STATISTICAL ANALYSIS PLAN

Nova Laboratories Limited

Relative bioavailability and comparative pharmacokinetics of 13-CRA oral liquid and extracted capsule formulation: a randomised, open label, multi-dose, cross-over clinical trial in patients requiring treatment cycles of 13-CRA.

Protocol: INV500

The author's signature indicates the document is no longer a draft and is ready for approval.

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The following signatures indicate the person signing has reviewed this document and approved it for use.

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1 Abbreviations

Abbreviation	Meaning
%CV	Coefficient of Variation
AE	Adverse Event
ATC	Anatomical Therapeutic Chemical
AUC	Area under the Plasma Concentration-Time Curve
BMI	Body Mass Index
CL/F	Clearance
Cmax	Maximum Plasma Concentration
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
HRNBL	High Risk Neuroblastoma
IMP	Investigational Medical Product
MedDRA	Medical Dictionary for Regulatory Activities
NBL	Neuroblastoma
NCI	National Cancer Institute
PK	Pharmacokinetics
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOC	System Organ Class
TEAE	Treatment Emergent Adverse Event
T1/2	Half Life
Tmax	Time to Maximum Concentration
V/F	Volume of Distribution
WHO	World Health Organization

2 Version Control

This Statistical Analysis Plan (SAP) is based on the protocol version 5.0 dated 08 December 2017 and Case Report Form (CRF) version 2 dated 26 March 2018.

The population pharmacokinetic (PK) analysis detailed in the protocol is not within the scope of this SAP.

3 Study Rationale

13-CRA is administered during the remission phase in the treatment of high risk neuroblastoma (HRNBL) between cycles of immunotherapy and also in patients with unresectable, intermediate risk neuroblastoma with unfavourable INPC histology and in patients with relapsed and refractory disease who have not already received 13-CRA. It is administered orally, currently using the capsule form licensed for severe acne.

It is clear that many young children will not take solid medicines (tablets, capsules). Potential solutions to this issue include developing a liquid for parenteral use, a medicine to be administered by the rectal route

and a transdermal drug. While there are other potential solutions, the oral liquid preparation meets important criteria for this medicine including flexible dosing, ease of use, minimal additional risk to patient and carer and patient comfort.

The dose administered is in accordance with approved practice for 13-CRA extracted capsules. This is based on previous studies which have shown that the dose obtained from extracted capsules needs to be adjusted to 200mg/m^2/day in order to achieve the same blood level as that seen for capsules that have been swallowed whole. Therefore the dose used in this trial will be 200mg/m^2/day for both test and reference product. Patients with a body weight of ≤12kg will receive a dose of 160 mg/m^2.

The pharmacokinetics of 13-CRA liquid (test product) and extracted capsule (reference product) will be evaluated in over a two month period to allow for two cycles of treatment in a cross over design (one test treatment period and one reference treatment period). The 14-day treatment period and two week break between treatment cycles is based upon previous studies and is the standard treatment period for cycles of 13-CRA. If patients require a longer period of time between treatments then this will be acceptable within the visit windows (i.e. treatment period 2 will be within 14 +up to 7 days of completion of treatment period 1).

4 Objectives

The aim of this trial is to investigate the PK, safety, tolerability and palatability of oral liquid 13-CRA in patients aged from 0 months – < 21 years.

4.1 Primary Objective

- To determine the relative bioavailability and PK of 13-CRA administered as oral liquid and extracted capsule formulations

4.2 Secondary Objectives

- To determine the safety and tolerability
- To assess palatability and acceptability

5 Endpoints

5.1 Primary Endpoint

Primary endpoints are:

Primary parameters:

- Relative bioavailability
- Clearance (CL/F)
- Volume of distribution (V/F)

Secondary parameters:

- Time to maximum concentration (T_{max})
- Maximum plasma concentration (C_{max})
- Area under plasma concentration time curve (AUC)
- Half life ($t_{1/2}$)
- Levels of the metabolite 4-oxo-13-cis-RA

5.2 Secondary Endpoints

Safety parameters:

- Evaluate the local (oro-pharyngeal) tolerability
- Skin toxicity
- Hypercalcaemia (noted as adverse events if intervention required)
- Hepatoxicity (noted as adverse events if intervention required)
- Triglycerides (noted as adverse events if intervention required)

Palatability and acceptability parameters:

