A single centre, single dose, open-label, randomised, two period crossover study to assess the bioequivalence of an oral azathioprine suspension 10mg/mL (Jayempi™) versus oral azathioprine tablet 50mg (Imurek®, Aspen Pharma Trading Limited, Dublin, Ireland) in at least 30 healthy adult subjects under fasting conditions.

Nova Laboratories Study No: INV 691
Medicines Evaluation Unit Study No: MEU 18/372
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Statistical Analysis Plan

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For Syne qua non Ltd – Lead Statistician

[redacted]

For Medicines Evaluation Unit

If signing manually, please include: Signature + Date + Full Name + Position

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Contents

LIST OF ABBREVIATIONS

AE	Adverse Event
$AUC_{0-\tau}$	Area Under the Plasma Concentration time curve to the Last quantified concentration
$AUC_{0-\infty}$	Area Under the Plasma Concentration time curve from time Zero to Infinity
BLQ	Below the Lower Limit of Quantification
BMI	Body Mass Index
BP	Blood Pressure
C_{max}	Maximum Observed Plasma Concentration
CI	Confidence Interval
ECG	Electrocardiogram
λ_z	Terminal Elimination Rate Constant
LLOQ	Lower Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
PK	Pharmacokinetic
PT	Preferred Term
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
$t_{½}$	Terminal Elimination Half-Life
TEAE	Treatment Emergent Adverse Event
t_{max}	Time of the Maximum Observed Plasma Concentration
WHO Drug	World Health Organization Drug Dictionary

1 INTRODUCTION

This document details the statistical analysis that will be performed for the Nova Laboratories study: A single centre, single dose, open-label, randomised, two period crossover study to assess the bioequivalence of an oral azathioprine suspension 10mg/mL (Jayempi™) versus oral azathioprine tablet 50mg (Imurek®, Aspen Pharma Trading Limited, Dublin, Ireland) in at least 30 healthy adult subjects under fasting conditions.

The proposed analysis is based on the contents of the Final Version of the protocol (dated 03-May-2019). In the event of future amendments to the protocol, this statistical analysis plan (SAP) may be modified to account for changes relevant to the statistical analysis.

The table, listing and figure shells are supplied in a separate document.

2 STUDY OBJECTIVES AND DESIGN

2.1 Study Objectives

The primary objective of the study is to assess whether Nova's oral azathioprine suspension (Jayempi™) and a marketed azathioprine tablet formulation (Imurek®) are bioequivalent.

The secondary objectives of the study are

- To assess the safety and tolerability of the test product, azathioprine oral 10mg/mL suspension
- To determine the plasma concentrations and pharmacokinetics of the metabolite mercaptopurine

2.2 Study Endpoints

The primary endpoints of the study are

- Maximum observed plasma concentration (C_{max})
- Area under the plasma concentration curve from zero to time of the last quantifiable concentration (AUC_{0-t})
- Area under the plasma concentration curve from zero extrapolated to infinity ($AUC_{0-\infty}$)

The secondary endpoint(s) are

- Time to maximum observed plasma concentration (t_{max})
- Terminal elimination rate constant (λ_z)
- Apparent terminal elimination half-life ($t_{½}$)

2.3 Study Design

This will be a single-dose, open-label, randomised, two-period crossover study with orally administered 1 x 5mL (50mg) of Jayempi™ Oral Suspension 10mg/mL versus oral azathioprine tablet 50mg (Imurek®, Aspen Pharma Trading Limited, Dublin,

Ireland) on two separate occasions conducted under fasting conditions in healthy male and female subjects at a single study centre.

The study will comprise:

- Thiopurine methyltransferase (TPMT) testing
- Screening period of maximum 28 days
- Two treatment periods (each of which will include a PK profile period of 12 hours) separated by a wash-out period of at least 3 calendar days (minimum number of days based on half-life of the analyte) and maximum of 14 calendar days between consecutive administrations of the IMP
- A post-study visit 7-10 days after the last dose of the last treatment period of the study

Subjects will be randomly assigned to treatment sequence, prior to the first administration of IMP.

2.4 Visit Structure

The visit structure and scheduled assessments are detailed in protocol section 7.1.3.

3 SAMPLE SIZE

Thirty (30) subjects will be randomised, to allow for dropouts or subjects who otherwise fail to complete the study with at least 28 evaluable subjects. The sample size for this study has been selected without any formal statistical considerations. However, similar bioequivalence studies

- AT/H/0270/001-002/DC
- UK/H/2846/004/DC
- UK/H/934/01/DC

for azathioprine have recruited between 24-30 subjects. This figure has been determined adequate to meet the study objectives.

