A Phase 2 Study to Assess the Effect of a Repeated Dose of Exeporfinium Chloride (XF-73) Nasal Gel on the Microbiological Burden of Commensal *Staphylococcus aureus* Nasal Carriage in Surgical Patients at Risk of Post-Operative Staphylococcal Infections

Destiny Pharma Study No: XF-73B07
[redacted] Study No: DMA18003

Statistical Analysis Plan

NCT03915470

Version: 3.0

Date: 8th February 2021

For [redacted] – Lead Statistician

For Destiny Pharma plc

Modification History

Version	Change History	Reason	Date
1.0	First Version	N/A	16SEP2019
2.0	• Section 3: Updated sample size section following protocol amendment. • Section 5: Removed text relating to interim analysis of efficacy data – only safety data was considered per Protocol Amendment 2.1 (dated 04JUN2020). • Section 6.4: Removed modified ITT from list of analysis sets (and throughout SAP) – no longer required for the analysis. • Section 6.4: Added microbiological ITT as the primary population for the efficacy analyses per Protocol Amendment 2.1 (dated 04JUN2020). • Section 6.11.1: Added details of new table – change from baseline by baseline CFU category and new plot - change in S. aureus log CFU/mL from baseline versus observed baseline S. aureus log CFU/mL scatterplot. • Minor clarifications to SAP text throughout.	All updates resulting from the changes in Protocol V2.1 (dated 04JUN20)	17SEP2020

3.0	• Section 6: New section for Follow-Up Review and re-numbered subsequent sections accordingly. • Section 7.3: updated to specify that study day will be calculated relevant to Day -1. • Section 7.4: added details to clarify process for reviewing protocol deviations leading to exclusion from the PPS. • Added MedDRA version that will be used to the relevant sections (v23.1) • Section 7.8.3: Added summary of number of patients receiving peri-operative prophylaxis interventions by the time at which they were recorded. • Sections 7.11.2.1 and 7.11.2.2: Added details of how missing data will be handled in the calculation of AUC. • Section 7.11.2.4: Added details of 2 new summary tables to support the figures for patients reaching a specific reduction in S. aureus carriage. • Section 7.11.2.6: Changed from Semi-quantitative scale to semi-quantitative categorization. • Section 7.11.3.1: Added that duration of post-operative staphylococcal antibiotics will be summarized. • Section 7.11.3.1: Added new plot of the individual patient data for S. aureus log10 CFU/mL over time. • Section 7.14: Text added to clarify how major protocol deviations leading to exclusion from the PPS are handled compared to the definition of major protocol deviations in the study protocol. • Other minor clarifications to SAP text.	All updates to provide clarification on current analyses or to provide additional supportive summaries.	05FEB2021

Contents

LIST OF ABBREVIATIONS

AE	Adverse Event
AIC	Akaike Information Criterion
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
BDRM	Blinded Data Review Meeting
BMI	Body Mass Index
B-SIT	Brief Smell Identification Test
CFU	Colony Forming Units
CI	Confidence Interval
CRF	Case Report Form
ENR	Enrolled Set
ENT	Ear, Nose and Throat
IDMC	Independent Data Monitoring Committee
ITT	Intent-to-treat
LS	Least square
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified Intent-to-Treat
MMRM	Mixed Model for Repeated Measures
PPS	Per Protocol Set
PT	Preferred Term
SAF	Safety Analysis Set
SAP	Statistical Analysis Plan
SD	Standard Deviation
SE	Standard Error
SOC	System Organ Class
SQS	Semi-Quantitative Scale
S. aureus	*Staphylococcus aureus*
TEAE	Treatment Emergent Adverse Event
TLF	Tables, Listings and Figures
WHO Drug	World Health Organization Drug Dictionary
XF-73	Exeporfinium Chloride

1 INTRODUCTION

This document details the statistical analysis of the data that will be performed for the Destiny Pharma study: A Phase 2 Study to Assess the Effect of a Repeated Dose of Exeporfinium Chloride (XF-73) Nasal Gel on the Microbiological Burden of Commensal *Staphylococcus aureus* Nasal Carriage in Surgical Patients at Risk of Post-Operative Staphylococcal Infections.

The proposed analysis is based on the contents of the Final Version (Version 2.1) of the protocol (dated 04 June 2020). 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 (TLF) shells are supplied in a separate document.

2 STUDY OBJECTIVES AND DESIGN

2.1 Study Objectives

The primary objective of the study is to demonstrate the efficacy of a 0.2% XF-73 nasal gel in reducing the microbiological burden of nasal *Staphylococcus aureus (S. aureus)* measured as change in colony forming units (CFU) per mL from baseline to immediately prior to surgery in a patient population at risk of post-operative staphylococcal infection.

The secondary objectives of the study are to:

- Determine the effect of a 0.2% XF-73 nasal gel on *S. aureus* nasal burden from baseline to pre-surgery and from baseline to immediately post-surgery measured as area under the curve (AUC) of *S. aureus* log CFUs/mL over time.
- Determine the efficacy of a 0.2% XF-73 nasal gel in their effect on *S. aureus* nasal burden measured as CFUs/mL in follow-up immediately after surgery, at approximately 48 hours after surgery and 7 days after surgery.
- Explore the efficacy of XF-73 in reducing the burden of *S. aureus* carriage at the patient level.
- Assess the effect of XF-73 on *S. aureus* nasal carriage in the prevention of postoperative staphylococcal infections (surgical site infections, blood stream infections and others) during the 30 days post-surgery (90 days in the case of a foreign implant).
- Describe the safety and tolerability of multiple administrations of a 0.2% XF-73 nasal gel in a population of surgical patients at risk of post-operative staphylococcal infections.

The exploratory objective of the study is to describe the use of post-operative anti-staphylococcal antibiotics, except those provided for prophylaxis as local standard of care, during the 30-day post-surgery period (90 days in the case of an object implant) reported separately.

2.2 Study Endpoints

The primary endpoint of the study is the change in *S. aureus* log CFU/mL from baseline to pre-surgery.

