ASTX727-01

Statistical Analysis Plan

(Phase 2 Dose Confirmation Stage and Fixed-Dose Combination Stage)

NCT02103478

A Phase 1-2 Pharmacokinetic Guided Dose-Escalation and Dose-Confirmation Study of ASTX727, a Combination of the Oral Cytidine Deaminase Inhibitor (CDAi) E7727 with Oral Decitabine in Subjects with Myelodysplastic Syndromes (MDS)

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Based on: Protocol Version 5.0

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ABBREVIATIONS AND DEFINITIONS

AE	adverse event
AML	acute myeloid leukemia
ANC	absolute neutrophil count
AUC	area under the curve
BQL	below the limit of quantification
BSA	body surface area
CDA	cytidine deaminase
CDAi	cytidine deaminase inhibitor
CI	confidence interval
C_{max}	maximum concentration
CMML	chronic myelomonocytic leukemia
CR	complete response
CRF/eCRF	case report form/electronic case report form
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose limiting toxicity
DNA	deoxyribonucleic acid
DSRC	Data and Safety Review Committee
ECG	electrocardiogram
GI	gastrointestinal
HI	hematological improvement
HI-E	erythroid response
HI-N	neutrophil response
HI-P	platelet response
HMA	hypomethylating agent
IPD	important protocol deviations
IPSS	International Prognostic Scoring System
IV	intravenous
IWG	International Working Group
LINE-1	long interspersed nucleotide elements-1
LVEF	left ventricular ejection fraction
mCR	marrow complete response
MDS	myelodysplastic syndromes
MedDRA	Medical Dictionary for Regulatory Activities
OR	overall response
PD	pharmacodynamic(s)
pH	a measure of the acidity or alkalinity of an aqueous solution
PK	pharmacokinetic(s)
PR	partial response
PT	preferred term
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SDTM	Standard Data Tabulation Model
SOC	system organ class
TEAE	treatment emergent adverse event
THU	tetrahydrouridine
T_{max}	time at which C_{max} occurs
WHO	World Health Organization

1.0 INTRODUCTION

This Statistical Analysis Plan (SAP) is based on ASTX727-01 protocol version 5 (dated 06 April 2017). Analyses and statistical reporting for ASTX727-01 will be conducted by Astex Pharmaceuticals, Inc. (Astex) Biometrics department using SAS version 9.4 or higher. The study consists of 3 stages: Phase 1 Dose Escalation Stage, Phase 2 Dose Confirmation Stage, and Fixed-Dose Combination Stage. This analysis plan describes the analyses only for Phase 2 Dose Confirmation Stage and Fixed-Dose Combination Stage.

1.1 Myelodysplastic syndromes

Myelodysplastic syndromes (MDS) are a heterogenous group of hematopoietic stem cell disorders characterized by dysplastic changes in myeloid, erythroid, and megakaryocytic progenitors and associated with cytopenias affecting one or more of the three lineages (Bennett 1982; Cheson et al 2000; Heaney and Golde 1999; Kantarjian and Estey 2001). Most MDS patients are elderly, and their prognosis (with high-risk factors) is poor. Patients often present with complications related to anemia (fatigue), neutropenia (infections), or thrombocytopenia (bleeding). In addition, variable blast expansion, and, less commonly, leukocytosis are observed. MDS may evolve into acute myeloid leukemia (AML) in one-third of patients (Shukron et al 2012).

MDS patients die either from complications associated with cytopenias (infections and bleeding) or from transformation to AML. In practice, "lower risk" MDS patients may be distinguished from "higher risk" MDS patients by their degree of pre-leukemic blast expansion, responses to therapeutic agents, disease outcomes, and prognosis (Benjanyan and Sekeres 2011). These factors have allowed the establishment of an International Prognostic Scoring System (IPSS) to predict survival and progression to AML (Greenberg et al 1997) as well as aiding treatment decisions.

Based on the IPSS scoring system, patients with lower risk (IPSS low risk or intermediate-1) MDS (approximately 70% of patients) have an expected median survival of 3.5 to 5.7 years. Median survival for higher risk patients (intermediate-2 and high-risk MDS) ranges from 0.4 to 1.2 years (Greenberg et al 1997).

1.2 Treatment Options

Hypomethylating agents (HMAs), such as decitabine and azacitidine, are effective treatment modalities for hematologic cancers and are FDA-approved for higher risk MDS and chronic myelomonocytic leukemia (CMML). Consecutive daily dosing for a minimum of 5 or 7 days in 28-day cycles is the labelled administration schedule and is necessary to achieve the desired effects. Relapse rapidly occurs in responders whose HMA treatment is suspended (Cabrero et al 2015). Treatment can therefore continue for several months or even years—a significant hardship for patients undergoing treatment because of the 5 to 7 clinic visits required each month, and the 1-hour IV infusion or large volume of subcutaneous injection. A possible consequence is non-compliance or early discontinuation of a beneficial treatment. More convenient oral administration of HMAs has historically proven difficult due to rapid metabolism by cytidine deaminase (CDA)

during passage through the gastrointestinal (GI) mucosa and liver. To achieve even modest exposures of azacitidine requires administration of large doses, which are associated with Grades 3 and 4 gastrointestinal toxicity (nausea, vomiting, and diarrhea) and high variability in exposures (Garcia-Manero et al 2011). Successful development of an oral HMA will alleviate the significant inconvenience of long-duration parenteral therapy, particularly for those patients who benefit the most.

1.2.1 Oral Decitabine and Cedazuridine (E7727)

Cedazuridine is a 2'-fluorinated analog of tetrahydrouridine (THU) and is a novel cytidine deaminase inhibitor (CDAi), developed for oral administration. The bioavailability of decitabine and of several other therapeutic cytidine analogs (eg, azacitidine, cytarabine, gemcitabine) is significantly limited by a rapid metabolism catalyzed by cytidine deaminase (CDA) which is highly expressed in the gut and liver. Cedazuridine is a potent CDAi with an IC_{50} of 0.281 μM. Cedazurdine is also characterized by an improved stability at lower pH which has been a challenge with THU.

Cedazuridine has negligible antiproliferative activity in human cancer cell lines, did not show any significant inhibition in a panel of 80 physiologically important human receptors and did not inhibit hERG potassium channel at up to 300 μM. In vitro studies have also characterized an epimer of cedazuridine (ER-849726) that has about 1/10th the activity of cedazuridine as determined by an IC_{50} of 5 μM against human recombinant CDA. After oral dosing in preclinical toxicology models (mouse and monkey), near-equivalent levels were detected for circulating cedazuridine and its epimer.

1.2.2 ASTX727 Fixed-Dose Combination

The fixed-dose combination (FDC) drug form of ASTX727 used in study ASTX727-01 is film-coated, oval, white, immediate-release, fixed-dose combination of 100 mg cedazuridine and 35 mg decitabine for oral administration.

1.2.3 Administration of ASTX727

ASTX727 was administered to subjects in the Dose Confirmation Stage initially as separate capsules of cedazuridine and decitabine. Subjects still on treatment with capsules as of 23 October 2017 were permitted to transition to treatment with the ASTX727 FDC tablet at that time (at the discretion of the investigator), as new supplies of cedazuridine and decitabine capsules were not supported after the supply expiry date (31 October 2017). In the FDC Stage, ASTX727 was administered to subjects as FDC tablets.

2.0 STUDY OBJECTIVES

2.1 Dose Confirmation Stage – Primary Objectives

- Confirm the dose of oral decitabine + cedazuridine identified in the Dose Escalation Stage administered daily×5 that achieves mean decitabine AUC (estimated from 5 days of dosing) and LINE-1 DNA demethylation comparable to that of IV decitabine 20 mg/m^2 daily×5.
- Assess response rate in all subjects.

2.2 Dose Confirmation Stage – Secondary Objectives

- Assess safety and tolerability of oral decitabine + cedazuridine.
- Assess duration of response, hematological improvement, rate of transfusion independence, time to AML, and overall survival.
- Evaluate other PK parameters of oral decitabine, cedazuridine, and cedazuridine-epimer (if needed).

2.3 Dose Confirmation Stage – Exploratory Objective

[redacted]

2.4 Fixed-Dose Combination Stage – Primary Objectives

- Confirm the ASTX727 FDC tablet achieves mean decitabine AUC (estimated from 5 days of dosing) and LINE-1 DNA demethylation similar to that for IV decitabine 20 mg/m^2 daily×5.
- Assess response rate in all subjects.

2.5 Fixed-Dose Combination Stage – Secondary Objectives

- Assess safety and tolerability of ASTX727 FDC.
- Assess duration of response, hematological improvement, rate of transfusion independence, time to AML, and overall survival.
- Evaluate other PK parameters of ASTX727 FDC.

2.6 Fixed-Dose Combination Stage – Exploratory Objective

[redacted]

3.0 STUDY DESIGN

3.1 Dose Confirmation Stage

The Dose Confirmation Stage (Figure 1) involving at least 42 evaluable subjects was conducted to confirm the dose level of oral decitabine + cedazuridine established in the Dose Escalation Stage. Subjects were randomized to the sequence of:

- Oral decitabine + cedazuridine daily×5 in Course 1 followed by IV decitabine daily×5 in Course 2, or
- IV decitabine daily×5 in Course 1 followed by ASTX727 daily×5 in Course 2.

Beginning with Course 3 and all subsequent courses, subjects continued to receive ASTX727 daily×5 of a 28-day course until disease progression or unacceptable toxicity, or the subject withdrew consent or was withdrawn from the study.

At the conclusion of the Dose Escalation Stage, the Data and Safety Review Committee (DSRC) made a decision on the fixed dose levels of cedazuridine (100 mg) and oral decitabine (35 mg) to be further investigated in the Dose Confirmation Stage.

Subjects ongoing in the Dose Confirmation Stage as of 23 October 2017 were transitioned from ASTX727 capsules to the ASTX727 FDC tablet.

3.2 Fixed-Dose Combination Stage

This stage is a comparison between a tablet form of ASTX727 and IV decitabine 20 mg/m^2. After enrollment in the Dose Confirmation Stage had completed, 18-24 subjects were planned to be randomized and treated in a crossover study to confirm that the ASTX727 FDC yields PK exposures and DNA demethylation similar to those for IV decitabine.

Subjects were randomized in a 1:1 ratio to receive the FDC tablet daily×5 in Course 1 followed by IV decitabine daily×5 in Course 2, or the converse. In Courses ≥3, all subjects received the FDC tablet daily×5 (in a 28-day course) until disease progression or unacceptable toxicity or withdrawal from the study. See Figure 2.

