A Phase 1/2, Multicenter, Open-Label, Single Arm, Dose Escalation and Expansion Study of Gilteritinib (ASP2215) Combined with Chemotherapy in Children, Adolescents and Young Adults with FMS-like Tyrosine Kinase 3 (FLT3)/Internal Tandem Duplication (ITD) Positive Relapsed or Refractory Acute Myeloid Leukemia (AML)

ISN/Protocol 2215-CL-0603
Version 8.0
Incorporating Substantial Amendment 7

22 Jul 2022

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Pediatric Investigational Plan EMEA-002064-PIP01-16

Sponsor:

Astellas Pharma Global Development, Inc. (APGD)

1 Astellas Way
Northbrook, IL 60062

Protocol History:

Version 1.0 Original [02 Jul 2018]
Version 2.0 Incorporating Substantial Amendment 1 [24 May 2019]
Version 3.0 [UK] Incorporating Country-specific Substantial Amendment 2 [02 August 2019]
Version 4.0 [CA] Incorporating Country-specific Substantial Amendment 3 [15 August 2019]
Version 5.0 [DE] Incorporating Country-specific Substantial Amendment 4 [31 October 2019]
Version 6.0 Incorporating Substantial Amendment 5 [17 Feb 2021]
Version 7.0 Incorporating Substantial Amendment 6 [07 Sep 2021]

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PROTOCOL AMENDMENT SUMMARY OF CHANGES

DOCUMENT HISTORY	
Document	**Date**
Version 7.0 Incorporating Substantial Amendment 6	07 Sep 2021
Version 6.0 Incorporating Substantial Amendment 5	17 Feb 2021
Version 5.0 [DE] Incorporating Country-specific Substantial Amendment 4	31 Oct 2019
Version 4.0 [CA] Incorporating Country-specific Substantial Amendment 3	15 Aug 2019
Version 3.0 [UK] Incorporating Country-specific Substantial Amendment 2	02 Aug 2019
Version 2.0 Incorporating Substantial Amendment 1	24 May 2019
Version 1.0 Original	02 Jul 2018

Amendment 7 [Substantial] 22 Jul 2022

This amendment is considered to be substantial based on the criteria set forth in Article 10(a) of Directive 2001/20/EC of the European Parliament and the Council of the European Union and applicable national laws and regulations because it potentially significantly impacts the safety or physical/mental integrity of participants or the scientific value of the study.

Overall Rationale for the Amendment:

The overall rationale for this amendment is based on regulatory feedback.

Summary of Changes

Substantial Changes

Section Number	Description of Change	Brief Rationale
IV, 2.2.1	For a dose to be considered biologically active, at least 7 of 9 subjects at the RP2D dose in each group must demonstrate CR, a high degree of gilteritinib biologic activity (as measured by PIA), or a combination of both with the following conditions for PIA: • Subjects that complete 2 induction cycles must achieve PIA of > 90% for at least 3 of 4 trough time points; or • Subjects that complete only 1 induction cycle must achieve PIA of > 90% at 2 of 2 trough time points.	The original intent of the study was for subjects to receive 2 cycles of treatment. However, subjects who achieve response after 1 cycle and have an available transplant donor, have proceeded to transplant. This modification will allow subjects who receive 1 cycle to be evaluable for biological activity based on CR, PIA or a combination of both and such subjects will not need to be replaced in RP2D determination.
IV, V, 2.1.1, 3.1, 3.2, 12.8	Update the phase 2 primary objective and inclusion criterion #4 for the phase 2 portion of the study to clarify that a subject must be refractory to or at the first hematologic relapse after first-line remission induction AML therapy (up to 2 induction cycles). Definition of line of therapy is added to Appendix 12.8.	The AML eligibility is updated for the phase 2 portion of the study to align with entry criteria for the phase 3 gilteritinib program; the flow chart for line of therapy is added to ensure clarity and understanding of the therapy definitions.

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Section Number	Description of Change	Brief Rationale
IV, 2.3.1	The primary phase 1 endpoint for determination of MTD and/or RP2D is updated to remove the following text: "…based on the DLT and biologic activity according to PIA." One of the primary endpoints for phase 2 (i.e., CR rates after 2 cycles of therapy) is updated to specify that the CR rate will be further described by the duration of CR (only for USA).	The MTD and/or RP2D determination will be based on the combination of both safety and efficacy data; this change also aligns with the updated definition of biological activity. To provide additional information regarding CR rate based on regulatory feedback.
IV, 2.3.2.2	DLT criteria are updated to clarify that nonhematologic DLT will be defined as grade 3 nonhematologic toxicity at least possibly related to protocol therapy that persists for > 48 hours without resolution to grade ≤ 2 or grade 4 nonhematologic toxicity, regardless of duration, at least possibly related to protocol therapy. Hy's law (as defined in [Section 12.5]) or treatment-related deaths will be considered as a DLT. Exceptions for the following toxicities commonly seen with intensive AML reinduction regimens are revised: grade 4 elevation in hepatic transaminases is removed; grade 4 fever with neutropenia, with or without infection, is removed; grade 4 infection is replaced with grade 4 infections expected as direct complication of cytopenia due to active underlying leukemia; and grade 4 mucositis is removed. Hematologic DLT will be evaluated at day 42 from the start of cycle 1 day 1.	To ensure subject safety.
IV, 3.2	Remove serum creatinine from inclusion criterion #8, but retain estimated glomerular filtration rate of > 60 mL/min/1.73 m^2.	To describe the minimal renal function criterion in terms of creatinine clearance or estimated glomerular filtration rate alone.

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Section Number	Description of Change	Brief Rationale
IV, 3.3	Update exclusion criterion #6 to exclude subjects who have active clinically significant GVHD or are on treatment with immunosuppressive drugs for treatment of active GVHD, with the exception of subjects being weaned from systemic corticosteroids where the subject is receiving ≤ 0.5 mg/kg of prednisone (or equivalent) daily dose for prior GVHD. Subjects who have received calcineurin inhibitors within 4 weeks prior to screening, unless used as GVHD prophylaxis.	To ensure subject safety.
2.2.1, 11	Safety monitoring rules are clarified. A Bayesian posterior probability for safety monitoring will be used for phase 2. Subjects in phase 2 will be continued to be monitored for ≥ grade 3 non-hematologic AEs considered at least possibly related to protocol therapy, and AEs of any grade leading to treatment discontinuation or death considered at least possibly related to protocol therapy. The event rate will be reviewed for the first 6 evaluable subjects at the end of cycle 1. After that, the event rate will be reviewed continuously. The estimated event rate based on the Bayesian beta-binomial model will be provided for safety monitoring. If the event rate is ≥ 20% with a posterior probability of at least 80%, then the enrollment to phase 2 will be paused and safety will be reassessed by the DEC. With a non-informative prior Beta(1,1) distribution, the numbers of subjects with event for certain evaluable subjects, which trigger the enrollment pause for safety review, are listed in [Table 4]. The algorithm for differentiation syndrome based on the [Montesinos et al, 2009] criteria, in addition to the preferred term of 'acute promyelocytic leukemia differentiation syndrome', will be used to search for potential cases of differentiation syndrome in every 6 subjects in phase 2.	To ensure subject safety.

Nonsubstantial Changes

Section Number	Description of Change	Brief Rationale
II, 13	Update clinical research contact identity, medical monitor/study physician information, and senior data science and statistical manager identity (sponsor's signature page).	To provide updated contact information.
IV	Planned study period is updated to end 3Q2026.	To update period based on current study enrollment projections.
IV, 2.2.1	Clarify that, in addition to pharmacokinetic parameters and biological activity, response assessment will also be evaluated during cycle 1 and/or cycle 2.	To clarify that response assessment is part of the data that will be considered when determining the optimal dose.
IV, V, 2.2.1, 5.1.1.1.3, 5.1.5	LTT is updated to clarify that subjects completing 1 or 2 treatment cycles of phase 1 or 2 will have the option to participate in LTT and that subjects receiving clinical benefit during phase 1 or 2 may initiate LTT only after discussion and approval from the Astellas medical monitor. Criteria clarifying clinical benefit is updated for all sites as follows: a) Completed 1 or 2 treatment cycles of phase 1 or 2. b) Subject did not develop intolerable or unacceptable toxicity. c) Subject has achieved remission, stable or improved disease, or symptom improvement, or decreased transfusion dependence. d) Any other reason that the investigator judges will benefit the subject from continued treatment with gilteritinib, must be reviewed and approved by the Astellas medical monitor. During the LTT period, subjects will receive gilteritinib once daily starting from day 1 through day 28 of a 28-day cycle at the same dose received during phase 1 or 2.	To provide a clear definition of clinical benefit to qualify for LTT.

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Section Number	Description of Change	Brief Rationale
IV, 2.2.1, 7.1.2	Operationally, 22 response evaluable subjects will be needed during the first stage, with 4 CR responders or 9 CRc responders after 1 or 2 cycles of therapy required to continue to a total of 52 response evaluable subjects. If there are less than 4 CR responders in stage 1, the enrollment will be stopped in the USA and the study will not proceed to stage 2 in the USA.	USA-specific response requirements are clarified based on regulatory feedback.
IV, 2.2.1, 11	In addition to safety data through the DLT observation period, the DEC will review response data and gilteritinib biologic activity (as measured by PIA) through the cycle 1 and/or cycle 2 of the evaluable subjects. The algorithm for differentiation syndrome based on the [Montesinos et al, 2009] criteria, in addition to the preferred term of 'acute promyelocytic leukemia differentiation syndrome', is added to search for potential cases of differentiation syndrome at every dose level in phase 1. The RP2D and/or MTD will be selected based on the DEC's review of all available data at each dose level, including safety data, pharmacokinetic data (if available), response data and gilteritinib biologic activity data, and the RP2D will become the minimum safe and biologically effective dose level.	To ensure subject safety.
5.5.2.1	This section describing "Always Serious Adverse Events" is removed.	This section described a sponsor process that is now obsolete and replaced with an updated process. As this is a sponsor process that does not impact reporting by the investigator site, it is removed from the protocol.
10.1	The responsibilities of the DEC are clarified and will also include previous IDMC responsibilities formerly described in Section 10.2.	A separate IDMC will not be utilized in this study; the DEC will fulfill the independent safety monitoring requirements.
Throughout	Minor editorial changes incorporated as necessary. Document history and protocol amendment summary of changes are placed at the beginning of the protocol.	Minor editorial changes incorporated to improve clarity. Changes incorporated per update to Astellas Global Protocol Format V11.0.

I. SIGNATURES

1. SPONSOR'S SIGNATURES

Required signatures (e.g., Protocol authors and contributors, etc.) are located in [Section 13 Sponsor's Signatures].

2. INVESTIGATOR'S SIGNATURE

A Phase 1/2, Multicenter, Open-Label, Single Arm, Dose Escalation and Expansion Study of Gilteritinib (ASP2215) Combined with Chemotherapy in Children, Adolescents and Young Adults with FMS-like Tyrosine Kinase 3 (FLT3)/Internal Tandem Duplication (ITD) Positive Relapsed or Refractory Acute Myeloid Leukemia (AML)

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Version 8.0, Incorporating Substantial Amendment 7

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I have read all pages of this protocol for which Astellas is the sponsor. I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local regulations. I will also ensure that subinvestigator(s) and other relevant members of my personnel have access to copies of this protocol and the ICH GCP guidelines to enable them to work in accordance with the provisions of these documents.

Principal Investigator:

Signature: _____

Date (DD Mmm YYYY)

Printed Name:----------
-

_____

<Insert name and qualification of the Investigator>

Address: _____

_____

II. CONTACT DETAILS OF KEY SPONSOR'S PERSONNEL

24h-Contact for Serious Adverse Events (SAEs) See [Section 5.5.4.1 Reporting of Serious Adverse Events] for SAE Fax Number and Email	PPD **, Oncology Medical Science** **Astellas Pharma Global Development** **1 Astellas Way, Northbrook, Illinois, USA- 60062** PPD **Please fax or email the SAE Worksheet to:** **Astellas Pharma Global Development, Inc.** **Pharmacovigilance** **North America Fax Number: 888-396-3750** **(North America Alternate Fax: 847-317-1241)** **International Fax Number: +44 800 471 5263** **Email: safety-us@astellas.com** **Investigational sites in Japan:** **Astellas Pharma Inc. – Japan** **Pharmacovigilance** **Fax Number: +81-3-3243-5747** **Email: rk-safety-jp@astellas.com** **PAREXEL International** **Global Monitoring Operations** **Fax: +81 36888 5377**
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III. LIST OF ABBREVIATIONS AND DEFINITION OF KEY TERMS

List of Abbreviations

Abbreviations	Description of abbreviations
$5HT_{2B}$	serotonin 5 hydroxytryptamine 2B
AE	adverse event
ALK	anaplastic lymphoma kinase
ALP	alkaline phosphatase
ALT	alanine aminotransferase (SGPT)
AML	acute myeloid leukemia
ANC	absolute neutrophil count
AST	aspartate aminotransferase (SGOT)
AUC	area under the concentration-time curve
AXL	AXL tyrosine kinase
BCRP	breast cancer resistance protein
C_{max}	maximum concentration
CNS	central nervous system
CA	Competent Authorities
cEC	concerned Ethics Committee
CL/F	oral clearance
COA	clinical outcome assessment
CR	complete remission
CRc	composite complete remission
CRh	complete remission with partial hematological recovery
CRF	case report form
CRi	complete remission with incomplete hematologic recovery
CRO	contract research organization
CRp	complete remission with incomplete platelet recovery
CYP	cytochrome P450
DILI	drug-induced liver injury
DLT	dose-limiting toxicity
DEC	Dose Escalation Committee
ECG	electrocardiogram
eCRF	electronic case report form
EFS	event-free survival
EOT	end of treatment
ERK	extracellular signal-regulated kinase
FAS	full analysis set
FLAG	fludarabine, cytarabine and granulocyte colony-stimulating factor

Abbreviations	Description of abbreviations
FLT3	FMS-like tyrosine kinase 3
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
G-CSF	granulocyte colony-stimulating factor
GGT	gamma-glutamyl transferase
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GVHD	graft-versus-host disease
HEK293	human embryonic kidney 293
hERG	human ether-à-go-go-related gene
HRT	hormone replacement therapy
HSCT	hematopoietic stem cell transplant
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IND	Investigational New Drug
INR	international normalized ratio
IRB	Institutional Review Board
ISN	international study number
ITD	internal tandem duplication
LA-CRF	liver abnormality case report form
LFT	liver function tests
LLN	lower limit of normal
LTK	leukocyte receptor tyrosine kinase
LTT	long-term treatment
MedDRA	medical dictionary for regulatory activities
MRD	minimal residual disease
MTD	maximum tolerated dose
NCI-CTCAE	National Cancer Institute's Common Terminology Criteria for Adverse Events
OATP	organic anion transporting polypeptide
OS	overall survival
P-gp	P-glycoprotein
PGx	pharmacogenomics
PIA	plasma inhibitory activity
PKAS	pharmacokinetic analysis set

Abbreviations	Description of abbreviations
PND	postnatal day
PRES	posterior reversible encephalopathy syndrome
QTcF	Fridericia-corrected QT interval
RBC	red blood cell
RP2D	recommended phase 2 dose
R/R	relapsed/refractory
RTK	receptor tyrosine kinase
SAE	serious adverse event
SOP	standard operating procedure
SUSAR	suspected unexpected serious adverse reactions
$t_{1/2}$	terminal elimination half-life
TBL	total bilirubin
TEAE	treatment-emergent adverse event
TKD	tyrosine kinase domain
t_{max}	time of maximum concentration
ULN	upper limit of normal
USM	urgent safety measure
V_d/F	apparent volume of distribution
WBC	white blood cell
XRT	X-ray treatment

Definition of Key Study Terms

Terms	Definition of terms
Baseline	Assessments of subjects as they enter a study before they receive any treatment.
Endpoint	Variable that pertains to the efficacy or safety evaluations of a study.
Enroll	To register or enter a subject into a clinical study. NOTE: Once a subject has received the study drug or placebo, the clinical study protocol applies to the subject.
Intervention	The drug, device, therapy or process under investigation in a clinical study that is believed to have an effect on outcomes of interest in a study (e.g., health-related quality of life, efficacy, safety, pharmacoeconomics).
Investigational period	Period of time where major interests of protocol objectives are observed, and where the test drug or comparative drug (sometimes without randomization) is usually given to a subject, and continues until the last assessment after completing administration of the test drug or comparative drug.
Post investigational period	Period of time after the last assessment of the protocol. Follow-up observations for sustained adverse events and/or survival are done in this period.
Randomization	The process of assigning study subjects to treatment or control groups using an element of chance to determine assignments in order to reduce bias.
Screening	A process of active consideration of potential subjects for enrollment in a study.
Screen failure	Potential subject who did not meet 1 or more criteria required for participation in a study.
Screening period	Period of time before entering the investigational period, usually from the time when a subject signs the consent until just before the test drug or comparative drug (sometimes without randomization) is given to a subject.
Study period	Period of time from the first site initiation date to the last site completing the study.
Subject/ Participant	An individual who participates in a clinical trial either as a recipient of the investigational product(s) or as a control. The term “subject” is part of the federal regulation and may be used interchangeably with participant.
Variable	Any entity that varies; any attribute, phenomenon or event that can have different qualitative or quantitative values.

IV. SYNOPSIS

Date and Version No of Protocol Synopsis:	22 Jul 2022/Version 8.0
Sponsor: Astellas Pharma Global Development Inc. (APGD)	**Protocol Number:** 2215-CL-0603
Name of Study Drug: Gilteritinib (ASP2215)	**Phase of Development:** Phase 1/2

Title of Study:
A Phase 1/2, Multicenter, Open-Label, Single Arm, Dose Escalation and Expansion Study of Gilteritinib (ASP2215) Combined with Chemotherapy in Children, Adolescents and Young Adults with FMS-like Tyrosine Kinase 3 (FLT3)/Internal Tandem Duplication (ITD) Positive Relapsed or Refractory Acute Myeloid Leukemia (AML)

Planned Study Period:
From 3Q2020 to 3Q2026

Study Objective(s)**:**

Primary Objectives:

The primary objectives are:

- **Phase 1 (Dose Escalation Phase):**
  To determine the maximum tolerated dose (MTD) and/or optimally safe and biologically active recommended phase 2 dose (RP2D) of gilteritinib given in sequential combination with fludarabine, cytarabine and granulocyte colony-stimulating factor (FLAG) in children, adolescents and young adults with relapsed/refractory (R/R) FMS-like tyrosine kinase 3 (FLT3) [internal tandem duplication (ITD) and/or tyrosine kinase domain (TKD)] acute myeloid leukemia (AML).
- **Phase 2 (Dose Expansion Phase):**
  To determine complete remission (CR) rates and composite complete remission (CRc) rates after 2 cycles of gilteritinib in sequential combination with FLAG in children, adolescents and young adults with FLT3 (ITD) AML who are refractory to or at the first hematologic relapse after first-line remission induction AML therapy (up to 2 induction cycles).

Secondary Objectives:

The secondary objectives are:

- To assess the safety, tolerability and toxicities of gilteritinib when given in sequential combination with FLAG in children, adolescents, and young adults with R/R FLT3/ITD AML.
- To evaluate FLT3 inhibition due to gilteritinib treatment
- To characterize gilteritinib pharmacokinetics.
- To perform serial measurements of minimal residual disease (MRD) and examine the relationship with study endpoints.
- To obtain preliminary estimates of 1-year event-free survival (EFS) and overall survival (OS) rate.
- To assess the acceptability and palatability of the formulation.

Exploratory Objectives:

The exploratory objectives are:

- To relate the clinical responses to gilteritinib therapy with FLT3 plasma inhibitory activity (PIA).
- To evaluate relationship between FLT3 ligand levels and clinical response.
- To assess the mechanisms of innate and acquired resistance to gilteritinib.

Planned Total Number of Study Centers and Location(s):

Approximately 30 sites in North America, Europe and Asia

Study Population:

Phase 1 (Dose Escalation Phase): Male and female children, adolescents and young adults from 6 months to less than 21 years of age with R/R FLT3 (ITD and/or TKD) AML.

Phase 2 (Dose Expansion Phase): Male and female children, adolescents and young adults from 6 months to less than 21 years of age with FLT3 (ITD) AML who are refractory to or at the first hematologic relapse after first-line remission induction AML therapy (up to 2 induction cycles).

Number of Subjects to be Enrolled/Randomized:

Up to 97* total subjects from 6 months to less than 21 years of age can be enrolled in the study.

Subject enrollment distribution during each phase of the study is outlined below:

- **Phase 1 (Dose Escalation Phase):**

  Group 1:

  Subjects from 2 years to less than 21 years of age: minimum 5 subjects from 2 years to 18 years, up to 15* subjects

  Group 2:

  Subjects from 1 year to less than 2 years of age: minimum 2 subjects, up to 15* subjects

  Group 3:

  Subjects from 6 months to less than 1 year of age: minimum 2 subjects, up to 15* subjects.

  *The number of subjects enrolled in phase 1 will depend on the number of dose levels evaluated, evaluation of biological activity, and the availability of time points for PIA assessments.

- **Phase 2 (Dose Expansion Phase):**

  Fifty-two (52) evaluable subjects from 6 months to less than 21 years of age can be enrolled with:

  - at least 2 subjects from 6 months to less than 6 years of age,
  - at least 6 subjects from 6 years to less than 12 years of age, and
  - at least 10 subjects from 12 years to less than 18 years of age.

Study Design Overview:

This study is an open-label, single-arm, phase 1/2 study to evaluate the safety, pharmacokinetics, and anti-leukemic activity of gilteritinib in children, adolescents and young adults with AML.

The study will consist of 2 phases:

- Phase 1 (Dose Escalation Phase)
- Phase 2 (Dose Expansion Phase)

One cycle is defined as 28 days of treatment. A subject completing 1 or 2 treatment cycles in phase 1 or 2 will have the option to participate in long-term treatment (LTT) with gilteritinib (for up to 2 years). The study treatment will continue until 1 of the discontinuation criteria is met.

Phase 1 (Dose Escalation Phase):

The primary objective of phase 1 will be to establish an optimally safe and biologically active RP2D and/or to determine MTD for gilteritinib in combination with FLAG. The RP2D will be a dose, which is safe (i.e., has an acceptable dose limiting toxicity [DLT] profile) and demonstrates CR, a high degree of gilteritinib biologic activity (as measured by PIA), or a combination of both.

Dose Escalation in phase 1 will be performed in 3 groups based on the age of the subject:

- **Group 1:** Dose Escalation in subjects from 2 years to less than 21 years of age
- **Group 2:** Dose Escalation in subjects from 1 year to less than 2 years of age
- **Group 3:** Dose Escalation in subjects from 6 months to less than 1 year of age

Induction therapy in this phase will consist of 2 cycles of gilteritinib plus FLAG. During each cycle, FLAG (cycle 1 and 2) chemotherapy will be administered on days -1 to 5 and gilteritinib will be administered once per day on days 8 to 21 at the assigned dose levels below:

Level	Dose
-1	1 mg/kg/day (maximum 60 mg/day) #
1	2 mg/kg/day (maximum 120 mg/day) *
2	3 mg/kg/day (maximum 180 mg/day) **
* Starting dose of gilteritinib for Group 1; # Starting dose of gilteritinib for Groups 2 and 3 ** To be evaluated only if there is lack of toxicity or acceptable DLT profile combined with the lack of sufficient gilteritinib activity observed at the Dose Level 1 (2 mg/kg/day). Not applicable for sites in USA.	

DLT assessment will occur during the first cycle only. Pharmacokinetic parameters, response assessment and biological activity will be evaluated during cycle 1 and/or cycle 2.

Dose Escalation Rules

Dose escalation, stay, or de-escalation between the dose levels in Groups 1, 2 and 3 will be guided by the 3 + 3 design.

Dose escalation rules during the DLT Observation Period are as follows:

Number of Subjects with DLT at the Given Dose	Escalation Decision Rules
0 of 3 or ≤ 1 of 6 subjects	Escalate and enter up to 3 subjects at the next dose level, if the next higher dose level is available
1 of 3 subjects	Enter up to 3 subjects at the same dose level
≥ 2 subjects	De-escalate, if the next lower dose level is available or stop escalating

Intra-subject dose escalation is not allowed during the study. Guidance for intra subject dose interruption or reduction for gilteritinib is outlined in [Section 5.1.2 Interruption or Reduction in Dose of the Study Drug].

The Dose Escalation Committee (DEC) will review safety data through the DLT observation period for 3 evaluable subjects at each dose level. In addition to safety data through the DLT observation period, the DEC will review response data and gilteritinib biologic activity (as measured by PIA) through the cycle 1 and/or cycle 2 of the evaluable subjects. The algorithm for differentiation syndrome based on the [Montesinos et al, 2009] criteria, in addition to the preferred term of 'acute promyelocytic leukemia differentiation syndrome', will be used to search for potential cases of differentiation syndrome at every dose level in phase 1. The decision will be made by the DEC to escalate to the next planned dose level, remain at the same dose level, de-escalate to the dose level below or stop escalation. The RP2D and/or MTD will be selected based on the DEC's review of all available data at each dose level, including safety data,

pharmacokinetic data (if available), response data and gilteritinib biologic activity data, and the RP2D will become the minimum safe and biologically effective dose level.

- ***Group 1:***

  Three subjects will be enrolled in the initial cohort starting at Dose Level 1 (2 mg/kg/day).

- ***Groups 2 and 3:***

  Three subjects will be enrolled in the initial cohort starting at Dose Level -1 (1 mg/kg/day).

  Enrollment of subjects in Groups 2 and 3 will be initiated following the decision by the DEC on the Dose Level 1 (2 mg/kg/day) evaluated in Group 1.

  Escalation to Dose Level 1 (2 mg/kg/day) in Groups 2 and 3 will not be performed if de-escalation to Dose Level -1 (1 mg/kg/day) has been performed in Group 1 or Dose Level -1 has been established as the MTD in Group 1.

Additional subjects will be accrued as needed at the RP2D of each group (Groups 1, 2 and 3) to ensure that gilteritinib activity is assessable in at least 9 subjects in the respective age group.

For the dose to be considered biologically active, at least 7 of 9 subjects at the RP2D dose in each group must demonstrate CR, a high degree of gilteritinib biologic activity (as measured by PIA), or a combination of both with the following conditions for PIA:

- Subjects that complete 2 induction cycles must achieve PIA of > 90% for at least 3 of 4 trough time points; or
- Subjects that complete only 1 induction cycle must achieve PIA of > 90% at 2 of 2 trough time points.

Alternative dose levels can be explored in the following cases:

- A higher Dose Level 2 (3 mg/kg/day) in the event there is lack of toxicity or acceptable DLT profile combined with the lack of sufficient gilteritinib activity at the Dose Level 1 (2 mg/kg/day), after discussion with the DEC. Dose Level 2 (3 mg/kg/day) will not be evaluated in subjects enrolled in USA.
- A lower Dose Level than Dose Level -1 (1 mg/kg/day) in the event the Dose Level -1 (1 mg/kg/day) demonstrates sufficient gilteritinib activity but DLTs are observed. Exploration of dose level lower that Dose Level -1 will be performed via an amendment to the current study protocol, after discussion with the DEC.

Continued participation in phase 2 portion of the study for each age group will be dependent on the determination of RP2D in Groups 1, 2 and 3, respectively.

Dose-limiting Toxicity (DLT) Observation Period

The DLT observation period will be 28 days from the start of cycle 1 day 1, i.e., for the first cycle only.

Dose-limiting Toxicity (DLT) Criteria

Nonhematologic Dose-limiting Toxicity will be defined as grade 3 nonhematologic toxicity at least possibly related to protocol therapy that persists for > 48 hours without resolution to grade ≤ 2 or grade 4 nonhematologic toxicity, regardless of duration, at least possibly related to protocol therapy. Hy's law (as defined in [Section 12.5]) or treatment-related deaths will be considered as a DLT. Gilteritinib dosing will be interrupted if nonhematologic DLT occurs. Exceptions will include the following toxicities commonly seen with intensive AML reinduction regimens:

- Alopecia, anorexia or fatigue.
- Grade 3 vomiting or diarrhea that resolves (with or without supportive care) to ≤ grade 2 within 48 hours

- Grade 3 nausea that resolves (with or without supportive care) to ≤ grade 2 within 7 days
- Grade 3 elevation in total bilirubin (TBL) that is asymptomatic and that returns to ≤ grade 2 elevation within 7 days
- Grade 3 elevation in hepatic transaminases (alanine aminotransferase [ALT], aspartate aminotransferase [AST] and gamma-glutamyl transferase [GGT]) or alkaline phosphatase (ALP) that returns to ≤ grade 2 elevation within 14 days
- Grade 3 fever with neutropenia, with or without infection
- Grade 3 infection or grade 4 infections expected as direct complication of cytopenia due to active underlying leukemia
- Grade 3 mucositis

Hematologic Dose-limiting Toxicity will be defined as failure to recover a peripheral absolute neutrophil count (ANC) > 500/µL and non-transfusion dependent platelet count > 20000/µL due to documented bone marrow aplasia/hypoplasia at day 42 from the start of cycle 1 day 1.

Failure to recover peripheral counts due to disease involvement of the bone marrow will not be considered dose-limiting toxicity.

Subject Replacement (Only for Phase 1 Dose Escalation)

A subject meeting fulfilling the inclusion/exclusion criteria and receiving 80% of the intended dose of the study treatment regimen (FLAG and gilteritinib) during the DLT observation period will be considered evaluable for DLT.

A subject that receives less than 80% of the intended dose of the study treatment regimen (for reasons other than a DLT) during the DLT observation period will not be evaluable for DLT and will be replaced by another subject in the dose level.

In addition, if after enrollment any subject is found not to fulfill any inclusion/exclusion criteria that would adversely affect safety or efficacy evaluation of that subject or are not evaluable for DLT, they may be replaced after discussion with the DEC.

Subjects without adequate sampling time points to assess biological activity for RP2D determination can be replaced.

Phase 2 (Dose Expansion Phase):

The phase 2 part of this study is the dose expansion phase, which will be initiated upon establishment of the optimally safe and biologically effective RP2D and/or MTD dose from Group 1 of phase 1.