The secondary endpoints are:

- Difference in AUC of *S. aureus* log CFU/mL from baseline to pre-surgery.
- Difference in AUC of *S. aureus* log CFU/mL from baseline to immediately post-surgery.
- Change in *S. aureus* log CFU/mL from baseline to immediately after surgery.
- Change in *S. aureus* log CFU/mL from baseline to 48 hours after surgery.
- Change in *S. aureus* log CFU/mL from baseline to 7 days after surgery.
- Percentage of patients reaching a specific reduction in *S. aureus* carriage prior to surgery, immediately post-surgery and on Day 6 ± 24hrs.
- Difference in the incidence of *S. aureus* post-operative infections during the 30-day period after surgery (90 days in the case of foreign implant).
- Incidence of Treatment Emergent Adverse Events (TEAEs) from the first dose of study medication to 7 days post last application of study medication.
- Changes in vital signs, safety clinical laboratory assessments, nasal examination and smell identification test.

The exploratory endpoint is the reason for post-operative prescribed anti-staphylococcal antibiotic use during the 30-day post-operative period (90 days in the case of an object implant).

2.3 Study Design

This is a Phase 2, multi-center, randomized, double-blind, parallel, placebo-controlled study of multiple applications of a single concentration of XF-73 nasal gel to assess the microbiological effect of XF-73 on commensal *S. aureus* nasal carriage in patients scheduled for surgical procedures deemed to be at high risk of post-operative *S. aureus* infection.

The study is divided in 4 periods: screening (Days -14 to -3), randomization (Days -10 to -1), treatment (Days -1 and 0) and follow-up (Day 1 to Day 30 or Day 90 if an implant is inserted during surgery). Day 0 is the calendar day in which surgery takes place. Only patients who test positive to *S. aureus* by a centrally performed rapid diagnostic test will be enrolled in the study. The maximum study duration will be 45 or 105 days for each individual (from screening to post-study follow-up visit) depending on whether a foreign implant was inserted during surgery.

Approximately 125 patients will be randomized in a double-blind manner (the investigators and patients are blind to the treatment assignment) in a 1:1 ratio to 0.2% w/w XF-73 nasal gel treatment OR placebo to match XF-73 nasal gel. Patients are planned to be recruited from approximately 10 sites in the USA, 10 sites in Georgia and 4 sites in Serbia. The randomization scheme will be stratified by site.

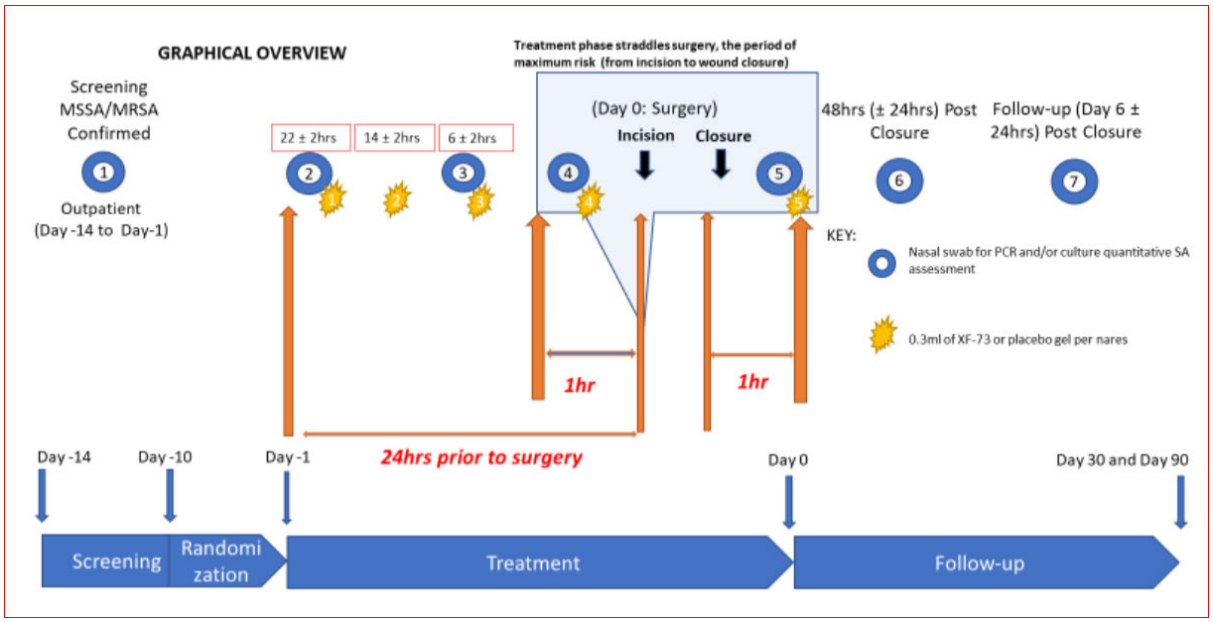
The study drug, 0.2% w/w XF-73 nasal gel, or matched placebo will be administered 4 times into each nostril over 24 hours prior to surgery and then a single application

immediately upon closure of surgical wound. Additionally, patients may undergo chlorhexidine skin decolonization ahead of surgery and receive perioperative prophylactic systemic antibiotics in accordance with local practice.

Seven nasal swabs will be collected: one at screening, three pre-surgery (between 24 hours and 1 hour prior to surgery) and three post-surgery (immediately after incision closure, 48 hours after surgery and 7 days after surgery).

2.4 Visit Structure

The visit structure and scheduled assessments are detailed Section 10, and Table 1.1 of the protocol. A diagrammatic explanation of the swab and treatment times can be found in Figure 6.1 of the protocol and is included below.



