STATISTICAL REPORTING AND ANALYSIS PLAN

A Randomized, Double-Blind, Placebo-Controlled, Parallel Arm, Steady-State Pharmacokinetic and Disposition Study Characterizing Diclofenac's Plasma and Knee Exposure in Osteoarthritis Subjects Undergoing Scheduled Arthroplasty after Treatment with Diclofenac Diethylamine 2.32% Gel

Protocol Number: 208901

Phase: Phase 1

Document History

Document	Version Date	Summary of Changes (New analysis or Change in planned analysis)
Original Analysis Plan	08 May 2019	Not applicable (N/A)

Table of contents

List of tables

List of figures

List of Abbreviations

Abbreviation	Description
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
b.i.d.	Twice Daily
BDRM	Blind Data Review Meeting
BMI	Body Mass Index
COX-2	Cyclo-oxygenase 2
CRF	Case Report Form
DDEA	Diclofenac Diethylamine
ECG	Electrocardiogram
GSKDRUG	GSK Drug Coding Dictionary
LOQ	Limit of Quantification
MedDRA	Medical Dictionary for Regulatory Activities
N/A	Not Applicable
OA	Osteoarthritis
PP	Per Protocol
PP-PD	Per Protocol Pharmacodynamic
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event

The purpose of this Statistical Reporting and Analysis Plan is to describe the planned analyses and outputs to be included in the Clinical Study Report for Protocol 208901.

1 Summary of Key Protocol Information

The purpose of this study is to evaluate the exposure of diclofenac in the plasma and in the knee joint following topical administration of 2.32% diclofenac diethylamine (DDEA) gel (Voltarol 12 Hour Emulgel P 2.32% Gel in the UK, Voltaren Schmerzgel forte 23.2 mg/g gel in Germany) 4 g applied twice daily (b.i.d.) for 7 days.

This will be a randomized, double-blind, multi-center, placebo-controlled clinical study investigating the concentration of topically applied diclofenac achieved in the knee joint synovial tissue and synovial fluid following b.i.d dosing for seven days.

The study will be performed in subjects diagnosed with osteoarthritis (OA) of the knee who are scheduled for arthroplasty of the knee as a treatment for their OA.