Figure 1: Dose Confirmation Stage Schema

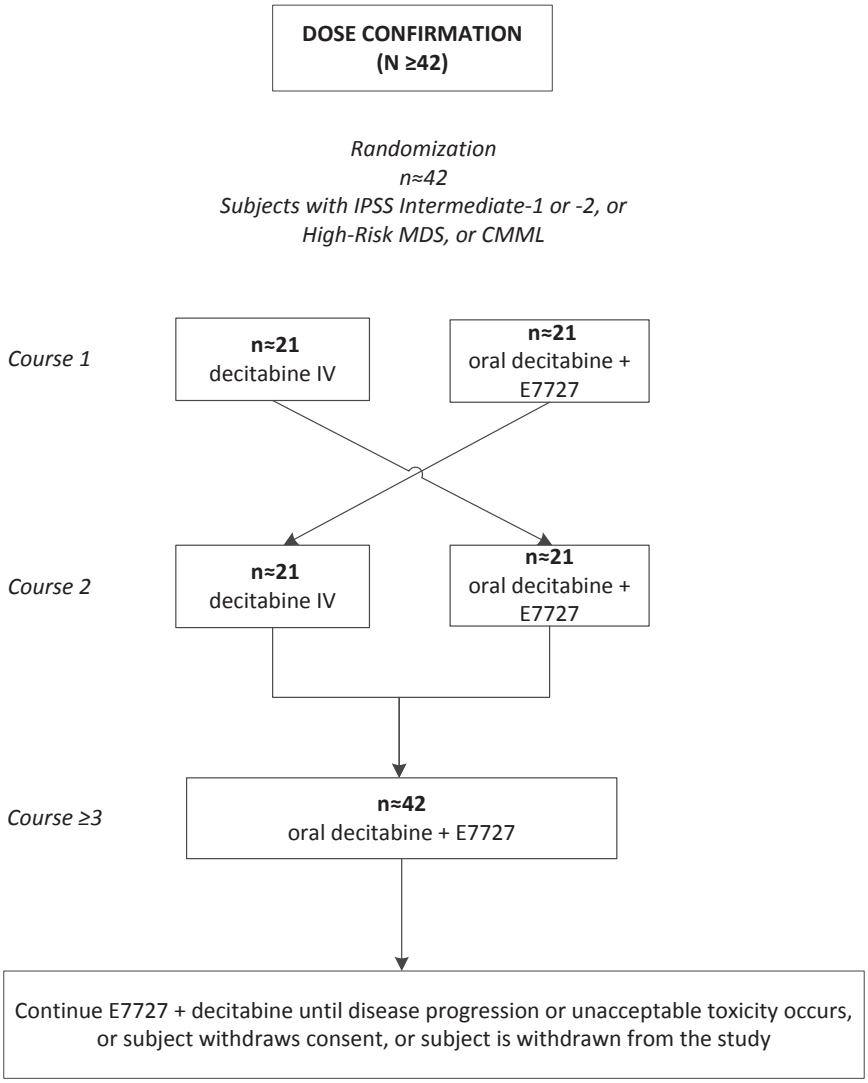
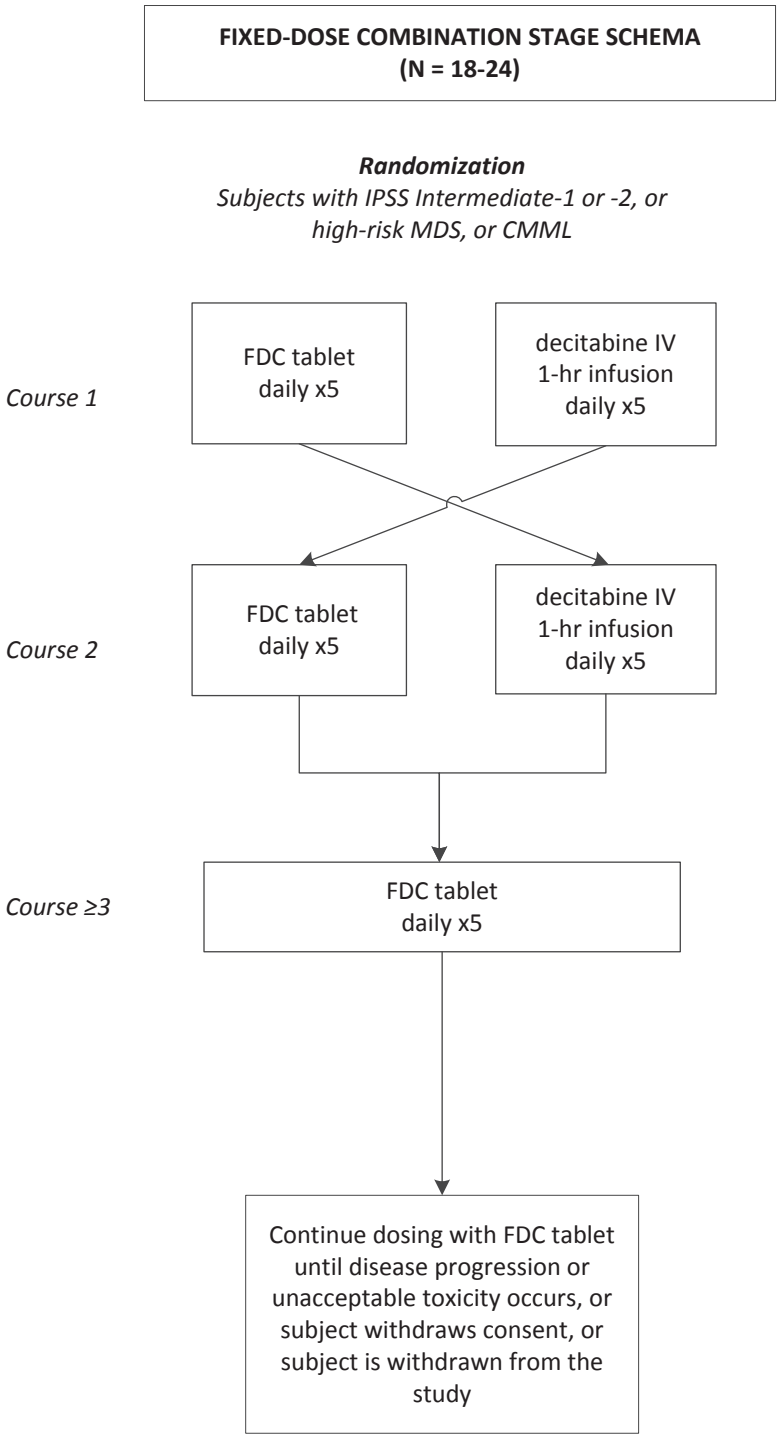


Figure 2: Fixed-Dose Combination Stage Schema



3.3 Discussion of Study Design

In the Dose Confirmation Stage, the primary objective was to confirm that the doses of oral decitabine + cedazurdine identified in the Dose Escalation Stage given as a daily×5 oral dosing regimen achieve a mean AUC (estimated from 5 days of dosing) comparable to that of IV decitabine given daily×5 at the approved dose of 20 mg/m^2. The standard 2×2 crossover design was chosen for the first two courses of the Dose Confirmation Stage in order to facilitate efficient evaluation of within-subject comparison of AUC between ASTX727 and IV decitabine. Because of the short half-life of decitabine, no appreciable carry-over effect of AUC is expected.

In the FDC stage, the goal was to confirm that the FDC tablet formulation is similar to IV decitabine in an intrapatient fashion and that PK and PD results are similar to results obtained with ASTX727 capsules in the Dose Confirmation Stage.

3.4 Study Endpoints

3.4.1 Dose Confirmation Stage – Primary Endpoints

- Mean decitabine AUC (estimated from 5 days of dosing) and mean maximum %LINE-1 demethylation after oral decitabine + cedazuridine compared with IV decitabine 20 mg/m^2.
- Response rate in all subjects.

3.4.2 Dose Confirmation Stage – Secondary Endpoints

- Incidence and severity grades of adverse events and clinically significant abnormal laboratory values.
- Duration of response, hematological improvement, rate of transfusion independence, time to AML, and overall survival.
- Other PK parameters of oral decitabine, cedazuridine, and cedazuridine-epimer (if needed).

3.4.3 Dose Confirmation Stage – Exploratory Endpoint

- [redacted]

3.4.4 Fixed-Dose Combination Stage – Primary Endpoints

- Mean decitabine AUC and mean maximum %LINE-1 demethylation after administration of ASTX727 FDC compared with IV decitabine 20 mg/m^2.
- Response rate in all subjects.

3.4.5 Fixed-Dose Combination Stage – Secondary Endpoints

- Incidence and severity grades of AEs and clinically significant abnormal laboratory values.
- Duration of response, hematological improvement, rate of transfusion independence, time to AML, and overall survival.
- Other PK parameters of ASTX727 FDC.

3.4.6 Fixed-Dose Combination Stage – Exploratory Endpoints

[redacted].

3.5 Data and Safety Review Committee

The Data and Safety Review Committee (DSRC) comprised the principal investigators (or nominated deputies), medical monitor, study director, PK director, and other study team members as appropriate. In the Dose Confirmation Stage, the DSRC evaluated safety, PK, and PD data (1) to assess PK AUC and LINE-1 demethylation comparability between IV decitabine and the combination of cedazuridine + oral decitabine and (2) to determine the final oral dose combination.

4.0 SAMPLE SIZE

4.1 Dose Confirmation Stage

At least 42 evaluable subjects were planned for enrollment into the Dose Confirmation Stage.

Forty-two evaluable subjects were needed to assess decitabine AUC (estimated from 5 days of dosing) and LINE-1 demethylation, using a standard 2×2 crossover design, between IV decitabine 20 mg/m^2 daily×5 and the recommended doses of oral decitabine + cedazuridine as determined in the Dose Escalation Stage. Evaluable subjects for this assessment of decitabine AUC and LINE-1 DNA demethylation were those who meet all the following criteria:

- Complete the dosing regimens in Courses 1 and 2 with sufficient PK data available.
- Have at least one bone marrow aspirate/biopsy after Course 2.

In an equivalence test of the mean decitabine AUCs of ASTX727 (estimated from 5 days of dosing) compared with IV decitabine using two one-sided tests on data from a 2×2 cross-over design, a sample size of 42 achieves 86% power at a 10% significance level when the true ratio of the means is 1.0, the coefficient of variation on the original, unlogged scale is 0.50 which was estimated from a previous study described in the Dacogen Prescribing Information (2014), and the equivalence limits of the mean ratio are 0.75 and 1.33.

This same sample size of 42 in the same 2×2 cross-over design achieves 80% power to detect the non-inferiority, with respect to the mean maximum %LINE-1 demethylation from baseline, of ASTX727 compared with IV decitabine using a one-sided t-test when the margin of non-inferiority is -0.05, the true mean difference is 0, the standard deviation (SD) is 0.15 and the significance level is 0.10. The SD of the paired differences of 0.15 was estimated from a previous Astex study.

4.2 Fixed-Dose Combination Stage

Subjects in a standard 2×2 crossover design were considered evaluable for equivalence analysis if those subjects complete treatment in Courses 1 and 2 with sufficient PK data. A total of 18-24 evaluable subjects included in the 2 one-sided equivalence tests for mean oral decitabine AUC compared with mean IV decitabine AUC will provide 75%-88% power at a 10% significance level

when the true ratio of means is 1.0, the coefficient of variation under an unlogged scale is 0.55, and the equivalence limits for the ratio of means are 0.65 and 1.539.

The 18-24 subjects who completed Courses 1 and 2 with sufficient LINE-1 data in the same 2×2 crossover design will provide 78%-86% power to detect non-inferiority, with respect to the mean maximum %LINE-1 demethylation from baseline, of the ASTX727 FDC tablet compared with IV decitabine using a one-sided t-test when the margin of non-inferiority is -0.075, the true mean difference is 0, the standard deviation (SD) is 0.15 and the significance level is 0.10.

5.0 ANALYSIS SETS

5.1 All Subject Analysis Set

This analysis set contains available data from all enrolled subjects, including those who did not receive any study treatment. This analysis set will be used for analysis of subject disposition.

5.2 Efficacy Analysis Set

The Efficacy Analysis Set includes data from all enrolled subjects who received any amount of study treatment.

5.3 Safety Analysis Set

The safety analysis set is the same as the Efficacy Analysis Set, which includes data from all enrolled subjects who received any amount of study treatment. In the safety analysis, no data exclusion will be allowed because of protocol deviations.

5.4 Pharmacokinetics Analysis Set

The PK Analysis Set will include data from all treated subjects for whom sufficient PK samples were collected to enable calculation of full AUCs on PK assessment days.

5.5 Pharmacodynamics and Biomarker Analysis Set

PD Analysis Set for LINE-1 methylation will be all subjects who received at least one course (Days 1-5) study treatment and had baseline and Day 8 or Day 15 LINE-1 methylation data. PD Analysis Set for other biomarkers will be based on those subjects who had biomarker data collected and analyzed.

6.0 SCHEDULE OF ANALYSES

In the Dose Confirmation Stage, the DSRC will review data from the first 6-12 subjects with complete PK and PD data from the first 2 courses to confirm the doses used for ASTX727 components.