3 SAMPLE SIZE

Individual CFU data is presented in the study of Verhoeven 2012[1] in the absence of treatment. As the primary population for the efficacy analysis is the microbiological Intent-to-Treat (micro-ITT) set, those values with zero CFU have been excluded and the resulting data have been converted to the $\log_{10}$ CFU scale. The standard deviation (SD) of these values has been used as the estimate of variability for an analysis of change from baseline whilst including baseline $\log_{10}$ CFU as a covariate. As a result, a trial including 80 evaluable patients (40 per arm) would have a greater than 90% power to detect a true treatment effect of a 2 $\log_{10}$ CFU and an 90% power to detect a true treatment effect of a 1.5 $\log_{10}$ CFU difference between XF-73 and placebo, assuming 2-sided alpha=0.05 and a between patient SD of 2 $\log_{10}$ CFUs taken from the Verhoeven study.

It is estimated that approximately 125 patients will need to be recruited in order to have 80 patients with a burden *S. aureus* nasal carriage which will allow for the detection of a microbiological effect of XF-73 nasal gel.

4 RANDOMISATION

Patients will be randomly allocated in a 1:1 ratio as follows: 0.2% w/w XF-73 nasal gel treatment OR placebo to match XF-73 nasal gel treatment prior to dosing.

A randomization scheme will be prepared where patients will be stratified by site.

Treatment packs will be prepared according to the random code, numbered for sequential allocation to eligible study patients.

5 IDMC SAFETY REVIEW

An interim analysis will be performed, depending on recruitment projections, when approximately 60% of evaluable patients have been recruited. The interim analysis will:

- Review the Safety data generated to date.
- Review the incidence of post-operative staphylococcal infections.
- Stop the study in case of serious concerns on patient's safety.
- Stop the study due to the incidence of post-operative staphylococcal infections.

All study staff including the Sponsor will remain blinded until the end of the study.

Full details of the objectives, timing, analyses and the role of the Independent Data Monitoring Committee (IDMC) will be provided in the IDMC charter and IDMC SAP.

The IDMC safety review has been completed. The meeting took place on 5th August 2020 and it was agreed that the study should continue without modification.

6 FOLLOW-UP REVIEW

The main analysis of primary and secondary endpoints will be performed once the last patient has completed their Day 6 visit. Following this, there will be a review of relevant, longer term data, as part of the secondary and exploratory objectives, once the last patient has reached their 30 or 90-day follow-up visit depending on whether the patient had a foreign implant inserted or not. TFLs to be included in the follow-up analysis will be indicated in the TFL Shells document and will include the following data:

- Concomitant Medications
- Adverse Events
- Staphylococcal infections
- Use of post-operative staphylococcal antibiotics

7 ANALYSIS PLAN

7.1 General

Summary statistics for continuous variables will consist of number of non-missing observations (n), mean, standard deviation (SD), minimum, median, maximum, 25th and 75th percentiles 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 patients in each category will be presented, based on the number of non-missing observations apart from disposition of patients, protocol deviations, background and demographic characteristics, prior and concomitant medications/procedures and adverse events where the percentage will be based on the number of patients in the analysis set.

All statistical tests will be performed using a two-tailed 5% overall significance level, unless otherwise stated. The null hypothesis at all times will be that the 0.2% w/w XF-73 nasal gel and the placebo are equivalent. All comparisons between the treatments will be reported with 95% confidence intervals for the difference.

7.2 Blinded Data Review Meeting

The Sponsor may convene a blinded data review meeting (BDRM) after the data has been cleaned and before the study is unblinded. This will be performed using blinded data taken directly from the database (i.e. TLF outputs will not be created).

The BDRM will make decisions that will include but will not be limited to assessment of protocol deviations, population assignment and if changes are required to the SAP. If required, after the BDRM and prior to database lock, a new version of the SAP will be issued.

7.3 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 study day

Throughout this SAP any references to “Day XX” refer to the pre-specified visits defined in the protocol (where Day 0 is the calendar day in which surgery takes place).

Data listings will additionally present study day. Study day will be calculated relative to the first date of administration of study drug (Study Day -1).

- Definition of baseline

Baseline is defined as the last non-missing value (including scheduled and unscheduled assessments) prior to the patient receiving study drug.

- 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 recorded in numeric field

In the case where a variable is recorded as ">x", "≥x", "<x" or "≤x" rather than an exact number, 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.

- Methods for handling withdrawals and missing data

In general, data will not be imputed. Where imputation may be deemed appropriate, information is detailed in the analysis descriptions.

7.4 Analysis Sets

The **Enrolled Set (ENR)** includes all patients who completed main study informed consent.

The **Safety Analysis Set** (SAF) consists of all patients who received at least one dose of study drug. Patients will be presented in the actual treatment group that they received. The primary population for the safety analyses will be the SAF set.

The **Intent-To-Treat** (ITT) set consists of all patients who were randomized. Patients will be presented in the treatment group they were randomly assigned to.

The **microbiological Intent-to-Treat** (micro ITT) set includes all patients who were randomized, received at least one dose of study drug, have at least one post-baseline assessment of S. aureus log10 CFU/mL and have a log CFU/mL result at baseline greater than 0. The primary population for the efficacy analyses will be the micro ITT. Patients will be presented in the treatment group that they were randomly assigned to.

The **Per-Protocol Set** (PPS) will be a subset of the micro ITT set consisting of those patients who do not have major protocol deviations considered to have a serious impact on the efficacy results. Patients will be presented in the actual treatment group that they received. Supportive efficacy analyses will also be performed on the PPS for key endpoints and at key time points.

All protocol deviations will be assessed and documented on a case-by-case basis by Destiny Pharma at the following intervals: monthly, prior to interim analysis, prior to database lock at Day 6 and prior to final data base lock. The protocol deviations will be classified as major or minor deviations. All major deviations will be reviewed prior to database lock, whilst blinded, to identify those considered to have a serious impact on the efficacy results, leading to exclusion from the PPS. Some categories of major protocol deviations that might be considered as having a serious impact on the efficacy results include but are not limited to:

- Patients failing specific eligibility criteria.
- Any patients with treatment administration errors.
- Taking inadmissible concomitant medication.
- Significant non-compliance with timing of study visits.
- Non-compliance of study drug administration. Patients will be considered compliant if they receive 5 scheduled applications (4 prior to surgery and 1 after surgery) of randomized study drug during the appropriate dosing windows.

