SYNOPSIS

Title of the Study

A COMPARATIVE, SINGLE CENTER, OPEN LABEL, LABORATORY-BLIND, RANDOMIZED, SINGLE DOSE, TWO PERIOD CROSSOVER STUDY TO DETERMINE THE RELATIVE BIOAVAILABILITY OF AN IMMEDIATE-RELEASE TABLET FORMULATION CONTAINING 500 MG FLUCYTOSINE AND A SUSTAINED-RELEASE PELLET FORMULATION OF FLUCYTOSINE IN HEALTHY MALES AND FEMALES UNDER FED CONDITIONS

Study Objectives

Primary Objective

To assess and compare the relative bioavailability of the test product, SR-5-FC pellets (single dose: 2 x 3000 mg at 0 hours) and the reference product, IR flucytosine (IR-5-FC) Ancotil® 500 mg tablets (3 x 500 mg at 0 hours and 3 x 500 mg at 6 hours after dosing) under fed conditions.

Secondary Objective

To evaluate the safety and tolerability of the test and reference products in healthy males and females under fed conditions.

Exploratory Objective

To assess the acceptability and palatability of the test formulation in healthy volunteers.

Study Endpoints

Pharmacokinetic Endpoints/Parameters

Primary PK parameters for flucytosine and 5-FU:

- Maximum observed plasma concentration (C_{max})
- Area under the plasma concentration versus time curve, from time zero to t, where t is the time of the last quantifiable concentration ($AUC_{(0-t)}$)

Secondary PK parameters for flucytosine and 5-FU:

- Time to maximum observed plasma concentration (t_{max})
- Area under the plasma concentration versus time curve, with extrapolation to infinity ($AUC_{(0-\infty)}$)
- Terminal elimination rate constant (λ_z)
- Apparent terminal elimination half-life ($t_{½}$)

Safety Endpoints

Frequency and cumulative incidence of adverse events (AEs) and serious adverse events (SAEs) for 5-FC test and reference product, assessed through clinical, electrocardiogram (ECG) and laboratory safety assessments (hematology, clinical chemistry and urinalysis) from baseline to post-study visit. Medical history and prior and concomitant medication will also be recorded.

Exploratory Endpoint

Acceptability and palatability will be assessed using questionnaires – an Investigator questionnaire (to assess acceptability) and a participant questionnaire (to assess palatability).

Study Design

This study will be an open-label, bioanalytical laboratory-blind, randomized, single dose, two-period crossover study with orally administered flucytosine IR tablets and SR pellets conducted under fed conditions in at least 30 evaluable healthy males and females at a single study center.

Subjects will receive the following treatments:

- **Reference (Treatment A):** Ancotil® 3000 mg IR tablets (twice daily [b.i.d] dose: 3 x 500 mg [0 hours] and 3 x 500 mg [6 hours])
- **Test (Treatment B):** Flucytosine 6000 mg SR pellets (single dose: 2 x 3000 mg [0 hours])

The study will comprise:

- a screening period of maximum 21 days,
- two treatment periods (each of which will include a PK profile period of 48 hours) separated by a washout period of 7 calendar days (5 half-lives are between 15 and 30 hours for the IR formulation and approximately 80 hours for the SR pellet formulation) to 21 calendar days (maximum number of days based on logistical arrangements and to allow interim safety analysis) between consecutive administrations of the IP. Subjects will be admitted to the study center on Day -1 and will remain in the study center for at least 48 hours after dosing.
- interim safety evaluations (hematology, clinical chemistry, and urinalysis) that will be performed at 30 hours post-dose in Treatment Periods 1 and 2, and 5 days after administration of IP in Treatment Period 1, and
- a post-study visit 7 to 10 days after completion of the last treatment period of the study,
- telephonic follow-up, 1 month (for females) and 3 months (for males) post last IP administration.

Procedures listed for the post-study visit and the telephonic follow-up will be performed in the event of early withdrawal from the study.

Subjects will be assigned randomly to treatment sequence, before the first administration of IP.

DMC Review of Safety Data of Initially Dosed 4 Subjects

As additional safety measure, 4 subjects will initially be enrolled, dosed, and monitored in the study. The Safety and Data Monitoring Committee (DMC) will review the emerging safety data from these 4 subjects after the post-study visit has been completed. Only after confirmation by the DMC that it is considered safe to continue with dosing of the other 32 subjects will they be enrolled and dosed.