Enrollment of subjects from 6 months to less than 2 years in phase 2 will depend upon the RP2D established in the respective age group (Groups 2 and 3) during the phase 1 portion of the study.

Applicable only for sites in Germany: Enrollment of subjects in phase 2 will be initiated in sites in Germany only after a positive assessment on the phase 1 data of each age group (Group 1, 2 or 3) is received from the competent authority of the country.

Phase 2 will be a single-arm, 2-stage, open-label design with 52 response evaluable subjects from 6 months to less than 21 years of age, with:

- at least 2 subjects from 6 months to less than 6 years of age,
- at least 6 subjects from 6 years to less than 12 years of age, and
- at least 10 subjects from 12 years to less than 18 years of age.

Induction therapy in this phase will consist of 2 cycles of gilteritinib plus FLAG. During each cycle (1 and 2), FLAG chemotherapy will be administered on days -1 to 5 and gilteritinib will be administered once per day on days 8 to 21 at the assigned dose.

Subjects are response evaluable if

1. they are confirmed FLT3/ITD mutation positive,
2. they receive at least 1 dose of gilteritinib, and
3. they are disease progression/recurrence free during cycle 1 and 2 of FLAG + gilteritinib and have the required bone marrow evaluations or have died of disease progression during the first 2 cycles.

Initially, 22 response evaluable subjects will be needed during the first stage, with 9 CRc responders or 4 CR responders after 1 or 2 cycles of therapy required to continue to a total of 52 response evaluable subjects.

Applicable only for sites in USA: If there are less than 4 CR responders in stage 1, the enrollment will be stopped in the USA and the study will not proceed to stage 2 in the USA.

Treatment Plan (Phases 1 and 2):

An overview of the treatment plan for cycle 1 and cycle 2 is presented below:

Cycle 1 & Cycle 2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6 & 7	Day 8 to 21	Day 28
Fludarabine		●	●	●	●	●			Disease evaluation
Cytarabine		●	●	●	●	●			
G-CSF	●	●	●	●	●	●			
Gilteritinib								●	
IT Cytarabine*	●								

G-CSF: granulocyte-colony stimulating factor; IT: induction therapy.

* Subject may receive prophylactic intrathecal cytarabine at the start of the cycle, as per institutional standards.

Criteria to Begin Cycle 2 (Phases 1 and 2):

Subjects should receive a second cycle of gilteritinib plus FLAG when all non-hematologic grade 3 and 4 toxicities have resolved to ≤ grade 2. It is suggested but not required that cycle 2 begin when ANC > 1000/µL and platelets are > 100000/µL in responding subjects.

Any subject who is unable to initiate cycle 2 after 50 days from the start of cycle 1 day 1 will be evaluated for participation in cycle 2 and/or replacement.

Response Assessment (Phases 1 and 2):

The end of cycle 1 bone marrow analysis will be performed upon count recovery but no later than day 28. For subjects whose response assessment is not evaluable due to hypocellularity, a repeat bone marrow analysis should be performed at least every 14 days until response determination is possible.

Long-term Treatment (LTT) (Phase 1 and 2):

In the event the principal investigator feels that a subject is receiving clinical benefit from treatment with gilteritinib in phase 1 or phase 2, subjects having completed 1 or 2 treatment cycles of phase 1 or 2 will have the option to participate in LTT with gilteritinib for up to 2 years (~26 cycles). LTT for any subject may only be initiated after discussion with the medical monitor. Subjects receiving clinical benefit during phase 1 or 2 may initiate LTT after discussion and approval from Astellas medical monitor. Clinical benefit is defined as follows:

a) Completed 1 or 2 treatment cycles of phase 1 or 2.
b) Subject did not develop intolerable or unacceptable toxicity .
c) Subject has achieved remission, stable or improved disease, or symptom improvement, or decreased transfusion dependence.
d) Any other reason that the investigator judges will benefit the subject from continued treatment with gilteritinib, must be reviewed and approved by the Astellas medical monitor.

During the LTT period, subjects will receive gilteritinib once daily starting from day 1 through day 28 of a 28-day cycle at the same dose received during phase 1 or 2.

End of Treatment (EOT) Visit:

An end of treatment (EOT) visit will be performed 7 days after last dose of gilteritinib, or prior to initiation of another anticancer therapy, whichever occurs earlier.

EOT_1 Visit (C2D28 for phase 1 or phase 2) will be performed for any subject participating in phase 1 or 2 and indicates the end of the respective phase.

EOT_2 will be performed for any subject participating in LTT phase and indicates the end of the LTT phase.

28-Day Follow-up Visit:

A follow-up visit will be performed 28 days following the EOT visit of phase 1 and phase 2 (i.e., EOT_1), in the event the subject is not eligible to continue for LTT. In the event it is determined that the subject is eligible to participate in the LTT, then a follow-up visit will only be performed 28 days following the LTT EOT visit (i.e., EOT_2).

Survival Follow-up:

Survival follow-up will be performed every 3 months for up to 2 years from the 28-day follow-up visit. Survival follow-up will include collection of data on subsequent anti-leukemic treatments/outcomes received by the subject, remission status and survival (cause of death and date of death) of the subject. In the event that the subject is not available for a clinic visit, follow-up via telephone or e-mail correspondence is acceptable.

Inclusion/Exclusion Criteria:

Inclusion:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved informed consent and privacy language as required per national regulations (e.g., Health Insurance Portability and Accountability Act Authorization for USA sites) must be obtained from the subject or subject's parent or legal guardian and if required child assent prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).
2. **Phase 1:** Subject is positive for FLT3 (ITD and/or TKD) mutation in bone marrow or blood as determined by the local institution.
   Phase 2: Subject is positive for the FLT3 (ITD) mutation in bone marrow or blood as determined by the local institution.
3. Subject is aged ≥ 6 months and < 21 years of age* at the time of signing informed consent and/or assent, as applicable.
   * For phase 2: Enrollment of subjects from 6 months to less than 1 year (Group 3) and 1 year to less than 2 years (Group 2) will be dependent on the establishment of RP2D in the respective for age groups during phase 1.
4. Subject has a diagnosis of AML according to The French–American–British (FAB) classification with ≥ 5% blasts in the bone marrow, with or without extramedullary disease (except subjects with active central nervous system [CNS] leukemia).
   a) In the phase 1 portion of the study, subject must be in first or greater relapse or refractory to induction therapy with no more than 1 attempt at remission induction (up to 2 induction cycles).
   b) For the phase 2 portion of the study, subject must be in first relapse or refractory to induction therapy with no more than 1 attempt at remission induction (up to 2 induction cycles) (see definition of line of therapy in Appendix 12.8).

5. Subject has fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study.
   - Myelosuppressive chemotherapy:
     - For subject who relapses while receiving cytotoxic therapy, at least 21 days must have elapsed since the completion of cytotoxic therapy and prior to screening, unless based on investigator's judgment the subject has recovered earlier than 21 days.
     - Cytoreduction with the following can be initiated and continued for up to 24 hours prior to the start of systemic protocol therapy (cycle 1 day -1) as determined by the investigator:
       - hydroxyurea,
       - low dose cytarabine (100 mg/m^2 per dose once daily for 5 days) or
       - other low dose/maintenance therapies as per local site practice.
     - Subject who has received other FLT3 inhibitors (e.g., lestaurtinib, sorafenib, etc) is eligible for this study.
   - Hematopoietic growth factors: at least 7 days must have elapsed since the completion of therapy with a growth factor and prior to screening.
   - Biologic (anti-neoplastic agent): at least 7 days must have elapsed since the completion of therapy with a biologic agent and prior to screening. For agents that have known adverse events (AEs) occurring beyond 7 days after administration, this period must be extended beyond the time during which AEs are known to occur. The duration of this interval must be discussed with the medical monitor.
   - X-ray treatment (XRT):
     - 14 days must have elapsed for local palliative XRT for CNS chloromas and prior to screening; no washout period is necessary for other chloromas;
     - Prior to screening, 90 days must have elapsed if the subject had a prior traumatic brain injury or has received craniospinal XRT.
6. For subject undergoing hematopoietic stem cell transplant (HSCT), at least 90 days must have elapsed since HSCT and subject must not have active graft-versus-host disease (GVHD).
7. Subject has Karnofsky score ≥ 50 (if the subject is of ≥ 16 years of age) or Lansky score of ≥ 50 (if the subject is < 16 years of age). A score < 50 is acceptable if related to the subject's leukemia in the investigator's judgment.
8. Subject must meet the following criteria as indicated on the clinical laboratory tests:
   - Serum AST and ALT ≤ 3.0 x upper limit normal (ULN) for age
   - Total serum bilirubin ≤ 1.5 x ULN for age
   - Estimated glomerular filtration rate of > 60 mL/min/1.73 m^2.
9. A female subject is eligible to participate if she is not pregnant (see [Appendix 12.3 Contraception Requirements]) and at least 1 of the following conditions applies:
   a) Not a woman of childbearing potential (WOCBP) as defined in [Appendix 12.3 Contraception Requirements]

      OR

   b) WOCBP who agrees to follow the contraceptive guidance as defined in [Appendix 12.3 Contraception Requirements] throughout the treatment period and for at least 180 days after the final study drug administration.
10. Female subject must agree not to breastfeed starting at Screening, and throughout the study period and for 60 days after the final study drug administration.

11. Female subject must not donate ova starting at Screening and throughout the study, and for 180 days after the final study drug administration.
12. A male subject with female partner(s) of childbearing potential must agree to use contraception as detailed in [Appendix 12.3 Contraception Requirements] during the treatment period and for at least 180 days after the final study drug administration.
13. A male subject must not donate sperm during the treatment period and for at least 120 days after the final study drug administration.
14. Male subject with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy or time partner is breastfeeding throughout the study period and for 180 days after the final study drug administration.
15. Subject and subject's parent(s) or legal guardian agrees not to participate in another interventional study while on treatment.
16. Live Vaccines: At least 6 weeks must have elapsed since the administration of the last dose of a live vaccine and prior to initiation of study treatment (cycle 1 day -1).

Waivers to the inclusion criteria will **NOT** be allowed.

Exclusion Criteria:

1. Subject has active CNS leukemia.
2. This criterion has been removed.
3. Subject has uncontrolled or significant cardiovascular disease, including:
    - Diagnosed or suspected congenital long QT syndrome or any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or torsades de pointes); any history of arrhythmia will be discussed with the sponsor's medical monitor prior to subject's entry into the study
    - Prolonged QTcF interval on pre-entry electrocardiogram (ECG) (≥ 450 ms)
    - Any history of second- or third-degree heart block (may be eligible if the subject currently has a pacemaker)
    - Heart rate < 50 beats/minute on pre-entry ECG
    - Uncontrolled hypertension
    - Complete left bundle branch block
4. Subject has systemic fungal, bacterial, viral or other infection that is exhibiting ongoing signs/symptoms related to the infection without improvement despite appropriate antibiotics or other treatment. The subject needs to be off pressors and have negative blood cultures for 48 hours.
5. Subject is receiving or plans to receive concomitant chemotherapy, radiation therapy, or immunotherapy other than as specified in the protocol.
6. Subject has active clinically significant GVHD or is on treatment with immunosuppressive drugs for treatment of active GVHD, with the exception of subjects being weaned from systemic corticosteroids where the subject is receiving ≤ 0.5 mg/kg of prednisone (or equivalent) daily dose for prior GVHD. Subject has received calcineurin inhibitors within 4 weeks prior to screening, unless used as GVHD prophylaxis.
7. Subject has active malignant tumors other than AML.
8. Subject has any significant concurrent disease, illness, psychiatric disorder or social issue that would compromise subject safety or compliance; interfere with consent, study participation, follow-up or interpretation of study results.

9. Subject has hypokalemia and/or hypomagnesemia at Screening (defined as values below institutional lower limit of normal [LLN]). Repletion of potassium and magnesium levels during the screening period is allowed.
10. Subject requires treatment with concomitant drugs that are strong inducers of cytochrome P450 (CYP)3A/P-glycoprotein (P-gp).
11. Subject is known to have human immunodeficiency virus infection.
12. Subject has active hepatitis B or C, or other active hepatic disorder.
    - Subjects with positive hepatitis B surface antigen (HBsAg) or detectable hepatitis B DNA are not eligible.
    - Subjects with negative HBsAg, positive hepatitis B core antibody and negative hepatitis B surface antibody will be eligible if hepatitis B DNA is undetectable.
    - Subjects with antibodies to hepatitis C virus will be eligible if hepatitis C RNA is undetectable.
13. This criterion has been removed.
14. Subject must wait for at least 5 half-lives after stopping therapy with any investigational agent and before starting gilteritinib.
15. Subject has known or suspected hypersensitivity to gilteritinib, cytarabine, fludarabine, G-CSF or any components of the formulations used.

Waivers to the exclusion criteria will **NOT** be allowed.

Investigational Product(s)**:**

Gilteritinib tablets containing 40 mg of active ingredient and mini-tablets containing 10 mg of active ingredient.

Dose(s):

- **Phase 1 (Dose Escalation Phase):**

  Gilteritinib will be administered once daily starting from day 8 through day 21 of a 28-day cycle starting at 2 mg/kg per day for Group 1. For Groups 2 and 3, the starting dose is Dose Level -1 (1 mg/kg/day).

Level	Dose (by body weight)
-1	1 mg/kg/day (for a maximum dose of 60 mg/day) #
1	2 mg/kg/day (for a maximum dose of 120 mg/day) *
2	3 mg/kg/day (for a maximum dose of 180 mg/day) **

* Starting dose of gilteritinib for Group 1; # Starting dose of gilteritinib for Groups 2 and 3
** To be evaluated only if there is lack of toxicity or acceptable DLT profile combined with the lack of sufficient gilteritinib activity observed at the Dose Level 1 (2 mg/kg/day). Not applicable for sites in USA.

Weight-based dosing (rounding to the nearest 10 mg) is proposed to be used in pediatric subjects up to a body weight of 60 kg. At weights of 60 kg and greater, a flat dose will be given (i.e., the maximum dose for the particular dose level).

Gilteritinib daily dose for any subject ≤ 5 kg weight will be discussed and approved by Astellas medical monitor.

- **Phase 2 (Dose Expansion Phase):**

  Gilteritinib will be administered once daily starting from day 8 through day 21 of a 28-day cycle at the RP2D or MTD established by phase 1 for the respective age groups.

- **Long-term Treatment Period:**

  Gilteritinib will be administered once daily starting from day 1 through day 28 of a 28-day cycle at the same dose the subject received during phase 1 or 2 for a period of up to 2 years.

Mode of Administration:

Gilteritinib will be administered orally.

Co-administered Drugs:

The co-administered drugs during phase 1 and phase 2 are as follows:

Cycle 1 and 2: FLAG on days -1 to 5

Dose(s):

Fludarabine: 30 mg/m^2 per day
Cytarabine: 2000 mg/m^2 per day
G-CSF: 5 µg/kg per day

Subject may receive prophylactic intrathecal cytarabine at the start of the cycle as per institutional standards.

FLAG dosage calculation per institutional practice (such as mg/kg/dose) for subjects less than 2 years of age, or below a certain weight or body surface area, is allowed.

Mode of Administration:

Intravenous

Duration of Treatment:

Treatment Period for Phase 1 and Phase 2:

Up to 2 cycles of 28 days each.

Cycle 1 and 2: FLAG on days -1 to 5 and gilteritinib on days 8 to 21.

Treatment Period for Long-term Treatment:

LTT with gilteritinib for up to 2 years (~ 26 cycles) may continue only after discussion with the medical monitor.

Concomitant Medication Restrictions or Requirements:

The following is allowed during the course of the study:

- Local practice for prophylaxis of conjunctivitis caused by high-dose cytarabine

The following are ***prohibited*** during the course of the study:

- Treatment with strong inducers of CYP3A/P-gp.
- Therapies to treat AML including, but not limited to:
- Chemotherapy
- Surgery
- Immunotherapy, cellular therapy or vaccines
  - **Exceptions:** Hydroxyurea (up to 5 g daily for up to 2 weeks to keep the absolute blast count below 50 x 10^9/L), intrathecal chemotherapy, cranial irradiation, localized radiation for palliation and leukapheresis are allowed.
- Vaccination with live vaccines (the prohibited duration starts from 6 weeks prior to the first dose of FLAG regimen and lasts until 6 weeks after the last dose of FLAG regimen)
- Any other investigational agent for AML.
- Participation in another interventional study while on treatment

Caution is advised when considering the concomitant use of the following medications:

- Treatment with Medications known to prolong QT or QTcF intervals. For concomitant drugs that have the potential to prolong QT or QTc intervals, a cardiology consult should be obtained as medically indicated.
- Strong inhibitors of P-gp and concomitant drugs that target serotonin 5 hydroxytryptamine 2B ($5HT_{2B}$) receptor or sigma nonspecific receptor are to be avoided with the exception of drugs that are considered absolutely essential for the care of the subject.
- Treatment with concomitant drugs that are strong inhibitors of CYP3A should be avoided with the exception of antibiotics, antifungals and antivirals that are used as standard of care to prevent or treat infections. If CYP3A inhibitors are used concomitantly, subjects should be closely monitored for AEs.
- Precaution should be used in treatment of gilteritinib with concomitant drugs that are substrates of P-gp (e.g., digoxin, dabigatran etexilate), BCRP (e.g., mitoxantrone, rosuvastatin) and OCT1 (e.g., metformin), since these transporters have been shown to be inhibited by gilteritinib in vitro.

Formal Stopping Rules:

Discontinuation Criteria

- Subject and/or parent(s) or legal guardian declines further study participation (i.e., withdrawal of consent)
- Subject develops an intolerable or unacceptable toxicity
- Investigator determines that continuation of the study treatment will be detrimental to the subject
- Subject has no response or progressive disease with no clinical benefit as defined by the investigator
- Subject becomes pregnant
- Subject is noncompliant with the protocol based on the investigator or medical monitor assessment
- Subject is found to have significantly deviated from any of the inclusion or exclusion criteria after enrollment (subjects having clinical benefit may be kept in the study after discussion with the medical monitor)
- Subject receives any antileukemic therapy other than the assigned treatment, with the exception of hydroxyurea up to 5 g daily for up to 2 weeks, prophylactic intrathecal chemotherapy, cranial irradiation, localized radiation for palliation or leukapheresis.

Resumption of Treatment After Hematopoietic Stem Cell Transplantation (HSCT)

Pre-HSCT preparation:

Subjects who have a donor identified and achieve a response allowing them to undergo HSCT per each institution's assessment can undergo HSCT without leaving the study if they have completed cycle 1 of phase 1 or 2. However, gilteritinib should be stopped for at least 1 week prior to start of the preparative regimen and a pre-HSCT visit should be performed prior to starting the conditioning regimen for HSCT.

Resumption of Treatment After HSCT:

Subjects returning to the study post HSCT can resume gilteritinib at the same dose prior to HSCT if the following conditions are met:

- Subject is between 30 to 90 days post HSCT
- Subject has had successful engraftment as demonstrated by ANC ≥ 500 /µL and platelets ≥ 20000/µL without transfusions
- Subject does not have ≥ grade 2 acute GVHD
- Subject is in CRc (CR, complete remission with incomplete hematologic recovery [CRi], or complete remission with incomplete platelet recovery [CRp])

For subjects resuming gilteritinib treatment, subjects will follow the procedures listed under subsequent visits (day 1) in the Schedule of Assessments [Table 1].

Endpoints for Evaluation:

Primary:

The primary endpoints are:

- Phase 1: Determination of MTD and/or RP2D
- Phase 2:
  - CRc rates (overall best response) after 2 cycles of therapy.
  - CR rates after 2 cycles of therapy; CR rate will be further described by the duration of CR (only for USA).

Secondary:

The secondary endpoints are:

- Inhibition of phosphorylated FLT3 (pFLT3) measured by PIA assay
- Gilteritinib plasma concentration
- Pharmacokinetic parameters (e.g., oral clearance [CL/F], apparent volume of distribution [V_d/F], maximum concentration [C_{max}], time of maximum concentration [t_{max}], area under the concentration-time curve [AUC]) of gilteritinib
- Safety, tolerability and toxicity assessments of gilteritinib when given in combination with FLAG
- EFS rate
- OS rate
- MRD assessment
- Acceptability and palatability assessment of the formulation

Exploratory:

The exploratory endpoints are:

- Correlation of clinical responses to gilteritinib therapy with FLT3 PIA levels
- Correlation of FLT3 PIA levels and clinical responses to gilteritinib therapy with FLT3 ligand levels before and after exposure to FLAG chemotherapy
- Assessment of mechanisms of innate and acquired resistance to gilteritinib

Statistical Methods:

Sample size justification:

Phase 1

Group 1:

Three subjects will be enrolled in a cohort at 1 of a series of doses of gilteritinib with a starting dose of 2 mg/kg/day according to the 3 + 3 design. Additional subjects will be accrued as needed to ensure that gilteritinib activity is assessable in at least 9 subjects.

The RP2D will be a safe dose of gilteritinib that demonstrates sufficient activity.

The number of subjects enrolled will depend on the number of dose levels evaluated, evaluation of biological activity, and the availability of time points for PIA assessments.

Groups 2 and 3:

Three subjects will be enrolled in a cohort at one of a series of doses of gilteritinib with a starting dose of 1 mg/kg/day according to the 3 + 3 design.

Additional subjects will be accrued as needed to ensure that gilteritinib activity is assessable in at least 9 subjects.

The RP2D will be a safe dose of gilteritinib that demonstrates sufficient activity.

The number of subjects enrolled will depend on the number of dose levels evaluated, evaluation of biological activity, and the availability of time points for PIA assessments.

Phase 2

The phase 2 portion of this study will be a single-arm, 2-stage, open-label design with a total of 52 response evaluable subjects. Subjects are response evaluable if (1) they are confirmed FLT3/ITD mutation positive, (2) they receive at least 1 dose of gilteritinib, and (3) they are progression/recurrence free during the first 2 cycles of FLAG + gilteritinib and have the required bone marrow evaluations or have died of disease progression during the first 2 cycles. Subjects who die during treatment will be counted as nonresponders for purposes of analysis.

In the available dataset (COG AAML 06P1, 1-BFM AML 2001-01, Costa dataset), there are 26 CRc responders out of 73 subjects (a CRc rate of about 35%). With a 1-sided Type 1 error rate of 2.5%, there will be about 80% power to detect a 30% CR rate (i.e., a 16% increase in CR rate from the null hypothesis value of 14%). The 52 response evaluable subjects will provide about 90% power to detect a 56% CRc rate (i.e., a 21% increase in CRc rate from the null hypothesis value of 35%), with 1-sided Type error rate of 5%. Operationally, 22 response evaluable subjects will be needed during the first stage, with 4 CR responders or 9 CRc responders after 1 or 2 cycles of therapy required to continue to a total of 52 response evaluable subjects. If there are less than 4 CR responders in stage 1, the enrollment will be stopped in the USA and the study will not proceed to stage 2 in the USA. Ultimately, 13 or more CR responders or 24 CRc responders will be required to meet the efficacy threshold of FLAG + gilteritinib. The sample size was calculated in East Version 6.4 for CR.

Efficacy:

The rates of CRc, CR, OS, and EFS will be summarized using descriptive statistics. The survival curve and median for time-to-event variables and EFS and OS rates at 6 months/1 year/2 years will be estimated using the Kaplan-Meier method and will be reported along with the corresponding 95% confidence interval.

Pharmacokinetics:

Gilteritinib plasma concentrations will be summarized by dose levels, study day and sample collection window using descriptive statistics, including number of subjects, mean, standard deviation, minimum, median, maximum, geometric mean, and coefficient of variation (CV) of the mean and geometric mean. Time-course of drug concentrations will be plotted as appropriate. If warranted, plasma concentrations for active metabolites will also be summarized in a similar manner.

Gilteritinib pharmacokinetic parameters (e.g., CL/F, V_d/F) will be estimated using population pharmacokinetic analysis. Simulation will be performed to predict pharmacokinetic parameters such as C_{max}, t_{max} and AUC. Pharmacokinetic parameters will be summarized by phase of the study and dose levels using descriptive statistics, including number of subjects, mean, standard deviation, minimum, median, maximum, geometric mean, and CV of the mean and geometric mean. If warranted, pharmacokinetic parameters for active metabolites will also be summarized in a similar manner.

Pharmacodynamics:

A PIA assay will be employed to determine FLT3 inhibition relative to baseline FLT3 levels. Data will be summarized by phase of the study and dose levels using descriptive statistics, including number of subjects, mean, standard deviation, minimum, median, maximum, geometric mean, and coefficient of variation (CV) of the mean and geometric mean.

Safety:

Safety analyses will include all subjects who receive at least 1 dose of the treatment regimen.

Safety analyses will consist of data summaries of AEs, DLTs, and other safety parameters. The number and percent of subjects experiencing 1 or more AE(s) will be summarized by the phase of the study and dose levels. The relationship to study drug and severity of AE will also be summarized. AEs will be coded to system organ class and preferred term using Medical Dictionary for Regulatory Activities (MedDRA) terminology and will be graded by the investigator using the National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI-CTCAE) severity grade (version 5.0).

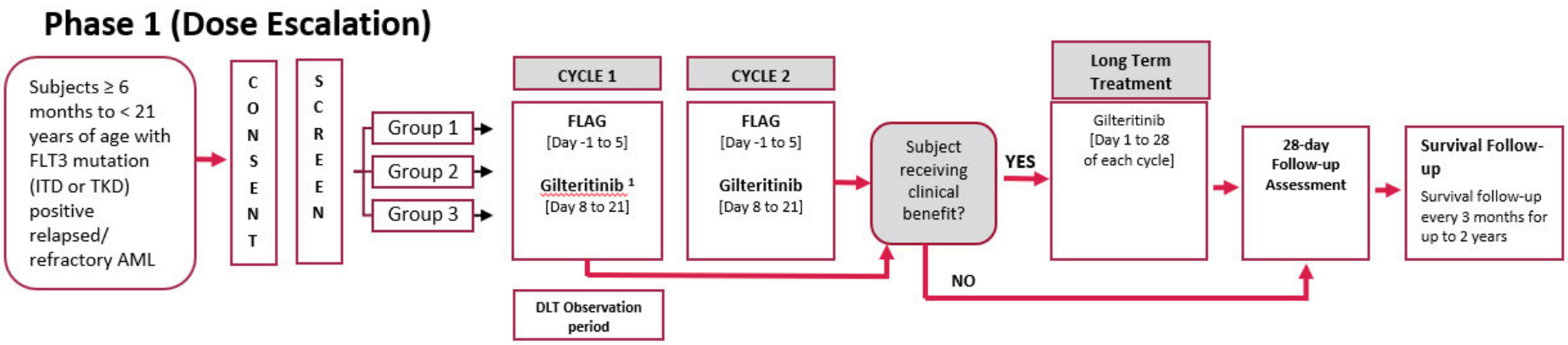
Laboratory parameters will be summarized by the phase of the study, dose levels and visits using descriptive statistics for shifts in change from baseline, and will be presented in listings of clinically significant abnormalities. Descriptive statistics will be used to summarize vital sign results and changes from baseline by phase of the study and dose level and visit. The 12-lead ECG results will be summarized by phase of the study, dose levels, visits and time points using descriptive statistics.

Interim Analyses:

The phase 2 portion of this study will be a 2-stage design [See Section 7.1.2]. No other formal interim analysis is planned.

V. FLOW CHART AND SCHEDULE OF ASSESSMENTS

Figure 1 Study Flow Chart



Group 1: subjects from 2 years to less than 21 years; **Group 2:** subjects from 1 year to less than 2 years of age; **Group 3:** subjects from 6 months to less than 1 year of age
[1] Starting Dose for Group 1 is 2 mg/kg/day and starting dose for Group 2 and 3 is 1 mg/kg/day.