This is a non-exhaustive list and additional protocol deviations other than those specified above may be identified. Protocol deviations leading to exclusion from the PPS, will be referred to as 'important' protocol deviations in the statistical outputs and

will be finalized at the population assignment meeting ahead of database lock and unblind.

The definitions for the SAF, ITT, and micro ITT analysis sets are sufficient to determine the patients included within these analysis sets and so do not require listing and agreeing prior to breaking the blind. The list of subjects included in the PPS will be agreed as far as possible prior to database lock and unblinding.

Actual study drug received will be determined using a kit list report obtained from [redacted] and provided to [redacted] once the blind has been broken. The report will be reconciled with the site records to confirm the correct kit was administered to each patient.

7.5 Data presentations

The data will be summarized in tabular form by treatment group apart from disposition of patients, protocol deviations and background and demographic data which will be summarized by treatment group and overall patients. Patients will not be presented for each site/country separately in the summaries unless otherwise specified. Similarly, patients will not be presented for each country separately in the listings.

Treatment group labels will be “0.2% w/w XF-73 Nasal Gel” and “Placebo XF-73 Nasal Gel” and presented in this order.

Visits and time points will be presented throughout as follows:

Case Report Form (CRF) Visit Name	**Time point**	**SAP and TLF Terminology**
Randomisation (Day -14 to Day -1)	-	Randomization
Treatment Period (Day -1 to Day 0)	First dose nasal swab Third dose nasal swab Fourth dose nasal swab Fifth dose nasal swab	Baseline Prior to third dose Immediately prior to surgery Within 1 hour of incision closure
48h post wound closure	-	48 hours after surgery
Follow up (Day 6)	-	6 days after surgery
Follow up (Day 30)	-	30 days after surgery
Follow up (Day 90)	-	90 days after surgery

Only scheduled post-baseline assessments will be tabulated, post-baseline repeat/unscheduled assessments will not be tabulated although they will be listed and in particular all clinically significant values will be noted.

Eligibility, consent, completion/withdrawal and analysis set listings will be based on the enrolled set, safety listings will be based on the SAF set, and all other listings will be based on the ITT set.

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

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

Analysis sets will be summarized using the enrolled set. Study completion/withdrawal will be summarized using the ITT set. Background and demographic characteristics will be summarized using the ITT set. The primary efficacy endpoint will be summarized using the micro ITT set and the PPS. Selected secondary endpoints may be summarized using the micro ITT set and the PPS but generally will be based on the micro ITT set only. Prior and concomitant medications, administration of study drug and exposure and safety will be summarized using the SAF set.

7.6 Disposition of patients

The number and percentage of all patients enrolled, included in the SAF set, ITT set, micro ITT set and PPS, who completed the study and prematurely discontinued the study and study duration will be summarized. The number and percentage of patients will be summarized by their reasons for withdrawal from the study. In addition, the table will include the number of patients screened and the number who failed at least one inclusion or exclusion criteria. These patients will be summarized by the inclusion criteria failed. Note that patients failing screening due to a negative polymerase chain reaction (PCR) result for MSSA/MRSA carriage are not entered into the database. Therefore, a separate list of these subjects will be obtained from the interactive randomization technology system ([redacted]) and will be included in an appendix to the CSR.

For patients with a positive PCR result, eligibility for each of the analysis sets along with reasons for exclusion will be listed. Study completion/withdrawal data will be listed. Eligibility for the study, together with the details of any failed criteria will also be listed.

Study duration will be derived as (date of study completion or the date of early study withdrawal - date of first administration of study drug) +1.

7.7 Protocol Deviations

Destiny Pharma will review the individual deviations at the following intervals: monthly, prior to interim analysis, prior to database lock at Day 6 and prior to final data base lock. The review of protocol deviations will be performed whilst blinded to randomized treatment. See Section 7.4 for further details.

The number and percentage of patients with at least one important protocol deviation will be summarized. The number of patients with each important protocol deviation will be summarized for each important deviation category. Important protocol deviations are major protocol deviations considered to impact the evaluation of efficacy and lead to exclusion from the PPS.

Details of all important protocol deviations will be listed.

7.8 Background and Demographic Characteristics

7.8.1 Demography

Demographic characteristics (age, sex, ethnic origin and race) and body measurements (height, weight and body mass index (BMI)) collected at screening (Randomization Day -14 to Day -1) will be summarized. The number of patients within each country and within each site will also be summarized.

BMI is calculated as (weight (kg)/height (m)^2).

All patient demographic data including the country where the site is based will be listed. Country will be determined from the site list relevant at the time of data extraction. Informed consent data will be listed.

7.8.2 Medical History

Medical history events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary version 23.1. This was updated to address the introduction of COVID-19 specific terms. The version used will be indicated in the data summaries and listings. The number and percentage of patients 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 patients with medical history events. Ear, nose and throat (ENT), Peri-operative and Other events will be presented in separate tables. All events will be listed.

7.8.3 Surgery

Summaries will be provided of the number of patients receiving surgery and whether this surgery involved a foreign implant. The number of patients receiving any peri-operative prophylaxis interventions and the type of intervention will also be summarized. This summary will be further broken down by the time at which peri-operative prophylaxis interventions were recorded – randomization, treatment period (Day -1 to Day 0) and 48 hours after surgery.

Measurements for blood glucose and body temperature will be taken between hospital admission and Dose 5. They will be entered in to the CRF under two separate collection periods (hospital admission to Dose 4 and Dose 1 to Dose 5). The collection period of interest for the listings and summaries will be Dose 1 to Dose 5.

For each patient, the percentage of blood glucose measurements over 200 mg/dL will be calculated as:

$$100 * \frac{number\ of\ measurements\ over\ 200\ mg/dL}{number\ of\ measurements\ taken\ between\ Dose\ 1\ and\ Dose\ 5}$$

A similar calculation will be performed for the number of body temperature measurements under 35.5°c for each patient. The percentage of blood glucose measurements over 200 mg/dL and the percentage of body temperature measurements under 35.5°c will be summarized for each treatment group.