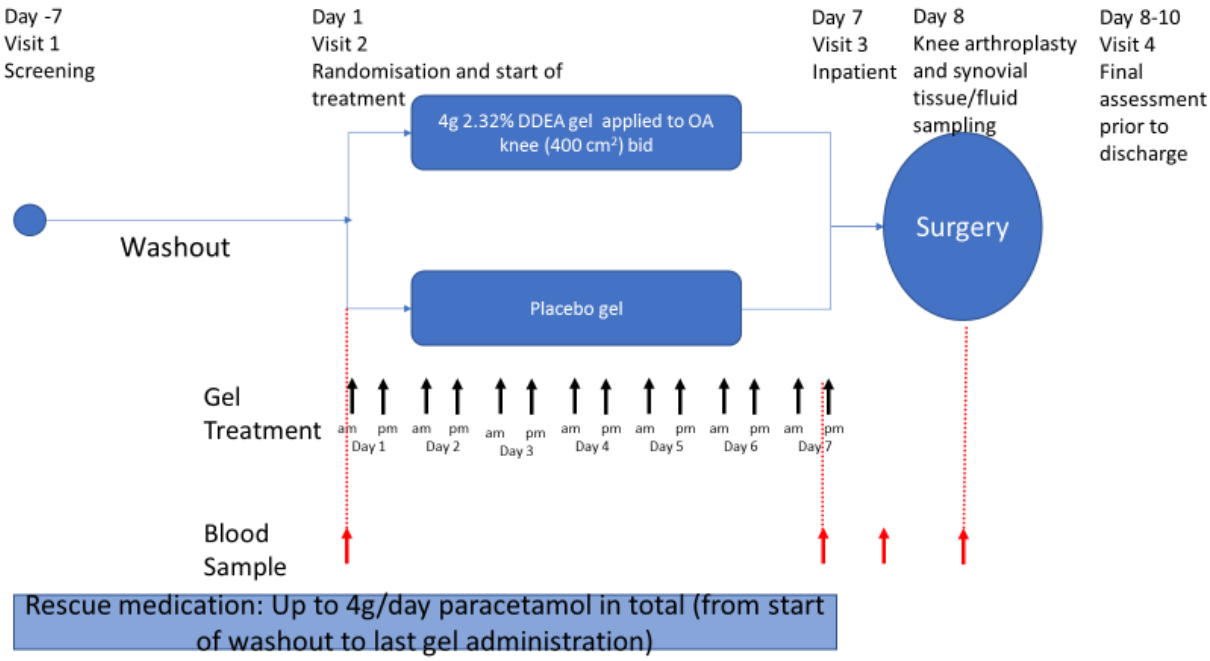
1.1 Study Design

Approximately fifty (50) male and female OA subjects, who, at the time of screening, are ≥50 years old, will be randomized in a 2:1 ratio: two thirds will receive DDEA 2.32% gel treatment and one third placebo gel.

After providing informed consent to participate in the study the subjects will need to forego any NSAID or cyclo-oxygenase 2 (COX-2) treatment for at least 7 days prior to starting study treatment (Visit 2) in order to allow wash-out of existing therapy and thus avoiding confounding the effect of the study treatments. Dosing will be scheduled to commence seven days prior (at minimum) to the scheduled surgery and will occur twice a day following dosing instructions. If surgery is delayed dosing with study treatment can continue up to 14 days.

A schematic of the study design is provided in Figure 1-1.

Figure 1-1 A schematic of the study design



1.2 Study Objectives

Objectives	Endpoints
Primary Objective	**Primary Endpoint**
• To determine whether diclofenac penetrates into treated knee joint following repeated topical administration of diclofenac diethylamine 2.32% gel.	• Diclofenac concentration in treated knee synovial tissue and synovial fluid after 7 days topical application of study treatment to the knee (12 hours after last application)
Secondary Objectives	**Secondary Endpoints**
• To evaluate the relative exposure of diclofenac in the knee joint vs. plasma.	• Ratio between diclofenac concentration in treated knee synovial tissue and diclofenac plasma concentration (sample taken during surgery) after 7 days topical application of study treatment to the knee (12 hours after last application) • Ratio between diclofenac concentration in treated knee synovial fluid and diclofenac plasma concentration (sample taken during surgery) after 7 days topical application of

Objectives	Endpoints
	study treatment to the knee (12 hours after last application)
Exploratory Objectives	**Exploratory Endpoints**
• To evaluate treatment effects upon COX-2 inhibition in the knee joint	• PGE_2 levels in treated knee synovial tissue and synovial fluid after 7 days topical application of study treatment to the knee (12 hours after last application)
• To evaluate treatment effects upon inflammatory cytokines in the knee joint	• IL-6 and TNFα levels in treated knee synovial tissue and synovial fluid after 7 days topical application of study treatment to the knee (12 hours after last application)

1.3 Treatments

The study includes one active treatment arm and one placebo control arm.

In the treatment arm, DDEA 2.32% gel will be applied to the knee planned for arthroplasty surgery (target knee). If the subject has bilateral knee OA, the treatment will be applied to the knee planned for surgery only and not to the contralateral knee. A placebo control arm is included to facilitate bioactivity comparisons. In the placebo control arm a placebo gel will be applied in a similar manner as the DDEA 2.32% gel on the knee planned for surgery (target knee).

1.4 Sample Size Calculation

A formal estimation of sample size was not carried out. The sample size selected (approximately 30 subjects on active, 15 subjects on placebo control) for the present study is in general agreement with similar studies (Benito 2005, Gallelli 2013, Gallelli 2012, Alvarez-Soria 2006, Fowler 1983, Efe 2014) and is deemed adequate to provide information on diclofenac concentration(s) in treated knee synovial tissue and synovial fluid.

Approximately 50 subjects will be randomized to account for 10% dropout and ensure evaluable data for 45 subjects who underwent the surgery.

2 Planned Analyses

2.1 Interim Analysis

No interim analysis is planned.

2.2 Final Analyses

The final planned primary analyses will be performed after the completion of the following sequential steps:

1. All subjects have completed the study as defined in the protocol.
2. All required database cleaning activities have been completed and database has been locked.

3 Considerations for Data Analyses and Data Handling Conventions

3.1 Baseline Definition

For all endpoints, the baseline value is the latest pre-dose assessment with a non-missing value.

3.2 Centers Pools

This study is a multicenter study. Data will be summarized for all centers combined. Statistical analyses will not be adjusted for centers.

4 Data Analysis

Data analysis will be performed by PPD. The statistical analysis software used will be SAS version 9.4 in a WINDOWS environment.