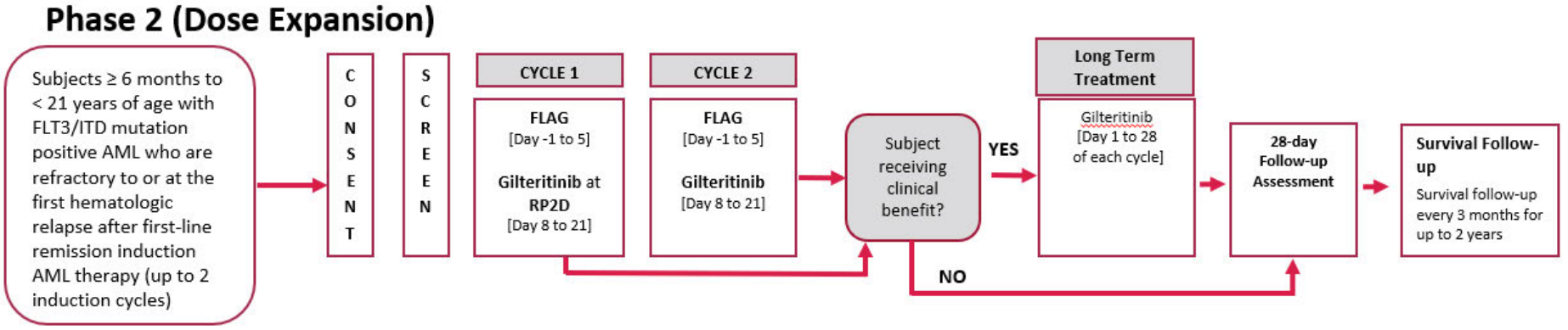


Table 1 Schedule of Assessments for Phase 1, Phase 2 and Long-term Treatment (LTT)

Assessments	Screening	PHASE 1/PHASE 2														LTT	EOT_2/ Pre-HSCT Visit[v]	28-day Follow-up[w]	Survival Follow-up[x]
			Cycle 1[a]							Cycle 2[a]						Cycle 1 & Subsequent Cycles			
Cycle Day →	Days -28 to -2	Day -1	Day 1	Day 4	Day 8	Day 15	Day 21	Day 28	Day -1	Day 1	Day 4	Day 8	Day 15	Day 21	Day 28 /EOT_1[v]	Day 1			
Visit Window (days) →				± 1	± 1	± 2	± 2	± 2			± 1	± 1	± 2	± 2	± 2	± 2	± 2	+ 7	± 7
Informed consent[b]	X																		
Eligibility criteria	X																		
Medical and disease history	X																		
Vital Signs[c]	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X		
Karnofsky/Lansky performance status	X														X	X	X		
Physical examination[d]	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X		
Pregnancy test for WOCBP[e]	X		X							X					X	X	X		
Chest x-ray (or chest CT)	X																		
12-lead ECG[f]	X		X		X	X	X			X		X	X	X	X	X	X		
Clinical laboratory tests (chemistry, hematology, coagulation, urinalysis) [g]	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X		
Thyroid function tests[h]	X		X							X					X	X			
MUGA or ECHO[i]	X														X				
PK sample collection[j]					X	X	X						X						
PGx[k]		X																	
Plasma Inhibitory Assay (PIA) & FLT3 Ligand [l]					X	X	X					X	X	X					
FLT3 mutational status[m]	X																		
Bone marrow aspirate/biopsy and MRD analysis[n, o]	X							X							X	X	X		
Gilteritinib dispensing[p]					X							X				X			
FLAG administration[q]		X	X	X					X	X	X								
DLT assessment[r]					X	X	X	X											

Table continued on next page

Assessments	Screening	PHASE 1/PHASE 2														LTT	EOT$_2$/ Pre-HSCT Visit[v]	28-day Follow-up[w]	Survival Follow-up[x]
			Cycle 1[a]							Cycle 2[a]						Cycle 1 & Subsequent Cycles			
Cycle Day →	Days -28 to -2	Day -1	Day 1	Day 4	Day 8	Day 15	Day 21	Day 28	Day -1	Day 1	Day 4	Day 8	Day 15	Day 21	Day 28 /EOT$_1$[v]	Day 1			
Visit Window (days) →				± 1	± 1	± 2	± 2	± 2			± 1	± 1	± 2	± 2	± 2	± 2	± 2	+ 7	± 7
Clinical Outcome Assesment[s]					X		X									X			
AE/SAE assessment[t]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Prior and concomitant medications[u]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Subsequent anti-leukemic treatments/outcomes																		X	X
Survival																		X	X

AE: adverse event; C: cycle; COA: clinical outcome assessment; CT: computed tomography; D: day; DLT; dose limiting toxicity; ECG: electrocardiogram; ECHO: echocardiogram; EOT: end of treatment; FLAG: fludarabine, cytarabine, and granulocyte colony-stimulating factor; FLT3: FMS-like tyrosine kinase 3; HSCT: hematopoietic stem cell transplant; ICF: informed consent form; LTT: long-term treatment; MRD: minimal residual disease; MUGA: multigated acquisition; PGx: pharmacogenomics; PIA: plasma inhibitory assay; PK: pharmacokinetic; SAE: serious adverse event; WOCBP: women of childbearing potential

Note: The day after day -1 is day 1. There is no day 0.

a. Visits for each cycle should be based upon day 1 of that cycle. Unscheduled visits may be performed at any time during the study whenever necessary to assess for or follow-up AEs or if deemed necessary by the investigator. Unscheduled visits will include assessment of AEs; additional assessments (e.g., laboratory testing) that may be performed as deemed appropriate by the investigator. Cycle 2 can begin once the subject meets the "Criteria to Begin Cycle 2 (Phases 1 and 2)" as outlined in [Section 2.2.1 Study Design].

b. ICF must be obtained prior to performing any study-specific procedures with the exception of procedures that are performed within the protocol specified windows as part of routine patient management.

c. Vital signs (includes blood pressure, respiratory rate, O_2 saturation, pulse rate and temperature) should be collected pre-dose as applicable. All vital sign measures will be obtained with the subject in the sitting or supine position.

d. A complete physical exam will be completed at screening and will be directed towards subject-reported symptoms and areas of disease, as per investigator judgment. Brief physical exams can be completed thereafter and must be obtained predose. Physical examination also includes measurements of height and weight. Weight and height will be collected at screening, day 1 of each cycle and EOT$_1$ (C2D28 for phase 1/phase 2).

e. WOCBP must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of human chorionic gonadotropin) within 72 hours prior to the start of each cycle.

Footnotes continued on next page

f. ECG assessment is required at screening. During treatment period, pre-dose ECG must also be obtained within 1 hour prior to dosing. The 12-lead ECGs will be recorded in triplicate (3 separate ECGs with 10 minutes resting prior to first ECG and at least 5 minutes apart per time point) and transmitted electronically for central reading. Triplicate ECGs must also be obtained within 1 hour prior to obtaining the time-matched PK samples, for a particular visit.

g. Laboratory tests performed will include hematology, serum chemistries, liver function, coagulation, urinalysis and uric acid.

   Coagulation and urinalysis: Screening only

   Uric acid: C1D1, C1D4, C1D8 and C1D15 only

h. For phase 1/phase 2: Thyroid function tests will be performed during screening, C1D1, C2D1 and C2D28 visit.

   For LTT: Thyroid function tests will be performed on C1D1 and repeated after every 2 cycles of therapy (C3D1, C5D1, C7D1, etc.).

i. MUGA scans or ECHO (per local standard of care) are to be performed at screening and C2D28/EOT_1 visit. **Note**: MUGA scans are not applicable to Germany.

j. PK samples will be collected in cycle 1 and cycle 2 at the following time points:

   - C1D8 - Predose
   - C1D15 (± 2 days) – Predose
   - C1D21 (± 2 day) – Predose and 4 to 6 hours
   - C2D15 (± 2 days) – Predose

   Predose samples should be collected within 1 hour prior to dosing.

k. Whole blood and buccal swab collected at C1D -1 (predose) for optional PGx study. Sample should be collected prior to the administration of induction chemotherapy.

l. PIA and FLT3 Ligand samples will be collected on C1D8 (predose), C1D15 (predose) and C1D21 (predose), C1D21 (4-6 hours post dose), C2D8 (predose), C2D15 (predose) and C2D21 (predose). Samples should be collected at or near the same time as PK samples. Predose samples should be collected within 1 hour prior to dosing.

m. Subjects is positive for the FLT3/ITD mutation in bone marrow or blood as determined by the local institution. A bone marrow aspirate or blood sample must be sent to the central laboratory for FLT3 mutational analysis during the screening period.

n. If bone marrow aspirate is unobtainable (e.g., dry tap), an additional EDTA tube of whole blood should be collected instead. Bone marrow aspirate is required, and bone marrow biopsy is preferred. In case of inadequate aspirate, bone marrow biopsy is required.

   Bone marrow samples are required during screening and end of each cycle (C1D28 and C2D28) for phase 1/phase 2.

   For LTT: Bone marrow assessments will be performed on C1D1 and repeated after every 3 cycles until 1 year of LTT, followed by every 6 cycles until 2 years of LTT (i.e., C1D1, C4D1, C7D1, C10D1, C13D1, C19D1, etc.). Bone marrow samples are also required at the end of treatment visit and as clinically indicated. Bone marrow assessments do not need to be performed on C1D1 of LTT and EOT2, if a bone marrow assessment was performed within 2 weeks during the previous study visit.

o. For subjects whose response assessment is not evaluable due to hypocellularity, a repeat bone marrow analysis should be performed at least every 14 days until response determination is possible.

p. For phase 1/phase 2: Gilteritinib will be dispensed on day 8 of each cycle and will be taken daily by the subject from day 8 to day 21 of each cycle.
   For LTT: Gilteritinib bottles will be dispensed on day 1 of each cycle and will be taken by the subject once daily for the 28-day cycle.

Footnotes continued on next page

q. FLAG will be administered on days -1 to 5 of cycle 1 and cycle 2. Refer to Table 5 Treatment Plan for cycle 1 and cycle 2 of phases 1 and 2 for detailed scheduled of FLAG administration.

r. DLT assessment is performed in cycle 1 of phase 1 only.

s. Clinical outcome assessment (COA) should be collected immediately after the administration of the study drug on that visit day. For LTT: COA will be collected only on C1D1 visit.

t. AE collection begins after the signing of the informed consent and will be collected until the 28-day Follow-up visit of the subject or the subject is determined to be a screen failure. For subjects who plan to proceed to HSCT and resume gilteritinib treatment after HSCT, AE collection will continue until the start of the HSCT conditioning regimen and AE collection will resume upon the resumption of gilteritinib treatment until 30 days after the last dose of study drug. For subjects who do not plan to resume gilteritinib treatment after HSCT, AE collection will continue until the start of the HSCT conditioning regimen or 30 days after the last dose of study drug, whichever comes first.

u. Includes medications taken within 28 days prior Screening.

v. An EOT visit [EOT1 or EOT2] will be performed 7 days after last dose of gilteritinib, or prior to initiation of another anticancer therapy, whichever occurs earlier. EOT1 visit (C2D28 for phase 1/phase 2) will be performed for any subject participating in phase 1 or 2 and indicates the end of the respective phase. EOT2 visit will be performed for any subject participating in LTT phase and indicates the end of LTT. For subjects who will undergo HSCT and plan to resume gilteritinib treatment after HSCT, a pre-HSCT visit will be performed.

w. A follow-up visit will be performed 28 days following the C2D28/EOT1 visit in the event the subject is not participating in the LTT phase. In the event the subject is participating in LTT, then the follow-up visit will be performed 28 days following the EOT2 visit.

x. Survival follow-up will be performed every 3 months for up to 2 years after the 28-day follow-up visit. In the event that the subject is not available for a clinic visit, telephone or e-mail correspondence is acceptable.

1 INTRODUCTION

1.1 Background

Acute myeloid leukemia (AML) is a group of biologically heterogeneous diseases that comprise 20% of pediatric and 80% of adult acute leukemias. It is estimated that 19940 people in the United States will be diagnosed with AML in 2020, and 11180 of these patients will die from AML [American Cancer Society, 2020]. While outcomes for children with de novo AML have improved over the past several decades, event-free survival (EFS) and overall survival (OS) remain suboptimal at approximately 60 and 70%, respectively. Relapsed disease and poor response to salvage therapy remain significant hurdles in achieving cure [Sexauer & Tasian, 2017].

FMS-like tyrosine kinase 3 (FLT3) is a member of the class III receptor tyrosine kinase (RTK) family that is normally expressed on the surface of hematopoietic progenitor cells. FLT3 and its ligand play an important role in proliferation, survival and differentiation of multipotent stem cells [Schlenk & Döhner, 2009].

Mutations in FLT3 are one of the most common genetic alterations in AML and are associated with high rates of relapse in adults and children. Activating FLT3 mutations are classified into 2 types [Sexauer & Tasian, 2017]:

1. internal tandem duplication (FLT3–ITD) mutations, which are 3 to 400 bp in-frame duplications located in the juxtamembrane domain and
2. activating point mutations, which are found in the tyrosine kinase domain (FLT3-TKD) and most often involve residue D835.

ITD and TKD mutations occur in approximately 25% and 10% of adult AML cases, respectively. Recent studies have reported similar incidence of ITD and activating TKD mutations in childhood AML. Numerous clinical studies have demonstrated inferior clinical outcomes in patients with FLT3-ITD AML [Sexauer & Tasian, 2017].

Children with FLT3–ITD AML treated on Children's Cancer Group and Pediatric Oncology Group studies fared similarly poorly with 30% 4-year EFS when treated with conventional multi-agent chemotherapy. Higher mutant-to-wild-type FLT3 allelic ratios have also been associated with increasingly inferior outcomes in children treated on Dutch Children's Oncology Group and Children's Oncology Group studies [Sexauer & Tasian, 2017].

Given the significant negative prognostic effects of FLT3–ITD mutations in AML and the relative frequency of these alterations, therapeutic targeting of aberrant FLT3 signaling has been a major research focus with goals of decreasing relapse and improving survival [Sexauer & Tasian, 2017].

AXL tyrosine kinase (AXL), an RTK, has been detected in AML and has been shown to play a role in mediating migration and invasiveness of cancer cells. Inhibition of AXL has been shown to increase apoptosis and inhibit proliferation of FLT3-ITD and FLT3 wild-type AML cell lines and primary AML cells in vitro and reduced tumor burden and prolonged survival in mouse models [Janning et al, 2015].

ASP2215 hemifumarate, also referred to as ASP2215 and gilteritinib, is a new chemical entity discovered by Astellas Pharma Inc. in collaboration with Kotobuki Pharmaceutical Co., Ltd. It is a third-generation oral FLT3 inhibitor under development for the treatment of AML. ASP2215 also has inhibitory activities for AXL, leukocyte receptor tyrosine kinase (LTK) and anaplastic lymphoma kinase (ALK).

Gilteritinib demonstrated favorable efficacy in a nonclinical AML model, with complete regression of tumors in the xenograft model mice transplanted with MV4-11, human AML cell line expressing FLT3-ITD, by repeated oral doses. In addition, gilteritinib inhibited the growth of cells expressing FLT3-ITD, FLT3-D835Y or FLT3-ITD-D835Y.

The efficacy of gilteritinib as monotherapy has been demonstrated in adult patients with R/R AML. Phase 1 and phase 2 studies of gilteritinib in combination with chemotherapy in newly diagnosed patients with AML are currently ongoing. A phase 2 maintenance study with gilteritinib in newly diagnosed patients with AML after first CR is ongoing. A phase 3 study of gilteritinib in comparison with azacitidine in newly diagnosed FLT3-mutated AML patients is ongoing, but was recently closed to enrollment post interim analysis.

Two phase 3 studies, one of gilteritinib compared with chemotherapy in FLT3-mutated patients with R/R AML and one of gilteritinib compared with placebo as a maintenance therapy following HSCT in patients with FLT3/ITD AML in first CR, are also ongoing.

XOSPATA (gilteritinib) tablets have been approved by the FDA, EMA, MHLW, Health Canada and other health authorities for the treatment of adult patients who have relapsed or refractory AML with an FLT3 mutation.

1.2 Nonclinical and Clinical Data

A detailed description of the chemistry, pharmacology, efficacy and safety of gilteritinib is provided in the current version of the [ASP2215 Investigator's Brochure].

1.3 Summary of Key Safety Information for Study Drugs

1.3.1 Gilteritinib (ASP2215) Data

The nonclinical and clinical studies that are referred to in this section are described in more detail in the current version of the [ASP2215 Investigator's Brochure].

1.3.1.1 Gilteritinib Nonclinical Data

Major findings in the safety pharmacology studies were vomiting, positive fecal occult blood and increased/decreased blood calcium ion in dogs, and decreased urination and defecation in rats. In the oral 13-week repeated dose toxicity study in rats, and the 4- and 13-week repeated dose toxicity studies in dogs, mortality occurred at 20, 10 and 5 mg/kg per day, respectively. With respect to other major target organ toxicities, effects on the urinary bladder, epithelial tissue, gastrointestinal tract, lymphohematopoietic system, eye, liver, kidney and/or lung were observed in rats and dogs at 2.5 mg/kg per day or more. All major findings were reversible and monitorable.

Gilteritinib has a potential to induce genotoxicity in vivo. Gilteritinib showed teratogenic potential and embryo-fetal deaths in the embryo-fetal development study in rats. Gilteritinib showed no potential to induce phototoxicity in cultured mammalian cells.

When gilteritinib was dosed to juvenile rats from PND 4 to 42, the minimum lethal dose level was 2.5 mg/kg per day and this dose level was lower than that (20 mg/kg per day) in adult rats in the 13-week dose study. In the preliminary (non-GLP) dose range finding study (dosing from PND 4 to 21), gastrointestinal bleeding detected as abnormal stool color (dark red) was noted at 10 mg/kg per day and higher. Gastrointestinal bleeding was suggested to be a target organ at 10 mg/kg per day or higher as in adult rats in the 13-week dose study. It should be noted that gilteritinib exposure, i.e., C_{max} and AUC_{24}, decreased after repeated dosing, which may be due to maturation of CYP450 isozymes over time; however, a definitive conclusion cannot be made as it has not been determined if and which rat-specific CYP isozymes are involved with gilteritinib metabolism.

Compared to adult rats treated with gilteritinib, juvenile rats treated with the same dose were exposed to higher gilteritinib levels, and juvenile rats at similar exposure (i.e., at 10 mg/kg per day in juvenile vs 20 mg/kg per day in adult rats) exhibited mortality and moribundity earlier during the treatment period.

1.3.1.2 Gilteritinib Clinical Data

Data from clinical studies 2215-CL-0101, 2215-CL-0102, 2215-CL-0103 and 2215-CL-0301 demonstrated efficacy in FLT3-mutation positive patients with R/R AML, with a tolerable safety profile.

Expected serious adverse drug reactions for gilteritinib include (by preferred term) pericardial effusion, pericarditis, cardiac failure, diarrhea, nausea, fatigue, asthenia, anaphylactic reaction, increased blood creatine phosphokinase, increased ALT, increased AST, ECG QT prolonged, arthralgia, posterior reversible encephalopathy syndrome, acute kidney injury, differentiation syndrome, dyspnea, cough, and hypotension [ASP2215 Investigator's Brochure; Section 5.2.3 and Appendix 1].

1.3.2 Co-administered Chemotherapy Regimens

Detailed information on the toxicities and common AEs associated with the co-administered chemotherapy regimens can be found within the Package Insert, Summary of Product Characteristics or local product information.

1.4 Risk Benefit Assessment

As in adults, the prognosis for children with R/R FLT3/ITD AML is particularly poor, even with the current standard of care.

The current standard of care for children with FLT3-ITD mutations is intensive induction chemotherapy followed by hematopoietic stem cell transplantation (HSCT) [Cooper et al, 2016; Brown and Small, 2004].

The FLAG (fludarabine, cytarabine and filgrastim) regimen is a non-anthracycline based chemotherapy and has been reported as effective and well tolerated in newly diagnosed as well as in resistant or relapsing AML patients [Montillo et al, 1998; Estey et al, 1993].

FLAG chemotherapy without an anthracycline (liposomal daunorubicin/idarubicin) is a relatively effective and well-tolerated regimen in children with relapsed or refractory AML [Kaspers et al, 2013; Lee et al, 2009].

The use of FLAG chemotherapy has allowed intensive post-remission therapy including HSCT in such patients [Lee et al, 2009].

Children with acute myeloid leukemia and internal tandem duplication mutations in the FLT3 receptor tyrosine kinase have a poor prognosis despite intensive chemotherapy and hematopoietic stem cell transplantation. FLT3 inhibition represents a promising therapeutic strategy for improving survival in this high-risk patient population [Cooper, 2016; Brown and Small, 2004].

Gilteritinib is a third-generation oral FLT3 inhibitor that has been approved by FDA and PMDA for the treatment of adult patients who have relapsed or refractory AML with an FLT3 mutation.

In an adult population (Study 2215-CL-0101), gilteritinib has resulted in CRc in over 40% of patients receiving 80 mg or higher dose in FLT3 mutation-positive patients. The majority of patients in the study received multiple treatments prior to receiving gilteritinib. Furthermore, gilteritinib was well tolerated at the proposed doses in this study. Refer to the current version of the [ASP2215 Investigator's Brochure] for further details.

Since the clinical progression of FLT3/ITD AML appears to be similar in children and adults, it is expected that the clinical benefit with inclusion of gilteritinib in pediatric FLT3/ITD AML should be similar to that demonstrated in adult studies.

An efficacious and safe dose of gilteritinib in pediatric patients has not yet been determined.

This is a first-in-child phase 1/2 study of gilteritinib and the primary objective of this study is to determine a safe and biologically active dose of gilteritinib given in sequential combination with FLAG chemotherapy regimen in in children, adolescents and young adults with R/R FLT3/ITD AML.

In the juvenile animal studies in rats (2215-TX-0015 and 2215-TX-0016), changes suggesting effects on the development of reproductive system, nervous system, kidney, lung, immune system, skeletal system and gastrointestinal system, which undergo significant postnatal development, were not observed. Based on the difference in the development of heart in humans and rats, effects on the cardiac development could not be investigated in the juvenile animal studies in rats, therefore, infants younger than 6 months will not be involved in this study [Hew & Keller, 2003].

The juvenile toxicity data suggest that the safety profile in children less than 2 years may differ from older children. Further, there may be differences in safety profiles for children between 6 months to less than 1 year old compared with 1 year to less than 2 year-old

children based on CYP450 ontogeny. Therefore, the phase 1 portion of the clinical study will have a separate sub-group focusing on the age group from 6 months to less than 2 years to establish the recommended phase 2 dose (RP2D) in this age group. The approach will provide evidence for safe dosing in this age group of children.

Subjects enrolled in this study may experience AEs, including previously unknown AEs related to study drug or may experience procedural complications, e.g., light skin irritation from the adhesive on the ECG electrodes. While the proposed dose regimen was established to minimize the number of subjects reaching exposures associated with increased toxicity in adults, some pediatric subjects could reach exposures that resulted in transaminase elevation and gastrointestinal (GI)-type adverse effects in adults. Subjects with prolonged QTcF are excluded from participation in this study. Pediatric subjects enrolled in this study will be closely monitored for AEs, especially for liver toxicity.

It is unknown if pediatric subjects participating in this clinical study will benefit from administration of gilteritinib. Overall, based on the target population selected for this study (children, adolescents and young adults with R/R FLT3-ITD AML), the exclusion criteria and the proposed dose regimen, which is expected to match adult efficacious exposures, the benefit and risk assessment associated with the participation of study population is worth exploring.

2 STUDY OBJECTIVE(S), DESIGN, AND ENDPOINTS

2.1 Study Objective(s)

2.1.1 Primary Objective

The primary objectives are:

- **Phase 1 (Dose Escalation Phase):**

  To determine the MTD and/or optimally safe and biologically active RP2D of gilteritinib given in sequential combination with FLAG in children, adolescents and young adults with R/R FLT3 (ITD and/or TKD) AML.

- **Phase 2 (Dose Expansion Phase):**

  To determine CR rates and CRc rates after 2 cycles of gilteritinib in sequential combination with FLAG in children, adolescents and young adults with FLT3 (ITD) AML who are refractory to or at the first hematologic relapse after first-line remission induction AML therapy (up to 2 induction cycles).

2.1.2 Secondary Objectives

The secondary objectives are:

- To assess the safety, tolerability and toxicities of gilteritinib when given in sequential combination with FLAG in children, adolescents, and young adults with R/R FLT3/ITD AML
- To evaluate FLT3 inhibition due to gilteritinib treatment
- To characterize gilteritinib pharmacokinetics

- To perform serial measurements of minimal residual disease (MRD) and examine the relationship with study endpoints
- To obtain preliminary estimates of 1-year event-free survival (EFS) and overall survival (OS) rate
- To assess the acceptability and palatability of the formulation.

2.1.3 Exploratory Objectives

The exploratory objectives are:

- To relate the clinical responses to gilteritinib therapy with FLT3 PIA.
- To evaluate relationship between FLT3 ligand levels and clinical response.
- To assess the mechanisms of innate and acquired resistance to gilteritinib.

2.2 Study Design and Dose Rationale

2.2.1 Study Design

This study is an open-label, single-arm, phase 1/2 study to evaluate the safety, pharmacokinetics, and anti-leukemic activity of gilteritinib in children, adolescents and young adults with AML.

The study will consist of 2 phases:

- Phase 1 (Dose Escalation Phase)
- Phase 2 (Dose Expansion Phase)

One cycle is defined as 28 days of treatment. A subject completing 1 or 2 treatment cycles in phase 1 or 2 will have the option to participate in long-term treatment (LTT) with gilteritinib (for up to 2 years). The study treatment will continue until 1 of the discontinuation criteria is met.

Phase 1 (Dose Escalation Phase):

The primary objective of phase 1 will be to establish an optimally safe and biologically active RP2D and/or to determine MTD for gilteritinib in combination with FLAG.

The RP2D will be a dose, which is safe (i.e., has an acceptable DLT profile) and demonstrates CR, a high degree of gilteritinib biologic activity (as measured by PIA), or a combination of both.

Dose Escalation during phase 1 will be performed in 3 groups based on the age of the subject:

- Group 1: Dose Escalation in subjects from 2 years to less than 21 years of age
- Group 2: Dose Escalation in subjects from 1 year to less than 2 years of age
- Group 3: Dose Escalation in subjects from 6 months to less than 1 year of age

Induction therapy in this phase will consist of 2 cycles of gilteritinib plus FLAG. During each cycle, FLAG chemotherapy will be administered on days -1 to 5 and gilteritinib will be administered once per day on days 8 to 21 at the assigned dose levels as outlined in below:

Table 2 Dose Levels

Level	Dose
-1	1 mg/kg/day (maximum 60 mg/day) #
1	2 mg/kg/day (maximum 120 mg/day) *
2	3 mg/kg/day (maximum 180 mg/day) **

* Starting dose of gilteritinib for Group 1; # Starting dose of gilteritinib for Groups 2 and 3

** To be evaluated only if there is lack of toxicity or acceptable DLT profile combined with the lack of sufficient gilteritinib activity observed at the Dose Level 1 (2 mg/kg/day). Not applicable for sites in USA. DLT assessment will occur during the first cycle only. Pharmacokinetic parameters, response assessment and biological activity will be evaluated during cycle 1 and/or cycle 2.

Dose Escalation Rules

Dose escalation, stay, or de-escalation between the dose levels in Groups 1, 2 and 3 will be guided by the 3 + 3 design.

Dose escalation rules to be followed during the DLT Observation Period are outlined in Table 3.

Table 3 Dose Escalation Rules during the DLT Observation Period

Number of Subjects with DLT at the Given Dose	Escalation Decision Rules
0 of 3 or ≤ 1 of 6 subjects	Escalate and enter up to 3 subjects at the next dose level, if the next higher dose level is available.
1 of 3 subjects	Enter up to 3 subjects at the same dose level
≥ 2 subjects	De-escalate, if the next lower dose level is available or stop escalating

DLT: dose-limiting toxicity

Intra-subject dose escalation is not allowed during the study. Guidance for intra subject dose interruption or reduction for gilteritinib is outlined in [Section 5.1.2 Interruption or Reduction in Dose of the Study Drug].

The Dose Escalation Committee (DEC) will review safety data through the DLT observation period for 3 evaluable subjects at each dose level. In addition to safety data through the DLT observation period, the DEC will review response data and gilteritinib biologic activity (as measured by PIA) through the cycle 1 and/or cycle 2 of the evaluable subjects. The algorithm for differentiation syndrome based on the [Montesinos et al, 2009] criteria, in addition to the preferred term of ‘acute promyelocytic leukemia differentiation syndrome’, will be used to search for potential cases of differentiation syndrome at every dose level in phase 1. The decision will be made by the DEC to escalate to the next planned dose level, remain at the same dose level, de-escalate to the dose level below or stop escalation. The RP2D and/or MTD will be selected based on the DEC’s review of all available data at each dose level, including safety data, pharmacokinetic data (if available), response data and gilteritinib biologic activity data, and the RP2D will become the minimum safe and biologically effective dose level.

- ***Group 1:***

  Three subjects will be enrolled in the initial cohort starting at Dose Level 1 (2 mg/kg/day).

- ***Groups 2 and 3:***

  Three subjects will be enrolled in the initial cohort starting at Dose Level -1 (1 mg/kg/day). Enrollment of subjects in Groups 2 and 3 will be initiated following the decision by the DEC on the Dose Level 1 (2 mg/kg/day) evaluated in Group 1.

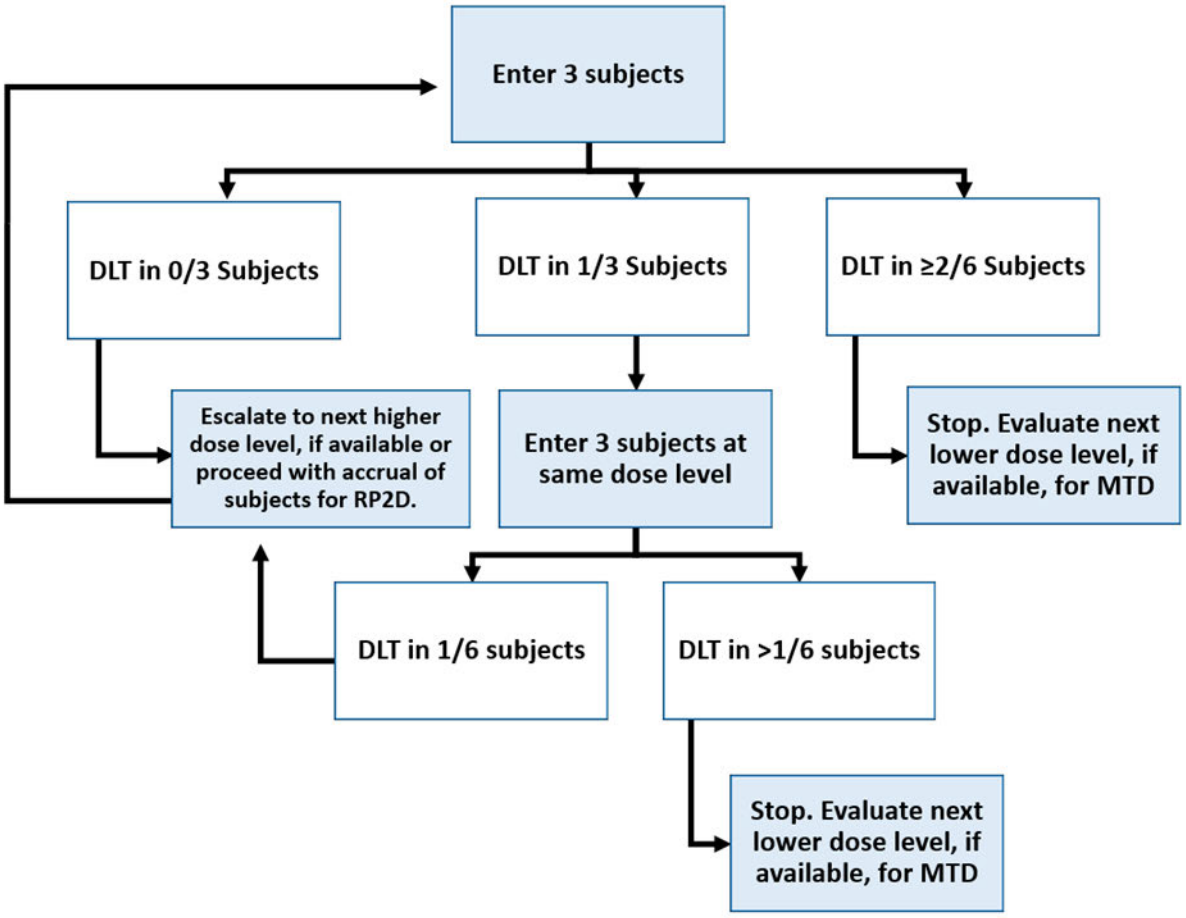
  Escalation to Dose Level 1 (2 mg/kg/day) in Groups 2 and 3 will not be performed if de-escalation to Dose Level -1 (1 mg/kg/day) has been performed in Group 1 or Dose Level -1 has been established as the MTD in Group 1.