DMC Review of Safety Data and Selected PK Samples Analyzed for Safety Reasons

An interim safety analysis will be performed on safety data and selected PK samples after all subjects have completed PK sampling in Treatment Period 1. Twelve PK samples per subject will be analyzed for flucytosine and 5-FU as part of the safety analysis and will be reviewed by the DMC before subjects may continue to Treatment Period 2. Data that will be collected/reviewed by the DMC for this interim safety analysis will be flucytosine and 5-FU plasma concentrations, time of IP administration, time of PK blood sampling, safety data (i.e., AEs, laboratory test results) and subject number. The PK parameters C_{max}, t_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ will be calculated. No formal statistical test will be performed and therefore there will be no impact on the overall risk alpha level of the study.

Entry Criteria

Subjects who meet all the inclusion criteria and none of the exclusion criteria will be considered eligible to participate in the study.

Inclusion Criteria:

- Healthy males and females, 18 to 55 years (both inclusive) at the time of signing of informed consent.
- Body mass index (BMI) between 18.5 and 30 kg/m^2 (both inclusive).
- Body weight not less than 50 kg for males and 51 kg for females.
- Medical history, vital signs, physical examination, standard 12-lead ECG (including QT interval corrected with the Fridericia formula [QTcF] of ≤ 450 msec), and laboratory investigations within laboratory reference ranges, or if outside reference ranges, the out-of-range results are considered as not clinically significant. Acceptable reference ranges will be agreed with the sponsor before study start.
- Normal blood pressure (BP): Systolic BP between 90 and 140 mmHg (inclusive), diastolic BP 45 to 90 mmHg (inclusive), measured after 10 min rest in supine position.
- Non-smokers or mild smokers (≤ 5 cigarettes or pipes per day).
- Females, if:
- *Not of childbearing potential*, e.g., has been surgically sterilized (hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy) or is postmenopausal (had no menses for 12 months without an alternative medical cause and a serum follicle stimulating hormone [FSH] concentration ≥40 IU/L).

  Note: In postmenopausal women, the value of the serum pregnancy test may be slightly increased. This test will be repeated to confirm the results. If there is no increase indicative of pregnancy, the female will be included in the study.

- *Of childbearing potential*, the following conditions are to be met:
  - Negative pregnancy test.

    If this test is positive, the subject will be excluded from the study. In the rare circumstance that a pregnancy is discovered after the subject received IP, every attempt must be made to follow her to term. The subject will also be informed of the teratogenic risk with Ancotil® and will be advised of the importance of careful prenatal and postnatal monitoring.
  - Not breastfeeding and must agree not to breastfeed while participating in the study and for up to 1 month after last administration of the IPs.
  - Abstaining from sexual activity (if this is the usual lifestyle of the subject) or are in a same-sex relationship or must agree to use a highly effective method of contraception from the time of screening or should already be using a highly effective method of contraception at screening, and agree to continue with the same method throughout the study and up to 1 month after discontinuation of IPs.

  - Examples of highly effective methods of contraception include:
    - combination (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: oral (documented that the dose has been stable for at least 1 month before the first intake of IP), intravaginal and transdermal
    - progestogen-only hormonal contraception associated with inhibition of ovulation: oral (documented that the dose has been stable for at least 1 month before the first intake of IP), injectable and implantable
    - intrauterine device
    - intrauterine hormone-releasing system
    - bilateral tubal occlusion
    - vasectomised partner
    - sexual abstinence (if this is the usual lifestyle of the subject)

  OR
    - Is in a same sex relationship and therefore may not be using any method of contraception.

  In this study the concomitant use of hormonal contraceptives is allowed.
- Male subjects (or their non-pregnant female partners of childbearing potential) must agree to use highly effective method of contraception during treatment and up to 3 months after the last administration of IPs.
- Written consent given for participation in the study.

Exclusion Criteria:

- Evidence of psychiatric disorder, antagonistic functioning, poor motivation, emotional or intellectual problems likely to limit the validity of consent to participate in the study or limit the ability to comply with protocol requirements.
- Current alcohol use > 14 units of alcohol per week for males and > 7 units of alcohol per week for females (1 unit is equal to approximately 330 mL of beer, one small glass [200 mL] of wine, or one measure [25 mL] of spirits).
- Regular exposure to substances of abuse (other than alcohol) within the past year.
- Use of any medication, prescribed or over-the-counter (including herbal remedies and St. John's wort [Hypericum perforatum]), within 2 weeks or 5 elimination half-lives of medication or remedies before the first administration of IP, whichever is longer.
- SARS-CoV-2 vaccination received within 2 weeks before the first administration of IP, or a dose of vaccination planned during the study period.
- Participation in another study with an experimental drug, where the last administration of the previous IP was within 8 weeks (or within 5 elimination half-lives for chemical entities or 2 elimination half-lives for antibodies or insulin, whichever is the longer) before administration of IP in this study.
- Participation in the DNDi-5FC-01-CM (fasted) study.