Data relating to the planned surgery (date, time and procedure), performed surgical procedure (date and time of incision, date and time of wound closure, whether the procedure involved a foreign implant), blood glucose and body temperature measurements (number of measurements taken between admission and Dose 4 and between dose 1 and dose 5 and the number of measurements over 200 mg/dL or under 35.5°c as applicable in the two periods) and the peri-operative prophylaxis interventions carried out will be listed.

7.9 Prior, Concomitant and Follow-up Period Medications

Medications will be coded using the World Health Organization Drug dictionary (WHO Drug) version 23.1. 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 drug. Medications that are either ongoing at the first administration of study drug, or started after time of first administration and on or before a patients' Day 6 visit will be deemed to be concomitant medications. If medication dates are incomplete and it is not clear whether the medication was concomitant, it will be assumed to be concomitant. Follow-up period medications are defined as those that started after a patient's Day 6 visit.

The number and percentage of patients taking concomitant medications will be summarized by medication class and standardized medication name, where medication class and standardized medication name will be presented in decreasing frequency of the total number of patients with medications. In summary tables, patients taking multiple medications in the same medication class or having the same standardized medication recorded multiple times in the study will be counted only once for that specific medication class and standardized medication name. Additional concomitant medications tables will also be summarized for patients with Peri-operative Prophylaxis Interventions and for patients with Post-operative Staphylococcal Antibiotics. These will be identified using the classification recorded in the CRF (those with 'Indication category' = Peri-operative prophylaxis or 'Indication category' = Post-operative anti-staphylococcal antibiotic). A medical review will be performed on an ongoing basis to ensure that indication categories are assigned correctly in the CRF.

Medication data will be listed, where medications will be flagged as prior, concomitant or follow-up period medications.

7.10 Administration of Study Drug and Exposure

Treatment exposure will be summarized by treatment group and will include the total number of patients and percentage of patients with each number of doses applied. Compliance will also be included in the summary table where a compliant subject is defined as having received all 5 doses of study drug all within the acceptable dosing windows.

Acceptable dosing windows for each dose are as follows:

- First dose: 22 hours ±2 hours before estimated incision time.
- Second dose: 14 hours ±2 hours before estimated incision time.
- Third dose: 6 hours ±2 hours before estimated incision time.
- Fourth dose: Within 1 hour before incision time.
- Fifth dose: Within 1 hour of incision closure time.

Details of the doses applied including the total number of doses applied will be listed.

7.11 EFFICACY EVALUATION

One average *S. aureus* CFU/mL value will be supplied for each patient at each swab collection time point. These data will be supplied on the original scale and will be log-transformed for analysis as appropriate. In the event that a *S. aureus* CFU/mL value is 0 (or no *S. aureus* has been detected), the value will be replaced with the next lowest meaningful value (1 CFU/mL) to enable the log transformation to be applied. Values between 0 and 1 CFU/mL are not expected.

For all efficacy endpoints the analyses will be performed on the micro ITT set. The primary efficacy endpoint will be repeated in the PPS.

For all analyses the baseline value is defined as the swab collected prior to the first dose of study drug at approximately 24 hours prior to surgery.

The null hypothesis is that there is no difference between the treatment means for the primary endpoint. The alternative hypothesis is that there is a difference between the two treatment means (0.2% w/w XF-73 Nasal Gel - Placebo XF-73 Nasal Gel). Denoting θ_1 and θ_2 as the mean of the response variable for each treatment group respectively, the hypotheses can alternatively be expressed as:

$$H_0: \theta_1 = \theta_2 \text{ vs. } H_1: \theta_1 \neq \theta_2$$

where H_0 is the null hypotheses and H_1 is the alternative hypothesis.

The null hypothesis of no difference in mean change between treatment groups will be assessed from the analysis of the primary endpoint. The null hypothesis will be rejected in favour of the alternative hypothesis should the probability of the test statistic, derived from observed data occurring if the null hypothesis were true (α), be less than 5% (or in other words, the two-sided p-value shows $p<0.05$).

7.11.1 Primary Endpoint

The primary endpoint of the study is the change in *S. aureus* $\log_{10}$ CFU/mL from baseline to immediately prior to surgery.

The baseline value is defined as the swab collected prior to the first dose of study drug at approximately 24 hours prior to surgery. The immediately prior to surgery value is defined as the pre-surgery swab collected up to 1 hour prior to surgery which is obtained following three doses of study drug and prior to the fourth dose of study drug.

Primary Analysis

The change in *S. aureus* $\log_{10}$ CFU/mL from baseline to immediately prior to surgery will be analyzed using an analysis of covariance (ANCOVA). The model will be fitted with the change in *S. aureus* $\log_{10}$ CFU/mL from baseline to immediately prior to surgery as the dependent variable and treatment group and country as fixed effects. The baseline *S. aureus* $\log_{10}$ CFU/mL value will be included as a covariate.

Adjusted Least square means (LS Means) for the placebo and 0.2% w/w XF-73 treatment groups estimated by the model will be presented, together with the associated standard errors (SE). The adjusted LS Mean, SE and 95% CI and two-sided p-value of the treatment difference will also be presented. All estimates provided by the ANCOVA model will be presented on the $\log_{10}$ scale.

The assumption is that the log10 CFU data are normally distributed. This assumption will be assessed using a Shapiro-Wilks' test alongside a visual inspection of the residual plots. If this assessment indicates a departure from log-normality, additional data transformations or a non-parametric analysis will be considered for further analysis post-hoc, as appropriate.

Sensitivity Analysis

In addition to the planned analysis in the PPS which requires patients to receive at least 4 doses of pre-surgery treatment, a sensitivity analysis will be performed which

will include all patients who receive at least 3 doses of pre-surgery treatment to confirm the main analysis conclusions.