Additional subjects will be accrued as needed at the RP2D of each group (Groups 1, 2 and 3) to ensure that gilteritinib activity is assessable in at least 9 subjects in the respective age group.

For the dose to be considered biologically active, at least 7 of 9 subjects at the RP2D dose in each group must demonstrate CR, a high degree of gilteritinib biologic activity (as measured by PIA), or a combination of both with the following conditions for PIA:

- Subjects that complete 2 induction cycles must achieve PIA of > 90% for at least 3 of 4 trough time points; or
- Subjects that complete only 1 induction cycle must achieve PIA of > 90% at 2 of 2 trough time points.

Figure 2 Schematic Representation of Dose Assignments of Each Dose Level in the 3 + 3 Design



DLTs: dose-limiting toxicities; MTD: maximum tolerated dose

Alternative dose levels can be explored in the following cases:

- A higher Dose Level 2 (3 mg/kg/day) in the event there is lack of toxicity or acceptable DLT profile combined with the lack of sufficient gilteritinib activity at the Dose Level 1 (2 mg/kg/day), after discussion with the DEC. Dose Level 2 (3 mg/kg/day) will not be evaluated in subjects enrolled in USA.
- A lower dose level than Dose Level -1 (1 mg/kg/day) in the event the Dose Level -1 (1 mg/kg/day) demonstrates sufficient gilteritinib activity but DLTs are observed. Exploration of dose level lower that Dose Level -1 will be performed via an amendment to the current study protocol, after discussion with the DEC.

Continued participation in phase 2 portion of the study for each age group will be dependent on the determination of RP2D in Groups 1, 2 and 3, respectively.

Subject Replacement (Only For Phase 1 -Dose Escalation)

A subject meeting fulfilling the inclusion/exclusion criteria and receiving 80% of the intended dose of the study treatment regimen (FLAG and gilteritinib) during the DLT observation period will be considered evaluable for DLT.

A subject that receives less than 80% of the intended dose of the study treatment regimen (for reasons other than a DLT) during the DLT observation period will not be evaluable for DLT and will be replaced by another subject in the dose level.

In addition, if after enrollment any subject is found not to fulfill any inclusion/exclusion criteria that would adversely affect safety or efficacy evaluation of that subject or are not evaluable for DLT, they may be replaced after discussion with the DEC.

Subjects without adequate sampling time points to assess biological activity for RP2D determination can be replaced.

Phase 2 (Dose Expansion Phase):

The phase 2 part of this study is the dose expansion phase, which will be initiated upon establishment of the optimally safe and biologically effective RP2D and/or MTD dose from Group 1 of phase 1.

Enrollment of subjects from 6 months to less than 2 years in phase 2 will depend upon the RP2D established in the respective age group (Groups 2 and 3) during the phase 1 portion of the study.

Applicable only for Germany: Enrollment of subjects in phase 2 will be initiated in sites in Germany only after a positive assessment on the phase 1 data of each age group (Group 1, 2 or 3) is received from the competent authority of the country.

Phase 2 will be a single-arm, 2-stage, open-label design with at least 52 response evaluable subjects from 6 months to less than 21 years of age, with:

- at least 2 subjects from 6 months to less than 6 years of age,
- at least 6 subjects from 6 years to less than 12 years of age, and
- at least 10 subjects from 12 years to less than 18 years of age.

Subjects are response evaluable if

1. they are confirmed FLT3/ITD mutation positive
2. they receive at least 1 dose of gilteritinib, and
3. they are disease progression/recurrence free during the first 2 cycles of FLAG + gilteritinib and have the required bone marrow evaluations, or have died of disease progression during the first 2 cycles.

Induction therapy in this phase will consist of 2 cycles of gilteritinib plus FLAG. During each cycle, FLAG chemotherapy will be administered on days -1 to 5 and gilteritinib will be administered once per day on days 8 to 21 at the assigned dose (RP2D).

Initially, 22 response evaluable subjects will be needed during the first stage, with 9 CRc responders or 4 CR responders after 1 or 2 cycles of therapy required to continue to a total of 52 response evaluable subjects.

Applicable only for USA: If there are less than 4 CR responders in stage 1, the enrollment will be stopped in the USA and the study will not proceed to stage 2 in the USA.

A Bayesian posterior probability for safety monitoring will be used for phase 2. Subjects in phase 2 will be continued to be monitored for ≥ grade 3 non-hematologic AEs considered at least possibly related to protocol therapy, and AEs of any grade leading to treatment discontinuation or death considered at least possibly related to protocol therapy. The event rate will be reviewed for the first 6 evaluable subjects at the end of cycle 1. After that, the event rate will be reviewed continuously. The estimated event rate based on the Bayesian beta-binomial model will be provided for safety monitoring. If the event rate is ≥ 20% with a posterior probability of at least 80%, then the enrollment to phase 2 will be paused and safety will be reassessed by the DEC. With a non-informative prior Beta(1,1) distribution, the numbers of subjects with event for certain evaluable subjects, which trigger the enrollment pause for safety review, are listed in [Table 4]. The algorithm for differentiation syndrome based on the [Montesinos et al, 2009] criteria, in addition to the preferred term of 'acute promyelocytic leukemia differentiation syndrome', will be used to search for potential cases of differentiation syndrome in every 6 subjects in phase 2.

Table 4 Safety Monitoring Rules for Phase 2

Number of Evaluable Participants	Minimum Number of Subjects with Event to Pause Enrollment
6	2
7-10	3
11-14	4
15-19	5
20-23	6
24-27	7
28-31	8
32-36	9
37-40	10
41-45	11
46-49	12
50-52	13

Treatment Plan (Phases 1 and 2):

An overview of the treatment plan is presented below.

Table 5 Treatment Plan for Cycle 1 and Cycle 2 of Phases 1 and 2

Cycles 1 and 2	Day -1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6 & 7	Day 8 to 21	Day 28
Fludarabine		●	●	●	●	●			Disease evaluation
Cytarabine		●	●	●	●	●			
G-CSF	●	●	●	●	●	●			
Gilteritinib								●	
IT Cytarabine*	●								

G-CSF: granulocyte-colony stimulating factor; IT: induction therapy

* Subject may receive intrathecal cytarabine at the start of the cycle, as per institutional standards.

Criteria to Begin Cycle 2 (Phases 1 and 2):

Subjects should receive a second cycle of gilteritinib plus FLAG when all non-hematologic grade 3 and 4 toxicities have resolved to ≤ grade 2. It is suggested, but not required, that cycle 2 begin when absolute neutrophil count (ANC) > 1000/µL and platelets are > 100000/µL in responding subjects.

Any subject who is unable to initiate cycle 2 after 50 days from the start of cycle 1 day 1 will be evaluated for participation in cycle 2 and/or replacement.

Response Assessment (Phases 1 and 2):

The end of cycle 1 bone marrow analysis will be performed upon count recovery but no later than day 28. For subjects whose response assessment is not evaluable due to hypocellularity, a repeat bone marrow analysis should be performed at least every 14 days until response determination is possible.

Long-term Treatment (LTT) (Phases 1 and 2):

In the event the principal investigator feels that a subject is receiving clinical benefit from treatment with gilteritinib in phase 1 or phase 2, subjects having completed 1 or 2 treatment cycles of phase 1 or 2 will have the option to participate in LTT with gilteritinib for up to 2 years (~ 26 cycles). Subjects receiving clinical benefit during phase 1 or 2 may initiate LTT after discussion and approval from the Astellas medical monitor. Clinical benefit (see [Section 5.1.5]) is defined as follows:

a) Completed 1 or 2 treatment cycles of phase 1 or 2.

b) Subject did not develop intolerable or unacceptable toxicity.

c) Subject has achieved remission, stable or improved disease, or symptom improvement, or decreased transfusion dependence.

d) Any other reason that the investigator judges will benefit the subject from continued treatment with gilteritinib, must be reviewed and approved by the Astellas medical monitor.

During the LTT period, subjects will receive gilteritinib once daily starting from day 1 through day 28 of a 28-day cycle at the same dose received during phase 1 or 2.

End of Treatment (EOT) Visit (Phase 1, Phase 2 and LTT):

An end of treatment (EOT) visit will be performed 7 days after last dose of gilteritinib, or prior to initiation of another anticancer therapy, whichever occurs earlier.

EOT_1 Visit (C2D28 for phase 1 or phase 2) will be performed for any subject participating in phase 1 or 2 and indicates the end of the respective phase.

EOT_2 will be performed for any subject participating in LTT phase and indicates the end of the LTT phase.

28-day Follow-up Visit:

A follow-up visit will be performed 28 days following the EOT visit of phase 1 and phase 2 (i.e., EOT_1), in the event the subject is not eligible to continue for LTT. In the event it is determined that the subject is eligible to participate in the LTT, then a follow-up visit will only be performed 28 days following the LTT EOT visit (i.e., EOT_2).

Survival Follow-up Visit:

Follow-up for survival will be performed every 3 months for up to 2 years from the 28-day follow-up visit. Survival follow-up visit will include collection of data on subsequent anti-leukemic treatments/outcomes received by the subject, remission status and survival (cause of death and date of death) of the subject. In the event that the subject is not available for a clinic visit, follow-up via telephone or e-mail correspondence is acceptable.

2.2.2 Dose Rationale

Gilteritinib has been evaluated in relapsed and refractory AML subjects in the USA (clinical study 2215-CL-0101) and Japan (clinical study 2215-CL-0102) at doses from 20 mg to 450 mg. Clinical efficacy data supports doses of 120 mg and greater to ensure efficacy in FLT3-mutation positive subjects. PIA has shown substantial reduction of phospho-FLT3, with > 90% inhibition at doses of 80 mg or greater.

The adult phase 1/2 study 2215-CL-0101 determined that daily doses of 80 and 120 mg of gilteritinib are expected to result in adequate drug exposure for clinical efficacy for adult patients with FLT3-mutation-positive R/R AML while providing and acceptable safety profile without the need for dose adjustment in patients receiving concomitant treatment with strong or moderate CYP3A4 inhibitors.

In study 2215-CL-0101 (USA) study, the MTD was determined to be 300 mg, and in study 2215-CL-0102 (Japan), the MTD was determined to be 200 mg; however, 120 mg was selected as the recommended phase 2/3 dose based on comparable efficacy, effective inhibition of target, and lower DLT rate. This dose is being used in all ongoing phase 3 studies.

Overall, 120 mg provides a good balance of ensuring effective drug levels for virtually all adult subjects with a low incidence of safety concerns.

Pediatric pharmacokinetic simulation was performed using the adult population PK model based on data from healthy volunteers and AML patient studies. The data indicate that pediatric daily dose of 3 mg/kg is predicted to result in comparable gilteritinib exposure observed to adults administered 120 mg once daily. Hence the pediatric starting dose for children from 2 years to less than 21 years of age is 2 mg/kg (1 dose level less than the RP3D for adults) to provide initial safety margin given the lack of clinical data in adults for the combination of gilteritinib and FLAG.

Based on the toxicology data in juvenile rats, the safety profile in children less than 2 years may differ from older children. Therefore, the phase 1 portion of the clinical study will have a separate sub-group focusing on the age group from 6 months to less than 2 years to establish the RP2D in this age group. The starting dose for children from 6 months to less than 2 years of age will be dependent on the tolerability observed in older children (2 years and above). This may be re-evaluated during the course of the phase 1 portion of the study depending on the safety response observed in children from 2 years to less than 21 years of age.

Weight-based dosing (rounding to the nearest 10 mg) is proposed to be used in pediatric subjects up to a body weight of 60 kg. At weights of 60 kg and greater, a flat dose will be given (i.e., the maximum dose for the particular dose level).

For Group 1: One dose de-escalation to 1 mg/kg (maximum of 60 mg) will be allowed.

For Groups 2 and 3: One dose escalation to 2 mg/kg (maximum of 120 mg) will be allowed, provided the dose level of 2 mg/kg/day has been established to be safe in Group 1.

Dose Level 2 (3 mg/kg/day) will be explored if there is lack of toxicity or acceptable DLT profile combined with the lack of sufficient gilteritinib activity observed at the Dose Level 1 (2 mg/kg/day). This would not apply for sites in the USA.

2.3 Endpoints

2.3.1 Primary Endpoints

The primary endpoints are:

- Phase 1: Determination of MTD and/or RP2D.
- Phase 2:
  - CRc rates (overall best response) after 2 cycles of therapy.
  - CR rates after 2 cycles of therapy; CR rate will be further described by the duration of CR (only for USA).

2.3.2 Definition of Dose-limiting Toxicity (DLT)

A DLT is defined as any of the events meeting the DLT criteria that occur during the DLT observation period and that is considered to be possibly or probably related to protocol therapy.

2.3.2.1 DLT Observation Period

The DLT observation period will be 28 days from the start of cycle 1 day 1; for the first cycle only.

2.3.2.2 DLT Criteria

Nonhematologic Dose-limiting Toxicity will be defined as grade 3 nonhematologic toxicity at least possibly related to protocol therapy that persists for > 48 hours without resolution to grade ≤ 2 or grade 4 nonhematologic toxicity, regardless of duration, at least possibly related to protocol therapy. Hy's law (as defined in [Section 12.5]) or treatment-related deaths will be considered as a DLT. Gilteritinib dosing will be interrupted if nonhematologic DLT occurs. Exceptions include the following toxicities commonly seen with intensive AML reinduction regimens:

- Alopecia, anorexia or fatigue.
- Grade 3 vomiting or diarrhea that resolves (with or without supportive care) to ≤ grade 2 within 48 hours
- Grade 3 nausea that resolves (with or without supportive care) to ≤ grade 2 within 7 days
- Grade 3 elevation in total bilirubin (TBL) that is asymptomatic and that returns to ≤ grade 2 elevation within 7 days
- Grade 3 elevation in hepatic transaminases (ALT/SGPT, AST/SGOT and gamma-glutamyl transferase [GGT]) or ALP that returns to ≤ grade 2 elevation within 14 days
- Grade 3 fever with neutropenia, with or without infection
- Grade 3 infection or grade 4 infections expected as direct complication of cytopenia due to active underlying leukemia
- Grade 3 mucositis

Hematologic Dose-limiting Toxicity will be defined as failure to recover a peripheral ANC > 500/µL and non-transfusion dependent platelet count > 20000/µL due to documented bone marrow aplasia/hypoplasia at day 42 from the start of cycle 1 day 1.

Failure to recover peripheral counts due to disease involvement of the bone marrow will not be considered as a DLT.

2.3.3 Secondary Endpoints

The secondary endpoints are:

- Inhibition of phosphorylated FLT3 (pFLT3) measured by PIA assay
- Gilteritinib plasma concentration
- Pharmacokinetic parameters (e.g., oral clearance [CL/F], apparent volume of distribution [V_d/F], C_{max}, t_{max}, AUC) of gilteritinib
- Safety, tolerability and toxicity assessments of gilteritinib when given in combination with FLAG
- EFS rate
- OS rate

- MRD assessment
- Acceptability and palatability assessment of the formulation

2.3.4 Exploratory Endpoints

The exploratory endpoints are:

- Correlation of clinical responses to gilteritinib therapy with FLT3 PIA levels
- Correlation of FLT3 PIA levels and clinical responses to gilteritinib therapy with FLT3 ligand levels before and after exposure to FLAG chemotherapy
- Assessment of mechanisms of innate and acquired resistance to gilteritinib

3 STUDY POPULATION

3.1 Selection of Study Population

Phase 1 (Dose Escalation Phase): Male and female children, adolescents and young adults from 6 months to less than 21 years of age with R/R FLT3 (ITD and/or TKD) AML.

Phase 2 (Dose Expansion Phase): Male and female children, adolescents and young adults from 6 months to less than 21 years of age with FLT3 (ITD) who are refractory to or at the first hematologic relapse after first-line remission induction AML therapy (up to 2 induction cycles).

Re-screening is allowed, with a limit of 2 re-screenings for any potential subject. Enrollment of a re-screened subject into the study will be permissible as long as they are re-consented to the most current version of the informed consent form (ICF) applicable at the site, fulfill all inclusion and exclusion criteria and the screening procedures are performed as per the Schedule of Assessments and within the protocol-defined timeframe.

3.2 Inclusion Criteria

Subject is eligible for the study if all of the following apply:

1. Institutional Review Board (IRB)/Independent Ethics Committee (IEC) approved informed consent and privacy language as required per national regulations (e.g., Health Insurance Portability and Accountability Act Authorization for USA sites) must be obtained from the subject or subject's parent or legal guardian and if required child assent prior to any study-related procedures (including withdrawal of prohibited medication, if applicable).

2. **Phase 1:** Subject is positive for FLT3 (ITD and/or TKD) mutation in bone marrow or blood as determined by the local institution.
Phase 2: Subject is positive for the FLT3 (ITD) mutation in bone marrow or blood as determined by the local institution.

3. Subject is aged ≥ 6 months and < 21 years of age* at the time of signing informed consent and/or assent, as applicable.

* For phase 2: Enrollment of subjects from 6 months to less than 1 year (Group 3) and 1 year to less than 2 years (Group 2) will be dependent on the establishment of RP2D in the respective age groups during phase 1.

4. Subject has a diagnosis of AML according to The French–American–British (FAB) classification with ≥ 5% blasts in the bone marrow, with or without extramedullary disease (except subjects with active CNS Leukemia).

a) In the phase 1 portion of the study, subject must be in first or greater relapse or refractory to induction therapy with no more than 1 attempt at remission induction (up to 2 induction cycles).
b) For the phase 2 portion of the study, subject must be refractory to or at the first hematologic relapse after first-line remission induction AML therapy (up to 2 induction cycles) (see definition of line of therapy in Appendix 12.8).

5. Subject has fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy prior to entering this study.

- Myelosuppressive chemotherapy:
  - For subject who relapses while receiving cytotoxic therapy, at least 21 days must have elapsed since the completion of cytotoxic therapy and prior to screening, unless based on investigator's judgment the subject has recovered earlier than 21 days.
  - Cytoreduction with the following can be initiated and continued for up to 24 hours prior to the start of systemic protocol therapy (cycle 1 day -1) as determined by the investigator:
    - hydroxyurea,
    - low dose cytarabine (100 mg/m^2 per dose once daily for 5 days) or
    - other low dose/maintenance therapies as per local site practice.
  - Subject who has received other FLT3 inhibitors (e.g., lestaurtinib, sorafenib, etc.) is eligible for this study.
- Hematopoietic growth factors: at least 7 days must have elapsed since the completion of therapy with a growth factor prior to screening.
- Biologic (antineoplastic agent): at least 7 days must have elapsed since the completion of therapy with a biologic agent prior to screening. For agents that have known AEs occurring beyond 7 days after administration, this period must be extended beyond the time during which AEs are known to occur. The duration of this interval must be discussed with the medical monitor.
- X-ray treatment (XRT):
  - 14 days must have elapsed for local palliative XRT for CNS chloromas and prior to screening; no washout period is necessary for other chloromas;
  - Prior to screening, 90 days must have elapsed if the subject had a prior traumatic brain injury or has received craniospinal XRT.

6. For subject undergoing hematopoietic stem cell transplant (HSCT), at least 90 days must have elapsed since HSCT and subject must not have active GVHD.

7. Subject has Karnofsky score ≥ 50 (if the subject is of ≥ 16 years of age) or Lansky score of ≥ 50 (if the subject is < 16 years of age). A score < 50 is acceptable if related to the subject's leukemia in the investigator's judgment.

8. Subject must meet the following criteria as indicated on the clinical laboratory tests:

- Serum AST and ALT ≤ 3.0 x upper limit normal (ULN) for age
- Total serum bilirubin ≤ 1.5 x ULN for age
- Estimated glomerular filtration rate of > 60 mL/min/1.73 m^2

9. A female subject is eligible to participate if she is not pregnant (see [Appendix 12.3 Contraception Requirements]) and at least 1 of the following conditions applies:

a) Not a woman of childbearing potential (WOCBP) as defined in [Appendix 12.3 Contraception Requirements]

OR

b) WOCBP who agrees to follow the contraceptive guidance as defined in [Appendix 12.3 Contraception Requirements] throughout the treatment period and for at least 180 days after the final study drug administration.

10. Female subject must agree not to breastfeed starting at Screening, and throughout the study period and for 60 days after the final study drug administration.

11. Female subject must not donate ova starting at Screening and throughout the study, and for 180 days after the final study drug administration.

12. A male subject with female partner(s) of childbearing potential must agree to use contraception as detailed in [Appendix 12.3 Contraception Requirements] during the treatment period and for at least 180 days after the final study drug administration.

13. A male subject must not donate sperm during the treatment period and for at least 120 days after the final study drug administration.

14. Male subject with a pregnant or breastfeeding partner(s) must agree to remain abstinent or use a condom for the duration of the pregnancy or time partner is breastfeeding throughout the study period and for 180 days after the final study drug administration.

15. Subject and subject's parent(s) or legal guardian agrees not to participate in another interventional study while on treatment.

16. Live Vaccines: At least 6 weeks must have elapsed since the administration of the last dose of a live vaccine and prior to initiation of study treatment (cycle 1 day -1).

Waivers to the inclusion criteria will **NOT** be allowed.

3.3 Exclusion Criteria

Subject will be excluded from participation if any of the following apply:

1. Subject has active CNS leukemia.

2. This criterion has been removed.

3. Subject has uncontrolled or significant cardiovascular disease, including:

- Diagnosed or suspected congenital long QT syndrome or any history of clinically significant ventricular arrhythmias (such as ventricular tachycardia, ventricular fibrillation, or torsades de pointes); any history of arrhythmia will be discussed with the sponsor's medical monitor prior to subject's entry into the study
- Prolonged QTcF interval on pre-entry ECG (≥ 450 ms)
- Any history of second or third degree heart block (may be eligible if the subject currently has a pacemaker)
- Heart rate < 50 beats/minute on pre-entry ECG
- Uncontrolled hypertension
- Complete left bundle branch block

4. Subject has systemic fungal, bacterial, viral or other infection that is exhibiting ongoing signs/symptoms related to the infection without improvement despite appropriate antibiotics or other treatment. The subject needs to be off pressors and have negative blood cultures for 48 hours.

5. Subject is receiving or plans to receive concomitant chemotherapy, radiation therapy, or immunotherapy other than as specified in the protocol.

6. Subject has active clinically significant GVHD or is on treatment with immunosuppressive drugs for treatment of active GVHD, with the exception of subjects being weaned from systemic corticosteroids where the subject is receiving ≤ 0.5 mg/kg of prednisone (or equivalent) daily dose for prior GVHD. Subject has received calcineurin inhibitors within 4 weeks prior to screening, unless used as GVHD prophylaxis.

7. Subject has active malignant tumors other than AML.

8. Subject has any significant concurrent disease, illness, psychiatric disorder or social issue that would compromise subject safety or compliance, interfere with consent, study participation, follow-up or interpretation of study results.

9. Subject has hypokalemia and/or hypomagnesemia at Screening (defined as values below institutional lower limit of normal [LLN]). Repletion of potassium and magnesium levels during the screening period is allowed.

10. Subject requires treatment with concomitant drugs that are strong inducers of cytochrome P450 (CYP)3A/P-gp.

11. Subject is known to have human immunodeficiency virus infection.

12. Subject has active hepatitis B or C, or other active hepatic disorder.

- Subjects with positive hepatitis B surface antigen (HBsAg) or detectable hepatitis B DNA are not eligible.
- Subjects with negative HBsAg, positive hepatitis B core antibody and negative hepatitis B surface antibody will be eligible if hepatitis B DNA is undetectable.
- Subjects with antibodies to hepatitis C virus will be eligible if hepatitis C RNA is undetectable.

13. This criterion has been removed.

14. Subject must wait for at least 5 half-lives after stopping therapy with any investigational agent and before starting gilteritinib.

15. Subject has known or suspected hypersensitivity to gilteritinib, cytarabine, fludarabine, G-CSF or any components of the formulations used.

Waivers to the exclusion criteria will **NOT** be allowed.

4 TREATMENT(S)

4.1 Identification of Investigational Product(s)

4.1.1 Study Drug(s)

The study drug is gilteritinib (ASP2215) available as a tablet containing 40 mg of active ingredient and mini-tablet containing 10 mg of active ingredient.

The tablets are packaged in a high-density polyethylene bottle. The study centers will be provided bottles of gilteritinib, each containing 30 tablets. The study site personnel will fill out the label to indicate the dispensing date, subject's gilteritinib dose and the corresponding number of tablets that need to be taken each day. The total dose must be dispensed using the tablet strength as appropriate for the child (e.g., a dose of 50 mg/day could be dispensed as one 40 mg tablet and one 10 mg tablet or five 10 mg tablets, as determined appropriate by the investigator). The gilteritinib product information is listed in Table 6.

Table 6 Test Drug (Gilteritinib Tablets)

Test Drug	**Gilteritinib Tablets**
Code name	ASP2215
Active ingredient	Chemical name: $(C_{29}H_{44}N_8O_3)_2 \cdot C_4H_4O_4$
Composition and dosage form	**40 mg Tablet:** One tablet contains 40 mg of gilteritinib in free form. Gilteritinib tablets are round light-yellow film-coated tablets. **10 mg Mini-tablet:** One tablet contains 10 mg of gilteritinib in free form. Gilteritinib mini tablets are round pale-yellow film-coated tablets.
Storage	Bottled gilteritinib should be stored according to labeled storage instructions. Store in original container.

4.1.2 Co-administered Chemotherapy Regimen

The co-administered drugs consist of induction therapy with FLAG regimen from day -1 to day 5 for cycle 1 and cycle 2 for both phase 1 and phase 2. The FLAG regimen will be administered per institutional guidelines for chemotherapy product preparation and administration. All cycles will be 28 days. The co-administered chemotherapy regimen will be supplied by the responsible site pharmacy of each investigational site or by the sponsor if applicable [Table 7].

Sites are permitted to use generic chemotherapy drug that is approved by the respective regulatory authority (e.g., FDA, European Commission or each country regulatory agency). Refer to the approved package insert, summary of product characteristics or local product information for co-administered chemotherapy drug product information and storage

condition supplied by the manufacturers. In the situation when co-administered chemotherapy products are supplied by the sponsor, co-administered chemotherapy products used in this study will be packaged by the manufacturer, but labeled and repackaged into white cartons (secondary packaging) under the responsibility of Astellas Pharma Global Development, Inc. (APGD)-Astellas United States Technologies (AUST) in accordance with APGD-AUST Standard Operating Procedures (SOPs), Good Manufacturing Practice (GMP) guidelines, International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) guidelines and applicable local laws/regulations.

Table 7 Co-administered Drug Products Supplied by the Sponsor

FLAG Induction Chemotherapy	
Fludarabine for IV use	Fludarabine Powder for Solution for Infusion
Cytarabine for IV use	Cytarabine Solution for Infusion
Cytarabine for IT use	Cytarabine Solution for Injection
G-CSF Filgrastim for SC/IV use	Filgrastim Solution for Injection or Infusion
Co-administered drug products supplied by sponsor can vary by brand and strength depending on market availability.	

FLAG: fludarabine, cytarabine and granulocyte colony-stimulating factor;
G-CSF: granulocyte-colony stimulating factor; IV: intravenous; SC: subcutaneous.

4.2 Packaging and Labeling

Gilteritinib used in this study will be prepared, packaged and labeled under the responsibility of qualified staff at APGD-AUST or sponsor's designee in accordance with APGD-AUST or sponsor's designee SOPs, GMP guidelines, ICH GCP guidelines and applicable local laws/regulations.

Each bottle will bear a label conforming to regulatory guidelines, GMP and local laws and regulations, which identifies the contents as investigational drug. A qualified person of Astellas Pharma Europe B.V. or sponsor's designee will perform the final release of the medication according to Directive 2003/94/EC annex 13.

In the situation when co-administered products are supplied by the sponsor, co-administered products used in this study will be labeled and repackaged into white cartons under the responsibility of APGD-AUST in accordance with APGD-AUST SOPs, GMP guidelines, ICH GCP guidelines and applicable local laws/regulations.

4.3 Study Drug Handling

Current ICH GCP Guidelines require the investigator to ensure that study drug deliveries from the sponsor are received by the investigator/or designee and that:

- Such deliveries are recorded,
- Study drug is handled and stored according to labeled storage conditions,
- Study drug with appropriate expiry/retest and is only dispensed to study subjects in accordance with the protocol, and

- Any unused study drug is returned to the sponsor.

Study drug inventory and accountability records will be maintained by the investigator or designee. Study drug accountability throughout the study must be documented and reconciled.

The following guidelines are therefore pertinent:

- The investigator or designee agrees not to supply study drug(s) to any persons except the eligible subjects in this study in accordance with the protocol.
- The investigator or designee will keep the study drugs in a pharmacy or other locked and secure storage facility under controlled storage conditions, accessible only to those authorized by the investigator to dispense these test drugs.
- The investigator or designee will store and take accountability of the study drug(s) in conforming to the procedures for handling the study drugs written by the sponsor.
- The investigator or designee will prepare and retain records of the study drug's receipt, the inventory at the study site, the use by each subject, and the return to the sponsor or alternative disposal of unused study drug(s) if approved by sponsor. These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the study drugs and subjects.
- At the conclusion or termination of this study, the investigator, or designee agrees to conduct a final drug supply inventory and to record the results of this inventory on the Drug Accountability Record. It must be possible to reconcile delivery records with those of used and/or returned/disposal of medication. Any discrepancies must be accounted for and documented. Appropriate forms of deliveries and returns/disposal must be signed by the site staff delegated this responsibility.
- The site must return used and unused gilteritinib and unused co-administered chemotherapy drugs supplied by sponsor, back to the sponsor or designee at the end of the study or upon expiration.

