpoints

CCI [redacted]

If data permit, TO_{max} and IC_{50} will be estimated using a turnover model. These PD endpoints are considered exploratory and will not be included in the CSR. An additional PKPD modelling results will be reported separately in a population modelling analyses report (PMAR), if necessary.

6.4. Subset Analyses

Not applicable.

6.5. Baseline and Other Summaries and Analyses

Demographic data collected at screening will be reported as part of the standard baseline summary tables. A breakdown of demographic data will be provided for age, race, weight, body mass index, and height. Each will be summarized by dose in accordance with the sponsor reporting standards.

6.5.1. Treatment and Disposition of Subjects

Subject evaluation groups will show end of study subject disposition and will show which subjects were analyzed for safety (adverse events and laboratory data). Frequency counts will be supplied for subject discontinuation(s) by dose. Data will be reported in accordance with the sponsor reporting standards.

6.5.2. Concomitant Medications and Nondrug Treatments

All concomitant medication(s) as well as non-drug treatment(s) will be provided in the listings.

7. INTERIM ANALYSES

No formal interim analysis will be conducted for this study. As this is an open-label study, the sponsor may conduct unblinded reviews of the data during the course of the study for the purpose of safety assessment, facilitating PK/PD modeling, and/or supporting clinical development.

7.1. Introduction

Not applicable.

7.2. Interim Analyses and Summaries

Available safety and PK data may be reviewed.

8. APPENDICES

Appendix 1. Categorical Classes for ECG and Vital Signs of Potential Clinical Concern

Categories for QTcF

Degree of Prolongation	Mild (msec)	Moderate (msec)	Severe (msec)
Absolute value	>450-480	>480-500	>500
Increase from baseline		30-60	>60

Categories for PR and QRS

PR (ms)	max. ≥300	
PR (ms) increase from baseline	Baseline >200 and max. ≥25% increase	Baseline ≤200 and max. ≥50% increase
QRS (ms)	max. ≥140	
QRS (ms) increase from baseline	≥50% increase	

Categories for Vital Signs

Systolic BP (mm Hg)	min. <90	
Systolic BP (mm Hg) change from baseline	max. decrease ≥30	max. increase ≥30
Diastolic BP (mm Hg)	min. <50	
Diastolic BP (mm Hg) change from baseline	max. decrease ≥20	max. increase ≥20
Supine pulse rate (bpm)	min. <40	max. >120

Measurements that fulfill these criteria are to be listed in the clinical study report.

Appendix 2. List of Abbreviations

Abbreviation	Term
AA	alopecia areata
AE	adverse event
AUC	area under the curve
AUC_{24}	area under the plasma concentration time profile from time 0 to 24 hours
AUC_{last}	area under the plasma concentration time profile from time 0 to the time of the last quantifiable concentration
AV	atrioventricular
BCR	B cell receptor
BLQ	below the limit of quantification
BMX	bone marrow tyrosine kinase gene in chromosome X
BP	blood pressure
BTK	Bruton's tyrosine kinase
C_{av}	Average plasma concentration from time 0 to the time of the last quantifiable concentration
CD	Crohn's disease
CI	confidence interval
C_{last}	Plasma concentration at 48 hours post-dose
C_{max}	maximum observed concentration
CFB	change from baseline
COVID-19	coronavirus disease 2019
CRF	case report form
CRU	clinical research unit
CSR	clinical study report
D	day
DMC	data monitoring committee
EC	ethics committee
ECG	electrocardiogram
HR	heart rate
IC_{50}	50% inhibitory concentration
ICD	informed consent document
IL	Interleukin
ITK	IL 2 inducible T-cell kinase
JAK	Janus kinase
LLQ	lower limit of quantification
CCI [redacted]	
msec	milli-second
N/A	not applicable
NK	natural killer

Abbreviation	Term
PBMC	Peripheral blood mononuclear cells
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PR	PR interval
QRS	QRS interval
QT	Q wave interval
QTc	corrected QT
QTcF	corrected QT (Fridericia method)
RA	rheumatoid arthritis
RLK	resting lymphocyte kinase
SAE	serious adverse event
SAP	statistical analysis plan
SoA	schedule of activities
SOP	standard operating procedure
TBNK	T cell, B cell, natural killer cell
TCR	T cell receptor
TEAE	treatment emergent adverse event
TEC	tyrosine kinase expressed carcinoma
T_{max}	time for C_{max}
TO	target occupancy
TO_{max}	maxmum target occupancy
TYK	tyrosine kinase
UC	Ulcerative colitis