A Mixed Model for Repeated Measures (MMRM) using all available data will be fitted in order to estimate the change from baseline to immediately prior to surgery. Further details on the model fitted can be found in Section 6.11.2.3 of this SAP.

Where conclusions resulting from these analyses provide substantive differences in the conclusions drawn, imputation methods may be used and additional analyses may also be considered for key secondary endpoints.

Summaries

Descriptive statistics including the geometric mean and geometric co-efficient of variation will be presented for all observed *S. aureus* CFU/mL values for each pre-surgery and post-surgery swab collected (baseline, prior to the third dose of study drug, immediately prior to surgery, within 1 hour of incision closure, 48 hours after surgery and 6 days after surgery). The change from baseline values will also be presented and summarized by CFU category. For both the observed and the change from baseline, descriptive statistics for the log-transformed values will also be presented (geometric mean and co-efficient of variation will not be presented as these are not relevant for log transformed data).

Box and whisker plots will be presented for each treatment group for the change from baseline in *S. aureus* $\log_{10}$ CFU/mL over time. The change in S. *aureus log CFU/mL* from baseline versus observed baseline S. *aureus log CFU/mL* will also be presented in a scatterplot.

The observed *S. aureus* CFU/mL and $\log_{10}$ CFU/mL values will be listed.

7.11.2 Secondary Endpoints

7.11.2.1 AUC of *S. aureus* from baseline to pre-surgery

An AUC of *S. aureus* CFU/mL will be calculated using the values collected at baseline, prior to the third dose of study drug and immediately prior to surgery. Data on the original scale will be used to determine the AUC. The AUC will be calculated using the trapezoidal method and will be $\log_{10}$-transformed for analysis.

The formula for the trapezoidal method is below:

	Baseline	**Prior to the third dose**	**Immediately prior to surgery**
Time	t_1	t_2	t_3
Response	a	b	c

$$AUC = (t_2 - t_1)\frac{(a+b)}{2} + (t_3 - t_2)\frac{(b+c)}{2}$$

AUC will only be calculated where a patient has non-missing results at both the baseline and immediately prior to surgery time points. An ANCOVA model will be fitted and presented as described in Section 6.11.1 where the baseline *S. aureus* $\log_{10}$ CFU/mL value will be included as the covariate and treatment group and site as fixed effects. If there is evidence that there is a departure from the assumptions of normality alternative transformations such as AUC of $\log_{10}$ S. *aureus* CFU/mL will be considered.

7.11.2.2 AUC of *S. aureus* from baseline to post-surgery

An AUC of *S. aureus* CFU/mL including values at all timepoints from baseline to within 1 hour of incision closure will be calculated. This AUC will include the following time points: at baseline, prior to the third dose, immediately prior to surgery and within 1 hour of incision closure. The AUC will be calculated as described in section 6.11.2.1 using the trapezoidal method where the equation will be expanded to include the appropriate post-surgery time point as:

$$AUC = (t_2 - t_1)\frac{(a+b)}{2} + (t_3 - t_2)\frac{(b+c)}{2} + \ldots + (t_n - t_{n-1})\frac{(r_{n-1}+r_n)}{2}$$

where t_n and r_n are the time and the response at the post-surgery timepoint respectively. The AUC will be $\log_{10}$-transformed for analysis. AUC will only be calculated where a patient has non-missing results at all of the following time points: baseline, immediately prior to surgery and within 1 hour of incision closure.

An ANCOVA model will be fitted and presented as described in Section 6.11.1 where the baseline *S. aureus* $\log_{10}$ CFU/mL value will be included as the covariate and treatment group and site as fixed effects.

Summaries

Descriptive statistics will be presented for the pre-surgery AUC and the post-surgery AUC.

Box and whisker plots will be presented for each treatment group for the AUC of *S. aureus* $\log_{10}$ CFU/mL over time.

The calculated AUC values will be listed on both the observed and $\log_{10}$ scale.

7.11.2.3 Change in *S. aureus* $\log_{10}$ CFU/mL from baseline to post-surgery

The change in *S. aureus* $\log_{10}$ CFU/mL between baseline and post-surgery will be assessed at each of the post-surgery time points (within 1 hour of incision closure, 48 hours after surgery and 6 days after surgery) using an MMRM with visit, treatment group, site and the treatment group by visit interaction as fixed effects, and the baseline *S. aureus* $\log_{10}$ CFU/mL value as a covariate. F-tests from PROC MIXED will be based on Kenward-Roger's adjusted degrees of freedom. The model will include data from all pre and post-surgery visits.

To select the best model, the following variance/covariance matrix structures for the repeated visits within a subject will be assessed: Compound symmetry, 1st order autoregressive, Toeplitz and unstructured. The variance/covariance matrix structure that results in the smallest Akaike information criterion (AIC), indicating the best model fit will be selected.

Results from the MMRM, for the estimate of the effect of each post-surgery time point, will be presented as described in Section 6.11.1. Results from the MMRM, for immediately prior to surgery will also be presented as a sensitivity analysis for the primary analysis (see section 6.11.1).

7.11.2.4 Patients Reaching a Specific Reduction in *S. aureus* Carriage

For each patient, the $\log_{10}$ CFU/mL *S. aureus* values obtained prior to third dose, immediately prior to surgery, within 1 hour of incision closure, 48 hours after surgery and 6 days after surgery will each be subtracted from their baseline $\log_{10}$ CFU/mL *S.*

aureus value to determine the reduction in their log_{10} CFU/mL *S. aureus* nasal carriage.

A plot of the proportion of patients with a range of specific reductions in log_{10} CFU/mL *S. aureus* nasal carriage from baseline will be presented for each treatment group for each time point of interest. Cut-offs in intervals of 0.5 log_{10} CFU/mL will be considered in the first instance, but other intervals will be considered as appropriate. The plot will present the percentage of patients with a reduction greater than or equal to the cut-off. Treatment groups will be represented by different symbols and line style. The reductions at the time-points of interest will be presented on separate plots. A supporting table containing the proportion of patients with a range of specific reductions in log_{10} CFU/mL *S. aureus* nasal carriage presented by treatment group and time point will also be produced.