4.4 Blinding

This section is not applicable as this is an open-label study.

4.5 Assignment and Allocation

Enrollment and study drug assignment will be performed via Interactive Response Technology.

5 TREATMENTS AND EVALUATION

5.1 Dosing and Administration of Study Drug(s) and Other Medication(s)

5.1.1 Dose/Dose Regimen and Administration Period

5.1.1.1 Gilteritinib (ASP2215)

Gilteritinib is an oral tablet that subjects will take. Subjects will be instructed to take the daily gilteritinib dose with water as close to the same time each morning as possible. Gilteritinib

can be taken without regard to food. Gilteritinib [40 mg and/or 10 mg tablet] will be self-administered at home when subjects are not scheduled for clinic visits.

- **Gilteritinib 40 mg tablet** cannot be crushed, chewed or compounded. They must be swallowed intact
- **Gilteritinib 10 mg tablet** cannot be crushed or chewed. They must be also be swallowed intact. Alternatively, they can be dissolved in white grape juice or water to form a suspension (liquid formulation) to drink. The compounding instructions will be available in the pharmacy manual of the study.

If a dose of gilteritinib is missed or not taken at the usual time:

- Administer the dose as soon as possible on the same day, and at least 12 hours prior to the next scheduled dose
- Return to the normal schedule the following day
- Do not administer 2 doses within 12 hours

If vomiting occurs after dosing, the subject should not receive another dose, but just wait until the next morning to dose.

Gilteritinib will be self-administered at home when subjects are not scheduled for clinic visits.

5.1.1.1.1 Phase 1 (Dose Escalation Phase)

Gilteritinib will be administered once daily starting from day 8 through day 21 of a 28-day cycle starting at 2 mg/kg per day for Group 1 [Table 8]. For Groups 2 and 3, the starting dose is Dose Level -1 (1 mg/kg/day).

Table 8 Dose Levels in Phase 1 with Correlation to Maximum Dose per Day

Dose Level	Dose (by body weight)
-1	1 mg/kg/day (for a maximum dose of 60 mg/day) #
1	2 mg/kg/day (for a maximum dose of 120 mg/day) *
2	3 mg/kg/day (for a maximum dose of 180 mg/day)**

* Starting dose of gilteritinib for Group 1; # Starting dose of gilteritinib for Groups 2 and 3.

** To be evaluated only if there is lack of toxicity or acceptable DLT profile combined with the lack of sufficient gilteritinib activity observed at the Dose Level 1 (2 mg/kg/day). Not applicable for sites in USA.

Weight-based dosing (rounding to the nearest 10 mg) is proposed to be used in pediatric subjects up to a body weight of 60 kg. At weights of 60 kg and greater, a flat dose will be given (i.e., the maximum dose for the particular dose level as per Table 8).

Gilteritinib daily dose for any subject ≤ 5 kg weight will be discussed and approved by Astellas medical monitor.

5.1.1.1.2 Phase 2 (Dose Expansion Phase)

Gilteritinib will be administered once daily starting from day 8 through day 21 of a 28-day cycle at the RP2D or MTD established by phase 1 for the respective age groups.

5.1.1.1.3 Long-term Treatment Period

Gilteritinib will be administered once daily starting from day 1 through day 28 of a 28-day cycle at the same dose the subject received during phase 1 or 2 for a period of up to 2 years.

5.1.1.2 Co-administered Chemotherapy Drugs

The co-administered chemotherapy drugs during each cycle of phase 1 (Dose Escalation Phase) and phase 2 (Dose Expansion Phase) are as follows:

- *Cycle 1 and 2:*
  FLAG on days -1 to 5
  - Fludarabine 30 mg/m^2 per day will be administered intravenously for 5 days (from day 1 to day 5).
  - Cytarabine 2000 mg/m^2 per day will be administered intravenously for 5 days (from day 1 to day 5).
  - G-CSF filgrastim 5 µg/kg per day will be administered by SC/intravenous for 6 days (from day -1 to day 5).
  - Subject may receive prophylactic intrathecal cytarabine at the start of the cycle as per site institutional standards.
    FLAG dosage calculation per institutional practice (such as mg/kg/dose) for subjects less than 2 years of age, or below a certain weight or body surface area, is allowed.

5.1.2 Interruption or Reduction in Dose of the Study Drug

Intra subject dose reduction or interruption, during any phase of the study, is as follows:

- ***Phase 1:***

  During the DLT observation period, the assigned dose levels of gilteritinib are not allowed to change. Any subject requiring dose reduction in cycle 1 may be considered for replacement. The gilteritinib dose may be reduced for subjects after cycle 1.

- ***Phase 2:***

  For the phase 2 portion of the study, dose reduction is allowed in cycle 1 or 2.

- ***LTT:***

  For LTT portion of the study, dose reductions are allowed in any cycle.

The guidance outlined in Table 9 should be followed for gilteritinib dose interruption or reduction. Dose interruption or reduction for any other reason other than that outlined in Table 9 must be discussed with the medical monitor. Note that dose reductions should occur in a step-wise manner, following the dose levels outlined in Table 9. After the initial dose reduction, additional dose reductions may occur unless stated otherwise. If no further dose reductions are available (below 1 mg/kg per day), study treatment will be discontinued.

Any subject who has been off treatment for more than 14 days, other than for HSCT, can only resume treatment only after discussion with the medical monitor.

Intra-subject dose escalation is not allowed in phase 1, 2 or LTT.

Table 9 Guidelines for Gilteritinib (ASP2215) Dose Interruption or Reduction

QTc Prolongation	
QTcF > 500 ms (based on central or local reading)	If the mean of the triplicate QTcF is > 500 ms at any time point (by either value on ECG tracing printout or central reading), then triplicate ECGs will be repeated (within 2 hours if based on value on ECG tracing printout and as soon as possible if based on central reading). Cardiology consult will be obtained as medically indicated. If the repeat ECG confirms a mean of the triplicate QTcF > 500 ms, dosing of gilteritinib will be interrupted for up to 14 days. While ASP2215 may be interrupted temporarily based on value on ECG tracing printout, the central reading should be used for final treatment decisions. If QTcF resolves to ≤ 480 ms within 14 days, the subject may resume dosing but at a reduced dose as long as they are not currently receiving 1 mg/kg. If QTcF does not resolve to ≤ 480 ms within 14 days, treatment with gilteritinib will be discontinued and an EOT visit should be performed.
Nonhematological Events	
Grade 3 clinically significant related to gilteritinib	Dosing will be interrupted for up to 14 days. If the AE resolves to ≤ grade 1 within 14 days, the subject may resume dosing but at a reduced dose as long as they are not currently receiving 1 mg/kg. If the AE does not resolve to ≤ grade 1 within 14 days, treatment with gilteritinib will be discontinued and an EOT visit should be performed.
Grade 4 clinically significant toxicity related to gilteritinib	Treatment with gilteritinib will be discontinued.
Posterior reversible encephalopathy syndrome (PRES)	Treatment with gilteritinib will be discontinued for subjects diagnosed with PRES
Differentiation Syndrome	If differentiation syndrome is suspected, initiate corticosteroids and hemodynamic monitoring until improvement. Taper corticosteroids after resolution of symptoms. Symptoms of differentiation syndrome may recur with premature discontinuation of corticosteroid treatment. If severe signs and/or symptoms of differentiation syndrome persist for more than 48 hours after initiation of corticosteroids, interrupt gilteritinib until signs and symptoms are no longer severe. Treatment with gilteritinib can be resumed at the same dose when signs and symptoms improve to Grade 2 or lower.
Myelosuppression	
CRp or CRi	Dose may be reduced without interruption if the following criteria are met: • Subject has received a minimum of 2 cycles of gilteritinib • Platelets < 25 x 10^9/L and/or ANC ≤ 0.5 x 10^9/L; • Marrow blasts < 5%; • No evidence of extramedullary disease; Further stepwise dose reduction is permitted if dosing 1 full cycle at the reduced dose has not resulted in the desired hematologic recovery.
AE: adverse event; ANC: absolute neutrophil count; CRi: complete remission with incomplete hematologic recovery; CRp: complete remission with incomplete platelet recovery ECG: electrocardiogram; EOT: end of treatment; LTT: long-term treatment; PRES: posterior reversible encephalopathy syndrome; QTcF: Fridericia-corrected QT interval	

5.1.3 Previous and Concomitant Treatment (Medication and Nonmedication Therapy)

All medications and concomitant treatments administered from 28 days prior to screening must be recorded in the Case Report Form (CRF). Concomitant medications should be collected for reported AE/serious adverse events (SAEs) through 30 days post dose for subjects who have discontinued. For subjects who undergo HSCT, concomitant medications should be collected for reported AE/SAEs through start of conditioning treatment or 30 days post dose, whichever comes first.

The following is ***allowed*** during the course of the study:

- Local practice for prophylaxis of conjunctivitis caused by high-dose cytarabine

The following are ***prohibited*** during the course of the study:

- Treatment with strong inducers of CYP3A/P-gp.
- Therapies to treat AML including, but not limited to:
  a) Chemotherapy
  b) Surgery
  c) Immunotherapy, cellular therapy or vaccines

  Exceptions: Hydroxyurea (up to 5 g daily for up to 2 weeks to keep the absolute blast count below 50 x 10^9/L), intrathecal chemotherapy, cranial irradiation, localized radiation for palliation and leukapheresis are allowed.
- Vaccination with live vaccines (the prohibited duration starts from 6 weeks prior to the first dose of FLAG regimen and lasts until 6 weeks after the last dose of FLAG regimen)
- Any other investigational agent for AML.
- Participation in another interventional study while on treatment

Caution is advised when considering the concomitant use of the following medications:

- Treatment with Medications known to prolong QT or QTcF intervals. For concomitant drugs that have the potential to prolong QT or QTc intervals, a cardiology consult should be obtained as medically indicated.
- Strong inhibitors of P-gp and concomitant drugs that target serotonin $5HT_{2B}$ receptor or sigma nonspecific receptor are to be avoided with the exception of drugs that are considered absolutely essential for the care of the subject.
- Treatment with concomitant drugs that are strong inhibitors of CYP3A should be avoided with the exception of antibiotics, antifungals and antivirals that are used as standard of care to prevent or treat infections. If CYP3A inhibitors are used concomitantly, subjects should be closely monitored for AEs.
- Precaution should be used in treatment of gilteritinib with concomitant drugs that are substrates of P-gp (e.g., digoxin, dabigatran etexilate), BCRP (e.g., mitoxantrone, rosuvastatin) and OCT1 (e.g., metformin), since these transporters have been shown to be inhibited by gilteritinib in vitro.

Common CYP3A inhibitors, CYP3A inducers, drugs targeting the serotonin receptor, P-gp inhibitors or inducers, and drugs known to prolong QT or QTc intervals are listed in [Appendix 12.4]. The investigator should consult individual labels for all drugs that the subject is taking to evaluate if they fall into any of the above-named categories.

5.1.4 Treatment Compliance

Study subjects should be counseled on the need to meet 100% compliance with study treatment. Investigator or designee should ensure that study subjects meet this goal throughout the study period. When study treatment is administered at the research facility, it will be administered under the supervision of study personnel.

Compliance of the study drug (gilteritinib) will be monitored by the accounting of unused medication returned by the subject at visits. Compliance will be documented.

If compliance is 80% or less, the investigator or designee is to counsel the subject and ensure steps are taken to improve compliance.

In phase 1 portion of the study, subjects who are less than 80% compliant with the study treatment regimen will not be evaluable for DLT and may be replaced.

Treatment compliance should be monitored closely and deviation in compliance should be reported to the sponsor.

5.1.5 Criteria for Continuation of Treatment

A subject completing 1 or 2 treatment cycles of phase 1 or 2 will have the option to participate in LTT with gilteritinib for up to 2 years (~ 26 cycles), in the event the principal investigator feels that a subject is receiving clinical benefit from treatment with gilteritinib in phase 1 or phase 2.

Subjects receiving clinical benefit during phase 1 or 2 may initiate LTT with gilteritinib for up to 2 years only after discussion and approval from the Astellas medical monitor. Clinical benefit is defined as follows:

a) Completed 1 or 2 treatment cycles of phase 1 or 2.

b) Subject did not develop intolerable or unacceptable toxicity.

c) Subject has achieved remission, stable or improved disease, or symptom improvement, or decreased transfusion dependence.

d) Any other reason that the investigator judges will benefit the subject from continued treatment with gilteritinib, must be reviewed and approved by the Astellas medical monitor.

5.1.6 Resumption of Treatment After Hematopoietic Stem Cell Transplantation

Pre-HSCT preparation:

Subjects who have a donor identified and achieve a response allowing them to undergo HSCT per each institution's assessment can undergo HSCT without leaving the study if they have completed cycle 1 of phase 1 or 2. However, gilteritinib should be stopped for at least

1 week prior to start of the preparative regimen and a pre-HSCT visit should be performed prior to starting the conditioning regimen for HSCT.

Resumption of Treatment after HSCT:

Subjects returning to the study post HSCT can resume gilteritinib at the same dose prior to HSCT if the following conditions are met:

- Subject is between 30 to 90 days post HSCT
- Subject has had successful engraftment as demonstrated by ANC $\geq 500/mm^3$ and platelets $\geq 20000/mm^3$ without transfusions
- Subject does not have ≥ grade 2 acute GVHD
- Subject is in CRc (CR, complete remission with incomplete hematologic recovery [CRi], complete remission with incomplete platelet recovery [CRp])

For subjects resuming gilteritinib treatment, subjects will follow the procedures listed under subsequent visits (day 1) in the Schedule of Assessments [Table 1].

5.2 Demographics and Baseline Characteristics

5.2.1 Demographics

Demographic information will be collected for all subjects and will include age, sex, race and ethnicity.

5.2.2 Medical History

Medical history includes all significant medical conditions other than AML that have resolved prior to informed consent. Conditions that are ongoing at the time of consent will be collected as baseline condition on the Medical History Electronic Case Report Form (eCRF).

Details that will be collected include the onset date and recovery date and NCI-CTCAE grade, if applicable for ongoing conditions.

5.2.3 Diagnosis of the Target Disease, Severity, and Duration of Disease (Optional for Healthy Volunteer Studies)

5.2.3.1 Disease History

AML diagnosis and studies related to AML subtype classification will be collected and will include date and method of diagnosis, bone marrow evaluations, histopathology, cytogenetics, immunophenotyping and cytochemistry, FLT3 mutation status performed using institutional assay, lumbar puncture results if performed (red blood cell [RBC], WBC and differential, cytospin results) and related genetic syndromes. Dates for diagnostic procedures will be collected.

Prior HSCT and AML therapy including induction, consolidation and maintenance chemotherapy will be collected. Response to HSCT and AML therapy, as well as the duration of the response will also be collected.

5.2.3.2 FMS-like Tyrosine Kinase (FLT3) Mutation Status

Only for subjects who have R/R AML and have not received chemotherapy within 1 month prior to screening, FLT3 mutation status obtained within 1 month as part of routine patient management prior to obtaining informed consent in the study may be used if documentation is available for eligibility assessment.

5.2.3.3 Performance Status

The Karnofsky Performance Score (KPS) [Karnofsky and Burchenal, 1949] will be used to assess performance status [Table 10] of a subject ≥ 16 years of age while and the Lansky play-performance scale [Lansky et al, 1987] will be used to assess performance status [Table 11] of a subject < 16 years of age.

Performance status will be obtained and recorded according to the Schedules of Assessments [Table 1].

Table 10 Karnofsky Performance Status

Index	Specific criteria
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity, minor signs or symptoms of disease
80	Normal activity with effort, some signs or symptoms of disease
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization is indicated although death not imminent.
20	Hospitalization necessary, very sick, active supportive treatment necessary.
10	Moribund, fatal processes progressing rapidly.
0	Dead

Table 11 Lansky Play-Performance Scale for Children

Index	Specific criteria
100	fully active, normal
90	minor restrictions in physically strenuous activity
80	active, but tires more quickly
70	both greater restriction of, and less time spent in, active play
60	up and around, but minimal active play; keeps busy with quieter activities
50	gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities
40	mostly in bed; participates in quiet activities
30	in bed; needs assistance even for quiet play
20	often sleeping; play entirely limited to very passive activities
10	no play: does not get out of bed
0	unresponsive

5.3 Efficacy Assessments

5.3.1 Response Definitions

Response to treatment will be defined per modified criteria [Cheson et al, 2003] as outlined below.

5.3.1.1 Complete Remission

For subjects to be classified as being in CR at a post-baseline visit, they must have bone marrow regenerating normal hematopoietic cells and achieve a morphologic leukemia-free state and must have an ANC $\geq 1 \times 10^9$/L and platelet count $\geq 100 \times 10^9$/L and normal marrow differential with < 5% blasts, and they will be RBC and platelet transfusion independent (defined as 1 week without RBC transfusion and 1 week without platelet transfusion). There should be no evidence of extramedullary leukemia.

5.3.1.2 Complete Remission with Incomplete Platelet Recovery

For subjects to be classified as being in CRp at a post-baseline visit, they must achieve CR except for incomplete platelet recovery ($< 100 \times 10^9$/L).

5.3.1.3 Complete Remission with Incomplete Hematologic Recovery

For subjects to be classified as being in CRi at a post-baseline visit, they must fulfill all the criteria for CR except for incomplete hematological recovery with residual neutropenia $< 1 \times 10^9$/L with or without complete platelet recovery. RBC and platelet transfusion independence is not required.

5.3.1.4 Composite Complete Remission

For subjects to be classified as being in CRc at a post-baseline visit, they must achieve CR, CRp or CRi at the visit.

Overall best response is defined as CRc after 2 cycles of therapy.

5.3.1.5 Complete Remission with Partial Hematologic Recovery

At a post-baseline visit, subjects will be classified as CRh if they have marrow blasts < 5%, partial hematologic recovery ANC ≥ 0.5 x 10^9/L and platelets ≥ 50 x 10^9/L, no evidence of extramedullary leukemia and cannot be classified as CR.

5.3.1.6 Partial Remission

For subjects to be classified as being in PR at a post-baseline visit, they must have bone marrow regenerating normal hematopoietic cells with evidence of peripheral recovery with no (or only a few regenerating) circulating blasts and with a decrease of at least 50% in the percentage of blasts in the bone marrow aspirate with the total marrow blasts between 5% and 25%. A value of less or equal than 5% blasts is also considered a PR if Auer rods are present.

5.3.1.7 Not Evaluable/No Response

In the situation where no bone marrow assessments are performed or myeloblast value is missing, blast value from peripheral blood is missing or ≤ 2%, and extramedullary leukemia is missing or not done, the response will be classified as not evaluable (NE). In any case response cannot be categorized as CR, CRp, CRi, PR or NE, it will be categorized as NR.

5.3.1.8 Relapse

Relapse after CR, CRh, CRp or CRi is defined as a reappearance of leukemic blasts in the peripheral blood or ≥ 5% blasts in the bone marrow aspirate not attributable to any other cause or reappearance or new appearance of extramedullary leukemia.

Relapse after PR is similarly defined with reappearance of significant numbers of peripheral blasts and an increase in the percentage of blasts in the bone marrow aspirate to > 25% not attributable to any other cause or reappearance or new appearance of extramedullary leukemia.

5.3.1.9 Best Response

Best response is defined as the best measured response to treatment for all visits (in the order of CR, CRp, CRi, PR, NR and NE) post-baseline. Subjects with best responses of CR, CRp, CRi or PR will be considered responders. Subjects who do not achieve at least a best response of PR will be considered nonresponders.

5.3.1.10 Complete Remission Rate

CR rate is defined as the number of subjects who achieve the best response of CR divided by the number of subjects in the analysis population.

5.3.1.11 Composite Complete Remission Rate

CRc rate is defined as the number of subjects who achieve the best response of CRc (CR, CRp or CRi) divided by the number of subjects in the analysis population.

5.3.1.12 Complete Remission with Partial Hematologic Recovery Rate

CRh rate is defined as the number of subjects who achieve CRh at any of the post-baseline visits and do not have best response of CR divided by the number of subjects in the analysis population.

5.3.1.13 Complete Remission and Complete Remission with Partial Hematologic Recovery Rate

CR/CRh rate is defined as the number of subjects who achieve either CR or CRh at any of the post-baseline visits divided by the number of subjects in the analysis population.

5.3.2 Survival Time, Duration and Other Efficacy Endpoints

5.3.2.1 Overall Survival

OS is defined as the time from the date of enrollment until the date of death from any cause. For a subject who is not known to have died by the end of study follow-up, OS is censored at the date of last contact.

Date of last contact is the latest date the subject is known to be alive by the cutoff date.

5.3.2.2 Event-free Survival

EFS is defined as the time from the date of enrollment until the date of documented relapse (excluding relapse after PR), treatment failure or death, whichever occurs first. If a subject experiences relapse or death, the subject is defined as having EFS event related to either “relapse” or “death”, and the event date is the date of relapse or death. If a subject fails to achieve any of the response of CR, CRp, CRi or PR during the treatment period, the subject is defined as having EFS event related to treatment failure, and the event date is the enrollment date. For a subject who is not known to have had a relapse or treatment failure or death event, EFS is censored at the date of last relapse-free disease assessment. Subject is not censored at HSCT.

5.3.2.3 Leukemia-free Survival

LFS is defined as the time from the date of first CRc until the date of documented relapse or death for subjects who achieve CRc. For a subject who is not known to have relapsed or died, LFS is censored on the date of last relapse-free disease assessment date.

5.3.2.4 Duration of Remission

Duration of Remission

Duration of remission includes duration of CRc, duration of CR/CRh, duration of CRh, duration of CR, duration of CRi, duration of CRp and duration of response (CRc + PR).

Duration of CRc

Duration of CRc is defined as the time from the date of first CRc until the date of documented relapse for subjects who achieve CRc. Subjects who die without report of relapse are considered nonevents and censored at their last relapse-free disease assessment

date. Other subjects who do not relapse on study are considered nonevents and censored at the last relapse-free disease assessment date.

Duration of CR/CRh, CRh, CR, CRp, CRi

Duration of CR/CRh, CRh, CR, CRp, CRi is defined similarly as duration of CRc.

Duration of Response

Duration of response is defined as the time from the date of either first CRc or PR until the date of documented relapse of any type for subjects who achieve CRc or PR. Subjects who die without report of relapse are considered nonevents and censored at their last relapse-free disease assessment date. Other subjects who do not relapse on study are considered nonevents and censored at the last relapse-free assessment date.

5.3.2.5 Transplantation Rate

Transplantation rate is defined as the percentage of subjects undergoing HSCT during the study period.

5.3.3 Bone Marrow Aspiration and/or Biopsy

Bone marrow samples are required during screening and end of each cycle (C1D28 and C2D28) for phase 1 and phase 2.

For subjects whose response assessment is not evaluable due to hypocellularity, a repeat bone marrow analysis should be performed at least every 14 days until response determination is possible.

For LTT, bone marrow assessments will be performed on C1D1 and repeated after every 3 cycles until 1 year of LTT, followed by after every 6 cycles until 2 years of LTT (i.e., C1D1, C4D1, C7D1, C10D1, C13D1, C19D1 etc.). Bone marrow samples are also required at the pre-HSCT/end of treatment visit and as clinically indicated. Bone marrow assessments on LTT C1D1 and EOT_2 can be avoided if the last bone marrow sample in the study was collected within 2 weeks of the visit.

If bone marrow aspirate is unobtainable (e.g., dry tap), an additional EDTA tube of whole blood should be collected instead. Bone marrow aspirate is required, and bone marrow biopsy is preferred. In case of inadequate aspirate, bone marrow biopsy is required.

5.3.4 Survival Status and Subsequent Antileukemic Treatments and Their Outcomes

Information on survival status, subsequent antileukemic treatments and outcomes will be collected for all subjects.

The first survival status will occur at the 28 day follow-up visit where telephone contact with the subject is sufficient unless any assessment must be repeated for resolution of treatment-related AEs.

After the 28 day follow-up visit, the subject or caregiver will continue to be contacted via telephone by site personnel for follow-up every 3 months for up to 2 years. Data may be supplemented by site records when available at the time of the contact (e.g., treatment

records, outcomes). Follow-up will continue until the final database lock, which is estimated to be up to 2 years of follow-up post completion of treatment.

If a subject death occurs during the SAE reporting period or if the death occurs after the SAE reporting period, but is determined by the investigator to be possibly related to study drug, then the associated AE with outcome of death will also be reported on the CRF and SAE form. If a subject death does not meet the criteria of an SAE, then death and antileukemic treatment and outcome up through the date of death should be collected and entered in CRF.

5.4 Safety Assessment

5.4.1 Vital Signs

Vital signs, including systolic and diastolic blood pressures (mmHg), radial pulse rate (beats per minute), respiratory rate, O_2 saturation and temperature will be obtained and recorded at the times specified in the Schedule of Assessments [Table 1]. All vital sign measurements will be obtained with the subject in the sitting or supine position.

If clinically significant vital sign changes from baseline (pretreatment) are noted, the changes will be documented as AEs on the AE page of the eCRF. Clinical significance will be defined as a variation in vital signs, which has medical relevance that could result in an alteration in medical care. The investigator will continue to monitor the subject until the parameter returns to grade ≤ 1 or to the baseline (pretreatment) value or until the investigator determines that follow-up is no longer medically necessary.

5.4.2 Laboratory Assessments

The laboratory tests that will be performed during the conduct of the study are described below [Table 12]. Safety assessments will be obtained using local laboratories. See Schedule of Assessments [Table 1] for study visit involving sample collection. Clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator/sub-investigator who is a qualified physician.

Table 12 Laboratory Tests Performed During the Conduct of the Study

Panel/Assessment	Parameters to be Analyzed	Estimated sample volume
Hematology	White Blood Cell Count (WBC) WBC Differential Red Blood Cell Count (RBC) Hemoglobin (Hgb) Hematocrit (Hct) Mean Corpuscular Volume Platelet Count MCHC MCH	1.0 mL of whole blood[1]
Table continued on next page		

Panel/Assessment	Parameters to be Analyzed	Estimated sample volume
Biochemistry	Sodium Potassium Chloride Bicarbonate Blood Urea Nitrogen Creatinine Glomerular filtration rate Uric acid[2] Glucose Calcium Phosphate Magnesium Albumin Total Protein Alkaline Phosphatase Lactate Dehydrogenase Creatine Phosphokinase Triglycerides Total Cholesterol Liver Function Tests including: Total Bilirubin Alanine Aminotransferase Aspartate Aminotransferase	1.1 mL of whole blood[1]
Thyroid Function Tests	TSH Free T4	2.5 of whole blood[2]
Serum Pregnancy Test	Human Chorionic Gonadotropin or alternatively pregnancy dipstick can be used	1.4 ml of whole blood[2]
Coagulation Profile	INR (with PT if reported) aPTT Fibrinogen (Screening Only) D-dimer (Screening Only)	2.0 mL of whole blood[2]
Urinalysis	Color Appearance Specific Gravity pH Bilirubin Blood Glucose Ketones Leukocyte Esterase Nitrite Protein Urobilinogen	Dipstick

Table continued on next page

Panel/Assessment	Parameters to be Analyzed	Estimated sample volume
Bone Marrow	Blast Count and Cell Counts [4] Flow Cytometry for Blasts MRD	Aspirate 1 to 3 mL in EDTA tube. If aspirate is unavailable, then biopsy and whole blood is required. 2 to 3 bedside smear slides in addition to the sample (aspirate/biopsy and whole blood).
Peripheral Blood	Disease Assessment for Blast Count and Manual Differential	Peripheral blood 2 mL and 2 to 3 bedside peripheral blood smear slides
Bone Marrow Aspirate and/or Blood[3]	FLT3 Mutation Analysis	Bone marrow aspirate 0.25 mL to 0.75 mL in a Sodium Heparin tube or 1 to 3 mL of peripheral blood in a Sodium Heparin tube
PIA & FLT3 Ligand	FLT3 inhibition (PIA) FLT3 Ligand	2 mL of whole blood
PK	Gilteritinib	1 mL of whole blood
PGx	Pharmacogenomics Analysis	3 mL of whole blood and a buccal swab sample

aPTT: activated partial thromboplastin time; eCRF: Electronic Case Report Form; FLT3: FMS-like tyrosine kinase; INR: international normalization ratio; MRD: minimal residual disease; PK: pharmacokinetics; PGx: Pharmacogenomics; PIA: plasma inhibitory assay; PT: prothrombin time; T4: thyroxin; TSH: thyroid stimulating hormone.

1 Estimated volumes are provided since local laboratories will be used for these assessments and the volume may vary depending on the site institutional practice.

2 On days 1, 4, 8 and 15 in cycle 1 of phase 1 or phase 2.

3 Screening only.

4 In addition to the central read of these values, available local results will also be entered into the electronic case report form.

5.4.3 Physical Examination

Standard, full physical examinations will be performed to assess general appearance, skin, eyes, ears, nose, throat, neck, cardiovascular, chest and lungs, abdomen, musculoskeletal, neurologic status, mental status, and lymphatic systems. Genitourinary and rectal system exam are to be performed only if clinically indicated. Physical examinations will be conducted at visits as outlined in the Schedule of Assessments. Each physical examination will include the observation and review of body system, weight and height at Screening and on day 1 of each cycle. If clinically significant worsening of findings from predose (day 1) is noted at any study visit, the changes will be documented as AEs on the Adverse-Event page of the CRF. Clinical significance is defined as any variation in physical findings, which has medical relevance that could result in an alteration in medical care. The investigator will continue to monitor the subject until the parameter returns to grade ≤ 1, or to the baseline (pretreatment) condition, or until the investigator determines that follow up is no longer medically necessary.

5.4.4 Electrocardiogram

A 12-lead ECG will be performed as outlined in the Schedule of Assessment [Table 1] using a central ECG reading laboratory. Predose assessments should be taken within 1 hour prior to study drug administration. The 12-lead ECGs will be recorded in triplicate (3 separate ECGs,

10 minutes resting prior to first ECG and at least 5 minutes apart per time point) and transmitted electronically for central reading. The mean of the triplicate ECG from central read should be used for all final treatment decisions and AE reporting.

If the mean triplicate QTcF is > 500 ms at any time point, the ECG will be repeated (within 2 hours if identified on machine read or as soon as possible if identified from central read).