- Evaluate the taste acceptability of the new oral formulation of 13-CRA

6 Study Design

This is an open label, randomised, multiple dose, cross-over relative bioavailability and PK trial of oral 13–CRA liquid administered to patients aged from 0-< 21 years. The decision to initiate 13-CRA therapy will be taken independently of the trial protocol. The trial will be conducted in patients who are attending the hospital for treatment for neuroblastoma requiring at least two cycles of 13-CRA. 13–CRA will be prescribed to patients according to the locally approved treatment protocols at a dose of 200mg/m^2/day for both test and reference product. Oral 13–CRA is administered for neuroblastoma (NBL) (this is usually at a dose of 160mg/m^2) for 14 days, every 4 weeks, for 6 months, however where the dose is extracted from capsules this dose is increased to 200mg/m^2. In this trial the dose of 13-CRA will be 200mg/m^2 as patients able to swallow the capsules will not be recruited to the trial. Patients with a body weight of ≤12kg will receive a dose of 160 mg/m^2. Patients who require their dose through a nasogastric tube or gastromy will be included in the trial, this will be recorded in the case record form.

Evaluation of the test and reference product will occur during two months of therapy.

Consent to take part in the trial will be obtained before any trial procedures are undertaken. Patients who are already receiving 13-CRA treatment will also be eligible for the trial, so long as they are able to commit to two cycles of the trial. The next planned 14-day treatment cycle will be classed as My-CRA Month 1. After consent to take part has been obtained and prior to the initiation of the next 14 day treatment patients will be randomised to receive either 13-CRA liquid (test product) or liquid extracted from capsule (reference product) in My-CRA Month 1. Randomisation will be done once eligibility has been confirmed and their next scheduled treatment visit is booked, in order that the appropriate medication can be made available for the patient's visit. Randomisation will be done centrally by Nova according to a randomisation list generated by the trial statistician, investigators will email (or telephone) Nova to receive randomisation details, this will be confirmed by email as "liquid first" or "extracted capsules first", pharmacy contacts will also be notified. After My-CRA month 1, patients will then cross over to the alternative formulation in My-

CRA Month 2. In all other treatment cycles patients will revert to standard therapy i.e. liquid extracted from capsules. The pharmacokinetics of 13-CRA will be evaluated during test and reference treatments (My-CRA Month 1 and 2).

When the patient attends hospital for initiation of 13-CRA treatment, it is anticipated that they will have a central line *in situ*. Patients will attend the clinic on Pharmacokinetic days; these will be Day 1 and Day 14 for each of the trial treatment periods. Blood samples for pharmacokinetic analysis will be taken over the course of six hours after which the patients will leave the clinic. Parents and patients will be asked if they are willing to return for an additional sample at 24-48 hours following their final dose on Day 14 of treatment.

7 Statistical Considerations

7.1 Sample Size

Up to 20 patients, both male and female, aged 0 – < 21 years of age will be recruited.

There are no power calculations in this trial. The objective of this trial is to estimate the population pharmacokinetics of 13-CRA following the administration of 13-CRA liquid (test product) and extracted capsule (reference product). From a statistical point of view, the collected plasma 13-CRA concentration data are expected to relate to a non-linear, mixed effects model involving repeated measures. The developed pharmacokinetic model will estimate the parameters of the model and their associated within- and between-patient variability.

In order to develop a robust population pharmacokinetic model (i.e. precisely estimated parameters), data from a minimum of 150 blood samples will be required. It is hoped that the required number of samples may be obtained from 12-18 patients. However, if problems are encountered in obtaining samples further patients may be included in the trial.

7.2 Randomisation

Prior to the initiation of the trial 13-CRA, patients will be randomised to receive either liquid or extracted capsule formulation in "My –CRA month 1". The patients will then cross-over to the alternative formulation in "My-CRA month 2". After the trial if a patient requires additional cycles of 13-CRA, the patient will revert to standard 13-CRA therapy i.e. extracted capsules according to local practice.

Randomisation will be done centrally by Nova according to a randomisation list generated be the trial statistician, investigators will email (or telephone) Nova to receive randomisation details, this will be confirmed by email as "liquid first" or "extracted capsules first", pharmacy contacts will also be notified.

8 Major Protocol Deviations

Protocol violations are any deviations from the procedures outlined in the protocol, for example, missed evaluations, incorrect timing of evaluations, non-compliance with investigation medical product (IMP) and intake of prohibited medications. It is the investigator's responsibility to make all reasonable efforts to avoid protocol violations in order to avoid possible exclusion of the patient from the trial and/or analyses.

Major protocol deviations are defined as those deviations from the study protocol that may have the ability to impact the study results.

