STATISTICAL ANALYSIS PLAN

Study Protocol Number:	E7389-G000-223
Study Protocol Title:	A Phase 2, multicenter, open-label study to assess safety and preliminary activity of eribulin mesylate in pediatric subjects with relapsed/refractory rhabdomyosarcoma (RMS), non-rhabdomyosarcoma soft tissue sarcoma (NRSTS) and Ewing sarcoma (EWS)
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2 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
AE	adverse event
ATC	anatomical therapeutic chemical
BOR	best overall response
BSA	body surface area
CI	confidence interval
CR	complete response
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DOR	duration of response
ECG	electrocardiogram
EWS	Ewing sarcoma
FAS	full analysis set
FDA	Food and Drug Administration
IV	intravenous
LLT	lower level term
MedDRA	Medical Dictionary for Regulatory Activities
NRSTS	non-rhabdomyosarcoma soft tissue sarcoma
ORR	objective response rate
OS	overall survival
PAS	pharmacokinetic analysis set
PD	progressive disease
PFS	progression-free survival
PK	pharmacokinetic
PR	partial response
PT	preferred term
QT	QT interval is the time between the start of the Q wave and the end of the T wave in the heart's electrical cycle
QTc	QT interval corrected for heart rate
QTcB	QT interval corrected for heart rate using Bazett's formula

Abbreviation	Term
QTcF	QT interval corrected for heart rate using Fridericia's formula
RECIST	Response Evaluation Criteria in Solid Tumors
RMS	rhabdomyosarcoma
RP2D	recommended phase 2 dose
SAE	serious adverse event
SAP	statistical analysis plan
SD	stable disease
SOC	system organ class
TEAE	treatment-emergent adverse event
TLG	tables, listings, and graphs
WHODD	World Health Organization Drug Dictionary

3 INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to describe the procedures and the statistical methods that will be used to analyze and report results for Eisai Protocol E7389-G000-223 titled "A Phase 2, multicenter, open-label study to assess safety and preliminary activity of eribulin mesylate in pediatric subjects with relapsed/refractory rhabdomyosarcoma (RMS), non-rhabdomyosarcoma soft tissue sarcoma (NRSTS) and Ewing sarcoma (EWS)".

3.1 Study Objectives

3.1.1 Primary Objective

- To conduct a preliminary assessment of activity of eribulin mesylate in pediatric subjects with relapsed/refractory RMS, NRSTS or EWS to determine whether each cohort warrants further investigation.

3.1.2 Secondary Objectives

- To evaluate the progression-free survival (PFS), using Response Evaluation Criteria in Solid Tumors (RECIST) 1.1, of eribulin mesylate in pediatric subjects with relapsed/refractory RMS, NRSTS, or EWS in all cohorts combined.

- To evaluate the safety and tolerability of eribulin in the pediatric subjects with relapsed/refractory RMS, NRSTS, or EWS in all cohorts combined.

- To evaluate the duration of response (DOR) of eribulin mesylate in pediatric subjects with relapsed/refractory RMS, NRSTS, or EWS.

- To evaluate the overall survival (OS) of pediatric subjects with relapsed/refractory RMS, NRSTS and EWS.

3.1.3 Exploratory Objectives

- To evaluate the exposure of eribulin mesylate in pediatric subjects with relapsed/refractory RMS, NRSTS, or EWS.

- To explore the relationship between exposure to eribulin mesylate and adverse events (AEs) and efficacy.

3.2 Overall Study Design and Plan

E7389-G000-223 is a Phase 2, multicenter, open-label study to conduct a preliminary assessment of the safety and activity of eribulin mesylate in pediatric subjects with relapsed/refractory RMS, NRSTS, or EWS.

Up to forty-five pediatric subjects, with approximately 15 subjects each with RMS, NRSTS, or EWS, will be enrolled to receive eribulin mesylate as an intravenous (IV) infusion at a dose

of 1.4 mg/m^2 (Recommended Phase 2 Dose [RP2D]) on Days 1 and 8 of each 21-day cycle as determined by the dose finding study ADVL1314 (Eisai Study E7389-A001-113).

