SYNOPSIS

Title of the Study

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

Study Objectives

Primary Objective

To assess and compare the relative bioavailability of each of the three test products (Test 1, Test 2 and Test 3), flucytosine sustained-release (SR 5-FC) pellets (single dose: 1 x 3000 mg at 0 hours) and the reference product, immediate-release flucytosine (IR 5-FC) Ancotil® 500 mg tablets (3 x 500 mg at 0 hours and 3 x 500 mg at 6 hours after first dosing) under fasting conditions.

Secondary Objective

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

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 ($t_{½}$)
- Evaluation of unexpected release characteristic (i.e., dose-dumping) in selected PK samples (8) of all subjects at the end of Treatment Period 1. No additional blood samples will be collected for these analyses.

Safety Endpoints

- Frequency and cumulative incidence of adverse events (AEs) and serious adverse events (SAEs) for 5-FC test and reference products, assessed through clinical, electrocardiogram (ECG) and laboratory safety assessments from baseline to post-study visit. Medical history and prior and concomitant medication will also be recorded.
- Frequency and incidence of AEs in all subjects will be analyzed at the end of Treatment Period 1 to evaluate potential for significant dose dumping.

Study Design

This will be an open-label, bioanalytical laboratory-blind, randomized, four-period crossover study with orally administered flucytosine IR tablets and SR pellets conducted under fasting conditions in at least 32 healthy males and females at a single study center.

Subjects will receive the following treatments:

- **Reference (Treatment A):** Ancotil® 500 mg IR tablets (twice daily [b.i.d] dose: 3 x 500 mg [0 hours] and 3 x 500 mg [6 hours])
- **Test 1 (Treatment B):** Flucytosine 3000 mg SR pellets (single dose: 1 x 3000 mg [0 hours])
- **Test 2 (Treatment C):** Flucytosine 3000 mg SR pellets (single dose: 1 x 3000 mg [0 hours])
- **Test 3 (Treatment D):** Flucytosine 3000 mg SR pellets (single dose: 1 x 3000 mg [0 hours])

The study will comprise:

- a screening period of maximum 21 days,
- four 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 immediate-release formulation) to 14 calendar days (maximum number of days based on logistical arrangements) between consecutive administrations of the investigational product (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, 2, 3 and 4, and 5 days after administration of IP in Treatment Periods 1, 2 and 3, and

- a post-study visit, 1 week 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 follow-up phone calls 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 IPs.

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 or bilateral oophorectomy) or is post-menopausal (had no menses for at least 12 months without an alternative medical cause and a serum follicle stimulating hormone [FSH] concentration ≥ 40 IU/L).

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.

- 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 one month after discontinuation of treatment.

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
- vasectomized partner
- sexual abstinence (if this is the usual lifestyle of the subject) except if they are in a same sex relationship

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 a 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 or herbal remedies, 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 second 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.
- Treatment within the previous 3 months before the first administration of IP with any drug with a welldefined 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 or 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 between 30 and 51 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 four 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 (PK) blood samples will be collected at the following time points: at predose (0 hours) and at 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 3 hours, 4 hours, 6 hours, 6 hours 30 minutes, 7 hours, 7 hours 30 minutes, 8 hours, 9 hours, 10 hours, 12 hours, 16 hours, 20 hours, 24 hours, 30 hours, 36 hours and 48 hours post dose (total: 21 samples per treatment period, a total of 84 samples for the entire study).

Note: For Treatment Period 1, selected PK samples of all subjects (i.e., pre-dose [0 hours], and 1 hour, 1 hour 30 minutes, 2 hours, 3 hours, 4 hours, 6 hours and 8 hours post-dose) will be analyzed for safety purposes to ensure that there is no significant dose dumping. No additional blood samples will be collected for these analyses.

Blood Volume

The total blood volume to be collected from each subject during the study is about 424.5 mL (repeat laboratory investigations are not included), which is about 10% 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®
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])
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 (Treatments B, C and D)

Generic name:	Flucytosine
Dosage form and strength:	3000 mg SR pellets
Study dose:	3000 mg (single dose: 1 x 3000 mg [0 hours])
Route of administration:	Oral
Manufacturer:	Viatris (previously known as Mylan)
Country of origin:	India

Analytes

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

Sample Size

Thirty-two subjects are required as completers in this clinical study. Sample size considerations are driven by the primary pharmacokinetic evaluation and planned pairwise comparison among treatments B, C and D (test products) versus treatment A (reference).

Assuming the intra-subject coefficient of variation (CV) being no more than 17%, with 32 subjects in total (i.e. 8 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 test mean to the standard

mean is above 1.25; i.e., that the test and standard are not equivalent, in favour 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, MSE (in scale) is 0.17 (the SD differences, σ (ln scale) is 0.24), 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 1.7% level. CV will be calculated with the ANOVA final analysis. If the test is significant, the power of the analysis will be calculated based on the final outputted CV%.
A total of 36 eligible subjects will be entered into the study to complete the study with at least 32 evaluable subjects. 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., 32).

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:

All subjects who complete the study and for whom primary PK parameters can be calculated for all 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. PK data of 5-FU (metabolite) will serve as supportive data only.

Interim analysis:

An interim analysis will be performed after Treatment Period 1. Eight flucytosine PK samples per subject will be analyzed for safety reasons to assess any potential dose dumping before commencement of Treatment period 2. Data collected for this interim analysis will be 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}, $AUC_{(0t)}$ and $AUC_{(0\infty)}$ will be calculated to study dose dumping. No formal statistical test will be performed and therefore there will be no impact on the overall risk alpha level of the study.

Safety population:

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.