Cardiology consult will be obtained as medically indicated. If QTcF > 500 ms is confirmed, then the investigator will interrupt and reduce gilteritinib per the interruption or reduction guidelines in [Section 5.1.2].

Triplicate ECGs must also be obtained within 1 hour prior to obtaining the time-matched pharmacokinetic samples, for a particular visit. For phase 1 and 2, all visits have a predose pharmacokinetic sample with the exception of the cycle 1 day 21 visit, where there is a predose and 4 to 6 hours post dose pharmacokinetic samples are collected. Thus, a triplicate ECG will be obtained within 1 hour of the predose pharmacokinetic sample and 4 to 6 hours post dose pharmacokinetic sample for cycle 1 day 21.

5.4.5 Imaging

5.4.5.1 Chest X-ray or Computed Tomography Scan

Local chest X-ray or computed tomography (CT) scan is to be performed at screening. A chest X- ray (or CT of chest) performed as part of routine patient management within 2 weeks prior to start of screening can be used if available.

5.4.5.2 Multigated Acquisition Scan (MUGA) or Echocardiogram (ECHO)

A local MUGA scan or ECHO is to be performed as outlined in the Schedule of Assessment [Table 1], i.e., at screening and EOT_1 for each subject. Additional MUGA scans or ECHO will be performed while on gilteritinib treatment when clinically indicated.

5.4.6 Order of Assessments

Predose assessments:

Predose indicates dosing prior to gilteritinib or chemotherapy. The following sequence order for study activities is recommended during cycle 1 and 2 of phase 1 or 2:

- Vitals signs
- Triplicate ECGs (within 1 hour prior to dosing)
- Any type of blood draw (includes clinical laboratory tests, thyroid function test, pharmacokinetic sample, PIA/FLT3 Ligand sample, Pregnancy test)

Postdose assessments:

The post dose assessments includes

- Pharmacokinetic samples and
- ECG: Triplicate ECGs are to be obtained within 1 hour prior to the time-matched pharmacokinetic sample during cycle 1 and 2 of phase 1 or 2.

5.5 Adverse Events and Other Safety Aspects

5.5.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a subject administered a study drug, and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product whether or not considered related to the medicinal product.

In order to identify any events that may be associated with study procedures and could lead to a change in the conduct of the study, Astellas collects AEs even if the subject has not received study drug treatment. AE collection begins after the signing of the informed consent and will be collected until 28 days after the last dose of study drug (or until the 28-day follow-up visit of the subject, whichever is later) or the subject is determined to be a screen failure.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the subject is the preferred method to inquire about AE occurrences.

5.5.1.1 Abnormal Laboratory Findings

Any abnormal laboratory test result (e.g., hematology, clinical chemistry, or urinalysis) or other safety assessment (e.g., ECGs, radiographic scans, vital signs measurements, physical examination), including those that worsen from baseline, that is considered to be clinically significant in the medical and scientific judgment of the investigator and not related to underlying disease, is to be reported as an (S)AE.

Any clinically significant abnormal laboratory finding or other abnormal safety assessment which is associated with the underlying disease does not require reporting as an (S)AE, unless judged by the investigator to be more severe than expected for the subject's condition.

Repeating an abnormal laboratory test or other safety assessment, in the absence of any of the above criteria, does not constitute an AE. Any abnormal test result that is determined to be an error does not require reporting as an AE.

5.5.1.2 Potential Cases of Drug-Induced Liver Injury

Refer to [Appendix 12.5 Liver Safety Monitoring and Assessment] for detailed instructions on Drug Induced Liver Injury (DILI). Abnormal values in AST and/or ALT concurrent or with abnormal elevations in TBL that meet the criteria outlined in [Appendix 12.5 Liver Safety Monitoring and Assessment], in the absence of other causes of liver injury, are considered potential cases of DILI (potential Hy's Law cases) and are always to be considered important medical events and reported per [Section 5.5.4.1 Reporting of Serious Adverse Events].

5.5.1.3 Disease Progression and Study Endpoints

Under this protocol, the following event(s) will not be considered as an(S)AE:

- Disease Progression: events including defined study endpoints that are clearly consistent with the expected pattern of progression of the underlying disease are not to be recorded as AEs. These data will be captured as efficacy assessment data as outlined in [Section 7.4 Analysis of Efficacy]. Progressive Disease should not be reported as an AE/SAE unless the disease progression is the cause of death. If there is any uncertainty as to whether an event is due to anticipated disease progression and/or if there is evidence suggesting a causal relationship between the study drug and the event, it should be reported as an (S)AE. All deaths up to 30 days after the last dose of study drug must be reported as an SAE, even if attributed to disease progression.
- Pre-planned and elective hospitalizations or procedures for diagnostic, therapeutic, or surgical procedures for a pre-existing condition that did not worsen during the course of the clinical study. These procedures are collected per the eCRFs Completion Guidelines.

5.5.2 Definition of Serious Adverse Events (SAEs)

An AE is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Results in death
- Is life-threatening (an AE is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an AE that, had it occurred in a more severe form, might have caused death)
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly, or birth defect
- Requires inpatient hospitalization (except for planned procedures as allowed per study) or leads to prolongation of hospitalization (except if prolongation of planned hospitalization is not caused by an AE). Hospitalization for treatment/observation/examination caused by AE is to be considered as serious.
- Other medically important events (defined in paragraph below)

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent 1 of the other outcomes listed in the definition above. These events, including those that may result in disability/incapacity, usually are considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

5.5.3 Criteria for Causal Relationship to Study Drug

A medically qualified investigator is obligated to assess the relationship between each study drug and each occurrence of each (S)AE. This medically qualified investigator will use medical judgment, as well as the Reference Safety Information 1.3 Summary of Key Safety Information for Study Drugs to determine the relationship. The causality assessment is 1 of the criteria used when determining regulatory reporting requirements.

The medically qualified investigator is requested to provide an explanation for the causality assessment for each (S)AE and must document in the medical notes that he/she has reviewed the (S)AE and has provided an assessment of causality.

Following a review of the relevant data, the causal relationship between the study drug and each (S)AE will be assessed by answering '**yes**' or '**no**' to the question "**Do you consider that there is a reasonable possibility that the event may have been caused by the study drug**".

When making an assessment of causality, the following factors are to be considered when deciding if there is evidence and/or arguments to suggest there is a 'reasonable possibility' that an (S)AE may have been caused by the study drug (rather than a relationship cannot be ruled out) or if there is evidence to reasonably deny a causal relationship:

- Plausible temporal relationship between exposure to the study drug and (S)AE onset and/or resolution. Has the subject actually received the study drug? Did the (S)AE occur in a reasonable temporal relationship to the administration of the study drug?
- Plausibility; i.e., could the event been caused by the study drug? Consider biologic and/or pharmacologic mechanism, half-life, literature evidence, drug class, preclinical and clinical study data, etc.
- Dechallenge/Dose reduction/Rechallenge:
    - Did the (S)AE resolve or improve after stopping or reducing the dose of the suspect drug? Also consider the impact of treatment for the event when evaluating a dechallenge experience.
    - Did the (S)AE reoccur if the suspected drug was reintroduced after having been stopped?
- Laboratory or other test results; a specific lab investigation supports the assessment of the relationship between the (S)AE and the study drug (e.g., based on values pre-, during and post-treatment)
- Available alternative explanations independent of study drug exposure; such as other concomitant drugs, past medical history, concurrent or underlying disease, risk factors including medical and family history, season, location, etc. and strength of the alternative explanation

There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to the sponsor/designee. However, it is very important that the medically qualified investigator always make an assessment of causality for every event before the initial transmission of the SAE data to sponsor/designee. With

limited or insufficient information about the event to make an informed medical judgment and in absence of any indication or evidence to establish a causal relationship, a causality assessment of 'no' is to be considered. In such instance, the investigator is expected to obtain additional information regarding the event as soon as possible and to re-evaluate the causality upon receipt of additional information. The medically qualified investigator may revise his/her assessment of causality in light of new information regarding the SAE and shall send an SAE follow-up report and update the eCRF with the new information and updated causality assessment.

5.5.4 Criteria for Defining the Severity of an Adverse Event

AEs, including abnormal clinical laboratory values, will be graded using the NCI-CTCAE guidelines (Version 5.0) [Table 13]. The items that are not stipulated in the NCI-CTCAE Version 5.0 will be assessed according to the criteria below and entered into the eCRF.

Table 13 NCI-CTCAE Version 5.0

Grade	Assessment Standard
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death related to AE.

A Semi-colon indicates 'or' within the description of the grade. A single dash (-) indicates a grade is not available. Not all grades are appropriate for all AEs.

Activities of Daily Living (ADL)

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

5.5.4.1 Reporting of Serious Adverse Events (SAEs)

The collection of AEs and the expedited reporting of SAEs will start following receipt of the informed consent and will continue until the 28-day Follow-up visit of the subject or until the subject is determined to be a screen failure.

In the case of a SAE, the investigator must complete and submit an SAE worksheet containing all information that is required by local and/or regional regulations to sponsor or designee by email or fax immediately (within 24 hours of awareness).

The SAE worksheet must be signed by a medically qualified investigator (as identified on Delegation of Authority Log). Signature confirms accuracy and completeness of the SAE data as well as the investigator causality assessment including the explanation for the causality assessment.

Specific for study sites in Japan:

In the case of a SAE, the investigator or subinvestigator must report to the head of the study site and must contact the sponsor/delegated CRO by fax or email immediately (within 24 hours of awareness).

The investigator should complete and submit JUTOKUNA YUUGAIJISHOU HOUKOKUSHO containing all information that is required by the appropriate regulatory authorities to the sponsor/delegated CRO by fax or email immediately (within 24 hours of awareness) and to the head of the hospital.

For contact details, see [Section II Contact Details of Key Sponsor's Personnel]. Fax or email the SAE/Special Situations Worksheet to:

Astellas Pharma Global Development – United States
North American Fax number: 888-396-3750
(North America Alternate Fax: 847-317-1241)
International Fax number: +44-800-471-5263
Email: safety-us@astellas.com

Specific for study sites in JAPAN:

JUTOKUNA YUUGAIJISHOU HOUKOKUSHO the SAE/special situations worksheet to:

Astellas Pharma Inc. – Japan
Pharmacovigilance
Fax number 81-3-3243-5747
Email: rk-safety-jp@astellas.com
PAREXEL International
Global Monitoring Operations
Fax: +81 36888 5377

If there are any questions, or if clarification is needed regarding the SAE, please contact the sponsor's medical monitor/study physician or his/her designee [Section II Contact Details of Key Sponsor's Personnel].

Follow-up information for the event should be sent promptly (within 7 days of the initial notification and for sites in Japan - within 2 days for the initial notification).

Full details of the SAE should be recorded on the medical records, SAE/Special Situation Worksheet and on the (e)CRF.

The following minimum information is required:

- International Study Number (ISN)/Study number,
- Subject number, sex and age,
- The date of report,
- A description of the SAE (event, seriousness criteria),
- Causal relationship to the study drug (including reason), and
- The drug provided

The sponsor or sponsor's designee will medically evaluate the SAE and determine if the report meets the requirements for expedited reporting based on seriousness, causality, and expectedness of the events (e.g., Suspected Unexpected Serious Adverse Reaction [SUSAR] reporting) according to current local/regional regulatory requirements in participating countries. The sponsor or sponsor's designee will submit expedited safety reports (e.g., IND Safety Reports, SUSAR, CIOMS-I [Council for International Organizations of Medical Sciences]) to Competent Authorities (CA) and concerned Ethics Committee (cEC) per current local regulations and will inform the investigators of such regulatory reports as required. Investigators must submit safety reports as required by their IRB/local IEC within timelines set by regional regulations (e.g., EMA, FDA) where required. Documentation of the submission to and receipt by the IRB/local IEC of expedited safety reports should be retained by the site.

The sponsor or designee will notify all investigators responsible for ongoing clinical studies with the study drug of all SUSARs, which require submission per local requirements.

The investigators should provide written documentation of IRB/IEC notification for each report to the sponsor.

The investigators may contact the sponsor's medical monitor/expert for any other problem related to the safety, welfare, or rights of the subject.

5.5.5 Follow-up of Adverse Events

All AEs occurring during or after the subject has discontinued the study are to be followed up until resolved or judged to be no longer clinically significant, or until they become chronic to the extent that they can be fully characterized by the investigator.

If after the protocol defined AE collection period [see Section 5.5.1 Definition of Adverse Event], an AE progresses to a SAE, or the investigator learns of any (S)AE including death, where he/she considers there is reasonable possibility it is related to the study drug treatment or study participation, the investigator must promptly notify the sponsor.

5.5.6 Monitoring of Common Serious Adverse Events

Common SAEs are SAEs commonly anticipated to occur in the study population independent of drug exposure. SAEs classified as "common" are provided in [Appendix 12.6 Common Serious Adverse Events] for reference. The list does NOT change the investigator's reporting obligations, nor his obligations to perform a causality assessment, or prevent the need to report an AE meeting the definition of an SAE as detailed above. The purpose of this list is to alert the investigator that some events reported as SAEs may not require expedited reporting to the regulatory authorities based on the classification of "common SAEs" as specified in [Appendix 12.6 Common Serious Adverse Events]. The sponsor will monitor these events throughout the course of the study for any change in frequency. Any changes to this list will be communicated to the participating investigational sites. Investigators must report individual occurrences of these events as stated in [Section 5.5.4.1 Reporting of Serious Adverse Events].

5.5.7 Special Situations

Certain Special Situations observed in association with the study drug(s), such as incorrect administration (e.g., wrong dose of study drug, comparator, or background therapy) are collected in the eCRF, as Protocol Deviation per [Section 8.3 Major Protocol Deviations] or may require special reporting, as described below. These Special Situations are not considered AEs, but do require to be communicated to Astellas as per the timelines defined below.

If a Special Situation is associated with, or results in, an AE, the AE is to be assessed separately from the Special Situation and captured as an AE in eCRF. If the AE meets the definition of a SAE, the SAE is to be reported as described in [Section 5.5.4.1 Reporting of Serious Adverse Events] and the details of the associated Special Situation are to be included in the clinical description on the SAE worksheet.

The Special Situations are:

- Pregnancy
- Medication Error, Overdose and “Off-label use”
- Misuse/abuse
- Occupational exposure
- (Suspicion of) Transmission of infectious agent
- Suspected Drug-Drug interaction

5.5.7.1 Pregnancy

If a female subject becomes pregnant during the study dosing period or within 180 days from the discontinuation of dosing, the investigator is to report the information to the sponsor according to the timelines in [Section 5.5.4.1 Reporting of Serious Adverse Events] using the Pregnancy Reporting Form and in the eCRF.

The investigator will attempt to collect pregnancy information on any female partner of a male subject who becomes pregnant during the study dosing period or within 120 days from the discontinuation of dosing and report the information to sponsor according to the timelines in [Section 5.5.4.1 Reporting of Serious Adverse Event**s**] using the Pregnancy Reporting Form.

The expected date of delivery or expected date of the end of the pregnancy, last menstruation, estimated conception date, pregnancy result and neonatal data etc., should be included in this information.

While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or termination (including elective termination) of a pregnancy is to be reported for a female study subject as an AE in the eCRF or SAE per [Section 5.5.4.1 Reporting of Serious Adverse Events]. For (S)AEs experienced by a female partner of a male subject, (S)AEs are to be reported via the Pregnancy Reporting Form.

Additional information regarding the outcome of a pregnancy when also categorized as an SAE is mentioned below:

- "Spontaneous abortion" includes miscarriage, abortion and missed abortion.
- Death of a newborn or infant within 1 month after birth is to be reported as an SAE regardless of its relationship with the study drug.
- If an infant dies more than 1 month after the birth, is to be reported if a relationship between the death and intrauterine exposure to the study drug is judged as "possible" by the investigator.
- Congenital anomaly (including anomaly in miscarried fetus).

Unless a congenital anomaly is identified prior to spontaneous abortion or miscarriage, the embryo or fetus should be assessed for congenital defects by visual examination. (S)AEs experienced by the newborn/infant should be reported via the Pregnancy Reporting Form. Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date.

5.5.7.2 Medication Error, Overdose and "Off-Label Use"

If a Medication Error, Overdose or "Off-Label Use" (i.e., use outside of what is stated in the protocol) is suspected, refer to [Section 8.3 Major Protocol Deviations]. Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 5.5.4.1 Reporting of Serious Adverse Events] together with the details of the medication error, overdose and/or "Off-Label Use".

In the event of suspected gilteritinib overdose, the subject should receive supportive care and monitoring. The medical monitor/expert should be contacted as applicable.

In the event of suspected overdose for FLAG regimen, refer to the approved Package Insert, Summary of Product Characteristics, or local product information supplied by the manufacturer for each agent.

5.5.7.3 Misuse/Abuse

If misuse or abuse of the study drug(s) is suspected, the investigator must forward the Special Situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of a SAE, the SAE is also to be reported as described in [Section 5.5.4.1 Reporting of Serious Adverse Events] together with details of the misuse or abuse of the study drug(s).

5.5.7.4 Occupational Exposure

If occupational exposure (e.g., inadvertent exposure to the study drug(s) of site staff whilst preparing it for administration to the patient) to the study drug(s) occurs, the investigator must forward the Special Situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs occurring to the individual associated with or resulting from the Special Situation are to be reported on the Special Situations worksheet.

5.5.7.5 (Suspicion of) Transmission of Infectious Agent

If transmission of an infectious agent associated with the study drug(s) is suspected, the investigator must forward the Special Situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness) and any associated (S)AEs are to be reported in the e-CRF. If the AE meets the definition of a SAE, the SAE is also to be reported as described in [Section 5.5.4.1 Reporting of Serious Adverse Events] together with the details of the suspected transmission of infectious agent.

5.5.7.6 Suspected Drug-Drug Interaction

If a suspected drug-drug interaction associated with the study drug(s) is suspected, the investigator must forward the Special Situation worksheet to the sponsor by fax or email immediately (within 24 hours of awareness). Any associated (S)AEs are to be reported in the eCRF. If the AE meets the definition of an SAE, the SAE is also to be reported as described in [Section 5.5.4.1 Reporting of Serious Adverse Events] together with details of the suspected drug-drug interaction.

5.5.8 Supply of New Information Affecting the Conduct of the Study

When new information becomes available necessary for conducting the clinical study properly, the sponsor will inform all investigators involved in the clinical study as well as the regulatory authorities. Investigators should inform the IRB/IEC of such information when needed.

The investigator will also inform the subjects, who will be required to sign an updated ICF in order to continue in the clinical study.

Specific to Japan:

1. When information is obtained regarding serious and unexpected adverse drug reactions (or other) that are specified in Article 273 of the Act on Securing Quality, Efficacy and Safety of Pharmaceuticals, Medical Devices, Regenerative and Cellular Therapy Products, Gene Therapy Products, and Cosmetics, in compliance with Article 80-2 Paragraph 6 of the Pharmaceutical Affairs Law, the sponsor should inform all investigators involved in the study, head of the study site and appropriate regulatory authorities of such information. The head of the study site who receives such information will decide whether the study should be continued after hearing the opinions of the IRB. The investigator will supply the new information to the subjects, in compliance with [Appendix 12.1.5.2 Supply of New and Important Information Influencing the Subject's Consent and Revision of the Written Information].
2. In addition, when the head of the study site receives the revisions of the Investigator's Brochure, protocol, written information, information on the matters covering the quality of the test product, efficacy and safety, information necessary for conducting the study properly or documents to be examined by the IRB, these documents should be sent to the IRB.

5.5.9 Urgent Safety Measures

An Urgent Safety Measure (USM) is an intervention which is not defined by the protocol and can be put in place with immediate effect without needing to gain prior approval by the sponsor, relevant CA, IRB/IEC, where applicable, in order to protect study subjects from any immediate hazard to their health and/or safety. Either the investigator or the sponsor can initiate an USM. The cause of an USM can be safety, product or procedure related.

5.5.10 Reporting Urgent Safety Measures

In the event of a potential USM, the investigator must contact the Astellas Study Physician *(**Specific to Japan:*** and/or Astellas team member) within 24 hours of awareness. Full details of the potential USM are to be recorded in the subject's medical records. The sponsor may request additional information related to the event to support their evaluation.

If the event is confirmed to be a USM, the sponsor will take appropriate action to ensure the safety and welfare of the subjects. These actions may include but are not limited to a change in study procedures or study treatment, halting further enrollment in the study, or stopping the study in its entirety. The sponsor or sponsor's designee will notify CA and cEC within the timelines required per current local regulations, and will inform the investigators as required. When required, investigators must notify their IRB/IEC within timelines set by regional regulations.

5.6 Test Drug Concentration

Plasma concentrations of gilteritinib will be evaluated for the escalation (phase 1) and expansion (phase 2) phases as outlined in Schedule of Assessments [Table 1].

For phase 1 and phase 2, pharmacokinetic samples will be collected in cycle 1 and cycle 2 at the following time points:

- Cycle 1 day 8 – Predose
- Cycle 1 day 15 (±2 days) – Predose
- Cycle 1 day 21 (±2 day) – Predose and 4 to 6 hours
- Cycle 2 day 15 (±2 days) – Predose

Predose samples should be collected within 1 hour prior to dosing. For each sample, 1.0 mL of blood will be collected and processed.

Plasma samples may also be used for metabolite profiling of gilteritinib. The reports for the metabolite profiling and identification will not be incorporated to the clinical study report.

Blood sampling, processing, storage and shipment instructions will be provided in the Lab manual. Samples will be shipped to and analyzed by a sponsor designated analytical laboratory.

Please refer to the Laboratory Manual for more detailed information on this topic.

5.7 Other Measurements, Assessments or Methods

5.7.1 Blood Sample for Banked Pharmacogenomic (PGx) Sample Analysis (Optional)

Pharmacogenomic (PGx) research may be conducted in the future to analyze or determine genes of relevance to clinical response, pharmacokinetics, and toxicity/safety issues. After enrollment (see Schedule of Assessments [Table 1]), a 3 mL sample of whole blood and buccal swab sample for possible retrospective PGx analysis will be collected from subjects who consent. Samples will be shipped to a sponsor designated banking contract research organization (CRO).

Details on sample collection, labeling, storage and shipment procedures will be provided in a separate laboratory manual.

See [Appendix 12.7, Retrospective PGx Sub-study] for further details on the banking procedures.

5.7.2 Exploratory Biomarker Analysis

Bone marrow samples:

Samples will be analyzed for MRD at the time points defined in the schedule of assessments [Table 1] and may be analyzed for MRD at other time points. Samples will be analyzed for FLT3 mutational status at screening/baseline and EOT_1 for phase 1 and phase 2, and may be analyzed for FLT3 mutational status at other time points. Samples may be analyzed for mutations in AML related genes and changes in proteins in relation to treatment effects at screening/baseline and EOT_1 for phase 1 and phase 2, and may be analyzed for mutations in AML related genes and changes in proteins in relation to treatment effects at other time points. Samples may be used for method development or validation of diagnostic assays related to study treatment.

Blood samples:

Samples will be analyzed for PIA and FLT3 ligand assay in relation to dose and clinical response. Samples may be analyzed for MRD at any time point. Samples may be analyzed for FLT3 mutational status at screening/baseline and EOT_1 for phase 1 and phase 2, and may be analyzed for FLT3 mutational status at other time points. Samples may be analyzed for mutations in AML related genes and changes in proteins in relation to treatment effects at screening/baseline and EOT_1 for phase 1 and phase 2, and may be analyzed for mutations in AML related genes and changes in protein expression in relation to treatment effects at other time points.

Bone marrow/blood sampling, processing, storage and shipment instructions will be provided in the Laboratory Manual. Samples will be shipped to and analyzed by a sponsor designated analytical laboratory. Samples will be stored for a period up to 15 years following study database hard lock. Please refer to the Laboratory Manual for more detailed information on this topic.

5.7.3 Clinical Outcome Assessment

A clinical outcome assessment (COA) using a 5-point Facial Hedonic scale will be used during the study period to report palatability and the acceptability of the study drug formulation.

The COA measure should be collected immediately after administration of the study drug on that visit day.

5.8 Total Amount of Blood

The estimated total blood volume to be collected for the entire phase of the study (phase 1 or phase 2) should not be more than 71.5 mL [Table 14].

Table 14 Estimated Total Volume of Blood Collected in Phase 1 or Phase 2

Parameter	Screening (mL)	Cycle 1 (mL)	Cycle 2 (mL)	Approximate total volume per parameter (mL)
Hematology	1[#]	6[#]	6[#]	13[#]
Chemistry	1.1[#]	6.6[#]	6.6[#]	14.3[#]
Thyroid Function	2.5[#]	2.5[#]	5[#]	10[#]
Coagulation	2[#]	0[#]	0[#]	2[#]
Pregnancy†	1.4[#]	1.4[#]	1.4[#]	4.2[#]
PK	0	4	1	5
FLT3 Ligand/PIA	0	8	6	14
Disease Assessment	2	2	2	6
PGx	0	3	0	3
Approximate total volume	10	33.5	28	71.5

FLT3: FMS-like tyrosine kinase 3; PGx: pharmacogenomics; PIA: plasma inhibitory activity; PK: pharmacokinetics

[#] Estimated volumes are provided since local laboratories will be used for these assessments and the volume may vary depending on the site institutional practice.

† Applicable for WOCBP or alternatively pregnancy dipstick can be used

The estimated total blood volume to be collected during each visit of the LTT phase should not be more than 8 mL.

6 DISCONTINUATION

6.1 Discontinuation of Individual Subject(s) From Study Treatment

A discontinuation from treatment is a subject who enrolled in the study and for whom study treatment is permanently discontinued for any reason. The reason for discontinuation from study treatment must be documented in the subject's medical records.

A subject must discontinue study treatment for any of the following reasons:

- Subject and/or parent(s) or legal guardian declines further study participation (i.e., withdrawal of consent)
- Subject develops an intolerable or unacceptable toxicity

- Investigator determines that continuation of the study treatment will be detrimental to the subject
- Subject has no response or progressive disease with no clinical benefit (see [Section 5.1.5]) as defined by the investigator
- Subject becomes pregnant
- Subject is noncompliant with the protocol based on the investigator or medical monitor assessment
- Subject is found to have significantly deviated from any of the inclusion or exclusion criteria after enrollment (subjects having clinical benefit [Section 5.1.5] may be kept in the study after discussion with the medical monitor)
- Subject receives any antileukemic therapy other than the assigned treatment, with the exception of hydroxyurea up to 5 g daily for up to 2 weeks, prophylactic intrathecal chemotherapy, cranial irradiation, localized radiation for palliation or leukapheresis.

All subjects who discontinue study treatment will remain in the study and must continue to be followed for protocol specific follow up procedures as outlined in Schedule of Assessments [Table 1]. The only exception to this is when the subject specifically withdraws consent for any further contact with him/her or persons previously authorized by the participant to provide this information. If a subject is discontinued from the study with an ongoing AE or an unresolved laboratory result that is significantly outside of the reference range, the investigator will attempt to provide follow-up until the condition stabilizes or no longer is clinically significant.

All subjects who discontinue study treatment are to be followed for up to 2 years after their 28-day Follow-up Visit, death or the final analysis whichever occurs first per Schedule of Assessments.

6.1.1 Lost to Follow-up

Every reasonable effort is to be made to contact any subject lost to follow-up during the course of the study to complete study-related assessments, record outstanding data, and retrieve study drug.

Following unsuccessful telephone contact, an effort to contact the subject by mail using a method that provides proof of receipt should be attempted. Contact via an alternate, preapproved contact is permissible if the subject is not reachable. Such efforts should be documented in the source documents.

6.2 Discontinuation of the Site

If an investigator intends to discontinue participation in the study, the investigator must immediately inform the sponsor (***Specific to Japan:*** and the head of the study site).

6.3 Discontinuation of the Study

The sponsor may terminate this study prematurely, either in its entirety or at any study site, for reasonable cause provided that written notice is submitted in advance of the intended termination. Advance notice is not required if the study is stopped due to safety concerns. If

the sponsor terminates the study for safety reasons, the sponsor will immediately notify the investigator and subsequently provide written instructions for study termination.

7 STATISTICAL METHODOLOGY

A statistical analysis plan (SAP) will be written to provide details of the analysis, along with specifications for tables, listings and figures to be produced. The SAP will be finalized before the database soft lock at the latest. Any changes from the analyses planned in SAP will be justified in the clinical study report (CSR).

In general, all data will be summarized with descriptive statistics (number of subjects, mean, SD, minimum, median and maximum) for continuous endpoints, and frequency and percentage for categorical endpoints, unless otherwise specified. Percentages by categories will be based on the number of subjects with no missing data, i.e., will add up to 100%.

Baseline will be defined as the last observation prior to first dose, unless otherwise specified.

7.1 Sample Size

7.1.1 Phase 1

Group 1:

Three subjects will be enrolled in a cohort at one of a series of doses of gilteritinib with a starting dose of 2 mg/kg/day according to the 3 + 3 design. Additional subjects will be accrued as needed at the determined RP2D of Group 1 to ensure that gilteritinib activity is assessable in at least 9 subjects.

The RP2D will be a safe dose of gilteritinib that demonstrates sufficient activity.

The number of subjects enrolled will depend on the dose levels evaluated, evaluation of biological activity, and the availability of time points for PIA assessments.

Groups 2 and 3:

Three subjects will be enrolled in a cohort at one of a series of doses of gilteritinib with a starting dose of 1 mg/kg/day according to the 3 + 3 design.

Additional subjects will be accrued as needed to ensure that gilteritinib activity is assessable in at least 9 subjects.

The RP2D will be a safe dose of gilteritinib that demonstrates sufficient activity.

The number of subjects enrolled will depend on the dose levels evaluated, evaluation of biological activity, and the availability of time points for PIA assessments.

7.1.2 Phase 2

The phase 2 portion of this study will be a single-arm, 2-stage, open-label design with a total of 52 response evaluable subjects. Subjects are response evaluable if (1) they are confirmed FLT3/ITD mutation positive; (2) they receive at least 1 dose of gilteritinib; and (3) they are progression/recurrence free during the first 2 cycles of FLAG + gilteritinib and have the

required bone marrow evaluations, or have died of disease progression during the first 2 cycles. Subjects who die during treatment will be counted as nonresponders for purposes of analysis.