In the FDC Stage, data from subjects who have completed PK and PD data from Courses 1 and 2 of the FDC Stage will be reviewed to confirm that data with the FDC tablet are similar to data with IV decitabine.

Formal analyses including all data to the data cutoff date June 05, 2018, in the Dose Confirmation Stage and the FDC Stage will be conducted for preparation of the clinical study report.

No formal interim analyses are planned for this study.

7.0 STATISTICAL ANALYSIS

Unless otherwise specified, all statistical tests and confidence intervals created will be two-sided with alpha = .05. The SAS® statistical package (version 9.4 or a later version) will be used for the analyses.

In general, the first dosing date is defined as the first date the subject received any study treatment. Within each stage, subject disposition, demographics and baseline characteristics will be summarized by treatment sequence and both sequences combined. Adverse events will be summarized by actual treatment received (Section 7.4.2). Efficacy analyses, laboratory tests, vital signs, ECG, and ECOG performance status will be summarized with both treatment sequences combined.

7.1 Subject Disposition

Subject disposition including numbers screened, treated, and treatment discontinuation by reason, as well as the reasons for study exit (ie, death or reasons that subjects were not followed for survival status) will be summarized using frequencies and percentages according to study visit, treatment discontinuation, and withdrawal from study case report form pages. Sample size for the Efficacy, Safety, PK, and PD Analysis sets will be clearly identified. Subjects in the All Subject Analysis Set will be included in the disposition analysis.

7.2 Demographic and Other Baseline Characteristics

The Efficacy Analysis Set, which is the same as the Safety Analysis Set (Section 5.1.2), will be used to summary demographic and baseline characteristics. The demographic variables consist of age, age category, sex, ethnicity, race, and geographical region. Baseline characteristics include height, weight, BSA, ECOG performance status, IPSS risk category, time since prior diagnosis, cytogenetic risk levels, peripheral blood (including blasts) counts, and BM blasts.

Baseline values are generally the last value pre-dose collected on or before the first dosing date unless otherwise specified.

Age at baseline, if not already provided through data collection, will be calculated as the integer part of (date of enrollment minus date of birth)/365.25.

Time since Diagnosis will be calculated as the (date of 1st dosing – date of diagnosis). If the day is missing for date of diagnosis, the 15th of the month will be used. If the month is missing, July 1st will be used. If the year is missing, the date will remain as missing.

Subject demographic and baseline characteristics will be summarized by mean, standard deviation, median, minimum, and maximum for continuous variables; and by counts and percentages for categorical variables.

7.3 Efficacy Variables and Analyses

Efficacy analyses will be based on the Efficacy Analysis Set (Section 5.1.2).

7.3.1 Response Rate

The evaluation of response (Table 1) is based on IWG 2006 MDS Response Criteria (Cheson et al 2006). Details of response assessments are described in Appendix 1.

Table 1: Modified IWG 2006 MDS Response Criteria

Complete Response (CR): the following for 4 weeks		
Peripheral: Normal peripheral counts with persistent granulocyte count ≥1.0×10^9/L, platelet ≥100×10^9/L and Hgb ≥11 g/dL.		
Marrow: Normal bone marrow with persistent marrow blasts ≤5%. Persistent dysplasia will be noted.		
Partial Response (PR): the following for 4 weeks		
Peripheral: Normal peripheral counts with granulocyte count ≥1.0×10^9/L, platelet count ≥100×10^9/L, and Hgb ≥11 g/dL.		
Marrow: Normal bone marrow and marrow blasts >5%, but were reduced by 50% or more.		
Marrow Complete Response (mCR): the following 4 weeks		
Reduction of bone marrow blasts to ≤5% and decrease by 50% or more without normalization of peripheral counts.		
Hematological Improvement (HI): lasts at least 8 weeks		
Erythroid Response (HI-E):	Major Response:	Hemoglobin increase ≥1.5 g/dL or RBC transfusion independence
Platelet Response (HI-P):	Major Response:	Absolute increase of platelet count from <20 to >20×10^9/L and by at least 100%, or if more than 20×10^9/L, by an absolute increase of at least 30×10^9/L.
Neutrophil Response (HI-N):	Major Response:	Granulocyte increase ≥100%, and by an absolute increase ≥0.5×10^9/L.

Baseline value is defined as the average of any values obtained before and on the first dosing date. If there is only one value before treatment then it will be used as the baseline value.

The following rates will be estimated using sample proportions and 95% Clopper-Pearson confidence interval based on the number of subjects in the Efficacy Analysis Set:

- CR.

- PR.
- mCR.
- CR+PR+mCR.
- CR+PR+mCR+HI.
- HI (HI-E, HI-P or HI-N).
  - HI-E.
  - HI-P.
  - HI-N.

If adequate baseline [redacted] is available, responses will also be summarized by selected baseline [redacted].

7.3.2 Overall Survival

Overall survival is defined as the number of days from the day the subject received the first dose of study treatment to the date of death (regardless of cause). Subjects without documentation of death will be censored on the last date of contact or the last date subject was confirmed alive in the Study Discontinuation CRF page, whichever is later.

The Kaplan-Meier plot will be provided based on the Efficacy Analysis Set. Estimated median survival and 95% confidence intervals will also be provided if available.

7.3.3 Time to AML

Time to AML is defined as the number of days from the day the subject received the first dose of study treatment to the date of MDS progression to AML as defined by ≥20% blasts in bone marrow or peripheral blood or death of any cause. The event date of time to AML will be based on the earliest date of following:

- Death date.
- AML conversion date defined as the earliest date of the following:
  - AML conversion date on the Conversion to AML CRF page.
  - AML conversion date on the Subject Information CRF page.
  - The first date when a record of ≥20% blasts in bone marrow was reported or 2 consecutive records of ≥20% blasts peripheral blood were reported.

Subjects without a time to AML event as described above will be censored on the date of last contact.

The Kaplan-Meier plot for time to AML will be provided based on the Efficacy Analysis Set. Estimated median survival and 95% confidence intervals will also be provided if available.

7.3.4 Duration of Response

Duration of response will be using Kaplan-Meir method only for responders separately for CR, PR, mCR, and CR+PR+mCR from the first time a response category (CR, PR, and mCR) is initiated, as addressed in the Implementation Guide of Response Assessment in Appendix 1, to the earliest date of disease progression defined as below:

- Death date.
- AML conversion as described in Section 7.3.3.
- Disease progression date on the Subject Information CRF page.
- Last treatment date if the reason for treatment discontinuation is Progressive Disease.
- Study exit date on the Study Discontinuation CRF page if the reason for study discontinuation is Progressive Disease.
- Date of disease progression on the Survival Follow-up CRF page.

In the absence of progressive disease, subjects will be censored on the last date of disease assessment including Survival Follow-up on which clearly shows progressive disease have not occurred. Subjects who receive alternative anti-cancer therapy for MDS/CMML or subsequent AML (except for bone marrow transplant) will also be censored on the last date of disease assessment as described above before receiving the alternative therapy or progressive disease, whichever occurs earlier.

7.3.5 Transfusion Independence

Transfusion dependence and independence is defined as follows:

- Transfusion dependence at baseline: documentation of 2 units or more of transfusion within 56 days of the first day of study treatment.
- Post-treatment transfusion independence: no transfusion for 56 consecutive days or more after the first dose of study treatment.

Post-treatment transfusion independence rate will be calculated separately for RBC transfusion independence and platelet transfusion independence as the number of subjects who are transfusion independent post treatment (n) divided by the number of subjects who were transfusion dependent at baseline (N). The 95% Clopper-Pearson confidence interval for transfusion independence rates will be provided. The same analyses will be performed for 84-day and 112-day transfusion independence, defined as no transfusion for 84 consecutive and 112 consecutive days respectively.

7.4 Safety Variables and Analyses

Safety Analysis will be performed using the Safety Analysis Set (Section 5.1.3). Safety will be assessed by subject-reported and investigator-observed AEs, along with physical examination, clinical laboratory tests (hematology, chemistries), vital signs, concomitant medications, and ECGs. Exposure to study treatment, reasons for discontinuation, deaths, and causes of deaths will be tabulated.

7.4.1 Study Treatment and Exposure

The number of courses completed or partially completed per subject will be summarized using descriptive statistics. Frequency count and percentage of dose delayed courses reported by investigator will be calculated based on the total number of treatment courses all subjects received. Dose reduced courses, defined as the courses in which any investigator-assigned dose is lower than the dose level determined in the Dose Escalation Stage (100 mg for cedazuridine and 35 mg for decitabine), will be summarized using frequency count and percentage based on the total number of courses all subjects received. Subject follow-up time will also be summarized using mean, standard deviation, median, and range.

7.4.2 Adverse Events

Adverse event (AE) terms reported by study subjects or observed by investigators will be mapped to the appropriate System Organ Class (SOC) and Preferred Term (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA). Severity of AE will be graded using Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Treatment-emergent AEs (TEAEs) are defined as events that first occurred or worsened, after the first dose of study treatment until 30 days after the last dose of study treatment or the start of a post-treatment alternative anti-cancer treatment for MDS/chronic myelomonocytic leukemia (CMML) and subsequent AML, whichever occurs first, with the following exceptions: events that occurred after 30 days beyond the last dose of study treatment or the start of a post-treatment alternative anti-cancer treatment for MDS/CMML and subsequent AML will also be considered treatment-emergent if the events are both serious and related to the study treatment. For the purpose of determining whether an AE is a treatment-emergent AE, incomplete AE start and stop dates will be imputed conservatively following the data programming standards as detailed in the Astex Data Programming Conventions.

All AE data collected in the study database will be listed, including those that are not treatment emergent. Only TEAEs will be included in the AE summary tables.

An overall safety summary table containing counts and percentages of subjects with any AE, any AE Grade ≥3, AE leading to treatment discontinuation, any SAE, and subcategories of SAEs (death vs not death) will be produced.

Related events are those that the Investigator considered to be related to study treatment as described in the study protocol. All summaries of AEs will be made separately in the following groups for each stage:

- IV decitabine.
- ASTX727 (ie, oral decitabine + cedazuridine for Dose Confirmation Stage or ASTX727 FDC for Fixed-dose Combination Stage) in Cycle 1 or Cycle 2.
- ASTX727 in Cycle 3 or later.
- Total for ASTX727.
- Total for the Stage.

The number and percentage of subjects experiencing AEs will be summarized by MedDRA SOCs (sorted alphabetically) with PTs sorted alphabetically within each SOC, and by CTCAE grade. The number and percentage of subjects experiencing AEs will also be summarized by PT and sorted by event frequency. Related AEs, serious AEs (SAEs), and related SAEs will be summarized similarly. In summarizing AEs, if the occurrence of a particular AE for a given subject is reported more than once, the event is only counted once with its worst CTCAE grade.

7.4.3 Concomitant Medications

Concomitant medications are the medications taken with a start date on or after the start of the administration of study treatment, or those with a start date before the start of the administration of study treatment and a stop date on or after the start of the administration of study treatment. Medications taken beyond 30 days from the last dose of study treatment or after the start of a post-treatment alternative anti-cancer treatment for MDS/CMML and subsequent AML are not considered concomitant medications, unless they are used for treating a related SAE. For the purpose of inclusion in the concomitant medication tables, incomplete medication start and stop dates will be imputed conservatively.

Concomitant medication will be coded by the latest version of WHO Drug Dictionary before the data download and summarized by Anatomical Therapeutic Chemical (ATC level 2) and drug name, sorted alphabetically, using counts and percentages.

7.4.4 Laboratory Tests

Laboratory values will also be graded, if possible, by CTCAE v4.0 in conjunction with the Harrison (18th edition) lab book normal values (Longo et al 2011). Shift tables will display (1) shift from baseline grade to the worst grade during the study, and (2) shift from baseline grade to the last grade at the end of study.

