Protocol No: AMZ001-008

STATISTICAL ANALYSIS PLAN

A Phase 1, Single-Center, Randomized, Open-Label, Bioavailability and Safety Three-Period Crossover Multiple-Dose Study Comparing Two Dose Levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001 (Diclofenac Sodium Gel 3.06%) and One Dose Level (32 g) of Voltaren Arthritis Pain Gel (Diclofenac Sodium Gel 1%) in Healthy Subjects

TKL Study Number P1980822

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Table of Contents

Table of Contents **2**

List of Abbreviations and Definitions of Terms **3**

1. INTRODUCTION **4**

2. STUDY OBJECTIVES **4**

3. STUDY DESIGN **4**

4. HARDWARE AND SOFTWARE **5**

5. STATISTICAL DATA REVIEW **6**

6. DATABASE CLOSURE **6**

7. SAMPLE SIZE DETERMINATION **6**

8. HANDLING OF MISSING DATA **7**

9. ANALYSIS POPULATIONS **7**

10. STATISTICAL EVALUATION **7**

10.1 General Comments 7
10.2 Subject Disposition 8
10.3 Demographic and Other Covariates 8
10.4 Dosing Compliance and Extent of Exposure 8
10.5 Pharmacokinetics Evaluations 9
10.6 Safety Evaluations 10

11. CHANGES FROM THE PROTOCOL AND PLANNED ANALYSES **12**

12. HEADINGS **12**

13. ARCHIVING AND RETENTION OF DOCUMENTS **12**

14 OUTLINE OF PROPOSED TABLES, FIGURES AND LISTINGS **13**

List of Abbreviations and Definitions of Terms

Abbreviation	Definition
ADaM	Analysis Data Model
AE	Adverse event
ANOVA	Analysis of variance
BLQ	Below the limit of quantification
CRF	Case report form
DMP	Data Management Plan
ECG	Electrocardiogram
EOS	End of study
EOT	End of treatment
ET	Early termination
ODS	Output Delivery System
PD	Pharmacodynamics
PK	Pharmacokinetics
PT	Preferred term
RTF	Rich-text format
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SDTM	Study Data Tabulation Model
SOC	System organ class
SOP	Standard operating procedure
TEAE	Treatment-emergent adverse event

1. INTRODUCTION

Study AMZ001-008 will be a Phase 1 pharmacokinetic (PK) and safety clinical trial comparing 2 dose levels of AMZ001, an experimental formulation of diclofenac sodium gel, with the marketed over-the-counter (OTC) formulation Voltaren Arthritis Pain 1% Gel (GSK Consumer Healthcare). The two doses of the new formulation were selected based on the outcomes of a previous PK and safety study (AMZ001-005) to provide systemic diclofenac exposure when applied once daily comparable to 4 daily applications of Voltaren Arthritis Pain gel. Voltaren Arthritis Pain gel is indicated for 4 daily applications for the topical treatment of arthritis pain. Study AMZ001-008 will characterize the rate and extent of diclofenac absorption from the two different doses of AMZ001 compared to Voltaren Arthritis Pain gel. This Statistical Analysis Plan (SAP) is based on the study protocol dated 23 March 2023.

2. STUDY OBJECTIVES

The primary objective of the study is to compare the bioavailability of 2 dose levels (6 mL [5.5 g] and 8 mL [7.4 g]) of AMZ001 (diclofenac sodium gel 3.06%) applied once daily as 3 mL and 4 mL per knee on both knees and one dose level of Voltaren Arthritis Pain Gel (32 g, diclofenac sodium gel 1%) applied 4 times daily as 4 g per knee on both knees after repeated topical administrations in healthy subjects for 7 days.

Secondary objectives are to determine the PK profiles of the 3 study products and to assess their safety and tolerability under the conditions of their intended use.

3. STUDY DESIGN

The study will follow a randomized open-label 3-way crossover design in which every subject will receive all 3 study products in one of the 6 possible treatment sequences. During each of the 3 study periods, subjects will receive daily treatment for 7 successive days. Subjects will be confined to the study site under supervision from the evening before the first treatment application of the period until 24 hours after the last application. The treatment periods will be separated by 2 washout periods of 21 days each.

Treatments and treatment sequences are defined as follows.