4 RANDOMISATION

The randomisation will be produced by SQN. The treatment will be allocated by staff at the site using the randomisation list provided.

Subjects who withdraw or are withdrawn from the study may be replaced (recruitment continued), if fewer complete the study than the estimated number of evaluable subjects. Replacements will be randomised to treatment (they will not necessarily be on the same treatment sequence).

5 INTERIM ANALYSIS

No interim analysis is planned for this study.

6 ANALYSIS PLAN

6.1 General

Summary statistics for continuous variables will consist of number of non-missing observations (n), arithmetic mean, standard deviation (SD), minimum, median and maximum, unless specified otherwise. The precision of these variables is defined in the table, figure and listing shells document.

For categorical variables the number and percentage of subjects in each category will be presented, based on the number of subjects in the analysis set, unless otherwise specified.

6.2 General Derivations

This section provides details of general derivations. Derivations specific to the parameter of interest are detailed within the specific SAP section.

- Definition of baseline

A within-period baseline is defined as the last non-missing value (including scheduled and unscheduled assessments) recorded prior to the subject receiving the respective study treatments for the respective period.

- Incomplete dates

For calculation purposes, incomplete dates will be completed using worst case. Further details are detailed in the relevant sections as required.

- Non-numeric values

In the case where a variable is recorded as “> x”, “≥ x”, “< x” or “≤ x”, then for analysis purposes a value of x will be taken. Where a range of values is quoted the midpoint of the range will be taken.

6.3 Analysis Sets

The **Enrolled set** includes all subjects who provide informed consent irrespective of whether they were randomised or received study treatment.

The **Safety analysis set** (SAF) consists of all subjects who take at least one administration of study treatment. Subjects will be analysed according to the treatment actually taken.

The **Pharmacokinetic (PK) set** will consist of all subjects who complete the study and for whom primary PK variables can be calculated for all treatments.

In case of events such as vomiting, diarrhoea and use of concomitant medication, which could render the plasma concentration time profile unreliable, such subjects may be excluded from the PK population after review of the severity of the event and the start and stop time in relation to dosing. When vomiting occurs within 2x the calculated median t_{max} of the reference (tablet) product, data from all treatments being compared will be excluded from PK and statistical evaluation. Subjects with pre-dose azathioprine concentrations ≥ 5% of C_{max} will be excluded from all statistical PK analysis (including descriptive statistics). The available concentration data of the

above subjects and those who do not complete the study will only be listed, not presented in descriptive statistics or included in PK or formal statistical analysis.

The list of subjects included in the PK will be agreed prior to database lock once all study data is available. The definitions for the enrolled set and SAF are sufficient to determine the subjects included within these analysis sets and so do not require listing and agreeing prior to database lock.

6.4 Data presentations

The data will be summarised in tabular form by treatment group ('Oral azathioprine tablet 50mg' and 'Oral azathioprine suspension 10mg/mL' with the following exceptions: disposition of subjects, background and demographic characteristics which will be summarised by treatment sequence ('Oral azathioprine tablet 50mg – Oral azathioprine suspension 10mg/mL' and 'Oral azathioprine suspension 10mg/mL – Oral azathioprine tablet 50mg') and overall subjects ('Overall') treatment group.

Only scheduled post-baseline laboratory, vital signs and ECG values will be tabulated, post-baseline repeat/unscheduled assessments will be disregarded, although they will be listed and in particular all clinically significant values will be noted.

Analysis sets, protocol deviations and eligibility listing will be summarised using the enrolled set, PK outputs will be summarised using the PK set and everything else will be summarised using the SAF.

Listings will be sorted by treatment sequence, subject number and date/time of assessment.

Graphical presentations of the data will also be provided where appropriate.

6.5 Disposition of subjects

The number and percentage of all subjects enrolled, included in the SAF, PK analysis set, who completed the study and prematurely discontinued the study, who completed study treatment and prematurely discontinued study treatment, study duration and treatment duration, will be summarised. The number and percentage of subjects will be summarised by their reasons for withdrawal from the study.

Study duration will be derived as the number of days between first visit and the date of study completion or the date of early study withdrawal. Treatment duration will be derived as the number of days between first administration of study treatment in period 1 and date of study completion or the date of early study withdrawal.

Eligibility for each of the analysis sets along with reasons for exclusion will be listed. Study completion/withdrawal data will be listed.

6.6 Protocol Deviations

Details of all protocol deviations (start and end dates, report type (Full report/Procedural), deviation category, specific details and cause) and subject eligibility will be listed.