Prior to database closure a Blind Data Review Meeting (BDRM) will be conducted in which various aspects of the trial will be discussed and agreed.

4.1 Populations for Analysis

Tables described in this section will be produced for all randomized subjects.

4.1.1 Subject Disposition

Subject disposition will be summarized as the number and percentage of subjects who complete the study, with the number who discontinue broken down by reason for discontinuation (Table 14.1.1). Subject disposition including date of informed consent, date of first and last dose, completion status (Yes/No), completion or termination date and reason for discontinuation will be listed in Listing 16.2.1.1 by treatment group.

A listing of screen failures including date of screening and reason(s) for screen failure will be provided (Listing 16.2.1.2).

4.1.2 Protocol Deviations

All protocol deviations (both significant and non-significant) will be entered and tracked in PPD Clinical Trial Management System (CTMS) by the study team throughout the conduct of the study in accordance with PPD's Study Deviation Rules Document.

Significant protocol deviations that would exclude subjects from the Per Protocol (PP) population may include (but are not limited to) the following:

- Major deviations in synovial tissue and synovial fluid sampling
- Subjects with poor compliance with study treatment
- Subjects taking prohibited medication or treatment during the study which is felt to affect the assessment of diclofenac concentration in synovial tissue and synovial fluid samples

Significant protocol deviations that would exclude subjects from the Per Protocol Pharmacodynamic (PP-PD) population may include (but are not limited to) the following:

- Major deviations in synovial tissue and synovial fluid sampling
- Subjects with poor compliance with study treatment
- Subjects taking NSAIDs or/and corticosteroids administered between screening and synovial samples collection
- Subjects taking other prohibited medications between screening and synovial samples collection which is felt to affect the assessment of pharmacodynamic biomarker

Data will be reviewed prior to closure of the database to ensure all significant deviations are captured and categorised.

Final list of significant protocol deviations leading to exclusion from the PP population (These were considered as major protocol deviations in protocol) and from the PP-PD population will also be determined by the study team.

The number and percentage of subjects with any significant protocol deviations and with each type of significant protocol deviations will be presented by treatment (Table 14.1.2) and listed in Listing 16.2.2.1. Any non-significant protocol deviations will be listed similarly (Listing 16.2.2.2).

4.1.3 Analysis Populations

Population	Definition / Criteria	Analyses Evaluated
Safety	• Comprised of all subjects who are randomized and have received at least one dose of investigational product. • This population will be based on the treatment the subject actually received.	• Safety
Analysable	• Comprised of all randomized subjects included in the safety population, who completed the surgery and have evaluable synovial tissue or synovial fluid sample. • This population will be based on the treatment the subject actually received.	• Primary, Secondary and Exploratory

Population	Definition / Criteria	Analyses Evaluated
Per Protocol (PP)	• Comprised of all randomized subjects from the analysable population who do not have any significant protocol deviations that could confound the interpretation of primary analyses conducted on the analysable population. • Protocol deviations that would exclude subjects from the PP population are defined in Section 4.1.2 (Protocol Deviations). • This population will be based on the treatment the subject actually received.	• None
Per Protocol Pharmacodynamic (PP-PD)	• Comprised of all randomized subjects from the analysable population who do not have any significant protocol deviations that could confound the interpretation of exploratory analyses for pharmacodynamic biomarkers (i.e. PGE_2, IL-6 and TNFα). • Protocol deviations that would exclude subjects from the PP-PD population are defined in Section 4.1.2 (Protocol Deviations). • This population will be based on the treatment the subject actually received.	• Exploratory

The numbers of subjects included in each of the analysis populations will be presented (Table 14.1.1). Subjects excluded from any of the analysis populations will be listed in Listing 16.2.3.1, with the reason for exclusion.

4.2 Subject Demographics and Other Baseline Characteristics

4.2.1 Demographic Characteristics

Categorical demographic variable includes sex. This variable will be summarized by the number and percentage of subjects with each relevant characteristic in each treatment group. Body Mass Index (BMI) will be calculated as weight (kg)/ [height (m)]^2. Age, weight, height and BMI will be summarized by the mean, standard deviation, median, minimum and maximum

values in each treatment group. All demographic information will be tabulated for the safety population (Table 14.1.3) and for the analysable population (Table 14.1.4). All demographic information will also be listed for safety population (Listing 16.2.4.1).