Major protocol deviations include (but are not limited to):

- Deviation from inclusion/exclusion criteria
- Withdrawal criteria met during the study but patient was not withdrawn
- Missed evaluations
- Incorrect timing of evaluations
- Prohibited concomitant medications
- Non-compliance with IMP

Where possible; major deviations will be programmatically generated. Listings of concomitant medications will be provided to the sponsor for review to identify prohibited medications prior to database freeze.

Major protocol deviations will result in exclusion from the pharmacokinetic population if they are considered likely to affect the determination of the pharmacokinetic parameters.

9 Analysis Populations

9.1 Pharmacokinetic Population

The pharmacokinetic population will include those patients who had at least one successful blood sample taken and complete the trial without significant protocol deviations/violations which are likely to affect the determination of the pharmacokinetic parameters. This population will be used for the analysis of the primary variable.

9.2 Safety Population

The safety population is defined as all patients who receive trial medication. This population is the primary population for all safety summaries.

10 Data Reporting Conventions

10.1 Descriptive Statistics

Unless otherwise stated, all continuous parameters will be summarized using standard summary statistics as appropriate (n, mean, standard deviation [SD], coefficient of variation [%CV], minimum, median and maximum). Summary statistics for categorical variables will include frequency counts and percentages.

In the presentation of descriptive summary statistics, the minimum and maximum will be presented to the same number of decimal places as the variable being reported. The mean and median will be reported to one extra decimal place; the SD to two extra decimal places.

Frequency counts will be provided for categorical variables (e.g., gender). Unless otherwise stated, this will consist of the number of patients in a particular category and the percentage of the total number of patients per dose group, presented to one decimal place.

Analyses will be performed using the validated statistical software SAS version 9.4 or higher.

10.2 Missing Data

Data will be presented as recorded in all listings. If there are partial dates which require imputation for calculations, the day and/or month and/or year will be imputed in a conservative manner i.e. for the start dates, if only the day is missing, it will be imputed with the first day of the month and if the month is also missing, it will be imputed with the 1st January. For the end dates, if only the day is missing, it will be imputed with the last day of the month and if the month is also missing, it will be imputed with the 31st December.

No further imputations for missing data will be made.

11 Patient Disposition and Baseline Information

11.1 Patient Disposition

Study completion and discontinuation details, eligibility, treatment schedule allocation, population assignment, and protocol deviations will be listed.

The number of patients screened and treated, and the primary reason for study treatment discontinuation will be tabulated by treatment sequence.

The number and percentage of patients in each analysis population will be tabulated by treatment sequence.

The number and percentage of patients with major protocol deviations by treatment sequence will be tabulated.

11.2 Baseline and Demographic Characteristics

Demographic parameters will include age, gender, race, height (cm), weight (kg) and body mass index (BMI) (kg/m^2). Demographics will be listed and tabulated by treatment sequence using descriptive statistics.

11.3 Medical History

Details of non-oncology medical history will be listed including condition, start and stop dates, and whether any medication was taken. Partial dates may be recorded and no imputations will be made for missing or partial dates in the listing.

Oncology history data will also be listed, including start and stop dates, and whether any medication was taken. Partial dates may be recorded and no imputations will be made for missing or partial dates in the listing.

Medical histories will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) dictionary version in effect at the time of study activation. A summary of non-oncology and oncology medical histories and number of patients with a medical history will be presented by treatment sequence, system organ class and preferred term.

11.4 Concomitant Medications

Concomitant medications recorded during the study will be coded using eMIMS. Details of concomitant medication will be listed.

Concomitant medications will be summarized according to whether they were being taken pre-study (prior medications) and/or during the study (concomitant). Prior medications are any that were being taken prior to the first dose of study medication (medication stop date prior to the first dose of study medication). Concomitant medications are any that were being taken on or after the first dose of study medication. If the start and stop dates of the concomitant medications do not clearly define the period during which a medication was taken, it will be assumed to be a concomitant medication.

Prior and concomitant medications will be summarized by treatment sequence, system class and sub-class.

12 Pharmacokinetic Assessments

The population PK analysis detailed in the protocol is not within the scope of this SAP.

Blood samples for measurement of 13-CRA and its metabolite 4-oxo-13-cis-RA will be obtained over the course of 6 hours after each dose. An additional sample 24-48 hours after day 14 will be obtained if possible.

Plasma concentrations will be expressed in ng/mL. All concentrations below the limit of quantification or missing data will be labelled as such in the concentration data listings. Concentrations below the limit of quantification will be treated as zero in summary statistics. Plasma concentrations will be listed and summarised by treatment group, day, and nominal time. In addition, individual plasma concentration-time plots, and mean plasma concentration-time plots by dose will be provided.