6.7 Background and Demographic Characteristics

6.7.1 Demography

Demographic characteristics (age, sex, ethnicity and race), body measurements (height, weight and BMI), average weekly consumption of alcohol and smoking history collected at Screening will be summarised.

Body mass index (BMI) in kg/m^2 is calculated as

$$\frac{\text{weight in kg}}{(\text{height in cm} / 100)^2}$$

All subject demographic data including informed consent will be listed.

6.7.2 Medical History

Medical history events will be coded using the latest MedDRA dictionary version. The version used will be indicated in the data summaries and listings. The number and percentage of subjects will be presented for ongoing conditions and previous conditions separately by system organ class (SOC), and preferred term (PT), where SOC and PT will be presented in decreasing frequency of the total number of subjects with medical history events. All events will be listed.

6.7.3 Thiopurine Methlytransferase Testing

The date, time and result in mU/L taken at screening will be listed.

6.8 Prior and Concomitant Medications

Medications will be coded using the latest World Health Organization Drug dictionary (WHO Drug) version. The version used will be indicated in the data summaries and listings.

Prior medications are defined as those that started and ended prior to the first administration of study treatment.

Medications that are ongoing at the first administration of study treatment or started after time of first administration will be deemed to be concomitant medications. Concomitant medications will be reported under each period they were concomitant to. For example; a medication starting and ending in period 1 will be assigned to period 1 only. A treatment starting after the first administration of study treatment in period 2 will be assigned to period 2 only. A treatment starting before the end of period 1 and continuing into period 2 will be reported under period 1 and period 2.

For the purposes of concomitant medication, period 1 is defined from day of first administration of study treatment for period 1 to the day before the first administration of study treatment for period 2. If medication dates are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant.

The number and percentage of subjects taking prior and concomitant medications will be summarised separately by medication class and standardised medication name, where medication class and standardised medication name will be presented in decreasing frequency of the total number of subjects with medications. In summary tables, subjects taking multiple medications in the same medication class or having the same standardised medication recorded multiple times in the study will be counted only once for that specific medication class and standardised medication name.

Medication data will be listed, where concomitant medications will be flagged.

6.9 Study Treatments

A listing will be provided detailing if the subject received any treatment for the period, date and time of administration, the treatment and dose received.

6.10 Pharmacokinetics

Summary statistics for PK outputs will be reported using n, arithmetic mean, geometric mean, median, coefficient of variation, standard deviation, minimum and maximum.

For purposes of analysis, the test product is Jayempi 50mg/mL (azathioprine oral suspension) and the reference is Imurek 50mg (oral azathioprine tablet).

Plasma concentrations of azathioprine (both oral and tablet) will be listed and summarised over time. Concentrations below the lower limit of quantification (LLOQ) of 0.2ng/ml will be indicated as below the lower limit of quantification (BLQ). For PK summaries and analyses, BLQ concentrations will be handled as follows:

- All BLQ values in the absorption phase, prior to the first reported concentration, will be substituted by zeros.
- The BLQ values between evaluable concentrations will be substituted by 0.5 × LLOQ, before the calculation of the PK variables.
- Terminal BLQ values will be set to missing.

These measures are taken to prevent an over-estimation of AUC. Values reported as 'NS' (no sample) will be set to "missing".

For the purposes of PK analysis, missing concentrations will be deleted, resulting in an interpolation between the nearest two concentration values. A table reflecting summary statistics per product will be provided for PK variables of azathioprine, including the number of points used to calculate λ_z (λ_z will, however, not be summarised).

A table reflecting summary statistics will be provided for the ratios (%test/reference) of the primary PK variables (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$), for $t_{½}$ and for the difference (test – reference) of t_{max}.

Analysis of t_{max} will be done using Wilcoxon's Signed Rank test. The median t_{max} of the test and reference will be tabulated together with the p-value from Wilcoxon's Test.

The test product (azathioprine only) will be compared to the reference product by means of statistical analysis with respect to the primary PK variables (C_{max}, AUC_{0-t} and $AUC_{0-\infty}$) using an analysis of variance (ANOVA) with sequence, subject(sequence), product and period effects after logarithmic transformation of the data. Point estimates and 90% confidence intervals for the "test/reference" geometric mean ratios of these variables will be tabulated.

Bioequivalence of the test and reference products will be assessed on the basis of the 90% confidence intervals for the geometric mean ratio of the primary PK variables of azathioprine in relation to the conventional bioequivalence range of 80.00% to 125.00%.

In case of outliers present in the data regarding the primary PK parameters, the analyses will be performed on both the complete data and on the data excluding the outliers. Bioequivalence will however be assessed on the complete data.