In the available dataset (COG AAML 06P1, 1-BFM AML 2001-01, Costa dataset), there are 26 CRc responders out of 73 subjects (a CRc rate of about 35%). With a 1-sided Type 1 error rate of 2.5%, there will be about 80% power to detect a 30% CR rate (i.e., a 16% increase in CR rate from the null hypothesis value of 14%). The 52 response evaluable subjects will provide about 90% power to detect a 56% CRc rate (i.e., a 21% increase in CRc rate from the null hypothesis value of 35%), with 1-sided Type error rate of 5%. Operationally, 22 response evaluable subjects will be needed during the first stage, with 4 CR responders or 9 CRc responders after 1 or 2 cycles of therapy required to continue to a total of 52 response evaluable subjects. If there are less than 4 CR responders in stage 1, the enrollment will be stopped in the USA and the study will not proceed to stage 2 in the USA. Ultimately, 13 or more CR responders or 24 CRc responders will be required to meet the efficacy threshold of FLAG + gilteritinib. The sample size was calculated in East Version 6.4 for CR.

7.2 Analysis Sets

Detailed criteria for analysis sets will be laid out in Classification Specifications and the allocation of subjects to analysis sets will be determined prior to database hard-lock.

7.2.1 Full Analysis Set (FAS)

The full analysis set (FAS) will consist of all subjects who are enrolled and receive at least 1 dose of the treatment regimen. This will be the primary analysis set for efficacy analyses.

7.2.2 Safety Analysis Set (SAF)

The safety analysis set (SAF) consists of all subjects who took at least 1 dose of the treatment regimen and will be used for safety analyses.

For the statistical summary of the safety data, the SAF will be used.

Note that the SAF and FAS are the same in this study.

7.2.3 Pharmacokinetic Analysis Set (PKAS)

The pharmacokinetic analysis set (PKAS) consists of the administered population for which sufficient plasma concentration data is available to facilitate derivation of at least 1 pharmacokinetic parameter and for whom the time of dosing on the day of sampling is known. Additional subjects may be excluded from the PKAS at the discretion of the pharmacokineticist. Any formal definitions for exclusion of subjects or time-points from the PKAS will be documented in the in the Classification Specifications and determined the Classification Meeting.

7.2.4 Pharmacodynamic Analysis Set (PDAS)

The PDAS consists of a subset of the SAF for which sufficient pharmacodynamic measurements were collected. Inclusion of subjects in the PDAS with missing data or major protocol deviations will be considered on a case-by-case basis.

The PDAS will be used for all summaries and analyses of pharmacodynamic data.

7.2.5 Minimal Residual Disease (MRD) Analysis Set (MAS)

The MRD Analysis Set (MAS) will consist of all subjects who were enrolled and received at least 1 dose of treatment regimen and had at least 1 post-baseline sample with MRD data.

7.3 Demographics and Baseline Characteristics

Demographics and other baseline characteristics will be summarized by dose levels for the SAF. Descriptive statistics will include number of subjects, mean, standard deviation, minimum, median and maximum for continuous endpoints, and frequency and percentage for categorical endpoints.

7.3.1 Subject Disposition

The number and percentage of subjects who completed and discontinued treatment and reasons for treatment discontinuation will be presented for all enrolled subjects and for subjects in the SAF by dose level and overall. Similar tables for screening disposition, investigational period disposition and follow-up disposition will also be presented for all enrolled subjects by dose level and overall. All disposition details and dates of first and last evaluations for each subject will be listed.

7.3.2 Previous and Concomitant Medications

All previous and concomitant medications will be presented in a listing.

7.3.3 Medical History

Medical history for each subject will be presented in a listing.

7.3.4 Treatment Compliance

Treatment compliance is defined as the total number of study drug actually taken by the subject divided by the number of study drug expected to be taken during the study multiplied by 100. Descriptive statistics for study drug compliance will be presented by dose level for the entire study period for the SAF.

7.3.5 Extent of Exposure

Exposure to treatment, measured by the duration of treatment in number of days will be summarized by dose level on SAF. Duration of exposure to a study drug is defined as: (the last date that subject took study drug – the first dose date + 1). Any duration of dose interruptions will be excluded from the exposure.

The following will be tabulated:

- the total dose administered
- number of intra subject dose reductions
- number and proportion of subjects with dose reductions

7.4 Analysis of Efficacy

Efficacy analysis will be conducted on the FAS. The interpretation of results from statistical tests will be based on the FAS.

7.4.1 Analysis of Primary Endpoint

The CR rate and the CRc (CR + CRp + CRi) rate will be summarized using descriptive statistics with 90% and 95% confidence intervals.

Hypothesis test for Phase 2 CR rate:

The null hypothesis H0 for CR is that the true CR rate p0 is ≤ 0.14, and the alternative hypothesis H1 is that the true CR rate p1 is ≥ 0.30.

H0: $p0 \leq 0.14$

H1: $p1 \geq 0.30$

Hypothesis test for Phase 2 CRc rate:

The null hypothesis H0 for CRc is that the true CRc rate p0 is ≤ 0.35, and the alternative hypothesis H1 is that the true CRc rate p1 is ≥ 0.56.

H0: $p0 \leq 0.35$

H1: $p1 \geq 0.56$

7.4.2 Analysis of Secondary Endpoints

MRD will be assessed in relation to the following efficacy variables:

- CR rate
- CRc rate
- Overall survival (OS)

MRD negative status will be defined as FLT3-ITD signal ratio $\leq 10^{-4}$. Other FLT3-ITD signal ratio cut points may be assessed.

The time of OS and EFS will be summarized using descriptive statistics. The survival curve and median for time-to-event variables and EFS and OS rates at 6 months/1 year/2 years will be estimated using the Kaplan-Meier method and will be reported along with the corresponding 95% confidence interval.

Acceptability and palatability of the formulation will be summarized. The analysis method will be detailed in the SAP of the study.

7.4.3 Analysis of Exploratory Endpoints

FLT3 mutation status and the mutational status of other AML-related genes at screening/baseline, EOT (for patients who relapse) will be assessed in relation to clinical efficacy.

Changes in FLT3 ligand levels as compared to baseline sampling will be summarized at each time point by dose levels.

Plasma inhibitory assay as compared to baseline sampling will be summarized at each time point by dose levels.

Further analyses may be performed to explore relationships between mutational status and changes in proteins in relation to pharmacodynamic parameters and clinical response.

7.5 Analysis of Safety

Safety analyses will consist of data summaries of AEs, DLTs, and other safety parameters on the SAF.

7.5.1 Adverse Events

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA).

Treatment Emergent Adverse Event (TEAE) is defined as an adverse event observed after starting administration of the study treatment and 30 days after the last dose of study treatment. The number and percentage of subjects with treatment-emergent AEs, SAEs, AEs leading to withdrawal of treatment, and AEs related to study drug will be summarized by system organ class, preferred term and treatment group. The number and percentage of AEs by severity will also be summarized. All AEs will be listed.

A study drug-related TEAE is defined as any TEAE with a causal relationship of YES by the investigator.

AEs will be coded using MedDRA. An AE with onset at any time from first dosing until last scheduled procedure will be classified as treatment-emergent for inclusion in the summary tabulations.

The number and percent of subjects experiencing 1 or more AE(s) will be summarized by phase of the study and dose levels. The relationship to study drug and severity of AE will also be summarized. AEs will be coded to system organ class and preferred term using MedDRA terminology and will be graded by the investigator using the NCI-CTCAE severity grade (version 5.0).

7.5.2 Laboratory Assessments

Laboratory parameters will be summarized by phase of the study, dose level and visit using descriptive statistics for shifts in change from baseline and will be presented in listings of clinically significant abnormalities.

For quantitative laboratory measurements descriptive statistics will be used to summarize results and change from baseline for subjects in the SAF by phase of the study, dose level and

visit. Shifts relative to normal ranges from baseline to each time point during treatment period in lab tests will also be tabulated. Laboratory data will be displayed in listings.

7.5.3 Vital Signs

Descriptive statistics will be used to summarize vital sign results and changes from baseline for subjects in the SAF by phase of the study and dose level and visit.

7.5.4 Physical Examination

Physical examination will be listed by phase of the study and dose level using descriptive statistics.

All clinically significant abnormal findings will be recorded as medical history or AEs and graded using NCI-CTCAE guidelines.

7.5.5 12-lead Electrocardiograms

The 12-lead ECG results will be summarized by phase of the study, dose levels, visits and time points using descriptive statistics.

All ECG interpretations will be displayed in listings.

7.5.6 Concentration-Response Relationship Analysis

An exploratory analysis of gilteritinib concentration and select pharmacodynamic and clinical response endpoints may be evaluated.

7.6 Analysis of Pharmacokinetics

7.6.1 Estimation of Pharmacokinetic Parameters

Population pharmacokinetic analysis will be performed on the data to characterize gilteritinib pharmacokinetics in pediatric patients with R/R AML. Details of model development, validation and simulations will be reported separately.

7.6.2 Statistical Analysis of Pharmacokinetic Parameters

Gilteritinib plasma concentrations will be summarized by study phase, dose levels, visit and sample collection window using descriptive statistics, including number of subjects, mean, standard deviation, minimum, median, maximum, geometric mean, and coefficient of variation (CV) of the mean and geometric mean. Time-course of drug concentrations will be plotted as appropriate. If warranted, plasma concentrations for active metabolites will also be summarized in a similar manner.

Gilteritinib pharmacokinetic parameters (e.g., CL/F, V_d/F) will be estimated using population pharmacokinetic analysis. Simulation will be performed to predict pharmacokinetic parameters such as C_{max}, t_{max} and AUC. Pharmacokinetic parameters will be summarized by phase of the study and dose levels using descriptive statistics, including number of subjects, mean, standard deviation, minimum, median, maximum, geometric mean, and CV of the mean and geometric mean. If warranted, pharmacokinetic parameters for active metabolites will also be summarized in a similar manner.

7.7 Analysis of Pharmacodynamics

A PIA assay will be employed to determine FLT3 inhibition relative to baseline FLT3 levels. Data will be summarized by phase of the study and dose levels using descriptive statistics, including number of subjects, mean, standard deviation, minimum, median, maximum, geometric mean, and CV of the mean and geometric mean.

7.8 Analysis of Biomarkers

Associations between biomarkers [Section 5.7.2 Exploratory Biomarker Analysis] and clinical results (efficacy, safety, or, pharmacodynamic) may be performed on subjects in the SAF who have the necessary baseline and on-study measurements to provide interpretable results for specific parameters of interest. Biomarkers may be summarized graphically or descriptively as they relate to clinical measures, as applicable. Summary statistics may be tabulated. Additional post-hoc analyses not specified in the protocol, such as alternative modeling approaches, may be conducted. All analyses described in this section are based on availability of the data.

7.9 Palatability and Acceptability Assessment

Scores from the COA will be summarized by phase of the study and dose level and visit.

7.10 Major Protocol Deviations and Other Analyses

Major protocol deviations as defined in [Section 8.3 Major Protocol Deviations] will be summarized for all enrolled subjects by dose level and total as well as by site, and will be provided in a data listing by site and subject.

The major protocol deviation criteria will be uniquely identified in the summary table and listing. The unique identifiers will be as follows:

PD1 - Entered into the study even though they did not satisfy entry criteria,

PD2 - Developed withdrawal criteria during the study and was not withdrawn,

PD3 - Received wrong treatment or incorrect dose,

PD4 - Received excluded concomitant treatment.

7.11 Interim Analysis (and Early Discontinuation of the Clinical Study)

The phase-2 portion of this study will be a 2-stage design [see Section 7.1.2 Phase 2]. No other formal interim analysis is planned.

7.12 Handling of Missing Data, Outliers, Visit Windows, and Other Information

Imputation methods for missing data, if applicable, and the definitions for windows to be used for analyses by visit will be outlined in the SAP.

8 OPERATIONAL CONSIDERATIONS

8.1 Data Collection

The investigator or site designee will enter data collected using an Electronic Data Capture system. In the interest of collecting data in the most efficient manner, the investigator or site designee should record data (including laboratory values, if applicable) in the eCRF within 5 days after the subject visit.

The investigator or site designee is responsible to ensure that all data in the eCRFs and queries are accurate and complete and that all entries are verifiable with source documents. These documents should be appropriately maintained by the site. The monitor should verify the data in the eCRFs with source documents and confirm that there are no inconsistencies between them.

Laboratory tests are performed at local and central laboratory; depending of the assessment evaluated. Central laboratory data will be transferred electronically to the sponsor or designee at predefined intervals during the study. The laboratory will provide the sponsor or designee with a complete and clean copy of the data.

ECG results are performed at a central ECG reading laboratory. Central ECG read data will be transferred electronically to the sponsor or designee at predefined intervals during the study. The central ECG laboratory will provide the sponsor or designee with a complete and clean copy of the data.

All procedures conducted under the protocol must be documented.

The investigator or designee will be responsible for eCRF completion and that all data and queries are accurate, complete and are verifiable with the source. The source should be appropriately maintained by the clinical unit.

Electronic data sources and any supporting documents should be available for review/retrieval by the sponsor/designee at any given time.

8.2 Screen Failures

For screen failures, the demographic data, reason for failing, informed consent, inclusion and exclusion criteria and AEs will be collected in the eCRF.

8.3 Major Protocol Deviations

A major protocol deviation is generally an unplanned excursion from the protocol that is not implemented or intended as a systematic change. The investigator is responsible for ensuring the study is conducted in accordance with the procedures and evaluations described in this protocol and must protect the rights, safety, and welfare of subjects. The investigator should not implement any deviation from, or changes of, the protocol, unless it is necessary to eliminate an immediate hazard to study subjects.

A protocol waiver is a documented prospective approval of a request from an investigator to deviate from the protocol. Protocol waivers are strictly prohibited. Refer to [Section 7.10 Major Protocol Deviations and Other Analyses] for the major protocol deviation criteria.

When a major deviation from the protocol is identified for an individual subject, the investigator or designee must ensure the sponsor is notified. The sponsor will follow-up with the investigator, as applicable, to assess the deviation and the possible impact to the safety, efficacy and/or pharmacokinetic parameters of the subject to determine subject continuation in the study.

If a major deviation impacts the safety of a subject, the investigator must contact the sponsor immediately.

The investigator will also assure that deviations meeting IRB/IEC and applicable regulatory authorities' criteria are documented and communicated appropriately. All documentation and communications to the IRB/IEC and applicable regulatory authorities will be provided to the sponsor and maintained within the trial master file.

9 END OF TRIAL

Study completion is defined as the conclusion of data collection for the defined study endpoints. The study may be closed within a participating country per local regulations once the study has completed and if all subjects enrolled in the country are no longer receiving study treatment and have completed all the protocol required visits/assessments or have discontinued from the study for any reason.

The end of the study is defined as the last visit or scheduled procedure shown in the Schedule of Assessments (including phase 1, phase 2 and LTT) for the last participant in the study.

10 STUDY ORGANIZATION

10.1 Dose Escalation Committee (DEC)

A DEC comprising of the sponsor, principal investigators and, if appropriate, expert consultants will be setup for this study. The DEC will be responsible for the review of safety data at specified time points in phase 1 in order to provide an assessment of whether dose escalation or de-escalation between the dose levels and/or to determine when maximum tolerated dose and/or RP2D has been reached in a given dose level. At each meeting, individual subject data will be reviewed for dose escalation or de-escalation decisions. Additional details regarding responsibilities and membership requirements will be included in the Subject Enrollment and Dose Escalation Plan.

The DEC will also be responsible for the review of subject safety data enrolled in the phase 2 portion of the study, enrollment rates, response rates and event (death) rates during periodic review. The DEC may recommend terminating enrollment for subjects from investigational sites based on safety evaluation.

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12 APPENDICES

12.1 Ethical, Regulatory, and Study Oversight Considerations

12.1.1 Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, ICH guidelines, applicable regulations and guidelines governing clinical study conduct and the ethical principles that have their origin in the Declaration of Helsinki.

12.1.2 Institutional Review Board (IRB)/Independent Ethics Committee (IEC)/Competent Authorities (CA)

GCP requires that the clinical protocol, any protocol amendments, the Investigator's Brochure, the informed consent and all other forms of subject information related to the study (e.g., advertisements used to recruit subjects) and any other necessary documents be reviewed by an IEC/IRB. The IEC/IRB will review the ethical, scientific and medical appropriateness of the study before it is conducted. IEC/IRB approval of the protocol, informed consent and subject information and/or advertising, as relevant, will be obtained prior to the authorization of drug shipment to a study site.

Any substantial amendments to the protocol will require IRB/IEC approval before implementation, except for changes necessary to eliminate an immediate hazard to subjects.

The investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

12.1.3 Protocol Amendment and/or Revision

Any changes to the study that arise after approval of the protocol must be documented as protocol amendments: substantial amendments and/or non-substantial amendments. Depending on the nature of the amendment, either IRB/IEC, CA approval or notification may be required. The changes will become effective only after the approval of the sponsor, the investigator, the regulatory authority, and the IRB/IEC (if applicable).

Amendments to this protocol must be signed by the sponsor and the investigator. Written verification of IRB/IEC approval will be obtained before any amendment is implemented. Modifications to the protocol that are administrative in nature do not require IRB/IEC approval, but will be submitted to the IRB/IEC for their information, if required by local regulations.

If there are changes to the informed consent, written verification of IRB/IEC approval must be forwarded to the sponsor. An approved copy of the new informed consent must also be forwarded to the sponsor.

12.1.4 Financial Disclosure (Optional)

Investigators and sub-investigators will provide the sponsor with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

12.1.5 Informed Consent of Subjects

12.1.5.1 Subject Information and Consent

The investigator or his/her representative will explain the nature of the study to the subject and/or parent(s) or legal guardian, and answer all questions regarding this study. Prior to any study-related screening procedures being performed on the subject, the IC statement/assent will be reviewed and signed (***Unique to Japan***: place a personal seal) and dated by the subject and/or parent(s) or legal guardian, the person who administered the IC/assent and any other signatories according to local requirements. A copy of the signed (***Unique to Japan***: or sealed) IC/assent form will be given to the subject and/or parent(s) or legal guardian and the original will be placed in the subject's medical record. An entry must also be made in the subject's dated source documents to confirm that IC/assent was obtained prior to any study-related procedures and that the subject and/or parent(s) or legal guardian received a signed copy.

The signed consent/assent forms will be retained by the investigator and made available (for review only) to the study monitor, auditor(s), regulatory authorities and other applicable individuals upon request.

12.1.5.2 Supply of New and Important Information Influencing the Subject's Consent and Revision of the Written Information

1. The investigator or his/her representative will immediately inform the subject and/or parent(s) or legal guardian orally whenever new information becomes available that may be relevant to the subject's consent or may influence the subject's willingness to continue to participate in the study (e.g., report of serious drug adverse drug reaction). The communication must be documented in the subject's medical records and whether the subject and/or parent(s) or legal guardian is willing to remain in the study or not must be confirmed and documented.
2. The investigator must update their ICF and submit it for approval to the IRB/IEC. The investigator or his/her representative must obtain written informed consent from the subject and/or parent(s) or legal guardian on all updated ICFs throughout their participation in the study. The investigator or his/her designee must re-consent subjects with the updated ICF/assent even if relevant information was provided orally. The investigator or his/her representative who obtained the written informed consent/assent

and the subject and/or parent(s) or legal guardian should sign and date the ICF/assent (***Specific to Japan:*** place a personal seal). A copy of the signed ***Specific to Japan:*** or sealed) ICF/assent will be given to the subject and/or parent(s) or legal guardian and the original will be placed in the subject's medical record. An entry must be made in the subject's records documenting the re-consent process.

12.1.6 Source Documents

Source data must be available at the site to document the existence of the study subjects and to substantiate the integrity of study data collected. Source data must include the original documents relating to the study, as well as the medical treatment and medical history of the subject.

The investigator is responsible for ensuring the source data are attributable, legible, contemporaneous, original, accurate and complete whether the data are hand-written on paper or entered electronically. If source data are created (first entered), modified, maintained, achieved, retrieved or transmitted electronically via computerized systems (and/or other kind of electric devices) as part of regulated clinical study activities, such systems must be compliant with all applicable laws and regulations governing use of electronic records and/or electronic signatures. Such systems may include, but are not limited to, electronic medical/health records, protocol related assessments, adverse event tracking, and/or drug accountability.

Paper records from electronic systems used in place of electronic format must be certified copies. A certified copy must be an exact copy and must have all the same attributes and information as the original. Certified copies must include signature and date of the individual completing the certification. Certified copies must be a complete and chronological set of study records (including notes, attachments, and audit trail information (if applicable). All printed records must be kept in the subject file and available for archive.

12.1.7 Record Retention

The investigator will archive all study data (e.g., subject identification code list, source data, CRFs, and investigator's file) and relevant correspondence. These documents are to be kept on file for the appropriate term determined by local regulation (for USA sites, 2 years after approval of the NDA or discontinuation of the IND). The sponsor will notify the site/investigator if the NDA/MAA/J-NDA is approved or if the IND/IMPD/CHIKEN TODOKE is discontinued. The investigator agrees to obtain the sponsor's agreement prior to disposal, moving, or transferring of any study-related records. The sponsor will archive and retain all documents pertaining to the study according to local regulations.

Data generated by the methods described in the protocol will be recorded in the subjects' medical records and/or study progress notes.

The records to be retained at the study sites are the ones listed as essential documents in GCP. These records shall be retained by the head of the study site or the record keeper designated by the head until notice issued by the sponsor on completion of the retention

period is received. These documents are also subject to direct access and should be provided upon request from the sponsor or regulatory authorities.

The head of the study site will retain the essential documents that should be stored at the study site in an appropriate manner according to the rules of the study site concerned until the date defined in 1 or 2 below, whichever comes later.

1. Approval date of marketing of the test drug (if development of the drug is stopped, until 3 years after the decision to discontinue development is notified)
2. Until 3 years after discontinuation or termination of the study.

Specific to Japan:

The following are the major documents to be retained at the study site.

a) Source documents (clinical data, documents and records for preparing the eCRF) hospital records, medical records, test records, memoranda, subject diary or checklists for evaluation, administration records, data recorded by automatic measuring instruments, reproductions or transcripts verified as precise copies, microfiche, negative films, microfilms/magnetic media, X-ray films, subject files and study-related records kept at either a pharmacy, a laboratory, or medical technical office, as well as subject registration forms, laboratory test slips including central measurement, worksheets specified by the sponsor, records of clinical coordinators and records related to the study selected from those verified in other departments or hospitals.
b) Study contracts, written ICFs, written information and other documents or their copies prepared by the study personnel. A letter of request for study (including a request for continuation/amendment), letter of request for review, notice of study contract, study contract, notification of discontinuation or completion of clinical study, written information for informed consent (including revisions), signed and dated written informed consent (including revisions), curriculum vitae of investigators, list of subinvestigators, list of signatures and print of seals (copy) and eCRF (copy), etc.
c) The protocol, documents obtained from the IRB related to the adequacy of conducting the study by the head of the study sites (Article 32-1, MHW Ordinance No. 28), documents obtained from the IRB related to the adequacy of conducting a study whose period exceeds 1 year or the adequacy of continuously conducting the study from which information on adverse drug reactions is obtained, and other documents obtained. A finalized protocol (including revisions), finalized Investigator's Brochure (including revisions), operational procedures for the investigator, materials and information supplied by the sponsor (e.g., AE report), matters reported by the investigator (revisions of the protocol, AE reports, etc.), operational procedures for the IRB, the list of names of the IRB members, materials for IRB review (including continuous deliberation), IRB review records (including continuous deliberation) and the review result report of the IRB (including continuous deliberation), etc.

d) Records of control for study drug and other duties related to the study. Procedure for controlling the study drug, drug inventory and accountability record, vouchers for the receipt and return of the study drug, and the prescriptions for concomitant medications.

12.1.8 Subject Confidentiality and Privacy/Data Protection

Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited unless otherwise the subject provides written consent or approval. Additional medical information may be given only after approval of the subject to the investigator or to other appropriate medical personnel responsible for the subject's well-being.

The sponsor shall not disclose any confidential information on subjects obtained during the performance of their duties in the clinical study without justifiable reasons.

Even though any individuals involved in the study, including the study monitors and auditors, may get to know matters related to subject's privacy due to direct access to source documents, or from other sources, they may not disclose the content to third parties.

The sponsor affirms the subject's right to protection against invasion of privacy. Only a subject identification number will identify subject data retrieved by the sponsor. However, the sponsor requires the investigator to permit the sponsor, sponsor's representative(s), the IRB/IEC and when necessary, representatives of the regulatory health authorities to review and/or to copy any medical records relevant to the study.

The sponsor agrees to comply and process personal data in accordance with all applicable privacy laws and regulations, including, without limitation, the Personal Information Protection Law in Japan and Privacy laws in the USA. If the services will involve the collection or processing of personal data (as defined by applicable data protection legislation) within the European Economic Area (EEA), then sponsor shall serve as the controller of such data, as defined by the European Union (EU) Data Protection Directive (DPD), and Investigator and/or third party shall act only under the instructions of the sponsor in regard to personal data. If sponsor is not based in the EEA, the sponsor must appoint a third party to act as its local data protection representative or arrange for a co-controller established in the EU for data protection purposes in order to comply with the DPD.

12.1.9 Arrangement for Use of Information and Publication of the Clinical Study

Information concerning the study drug, patent applications, processes, unpublished scientific data, the Investigator's Brochure and other pertinent information is confidential and remains the property of the sponsor. Details should be disclosed only to the persons involved in the approval or conduct of the study. The investigator may use this information for the purpose of the study only. It is understood by the investigator that the sponsor will use the information obtained during the clinical study in connection with the development of the drug and therefore may disclose it as required to other clinical investigators or to regulatory agencies. In order to allow for the use of the information derived from this clinical study, the

investigator understands that he/she has an obligation to provide the sponsor with all data obtained during the study.

Publication of the study results is discussed in the clinical study agreement.

12.1.10 Insurance of Subjects and Others (UNIQUE to JP/Studies enrolling subjects in EU)

The sponsor has covered this study by means of an insurance of the study according to national requirements. The name and address of the relevant insurance company, the certificate of insurance, the policy number and the sum insured are provided in the investigator's file.

Specific to Japan:

If a subject suffers any study-related injury, the sponsor will compensate the subject appropriately according to the severity and duration of the damage. However, if the injury was caused intentionally or was due to gross negligence by the study site, the sponsor will consult with the study site about handling the injury, based on the agreed study contract. Compensation for the study-related injury is provided by the following procedures:

1. If a subject incurs an injury as a result of participation in the study, the study site should provide medical treatment and other necessary measures. The sponsor should be notified of the injury.
2. When the subject claims compensation from the study site for the above study-related injury, or such compensation may be claimed, the study site should immediately communicate the fact to the sponsor. Both parties should work together towards a compensation settlement.
3. The sponsor shall pay compensation or indemnification and bear expenses necessary for the settlement as provided in the study contract.
4. The sponsor shall make an arrangement for insurance and take measures necessary to ensure the compensation or indemnification mentioned above.

12.1.11 Signatory Investigator for Clinical Study Report

ICH E3 guidelines recommend and EU Directive 2001/83/EC requires that a final study report which forms part of a marketing authorization application be signed by the representative for the coordinating investigator(s) or the principal investigator(s). The representative for the coordinating investigator (s) or the principal investigator(s) will have the responsibility to review the final study results to confirm to the best of his/her knowledge it accurately describes the conduct and results of the study. The representative for coordinating investigator(s) or the principal investigator(s) will be selected from the participating investigators by the sponsor prior to database lock.

12.2 Procedure for Clinical Study Quality Control

12.2.1 Clinical Study Monitoring

The sponsor or delegated CRO is responsible for monitoring the clinical study to ensure that subject's human rights, safety, and well-being are protected, that the study is properly conducted in adherence to the current protocol and GCP, and study data reported by the investigator/sub-investigator are accurate and complete and that they are verifiable with study-related records such as source documents. The sponsor is responsible for assigning study monitor(s) to this study for proper monitoring. They will monitor the study in accordance with planned monitoring procedures.

12.2.2 Direct Access to Source Data/Documents

The investigator and the study site must accept monitoring and auditing by the sponsor or delegated CRO, as well as inspections from the IRB/IEC and relevant regulatory authorities. In these instances, they must provide all study-related records including source documents when they are requested by the sponsor monitors and auditors, the IRB/IEC, or regulatory authorities. The confidentiality of the subject's identities shall be well protected consistent with local and national regulations when the source documents are subject to direct access.

12.2.3 Data Management

Data Management will be coordinated by the Data Science or designee of the sponsor in accordance with the SOPs for data management. All study-specific processes and definitions will be documented by Data Management. eCRF completion will be described in the eCRF instructions. Coding of medical terms and medications will be performed using MedDRA and World Health Organization (WHO) Drug Dictionary, respectively.

12.2.4 Quality Assurance

The sponsor is implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that studies are conducted and data are generated, documented, recorded, and reported in compliance with the protocol, GCP, and applicable regulatory requirement(s). Where applicable, the quality assurance and quality control systems and written SOPs of the CRO will be applied.

The sponsor or sponsor's designee may arrange to audit the clinical study at any or all investigational sites and facilities. The audit may include on-site review of regulatory documents, case report forms, and source documents. Direct access to these documents will be required by the auditors.

12.3 Contraception Requirements

WOCBP participants who choose complete abstinence must continue to have pregnancy tests, as specified in the Schedule of Assessments.

WOMEN OF CHILDBEARING POTENTIAL (WOCBP) DEFINITIONS AND METHODS OF CONTRACEPTION DEFINITIONS

A female is considered fertile (i.e., WOCBP) following menarche and until becoming postmenopausal unless permanently sterile.

Women in the following categories are not considered WOCBP:

- Premenarchal
- Premenopausal female with 1 of the following:
  a) Documented hysterectomy
  b) Documented bilateral salpingectomy
  c) Documented bilateral oophorectomy
- Postmenopausal

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

In case the time of last regular menstrual bleeding cannot be clearly determined, confirmation with repeated FSH measurements of at least > 40 IU/L (or higher per local institutional guidelines) is required.