- Treatment within the previous 3 months before the first administration of IP with any drug with a well-defined potential for adversely affecting a major organ or system.
- Treatment with potent inhibitors of dihydropyrimidine dehydrogenase (DPD) in the previous 4 weeks: Antiviral antiherpetic nucleoside agents (e.g. brivudine, sorivudine and their analogues) or Uracil.
- Dihydropyrimidine dehydrogenase (DPD) deficiency defined as a plasma uracil level of > 16 ng/mL.
- Treatment with a not recommended and contraindicated medication as per Ancotil® SmPC within 2 weeks before the first administration of IP.
- A major illness during the 3 months before commencement of the screening period.
- History of hypersensitivity or allergy to the IP, its excipients or any related medication.
- History of bronchial asthma or any other bronchospastic disease.
- History of convulsions.
- History of porphyria.
- Relevant history or laboratory or clinical findings indicative of acute or chronic disease, likely to influence study outcome or patient safety, in the opinion of investigator.
- Total bilirubin above the normal range; transaminases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) deviating 15% or more above the upper limit of normal (ULN), ≥ 1.15 x ULN.
- Donation or loss of blood equal to or exceeding 500 mL during the 8 weeks before the first administration of IP.
- Resting pulse of > 100 beats per minute or < 40 beats per minute during the screening period, either supine or standing.
- Positive testing for human immunodeficiency virus (HIV), hepatitis B and/or hepatitis C.
- Positive urine screen for drugs of abuse. In case of a positive result the urine screen for drugs of abuse may be repeated once at the discretion of the investigator.
- Hemoglobin count deviating more than 10% of the lower limit of normal.
- Veins unsuitable for venous blood collection.
- Difficulty in swallowing.
- Estimated Glomerular Filtration Rate (eGFR < 90 mL/min /1.73m^2) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.
- Female subjects who plan to procreate within 1 month after IP administration, or who are not willing to practice effective methods of contraception from screening and for at least 1 month after the last dose of IP.
- Male subjects who plan to procreate within 12 weeks (3 months) after the last dose of IP or who are not willing to practice effective forms of contraception during the study and for at least 3 months, after the last dose of IP.
- Any safety concern with regards to the active ingredient (flucytosine) and as per the Ancotil® SmPC.
- Vulnerable subjects, e.g., persons in detention.
- Employees or close relatives of the contract research organization, the sponsor, 3rd party vendors or affiliates of the above-mentioned parties.

Study Duration

The study will include a screening period of 21 days. The duration of this study is expected to be approximately 16 to 30 days per subject (excluding the screening period). The actual overall study duration and study recruitment time may vary.

In house Stay

In each of the two treatment periods subjects will be admitted to the study center on Day -1 and will remain in the study center for at least 48 hours after dosing.

Pharmacokinetic Sampling Times

Pharmacokinetic blood samples for flucytosine and 5-FU analyses will be collected at the following time points: at pre-dose (0 hours) and at 30 minutes, 1 hour, 2 hours, 3 hours, 4 hours, 6 hours, 7 hours, 8 hours, 10 hours, 12 hours, 16 hours, 20 hours, 24 hours, 30 hours, 36 hours and 48 hours post-dose (total: 17 samples per treatment period, a total of 34 samples for the entire study). The collection of PK blood samples takes precedence over other assessments at a scheduled time point.

Interim Safety analysis of PK samples: Flucytosine and 5-FU concentrations will be analyzed using PK samples from all subjects at selected time points in Treatment Period 1 for safety reasons: at pre-dose (0 hours) and at 30 minutes, 1 hour, 3 hours, 4 hours, 6 hours, 8 hours, 12 hours, 16 hours, 24 hours, 36 hours and 48 hours post-dose (total: 12 samples).

Blood Volume

The total blood volume to be collected from each subject during the study is about 185 mL (repeat laboratory investigations are not included), which is less than the volume given as a single donation at the South African Blood Transfusion Service (i.e., 480 mL).

Study Population

The study population will consist of healthy male and female subjects, 18 to 55 years old with a body mass not less than 50 kg for males and 51 kg for females and a body mass index (BMI) of 18.5 to 30 kg/m^2 (both inclusive).

Subjects who meet all the inclusion criteria and none of the exclusion criteria will be considered eligible to participate in the study.