PK parameters will be listed. Additionally, PK parameters will be summarised by treatment group and day.

13 Safety

Safety will be assessed from records of adverse events (AEs), oro-pharyngeal tolerability, vital signs and results of physical examination.

13.1 Treatment Exposure

Details of study drug administration will be listed.

The total dose received for each treatment, and time on treatment will be summarized by treatment, and listed.

Time on treatment for each treatment will be defined as last dose date for that treatment – first dose date for that treatment +1.

13.2 Adverse Events

AEs will be coded according to the latest version of the MedDRA dictionary. The severity of AEs will be coded according to the National Cancer Institute (NCI)-Common Terminology Criteria for Adverse Events (CTCAE) coding system version 4.03.

AEs will be deemed treatment emergent if they start on or after the time of the first dose of study medication.

All treatment emergent adverse events (TEAEs) and serious adverse events (SAEs) will be listed.

An overall adverse event summary will be presented for the categories: any TEAEs, any drug-related AEs, any Grade 3 or greater AEs, any Grade 3 or greater drug-related AEs, any TEAEs leading to death, any drug-related TEAEs leading to death, any serious TEAEs, any drug-related serious TEAEs, any TEAEs leading to discontinuations, and any drug-related TEAEs leading to discontinuations. Both number of events and number of patients will be tabulated. For patients, percentages will be calculated from the total number of patients per treatment.

Summaries of TEAEs and serious TEAEs will be tabulated by system organ class and preferred term sorted by decreasing frequency within system organ class (SOC). Both number of events and number of patients will be tabulated. For patients, percentages will be calculated from the total number of patients per treatment.

In addition, TEAES and serious TEAEs will be summarized by maximum severity, system organ class and preferred term, and strongest relationship, system organ class and preferred term. If a patient experiences an adverse event more than once the event with the worst severity or at the most related to IMP occurrence will be considered. Patients will be included only once at each level where they experienced one or more events.

Oro-pharyngeal tolerability and symptoms will be separately assessed. Dry skin, peeling skin, cracked lips and dry eyes will be assessed by the investigator on a four point scale (0 = none, 1 = mild, 2 = moderate, and 3 = severe). Oro-pharyngeal symptoms will be assessed at baseline and following administration of 13-CRA during the Pharmacokinetic Day 14. Each symptom will be summarised by treatment and study day, presenting frequency counts and percentages for each value on the scale.

13.3 Pregnancy Test

A null listing will be presented for pregnancy test results , as this will not be applicable for patients in this study.

13.4 Vital Signs

Vital signs will be collected at Day 1 and Day 14 of each treatment. Baseline vital signs will be determined as those at Day 1 for each treatment.

The result and change from baseline of each treatment in vital signs parameters (systolic and diastolic blood pressure, pulse and temperature) will be listed and summarized descriptively for each scheduled time-point during the study.

13.5 Physical Examination

Physical examinations data will be listed.

Visit dates will be listed.

14 Palatability

At the end of each of the two treatment cycles patients (together with their parents) will be surveyed using a standard questionnaire for their views of the palatability and acceptability of the 13-CRA. In the case of patients <6 years of age, the researcher will survey the view of one or both parents. For patients from 6 years of age, the researcher will seek the views of one or both parents and the patient.

The questionnaire will probe the taste and general acceptability (willingness to continue with treatment, ease of dose administration etc.). After five patients have completed the survey the use of the questionnaire will be reviewed, by both the investigator and Nova, to ensure that it is working satisfactorily.

Data on the following will be collected using a visual (hedonic) analogue scale and verbal responses:

- Taste on first administration
- Residual after taste
- Smell
- Any incidences of spitting medicine out or vomiting
- Willingness to take 13-CRA on a daily basis
- Ease of dose administration using the bottle and oral syringe
- Preference between extracted capsule and liquid medicines

All palatability data will be listed. Each question recorded on a VAS scale will be summarised by treatment, presenting summary statistics for the length of line. Each categorical question will be summarised by treatment, presenting frequency counts and percentages

15 Interim Analysis

There is no planned interim analysis.

16 Changes to Planned Methodology

It was planned in the protocol to summarise tables by age group (1-11 years, 12-<21 years). However as all patients in the study were under 12, all patients were summarised together in a "total" treatment group.

17 Tables, Figures and Listings

Tables and listings will be presented landscape. A font size of 8 and a font of courier new will be used throughout for tables and listings. Tables and listings will be provided as a bookmarked pdf. Figures will be provided as individual files.