Plots will be produced for plasma concentration versus actual time as follows:

- Individual subjects plotted together on the same axis per product using a log-linear and linear-linear scales.
- Distinct individual subject plots per product using a log-linear and linear-linear scales.
- Arithmetic and geometric mean plots with both products on same axis using log-linear and linear-linear scales.

All PK data will be listed.

6.11 SAFETY EVALUATION

6.11.1 Tolerability

Tolerability will be evaluated by monitoring adverse event, laboratory, vital signs and ECGs performed at protocol-specified visits. Details regarding summarisation of these data are given in the relevant sections below.

6.11.2 Adverse Events

Adverse events (AEs) will be coded using the latest MedDRA dictionary version. The version used will be indicated in the data summaries and listings.

A treatment-emergent adverse event (TEAE) is defined as an AE that started on or after the start of the administration study treatment. If adverse event dates and times are incomplete and it is not clear whether the adverse event was treatment-emergent, it will be assumed to be treatment-emergent.

Adverse events are treatment-emergent and accountable to period 1 treatment if they started on or after the start of the administration study treatment in period 1 up to the first administration of study treatment in period 2. However, for period 2, adverse events are treatment-emergent and accountable to period 2 treatment if they started on or after the start of the administration study treatment period 2 up to the end of the follow-up period. If the start date and time, and other data mean that the event could have occurred in more than one period, then the event will be recorded under both periods.

A treatment-related TEAE is defined as a TEAE that is certain, probable/likely or possibly related to the study treatment. If the TEAE has a missing relationship it is assumed to be related to the study treatment for analysis purposes.

A summary table will present the following:

- TEAEs (events and subjects).
- Serious TEAEs (events and subjects).
- Serious study treatment-related TEAEs (events and subjects).
- TEAEs by severity (mild/moderate/severe) (events and subjects).

- TEAEs by relationship to study treatment and the pooled study treatment related category (events and subjects).
- TEAEs leading to withdrawal from study (subjects only).
- TEAEs leading to discontinuation of study treatment (subjects only).
- Study treatment-related TEAEs leading to discontinuation of study treatment (subjects only).
- TEAEs leading to death (subjects only).

In the above summaries, if a subject experienced more than one TEAE, the subject will be counted once using the most related event for the "by relationship to study treatment" and "related to study treatment" summaries and at the worst severity for the "by severity" summary.

The following tables will be presented:

- TEAEs by system Organ Class (SOC) and Preferred Term (PT).
- TEAEs by SOC, PT and relationship to study treatment and the pooled related categories (related/unrelated).
- TEAEs by SOC, PT and severity (mild/moderate/severe)

For all of the above, SOC and PT will be presented in decreasing frequency of the total number of subjects with TEAEs.

Further details of the above tables are given below:

1. If a subject experienced more than one TEAE assigned to the same treatment, the subject will be counted once for each SOC and once for each PT within that treatment.
2. If a subject experienced more than one TEAE assigned to the same treatment, the subject will be counted once for each PT within that treatment.
3. If a subject experienced more than one TEAE assigned to the same treatment, the subject will be counted once for each SOC and once for each PT using the most related event within that treatment.
4. If a subject experienced more than one TEAE assigned to the same treatment, the subject will be counted once for each SOC and once for each PT at the worst severity within that treatment.

Adverse event data will be listed in full and this will also include a treatment emergent flag, the treatment the adverse event is assigned to, the time of onset and cessation of event relative to first dosing of study treatment and duration of AE. A flag will also be used for AEs related to vomiting that occurred within 2 × the calculated median t_{max}.

6.12 Clinical Laboratory Evaluation

Listings of haematology, biochemistry and urinalysis laboratory measurements recorded throughout the study will be provided.

Each haematology, coagulation biochemistry and urinalysis parameter will be classed as low, normal, high, missing based on the reference ranges and will be reported in separate listings.

Observed values in biochemistry and haematology will be summarised by visit. If the test results are reported in categorical format, the results will be summarised by subject counts and percentage for each category.

6.13 Vital Signs

All vital sign data will be listed.

6.14 Electrocardiography

All ECG data will be listed.

6.15 Physical Examination

Details of timings of physical examinations will be listed.

6.16 Pregnancy Test

Pregnancy test details will be listed.

6.17 Urine Screen and Alcohol Breath Test

Results of urine drugs of abuse, alcohol breath test and urine cotinine test will be listed.

6.18 Palatability Questionnaire

Visual analogue scale questions (Questions 1, 3, 4 and 5) captured as lengths in mm will be summarised using summary statistics. The categorical question (Question 2) will be summarised using number and percent.

All data related to palatability questionnaire will be listed.

6.19 Changes from the Protocol Planned Analysis

Not applicable.