Investigational Products

Reference Product (Treatment A)

Generic name:	Flucytosine
Trade name:	Ancotil®,[4]
Dosage form and strength:	500 mg IR tablets
Study dose:	3000 mg (b.i.d. dose: 3 x 500 mg [0 hours] and 3 x 500 mg [6 hours]) under fed conditions On profile days subjects will receive a high-fat, high-calorie meal prior to each dosing.
Route of administration:	Oral
Manufacturer:	ICN Polfa Rzeszów S.A ul. Przemysłowa 2 35-959 Rzeszów Poland
Country of origin:	Poland
Marketing Authorization Holder	Mylan Medical SAS (France)

Test Product (Treatment B)*

Generic name:	Flucytosine
Dosage form and strength:	Bottle of 3000 mg SR pellets
Study dose:	6000 mg (single dose: 2 x 3000 mg [0 hours]) under fed conditions On profile days subjects will receive a high-fat, high-calorie meal prior to dosing.

Route of administration:	Oral
Manufacturer:	Viatris (previously known as Mylan)
Country of origin:	India

Analytes

Flucytosine and metabolite, 5-fluorouracil (5-FU)

Sample Size

Based on available information on IR and SR flucytosine, it is estimated that up to 36 eligible subjects will be entered into the study to complete the study with at least 30 evaluable subjects.

Sample size considerations are driven by the primary pharmacokinetic evaluation and the planned comparison between the test and reference treatments.

Assuming the intra-subject coefficient of variation (CV) being no more than 28%, with 30 subjects in total (i.e., 15 subjects per sequence), a crossover design will have 80% power to reject both the null hypothesis that the ratio of the test mean to the standard mean is below 0.8 and the null hypothesis that the ratio of the test mean to the standard mean is above 1.25; i.e., that the test and standard are not equivalent, in favor of the alternative hypothesis that the means of the two treatments are equivalent, assuming that the expected ratio of means is 1, the crossover ANOVA $\sqrt{MSE}$ (in scale) is 0.28 (the SD differences, σ(ln scale) is 0.396), that data will be analyzed in the natural log scale using t-tests for differences in means and that each t-test is made at the 5% level. CV will be calculated with the ANOVA final analysis. If the test is significant, the power of the analysis will be re-calculated based the final outputted CV% assuming the same initial design characteristics (type 1 error, null hypothesis, alternative hypothesis).

Options for sample size are presented in the table below for different scenarios of within-subject CV%.

Options for sample Size Based on Different Scenarios of Within-subject Coefficients of Variation

Within-subject coefficient of variation (CV%)	20%	22%	25%	28%	30%
Required sample size	16	20	24	30	34
Sample size for enrolment, to account for possible drop-outs/ withdrawals	20	24	28	36	40

Sample sizes for enrolment are increased by approximately 15% to account for possible drop-outs/withdrawals. Subjects who withdraw or are withdrawn from the study will not be replaced, unless fewer complete the study than the estimated number of evaluable subjects (i.e., 30).

Statistical Analysis

Randomization:

A randomization schedule will be provided by Biostatistics. The randomization schedule will be generated utilizing the PROC PLAN procedure of SAS® software or appropriate equivalent.

Pharmacokinetic population and analysis:

All subjects who complete the PK sampling in both periods, and for whom primary PK parameters can be calculated for both treatment periods and who have no major protocol deviations thought to impact the analysis of the PK data will be included in the statistical PK analysis of the study.

Relative bioavailability of the test and reference products will be assessed on the basis of the 90% confidence intervals (CIs) for estimates of the geometric mean ratios between the primary PK parameters of the test and reference products for flucytosine.

Results for 5-FU will serve as supportive data only.

Safety population and analysis:

All subjects who received at least one dose of IP will be included in the safety analysis of the study.

Safety data will be listed as described in the statistical analysis plan.

Demographic characteristics and AEs will be summarized by treatment group.

Interim safety analysis:

An interim safety analysis will be performed on safety data and on the PK samples at selected time points after all subjects have completed PK sampling in Treatment Period 1. Twelve PK samples per subject will be analyzed for flucytosine and 5-FU as part of the safety evaluation and will be reviewed by the DMC before subjects may continue to Treatment Period 2. Data that will be collected/reviewed by the DMC for this safety analysis will be flucytosine and 5-FU plasma concentrations, time of IP administration, time of PK blood sampling, safety data (i.e., AEs, laboratory test results) and subject number. The PK parameters C_{max}, t_{max}, $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ will be calculated. No formal statistical test will be performed and therefore there will be no impact on the overall risk alpha level of the study.

Exploratory analysis:

Acceptability and palatability analyses will be performed in subjects in the safety population who have received the test product. Results (scores) obtained from the Investigator and participant questionnaires, respectively, will be presented as indicated in the SAP.