Laboratory values recorded as an interval such as "≥x", "<x", or "2+" will be handled, if necessary for calculation purposes, following the data programming standards as detailed in the Astex Data Programming Conventions.

7.4.5 Vital Signs

Vital Signs will be summarized by visit and treatment sequence using proportion of subjects with each vital sign being too high or too low according to conventionally accepted vital sign normal ranges as following:

- Pulse rate 110 bpm or greater.
- Pulse rate 50 bpm or less.
- Diastolic blood pressure 110 mmHg or greater.
- Diastolic blood pressure 55 mmHg or less.
- Systolic blood pressure 180 mmHg or greater.
- Systolic blood pressure 80 mmHg or less.
- Respiration rate 20 breaths/min or greater.
- Body temperature 39°C or greater.

7.4.6 Electrocardiogram (ECG)

QTc values will be graded (grade 1, 2, and ≥3) based on CTCAE 4.0, and the shift table showing the shift from baseline grade to the worst grade, and from baseline grade to the last available grade will be provided.

7.4.7 ECOG Performance Status

Shift tables for ECOG from baseline to the worst grade, and from the baseline to the last available grade will be provided.

7.4.8 Physical Examination

Physical examination data will be preserved in a Study Data Tabulation Model (SDTM) dataset. No particular analysis will be conducted.

7.5 Pharmacokinetics Analysis

The PK Analysis Set (Section 5.1.4) will be used for analysis of all PK parameters. PK parameters of decitabine, cedazuridine, and cedazuridine-epimer will be derived for each subject using a non-compartmental approach. The details of PK analysis plan will be covered under a separate PK Analysis Plan document.

7.5.1 AUC Equivalence Analysis

Decitabine 5-day AUC_{0-t} will be analyzed in the log scale using a mixed effect model including fixed effects of sequence, period, and treatment, and a random effect of subject within sequence (EMA 2010; EMA 2015). The factors to be included in the model will be based on the actual treatment sequence. A 90% CI for the geometric mean ratio of 5-day AUC_{0-t} will be obtained by applying the exponential function on the 2-sided 90% CI for the difference of mean 5-day AUC_{0-t} between ASTX727 and IV decitabine in the log scale from the mixed effect model.

This analysis will be based on actual treatment. Subjects without 5-day AUC_{0-t} data in both of ASTX727 and IV decitabine will not be included.

7.6 Pharmacodynamic/Biomarker Analyses

The PD and Biomarker Analysis Set (Section 5.1.5) will be used for analyses of LINE-1 methylation and other biomarkers. All data will be summarized descriptively (using mean, standard deviation, median, and range for continuous variables and counts and percentages for categorical variables) by treatment sequence and visit for each biomarker.

Maximum %LINE-1 demethylation (defined as the largest percent decrease from baseline in methylation values within a subject between Day 8 and Day 22 of each treatment course) will be analyzed separately for Course 1 and Course 2 with baseline methylation defined as below:

- For Course 1, baseline is defined as last value obtained before or on Day 1 pre-dose.
- For Course 2, baseline is defined as the value obtained on Day 1.

Within each course, subjects to be included for analysis need to complete whole course of treatment. The 95% CIs for the difference of mean maximum %LINE-1 demethylation between ASTX727 and IV decitabine will be generated using an analysis of variance (ANOVA) model separately for Course 1 and Course 2.

7.7 Handling of Missing Data and Other Data Anomalies

No missing data imputations are planned for the study, except as specified. Subjects lost to follow-up will be included in statistical analyses to the point of the last evaluation.

7.8 Handling of Protocol Deviations

Important Protocol Deviations (IPDs) categories will be defined by the study team during the IPD reviews throughout the study prior to the data lock date. IPD will be tabulated by IPD category and listed.

8.0 REFERENCES

Bejanyan N, Sekeres MA. The revolution of myelodysplastic syndromes. Therapeutic advances in hematology. 2011;2(1):33–43.

Bennett JM. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51(2):189–199.

Beumer J, Eiseman J, Gilbert J, Holleran J, Yellow-Duke A, Clausen D, et al. Plasma pharmacokinetics and oral bioavailability of the 3,4,5,6-tetrahydrouridine (THU) prodrug, triacetyl-THU (taTHU), in mice. Cancer Chemother Pharmacol. 2011;67(2):421–430.

Beumer JH, Eiseman JL, Parise RA, Joseph E, Covey JM, Egorin MJ. Modulation of gemcitabine (2',2'-difluoro-2'-deoxycytidine) pharmacokinetics, metabolism, and bioavailability in mice by 3,4,5,6-tetrahydrouridine. Clin Cancer Res. 2008;14(11):3529–3535.

Beumer JH, Eiseman JL, Parise RA, Joseph E, Holleran JL, Covey JM, et al. Pharmacokinetics, metabolism, and oral bioavailability of the DNA methyltransferase inhibitor 5-fluoro-2'-deoxycytidine in mice. Clin Cancer Res. 2006;12(24):7483–7491.

Beumer JH, Parise RA, Newman EM, Doroshow JH, Synold TW, Lenz HJ, et al. Concentrations of the DNA methyltransferase inhibitor 5-fluoro-2'-deoxycytidine (FdCyd) and its cytotoxic metabolites in plasma of patients treated with FdCyd and tetrahydrouridine (THU). Cancer Chemother Pharmacol. 2008;62(2):363–368.

Cabrero M, Jabbour E, Ravandi F, Bohannan Z, Pierce S, Kantarjian HM, et al. Discontinuation of hypomethylating agent therapy in patients with myelodysplastic syndromes or acute myelogenous leukemia in complete remission or partial response: retrospective analysis of survival after long-term follow-up. Leuk Res. 2015;39(5):520–524.

Chabot GG, Bouchard J, Momparler RL. Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside. Biochem Pharmacol. 1983;32(7):1327–1328.

Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood. 2000;96(12):3671–3674.

Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood. 2006;108(2):419–425.

Dacogen. Dacogen (decitabine for injection) Prescribing Information. 2014.

European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Guideline on the Investigation of Bioequivalence. London, UK. CPMP/EWP/QWP/1401/98 Rev. 1/ Corr. 2010.

European Medicines Agency. Committee for Medicinal Products for Human Use (CHMP). Questions & Answers: positions on specific questions addressed to the Pharmacokinetics Working Party (PKWP). EMA/618604/2008 Rev 13. 2015.

Garcia-Manero G, Gore SD, Cogle C, Ward R, Shi T, MacBeth KJ, et al. Phase I study of oral azacitidine in myelodysplastic syndromes, chronic myelomonocytic leukemia, and acute myeloid leukemia. J Clin Oncol. 2011;29(18):2521–2527.

Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079–2088.

Heaney ML, Golde DW. Myelodysplasia. N Engl J Med. 1999;340(21):1649–1660.

Kantarjian H, Estey E. Myelodysplastic syndromes. 6th ed. DeVita VT, Hellman S, Rosenberg SA, editors: Lippincott Williams & Wilkins; 2001.

Karahoca M, Momparler RL. Pharmacokinetic and pharmacodynamic analysis of 5-aza-2'-deoxycytidine (decitabine) in the design of its dose-schedule for cancer therapy. Clin Epigenetics. 2013;5(1):3.

Longo DL et al. 2011. Harrison's principles of internal medicine (18th edition). New York: McGraw-Hill Medical Publishing Division.

Mistry B, Jones M, Kubiak P. A phase 1 study to assess the absolute bioavailability and safety of an oral solution of decitabine in subjects with Myelodysplastic Syndromes (MDS). Blood. 2011;118:Abstract 3801.

Shukron O, Vainstein V, Kündgen A, Germing U, Agur Z. Analyzing transformation of myelodysplastic syndrome to secondary acute myeloid leukemia using a large patient database. Am J Hematol. 2012;87:853–860.

Steensma DP, Baer MR, Slack JL, Buckstein R, Godley LA, Garcia-Manero G, et al. Multicenter study of decitabine administered daily for 5 days every 4 weeks to adults with myelodysplastic syndromes: the alternative dosing for outpatient treatment (ADOPT) trial. J Clin Oncol. 2009;27(23):3842–3848.

Vidaza. Vidaza (azacitidine for injection) for SC or IV use. Prescribing information. 2012.

APPENDIX 1: Implementation Guide For Response Assessment

The rules for determination of MDS responses and the corresponding durations of responses are described in the following sections. These rules are based on the "best" same-day blood counts (PB blasts, neutrophils, platelets, and Hgb) measured within a treatment course and the transfusion-free period requirement. The "best" was defined in the sense that blood count criteria were met for a particular response status. For BM blasts, if the current course did not have a BM evaluation, the immediately preceding BM evaluation was used.

Rules for CR

The subject must have one of the following baseline values to be eligible for a CR.

Hgb <11 g/dL

Platelets $<100 \times 10^9$/L

Neutrophils $<1.0 \times 10^9$/L

BM Blasts >5%

PB Blasts >5%

To be a CR, the subject must have fulfilled all of the following blood count criteria (the same-day counts for PB blasts, neutrophils, platelets, and Hgb were required) and have been transfusion free, with confirmation period ≥28 days:

Hgb ≥11 g/dL

Platelets $\geq 100 \times 10^9$/L

Neutrophils $\geq 1.0 \times 10^9$/L

BM Blasts ≤5%

PB Blasts absent (no value of PB blasts in the database is interpreted as absent)

The start date of CR must have been >7 days after any RBC transfusion and >3 days after any platelet transfusion.

The duration of confirmation period for CR was calculated from the first time a subject fulfilled these criteria to the earliest time point when BM blasts became >5%, PB blasts > 0%, or when any of the blood counts no longer met the above criteria, the subject received an RBC or platelet transfusion, or the subject showed a clinical progression as determined by the investigator.

In determining duration of CR, if one measurement of BM blasts increased above the 5% level and then decreased to this level or lower without receiving an alternative treatment, the subject was considered to have maintained the ≤5% level.

Rules for PR

To be a PR, subjects must have demonstrated all CR criteria with a relaxed requirement for BM blasts level. The BM blasts must have decreased by 50% or more compared with the pretreatment level but were still above 5%. The duration of confirmation period for PR is calculated from the first time a subject fulfilled these criteria to the earliest time point when BM blasts, peripheral blasts or any of the blood counts no longer met the above criteria, the subject received an RBC or platelet transfusion, or the subject showed a clinical progression as determined by the investigator.

Rules for mCR

To be eligible for mCR, subjects must have had a baseline BM blast >5%. An mCR could be declared if post treatment BM blasts become ≤5% and decreased by ≥50% over the pretreatment level with confirmation period ≥28 days until disease progression.

End of confirmation period for mCR was considered the earliest time point when BM blasts become >5% or a clinical progression was determined by the investigator. In determining duration of mCR, if one measurement of BM blasts were above the 5% level and then decreased to this level or lower without receiving an alternative treatment, the subject was considered to have maintained the ≤5% level.

Rules for CR+PR+mCR

Subjects who have CR, PR, or mCR will be considered a responder for CR+PR+mCR. The duration of CR+PR+mCR is measured from the initiation of CR, PR, or mCR, to the day that the subject do not meet criteria for CR, PR, and mCR.

Rules for HI-E

HI-E status was determined based on RBC transfusion and Hgb level at baseline and during the posttreatment period. To be eligible for HI-E, the subject must have had a baseline RBC transfusion dependence (any RBC transfusion within 4 weeks prior to C1D1) or had a baseline Hgb <11.0 g/L.

For subjects with RBC transfusion dependence at baseline, an HI-E was declared if the subjects did not receive any RBC transfusion for a ≥56-day period of confirmation posttreatment, regardless of Hgb level.

For subjects without RBC transfusion dependence at baseline but with a baseline Hgb <11.0 g/L, an HI-E was declared if subjects had an Hgb increase by ≥1.5 g/L for ≥56 days (at least one count

per course above threshold) and did not receive any RBC transfusions for ≥56 days. The start date of Hgb increase must have been >7 days after any RBC transfusion.