4.2.2 General Medical History

Medical history will be listed for the safety population by subject, with start date and end date or ongoing at the start of treatment with study drug (Listing 16.2.4.2).

4.3 Treatments (Study Drug, Rescue Medication, other Concomitant Therapies, Compliance)

Compliance data will be summarized for the analysable population. Exposure and other medications will be summarized on the safety population.

4.3.1 Study Drug Compliance

Study drug compliance is evaluated by the total number of applications and percentage of scheduled applications made. Percentage of scheduled applications made is defined as the number of applications made divided by the number of applications planned. The study drug will start to be applied on the day of randomization (Day 1) and continue to be applied twice daily until and including the day before surgery. The number of applications planned will be calculated as 2*(date of last application-date of first application +1). Both compliance variables will be summarized descriptively for the analysable population. In addition, number and percentage of subjects with a minimum of 12 applications will be also presented (Table 14.2.1.1). Study drug accountability (Listing 16.2.5.1) and study drug administration (Listing 16.2.5.2) will also be listed for the safety population.

4.3.2 Study Drug Exposure

Study drug exposure will be summarized descriptively by treatment group for the Safety population, including the following summaries: total number of applications and total weight of gel used (Table 14.3.1).

4.3.3 Prior and Concomitant Medication

Any medications taken by subject within 90 days before the screening visit (Visit 1) and up to the last study visit (Visit 4) during the study will be recorded in the case report form (CRF).

All medications will be coded using the GSK Drug coding dictionary (GSKDRUG), which will be updated whenever available throughout the life of the study.

For purposes of analysis, prior medications are defined as any medications with a stop date that is prior to the initial study drug dosing date (Day 1) and concomitant medications are defined as any medications with a start date on or after the initial study drug dosing date (Day 1) or any medications with a start date prior to the initial study drug dosing date and a stop date after the initial study dosing date.

For medications with partial start or stop dates, the partial dates will be imputed according to Table 4-1 and then will be categorized as prior medication or concomitant medication according to the definitions. Medications with completely missing dates will be included in the concomitant medication summary.

Imputation rules for the start or stop date of missing or incomplete medications are presented in Table 4-1.

Table 4-1 Imputation rules for the start or stop date of missing or incomplete medications

	Missing	Imputation	Exception
Start date (concomitant medication)	Day	01	Default to Study Day 1 if an event starts the same year and month as Day 1 and the stop date contains a full date and the stop date is later than Day 1; Otherwise If the stop date is prior to Day 1, default to 1 day prior to the stop date
	Day/Month	01Jan	Default to Study Day 1 if an event started the same year as Day1 and the stop date contains a full date and the stop date is later than Day 1; Otherwise If the stop date is prior to Day 1, default to 1 day prior to the stop date
	Day/Month/Year	No imputation	Assume start prior to study entry.
Stop date (concomitant medication)	Day	Last day of the month	Default to the End of Study Date if the concomitant medication stopped the same year and month as the End of Study Date.
	Day/Month	31DEC	Default to the End of Study Date if the concomitant medication stopped the same year as the End of Study Date.
	Day/Month/Year	No imputation	Assume stops after study end.

Tables and listings described in this section will be presented for the safety population. The prior and concomitant medications listing will include the preferred term, indication, a flag indication of prior or concomitant, single dose, frequency, unit, route, start date, and end date or ongoing at final visit (Listing 16.2.5.7). The significant non-drug therapies listing will

include the name of non-drug therapy, a flag indication of prior or concomitant, start date, and end date or ongoing at final visit (Listing 16.2.5.8).

Concomitant medications will be reported by GSK-Drug Anatomical Therapeutic Chemical (ATC) classification level 1 (Body System) and ingredient (Table 14.3.2). Drugs that are composed of a combination of ingredients will be displayed according to the ATC classifications of the ingredients, not of the combination.

4.3.4 Rescue Medication

Rescue medications used during the study treatment period (number of subjects that used paracetamol, number of days on paracetamol, percentage of days on paracetamol and total dose of paracetamol taken) will be presented by treatment group for the analysable population (Table 14.2.1.2). This summary will only include those subjects who used rescue medications during the study treatment period.

By-subject listing of all rescue medications, used before start of the study treatment and during the study treatment, will be produced for the safety population (Listing 16.2.5.3).

4.4 Analysis of Primary Objective

The primary objective of this study is to determine whether diclofenac penetrates into the treated knee joint following repeated topical administration of diclofenac diethylamine 2.32% gel.