The patient subset being utilized in the table will be indicated in the table title. Page numbers and dates of production will be included in an appropriate place at the bottom of the output. Listings which support the table will also be detailed in an appropriate place at the bottom of the output.

Tables will only be generated where there is sufficient data to warrant the inclusion of the table.

17.1 List of Tables

Table Number	Table Title	Population
Table 14.1.1	Patient Disposition	Safety Population
Table 14.1.2	Major Protocol Deviations	Safety Population
Table 14.1.3	Demography	Safety Population
Table 14.1.4	Oncology Medical History	Safety Population
Table 14.1.5	Non-Oncology Medical History	Safety Population
Table 14.1.6	Prior Medications	Safety Population
Table 14.1.7	Concomitant Medications	Safety Population
Table 14.2.1	Summary of 13-CRA Plasma Concentrations	PK Population
Table 14.2.2	Summary of 4-oxo-13-cis-RA Plasma Concentrations	PK Population
Table 14.2.3	Summary of 13-CRA Pharmacokinetic Parameters	PK Population
Table 14.3.1	Study Drug Exposure	Safety Population
Table 14.3.2.1	Overall Adverse Event Summary	Safety Population
Table 14.3.2.2	Treatment Emergent Adverse Events by System Organ Class and Preferred Term	Safety Population
Table 14.3.2.3	Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Maximum Severity	Safety Population
Table 14.3.2.4	Treatment Emergent Adverse Events by System Organ Class, Preferred Term and Strongest Relationship	Safety Population
Table 14.3.2.5	Treatment Emergent Serious Adverse Events by System Organ Class and Preferred Term	Safety Population
Table 14.3.2.6	Treatment Emergent Serious Adverse Events by System Organ Class, Preferred Term and Maximum Severity	Safety Population
Table 14.3.2.7	Treatment Emergent Serious Adverse Events by System Organ Class, Preferred Term and Strongest Relationship	Safety Population

Table Number	Table Title	Population
Table 14.3.2.8	Oro-pharyngeal Tolerability	Safety Population
Table 14.3.3	Summary of Result and Change from Baseline Value for Vital Sign Parameters	Safety Population
Table 14.3.4	Summary of Palatability Questions	Safety Population

17.2 List of Figures

Figure Number	Figure Title	Population
Figure 14.2.4	Individual 13-CRA Plasma Concentration-Time Curves	PK Population
Figure 14.2.5	Individual 4-oxo-13-cis-RA Plasma Concentration-Time Curves	PK Population
Figure 14.2.6	Mean 13-CRA Plasma Concentration-Time Curve	PK Population
Figure 14.2.7	Mean 4-oxo-13-cis-RA Plasma Concentration-Time Curve	PK Population

17.3 List of Listings

Listings will be sorted by treatment sequence, patient number and treatment.

Listing Number	Listing Title	Population
Listing 16.2.1.1	Patient Disposition	All Patients
Listing 16.2.1.2	Informed Consent / Randomisation	All Patients
Listing 16.2.1.3	Pre-Trial Eligibility	All Patients
Listing 16.2.1.4	Pre-Treatment Eligibility	All Patients
Listing 16.2.2	Major Protocol Deviations	All Patients
Listing 16.2.3	Population Allocation	All Patients
Listing 16.2.4.1	Demography	All Patients
Listing 16.2.4.2	Oncology Medical History	All Patients
Listing 16.2.4.3	Non-Oncology History	All Patients

Listing Number	Listing Title	Population
Listing 16.2.4.4	Prior and Concomitant Medications	All Patients
Listing 16.2.5.1	Study Drug Administration	All Patients
Listing 16.2.5.2	Overall Study Drug Exposure	All Patients
Listing 16.2.6.1	13-CRA Plasma Concentrations	All Patients
Listing 16.2.6.2	4-oxo-13-cis-RA Plasma Concentrations	All Patients
Listing 16.2.6.3	13-CRA Pharmacokinetic Parameters	All Patients
Listing 16.2.7.1	Adverse Events	All Patients
Listing 16.2.7.2	Serious Adverse Events	All Patients
Listing 16.2.7.3	Oro-pharyngeal Tolerability	All Patients
Listing 16.2.8.1	Pregnancy Tests	All Patients
Listing 16.2.9.1	Vital Signs	All Patients
Listing 16.2.9.2	Physical Examination	All Patients
Listing 16.2.9.3	Visit Dates	All Patients
Listing 16.2.9.4	Palatability Data	All Patients