Outline

- Pediatric subjects with relapsed/refractory RMS, NRSTS or EWS will be enrolled to receive therapy with eribulin mesylate.

- For each histology type, approximately 15 subjects will be treated. Data will be monitored on an ongoing basis; depending on evaluation by the investigators and sponsor, if sufficient responses (PRs or CRs) are observed, then this histology type will be recommended for inclusion in the subsequent study.

- Subjects will continue study therapy until progression of disease (per RECIST 1.1), intolerable toxicity or withdrawal of consent.

Pre-study Phase:

Day -28 to -1, computed tomography (CT) / magnetic resonance imaging (MRI) scans must be performed within 28 days prior to study drug administration. All clinical and laboratory test results to determine eligibility must be performed within 7 days prior to study drug administration, unless otherwise indicated.

Treatment Phase:

The treatment phase will start on Day 1 of Cycle 1.

Follow-up:

The follow-up period will begin immediately after the End of Treatment visit until death or informed consent is withdrawn, unless the study is terminated by the sponsor. Subjects will be followed for survival approximately every 12 weeks for 1 year and then annually thereafter. As well as follow-up for survival, the annual surveillance will also assess any long-term effects of the study treatment.

During the follow-up period, subjects who have gone off study treatment without progression should have tumor assessments every 6 - 12 weeks, at the investigator's discretion, from the date of last tumor assessment until disease progression is documented, death, or initiation of another anticancer therapy, whichever occurs first, unless the study is terminated by the sponsor. Follow-up data will be required unless consent is withdrawn.

The schedules of procedures/assessments are presented in Table 9 of the protocol.

4 DETERMINATION OF SAMPLE SIZE

A total of up to 45 subjects will be enrolled. In each of the histology groups of RMS, NRSTS, and EWS, approximately 15 subjects will be enrolled and treated with eribulin mesylate. The

sample size is considered sufficient based on clinical judgment and is not obtained from statistical calculation.

5 STATISTICAL METHODS

All descriptive statistics for continuous variables will be reported using n, mean, standard deviation, median, 25th percentile (Q1), 75th percentile (Q3), minimum, and maximum. Categorical variables will be summarized as number and percentage of subjects.

All summary tables will be presented by histology type and overall.

5.1 Study Endpoints

5.1.1 Primary Endpoint

- Objective response: number of subjects achieving a best overall response of partial or complete response (PR or CR), by up to 24 weeks after all subjects have completed response assessment. Response assessment will be determined by investigators.

5.1.2 Secondary Endpoints

- PFS: defined as the time from the first dose date to the date of disease progression or date of death (whichever occurs first).
- Safety and tolerability: AEs, SAEs, clinical laboratory values, ECG parameters, vital sign measurements, and performance status.
- DOR: defined as the time from the first date of documented PR or CR to the date of disease progression or date of death (whichever occurs first).
- OS: defined as the time from the first dose date to the date of death.

5.1.3 Exploratory Endpoint

- Exposure of eribulin mesylate.

5.2 Study Subjects

5.2.1 Definitions of Analysis Sets

The **Full Analysis Set** (FAS) will consist of all subjects who receive at least 1 dose of study drug.

The **Safety Analysis Set** will consist of all subjects who receive at least 1 dose of study drug.

The **Pharmacokinetic Analysis Set** (PAS) will include subjects who have at least 1 evaluable plasma concentration and sufficient dosing information.

5.2.2 Subject Disposition

Screening disposition will be summarized by number and percentage of subjects with reasons.

Study treatment disposition will be summarized by number and percentage of subjects with reasons for treatment discontinuation.

5.2.3 Protocol Deviations

Protocol deviations will be determined prior to database lock. The protocol deviations data as collected in the clinical database will be presented in tables and/or listings as appropriate.