Females on hormone replacement therapy (HRT) will be required to use 1 of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study.

Documentation of any of these categories can come from the site personnel's review of the female subject's medical records, medical examination, or medical history interview.

CONTRACEPTION GUIDANCE FOR FEMALE PARTICIPANTS OF CHILD BEARING POTENTIAL

Female subjects of childbearing potential are eligible for participation in the study if they agree to use 1 of the highly effective methods of contraception listed below from the time of signing the ICF and until the end of relevant systemic exposure, defined as 180 days after the final study drug administration[a]

Highly effective methods of contraception (failure rate of < 1% per year when used consistently and correctly)[b]:

- **Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation**
  a) Oral
  b) Intravaginal
  c) Transdermal
- **Progestogen-only hormonal contraception associated with inhibition of ovulation**
  a) Oral
  b) Injectable
  c) Implantable
- **Other combined (estrogen- and progesterone-containing) methods**
  a) Vaginal ring
  b) Injectable
  c) Implantable
  d) Intrauterine hormone-releasing system or intrauterine device
- **Bilateral tubal occlusion**
- **Vasectomized partner**

  A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.

- **Sexual abstinence**

  Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the test product. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the subject. It is not necessary to use any other method of contraception when complete abstinence is elected.

  Periodic abstinence (calendar, ovulation, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhea method are not acceptable methods of contraception.

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

[b] Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for subjects participating in clinical studies.

CONTRACEPTION GUIDANCE FOR MALE PARTICIPANTS WITH PARTNER(S) OF CHILD BEARING POTENTIAL.

Male subjects with female partners of childbearing potential are eligible to participate if they agree to the following during treatment and until the end of relevant systemic exposure defined as 180 days after final drug administration[a]

- Inform any and all partner(s) of their participation in a clinical drug study and the need to comply with contraception instructions as directed by the investigator
- Use a condom
- Female partners of male subjects who have not undergone a vasectomy with the absence of sperm confirmed or a bilateral orchiectomy should consider use of effective methods of contraception

[a] Local laws and regulations may require use of alternative and/or additional contraception methods.

12.4 List of Excluded Concomitant Medications

Strong CYP3A Inhibitors

Treatment with concomitant drugs that are strong inhibitors of CYP3A should be **avoided** with the exception of antibiotics, antifungals and antivirals that are used as standard of care to prevent or treat infections.

The following list describes medications and foods that are common strong inhibitors of CYP3A. This list should not be considered all inclusive. Consult individual drug labels for specific information on a compound's propensity to inhibit CYP3A.

Drug Type	Generic Drug Name
Human Immunodeficiency Virus Protease Inhibitors	Indinavir Nelfinavir Lopinavir/ritonavir Ritonavir Saquinavir
Food/Juice	Grapefruit juice
Others	Boceprevir Clarithromycin Conivaptan Itraconazole Ketoconazole Nefazodone Posaconazole Telaprevir Telithromycin Voriconazole

CYP: cytochrome P450.

Source: University of Washington Metabolism and Transport Drug Interaction Database (2021): http://www.druginteractioninfo.org/

Strong CYP3A/P-gp Inducers

Treatment with concomitant drugs that are strong inducers of CYP3A/P-gp are **prohibited.**

The following list describes medications and foods that are common strong inducers of CYP3A/P-gp. This list should not be considered all inclusive. Consult individual drug labels for specific information on a compound's propensity to induce CYP3A/P-gp.

Drug Type	Generic Drug Name
Antiepileptic, Anticonvulsant	Carbamazepine Phenytoin
Antibiotic	Rifampicin
Food/Juice Supplement	St. John's Wort

CYP: cytochrome P450.

Source: University of Washington Metabolism and Transport Drug Interaction Database (2021): http://www.druginteractioninfo.org/

Drugs Targeting Serotonin Receptors

Treatment with concomitant drugs that target serotonin $5HT_{2B}$ receptor are to be **avoided** with the exception of drugs that are considered absolutely essential for the care of the subject.

The following list describes medications that target serotonin receptors. This list should not be considered all inclusive. Consult individual drug labels for specific information on whether a compound targets serotonin receptors.

Drug Type	Generic Drug Name
Affinity or function to $5HT_{2B}$ receptor	Eletriptan Hydrobromide

$5HT_{2B}$: 5-hydroxytryptamine 2B

P-gp Inhibitors

Treatment with strong inhibitors of P-gp are to be avoided with the exception of drugs that are considered absolutely essential for the care of the subject.

The following list describes medications and foods that are common inhibitors of P-gp. This list should not be considered all inclusive. Consult individual drug labels for specific information on a compound's propensity to inhibit P-gp.

Transporter	Gene	Inhibitor
P-gp	*ABCB1*	amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir and ritonavir, quercetin, quinidine, ranolazine, verapamil

P-gp: P-glycoprotein

Source: University of Washington Metabolism and Transport Drug Interaction Database (2021): http://www.druginteractioninfo.org/

Drugs Targeting Sigma (nonspecific) Receptor (sigma R)

Treatment with concomitant drugs that target non-sigma specific receptor are to be **avoided** with the exception of drugs that are considered absolutely essential for the care of the subject.

No list of drugs that target sigma nonspecific receptor is provided. Please consult individual drug labels for specific information on whether a compound targets sigma nonspecific receptors.

Drugs That May Prolong QT or QTc

For concomitant drugs that have the potential to prolong QT or QTc intervals, a cardiology consult should be obtained as medically indicated.

The following list describes drugs that are known to prolong QT or QTc. This list should not be considered all inclusive. Consult individual drug labels for specific information on whether a compound is known to prolong QT or QTc.

Drug Type	Generic Drug Name
Class IA antiarrhythmics	Quinidine Procainamide Disopyramide
Class IC antiarrhythmics	Flecainide Propafenone Moricizine
Class III antiarrhythmics	Amiodarone Sotalol Bretylium Ibutilide Dofetilide
Table continued on next page	

Drug Type	Generic Drug Name
Antipsychotics	Thioridazine Mesoridazine Chlorpromazine Prochlorperazine Trifluoperazine Fluphenazine Perphenazine Pimozide Risperidone Ziprasadone Haloperidol
Antimanics	Lithium
Tricyclic/tetracyclic antidepressants	Amitriptyline Desipramine Doxepin Dosulepin hydrochloride Imipramine Maprotiline
Selective serotonin and norepinephrine reuptake inhibitors (SSNRIs) antidepressants	Venlafaxine
Macrolide antibiotics	Azithromycin Erythromycin Clarithromycin Dirithromycin Roxithromycin Tulathromycin
Fluoroquinolone antibiotics	Moxifloxacin Gatifloxacin
Azole antifungals	Ketoconazole Fluconazole Itraconazole Posaconazole Voriconazole
Table continued on next page	

Drug Type	Generic Drug Name
Antimalarials	Amodiaquine Atovaquone Chloroquine Doxycycline Halofantrine Mefloquine Proguanil Primaquine Pyrimethamine Quinine Sulphadoxine
Antiprotozoals	Pentamidine
Antiemetics	Droperidol Dolasetron Granisetron Ondansetron
Antiestrogens	Tamoxifen
Immunosuppressants	Tacrolimus

12.5 Liver Safety Monitoring and Assessment

Any subject enrolled in a clinical study with active drug therapy and reveals an increase of serum aminotransferases (AT) to > 3 x ULN or bilirubin > 2 x ULN should undergo detailed testing for liver enzymes (including at least ALT, AST, ALP and TBL). Testing should be repeated within 72 hours of notification of the test results. For studies for which a central laboratory is used, alerts will be generated by the central laboratory regarding moderate and severe liver abnormality to inform the investigator and study team. Subjects should be asked if they have any symptoms suggestive of hepatobiliary dysfunction.

Definition of Liver Abnormalities

Confirmed abnormalities will be characterized as moderate and severe where ULN:

	ALT or AST		**Total Bilirubin**
Moderate	> 3 x ULN	or	> 2 x ULN
Severe	> 3 x ULN	and	> 2 x ULN

In addition, the subject should be considered to have severe hepatic abnormalities for any of the following:

- ALT or AST > 8 x ULN.
- ALT or AST > 5 x ULN for more than 2 weeks.
- ALT or AST > 3 x ULN and International Normalized Ratio (INR) > 1.5 (If INR testing is applicable/evaluated).
- ALT or AST > 3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%).

The investigator may determine that abnormal liver function results, other than as described above, may qualify as moderate or severe abnormalities and require additional monitoring and follow-up.

Follow-up Procedures

Confirmed moderate and severe abnormalities in hepatic functions should be thoroughly characterized by obtaining appropriate expert consultations, detailed pertinent history, physical examination and laboratory tests. The site staff is to complete the liver abnormality case report form (LA-CRF). Subjects with confirmed abnormal liver function testing should be followed as described below.

Confirmed moderately abnormal LFTs should be repeated 2 to 3 times weekly then weekly or less if abnormalities stabilize or the study drug has been discontinued and the subject is asymptomatic.

Severe hepatic liver function abnormalities as defined above, in the absence of another etiology, may be considered an important medical event and may be reported as a SAE. The sponsor should be contacted and informed of all subjects for whom severe hepatic liver function abnormalities possibly attributable to study drug are observed.

To further assess abnormal hepatic laboratory findings, the investigator is expected to:

- Obtain a more detailed history of symptoms and prior or concurrent diseases. Symptoms and new-onset diseases is to be recorded as "AEs" within the (e)CRF. Illnesses and conditions such as hypotensive events, and decompensated cardiac disease that may lead to secondary liver abnormalities should be noted. Nonalcoholic steatohepatitis is seen in obese hyperlipoproteinemic and/or diabetic patients, and may be associated with fluctuating AT levels. The investigator should ensure that the medical history form captures any illness that predates study enrollment that may be relevant in assessing hepatic function.
- Obtain a history of concomitant drug use (including nonprescription medication, complementary and alternative medications), alcohol use, recreational drug use and special diets. Medications, is to be entered in the (e)CRF. Information on alcohol, other substance use and diet should be entered on the LA-CRF or an appropriate document.
- Obtain a history of exposure to environmental chemical agents.
- Based on the subject's history, other testing may be appropriate including:
  a) Acute viral hepatitis (A, B, C, D, E or other infectious agents),
  b) Ultrasound or other imaging to assess biliary tract disease,
  c) Other laboratory tests including INR, direct bilirubin.
- Consider gastroenterology or hepatology consultations.
- Submit results for any additional testing and possible etiology on the LA-CRF or an appropriate document.

Study Treatment Discontinuation

In the absence of an explanation for increased LFTs, such as viral hepatitis, preexisting or acute liver disease, or exposure to other agents associated with liver injury, the subject may be discontinued from study treatment. The investigator may determine that it is not in the subject's best interest to continue study treatment. Discontinuation of study treatment should be considered if:

- ALT or AST > 8 x ULN.
- ALT or AST > 5 x ULN for more than 2 weeks.
- ALT or AST > 3 x ULN and TBL > 2 x ULN or INR > 1.5) (If INR testing is applicable/evaluated).
- ALT or AST > 3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash and/or eosinophilia (> 5%).

In addition, if close monitoring for a subject with moderate or severe hepatic laboratory tests is not possible, study treatment should be discontinued.

***Hy's Law Definition:**

Drug-induced jaundice caused by hepatocellular injury, without a significant obstructive component, has a high rate of bad outcomes, from 10 to 50% mortality (or transplant).

The 2 “requirements” for Hy’s Law are:

1. Evidence that a drug can cause hepatocellular-type injury, generally shown by an increase in transaminase elevations higher 3 x ULN (“2 x ULN elevations are too common in treated and untreated patients to be discriminating”).
2. Cases of increased bilirubin (at least 2 x ULN) with concurrent transaminase elevations at least 3 x ULN and no evidence of intra- or extra-hepatic bilirubin obstruction (elevated ALP) or Gilbert’s syndrome [Temple, 2006].

Briefly, Hy’s Law cases have the following three components:

a. The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (nonhepatotoxic) control drug or placebo.

b. Among study subjects showing such AT elevations, often with ATs much greater than 3 x ULN, 1 or more also show elevation of serum TBL to >2 x ULN, without initial findings of cholestasis (elevated serum ALP).

c. No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury [FDA guidance, July 2009].

References

Temple R. Hy’s law: Predicting Serious Hepatotoxicity. Pharmacoepidemiol Drug Saf. 2006 April;15(Suppl 4):241-3.

Guidance for Industry titled “Drug-Induced Liver Injury: Premarketing Clinical Evaluation” issued by FDA on July 2009.

12.6 Common Serious Adverse Events

The following is a list of SAEs that the sponsor considers to be associated with the disease state being studied. **The list does NOT change the reporting obligations or prevent the need to report an AE meeting the definition of an SAE as detailed in [Section 5.5.2 Definition of Serious Adverse Events]**. The purpose of this list is to alert you that some events reported as SAEs may not require expedited reporting to the regulatory authorities based on the classification of "common SAEs." Investigators are required to follow the requirements detailed in [Section 5.5.4.1, Reporting of Serious Adverse Events].

For IND safety reporting, single occurrences of the following events may be excluded from expedited reporting to the FDA. If aggregate analysis of these events indicate they occur more frequently with study drug, an expedited IND safety report may be submitted to the FDA.

Serious Adverse Events Caused by AML	Grades Usually Observed with AML
Hematologic AE	
Anemia	0 - 4
Bone marrow hypocellular	0 - 4
CD4 lymphocytes decreased	0 - 4
Disseminated intravascular coagulation	0 - 3
Leukocytosis	0 - 4
Lymphocyte count decreased	0 - 4
Lymphocyte count increased	0 - 4
Neutropenia	0 - 4
Neutrophil count decreased	0 - 4
Platelet count decreased	0 - 4
Purpura	0 - 3
Thrombocytopenia	0 - 4
White blood cell decreased	0 - 4
Infection-related AE	
Bacterial infection (regardless of organ-system involved or specific bacterial cause)	0 - 3
Chills	0 - 3
Cough	0 - 3
Fever	0 - 5
Flu-like symptoms	0 - 3
Fungal infections (regardless of organ-system involved or fungal cause)	0 - 3
Mucositis	0 - 4
Periodontal disease	0 - 3
Pneumonia	0 - 5
Sepsis/septicemia/bacteremia (all causes)	0 - 5
Sinusitis	0 - 4
Sore throat	0 - 3
Table continued on next page	

Serious Adverse Events Caused by AML	Grades Usually Observed with AML
Psychiatric and Nervous System Related AE	
Anxiety	0 - 2
Cognitive disturbance	0 - 3
Confusion	0 - 5
Depressed level of consciousness	0 - 5
Depression	0 - 3
Libido decreased	0 - 2
Meningismus	0 - 5
Seizure	0 - 5
Somnolence	0 - 5
Syncope	3
Other AE	
Activated partial thromboplastin time prolonged	0 - 2
Alanine aminotransferase increased	0 - 2
Alkaline phosphatase increased	0 - 2
Anorexia	0 - 2
Abdominal pain	0 - 2
Aspartate aminotransferase increased	0 - 2
Blood bilirubin increased	0 - 2
Bone and/or joint pain	0 - 2
Bruising	0 - 2
Bleeding/hemorrhage	0 - 5
Constipation	0 - 2
Diarrhea	0 - 3
Dyspnea	0 - 5
Fatigue	0 - 3
Febrile neutropenia	0 - 4
Flushing	0 - 2
Gamma-glutamyltransferase increased	0 - 1
GVHD-acute and chronic	0 - 2
Hypertrophied gums	0 - 1
Hyperuricemia	0 - 1
Hypokalemia	0 - 2
Hypotension	0 - 2
Hypoxia	0 - 3
INR increased	0 - 1
Lactate dehydrogenase increased	0 - 2
Malaise	0 - 2
Multiorgan failure	0 - 5
Nausea	0 - 2
Oral dysesthesia	0 - 2
Petichiae	0 - 2
Pruritus	0 - 3
Skin and subcutaneous tissue disorders	0 - 3
Transient ischemic attacks	0 - 2
Tumor lysis syndrome	3 - 5
Vasculitis	0 - 5
Vomiting	0 - 3
Weight loss	0 - 2

AE: adverse event; AML: acute myeloid leukemia; GVHD: graft-versus-host disease; INR: international normalization ratio

12.7 Pharmacogenomic (PGx) Analysis With Banked Sample (Optional)

INTRODUCTION

PGx research aims to provide information regarding how naturally occurring differences in a subject's gene and/or expression of genes based on genetic variation may impact what treatment options are best suited for the subject. Through investigation of PGx by technologies such as genotyping, gene sequencing, statistical genetics and Genome-Wide Association Studies, the relationship between gene profiles and a drug's kinetics, efficacy or toxicity may be better understood. As many diseases may be influenced by 1 or more genetic variations, PGx research may identify which genes are involved in determining the way a subject may or may not respond to a drug.

OBJECTIVES

The PGx research that may be conducted in the future with acquired blood samples is exploratory. The objective of this research will be to analyze or determine genes of relevance to clinical response, pharmacokinetics and/or toxicity/safety issues.

By analyzing genetic variations, it may be possible to predict an individual subject's response to treatment in terms of efficacy and/or toxicity.

SUBJECT PARTICIPATION

Subjects who have consented to participate in this study will participate in this PGx substudy. Subjects must provide written consent prior to providing any blood samples that may be used at a later time for PGx analysis.

SAMPLE COLLECTION AND STORAGE

Subjects who consent to participate in this sub-study will provide 1 tube of whole blood per Astellas' instructions. Each sample will be identified by the unique subject number. Samples will be shipped to a designated banking CRO as directed by Astellas.

PGx ANALYSIS

Details on the potential PGx analysis cannot be established yet. Astellas may initiate the PGx analysis if evidence suggests that genetic variants may be influencing the drug's kinetics, efficacy and/or safety.

DISPOSAL OF PGx SAMPLES/DATA

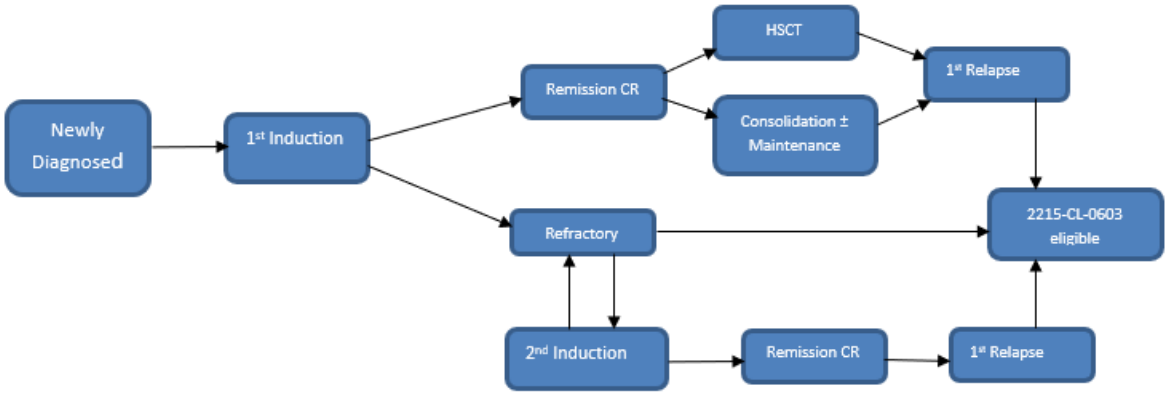
All PGx samples collected will be stored for a period of up to 15 years following study database hardlock. If there is no requirement for analysis, the whole blood sample will be destroyed after the planned storage period. The subject has the right to withdraw consent at any time. When a subject's withdraw notification is received, the PGx sample will be destroyed. The results of any PGx analysis conducted on a sample prior to its withdrawal will be retained at Astellas indefinitely unless otherwise specified by local regulation.

INFORMATION DISCLOSURE TO THE SUBJECTS

Exploratory PGx analysis may be conducted following the conclusion of the clinical study, if applicable. The results of the PGx analysis will not be provided to any investigators or subjects, nor can the results be requested at a later date. Any information that is obtained from the PGx analysis will be the property of Astellas.

12.8 Definitions of Line of Therapy and Tools to Determine Study Eligibility

Schematic representation of AML treatment and eligible path for study participation after treatment with 1 line of therapy.



Below are examples of the treatment paths that would qualify the patient to participate in the study:

- First induction → Refractory
- First induction → Refractory → Second Induction*→ Refractory
- First induction → Refractory → Second Induction*→ Remission→ Relapse
  (*can include different treatment from first induction)
- First induction → Remission → Consolidation/Maintenance with HSCT→ Relapse
- First induction → Consolidation/Maintenance without HSCT → Relapse

Please note: Induction with consolidation/maintenance followed by HSCT is considered as 1 line of therapy. HSCT by itself and hydroxyurea are not considered to be lines of therapy.

12.9 Clinical Study Continuity

INTRODUCTION

The purpose of this appendix is to provide acceptable alternate methods to assess safety and efficacy parameters, as appropriate, in the event the clinical study is interrupted at the country, state, site or participant level during the COVID-19 pandemic.

BENEFIT-RISK RATIONALE

Maintaining the safety of clinical study participants and delivering continuity of care in the clinical study setting is paramount during the COVID-19 pandemic. The site is expected to follow the protocol and associated Schedule of Assessments [Table 1 and Table 5] unless the site Principal Investigator (PI) discusses the need with the Astellas medical monitor to implement the alternate measures.

The approach outlined within this appendix defines which assessments are required to maintain a favorable benefit/risk to the participant, to maintain overall study integrity and to provide acceptable alternate methods to complete the study required assessments and procedures if study activities are unable to be performed as described in [Section 5 Treatments and Evaluation] due to the COVID-19 pandemic.

INFORMED CONSENT AND/OR ASSENT

Participants who need to follow any or all of the alternate measures outlined in this appendix will be required to provide informed consent, which explicitly informs them of the nature of and rationale for these changes and gain their agreement to continue participation in the study prior to the implementation of any of these changes. In the event the urgency of implementing the alternate measures does not allow for the participant to provide written consent prior to implementation, the principal investigator or designee will obtain oral agreement from the participant followed by written documentation as soon as is feasible. A separate addendum to the study informed consent will be provided to document the participant's consent of the changes, in the event the ICF/Assent Form does not contain the details.

PARTICIPANT PROCEDURES ASSESSMENT

Sites with participants who are currently enrolled into this clinical study may consider implementing the alternate methods outlined below if one or more of the following conditions are met due to the COVID-19 pandemic:

- Regional or local travel has been restricted, inclusive of mandatory shelter in place measures, which makes participant travel to/from the study site nearly impossible
- Site facilities have been closed for clinical study conduct.
- Site has been restricted to treating patients with conditions outside of the scope of the study.
- Site personnel have temporarily relocated the conduct of the study to a location that place a burden on the participant with respect to time and travel.

- Participant(s) have temporarily relocated from the current study site to an alternate study site to avoid placing a burden on the participant with respect to travel.
- Participant(s) have temporarily relocated from their home location and the new distances from the site would cause undue burden with respect to time and travel.
- Participant has risk factors for which traveling to the site poses an additional risk to the participant's health and safety.

Adherence to the original protocol as reflected in the Schedule of Assessments [Table 1 and Table 5] is expected, where plausible, in the case of the COVID-19 pandemic. The alternate measures as noted in below are only permissible in the event of the COVID-19 pandemic, and after discussing the need with the Astellas medical monitor to implement the alternate measures. This is to allow for continuity of receiving Investigational Medicinal Product (IMP) and maintaining critical safety and efficacy assessments for patients participating in the study at a time of the COVID-19 pandemic.

If one or more of the alternate measures noted below is implemented for a participant, the site should document in the participant's source document the justification for implementing the alternate measure and the actual alternate measures that were implemented, along with the corresponding time point(s).

ALTERNATIVE SCHEDULE OF ASSESSMENTS IN RESPONSE TO A CRISIS

No alternative measures are permissible for cycle 1 and cycle 2 of phase 1 or phase 2 as several assessments and dosing of FLAG and gilteritinib need to be performed on-site at the clinic.

The table below provides the alternative measures that are permissible for each assessment and the visit days on which those assessments are performed for the LTT and Survival Follow-up period. For detailed guidance on the conduct of the study assessments, please refer to [Table 1] and its footnotes and [Section 5] of the study protocol.

Table 15 Alternative Approaches/Measures Permissible for Protocol Assessments

Assessments	Alternate Approach(es)	LTT Cycle 1 & Subsequent Cycles	EOT_2/ Pre-HSCT Visit	28-day Follow-up	Survival Follow-up
	Cycle Day (Visit Window in days) →	**Day 1 (± 2)**	**(± 2)**	**(+ 7)**	**(± 7)**
Informed consent	Not Applicable				
Eligibility criteria	Not Applicable				
Medical and disease history	Not Applicable				
Vital Signs	Vitals are linked to whether patient can be dosed that day in clinic. For D1 of each cycle, if ASP2215 dosing is given in the clinic then vitals can be drawn in the clinic. If ASP2215 dosing is not performed in the clinic then the assessment performed at local facility is acceptable if results can be made available to investigative site. Most vital sign parameters may be assessed and captured via phone contact if a patient/parent/guardian has adequate monitoring instruments and is knowledgeable and able to obtain measurements.	X	X		
Karnofsky/Lansky performance status	For D1 of each cycle, if ASP2215 dosing is given in the clinic then the assessment can be drawn in the clinic. If ASP2215 dosing is not performed in the clinic then the assessment can be performed remotely (Virtual/Telephone) by the investigator is acceptable if results are captured appropriately in the site source documents.	X	X		
Physical examination	Physical exam is linked to whether patient can be dosed that day in clinic. Targeted exam would be acceptable. Weight is needed on D1 of each cycle when patient will be in clinic to determine the actual dose of gilteritinib to be taken for the cycle based on the weight of the participant. If ASP2215 dosing is not performed in the clinic then the weight and height measurement performed at local facility or patient home is acceptable if results can be made available to investigative site.	X	X		
Pregnancy test for WOCBP	For D1 of each cycle, if ASP2215 dosing is given in the clinic then labs can be drawn in the clinic. If ASP2215 dosing is not performed in the clinic then the assessment performed at local facility is acceptable if results can be made available to investigative site.	X	X		
Chest x-ray (or chest CT)	Not Applicable				

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Assessments	Alternate Approach(es)	LTT Cycle 1 & Subsequent Cycles	EOT_2/ Pre-HSCT Visit	28-day Follow-up	Survival Follow-up
	Cycle Day (Visit Window in days) →	**Day 1 (± 2)**	**(± 2)**	**(+ 7)**	**(± 7)**
12-lead ECG	For D1 of each cycle, if ASP2215 dosing is given in the clinic then the assessment can be performed in the clinic. If the site is not allowed to use sponsor supplied ECG machine but can use site ECG machine, then ECG in triplicates performed using site machine is acceptable as long as it is uploaded for central read. If ASP2215 dosing is not performed in the clinic then ECG in triplicates performed in local facility is acceptable if results can be made available to investigative site.	X	X		
Clinical laboratory tests (chemistry, hematology, coagulation, urinalysis)	For D1 of each cycle, if ASP2215 dosing is given in the clinic then the labs can be performed in the clinic. If ASP2215 dosing is not performed in the clinic then lab assessments performed at local facility is acceptable if results can be made available to investigative site.	X	X		
Thyroid function tests	For D1 of each cycle, if ASP2215 dosing is given in the clinic then the labs can be performed in the clinic. If ASP2215 dosing is not performed in the clinic then lab assessments performed at local facility is acceptable if results can be made available to investigative site.	X			
MUGA or ECHO	Not Applicable				
PK Sample Collection	Not Applicable				
PGx	Not Applicable				
Plasma Inhibitory Assay (PIA) & FLT3 Ligand	Not Applicable				
FLT3 mutational status	Not Applicable				
Bone marrow aspirate/biopsy and MRD analysis	For D1 of each cycle, if ASP2215 dosing is given in the clinic then the labs can be performed in the clinic. If ASP2215 dosing is not performed in the clinic then lab assessments performed at local facility is acceptable if results can be made available to investigative site.	X	X		
Gilteritinib dispensing	For D1 of each cycle, it is preferred to have ASP2215 dosing provided to the participant in the clinic. If in-clinic visit is not possible, ASP2215 could be delivered from the hospital to the patient.	X			
FLAG administration	Not Applicable				

Table continued on next page

Assessments	Alternate Approach(es)	LTT Cycle 1 & Subsequent Cycles	EOT_2/ Pre-HSCT Visit	28-day Follow-up	Survival Follow-up
	Cycle Day (Visit Window in days) →	**Day 1 (± 2)**	**(± 2)**	**(+ 7)**	**(± 7)**
DLT assessment	Not Applicable				
Clinical Outcome Assessment	Only required for C1D1 of LTT. For D1 of each cycle, if ASP2215 dosing is given in the clinic then the assessment can be performed in the clinic. If ASP2215 dosing is not performed in the clinic then Clinical Outcome Assessment worksheet completed by the participant, parent or observer remotely can be sent to the site.	X			
AE/SAE assessment	Remote/Virtual/Telemedicine Visits allowed if ASP2215 dosing is not performed in the clinic	X	X	X	
Prior and concomitant medications	Remote/Virtual/Telemedicine collection of the Visits allowed for non-dosing visits.	X	X	X	
Subsequent anti-leukemic treatments/outcomes	Remote/Virtual/Telemedicine Visits allowed			X	X
Survival	Remote/Virtual/Telemedicine Visits allowed			X	X

AE: adverse event; C: cycle; CT: computed tomography; D: day; DLT; dose limiting toxicity; ECG: electrocardiogram; ECHO: echocardiogram; EOT: end of treatment; FLAG: fludarabine, cytarabine, and granulocyte colony-stimulating factor; FLT3: FMS-like tyrosine kinase 3; HSCT: hematopoietic stem cell transplant; ICF: informed consent form; LTT: long- term treatment; MRD: minimal residual disease; MUGA: multigated acquisition; PGx: pharmacogenomics; PIA: plasma inhibitory assay; PK: pharmacokinetic; SAE: serious adverse event; WOCBP: women of childbearing potential

13 SPONSOR'S SIGNATURES

Astellas Signatories

(Electronic signatures are attached at the end of the document.)

PPD, Oncology — Medical Science

PPD, Biostatistics — Data Science