7.11.2.5 Proportion of patients with nasal carriage below a specific cut-off

The proportion of patients with a nasal carriage of log_{10} CFU/mL *S. aureus* considered in intervals of 0.5 will be presented using methods described in section 6.11.2.4, presenting the percentage of patients below the cut-off. Alternative intervals will be used as appropriate.

7.11.2.6 Nasal carriage according to semi-quantitative categorization

A semi-quantitative categorization will be used to categorize *S. aureus.* nasal carriage. The categories are:

S. aureus nasal carriage (CFU/mL)	Semi-Quantitative Scale Category
0	Negative
1-10	0
>10-100	1
>100-1,000	2
>1,000-10,000	3
>10,000-100,000	4
> 100,000	5

The category for each *S. aureus* nasal carriage value at all time points will be listed.

7.11.2.7 Proportion of patients with a 2-log drop in *S. aureus*

A 2-log drop in log_{10} CFU/mL *S. aureus* is defined as a value which is 100 times smaller than their baseline *S. aureus* value. The 2-log drop in *S. aureus* will be determined from the values obtained from prior to the third dose through to 6 days after surgery.

The percentage of patients with a 2-log drop in log_{10} CFU/mL *S. aureus* will be summarized.

A flag will be added to the listing of *S. aureus* data to indicate patients identified as having a 2-log drop in *S. aureus* nasal carriage.

7.11.2.8 Difference in *S. aureus* Post-operative Infections

The number of patients experiencing post-operative infections will be calculated from study treatment to 30 days after surgery (or 90 days after surgery in the case of an object implant). Post-operative infections (as recorded on the 'Post-Operative

Infection' CRF page) include, but are not limited to, surgical site infection, bloodstream infections, catheter-related infections and implant infections. Descriptive statistics for the number of post-surgery days per patient will also be presented. For patients who have not reached 30 days after surgery at the time of analysis (or 90 days after surgery in the case of an object implant), the number of post-surgery days per patient will be calculated as (date of early study withdrawal – date of surgery) + 1. The number and percentage of subjects not reaching 30 days (or 90 days in the case of an object implant) will also be presented.

The number and percentage of patients with post-operative infections will be presented for each treatment group together with the severity of these infections and the bacterial strains identified.

All post-operative infection data will be listed.

7.11.3 Exploratory Endpoints

7.11.3.1 Post-operative Antibiotic Use

The reason for post-operative prescribed anti-staphylococcal antibiotic use during the 30-day post-operative period (90 days in the case of an object implant) will be summarized by treatment group.

The reason for the post-operative antibiotic use will be obtained from the indication field of the concomitant medication page of the CRF. A medical review will be performed on an ongoing basis to ensure that indication field in the CRF is correct. The number and percentage of patients within each category will be presented in a summary table and categories assigned will be included in the medication listing. Duration of post-operative staphylococcal antibiotics will also be summarized. Where a patient took more than one post-operative staphylococcal antibiotic medication, the total duration of all post-operative staphylococcal medications taken by the patient will be summarized.

7.11.3.2 Change in *S. aureus* log CFU/mL following surgery

The change in *S. aureus* $\log_{10}$ CFU/mL from within 1 hour of incision closure will be described graphically to identify any rebound effects.

A plot of the mean *S. aureus* $\log_{10}$ CFU/mL with error bars will be presented over time for each treatment group. The plot will display both treatment groups on the same graph. Each pre-surgery and post-surgery time point (baseline, prior to the third dose of study drug, immediately prior to surgery, within 1 hour of incision closure, 48 hours after surgery and 6 days after surgery) will be included to provide a complete depiction of the S. aureus $\log_{10}$ CFU/mL values over time.

Additionally, a plot of the individual patient data for S. aureus $\log_{10}$ CFU/mL will be presented over time. A separate plot will be produced for each treatment group with each patient represented by a separate line on the same plot. Each pre-surgery and post-surgery time point (baseline, prior to third dose, immediately prior to surgery, within 1 hour of incision closure, 48 hours after surgery and 6 days after surgery) will be included.

7.12 Multiplicity

All secondary endpoints will be considered as descriptive evidence of efficacy and will be analyzed without any procedures to account for multiple comparisons.

7.13 SAFETY EVALUATION

The safety and tolerability of 0.2% w/w XF-73 will be assessed based on adverse events, nasal examinations, brief smell identification test and safety evaluations including clinical laboratory evaluations and vital signs.

7.13.1 Adverse Events

Adverse events (AEs) will be coded using the MedDRA dictionary version 23.1. The version used will be indicated in the data summaries and listings.

AEs will be collected between screening and 6 days after surgery.

An AE occurring after written screening consent has been obtained but before the first dose of study drug, the AE will be considered a non-treatment emergent AE. These events will be reported in the medical history summaries.

A TEAE is defined as an AE that occurs from the time the patient receives their first dose of the study drug until the patient's Day 6 visit, or for serious AEs (SAEs) considered related to study drug, until the final follow-up visit. Note only AEs meeting the serious SAE criteria and considered related to study drug will be recorded between the Day 6 visit and the final follow up visit (Day 30 or Day 90 in the case of an object implant).

If a subject discontinues treatment but has an AE occurring from the time of first dose and on or prior to Day 6 (Date of first dose (Day 0) + 6 days) then this will be considered a TEAE. Any AE occurring after Day 6 for a discontinued subject will not be considered treatment-emergent unless it meets the SAE criteria.

If adverse event dates are incomplete and it is not clear whether the adverse event was treatment-emergent, it will be assumed to be treatment-emergent. If the TEAE has a missing relationship it is assumed to be related to the study drug for analysis purposes.