5.2.4 Demographic and Other Baseline Characteristics

Demographic and other baseline characteristics for the Full Analysis Set will be summarized for each histology group and overall using descriptive statistics. Continuous demographic and baseline variables include age (years), height (cm), weight (kg), BSA (m^2), and vital signs, categorical variables include country, sex, age group, ethnicity, race, Karnofsky (for age>16) / Lansky (for age<=16) performance status score.

For continuous variables, the number of non-missing observations, mean, standard deviation, median, 25^{th} percentile (Q1), 75^{th} percentile (Q3), minimum, and maximum will be presented. For categorical variables, the number and percentage of patients will be presented.

5.2.4.1 Medical History

Medical history will be coded by MedDRA (Version 21.1 or higher), the linked MedDRA preferred term (PT) and primary system organ class (SOC) will also be captured in the database. The number (percentage) of subjects in the Full Analysis Set reporting a history of any medical condition, will be summarized by SOC and PT for each histology group and overall. A subject data listing of medical history will be provided.

5.2.4.2 Disease History and Characteristics

Disease history and characteristics for the Full Analysis Set will be summarized for each histology group and overall using descriptive statistics.

Continuous variables include:

- Time from first diagnosis to first dose (months);
- Age at first diagnosis (years);
- Time from last disease progression to first dose (days);

Categorical variables include:

- Location of primary tumor;

- Target lesions at baseline (Lymph node only, Non-lymph node only, Lymph node and non-lymph node);
- Non-target lesions at baseline (Yes and No);

A subject data listing of disease history and characteristics will be provided.

5.2.5 Prior and Concomitant Therapy

All investigator terms for medications recorded in the CRF will be coded to an 11-digit code using the World Health Organization Drug Dictionary (WHODDMAR18_HD_B2). The number (percentage) of subjects who took prior and concomitant medications will be summarized on the Full Analysis Set by histology group, Anatomical Therapeutic Chemical (ATC) class (i.e., anatomical class, pharmacologic class), and WHODD preferred term.

Prior medications are defined as medications that stopped before the first dose of study drug. Concomitant medications are defined as medications that (1) started before the first dose of study drug and were continuing at the time of the first dose of study drug, or (2) started on or after the date of the first dose of study drug up to 28 days after the subject's last dose. A medication that cannot be determined as prior/concomitant/post-treatment due to missing/incomplete dates will be regarded as a concomitant medication.

Prior and concomitant medications will be summarized respectively. All medications will be presented in subject data listings.

Previous anticancer therapies

Number of regimens, type of regimens, duration of last regimen (months), time from end of last regimen to first dose (months) and best response to the last regimen will be summarized for prior anticancer medications.

The number (percentage) of subjects who took prior anticancer medications will be summarized on the Full Analysis Set by histology group, Anatomical Therapeutic Chemical (ATC) class (i.e., anatomical class, pharmacologic class), and WHODD preferred term.

Number of radiotherapy, sites of radiotherapy, total dose (cGy) per subject, time from end of last radiotherapy to first dose (months) and tumor lesion progressed since most recent radiotherapy, will be summarized for prior anticancer radiotherapy.

Previous anticancer medication and radiotherapy will be summarized/listed separately.

5.2.6 Treatment Compliance

Not applicable.

5.3 Data Analysis General Considerations

5.3.1 Pooling of Centers

This study is a multicenter study. Subjects from all centers will be pooled for all analyses.

5.3.2 Adjustments for Covariates

Not applicable.

5.3.3 Multiple Comparisons/Multiplicity

Not applicable.

5.3.4 Examination of Subgroups

No subgroup analyses are planned for this study.

5.3.5 Handling of Missing Data, Dropouts, and Outliers

In general, missing data including dates will be treated as missing and no data imputation will be applied, unless otherwise specified. Data that are potentially spurious or erroneous will be queried and examined during the review of the study data.