Duration of confirmation period for HI-E was calculated from the first time the HI-E criteria was fulfilled until the subject no longer met the HI-E criteria or disease progression.

Rules for HI-P

To be eligible for HI-P, a subject had to have a baseline platelet count $<100 \times 10^9$/L.

To be considered an HI-P a subject had to remain independent of platelet transfusions, and have platelet counts meet the criteria as described below, for a ≥56-day period of confirmation posttreatment. The start date of response must have been >3 days after any platelet transfusion.

- If the baseline platelet count was $<20 \times 10^9$/L, the count must have increased to $\geq 20 \times 10^9$/L and by at least 100%.
- If the baseline count was $\geq 20 \times 10^9$/L, the count must have increased by 30×10^9/L.

The duration of confirmation period for HI-P was calculated from the first time the HI-P criteria was fulfilled until the subject no longer met the HI-P criteria or disease progression.

Rules for HI-N

G-CSF or GM-CSF was not to be administered. Clinical review of concomitant medication data listings was conducted to confirm that there were no such instances of subject use.

The subject must have had a baseline ANC of $<1.0 \times 10^9$/L to be eligible for an HI-N response.

To have a response, ANC must have increased by ≥100% with an absolute increase of $\geq 0.5 \times 10^9$/L and been maintained for a 56-day period of confirmation (at least one count per course above threshold).

The duration of confirmation period for HI-N was calculated from the first time the HI-N criteria was fulfilled until the subject no longer met the HI-N criteria or disease progression.

ASTX727-01

Statistical Analysis Plan

(Phase 1 Dose Escalation Stage)

NCT02103478

A Phase 1-2 Pharmacokinetic Guided Dose-Escalation and Dose-Confirmation Study of ASTX727, a Combination of the Oral Cytidine Deaminase Inhibitor (CDAi) E7727 with Oral Decitabine in Subjects with Myelodysplastic Syndromes (MDS)

Date: 12 July 2017

Based on: Protocol Version 5.0

AUTHORED BY:

[redacted]

APPROVED BY:

[redacted]

[redacted]

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ABBREVIATIONS AND DEFINITIONS

AE	adverse event
AML	acute myeloid leukemia
ANC	absolute neutrophil count
AUC	area under the curve
BQL	below the limit of quantification
BSA	body surface area
CDA	cytidine deaminase
CDAi	cytidine deaminase inhibitor
CI	confidence interval
C_{max}	maximum concentration
CMML	chronic myelomonocytic leukemia
CR	complete response
CRF/eCRF	case report form/electronic case report form
CTCAE	Common Terminology Criteria for Adverse Events
DLT	dose limiting toxicity
DNA	deoxyribonucleic acid
DSRC	Data and Safety Review Committee
ECG	electrocardiogram
FDA	Food and Drug Administration
HI	hematological improvement
HI-E	erythroid response
HI-N	neutrophil response
HI-P	platelet response
HMA	hypomethylating agent
IPD	important protocol deviations
IPSS	International Prognostic Scoring System
IV	intravenous
IWG	International Working Group
LINE-1	long interspersed nucleotide elements-1
LVEF	left ventricular ejection fraction
mCR	marrow complete response
MDS	myelodysplastic syndromes
MedDRA	Medical Dictionary for Regulatory Activities
OR	overall response
PD	pharmacodynamic(s)
pH	a measure of the acidity or alkalinity of an aqueous solution
PK	pharmacokinetic(s)
PR	partial response
PT	preferred term
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SD	standard deviation
SOC	system organ class
TEAE	treatment emergent adverse event
THU	tetrahydrouridine
T_{max}	time at which C_{max} occurs
WHO	World Health Organization

1.0 INTRODUCTION

This Statistical Analysis Plan (SAP) is based on ASTX727-01 protocol version 5. Analyses and statistical reporting for ASTX727-01 will be conducted by Astex Pharmaceuticals Biometrics department using SAS version 9.4 or higher. This analysis plan describes the analyses only for the Dose Escalation Stage.

1.1 Myelodysplastic syndromes

Myelodysplastic syndromes (MDS) are a heterogenous group of hematopoietic stem cell disorders characterized by dysplastic changes in myeloid, erythroid, and megakaryocytic progenitors and associated with cytopenias affecting one or more of the three lineages (Bennett 1982; Cheson et al 2000; Heaney and Golde 1999; Kantarjian and Estey 2001). Most MDS patients are elderly, and their prognosis (with high-risk factors) is poor. Patients often present with complications related to anemia (fatigue), neutropenia (infections), or thrombocytopenia (bleeding). In addition, variable blast expansion, and, less commonly, leukocytosis are observed. MDS may evolve into acute myeloid leukemia (AML) in 10% to 70% of patients.

Prognosis for MDS patients is poor; patients die either from complications associated with cytopenias (infections and bleeding) or from transformation to AML. In practice, "lower risk" MDS patients may be distinguished from "higher risk" MDS patients by their degree of pre-leukemic blast expansion, responses to therapeutic agents, disease outcomes, and prognosis (Benjanyan and Sekeres 2011). These factors have allowed the establishment of an International Prognostic Scoring System (IPSS) to predict survival and progression to AML (Greenberg et al 1997) as well as aiding treatment decisions.

Based on the IPSS scoring system, patients with lower risk (IPSS low risk or intermediate-1) MDS (approximately 70% of patients) have an expected median survival of 3.5 to 5.7 years. Median survival for higher risk patients (intermediate-2 and high-risk MDS) ranges from 0.4 to 1.2 years (Greenberg et al 1997).

1.2 Treatment Options

The standard for MDS therapy for many years has been supportive care (Heaney and Golde 1999; Kantarjian and Estey 2001). Intensive chemotherapy has been associated with complete response rates of 40% to 60%, induction mortality rates of 10% to 40%, and no improvement in survival (Kantarjian and Estey 2001).

Within the previous 10 years, three agents have been approved by the US FDA for the treatment of MDS: the immunomodulating agent lenalidomide, and the hypomethylating agents (HMAs) azacitidine and decitabine. Patients administered azacitidine at a subcutaneous dose of 75 mg/m^2 daily×7 showed an overall response rate (CR+PR) of 15.7%, which was significantly greater than the response rate of 0% in the observation group ($p<0.0001$) (Vidaza 2012). Similarly, patients with intermediate- or high-risk MDS who were administered IV decitabine (Dacogen®) at a dose of 20 mg/m^2 daily×5 showed an overall response rate of 16% (Dacogen 2014).

1.2.1 Oral Decitabine and E7727

E7727 is a 2'-fluorinated analog of tetrahydrouridine (THU) and is a novel cytidine deaminase inhibitor (CDAi), developed for oral administration. The bioavailability of decitabine and of several other therapeutic cytidine analogs (eg, azacitidine, cytarabine, gemcitabine) is significantly limited by a rapid metabolism catalyzed by cytidine deaminase (CDA) which is highly expressed in the gut and liver. E7727 is a potent CDAi with an IC_{50} of 0.281 μM. E7727 is also characterized by an improved stability at lower pH which has been a challenge with THU.

E7727 has negligible antiproliferative activity in human cancer cell lines, did not show any significant inhibition in a panel of 80 physiologically important human receptors and did not inhibit hERG potassium channel at up to 300 μM. In vitro studies have also characterized an epimer of E7727 (ER-849726) that has about 1/10th the activity of E7727 as determined by an IC_{50} of 5 μM against human recombinant CDA. After oral dosing in preclinical toxicology models (mouse and monkey), near-equivalent levels were detected for circulating E7727 and its epimer.

2.0 STUDY OBJECTIVES

2.1 Primary Objective

- Assess safety, tolerability, and pharmacokinetics (PK) of concomitant orally administered decitabine + E7727.
- Identify a dose of oral decitabine + E7727 that together achieve a target mean decitabine AUC comparable to that from decitabine given by IV infusion (20 mg/m^2) for 1 hour.

2.2 Secondary Objectives

- Determine percent change of long interspersed nucleotide elements-1 (LINE-1) demethylation in Course 1 and Course 2.
- Assess preliminary efficacy, as determined by response rate, duration of response, hematological improvement, rate of transfusion independence, time to AML, and overall survival.
- Evaluate other PK parameters of decitabine, E7727, and E7727-epimer.

2.3 Exploratory Objectives

[redacted]

3.0 STUDY DESIGN

3.1 Overall Study Design

Stage 1 of ASTX727-01 was a phase 1 pharmacokinetic guided dose-escalation study of a combination of E7727 with oral decitabine in subjects with myelodysplastic syndromes (MDS). The starting dose for cohort 1 for E7727 was 40 mg, and the starting dose of oral decitabine was

20 mg. All subsequent cohort dose levels and cohort sizes were determined by the Data and Safety Review Committee (DSRC) based on both safety and PK data review from at least 3 evaluable subjects in each cohort; see below for dose escalation guidance and Section 3.4 for description of DSRC functions.

The dosing schedule for all cohorts in the Dose Escalation Stage is as follows:

Course 1:

- Day -3 (± 2 days): Single dose oral decitabine.
- Day 1: IV decitabine administered at 20 mg/m^2.
- Days 2-5: Concomitant oral administration of oral decitabine + E7727.

Course 2:

- Day -3 (± 2 days): Single dose E7727.
- Days 1-5: Concomitant oral administration of E7727 + decitabine.

The doses of single oral decitabine or oral E7727 given on Day -3 (±2 days) in Course 1 and Course 2 were the same doses, respectively, that were given concomitantly on Days 2-5 in Course 1 and Days 1-5 in Course 2.

Course ≥3:

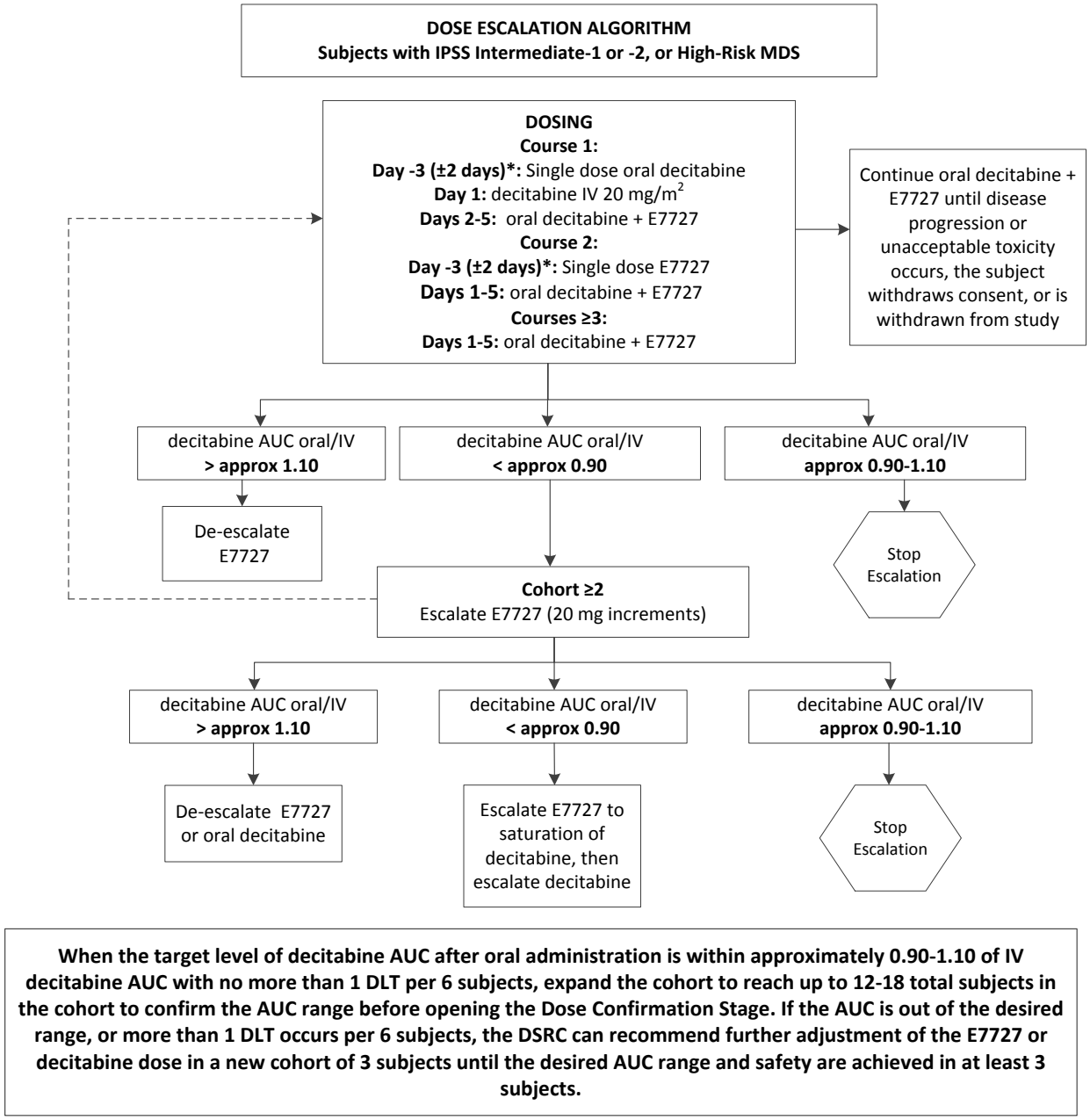
- Days 1-5: Concomitant oral administration of E7727 + decitabine (note, there are no Day -3 visits beginning with Course 3).