Sequence Number	Period 1	Period 2	Period 3
1	A	B	C
2	B	C	A
3	C	A	B
4	B	A	C
5	A	C	B
6	C	B	A

Treatment A: 6 mL (5.5 g) daily, 2 doses of 1.5 mL on each knee QD
Treatment B: 8 mL (7.4 g) daily, 4 doses of 1 mL on each knee QD
Treatment C: Voltaren Arthritis Pain Gel, 32 g daily, 4 g on each knee 4 times daily

At each application, subjects will spread the gel directly to pre-defined treatment areas of approximately 400 cm^2 (Treatments A and C) and 500 cm^2 (Treatment B) on each knee following prescribed application instructions.

On Day 1 and Day 7, blood samples for PK analyses will be collected within 1 hour prior to application and at 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, and 24 hours after application. Trough level samples will be collected before application on Days 3, 4, 5, and 6. At the end of Period 3, additional PK blood samples will be taken at 3, 4, and 5 weeks after the last application in order to characterize and to compare the elimination kinetics of the 3 study products.

Subjects will be healthy males or females 18 to 65 years old, determined by the Investigator to be in good general health based on medical history and physical examination, including vital signs, 12-lead ECG, and clinical laboratory tests. Approximately 75 subjects will be randomized to ensure at least 60 evaluable subjects.

4. HARDWARE AND SOFTWARE

Statistical analysis will be performed following TKL standard operating procedures (SOPs) and on the TKL computer network. All statistical analyses will be performed using SAS Version 9.4 or higher with program code prepared specifically for the project by qualified TKL statisticians and SAS programmers.

The SAS programs will generate rich-text-formatted (RTF) output with the “RTF” extension using the SAS Output Delivery System (ODS). The summary tables and listings will be

formatted using the Times New Roman font. The RTF output is included in report documents prepared with Microsoft Word and converted to PDF format without typographical change.

Study data tabulation model (SDTM) data sets and required analysis data model (ADaM) data sets will be created and taken as input to validated SAS programs to generate the report-ready tables, listings, and figures. Each output display will show the names of the data sets and SAS program used to produce it. Upon completion of the study report, the data sets will be provided to the sponsor as SAS XPT transport files with define.xml files including details of all derivations and imputations used.

5. STATISTICAL DATA REVIEW

Data verification activities to be performed prior to delivery of the SAS data sets to the project statistician are described in the approved Data Management Plan (DMP). After completion of the data verification activities, the SAS data sets will be reviewed by the project statistician along with documentation of any unresolved queries and data conventions applied that are not fully explained in the data or in the DMP. The project statistician will perform completeness and self-consistency checks of the study data. These checks will be selected to ensure the self-consistency of dates and events in the subject record, the consistency of all reported data values with expected ranges, and, if not within range, the presence in the subject record of explanatory comments or other corroborative data. Overall, each subject's record in the study database must convey a full but concise description of the subject's experience with respect to protocol-specific events. Questions will be issued to the Data Manager and resolved before closure of the database.

6. DATABASE CLOSURE

After completion of all data review procedures, validation of the project database (ie, all those activities and operations designed and performed to ensure that the database is a complete and accurate representation of protocol-specific events), and approval of the data review document by the study sponsor, the clinical database will be closed (ie, "locked"). Any change to the clinical database after this time will require written authorization, with explanation, by the Sponsor and the Biostatistician, in conformance with TKL SOPs.

7. SAMPLE SIZE DETERMINATION

The sample size of 60 subjects was determined to provide 80% power in a standard two 1-sided test of bioequivalence assuming an intrasubject coefficient of variation (CV) of 61% and a true

ratio of geometric means 1.05. These assumptions were derived by averaging historical data and the results of Study 000084. For the purposes of the sample size estimate, bioequivalence was defined as the inclusion of a 2-sided 95% confidence interval (1-sided 97.5%) on the ratio of geometric means (Test/Reference) within the equivalence margin of 80% to 125%. The wider confidence interval was used to account for the multiplicity of tests planned in the study. The plan to enroll 75 subjects assumes that 20% of enrolled subjects will be incomplete or not evaluable.

8. HANDLING OF MISSING DATA

In this study, there will be no imputations of missing data points. All data will be reported and analyzed as observed values only.

9. ANALYSIS POPULATIONS

The Safety population will include randomized subjects who received at least one dose of AMZ001 or Voltaren Arthritis Pain Gel. The Safety population will be used for safety analysis, presentation of compliance and all baseline characteristics (demographics, medical history, prior and concomitant medication, and physical examination).