For efficacy data summarized for the FAS, subjects with missing response status (subjects whose baseline or post-baseline tumor assessment is missing) will be considered as non-responders and will be included in the denominator when calculating the response rate.

5.3.6 Other Considerations

Not applicable.

5.4 Efficacy Analyses

Efficacy analyses will be performed on the Full Analysis Set.

5.4.1 Primary Efficacy Analyses

The primary analysis will be based on investigator assessments of tumor response according to RECIST 1.1 (Eisenhauer, et al. 2009).

The primary analysis is to examine the number of responders (CR or PR) within each histology subject cohort. Confirmed response (PR or CR) will be used in this analysis. All responses must be confirmed no less than 28 days following the initial assessment of response. In order for stable disease (SD) to be considered the best overall response, it must occur ≥ 5 weeks following the first dose of study drug.

Objective response rate (ORR) is defined as the proportion of subjects achieving the best overall response (BOR) of CR or PR as per RECIST 1.1. ORR will be calculated with a 2-sided, exact 95% confidence interval (CI), using the Clopper-Pearson method.

5.4.2 Secondary Efficacy Analyses

The secondary analyses will be to estimate the PFS, DOR, and OS in all cohorts combined. Median PFS, DOR, and OS with the corresponding 95% confidence intervals (CI) will be estimated using Kaplan-Meier method (Kaplan; Meier. 1958). Descriptive statistics will be provided. Duration of response (DOR) will be calculated for subjects with an objective response of PR or CR.

Censoring rules for PFS/DOR are listed in section 8: definitions and conventions for data handling. For OS, subjects who were alive at the data cutoff date will be censored at the data cutoff date, and subjects who were lost to follow-up, withdrew consent, or had survival status collected as "Study terminated by Sponsor" will be censored at the date the subject was last known to be alive.

5.4.3 Other Efficacy Analyses

No other efficacy analyses are planned for this study.

5.5 Pharmacokinetic, Pharmacodynamic, Pharmacogenomic, and Other Biomarker Analyses

5.5.1 Pharmacokinetic Analyses

Blood samples will be collected for the PK analysis of eribulin as follows:

1. Immediately post-infusion on Day 1 of each cycle for the first 3 cycles;
2. Pre-infusion and immediately post-infusion on Day 8 of each cycle for the first 3 cycles.

Plasma concentrations of eribulin will be tabulated and summarized by histology and time.

5.5.2 Pharmacodynamic, Pharmacogenomic, and Other Biomarker Analyses

Exposure-response Analyses

Following FDA guidance, PK sampling will be conducted in subjects across the pediatric program at regular intervals in order to adequately characterize the PK of eribulin in the pediatric population in the various age cohorts to explore exposure-response (ER) relationships for efficacy and safety (AE). The relationship between exposure to eribulin mesylate and AE or efficacy will be explored graphically and may be modelled if a relationship is deemed to exist.

5.6 Safety Analyses

All safety analyses will be performed on the Safety Analysis Set. Safety data, presented by histology type, will be summarized on an "as treated" basis using descriptive statistics (e.g., n, mean, standard deviation, median, minimum, Q1, Q3 and maximum for continuous variables; n [%] for categorical variables). Safety variables include treatment-emergent adverse events (TEAEs), clinical laboratory parameters, vital signs, 12-lead ECG results, and performance status.

Study Day 1 for all safety analyses is defined as the date of the first dose of study drug.

5.6.1 Extent of Exposure

The number of cycles administered, duration of treatment, number of doses, total dose (mg/m^2) per subject, actual dose intensity (mg/m^2/week) per subject and relative dose intensity per subject will be summarized for eribulin mesylate and be presented by n, mean with standard deviation, median, minimum, Q1, Q3 and maximum.

Duration of Treatment (weeks) = (Date of first dose of last cycle + 21 - date of first dose of study drug) / 7.

Actual Dose Intensity (mg/m^2/week) = Total dose (mg/m^2) / Duration of treatment (weeks).