A summary table will present the following:

- TEAEs (number of events and number of patients).
- Serious TEAEs (number of events and number of patients).
- Serious study drug-related TEAEs (number of events and number of patients).
- TEAEs by severity (mild/moderate/severe) (number of events and number of patients).
- TEAEs by relationship to study drug category (Not related/Related) (number of events and number of patients).
- TEAEs leading to withdrawal of study drug (number of patients only).
- Study drug-related TEAEs leading to withdrawal of study drug (number of patients only).
- TEAEs leading to death (number of patients only).

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

The following tables will be presented for events and patients:

1. TEAEs by System Organ Class (SOC) and Preferred Term (PT).
2. TEAEs by SOC and PT –ENT flagged events (as indicated on the 'Adverse Events' CRF page).
3. TEAEs by PT.
4. TEAEs by SOC, PT and severity.
5. TEAEs by SOC, PT and relationship to study drug.

For all of the above, SOC and PT will be presented in decreasing frequency of the total number of patients in the 0.2% XF-73 nasal gel treatment group with TEAEs.

Further details of the above five tables are given below:

1. If a patient experienced more than one TEAE, the patient will be counted once for each SOC and once for each PT.
2. If a patient experienced more than one ENT TEAE, the patient will be counted once for each SOC and once for each PT.
3. If a patient experienced more than one TEAE, the patient will be counted once for each PT.
4. If a patient experienced more than one TEAE, the patient will be counted once for each SOC and once for each PT at the worst severity.
5. If a patient experienced more than one TEAE, the patient will be counted once for each SOC and once for each PT using the most related event.

Adverse event data will be listed in full and this will also include a treatment emergent flag, an ENT finding flag, the time of onset and cessation of event relative to first dosing of study drug and duration of AE.

7.13.2 Clinical Laboratory Evaluation

Observed values and change from baseline in hematology, biochemistry and urinalysis assessments will be summarized over time. If the test results are reported in categorical format, the results will be summarized by patient counts and percentage for each category.

Each hematology and biochemistry parameter will be classed as low, normal, high or missing based on the reference ranges. Shift tables in relation to the normal range from baseline over time will be presented.

Hematology, biochemistry and urinalysis data will be listed separately including change from baseline, reference ranges flagging all out of range values and their clinical significance.

Scatterplots of key hematology parameters (Hemoglobin, White blood cell count, Neutrophils count, Lymphocyte count and Platelets) and key biochemistry parameters (Creatinine, Alanine aminotransferase, Aspartate aminotransferase, Total bilirubin and Alkaline phosphatase) will be produced, with measured value appearing on the y-axis and time (days since first administration of study drug) on the x-axis. The relevant reference range will be overlaid on the plot as a reference line. Parameters will be presented in the same order as the CRF.

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

7.13.3 Vital Signs

Vital sign observed values and change from baseline by parameter will be summarized over time.

Parameters will be presented in the same order as the CRF.

All vital sign data will be listed including change from baseline and flags for substantial changes from baseline and reference ranges flagging all out of range values.

7.13.4 Physical Examination

Details of timings of physical examinations will be listed.

7.13.5 Pregnancy Test

Pregnancy test details will be listed.

7.13.6 Nasal Examination

Nasal examinations will be carried out by an ear nose and throat (ENT) specialist at Baseline and 48 hours after surgery.

The number of patients with a clinically significant nasal examination will be summarized at each time point by treatment group.

All data from the nasal examinations will be listed.

7.13.7 Brief Smell Identification Test™ (B-SIT)

The Brief Smell Identification Test (B-SIT) is a measure of olfactory function which is self-administered in a 'scratch and sniff' format. An accompanying questionnaire has 4 possible options for each of the 12 odorants tested. If the patient does not perceive an odor a response must still be entered.

A patient scores 1 point for every correctly identified scent and the overall score is derived as the total correct scents identified out of 12, with higher scores indicating a better olfactory performance.

The percentile rank of the score is determined using the B-SIT interpretation matrix which is divided into age groups and differs between the sexes. This percentile rank is then used by the investigator to attribute the patient to one of the following three categories: abnormal relative to age, deficit relative to younger persons and normal. The data will be presented as collected on the CRF. If the score is so low that the percentile rank is not quantifiable the percentile and the category will both be entered as 'below percentile rank'.

B-SIT data will be summarized using shift tables, in relation to the normal category, from baseline over time.

A medical review will be performed on an ongoing basis in the CRF to identify medical history and adverse events linked to subjective impact on smell. The baseline B-SIT score will be compared to any smell related medical history events (those with 'Was this medical history an ENT finding?' = Yes) and the Day 6 B-SIT score will be compared to any smell related TEAEs (those with 'Was this event an ENT finding?' = Yes).

All B-SIT data will be listed including change from baseline for the B-SIT score.

7.14 Changes from the Protocol Planned Analysis

For the analysis of the change in log10 CFU from baseline to post-surgery an MMRM model has been chosen in favor of the ANCOVA specified in protocol section 11.7.

The AUC of S. aureus from baseline to pre-surgery will be calculated using values on the original scale and will then be log_{10}-transformed, as opposed to calculating the AUC on the log_{10}-transformed values as described in protocol section 11.7.

The proportional odds model described in protocol section 11.7 for the nasal carriage according to SQS has been removed. Summary statistics are deemed to be sufficient.

Any reference to the modified Intent-to-Treat (mITT) set has been removed from the SAP as analysis will no longer be performed in this population.

The major protocol deviations leading to exclusion from the PPS has been clarified in Section 7.4. Major protocol deviations are defined more broadly in the protocol, so include deviations which are important to report, but would not impact the evaluation of efficacy. Therefore, the SAP has made clear that only those major protocol deviations considered to impact the evaluation of efficacy (i.e. deemed important) will be excluded from the PPS.

7.15 References

1. Verhoeven PO, Grattard F, Carricajo A, Lucht F, Cazorla C, Garraud O, Pozzetto B, Berthelot P. An algorithm based on one or two nasal samples is accurate to identify persistent nasal carriers of Staphylococcus aureus. Clin Microbiol Infect. 2012 Jun;18(6):551-7. doi: 10.1111/j.1469-0691.2011.03611.x.