4.4.1 Primary Endpoints

4.4.1.1 Primary Endpoints Definition

The primary endpoints are diclofenac concentrations in treated knee synovial tissue and synovial fluid after 7 days topical application of study treatment to the knee (12 hours after last application).

4.4.1.2 Statistical Hypothesis, Model, and Method of Analysis

The success criterion of this study is that diclofenac can be detected within the treated knee synovial tissue or synovial fluid after 7 days treatment.

Synovial tissue and synovial fluid diclofenac concentrations will be summarized descriptively by treatment group in both the arithmetic and the logarithmic scale (Table 14.2.2.1 and Table 14.2.3.1). The tables will present the proportion of subjects with values above the limit of quantification (LOQ) with its two-sided 95% confidence interval estimated by Wilson score interval. The mean, SD, min, P10, Q1, median, Q3, P90, max will be calculated after replacing values below the LOQ by LOQ/2 for treatment group and by 0 for placebo group. The geometric mean will also be calculated for treatment group with a two-sided 95% confidence interval assuming data on the logarithmic scale are normally distributed. Boxplots for study treatment group will be produced on the original data (Figure 14.2.1 and Figure 14.2.3). There is no formal hypothesis testing to be performed.

The analyses will be performed on the analysable population.

4.4.2 Handling of Missing Values/Censoring/Discontinuations

Missing data will not be imputed. Values that are below the limit of quantification (LOQ) will be replaced by LOQ/2 for treatment group and be replaced by 0 for placebo group.

4.5 Analysis of Secondary Objective

The secondary objective is to evaluate the relative exposure of diclofenac in the knee joint vs. plasma.

4.5.1 Secondary Endpoints

The secondary endpoints are

- Ratio between diclofenac concentration in treated knee synovial tissue and diclofenac plasma concentration (sample taken during surgery) after 7 days topical application of study treatment to the knee (12 hours after last application)
- Ratio between diclofenac concentration in treated knee synovial fluid and diclofenac plasma concentration (sample taken during surgery) after 7 days topical application of study treatment to the knee (12 hours after last application)

Following the same approach as for the primary endpoints, the ratios between diclofenac concentration in synovial tissue / fluid and diclofenac plasma concentration (last sample taken during surgery) will be summarized descriptively by treatment group in both the arithmetic and the logarithmic scale. Geometric means will be calculated with two-sided 95% confidence intervals (Table 14.2.4.1 and Table 14.2.5.1). Boxplots for study treatment group will be produced (Figure 14.2.5 and Figure 14.2.7). The analyses will be performed on the analysable population.

4.5.2 Handling of Missing Values/Censoring/Discontinuations

Missing data will not be imputed. For diclofenac concentrations in treated knee synovial tissue or synovial fluid that are below the limit of quantification (LOQ), refer to Section 4.4.2 for imputation. For diclofenac plasma concentrations that are below the limit of quantification (LOQ), refer to Section 4.8.2 for imputation. For placebo group the ratios will be set as missing.

4.6 Analysis of Exploratory Objectives

The exploratory objectives are

- To evaluate treatment effects upon COX-2 inhibition in the knee joint
- To evaluate treatment effects upon inflammatory cytokines in the knee joint

4.6.1 Exploratory Endpoints

The exploratory endpoints are

- PGE_2 levels in treated knee synovial tissue and synovial fluid after 7 days topical administration to the knee (12 hours after last application)
- IL-6 and TNFα levels in treated knee synovial tissue and synovial fluid after 7 days topical administration to the knee (12 hours after last application)

The success criteria for exploratory objectives are

- diclofenac reduces PGE_2 levels in treated knee synovial tissue or synovial fluid after 7 days topical administration to the knee compared to placebo gel
- diclofenac reduces levels of inflammatory cytokines i.e. TNFα and/or IL-6 associated with OA in treated knee synovial tissue or synovial fluid after 7 days topical administration to the knee compared to placebo gel

PGE_2 and inflammatory cytokines levels will be summarized descriptively in both the arithmetic and the logarithmic scale (Table 14.2.6 - Table 14.2.11). The mean, SD, min, P10, Q1, median, Q3, P90 and max will be summarized.

For each endpoint, the success criterion will be addressed by a two-sided test for superiority at level alpha=0.05 in an exploratory manner. Log-transformed mean levels will be compared between treatment groups using an analysis of variance including treatment as a fixed effect. The two-sided 95% confidence interval for the ratio of geometric means on the original scale will be derived by back-transforming the confidence interval for the difference between treatment groups on the log-transformed scale obtained from the analysis.