Relative Dose Intensity (%) = Actual Dose intensity (mg/m^2/week)/ Planned dose intensity * 100, where Planned dose intensity is 0.933 mg/m^2/week = 1.4 mg/m^2 * 2 / 3 weeks.

The number of subjects who had dose changes will be summarized for eribulin mesylate.

5.6.2 Adverse Events

The AE verbatim descriptions (investigator terms from the CRF) will be classified into standardized medical terminology using the Medical Dictionary for Regulatory Activities (MedDRA). Adverse events will be coded to the MedDRA (Version 21.1 or higher) lower level term (LLT) closest to the verbatim term. The linked MedDRA preferred term (PT) and primary system organ class (SOC) will also be captured in the database.

A treatment-emergent adverse event (TEAE) is defined as an AE that emerged during treatment, having been absent at pretreatment (Baseline) or

- Reemerged during treatment, having been present at pretreatment (Baseline) but stopped before treatment, or
- Worsened in severity during treatment relative to the pretreatment state, when the AE was continuous.

Only those AEs that are treatment emergent will be included in summary tables. All AEs, treatment emergent or otherwise, will be presented in subject data listings.

The TEAEs will be summarized by histology group. The incidence of TEAEs will be reported as the number (percentage) of subjects with TEAEs by SOC and PT. A subject will be counted only once within a SOC and PT, even if the subject experienced more than 1 TEAE within a specific SOC and PT. The number (percentage) of subjects with TEAEs will also be summarized by highest Common Terminology Criteria for Adverse Events (CTCAE) V4.03 grade.

The number (percentage) of subjects with TEAEs will also be summarized by relationship to study drug (Yes [related] and No [not related]).

The number (percentage) of subjects with TEAEs with fatal outcomes will be summarized by MedDRA SOC and PT for each histology type. A subject data listing of all AEs with fatal outcomes will be provided.

The number (percentage) of subjects with treatment-emergent serious adverse events (SAEs) will be summarized by MedDRA SOC and PT for each histology type. A subject data listing of all SAEs was provided.

The number (percentage) of subjects with TEAEs leading to discontinuation from study drug will be summarized by MedDRA SOC and PT for each histology type. A subject data listing of all AEs leading to discontinuation from study drug will be provided.

5.6.3 Laboratory Values

Laboratory results will be summarized using Système International (SI) units, as appropriate. For all hematology and chemistry parameters listed in the protocol Section 9.5.1.6.3 (Table 8), the actual value and the change from baseline to each post-baseline visit will be summarized using descriptive statistics (n, mean, standard deviation, median, Q1, Q3, minimum, maximum).

Laboratory test results will be assigned a classification according to CTCAE grade (Appendix 1 in the protocol). Shifts of CTCAE grade from baseline to worst post-baseline will be presented. Percentages will be based on the number of subjects with both non-missing baseline and at least one post-baseline result for relevant parameters.

Urinalysis and pregnancy test data will be listed.

5.6.4 Vital Signs

Descriptive statistics for vital signs parameters (i.e., systolic and diastolic blood pressure (BP), pulse, temperature, weight, and height) and changes from baseline will be presented by visit.

A listing of all vital sign measurements will be provided.

5.6.5 Electrocardiograms

ECG assessments will be performed. QT intervals will be measured from Lead II and will be corrected for heart rate (QTc) using Fridericia's (QTcF) and Bazett's (QTcB) correction factors. The primary QTc parameter will be QTcF. Secondary parameters will be QTcB, QT, QRS, and heart rate.

Shift tables will present shifts from baseline to end of treatment in ECG interpretation (categorized as normal; abnormal, not clinically significant; and abnormal, clinically significant).