All subjects continued to receive oral decitabine + E7727 daily×5 of a 28-day course in Course 3 and thereafter until disease progression, unacceptable toxicity, subject withdrawal of consent, or withdrawal from the study.

Dosing for Cohort ≥2: The dosing schedule for Cohort ≥2 was the same as for Cohort 1; however, the dose levels of either E7727 or oral decitabine could have been modified based on the decision of the DSRC guided by the algorithm in Figure 1. Only one of the 2 components could have been escalated at any one time. If 1 dose limiting toxicity (DLT) occurred in the first 3 subjects in the cohort, 3 more subjects could have been added in that cohort to assess safety and AUC range. In the absence of DLTs in more than 1 of the first 6 subjects in any cohort, the dose of E7727 was to be escalated in 20 mg increments until the cohort mean decitabine oral AUC/IV decitabine AUC approached unity (within 0.90-1.10, inclusive). If E7727 dose adjustments failed to produce the desired oral decitabine AUC levels, or a smaller dose adjustment was required (5 mg instead of 20 mg), the decitabine dose (increments or decrements of 20 mg or 5 mg) was to be adjusted until the desired AUC levels were achieved. More details of dose adjustment are described in protocol section 4.4.1.

When the target level of decitabine AUC after oral administration was within 0.90-1.10 of IV decitabine AUC with no more than 1 DLT in 3-6 evaluable subjects, additional evaluable subjects were added to the cohort to reach up to 12-18 subjects in that cohort to confirm the AUC range at 0.90-1.10 before opening the Dose Confirmation Stage of the trial. If the AUC range in the first 12 subjects was out of the desired range, or more than 1 DLT occurred in 6 subjects, the DSRC could recommend further adjustment of the E7727 or decitabine dose in a new cohort of at least 3 subjects until the desired AUC range (0.90-1.10) and safety (no more than 1 DLT) were achieved. If more than one DLT in 6 subjects was observed in any cohort, no further escalation would take place, and the DSRC would decide whether de-escalation of one or more components (oral decitabine and/or E7727) was necessary.

Figure 1: Dose Escalation Stage Algorithm



*The doses of oral decitabine or oral E7727 given on Day -3 (±2 days) in Course 1 and Course 2 are the same doses, respectively, that are given on Days 2-5 in each cohort.

3.2 Discussion of Study Design

The study was not intended to identify a maximum tolerated dose of oral decitabine + E7727. Rather, dose escalation targeted a dose of the 2 components that approximately achieved unity in decitabine AUC exposures compared with AUC of IV decitabine 20 mg/m^2 (AUC ratio within

0.90-1.10). Escalation occurred only after review of the PK and safety data from at least 3 evaluable subjects in each cohort and a recommendation from the DSRC (see Section 3.4).

3.3 Study Endpoints

3.3.1 Primary Endpoint

- Mean decitabine AUC oral/IV: Mean AUC of oral decitabine + E7727 (Days 2 and/or 5) compared with IV decitabine 20 mg/m^2 (Day 1).
- Incidence and severity grades of DLTs.

3.3.2 Secondary Endpoints

- AUC, C_{max}, T_{max}, and other PK parameters of oral decitabine, E7727, and E7727-epimer.
- Overall incidence and severity of adverse events and clinically significant abnormal laboratory values.
- Mean maximum %LINE-1 demethylation of oral decitabine + E7727 by cohort.
- Overall response rate as measured by the International Working Group (IWG) 2006 MDS Response Criteria, duration of response, hematological improvement, rate of transfusion independence, time to AML, and overall survival.

3.3.3 Exploratory Endpoints

[redacted]

3.4 Data and Safety Review Committee

A thorough review of PK and safety data by the DSRC was regularly conducted, particularly after every cohort of 3-12 evaluable subjects during the Dose Escalation Stage.

The DSRC conferred when the last of at least 3 evaluable subjects in a cohort completed Course 1, Day 28. This committee comprised the principal investigators (or nominated deputies), medical monitor, study director, PK director, and other study team members as appropriate and reviewed available safety, PK, and PD data. The DSRC recommended whether to continue dosing based on the oral decitabine + E7727 dose escalation algorithm (Figure 1) and the safety data (DLTs). The DSRC made decisions regarding continuing dose escalation or de-escalation, to better assess the PK and safety of oral decitabine + E7727 during the Dose Escalation Stage. The DSRC decided on the exact next dose level based on the escalation scheme provided in Figure 1. DSRC could have also decided to escalate/de-escalate either drug component (E7727 or oral decitabine) if AUC levels or safety data at any cohort warranted such escalation/de-escalation.

3.5 Definition of Dose-Limiting Toxicities

A DLT occurring in the first course of treatment for all subjects at any cohort during dose escalation guided the dose escalation decision. At subsequent courses, DLTs guided dose adjustment or dose delays as discussed in protocol Section 7.3. DLTs were defined using the Common Terminology Criteria for Adverse Events Version 4.03 (CTCAE v4.03).

- ≥ Grade 3 non-hematologic toxicity (unless the DSRC determines that it is clearly related to an underlying disease or to disease progression), except the following:
  - Grade 3 nausea or vomiting that is controllable by anti-emetics.
  - Grade 3 diarrhea controllable by optimal therapy such as loperamide.
- Grade 3 laboratory tests other than serum creatinine, bilirubin, AST, or ALT will not be considered a DLT unless the abnormal result(s) is/are associated with clinical manifestations.
- Related prolonged Grade 4 thrombocytopenia that was not present prior to dosing, does not resolve within 14 days, and is not related to underlying disease.
- Related febrile neutropenia or Grade 4 neutropenia that was not present prior to dosing, does not resolve within 14 days, and is not related to underlying disease.
- Any toxicity (related to study treatment) that results in treatment delays of >4 weeks after Day 28.

4.0 SAMPLE SIZE

For this stage, no formal statistical tests of hypotheses were performed to calculate sample size. The Dose Escalation Stage contained multiple cohorts with a cohort size of 3-12 evaluable subjects (and could have been extended to 18 subjects to confirm the AUC range if recommended by the DSRC) for each cohort. The enrollment of subjects in the Dose Escalation Stage continued until the desired decitabine AUC level (AUC ratio of 0.90 to 1.10 of oral decitabine to IV decitabine) and safety were achieved, as described in Section 3.1. A subject was considered evaluable for dose escalation if all the criteria below were met:

- Completed Course 1 dosing regimen with sufficient PK data available.
- Had safety assessment for the 28 days of Course 1.

5.0 ANALYSIS SETS

5.1.1 All Subject Analysis Set

This analysis set contains available data from all enrolled subjects, including those who did not receive any study treatment. This analysis set will be used for analysis of subject disposition.

5.1.2 Efficacy Analysis Set

The Efficacy Analysis Set includes data from all enrolled subjects who receive any amount of study treatment.

5.1.3 Safety Analysis Set

The safety analysis set is the same as the Efficacy Analysis Set, which includes data from all enrolled subjects who received any amount of study treatment. In the safety analysis, no data exclusion will be allowed because of protocol deviations.

5.1.4 Pharmacokinetics Analysis Set

The PK Analysis Set will include data from all treated subjects for whom sufficient PK samples were collected to enable calculation of full AUCs on PK assessment days.

5.1.5 Pharmacodynamics and Biomarker Analysis Set

PD Analysis Set for LINE-1 methylation will be all subjects who received at least one course (Days 1-5) study treatment and had baseline and Day 8 or Day 15 LINE-1 methylation data. PD Analysis Set for other biomarkers will be based on those subjects who had biomarker data collected and analyzed.

6.0 SCHEDULE OF ANALYSES

Analyses of safety, efficacy, PD, and PK data will be regularly performed for review by the DSRC for the purpose of guiding decisions such as dose escalation, de-escalation, and change in cohort size, determination of subsequent dose level during the Dose Escalation Stage. Formal analyses including all data collected up to June 1, 2017, will be performed for preparation of the clinical study report for the dose escalation stage of ASTX727-01. Additional analyses will be conducted when all subjects are out of study.

No formal interim analyses are planned for this study.

7.0 STATISTICAL ANALYSIS

Unless otherwise specified, all statistical tests and confidence intervals created will be two-sided with alpha = .05. The SAS® statistical package (version 9.4 or a later version) will be used for the analyses.

In general, the first dosing date is defined as the first date the subject received decitabine IV, oral decitabine, or E7727. All analyses will be conducted by cohort and all cohorts combined unless otherwise specified.

7.1 Subject Disposition

Subject disposition including numbers screened, treated, and treatment discontinuation by reason, as well as the reasons for study exit (ie, death or reasons that subject were not followed up for survival status) will be summarized using frequencies and percentages according to the study visit, treatment discontinuation, and withdrawal from study case report form pages. Sample size for the Efficacy, Safety, PK, and PD Analysis sets will be clearly identified. Subjects in the All Subject Analysis Set will be included in the disposition analysis.

7.2 Demographic and Other Baseline Characteristics

The Efficacy Analysis Set (Section 5.1.2) will be used to summary demographic and baseline characteristics. The demographic variables consist of age, age category, sex, ethnicity, race, and geographical region. Baseline characteristics include height, weight, BSA, ECOG performance status, IPSS risk category, time since prior diagnosis, cytogenetic risk levels, peripheral blood (including blasts) counts, and BM blasts.

Baseline values are generally the last value collected on or before the 1st dosing date unless otherwise specified.

Age at baseline, if not already provided through data collection, will be calculated as the integer part of (date of enrollment- date of birth)/365.25.

Time since Diagnosis will be calculated as the (date of 1st dosing – date of diagnosis). If the day is missing for date of diagnosis, the 15th of the month is used. If the month is missing, July 1st is used. If the year is missing, the date is left as missing.

Subject demographic and baseline characteristics will be summarized by mean, standard deviation, median, minimum, and maximum for continuous variables; and by counts and percentages for categorical variables.

7.3 Efficacy Variables and Analyses

Efficacy analyses will be based on the Efficacy Analysis Set (Section 5.1.2).

7.3.1 Response Rate

The evaluation of response (Table 1) is based on IWG 2006 MDS Response Criteria (Cheson et al 2006). Details of response assessments are described in Appendix 1.