The analyses will be performed on the analysable population.

The analyses will be repeated for the PP-PD (Per Protocol Pharmacodynamic) Population (Table 14.2.13 - Table 14.2.18).

4.6.2 Handling of Missing Values/Censoring/Discontinuations

Missing data will not be imputed. Values that are below the limit of quantification (LOQ) will be replaced by LOQ/2.

4.7 Analysis of Safety

4.7.1 Adverse Events and Serious Adverse Events

Adverse events (AEs) will be summarized (Table 14.3.1.1) by presenting, for each treatment group, the number and percentage of subjects having any AE, any Treatment Emergent adverse events (TEAE), any serious TEAE, any study drug-related TEAE, any study drug-related serious TEAE, any TEAE leading to treatment discontinuation or study discontinuation, or any AE leading to death. TEAE is defined as an AE that starts on or after the date of first study treatment. For an AE with an incomplete start date where the day is missing while the year and month are present and the same as the date of first study treatment administration, the date of first study treatment will be assumed to be the AE start date.

TEAEs will be summarized by presenting, for each treatment group, the number and percentage of subjects having any TEAE, any TEAE in each MedDRA primary system organ class (SOC) and having each individual TEAE (using MedDRA preferred term).

This will be done separately for all TEAEs by SOC/Preferred term (Table 14.3.1.2) and for TEAEs that are suspected to be drug-related by SOC/Preferred term (Table 14.3.1.4). All TEAEs will also be tabulated in corresponding fashion by SOC/Preferred term/Intensity (Table 14.3.1.3 and Table 14.3.1.5). Any other information collected (e.g. action taken, duration, outcome, seriousness) will be listed as appropriate.

Additionally, all adverse events will be listed (listing 16.2.7.1).

Deaths occurring during treatment (if any) will be listed by treatment, including the date and study day of death, and the principal cause of death (listing 14.3.2.1). Non-fatal serious AEs and TEAEs leading to study treatment discontinuation will be listed (listing 14.3.2.2 and Listing 14.3.2.3).

4.7.2 Laboratory Tests

Chemistry and hematology results at Screening, Visit 3 and at Visit 4 will be summarized by the mean, standard deviation, median, minimum and maximum values in each treatment group (Tables 14.3.3). Shift tables (between baseline and Visit 3, between Visit 3 and Visit 4) will also be presented (Table 14.3.4 and Table 14.3.5). Laboratory normal ranges will be listed in Listing 16.2.8.1 and all laboratory (chemistry, hematology and urinalysis) test results will be listed in Listings 16.2.8.2 – 16.2.8.4.

Note: Laboratory tests were performed by the local hospital laboratories. Normal ranges provided prior to study start were recorded in the eCRF. During the study the normal ranges had shifted slightly for some of the tests at some of the local laboratories, but the shifted normal ranges were not recorded in the eCRF. The shift tables will be based on the normal ranges provided prior to study start.

4.7.3 Vital Signs

Vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate, body temperature) recorded at Visit 3 and changes in vital signs from Visit 2 (Baseline) to Visit 3 will be summarized by the mean, standard deviation, median, minimum and maximum values in each treatment group (Tables 14.3.6). Vital signs at each assessment will also be listed (Listing 16.2.9.1).

4.7.4 Findings on Physical Examination

A full physical examination will include head, ears, eyes, nose, mouth, skin, heart and lung examinations, lymph nodes, gastrointestinal, musculoskeletal, vascular and neurological systems.

The findings on the physical examination performed at Visit 1-Visit 4 will be listed (Listing 16.2.9.2).

4.7.5 ECG

The findings on the electrocardiogram (ECGs) performed at Visit 1, Visit 3 and Visit 4 will be listed (Listing 16.2.9.3).

4.8 Analysis of Other Variables

4.8.1 Diclofenac Plasma Concentrations

Diclofenac plasma concentrations will be summarized (Table 14.2.12) on the analysable population by calculating the mean, standard deviation, median, minimum and maximum for the following timepoints: Visit 2 (baseline), Visit 3 (pre-dose), Visit 3 (sample taken between last application and surgery), Visit 3 (last sample taken during surgery). Mean of diclofenac plasma concentrations will also be plotted by visits (Figure 14.2.9).