In addition, the number and percentage of subjects with at least 1 post-baseline abnormal ECG result in QTc Fridericia and Bazett's during the treatment period will be summarized. Clinically abnormal ECG results in QTc Fridericia and Bazett's will be categorized as follows:

Absolute QTc interval prolongation:

- QTc interval >450 ms
- QTc interval >480 ms
- QTc interval >500 ms

Change from baseline in QTc interval:

- QTc interval increase from baseline >30 ms
- QTc interval increase from baseline >60 ms

5.6.6 Other Safety Analyses

The changes of Karnofsky and Lansky performance status scores from baseline to worst post-baseline will be presented in shift tables.

5.7 Other Analyses

Not applicable.

5.8 Exploratory Analyses

Exploratory analyses may be conducted as appropriate. Any exploratory analyses that are performed will be appropriately titled and labeled as exploratory and will be clearly distinguished from planned analyses when results are reported in the Clinical Study Report.

6 INTERIM ANALYSES

Data will be monitored on an ongoing basis; depending on evaluation by the investigators and sponsor, if sufficient responses (PR or CR) are observed, then this histology type will be recommended for inclusion in the subsequent study.

7 CHANGES IN THE PLANNED ANALYSES

Not applicable.

8 DEFINITIONS AND CONVENTIONS FOR DATA HANDLING

Censoring Rules for PFS/DOR

The PFS censoring rules in this SAP and definition of progression date follow the Food and Drug Administration (FDA) "Guidance for Industry Clinical Trial Endpoints for the Approval of Cancer Drugs and Biologics (2007)".

Table 1 Censoring Rules for Progression-free Survival Endpoint

Situation	End Date	Censored
Investigator documented PD	Date of the first assessment of the series of radiologic tests that determined PD	No
Death during the study before first PD	Date of death	No
No baseline or post-baseline tumor assessments	Date of first dose	Yes
No progression and no death at the time of data cutoff	Date of last adequate tumor assessment* prior to data cutoff	Yes
Non-study anticancer treatment initiated before progression or death	Date of last adequate tumor assessment* prior to or on date of non-study anticancer treatment	Yes
Death or progression after more than one missed tumor assessments**	Date of last adequate tumor assessment* prior to missed tumor assessments	Yes

PD = progressive disease.

* Adequate tumor assessment is radiologic assessment of CR, PR, SD, non-CR/non-PD or PD as determined by investigators at regular interval as defined in the protocol. Any tumor assessments after new anticancer treatment starts will be removed in the definition of PFS.

**More than one missed visit/adequate tumor assessment is defined as having the duration between the last adequate tumor assessment and PD or death being longer than the gap limit.

a) The gap from baseline to the 1st assessment post-baseline (calculated from C1D1), gap limit = 2*6 weeks + 2*3 Days = 90 days.

b) The gap from the 1st assessment post-baseline, or from any later assessment -- call it "previous assessment", to the next assessment.

- if Study Day of the previous assessment < (18 weeks - 3 days), then gap limit = 2*6 weeks + 2*3 days = 90 days;
- if (18 weeks - 3 days) <= Study Day of the previous assessment < 24 weeks - 3 days, then gap limit = 6 weeks + 9 weeks + 2*3 days = 111 days;
- if Study Day of the previous assessment >= 24 weeks - 3 days, the gap limit = 2*9 weeks + 2*3 days = 132 days.

9 PROGRAMMING SPECIFICATIONS

The rules for programming derivations and dataset specifications are provided in separate documents.

10 STATISTICAL SOFTWARE

All statistical analyses will be performed using SAS v9.4.

11 MOCK TABLES, LISTINGS, AND GRAPHS

The study TLG shells will be provided in a separate document, which will show the content and format of all tables, listings, and graphs in detail.

12 REFERENCES

Kaplan, E. L.; Meier, P. (1958). "Nonparametric estimation from incomplete observations". Journal of the American Statistical Association. 53 (282): 457–481.

Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009;45(2):228-47.

Cancer Therapy Evaluation Program, Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 [published 28 May 2009 (v4.03: June 14, 2010)]. Available from: http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf.

13 APPENDICES

Not applicable.

SIGNATURE PAGE

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