Table 1: Modified IWG 2006 MDS Response Criteria

Complete Response (CR): the following for 4 weeks		
Peripheral: Normal peripheral counts with persistent granulocyte count ≥1.0×10^9/L, platelet ≥100×10^9/L and Hgb ≥11 g/dL.		
Marrow: Normal bone marrow with persistent marrow blasts ≤5%. Persistent dysplasia will be noted.		
Partial Response (PR): the following for 4 weeks		
Peripheral: Normal peripheral counts with granulocyte count ≥1.0×10^9/L, platelet count ≥100×10^9/L, and Hgb ≥11 g/dL.		
Marrow: Normal bone marrow and marrow blasts >5%, but were reduced by 50% or more.		
Marrow Complete Response (mCR): the following 4 weeks		
Reduction of bone marrow blasts to ≤5% and decrease by 50% or more without normalization of peripheral counts.		
Hematological Improvement (HI): lasts at least 8 weeks		
Erythroid Response (HI-E):	Major Response:	Hemoglobin increase ≥1.5 g/dL or RBC transfusion dependence
Platelet Response (HI-P):	Major Response:	Absolute increase of platelet count from <20 to >20×10^9/L and by at least 100%, or if more than 20×10^9/L, by an absolute increase of at least 30×10^9/L.
Neutrophil Response (HI-N):	Major Response:	Granulocyte increase ≥100%, and by an absolute increase ≥0.5×10^9/L.

Baseline value is defined as the average of any values obtained before and on the first dosing date. If there is only one value before treatment then it will be used as the baseline value.

The following rates will be estimated using sample proportions and 95% Clopper-Pearson confidence interval based on the number of subjects in the Efficacy Analysis Set:

- Overall response (OR [CR+PR+mCR+HI]).
- CR+PR+mCR.
- CR.
- PR.
- mCR.
- HI (HI-E, HI-P or HI-N).
  - HI-E.
  - HI-P.
  - HI-N.

If adequate baseline [redacted] is available responses will also be summarized by selected baseline [redacted].

7.3.2 Overall Survival

Overall survival is defined as the number of days from the day the subject received the first dose of study treatment to the date of death (regardless of cause). Subjects without documentation of death will be censored on the last date of contact or the last date subject was confirmed alive in the Study Discontinuation CRF page, whichever is later.

The Kaplan-Meier plot will be provided based on the Efficacy Analysis Set. Estimated median survival and 95% confidence intervals will also be provided if available.

7.3.3 Time to AML

Time to AML is defined as the number of days from the day the subject received the first dose of study treatment to the date of MDS progression to AML as defined by ≥20% blasts in bone marrow or peripheral blood or death of any cause. The event date of time to AML will be based on the earliest date of following:

- Death date.
- AML conversion date defined as the earliest date of the following:
  - AML conversion date on the Conversion to AML CRF page.
  - AML conversion date on the Subject Information CRF page.
  - The first date when a record of ≥20% blasts in bone marrow was reported or 2 consecutive records of ≥20% blasts peripheral blood were reported.

Subjects without a time to AML event as described above will be censored on the date of last contact.

The Kaplan-Meier plot for time to AML will be provided based on the Efficacy Analysis Set. Estimated median survival and 95% confidence intervals will also be provided if available.

7.3.4 Duration of Response

Duration of response will be calculated separately for CR, PR, mCR, and CR+PR+mCR from the first time a response category (CR, PR, and mCR) is initiated, as addressed in the Implementation Guide of Response Assessment in Appendix 1, to the earliest of the following dates:

- Death date.
- End of response as addressed in Appendix 1, where date of disease progression is defined as the earliest date of:
  - AML conversion as described in Section 7.3.3.
  - disease progression date on the Subject Information CRF page.
  - last treatment date if the reason for treatment discontinuation is Progressive Disease.

- study exit date on the Study Discontinuation CRF page if the reason for study discontinuation is Progressive Disease.
- date of disease progression on the Survival Follow-up CRF page.

Duration of response will be summarized using mean, standard deviation, standard error, minimum, median, and maximum for subjects who achieved a corresponding response during the study. The longest duration will be selected within a subject for summary if multiple responses occurred in a subject.

7.3.5 Transfusion Independence

Transfusion dependence and independence is defined as follows:

- Transfusion dependence at baseline: documentation of 2 units or more of transfusion within 56 days of the first day of study treatment.
- Post-treatment transfusion independence: no transfusion for 56 consecutive days or more after treatment.

Post-treatment transfusion independence rate will be calculated separately for RBC transfusion independence and platelet transfusion independence as the number of subjects who are transfusion independent post treatment (n) divided by the number of subjects who were transfusion dependent at baseline (N). The 95% Clopper-Pearson confidence interval for transfusion independence rates will be provided. Analyses will also be provided by duration of transfusion follow-up (≥56 days, ≥84 days and more). The same analyses will be performed for 84-day and 112-day transfusion independence, defined as no transfusion for 84 consecutive and 112 consecutive days respectively.

7.4 Safety Variables and Analyses

Safety Analysis will be performed using the Safety Analysis Set (Section 5.1.3). Safety will be assessed by subject-reported and investigator-observed AEs, along with physical examination, clinical laboratory tests (hematology, chemistries), vital signs, concomitant medications, and ECGs. Exposure to study treatment, reasons for discontinuation, deaths, and causes of deaths will be tabulated.

7.4.1 Study Treatment and Exposure

The number of courses received per subject will be summarized using descriptive statistics. Frequency count and percentage of dose delayed courses reported by investigator will be calculated based on the total number of treatment courses all subjects received. Dose reduced courses, defined as the courses in which the dose is reduced by 20% or more relative to the planned dose within the course, will be summarized using frequency count and percentage for oral decitabine, E7727, and IV decitabine separately based on the total number of courses all subjects received for each study medication.

7.4.2 Adverse Events

Adverse event (AE) terms reported by study subjects or observed by investigators will be mapped to the appropriate System Organ Class (SOC) and Preferred Term (PT) according to the Medical Dictionary for Regulatory Activities (MedDRA). Severity of AE will be graded using Common Terminology Criteria for Adverse Events (CTCAE) version 4.03.

Treatment-emergent AEs (TEAEs) are defined as events that first occurred or worsened, after the first dose of study treatment until 30 days after the last dose of study treatment or the start of an alternative anti-cancer treatment for MDS/chronic myelomonocytic leukemia (CMML) and subsequent AML, whichever occurs first, with the following exceptions: events that occurred after 30 days beyond the last dose of study treatment or the start of an alternative anti-cancer treatment for MDS/CMML and subsequent AML will also be considered treatment-emergent if the events are both serious and related to the study treatment. For the purpose of determining whether an AE is a treatment-emergent AE, incomplete AE start and stop dates will be imputed conservatively following the data programming standards as detailed in the Astex Data Programming Conventions.

All AE data collected in the study database will be listed, including those that are not treatment emergent. Only TEAEs will be included in the AE summary tables.

An overall safety summary table containing counts and percentages of subjects with any AE, any AE Grade ≥3, AE leading to treatment discontinuation, any SAE, and subcategories of SAEs (death vs not death) will be produced. A similar table with related AE counts will also be produced. Related events are those that the Investigator considered to be suspected to be related to study treatment as described in the study protocol.

The number and percentage of subjects experiencing AEs will be summarized by MedDRA SOCs (sorted alphabetically) with PTs sorted alphabetically within each SOC, and by CTCAE grade. The number and percentage of subjects experiencing AEs will also be summarized by PT and sorted by event frequency. Related AEs, serious AEs, and related serious AEs will be summarized similarly. In summarizing AEs, if the occurrence of a particular AE for a given subject is reported more than once, the event is only counted once with its worst CTCAE grade.

7.4.3 Dose-Limiting Toxicity

Protocol specified DLTs were identified based on CTCAE v4.03 (Section 3.5). Frequency count and percentage of DLTs will be summarized by cohort. DLTs will also be identified in the listing of AEs.

7.4.4 Concomitant Medications

Concomitant medications are the medications taken with a start date on or after the start of the administration of study treatment, or those with a start date before the start of the administration of study treatment and a stop date on or after the start of the administration of study treatment.

Medications taken beyond 30 days from the last dose of study treatment or after the start of an alternative anti-cancer treatment for MDS/CMML and subsequent AML are not considered concomitant medications, unless they are used for treating a related SAE. For the purpose of inclusion in the concomitant medication tables, incomplete medication start and stop dates will be imputed conservatively.

Concomitant medication will be coded by the latest version of WHO Drug Dictionary before the data download and summarized by Anatomical Therapeutic Chemical (ATC level 2) and drug name, sorted alphabetically, using counts and percentages.

7.4.5 Laboratory Tests

Laboratory values will also be graded, if possible, by CTCAE v4.03 in conjunction with the Harrison (18th edition) lab book normal values (Longo et al 2011). Shift tables will display (1) shift from baseline grade to the worst grade during the study, and (2) shift from baseline grade to the last grade at the end of study.

Laboratory values recorded as an interval such as "≥x", "<x", or "2+" will be handled, if necessary for calculation purposes, following the data programming standards as detailed in the Astex Data Programming Conventions.

7.4.6 Vital Signs

Vital Signs will be summarized by visit and treatment group using proportion of subjects with each vital sign being too high or too low according to conventionally accepted vital sign normal ranges as following:

- Pulse rate 110 bpm or greater.
- Pulse rate 50 bpm or less.
- Diastolic blood pressure 110 mmHg or greater.
- Diastolic blood pressure 55 mmHg or less.
- Systolic blood pressure 180 mmHg or greater.
- Systolic blood pressure 80 mmHg or less.
- Respiration rate 20 breaths/min or greater.
- Body temperature 39°C or greater.

7.4.7 Electrocardiogram (ECG)

Abnormal ECG findings will be summarized by visit. Subjects with any post-baseline abnormal ECG findings will also be tabulated.

QTc values will be graded based on CTCAE 4.0.3, and the shift table showing the shift from baseline grade to the worst grade, and from baseline grade to the last available grade will be provided.

7.4.8 ECOG Performance Status

Shift tables for ECOG from baseline to the worst grade, and from the baseline to the last available grade will be provided.

7.4.9 Echocardiogram / Multigated Acquisition (ECHO/MUGA)

Summary statistics for left ventricular ejection fraction (LVEF, %) and abnormality (normal or abnormal) will be presented at baseline and last visit. Summary statistics for change from baseline of LVEF (%) will also be presented for subjects with valid baseline and any post-baseline LVEF data.

7.4.10 Physical Examination

Physical examination data will be presented in a data listing.

7.5 Pharmacokinetics Analysis

The PK Analysis Set (Section 5.1.4) will be used for analysis of all PK parameters. PK parameters of decitabine, E7727, and E7727-epimer will be derived for each subject using a non-compartmental approach.

7.5.1 Calculation of Decitabine AUC

7.5.1.1 Calculation of Decitabine Daily AUCs

AUCs (the area under the concentration-time curve) will be calculated by the linear up/log down method using the measured concentration-time values above the BQL (below the limit of quantification). In particular, AUC_{0-t} (the area under the concentration-time curve from time zero to the time of the last (t_{last}) quantifiable concentration (C_t)) by dose/cohort and course/days will be used for estimating decitabine cumulative 5-day AUC_{0-t} exposures.

7.5.1.2 Decitabine 5-day AUC_{0-t}

For estimating decitabine 5-day cumulative AUC_{0-t} exposures in the Dose Escalation stage after oral ASTX727 combination, the following assumptions were used:

- Steady state is reached on the 2nd day of dosing with oral E7727+decitabine combination.
- Based on steady state achievement on Day 2, decitabine AUC_{0-t} from Day 5 in Dose Escalation (4th dosing day with combination) would be representative of daily AUC_{0-t} on Days 2 through 5 in a putative 5-day dosing with ASTX727.