The PK population is defined as all treated subjects who have at least one post-dose measured plasma drug concentration, and who have no major protocol deviations that might affect PK. Two PK analysis sets will be considered for this clinical trial. PKAS1 includes all subjects in the PK population for which at least $AUC_{0-\tau}$ or C_{max} can be determined for the reference treatment (Treatment C) along with at least one of Treatments A or B. This analysis set will be used in all PK summaries and PK analysis. PKAS2 includes the subset of subjects in PKAS1 who show a pre-dose diclofenac concentration no greater than 5% of the subsequent maximum concentration. This second analysis set will be used for sensitivity analysis.

10. STATISTICAL EVALUATION

10.1 General Comments

Summary tables (descriptive statistics and/or frequency tables) will be provided for baseline variables, PK variables, and safety variables. In general, continuous variables will be described by descriptive statistics (mean, median, standard deviation, minimum, and maximum). Frequency counts and percentage of subjects within each category will be provided for categorical data.

10.2 Subject Disposition

Subject disposition will be summarized by showing the number of subjects screened, and the number and percent of screen failures and enrolled subjects; the number and percent of subjects completed and discontinued, overall and by treatment, along with the primary reason for discontinuation; and the number and percentage of subjects in each analysis population. By-subject listings will include the date of informed consent, date of screening, date of randomization, date of last visit, date of last contact (if applicable), number of days on study, and reason for discontinuation. Individual subject data for analysis population inclusion and protocol deviations will also be presented in a by-subject listing.

10.3 Demographic and Other Covariates

Subject age will be summarized as mean, standard deviation, median, minimum, and maximum. Sex, race, and ethnicity will be summarized as number and percent of subjects in each category. Subject listings will include age, sex, race, ethnicity, weight, height, and body mass index.

Data listings will also be provided to show prior and concomitant medications, medical history, childbearing potential and pregnancy test results, and general investigator comments. Medication terms will be coded and classified according to the WHO Drug Dictionary, Version B3 (September 2022).

10.4 Dosing Compliance and Extent of Exposure

Descriptive statistics will be used to summarize the total number of applications for the Safety and PK populations. Subjects who apply at least 80% of the expected total number of applications and have no other evidence of material dosing non-compliance will be considered to be compliant with dosing. Duration of treatment (days), number of applications, total weight of product used (g), total diclofenac dose (mg), and average weight per application (g) will be summarized by mean, standard deviation, median, minimum, and maximum.

10.5 Pharmacokinetics Evaluations

Individual plasma diclofenac concentrations will be listed by treatment and time point. These data will be plotted for each individual subject. Generally, the concentration vs time curves may be displayed separately for Day 1 0 to 24 hours, Day 7 0 to 24 hours, and Day 1 to Day 8 (ie, the 24-hour time point for the Day 7 dose) trough levels. Treatments will be overlayed in each of these 3 plots. A similar format will be followed for plots of the treatment means. The concentration data will be summarized for each time point and treatment by mean, standard deviation, median, minimum and maximum. Concentrations reported as below the limit of quantitation (50 pg/mL in the planned assay method) will be included as zero for the purposes of the PK analysis.

The following PK parameters will be calculated for each subject and treatment.

Symbol	**CDISC terms**	**Definition**
$AUC_{0-\tau}$	AUCTAU (AUC Over Dosing Interval)	The area under the plasma concentration curve from time 0 to 24 hours calculated by the trapezoidal rule.
C_{max}	CMAX (Max Conc)	The maximum observed plasma concentration in the dosing interval.
C_{min}	CMIN (Min Conc)	The minimum observed plasma concentration in the dosing interval.
PTF	PTROUGHR (Peak Trough Ratio)	CMAX/CMIN
T_{max}	TMAX (Time of CMAX)	The (nominal) time of CMAX
T_{min}	TMIN (Time of CMIN Observation)	The (nominal) time of CMIN
λ_z	LAMZ (Lambda z)	The elimination rate constant calculated as the negative of the slope of the log-linear phase of the concentration curve using linear regression
$T_{½}$	LAMZHL (Half-Life Lambda z)	Log(2)/LAMZ

The parameters $AUC_{0-\tau}$, C_{max}, and T_{max} will be determined separately for Day 1 and Day 7. All other parameters will be determined for Day 7 only.

Elimination rate constant and half-life will be determined whenever the elimination phase after the Day 7 dose appears to be adequately sampled based on a visual inspection of the log-linear plots.

The parameters $AUC_{0-\tau}$, C_{max}, and C_{min} will be summarized by treatment group as geometric mean and coefficient of variation (calculated as 100*SQRT(EXP(VAR) − 1) with VAR= the variance of the log-transformed values), minimum, and maximum. The parameters PTF, T_{max}, T_{min}, λ_z, and $T_{½}$ will be summarized by treatment as mean, standard deviation, median, minimum, and maximum.