4.8.2 Handling of Missing Values/Censoring/Discontinuations

Missing data will not be imputed. For diclofenac plasma concentrations in active treatment group, values that are below the limit of quantification (LOQ) will be replaced by LOQ/2 for post-baseline visits and be replaced by 0 for baseline visit. For diclofenac plasma concentrations in placebo group, values that are below the limit of quantification (LOQ) will be replaced by 0 for all visits.

5 Changes to the Protocol Defined Statistical Analysis Plan

Any changes from the originally planned statistical analysis specified in the protocol are outlined in Table 5-1.

Table 5-1 Changes to Protocol Defined Analysis Plan

Protocol	Reporting & Analysis Plan	
Statistical Analysis section	Statistical Analysis Plan	Rationale for Changes
• No analysis was planned for Per Protocol Pharmacodynamic population in protocol	• Analyses for Per Protocol Pharmacodynamic population are added for exploratory analyses.	• Per Protocol Pharmacodynamic population analyses are added for sensitivity analyses.
• Values that are below the limit of quantification (LOQ)	• For diclofenac concentrations in treated knee synovial tissue or synovial fluid after 7 days	• For active treatment group, we don't expect to see diclofenac concentrations before

Protocol	Reporting & Analysis Plan	
Statistical Analysis section	Statistical Analysis Plan	Rationale for Changes
will be replaced by LOQ/2.	of topical application, values that are below the limit of quantification (LOQ) will be replaced by LOQ/2 for active treatment group and be replaced by 0 for placebo group. • For diclofenac plasma concentrations in active treatment group, values that are below the limit of quantification (LOQ) will be replaced by LOQ/2 for post-baseline visits and be replaced by 0 for baseline visits. For diclofenac plasma concentrations in placebo group, values that are below the limit of quantification (LOQ) will be replaced by 0 for all visits. • For PGE_2, IL-6 and TNFα, values that are below the limit of quantification (LOQ) will be replaced by LOQ/2.	first application of study treatment. For placebo group, we don't expect to see diclofenac concentrations at any visit.

6 References

1. [Álvarez-Soria, M.A., Largo, R., Santillana, J., Sánchez-Pernaute, O., Calvo, E., Hernandez, M., Egido, J. and Herrero-Beaumont, G., 2006]. Long term NSAID treatment inhibits COX-2 synthesis in the knee synovial membrane of subjects with osteoarthritis: differential proinflammatory cytokine profile between celecoxib and aceclofenac. Annals of the rheumatic diseases, 65(8), pp.998-1005.

2. [Benito, M.J., Veale, D.J., FitzGerald, O., van den Berg, W.B. and Bresnihan, B., 2005]. Synovial tissue inflammation in early and late osteoarthritis. Annals of the rheumatic diseases, 64(9), pp.1263-1267.

3. [Efe, T., Sagnak, E., Roessler, P.P., Getgood, A., Patzer, T., Fuchs-Winkelmann, S., Peterlein, C.D. and Schofer, M.D., 2014]. Penetration of topical diclofenac sodium 4% spray gel into the synovial tissue and synovial fluid of the knee: a randomised clinical trial. Knee Surgery, Sports Traumatology, Arthroscopy, 22(2), pp.345-350.

4. [Fowler, P.D., Shadforth, M.F., Crook, P.R. and John, V.A., 1983]. Plasma and synovial fluid concentrations of diclofenac sodium and its major hydroxylated metabolites during long-term treatment of rheumatoid arthritis. European journal of clinical pharmacology, 25(3), pp.389-394.

5. [Gallelli, L., Galasso, O., Falcone, D., Southworth, S., Greco, M., Ventura, V., Romualdi, P., Corigliano, A., Terracciano, R., Savino, R. and Gulletta, E., 2013]. The effects of nonsteroidal anti-inflammatory drugs on clinical outcomes, synovial fluid cytokine concentration and signal transduction pathways in knee osteoarthritis. A randomized open label trial. Osteoarthritis and Cartilage, 21(9), pp.1400-1408.

6. [Gallelli, L., Galasso, O., Urzino, A., Saccà, S., Falcone, D., Palleria, C., Longo, P., Corigliano, A., Terracciano, R., Savino, R. and Gasparini, G., 2012]. Characteristics and clinical implications of the pharmacokinetic profile of ibuprofen in subjects with knee osteoarthritis. Clinical drug investigation, 32(12), pp.827-833.