Thus, to estimate total 5-day oral decitabine AUC_{0-t} exposures using PK data from Dose Escalation, Day 2 AUC (first oral combination dose) was added to Day 5 $AUC_{0-t} \times 4$.

For estimating decitabine 5-day AUC_{0-t} exposures after IV infusion, Day 1 (IV-infusion) AUC_{0-t} was multiplied by 5 based on information from Dacogen prescribing information that there was no accumulation on Day 5 vs Day1 for 5-day IV infusion.

7.5.2 Calculation of the Ratio for Decitabine 5-day AUC_{0-t} for Oral:IV

To compare decitabine cumulative 5-day systemic exposures between Oral E7727+decitabine combination and IV infusion, the ratio of 5-day AUC_{0-t} exposure estimates for Oral and IV administration was derived (Oral:IV) for each dose cohort. This ratio was used to assess the progress in dose escalation for achieving targeted IV AUC_{0-t} using oral ASTX7272.

7.5.3 Dose Proportionality

PK dose proportionality will be tested using linear regression between dose and dose-adjusted parameter estimates. A "Power model" will be applied to each of the PK parameters including AUC_{0-inf}, AUC_{0-t} and C_{max} for decitabine and E7727, when applicable.

7.5.4 PK Summary

Daily decitabine AUC will be summarized descriptively using mean, standard deviation, median, and range by dose cohort and course/days if deemed necessary. Decitabine 5-day AUC_{0-t} will be summarized separately for ASTX727+E7727 and IV decitabine using descriptive statistics by cohort.

7.6 Pharmacodynamic/Biomarker Analyses

The PD and Biomarker Analysis Set (Section 5.1.5) will be used for analyses of LINE-1 methylation and other biomarkers. All data will be summarized descriptively (using mean, standard deviation, median, and range for continuous variables and counts and percentages for categorical variables) by dose cohort and visit for each biomarker.

Maximum %LINE-1 demethylation (defined as the largest percent decrease from baseline in methylation values within a subject between Day 8 and Day 22 of the first treatment course) will be summarized using mean, standard deviation, standard error, minimum, median, and maximum.

Additional biomarker analysis will include assessment of baseline CDA activity and changes in circulating [redacted] levels post-treatment. Changes in [redacted] after treatment with E7727 will be analyzed by comparing post-dose [redacted] levels to pre-dose values at each dosing day of E7727 per subject. Mean change of [redacted] will be presented by E7727 dosing day.

7.7 Handling of Missing Data and Other Data Anomalies

No missing data imputations are planned for the study, except as specified. Subjects lost to follow-up will be included in statistical analyses to the point of the data cut-off date.

7.8 Handling of Protocol Deviations

Important Protocol Deviations (IPDs) categories are defined by the study team before the first subject visit and updated during the IPD reviews throughout the study prior to data cut-off date. IPDs will include violation of major inclusion or exclusion criteria. IPD will be tabulated by cohort and listed by cohort and subject.

8.0 REFERENCES

Bejanyan N, Sekeres MA. The revolution of myelodysplastic syndromes. Therapeutic advances in hematology. 2011;2(1):33-43.

Bennett JM. Proposals for the classification of the myelodysplastic syndromes. Br J Haematol. 1982;51(2):189-199.

Beumer J, Eiseman J, Gilbert J, Holleran J, Yellow-Duke A, Clausen D, et al. Plasma pharmacokinetics and oral bioavailability of the 3,4,5,6-tetrahydrouridine (THU) prodrug, triacetyl-THU (taTHU), in mice. Cancer Chemotherapy and Pharmacology. 2011;67(2):421-430.

Beumer JH, Eiseman JL, Parise RA, Joseph E, Covey JM, Egorin MJ. Modulation of gemcitabine (2',2'-difluoro-2'-deoxycytidine) pharmacokinetics, metabolism, and bioavailability in mice by 3,4,5,6-tetrahydrouridine. Clin Cancer Res. 2008;14(11):3529-3535.

Beumer JH, Eiseman JL, Parise RA, Joseph E, Holleran JL, Covey JM, et al. Pharmacokinetics, metabolism, and oral bioavailability of the DNA methyltransferase inhibitor 5-fluoro-2'-deoxycytidine in mice. Clin Cancer Res. 2006;12(24):7483-7491.

Beumer JH, Parise RA, Newman EM, Doroshow JH, Synold TW, Lenz HJ, et al. Concentrations of the DNA methyltransferase inhibitor 5-fluoro-2'-deoxycytidine (FdCyd) and its cytotoxic metabolites in plasma of patients treated with FdCyd and tetrahydrouridine (THU). Cancer Chemother Pharmacol. 2008;62(2):363-368.

Chabot GG, Bouchard J, Momparler RL. Kinetics of deamination of 5-aza-2'-deoxycytidine and cytosine arabinoside by human liver cytidine deaminase and its inhibition by 3-deazauridine, thymidine or uracil arabinoside. Biochem Pharmacol. 1983;32(7):1327-1328.

Cheson BD, Bennett JM, Kantarjian H, Pinto A, Schiffer CA, Nimer SD, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. Blood. 2000;96(12):3671-3674.

Cheson BD, Greenberg PL, Bennett JM, Lowenberg B, Wijermans PW, Nimer SD, et al. Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. Blood. 2006;108(2):419-425.

Dacogen. Dacogen (decitabine for injection) Prescribing Information. 2014.

Greenberg P, Cox C, LeBeau MM, Fenaux P, Morel P, Sanz G, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89(6):2079-2088.

Heaney ML, Golde DW. Myelodysplasia. N Engl J Med. 1999;340(21):1649-1660.

Kantarjian H, Estey E. Myelodysplastic syndromes. 6th ed. DeVita VT, Hellman S, Rosenberg SA, editors: Lippincott Williams & Wilkins; 2001.

Karahoca M, Momparler RL. Pharmacokinetic and pharmacodynamic analysis of 5-aza-2'-deoxycytidine (decitabine) in the design of its dose-schedule for cancer therapy. Clin Epigenetics. 2013;5(1):3.

Longo DL et al. 2011. Harrison's principles of internal medicine (18th edition). New York: McGraw-Hill Medical Publishing Division.

Mistry B, Jones M, Kubiak P. A phase 1 study to assess the absolute bioavailability and safety of an oral solution of decitabine in subjects with Myelodysplastic Syndromes (MDS). Blood. 2011;118:Abstract 3801.

Steensma DP, Baer MR, Slack JL, Buckstein R, Godley LA, Garcia-Manero G, et al. Multicenter study of decitabine administered daily for 5 days every 4 weeks to adults with myelodysplastic syndromes: the alternative dosing for outpatient treatment (ADOPT) trial. J Clin Oncol. 2009;27(23):3842-3848.

Vidaza. Vidaza (azacitidine for injection) for SC or IV use. Prescribing information. 2012.

APPENDIX 1: Implementation Guide For Response Assessment

The rules for determination of MDS responses and the corresponding durations of responses are described in the following sections. These rules are based on the "best" same-day blood counts (PB blasts, neutrophils, platelets, and Hgb) measured within a treatment course and the transfusion-free period requirement. The "best" was defined in the sense that blood count criteria were met for a particular response status. For BM blasts, if the current course did not have a BM evaluation, the immediately preceding BM evaluation was used.

Rules for CR

The subject must have one of the following baseline values to be eligible for a CR.

Hgb <11 g/dL

Platelets $<100 \times 10^9$/L

Neutrophils $<1.0 \times 10^9$/L

BM Blasts >5%

PB Blasts >5%

To be a CR, the subject must have fulfilled all of the following blood count criteria (the same-day counts for PB blasts, neutrophils, platelets, and Hgb were required) and have been transfusion free, for ≥28 days:

Hgb ≥11 g/dL

Platelets $\geq 100 \times 10^9$/L

Neutrophils $\geq 1.0 \times 10^9$/L

BM Blasts ≤5%

PB Blasts absent (no value of PB blasts in the database is interpreted as absent)

The start date of CR must have been >7 days after any RBC transfusion and >3 days after any platelet transfusion.

The duration of CR was calculated from the first time a subject fulfilled these criteria to the earliest time point when BM blasts became >5%, PB blasts > 0%, or when any of the blood counts no longer met the above criteria, the subject received an RBC or platelet transfusion, or the subject showed a clinical progression as determined by the investigator.

In determining duration of CR, if one measurement of BM blasts increased above the 5% level and then decreased to this level or lower without receiving an alternative treatment, the subject was considered to have maintained the ≤5% level.

Rules for PR

To be a PR, subjects must have demonstrated all CR criteria with a relaxed requirement for BM blasts level. The BM blasts must have decreased by 50% or more compared with the pretreatment level but were still above 5%. The duration of PR is calculated from the first time a subject fulfilled these criteria to the earliest time point when BM blasts, peripheral blasts or any of the blood counts no longer met the above criteria, the subject received an RBC or platelet transfusion, or the subject showed a clinical progression as determined by the investigator.

Rules for mCR

To be eligible for mCR, subjects must have had a baseline BM blast >5%. An mCR could be declared if post treatment BM blasts become ≤5% and decreased by ≥50% over the pretreatment level for ≥28 days until disease progression.

End of mCR was considered the earliest time point when BM blasts become >5% or a clinical progression was determined by the investigator. In determining duration of mCR, if one measurement of BM blasts were above the 5% level and then decreased to this level or lower without receiving an alternative treatment, the subject was considered to have maintained the ≤5% level.

Rules for CR+PR+mCR

Subjects who have CR, PR, or mCR will be considered a responder for CR+PR+mCR. The duration of CR+PR+mCR is measured from the initiation of CR, PR, or mCR, to the day that the subject do not meet criteria for CR, PR, and mCR.

Rules for HI-E

HI-E status was determined based on RBC transfusion and Hgb level at baseline and during the posttreatment period. To be eligible for HI-E, the subject must have had a baseline RBC transfusion dependence (any RBC transfusion within 4 weeks prior to C1D1) or had a baseline Hgb <11.0 g/L.

For subjects with RBC transfusion dependence at baseline, an HI-E was declared if the subjects did not receive any RBC transfusion for a ≥56-day period posttreatment, regardless of Hgb level.

For subjects without RBC transfusion dependence at baseline but with a baseline Hgb <11.0 g/L, an HI-E was declared if subjects had an Hgb increase by ≥1.5 g/L for ≥56 days (at least one count per course above threshold) and did not receive any RBC transfusions for ≥56 days. The start date of Hgb increase must have been >7 days after any RBC transfusion.

Duration of HI-E was calculated from the first time the HI-E criteria was fulfilled until the subject no longer met the HI-E criteria or disease progression.

Rules for HI-P

To be eligible for HI-P, a subject had to have a baseline platelet count $<100 \times 10^9$/L.

To be considered an HI-P a subject had to remain independent of platelet transfusions, and have platelet counts meet the criteria as described below, for a ≥56-day period posttreatment. The start date of response must have been >3 days after any platelet transfusion.

- If the baseline platelet count was $<20 \times 10^9$/L, the count must have increased to $\geq 20 \times 10^9$/L and by at least 100%.
- If the baseline count was $\geq 20 \times 10^9$/L, the count must have increased by 30×10^9/L.

The duration of HI-P was calculated from the first time the HI-P criteria was fulfilled until the subject no longer met the HI-P criteria or disease progression.

Rules for HI-N

G-CSF or GM-CSF was not to be administered. Clinical review of concomitant medication data listings was conducted to confirm that there were no such instances of subject use.

The subject must have had a baseline ANC of $<1.0 \times 10^9$/L to be eligible for an HI-N response.

To have a response, ANC must have increased by ≥100% with an absolute increase of $\geq 0.5 \times 10^9$/L and been maintained for a 56-day period (at least one count per course above threshold).

The duration of HI-N was calculated from the first time the HI-N criteria was fulfilled until the subject no longer met the HI-N criteria or disease progression.