The log-transformed parameters $AUC_{0-\tau}$ and C_{max} for Day 1 and Day 7 will be compared across treatments using the analysis of variance (ANOVA) model with effects of sequence, subject within sequence, period, and treatment. Sequence will be tested using subject within sequence for error. Period and treatment will be tested against the residual error. Least squares geometric means and standard errors will be provided by treatment. The ratio of geometric means will be determined for Treatments A and B using Treatment C as Reference. A 90% 2-sided confidence interval (ie, two 95% 1-sided tests) will be calculated. The object of the tests is to determine comparability of the rate (C_{max}) and extent ($AUC_{0-\tau}$) of absorption after a single dose and after a week of daily dosing.

10.6 Safety Evaluations

Adverse Events

Adverse event terms will be coded using the MedDRA version 25.1 (2022) dictionary. All AEs will be presented in a by-subject listing, detailing the verbatim term given by the investigator, the preferred term (PT), system organ class (SOC), onset date and time, end date and time, intensity, outcome, relationship to study drug, action taken with study drug, other action taken, seriousness and criteria for seriousness. A separate listing for any serious adverse events and adverse events Leading to study discontinuation will also be provided.

A summary table of treatment-emergent adverse events (TEAE) will be presented including for each treatment the number of subjects reporting an AE, the percentage of subjects with an AE, and the number of events reported, for the following categories: all AEs, severe AEs, serious AEs (SAEs), adverse drug reactions (ADRs, defined as AEs considered by the Investigator to be at least possibly related to the study drug), AEs leading to subject withdrawal, and deaths. For the purpose of the summary, each AE will be counted according to the most recently applied treatment at the time of onset.

Missing values will be treated as missing except for causality, intensity, seriousness, and outcome of an AE, at which occurrence a “worst case” approach will be taken. Thus, if causality is missing the AE will be regarded as related to the study drug, if the intensity is missing the intensity of the AE will be regarded as severe, if seriousness is missing the AE will

be regarded as an SAE, and if the outcome is missing and no date of outcome is present the outcome is regarded as "Not Recovered."

Vital Signs

Blood pressure, heart rate (pulse), and body temperature will be recorded at Screening, Day −1 pre-dose of each treatment period, and Day 8 of each treatment period. These values will be reported in by-subject listings and summarized by time point as well as change from Day −1 to Day 8 as mean, standard deviation, minimum, and maximum.

Physical Examination

Complete physical examinations will be performed at Screening, Day −1 pre-dose of each treatment period, and Day 8 of each treatment period. The results of these examinations will be presented in by-subject listings.

Administration Site Reactions

Administration site reactions will be recorded daily during each treatment period as 0=no reaction (negative documentation), 1=doubtful reaction, 2=mild erythema, 3=strong erythema, or 4=very strong erythema and edema. These scores will be summarized by frequency counts and percentages by treatment and treatment day, as well as worst overall score by treatment.

Clinical Laboratory Tests

Clinical laboratory panels will be performed at Screening, Day −1 pre-dose of each treatment period, and Day 8 of each treatment period. All test results will be displayed in by-subject listings. Results outside of the laboratory normal range will be highlighted as well as listed separately for review. Results will be summarized for each treatment as shift tables for each test parameter, showing the number and percent of subjects below normal range, within normal range, and above normal range at Day −1 and Day 8.

Electrocardiogram

Electrocardiogram measurements will be performed at Screening only and will be displayed in a by-subject listing along with the investigator's interpretations and comments.

11. CHANGES FROM THE PROTOCOL AND PLANNED ANALYSES

All exceptions to the planned analyses as described herein will be clearly indicated and explained in the study report.

12. HEADINGS

Each page of the analysis will show the sponsor's name, the investigational product, and the protocol number. Report tables will be embedded in the MS Word report document from SAS program output without change. The footer of each table will show the name of the SAS program module which generated it, the names of all data sets providing input data in the program and the date and time the table was generated.

13. ARCHIVING AND RETENTION OF DOCUMENTS

After finalization of the analysis, the following will be archived at TKL Research, Inc and/or with the study sponsor:

- SAP and any amendments
- DMP and Database Specification
- All SAS code used in the project for statistical analysis, report tables generation, and analysis data set creation
- Tables, listings and figures as included in the clinical study report
- SAS SDTM and ADaM datasets
- Relevant correspondence
- Any other pertinent study document (ie, study protocol, investigator's brochure, correspondence, study report, etc).